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Microsatellite Instability in Chronic Gastritis Associated with Gastric Cancer

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Aim: to assess the microsatellite instability status in gastric mucosa to determine the possibility of its use as a predictive marker of carcinogenesis.
Material and methods. The study included two groups of gastric mucosa specimens: Group 1 — 155 mucosa fragments of the distant tumor growth zone obtained from stomachs resected for malignant neoplasms; Group 2 — 100 fragments with chronic gastritis taken from patients with dyspeptic complaints. 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 Inc., USA), presented using descriptive, analytical statistics.
Results. Immunohistochemical examination revealed 8 microsatellite-unstable cases in gastric mucosa specimens of Group 1 and 0 cases in Group 2 (statistically significant differences, $p = 0.024$). All studied gastric tissue samples were assessed as microsatellite-stable based on PCR results.
Conclusion. Detection of MMR deficiency in gastric mucosa of the distant tumor growth zone and its absence in morphologically comparable specimens obtained from patients with chronic gastritis may be considered as confirmation of the hypothesis of disturbances in the MMR system as an early event in carcinogenesis. This, in turn, may indicate the potential for studying MMR status as a component of a decision support system for assessing the risk of developing microsatellite-associated gastric cancer.
Keywords: microsatellite instability, chronic gastritis, precancerous lesions, intestinal metaplasia, atrophy, gastric cancer, cancer prediction
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Микросателлитная нестабильность при хроническом гастрите, ассоциированном с раком желудка

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Цель: оценка статуса микросателлитной нестабильности в слизистой оболочке желудка для определения возможности ее использования как предиктивного маркера канцерогенеза.
Материал и методы. В исследование включено две группы биоптатов слизистой оболочки желудка: группа 1 — 155 фрагментов слизистой оболочки дистантной зоны опухолевого роста, полученных из желудков, резецированных по поводу злокачественного новообразования; группа 2 — 100 фрагментов с признаками хронического гастрита, взятых у пациентов с диспептическими жалобами. 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 Inc., США), представлены с использованием методов описательной, аналитической статистики.

Результаты. При иммуногистохимическом исследовании во фрагментах слизистой оболочки желудка группы 1 выявлено 8 микросателлит-нестабильных случаев, в группе 2 — 0 случаев (отличия статистически значимы, $p = 0{,}024$). Все исследованные образцы ткани желудка расценили как микросателлит-стабильные по результатам ПЦР.

Выводы. Обнаружение MMR-дефицита в слизистой оболочке желудка дистантной зоны опухолевого роста и его отсутствие в морфологически сопоставимых фрагментах, полученных от пациентов с хроническим гастритом, может рассматриваться как подтверждение гипотезы о нарушениях в системе MMR как о раннем событии в канцерогенезе. Это, в свою очередь, может указывать на наличие потенциала исследования MMR-статуса как компонента системы поддержки принятия решения оценки риска развития рака желудка, ассоциированного с микросателлитной нестабильностью.

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

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Microsatellite instability (MSI) is a consequence of an absolute deficiency or defect in the function of the DNA mismatch repair system (MMR) genes: *MSH2, MSH3, MSH5, MSH6, MLH1, PMS1* (*MLH2*), *MLH3* and/or *PMS2* (*MLH4*) [1, 2]. The MMR system proteins are combined in pairs to detect and eliminate the DNA structure errors appearing during replication in tandem stereotypical repeats of nucleotide groups — microsatellites and leading to the excess chains of unpaired nucleotides formation [2, 3]. The MMR system defect leads to the accumulation of mutations in microsatellites and to a state of genetic instability — hypermutability or MSI [1–4]. The most widely used methods for MSI detection are immunohistochemistry (IHC) and polymerase chain reaction (PCR). Their results may vary due to heterogeneity of IHC staining within a single tumor, false-positive reactions due to defects in the preanalytical stage of IHC studies, missense mutations in the *MLH1* or *MSH6* genes leading to the presence of translated but non-functional proteins with normal affinity for antibodies and the difficulty of interpreting MSH6 expression associated with focal loss of expression and weak staining in tumor cells.

Dysfunction of one or more MMR system genes leading to MMR deficiency (dMMR) most often occurs in tumors due to sporadic or inherited mutations [2, 5, 6]. MSI is detected in a large number of tumors including gastric cancer [5–7]. Numerous genetic abnormalities occur during the gastric cancer development and progression leading to its high molecular diversity [7]. There is a number of gastric cancer molecular classifications. The most commonly used are the Asian Cancer Research Group (ACRG) and the Cancer Genome Atlas (TCGA) classifications [8, 9]. Interestingly, despite their differences both of them distinguish a separate category — gastric cancer with MSI. It is also distinguished in the first Russian gastric cancer molecular classification [10]. This decision is not accidental and is determined by the possibility of targeted therapy for microsatellite-unstable tumors [2, 11].

According to various studies, gastric cancer with MSI accounts for 8 to 25 % of observations depending on the studied cohort of patients and genetic changes detection methods [7, 8]. Along with the molecular phenotype they are united by a number of common features: more often associated with old age (≥ 65 years), female gender

and tumor localization in the middle or lower third of the stomach corpus. The MSI presence does not have reliable phenotypic manifestations, although some researchers report that more often MSI-positive tumors phenotypically correspond to mucinous adenocarcinoma, have pronounced stromal infiltration with lymphocytes and characterized by the presence of pronounced cell polymorphism and expression of mucin 6 [7, 12, 13].

However, a number of studies have demonstrated MSI can be found not only in cancer, but also in the gastric mucosa (GM) precancerous lesions. According to published data, the MSI level in intestinal metaplasia varies from 3.2 to 63.3 %, while in some cases an identical MSI profile is determined in the foci of intestinal metaplasia near an already established tumor and tumor tissue [14–18]. All the studies cited have stated that the MSI level gradually increases as precancerous lesions progress toward intestinal-type gastric cancer. This observation naturally raises the question: could MSI be the cause of tumor transformation of foci of incomplete intestinal metaplasia which is often associated with adenocarcinoma according to epidemiological studies or could genetic instability present in the GM even in the absence of tumor growth?

The aim of the study was to assess the microsatellite instability status in the gastric mucosa to determine the possibility of its use as a predictive marker of carcinogenesis.

Materials and methods

The study was conducted at one stage using the cross-sectional method. The objects of the study were GM biopsy samples. Group 1 included 155 GM specimens of the distant tumor growth zone obtained from stomachs resected for an established gastric cancer. Macroscopically unchanged GM at a distance of ≥ 1.0 cm from the visualized tumor edge was considered as a distant zone. Group 2 consisted of 100 GM specimens with chronic gastritis taken during fibrogastroduodenoscopy in patients with dyspeptic complaints.

In Group 1 the median age of patients (56 women, 99 men) at the time of surgery was 63 years with the minimum value 34 years, the maximum — 82 years. In Group 2 (51 women, 49 men) the age of patients at the time of biopsy sampling was lower ($p = 0.001$) compared to Group 1 with an age median of 58 years, a minimum age of 23 years and a maximum of 81 years. Men were more common in Group 1 ($p = 0.02$).

The sampling points for GM biopsy in both groups complied with the international protocol of the modified Maastricht VI guidelines [19]. The study included GM samples of sufficient volume containing at least 10 glands. The gastric biopsy samples were oriented using an adhesive orientation pad made of cellulose acetate [20]. The correct orientation criterion was sections perpendicular to the GM surface including the gastric ridges, the bottom sections of the glands and the muscularis mucosa. Histological processing of the material, embedding in paraffin, preparation of paraffin sections and staining with hematoxylin and eosin were carried out according to the generally accepted method.

Tissue matrices (multiblocks) were formed using the tissue microarray method [21]. Each multiblock contained 20 GM fragments as well as an external control (a tissue of microsatellite-unstable colon adenocarcinoma) installed in an asymmetric position to allow navigation on the tissue sections. 3–5 μm sections were formed from the multiblocks.

All samples of Groups 1 and 2 stained with hematoxylin and eosin were examined histologically according to the visual analogue scale of the modified Sydney system for chronic gastritis with a semi-quantitative (from 0 to 3) assessment of the severity and activity of inflammation, the presence of atrophy, intestinal metaplasia [22].

Histochemical examination with Alcian blue, iron diamine and PAS reaction staining was used for intestinal metaplasia typing. Alcian blue at pH 2.5 verified the presence of goblet cells regardless of the metaplasia type staining their cytoplasm blue due to the accumulation of mucins. A PAS-positive brush border of tall bordered cells between the goblet cells indicated intestinal metaplasia type I (complete small intestinal-type). Type II intestinal metaplasia (complete colonic-type) was characterized by the blue staining of the apical parts of tall cylindrical cells between the goblet cells with Alcian blue (pH 2.5). Type III (incomplete colonic-type) corresponded to the detection of inclusions brown stained with iron diamine in the apical parts of the cytoplasm of epithelial cells located between goblet cells.

No statistically significant differences were found comparing the distribution of the studied groups samples morphological characteristics (Table 1).

Table 1. Summary morphological characteristics of the corresponding groups samples
Таблица 1. Сводная морфологическая характеристика образцов исследуемых групп

Features ***Показатели***	**Group 1 (gastric mucosa of distant tumor growth zone)** ***Группа 1 (СОЖ дистантной зоны опухолевого роста)*** **$n = 155$**	**Group 2 (gastric ulcer with signs of chronic gastritis)** ***Группа 2 (СОЖ с признаками хронического гастрита)*** **$n = 100$**	**Fisher's exact test** ***Точный критерий Фишера***
Inflammation / *Воспаление*			
0 (no / *нет*)	3 (2 %)	11 (11 %)	$p = 0.79$
1 (mild / *слабое*)	51 (33 %)	54 (54 %)	
2 (moderate / *умеренное*)	88 (57 %)	32 (32 %)	
3 (severe / *выраженное*)	13 (8 %)	3 (3 %)	
Activity / *Активность*			
0 (no / *нет*)	118 (76 %)	84 (84 %)	$p = 0.38$
1 (mild / *слабое*)	33 (21 %)	16 (16 %)	
2 (moderate / *умеренное*)	4 (3 %)	1 (1 %)	
Atrophy / *Атрофия*			
0 (no / *нет*)	62 (40 %)	58 (58 %)	$p = 0.82$
1 (mild / *слабое*)	31 (20 %)	27 (27 %)	
2 (moderate / *умеренное*)	17 (11 %)	10 (10 %)	
3 (severe / *выраженное*)	45 (29 %)	6 (6 %)	
Intestinal metaplasia / *Кишечная метаплазия*			
no / *нет*	79 (51 %)	83 (83 %)	$p = 0.44$
type I / *тип I*	65 (42 %)	12 (12 %)	
type II / *тип II*	8 (5 %)	4 (4 %)	
type III / *тип III*	3 (2 %)	1 (1 %)	

IHC study was performed on paraffin sections of all studied samples using mouse monoclonal antibodies (Diagnostic Bio Systems, USA) to the MMR system proteins: MLH-1 (clone G168-15, dilution 1:50), MSH2 (clone DBM15.82, dilution 1:100), MSH6 (clone 44, 1:50), PMS2 (clone A16-4, ready for use); PolyVuePlus HRP/DAB detection system (Diagnostic Bio Systems, USA). Deparaffinization and rehydration, high-temperature unmasking of antigens using EDTA buffer (pH 8.0), incubation with antibodies were performed according to the manufacturer's protocol. The inflammatory infiltrate lymphocytes were used as an internal positive control of the reaction.

Samples with diffuse nuclear expression of all four MMR system proteins (MLH1, PMS2, MSH2 and MSH6) in epithelial cells and lymphocytes of the GM lamina propria inflammatory infiltrate were considered as a case with an intact MMR system (MMR-positive, pMMR). In the absence of IHC expression of ≥ 1 MMR system protein in the epithelial cells nuclei and the presence of nuclear staining in the inflammatory infiltrate lymphocytes the case was considered as having the MMR system defect (MMR-deficient, dMMR). The staining intensity was not considered.

MSI status was assessed by the multiplex PCR method on preparations from 5 microtome sections from paraffin block with a thickness of 4 μm with the prevention of cross-contamination of the samples. A total of 100 samples from Group 1 and 25 samples from Group 2 were analyzed. DNA extraction was performed using manual sample preparation with the ParaMag nucleic acid extraction kit, followed by multiplex amplification of the extracted DNA to obtain DNA fragments of microsatellites tested for MSI diagnostics (NR-21, NR-24, NR-27, BAT-25, BAT-26). The resulting mixture of amplicons was analyzed by capillary electrophoresis on an Applied Biosystems 3500 genetic analyzer with POP-7 polymer and 50-cm capillaries.

The obtained data were visualized using the Gene Mapper program; microsatellite markers were visualized in the blue (NR-24, BAT-26) and yellow (NR-27, NR-21, BAT-25) detection channels. In this case, instability in one or more markers was considered as the presence of MSI. In the absence of unstable markers the case was assessed as microsatellite-stable.

Statistical data processing was performed in Microsoft Office Excel (Microsoft Corp., USA), Statistica 10.0 package (StatSoft Inc., USA). Descriptive statistics were presented as a median, maximum and minimum values (quantitative data, distribution different from normal) and percentage (nominal data). To analyze the obtained data Pearson's chi-square (χ^2), Fisher's exact test, Mann — Whitney test, contingency tables were used.

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

Results

All GM samples of Group 1 (GM of the distant tumor growth zone) were examined by IHC to evaluate the MMR system proteins. In most cases expression of all 4 proteins was preserved but in 8 cases focal loss of expression and accordingly the MMR system deficiency were determined. In 5 cases loss of expression in the MSH2/MSH6 pair was noted, in 1 case — loss of expression in the MLH1/PMS2 pair, 1 case combined loss of expression in the MLH1/PMS2 pair and disappearance of the MSH6 protein and in 1 case isolated loss of MSH6 protein expression was detected in the focus of intestinal metaplasia (Fig. 1).

In Group 1 of the distant zone GM valid results in the MSI status PCR assessment were obtained for 95 samples. All distant zone GM samples were assessed as microsatellite-stable regardless of the presence of atrophy, presence and type of intestinal metaplasia and severity of inflammation according to the results of the MSI status assessment (Fig. 2).

The distribution of MMR-deficient samples was assessed depending on clinical and morphological characteristics.

The ratio of male to female patients was 3:1 (6 men, 2 women) in the MMR-deficient subgroup, while in the MMR-surplus group it was 1.7:1 (93 men, 54 women), but this difference was statistically insignificant ($p = 0.43$). The age of patients in the dMMR subgroup was slightly higher (from 49 to 76 years, median — 58 years) compared to the pMMR subgroup (minimum value — 34 years, maximum — 82 years, median — 63 years); the differences are also statistically insignificant ($p = 0.65$).

A comparison of the morphological characteristics of the distant zone GM samples was carried out. A predominance of cases with moderate inflammation was noted in both groups, as well as the presence of activity in microsatellite-stable cases and the absence of it in cases with MMR system deficiency; however, the difference in the distribution is statistically insignificant ($p = 0.37$ and $p = 0.25$,

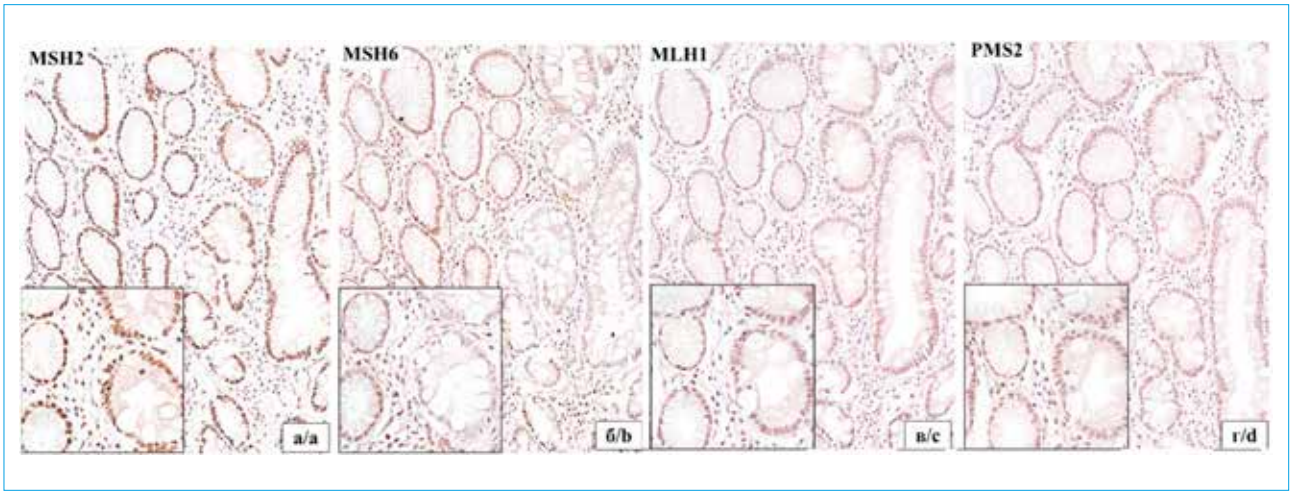

Figure 1. A gastric antral mucosa specimen (distant tumor growth zone) with focal loss of MSH6 protein immunohistochemical expression: a — preserved nuclear expression of MSH2 protein; b — loss of MSH6 protein expression in the intestinal metaplasia focus; c — preserved nuclear expression of MLH1 protein; d — preserved nuclear expression of PMS2 protein. Magnification ×200 (inserts ×400)

Рисунок 1. Фрагмент слизистой оболочки антрального отдела желудка (дистантная зона опухолевого роста) с очаговой утратой иммуногистохимической экспрессии белка MSH6: а — сохранная ядерная экспрессия белка MSH2; б — утрата экспрессии белка MSH6 в очаге кишечной метаплазии; в — сохранная ядерная экспрессия белка MLH1; г — сохранная ядерная экспрессия белка PMS2. Увеличение ×200 (врезки ×400)

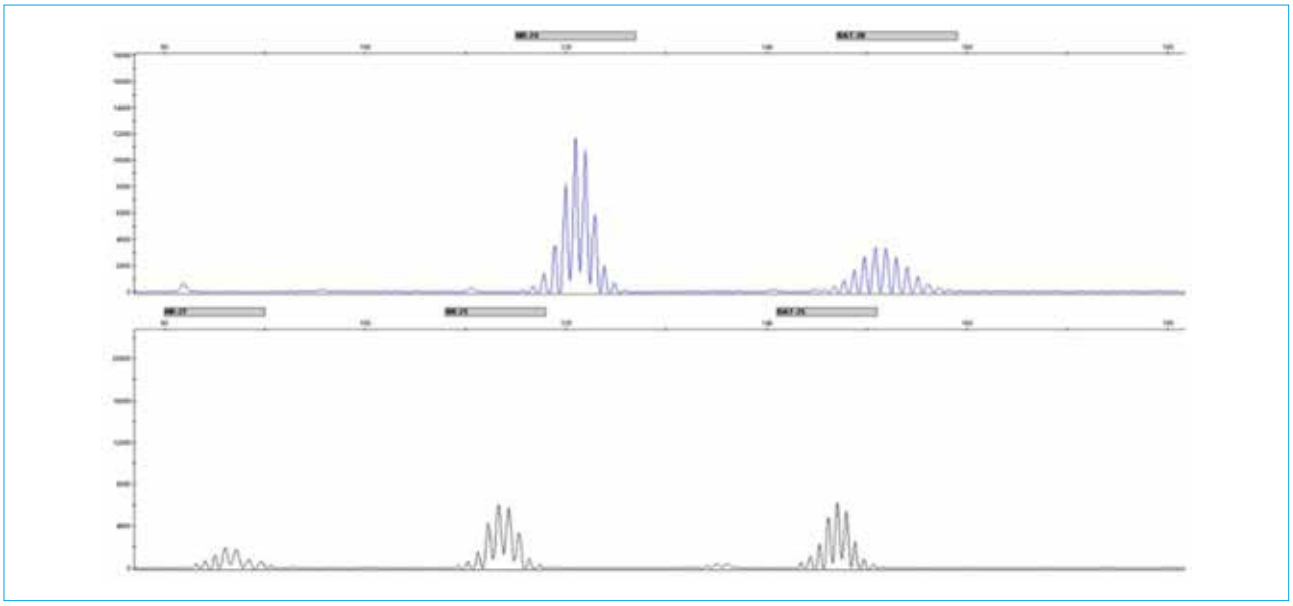

Figure 2. Absence of microsatellite instability (MSS) in a gastric mucosa specimen of the distant zone with the presence of severe atrophy, complete (type I) intestinal metaplasia

Рисунок 2. Отсутствие микросателлитной нестабильности (MSS) в образце слизистой оболочки желудка дистантной зоны с наличием выраженной атрофии желез, полной (тип I) кишечной метаплазии

respectively). Of interest is the statistically significantly higher frequency of incomplete intestinal metaplasia (type II and type III) in samples with MMR deficiency ($p = 0.012$). At the same time the total distribution of atrophy (absolute and relative (metaplastic)) between the subgroups demonstrates comparable values ($p = 0.64$) (Table 2).

Preserved IHC expression of MLH1, MSH2, MSH6, PMS2 proteins and accordingly an MMR excess was noted in all 100 GM samples of the second group regardless of the severity and activity of the inflammation, the presence and severity of absolute atrophy, the presence and type of intestinal metaplasia in chronic non-atrophic and atrophic gastritis (Fig. 3).

The MSI status in GM biopsies in chronic gastritis was assessed using the PCR method in 25 biopsies. Valid results were obtained for 23 biopsies, two cases were excluded from further stages of the study. Based on the results of the analysis, all cases were assessed as microsatellite-stable regardless of the severity and activity of the inflammation, presence and severity of absolute atrophy, presence and type of intestinal metaplasia (Fig. 4).

Thus, pMMR and MSS were noted in all the studied cases of the second group (GM biopsy specimens with chronic gastritis) regardless of the severity of the morphological features of chronic gastritis, including the presence of intestinal metaplasia.

The next step was a comparative assessment of the MMR system status distribution between Group 1 (GM of the distant tumor growth zone) and Group 2 (GM biopsies with chronic gastritis). According to the analysis results, statistically significant differences in the MSI distribution in the GM distant zone group were revealed compared to the chronic gastritis group, where such cases were absent ($p = 0.024$) (Table 3).

Discussion

When comparing the study groups by the main clinical and morphological characteristics, the only indicator with reliable differences was the age and gender distribution: an older age and a predominance of men were noted in the distant tumor growth zone group compared to the chronic gastritis group. No significant differences were found in the chronic gastritis presence and severity including atrophy and/or intestinal metaplasia.

Thus, it can be concluded that the samples of Groups 1 and 2 demonstrate comparable clinical and morphological characteristics that do not allow to divide them into groups of high and low risk of gastric cancer development based on

Table 2. Comparison of microsatellite status with morphological characteristics of the distant tumor growth zone specimens

Таблица 2. Сопоставление микросателлитного статуса с морфологическими характеристиками образцов группы дистантной зоны

Features ***Показатели***	**Microsatellite stable cases (MSS)** ***Микросателлит-стабильные случаи (MSS)*** ***n* = 147**	**Microsatellite unstable cases (MSI)** ***Микросателлит-нестабильные случаи (MSI)*** ***n* = 8**	**Fisher's exact test** ***Точный критерий Фишера***
Inflammation / *Воспаление*			
0 (no / *нет*)	3 (2 %)	0	$p = 0.37$
1 (mild / *слабое*)	50 (34 %)	1 (12.5 %)	
2 (moderate / *умеренное*)	81 (55 %)	7 (87.5 %)	
3 (severe / *выраженное*)	13 (9 %)	0	
Activity / *Активность*			
0 (no / *нет*)	110 (75 %)	8 (100 %)	$p = 0.25$
1 (mild / *слабое*)	33 (22 %)	0	
2 (moderate / *умеренное*)	4 (3 %)	0	
3 (severe / *выраженное*)	0	0	
Atrophy / *Атрофия*			
0 (no / *нет*)	60 (41 %)	2 (25 %)	$p = 0.64$
1 (mild / *слабое*)	30 (20 %)	1 (12.5 %)	
2 (moderate / *умеренное*)	16 (11 %)	1 (12.5 %)	
3 (severe / *выраженное*)	41 (28 %)	4 (50 %)	
Intestinal metaplasia / *Кишечная метаплазия*			
no / *нет*	76 (52 %)	3 (37.5 %)	$p = 0.012$
type I / *тип I*	62 (42 %)	3 (37.5 %)	
type II / *тип II*	7 (5 %)	1 (12.5 %)	
type III / *тип III*	2 (1 %)	1 (12.5 %)	

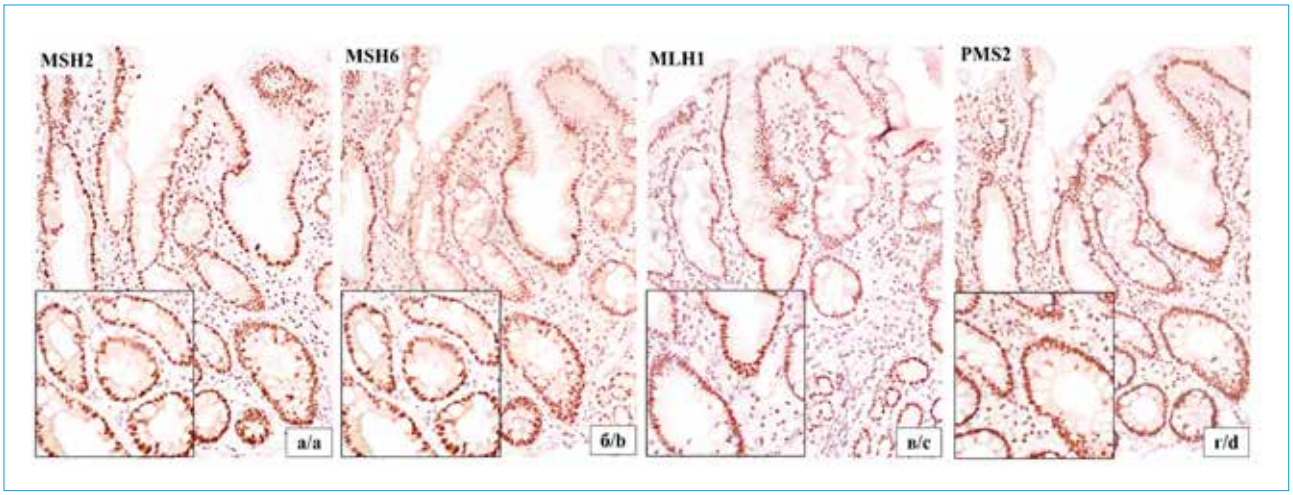

Figure 3. A gastric antral mucosa specimen with complete (type I) intestinal metaplasia: a — preserved nuclear expression of MSH2 protein; b — preserved nuclear expression of MSH6 protein; c — preserved nuclear expression of MLH1 protein; d — preserved nuclear expression of PMS2 protein. Magnification ×200 (inserts ×400)

Рисунок 3. Фрагмент слизистой оболочки антрального отдела желудка с полной (тип I) кишечной метаплазией: а — сохранная ядерная экспрессия белка MSH2; б — сохранная ядерная экспрессия белка MSH6; в — сохранная ядерная экспрессия белка MLH1; г — сохранная ядерная экспрессия белка PMS2. Увеличение ×200 (врезки ×400)

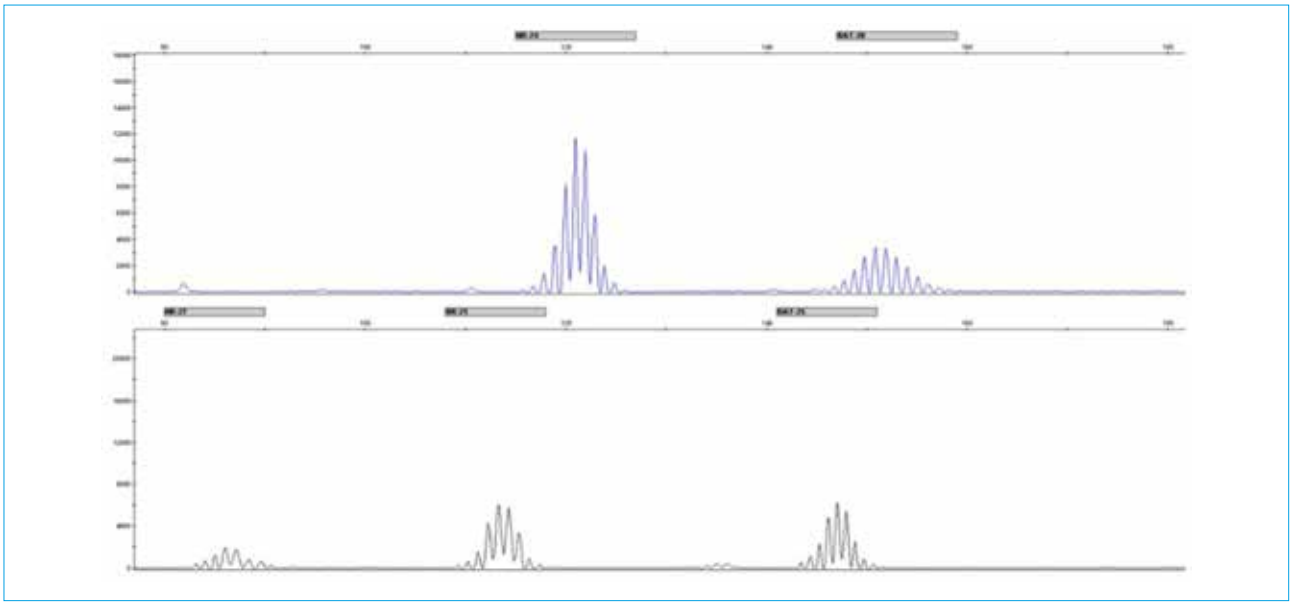

Figure 4. Absence of microsatellite instability (MSS) in a chronic gastritis mucosa specimen with mild inflammation without activity, with the presence of mild atrophy without intestinal metaplasia

Рисунок 4. Отсутствие микросателлитной нестабильности (MSS) в биоптате слизистой оболочки желудка при хроническом гастрите со слабым воспалением без признаков активности, с наличием слабой атрофии желез без кишечной метаплазии

Table 3. Comparison of MMR status of distant zone samples and chronic gastritis mucosa specimens

Таблица 3. Сопоставление MMR-статуса образцов дистантной зоны и биоптатов слизистой оболочки желудка при хроническом гастрите

	Group 1 (gastric mucosa of distant tumor growth zone) ***Группа 1 (СОЖ дистантной зоны опухолевого роста)***	**Group 2 (gastric ulcer with signs of chronic gastritis)** ***Группа 2 (СОЖ с признаками хронического гастрита)***	**Fisher's exact test** ***Точный критерий Фишера***
dMMR	8	0	$p = 0.024$
pMMR	147	100	

the atrophy and intestinal metaplasia assessment. This, in turn, allows us to state that the MMR status difference may be due to the association of distant zone samples with adenocarcinoma, which a priori marks a higher risk of gastric cancer development.

The absence of MSI cases in the chronic gastritis group to some extent diverges from the available publications indicating MSI in intestinal metaplasia in the absence of tumor growth. However, along with such studies there are also publications indicating the absence of MSI in GM precancerous lesions even in the presence of an established tumor, which demonstrates the inconsistency and ambiguity of existing data [14, 15].

In the distant tumor growth zone GM samples with MSI detected both with IHC and PCR an additional IHC assessment of protein expression and MSI status was performed using PCR in tumor tissue. The result was the MMR system deficiency detection in 7 of 8 samples and MSI detection in 6 cases, respectively. All detected dMMR and MSI cases were histologically consistent with well- or poorly differentiated tubular adenocarcinoma. In the only case that demonstrated microsatellite stability in the PCR study and pMMR on the IHC assessment of the tumor tissue was signet ring cell carcinoma. This case of the MMR system deficiency in the distant zone GM and stability in the tumor tissue should probably be interpreted as an example of parallel development of different gastric carcinogenesis molecular cascades considering the existing information on the differences in the molecular events underlying the development

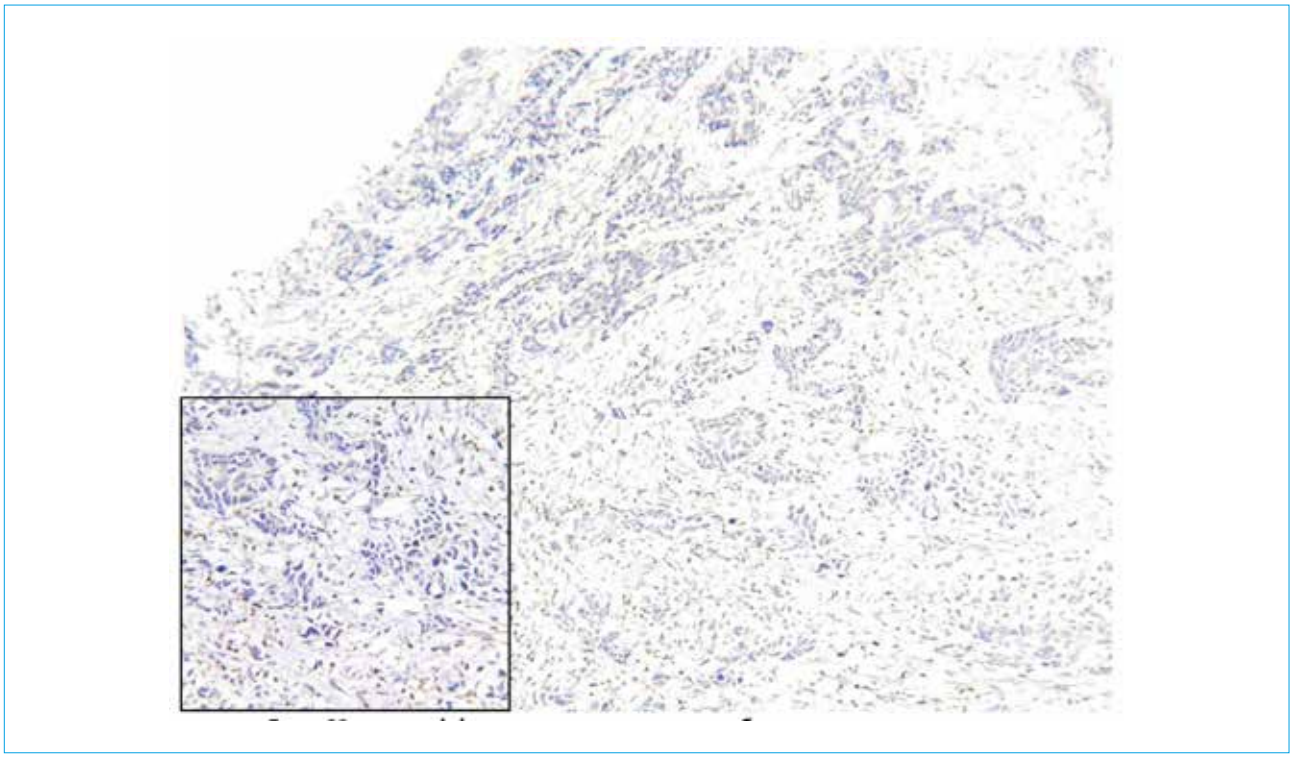

Figure 5. Poorly differentiated tubular adenocarcinoma. Loss of MSH6 protein expression. Magnification ×200 (insert ×400). Case corresponding to distant zone gastric mucosa specimen with focal loss of MSH6 protein expression

Рисунок 5. Низкодифференцированная тубулярная аденокарцинома. Утрата экспрессии белка MSH6. Увеличение ×200 (врезка ×400). Случай, соответствующий образцу слизистой оболочки дистантной зоны с очаговой утратой экспрессии белка MSH6

of gastric discohesive carcinomas and MSI-associated cancer.

The second case characterized by a microsatellite-stable status with a deficiency of the MMR system was due to an isolated disappearance of MSH6 protein expression corresponded to a poorly differentiated tubular adenocarcinoma (Fig. 5).

The MSI absence according to the PCR in this and other samples that showed a deficiency of the MMR system, but microsatellite stability, can probably be partly explained by the replacement of their functions by other proteins of the MMR system and accordingly the prevention of the microsatellite repeats formation. Thus, a number of publications reflect the discrepancy between MMR and MSI status associated with mutations in the *MSH6* gene leading accordingly to the loss of its protein IHC expression [23–25]. At the same time the absence of microsatellite instability in the PCR study was noted comparable with our results.

The distribution of the MMR-deficient cases significantly differs in the distant zone GM group from the results obtained in the chronic gastritis group with comparable morphological changes. The MMR system deficiency in distant zone GM according to the tumor field theory associated with adenocarcinoma can be considered as a confirmation of the hypothesis that the MMR system disturbances are an early event in carcinogenesis and indicate a high risk of developing MSI-associated gastric cancer.

Of interest is the polymorphism of the NR-21 marker, detected in one sample of distant zone tumor growth with chronic gastritis (mild inflammation and activity, moderate atrophy of glands with the presence of complete intestinal metaplasia) (Fig. 6).

This finding is consistent with the data available in a comparable study on the predominant detection of NR-21 marker polymorphism in GM samples in chronic gastritis in the absence of MSI [26]. The tumor fragment corresponding to this sample was histologically assessed as poorly differentiated tubular adenocarcinoma and also turned out to be microsatellite-stable. Both samples had preserved expression of all 4 proteins in the IHC study and accordingly were assessed as pMMR.

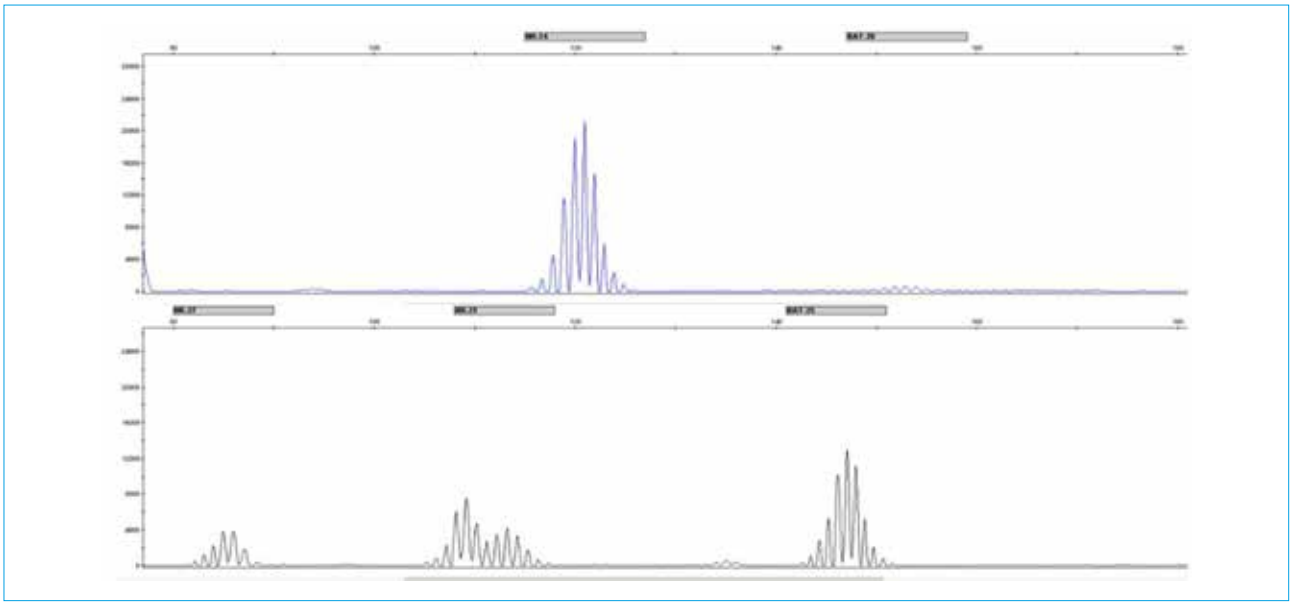

Figure 6. A gastric mucosa specimen of the distant tumor growth zone with moderate atrophy, complete (type I) intestinal metaplasia. MSS in the presence of NR-21 marker polymorphism

Рисунок 6. Образец дистантной зоны опухолевого роста с наличием умеренной атрофии желез, полной (тип I) кишечной метаплазии. MSS при наличии полиморфизма маркера NR-21

Cases of marker polymorphism can graphically simulate MSI which in some cases can be mistakenly recognized as the presence of MSI and thus be the basis for the false-positive cases occurrence if interpreted incorrectly.

Thus, the obtained results suggest that high accuracy and specificity make the determination of microsatellite status by PCR the optimal choice for diagnosing MSI in tumor tissue for the purpose of targeted therapy. At the same time, the possibility of using PCR method for screening assessment of the gastric cancer risk development at early, possibly reversible stages of the carcinogenesis cascade, is excluded, whereas IHC allows to identify the MMR system deficiency in early precancerous lesions such as chronic atrophic gastritis. It indicates the possible potential of IHC assessment of the MMR system as a component of a decision support system for assessing the risk of developing MSI-associated gastric cancer.

Conclusion

Detection of MMR deficiency in the distant tumor growth zone gastric mucosa and its absence in morphologically comparable specimens obtained from patients with chronic gastritis may be considered as confirmation of the hypothesis of disturbances in the MMR system as an early event in carcinogenesis. This, in turn, may indicate the potential for studying MMR status as a component of a decision support system for assessing the risk of developing microsatellite-associated gastric cancer.

Microsatellite instability is a consequence and, in a sense, a secondary event to the disruption of the MMR system proteins; its PCR assessment demonstrates less potential as a method for the risk of developing MSI-associated gastric cancer evaluation compared to immunohistochemical assessment of the expression of MMR proteins according to the obtained data.

References / Литература

1. *Luchini C., Bibeau F., Ligtenberg M.J.L., Singh N., Nottegar A., Bosse T., et al.* ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. *Ann Oncol*. 2019;30(8):1232–43. DOI: 10.1093/annonc/mdz116
2. *Yamamoto H., Watanabe Y., Arai H., Umemoto K., Tateishi K., Sunakawa Y.* Microsatellite instability: A 2024 update. *Cancer Sci.* 2024;115(6):1738–48. DOI: 10.1111/cas.16160
3. *Olave M.C., Graham R.P.* Mismatch repair deficiency: The what, how and why it is important. *Genes Chromosomes Cancer.* 2022;61(6):314–21. DOI: 10.1002/gcc.23015

4. *Randrian V., Evrard C., Tougeron D.* Microsatellite instability in colorectal cancers: Carcinogenesis, neo-antigens, immuno-resistance and emerging therapies. *Cancers*. 2021;13(12):3063. DOI: 10.3390/cancers13123063
5. *Le D.T., Durham J.N., Smith K.N., Wang H., Bartlett B.R., Aulakh L.K., et al.* Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade. *Science*. 2017;357(6349):409–13. DOI: 10.1126/science.aan6733
6. *Le D.T., Uram J.N., Wang H., Bartlett B.R., Kemberling H., Eyring A.D., et al.* PD-1 blockade in tumors with mismatch-repair deficiency. *N Engl J Med*. 2015;372(26):2509–20. DOI: 10.1056/NEJMoa1500596
7. *The WHO Classification of Tumours Editorial Board, eds.* Digestive system tumours. WHO Classification of Tumours. 5th ed. IARC, 2019.
8. *Cancer Genome Atlas Research Network.* Comprehensive molecular characterization of gastric adenocarcinoma. *Nature*. 2014;513(7517):202–9. DOI: 10.1038/nature13480
9. *Cristescu R., Lee J., Nebozhyn M., Kim K.M., Ting J.C., Wong S.S., et al.* Molecular analysis of gastric cancer identifies subtypes associated with distinct clinical outcomes. *Nat Med*. 2015;21(5):449–56. DOI: 10.1038/nm.3850
10. *Данилова Н.В., Чайка А.В., Хомяков В.М., Олейникова Н.А., Андреева Ю.Ю., Мальков П.Г.* Микросателлитная нестабильность в раке желудка — предиктор благоприятного прогноза. *Архив патологии.* 2022;84(6):5–15. [*Danilova N.V., Chayka A.V., Khomyakov V.M., Oleynikova N.A., Andreeva Yu.Yu., Malkov P.G.* Microsatellite instability in gastric cancer is a predictor of a favorable prognosis. *Arkhiv Patologii.* 2022;84(6):5–15. (In Russ.)]. DOI: 10.17116/patol2022840615
11. *Ajani J.A., D'Amico T.A., Bentrem D.J., Chao J., Cooke D., Corvera C., et al.* Gastric cancer, version 2.2022, NCCN Clinical Practice Guidelines in Oncology. *J Natl Compr Canc Netw*. 2022;20(2):167–92. DOI: 10.6004/jnccn.2022.0008
12. *Kim J.-Y., Shin N.R., Kim A., Lee H.J., Park W.Y., Kim J.Y., et al.* Microsatellite instability status in gastric cancer: A reappraisal of its clinical significance and relationship with mucin phenotypes. *Korean J Pathol*. 2013;47(1):28–35. DOI: 10.4132/KoreanJPathol.2013.47.1.28
13. *Mathiak M., Warneke V.S., Behrens H.-M., Haag J., Böger C., Krüger S., et al.* Clinicopathologic characteristics of microsatellite instable gastric carcinomas revisited: Urgent need for standardization. *Appl Immunohistochem Mol Morphol*. 2017;25(1):12–24. DOI: 10.1097/PAI.0000000000000264
14. *Fang D.C., Jass J.R., Wang D.X., Zhou X.D., Luo Y.H., Young J.* Infrequent loss of heterozygosity of APC/MCC and DCC genes in gastric cancer showing DNA microsatellite instability. *J Clin Pathol*. 1999;52(7):504–8. DOI: 10.1136/jcp.52.7.504
15. *Hamamoto T., Yokozaki H., Semba S., Yasui W., Yunotani S., Miyazaki K., et al.* Altered microsatellites in incomplete-type intestinal metaplasia adjacent to primary gastric cancers. *J Clin Pathol*. 1997;50(10):841–6. DOI: 10.1136/jcp.50.10.841
16. *Kobayashi K., Okamoto T., Takayama S., Akiyama M., Ohno T., Yamada H.* Genetic instability in intestinal metaplasia is a frequent event leading to well-differentiated early adenocarcinoma of the stomach. *Eur J Cancer*. 2000;36(9):1113–9. DOI: 10.1016/s0959-8049(00)00066-6
17. *Leung W.K., Kim J.J, Kim J.G., Graham D.Y., Sepulveda A.R.* Microsatellite instability in gastric intestinal metaplasia in patients with and without gastric cancer. *Am J Pathol*. 2000;156(2):537–43. DOI: 10.1016/S0002-9440(10)64758-X
18. *Garay J., Bravo J.C., Correa P., Schneider B.G.* Infrequency of microsatellite instability in complete and incomplete gastric intestinal metaplasia. *Hum Pathol*. 2004;35(1):102–6. DOI: 10.1016/j.humpath.2003.08.023
19. *Malfertheiner P., Megraud F., Rokkas T., Gisbert J.P., Liou J.M., Schulz C., et al.; European Helicobacter and Microbiota Study group.* Management of *Helicobacter pylori* infection: The Maastricht VI/Florence consensus report. *Gut*. 2022. DOI: 10.1136/gutjnl-2022-327745
20. *Парыгина М.Н., Поморгайло Е.Г., Шиманская А.Г., Панюшкин Л.В., Мозговой С.И., Кононов А.В.* Сравнительная оценка способов ориентировки биопсийного материала слизистой оболочки желудка. *Вестник новых медицинских технологий. Электронное издание.* 2020;14(1):151–6. [*Parygina M.N., Pomorgailo E.G., Shimanskaya A.G., Panyushkin L.V., Mozgovoy S.I., Kononov A.V.* A comparative assessment of technics for the orientation of gastric biopsy samples. *Journal of New Medical Technologies. eEdition* 2020;14(1):151–6. (In Russ.)]. DOI: 10.24411/2075-4094-2020-16588
21. *Koo M., Squires J.M., Ying D., Huang J.* Making a tissue microarray. *Methods Mol Biol*. 2019;1897:313–23. DOI: 10.1007/978-1-4939-8935-5_27
22. *Dixon M.F., Genta R.M., Yardley J.H., Correa P.* Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994. *Am J Surg Pathol*. 1996;20(10):1161–81. DOI: 10.1097/00000478-199610000-00001
23. *Puliga E., Corso S., Pietrantonio F., Giordano S.* Microsatellite instability in gastric cancer: Between lights and shadows. *Cancer Treat Rev*. 2021;95:102175. DOI: 10.1016/j.ctrv.2021.102175
24. *Luchini C., Bibeau F., Ligtenberg M.J.L., Singh N., Nottegar A., Bosse T., et al.* ESMO recommendations on microsatellite instability testing for immunotherapy in cancer, and its relationship with PD-1/PD-L1 expression and tumour mutational burden: A systematic review-based approach. *Ann Oncol*. 2019;30(8):1232–43. DOI: 10.1093/annonc/mdz116
25. *van der Werf-'t Lam A.S., Terlouw D., Tops C.M., van Kan M.S., van Hest L.P., Gille H.J.P., et al.* Discordant staining patterns and microsatellite results in tumors of MSH6 pathogenic variant carriers. *Mod Pathol*. 2023;36(9):100240. DOI: 10.1016/j.modpat.2023.100240
26. *Najjar Sadeghi R., Sahba N., Vahedi M., Reza Mohebbi S., Reza Zali M.* Association of intron and exon polymorphisms of p53 gene in Iranian patients with gastritis. *Gastroenterol Hepatol Bed Bench*. 2013;6 (Suppl 1): S45–51.

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