

## Article

# Differentiation between Gastric and Colorectal Adenocarcinomas Based on Maspin, MLH1, PMS2 and K-Ras Concentrations Determined Using Stochastic Sensors

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**Abstract:** Background: Gastrointestinal adenocarcinomas are a worldwide and some of the most important causes of death related to cancers. MLH1, PMS2, and K-Ras are some of the main molecules responsible for the control of cellular proliferation. They are widely used as biomarkers for the evaluation of the features of tumoral processes and the clinicopathological characteristics. They depend on the type of cells implied in the tumoral process, and it can be observed in the concentrations of them in different biological fluids. Maspin, also known as peptidase inhibitor 5 or serpin B5 is a tumor suppressor which inhibits invasion and angiogenesis and also regulates apoptosis, but it can also present oncogenic activity depending on tumor location and histology and on the subcellular maspin localization. Its correlations with gastric and colorectal carcinomas have been emphasized in a series of articles, and in this work, a method is used to quantify the concentrations of maspin in three biological fluids, allowing correlations with pathological features. Methods: Patients with their clinical and pathological features were selected from the database of the project GRAPHSENSGASTROINTES and used accordingly with the Ethics committee approval nr. 32647/2018 awarded by the County Emergency Hospital from Targu-Mures. Three kinds of samples have been analyzed (saliva, whole blood, and urine) using a stochastic method using stochastic microsensors. Results: The results obtained using stochastic sensors were correlated with the location of cancer, and there have been elaborated a series of criteria to differentiate gastric cancers from colorectal ones. Conclusions: There can be differentiation between the two types of cancers by using the concentrations of MLH1, PMS2, and K-Ras in saliva and urine samples or the levels of maspin in whole blood and urine or in whole blood, urine, and saliva. The data analysis led to a series of criteria for evaluation of the cancer location. Using only MLH1 and PMS2 concentrations in one of the two kinds of samples was only indicative and did not cover most cases. The use of the criteria only for MLH1 and PMS2 increased the probability of finding out the location, but the best results require the concentrations of K-Ras in the two kinds of samples as additional criteria.

**Keywords:** gastric cancer; colorectal cancer; maspin; MLH1; PMS2; KRAS; stochastic method; digital pathology; fast diagnosis



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## 1. Introduction

Maspin, a protease also known as peptidase inhibitor 5 or serpin B5 is a member of the serpin (serine protease inhibitor) superfamily [1,2] whose gene was described earlier [3–5]. The chemical structure of maspin is similar to that of  $\alpha_1$ -antitrypsin but also with the neutrophil-monocyte elastase inhibitors [2,5,6]. In terms of biochemistry, maspin is an

ovalbumin-like soluble protein with nine alpha helices (A–I), three  $\beta$ -sheets (named A, B, C), and eight cysteine residues. These aspects give its structural properties and make it determinable using the stochastic method [5,6]. Maspin can be found in epithelial structures and also in non-epithelial ones [3]. In pathology practice, it is used to mark squamous epithelium, urothelium, basal cells, fibroblasts, myoepithelial cells, mucosa of the gastrointestinal tract, and endothelial cells, mainly in the cytoplasm, in secretory vesicles and in the cell membrane [1,2,4,6–9]. In tumor pathology, maspin is a tumor suppressor that inhibits invasion and angiogenesis and also regulates apoptosis [3,7,8], but it can also present oncogenic activity depending on tumor location and histology [6] and on the subcellular maspin localization [3,8,9]. Both in gastric carcinomas and colorectal carcinomas (CRC), cytoplasmic immunohistochemical maspin positivity is considered to be an indicator for low metastatic risk and for late recurrence, but nuclear immunohistochemical positivity is related to early recurrence, especially in advanced stage carcinomas [8,10–19]. In early stages, nuclear maspin marking is related to the risk for lymph node metastases in early stages [16]. In human gastric cancer, maspin expression is considered to be an effective and objective biomarker to reveal biological behaviors. Maspin immunohistochemical expression presents a negative correlation with metastasis, invasive depth, microvessel density, and histological grade but a positive correlation with the expression of Caspase-3 [18,19]. In human colorectal carcinomas, maspin immunohistochemical cytoplasmic positivity is associated with negativity for the p53 protein [11–13]. Maspin is co-expressed with carcinoembryonic antigen (CEA) both in the cytoplasm and cell membrane of the tumor cells, being considered a CEA-interacting biomarker [6]. Maspin presents a sequential decreasing expression rate from adenomas to metastatic colorectal carcinomas and also an inverse correlation with the p53 and microvessel density [20–22].

Cancer biomarkers represent biomolecules that are produced by the tumor's cells or in response to the tumor by other cells. They relate to cells' unique molecular signature and other identifiable features, which explains their clinical utility for cancer patients. Mismatch repair deficiency (dMMR) involves the accumulation of DNA sequence errors located in microsatellite regions, which result in microsatellite instability (MSI), and it is caused by the inactivation of MLH1, MSH2, MSH6, and PMS2 genes, which are the mismatch repair (MMR) genes [23]. The MLH1 gene provides information for making a protein, which plays an essential role in the repairing process of DNA. MLH1 helps fix errors made during DNA replication in preparation for cell division. In vivo, the MLH1 protein joins PMS2 (from the PMS2 gene) in order to form a dimer that coordinates the activities of other proteins, which repair the errors by removing the sections of DNA and replacing them with a correct DNA sequence [24].

As a component of the heterodimer called MutL $\alpha$  (MLH1 + PMS2), the PMS2 protein (postmeiotic segregation increased 2) is the central element in the mechanism of postreplicative human DNA mismatch repair (MMR). Lynch syndrome is the most common cancer predisposition syndrome in human beings, and it is caused by heterozygous germline mutations related to MMR genes [25].

The KRAS gene provides instructions for the K-Ras protein, which is part of the RAS/MAPK signaling pathway, relaying signals from outside the cell to the cell's nucleus, inducing the cell's growth and division [26]. The KRAS gene is an oncogene from the Ras family that has the potential to cause normal cells to become cancerous when it is mutated [27]. Gastric cancer, one of the major causes of death related to cancers, can present MSI, and in this case, it associates certain clinicopathological features like no lymph metastasis, older age of presentation, early disease staging, and also a better prognosis [28]. For gastric cancer, unfortunately, the recurrence rate is high, and it requires preoperative and/or postoperative chemotherapy, but there have been identified a few predictive biomarkers for the response to chemotherapy [29].

In gastric cancer, the loss of MLH1 expression is a negative predictor of prognostic for the benefit of the fluorouracil-based adjuvant chemotherapy but a significant predictive biomarker of good prognosis [30]. In colorectal cancer, MSI-H (high microsatellite

instability) is a specific feature of hereditary nonpolyposis colorectal cancer (HNPCC). MSI-H status is associated with a good prognosis but a poor response to fluorouracil-based adjuvant chemotherapy [29].

These three biomarkers evaluate the capacity of the cells to respond to the tumoral process, and their release into biological fluids depends on the cellular type. As a consequence, their concentrations in different fluids can be correlated with the location of the tumor.

This paper aims to present two different ways to differentiate gastric cancers from colorectal cancers based on the concentration of maspin, MLH1, PMS2, and KRAS proteins in urine, whole blood, and saliva. There will be presented two different algorithms, which are the result of two studies. The first one is based on maspin levels in different biological fluids, and the second one is based on MLH1, PMS2, and K-Ras protein levels in different biological fluids. The proposed methods are non-invasive, present cost-effectiveness, and the results can be obtained in a very short time. Moreover, the levels of the proposed biomarkers can be further used for establishing the clinicopathological features with high probability even before surgery and pathology processing. All the advantages make this method very useful in practice, and it can give the surgeon and the oncologist very useful information for patient's management.

## 2. Results and Discussion

### 2.1. Differentiation Based on Maspin Levels

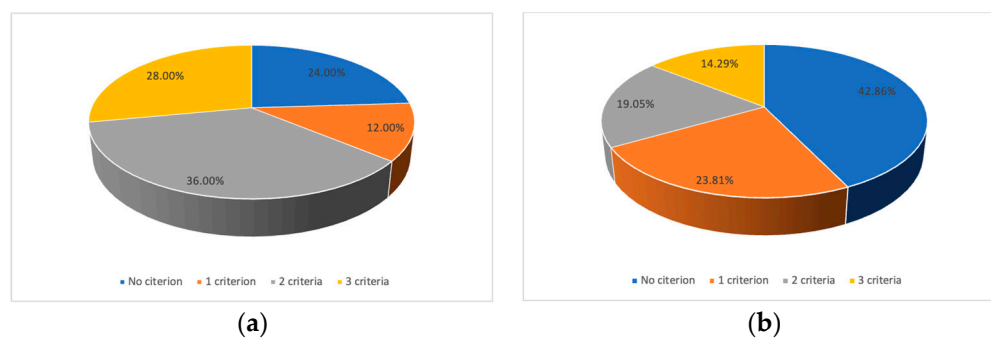
There have been proposed two algorithms for differentiating gastric and colorectal carcinomas, first by using online whole blood maspin concentration and urine maspin concentration, and the second by adding the maspin concentration in saliva.

#### 2.1.1. Differentiation Using Only Whole Blood and Urine Maspin Concentrations

By analyzing available data, there have been proposed the next three criteria for differentiation:

1. whole blood maspin concentration  $< 100$  pg/mL;
2. the ratio of whole blood maspin concentration and urine maspin concentration  $< 0.3$ ;
3. urine maspin concentration  $> 300$  pg/mL.

Using these criteria, there have been concluded that 16 out of the 25 gastric cancer patients (64%) meet at least two criteria mentioned above, and 7 out of 25 (28%) meet all three criteria mentioned above, while for colorectal cancer patients, only 7 out of 21 (33.3%) meet the at least two criteria mentioned above and only 3 out of 21 patients (14.3%) meet all three criteria. On the other side, 6 out of 25 gastric cancer patients (24%) meet no criteria, while 9 out of 21 colorectal cancer patients (42.9%) meet no criteria. These results are observed in Figure 1a (for gastric cancer patients) and Figure 1b (for colorectal cancer patients).



**Figure 1.** Gastric cancer patients in the first algorithm (a) and colorectal cancer ones in the first algorithm (b).

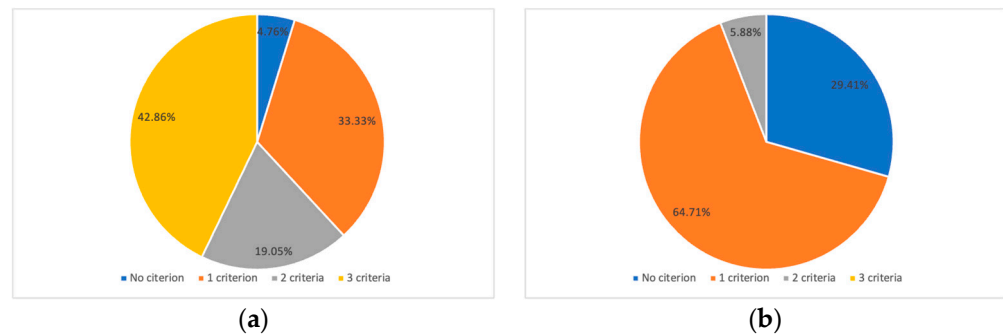
The proposed algorithm can only orientate, but for better results, it is necessary to use the saliva sample, which has a second proposed algorithm.

2.1.2. Differentiation Using All the Three Maspin Levels in Urine, Whole Blood and Saliva

In this case, there are also three criteria as follows:

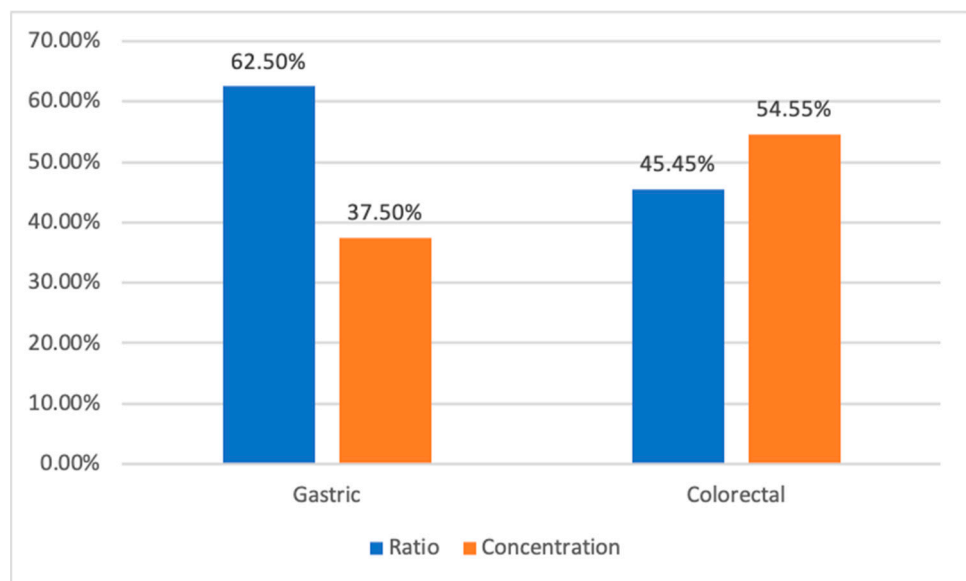
1. whole blood maspin concentration < 100 pg/mL;
2. the ratio of saliva maspin concentration and urine maspin concentration < 0.3;
3. the ratio of saliva maspin concentration and whole blood maspin concentration < 1.3.

Using these criteria, there have been obtained that 13 out of the 21 gastric cancer patients (61.9%) have at least two criteria and 9 out of 21 (42.9%) have all three criteria, while for colorectal cancer patients, only 1 out of 17 (5.9%) have at least two criteria and no patient has all the three criteria. On the other side, only 1 out of 21 gastric cancer patients (4.8%) have no criteria, while 5 out of 17 (29.4%) have no criteria. These results are observed in Figure 2a (for gastric cancer patients) and Figure 2b (for colorectal cancer patients).



**Figure 2.** Gastric cancer patients in the second algorithm (a) and colorectal cancer ones in the second algorithm (b).

The main problem remains for the cases where there is only one criterion. In this case, are 33.3% of the gastric cancer patients and 64.7% of the colorectal cancer patients. For this situation, there is proposed the next classification of patients—the patients where the only criterion is a ratio and the ones where the only criterion is the whole blood maspin concentration. For 5 out of 8 gastric cancer patients (62.5%), the only criterion is a ratio, while for 6 out of 11 colorectal cancer patients (54.5%), the only criterion is whole blood maspin concentration < 100 pg/mL. These results are given in Figure 3.



**Figure 3.** Gastric and colorectal cancer patients with one criterion.

In conclusion, if there are respected 2 or 3 criteria or, in the case of one criterion, it is a ratio, the tumor location is probably a gastric one. Otherwise, it is a colorectal one.

### 2.1.3. Further Uses of Maspin Levels for Clinicopathological Features

Starting from our previous work, it is known that a series of useful information on the tumor process can be obtained from maspin concentrations in different biological fluids. After establishing the location with a good probability and from other imaging tests, a tissue sample can be obtained and analyzed for maspin concentration in order to gain more information about the pathological features. In this work, there are proposed a series of steps for both types of cancer:

#### 1. First step—differentiation between gastric and colorectal adenocarcinomas:

Using the information discussed above, there is a high probability that the tumor is a gastric or a colorectal one.

#### 2. Second step—the location of the tumor in the organ where it is located:

For gastric cancer patients, there are necessary urine and saliva samples. For a proximal location, urine maspin concentrations are  $>150$  pg/mL in 5 out of 8 patients (62.5%), and saliva maspin concentrations are  $<30$  pg/mL in 6 out of 8 patients (75%).

For colorectal cancer patients, the most important for this step is the whole blood maspin concentration. For ascending and transverse colon, seven out of nine patients (77.8%) have whole blood maspin concentrations  $> 180$  pg/mL, while for the left colon, 13 of 22 patients (59.09%) are related to whole blood maspin concentrations  $< 180$  pg/mL.

After confirming the location with imaging techniques, a tissue sample maspin concentration is extremely useful for other features.

#### 3. Third step—maximum diameter of the tumor:

For gastric cancer patients, the concentrations that are needed are whole blood, tissue, and urine. There is proposed the maspin equation given below, which approximates the maximum diameter:

$$S \text{ (pg/mL)} = 2.1 \times C_1 \text{ (pg/mL)} + 1.45 \times C_2 \text{ (pg/mL)} + 1.05 \times C_3 \text{ (pg/mL)} \quad (1)$$

where  $C_1$  is whole blood maspin concentration,  $C_2$  is tissue maspin concentration, and  $C_3$  is urine maspin concentration.

The maximum diameter can be obtained from  $S$  by using the next formulae ( $R^2 = 0.6191$ ):

$$D \text{ (mm)} = (1061.2 - S \text{ (pg/mL)})/11.361 \quad (2)$$

For colorectal cancer patients, the concentration used is the tissue sample one. For 22 out of 29, it was obtained a linear regression for maximum diameter as a function of tissue sample maspin concentration ( $R^2 = 0.6765$ ) by using the next formulae:

$$D \text{ (mm)} = 15.969 + 0.089 \times C \text{ (pg/mL)} \quad (3)$$

where  $C$  is tissue sample maspin concentration.

#### 4. Fourth step—a first microscopic feature:

For gastric cancer patients, the presence or absence of mucus is related to saliva and whole blood concentrations. In saliva, 60% of values associated with the presence of mucus are under 20 pg/mL, while in the absence of mucus, there are only 23.53%. Regarding whole blood samples, 60% of values associated with the presence of mucus are under 25 pg/mL, while in the absence of mucus, there is only 25%.

Colorectal cancer patients can be evaluated by using whole blood and saliva maspin concentrations.

It is observed that most patients with a whole blood maspin concentration  $< 160$  pg/mL have a budding of 3 or 4 (85.71%), so a smaller value can be correlated with a bigger budding

value. On the other side, 7 of 9 (77.77%) of the patients with budding of 0 and 1 have a value of maspin > 160 pg/mL. For a maspin value > 160 pg/mL, the only assumption can be that it is correlated to budding 1 and 2 (11 of 15 patients).

For saliva samples, a greater value is associated with a higher budding. For a budding value of 3, it was observed that 75% of the patients were related to saliva maspin levels over 250 pg/mL, and only 25% of them presented levels under 250 pg/mL. On the other hand, for the budding value of 1, 80% of colorectal cancer patients have under 250 pg/mL, and only 20% of them have over 250 pg/mL.

#### 5. Fifth step—a second microscopic feature:

For gastric cancer patients, the whole blood maspin concentration is related to vascular, lymphatic, and perineural invasions.

For vascular invasion, 81.82% of the patients with invasion have maspin concentrations < 50 pg/mL, while 66.67% of the patients with no invasion have maspin concentrations > 50 pg/mL.

For lymphatic invasion, the proposed Lymphatic Maspin Equation is as follows:

$$\text{Dist} = |180 - C \text{ (pg/mL)}| / 80 \quad (4)$$

where C is whole blood maspin concentration.

The parameter Dist is <1 for 66.66% of the patients with no lymphatic invasion and >1 for 88.24% of the patients with lymphatic invasion.

For perineural invasion, 80% of the patients with invasion have concentrations < 65 pg/mL, while 62.5% of the patients with no invasion have concentrations > 65 pg/mL.

For colorectal cancer patients, the molecular subtype is related to whole blood maspin concentration, but the only observations are that concentrations < 100 pg/mL are related to epithelial molecular subtype and all the hybrid and mesenchymal molecular subtypes are related to concentrations > 100 pg/mL. Unfortunately, this information is not enough for differentiation because there are many patients with an epithelial molecular subtype and concentrations > 100 pg/mL.

#### 6. Sixth step—TNM staging:

For gastric cancer patients, pT value is related to whole blood and tissue sample maspin concentrations (most values < 55 pg/mL are related to pT1 and pT4 (14 out of 17 in whole blood samples and 16 of 24 in tissue samples)) and pN value is related to whole blood concentration (7 out of 11 patients (63.3%) with pN0 have concentrations > 60 pg/mL while all the patients with pN3 have concentrations < 60 pg/mL and there exists a tendency of decreasing values of maspin concentration with increase in pN value).

For colorectal cancer patients, pT value is related to urine maspin concentration and lymph node metastases are related to tissue sample concentration. Regarding pT value, among the patients with pT4, 10 out of 12 patients (83.33%) have values over 100 pg/mL, and all have over 20 pg/mL, while among the ones with pT3, only 2 out of 11 patients (18.18%) have values over 100 pg/mL, and the others have values between 10–100 pg/mL. Regarding lymph node metastases, among the patients with concentrations < 250 pg/mL, 11 out of 18 have lymph node metastases, while among the ones with values > 250 pg/mL, only 4 out of 15 have lymph node metastases.

### 2.2. Differentiation Based on MLH1, PMS2 and KRAS Levels

The aim of this paper is to differentiate between gastric and colorectal cancer. In order to realize this, there have been successively elaborated a series of criteria for different situations depending on the available data. Six possibilities were analyzed as follows:

1. MLH1 and PMS2 concentrations in saliva
2. MLH1 and PMS2 concentrations in urine
3. MLH1 and PMS2 concentrations in both samples

4. MLH1 and PMS2 concentrations in both samples + K-Ras concentration in saliva
5. MLH1 and PMS2 concentrations in both samples + K-Ras concentration in urine
6. MLH1, PMS2, and K-Ras concentrations in both samples

#### 2.2.1. MLH1 and PMS2 Concentrations in Saliva

For this case, there are proposed two parameters called Crit1 and Crit2 defined as follows:

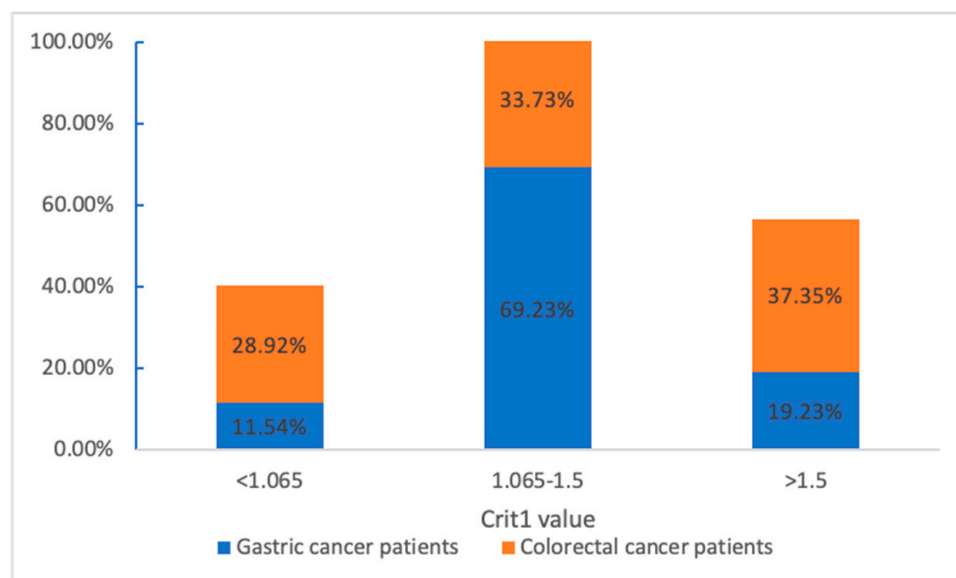
$$\text{Crit1} = ([\text{MLH1}] + [\text{PMS2}]) / [\text{MLH1}]$$

$$\text{Crit2} = [\text{MLH1}] / [\text{PMS2}]$$

where [X] is the concentration of X in the biological fluid.

The values of the two parameters have been linked to each location, and there have been proposed intervals of reference for both. For Crit1, it was chosen 1.065–1.5, and for Crit2, it was chosen >1. These are considered criteria.

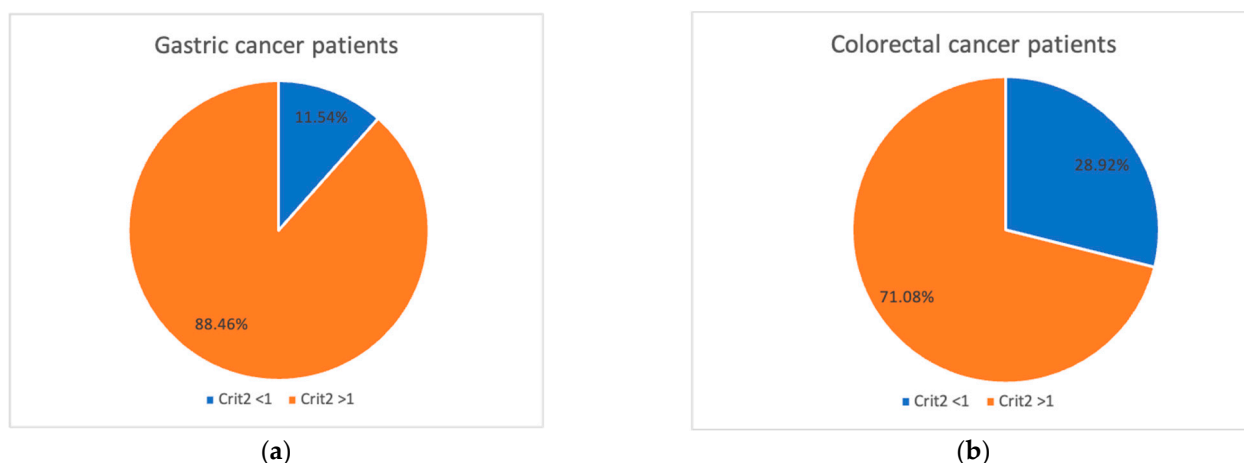
For Crit1 < 1.065, 28.29% of the colorectal cancer patients while only 11.54% of the gastric cancer patients. For Crit1 > 1.5, there are involved 37.35% of the colorectal cancer patients while only 19.23% of the gastric cancer patients. In contrast to the first two observations, most of the gastric cancer patients (69.23%) have a Crit1 value between 1.065 and 1.5, while only 33.73% of the colorectal cancer patients have this Crit1 value. In conclusion, a value for Crit1 between 1.065–1.5 is more characteristic of gastric cancer (Figure 4).



**Figure 4.** Classification of patients using the value of Crit1 in saliva.

For Crit2 < 1, there are 28.92% of colorectal cancer patients while only 11.54% of gastric cancer patients, so a value of Crit2 less than 1 is more frequent in colorectal cancer patients than in gastric cancer ones. Most of the patients have values Crit2 > 1 for both locations (Figure 5a,b).

Combining the two parameters, for Crit1 value outside of the interval 1.065–1.5 and Crit2 > 1, there are 5 out of 28 gastric cancer patients (17.85%) while 31 out of 83 colorectal cancer patients (37.35%). For Crit1 value outside of the interval 1.065–1.5 and Crit2 < 1, there are 3 out of 28 gastric cancer patients (10.71%) while 24 out of 83 colorectal cancer patients (28.91%).



**Figure 5.** Crit2 in saliva in gastric cancer patients (a) and in colorectal cancer patients (b).

### 2.2.2. MLH1 and PMS2 Concentrations in Urine

For this case, there are proposed the same two parameters called Crit1 and Crit2 defined as follows:

$$\text{Crit1} = ([\text{MLH1}] + [\text{PMS2}]) / [\text{MLH1}]$$

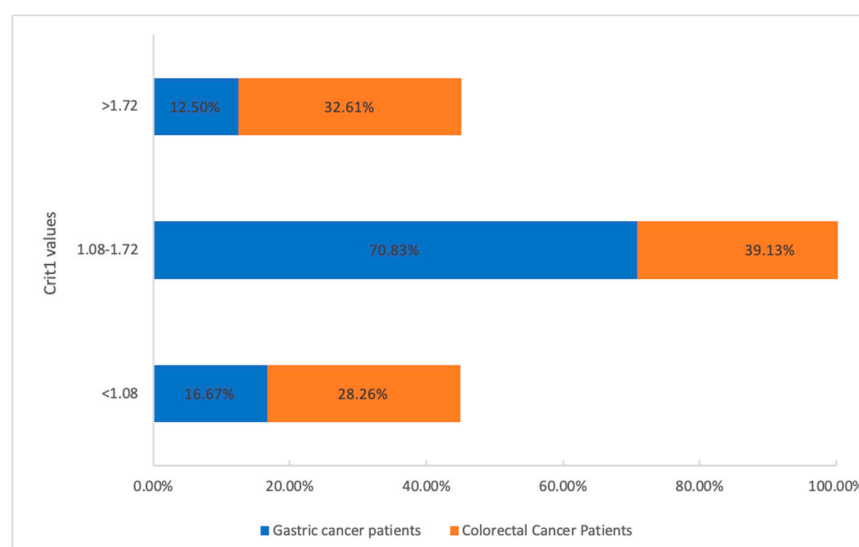
$$\text{Crit2} = [\text{MLH1}] / [\text{PMS2}]$$

where [X] is the concentration of X in the biological fluid.

The values of the two parameters have also been linked to each location, and there have been proposed intervals of reference for both. For urine samples, for Crit1, the chosen interval was 1.08–1.72, and for Crit2, it was chosen >1.4. These are considered the criteria for urine samples.

For Crit1 < 1.08, there are involved 26 out of the 92 colorectal cancer patients (28.26%) while only 4 out of the 24 gastric cancer patients (16.66%). For Crit1 > 1.72, there are involved 30 of the 92 colorectal cancer patients (32.61%) while only 3 out of 24 were gastric cancer patients (12.5%).

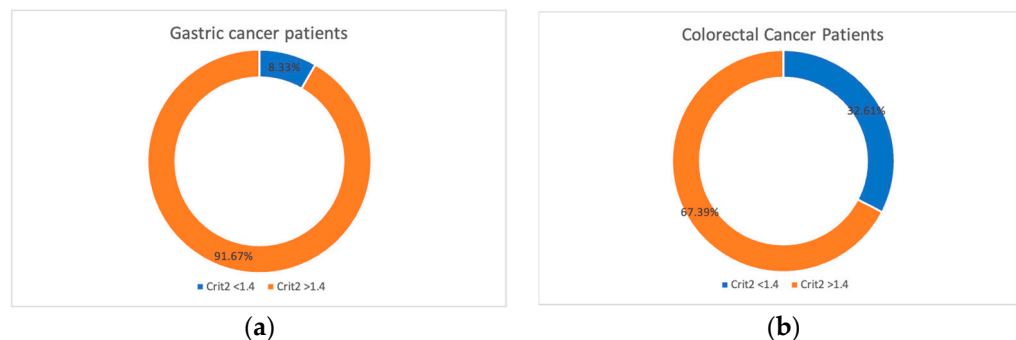
In contrast to the first two observations, most of the gastric cancer patients (75%) have a Crit1 value between 1.08 and 1.72, while only 39.13% of the colorectal cancer patients have the Crit1 value in this interval. In conclusion, a value for Crit1 between 1.08–1.72 is more characteristic of gastric cancer (Figure 6).



**Figure 6.** Classification of patients using the value of Crit1 in urine.



For  $\text{Crit2} < 1.4$ , there are involved 30 out of the 92 colorectal cancer patients (32.61%) while only 2 out of the 24 gastric cancer patients (8.33%), so a value of  $\text{Crit2}$  less than 1.4 is more frequent in colorectal cancer patients than in gastric cancer ones. Most of the patients have values  $\text{Crit2} > 1.4$  for both locations (Figure 7a,b).



**Figure 7.**  $\text{Crit2}$  in urine in gastric cancer patients (a) and in colorectal cancer patients (b).

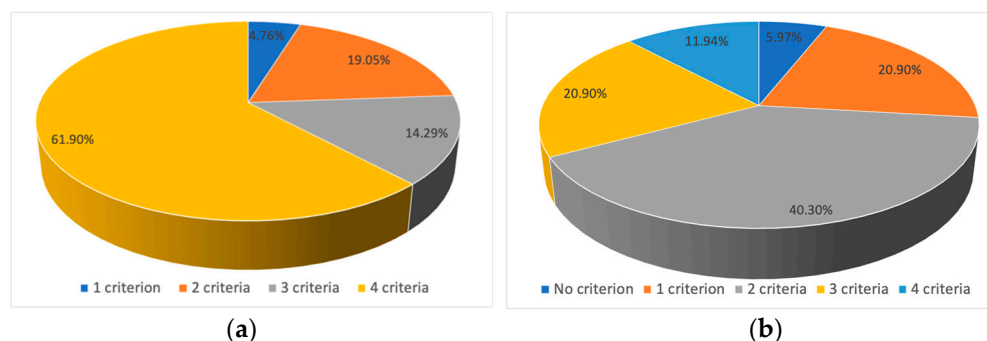
Combining the two parameters, for  $\text{Crit1}$  value outside of the interval 1.08–1.72 and  $\text{Crit2} > 1.4$ , there are 4 out of 24 gastric cancer patients (16.66%) while 26 out of 92 colorectal cancer patients (28.26%). For  $\text{Crit1}$  value outside of the interval 1.08–1.72 and  $\text{Crit2} < 1.4$ , there are 2 out of 24 gastric cancer patients (8.33%) while 30 out of 92 colorectal cancer patients (32.61%).

### 2.2.3. MLH1 and PMS2 Concentrations in Both Samples

If they have the same two parameters, and they can be determined in both kinds of samples, the results can be combined, and the gastric-located cancer can be better differentiated from the colorectal-located one. In this case, there have been analyzed the results for 67 colorectal cancer patients and 21 gastric cancer patients.

For these patients, there have been calculated the values of  $\text{Crit1}$  and  $\text{Crit2}$  in urine ( $\text{Crit1u}$  and  $\text{Crit2u}$ ) and saliva ( $\text{Crit1s}$  and  $\text{Crit2s}$ ). The same interval values are kept for these four parameters: 1.08–1.72 for  $\text{Crit1u}$ ,  $>1.4$  for  $\text{Crit2u}$ , 1.065–1.5 for  $\text{Crit1s}$  and  $>1$  for  $\text{Crit2s}$ . For better differentiation, all four parameters were used. These four parameters and their proposed values are considered criteria for the differentiation.

Among the 21 gastric cancer patients, 1 (4.76%) has one criterion, four (19.05%) have two criteria, three (14.29%) have three criteria, and 13 (61.9%) have all four criteria. All the patients have at least one criterion (Figure 8a).



**Figure 8.** Number of criteria in gastric cancer patients (a) and in colorectal cancer patients (b).

Among the 67 colorectal cancer patients, 4 (5.97%) have no criterion, 14 (20.9%) have one criterion, 27 (40.3%) have two criteria, 14 (20.9%) have three criteria and only 8 (11.94%) have all the four criteria. In the case of colorectal cancer, there exist patients with no criterion (Figure 8b).

It can be observed that most gastric cancer patients have more criteria, while most colorectal cancer patients have fewer criteria. Starting from this observation, there have been analyzed the number of patients who respect at least a given number of criteria for each kind of cancer. It has been found that 100% of gastric cancer patients vs. 94.03% of colorectal cancer patients have at least one criterion, 95.24% of gastric cancer patients vs. 73.13% of colorectal cancer patients have at least two criteria, 76.19% of gastric cancer patients vs. 32.84% of colorectal cancer patients have at least three criteria and 61.9% of gastric cancer patients vs. 11.94% of colorectal cancer patients have all the four criteria as observed in Figure 9.

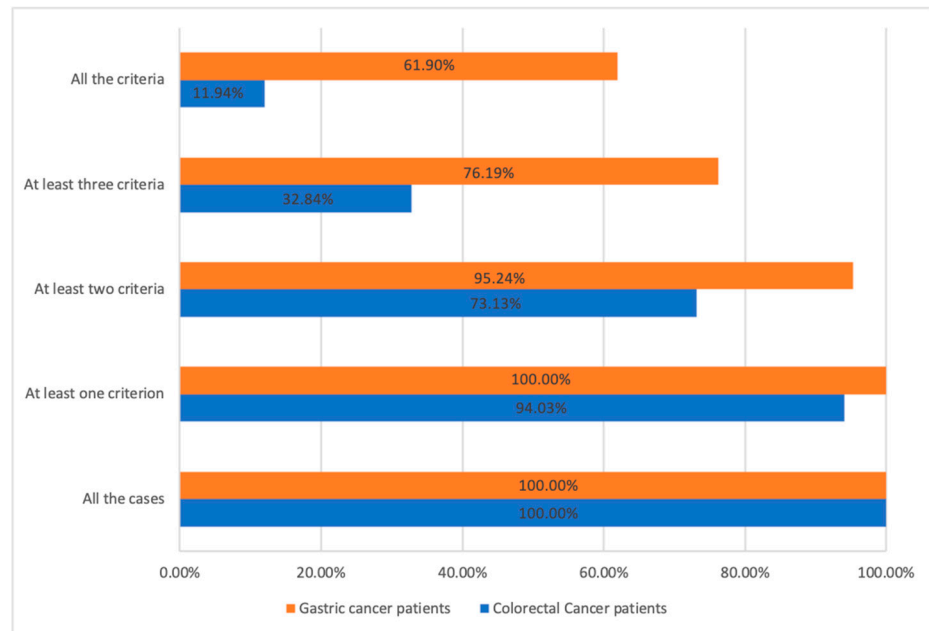


Figure 9. Classification of cancer patients using the least number of criteria.

2.2.4. MLH1 and PMS2 Concentrations in Both Samples with addition of K-Ras Concentrations Criteria

For the 67 colorectal cancer patients and the 21 gastric cancer patients, there has been proposed two additional criteria given by K-Ras concentration in saliva over 5.6 µg/mL and in urine over 12.5 µg/mL (Table 1). These criteria will be evaluated separately and together.

Table 1. Percent of patients for each combination, including K-Ras criteria.

Least Number of MLH1 and PMS2 Criteria	K-Ras in Saliva Criterion		K-Ras in Urine Criterion		K-Ras in Both Samples Criteria	
	Gastric	Colorectal	Gastric	Colorectal	Gastric	Colorectal
1	100.00%	40.30%	100.00%	11.94%	100.00%	4.48%
2	95.24%	31.34%	95.24%	11.94%	95.24%	4.48%
3	76.19%	16.24%	76.19%	7.46%	76.19%	2.99%
4	61.90%	5.97%	61.94%	0.00%	61.94%	0.00%

The proposed algorithms consist of the number of patients who have one or both of these additional criteria and at least a given number of the four criteria discussed above.

If only K-Ras concentration in saliva is used, it can be observed that this criterion helps for better differentiation between the two locations. The criteria are met more by gastric cancer patients than the colorectal cancer patients for salivary K-Ras criterion and at least one other criterion (100% vs. 40.3%), at least two other criteria (95.24% vs. 31.34%), at least three other criteria (76.19% vs. 16.42%) or all the other four criteria (61.9% vs. 5.97%).

The concentration of K-Ras only in urine can also help for better differentiation between the two locations. The criteria are also met more by gastric cancer patients than the colorectal cancer patients for urinary K-Ras criterion and at least one other criterion (100% vs. 11.94%), at least two other criteria (95.24% vs. 11.94%), at least three other criteria (76.19% vs. 7.46%) or all the other four criteria (61.9% vs. 0%—no colorectal patient met all the criteria).

The best way to differentiate gastric-located cancer from colorectal-located cancer is to use both K-Ras criteria, especially in the case of both K-Ras concentration and at least other two criteria from the four MLH1 and PMS2 criteria where 20 out of the 21 gastric cancer patients (95.24%) met the criteria and only 3 out of 67 colorectal cancer patients (4.48%) met the criteria.

### 3. Materials and Methods

#### 3.1. Patients Description

In this paper, two different studies have been performed to make the difference between gastric cancer patients and colorectal cancer patients. The first one regards using only the levels of maspin in different biological fluids. For this study, there were collected the four types of samples from 28 patients confirmed with gastric cancer (20 whole blood samples, 21 saliva samples, 19 urine samples, and 14 tissue samples) and from 31 patients confirmed with colorectal cancer (31 whole blood samples, 22 saliva samples, 23 urine samples, and 30 tissue samples). The second study regards using MLH1, PMS2, and KRAS levels in urine and saliva. For this study, the two kinds of samples (whole blood and saliva) were collected from 116 colorectal cancer patients (92 urine samples, 83 saliva samples) and from 28 patients confirmed with gastric cancer (19 urine samples, 21 saliva samples). The patients were selected from the database of the project GRAPHSENSGASTROINTES and used accordingly with the Ethics committee approval nr. 32647/2018 awarded by the County Emergency Hospital from Targu-Mures. Informed consent has been received from all the patients.

#### 3.2. Materials and Reagents

Highly pure chemicals were used for the design of the stochastic sensors and for the solutions. Biomarkers were bought from Sigma Aldrich. Stochastic microsensors designed for the assay of maspin, K-Ras, MLH1, and PMS2 were used for the determination of the concentrations of maspin, K-Ras, MLH1, and PMS2 [31–33].

#### 3.3. Apparatus

An Autolab PGSTAT 302 (Metrohm, Utrecht, The Netherlands) was used to perform all measurements using an electrochemical cell comprising a stochastic microsensor, Ag/AgCl electrode, and the Pt wire.

#### 3.4. Stochastic Method

After the application of a constant potential of 125 mV vs. Ag/AgCl, the diagrams were recorded. Maspin, K-Ras, MLH1, and PMS2 were identified in the diagrams based on their signatures (marked in the diagram as  $t_{\text{off}}$  values). The quantification of the analyte has been performed using the  $t_{\text{on}}$  values. The calibration equations of the microsensors were used to determine the concentrations of maspin, MLH1, PMS2, and K-Ras [31–33]. The concentration of MLH1 was determined from the equation of calibration:  $1/t_{\text{on}} = 0.03 + 3.58 \times 10^6 C_{\text{MLH1}}$ , with a signature of MLH1 ( $t_{\text{off}}$  value) of 3.9 s. Regarding PMS2 concentration, the values of  $t_{\text{on}}$  were introduced into the equation:  $1/t_{\text{on}} = 0.02 + 1.06 \times 10^5 C_{\text{PMS2}}$ , and the signature of PMS2 ( $t_{\text{off}}$  value) was 2.2 s [31]. For the determination of the concentration of K-Ras, the following equation of calibration was used:  $1/t_{\text{on}} = 0.03 + 1.99 C_{\text{K-Ras}}$ , the signature of K-Ras being 3.4 s [31]. The signature of maspin was 3.6 s, and the equation of calibration was  $1/t_{\text{on}} = 0.03 + 2.95 \times 10^4 C_{\text{Maspin}}$  [33].

#### 4. Conclusions

Preliminary studies for the differentiation of two main cancers of the gastrointestinal system based on the determination of the concentrations of maspin, MLH1, PMS2, and KRAS in saliva and urine were made.

Two algorithms for differentiating gastric and colorectal carcinomas, first based on whole blood maspin concentration and urine maspin concentration and the second based on the concentration of maspin on whole blood, urine, and saliva samples. It was observed that the use of MLH1 and PMS2 is indispensable, while the use of KRAS is for better and more conclusive differentiation. The best way to make the difference is using KRAS in both samples and all the criteria of MLH1 and PMS2. If one criterion is respected from the ones of MLH1 and PMS2 and both the criteria of KRAS in saliva and urine, there can be selected 100% of the gastric cancer patients compared to only 4.48% of the ones with colorectal cancer.

This differentiation can be extremely useful when there is suspected metastasis of a primary tumor from the gastrointestinal system, and it aims to help the surgeon and the oncologist for the best management of the patient.

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