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Validation of a Screening Test, based on Simultaneous Detection of CEA, CA19-9 and p53, for Fast Diagnosis of Gastric Cancer: A Pilot Study

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Abstract

Background: Fast screening tests are a need for fast and early diagnosis of gastric cancer.

Method: This study showed the results of validation of a screening test that used eight stochastic sensors as new tools for the identification and quantification of three cancer biomarkers: CEA, CA19-9, and p53. The biomarkers were determined from different biological samples: whole blood, urine, saliva, and fresh tissue.

Results: Results obtained using the proposed stochastic sensors were compared with those obtained using the standard methods ELISA (for CEA and CA19-9) and chemiluminescence (for p53). The t-tests shown that there is no significant difference between the results obtained using the proposed tools and the standard methods.

Conclusions: The fast screening method based on stochastic sensors can be successfully used for screening tests and fast diagnosis of gastric cancer.

Keywords: Stochastic sensors; Fast screening method; Gastric cancer

Introduction

According to the 2018 statistics of the International Agency for Research on Cancers (GLOBOCAN 2018), gastric cancer is the fourth type of cancer that affects the globe's population. Gastric cancer results from a mix of dietary, lifestyle and environmental factors and accumulation of specific genetic alterations [1]. Early and fast diagnosis was always a high priority. ELISA and chemiluminescence were usually used to date, as standard methods for assay of biomarkers; these techniques are very expensive, and they can only be used for the assay of one biomarker at a time, also extensive processing of the sample is needed.

Besides the biomarkers used in clinical laboratories for gastric cancer diagnosis, carbohydrate antigen 19-9 (CA19-9), carcinoembryonic antigen (CEA), and p53 are the most used in screening tests [2]. Carcinoembryonic antigen (CEA) is a biomarker used for diagnosis and follow-up of patients with gastrointestinal cancer [3]. It is correlated with the tumor stage and number of circulating tumor cells [4]. Carbohydrate antigen 19-9 (CA19-9) is a glycoprotein which is usually utilized as a clinical marker for the diagnosis of pancreatic cancer but high serum values were also reported in patients with colorectal and gastric carcinomas [5,6]. Elevated levels of CA19-9 in biological fluids and presence of CA19-9 positivity in tissue samples are considered indicators of aggressive behavior [7]. One of the most studied biomarkers among cancers is p53. In 2011, Zhou et al. [8] reported that there has been a notable growth in the p53 serum protein level in a diversity of human malignancies. The quantitative identification of the p53 protein has turned into an incredible method for the early determination and identification of different carcinomas [9].

Simultaneous assay of CA19-9, CEA, and p53 using stochastic sensors based on graphene was employed by the group of Stefan-van Staden for the past two years [10-12]. The advantages of utilization of stochastic sensors as tools for such screening tests are: the sensors are able to simultaneously identify three biomarkers (CA19-9, CEA, and p53) in biological samples; no pretreatment of the samples is needed before the analysis; the cost of analysis is far lower than the cost of any kit used for analysis of one biomarker.

The eight stochastic sensors developed earlier by the group of Stefan-van Staden had lower determination limits, large linear concentration range, and high sensitivities [10-12]. Therefore, they were employed as new tools for fast screening tests of biological samples for early detection of gastric cancer. Preliminary results of the pilot study used for the validation of the fast screening method of biological samples are shown in this article.

Materials and Methods

The pilot study was carried out using the following biological samples: 17 samples of whole blood, 17 samples of saliva, 16 samples of urine and 6 fresh tumor tissue samples from patients confirmed with gastric cancer.

ELISA was performed as standard method for the determination of CA19-9 and CEA, and the determination of p53 was done by chemiluminescence.

For the fast screening test, eight stochastic sensors based on graphenes were used as new tools for simultaneous identification and quantification of CA19-9, CEA, and p53 in the biological samples.

Preparation of the biological samples

Whole blood, saliva, urine and tissue samples were recieved from the Clinical County Emergency Hospital of Targu-Mures, Romania. The examined samples provided from consecutive patients with gastric carcinomas diagnosed between 2019-2020. For any patient, 2 ml of blood, saliva and urine were taken before performing surgery. No preoperative chemotherapy was done in any of the included patients. Fresh tumor tissue was taken from surgical specimens, in those cases

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in which DNA was necessary for molecular examinations. All patients underwent surgical resection.

The study was performed according to the procedures specified in the Ethics committee approval number 32647/2018 awarded by the Clinical County Emergency Hospital from Targu-Mures. Written consent was obtained from all patients.

No sample pre-treatment was performed when stochastic sensors were used.

Apparatus and methods

For ELISA and chemiluminescence, the samples were processed accordingly with the standard protocols used in the accredited clinical laboratory.

For all of the measurements, a potentiostat/galvanostat AUTOLAB/PGSTAT 302 (Methrom, Utrecht, The Netherlands) with a multichannel module, connected to a personal computer with a GPES software installed was used. An electrochemical cell, containing a multisensor cell containing 8 sets of stochastic sensors, each set containing the stochastic sensor, a Pt wire as counter electrode, and a Ag/AgCl electrode as reference electrode.

Stochastic mode

The stochastic mode was used, in order to perform all these measurements. The principle of the stochastic sensors is based on the channel conductivity, when a constant potential of 125 mV is applied and the current is recorded. The time needed for the screening test of whole blood, saliva, and tissue was 10 min; while for urine (a more complex sample) was 20 min. The stochastic sensors were used with good results for more than 100 measurements. The surface of the sensors was washed with distilled water, and dried with soft paper between measurements.

Results and Discussion

The response characteristics of the stochastic sensors made possible their utilization for the screening test of whole blood, saliva, urine and tissue samples and simultaneous identification and quantification of: p53, CEA and CA19-9. The eight sensors were used simultaneous for the measurements of the three biomarkers. The results obtained using the new screening method and the standard methods of analysis (ELISA. chemiluminescence) shown that there is a good correlation between the results obtained by utilization of these methods (Tables 1-4).

Sample no.	Method	CEA (ng mL^{-1})	CA 19-9 (U mL^{-1})	p53 (ng mL^{-1})
1	Screening method	28.51 ± 0.37	38.25 ± 0.64	9.61 ± 0.20
	Standard method	27.92 ± 2.50	37.21 ± 2.31	9.54 ± 1.20
2	Screening method	18.51 ± 0.24	57.80 ± 0.36	7.60 ± 0.27
	Standard method	18.20 ± 2.20	56.93 ± 2.12	7.54 ± 1.13
3	Screening method	15.58 ± 0.25	221.53 ± 0.53	2.48 ± 0.32
	Standard method	15.25 ± 2.19	220.76 ± 2.98	2.50 ± 1.02
4	Screening method	18.83 ± 0.98	55.20 ± 0.25	4.32 ± 0.25
	Standard method	19.02 ± 2.14	54.98 ± 2.12	4.28 ± 1.43
5	Screening method	13.27 ± 0.25	73.26 ± 0.12	4.46 ± 0.18
	Standard method	13.12 ± 2.20	74.00 ± 2.18	4.32 ± 1.15
6	Screening method	24.55 ± 0.10	185.77 ± 0.32	2.65 ± 0.26
	Standard method	23.98 ± 2.65	186.12 ± 2.12	2.50 ± 1.10
7	Screening method	26.22 ± 0.11	172.77 ± 0.25	2.50 ± 0.19
	Standard method	25.34 ± 2.43	170.98 ± 2.20	2.21 ± 1.07
8	Screening method	41.76 ± 0.14	202.95 ± 0.19	8.37 ± 0.27
	Standard method	42.02 ± 2.34	201.94 ± 2.54	8.20 ± 1.14
9	Screening method	35.06 ± 0.28	103.94 ± 0.12	22.80 ± 0.18
	Standard method	34.76 ± 2.54	104.19 ± 2.13	21.20 ± 1.95
10	Screening method	21.34 ± 0.18	65.47 ± 0.24	9.46 ± 0.26
	Standard method	21.12 ± 2.11	66.08 ± 2.65	9.40 ± 1.30
11	Screening method	45.87 ± 0.11	110.76 ± 0.54	44.62 ± 0.10
	Standard method	46.00 ± 2.33	108.23 ± 2.22	43.12 ± 1.27
12	Screening method	27.47 ± 0.19	89.91 ± 0.43	5.34 ± 0.12
	Standard method	27.27 ± 2.89	90.12 ± 2.21	5.30 ± 1.19
13	Screening method	35.66 ± 0.13	110.43 ± 0.21	7.23 ± 0.12
	Standard method	34.24 ± 2.76	109.04 ± 2.67	7.07 ± 1.87
14	Screening method	54.19 ± 0.13	145.23 ± 0.20	28.29 ± 0.08
	Standard method	55.08 ± 2.20	144.12 ± 2.12	27.49 ± 1.94

15	Screening method	30.30 ± 0.17	123.98 ± 0.54	32.91 ± 0.76
	Standard method	29.84 ± 2.13	120.78 ± 2.90	32.00 ± 1.32
16	Screening method	60.42 ± 0.12	165.87 ± 0.21	2.57 ± 0.19
	Standard method	60.60 ± 2.12	164.90 ± 2.43	2.20 ± 1.09
17	Screening method	34.34 ± 0.16	174.89 ± 0.37	34.71 ± 0.27
	Standard method	34.20 ± 2.93	175.00 ± 2.98	34.21 ± 1.90
Paired t-test		2.94	2.73	2.19

Screening method-is the proposed method using 8 stochastic sensors.

Standard method-for CA and CA19-9 is ELISA, and for p53 is chemiluminescence.

The values are the average of 80 measurements performed with the eight stochastic sensors based on graphenes (10 measurements were performed with each stochastic sensor).

Table 1: Determination of CA19-9, CEA, and p53 in whole blood samples from confirmed patients with gastric cancer.

Sample no.	Method	CEA (ng mL^{-1})	CA 19-9 (U mL^{-1})	p53 (ng mL^{-1})
1	Screening method	102.48 ± 0.46	1.56 ± 0.25	11.45 ± 0.27
	Standard method	101.76 ± 2.32	-*	11.23 ± 1.23
2	Screening method	121.03 ± 0.85	108.13 ± 0.61	2.33 ± 0.16
	Standard method	120.84 ± 2.34	110.02 ± 2.53	2.12 ± 1.11
3	Screening method	151.44 ± 0.26	155.64 ± 0.93	1.34 ± 0.32
	Standard method	150.12 ± 2.45	153.23 ± 2.24	1.29 ± 1.02
4	Screening method	36.12 ± 0.16	247.53 ± 0.10	6.69 ± 0.21
	Standard method	35.29 ± 2.27	244.98 ± 2.30	6.50 ± 1.20
5	Screening method	38.48 ± 0.14	43.68 ± 0.74	3.43 ± 0.30
	Standard method	37.69 ± 2.18	43.12 ± 2.23	3.12 ± 1.10
6	Screening method	10.17 ± 0.31	32.26 ± 0.57	1.40 ± 0.11
	Standard method	9.97 ± 2.12	30.12 ± 2.23	1.27 ± 1.02
7	Screening method	24.20 ± 0.34	164.20 ± 0.36	1.51 ± 0.28
	Standard method	23.15 ± 2.12	165.05 ± 2.43	1.24 ± 1.00
8	Screening method	20.62 ± 0.20	74.65 ± 0.32	1.53 ± 0.27
	Standard method	19.46 ± 2.18	73.23 ± 2.30	1.36 ± 1.05
9	Screening method	27.23 ± 0.16	98.43 ± 0.32	33.01 ± 0.15
	Standard method	27.30 ± 2.54	98.12 ± 2.54	32.76 ± 1.33
10	Screening method	17.33 ± 0.16	43.15 ± 0.32	10.35 ± 0.85
	Standard method	17.12 ± 2.21	43.20 ± 2.13	9.76 ± 1.54
11	Screening method	31.06 ± 0.16	107.43 ± 0.20	64.78 ± 0.15
	Standard method	32.10 ± 2.87	106.21 ± 2.55	64.20 ± 1.35
12	Screening method	22.71 ± 0.34	95.47 ± 0.17	13.25 ± 0.48
	Standard method	22.03 ± 2.53	94.94 ± 2.27	13.20 ± 1.23
13	Screening method	36.31 ± 0.12	89.38 ± 0.14	26.78 ± 0.83
	Standard method	36.19 ± 2.25	88.37 ± 2.12	26.20 ± 1.77
14	Screening method	27.28 ± 0.11	140.30 ± 0.55	13.46 ± 0.46
	Standard method	27.19 ± 2.27	141.12 ± 2.32	13.08 ± 1.25
15	Screening method	31.69 ± 0.12	105.23 ± 0.28	16.76 ± 0.44
	Standard method	30.98 ± 2.28	103.87 ± 2.43	16.16 ± 1.47

16	Screening method	31.98 ± 0.59	153.20 ± 0.37	6.38 ± 0.12
	Standard method	32.08 ± 2.24	150.94 ± 2.24	6.40 ± 1.28
17	Screening method	29.57 ± 0.12	165.35 ± 0.22	18.97 ± 0.50
	Standard method	29.23 ± 2.15	163.20 ± 2.12	17.65 ± 1.23
Paired t-test		3.01	2.84	2.12
Screening method-is the proposed method using 8 stochastic sensors Standard method -for CA and CA19-9 is ELISA, and for p53 is chemiluminescence. The values are the average of 80 measurements performed with the eight stochastic sensors based on graphenes (10 measurements were performed with each stochastic sensor). *No value was obtained using the standard method for this biomarker.				

Table 2: Determination of CA19-9, CEA, and p53 in saliva samples from confirmed patients with gastric cancer.

Sample no.	Method	CEA (ng mL^{-1})	CA 19-9 (U mL^{-1})	p53 (ng mL^{-1})
1	Screening method	40.55 ± 0.23	39.03 ± 0.85	8.51 ± 0.27
	Standard method	40.23 ± 2.38	38.15 ± 2.20	8.23 ± 1.37
2	Screening method	34.07 ± 0.14	134.24 ± 0.12	8.44 ± 0.33
	Standard method	33.98 ± 2.12	132.97 ± 2.23	8.30 ± 1.11
3	Screening method	25.12 ± 0.11	314.60 ± 0.12	4.60 ± 0.26
	Standard method	25.25 ± 2.16	312.76 ± 2.32	4.23 ± 1.12
4	Screening method	21.87 ± 0.68	72.24 ± 0.31	8.17 ± 0.28
	Standard method	21.12 ± 2.20	72.98 ± 2.43	7.94 ± 1.54
5	Screening method	23.14 ± 0.36	89.49 ± 0.27	6.50 ± 0.31
	Standard method	22.87 ± 2.23	89.00 ± 2.43	6.12 ± 1.47
6	Screening method	33.12 ± 0.61	41.53 ± 0.35	10.24 ± 0.33
	Standard method	32.85 ± 2.12	40.39 ± 2.42	9.67 ± 1.76
7	Screening method	17.32 ± 0.36	286.50 ± 0.13	9.35 ± 0.16
	Standard method	16.69 ± 2.54	285.20 ± 2.54	8.90 ± 1.74
8	Screening method	37.80 ± 0.21	67.59 ± 0.12	12.79 ± 0.30
	Standard method	37.37 ± 2.20	66.12 ± 2.31	12.80 ± 1.03
9	Screening method	38.60 ± 0.12	87.53 ± 0.33	33.10 ± 0.52
	Standard method	38.02 ± 2.09	86.54 ± 2.23	33.00 ± 1.98
10	Screening method	38.98 ± 0.79	98.98 ± 0.37	53.98 ± 0.50
	Standard method	38.79 ± 2.15	98.23 ± 2.33	52.90 ± 1.84
11	Screening method	32.72 ± 0.19	77.20 ± 0.43	3.55 ± 0.12
	Standard method	32.17 ± 2.21	75.98 ± 2.47	3.20 ± 1.11
12	Screening method	26.80 ± 0.12	99.54 ± 0.34	18.28 ± 0.70
	Standard method	26.53 ± 2.73	98.24 ± 2.32	18.07 ± 1.27
13	Screening method	82.21 ± 0.55	132.18 ± 0.11	27.57 ± 0.34
	Standard method	82.18 ± 2.20	131.14 ± 2.20	27.07 ± 1.31
14	Screening method	38.07 ± 0.69	112.87 ± 0.32	67.65 ± 0.25
	Standard method	37.97 ± 2.43	112.12 ± 2.32	65.45 ± 1.87
15	Screening method	39.16 ± 0.20	143.98 ± 0.32	1.29 ± 0.16
	Standard method	38.48 ± 2.27	142.32 ± 2.55	1.21 ± 1.00

16	Screening method	35.38 ± 0.84	98.32 ± 0.30	14.59 ± 0.12
	Standard method	34.20 ± 2.47	96.32 ± 2.21	14.12 ± 1.69
Paired t-test		3.01	2.85	2.15
Screening method-is the proposed method using 8 stochastic sensors Standard method -for CA and CA19-9 is ELISA, and for p53 is chemiluminescence. The values are the average of 80 measurements performed with the eight stochastic sensors based on graphenes (10 measurements were performed with each stochastic sensor).				

Table 3: Determination of CA19-9, CEA, and p53 in urine samples from confirmed patients with gastric cancer.

Sample no.	Method	CEA (ng mL^{-1})	CA 19-9 (U mL^{-1})	p53 (ng mL^{-1})
1	Screening method	27.50 ± 0.38	392.43 ± 0.64	7.53 ± 0.53
	Standard method	27.23 ± 2.48	390.65 ± 2.45	7.23 ± 1.87
2	Screening method	48.49 ± 0.17	397.59 ± 0.92	11.48 ± 0.32
	Standard method	47.32 ± 2.18	395.23 ± 2.47	11.50 ± 1.20
3	Screening method	22.68 ± 0.12	315.45 ± 0.21	12.40 ± 0.67
	Standard method	22.32 ± 2.46	312.07 ± 2.53	12.12 ± 1.44
4	Screening method	32.18 ± 0.60	119.32 ± 0.37	1.71 ± 0.09
	Standard method	33.10 ± 2.32	117.23 ± 2.52	1.65 ± 1.02
5	Screening method	37.62 ± 0.11	127.87 ± 0.43	7.60 ± 0.14
	Standard method	37.53 ± 2.23	126.05 ± 2.12	7.55 ± 1.87
6	Screening method	59.51 ± 0.18	121.14 ± 0.42	6.16 ± 0.05
	Standard method	59.12 ± 2.06	119.27 ± 2.13	6.13 ± 1.23
Paired t-test		2.12	2.85	2.02
Screening method-is the proposed method using 8 stochastic sensors Standard method-for CA and CA19-9 is ELISA, and for p53 is chemiluminescence. The values are the average of 80 measurements performed with the eight stochastic sensors based on graphenes (10 measurements were performed with each stochastic sensor).				

Table 4: Determination of CA19-9, CEA, and p53 in tumor tissue samples from confirmed patients with gastric cancer.

Paired t-test was performed for each biomarker, and each type of sample in order to statistically compare the results, and also to see if there is any significant difference between the results obtained using the fast screening method with stochastic sensors, and the standard methods, e.g. ELISA and chemiluminescence. The test was performed at 99.00% confidence level. At this level, the tabulated theoretical value was 4.032. The values shown in Tables 1-4 proved that there is no statistically significant difference between the results obtained using the proposed method and the standard method (ELISA or chemiluminescence), because all the calculated values were lower than the tabulated value (4.032). Accordingly, the proposed method can be reliable used for fast screening of bioloical samples such as whole blood, saliva, urine, and tissue samples, for CA19-9, CEA, and p53. The test will help to the fast diagnostic of gastric cancer.

Conclusion

Eight stochastic sensors were used as new tools for the screening of biological samples for gastric cancer. The values obtained for the test shown that there is no significant differences between the values obtained using the stochastic sensors and the values obtained standard methods such as ELISA and chemiluminescence. The results obtained in the pilot study for the analysis of whole blood, saliva, urine and tissue samples shown that the new method can be validated for the fast screening of these biological samples and diagnosis of gastric cancer.

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