# Stress-induced gastric adenocarcinoma: fluorescent and electrical measurements

MATVEY KANEVSKIY[a], EKATERINA BORISOVA[a, b,*], IRINA MIRONOVA[a], SVETLANA KONNOVA[a], ANNA GALITSKAYA[a], ALEXANDER KHOROVODOV[a], ILANA AGRANOVICH[a], PETYA PAVLOVA[c], LATCHEZAR AVRAMOV[b], OXANA SEMYACHKINA-GLUSHKOVSKAYA[a]
[a]*Saratov State University, 83, Astrakhanskaya str., 410012 Saratov, Russia*
[b]*Institute of Electronics, Bulgarian Academy of Sciences, 72, Tsarigradsko Chaussee Blvd., 1784, Sofia, Bulgaria*
[c]*Technical University - Sofia, branch - Plovdiv, 25, Tsanko Dyustabanov str., 4000 Plovdiv, Bulgaria*

We studied gender differences in gastric tumor in rats using original model of gastric adenocarcinoma and photodiagnostics with delta-aminolevulinic acid, and impedance measurements of the stomach, liver and red blood cells in rats of both genders. Highly heterogeneous gastric adenocarcinoma in rats of both genders was induced by means of a prolonged exposure to social and chemical stresses. Exogenous fluorescence mapping using 5-aminolevulinic acid / protoporphyrin IX was performed on the developed tumors, and the suspicious areas were investigated and compared with the surrounding intact tissues by electrical bio-impedancemetry techniques. The histological data revealed significant gender differences in as the number of male rats with neoplasia was higher by a factor of 1.5. Exogenous fluorescence maps were detected in gastric cancerous areas, but no gender correlation was observed. Significant gender differences were revealed when bioelectrical impedance and polarization coefficients patterns were measured. In some cases, photodiagnosis yielded a false-positive exogenous fluorescence due to dye accumulation in benign and inflammatory mucosal areas and the application of additional methods, such as impedance measurement of gastric tissues and red blood cells might prove to be a promising way of improving the diagnostic efficiency of photodiagnosis, including gender-related aspects.

(Received August 21, 2019; accepted June 16, 2020)

*Keywords*: Chronic stress, Gastrointestinal cancer, Fluorescent detection, Tissue impedance

## 1. Introduction

According to the data provided by the International Agency for Research on Cancer (IARC) [1], the incidence rate of cancer pathologies is growing at an alarming rate. As stated in GLOBOCAN 2012 (http://globocan.iarc.fr – IARC online database), gastric cancer (GC) is the fifth most common malignant tumor in the world. The highest incidence rates of GC are observed in Japan and the developing regions of China, in the Middle East, Central America and South America. [2] Moreover, gender differences exist in the prevalence of this disease: the incidence of gastric cancer is twice as high in men as in women, and the mortality rate among men is also 1.5 times higher than in women. [3-5]

Various assumptions have been put forward regarding the reasons for gender differences in gastric cancer development, including differences in dietary patterns, distinct characteristics of microelement composition, and diversity in nitrite food usage, smoking, consumption of excess salt, fried and smoked products, excess weight, infection with Helicobacter pylori [6-9], etc. Obviously, in-depth studies are needed of the interaction between synergistic risk factors that lead to male population's increased morbidity and high mortality due to gastrointestinal cancer.

Lately, photodynamic diagnostics (PDD) has been applied more frequently in the clinical practice to evaluate the state of the stomach. [10, 11] For this purpose, delta aminolevulinic acid (5-ALA) is used, which by itself is not a fluorophore compound, but a precursor of protoporphyrin IX, which accumulates specifically in tumour cells and emits fluorescence, when the tumour is irradiated with appropriate excitation light.

However, the major problem of gastric cancer PDD is its low specificity arising from the false-positive fluorescence signal of exogenous fluorophores accumulated in benign and inflammatory mucosal areas. [12, 13] This fact suggests that PDD alone is not sufficient for correct diagnosis of gastric neoplasia; thus, the application of additional methods for early detection of cancerous changes in the stomach could significantly increase the diagnostic accuracy of the gastric cancer PDD technique.

One of the possible ways of improving the PDD diagnostic efficiency is adding electrical impedance parameters measurements of the blood and evaluating the stomach tissues' polarization coefficients. Based on the tissue impedance measurements (from a patient biopsy taken for diagnosis, or from the red blood cell mass), it is possible to infer the presence of malignant tumors and necrotic changes in the tissues, while a reduction in the polarization coefficient is a telling evidence concerning the viability of body tissues and the time of death. [14, 15]

It has been established that the electrical properties of biological tissues depend on their morpho-functional state.

[16]. The total electrical resistance (impedance) of tissues, measured at high frequencies, allows one to evaluate the intracellular changes, while in the low-frequency range it is correlated with the size of the intercellular spaces, the level of blood filling and tissue hydration. In practice, relative impedance indices are analyzed, comparing the healthy tissues' impedance (the physiological norm) with the impedance of an affected tissue site.

In this way, the usage of PDD is appropriate to a higher degree in the detection and visualization of tumors boundaries, while the impedance method could evaluate more precisely the state of the tumor and the depth of tissue damage.

The development of technologies for early detection of gastric cancer has been impeded by the lack of suitable animal models. Currently, a large number of models of carcinogenesis induction have been proposed, but none of them can be unambiguously transferred to the development of tumor processes in humans. [17-22]. Thus, new models are required for a more in-depth study of the processes of carcinogenesis and the investigation of changes occurring in the initial stages.

In our preliminary studies, we showed that prolonged (nine-month) combination of chronic stress (overpopulation) and daily diet with nitrite in water and aromatic amine in food is accompanied by formation of metastatic adenocarcinoma in rats. [23]

Using our novel rat model of highly heterogeneous adenocarcinoma, we investigated the effectiveness of PDD for early detection of gastric cancer with the help of 5-ALA/PpIX, and measured the impedance in the stomach, liver and red blood cells. The gender differences in rat tissue' electrical parameters were also observed and estimated.

## 2. Materials and methods

The study was carried out on 100 outbred white female and male rats weighing 220-250 g in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996), the protocols were approved by the Institutional Review Boards of the Saratov State University (Protocol No. 7, 07.02.2017). The rats were housed at 25±2C, 55% relative humidity, and 12:12 h light - dark cycle. Food and water were given ad libitum.

The animals were divided into four groups: 1) and 2) were the control groups included healthy female rats (n=10) and male rats (n=10) living under standard conditions; 3) and 4) were the experimental groups included female rats (n=40) and male rats (n=40), which were subjected to chronic stress and daily diet with nitrites and aromatic amine.

To induce gastric cancer in rats, we used our original model of gastric highly heterogeneous adenocarcinoma [23], namely, chronic stress (overpopulation during nine months) and diet including the daily use of toluidine (25 mg/kg) in food, and water with nitrites (2 g/l). This model is close to the real natural factors such as stress and nutrition, which during long time (in our model during nine months) provoke development of cancer in gastrointestinal tract in humans. [24-26] Our model allows to study the formation of adenocarcinoma from pre-cancerous stages till cancer with spread of metastases in lungs and in liver that are also similar clinical signs of gastric tumor in humans. [27]

The histological studies were carried out according to the standard methods with the manufacture of paraffin blocks and staining of thin sections (3-5 μm thickness) with hematoxylin and eosin.

The tissue samples and areas of the normal and abnormal gastrointestinal tissues were evaluated using fluorescent techniques.

Delta-aminolevulinic acid (5-ALA) in a dose of 20 mg/kg (ALASENS, Niopik Inc., Russia) was applied 2 hours before the spectroscopic observation and evaluation of gastric and liver tissues. The organs were investigated *in vivo* – for initial addressing and *ex vivo*, after decapitation of the animals, using excitation at 405 nm (AFS-405 LED light source, FWHM = 20 nm, Popt=25 mW, Polironik Ltd., Russia), a USB4000 microspectrometer (OceanOptics Inc., Dunedin, USA) for the 1-D measurements, and a DinoLite digital microscope (model AM 4013 T-FWV, IDCP B.V., The Netherlands) for 2-D images of the samples. The suspicious areas (with exogenous fluorescence observed) were placed in the groups of "cancerous mucosa" and "metastasis" respectively, for the stomach and liver. The absence of exogenous fluorescent signal from delta-aminolevulinic acid/ protoporphyrin IX was an indicator for healthy tissue areas in both organs. All tissue areas addressed spectrally were histologically verified in posteriori as well.

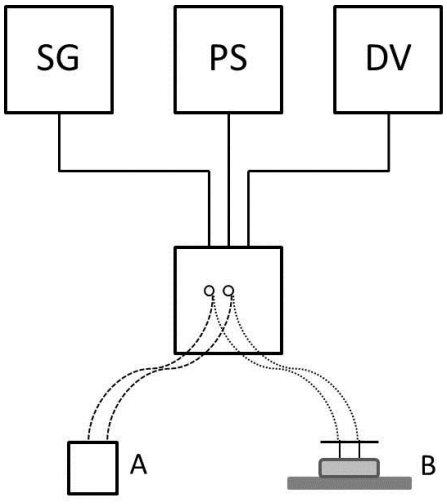


*Fig. 1. Block-scheme of the electric bio-impedance measurements unit, consisting of SG-signal generator, PS – power supply, DV- digital voltmeter, A - cuvette with a platinum electrode for a liquid media, and B - needle-shaped electrodes for solid media measurements of a submerged type fixed on a common base*

The electric bio-impedance measurements were carried out on *ex vivo* tissue samples of the stomach and liver and on washed red blood cells, washed twice with

NaCl (9 g/l), by means of a multi-frequency analyzer consisting of a G6-27 signal generator, a B7-27 A/1 digital voltmeter, a K762 power supply unit, and a cuvette, see Fig. 1.

The device is designed to measure the magnitude of the impedance in the 1 Hz - 1 MHz frequency range. Our electrical resistance measurements were conducted at six discrete frequencies from 50 Hz to 100 KHz using a 2-ml cuvette with a platinum electrode for a liquid medium and needle-shaped electrodes of a submerged type fixed on a common base. The length of the immersed part was 2 mm, with a 15-mm distance between the needles. Then, we calculated the polarization, or dispersion, coefficient, Cp, namely, the ratio of the impedances at two fixed frequencies (100 Hz and 100 kHz).

The statistical analysis of the data was performed using the Mann-Whitney test, with the differences considered significant for $p < 0.05$. The data obtained are presented as mean ± standard mean error.

## 3. Results

### 3.1. Histological data

Using our original model of gastric cancer in rats, we clearly demonstrated the development of gastric tumors in animals having lived for nine months under a combination of chronic stress (overpopulation) and a diet with nitrites+toluidine. The histological data revealed the signs of a highly-differentiated adenocarcinoma in 56% of rats (45 of 80) (Fig. 2d). Among these rats, 28 were males (70 %, 28 of 40) and 17 females (42%, 17 of 40), i.e. the number of males with gastric cancer was higher by a factor of 1.6 than that of females. Further, 35 % of the rats (14 females (35 %) and 12 males (30 %)) have exhibited atrophic gastritis and dysplasia of the glands in the stomach, which are precancerous indicators (Fig. 2b and 2c). Metastases to the liver and lungs were observed in 70 % of the rats with adenocarcinoma of the stomach independently of the animals' gender (Fig. 2e & 2f). Metastatic nodes were represented by clusters of atypical cells with large hyperchromatic nuclei. Moreover, 23 % of female rats (9 of 40) developed peptic ulcers without precancerous or cancerous changes in the stomach. Figure 2a shows normal gastric tissues.

### 3.2. Photodiagnosis of gastric cancer in rats

The spectra of normal and precancerous stomach mucosa are presented on Fig. 3a; the fluorescent signals of normal and metastatic liver areas are presented on Fig. 3b.

To assess not only the appearance of cancerous areas, but also the boundaries of such lesions, 2-D images in a fluorescent mode were also acquired. Examples of such observations are presented in figure 3C and figure 3D, respectively, for the stomach and the liver.

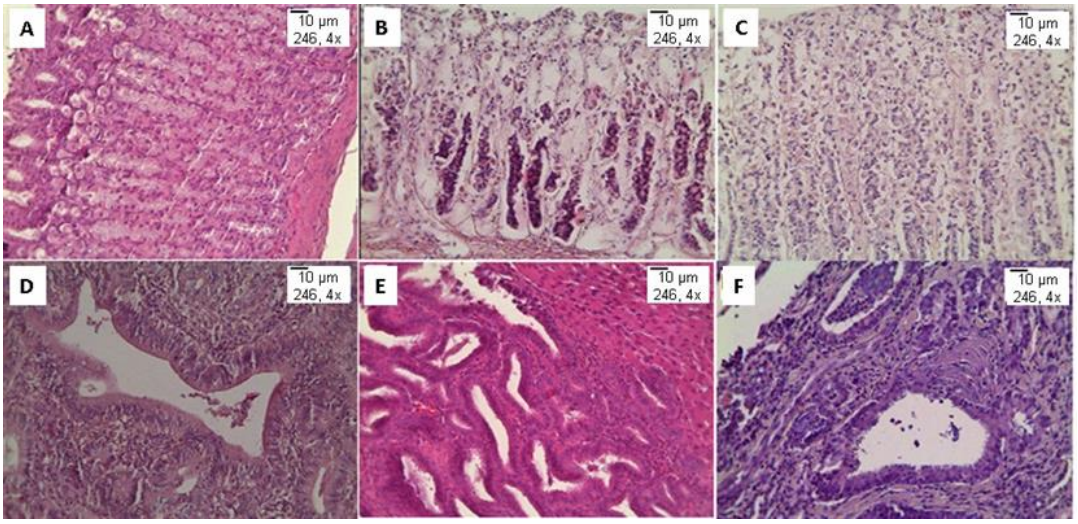


*Fig. 2. Histological analysis of various organs of rats: (a) normal gastric mucosa; (b) dysplasia of the glands in the stomach; (c) atrophic gastritis; (d) highly differentiated adenocarcinoma of the stomach; (e) metastases in the liver; (f) metastases in the lung. Hematoxylin & Eosin staining. Bars represent 10 µm (246.4X) (color online)*

The red spots observed correspond to exogenous fluorescence of 5-ALA/PpIX, while the blue signal is associated with endogenous fluorescence of healthy mucous membrane and corresponds to the signal from elastin, collagen and NADH and flavins coenzymes, which are emitting in the blue-green spectral region. [12, 28]

One can clearly distinguish the different accumulation of 5-ALA/PpIX in the abnormal areas, with a well-pronounced fluorescence in the 630 - 710 nm region and the PpIX-specific maxima at 635 nm and 704 nm, Figs. 3a and 3b. The integral intensity of the autofluorescence of normal gastric mucosa is higher in the visible spectral range; therefore, in Fig. 3c, presenting the fluorescent image of the stomach *ex vivo*, the bright light-blue signal is emitted by the normal mucosa, as opposed to the red

fluorescence that originates from the cancerous areas of the stomach.

The intermediate areas with pinky fluorescence observed (lower left-hand side of the image in Fig. 3c) are addressed a posteriori by the histological analysis as being inflammatory areas in the gastric mucosa.

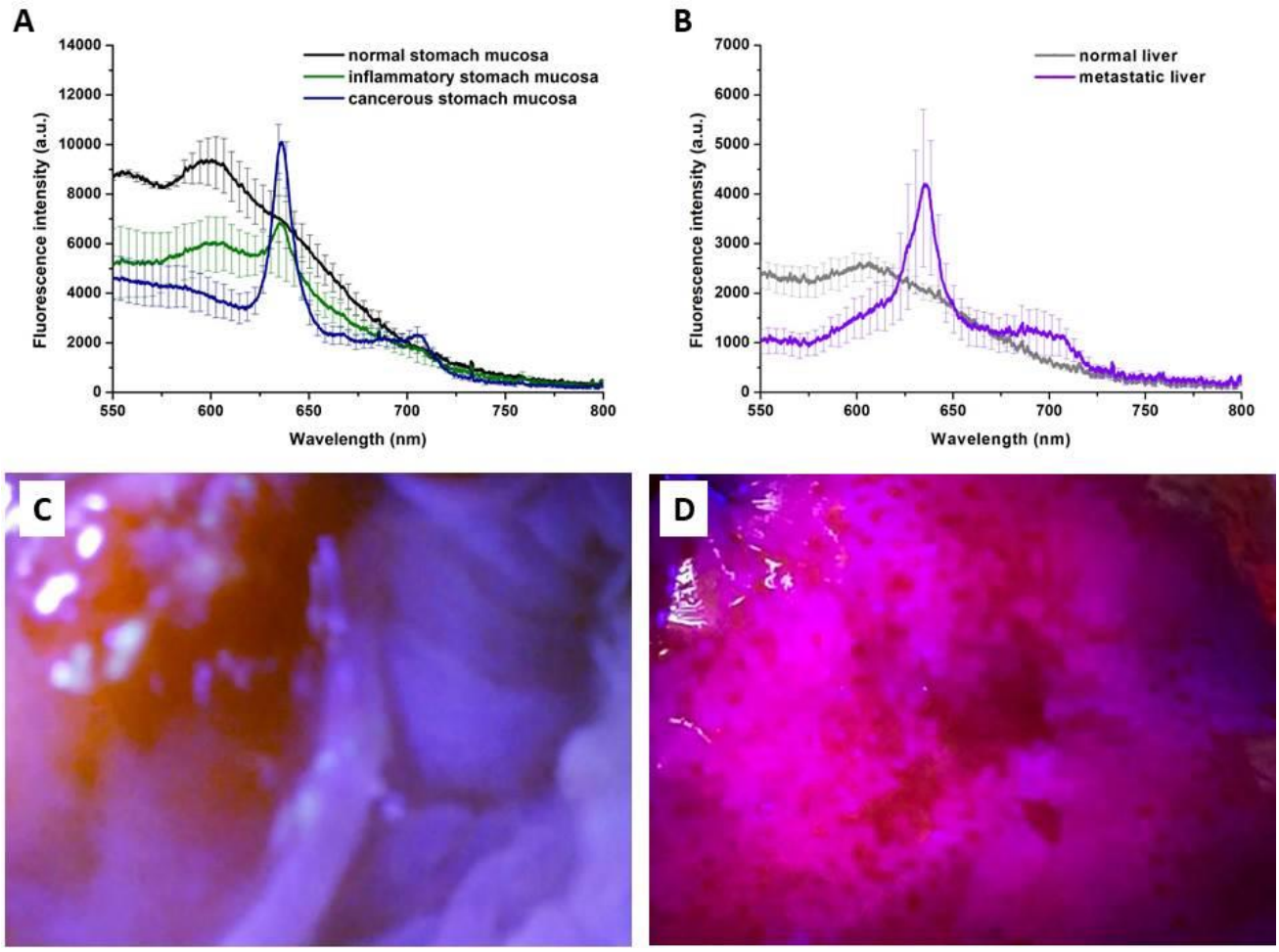


*Fig. 3. Photodiagnosis of gastric cancer in rats with highly heterogeneous adenocarcinoma using 5-ALA/PpIX: (a) Comparison of the fluorescence spectra of normal, inflammatory and cancerous stomach mucosa; (b) Comparison of the fluorescence spectra of normal and metastatic liver tissues; (c) Image in fluorescent mode of stomach mucosa with cancerous (red) and normal (blue) mucosa; (d) Image in fluorescent mode of liver tissue with metastatic area (red spots) observed (color online)*

Thus, false-positive fluorescence of exogenous fluorophores 5-ALA/PpIX is observed; this could give rise to problems in the case of applying 5-ALA/PpIX as a contrast agent for evaluation of gastrointestinal tumors [29] due to its accumulation in inflammatory areas as well. However, the intensity of the fluorescence in the 630 - 700 nm range originating from such areas is much lower than that from the cancerous sites, which allows one to discriminate between them using a 1-D spectroscopic measurement technique.

Fig. 3b presents a comparison of a normal and a metastatic liver, where the accumulation of 5-ALA/PpIX in the damaged tissues was also detected and the exogenous emission was used as an indicator of pathological alterations in the liver tissue.

The blue-violet fluorescence is addressed to the normal liver tissue, while the dissipative red fluorescent spots correspond to the metastases observed in the organ.

We should emphasize the lack of gender differences in the fluorescent signals detected during the examination of gastric tissues using the photodynamic diagnostics approach.

### 3.3. Impedance measurements

The fluorescent data obtained were validated by impedance measurements conducted *ex vivo* on the stomach and liver samples in normal and neoplastic state of the tissues (Table 1). The analysis of the electrical parameters of erythrocyte mass of animals of both genders revealed a significant decrease in the polarization coefficient with respect to the control groups in both males and females.

*Table 1. Indicators of impedance and Cp of erythrocyte mass, gastric mucosa and liver tissues at two fixed frequencies in the control and experimental group for male (♂) and female (♀) animals*

| | | | Z, kOm (F=100 Hz) | Z, kOm (F=100 kHz) | Cp |
|---|---|---|---|---|---|
| Erythrocyte mass | ♂ | Control group | 1,42±0,06† | 0,98±0,12† | 1,29±0,09† |
| | | Experimental group | 0,55±0,18*† | 0,51±0,03*† | 1,07±0,03*† |
| | ♀ | Control group | 1,87±0,03† | 1,21±0,06† | 1,54±0,06† |
| | | Experimental group | 1,59±0,12*† | 1,10±0,09*† | 1,45±0,06*† |
| Gastric mucosa | ♂ | Control group | 2,06±0,15† | 0,92±0,09† | 2,22±0,12† |
| | | Experimental group | 1,23±0,30*† | 0,82±0,12† | 1,26±0,15*† |
| | ♀ | Control group | 2,34±0,12† | 0,93±0,03† | 2,51±0,15† |
| | | Experimental group | 1,88±0,21*† | 1,16±0,06*† | 1,62±0,21*† |
| Liver tissue | ♂ | Control group | 4,25±0,03† | 0,91±0,06† | 4,62±0,36† |
| | | Experimental group | 2,31±0,12*† | 1,00±0,03*† | 2,31±0,06*† |
| | | Experimental animals with revealed metastases | 1,48±0,03*† | 1,10±0,06*† | 1,34±0,09*† |
| | ♀ | Control group | 4,61±0,36† | 1,21±0,24† | 3,81±0,33† |
| | | Experimental group | 3,00±0,45*† | 1,37±0,18† | 2,17±0,39*† |

($p < 0.05$): * – vs. the control group; †- between females and males.

Electrical impedance measurements of the gastric mucosa have been successfully used to diagnose forms of gastritis and benign and malignant tumors. Thus, according to the literature data, Cp has a characteristic interval for malignant tumors, namely, a reduction in the 15 - 40 % range, compared with healthy mucous membranes. [30, 31]

The data obtained in our experiments indicated a decrease in the Cp of the gastric mucosa by 43 % in males and 36 % in females, which might indicate a development of a pathological process in these tissues.

Our studies also revealed a significant difference between the impedance values of normal and pathological liver tissues in both males and females, namely, the Cp values decreased by 50% and 43%, respectively.

In the low-frequency range of measurements, the absolute resistance index of a liver where tumor metastases were diagnosed was lower by 67 % than the control values in a normal liver tissue, while the Cp values were reduced by 70%.

We assume that the values registered of the liver tissues' electrical characteristics may be the result of the prolonged exposure to toxicants by way of disrupting the membrane barrier function and the ion balance and altering the trans-membrane potential. The systematic application of toxic substances (nitrites and amines) causes a compensatory re-organization of the metabolism and leads to the development of a pathological type of metabolic processes, which is reflected in the nature of the impedance frequency dependence.

## 4. Discussion

We studied the development of gastric cancer in female and male rats using our original model of stress-induced metastatic adenocarcinoma. Our results demonstrate that long-term (during nine months) combination of such factors as social stress and food provoke development of gastric adenocarcinoma in rats that confirms hypothesis about an important carcinogenic nature of stress and nitrosamines in humans. [24-26]

It could be noticed that most inbred strains of animals have resistance to gastric carcinogenesis. Helicobacter infection and carcinogen treatment have been used to establish rodent models that exhibit phenotypes similar to those of human gastric cancer. [17, 30]

A large number of transgenic and knockout mouse models of gastric cancer have been developed using genetic engineering. However, these models cannot reproduce real scenarios of human gastric cancer, which include not only genetic mechanisms, but mostly influences of ecological and stressful factors. A combination of carcinogens and gene manipulation has been applied to facilitate development of advanced gastric cancer; as well, there is no animal models of gastric cancer that reproduce metastatic aggressiveness of gastric cancer that is typical for human.

Our data show that model of stress-induced gastric adenocarcinoma is associated with pre-cancerous stage and cancerous processes with spread of metastasis in lungs and in liver that is similar to the clinical signs of aggressiveness of gastric tumor in humans. [27]

Thus, our model of stress-induced gastric adenocarcinoma is useful for the study of formation and progression of gastric cancer that is important tool for development and improving of photodynamic diagnostic methods.

Our results also clearly demonstrate significant gender differences in the resistance to stress-induced gastric cancer. Indeed, the number of males with adenocarcinoma was 1.6-fold higher than females (70% of males and 42% females). Only among females we observed animals (23 %) resistant to stress and showing no cancerous changes in the stomach. Thus, the rat adenocarcinoma model chosen provided an informative platform for further analysis of gender differences in impedance measurements.

Fluorescence spectra showed no gender differences, in contrast with the results of the impedance measurements. However, the spectral observations could be used for preliminary addressing for the further electric measurements, giving information about the suspicious tissue alterations and their boundaries. As it is known, the accumulation of a photosensitizer in the cells depends on the state of the tissue: normal, inflammation / precancerous condition, and tumor, but does not depend on the animal' gender. Therefore, the fluorescent method is useful for preliminary mapping of the tissue. [12, 28, 29], while measuring the impedance allows a more accurate determination of the tissue' physiological state. [14]

Limitations of impedance measurement in the diagnosis of tumors lie in the fact that this method alone does not allow determining the localizations of the tumour in the body and requires prior mapping of the tissue investigated.

However, impedance measurements usage allows us to estimate the degree of tissue damage in the course of the pathological process. Electrical impedance detection could be used as a non-invasive method in the case of the electrical parameters of erythrocyte mass measurements. It is preferable to estimate the Cp, since it is relative value, does not require a comparison with the healthy tissue impedance and allows assessing the degree of the destructive pathological alterations. This indicator is also convenient for observing the dynamics of the pathological process development or to estimate the efficacy of therapies applied.

Moreover, during surgical interventions, measurement of tissue impedance at one frequency could be applied, which allows to assess the physiological state of the tissue during excision procedure. [31]

Analyzing the electrical parameters of tissues studied, we found that a greater decrease in the impedance and polarization coefficient (up to 65% in animals with diagnosed tumors) was typical for males, while for females this indicator did not exceed 45% (Table 1). This fact points to a more intensive course of development of pathological processes in males, as compared with females, when the stress conditions being kept the same. In particular, in males we determined a stronger tissue damage of the stomach and a decrease in the resistance of the erythrocyte mass and liver tissue, in agreement with [7]. We, therefore, believe that the rate of the inflammation process and neoplasm growth under the same conditions is higher in males than in females, so that using impedance measurements one could make gender-specific prognoses.

Our results are consistent with the data of clinical studies; for example, a study of more than a thousand Moroccan gastric biopsies showed that the most virulent vacA alleles (s1, i1 and m1) of *Helicobacter pylori* were found in specimens of men, rather than of women. [32] The authors explain these gender differences with the different activity of the immune system. They showed higher pro-inflammatory reactions in women compared with men, with significantly higher concentration values of inflammatory factors, such as TNF-α and IL-6 in plasma. [33] A correlation between the overexpression of the HER2 onco-gene (human epidermal growth factor receptor 2) in the male gender and diffuse-type tumors was found in the study of the Malaysia population. [34, 35].

The diagnostic significance of the analysis of tissue bio-impedance is associated with the high information content of its frequency dependencies; this is why we estimated Cp, defined as the ratio of the impedances at two fixed frequencies, low and high. It characterizes the general state of structural organization of a tissue. With the destruction of the structure, the resistances at these frequencies tend to one another and the Cp value approaches unity. Therefore, it is possible to estimate the viability of a tissue in accordance with its bio-impedance indices. [16, 31]. In *in vivo* studies, a preliminary mapping of the suspicious areas must be applied in view of focusing more accurately the impedance measurements. Such mapping could be conducted by using spectroscopic techniques, such as fluorescence detection of tumors with or without exogenous markers applied. [12, 36]

The impedance measurement is an express method for tissue analysis [37] and can be used as a tool complementary to the exogenous fluorescence observations with regard to increasing the diagnostic accuracy and discriminating between malignant and benign gastric changes. We found a decrease in the absolute impedance values at low frequencies in metastases and foci of hepatocellular carcinoma. These data obtained on experimental animal model correlate well with the results obtained earlier on humans with liver pathologies. [38, 39]

The alterations of tissue' electrical parameters correlated with the complex of factors that determine the preferred development of gastric cancer in males. Therefore, the impedance measurement technique can be used as a simple method of monitoring the dynamics of development of malignant neoplasms and, thus, of making gender-specific predictions about changes in a patient's condition.

In our studies 5-ALA was applied as a precursor of exogenous fluorescent marker – PpIX, as an approved drug for clinical applications, which is highly selective to the neoplastic lesions in gastrointestinal tract due to the specific metabolism of cancer cells, [12, 23, 28] Protoporphyrin IX has bright fluorescence in the red spectral region, far from the endogenous fluorescence of proteins and co-enzymes, typical fluorophores of the gastrointestinal mucosa, which is situated at the blue-green spectral range. [12, 28] In this way, a good contrast could be achieved between normal and neoplastic mucosa and the lesions areas and borders would be clearly observed and addressed for the following electrical measurements of the impedance properties of the cancerous and normal tissue sites.

## 5. Conclusions

Diagnosing gastric cancer in its early stages is still a very difficult clinical task. The search for new detection modalities or combined techniques that will improve the gastrointestinal tumors identification and evaluation of their relations with the patients' biochemical, physiological, genetic or other fingerprints is a challenging task, as many parameters are to be investigated.

In our study, we found that the combination of long-term social and chemical stresses brings forth the development of highly heterogeneous gastric adenocarcinoma in more male rats vs. female rats (incidence rate higher by about a factor of 1.5). The photodynamic diagnostics of the gastric cancer revealed precancerous and cancerous areas in the rat stomach, but no sex differences in the fluorescent mapping of the gastric tissues. Finding gender correlations was only possible by using impedance measurements of the stomach, liver and red blood cells. It is worth noting that, although exogenous fluorescence spectroscopy has recently become an advanced tool for endoscopic observation of gastrointestinal neoplasia, it is

characterized by a high sensitivity but a moderate specificity of diagnosis. In certain cases, PDD provides a false-positive fluorescence of exogenous fluorophores due to the latter accumulation in benign and inflammatory mucosal areas. This fact suggests that PDD alone is not sufficient for correct diagnosis of gastric cancer, particularly when a gender-specific approach is needed. Therefore, the application of additional methods, such as measuring the impedance of gastric tissues and red blood cells, could prove to be a novel way of improving the diagnostic efficiency of PDD, including when a gender-related aspect is sought for.

**Acknowledgements**

The research was funded by the Russian Science Foundation under grant No. 18-15-00139, spectroscopic and imaging equipment usage was supported in the frames of the Bulgarian National Science Fund grants #KP06-N28/11/2018 and #KP06-N38/13/2019 respectively.

## References

[1] B. Stewart, C. Wild (eds.): World cancer report 2014, Lyon, France: International Agency for Research on Cancer, (2014).
[2] A. Jemal, F. Bray, M. Center, J. Ferlay, E. Ward, D. Forman, CA Cancer J. Clin. **61**, 69 (2011).
[3] R. Siegel, J. Ma, Z. Zou, A. Jemal, CA Cancer J. Clin. **64**, 9, (2014).
[4] M. Arnold, M. Sierra, M. Laversanne, I. Soerjomataram, A. Jemal, F. Bray, Gut **66**, 683 (2017).
[5] A. Maguire, M. Porta, J. Sanz-Anquela, I. Ruano, N. Malats, J. Pinol, Eur. J. Cancer **32**, 1303 (1996).
[6] V. Dvoirin, N. Tranezinkov, Bulletin of the Cancer Center of the Russian Academy of Medical Sciences **6**, 3 (1995).
[7] H. Yatsuya, H. Toyoshima, T. Mizoue, T. Kondo, K. Tamakoshi, Y. Hori, N. Hayakawa, Intern. J. Cancer **97**, 688 (2002).
[8] H. Song, H. Kim, N. Choi, S. Hahn, Y. Cho, B. Park, Alcohol **42**, 363 (2008).
[9] M. Alonso-Amelot, Gastrointest. Can. Res. Ther. **3**, 1027 (2018)
[10] B. Nokes, M. Apel, C. Jones, G. Brown, E. Lang, J. Surg. Res. **181**, 262 (2013).
[11] M. Nakamura, A. Goto, A. Nishikawa, H. Shibata, World J. Gastroenterol. **21**, 6706 (2015).
[12] E. Borisova, B. Vladimirov, R. Ivanova, L. Avramov, in New Tech. Gastrointest. Endoscopy, O. Pascu, A. Seicean (ed.), InTech, 231 (2011).
[13] X. Yang, P. Pratheeba, D. Kraus, B. Chen, Int. J. Mol. Sci. **16**, 25865 (2015).
[14] A. Zuev, V. Mishlanov, Perm Med. J. **1**, 13 (2008).
[15] A. Rodin, A Pathology, Physiology and Experimental Therapy **2**, 80 (2014).
[16] S., Leonov, D. Panchenkov, A. Barsukov, Analyses of Surgical Hepatology **16**, 60 (2011).
[17] A. Poh, R. ODonoghue, M. Ernst, T. Putoczki, J. Gastroenter. Hepatol. **31**, 1257 (2016).
[18] K. Sintara, D. Thong-Ngam, Thai. J. Physiol. Sci. **21**, 31 (2008).
[19] T. Yamachika, H. Nakanishi, K. Inada, T. Tsukamoto, N. Shimidzu, K. Kobayashi, S. Fukushima, M. Tatematsu, Jpn. J. Cancer Res. **89**, 385 (1998).
[20] X. Cai, J. Carlson, C. Stoicov, H. Li, T. Wang, J. Houghton, Gastroenterol **128**, 1937 (2005),
[21] M. Gut, S. Parkkila, Z. Vernerová, E. Rohde, J. Závada, M. Hocker, J. Pastorek, T. Karttunen, A. Gibadulinova, Z. Zavadova, K. Knobeloch, B. Wiedenmann, J. Svoboda, I. Horak, S. Pastoreková, Gastroenterol. **123**, 1889 (2002).
[22] K. Ray, K. Bell, J. Yan, PLoS One **6**, e16786 (2011).
[23] A. Khorovodov, I. Agranovich, N. Shushunova, N. Navolokin, A. Telegin, A. Shnitenkova, M. Sagatova, I. Trishkina, M. Ulanova, E. Borisova, O. Semyachkina- Glushkovskaya, United Eur. Gastroenterol. J. **5**(5S), 369 (2017).
[24] S. Galić, Z. Glavić, M. Cesarik, Acta Clin. Croat. **53**, 279 (2014).
[25] S. Lee, I. Sung, J. Kim, S. Lee, H. Park, C. Shim, J. Neurogastroenterol. Motil. **21**, 273 (2015).
[26] P. Song, L. Wu, W. Guan, Nutrients **7**, 9872 (2015).
[27] K. Hemminki, Oncotarget **7**, 52307 (2016)
[28] L. Bachmann, D. Zezell, A. da Costa Ribeiro, L. Gomes, A. Ito, Appl. Spectr. Rev. **41**, 575 (2006).
[29] E. Borisova, O. Semyachkina-Glushkovskaya, N. Navolokin, V. Mantareva, I. Angelov, I. Agranovich, A. Khorovodov, N. Shushunova, A. Bodrova, I. Fedosov, A. Namykin, A. Abdurashitov, L. Avramov, Proc. SPIE **10501**, 1E (2018).
[30] Y. Hayakawa, J. Fox, T. Gonda, D. Worthley, S. Muthupalani, T. Wang, Cancers (Basel) **5**, 92 (2013).
[31] N. Abdullaev, S. Balakhnin, G. Bushmanova, Basic research **1**, 1775 (2015).
[32] M. El Khadir, S. Alaoui Boukhris, D. Benajah, K. El Rhazi, S. Ibrahimi, M. El Abkari, PLoS ONE **12**, e0170616 (2017).
[33] A. Wegner, S. Benson, L. Rebernik, I. Spreitzer, M. Jäger, M Schedlowski, S. Elsenbruch, H. Engler, Innate Immun. **23**, 432 (2017).
[34] R. Pathmanathan, F. Ho Kean, C. Foo Yoke, J. Gastrointest. Canc. **49**, 150 (2018).
[35] M. Wesołowska, P. Pawlik, P. Jagodzinski, Biomed. Pharmacother. **83**, 314 (2016).
[36] V. Subramanian, K. Ragunath, Clin. Gastroenterol. Hepatol. **12**, 368 (2014)
[37] Yu. Tornuev, D. Nepomnyaschikh, D. Nikityuk, G. Lapiy, O. Young, R. Nepomnyaschikh, E. Koldysheva, Yu. Krinitsyna, S. Balakhnin, R. Manvelidze, D. Semenov, B. Churin, Basic Research **10**, 782 (2014).
[38] A. Buyever, Hepatology Today **12**, 21 (2002).
[39] D. Panchenkov, S. Leonov, Yu. Ivanov, N. Solovev, A. Nechunaev, A. Rodin, Endoscopic Surgery **21**, 30 (2015).

* Corresponding author: borisova@ie.bas.bg