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FECAL CALPROTECTIN DOSAGE VALUE AS A DIAGNOSTIC AND POSTOPERATIVE MARKER IN DIABETIC PATIENTS WITH COLORECTAL CANCER

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Abstract

Background and Aims: *We evaluated fecal calprotectin values in patients with colorectal neoplasms undergoing surgery, comparatively in patients with and without diabetes mellitus.* ***Material and Methods:*** *We studied 40 patients operated for colorectal neoplasm, divided into two groups: one group of 20 patients with insulin-treated type 2 diabetes and another group of 20 patients without diabetes.* ***Results:*** *Patients had a high percentage of preoperative calprotectin test positivity (90%, 36 patients). A total of 19 patients in group 1 and 17 patients in group 2 had a positive calprotectin test. Postoperatively at 3 months, fecal calprotectin values remained elevated in 7 patients from group 1 and 4 patients from group 2. At 6 months postoperatively, fecal calprotectin values remained elevated in 2 patients from group 1 and 1 patient from group 2.* ***Conclusions:*** *Calprotectin values in faeces from patients with colorectal cancer were significantly increased, with a trend towards post-operatory normalization, slower in patients with diabetes. Fecal calprotectin value as a screening marker was almost equal compared to the hemoccult test, and better compared to that of the carcinoembryonic antigen.*

key words: *fecal calprotectin, colorectal cancer, diabetes mellitus*

Background and Aims

Cancer, a disease with a worldwide rapid growth in prevalence, has become an important cause of mortality in Romania. Although we are only on the 11th place in Europe in what concerns the number of cases reported [1], the increasing number of newly reported cancer cases and deaths from year to year makes this disease an important public health problem, requiring future solutions.

According to GLOBOCAN in 2012, the most commonly diagnosed cancers worldwide were: lung cancer (1.8 million, 13.0% of the total), breast cancer (1.7 million, 11.9% of the total), colorectal cancer (1.4 million, 9.7% of the total) [1]. Colorectal cancer is an increasingly common disease in the modern society and one of the most important causes of death, despite the relatively lower cost of screening compared to other cancers [2]. According to the same statistics, (GLOBOCAN 2012) in Romania,

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colorectal cancer currently represents the second neoplastic disease in incidence (Figure 1) and the second leading cause of cancer death, both in men and women, with a spectacular growth in recent years, in 2002 it ranked third place nationally.

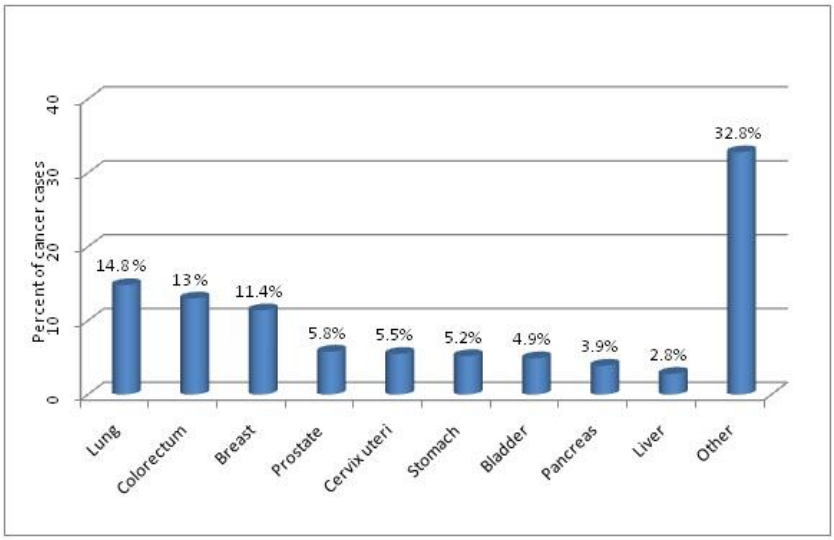

Figure 1. *Incidence of cancer in Romania according to cancer statistics GLOBOCAN 2012 (adapted from* [1]*).*

Before discussing the treatment regimen, the primary aim is the detection of cancer in its early stages, the only solution to increase life expectancy and quality of life, knowing the good evolution of the disease diagnosed and treated in its early stages. We considered that besides the hemoccult test, screening tests for colorectal neoplasms may include the fecal calprotectin, a new test for the screening of this type of neoplasia [3].

Calprotectin is a non – glycosylated protein from the S100 class. It can be found in the cytoplasm of leucocytes, more precisely of polymorphonuclears (PMN) and monocytes, representing about 60% from the proteins dissolved in their cytosol. Calprotectins have the capacity of fastening the intracellular calcium [4], having an important role in the protection of the cell against the action of the bacterial catalytic enzymes [5]. They also present the capacity of intracellularly binding zinc with a role in the activation of the microbial vital enzymes and the induction of apoptosis [5]. It is released in the lumen of the intestine together with the activation of the leucocytes or as a result of their degradation, having an adjusting role in the inflammatory process, antimicrobial and anti-proliferative function [6]. Fecal calprotectin may be used to differentiate the irritable bowel syndrome from the organic diseases, noticing that its level is seldom normal with patients with inflammatory intestinal diseases and colorectal cancer [7].

In this study, we aimed to evaluate the changes in fecal calprotectin excretion in operated colorectal cancer patients, tested preoperatively and postoperatively. We also aimed to study these changes comparatively in patients with associated diabetes mellitus (DM) versus those without diabetes. Finally we aimed to compare the value of fecal calprotectin as a screening marker with that of the hemoccult test and carcinoembryonic antigen.

Material and methods

We studied a total of 40 patients, who underwent surgery for colorectal neoplasms in the Clinical Hospital CF Oradea between 2012 and 2014. The study group was divided in two sub-groups as follows: group 1 included a total

of 20 patients with insulin-treated type 2 diabetes, while group 2 included a total of 20 patients with colorectal cancer without diabetes or any other form of glucose metabolism abnormality. Cancer diagnosis was confirmed in all patients by histopathological examination of biopsies taken at colonoscopy prior to surgery, all presenting colorectal adenocarcinoma.

Fecal calprotectin was determined preoperatively and postoperatively in all patients from the 2 groups. Also, patients were investigated using laboratory test for carcinoembryonic antigen. Hemoccult tests were performed by colonoscopy in all studied patients.

The test used to detect fecal calprotectin was a rapid type strip-test Cal-Detect® SOFAR, produced by *Sofar Farmaceutici*, Italy. It is a semi-quantitative immunochromatographic test, easy to use and affordable, which can be used in the general practitioner's office. Interpretation of this test results is done in about 3 minutes by the appearance of one, two or three lanes on the test strip as shown in Figure 2.

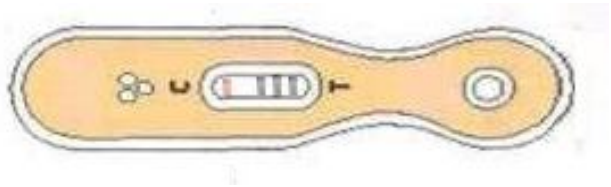

Figure 2. *The aspect of the card test for the assessment of fecal calprotectin Cal-Detect® SOFAR (adapted from* [8])

The first band means a concentration below 15 mg/g of fecal calprotectin and is unrepresentative for intestinal inflammation (normal test); the appearance of two bands signifies a concentration of 15-60 mg/g of fecal calprotectin and is associated with acute inflammation of the intestinal mucosa; the appearance of three bands indicates a high degree of intestinal mucosal inflammation [8]. All patients stopped any treatment with non steroidal anti inflammatory drugs at least two days before each test.

The assessment of the carcinoembryonic antigen values was made with the immunochemical method with electrochemi-lumiscence detection (ECLIA), with reference values of less than 3.4 ng/mL[3] for nonsmokers, and less than 4.3 ng/mL[3] for smokers, and the detection limit of 0.20 ng/mL[3].

The test used for the detection of occult hemorrhages in the stool was Hemoccult test in vitro. The test consists in the color reaction which appears on the paper impregnated with Guajac reactive on which a sample of stool and H2O2 developer are placed.

Statistical analysis: The statistic analysis was achieved with the help of SPSS 19 programme. The test of statistic significance χ^2 was used. Data were considered significant if $p<0.05$. Sensibility, specificity, positive and negative predictive value, accuracy index and Youden index for fecal calprotectin, carcinoembryonic antigen and hemoccult test were calculated.

Results

We observed that patients with colorectal neoplasms had a high percentage of calprotectin test positivity (90% of cases, representing a total of 36 patients) as shown in Figure 3, values consistent with the data existing in literature [9]. The distribution of these patients in the two study groups was as follows: 19 patients (95%) in the diabetes group and 17 patients (85%) in the non-diabetic group. 4 patients (20%) had normal fecal calprotectin values (below 15 mg/g): one from group 1 (5%) and 3 (15%) from group 2, as detailed in Figure 4. From the statistic point of view there are significant differences between the diabetic and nondiabetic patients in what concerns the values of the fecal calprotectin (p=0,005).

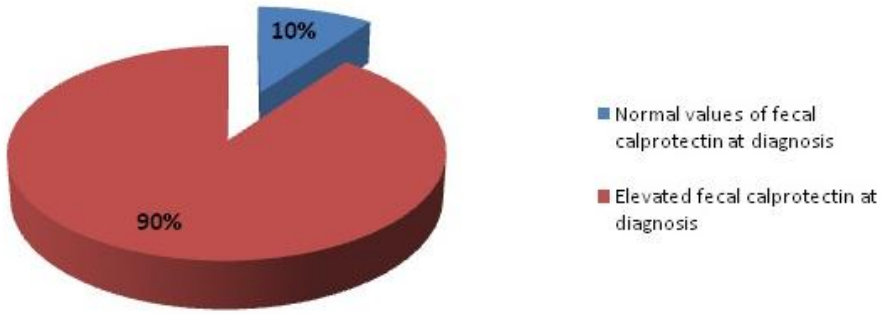

Figure 3. *Fecal calprotectin test results for the whole study group.*

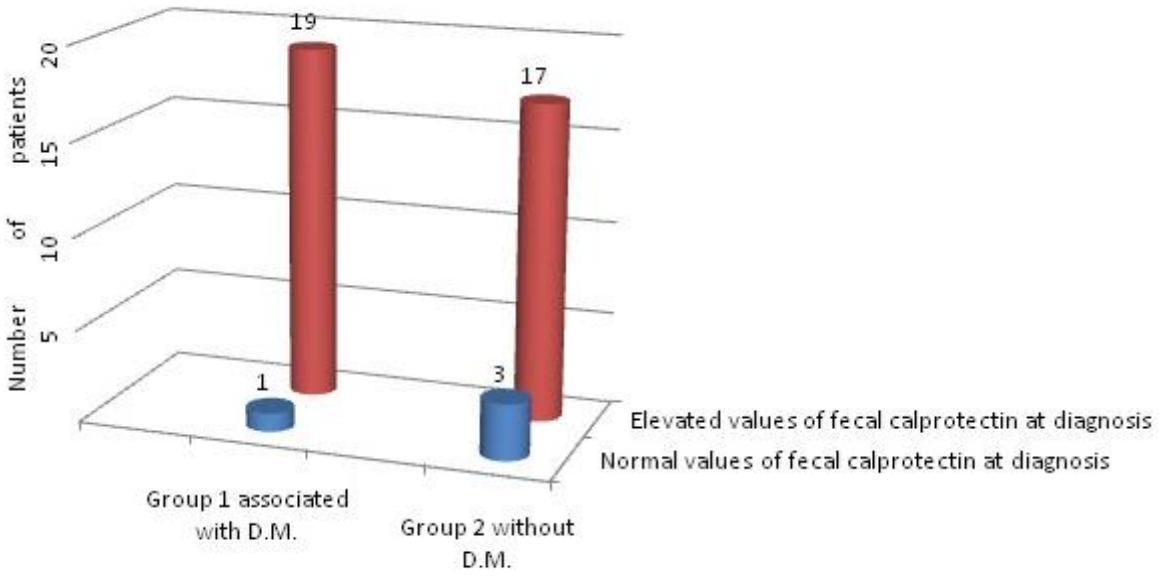

Figure 4. *Fecal calprotectin test results in the two study sub-groups.*

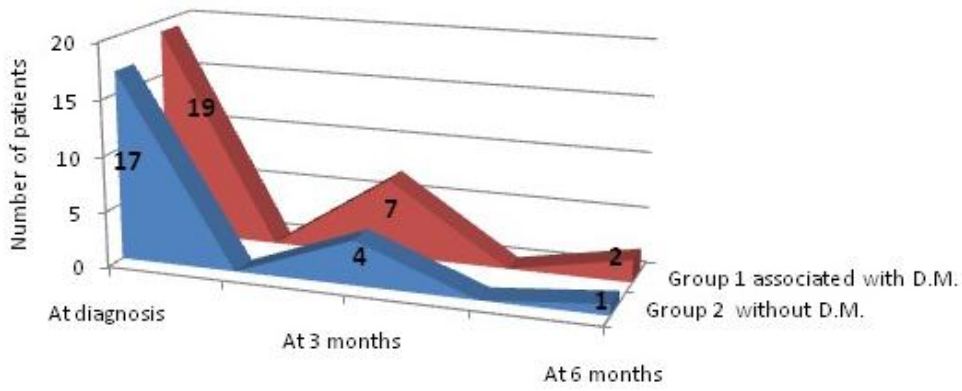

Figure 5. *Postoperative evolution of faecal calprotectin values in the 2 groups at 3 and 6 months.*

Postoperatively, the percentage of patients with elevated fecal calprotectin decreased at 3 months and 6 months. We noticed a trend of slower normalization of the fecal calprotectin values in patients who suffered from diabetes comparatively with non-diabetics, as detailed in Figure 5.

Thus, at 3 months postoperatively, in group 1 fecal calprotectin values remained elevated in 7 patients (i.e. 35%), while in group 2 only 4 patients (representing a total of 20%) maintained elevated fecal calprotectin values (p<0,001).

At 6 months postoperatively, in group 1 fecal calprotectin values remained elevated in 2 patients (representing 10%), while in group 2 only 1 patient (representing 5% of patients) maintained elevated fecal calprotectin values (p=0,022).

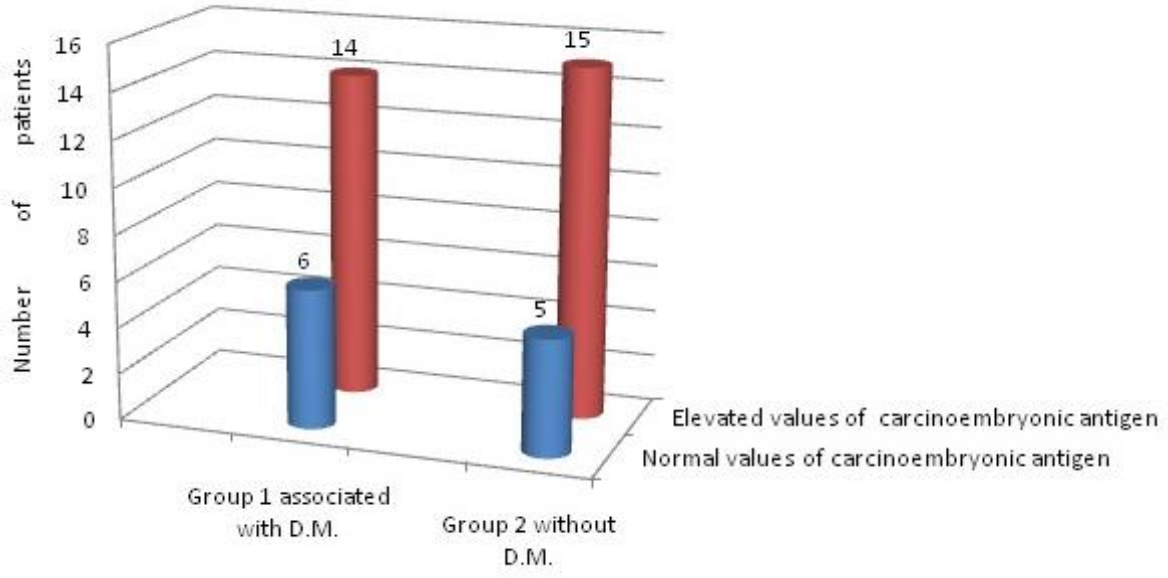

Figure 6. *Carcinoembryonic antigen results in the two study sub-groups.*

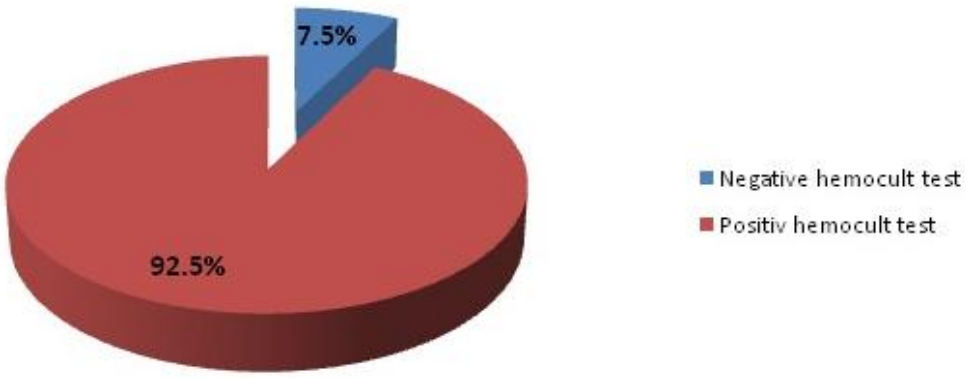

Figure 7. *Hemoccult test results for the whole study group.*

Increased values of the carcinoembryonic antigen were recorded in 29 patients (i.e. 72.5%) with a relatively equal distribution in the two study groups, as shown in Figure 6. In the first

group, associated with DM, we recorded increased values of the carcinoembryonic antigen in 14 patients (70%), and in the second group, in 15 patients (75%). From the statistical point of view there are no significant differences between the diabetic and nondiabetic patients in what concerns the values of the carcinoembryonic antigen (p=0,248).

Hemoccult test was also positive in a significant proportion of patients, i.e. 37 patients, representing 92.5% of all patients included in the study as detailed in Figure 7.

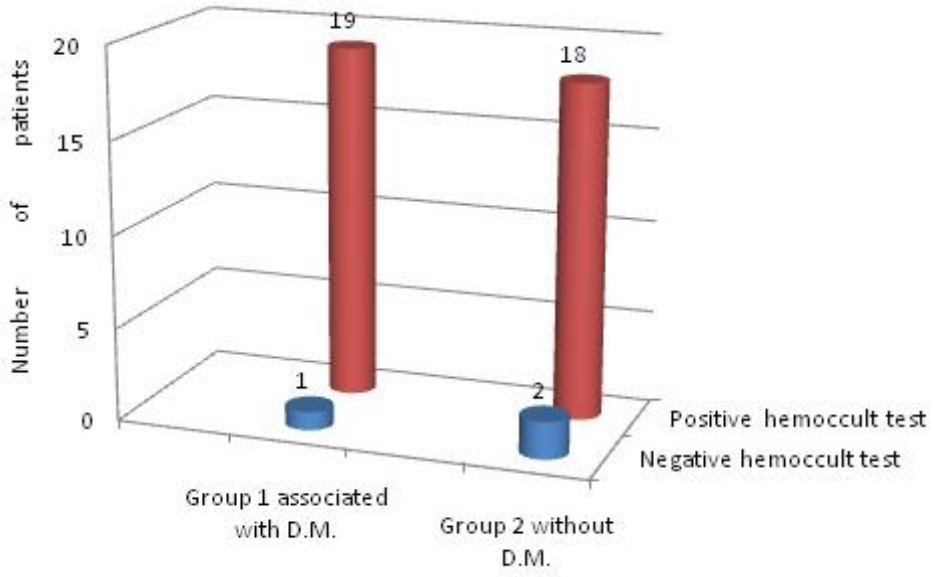

Figure 8. *Hemoccult test results in the two study sub-groups.*

The distribution of patients with positive hemoccult test in the two study sub-groups was similar: 19 patients from group 1 (95%) and 18 from group 2 (90%) as shown in Figure 8. From the statistical point of view there are no significant differences between the diabetic and nondiabetic patients in what concerns the values of the hemoccult test (p=0,105).

Statistic evaluation (sensibility, specificity, positive and negative predictive value, accuracy index and Youden index) for fecal calprotectin, carcinoembryonic antigen and hemoccult test in patients from group 1 (diabetics with colorectal cancer) are detailed in Table 1.

Table 1. Statistic evaluation of the fecal calprotectin, carcinoembryonic antigen and hemoccult test values in the patients from group 1 (diabetics with colorectal cancer)

	Fecal calprotectin	**Carcinoembryonic antigen**	**Hemoccult test**
Sensitivity (%)	90.24	73.17	92.68
Specificity (%)	83.54	83.54	91.14
Positive predictive value (%)	74.00	69.77	84.44
Negative predictive value (%)	94.29	85.71	96.00
Accuracy (%)	85.83	80.00	91.67
Youden index (%)	2.74	2.57	2.84

Discussion

Analyzing the obtained results we noticed increased values of the calprotectin in the faeces of patients with colorectal cancer, showing an increased sensibility of the fecal calprotectin for the colorectal cancer detection. The increase of

fecal calprotectin values was more important in the group of diabetic patients with colorectal cancer. This is associated with the already known etiological data which recognize common risk factors for both diseases [10-13], but there are studies which suggest that hyperglycemia is an independent risk factor for colorectal cancer [10]. The evolution in dynamics of the fecal calprotectin levels assessed at 3 and 6 months post-surgery highlighted a tendency towards a slower normalization in patients with diabetes, observation which could be explained by the important changes that diabetes induce upon the vascular endothelium, with significant repercussions on the status of the intestinal mucosa, favoring chronic inflammations.

As compared with the hemoccult test (recognized as part of the current screening and diagnosis of colorectal cancer), the results of fecal calprotectin were comparable, although the occurrence of blood and calprotectin in faeces recognize different mechanisms [14]. This demonstrates the existence of an increased sensitivity of fecal calprotectin for the detection colorectal cancer.

According to the results of our study, the sensitivity of fecal calprotection is much higher compared to the sensitivity of carcinoembryonic antigen. Thus, fecal calprotectin was positive in 90% of patients while carcinoembryonic antigen only in 72.5%. This can be explained by the fact that the carcinoembryonic antigen represents the systemic expression of colorectal cancer, with a decreased specificity and comparable values in what concerns the sensitivity for the detection of colorectal cancer, pulmonary, mammary, ovary and cervical neoplasms. In contrast, fecal calprotectin represents the expression of the local intensity of the disease and directly measures intestinal inflammation.

Conclusion

At the end of the study, we noticed that the values of the calprotectin in faeces in the patients with colorectal cancer were increased, with greater percentage values in diabetic patients than in the nondiabetic ones.

The postsurgical evolution of the fecal calprotectin values emphasizes a tendency towards a slower normalization in patients with hyperglycemia and colorectal cancer as compared to those without hyperglycemia.

The percentage of the patients with increased values of the fecal calprotectin was slightly equal with that of the patients with positive hemoccult test.

Fecal calprotectin presents an increased sensibility in colorectal cancer as compared to the carcinoembryonic antigen.

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REFERENCES

1. **Ferlay J, Soerjomataram I, Ervik M at al.** GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. *Lyon, France: International Agency for Research on Cancer*; 2013. Available from: http://globocan.iarc.fr, accessed on 05.11.2014

2. **Hart AR, Wicks AC, Mayberry JF.** Colorectal cancer screening in asymptomatic populations. *Gut* 36: 590-598, 1995.

3. **Røseth AG, Kristinsson J, Fagerhol MK at al.** Faecal calprotectin: a novel test for the diagnosis of colorectal cancer? *Scand J Gastroenterol* 28: 1073-1076, 1993.

4. **Gurban CV, Goţia, Creţu O at al.** Rolul calprotectinei fecale ca marker non-invaziv in cancerul colorectal. *Revista Română de Medicină de Laborator* Vol. 19, Supliment la Nr. 2/4, S34-S35, 2011 (Abstract)

5. Fagerhol MK, Dale I, Anderson T. Release of and quantitation of a leukocyte derived protein (L1). *Scand J Haemotol* 24: 393-398, 1980.

6. Steinbakk M, Naess-Andresen CF, Lingaas E, Dale I, Brandtzaeg P, Fagerhol MK. Antimicrobial actions of calcium binding leukocyte L1 protein, calprotectin. *Lancet* 336: 763-765, 1990.

7. El Saadany S, Mohamed WF, Mohamed AA, Hammoudah SAF. Fecal calprotectin as a marker in differentiating irritable bowel syndrome from organic intestinal disease. Available from: http://elsadany66.wordpress.com/article/fecal-calprotectin-as-a-marker-in-i2p6c6e8rrui-7/

8. Cal-Detect® SOFAR Available from: http://www.sofar.ro/sisteme-diagnosticare/cal-detect.html#sthash.Vu8dv08G.dpuf

9. Kristinsson J, Røseth A, Fagerhol MK at al. Fecal calprotectin concentration in patients with colorectal carcinoma. *Dis Colon Rectum* 41: 316-321, 1998.

10. Larsson SC, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. *J Natl Cancer Inst*. 97:1679–1687, 2005.

11. Yang YX, Hennessy S, Lewis JD. Insulin therapy and colorectal cancer risk among type 2 diabetes mellitus patients. *Gastroenterology* 127: 1044–1050, 2004.

12. Giovannucci E. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J Nutr* 131 (11 Suppl): 3109S–3120S, 2001.

13. Vinikoor LC, Long MD, Keku TO, Martin CF, Galanko JA, Sandler RS. The association between diabetes, insulin use, and colorectal cancer among Whites and African Americans. *Cancer Epidemiol Biomarkers Prev* 18: 1239–1242, 2009.

14. Gilbert JA, Ahlquist DA, Mahoney DW, Zinsmeister AR, Rubin J, Ellefson RD. Fecal marker variability in colorectal cancer: calprotectin versus hemoglobin. *Scand J Gastroenterol* 31: 1001-1005, 1996.