

THE EVALUATION OF DIAGNOSTIC VALUE OF THE TUMOR MARKERS: CCSA-2 AND CEA IN COLORECTAL CANCER

BARTŁOMIEJ KNYCHALSKI, TADEUSZ ŁUKIEŃCZUK

First Department and Clinic of General, Gastroenterological and Endocrinological Surgery,
Medical University in Wrocław
Kierownik: prof. dr hab. *T. Łukieńczuk*

Finding the biomarker or biomarkers with high sensitivity and specificity in colorectal cancer, and thus a high diagnostic value will determine their clinical usefulness in clinical practice. An effective noninvasive blood test would be an ideal method to detect colorectal cancer. Discovered in 2007 a novel tumor marker CCSA-2 shows a promising results in patients with colorectal cancer.

The aim of the study was the evaluation of diagnostic and clinical value of a novel marker – colon cancer specific antigen-2 (CCSA-2) in colorectal adenocarcinoma in comparison to carcinoembryonic antigen (CEA) in patients operated during the years 2008 to 2010 at Wrocław Medical University 1st Department and Clinic of General, Gastroenterological and Endocrinologic Surgery.

Material and methods. The study was performed on 40 patients with colorectal cancer and 40 patients in control group consisted of healthy subjects who had colonoscopy examinations with negative results (no pathology in the colon was found). The obtained results were statistically analyzed using nonparametric tests – Mann Whitney U and Kruskal-Wallis and Spearman's rank correlation coefficients. To determine the clinical value of CCSA-2 and CEA in those groups, their sensitivity and specificity was evaluated using ROC analysis. This analysis determines the accuracy and diagnostic value of both tests.

Results. There was a positive correlation between markers in patients with colorectal cancer and a statistically significant relationship according to which respondents with higher concentrations of CCSA-2 also have higher concentrations of CEA ($R=0.754$, $p<0,001$). Concentrations of tumor markers increase and correlate with the clinical progression of the disease. Accuracy of CCSA-2 test using ROC analysis showed a slightly lower measurement of antigen CCSA-2 as diagnostic value in colorectal cancer in comparison to measurement of antigen CEA (accuracy of tests: CCSA-2 – 52%, CEA – 60%).

Conclusions. CCSA-2 as a single tumor marker has a low diagnostic value in colorectal cancer because of low sensitivity and specificity. The diagnostic value of novel marker is slightly lower than previously understood and accepted in clinical practice – CEA.

Key words: colorectal cancer, CCSA-2, CEA, tumor biomarkers

Colorectal cancer is a growing oncological and health problem in Poland as well as in industrialised Western European countries and the USA. It is one of the most common malignant neoplasms globally. The number of newly diagnosed colorectal cancer cases worldwide stands at close to one million annually (1, 2). Colorectal cancer is the second, in terms of prevalence, malignant neoplasm in both genders in Poland. Each year, there are diagnosed over 14,000 new cases. In addition, it is

the causative factor of over 9,000 deaths, the second most common cause of death in males, behind lung cancer, and third in females – behind breast cancer and lung cancer (3).

For many years now there have been held discussions on the selection of the best diagnostic methods enabling the earliest possible diagnosis of colorectal cancer, the selection of correct additional treatment and the monitoring of its course. An ideal non-invasive screening blood serum test might prove an effective

method of colorectal cancer diagnosis. The work on the identification of a biomarker (or biomarkers) that would fulfil all the above criteria is ongoing. Perhaps combined assaying of many specific tumour markers, the diagnostic usefulness of which in colorectal cancer is continuously studied, might improve the efficiency of its diagnosis. To date, no ideal marker specific for all neoplasm types, including colorectal cancer, with 100% sensitivity and specificity, has been identified. Only such a test value enables the determination of its efficiency and clinical usefulness. So far, the carcinoembryonic antigen (CEA) has been the only tumour marker among many that found use in everyday clinical practice in patients with colorectal cancer. As a highly specific marker of proliferation in the large intestine, it has found acceptance in medical circles, and the recommendation of international oncological societies for its use has made it an inherent part of everyday clinical practice, albeit mainly in the monitoring of neoplasm recurrence (4-8).

Recent years have also brought promising data on novel tumour markers. There have already been identified tumour antigens specific for colorectal cancer and other neoplasms, such as breast, prostate, bladder, lung, ovarian cancer and others (9, 10). Those markers belong to the family of the so-called nuclear matrix proteins (NMPs). Their characteristic features are changes in the structure and function of cell nucleus, which may be detected with the use of the latest tests. A thorough analysis of nuclear matrix proteins has led to the identification of colon cancer specific antigens (CCSAs) encompassing the family of CCSA-1, -2, -3, -4, -5, -6a and -6b proteins with molecular mass of 20-60 kDa. Among the nuclear matrix proteins specific for colorectal cancer, particular interest has been placed on colon cancer specific antigen-2 (CCSA-2), the high expression of which has been found in highly dysplastic adenomas and invasive cancers.

The analysis of amino acid sequence of CCSA-2 has indicated that some of its regions

are common for other proteins, which has led to research on a novel, poorly characterised, tumour marker the assaying of which in colorectal cancer might prove useful in everyday clinical practice. So far, the individual stages of biochemical process by which CCSA-2 appears in the blood serum have not been identified or studied. It is suspected that the protein becomes detectable in the blood as a result of neoplastic cell degradation (11, 12). In 2008, American scientists presented preliminary data at the Gastrointestinal Cancers Symposium, indicating that CCSA-2 was characterised by high sensitivity and specificity and that the accuracy (ACC) of its determination in colorectal cancer was highest among the tumour markers identified so far, standing at 80-90% (13, 14).

The aim of the study was the assessment of the diagnostic value and clinical usefulness of a novel specific antigen (CCSA-2) in colorectal adenocarcinoma at various advancement stages as compared with the carcinoembryonic antigen (CEA) in patients undergoing surgery in the years 2008-2010 at the First Department and Clinic of General, Gastroenterological and Endocrinological Surgery, Wrocław Medical University.

MATERIAL AND METHODS

The study was conducted on a group of patients with colorectal cancer and on a control group (80 individuals). The former encompassed 40 patients who had undergone surgery due to colon cancer ($n = 28$) and rectal cancer ($n = 12$). The procedures had been performed at the First Department and Clinic of General, Gastroenterological and Endocrinological Surgery, Wrocław Medical University in the period between July 2008 and January 2010 (tab. 1). The studied individuals included 22 males and 18 females (mean age: 65 ± 10.7). Most of the patients – 18 (45%) – were graded at stage 3 of clinical advancement of colorectal cancer, while the smallest number – 4 (10%) – at stage 1 of advancement. Intermediate figures were determined for patients at stage

Table 1. Locations of cancer in the large intestine in the study group

Location of colorectal cancer	Men	Women	Total
Colon	14 (35%)	14 (35%)	28 (70%)
Rectum	8 (20%)	4 (10%)	12 (30%)
Total	22 (55%)	18 (45%)	40 (100%)

2 of advancement – 11 (27.5%) – and stage 4 of neoplastic disease advancement with metastatic lesions in lymph nodes and distant metastases – 7 (17.5%) (tab. 2).

The control group encompassed 40 healthy individuals without the presence of colorectal disease, as evidenced by a previously performed colonoscopy (normal endoscopy picture of the large intestine). This group included 23 males and 17 females aged 46-88. The mean age for both groups was 63 ± 11.5 .

The entire study involved 2 parts:

- 1) pre-operative determination of the colon cancer specific antigen (CCSA-2) level and carcinoembryonic antigen level in the blood serum of patients with colorectal cancer and individuals from the control group,
- 2) histopathological evaluation of tumour advancement stage as per the TNM system (pTNM).

CCSA-2 was assayed by means of the ELISA immunoenzymatic reaction with the use of ready reagent kits by USCNLIFE (Cat. No 1649h), while the CEA level was determined using the ARCHITECT apparatus by ABBOT and ready reagent kits (Cat. No 7K68). The study was approved by the Bioethics Committee. The obtained results were subject to statistical analysis by nonparametric methods (Mann-Whitney *U* test and Kruskal-Wallis test) and Spearman's rank correlation coefficient

(*R*). The statistical analysis was performed with the use of STATISTICA 9.0 software. The adopted statistical significance threshold was $p < 0.05$. For the evaluation of clinical usefulness of the performed CCSA-2 and CEA determinations in the studied group, they were examined for sensitivity and specificity by means of receiver operating characteristic (ROC) curve enabling the determination of the so-called accuracy (ACC) and thus the diagnostic value of the tests.

RESULTS

Table 3 presents the mean values (in ng/ml) and standard deviations for pre-operative levels of CCSA-2 and CEA in the blood serum according to the advancement stage of colorectal cancer.

The lowest level of CCSA-2 in the group of patients with colorectal cancer was seen for patient No 8 and it was close to 0.0 ng/ml, while the highest value was found in patient No 2 – 182.2 ng/ml. The mean CCSA-2 level for the entire group was 40.8 ± 48.9 ng/ml. In the control group, the lowest value was observed for individuals No 16 and 27, at close to 0 ng/ml, while the highest one was found in individual No 11 – 96.6 ng/ml. The mean CCSA-2 level in this group stood at 27.3 ± 25.2 ng/ml.

Table 2. The division of the study group due to the clinical stage of colorectal cancer

TNM classification of colorectal cancer	Men	Women	Total
0° (Tis, N0, M0)	0 (0%)	0 (0%)	0 (0%)
I°(T1 / T2, N0, M0)	3 (7,5%)	1 (2,5%)	4 (10%)
II°(T3 / T4, N0, M0)	4 (10%)	7 (17,5%)	11 (27,5%)
III°(T, N1, M0; T, N2, N3, M0)	10 (25%)	8 (20%)	18 (45%)
IV°(T i N, M1)	5 (12,5%)	2 (5%)	7 (17,5%)
Total	22 (55%)	18 (45%)	40 (100%)

Table 3. Comparative analysis of concentration of CCSA-2 and CEA according to the clinical stage of colorectal cancer

TNM classification of colorectal cancer	I°T1 / T2, N0, M0	II°T3 / T4, N0, M0	Each T, N1, M0 each T, N2, N3, M0	Each T and N, M1	Total
Number of patients	4	11	18	7	40
The average concentration of CCSA-2 (ng / ml) and standard deviation	$8,2 \pm 4,3$	$40,8 \pm 43,6$	$37,0 \pm 47,1$	$69,2 \pm 66,2$	$40,8 \pm 48,9$
The average concentration of CEA (ng / ml) and standard deviation	$1,2 \pm 0,3$	$6 \pm 9,2$	$21,9 \pm 49,5$	$365,6 \pm 812,2$	$75,6 \pm 347,8$

As regards the CEA level in the group of patients with colorectal cancer, the lowest value was found for patient No 7 – 0.3 ng/ml, and the highest for patient No 21 – 2,186.5 ng/ml. The mean CEA level for the entire group was 75.6 ± 347.8 ng/ml. In the control group, the lowest value was observed for individual No 16 – 0.42 ng/ml, and the highest for individual No 13 – 7 ng/ml. The mean CEA level in this group stood at 1.8 ± 1.2 ng/ml.

Table 4 presents the mean values and standard deviations for CCSA-2 and CEA according to the localisation of colorectal cancer.

The analysis of mean CCSA-2 levels indicated that its lowest value was present in patients with ascending colon cancer – 0.4 ± 0.6 ng/ml. The highest CCSA-2 levels were observed in patients with caecal cancer – 58.9 ± 57 ng/ml. No significant differences were found between the mean CCSA-2 levels determined for 2 sections of the large intestine: for rectal cancer the said value stood at 42 ± 38.5 ng/ml, while for colon cancer at 40.3 ± 53.4 ng/ml.

The lowest CEA levels were found in patients with ascending colon cancer – 0.9 ± 0.5 ng/ml, transverse colon cancer – 1 ± 0.3 ng/ml and descending colon cancer – 3 ± 0 ng/ml. The highest mean CEA level was seen in patients with splenic flexure cancer – 737 ± 1255.3 ng/ml. Upon considering the two sections of the large intestine – colon and rectum – separately, it was determined that the mean CEA value in patients with rectal cancer stood at 7.7 ± 11.7 ng/ml, while in individuals with colon cancer at 104.7 ± 414.4 ng/ml.

The CCSA-2 levels in the group of cancer patients and the control group were compared with the use of nonparametric Mann-Whitney U test. The CCSA-2 values in the cancer patient group were higher than in the control one, yet they did not differ statistically significantly ($p = 0.60$). Furthermore, there were no statistically significant differences found between the CCSA-2 levels in females and males, either in the cancer patient group ($p = 0.41$) or in the control group ($p = 0.45$). It was determined that the higher the clinical advancement stage was, the higher the CCSA-2 levels were present in patients. The small sizes of subgroups with stage 1 (4 individuals) and stage 4 (7 individuals) of advancement hindered the statistical analysis. The Kruskal-Wallis test did not demonstrate any difference between the groups (p

$= 0.23$). Upon combining the subgroups with stages 1 and 2, and subgroups with stages 3 and 4 of clinical advancement, no statistically significant difference was found either ($p = 0.31$; Mann-Whitney U test). Moreover, there was observed no statistically significant difference in the CCSA-2 level between the distinguished two localisations of colorectal cancer ($p = 0.39$; Mann-Whitney U test).

The CEA levels were also compared between the cancer patient and control groups with the use of nonparametric Mann-Whitney U test. Higher CEA values were found in the cancer patient group, yet no statistically significant difference was seen ($p = 0.13$). Furthermore, there were no statistically significant differences in CEA levels observed between males and females, either in the cancer patient group ($p = 0.22$) or the control group ($p = 0.56$). It was determined that the higher the clinical advancement stage was, the higher the CEA levels were present in patients. The small sizes of subgroups with advancement stage 1 (4 individuals) and 4 (7 individuals) hindered the statistical analysis. The Kruskal-Wallis test did not demonstrate any difference between the groups, although $p = 0.1$ only slightly exceeded the significance threshold of 0.05. Upon combining the subgroups with stages 1 and 2, and subgroups with stages 3 and 4 of clinical advancement, no statistically significant difference was found either ($p = 0.26$; Mann-Whitney U test). No statistically significant difference in the CEA level was observed between the subgroup of patients with rectal cancer and those with colon cancer ($p = 0.88$; Mann-Whitney U test).

The correlation between the age and the CCSA-2 and CEA levels was examined by means of Spearman's rank correlation coefficient (R) (tab. 5). A statistically significant correlation coefficient was determined for the association between age and CEA level in the control group ($R = 0.314$, $p = 0.049$). At the same time, highly significant correlation was found between the CCSA-2 and CEA levels in the group of patients with colorectal cancer ($R = 0.754$, $p < 0.001$) and the control group ($R = 0.318$, $p = 0.046$).

The analysis of accuracy (ACC) of the CCSA-2 test by means of the ROC curve demonstrated a slightly lower usefulness of CCSA-2 assaying in the studied group as a diagnostic value as compared with the CEA

Table 4. Comparative analysis concentrations of CCSA-2 and CEA in different locations of colorectal cancer

The location of colorectal cancer	The average concentration of CCSA-2 (ng / ml) and standard deviation	The average concentration of CEA (ng / ml) and standard deviation	Number of patients
Rectum	42 ± 38,5	7,7 ± 11,7	12
Colon	40,3 ± 53,4	104,7 ± 414,4	28
Total	40,8 ± 48,9	75,6 ± 347,8	40

Table 5. Spearman R correlation coefficients between age, CCSA-2 and CEA

	Number	R Spearman	p value
Patients with colorectal cancer			
age and CCSA-2	40	0,06	0,72
age and CEA	40	0,08	0,64
CCSA-2 and CEA	40	0,75	<0,001
Control group			
age and CCSA-2	40	0,04	0,82
age and CEA	40	0,31	0,05
CCSA-2 and CEA	40	0,32	<0,05

determination. The test accuracy (area under curve – AUC) of 0.522 meant that the differentiation between ill and healthy individuals could have been performed with 52% accuracy. The highest value of Youden's index, standing at just 0.15 (preferred: 0.60), was obtained for CCSA-2 of 97 ng/ml (cut-off limit) at which the test sensitivity was 0.15 and specificity – 1. The above indicates that only every sixth patient had the CCSA-2 level higher than the highest value observed in the group of healthy individuals.

The analysis of accuracy (ACC) of the CEA test by means of the ROC curve demonstrated insufficient usefulness of CEA assaying in the studied group as a diagnostic value. The test accuracy (area under curve – AUC) of 0.604 meant that the differentiation between ill and healthy individuals could have been performed with 60% accuracy. The highest value of Youden's index, standing at just 0.275 (preferred: 0.60), was obtained for CEA of 2.7 ng/ml (cut-off limit) at which the test sensitivity was 0.40 and specificity – 0.875. At the above cut-off limit for CEA = 7 ng/ml the test sensitivity stood at 0.275, specificity at 1, and the Youden's index at 0.275 as well. The above indicates that slightly more than every fourth patient had the CEA level higher than the highest value observed in the group of healthy individuals.

Figure 1 presents the ROC curves for the levels of both antigens (CCSA-2 and CEA) in the blood serum of studied individuals.

DISCUSSION

The appearance of the so-called nuclear matrix proteins, abnormal and specific for the given type of neoplastic cells, has led to the analysis of their structure in different types of neoplasm with the aim of determining whether the above proteins could serve as diagnostic

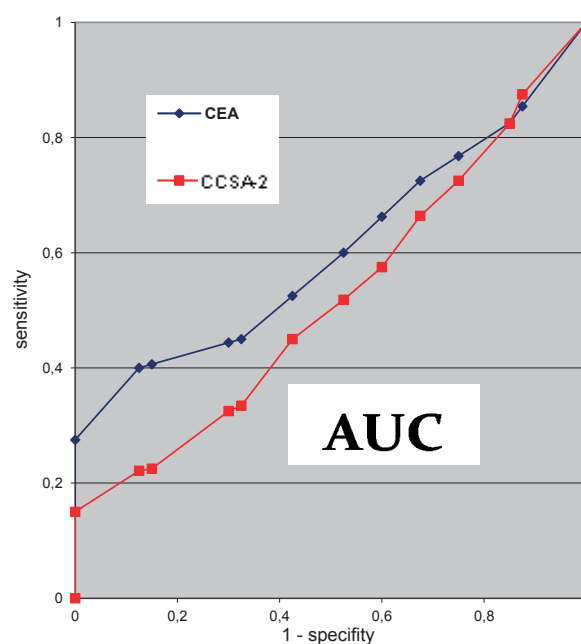


Fig. 1. ROC curves for concentrations of CCSA-2 and CEA in the study group

accuracy (ACC) for CCSA-2 test – 52%, accuracy (ACC) for CEA test – 60%
AUC – area under curve

and prognostic markers of malignant neoplasms, including colorectal cancer. Among the nuclear matrix proteins specific for colorectal cancer, particular interest has been placed on CCSA-2, for which the assaying accuracy in colorectal cancer, as per the preliminary results, has been high and close to the value for ideal markers characterised by nearly 100% sensitivity and specificity. The present study analysed the variables for a novel marker, CCSA-2, promising in terms of diagnostic value, as well as determined whether its clinical usefulness in colorectal cancer is higher than that of the only recommended marker to date, namely CEA.

Based on the accuracy analysis of CCSa-2 assay in the studied group, it was determined that it demonstrated a moderate clinical usefulness in colorectal cancer (ACC: 52%). The above value of the test differs significantly from the values obtained by American researchers who have evidenced the accuracy of its determination in the analysed groups at 80-90% (13, 14). The American studies on CCSA-2 conducted to date have indicated that it was expressed in 80% of colorectal cancer cells. The majority of patients in the studied group did not exhibit CCSA-2 expression above the determined cut-off limit (> 97 ng/ml – expression was present in 6 cancer patients only), indicating that the above research requires a thorough analysis, extension of the study over a larger representative patient group, and the determination of other nuclear matrix proteins, such as CCSA-3 or CCSA-4, the assaying of which in combination with CCSA-2 might increase their clinical usefulness in the colorectal cancer diagnostics.

On the other hand, the accuracy analysis of CEA assay indicated its moderate clinical usefulness in diagnosed colorectal cancer (ACC: 60%), although the diagnostic value of the above test in the studied group was slightly higher than that of CCSA-2 (ACC: 52%). The sensitivity and specificity values for CEA obtained at the determined cut-off limits did not differ from those reported by other researchers (15, 16). Hence, the European Group on Tumour Markers, in compliance with the recommendations of the National Institute of Health and the American Society of Clinical Oncology, has abandoned CEA assaying in screening tests due to its unsatisfactory sen-

sitivity and specificity, particularly in the early phases of colorectal cancer.

In the study results summary it was stated that the accuracy of differentiation between colorectal cancer patients and healthy individuals in the studied group with the use of CCSA-2 assaying was slightly lower than that by means of CEA determination. By using the CCSA-2 assay, colorectal cancer was confirmed in every sixth patient, while with the use of CEA assay cancer was confirmed in every fourth patient. Therefore, the accuracy of CEA test was slightly higher than that of CCSA-2 assay. Both of the above-stated assaying accuracy values are far from those characterising ideal markers. Based on the statistical analysis of study results it was determined that the studied novel antigen CCSA-2, when assayed alone, would most likely be of little usefulness in the diagnostics of colorectal cancer due to its low sensitivity and specificity. Moreover, it has a slightly lower value than CEA, currently recognised and accepted in clinical practice. The accuracy of CCSA-2 assaying stood at 52% and of CEA – at 60%. Such low statistical evaluation of CCSA-2 and the study conclusions might stem from the too small group of studied patients. The two reports on CCSA-2 published internationally to date and their results in colorectal cancer patient groups are extremely promising (ACC: 80-90%) (13, 14).

CONCLUSIONS

1. Higher CCSA-2 and CEA levels were found in the group of patients with colorectal cancer than in the control group.
2. The levels of tumour markers (CCSA-2 and CEA) grow higher and correlate with the clinical advancement stage of colorectal cancer.
3. The combined analysis of pre-operative CCSA-2 and CEA levels indicated a slightly lower usefulness of CCSA-2 assaying as a diagnostic value in diagnosed colorectal cancer (accuracy test – ACC: 52%) as compared with the CEA determination (accuracy test – ACC: 60%).
4. The low sensitivity and specificity of the above tests in diagnosed colorectal cancer, and thus their low diagnostic value, do not allow currently their full use in everyday clinical practice.

REFERENCES

1. *Steward BW, Kleihues P*: World cancer report. IARC Press, Lyon, France 2003: 198.
2. *Al-Mulla F, Behbehani AI, Bitar MS et al.*: Genetic profiling of stage I and II colorectal cancer may predict metastatic relapse. *Mod Pathol* 2006; 19: 648-58.
3. *Wojciechowska U, Didkowska J, Zatoński W*: Nowotwory złośliwe w Polsce w 2006 roku. Centrum Onkologii – Instytut, Warszawa 2006.
4. *Spann SJ, Rozen P, Young GP et al.*: Colorectal cancer: how big is the problem, why prevent it, and how might it present? In: Rozen P, Young GP, Levin B, Spann SJ, editors. *Colorectal Cancer in Clinical Practice*. London: Martin Dunitz Ltd 2002: 1-18.
5. *Eriksen MT, Wibe A, Haffner J et al.*: Prognostic group in 1,676 patients with T3 rectal treated without preoperative radiotherapy. *Dis Colon Rectum* 2007; 50: 156-67.
6. *Bębenek M, Pudętko M, Cisarz K et al.*: Therapeutic results in low-rectal cancer patients treated with abdominosacral resection are similar to those obtained by means of anterior resection in mid- and upper-rectal cancer cases. *Eur J Surg Oncol* 2007; 33: 320-23.
7. *Duffy MJ, van Dalen A, Haglund C et al.*: Tumor markers in colorectal cancers: European Group on Tumour Markers (EGTM) guidelines for clinical use. *Eur J Cancer* 2007; 43: 1348-60.
8. *Compton C, Fenoglio-Preiser CM, Pettigrew N et al.*: American Joint Committee on Cancer Prognostic Factors Consensus Conference: Colorectal Cancer Working Group. *Cancer* 2000; 88: 1739-57.
9. *Konety BR, Getzenberg RH*: Nuclear structural proteins as biomarkers of cancer. *J Cell Biochem* 1999; Suppl 32-33: 183-91.
10. *Leman ES, Getzenberg RH*: Nuclear structure as a source of cancer specific biomarkers. *J Cell Biochem* 2008; 104(6): 1988-93.
11. *Brunagel G, Schoen RE, Getzenberg RH*: Colon cancer specific nuclear matrix protein alterations in human colonic adenomatous polyps. *J Cell Biochem* 2004; 91: 365-74.
12. *Miller TE, Beausang LA, Winchell LF et al.*: Detection of nuclear matrix proteins in serum from cancer patients. *Cancer Res* 1992; 52: 422-27.
13. *Leman ES, Schoen RE, Magheli A et al.*: Evaluation of colon cancer – specific antigen 2 as a potential serum marker for colorectal cancer. *Clin Cancer Res* 2008; 14(5): 1349-54.
14. *Walgenbach-Brunagel G, Burger B, Leman ES et al.*: The use of colon cancer associated nuclear antigen CCSA-2 for the blood based detection of colon cancer. *J Cell Biochem* 2008; 104: 286-94.
14. *Goslin R, O'Brien MJ, Steele G et al.*: Correlation of plasma CEA and CEA tissue staining in poorly differentiated colorectal cancer. *Am J Med* 1981; 71: 246-53.
15. *Von Kleist S, Hesse Y, Kananeeh H*: Comparative evaluation of 4 tumor markers, CA 242, CA 19/9, TPA and CEA in carcinomas of the colon. *Anticancer Res* 1996; 16: 2325-31.

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Adress correspondence: 50-369 Wrocław, ul. M. Skłodowskiej-Curie 66