

# Blood serum levels of E-cadherin in patients with colorectal cancer

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## Abstract

**Introduction:** Colorectal cancer is the second most common cancer in terms of incidence in Poland. It is also the second most common cause of cancer deaths in men and the third women. In 75–80% of cases, depending on sources, it is of an occasional nature, and in the remaining 20–25% it has a hereditary character.

**Aim:** To compare the levels of E-cadherin in blood serum with some histopathological and clinical features. E-cadherin is an adhesion molecule, loss of function of which is suspected to influence both cancer progression and metastasis.

**Material and methods:** The study group comprised 48 patients diagnosed with colorectal cancer treated surgically in the Second Department of General and Gastroenterological Surgery, Medical University Hospital in Białystok.

**Results:** As has been shown here, there is no statistically significant relationship between the levels of E-cadherin in blood serum and the possible prognosis to the progression of colorectal cancer. However, it was indicated that there appears to be a statistically significant relationship between blood serum E-cadherin levels and the levels of alanine aminotransferase and aspartate aminotransferase in patients with colorectal cancer.

**Conclusions:** The authors suggest that this significance may require further study.

## Introduction

Colorectal cancer, also known as colon or bowel cancer, takes second place in Poland in terms of the number of new cases diagnosed every year [1]. It is also listed as the second and third cause of cancer deaths for men and women per year, respectively. In 75–80% of cases, depending on source, the colorectal cancer has a self-contained character and in the remaining 20–25% the cause is hereditary [1–3].

The adhesion phenomenon plays a crucial role in the regulation of vital biological mechanisms on a single-cell level, such as growth, differentiation, and migration. The proteins involved in the process of cellular adhesion are the so-called cellular adhesion molecules (CAMs) [4, 5]. These take part as a go-between in side-to-side relations of cells and between cells and extracellular matrix. There is strong evidence that inappropriate adhesion causes neoplastic cells to become invasive [6].

Cadherins are a large group of adhesion proteins, which, mostly by calcium ion binding in their structure, take part in cellular relations [6, 7]. These transmembrane proteins are able to recognise other molecules of the same type and create junctions with them. Classical cadherins: N-cadherin, E-cadherin, and P-cadherin, build the so-called adherence junctions and are needed for proper functioning of cell-to-cell adhesion mechanisms. E-cadherin belongs to a super-family of adhesion molecules dependent on calcium ions [6]. It is suggested that functional disorders of this molecule lead to neoplastic growth [6, 7]. On the other hand, the existence of metastases depends on E-cadherin's loss of function, what has already been proven [8]. These relations had been described in cases of prostate and lung cancer [9]. It is also suggested that E-cadherin disorders have a strong influence on the progression of neoplastic diseases of the stomach, pancreas, and the large intestine [10–12].

## Aim

The goal of this study was to point out the correlations between blood serum levels of E-cadherin and clinical, anatomical, and histopathological staging of the disease in patients with colorectal cancer.

## Material and methods

### The study group

The study group comprised 48 patients diagnosed with colorectal cancer treated surgically in the Second Department of General and Gastroenterological Surgery, Medical University Hospital in Białystok. In the study group there were 14 women and 34 men. Mean age was 67 years. In 28 cases the lesion was located in the colon, and in the rectum in 20 others. In 14 patients of the study group there were coexisting, confirmed liver metastases. In 45 cases the tumour had been described as a middle-stage histological grading (G2) and in the other three cases it was poorly differentiated (G3). Distribution of patients according to the TNM classification was as follows: stage I was diagnosed in 3 patients, II in the next 14, III was diagnosed in 18 patients, and IV in 13 patients. Table I shows the clinical characteristics of the patients in the study group.

Blood samples from patients with colorectal cancer were collected to clot before every surgery. Standard sample volume was 3–5 ml, which allowed to obtain up to 1.5 ml of serum after clotting was completed. Clotting time was approximately 120 min at room temperature. Samples were left in a refrigerator for about 2–4 h for the clot to be fully formed. The samples were then transported to a laboratory, where centrifugation was performed at 3000 rpm (rounds-per-minute) for 10 min at room temperature. After centrifugation, the serum was secured at –80°C until assayed.

### The control group

The control group consisted of 24 healthy subjects (13 men and 11 women, mean age of 60 years for women and 58 for men). Tests were carried out after obtaining the consent of the Bioethics Committee. Each of the

patients received information on the method and purpose of research and gave written informed consent to participate in the research.

### Enzyme-linked immunoabsorbent assays (ELISA)

E-cadherin concentrations were measured with a quantitative sandwich enzyme immunoassay (R&D Systems, Abingdon, UK). Serum samples were incubated on microtitre plates pre-coated with a murine monoclonal antibody against E-cadherin. The platelets were washed, and an enzyme-linked polyclonal antibody (part of the ELISA kit) specific for E-cadherin was added to the wells. After the second washing procedure, a substrate solution was added. This substrate coloured in proportion to the amount of E-cadherin, which was bound to the microtitre plate. The samples were tested in duplicate and there were no statistically significant differences between assays. Human E-cadherin Quantikine Colorimetric Sandwich ELISA Kit by R&D Systems recognised recombinant and natural human E-cadherin. The results are presented in nanograms per millilitre.

### Statistical analysis

Statistical analysis was performed based on the program Statistica 8.0. The *U* Mann-Whitney and Kruskal-Wallis tests were used for comparison of the two groups. Non-parametric Spearman's correlations were used to determine the relation between the level of E-cadherin, clinicopathological factors, and morphological parameters of blood. A *p*-value < 0.05 was considered statistically significant.

## Results

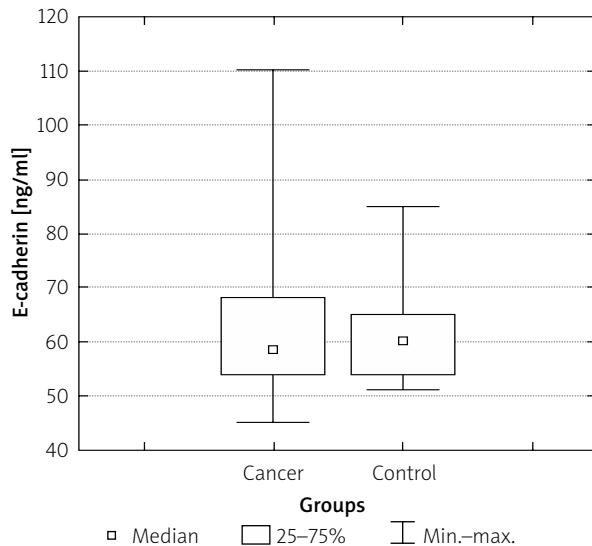
There were no statistically significant differences between the levels of E-cadherin in blood serum of patients with colorectal cancer and the control group, *p* > 0.05. Magnitude control group values ranged from 51.2 to 85.1 ng/ml with an average of 61.4 ng/ml, and the group of colorectal cancer ranged from 42.2 to

**Table I.** Clinical characteristics of the patients

Parameter		N	Parameter		N	Parameter		N
Gender	F	14	T feature	T1	1	M feature	M0	30
	M	34		T2	10		M1	14
G feature	G2	45	N feature	T3	37	TNM staging	I	3
	G3	3		N0	23		II	14
Location of primary tumour	Rectum	20	N1	15	III		18	
	Colon	28	N2	10	IV		13	

**Table II.** A comparison of the E-cadherin between the study and control groups

Group	N	E-cadherin [ng/ml]				P-value
		Mean	Median	SD	Range	
Normal	24	61.44	60	8.84	51.18–85.08	0.671
Tumour	48	61.71	58.51	12.75	45.22–110.26	

**Figure 1.** A graphic representation of E-cadherin levels in the serum of patients with colorectal cancer in comparison with control groups

110.3 ng/ml, with an average of 61.7 ng/ml. The following relation is hereby shown in Table II and the graphic representation is in Figure 1.

There were also no significant correlations between the levels of E-cadherin in blood serum of patients with

colorectal cancer and the level of malignancy in histopathological changes,  $p > 0.05$ , as shown in Table III.

There were also no statistically significant differences in the levels of blood serum E-cadherin and tumour stage (Table IV), the occurrence of lymph node metastasis (Table V), or liver metastases (Table VI).

The results of the concentration levels of blood serum E-cadherin were also correlated with such characteristics as age, the assessment of biochemical parameters (haematocrit, haemoglobin concentration, WBC, RBC, PLT), prothrombin time, prothrombin index, international normalise ratio (INR), total protein, albumin, and liver enzymes: alanine aminotransferase (ALT), aspartate aminotransferase (AST). The results of these comparisons are shown in Table VII. The nonparametric Spearman correlations were used to assess the statistical relationships.

Statistically significant correlation was observed between the level of E-cadherin and the ALT ( $p = 0.044$ ) and AST ( $p = 0.006$ ) levels in the blood serum of these patients. Graphic representations of these relationships are shown in Figures 2 and 3.

## Discussion

Disorders of E-cadherin expression are currently a subject being raised by many authors, considering

**Table III.** Comparing the levels of E-cadherin in blood serum of patients with colorectal cancer and the degree of malignancy of histopathological changes

G	N	Mean	Standard deviation	Minimum	Median	Maximum	P-value
2	42	0.459	0.145	0.271	0.417	0.971	0.460
3	6	0.381	0.083	0.288	0.411	0.445	

**Table IV.** Comparing the levels of E-cadherin in blood serum of patients with colorectal cancer and the staging of the tumour (Mann-Whitney test)

pT	N	Mean	Standard deviation	Minimum	Median	Maximum	P-value
1	1	0.398		0.398	0.398	0.398	0.539
2	8	0.389	0.116	0.316	0.328	0.523	
3	39	0.459	0.146	0.271	0.417	0.971	

**Table V.** Comparing the level of E-cadherin in blood serum of patients with colorectal cancer and the occurrence of metastases to the lymph nodes (Kruskal-Wallis test)

pN	N	Mean	Standard deviation	Minimum	Median	Maximum	P-value
0	23	0.456	0.161	0.288	0.412	0.971	0.895
1	15	0.459	0.134	0.271	0.463	0.695	
2	10	0.430	0.106	0.288	0.416	0.661	

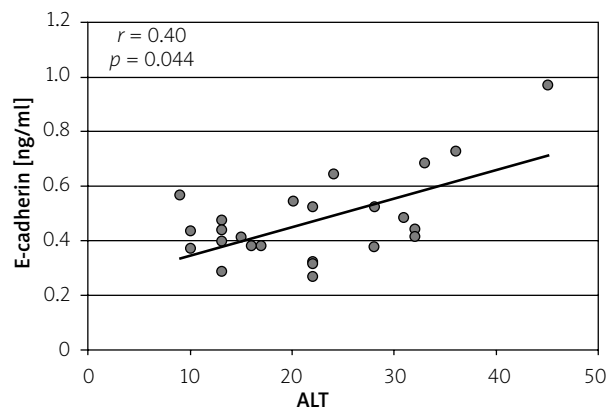
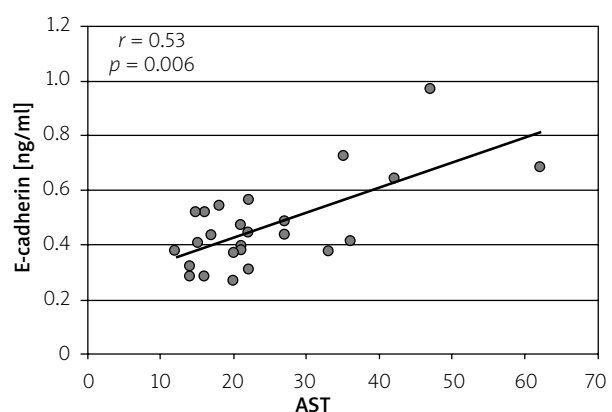
**Table VI.** Comparing the level of E-cadherin in blood serum of patients with colorectal cancer and the occurrence of liver metastases (Mann-Whitney test)

pM	N	Mean	Standard deviation	Minimum	Median	Maximum	P-value
0	34	0.452	0.144	0.271	0.417	0.971	0.939
1	14	0.450	0.133	0.288	0.399	0.695	

**Table VII.** Correlation between E-cadherin levels in the blood serum of the patients and age, and the results of the assessment of biochemical parameters

Parameter	R	P-value
Age [years]	0.23	0.110
Ht (%)	0.14	0.413
Hb [g/dl]	0.24	0.164
WBC [ $10^3/\mu\text{l}$ ]	0.11	0.542
PLT [ $10^3/\mu\text{l}$ ]	-0.25	0.141
RBC [ $10^6/\mu\text{l}$ ]	-0.03	0.850
Prothrombin time [s]	0.19	0.285
Prothrombin index (%)	-0.11	0.547
INR	0.21	0.218
Total protein [g/dl]	0.06	0.802
Albumins (%)	0.03	0.882
AST [IU]	0.53	0.006
ALT [IU]	0.40	0.044

both colorectal cancer and lung cancer, as well as gastric cancer and cancers of the thyroid. Many note the decrease in expression of E-cadherin in the course of colorectal cancer, which is associated with a decrease of its expression in the cell membrane [10–12]. However, in some cases we also have to deal with its overexpression, which is related to its accumulation in the cytoplasm and a decrease of E-cadherin at the cellular membrane [13, 14]. Most previous studies on the level of expression of E-cadherin were carried out within the primary tumour, weather further examination of the

**Figure 2.** The relationship between the level of E-cadherin and serum alanine aminotransferase (ALT) values**Figure 3.** The relationship between the level of E-cadherin in the serum and the value of aspartate aminotransferase (AST)

expression of E-cadherin was assessed in metastatic lymph nodes or in the secondary changes in the liver [12–15]. Based on these studies a thesis was established that the decrease in the expression of E-cadherin in the primary tumour is associated with its ability to spread, and it can be a prognostic factor for the further course of cancer. Some researchers also suggest that the decrease in the expression of E-cadherin is associated with low histological differentiation of the tumour. It has been shown that in the case of a metastatic lesion, the level of expression of E-cadherin is significantly higher than in the primary tumour. In lymph node metastases the level was higher by about 40%, and for changes in the liver by 45% compared to the primary tumour. This may suggest that cancer cells after the occupation of a lymph node or liver acquire the ability to elevated expression of E-cadherin, which allows them to further develop.

These observations were confirmed only among patients who underwent surgical treatment, where the level of E-cadherin was determined in tissues removed [13–15]. In the above work the authors focus on another aspect related to the level of expression of E-cadherin, associated with the designated E-cadherin levels in serum of patients with colorectal cancer. This study could be important in detecting colorectal cancer and eventually in the evaluation of treatment efficacy. However, there was no difference in the level of E-cadherin expression between the test and the control group. This precludes the use of E-cadherin levels in blood serum as a potential tumour marker.

An analysis was also carried out, taking into consideration the relationship between the levels of E-cadherin and the primary tumour histopathological features, the presence of lymph node metastasis, and distant metastases to the liver. There were no significant relations that would indicate that the level of expression of E-cadherin may be predictive as to the exponent of the disease. Also, patient age did not affect the test result.

The authors observed the relationship between the level of expression of E-cadherin in blood serum and the ALT and AST levels. It may be associated with functional damage to the liver, but there arises another thesis that a similar correlation should exist in the case of metastases to the liver, and this has not been demonstrated.

To sum up, the level of expression of E-cadherin determined in the primary tumour and eventual metastatic lesions might be a relevant factor to a possible outcome, it can also lead to further research into improving the treatment. In contrast, the level of expression of E-cadherin in blood serum of patients with colorectal cancer at present has no diagnostic significance.

As for the correlations between serum E-cadherin levels and the levels of ALT and AST, as shown in this observation, they require further study.

## Conclusions

Determination of the level of expression of E-cadherin in the serum of patients with colorectal cancer to date shows no clinical significance. The relationship between the levels of blood serum expression of E-cadherin and the levels of AST and ALT in patients with colorectal cancer requires further study.

## Conflict of interest

The authors declare no conflict of interest.

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