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Original research article

Serum TEM5 and TEM7 concentrations correlate with clinicopathologic features and poor prognosis of colorectal cancer patients

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ABSTRACT

Purpose: Colorectal cancer (CRC) is a serious threat worldwide; therefore, discovering sensitive and specific serum biomarkers for early stages of CRC is a great challenge. In this study, we evaluated whether tumour endothelial marker 5 (TEM5) and 7 (TEM7) circulating in blood serum can be useful as blood-based markers for detection, progression, and prognosis in CRC patients. Moreover, their specificity and sensitivity in the early diagnosis of CRC were compared with common carcinoma diagnostic markers, *i.e.* CEA and Ca 19-9.

Materials and methods: The study included 45 CRC patients and 35 healthy individuals. The serum concentration of TEM5 and TEM7 were quantified using sandwich ELISA.

Results: The mean TEM5 and TEM7 serum concentrations were statistically significantly higher in the CRC patients than in the healthy controls. Moreover, the mean TEM5 and TEM7 concentrations were statistically significantly higher in the serum of patients with late stage (III/IV) compared to early stage (I/II) cancer. The TEM5 and TEM7 values increased along the development of the T, N, and M stages. The TEM5 and TEM7 sensitivity and specificity in CRC detection were higher than routinely used blood markers (CEA, Ca19-9). The high TEM5 and TEM7 concentrations were associated with worse overall survival compared to CRC patients of low TEM5 and TEM7 concentrations.

Conclusions: Taken together, these findings suggest that TEM5 and TEM7 serum concentrations can be considered as useful biomarkers for the detection of CRC patients and for monitoring cancer progression and identifying patients with a high possibility of poor survival.

1. Introduction

Colorectal cancer (CRC) is a serious threat worldwide and the third most commonly occurring cancer in men and the second in women [1]. A significant 60% increase in CRC incidents is still expected, *i.e.* to more than 2.2 million new cases and 1.1 million deaths in 2030 [2]. Incidents and mortality statistics of CRC correlate with geographical regions and human development levels; the highest rates are reported in industrialized areas *i.e.* in Australia and New Zealand, while the lowest values are noted in developing African countries [3]. It is evidenced that CRC occurrence is related to the western lifestyle associated with high consumption of red meat and saturated fats, diabetes mellitus, long-term inflammatory bowel disease, lack of physical activity, and obesity [4]. Therefore, prevention and detection of early cancers and pre-cancerous stages are required to reduce the number of CRC patients in future decades [5]. Diagnostic and analytical methods are in progress; however, a high number of CRC cases is still identified in later stages (III and IV) characterised by spread to lymph nodes and poor outcomes [6]. Currently, carcinoembryonic antigen (CEA) and carbohydrate antigen (Ca 19-9) serum markers are regularly used for diagnosis and checking the progress in CRC patients [7,8]. The disadvantage and weakness of these markers are related to insufficient sensitivity, especially at early stages of CRC development [9]. Therefore, discovering sensitive and specific serum biomarkers for early stages of CRC is a great challenge.

The CRC tumour growth and progression is dependent on angiogenesis [10]. Tumour blood vessels are characterised by abnormal morphology and function, including expression of tumour-specific molecules [11]. A 20-fold expression of unique proteins referred to as tumour endothelial markers (TEMs) has been reported in human tumour endothelium compared to normal endothelium [10]. Therefore, targeting unique TEM expression is a major avenue in the diagnosis and

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monitoring of CRC [12,13].

Particularly attractive candidates that may be a great chance as potential biomarkers for CRC patients are TEM5 (G-protein-coupled receptor 124; GPR124) and TEM7 (plexin domain containing 1; PLXDC1) [14,15]. These molecules belong to a group of integral transmembrane proteins. They are involved in cell-cell and cell-matrix interactions happening during capillary morphogenesis and engaged in proliferation and maintenance of neovascular endothelial cells in fibrovascular membranes (FVMs) [16–18]. Moreover, TEM5 and TEM7 overexpression in tumour vasculature has already been documented [18–20].

The aim of the present study was to evaluate whether TEM5 and TEM7 circulating in blood serum can be used as blood-based markers for detection, progression, and prognosis in CRC patients. Moreover, their specificity and sensitivity in the early diagnosis of CRC were compared with common carcinoma diagnostic markers, *i.e.* CEA and Ca 19-9.

2. Materials and methods

2.1. Study cohorts

Between January 2014 and December 2014, 45 patients (more males n = 29 than females n = 16) at a mean age [± standard deviation (SD)] of 68.91 ± 10.95 years were enrolled among those admitted to the General, Oncological, and Minimally Invasive Surgery Department of the 1st Military Clinical Hospital with the Outpatient Clinic in Lublin, Poland (Table 1).

In all cases, the CRC was confirmed by histopathological examination. The tumour was located in the colon (n = 23; 51%) or in the rectum (n = 22; 49%). The stages of the tumour were determined by a pathologist using histological staining examination according to the rules of the American Joint Commission on Cancer Staging (AJCCS) system. None of the patients had been administered radiotherapy, chemotherapy, or chemoradiotherapy preoperatively. The patients were followed-up at our clinic or by phone calls at 3, 6, 12, 24, 30, and 36 months after surgery.

The control group included healthy volunteers (n = 35; male:female = 19:16) with no clinical evidence of CRC at the time of the study. None of the control participants had been on medication at the time of the study. Healthy blood donors matched in terms of age and body mass index (BMI) with the CRC patients (Table 1).

2.2. Collection and preparation of blood samples

The blood samples were collected into commercially available anticoagulant-treated tubes (EDTA) from all participants, *i.e.* preoperatively from the CRC patients and from the healthy individuals by venipuncture (˜10 ml). The samples were immediately centrifuged at 1000 × g for 10 min at 4 °C. Subsequently, the collected serum (3–4 ml) was aliquoted into clean polypropylene tubes and stored at −80 °C until further use. All samples were processed at room temperature within 3 h from the time of blood extraction. Hemolyzed samples were discarded from further analysis.

Table 1
General characteristics of CRC and control groups.

Characteristics	Control group (n = 35)	CRC group (n = 45)	*p*
Age, years			
Mean ± SD	65.23 ± 8.76	68.91 ± 10.95	0.327[a]
Median (min-max)	64 (45-85)	68 (45-88)	
Gender, n (%)			0.251[a]
Male	19 (54%)	29 (64%)	
Female	16 (46%)	16 (36%)	
Body Mass Index, n (%)			0.407[b]
≤24.99	14 (40%)	14 (31%)	
25.00 – 29.99	10 (28%)	17 (38%)	
≥30.00	11 (32%)	14 (31%)	

[a] *p* in Mann-Whitney *U* test.
[b] *p* in Kruskal Wallis test; CRC – colorectal cancer.

2.3. Biomarkers assay

2.3.1. Quantification of TEM5 and TEM7

Concentrations of TEM5 and TEM7 in the serum samples were quantified with the use of sandwich ELISA according to the manufacturer's instructions (MyBioSource, San Diego, USA). The samples were centrifuged again after thawing before the assay. We added 100 µl of standards and samples to appropriate wells covered with an adhesive strip, and incubated for 2 h at 37 °C. After incubation, the liquid was removed from each well. In the next step, 100 µl of biotin-antibody was added to the wells, covered with a new adhesive strip, and incubated for 1 h at 37 °C. The plate was washed with the use of an automatic washer TriNEST (Perkin Elmer, MA, USA). Then, 100 µl of HRP-avidin was added to the wells, covered with a new adhesive strip, and incubated for 1 h at 37 °C. After incubation, the plate was automatically washed and 90 µl of TMB substrate was added and incubated for 15 min at 37 °C. In the last step, 50 µl of a stop solution was added into the wells. The optical density was read at 450 nm wavelength using a microplate reader Victor (Perkin Elmer, MA, USA). The standard curve was plotted by reducing the data with computer software capable of generating a four parameter logistic (4-PL) curve-fit. The detection range for the TEM5 assay was 62.5 pg/ml - 4000 pg/ml and the sensitivity limits were lower than 15.6 pg/ml. In turn, the detection range for the TEM7 assay was 78 pg/ml - 5000 pg/ml and the sensitivity limits were lower than 19.5 pg/ml.

2.3.2. Measuring CEA and Ca 19-9 markers

The CEA and Ca 19-9 serum markers were assessed using a Cobas 6000 analyser (Roche Diagnostic, North America). The normal values for the CEA marker are less than 3.8 ng/ml (age 20–39 years) and 5.0 ng/ml (age 40–69 years) for nonsmokers and less than 5.5 ng/ml (age 20–39 years) and 6.5 ng/ml (age 40–69 years) for smokers, while the normal value for the Ca 19-9 marker is 0.6–39.00 ng/ml.

2.4. Statistical analysis

The data were analysed descriptively (mean ± SD; median with minimum and maximum values) and statistically using SPSS software (SPSS 15.0, Chicago, IL, USA) and XLSTAT 2018; Data Analysis and Statistical Solution for Microsoft Excel (Addinsoft, Paris, France, 2017). After homogeneity testing, the Mann-Whitney nonparametric U-test was applied to assess the difference in the TEM5 and TEM7 concentrations between the two cohorts – the CRC patients and healthy controls. Within the CRC cohort, the Kruskal Wallis test was applied to compare the TEM5 and TEM7 concentrations among 4 groups of the depth of invasion (T1,T2,T3,T4). The TEM5 and TEM7 concentrations across 2 groups of lymph nodes (N0, N1 + 2) and distal metastases (M0, M1) were compared with the Mann-Whitney U-test.

The Spearman rank correlation coefficient (r) was used to test the association between the concentration of the studied markers (TEM5, TEM7, CEA, and Ca19-9) and T-stage as well as between the TEM5 and TEM7 concentrations and between the TEM5/CEA; TEM5/Ca19-9; TEM7/ CEA, and TEM7/Ca19-9 concentrations.

Receiver-operating characteristic (ROC) curves were constructed to determine the sensitivity and specificity of serum TEM5, TEM7, CEA, and Ca19-9. Survival curves were assessed using the Kaplan-Meier curve and the differences in survival times among the subgroups were evaluated by the log-rank test. P values < 0.05 were considered statistically significant.

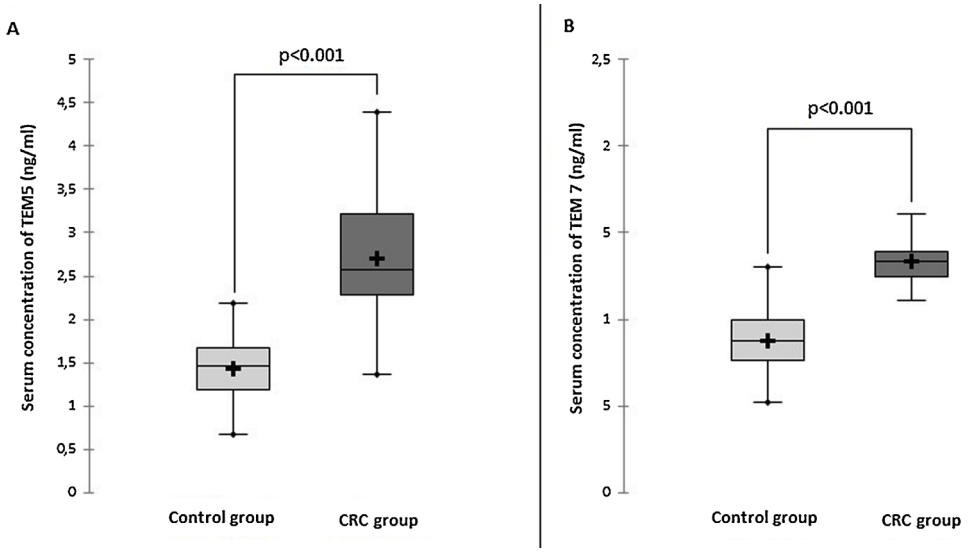

Fig. 1. Serum TEM5 (A) and TEM7 (B) concentrations in the CRC and control groups.

2.5. Ethical issues

The study was performed according to the Helsinki Declaration 1964 with its later amendments and approved by the Ethical Committee of the Medical University of Lublin, Poland (decision no. KE-0254/240/2008).

3. Results

3.1. Serum TEM5 and TEM7 concentrations in the CRC and control group

TEMs were detectable in all serum samples. The TEM5 values ranged from 1.36 ng/ml to 4.40 ng/ml in the CRC patients and from 0.67 ng/ml to 2.19 ng/ml in the healthy controls (Fig. 1). The TEM7 concentration ranged from 0.83 ng/ml to 2.19 ng/ml in the CRC patients and from 0.52 ng/ml to 1.30 ng/ml in the healthy controls. The TEM5 and TEM7 levels were statistically significantly increased in the CRC patients. The mean TEM5 concentration in the CRC individuals was nearly 2-fold higher than in the healthy control group (2.70 ± 0.76 ng/ml *vs.* 1.42 ± 0.35 ng/ml; $p < 0.001$). The TEM7 concentration was 1.5-fold higher in the CRC patients compared to the control group (1.33 ± 0.20 ng/ml *vs.* 0.88 ± 0.19 ng/ml; $p < 0.001$).

3.2. Association of TEM5 and TEM7 concentrations with clinicopathological characteristics of the CRC group

The mean TEM5 and TEM7 concentrations were statistically significantly higher in the serum of patients with the late stage (III/IV) compared to the early stage (I/II) cancer (Table 2). Moreover, the TEM5 and TEM7 concentrations were T stage-dependent. The TEM5 and TEM7 values increased along the development of T stages ($p < 0.001$). The mean ± SD levels of TEM5 in stages T1, T2, T3, and T4 were 1.61 ± 0.14 ng/ml, 1.89 ± 0.11 ng/ml, 2.82 ± 0.58 ng/ml, and 3.27 ± 0.70 ng/ml, respectively. The mean ± SD levels of TEM7 in stages T1, T2, T3, and T4 were 0.90 ± 0.06 ng/ml, 1.11 ± 0.13 ng/ml, 1.35 ± 0.10 ng/ml, and 1.48 ± 0.30 ng/ml, respectively.

The TEM5 and TEM7 concentrations in blood serum also showed a statistically significant association with the lymph node metastases (N stages; $p < 0.001$ for TEM5 and TEM7). The TEM5 and TEM 7 levels were higher in the M1 subgroup, compared to the M0 subgroup ($p = 0.018$ for TEM5 and $p = 0.002$ TEM7).

There was no statistically significant correlation between the TEM5 and TEM7 concentrations in blood serum and the sex, BMI, tumour location, and tumour size.

There was a statistically significant correlation between the T-stage and TEM5 (Spearman correlation coefficient $r = 0.407$; $p < 0.001$) and between the T-stage and TEM7 ($r = 0.366$; $p < 0.001$), which indicates an increase in TEM5 and TEM7 blood serum concentrations with an increase in the T-stage. There was no statistically significant correlation of the T-stage with the CEA and Ca19-9 blood concentrations. Additionally, a statistically significant positive correlation was found between the TEM5 and TEM7 concentrations ($r = 0.636$; $p < 0.001$). The correlations between TEM5 and CEA, as well as TEM5 and Ca19-9 were not statistically significant (TEM5/CEA: $r = 0.255$; $p = 0.091$; TEM5/Ca19-9: $r = 0.067$; $p = 0.659$). Similarly, no statistically significant correlations were observed between TEM7 and CEA ($r = 0.190$; $p = 0.210$), as well as TEM7 and Ca19-9 ($r = 0.046$; $p = 0.762$).

3.3. Performance of TEM5 and TEM7 in detecting CRC patients

The serum TEM5 and TEM7 concentrations were compared between CRC patients with an early TNM stage (I/II) and the control group. Both TEM5 and TEM7 concentrations were statistically significantly higher in the early stage CRC patients than in the control group (TEM5: 2.21 ± 0.44 ng/ml *vs.* 1.42 ± 0.35 ng/ml; $p < 0.001$; TEM7: 1.22 ± 0.13 ng/ml *vs.* 0.88 ± 0.19 ng/ml; $p < 0.001$).

To evaluate the performance of TEM5 and TEM7, ROC curves were developed and presented in Fig. 2. TEM5 predicted the diagnosis of the CRC patients with AUC of 0.946 at a cut-off point of 1.89 ng/ml. This cut-off point provided 84.4% sensitivity and 90.9% specificity. TEM7 differentiated the CRC patients from the control group with 95.6% sensitivity and 90.9% specificity at a cut-off point of 1.11 ng/ml. AUC was 0.956.

The value of TEM5 and TEM7 in the clinical application was evaluated by contrasting the diagnostic power of TEM5 and TEM7 with commonly used markers: CEA and Ca 19-9. Both markers CEA and Ca 19-9 showed lower sensitivity and specificity compared to TEM5 and TEM7. The results are presented in Table 3.

3.4. Association of TEM5 and TEM7 concentrations with the overall survival of CRC patients

Kaplan-Meier analysis was used to evaluate the overall survival of the CRC patients and TEM5 and TEM7 serum concentrations. Results

Table 2
Serum TEM5 and TEM7 concentrations in relation to the clinicopathological features of the colorectal cancer group.

Characteristics		TEM5 (ng/ml)			TEM7 (ng/ml)		
	n (%)	mean (SD)	median (min-max)	*p*	mean (SD)	median (min-max)	*p*
Gender				0.569[a]			0.380[a]
Male	29 (64%)	2.66 (0.75)	2.55 (1.52-4.40)		1.32 (0.21)	1.33 (0.83-2.19)	
Female	16 (36%)	2.77 (0.79)	2.84 (1.36-3.93)		1.35 (0.19)	1.34 (0.90-1.61)	
Body Mass Index				0.401[b]			0.181[b]
≤24.99	14 (31%)	2.57 (0.73)	2.76 (1.36-3.93)		1.29 (0.10)	1.38 (0.90-1.60)	
25.00 – 29.99	17 (38%)	2.86 (1.05)	2.55 (1.52-3.77)		1.37 (0.19)	1.29 (1.11-1.49)	
≥30.00	14 (31%)	2.93 (0.65)	2.55 (1.65-4.40)		1.38 (0.37)	1.34 (0.83-2.19)	
Tumour site				0.666[a]			0.229[a]
Colon	23 (51%)	2.64 (0.69)	2.55 (1.52-4.13)		1.37 (0.24)	1.34 (0.90-2.19)	
Rectum	22 (49%)	2.77 (0.82)	2.84 (1.36-4.40)		1.29 (0.15)	1.29 (0.83-1.56)	
Tumour size				0.884[a]			0.961[a]
≤5.0 cm	23 (51%)	2.65 (0.81)	2.66 (1.36-4.13)		1.35 (0.25)	1.34 (0.90-2.19)	
> 5.0 cm	22 (49%)	2.76 (0.76)	2.55 (1.52-4.40)		1.32 (0.16)	1.32 (0.83-1.61)	
TNM Stage				**< 0.001**[a]			**< 0.001**[a]
I-II	26 (58%)	2.21 (0.44)	2.31 (1.36-2.94)		1.22 (0.13)	1.25 (0.83-1.39)	
III-IV	19 (42%)	3.38 (0.53)	3.26 (2.36-4.40)		1.48 (0.19)	1.41 (1.34-2.19)	
Depth of invasion				**< 0.001**[b]			**< 0.001**[b]
T_1	5 (11%)	1.61 (0.14)	1.89 (1.36-2.23)		0.90 (0.06)	0.87 (0.83-0.97)	
T_2	10 (22%)	1.89 (0.11)	1.65 (1.54-1.78)		1.11 (0.13)	1.14 (0.96-1.20)	
T_3	18 (40%)	2.82 (0.58)	2.66 (2.04-4.40)		1.35 (0.10)	1.34 (1.23-1.60)	
T_4	12 (27%)	3.27 (0.70)	3.23 (2.33-4.13)		1.48 (0.30)	1.39 (1.18-2.19)	
Lymph node metastasis				**< 0.001**[a]			**< 0.001**[a]
Absent	26 (58%)	2.21 (0.44)	2.31 (1.36-2.94)		1.26 (0.13)	1.25 (0.83-1.39)	
Present	19 (42%)	3.38 (0.53)	3.26 (2.36-4.40)		1.48 (0.19)	1.41 (1.33-2.19)	
Distant metastasis				**0.018**[a]			**0.002**[a]
Absent	43 (96%)	2.64 (0.72)	2.56 (1.36-4.40)		1.31 (0.15)	1.32 (0.83-1.60)	
Present	2 (4%)	4.02 (0.16)	4.02 (3.91-4.13)		1.90 (0.41	1.90 (1.61-2.19)	
Lymphovascular invasion				**< 0.001**[a]			**< 0.001**[a]
Absent	26 (58%)	2.31 (0.58)	2.34 (1.36-3.91)		1.24 (0.15)	1.26 (0.83-1.61)	
Present	19 (42%)	3.23 (0.64)	3.23 (2.28-4.40)		1.45 (0.20)	1.39 (1.24-2.19)	

[a] *p* in Mann-Whitney *U* test.
[b] *p* in Kruskal Wallis test.

showed that patients with high TEM5 and TEM7 concentrations had worse overall survival, compared to the CRC patients of low TEM5 and TEM7 concentrations (Fig. 3). The postoperative mean overall survival time of patients with low serum TEM5 and TEM7 concentrations was 34.00 months and 33.82 months (95% CI: 33.34–34.30), respectively, while that of patients with high TEM5 and TEM7 concentrations was 25.68 months (95% CI: 22.61–28.74) and 24.74 months (95% CI: 21.45–28.02), respectively.

4. Discussion

In spite of advances in the diagnosing CRC patients mainly by means of colonoscopy and imaging techniques, early diagnosis of cancer still remains ineffective [21]. Therefore, one of the major approaches is to focus on indicators of the presence of a tumour and develop markers that enhance the early selection of colon and rectal cancer patients [7,9]. In this study, we tried to find out whether measuring TEM5 and TEM7 in the blood serum can be useful to differentiate between CRC and healthy individuals and whether these markers are useful for the early diagnosis and/or disease progression. High expression of TEM5 was observed in endothelial cells and tumour stroma in the human colon carcinoma xenograft, compared to normal colonic tissue [19]. TEM7 was recognized as the most abundant marker among TEMs isolated from colorectal tissues [15]. In recent years, both TEM5 and TEM7 proteins were identified to contain soluble fragments [15,22]. Therefore, we hypothesized that these proteins can be detected in the blood.

Here, we demonstrated that TEM5 and TEM7 were easy to identify in blood serum using common type ELISA assays. In the available literature, there are no reports on the determination of TEM concentrations in blood serum. We proved elevated concentrations of TEM5 and TEM7 proteins in the serum of the CRC patients, compared to the healthy control cohort. Therefore, the TEM5 and TEM7 proteins can be regarded as independent prognostic markers in CRC patients. The precise cause of the high concentrations of TEM5 and TEM7 proteins in serum is unclear; however, it can be linked with overexpression of TEMs in the blood vessels of tumour tissues and in tumour endothelial cells (TECs) [18–20]. The role of TEM5 and TEM7 in tumour angiogenesis, endothelial cell migration, invasion, and proliferation has been evidenced [20,23]. High TEM5 and/or TEM7 levels have been reported in patients with lung, pancreas, breast, and brain cancer as well as osteogenic sarcoma and colorectal cancer [15,19,24]. Moreover, the number of transcripts of TEM7 was associated with cancer advancement and lymph nodes involvement, while knockdown of both TEM5 and TEM7 resulted in reduction of cancer cell migration and invasion [23,25]. Therefore, we speculate that the elevated TEM5 and/or TEM7 serum levels are associated with the expression of TEM5 and TEM7 in the endothelium of cancer tissue.

Moreover, our results indicate that the sensitivity of both TEM5 and TEM7 molecules is higher than that of CEA and Ca 19-9, i.e, markers that are commonly used in CRC detection. However, in the CRC patients with an early cancer stage (I/II), we observed lower sensitivity of TEM5, compared to the sensitivity of TEM7, CEA, and Ca19-9. The sensitivity of TEM7 was higher than that of the CEA and Ca19-9 markers. Therefore, we suggest that TEM5 and TEM7 can be considered as potential biomarkers for CRC diagnosis. The preliminary character of the present study suggests that TEM5 and TEM7 can be beneficial in the CRC clinical diagnosis. We propose a combined use of TEM5 and TEM7 with CEA and Ca19-9 markers for clinical management of CRC. However, the assessement of the use of the TEM5 and TEM7 markers in the differential diagnosis requires carefully designed studies focused specifically on validation and standardization.

Approximately 20%–50% of stage II and stage III CRC will progress to stage IV, which is associated with dismal prognosis. Therefore,

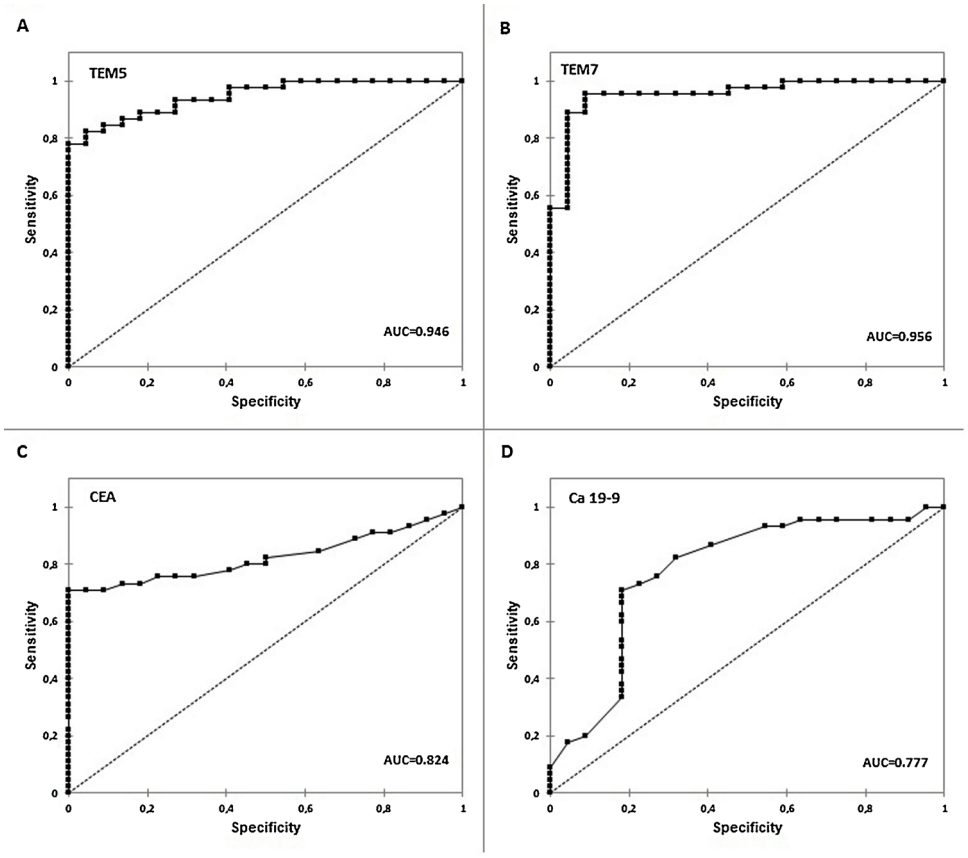

Fig. 2. ROC curve analysis of TEM5 (A), TEM7 (B), CEA (C), and Ca19-9 (D) serum concentrations in the CRC patients and the control group.

increased attention has recently been paid to clinical and molecular progression and prognostic factors in CRC [26–28].

In this study, the TEM5 and TEM7 concentrations were statistically significantly higher in the advanced stage (III/IV) of the disease than in the early stage (I/II). Moreover, the TEM5 and TEM7 levels were strongly positively linked with the TNM staging classification. These findings suggest that the TEM5 and TEM7 serum concentrations may be potentially useful as a biomarker of cancer progression. Additionally, we have evidenced that the TEM5 and TEM7 concentrations in the serum possess an adverse prognostic value and are related to unfavourable prognosis, *i.e.* patients with high levels of TEM5 and TEM7 exhibited statistically significantly shorter overall survival time than patients with low TEM5 and TEM7 levels in the blood serum.

4.1. Limitations of the study

The present study has some limitations, including its retrospective nature and the limited sample size of the CRC patients (n = 45). However, it was a homogenous sample with CRC recognized by colonoscopy. The patients were immediately admitted to the Surgical Department once the CRC was histopathologically confirmed. Moreover, the patients were treated surgically before any chemotherapy or radiotherapy was delivered. The mean time from the first CRC detection (colonoscopy) to the surgical treatment and blood sample collections was 14 days. Therefore, the time from detection to the blood sample collection should not have any significant effects on the serum concentration of the studied markers. Furthermore, a high

Table 3
Diagnostic performance of independent markers: TEM5, TEM7, CEA, and Ca 19-9.

	AUC (95%CI)	Sensitivity (%)	Specificity (%)	Cut-off value (ng/ml)
TEM5				
TNM I-IV	0.946 (0.901-0.992)	84.4	90.9	1.89
TNM I/II	0.907 (0.498-0.835)	69.2	90.9	1.89
TNM III/IV	1.000 (0.798-1.000)	100	95.5	1.92
TEM7				
TNM I-IV	0.956 (0.906-1.000)	95.6	90.9	1.11
TNM I/II	0.923 (0.745-0.988)	92.3	90.9	1.11
TNM III/IV	1.000 (0.798-1.00)	100	95.5	1.31
CEA				
TNM I-IV	0.824 (0.726-0.922)	73.3	86.4	6.0
TNM I/II	0.870 (0.769-0.892)	76.9	90.9	6.23
TNM III/IV	0.761 (0.409-0.808)	40.9	90.9	6.0
Ca 19-9				
TNM I-IV	0.777 (0.645-0.908)	71.1	81.8	7.98
TNM I/II	0.747 (0.615-0.918)	80.8	68.2	6.12
TNM III/IV	0.817 (0.614-0.951)	84.2	81.8	11.76

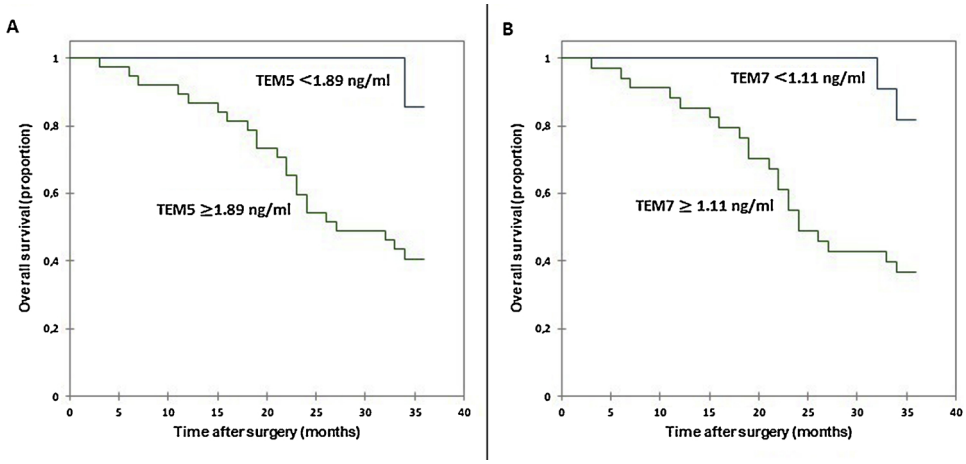

Fig. 3. Kaplan-Meier survival curves of CRC patients according to serum TEM5 (A) and TEM7 (B) concentrations.

number of advanced CRC cases was included in the study due to the very low response of Polish patients to the CRC screening programs, which results in the high number of late-stage CRC patients.

5. Conclusions

In conclusion, TEM5 and TEM7 serum concentrations can be considered to be useful biomarkers for detecting CRC patients and for monitoring cancer progression and identifying patients with a high possibility of poor survival. To the best of our knowledge, this study presents the first analysis of TEM5 and TEM7 in the blood serum. However, we assume that the optimistic results suggesting a biomarker character of TEM5 and TEM7 in CRC are preliminary and need to be confirmed in further studies.

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Conflict of interest

The authors declare they have no conflict of interest.

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The author contribution

Study design: Łukasz Pietrzyk
Data collection: Łukasz Pietrzyk
Analytical and statistical analysis: Łukasz Pietrzyk, Paulina Wdowiak
Data interpretation: Łukasz Pietrzyk
Manuscript Preparation: Łukasz Pietrzyk
Literature search: Łukasz Pietrzyk
Funds Collection: Łukasz Pietrzyk.

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