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RESEARCH ARTICLE

Novelty in Colorectal Cancer Biomarkers: The Predictive Value and Clinical Utility of the Carcinoembryonic Antigen and Aldehyde Dehydrogenase 1B1 Autoantibodies for Assessing Tumour Biology and the Cancer Stem Cell Burden

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Abstract

Background: Colorectal cancer (CRC) is responsible for nearly 10% of cancer cases and deaths and is emerging as one of the most prominent malignant diseases worldwide. Carcinoembryonic antigen (CEA) is the most widely used serum biomarker for CRC, but its limited sensitivity and specificity highlight the need for additional diagnostic and prognostic markers. The mitochondrial enzyme aldehyde dehydrogenase 1B1 (ALDH1B1) is highly expressed in CRC and cancer stem cells (CSCs), representing a novel biomarker, especially as its overexpression triggers an autoantibody response detectable in a patient's serum. This study aims to quantify serum concentration of CEA and ALDH1B1 autoantibodies in patients with colorectal cancer (CRC) and to investigate the relationship between them. It further seeks to evaluate their prognostic potential as circulating biomarkers for CRC. The underlying hypothesis proposes that elevated CEA and ALDH1B1 autoantibody levels are positively correlated and collectively reflect the underlying cancer stem cell (CSC) burden. This correlation is assessed through a predictive equation developed in the study, providing a novel, noninvasive indicator of tumor progression and aggressiveness. **Method:** Blood samples were collected from 75 newly diagnosed CRC patients (stages II–IV) and 25 healthy controls. CEA and ALDH1B1 autoantibodies were measured using ELISA techniques. Statistical analyses include analysis of variance (ANOVA), paired t-tests, Duncan's test, regression analysis, and receiver operating characteristic (ROC) curve assessments using SPSS software. **Results:** The findings show significantly higher CEA and ALDH1B1 autoantibody levels in CRC patients compared to the controls, though neither marker varied significantly across tumour stages, emphasising their role as indicators of tumour biology rather than tumour burden. The regression analysis revealed a significant direct relationship between CEA and ALDH1B1 autoantibody levels ($\beta = 0.026$, $p < 0.001$), as CEA explains 87% of the variability in ALDH1B1 autoantibody levels. The ROC analysis indicated a good diagnostic performance for CEA (AUC = 0.88) and a fair performance for ALDH1B1 autoantibodies (AUC = 0.67). **Conclusion:** The strong predictive relationship between CEA and ALDH1B1 autoantibodies suggests that CEA levels may indirectly reflect CSC activity, potentially guiding personalised treatment strategies targeting both bulk tumours and CSCs.

Keywords: CRC- CEA- ALDH1B1- autoantibodies- ROC test

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Introduction

Colorectal cancer (CRC) is a general term encompassing colon and rectal cancer, characterised by malignant epithelial tumours located in the cecum, colon, rectum and anal canal. These tumours vary in form, location and histological structure, with adenocarcinoma as the most frequent, accounting for >90% of CRC cases worldwide [1, 2]. In 2022 alone, over 1.9 million new cases were reported, resulting in more than 900,000 deaths annually, highlighting its significant global impact. It is ranked as the world's third most commonly diagnosed cancer in males and females (9.6% of all cancers globally) and the second most fatal cancer globally. According to the last update from the International Agency for Research on Cancer, approximately 9.3% of cancer-related deaths in

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2022 [3] were due to CRC.

Carcinoembryonic antigen (CEA) is a non-specific serum biomarker that is raised in many malignancies, including colorectal cancer. It is usually derived from foetal embryonic endodermal epithelium, controlled by foetal oncogenes, and then disappears from serum after birth. However, trace amounts of CEA may remain in the colon tissues. In healthy individuals, CEA levels typically remain below 5.0 ng/mL, providing a baseline for comparison in cancer diagnostics [4].

According to previous research, CEA was the single indicator that had the most efficient screening for colorectal cancer (CRC), with a specificity level of up to 91.00%. However, CEA sensitivity was discovered to be just 70.59%. It is thought that the combination of other potential clinical blood markers is essential for the reduction of missed diagnosis and misdiagnosis rates in colorectal cancer screening [5].

Among these markers, aldehyde dehydrogenase 1B1 (ALDH1B1) is a mitochondrial isoform of the ALDH family that catalyses the oxidation of reactive aldehydes, contributing to cellular detoxification and metabolic homeostasis in normal tissues [6]. It is much expressed in human colonic adenocarcinomas and colonic stem cells, where it plays a pivotal role in colorectal cancer (CRC) tumourigenesis. Its upregulation is closely linked to cancer stem cells (CSC), a distinct subpopulation within tumours, which are defined by their self-renewal, unlimited proliferative capacity and multipotent differentiation. Although CSCs constitute a small proportion of the tumour mass, they are critically involved in tumour initiation, metastasis, chemoresistance and recurrence [7].

Despite its role in tumour development, recent findings demonstrate that ALDH1B1 overexpression renders it immunogenic. This immunogenicity can elicit an immune response, leading to the production of autoantibodies against it. A study by Wang et al. [8] identified serum autoantibodies against ALDH1B1 in patients with CRC and advanced adenomas, demonstrating its potential as a non-invasive biomarker for early CRC detection This study aims to quantify CEA serum concentration as a tumor-associated antigen and to assess the autoantibody response against ALDH1B1, a proposed marker of cancer stem cell (CSC) activity, in patients with CRC. The central hypothesis proposes that elevated levels of CEA and ALDH1B1 autoantibodies are positively correlated, reflecting the underlying CSC burden and, consequently, tumor aggressiveness. To test this hypothesis, a regression equation will be derived to predict ALDH1B1 autoantibody levels based on CEA titers, thereby providing a mathematical and noninvasive means of estimating CSC-associated tumor progression. Ultimately, the study evaluates the prognostic potential and clinical applicability of these autoantibodies as circulating biomarkers for CRC.

Materials and Methods

Patient recruitment and sample collection

Samples were collected from newly diagnosed CRC patients under the supervision of expert oncologists before the initiation of any medical treatment. The patients were recruited from the General Oncology and Nuclear Medicine Hospital, Al-Salam Teaching Hospital, and Ibn Sina Hospital, as well as from a range of outpatient clinics and endoscopy centres. The collection process was carried out over the period from September 2024 to March 2025. The study received ethical approval from the Iraqi Ministry of Health and the Scientific and Ethical Committee of the University of Mosul, Iraq.

Study subject

A total of 75 blood samples were obtained from newly diagnosed colorectal cancer patients – 36 males and 39 females. Participants were divided into three groups according to the tumour, node, metastasis (TNM) staging system. The first group comprised 15 patients diagnosed with stage II CRC, the second group contained 38 patients with stage III CRC, and the third group comprised 22 patients with stage IV CRC. A detailed form was completed for each participant, capturing key clinical and demographic information, such as name, age, gender, weight, height, diagnosis, treatment stage, major symptoms, medical history, duration of symptoms, and any known genetic predisposition. A total of 25 control blood samples were obtained from healthy individuals – 16 females and 9 males. These participants were free from any diagnosed diseases, were not taking any form of medication at the time of sampling, and had no known family history of colorectal cancer.

Serum collection

A venous blood sample of 3 mL was taken from each individual in a gel tube, and the samples were centrifuged at 4,500 rpm for 10 minutes. The serum obtained was transferred into Eppendorf tubes for analysis and kept at -20°C.

ELISA measurement of CEA

The CEA assay was performed using a Human CEA ELISA Kit (Sunlong Biotech Co., LTD, China, Cat. No. SL2426Hu). This assay works on the principle of a sandwich ELISA, in which the CEA present in serum samples will react with an antibody specific to it that had previously been fixed to the surface of a polystyrene plate. Then, a CEA-specific horseradish peroxidase (HRP)-conjugated antibody is added to bind to the antigen–antibody complex. After incubation, washing is carried out. After that, a substrate solution (TMB) is added to each well. The intensity of the blue colour will increase depending on the existence of CEA. The colour will turn yellow after the stop solution is added, then the absorbance is red at 450 nm [9-11].

Procedure

1. Diluted standards were prepared in small tubes. Two wells were filled with 50 µl from each of the tubes (10 in total).

2. A blank control was created from the one empty well: 40 µl of the diluted sample buffer and 10 µl of the sample were added to each well (1:5 dilution). Finally, the plate was sealed and incubated at 37 °C for 30 minutes. After incubation, the wells were aspirated and filled with wash buffer, which was removed after 30 seconds. Washing was repeated five times.

3. The plate was incubated again at 37 °C for 30 minutes after adding 50 µl of the HRP conjugate to all wells except the blank. Washing was repeated as described in the previous step.

4. Each well received 50 µL of chromogen solution A and 50 µL of solution B. The plate was carefully shaken and kept at 37°C for 15 minutes in the dark.

5. The addition of 50 µl of stop solution changed the colour from blue to yellow.

6. Absorbance was read at 450 nm using a plate reader. The optical density (OD) of the blank well was set to zero. The absorbance measurement was recorded within fifteen minutes of the stop solution being added.

Measurement of the human aldehyde dehydrogenase family 1, member B1 autoantibody (ALDH1B1-Ab) by ELISA

The assay is performed on a double-antigen sandwich ELISA principle using a Human ALDH1B1-Ab ELISA Kit (Sunlong Biotech Co., LTD., China, Cat. No. SL4489Hu). In this approach, an ALDH1B1-specific antigen is first immobilised on the surface of a polystyrene microplate. When serum samples are added, any ALDH1B1 autoantibodies present will bind to the coated antigen. A second HRP-conjugated ALDH1B1 antigen is then introduced, which binds to the captured autoantibodies, forming a sandwich complex. Following incubation, the washing is done, and the TMB substrate solution is administered to each well. The blue colour will develop with intensity proportional to the level of ALDH1B1-Ab in the sample. The colour will turn yellow after the stop solution is added, then the absorbance is red at 450 nm [12].

Procedure

1. Diluted standards were prepared in small tubes. Two wells were filled with 50 µl from each of the tubes (10 in total).

2. A blank control was created from the one empty well: 40 µl of the diluted sample buffer and 10 µl of the sample were added to each well (1:5 dilution). Finally, the plate was sealed and incubated at 37 °C for 30 minutes. After incubation, the wells were aspirated and filled with wash buffer, which was removed after 30 seconds. Washing was repeated five times.

3. The plate was incubated again at 37°C for 30 minutes after adding 50 µl of HRP-conjugate to all wells except the blank. Washing was repeated as described in the previous step.

4. Each well received 50 µL of chromogen solution A and 50 µL of solution B. The plate was carefully shaken and kept at 37°C for 15 minutes in the dark.

5. The addition of 50 µl of stop solution changed the colour from blue to yellow.

6. Absorbance was read at 450 nm using a plate reader. The OD of the blank well was set to zero. The absorbance measurement was recorded within fifteen minutes of the stop solution being added.

Data analysis

SPSS version 24 was used to conduct the statistical analysis at the consulting office of the Department of Statistics and Informatics, University of Mosul. The most important statistical tests included the analysis of variance (ANOVA) t-test, the Duncan test, and regression coefficient analysis in addition to the curve and cutoff analysis tests and the standardised Z-score test. All tests were conducted at a significance level of $P \leq 0.05$.

Results and Discussion

Statistical comparisons

The results of the paired comparisons T-test showed a significant increase in the CEA concentration in the serum of the CRC patients (mean ± SD 33.57 ± 21.32) compared to the control sample (mean ± SD 21.18 ± 3.76) at 0.015 when $P \leq 0.05$. The results of the T-test also showed a significant increase in the serum concentration of ALDH1B1 autoantibodies in the CRC patients (mean ±SD 1.12 ± 0.59) compared to the control sample (mean ±SD 0.80 ± 0.34) at 0.031 when $P \leq 0.05$, as shown in Table 1.

The results of Duncan's paired comparison test showed no significant differences in CEA and ALDH1B1 autoantibody concentrations among the CRC patients, with a probability value of 0.093 and 0.112, respectively,

Table 1. Statistical Indicators and Two Independent Sample t-test for Pairwise Comparisons between Patients and Controls Groups for CEA and ALDH1B1 Variants

Parameters	Groups	N	Mean	SD	Min	Max	T	P
CEA picogram/milliliter	Patients	75	33.57	21.32	20.77	112.84	2.504	0.015
	Control	25	21.18	3.762	12.22	27.36		
ALDH1B1 nanogram/milliliter	Patients	75	1.12	0.59	0.78	4.11	2.214	0.031
	Control	25	0.8	0.34	0.02	1.1		

Abbreviations: CEA, carcinoembryonic antigene; ALDH1B1, aldehyde dehydrogenase 1B1; P, probability value <0.05; T, test value

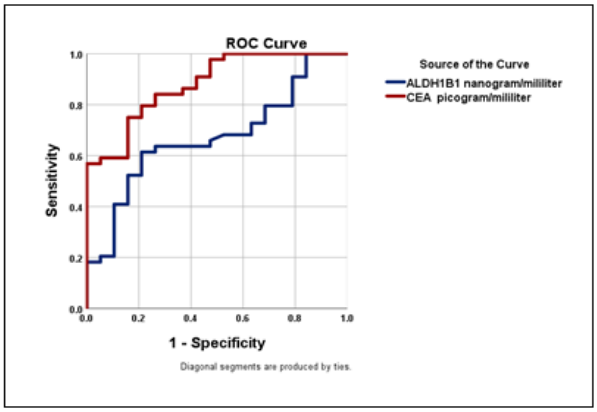

Figure 1. ROC Test for Predictive Values for CEA and ALDH1B1

at P ≤ 0.05, as shown in Table 2.

ROC Test

The results of the ROC curve test indicate that the CEA index was stronger than the ALDH1B1 index in its ability to classify patients as diseased or non-diseased. The CEA index had an area value of 0.88, which is a good classification value, while the area value of 0.67 was an average classification value, as shown in Table 3 and Figure 1.

Standardised Z-score test

The standardised Z-score test was chosen to determine the presence of both CEA and ALDH1B1 Auto-Ab in the serum of CRC patients, the elevations of both biomarkers occur in parallel, meaning they increase simultaneously across patient groups compered to controls. This parallel pattern reflects their close association and strong relationship, highlighting the coordinated increase of CEA and ALDH1B1 autoantibodies in CRC. The use of standardized scores not only enables clear visualization of this co-elevation but also reinforces the observed statistical correlation between the two biomarkers, supporting their potential combined relevance as indicators of tumor biology. as shown in Figure 2.

Regression coefficient test

This analysis tests the relationship between the independent variable and the dependent variable. Each of the following is shown in Table 4 and Figure 3.

1. The value of the regression coefficient estimate (β), which reached 0.026, shows that there is a direct effect of CEA on ALDH1B1, and the effect is significant, as the P-value reached 0.000, which is less than 0.05, and indicates the same as the tCal value of 17.154, which is greater than the tTab value, which reached 1.96 and also shows the same thing. Therefore, we can say that when the CEA value increases by one unit, the ALDH1B1 value will increase by 0.026.

2. The coefficient of determination (R-squared) showed that 87% of ALDH1B1 changes are due to CEA, and 13% is due to other variables not in the regression model.

The key findings of this study demonstrate that both CEA and ALDH1B1 markers are significantly elevated in colorectal cancer (CRC) patients compared to healthy controls, but neither marker shows significant variation across different CRC stages. Additionally, a strong regression relationship between CEA levels and ALDH1B1 autoantibody levels suggests a novel biological connection between these markers.

CEA Findings and Stage Correlation

the CEA level analysis revealed that patients with a CRC diagnosis had significantly higher levels than those of the healthy controls. However, no significant differences in CEA levels were observed across different CRC stages (Table 1 and 2), a finding that is consistent with previous research by Saputri et al. [13].

Elevated levels of CEA in CRC patients do not result from a single, specific cause but rather reflect a combination of tumour-related abnormalities. One major contributing factor is the loss of normal epithelial polarity, a common feature in malignant transformation. In healthy colon tissue, CEA is localised to the apical surface of cells facing the intestinal lumen, preventing their release into circulation. However, in CRC, disruption of this polarisation allows CEA to be misdirected and released into the bloodstream, increasing serum levels [14]. Additionally, genetic and epigenetic alterations can lead to transcriptional upregulation of the CEA gene CEACAM5, further elevating CEA production. These changes may be driven by oncogenic signalling pathways, inflammatory response and environmental factors [15].

The absence of a significant elevation in CEA levels across CRC stages may be attributed to the complex and multifactorial nature of CEA expression in CRC. While integrating CEA levels with the TNM staging system (a system used globally to describe the extent and spread of cancer in a standardised way) enhances prognostic

Table 2. Duncan's paired Comparison Test and Significance Level Test for CEA and ALDH1B1

Variable	Stage	N	Mean ±SD	Duncan test	P-value
CEA	2	15	27.88 ±6.55	27.88	
	3	38	30.83 ±17.46	30.83	0.093
	4	22	42.14 ±30.82	42.14	
	2	15	0.95 ±0.14	0.95	
ALDH1B1	3	38	1.05 ±0.43	1.05	0.112
	4	22	1.37 ±0.92	1.37	

Abbreviations: CEA, carcinoembryonic antigen; ALDH1B1, aldehyde dehydrogenase 1B1; N-number of samples; SD- standard deviation; P. value, probability value ≤0.05

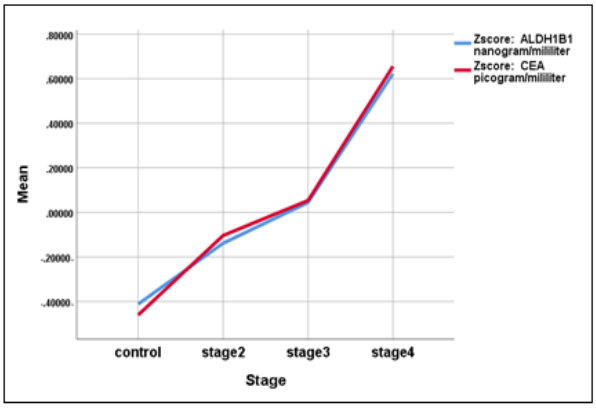

Figure 2. Z-score Standardized Values of ALDH1B1 and CEA

accuracy in colorectal cancer. Evidence indicates that CEA does not consistently increase linearly across tumour stages. For example, Liu et al. [16] demonstrated that patients classified as C1 (elevated CEA in the early stages) may exhibit similar or even worse prognoses compared to certain N-stage (more advanced stage) patients, highlighting that elevated CEA levels provide independent prognostic information beyond traditional staging.

This non-linear pattern is further supported by the findings of Xie et al. [17], who reported that while higher continuous CEA levels were associated with increased mortality risk, this did not align proportionally with tumour burden or stage. Instead, patients with elevated CEA levels showed significantly reduced overall survival across all TNM stages, suggesting that CEA reflects biological aggressiveness rather than merely tumour size or extent. Therefore, although CEA is a valuable prognostic marker, it should not be interpreted as a direct surrogate for tumour stage, as its levels remain relatively inconsistent across CRC progression [18].

ALDH1B1 Autoantibody Findings and Stage Correlation

Our results also showed significantly elevated levels of ALDH1B1 in CRC patients compared to the healthy controls (Table 1). This finding aligns with previous research by Singh et al. [19], which demonstrated that ALDH1B1 overexpression is associated with regulating oncogenic signalling pathways (Wnt/β-catenin, Notch and PI3K/Akt) essential for CRC progression and cancer stem cell maintenance. Moreover, silencing ALDH1B1 in their study led to impaired tumour growth and spheroid formation, underscoring its functional importance in CRC pathophysiology.

Additionally, Tsochantaridis et al. [20] indicated that elevated levels of ALDH1B1 are linked to the DNA damage response and repair mechanisms. Overexpression of ALDH1B1 was shown to enhance the phosphorylation of p53 and H2AX, promoting cell survival under genotoxic stress. It also activated the transcription of various DNA repair genes, contributing to chemoresistance and G2/M cell cycle arrest. These findings suggest that ALDH1B1 may provide a survival advantage in colorectal tumourigenesis and contribute to therapeutic resistance. The overexpression of ALDH1B1 in neoplastic tissues is also likely to induce a humoral immune response, leading to the generation of autoantibodies because the immune system recognises ALDH1B1 as a tumour-associated antigen [8].

Notably, although ALDH1B1 is upregulated in both colorectal adenomas and carcinomas, its expression does not exhibit significant variation across different stages of CRC (Table 2). Wang et al. [21] reported that ALDH1B1 protein and mRNA levels are elevated in tumour tissues relative to adjacent normal tissues but remain relatively stable from stage I of the disease through to stage IV. This stability may be attributed to the role of ALDH1B1 as a cancer stem cell marker in colorectal cancer.

CSCs represent a distinct, relatively stable tumour subpopulation responsible for sustaining tumour growth, metastasis, and therapy resistance. The expression of ALDH1B1 reflects the presence and function of these CSCs, which remain largely constant across different stages of tumour progression, thereby explaining the non-progressive pattern of ALDH1B1 expression [22]. This lack of stage-specific variation may contribute to the observation that circulating autoantibody levels against ALDH1B1 do not significantly increase with disease progression.

Another acceptable explanation involves B-cell exhaustion, a state characterised by the functional impairment of B lymphocytes due to sustained antigenic stimulation. Chronic exposure to tumour-associated antigens within an immunosuppressive tumour microenvironment – frequently exhibiting features of immune dysregulation – may attenuate B-cell effector functions, including antibody production [23]. Additionally, advanced tumours often acquire immune-evasive properties, including the upregulation of immune checkpoint molecules such as PD-L1, which further suppress anti-tumour immune responses, including

Table 3. ROC Test for Predictive Values for CEA and ALDH1B1

Test Result Variable	Area Under the Curve				
	Area	Asymptotic 95% CI		Max	Cutoff value
		Lower limit	Upper limit		
CEA	0.88	0.795	0.966	Sensitivity=0.744	24.24
				Specificity=0.842	
ALDH1B1	0.67	0.532	0.809	Sensitivity=0.614	0.95
				Specificity=0.789	

Area: 0.90-1 Excellent, 0.80-0.90 Good, 0.70-0.80 Fair ,0.60-0.70 Poor,0.50-0.60 Fail. Abbreviations: CEA, carcinoembryonic antigen; ALDH1B1, aldehyde dehydrogenase 1B1; CI, Confidence Interval; Max, maximum

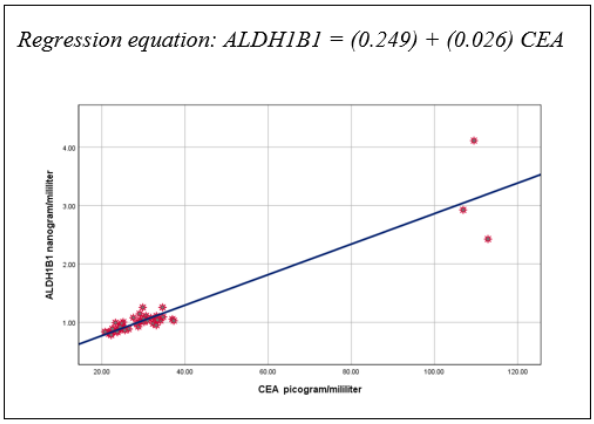

Figure 3. Regression Coefficient test CEA as Independent Variable in ALDH1B1 as Dependent Variable

autoantibody production [24].

Interrelationship Between CEA and ALDH1B1 Expression in Colorectal Cancer

A principal finding of the present study is the significant positive correlation between serum carcinoembryonic antigen (CEA) concentrations and ALDH1B1 autoantibody titers in patients with colorectal cancer (CRC). This association is unlikely to be coincidental; rather, it appears to reflect their shared involvement in tumor aggressiveness, metabolic adaptation, and cancer stem cell (CSC) biology. Both CEA and ALDH1B1 are influenced by convergent oncogenic signaling pathways and tumor microenvironmental cues. Aberrant activation of Wnt/β-catenin signaling, a hallmark of CRC, has been shown to upregulate CEACAM5 transcription and promote ALDH1B1 expression within CSC-enriched compartments. Similarly, inflammatory mediators (e.g., IL-6, TNF-α) and hypoxia-inducible factor-1α (HIF-1α) signaling can synergistically enhance both CEA production and ALDH1B1 activity, thereby reinforcing tumor progression under oxidative and metabolic stress.

Within this altered microenvironment, characterized by loss of epithelial polarity, chronic inflammation, and sustained proliferative signaling, CSCs coexist with differentiated tumor cells in a mutually supportive niche. Elevated CEA secretion contributes to immune modulation and tumor–stroma interactions, while ALDH1B1 upregulation provides metabolic protection through detoxification of reactive aldehydes and maintenance of redox balance. The concurrent elevation of these markers thus represents a coordinated adaptive response that promotes tumor survival, CSC maintenance, and disease persistence. Consequently, the observed correlation between CEA and ALDH1B1 may serve as a molecular signature of integrated epithelial and stem-like tumor activity, highlighting a biologically meaningful axis of colorectal cancer pathogenesis.

Clinical implication and ROC anlysis

The variation of cancer cells within a single tumour bulk has given rise to multiple hypotheses regarding tumour progression. Two models are commonly accepted, which are not necessarily contradictory: the clonal or stochastic model and the hierarchical or cancer stem cell model [25, 26]. According to the clonal model, it is the genetic and epigenetic instability of cancer cells that drives tumour heterogeneity. As a consequence, all cancerous cells initially possess a similar capacity to obtain the characteristics necessary for guiding the formation of a new tumour [27]. In the CSC model, tumours have an orderly structure similar to normal tissue, where only a small fraction of cells (CSCs) give rise to the tumour and trigger dissemination and/or recurrence [28].

CSCs may only account for a small percentage of cancer cells, but their contribution to the disease's clinical course is significant. These cells have greater radio/chemo resistance and improved tumourigenic and metastatic abilities. The CSC population isn't effectively targeted by conventional therapies. Such cells may give rise to new tumours that drive the recurrence and/or metastasis of the disease [29].

From this standpoint, we assess the CSC burden inside CRC by examining the relationship between CEA levels and anti-ALDH1B1 autoantibody levels in patient serum, using a regression coefficient test to derive a numerical estimate of ALDH1B1 autoantibody levels based on CEA values, thus estimating the CSC burden only from one

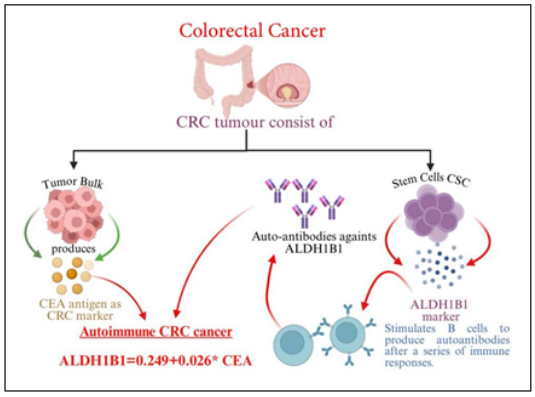

Figure. 4. A Diagram Showing the Interrelationship between CEA Antigen and ALDH1B1 Auto-Ab and the simple linear regression equation linking the two variables.

Table 4. Regression Coefficient test CEA as Independent Variable in ALDH1B1 as Dependent Variable

Independent Variable	Direction of influence	Dependent Variable	Regression coefficient Estimate (β)	Standard error of the regression coefficientSe.(β)	Coefficient of determination R-square	Calculated value (tCal.)	P-value
Constant			0.249	0.06		4.125	0
CEA (picogram/milliliter)	→	ALDH1B1 nanogram/milliliter			0.87		
			0.026	0.002		17.154	0

(1.96=tTab) Table value; Abbreviations: CEA, carcinoembryonic antigene; ALDH1B1, aldehyde dehydrogenase 1B1; P.value, probability value <0.05

test and eliminating the need for direct anti-ALDH1B1 autoantibody testing by conducting a regression equation. Based on this assessment, clinicians may be better equipped to select or adjust treatment protocols aimed at targeting both the bulk tumour and the CSC population, potentially improving patient outcomes and reducing the risk of relapse (Figure 4).

It is important to acknowledge that this approach does not directly quantify cancer stem cells (CSCs) but rather provides an indirect reflection of their biological influence on tumor behavior and the associated humoral immune response. In this context, the term "CSC burden" denotes the inferred impact of CSC-related processes such as self-renewal, tumor aggressiveness, and immune modulation rather than an absolute measure of CSC quantity. Accordingly, the proposed regression equation represents a novel yet inferential framework for estimating CSC-associated tumor activity. Its interpretation should therefore be made with caution and supported by future validation using direct cellular or molecular assessments of CSCs. Clinically, this model may help identify patients with tumors exhibiting greater CSC-driven aggressiveness, offering potential guidance for therapeutic strategies that target both the differentiated tumor mass and the CSC compartment to improve treatment outcomes and reduce recurrence risk .

ROC curve analysis is commonly used to determine the diagnostic value of CEA. These analyses typically show areas under the curve (AUC) values between 0.70 and 0.80, indicating good but not excellent performance in the discrimination between colorectal cancer and non-cancerous conditions. Sensitivity (~40–60%) and specificity (~70–90%) also vary depending on the cutoff used (usually 5 ng/mL) [30,31]. This is consistent with the results of the current study, where the AUC was 0.88, indicating good discrimination.

Combining CEA testing with other biomarkers or clinical factors improves diagnostic accuracy. The study suggests that CEA testing is not recommended as a primary screening tool for CRC (colonoscopy/FIT remains the standard). However, it is considered a promising tool for monitoring disease recurrence after resection and evaluating treatment response in cases of advanced CRC [32].

The results of the ROC test for the adoption of ALDH1B1 as a predictive marker showed that the AUC values ranged between 0.60 and 0.70 (Table 4 and Figure 1), which is considered fair. ALDH1B1 shows great potential as a biomarker for CRC, with emerging evidence supporting its role in tissue-based detection and early serum-based prediction. ALDH1B1 is strongly expressed in colon and rectal tumour tissue compared to normal tissue, making it a reliable prognostic biomarker. Studies using microtissue arrays have shown the ability of ALDH1B1 to distinguish CRC from normal tissue more effectively than other forms of ALDH, such as ALDH1A1. ALDH1B1 promotes the development of CRC by modulating carcinogenic pathways (Wnt/β-catenin, Notch and PI3K/Akt). Its expression is associated with highly carcinogenic cells (such as $ALDH^{+}CD44^{+}$ cells) [33].

Autoantibodies in serum against ALDH1B1 have been identified as promising tools for the early detection of CRC. A study using microarrays and ELISA verification reported 75.68% sensitivity and 63.06% specificity for the detection of colorectal cancer, with an AUC value of 0.70. For advanced adenoma (a precancerous lesion), the sensitivity was 62.31% with a specificity of 73.87% (AUC = 0.74) [8].

In conclusion, • Elevated biomarkers (not stage dependent): In CRC patients, CEA and ALDH1B1 autoantibody levels were significantly higher than those of the healthy controls. However, neither marker showed significant variation across different tumour stages, indicating that their levels reflect tumour biology rather than tumour burden or disease progression 1.

• Strong predictive relationship: Regression analysis revealed a significant, direct relationship between CEA and ALDH1B1 autoantibody levels, with CEA explaining 87% of the variance in ALDH1B1 autoantibody levels. This suggests that CEA may indirectly reflect the burden of CSCs, which are associated with ALDH1B1 expression. Deriving a statistical equation enables the treating physician to extract a value for ALDH1B1 autoantibodies mathematically through a practical CEA value.

• Diagnostic and prognostic value: ROC analysis indicated good diagnostic performance for CEA (AUC = 0.88) and fair performance for ALDH1B1 autoantibodies (AUC = 0.67). This highlights the established role of CEA in CRC diagnostics and monitoring, while also positioning ALDH1B1 autoantibodies as a novel, complementary biomarker with the potential for early detection and monitoring.

• Clinical implications: The findings suggest that monitoring these biomarkers could help clinicians assess both the bulk tumour and the CSC population, potentially guiding more personalised treatment strategies and improving outcomes by targeting the sources of tumour recurrence and resistance.

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Conflict of interests

The authors declare that there is no competing interest.

Data Availability Statement

The data underpinning this study's results are available from the corresponding author upon reasonable request.

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