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# Expression of Serum DCLK1 in Gastric Cancer Patients and Its Relationship with CEA, CA19-9, and CA72-4

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**Abstract: Objective:** To investigate the expression of Doublecortin-like kinase-1 (DCLK1) in the serum of gastric cancer patients and its relationship with carcinoembryonic antigen (CEA), carbohydrate antigen 19-9 (CA19-9), and carbohydrate antigen 72-4 (CA72-4). **Methods:** Fifty patients diagnosed with gastric cancer at the hospital from January to December 2021 were selected as the gastric cancer group, and 50 patients diagnosed with chronic atrophic gastritis during the same period were selected as the control group. The serum concentrations of DCLK1, CEA, CA19-9, and CA72-4 were measured in both groups. Cut-off values and AUC (Area Under the Curve) were determined based on the ROC curve, and the expression of DCLK1 and its relationship with CEA, CA19-9, and CA72-4 were analyzed. **Results:** The average concentrations of DCLK1, CEA, CA19-9, and CA72-4 in the serum of gastric cancer patients were significantly higher than those in the control group ($P < 0.05$). CA72-4 had the highest sensitivity (62%), CEA had the highest specificity (98%), and DCLK1 had the largest AUC (0.709). The combined diagnosis of gastric cancer using DCLK1, CEA, and CA19-9 resulted in the largest AUC (0.826), with a sensitivity of 82% and a specificity of 76%. **Conclusion:** The expression of DCLK1, CEA, CA19-9, and CA72-4 in the serum of gastric cancer patients is significantly higher than that in the control group. The combined detection of DCLK1, CEA, and CA19-9 offers better sensitivity and specificity for the diagnosis of gastric cancer.

**Keywords:** DCLK1; CEA; CA19-9; CA72-4; Gastric Cancer; Expression

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## 1. Introduction

The International Agency for Research on Cancer (IARC) conducted a study on the global cancer burden across 185 countries. The data from 2020 show that new cases of gastric cancer and deaths amounted to approximately 1.089 million and 769,000, respectively, accounting for the fifth (5.6%) and fourth (7.7%) positions in cancer incidence and mortality rates [1]. Despite the current comprehensive treatment strategies, including surgical resection, chemotherapy, radiotherapy, and gene therapy, the overall survival rate for gastric cancer remains low. The primary method for diagnosing gastric cancer is pathological diagnosis through endoscopic biopsy,

however, most cases are detected at an advanced stage. Tumor markers serve as an auxiliary tool for tumor diagnosis, efficacy evaluation, and recurrence monitoring, offering certain guidance in clinical diagnosis and treatment. CA19-9, CA72-4, and CEA are commonly used serum markers in the diagnosis of gastric cancer, but these markers lack organ specificity, and their sensitivity and specificity are not ideal when tested individually. Studies have reported that Doublecortin-like kinase-1 (DCLK1) is a molecular marker of gastrointestinal tumor stem cells and is highly expressed in gastric cancer tissues [2], but the expression of this protein in the serum of gastric cancer patients remains unclear. This study aims to investigate the expression of DCLK1 in the serum of gastric cancer patients and analyze its relationship with CA19-9, CA72-4, and CEA.

## 2. Materials and methods

### 2.1. General data

Fifty newly diagnosed gastric cancer patients admitted to our department from January to December 2021 were selected, including 23 males and 27 females, with an average age of 56.37 ± 6.45 years. All patients were confirmed by endoscopic pathology. Additionally, 50 patients with chronic atrophic gastritis (CAG) admitted during the same period were selected as the control group, including 26 males and 24 females, with an average age of 54.17 ± 7.29 years. All patients were excluded from other malignancies. Statistical analysis showed no significant differences in general data such as gender and age between the gastric cancer group and the control group ($P > 0.05$).

### 2.2. Detection methods and result determination

All patients had 5 mL of venous blood drawn into a non-anticoagulant blood collection tube in the morning after fasting. The blood was left to stand at 4 ℃ until coagulation, then centrifuged at 3000 r/min for 10 minutes, after which the serum was carefully collected, aliquoted, and stored frozen at -20 ℃ for later use. The Roche Cobas e6801 analysis system and corresponding CEA, CA19-9, and CA72-4 quantitative detection kits were used to measure the levels of these markers in the serum of patients in the gastric cancer and control groups. The DCLK1 content in the serum of both groups was measured using a Wuhan USCN enzyme-linked immunosorbent assay (ELISA) kit. All operations were conducted strictly according to the instructions. The cut-off values for DCLK1, CEA, CA19-9, and CA72-4 were determined using the receiver operating characteristic (ROC) curve to interpret the results. For the combined detection of DCLK1, CEA, CA19-9, and CA72-4, a positive result for any one marker was considered a positive result for the combined detection.

### 2.3. Statistical analysis

Data processing and statistical analysis were performed using SPSS 26.0 software. The mean and standard deviation (SD) were used to describe normally distributed measurement data and the independent samples t-test was used to compare means between groups. M (P25, P75) was used to describe skewed distribution data, and the rank-sum test was used to compare medians between groups. Prediction analysis was conducted based on the ROC curve, with a significance level of $\alpha = 0.05$.

## 3. Results

### 3.1. Expression of DCLK1, CEA, CA19-9, and CA72-4 in serum of gastric cancer and control groups

SPSS 26.0 was used to analyze the data from the two groups. The serum concentration of DCLK1 followed a

normal distribution in both groups, while the other data exhibited a skewed distribution. Therefore, the DCLK1 data are presented as mean ± standard deviation (SD), and the CEA, CA19-9, and CA72-4 data are presented as median and percentiles (Table 1). A significance test of the differences in the results between the two groups showed that the expression levels of DCLK1, CEA, CA19-9, and CA72-4 in the serum of gastric cancer patients were significantly higher than those in the CAG group ($P < 0.05$) (Table 1).

**Table 1. Serum concentrations of DCLK1, CEA, CA19-9, and CA72-4 in the two groups [mean ± SD/ M(P25, P75)]**

| Group | DCLK1 (ng/mL) | CEA (ng/mL) | CA19-9 (ng/mL) | CA72-4 (U/mL) |
|---|---|---|---|---|
| Control | 11.50 ± 5.79 | 1.765 (1.13, 3.14) | 10.10 (6.43, 15.65) | 2 (1.5, 3) |
| Gastric Cancer | 16.47 ± 6.03 | 3.62 (1.45, 11.76) | 13.76 (8.33, 32.41) | 3 (2, 6.3) |
| z/t-value | -4.198 | -3.468 | -2.585 | -2.763 |
| p-value | < 0.001 | 0.001 | 0.01 | 0.006 |

## 3.2. AUC and cut-off values for independent diagnosis of gastric cancer by DCLK1, CEA, CA19-9, and CA72-4

SPSS 26.0 was used to analyze the data from the two groups, calculate the ROC curve, and derive the area under the curve (AUC) and cut-off values. The results showed that the AUCs of DCLK1, CEA, CA19-9, and CA72-4 were all greater than 0.5, with the AUCs for DCLK1 and CEA both exceeding 0.7, specifically 0.709 and 0.701, respectively (Table 2).

**Table 2. AUC and cut-off values for DCLK1, CEA, CA19-9, and CA72-4**

| Marker | AUC | Cut-off value |
|---|---|---|
| DCLK1 | 0.709 | 15.6 |
| CEA | 0.701 | 5.075 |
| CA19-9 | 0.65 | 24.38 |
| CA72-4 | 0.659 | 2.85 |

## 3.3. Sensitivity and specificity of independent diagnosis of gastric cancer by DCLK1, CEA, CA19-9, and CA72-4

Based on the cut-off values determined by the ROC curve, the sensitivity and specificity of DCLK1, CEA, CA19-9, and CA72-4 for independent diagnosis of gastric cancer were calculated. CA72-4 had the highest sensitivity (62%), while CA19-9 had the lowest sensitivity (38%). CEA had the highest specificity (98%), while CA72-4 had the lowest specificity (74%). The accuracy results for gastric cancer diagnosis showed that CEA had the highest accuracy (73%), while DCLK1 and CA19-9 had the lowest accuracy, both at 66% (Table 3).

**Table 3. Sensitivity, specificity, and accuracy of DCLK1, CEA, CA19-9, and CA72-4 for gastric cancer detection (%)**

| Marker | Control group | Gastric cancer group | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|---|
| DCLK1 | | | | | |
| + | 10 | 26 | 52% | 80% | 66% |
| - | 40 | 24 | | | |

**Table 3 (Continued)**

| Marker | Control group | Gastric cancer group | Sensitivity | Specificity | Accuracy |
|---|---|---|---|---|---|
| CEA | | | | | |
| + | 1 | 24 | 48% | 98% | 73% |
| - | 49 | 26 | | | |
| CA19-9 | | | | | |
| + | 3 | 19 | 38% | 94% | 66% |
| - | 47 | 31 | | | |
| CA72-4 | | | | | |
| + | 13 | 31 | 62% | 74% | 68% |
| - | 37 | 19 | | | |

### 3.4. Combined detection of DCLK1, CEA, CA19-9, and CA72-4 in the diagnosis of gastric cancer

Various combinations of DCLK1, CEA, CA19-9, and CA72-4 were used to detect gastric cancer, with a positive diagnosis defined as any one of the markers reaching or exceeding the diagnostic standard. The sensitivity, specificity, and AUC of each combination were calculated (Table 4). The results showed that the combination of all four markers had the highest sensitivity (90%), but the lowest specificity (60%). The accuracy of each combination was above 70%, with the highest accuracy for the CEA+CA19-9 combination (80%) and the lowest accuracy for the DCLK1+CA19-9 combination (71%). The AUC of each combination was above 0.7 and greater than the AUC of any single marker, with the combination of DCLK1+CEA+CA19-9 having the largest AUC (0.826), followed closely by the combination of all four markers (0.825), which was only 0.001 less than the three-marker combination (Table 4, Figure 1).

**Table 4. Sensitivity, specificity, accuracy, and AUC of DCLK1, CEA, CA19-9, and CA72-4 for gastric cancer detection**

| Marker combination | Sensitivity | Specificity | Accuracy | AUC |
|---|---|---|---|---|
| DCLK1+CEA | 74% | 72% | 76% | 0.794 |
| DCLK1+CA72-4 | 82% | 64% | 73% | 0.744 |
| DCLK1+CA19-9 | 64% | 78% | 71% | 0.754 |
| CEA+CA72-4 | 72% | 72% | 72% | 0.718 |
| CEA+CA19-9 | 68% | 92% | 80% | 0.786 |
| CA72-4+CA19-9 | 76% | 70% | 73% | 0.702 |
| DCLK1+CEA+CA72-4 | 84% | 62% | 73% | 0.795 |
| DCLK1+CEA+CA19-9 | 82% | 76% | 79% | 0.826 |
| DCLK1+CA72-4+CA19-9 | 86% | 62% | 74% | 0.784 |
| CEA+CA72-4+CA19-9 | 82% | 68% | 75% | 0.786 |
| DCLK1+CEA+CA72-4+CA19-9 | 90% | 60% | 75% | 0.825 |

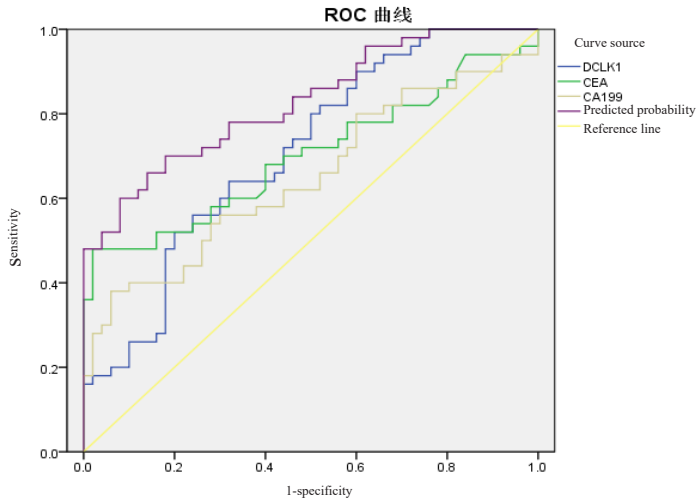


**Figure 1. ROC curves for combined detection of three gastric cancer markers.**

## 4. Discussion

During the development of malignant tumors, substances that are not expressed or are expressed at significantly higher levels in normal tissues can be produced or secreted. These substances can be released into the serum or other body fluids, reflecting the presence and status of tumors to some extent. Because of their association with malignant tumors, they are referred to as tumor markers [3]. An ideal tumor marker should have the following characteristics: (1) high sensitivity; (2) strong specificity; (3) organ specificity; (4) relevance to tumor occurrence and development; (5) presence in body fluids, making it easy to detect; (6) correlation with therapeutic effects; and (7) ability to assess prognosis [4]. Although tumor markers hold great clinical potential, markers with both very high sensitivity and specificity have yet to be applied in clinical practice. Moreover, apart from alpha-fetoprotein and prostate-specific antigen, no other tumor markers with strong organ specificity have been identified. Clinically, a particular marker may appear in multiple types of tumors, and various markers may also be present in a single tumor. Therefore, there is a need to explore new tumor markers actively.

Tumor stem cells are a small subset of tumor cells within tumor tissues that have the potential for self-renewal, and multi-lineage differentiation, and can induce the formation of tumor heterogeneity. These cells are closely associated with biological characteristics such as tumor resistance and recurrence [5,6]. Doublecortin-like kinase-1 (DCLK1) is a member of the protein kinase superfamily and the doublecortin (DCX) family. It is primarily expressed in fetal and adult brain tissues under normal conditions [7]. Still, it is also expressed in the heart, liver, spleen, thymus, prostate, testes, ovaries, small intestine, and colon [8]. Recent studies suggest that DCLK1 is a tumor stem cell marker, closely related to the development of tumors such as pancreatic cancer, colon cancer, and breast cancer [9–11]. It is also increasingly expressed in tumors and can be detected in the blood, making it a potential new marker for gastrointestinal tumors [12,13]. In this study, the average concentration of DCLK1 in the serum of gastric cancer patients was significantly higher than that in patients with chronic atrophic gastritis ($P < 0.001$). Its sensitivity for diagnosing gastric cancer was 52%, with a specificity of 80%, indicating relatively high specificity but low sensitivity. This result suggests that the tumor stem cell marker DCLK1 can be detected in serum and is related to tumor occurrence and development to some extent, but it cannot be considered an ideal tumor marker for gastric cancer when tested alone and must be combined with other markers. Studies have shown that DCLK1 is also expressed at higher levels in the serum of patients with

liver cancer and esophageal cancer than in normal subjects [12,13] and combined with the results of this study, it indicates that this gene is overexpressed in the serum of tumor patients but lacks organ specificity.

Carcinoembryonic antigen (CEA) is a glycoprotein expressed in embryonic cells, consisting of 641 amino acid residues with embryonic antigenic determinant clusters. CEA can be secreted by the digestive tract of a two-month-old embryo and disappears after birth, resulting in very low serum levels in normal individuals. However, when digestive tract tumors occur, tumor cells can re-express CEA, increasing its serum levels in patients. CA19-9 and CA72-4 are glycoprotein tumor markers. Normally, cell membranes are rich in glycoproteins, but when cells undergo malignant transformation, the surface glycoproteins change, forming specific antigens different from normal cell antigens, which can reflect cellular changes to some extent. CA19-9 and CA72-4 are mostly distributed in epithelial tissues of the pancreas, stomach, and intestines, and are common markers for gastrointestinal tumors. Several studies have shown that the expression of CEA, CA19-9, and CA72-4 in the serum of gastric cancer patients is higher than in non-gastric cancer patients, but their sensitivity and specificity are not high when tested individually, and there are no significant differences in the expression of these three markers in the serum of gastric cancer patients [14–16]. In this study, the sensitivity of individual marker tests ranged from 38% to 62%, and specificity ranged from 74% to 98%. The sensitivity of combined marker tests ranged from 64% to 82%, and specificity ranged from 64% to 92%. The sensitivity of three-marker combined tests ranged from 82% to 86%, and specificity ranged from 62% to 76%. The sensitivity of four-marker combined tests was 90%, with a specificity of 60%. These results indicate that as the number of combined markers increases, sensitivity gradually increases, but specificity decreases. A meta-analysis showed that the sensitivity and specificity of combined detection of CEA, CA19-9, and CA72-4 for gastric cancer are superior to those of individual markers [17], consistent with the results of this study.

In tumor screening, higher sensitivity is needed to avoid missed diagnoses, while in tumor diagnosis, higher specificity is required to avoid misdiagnosis. Combined marker detection can improve both sensitivity and specificity, but it also increases the financial burden on patients. Therefore, it is necessary to determine the appropriate sensitivity and specificity to determine the number of markers to test. The area under the ROC curve (AUC) is an indicator of diagnostic accuracy, with larger values indicating higher prediction accuracy. It is generally believed that when AUC exceeds 0.8, the diagnosis has practical application value [18]. In this study, the combined detection of DCLK1, CEA, and CA19-9 had the largest AUC (0.826), with a sensitivity of 82%, specificity of 76%, and accuracy of 79%. These results suggest that DCLK1 has potential as a serological marker for gastric cancer, and among the three markers CEA, CA199, and CA724, its combination with CEA and CA199 offers the best detection results.

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## Disclosure statement

The authors declare no conflict of interest.

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