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ORIGINAL RESEARCH

Interleukin-6 as a Predictive Marker for Lymph Node and Distant Metastasis in Colorectal Cancer: A Retrospective Cohort Study

Jianjun Xiao, Muyou Tian, Guoyu Chen, Huifen Li

Department of Medical Oncology, Zhongshan City People's Hospital, Zhongshan, Guangdong, People's Republic of China

Correspondence: Huifen Li, Department of Medical Oncology, Zhongshan City People's Hospital, No. 2 Sunwen East Road, Zhongshan, Guangdong, 528404, People's Republic of China, Tel +86 13450956484, Fax +86 760-88823566, Email LHF20250110@outlook.com

Background: Metastasis is the leading cause of death in colorectal cancer (CRC). While interleukin-6 (IL-6), a key inflammatory cytokine, is implicated in tumor metastasis, its specific association with lymph node metastasis (LNM) and distant metastasis (DM) in CRC remains unclear.

Methods: We retrospectively analyzed clinical data and serum levels of carcinoembryonic antigen (CEA) and cytokines (including IL-6) in 427 CRC patients, stratified by metastatic status. Statistical analyses assessed the predictive value of IL-6 for metastasis.

Results: Elevated serum IL-6 levels were significantly associated with both LNM and DM ($P<0.05$). IL-6 positively correlated with CEA levels (Spearman correlation). Although IL-6 alone showed modest predictive power for LNM (AUC=0.555), it outperformed CEA (AUC=0.525). Combining IL-6 and CEA improved diagnostic accuracy for LNM (AUC=0.583). Notably, IL-6 demonstrated greater sensitivity than CEA in predicting DM (77.30% vs 67.40% at optimal cutoff).

Conclusion: These findings demonstrate that IL-6 holds significant predictive value for metastasis in CRC, particularly excelling in the prediction of LNM. The detection of IL-6 offers a valuable complementary approach to the existing clinical prediction paradigm for CRC metastasis risk.

Keywords: IL-6, colorectal cancer, lymph node metastasis, distant metastasis

Introduction

Colorectal cancer (CRC) is a highly heterogeneous malignancy whose development involves complex interactions between genetic and epigenetic factors.[1,2] In recent years, its incidence has shown a rising trend among younger populations, contributing to an increasing global disease burden. As of 2023, CRC has become the third most commonly diagnosed cancer globally, while its mortality rate surpasses that of the second-ranking cancer.[3] The situation is particularly urgent in China. Data from 2022 indicate that the country recorded 517,100 new CRC cases, accounting for 10.7% of all new malignant tumor cases and representing the second highest incidence rate. Concurrently, CRC caused 240,000 deaths, constituting 9.3% of total cancer mortality and ranking as the fourth leading cause of cancer death. These alarming statistics underscore the critical and urgent need to enhance research into CRC prevention, control, and therapeutic strategies.

For patients with CRC, the primary cause of mortality stems not from the primary lesion itself, but rather from the metastatic dissemination of tumor cells to regional lymph nodes and distant organs.[4] CRC confined to the primary site is typically amenable to curative surgical resection. However, the therapeutic regime becomes substantially more complex upon lymph node metastasis. Even with multimodal therapy combining surgery and adjuvant chemotherapy, the cure rate approximates only 70%.[5] If distant organ metastasis occurs (eg, to the liver or lungs), the likelihood of cure plummets, leaving most patients with limited curative options.[6,7] Consequently, the early and accurate prediction of metastatic risk in CRC is critical for developing personalized treatment strategies and improving patient prognosis.

Current clinical methods for predicting CRC metastasis include serum tumor biomarker testing, imaging evaluation, molecular biological testing, and analysis of pathological factors. Among these, serum biomarkers are widely utilized due

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to their simplicity and relatively low cost. Carcinoembryonic antigen (CEA) is a commonly employed biomarker for predicting CRC metastatic recurrence, monitoring disease progression, and assessing treatment response.[8] However, approximately 20% of CRC patients exhibit CEA negativity, significantly limiting its predictive efficacy.[9] Therefore, there is an urgent need to identify more effective novel biomarkers to improve prediction accuracy. In this context, multiplex cytokine panels (such as 12 cytokines profiling) offer a new perspective and a potential tool for predicting metastatic risk, as they provide a more comprehensive reflection of the tumor microenvironment status and systemic inflammatory response.

Research indicates that the cytokine network plays a pivotal role in tumor metastasis, driving the metastatic process through multiple mechanisms, including promoting tumor growth, shaping the pre-metastatic microenvironment, and suppressing immune responses.[10,11] Among the various cytokines, Interleukin-6 (IL-6), as a central pro-inflammatory cytokine, plays a significant role in promoting early cancer cell proliferation and tumor growth.[12] Clinically, elevated serum IL-6 levels are frequently observed in patients with advanced and metastatic CRC, suggesting a strong correlation between IL-6 levels and clinical stage as well as disease progression.[13] Mechanistic studies reveal that multiple infiltrating cells within the tumor microenvironment (TME), such as T cells, macrophages, and fibroblasts, are capable of secreting IL-6.[14–16] Upon binding to its receptor, IL-6 activates the downstream signal transducer and activator of transcription 3 (STAT3) pathway. This activation subsequently drives cancer cell proliferation, angiogenesis, and tumor progression,[15–17] ultimately contributing to adverse outcomes such as liver metastasis and worsened prognosis.[13,18]

While the role of IL-6 in CRC progression and advanced metastasis has been partially elucidated, its specific value in predicting the risk of lymph node metastasis (LNM) and distant metastasis (DM) has not yet been systematically and thoroughly investigated or reported. Given this gap, this study aims to comprehensively investigate the predictive value of serum IL-6 levels for LNM and DM occurrence in CRC patients. By enabling the more precise identification of patients at high risk of metastasis at an early disease stage, this research is expected to provide more accurate and effective evidence for subsequent clinical decision-making (such as intensified adjuvant therapy, intensified surveillance strategies, etc)., thus ultimately contributing to improved survival outcomes for CRC patients.

Materials and Methods

Patients and Samples

In this retrospective study, we collected clinical data from 427 CRC patients treated at the Department of Oncology and Chemotherapy of our hospital between January 31, 2021, and March 31, 2023.

To ensure the accuracy of the study, we divided the patients into two groups based on strict diagnostic criteria, which were established through image-guided percutaneous puncture sampling or lymph node specimen acquisition via direct endoscopic visualization and subsequent pathological analysis: Group A comprised 143 CRC patients without LNM, while Group B included 284 CRC patients with LNM. Additionally, based on the results of biopsy and pathology analysis, patients were categorized into Group C, which consisted of 194 CRC patients without DM, and Group D, which included 233 CRC patients with DM. The clinical characteristics of all patients are summarized in Tables 1 and 2.

The following inclusion criteria were strictly adhered to in this study: (1) patients diagnosed with primary colorectal cancer; (2) the diagnostic process and initial surgical treatment were conducted at our hospital; and (3) complete clinical and pathological data were available. Additionally, we established stringent exclusion criteria to ensure the accuracy of the study data, which included: (1) patients with uncertain T-stage; (2) patients who had received chemotherapy or radiotherapy following diagnosis; (3) patients who had undergone targeted immunotherapy; (4) patients with infectious diseases; (5) patients with inflammatory bowel disease; (6) patients with two or more concurrent intestinal malignant tumors or other systemic malignant tumors; (7) patients with autoimmune-related diseases or those on long-term hormone therapy; and (8) patients with psychiatric disorders. The study protocol received formal approval from Clinical Research and Animal Experiment Ethic Committee of our hospital.

During the data collection process, we recorded the demographic information and clinical characteristics of each CRC patient, including gender, age, TNM stage (as defined by the 8th edition of the AJCC staging criteria), degree of differentiation, and overall stage. Additionally, we arranged for a qualified and authorized nurse to collect venous blood

samples from each patient, which were placed in vacuum-dried tubes. All blood samples were collected within two hours of admission. The results for CEA and 12 cytokines for all patients were obtained prior to any therapeutic interventions.

CEA levels in patients were quantified using the Beckman UniCel DxI 800 (California, USA). Additionally, 12 cytokines in the serum of CRC patients were measured using the Luminex 200 (Texas, USA). Detailed assay validation data, including precision, linearity, reference ranges, and quality control procedures, are provided in the Supplementary Material (Supplementary Material 1 and Supplementary Tables 1–4). All samples were processed within two hours of collection. The testing instruments were rigorously maintained and operated by specialized laboratory technicians. Instruments were under control for the duration of the assay.

Statistical Analysis

All statistical analyses were performed using SPSS 25.0 for Windows (IBM, Chicago, USA). Data are represented as mean ± standard deviation. Categorical variables were compared using parametric analysis of variance (ANOVA), while continuous variables were compared using the independent samples *t*-test, to assess differences between CRC patients with and without LNM, and with and without DM. The correlation between CEA and IL-6 was evaluated using Spearman's rank correlation test. Binary logistic regression analysis was employed to control for confounding variables and identify influencing factors. To assess the predictive relationship of multiple independent variables with LNM/DM in CRC and to ensure model reliability, we performed multicollinearity diagnostics. To evaluate the diagnostic performance of IL-6 and CEA, receiver operating characteristic (ROC) curves were generated. The area under the curve (AUC) was calculated to assess the discriminative ability (with a higher AUC indicating better discrimination). Sensitivity and specificity for each biomarker individually, as well as for their combination, were analyzed.

Result

Characteristics of Included Subjects

In the comparative analysis, no statistically significant differences were observed between Group A and B or between Group C and D regarding gender, degree of differentiation, or age. However, significant differences in tumor infiltration

Table 1 The Baseline Characteristics of Study Population

	Group A (n=143)	Group B (n=284)	χ2	P-value	Group C (n=194)	Group D (n=233)	χ2	P-value
Gender, n (%)			0.017	0.895			0.160	0.689
Male	78 (54.55%)	153 (53.87%)			107 (55.15%)	124 (53.22%)		
Female	65 (45.45%)	131 (46.13%)			87 (44.85%)	109 (46.78%)		
T stage, n (%)			21.109	0.000**			25.079	0.000**
T0-1, T2	58 (40.56%)	56 (19.72%)			29 (14.95%)	85 (36.48%)		
T3, T4	85 (59.44%)	228 (80.28%)			165 (85.05%)	148 (63.52%)		
N stage, n (%)			-	-			12.213	0.000**
N0	143 (100%)	-			48 (24.74%)	95 (40.77%)		
N1, N2	-	284 (100%)			146 (75.26%)	138 (59.23%)		
M stage, n (%)			12.213	0.000**			-	-
M0	48 (33.57%)	146 (51.41%)			194 (100%)	-		
M1	95 (66.43%)	138 (48.59%)			-	233 (100%)		
Differentiation degree, n (%)			0.903	0.342			0.613	0.434
Low, Moderate	132 (92.31%)	254 (89.44%)			173 (89.18%)	213 (91.42%)		
High	11 (7.69%)	30 (10.56%)			21 (10.82)	20 (8.58%)		
Overall stage, n (%)			-	-			-	-
I, II	47 (32.87%)	1 (0.35%)			48 (24.74%)	-		
III, IV	96 (67.13%)	283 (99.65%)			146 (75.26%)	233 (100%)		
	Group A (n=143)	**Group B (n=284)**	**t**	**P-value**	**Group C (n=194)**	**Group D (n=233)**	**t**	**P-value**
Age, years	59.190±9.899	57.260±10.317	1.844	0.066	56.980±9.952	58.680±10.375	−1.711	0.088
CEA (ng/mL)	3.195±1.757	3.300±1.763	−0.580	0.562	2.499±1.385	3.902±1.786	−8.931	0.000**

Note: **Represents a significantly statistical difference (P < 0.01).

depth (T-stage) were found both between Group A and B and between Group C and D ($P < 0.01$). Furthermore, lymph node metastasis status (N-stage) differed significantly between Group C and D ($P < 0.01$), while a statistically significant difference in distant metastasis status (M-stage) was noted between Group A and B ($P < 0.01$). Regarding CEA levels, no significant difference was detected between Group A and B, whereas a statistically significant difference was observed between Group C and D ($P < 0.01$). Detailed demographic and clinical characteristics of the CRC patients across all groups are summarized in Table 1.

The Level of Cytokines in LNM/DM

Table 2 demonstrates the comparison of serum levels for 12 cytokines between patients without LNM (Group A) and those with LNM (Group B). The data indicate that IL-6 levels were significantly higher in Group B compared to Group A ($P < 0.05$). Conversely, TNF-α levels were significantly lower in Group B than in Group A ($P < 0.05$).

Table 3 presents a comparison of serum levels of 12 cytokines between patients without DM (group C) and those with DM (group D). The analysis revealed that IL-6 levels were significantly elevated in group D compared to group C ($P<0.01$).

Table 2 The Correlation Between the Cytokines and LNM of CRC

Cytokine Profiles	Group A (n=143)	Group B (n=284)	P-value
IL-1β (pg/mL)	2.401±2.587	3.391±14.462	0.418
IL-2 (pg/mL)	7.846±20.667	6.555±17.105	0.494
IL-4 (pg/mL)	10.556±24.532	8.781±15.609	0.364
IL-5 (pg/mL)	3.143±3.544	3.291±3.414	0.676
IL-6 (pg/mL)	13.115±21.660	24.405±58.030	0.025*
IL-8 (pg/mL)	5.444±13.988	7.097±20.929	0.394
IL-10 (pg/mL)	9.206±18.523	6.825±15.109	0.156
IL-12p70 (pg/mL)	9.002±20.840	6.022±12.193	0.064
IL-17 (pg/mL)	25.258±71.808	16.866±39.756	0.121
IFN-α (pg/mL)	6.845±11.961	5.152±7.552	0.075
IFN-γ (pg/mL)	7.655±19.066	5.401±9.855	0.108
TNF-α (pg/mL)	7.165±16.717	4.298±7.298	0.014*

Note: *Represents a statistical difference ($P < 0.05$).
Abbreviations: LNM, Lymph Node Metastasis; CRC, Colorectal Cancer.

Table 3 The Correlation Between the Cytokines and DM of CRC

Cytokine Profiles	Group C (n=194)	Group D (n=233)	P-value
IL-1β (pg/mL)	2.552±5.354	3.482±15.342	0.422
IL-2 (pg/mL)	6.456±16.468	7.430±19.823	0.586
IL-4 (pg/mL)	9.368±19.034	9.382±19.117	0.994
IL-5 (pg/mL)	3.197±3.652	3.278±3.289	0.810
IL-6 (pg/mL)	13.396±40.685	26.642±54.682	0.005**
IL-8 (pg/mL)	6.059±20.051	6.947±17.899	0.629
IL-10 (pg/mL)	8.065±18.084	7.254±14.779	0.610
IL-12p70 (pg/mL)	7.191±15.197	6.878±16.083	0.838
IL-17 (pg/mL)	20.389±52.725	19.083±52.910	0.799
IFN-α (pg/mL)	5.799±9.674	5.653±8.970	0.872
IFN-γ (pg/mL)	5.127±8.776	7.012±16.654	0.156
TNF-α (pg/mL)	4.860±8.840	5.589±13.196	0.512

Note: **Represents a significantly statistical difference ($P < 0.01$).
Abbreviation: DM, Distant Metastasis.

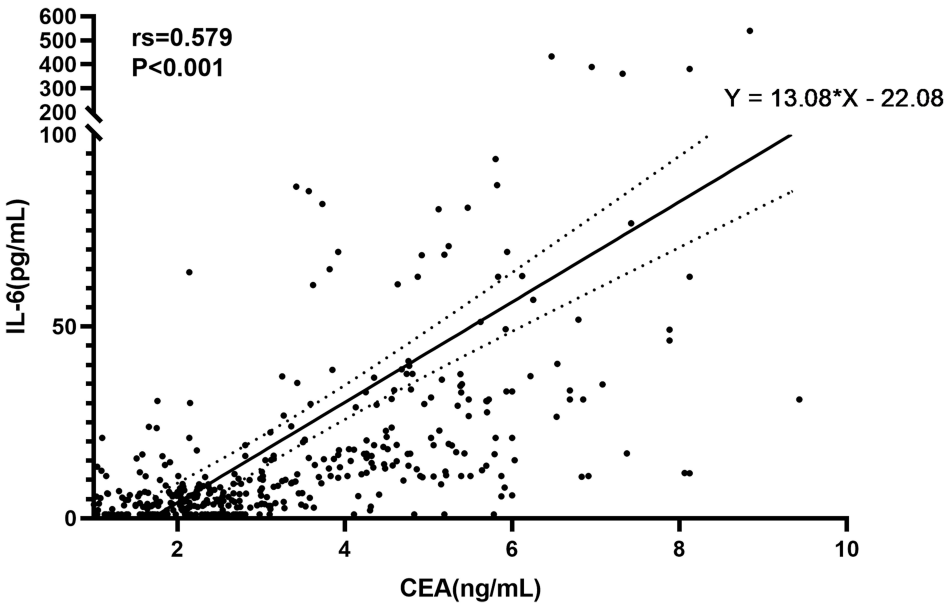

Figure 1 Spearman correlation between IL-6 and CEA in all participants (High correlation:0.5 to 1.0).

Correlation of CEA with IL-6

Spearman correlation analysis revealed a significant correlation between CEA and IL-6 (rs=0.579, P<0.001) (Figure 1). All participants were included in this analysis (Figure 1).

Association Between CEA/IL-6 and Metastasis

In the binary logistic regression model adjusting for baseline characteristics of CRC patients—including age, gender, overall stage, differentiation degree, and distant metastasis status—we evaluated the associations of CEA and IL-6 levels with LNM (Table 4). The analysis demonstrated that elevated IL-6 levels were significantly associated with increased risk of LNM (adjusted odds ratio [AOR] = 1.015; 95% confidence interval [CI]: 1.006–1.024). In contrast, CEA was not identified as an independent risk factor for LNM in CRC.

Furthermore, we assessed the predictive value of CEA and IL-6 for DM risk in CRC patients, with additional adjustment for lymph node metastasis status (Table 4). Among patients without baseline DM, elevated IL-6 levels were associated with higher risk of developing DM (AOR = 1.025; 95% CI: 1.011–1.038). In this same cohort, increased CEA levels independently predicted higher likelihood of DM (AOR = 1.697; 95% CI: 1.436–2.005).

Table 4 Association Between CEA/IL-6 and Metastasis

	Odds Ratio (95% CI) Unadjusted	P-value	Odds Ratio (95% CI) Adjusted[a,b,c]	P-value
Lymph node metastasis				
IL-6	1.012(1.001–1.021)	0.039*	1.015(1.006–1.024)[a,b]	0.001**
CEA	1.035(0.922–1.161)	0.561	1.122(0.961–1.309)[a,b]	0.145
Distant metastasis				
IL-6	1.011(1.002–1.020)	0.016	1.025(1.011–1.038)[a,c]	0.000**
CEA	1.766(1.524–2.046)	0.000**	1.697(1.436–2.005)[a,c]	0.000**

Notes: [a]Logistic regression with generalized estimating equations modeled the associations accounting for CRC patients correlations. [b]Adjusted models included: Age, gender, T-stage, overall stage, differentiation degree and distant metastasis. [c]Adjusted models included: Age, gender, T-stage, overall stage, differentiation degree and lymph node metastasis. *Represents a statistical difference (P < 0.05), **Represents a significantly statistical difference (P < 0.01).
Abbreviation: CI, Confidence Interval.

Diagnostic Evaluation of Multicollinearity Among Variables of Lymph Node Metastasis/ Distant Metastasis in Colorectal Cancer

Multicollinearity diagnostics were performed for all logistic regression models. When predicting LNM (dependent variable) using age, gender, differentiation degree, overall stage, IL-6, CEA, T-stage, and DM status (independent variables), all variance inflation factors (VIFs) were below 1.539, indicating no significant multicollinearity (Table 5). Similarly, for the model predicting DM using age, gender, differentiation degree, overall stage, IL-6, CEA, T-stage, and LNM status (independent variables), maximum VIF was 1.464, confirming absence of multicollinearity (Table 6).

Table 5 Diagnostic Evaluation of Multicollinearity Among Variables of LNM in CRC

Variables	Regression Coefficient	β - Beta	Tolerance	VIF
Age	−0.001	−0.031	0.959	1.043
Gender	−0.020	−0.021	0.987	1.013
T stage	0.135	0.127	0.899	1.112
M stage	−0.382	−0.403	0.701	1.427
Differentiation degree	0.104	0.065	0.978	1.023
Overall stage	0.959	0.642	0.825	1.211
IL-6	0.001	0.155	0.767	1.304
CEA	−0.001	−0.005	0.650	1.539

Abbreviation: VIF, Variance Inflation Factor.

Table 6 Diagnostic Evaluation of Multicollinearity Among Variables of DM in CRC

Variables	Regression Coefficient	β - Beta	Tolerance	VIF
Age	0.002	0.043	0.960	1.042
Gender	−0.026	−0.026	0.988	1.013
T stage	−0.119	−0.106	0.892	1.121
N stage	−0.436	−0.413	0.683	1.464
Differentiation degree	−0.001	−0.001	0.971	1.030
Overall stage	0.843	0.535	0.692	1.445
IL-6	0.000	0.037	0.744	1.344
CEA	0.073	0.259	0.702	1.425

Diagnostic Accuracy of IL-6 and CEA

Figure 2A presents the ROC curves analysis for IL-6 and CEA in evaluating LNM in CRC. Figure 2B compares their ROC curves for assessing DM in CRC.

For the assessment of LNM, setting the IL-6 cutoff at 11.040 pg/mL yielded a sensitivity of 46.10% and a specificity of 69.90%. The positive predictive value (PPV) was 75.30% and the negative predictive value (NPV) was 39.50%, resulting in an AUC of 0.555 ($P > 0.05$). For CEA, the optimal cutoff was 3.575 ng/mL, yielding an AUC of 0.525 ($P > 0.05$), a sensitivity of 39.80%, and a specificity of 72.00%. The PPV and NPV for CEA were 73.90% and 37.60%, respectively. When IL-6 and CEA were used in combination, sensitivity for LNM assessment decreased to 28.20%; however, specificity significantly increased to 86.70%. The PPV reached 80.80%, the NPV was 37.80%, and the AUC increased to 0.583 ($P < 0.01$) (Table 7).

For the assessment of DM, setting the IL-6 cutoff at 4.545 pg/mL resulted in a sensitivity of 77.30%, a specificity of 44.90%, a PPV of 62.70%, an NPV of 62.10%, and an AUC of 0.642 ($P < 0.01$). Using CEA, the optimal cutoff was 2.720 ng/mL, resulting in a higher AUC of 0.739 ($P < 0.01$), a sensitivity of 67.40%, a specificity of 70.10%, a PPV of 73.00%, and an NPV of 64.20%. Notably, the combination of IL-6 and CEA decreased sensitivity for DM assessment to 56.20% but increased specificity to 82.00%. The PPV reached 78.90%, the NPV was 60.90%, and the AUC was 0.737 ($P < 0.01$) (Table 8).

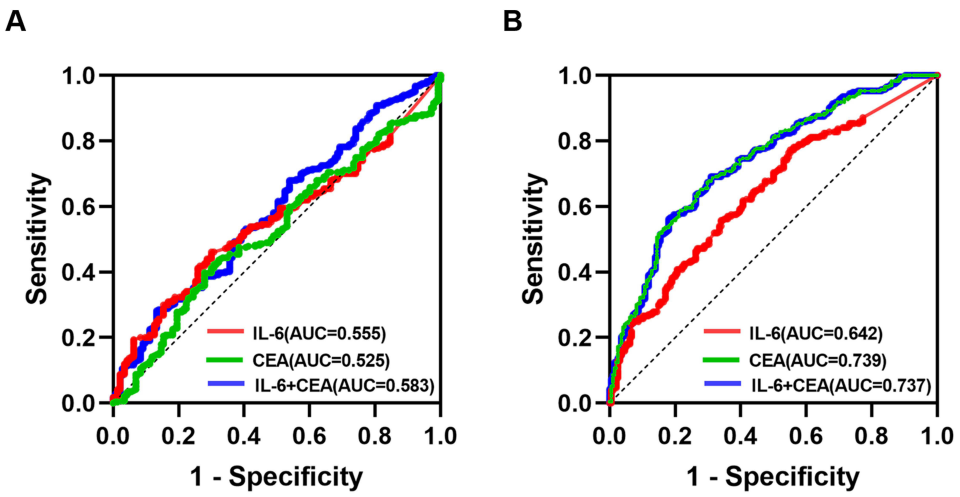

Figure 2 The receiver operating characteristic (ROC) curves of single and combined markers to evaluate diagnostic accuracy. (**A**) ROC curves analysis for IL-6 and CEA in evaluating LNM in CRC. (**B**) ROC curves analysis for IL-6 and CEA in evaluating DM in CRC.

Discussion

CRC ranks as the third most prevalent malignancy globally, with metastatic dissemination constituting the primary cause of cancer-related mortality.[19] The spread of cancer cells from the primary site to regional lymph nodes or distant organs is strongly associated with a significant decline in patient survival. Epidemiological data indicate a 5-year overall survival (OS) rate approaching 90% for stage I CRC patients, decreasing to 82.5% for stage II patients. However, a marked reduction to 59.5% is observed in stage III patients, plummeting to a mere 10–15% for those with stage IV disease.[20] Notably, recent population-based studies have revealed a critical phenomenon, DM occurs in 40% to 63% of CRC patients in the absence of detectable regional LNM. This finding strongly suggests that LNM and DM are not invariably linked by a linear, sequential progression.[21–23] Consequently, the identification of robust biomarkers capable of effectively predicting either LNM or DM holds substantial significance for optimizing clinical staging, guiding personalized therapeutic decisions, and ultimately improving patient outcomes.

CEA, a broad-spectrum tumor marker, exhibits elevated serum levels significantly correlated with CRC metastasis, particularly hepatic metastasis.[24] Our findings partially corroborate this association: we observed significantly elevated

Table 7 Diagnostic Accuracy of the IL-6 and CEA in LNM

Marker	Cutoff Value	Sensitivity	Specificity	PPV	NPV	AUC	P value
IL-6	11.040	0.461	0.699	0.753	0.395	0.555	0.064
CEA	3.575	0.398	0.720	0.739	0.376	0.525	0.396
IL-6+CEA	-	0.282	0.867	0.808	0.378	0.583	0.005**

Note: **Represents a significantly statistical difference (P < 0.01).
Abbreviations: PPV, Positive Predictive Value; NPV, Negative Predictive Value; AUC, Area Under the Curve.

Table 8 Diagnostic Accuracy of the IL-6 and CEA in DM

Marker	Cutoff Value	Sensitivity	Specificity	PPV	NPV	AUC	P value
IL-6	4.545	0.773	0.449	0.627	0.621	0.642	0.000**
CEA	2.720	0.674	0.701	0.730	0.642	0.739	0.000**
IL-6+CEA	-	0.562	0.820	0.789	0.609	0.737	0.000**

Note: **Represents a significantly statistical difference (P < 0.01).
Abbreviations: PPV, Positive Predictive Value; NPV, Negative Predictive Value; AUC, Area Under the Curve.

serum CEA levels in CRC patients with DM, yet its utility in predicting LNM proved limited. This result underscores the pressing clinical need for more readily accessible biomarkers with superior predictive power for metastasis, especially LNM.

Within our institutional clinical practice, we routinely assess a 12-cytokine profile in CRC patients preoperatively. This strategy systematically evaluates the patient's systemic immune status, thereby elucidating host anti-tumor immune responses and providing crucial immunological insights to inform more precise surgical planning and adjuvant therapy strategies (eg, selection and intensity of chemotherapy or immunotherapy).

Tumor metastasis is a complex process involving dynamic interactions between tumor cells and the microenvironment.[25] In CRC patients with LNM, the tumor microenvironment (TME) undergoes significant remodeling. This remodeled TME is characterized by the enrichment of immunosuppressive cells (eg, regulatory T cells, myeloid-derived suppressor cells) and immunosuppressive factors, which collectively suppress the activation of immune cells and the production of functional cytokines.[26,27] Furthermore, hypoxic and acidic conditions within the TME can further impair immune cell function and alter cytokine secretion profiles.[28] In this study, we observed significantly reduced levels of the pro-inflammatory cytokine TNF-α in the peripheral blood of CRC patients with LNM compared to those without LNM. This phenomenon likely reflects reduced TNF-α secretion resulting from the immunosuppressive state within the TME during the process of LNM.

IL-6 is a pleiotropic cytokine that plays a complex and often dualistic role within the TME, exhibiting both pro-inflammatory and pro-tumorigenic potential.[29,30] IL-6 signaling, initiated upon binding to its receptor complex (IL-6Rα/gp130) and activating downstream pathways (eg, JAK/STAT3), has been demonstrated to promote epithelial-mesenchymal transition (EMT). EMT is a critical step conferring invasive and migratory capabilities to tumor cells, thereby driving metastatic progression.[31,32] A central finding of our study is the significant elevation of serum IL-6 levels in CRC patients, irrespective of the presence of LNM or DM. This result strongly supports an active, pro-metastatic role for IL-6 in CRC and suggests its potential value as a biomarker for predicting both LNM and DM. Notably, compared to CEA, which demonstrates greater prominence in predicting DM, IL-6 exhibits a relative advantage in predicting LNM (though its standalone predictive power requires further enhancement). This complements the limitation of CEA in LNM prediction.

Given CEA's established strength in predicting DM, we further investigated the relationship between IL-6 and CEA. Spearman correlation analysis confirmed a significant positive correlation between the two markers in CRC patients (rs = 0.579, $P < 0.001$). This correlation suggests that IL-6 not only serves as an effective complementary marker to CEA for DM prediction but also partially mitigates CEA's deficiency in predicting LNM.

To establish the independent predictive value of IL-6 and CEA for CRC metastasis (LNM/DM), we performed multivariate binary logistic regression analyses. Prior to model construction, we noted a significant difference in the depth of primary tumor invasion (T stage) between comparison groups. T stage (T1-T4) directly quantifies the extent of tumor penetration through the bowel wall. Deep invasion represents a significant risk factor for lymphatic/vascular invasion and more directly influence cytokine production (eg, IL-6) within the local TME.[33,34] Consequently, in the final models, we adjusted for potential confounding variables, including age, gender, overall stage, tumor differentiation degree, T stage, and metastasis status (LNM/DM). The key multivariate analysis results revealed that elevated serum IL-6 level was an independent risk factor for LNM in CRC patients (OR = 1.015, 95% CI 1.006–1.024, P = 0.001) after adjusting for the aforementioned variables. This finding provides statistically adjusted evidence supporting its application value in LNM prediction. For DM prediction, both IL-6 and CEA were identified as independent risk factors. All included independent variables exhibited variance inflation factors (VIF) substantially below 5 (maximum VIF = 1.539 for LNM model; maximum VIF = 1.464 for DM model), effectively excluding significant multicollinearity within the models. These results collectively demonstrate that serum IL-6 level is an independent and valuable indicator for assessing metastatic risk in CRC, laying a crucial foundation for developing future precision clinical prediction models integrating multi-dimensional information.

We further employed ROC curves analysis to evaluate the diagnostic performance of IL-6 and CEA for LNM and DM. For LNM prediction, the AUC for IL-6 alone was 0.555 (P = 0.064). While this did not reach the conventional threshold for statistical significance ($P < 0.05$) – potentially attributable to the single-center design and relatively limited sample size of this study – the AUC value was nonetheless higher than that of CEA. More importantly, the combination

of IL-6 and CEA significantly enhanced predictive performance (AUC = 0.583, P < 0.01), demonstrating the advantage of a multi-marker approach. For DM prediction, CEA exhibited higher diagnostic performance (AUC = 0.739), while IL-6 demonstrated greater sensitivity. Combined detection further improved diagnostic specificity. Taken together, the ROC curves analyses indicate that IL-6 shows potential superiority in predicting CRC metastasis, particularly LNM, while the combined application of IL-6 and CEA significantly optimizes diagnostic efficacy, demonstrating its complementary diagnostic value for both LNM and DM prediction, thereby providing reliable support for clinical decision-making.

Through systematic retrospective analysis of preoperative clinical data from CRC patients, this study elucidates the potential value of IL-6 in predicting CRC metastasis and its complementarity with CEA. However, several limitations warrant acknowledgment: (1) Strict inclusion/exclusion criteria resulted in a relatively small final sample size, potentially affecting statistical power and partially explaining why some predictive performances (eg, the AUC for IL-6 alone predicting LNM) did not reach optimal levels. Future studies with expanded cohorts are needed to validate the robustness of these findings. (2) All participants in this study were recruited from a single center in China. The single geographic origin, coupled with potential cultural, environmental, and genetic background differences, may limit the generalizability of the findings. A planned multi-center collaborative study will help enhance the external validity and robustness of the results. (3) This study did not perform subgroup analyses stratifying IL-6 levels based on specific metastatic target organs (eg, liver, lung, peritoneum). Future research should investigate whether IL-6 levels correlate with organ-specific metastatic tropism to deepen understanding of its role in metastatic organotropism. (4) While focused on preoperative prediction, longitudinal monitoring of postoperative IL-6 levels, analyzing their relationship with metastatic recurrence risk and survival prognosis, holds promise for informing IL-6-based prognostic assessment and intervention strategies.

Despite these limitations, the results of this study support the integration of serum IL-6 testing, particularly in combination with CEA, as a valuable complement to existing clinical tools for a more comprehensive assessment of metastatic risk in CRC patients. This approach has the potential to facilitate more precise personalized treatment decisions.

Data Sharing Statement

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics Statement

The study protocol was reviewed and approved by the Zhongshan City People's Hospital Clinical Research and Animal Experimentation Ethic Committee (Approval No. 2025-005) and was conducted in accordance with the 1964 Declaration of Helsinki and its subsequent amendments or similar ethical standards. This study was a retrospective study that did not involve the disclosure of patients' private information, and informed consent was waived by our institutional review board. The authors solemnly promise to strictly comply with relevant laws and regulations to ensure the security and confidentiality of patients' information and to respect their right to privacy. The authors are well aware that any information that identifies a patient is sensitive information, and therefore promise not to disclose any patient identifying information in public information.

Informed Consent

For a retrospective study of anonymized data, informed consent is not required.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Xie S, Cai Y, Chen D, et al. Single-cell transcriptome analysis reveals heterogeneity and convergence of the tumor microenvironment in colorectal cancer. *Front Immunol*. 2022;13:1003419. doi:10.3389/fimmu.2022.1003419
2. Joung JG, Oh BY, Hong HK, et al. Tumor heterogeneity predicts metastatic potential in colorectal cancer. *Clin Cancer Res*. 2017;23(23):7209–7216. doi:10.1158/1078-0432.CCR-17-0306
3. Cercek A, Jemal A, Cercek A, Smith RA, Jemal A. Colorectal cancer statistics, 2023. *Ca A Cancer J Clin*. 2023;73(3):233–254. doi:10.3322/caac.21772
4. Cervantes A, Adam R, Roselló S, et al. Metastatic colorectal cancer: ESMO clinical practice guideline for diagnosis, treatment and follow-up. *Ann Oncol*. 2023;34(1):10–32. doi:10.1016/j.annonc.2022.10.003
5. Lee K, Kim HJ, Oh ST, Lee YS, Kwon TS. The prediction and prognosis of early recurrence in patients with colorectal cancer. *Ann Oncol*. 2013;24:iv99–iv99. doi:10.1093/annonc/mdt203.221
6. Fakih MG. Metastatic colorectal cancer: current state and future directions. *J Clin Oncol*. 2015;33(16):1809–1824. doi:10.1200/JCO.2014.59.7633
7. Ling H, Pickard K, Ivan C, et al. The clinical and biological significance of MIR-224 expression in colorectal cancer metastasis. *Gut*. 2016;65(6):977–989. doi:10.1136/gutjnl-2015-309372
8. Shinkins B, Primrose JN, Pugh SA, et al. Serum carcinoembryonic antigen trends for diagnosing colorectal cancer recurrence in the FACS randomized clinical trial. *Br J Surg*. 2018;105(6):658–662. doi:10.1002/bjs.10819
9. Nicholson BD, Shinkins B, Pathiraja I, et al. Blood CEA levels for detecting recurrent colorectal cancer. *Cochrane Database Syst Rev*. 2015;2015(12):CD011134. doi:10.1002/14651858.CD011134.pub2
10. Huan-Xiang L, Wang S, Lian Z, Deng S, Kun Y. Relationship between tumor infiltrating immune cells and tumor metastasis and its prognostic value in cancer. *Cells*. 2022;12(1):64. doi:10.3390/cells12010064
11. Elinav E, Nowarski R, Thaiss CA, Hu B, Jin C, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat Rev Cancer*. 2013;13(11):759–771. doi:10.1038/nrc3611
12. Ünver N, McAllister F. IL-6 family cytokines: key inflammatory mediators as biomarkers and potential therapeutic targets. *Cytokine Growth Factor Rev*. 2018;41:10–17. doi:10.1016/j.cytogfr.2018.04.004
13. Guthrie GJ, Roxburgh CS, Richards CH, Horgan PG, McMillan DC. Circulating IL-6 concentrations link tumour necrosis and systemic and local inflammatory responses in patients undergoing resection for colorectal cancer. *Br J Cancer*. 2013;109(1):131–137. doi:10.1038/bjc.2013.291
14. Xu H, Lai W, Zhang Y, et al. Tumor-associated macrophage-derived IL-6 and IL-8 enhance invasive activity of LoVo cells induced by PRL-3 in a KCNN4 channel-dependent manner. *BMC Cancer*. 2014;14(1):330. doi:10.1186/1471-2407-14-330
15. Oike T, Kanagawa H, Sato Y, et al. IL-6, IL-17 and stat3 are required for auto-inflammatory syndrome development in mouse. *Sci Rep*. 2018;8(1):15783. doi:10.1038/s41598-018-34173-5
16. Nagasaki T, Hara M, Nakanishi H, Takahashi H, Sato M, Takeyama H. Interleukin-6 released by colon cancer-associated fibroblasts is critical for tumour angiogenesis: anti-interleukin-6 receptor antibody suppressed angiogenesis and inhibited tumour-stroma interaction. *Br J Cancer*. 2014;110(2):469–478. doi:10.1038/bjc.2013.748
17. Rodriguez JA, Huerta-Yepez S, Law IK, et al. Diminished expression of CRHR2 in human colon cancer promotes tumor growth and EMT via persistent IL-6/Stat3 signaling. *CMGH*. 2015;1(6):610–630. doi:10.1016/j.jcmgh.2015.08.001
18. Belluco C, Nitti D, Frantz M, et al. Interleukin-6 blood level is associated with circulating carcinoembryonic antigen and prognosis in patients with colorectal cancer. *Ann Surg Oncol*. 2000;7(2):133–138. doi:10.1007/s10434-000-0133-7
19. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. *Ca A Cancer J Clinicians*. 2022;72(1):7–33. doi:10.3322/caac.21708
20. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. *Ca A Cancer J Clinicians*. 2015;65(1):5–29. doi:10.3322/caac.21254
21. Riihimäki M, Hemminki A, Sundquist J, Hemminki K. Patterns of metastasis in colon and rectal cancer. *Sci Rep*. 2016;6(1):29765. doi:10.1038/srep29765
22. Han JH, Lee KY, Kim NK, Min BS. Metachronous metastasis confined to isolated lymph node after curative treatment of colorectal cancer. *Int J Colorecl Dis*. 2020;35(11):2089–2097. doi:10.1007/s00384-020-03695-8
23. Nagtegaal ID, Schmoll HJ. Colorectal cancer: what is the role of lymph node metastases in the progression of colorectal cancer? *Nat Rev Gastroenterol Hepatol*. 2017;14(11):633–634. doi:10.1038/nrgastro.2017.122
24. Peltonen R, Gramkow MH, Dehlendorff C, Osterlund PJ, Johansen JS, Isoniemi H. Elevated serum YKL-40, IL-6, CRP, CEA, and CA19-9 combined as a prognostic biomarker panel after resection of colorectal liver metastases. *PLoS One*. 2020;15(8):e0236569. doi:10.1371/journal.pone.0236569
25. Li YL, Hung WC. Reprogramming of sentinel lymph node microenvironment during tumor metastasis. *J Biomed Sci*. 2022;29(1):84. doi:10.1186/s12929-022-00868-1
26. Gao X, Sui H, Zhao S, Gao X, Su Y, Qu P. Immunotherapy targeting Myeloid-Derived Suppressor Cells (MDSCs) in tumor microenvironment. *Front Immunol*. 2021;11:585214. doi:10.3389/fimmu.2020.585214
27. Li L, Yu R, Cai T, et al. Effects of immune cells and cytokines on inflammation and immunosuppression in the tumor microenvironment. *Int Immunopharmacol*. 2020;88:106939. doi:10.1016/j.intimp.2020.106939
28. Wu Q, You L, Nepovimova E, et al. Hypoxia-inducible factors: master regulators of hypoxic tumor immune escape. *J Hematol Oncol*. 2022;15(1):77. doi:10.1186/s13045-022-01292-6

29. Sharma BR, Karki R, Sundaram B, Wang Y, Vogel P, Kanneganti T. The transcription factor IRF9 promotes colorectal cancer via modulating the IL-6/STAT3 signaling axis. *Cancers*. 2022;14(4):919. doi:10.3390/cancers14040919
30. Taniguchi K, Karin M. IL-6 and related cytokines as the critical lynchpins between inflammation and cancer. *Semin Immunopathol*. 2014;26 (1):54–74. doi:10.1016/j.smim.2014.01.001
31. Taher MY, Davies DM, Maher J. The role of the interleukin (IL)-6/IL-6 receptor axis in cancer. *Biochem Soc Trans*. 2018;46(6):1449–1462. doi:10.1042/BST20180136
32. Huang Y, Chen H-K, Hsu Y, et al. Src-fak signaling mediates interleukin 6-induced HCT116 colorectal cancer epithelial–mesenchymal transition. *Int J Mol Sci*. 2023;24(7):6650. doi:10.3390/ijms24076650
33. Tong F, Dai L-J, Siyu W, Xiao Y, Ding M, Jiang Y. Spatial architecture of the immune microenvironment orchestrates tumor immunity and therapeutic response. *J Hematol Oncol*. 2021;14(1). doi:10.1186/s13045-021-01103-4
34. Borowczak J, Szczerbowski K, Maniewski M, et al. The role of inflammatory cytokines in the pathogenesis of colorectal carcinoma—recent findings and review. *Biomedicines*. 2022;10(7):1670. doi:10.3390/biomedicines10071670