Correlation Between the Glasgow Prognostic Score and the Serum Cytokine Profile in Taiwanese Patients with Colorectal Cancer

Yen-Lin Yu1, Chung-Wei Fan1, Wen-Ko Tseng1, Pei-Hung Chang2, Hsuan-Chih Kuo2, Yi-Ping Pan3 and Kun-Yun Yeh2

Abstract
Background: The Glasgow Prognostic Score and circulating cytokine levels are related to the prognosis of colorectal cancer and the severity of chronic inflammation. The association between the Glasgow Prognostic Score and circulating cytokines in colorectal cancer remains unclear.

Methods: The levels of 10 circulating cytokines (TNF-α, TGF-β, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13, and IL-23) were measured in 128 patients with colorectal cancer. The relationship between the Glasgow Prognostic Score, clinicopathologic variables, and cytokine levels was assessed by univariate and multivariate logistic regression analyses. The correlation among cytokines was also examined.

Results: Patients with advanced stage colorectal cancer had lower levels of albumin (P = 0.003), higher levels of C-reactive protein (CRP; P < 0.001), carcinoembryonic antigen (CEA; P < 0.001), interferon (IFN)-γ (P < 0.001), and interleukin (IL)-10 (P = 0.006), and shorter survival outcomes (P < 0.001). Patients with a high Glasgow Prognostic Score (1 or 2) had lower 5-year progression-free survival and poor overall survival (log-rank P < 0.001). A high Glasgow Prognostic Score was significantly correlated with abnormal CEA levels (CEA > 5 ng/mL, P = 0.033), and higher levels of tumor necrosis factor (TNF-α) (TNF-α ≥ 53.9 pg/mL, P = 0.035) and IL-10 (IL-10 ≥ 75.95 pg/mL, P = 0.008). TNF-α, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-13, and IL-23 were significantly correlated with each other (all P < 0.05). Only IL-10 was correlated with abnormal CEA levels (P < 0.001).

Conclusion: The Glasgow Prognostic Score and level of circulating cytokines have an intergroup correlation, and there is a close association among cytokines in colorectal cancer.

Keywords
Colorectal cancer, Glasgow Prognostic Score, tumor necrosis factor-α, interleukin-10, carcinoembryonic antigen, cytokines

Introduction
Colorectal cancer (CRC) is the third most common cancer and the second most common cause of cancer death worldwide. In 2018, approximately 1.8 million people were diagnosed with CRC, with 0.86 million deaths.1 Only 2%–5% of patients with CRC are diagnosed with inherited syndromes, such as familial adenomatous polyposis, hereditary non-polyposis CRC, and hamartomatous polyposis syndrome,2 whereas most CRC develops from environmental and non-hereditary etiologies. Both inherited and
non-inherited CRC share a common pathogenesis of cancer development: chronic inflammation.1

During the inflammatory response, mediators such as cytokines, reactive oxygen species, and reactive nitrogen species may lead to gene mutation, affect nucleic acid repair mechanisms, block apoptosis, and alter the growth of cancer cells, all of which can facilitate cancer cell proliferation and spreading.3,4 CRC is considered the best example to delineate the relationship between chronic inflammation and cancer development. Patients with inflammatory bowel disease have an increased risk of CRC.5 In contrast, the anti-inflammatory approaches, including the use of non-steroidal anti-inflammatory drugs, may reduce the risk of CRC.6

The Glasgow Prognostic Score (GPS), an inflammation-based model that combines the levels of serum C-reactive protein (CRP) and albumin, reflects the systematic inflammatory and nutritional status of patients with various types of cancer.7 The pretreatment GPS is associated with prognostic outcomes and can be used as a biomarker for cancer management in patients with CRC.8 A meta-analysis assessing the relationship between the GPS and survival outcomes of 9839 patients with CRC reported that an elevated GPS was associated with poor overall survival and cancer-specific survival.8 To further clarify, CRP produced by hepatocytes is a biomarker for the inflammatory response and can be applied to predict the incidence and survival of patients with CRC.9,10 Likewise, the serum albumin level represents nutritional status, acts as an acute-phase protein that is affected by inflammation stimuli,11 and has a positive correlation with the long-term survival of patients with CRC.12

In addition to acute-phase proteins such as CRP and albumin, the other hallmark of chronic inflammation in cancer is an increased production of circulating cytokines.4 Recently, accumulating evidence has shown that circulating cytokines act through a range of inflammatory pathways that potentially impact CRC pathogenesis. Indeed, some of these pathways have been identified as independent prognostic factors in CRC, including interleukin (IL)-6, tumor necrosis factor-alpha (TNF-α), and IL-1β.5,13 A meta-analysis that enrolled 1622 CRC patients who underwent surgery showed that a high expression of transforming growth factor-beta (TGF-β) had a favorable outcome on overall survival.14 In addition, CRC patients with worse prognosis tend to have higher levels of circulating IL-10 than those with a better prognosis.15 Moreover, IL-23, which contributes to the progression of chronic inflammation by promoting the maturation and maintenance of T helper 17 cells,16 is gradually elevated with tumor stage progression in patients with CRC.17 Furthermore, since multiple cytokines have been shown to actively participate in promoting the growth and metastasis of CRC, cytokine profiling has been applied to classify CRC patients into good and poor prognostic subgroups.18-20 IL-4, IL-12, IL-13, and interferon-gamma (IFN-γ) have been shown to be involved in these cytokine profiles.18-20 Taken together, measurement of circulating cytokines may contribute to prognostic prediction, the relationship between the GPS and circulating cytokines remains unclear. The aim of this study was to investigate the association between GPS and clinicopathologic features and nutritional index in Taiwanese patients with CRC. The following clinicopathologic features were examined: age, sex, tumor node metastasis (TNM) stage, tumor location, histologic differentiation, body mass index (BMI), hemoglobin (Hb), total lymphocyte count (TLC), carcinoembryonic antigen (CEA), 10 circulating cytokines (TNF-α, TGF-β, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13, IL-23), 5-year progression-free survival (PFS), and overall survival (OS). In addition, we attempted to decipher the correlations among the 10 circulating cytokines analyzed.

Material and methods

Patients

In this retrospective study, data were collected from 164 patients with CRC who underwent standard treatment at Keelung Branch of Chang Gung Memorial Hospital Taiwan, between January 2009 and December 2011. The patients’ clinical parameters and the results of clinical laboratory tests were obtained from the patients’ medical records, and included age, sex, TNM stage, tumor location, histologic differentiation, BMI, complete and differential blood count, CEA, albumin, CRP, 5-year PFS and OS. Thirty-six patients were excluded due to lack of CRP (32 patients) or albumin (5 patients) data. Finally, 128 patients were included in the analysis. The GPS was defined based on the presence of hypoalbuminemia (< 3.5 g/dL) and elevated CRP (> 10 mg/L) as follows: If both were abnormal, the score was 2; if either was abnormal, the score was 1; if neither was abnormal, the score was 0.21 Serum cytokines, including TNF-α, TGF-β, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-12, IL-13, and IL-23, were collected from patients before treatment. The serum samples were stored at −80°C in pyrogen-free plastic tubes until cytokine analysis by enzyme-linked immunosorbent assay. The serum cytokine concentrations were determined using a DuoSet ELISA Development Kit following the manufacturer’s instructions (R&D Systems, Minneapolis, MN, USA). The final concentrations were determined using a bioluminescence counter (Packard Instrument Co., Inc., Downers Grove, IL, USA). All blood samples were only thawed once and were assayed in triplicate.

Tumor staging was classified retrospectively according to the 7th edition of the American Joint Committee on
Cancer Staging System based on the findings of physical examination, routine laboratory tests, and computed tomography of the chest and abdomen. The diagnoses and treatment goals of all enrolled patients were reviewed and confirmed by the CRC committee at our hospital. The committee members included three colorectal surgeons, three medical oncologists, one general surgeon, one chest surgeon, one radiation oncologist, one radiologist, and one pathologist. The OS and 5-year PFS were determined as the time between the diagnosis date and mortality, and as the time between the date of diagnosis and the first evidence of cancer progression, respectively. The patients were continuously followed up until April 2019 or death, whichever occurred first. The study was approved by the Institutional Review Board (IRB) of Chang Gung Memorial Hospital in Taiwan (IRB number: 202001858B0).

**Data analysis**

Statistical analyses were performed using the Statistical Package for the Social Sciences version 26 (SPSS Inc., Chicago, IL, USA). The associations between categorical variables were examined using Pearson’s chi-square test. After assessment by the Kolmogorov–Smirnov normality test, the continuous variables were compared using analysis of variance (ANOVA) with Bonferroni adjustments or non-parametric statistics with the Kruskal–Wallis H test where appropriate. The optimal cutoff values were determined for continuous variables according to the values published in previous reports, including old age (> 65 years old),22 underweight (BMI < 18.5 kg/m²),23 anemia (Hb < 10 g/dL),24 malnutrition status (albumin < 3.5 g/dL and TLC < 1,500 cells/mm³)25,26 elevated inflammatory status (CRP > 10 mg/L),27 and abnormal CEA level (CEA > 5 ng/mL),28 or determined according to the Youden’s index based on receiver operating characteristic (ROC) curve analysis for serum cytokine levels. If the optimal cutoff values of the serum cytokines could not be acquired by ROC curve analysis, the median level of each cytokine was applied for stratification in the study. The associations between survival outcomes and clinicopathological characteristics (age, sex, TNM stage, tumor location, histologic differentiation), GPS, BMI, Hb, TLC, CEA, 10 circulating cytokines studied, and treatment setting were analyzed using Cox proportional hazard models. Forward stepwise selection was used in the univariate and multivariate analyses for different variables. All independent variables significantly associated with 5-year PFS and OS (P = 0.05) in the univariate analysis were included in the multivariate analysis (Supplementary Table 1). Variance inflation factors were used to test for variables collinearity. The Kaplan–Meier method and log-rank test were used to analyze 5-year PFS and OS. In Table 2, the variables with statistical significance (P < 0.05) in the univariate analysis were entered into a multivariate logistic regression to identify the independent variables associated with GPS in patients with CRC. Cytoscape, an open-source software platform for creating a two-dimensional visualization of the relationships between GPS, CEA, and serum cytokines was used, utilizing a preface force-directed algorithm weighted by the statistical significance of the correlations between individual variables.29 P-values < 0.05 were considered statistically significant.

**Results**

The clinical characteristics of the 128 patients with CRC are shown in Table 1. The study population comprised 82 (64.1%) males and 46 (35.9%) females, with a mean age of 65.52 years (range, 18–94). Twenty-eight (21.8%) patients had stage I CRC, 34 (26.5%) had stage II, 44 (34.3%) had stage III, and 22 (17.1%) had stage IV. The tumor was located in the colon in 87 cases (67.9%) and in the rectum in 41 cases (32.1%). In all patients, the histology indicated adenocarcinoma. The histologic grade was assessed according to the criteria of the World Health Organization; 40 patients (31.2%) had well-differentiated disease, 79 (61.7%) had moderately differentiated disease, and 9 (7.0%) had poorly differentiated disease. All patients (100%) received standard surgery to remove the primary tumor and regional lymph nodes. Two (7.1%) stage I patients, 21 (61.8%) stage II patients, and 26 (56.1%) stage III patients had adjuvant chemotherapy; 18 (40.9%) stage III rectal cancer patients had adjuvant chemoradiotherapy. For stage IV patients, 13 (59.1%) patients had palliative chemotherapy, 5 (22.7%) had palliative chemoradiotherapy, and 4 (18.2%) had no postoperative treatment.

Patients were stratified according to stage; advanced stage was associated with hypoalbuminemia (P = 0.003), higher levels of CRP (P < 0.001), CEA (P < 0.001), IFN-γ (P < 0.001), and IL-10 (P = 0.006), as well as shorter 5-year PFS (P < 0.001) and OS (P < 0.001). There was no statistical difference in sex, age, tumor location, histologic differentiation, BMI, Hb, TLC, TNF-α, TGF-β, IL-1β, IL-4, IL-6, IL-12, IL-13, and IL-23 levels among the various tumor stages (Table 1 and Figure 1).

Patients were next classified into two groups according to GPS: 47 patients with a GPS of 0 (low GPS group; 36.71%) and 81 patients with a GPS of 1 or 2 (high GPS group; 63.29%). The high GPS group had higher levels of IFN-γ (P < 0.05), and IL-10 (P < 0.05) (Figure 2). When we investigated the cutoff values for serum cytokines using ROC curve analysis, only three cytokines achieved the optimal cutoff values: TNF-α (53.9 pg/mL; area under curve (AUC): 0.624; P = 0.046), IFN-γ (35 pg/mL; AUC: 0.643; P = 0.007) and IL-10 (75.9 pg/mL; AUC: 0.678; P = 0.001). Consequently, we arbitrarily stratified patients according to the median level as a cutoff value in other cytokines for analysis (TGF-β: median, 2896 pg/mL; IL-1β: 14.3 pg/mL; IL-4: 8.8 pg/mL; IL-6: 6.2 pg/mL; IL-12: 336.2 pg/mL; IL-13: 301.2 pg/mL; and IL-23: 51.3 pg/mL).
| Variables expressed as number (%) or mean ± SD (median) | All | Stage I | Stage II | Stage III | Stage IV | P-value |
|--------------------------------------------------------|-----|---------|---------|---------|---------|---------|
| Patient number                                         | 128 (100) | 28 (21.8) | 34 (26.6) | 44 (34.8) | 22 (16.8) |          |
| Sex (male/female)                                      | 82 (64.0)/46.36.0 | 21 (75.0)/25.0 | 20 (58.9)/41.1 | 28 (63.6)/16.36.4 | 13 (59.0)/41.0 | 0.552a |
| Age (median)                                           | 65.5 ± 13.6 (67.0) | 62.4 ± 8.6 (63.0) | 68.6 ± 15.1 (70.0) | 65.1 ± 14.8 (70.0) | 65.4 ± 13.6 (67.0) | 0.150a  |
| Location (colon:rectum)                               | 87 (67.9)/41.32.1 | 21 (75.0)/25.0 | 28 (82.3)/6.17.7 | 26 (59.1)/18 (40.9) | 21 (95.4)/4.6 | 0.063a  |
| Histologic differentiation (well:moderate:poor)        | 40 (31.2)/76 (91.7)/9(7.1) | 14 (50.0)/14 (50.0)/0 (0.0) | 10 (29.4)/22 (64.7)/2 (5.9) | 12 (72.7)/28 (63.6)/4.9 | 2 (18.2)/15 (68.2)/3 (13.6) | 0.167a  |
| BMI (kg/m²)                                            | 23.1 ± 4.1 (23.2) | 23.7 ± 3.9 (24.5) | 23.2 ± 4.8 (23.0) | 23.1 ± 4.1 (23.4) | 22.0 ± 3.7 (21.4) | 0.560a  |
| Hb (g/dL)                                              | 11.9 ± 1.8 (11.8) | 12.4 ± 3.13.12.6 | 11.4 ± 1.7 (11.1) | 12.0 ± 1.9 (12.1) | 11.7 ± 1.3 (11.9) | 0.211a  |
| TLC (cells/mm³)                                        | 1341 ± 678 (1088) | 1453 ± 703 (1176) | 1321 ± 607 (1113) | 1377 ± 685 (1101) | 1160 ± 740 (734) | 0.482a  |
| Albumin (g/dL)                                         | 3.6 ± 0.7 (3.7) | 3.9 ± 0.4 (4.0) | 3.4 ± 0.9 (3.5) | 3.6 ± 0.5 (3.8) | 3.2 ± 0.6 (3.4) | 0.003a  |
| CRP (mg/L)                                             | 26.2 ± 52.1 (5.9) | 2.4 ± 2.2 (2.0) | 7.3 ± 10.9 (10.3) | 8.2 ± 10.5 (6.0) | 3.442 ± 1427 (23.4) | <0.001a  |
| GPS (0:1:2)                                            | 47 (36.7)/43 (33.6)/38 (29.7) | 18 (62.1)/9 (20.9)/2 (6.9) | 10 (29.4)/12 (35.3)/12 (35.3) | 17 (39.5)/13 (30.2)/13 (30.2) | 2 (9.1)/9 (40.9)/11 (50.0) | 0.004 |
| CEA (ng/mL)                                            | 64.4 ± 594.3 (3.3) | 2.4 ± 2.1 (1.4) | 7.3 ± 10.9 (3.2) | 8.2 ± 10.5 (2.9) | 3.442 ± 1427 (15.9) | <0.001a  |
| TGF-β (pg/mL)                                          | 81.8 ± 86.5 (67.8) | 65.9 ± 61.6 (64.3) | 105.8 ± 157.8 (73.5) | 80.2 ± 44.5 (67.8) | 68.0 ± 16.5 (66.2) | 0.114a  |
| IFN-γ (pg/mL)                                          | 3773 ± 2547 (2896) | 3456 ± 2745 (2516) | 3659 ± 1986 (3332) | 3834 ± 2861 (2806) | 4233 ± 2490 (3194) | 0.746a  |
| IL-1 α (pg/mL)                                         | 12.2 ± 19.8 (8.8) | 9.5 ± 6.7 (6.0) | 17.3 ± 32.9 (9.3) | 12.2 ± 13.7 (9.9) | 7.9 ± 5.5 (9.9) | 0.291a  |
| IL-6 (pg/mL)                                           | 126 ± 27.4 (62.6) | 6.7 ± 3.3 (5.8) | 17.5 ± 45.8 (6.0) | 10.3 ± 15.2 (6.7) | 17.0 ± 24.9 (8.5) | 0.734a  |
| IL-10 (pg/mL)                                          | 2090 ± 633.5 (76.3) | 787 ± 75.0 (50.3) | 3167 ± 1070.9 (81.6) | 2568 ± 521.7 (72.1) | 1128 ± 692 (91.6) | 0.006<sup>a</sup> |
| IL-12 (pg/mL)                                          | 3267 ± 1199.3 (366.2) | 3385 ± 1365.3 (393.3) | 3397 ± 1265.3 (301.1) | 3212 ± 1153.3 (344.0) | 3022 ± 974.3 (325.5) | 0.646<sup>a</sup> |
| IL-13 (pg/mL)                                          | 3987 ± 677.6 (301.2) | 2748 ± 71.1 (255.7) | 5413 ± 1249.5 (315.2) | 4104 ± 348.1 (315.2) | 3128 ± 867.8 (303.3) | 0.175<sup>a</sup> |
| IL-23 (pg/mL)                                          | 1057 ± 297.4 (51.3) | 679 ± 95.8 (36.2) | 1878 ± 544.4 (62.6) | 940 ± 140.6 (53.3) | 504 ± 21.7 (47.0) | 0.117<sup>a</sup> |
| Treatment modality                                     |       |       |       |       |       |       |
| Surgery                                                | 128 (100) | 28 (21.8) | 34 (26.6) | 44 (34.8) | 22 (16.8) | 0.999 |
| Adjuvant setting                                       |       |       |       |       |       |       |
| C/T                                                    | 49 (38.3) | 2 (7.1%) | 21 (61.8%) | 26 (59.1%) | 0 (0.0%) | <0.001<sup>a</sup> |
| C/T + R/T                                               | 18 (14.1%) | 0 (0.0%) | 0 (0.0%) | 18 (40.9%) | 0 (0.0%) | <0.001<sup>a</sup> |
| Palliative setting                                     |       |       |       |       |       |       |
| C/T                                                    | 13 (10.2%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 13 (59.1%) | <0.001<sup>a</sup> |
| C/T + R/T                                               | 5 (3.9%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 5 (22.7%) | <0.001<sup>a</sup> |
| No Adjuvant or palliative treatment                    | 4 (3.1%) | 0 (0.0%) | 0 (0.0%) | 0 (0.0%) | 4 (18.2%) | <0.001<sup>a</sup> |
| 5-year progression-free survival (%)                   | 72.7 | 96.4 | 94.1 | 72.7 | 9.1 | <0.001<sup>a</sup> |
| Overall survival (%)                                   | 74.2 | 92.9 | 94.1 | 75.0 | 18.2 | <0.001<sup>a</sup> |

The P-value was determined by *(ANOVA using Bonferroni adjustments (for BMI, Hb, TLC, TGF-β, IL-1, IL-4, and IL-12), *non-parametric statistics with Kruskal-Wallis H test (for age, albumin, CRP, CEA, TNF-α, IFN-γ, IL-6, IL-10, IL-13, and IL-23), *chi-square test (for sex, location, histologic differentiation, GPS, surgery, C/T, C/T + R/T, and no treatment), and *log rank test (for 5-year progression-free survival and overall survival) for multiple comparisons. CRC: Colorectal cancer; GPS: Glasgow Prognostic Score; C/T: chemotherapy; R/T: radiotherapy.

Prognostic Score: *P < 0.05.
To assess the role of GPS in survival outcome, we examined the interactive effect among clinical variables, GPS, and cytokines studied on 5-year PFS and OS rates. On univariate analysis, tumor location, tumor stage, GPS, BMI, CEA, and treatment setting showed significance for 5-year PFS; tumor stage, GPS, BMI, TLC, CEA, TNF-α, and treatment setting showed significance for OS (Supplementary Table 1). On multivariate analysis, tumor location \( (P = 0.014) \), tumor stage \( (P = 0.001) \), GPS \( (P = 0.026) \), BMI \( (P = 0.002) \), and treatment setting \( (P = 0.001) \) were independent prognostic factors for 5-year PFS; tumor stage \( (P < 0.001) \), GPS \( (P = 0.007) \), and treatment setting \( (P = 0.001) \) were independent prognostic factors for OS. (Supplementary Table 1). Patients with a high GPS (1 or 2) had lower 5-year PFS and poor OS (log-rank \( P < 0.001 \) ) (Supplementary Figure 1).

The results of univariate analysis demonstrated that abnormal CEA levels and high serum levels of TNF-α, IFN-γ, IL-6, and IL-10 had a probable association with high GPS (Table 2). Moreover, we found no association between high and low GPS in terms of sex, age, tumor location, histologic differentiation, BMI, Hb, TLC, TGF-β, IL-1β, IL-4, IL-12, IL-13, and IL-23. The multivariate logistic regression model after adjustment of the covariate effects demonstrated that in addition to CEA > 5 ng/mL, higher circulating TNF-α \( (\geq 53.9 \text{ pg/mL}) \) and IL-10 levels \( (\geq 75.95 \text{ pg/mL}) \) were independently correlated with high GPS (Table 2).

To facilitate the comprehension of correlation data, we utilized the Cytoscape software platform to create a two-dimensional visualization for a more detailed analysis of the interplay between cytokines, high GPS, and abnormal CEA. Figure 3 shows that multiple serum cytokines,

---

**Figure. 1.** Box plots show the concentrations of 10 cytokines stratified by TNM stage. X-axis, TNM stage; Y-axis, individual cytokine concentration (pg/mL); * denotes \( P < 0.05 \), considered significant between two groups. TNM: Tumor node metastasis.

**Figure. 2.** Box plots show the concentrations of ten cytokines stratified by GPS status. X-axis, GPS, 0 or GPS \( \geq 1 \); Y-axis, individual cytokine concentration (pg/mL); * denotes \( P < 0.05 \), considered significant between two groups. GPS: Glasgow Prognostic Score.
including TNF-α, IFN-γ, IL-1β, IL-4, IL-6, IL-10, IL-13, and IL-23, were significantly correlated with each other. TGF-β was only related to IL-12 but not to other cytokines. Abnormal CEA levels were only correlated with IL-10 levels ($P < 0.001$).

### Discussion

The present study demonstrated that abnormal CEA level ($>5$ ng/mL), and higher circulating TNF-α ($\geqslant 53.9$ pg/mL) and IL-10 ($\geqslant 75.95$ pg/mL) levels were independently correlated with high GPS after adjustment of the clinicopathologic parameters. In addition, a high GPS (1 or 2) was associated with shorter 5-year PFS and OS. Our finding partially supports the result of a previous study in that the GPS was positively correlated with the CEA level and survival outcomes, but was not associated with age, sex, tumor stage, and tumor differentiation. Our results further showed that at least two circulating cytokines (TNF-α and IL-10) affect the expression of either CRP or serum albumin, GPS parameters, in patients with CRC.

CRP can be regulated by IL-1β, IL-6, and TNF-α during the inflammatory response, and the CRP level is significantly correlated with IL-1β and IL-6 expression. Moreover, in animal models, high CRP concentration can

---

**Table 2.** Univariate and multivariate analyses of clinicopathologic features associated with a GPS $\geqslant 1$ in 128 patients with CRC.

| Variables                        | Univariate analysis | Multivariate analysis |
|----------------------------------|---------------------|-----------------------|
|                                  | Odds ratio (95% CI) | $P$-value             | Odds ratio (95% CI) | $P$-value |
| Sex (ref: female)                | 1.323 (0.619, 2.827) | 0.471                 | 2.580 (1.080, 6.162) | 0.033*    |
| Age (ref: $<65$ years)           | 1.348 (0.652, 2.784) | 0.421                 |                      |           |
| Location (ref: rectum)           | 1.179 (0.542, 2.564) | 0.679                 |                      |           |
| Differentiation (ref: well differentiated) | 1.685 (0.306, 9.272) | 0.549                 |                      |           |
| Tumor stage (ref: stage I and II) | 1.774 (0.858, 3.668) | 0.122                 |                      |           |
| BMI (ref: $<18.5$ kg/m²)         | 0.238 (0.051, 1.105) | 0.067                 |                      |           |
| Hb (ref: $<10$ g/dL)             | 0.592 (0.177, 1.977) | 0.394                 |                      |           |
| TLC (ref: $<1500$ cells/mm³)     | 0.644 (0.278, 1.493) | 0.305                 |                      |           |
| CEA (ref: $<5$ ng/mL)            | 3.610 (1.585, 8.222) | $<0.002*$             |                      |           |
| TNF-α (ref: $<53.9$ pg/mL)       | 4.485 (1.657, 12.139) | $0.003*$              | 3.178 (1.087, 9.290) | 0.035*    |
| TGF-β (ref: $<2896$ pg/mL)       | 0.623 (0.302, 1.286) | 0.201                 |                      |           |
| IFN-γ (ref: $<35$ pg/mL)         | 2.889 (1.374, 6.074) | $0.005*$              |                      |           |
| IL-1β (ref: $<14.3$ pg/mL)       | 0.855 (0.456, 1.919) | 0.935                 |                      |           |
| IL-4 (ref: $<8.8$ pg/mL)         | 1.109 (0.540, 2.277) | 0.779                 |                      |           |
| IL-6 (ref: $<6.2$ pg/mL)         | 2.319 (1.107, 4.860) | $0.026*$              |                      |           |
| IL-10 (ref: $<75.9$ pg/mL)       | 4.007 (1.854, 8.662) | $<0.001*$             | 3.017 (1.339, 6.799) | 0.008*    |
| IL-12 (ref: $<336.2$ pg/mL)      | 1.286 (0.626, 2.642) | 0.494                 |                      |           |
| IL-13 (ref: $<301.2$ pg/mL)      | 0.935 (0.456, 1.919) | 0.855                 |                      |           |
| IL-23 (ref: $<51.3$ pg/mL)       | 0.817 (0.398, 1.679) | 0.582                 |                      |           |

$P$-value was determined by binary logistic regression for univariate and multivariate analyses.

BMI: Body mass index; CEA: carcinoembryonic antigen; CI: confidence interval; CRC: colorectal cancer; GPS: Glasgow Prognostic Score; Hb: hemoglobin; IFN: interferon; IL: interleukin; TGF: transforming growth factor; TLC: total lymphocyte count; TNF: tumor necrosis factor.

* $P < 0.05$. 

---

**Figure 3.** Two-dimension visualization of the interrelationships between serum cytokine levels and Glasgow Prognostic Scores (GPS). Individual variables are represented by nodes, and their associations are represented by edges (connecting lines). Red solid edges indicate significant associations ($P < 0.05$) between two cytokine variables or between cytokine variables and GPS or CEA. Green dash edges indicate no correlation between two cytokines.

CEA: Carcinoembryonic antigen.
enhance IL-10 synthesis.\(^{33,34}\) Regarding the expression of serum albumin, Brenner et al.\(^{35}\) revealed that elevated circulating TNF-\(\alpha\) levels downregulate albumin gene transcription and decrease albumin production in the liver. Higher IL-10 levels are also reported to be associated with hypoalbuminemia in critically ill patients and those with Hodgkin lymphoma.\(^{36,37}\) In clinical settings, cancer cachexia, which is clinically manifested by fatigue, anorexia, weight loss, hypoalbuminemia, and decreased muscle mass/strength, is a practical scenario that reflects the relationship between circulating cytokines and the GPS. It is clinically manifested by fatigue, anorexia, weight loss, hypoalbuminemia, and decreased muscle mass/strength in cancer patients.\(^{38}\) Cachexia is triggered and aggravated by systemic inflammatory responses via multiple cytokine pathways, including TNF-\(\alpha\), IL-6, IL-1, and IFN-\(\gamma\).\(^{39}\) Cachexia is usually found in patients with CRC both at diagnosis and during treatment.\(^{40,41}\) Furthermore, the extent of increased CRP and lower serum albumin levels also reveals the severity of cachexia in a variety of cancers, including CRC.\(^{38}\) Taken together, these studies, including the current one, demonstrate that the GPS is indeed correlated with the expression of circulating cytokines in patients with CRC.

The other salient feature of the present study was the intimate and intricate network of circulating cytokines, which is related to high GPS and abnormal CEA (Figure 3). A few similar studies have been reported previously.\(^{20,42-44}\) Chen et al.\(^{20}\) retrospectively analyzed 1180 patients with CRC using the neutrophil-to-lymphocyte ratio (NLR) derived from the routine complete blood cell count with the differential as an indicator to evaluate the systemic inflammation response. They found that in addition to a reliable prognostic factor of CRC, a high NLR is correlated with at least 20 cytokines clustered into four categories: inflammatory cytokines (IL-6), angiogenic cytokines (IL-8), epidermal growth factor ligands, and vascular epidermal growth factor.\(^{20}\) Moreover, a prospective study conducted in Finland examined the expression of circulating matrix metalloproteinase-8 (MMP-8) released from neutrophils under inflammatory stimuli in 271 patients with CRC. They found that MMP-8 is associated with modified GPS (mGPS) and NLR, and is a good indicator of systemic inflammation status and survival outcomes of patients with CRC. The MMP-8 level is closely associated with several circulating cytokines including IL-1 receptor antagonist (IL-1RA), IL-6, IL-7, and IL-8.\(^{43}\) Nonetheless, Sharma et al.\(^{45}\) analyzed 19 circulating cytokines in 52 patients with stage IV CRC and found no association between GPS and all studied cytokines, except that a GPS of 2 was correlated with elevated IL-6 levels compared to a GPS of 0 or 1. Park et al.\(^{28}\) also showed a weak association between a panel of circulating cytokines (TNF-\(\alpha\), IL-1\(\beta\), IL-6, IL-8, IL-9, and IL-10) and systemic inflammation markers such as the mGPS and the NLR. Moreover, Chang et al.\(^{46}\) measured three cytokines (TNF-\(\alpha\), IL-1\(\beta\), and IL-6) and incorporated all three into the cytokine score, which predicted increased tumor recurrence-free survival of patients with CRP < 5 mg/L than those with CRP > 5 mg/L. Although these reports imply that the relationship between cytokine and inflammatory status in patients with CRC is negligible or limited if present, the interpretation and application of the discrepant results should made with caution depending on the study design (retrospective, prospective, or meta-analysis), enrollment patient status (cancer stage and treatment modality), panels, and tools used for cytokine measurements (multiplex bead immunoassay, real-time polymerase chain reaction, or enzyme-linked immunosorbent assay).

Some intriguing observations and potential study limitations merit further discussion. The underlying mechanism by which IL-10 outperforms other cytokines in association with abnormal CEA levels remains unclear. Some studies have proposed that, in certain circumstances, CEA may preferentially stimulate specific cytokine production.\(^{47,48}\) Indeed, Jessup et al.\(^{47}\) observed that CEA has a tendency to stimulate secretion of IL-10, not IL6, and consequently promotes the short-term survival of weakly metastatic human CRC in an in vitro ischemic-reoxygenated liver model. Furthermore, Roselli et al.\(^{48}\) examined the blood levels of TNF-\(\alpha\), IL-1\(\beta\), IL-6, and CEA in 194 patients with CRC and found that TNF-\(\alpha\) levels were positively correlated with the presence of distant metastasis and CEA levels, but not IL1\(\beta\) or IL-6; these findings suggest that CEA plays a role in the induction of TNF-\(\alpha\) production in CRC. Additionally, the levels of TNF-\(\alpha\), IFN-\(\gamma\), and IL-10 were higher in patients with stage II and III tumors than those with stage IV in this study. However, statistical analysis found that—except for IFN-\(\gamma\) and IL-10—the levels of the other eight cytokines had no significant difference among varied stages (Figure 1). The levels of both IFN-\(\gamma\) and IL-10 had statistical difference between stage I, stage II, and stage III. We could not detect the difference between stage II, III, and IV, even though the levels of TNF-\(\alpha\), IFN-\(\gamma\), and IL-10 were seemingly higher in stage II and III. Further, our institution actively promoted occult blood stool tests for the early screening of CRC from 2007. All 22 (16.8%) stage IV patients were diagnosed with no obvious clinical symptoms, such as cachexia, body weight loss, or decreased BMI, so their paraneoplastic cytokine levels or related immune reactions may not be worse than those in non-metastatic CRC patients. Although most studies favor that the serum IL-6 level is increased with CRC stage,\(^{49}\) the correlation between serum levels of other cytokines and tumor stages remains equivocal.\(^{49,50}\) Taken together, the reasons why the levels of TNF-\(\alpha\), IFN-\(\gamma\), and IL-10 were seemingly higher
in patients with stage II and III CRC in our study could be attributed to the better medical status of stage IV patients. Another possibility, from our own perspective, is that not all cytokine levels are closely related to tumor stages. Also, we did not include RAS/RAF status, two of the major genes related to survival outcomes in CRC, into survival analysis since our data were collected and both RAS and RAF mutational tests were not standard requirements for the pathologic diagnosis of CRC in our institution during 2009–2011. Although Chen et al. observed that NLR is associated with the prognosis in metastatic CRC independent of RAS/RAF status, the interactive effect between GPS and RAS/RAF status on survival outcomes of patients with CRC needs further investigation. Lastly, the retrospective design is the major limitation of this study given that—by its nature—excludes patients with incomplete data, which has the potential to affect the data analysis. As we enrolled all of the study participants from a single medical institution, had a small sample size, and examined the relationship between GPS and 10 cytokines only with no normal healthy controls, the extrapolation of the current results to patients with CRC who have been assessed using other cytokine and inflammatory indicator panels remains a concern. To understand the entire landscape of the interaction between circulating mediators and inflammatory status, a prospective study with more patients and healthy people, which includes the assessment of a comprehensive panel of cytokines, growth factors, and chemokines involved in CRC pathogenesis is warranted.

Conclusions

Inflammatory status plays an important role in the development, severity, and prognosis of CRC. GPS, an inflammation-based score, is significantly related to the 5-year PFS, OS, abnormal CEA level, and higher levels of TNF-α and IL-10 levels in Taiwanese patients with CRC. There is a close association between circulating cytokines, some of which are related to high GPS and abnormal CEA levels.

Authors’ contributions

Conceptualization: Kun-YunYeh; data curation: Yen-Lin Yu, Chung-Wei Fan, Wen-Ko Tseng, Pei-Hung Chang, Hsuan-Chih Kuo, and Yi-Ping Pan; formal analysis: Yen-Lin Yu, Pei-Hung Chang, and Yi-Ping Pan; investigation: Chung-Wei Fan, Wen-Ko Tseng, Pei-Hung Chang, Hsuan-Chih Kuo, and Yi-Ping Pan; methodology: Kun-YunYeh; validation: Kun-YunYeh; original draft: Kun-YunYeh and Yen-Lin Yu; writing, review, and editing: Kun-YunYeh and Yen-Lin Yu.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was partially supported by grants (CMRPG2C0161, CMRPG290111, CMRPG2A0351 and CMRPG2A0352) from the Chung Gang Memorial Hospital, Keelung, Taiwan.

ORCID iDs

Yen-Lin Yu https://orcid.org/0000-0003-3909-1715
Kun-Yun Yeh https://orcid.org/0000-0001-5572-7897

Supplemental material

Supplemental material for this article is available online.

References

1. Bray F, Ferlay J, Soerjomataram I, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin 2018; 68: 394–424.
2. Ma H, Brosens LAA, Offerhaus GJA, et al. Pathology and genetics of hereditary colorectal cancer. Pathology 2018; 50: 49–59.
3. Terzic J, Grivennikov S, Karin E, et al. Inflammation and colon cancer. Gastroenterology 2010; 138: 2101–2114 e2105.
4. Tuomisto AE, Makinen MJ and Väyrynen JP. Systemic inflammation in colorectal cancer: Underlying factors, effects, and prognostic significance. World J Gastroenterol 2019; 25: 4383–4404.
5. Lukas M. Inflammatory bowel disease as a risk factor for colorectal cancer. Dig Dis 2010; 28: 619–624.
6. Kuo CN, Pan JJ, Huang YW, et al. Association between nonsteroidal anti-inflammatory drugs and colorectal cancer: a population-based case-control study. Cancer Epidemiol Biomarkers Prev 2018; 27: 737–745.
7. McMillan DC. The systemic inflammation-based Glasgow Prognostic Score: a decade of experience in patients with cancer. Cancer Treat Rev 2013; 39: 534–540.
8. Lu X, Guo W, Xu W, et al. Prognostic value of the Glasgow prognostic score in colorectal cancer: a meta-analysis of 9,839 patients. Cancer Manag Res 2019; 11: 229–249.
9. Goyal A, Terry MB, Jin Z, et al. C-reactive protein and colorectal cancer mortality in U.S. adults. Cancer Epidemiol Biomarkers Prev 2014; 23: 1609–1618.
10. Nozoe T, Mori E, Takahashi I, et al. Preoperative elevation of serum C-reactive protein as an independent prognostic indicator of colorectal carcinoma. Surg Today 2008; 38: 597–602.
11. Kim S, McClave SA, Martindale RG, et al. Hypoalbuminemia and clinical outcomes: what is the mechanism behind the relationship? Am Surg 2017; 83: 1220–1227.
12. Lai CC, You JF, Yeh CY, et al. Low preoperative serum albumin in colon cancer: a risk factor for poor outcome. Int J Colorectal Dis 2011; 26: 473–481.
13. Gunawardene A, Dennett E and Larsen P. Prognostic value of multiple cytokine analysis in colorectal cancer: a systematic review. J Gastrointest Oncol 2019; 10: 134–143.
14. Chen XL, Chen ZQ, Zhu SL, et al. Prognostic value of transforming growth factor-beta in patients with colorectal cancer who undergo surgery: a meta-analysis. *BMC Cancer* 2017; 17: 240.

15. Abtahi S, Davani F, Mojtahedi Z, et al. Dual association of serum interleukin-10 levels with colorectal cancer. *J Cancer Res Ther* 2017; 13: 252–256.

16. Langrish CL, Chen Y, Blumenschein WM, et al. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; 201: 233–240.

17. Yan G, Liu T, Yin L, et al. Levels of peripheral Th17 cells and serum Th17-related cytokines in patients with colorectal cancer: a meta-analysis. *Cell Mol Biol (Noisy-le-grand)* 2018; 64: 94–102.

18. Vayrynen JP, Kantola T, Vayrynen SA, et al. The relationships between serum cytokine levels and tumor infiltrating immune cells and their clinical significance in colorectal cancer. *Int J Cancer* 2016; 139: 112–121.

19. Chen ZY, He WZ, Peng LX, et al. A prognostic classifier consisting of 17 circulating cytokines is a novel predictor of overall survival for metastatic colorectal cancer patients. *Int J Cancer* 2015; 136: 584–592.

20. Chen ZY, Raghav K, Lieu CH, et al. Cytokine profile and prognostic significance of high neutrophil-lymphocyte ratio in colorectal cancer. *Br J Cancer* 2015; 112: 1088–1097.

21. Read JA, Choy ST, Beale PJ, et al. Evaluation of nutritional and inflammatory status of advanced colorectal cancer patients and its correlation with survival. *Nutr Cancer* 2006; 55: 78–85.

22. World Health Organization. Definition of an older or elderly person. www.who.int/healthinfo/survey/ageingdefnolder/en/ (2016, accessed 26 July 2016).

23. National Heart, Lung, and Blood Institute. Assessing your weight and health risk. www.nhlbi.nih.gov/health/educational/losewt/risk.htm (2012, accessed 2 April 2012).

24. Bohlius J, Weingart O, Trelle S, et al. Cancer-related anemia and recombinant human erythropoietin—an updated overview. *Nat Clin Pract Oncol* 2006; 3: 152–164.

25. Araki K, Ito Y, Fukada I, et al. Predictive impact of absolute lymphocyte counts for progression-free survival in human epidermal growth factor receptor 2-positive advanced breast cancer treated with pertuzumab and trastuzumab plus eribulin or nab-paclitaxel. *BMC Cancer* 2018; 18: 982.

26. Pan YP, Chang PH, Fan CW, et al. Relationship between pre-treatment nutritional status, serum glutamine, arginine levels and clinicopathological features in Taiwan colorectal cancer patients. *Asia Pac J Clin Nutr* 2015; 24: 598–604.

27. Ghuman S, Van Hemelrijck M, Garmo H, et al. Serum inflammatory markers and colorectal cancer risk and survival. *Br J Cancer* 2017; 116: 1358–1365.

28. Park JW, Chang HJ, Yeo HY, et al. The relationships between systemic cytokine profiles and inflammatory markers in colorectal cancer and the prognostic significance of these parameters. *Br J Cancer* 2020; 123: 610–618.

29. Vayrynen JP, Tuomisto A, Klintrup K, et al. Detailed analysis of inflammatory cell infiltration in colorectal cancer. *Br J Cancer* 2013; 109: 1839–1847.

30. Kim IH, Lee JE, Yang JH, et al. Clinical significance of changes in systemic inflammatory markers and carcinoembryonic antigen levels in predicting metastatic colorectal cancer prognosis and chemotherapy response. *Asia Pac J Clin Oncol* 2018; 14: 239–246.

31. Choi KW, Hong SW, Chang YG, et al. Inflammation-based score (Glasgow prognostic score) as an independent prognostic factor in colorectal cancer patients. *Ann Surg Treat Res* 2014; 86: 309–313.

32. Miki C, Konishi N, Ojima E, et al. C-reactive protein as a prognostic variable that reflects uncontrolled up-regulation of the IL-1-IL-6 network system in colorectal carcinoma. *Dig Dis Sci* 2004; 49: 970–976.

33. Mold C, Rodriguez W, Rodic-Polic B, et al. C-reactive protein mediates protection from lipopolysaccharide through interactions with Fe gamma R. *J Immunol* 2002; 169: 7019–7025.

34. Szalai AJ, Nataf S, Hu XZ, et al. Experimental allergic encephalomyelitis is inhibited in transgenic mice expressing human C-reactive protein. *J Immunol* 2002; 168: 5792–5797.

35. Brenner DA, Buck M, Feitellberg SP, et al. Tumor necrosis factor-alpha inhibits albumin gene expression in a murine model of cachexia. *J Clin Investig* 1990; 85: 248–255.

36. Quispe EA, Li XM and Yi H. Comparison and relationship of thyroid hormones, IL-6, IL-10 and albumin as mortality predictors in case-mix critically ill patients. *Cytokine* 2016; 81: 94–100.

37. Sarris AH, Kliche KO, Pethambaram P, et al. Interleukin-10 levels are often elevated in serum of adults with Hodgkin’s disease and are associated with inferior failure-free survival. *Ann Oncol* 1999; 10: 433–440.

38. Fearon KC, Glass DJ and Guttridge DC. Cancer cachexia: mediators, signaling, and metabolic pathways. *Cell Metab* 2012; 16: 153–166.

39. Porporato PE. Understanding cachexia as a cancer metabolism syndrome. *Oncogenesis* 2016; 5: e200.

40. Shibata M, Fukahori M, Kasamatsu E, et al. A retrospective cohort study to investigate the incidence of cachexia during chemotherapy in patients with colorectal cancer. *Adv Ther* 2020; 37: 5010–5022.

41. Dewys WD, Begg C, Lavin PT, et al. Prognostic effect of weight loss prior to chemotherapy in cancer patients. Eastern Cooperative Oncology Group. *Am J Med* 1980; 69: 491–497.

42. Vayrynen JP, Vayrynen SA, Sirnio P, et al. Platelet count, aspirin use, and characteristics of host inflammatory responses in colorectal cancer. *Translat Med* 2019; 17: 199.

43. Sirnio P, Tuomisto A, Tervahartiala T, et al. High-serum MMP-8 levels are associated with decreased survival and systemic inflammation in colorectal cancer. *Br J Cancer* 2018; 119: 213–219.

44. Vayrynen JP, Tuomisto A, Vayrynen SA, et al. Preoperative anemia in colorectal cancer: relationships with tumor characteristics, systemic inflammation, and survival. *Sci Rep* 2018; 8: 1126.

45. Sharma R, Zucknick M, London R, et al. Systemic inflammatory response predicts prognosis in patients with advanced-stage colorectal cancer. *Clin Colorectal Cancer* 2008; 7: 331–337.

46. Chang PH, Pan YP, Fan CW, et al. Pretreatment serum interleukin-1beta, interleukin-6, and tumor necrosis factor-alpha levels predict the progression of colorectal cancer. *Cancer Med* 2016; 5: 426–433.
47. Jessup JM, Laguinge L, Lin S, et al. Carcinoembryonic antigen induction of IL-10 and IL-6 inhibits hepatic ischemic/reperfusion injury to colorectal carcinoma cells. *Int J Cancer* 2004; 111: 332–337.

48. Roselli M, Guadagni F, Martini F, et al. Association between serum carcinoembryonic antigen and endothelial cell adhesion molecules in colorectal cancer. *Oncology* 2003; 65: 132–138.

49. Lippitz BE and Harris RA. Cytokine patterns in cancer patients: A review of the correlation between interleukin 6 and prognosis. *Oncoimmunology* 2016; 11: e1093722.

50. Kim YW, Kim SK, Kim CS, et al. Association of serum and intratumoral cytokine profiles with tumor stage and neutrophil lymphocyte ratio in colorectal cancer. *Anticancer Res* 2014; 34: 3481–3488.