CLINICAL SIGNIFICANCE OF SERUM HEPATOCYTE GROWTH FACTOR (HGF) AND ITS RECEPTOR CMET LEVELS IN COLORECTAL CANCER PATIENTS

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ABSTRACT

Objective: The cMET receptor tyrosine kinase and its ligand hepatocyte growth factor (HGF) regulate many signaling pathways involved in proliferation and cell motility, invasion and angiogenesis. Deregulation of HGF/cMET system by different biological mechanisms may contribute to the tumor development in many types of cancers. Therefore, the present study was performed to investigate clinical significance of serum patterns of both HGF and cMET in colorectal cancer (CRC) patients.

Materials and methods: One hundred and three CRC patients were enrolled in this study. Serum HGF and cMET levels were measured by the solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) method. Age- and sex-matched 30 healthy control patients were included in the analysis.

Results: The median age of the patients was 60 years old, range 24 to 84 years. Most of the tumor localization areas were colon (n= 57, 55%). Median follow-up time was 14 months. While thirty-one patients (30 %) experienced disease progression, twenty-three of the remaining patients (22 %) died because of the disease. The estimated 2-year overall (OS) and 1-year progression-free survival (PFS) rates for the whole patient groups were 70.1 % (95% confidence interval (CI) = 57.2-83.0) and 23.3 % (95% CI = 8.2-38.4), respectively. The baseline median serum HGF and cMET levels were significantly higher in metastatic CRC patients than in the healthy control group (p<0.001). Furthermore, worse performance status and metastatic disease were associated with higher serum HGF concentrations all patients with CRC (p=0.03 and p=0.03, respectively). Clinical variables including metastatic disease, greater pathologic tumor status, and colonic site were found to be correlated with higher serum cMET concentrations all patients with CRC (p=0.01, p=0.05, and p=0.04, respectively). A correlation was determined between HGF and cMET levels in metastatic CRC patients (rs=0.286, n=47, and p=0.05), (Spearman’s correlation). Our study results did not show a statistically significant serum HGF and cMET concentrations regarding PFS and OS.

Conclusion: Serum levels of HGF and cMET may be diagnostic markers in CRC patients. However, their predictive and prognostic values were not determined.

Key words: HGF, cMET, serum, diagnostic, colorectal cancer.

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Introduction

Colorectal cancer (CRC) is the third most common malignancy and the second leading cause of cancer deaths in the western world, with an ever increasing global incidence(1). cMET is a proto-oncogene that encodes for the receptor tyrosine kinase, also known as hepatocyte growth factor receptor (HGF)(2).

The only known ligand for cMET is HGF; both cMET and HGF are upregulated in a number of malignancies and are associated with a poor prognosis and an early predictor of further metastasis(3). Specifically, cMET is involved in the regulation of proliferation, motility, invasion and metastasis via its phosphorylation and activation of downstream signaling pathways(4). The expression of cMET and its ligand HGF play a critical role in cell
proliferation and are involved in numerous malignancies\(^6\).

Furthermore, expression of both proteins have been correlated with a shorter patient survival period in CRC\(^5\), and another study showed that cMET activation is associated with an increase in CRC metastasis\(^6\). In CRC, MET is considered important for the metastatic potential to the liver and represents a powerful prognostic indicator for early stage invasion and metastasis; high expression of MET in CRC associates with development of distant metastases and with shorter metastasis-free survival\(^7\).

Elevated serum HGF level demonstrated a significant association with poor survival, and was only an independent risk factor for poor survival in Stage II and/or III CRC patients. Elevated serum HGF level is significantly associated with CRC development, lymphatic or distant invasive phenotypes and survival, especially in Stage II or III patients\(^8\).

Increasing evidence indicates that MET, the tyrosine kinase receptor for HGF, is frequently implicated in resistance to EGFR-targeted therapies, including EGFR tyrosine kinase inhibitors and EGFR antibodies\(^9\)-\(^11\).

A recent study has demonstrated that HGF-dependent MET activation contributes to cetuximab resistance in colon cancer\(^12\).

The HGF/cMET signaling pathway may be involved in the pathogenesis and progression of colon cancer. cMET overexpression can be used as a useful parameter to evaluate the prognosis of colon cancer\(^13\).

Our aim in this study was to evaluate the clinical significance of serum patterns of both HGF and cMET in CRC patients.

**Materials and methods**

**Study design and eligibility criteria**

The serum samples of the 103 consecutive patients who referred to Istanbul University, Institute of Oncology and Bakirkoy Dr. Sadi Konuk Training and Research Hospital from 2011 to 2014 were obtained. All patients were staged using seventh edition of the American Joint Committee on Cancer Tumor-Node-Metastasis systems by radiologic and pathologic basis.

All of the patients were treated with multidisciplinary approach. Patients with colon cancer who were undergone surgery including segmental colon resection were treated with adjuvant chemotherapy (CTx) according to their stages. Patients with rectum cancer who received neoadjuvant radiochemotherapy (RCTx) or radiotherapy (RT), were undergone low anterior resection or abdomino-perineal resection. Some patients were undergone palliative surgery and stage IV patients received palliative CTx with or without targeted therapy (bevacizumab or cetuximab). The pretreatment evaluation included detailed clinical history and physical examination with a series of biochemistry tests and complete blood cell counts. Selection for treatment required an Eastern Cooperative Oncology Group (ECOG) performance score (PS) of 0-2, and appropriate bone marrow (absolute neutrophil count > 1500/µL, and platelet count > 100,000/µL), cardiac, renal and hepatic function. Patients were treated with various CTx regimens including single agent or combination therapy. Regimens of single or combination CTx were selected according to the PS of patients and extension of disease.

Patients received one of the following treatment regimens: simplified LV5FU2 (leucovorin 400 mg/m², followed by 5-fluorouracil as a 400 mg/m² bolus and a 2400 mg/m² infusion over 46 hours every 2 weeks), capecitabine (1000 mg/m²/b.i.d., p.o. for 14 days of each 21-day cycle), modified FOLFOX regimen (simplified LV5FU2 regimen plus oxaliplatin 85 mg/m² every 2 weeks), FOLFIRI (simplified LV5FU2 regimen plus irinotecan 180 mg/m² every 2 weeks), XELOX (capecitabine 1000 mg/m²/b.i.d., p.o. for 14 days plus oxaliplatin 130 mg/m² every 3 weeks), or XELIRI (capecitabine 1000 mg/m²/b.i.d., p.o. for 14 days plus irinotecan 240 mg/m² every 3 weeks). Bevacizumab was given at a dose schedule of of either 5 mg/kg every 2 weeks or 7.5 mg/kg every 3 weeks. Cetuximab 500 mg/m² was administered intravenously every 2 weeks.

All patients had pretreatment imaging of primary tumors with magnetic resonance imaging (MRI) or computed tomography (CT). For patients with evaluable imaging studies before and after treatment, radiologic response was recorded according to Response Evaluation Criteria in Solid Tumors v. 1.1, and classified as follows: complete response (CR), partial response (PR), stable disease (SD), or progressive disease (PD). The tumor response after 2 months of CTx was used for statistical analysis. Follow-up programs of metastatic disease consisted of clinical, laboratory, and using a CT or MRI depending on which imaging methods were utilized.
were used at baseline and performed at 8-week intervals during CTx or every 12 weeks for no anti-cancer treatment. Patients with either CR or PR were classified as responders, and patients with SD or PD were considered non-responders.

The study was approved by the Institutional Review Board (IRB) of Istanbul University, Institute of Oncology. Baseline demographic, clinical, and laboratory data including age, gender, PS, tumor marker levels, KRAS mutation status, and treatment details were collected retrospectively for all patients using uniform database templates to ensure consistent data collection. The comorbid diseases of patients were cardiac and metabolic diseases.

The control group consisted of age- and sex-matched 30 healthy women with no previous history of malignancy or autoimmune disorders. Serum samples were obtained on the first admission before any adjuvant and metastatic treatment was given or follow-up patients. Blood samples of healthy controls were taken into dry tubes and sera separated from cellular elements by centrifugation (at 4000 rpm for 10 minutes) within half an hour after blood samples were stored at -80°C until analysis. All of the samples were collected under the approval of the IRB and with adequate informed consents.

**Measurement of serum HGF and cMET levels**

HGF and cMET, enzyme-linked immunosorbent assay (ELISA) (Shanghai Yehua Biological Technology Co., Ltd) uses a double-antibody sandwich ELISA to determine the level of Human HGF and cMET in samples. Serum samples and standards are added to the wells which are pre-coated with Human HGF and cMET monoclonal antibody, Streptavidin-HRP are added to form immune complex and allowed to incubate 37°C for one hour. Unbound material is washed away. Chromogen solution is added and incubate 37°C for 10 minutes (protect from light) for the conversion of the colorless solution to a blue solution, the intensity of which is proportional to the amount of HGF and cMET in the sample. As the effect of the acidic stop solution, the color has become yellow. The colored reaction product is measured using an automated ELISA reader (ChroMate® 4300 microplate awareness technology) at 450 nm. The results were expressed as ng/L.

**Statistical analysis**

Statistical Package for the Social Sciences (SPSS) for Windows version 21.0 (SPSS Inc., Chicago, IL., USA) was employed for data analysis. Continuous variables were categorized using median values as cut-off point. For group comparison of categorical variables, Chi-square tests or One-Way Anova tests were used and for comparison of continuous variables, Mann–Whitney U test or Kruskall-Wallis tests was accomplished. Spearman’s rank order correlation was used for correlation analysis. Progression-free survival (PFS) was calculated from the date of admission to the date of first radiologic progression with/without elevated serum tumor marker. Overall survival (OS) was calculated from the date of first admission to the clinics to disease-related death or date of last contact with the patient or any family member. Kaplan-Meier method was used for estimation of survival distribution and differences in PFS and OS were assessed by the log-rank statistics. All statistical tests were carried out two-sided and a p value ≤0.05 was considered statistically significant.

**Results**

One hundred and three patients who were pathologically diagnosed as CRC from May 2011 to August 2014 were included in the current study. Baseline demographic features and histopathological/laboratory characteristics of patients are listed in Table 1.

Thirty-one patients had family history of cancer including 8 lung cancers and 10 CRC. Median age at diagnosis was 60 years old, range 24 to 84 years, where male patients constituted majority of the group (n=72, 70%). The cancer localization was rectum in 45% (n=46) and colon in 55% (n=57) of patients (right colon; n=12, hepatic flexura; n=3 transvers colon; n=4, descendont colon; n=9, sigmoid colon; n=27, multipl colon synchronous tumor; n=2, rectosigmoid junction tumor; n=2, rectum; n=44). The most frequent metastatic sites were liver (n=32, 68.1%) and peritoneum (n=6, 12.7%) in 47 patients with metastasis. Synchronous metastasis was observed in 28 patients (59.6%) while metachronous metastasis was detected in 19 patients (40.4%). Of the 31 patients who had neoadjuvant treatment with rectal cancer, 22 were applied fluoropyrimidine-based RCTx while the remaining 9 received short-course RT. Fifty-two patients who had adjuvant CTx received one of the following treatment regimens: simplified LV5FU2/capecitabine (n=10), mFOLFOX regimen (n=22), or XELOX (n=20). Palliative CTx were
preferred oxaliplatin-based, irinotecan-based combination CTx regimens and single agent fluoropyrimidine in 16, 20, and 6 patients, respectively. Bevacizumab were given to 26 patients whereas 14 patients had cetuximab as targeted agents Thirty-one percent of 42 metastatic patients who received palliative CTx were CTx-responsive.

The levels of the whole group serum HGF and cMET in CRC patients and healthy controls are shown in Table 2.

![Table 2](image)

### Table 2: The values of serum cMET and HGF levels in CRC patients and healthy controls.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>cMET level (ng/L)</th>
<th>HGF level (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Median (range)</td>
<td>Median (range)</td>
</tr>
<tr>
<td>All patients</td>
<td>103</td>
<td>499.26 (228.84-1473.38)</td>
<td>3361.31 (996.40-7525.48)</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>375.78 (165.69-919.02)</td>
<td>3050.00 (796.56-7462.96)</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>0.03**</td>
<td>0.002**</td>
</tr>
<tr>
<td>Non-metastatic patients*</td>
<td>36</td>
<td>362.56 (265.64-1070.40)</td>
<td>2981.94 (1298.46-7217.67)</td>
</tr>
<tr>
<td>Controls</td>
<td>30</td>
<td>375.78 (165.69-919.02)</td>
<td>3050.00 (796.56-7462.96)</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>0.56</td>
<td>0.07</td>
</tr>
<tr>
<td>Metastatic patients</td>
<td>47</td>
<td>652.01 (275.99-1473.38)</td>
<td>4194.31 (996.40-7525.48)</td>
</tr>
<tr>
<td>Controls</td>
<td>20</td>
<td>375.78 (165.69-919.02)</td>
<td>3050.00 (796.56-7462.96)</td>
</tr>
<tr>
<td>F value</td>
<td></td>
<td>0.001**</td>
<td>0.001*</td>
</tr>
</tbody>
</table>

The median cMET levels were found to be significantly higher in all patients with CRC (469.26 ng/L) compared to healthy controls (357.78 ng/L), (p=0.03). Moreover, the median HGF levels were found to be significantly higher in all patients with CRC (3360.31 ng/L) compared to healthy controls (3036.03 ng/L), (p=0.002). The baseline serum cMET and HGF levels of the non-metastatic group (stage II and III) CRC patients were no significantly higher than the control group (362.56, 2981.94 ng/L, and p=0.56, p=0.07, respectively). The baseline serum cMET and HGF levels of the metastatic group CRC patients were significantly higher than the control group (652.01, 4194.31 ng/L, and p=0.001, p<0.001, respectively) (Fig. 1 and 2).

![Figure 1](image)

**Figure 1:** The values of serum cMET assays in all, non-metastatic (stage II or III), and metastatic CRC patients and controls (p=0.03, p=0.56, and p=0.001, respectively).

Table 3 and 4 shows the correlation between the serum levels cMET and HGF and clinico-pathological factors. Metastatic disease, greater pathologic tumor status (pT stage) and colonic site were found to be correlated with higher serum cMET.
concentrations all patients with CRC (p=0.01, p=0.05, and p=0.04, respectively). Alcohol intake was found to be correlated with higher serum cMET concentrations for metastatic patients (p=0.04). Greater pT stage was found to be correlated with higher serum cMET concentrations for non-metastatic patients (p=0.03 and p=0.03, respectively). Worse PS, smoking and alcohol intake were associated with higher serum HGF concentrations for metastatic CRC patients (p=0.05, p=0.01, and p=0.03, respectively). However, known clinical variables were not found to be correlated with serum HGF concentrations for non-metastatic CRC patients (p=0.05).

Figure 2: The values of serum HGF assays in all, non-metastatic (stage II or III), and metastatic CRC patients and controls (p=0.002, p=0.07, and p<0.001, respectively).

The correlation was found between serum cMET and HGF levels in all CRC patients (rs=0.183, n=103, p=0.04). The HGF was not found between serum cMET and HGF levels in non-metastatic CRC patients (n=56, p=0.90). The correlation was found between serum cMET and HGF levels in metastatic CRC patients (rs=0.194, n=47, p=0.05) (Fig. 3).

Median follow-up time was 14.0 months (range 1-33 months), while thirty-one patients (30%) experienced disease progression, twenty-three of the remaining patients (22%) died. Median PFS and OS of the whole group were 6.8 ± 1.0 months (95% CI=5-9 months) and 26.1 ± 1.3 months (95% CI = 24-29 months), respectively. While 1-year PFS rates were 23.3% (95% CI = 8.2-38.4), 1- and 2-year OS rates were 81.5% (95% CI = 73.7-89.3) and 70.1% (95% CI = 57.2-83.0), respectively. A significant relationship between other clinico-pathologic variables including worse PS (p=0.005), rectum involvement (p=0.003), no surgical resection (p=0.05), CTx-unresponsive (p=0.002), high serum levels carcinoembryonic antigen (CEA) (p=0.03), and carbohydrate antigen (CA) 19-9 (p=0.003) poorer PFS was determined (Table 5). Clinico-pathologic variables including localization of rectum (p=0.007), presence of metastasis (p<0.001), poor grade (p=0.002), no surgical resection (p<0.001), CTx-unresponsiveness (p=0.007), higher serum levels of lactate dehydrogenase (LDH) (p=0.01), CEA (p<0.001), and CA 19-9 (p<0.001) found to be correlated with poorer OS (Table 6).

Table 3: Results of comparisons between serum cMET assays and various demographic and disease characteristics.

*Stage II or III, ** p≤0.05, NR not reached, aa In 56 non-metastatic disease of patients, aaa In 47 patients with metastatic CRC, aaaa In 31 patients with rectal cancer who received neoadjuvant treatment
However, serum cMET levels showed no significantly adverse effect on PFS and OS (p>0.05). Moreover, neither metastatic nor non-metastatic CRC patients’ serum cMET levels showed significant adverse effect on PFS and OS (p>0.05).

**Discussion**

To date, we knew that HGF and its receptor cMET played an important role in tumor proliferation, invasion and metastasis. They have been...
investigated in various kinds of tumours and their role for potency to metastasis and their importance for treatment decision (14-17).

<table>
<thead>
<tr>
<th>Variables</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Age group, Tumor (15)</td>
<td>NS</td>
</tr>
<tr>
<td>Gender, Male</td>
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</tr>
<tr>
<td>T stage</td>
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<td>Chemotherapy, Yes</td>
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<tr>
<td>Surgery, Yes</td>
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</tr>
<tr>
<td>pT stage</td>
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<tr>
<td>pN stage</td>
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<tr>
<td>Metastasis, Yes*</td>
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<tr>
<td>Response to CTx, Yes (23, 24)</td>
<td>No (23, 24)</td>
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<td>Size of lesion, Cm, 5-6</td>
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<tr>
<td>Histology, Adenocarcinoma</td>
<td>Mucoadenoma</td>
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<tr>
<td>Regression score</td>
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<td>KRAS mutation status, Mutated</td>
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<td>LGI</td>
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<td>Staged, High</td>
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<tr>
<td>Adenoma, Normal</td>
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<tr>
<td>CEA, Normal</td>
<td>High</td>
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<tr>
<td>CA19-9, Normal</td>
<td>High</td>
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<tr>
<td>AMET total patients</td>
<td>Low</td>
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<tr>
<td>AMET of non-metastatic patients*</td>
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<tr>
<td>AMET of metastatic patients*</td>
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<tr>
<td>HGF total patients</td>
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<td>HGF of non-metastatic patients*</td>
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</tr>
<tr>
<td>HGF of metastatic patients*</td>
<td>Low</td>
</tr>
</tbody>
</table>

Table 6: Univariate analyses of overall survival. *Stage II or III, **p<0.05, NS not significantly.

The expression of HGF/cMET in CRC patients were found to be higher in patients with especially liver metastasis. These results suggest that c-MET may play an important role in the growth and scattering of CRC cells (18).

Activated cMET and IGF1R-driven PI3K signaling predicts poor survival in CRC independent of KRAS mutational status (19).

In another study; inhibition of HGF/cMET expression were found to prevent the distant recurrence of rectal cancer after preoperative RCTx (20).

![Figure 4](image1.png): Progression-free survival curves in CRC patients according to serum cMET levels (p=0.51).

![Figure 5](image2.png): Overall survival curves in CRC patients according to serum cMET levels (p=0.16).

![Figure 6](image3.png): Progression-free survival curves in CRC patients according to serum HGF levels (p=0.79).

![Figure 7](image4.png): Overall survival curves in CRC patients according to serum HGF levels (p=0.13).

Serum HGF levels were found to be prognostic for stage II or III CRC patients and CRC patients with synchronous liver metastasis, HGF/cMET levels were significantly higher (8, 21-23).

Another research on cMET signaling pathway in metastatic CRC patients, it has been found that
MET signaling in colon cancer stem-like cells blunts the therapeutic response to EGFR inhibitors. Moreover, it appears that cMET tumour expression could be a predictive marker of response to these targeted therapies for some gastrointestinal tumours. Nowadays, circulating HGF is correlated with resistance to cetuximab in metastatic CRC.

In our study; the median cMET and HGF levels were found to be significantly higher in all patients with CRC compared to healthy controls as in the literature and metastatic disease, greater pT stage and colonic site were found to be correlated with higher serum cMET concentrations all patients with CRC. Worse PS and metastatic disease were associated with higher serum HGF concentrations all patients with CRC. Worse PS, smoking and alcohol intake were associated with higher serum HGF concentrations for metastatic CRC patients. However, serum HGF/cMET levels showed no significantly adverse effect on PFS and OS.

Finally, serum levels of HGF and cMET may be diagnostic markers in CRC patients. However, their predictive and prognostic values were not determined. We had taken a large study group, clinicopathologic features of the patients were well characterized, but we couldn’t confirm their predictive and prognostic roles. Larger clinical trials should be done to emphasize the roles of these markers.

References


