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# Prognostic Significance of Targetable Angiogenic and Growth Factors in Patients Undergoing Resection for Gastric and Gastroesophageal Junction Cancers

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## ABSTRACT

**Background.** Circulating factors in patients with gastric/gastroesophageal junction (GEJ) cancers may promote tumor progression and metastasis and may be targeted for therapy.

**Methods.** Serum levels of ligands—vascular endothelial growth factor A (VEGF-A), fibroblast growth factor 2 (FGF2), epidermal growth factor (EGF), hepatocyte growth factor (HGF)—from four targetable pathways were measured before surgery, and levels were correlated to clinicopathologic characteristics and overall survival (OS).

**Results.** In 147 patients who underwent potentially curative resection for gastric/GEJ adenocarcinoma, VEGF-A levels were higher in patients with R1 versus R0 resection ($p = 0.037$). High EGF levels were associated with poorly differentiated tumors ($p = 0.02$). Elevated FGF2 levels were found in Lauren diffuse-type tumors ($p = 0.017$) and tumors with seven or more metastatic nodes (N3) ($p < 0.042$). Patients with advanced-staged tumors had higher HGF levels ($p = 0.012$). At a median follow-up of 35 months, 46 patients (31 %) had died. Increased VEGF and HGF levels were correlated with decreased OS ($p = 0.009$ and 0.005). An adjusted total value (ATV) of all factors was better than any single factor in stratifying patients into good and poor prognosis groups (5-year OS 84.1 vs. 53.9 %, $p = 0.005$). By multivariate analysis, serum VEGF-A and ATV were significant independent prognostic factors (along with T and N category) for OS ($p = 0.028$ and 0.013, respectively).

**Conclusions.** In patients undergoing resection for gastric and GEJ cancer, high levels of angiogenic and growth factors are associated with unfavorable tumor characteristics and poorer overall survival. Thus levels of these factors can help delineate tumor biology and stratify prognosis.

It is estimated that there are more than one million cases of gastric cancer worldwide per year and more than 700,000 deaths each year.[1] The pathways that drive the initiation, progression, and metastases of gastric cancers continue to be better delineated. Deng et al. performed a comprehensive survey of genomic alterations in gastric cancer and found the existence of five distinct subgroups defined by signature genomic alterations in fibroblast growth factor receptor 2 (FGFR2), V-Ki-ras Kirsten rat sarcoma viral oncogene homolog, epidermal growth factor receptor (EGFR), human epidermal growth factor receptor 2 (HER2), and c-MET.[2] The primary ligands for FGFR2 is fibroblast growth factor 2 (FGF2), for EGFR is EGF, and for c-MET is hepatocyte growth factor (HGF).[3] HER2 has no known ligand but can heterodimerize with other receptors in the EGFR family. In addition to these five

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pathways for gastric cancer tumor growth, the vascular endothelial growth factor A (VEGF-A) pathway plays an important role in driving tumor angiogenesis in gastric cancers.[4]

In the search for biomarkers that can delineate tumor biology, response to therapy, and/or prognosis, tumor tissue often is more difficult to obtain than circulating biomarkers. Many studies have examined circulating biomarkers for gastric cancer,[5] but no study has examined a panel of circulating growth factors that represent targetable pathways in a large number of gastric cancer patients undergoing potentially curative surgery. There are currently drugs approved or in clinical trials targeting the VEGF-A, FGF2, EGF, and HGF pathways for gastric cancer. Thus in this retrospective study, we sought to examine the circulating levels of VEGF-A, FGF2, EGF, and HGF before potentially curative surgical resection in patients with gastric adenocarcinoma.

## METHODS

### *Patients*

A total of 147 patients with gastric and gastroesophageal junction (GEJ) Siewert type II or III cancer who underwent radical gastrectomy or esophagogastrectomy with potentially curative intent (R0 and R1) were included from May 2006 to March 2012. Locally advanced or metastatic cancers treated with palliative resection (R2) were excluded. The Memorial Sloan-Kettering Cancer Center Institutional Review Board approved this study, and informed consents for study of blood and tumor tissue were obtained preoperatively from all patients. Clinical and pathological data were obtained from the institution's prospective gastric cancer database. Cancers were classified as intestinal, diffuse, or mixed according to Lauren classification and graded as differentiated (well or moderately differentiated) or undifferentiated (poorly differentiated). Tumor staging was determined form the surgical specimen and was based on the 7th edition of American Joint Committee on Cancer TNM staging system.

### *Blood Sample Collection and Enzyme-Linked Immunosorbent Assay*

All patients had blood samples drawn before any treatment. Blood samples were collected in plain vacuum tubes and coagulated at room temperature. They were centrifuged at 1,000×g for 10 min followed by serum collection. Serums were aliquoted and stored at −80 °C until analyses were performed. Serum samples were measured for VEGF-A, EGF, FGF2, and HGF using the following commercially available ELISA kits: Human VEGF Duoset, Human EGF Duoset, Human FGF2 Duoset, and Human HGF Duoset (all from R&D Systems, Minneapolis, MN). Manufacturer's protocols were followed, and samples were measured in duplicate. Enzyme-linked immunosorbent assay (ELISA) plates were read using the Emax Precision Microplate Reader (Molecular Devices, Sunnyvale, CA), and sample values were determined against a four parameter standard curves. The mean value of duplicate samples was used as the final concentration. Intraassay and interassay validation also were performed and found to be less than 15 and 15 %, respectively. Samples with angiogenic factor levels below the sensitivity of the VEGF-A and FGF2 assays were assigned a value midway between 0 and the lower limit of detection of the assay (31.2 pg/ml for VEGF-A and 50.0 pg/ml for FGF2). No lower limit of detection was found for the EGF and HGF assays.

To calculate a preoperative adjusted total value (ATV) for all four factors for each patient, the mean of each factor was subtracted from the individual factor and then divided by the standard deviation to obtain a standardized value (SV). The SVs of all four factors were then totaled for each patient to give an ATV:

$$\mathrm{ATV} = \mathrm{SV}_{\mathrm{VEGF}} + \mathrm{SV}_{\mathrm{FGF2}} + \mathrm{SV}_{\mathrm{EGF2}} + \mathrm{SV}_{\mathrm{HGF}}.$$

### *Statistical Analysis*

Pearson correlation coefficients were calculated by bivariate correlation analysis to evaluate correlations between angiogenic factors. Levels of serum angiogenic factors between patients were compared by the Kruskal–Wallis and Mann–Whitney $U$ test. Overall survival curves were plotted by the Kaplan–Meier method and compared by using log-rank test. Cutoff values for angiogenic factors and for ATV were determined by analyzing receiver operating characteristics curves (Supplementary Fig. 1). A Cox proportional hazards regression was used for univariate and multivariate analyses of prognostic factors for overall survival. $P < 0.05$ was considered as statistically significant. Analyses were performed using SPSS® software for Windows version 21 (SPSS, Chicago, IL).

## RESULTS

### *Patient Characteristics and Treatment*

Serum samples were obtained from 147 patients with gastric and gastroesophageal junction (type II or III) adenocarcinomas before potentially curative surgical resection. The ratio of male to female was 1.4–1, and median age was 67 (range 26–94) years. The majority of patients were Caucasian (74.8 %) followed by the Asian (12.9 %), Hispanic (6.8 %), and African-American

(4.1 %). Tumors were mainly located at the lower and middle third of the stomach (59.2 %); 19 % of tumors were located in the proximal stomach and 19 % were located at the GEJ. Neoadjuvant chemotherapy or chemoradiation was delivered in 36 patients (24.4 %), and adjuvant chemotherapy or chemoradiation was delivered in 47 patients (32 %). Distal gastrectomy was performed in 66 patients (44.9 %), proximal gastrectomy in 4 patients (2.7 %), total gastrectomy in 52 patients (35.4 %), and esophagogastrectomies in 25 patients (17 %). The types of esophagogastrectomy performed were transhiatal ($n = 7$) and Ivor-Lewis ($n = 18$).

### *Levels of Circulating Angiogenic and Growth Factors*

Mean and median values with standard deviation, interquartile range, and overall range for VEGF-A, EGF, FGF2, and HGF levels in these 147 patients are summarized in Supplemental Table 1. Scatter plots of the four factors are shown in Fig. 1a–d. Mean levels of HGF (1,353.9 pg/ml) were the highest of the four factors. There were large variations in HGF levels, and four patients had HGF levels > 17,000 pg/ml (Fig. 1d). Mean levels of the other three factors were between 183.8 and 319.1 pg/ml, with levels of VEGF-A having a wider range than levels of EGF or FGF2. Three patients had VEGF-A levels > 8,000 pg/ml. Supplemental Table 2 shows Pearson's correlation coefficients between the four angiogenic factors. VEGF-A, FGF2, and HGF were most strongly correlated with each other (Pearson's correlation coefficients 0.669–0.738).

Three patients had VEGF-A levels > 8,000 pg/ml; the remaining 144 patients had levels < 2,000 pg/ml. Similarly, three patients had FGF2 levels and four patients had HGF levels well above those of the remaining patients. In examining these patients, one patient with a Lauren diffuse-type tumor involving the entire stomach, had high levels of all three factors, and died of disease 7 days after surgery likely from a colon infarction.

### *Correlation between Factors and Clinical and Pathological Characteristics*

There was no statistically significant correlation between factor levels and patient and treatment variables (Table 1). However, there were significant correlations between factor levels and pathological characteristics (Table 2). Higher VEGF-A levels were found in patients having R1 resection compared with patients having R0 resection ($p = 0.037$). Higher EGF levels were found in patients with poorly or undifferentiated tumors ($p = 0.02$). Higher FGF2 levels correlated with a number of pathological variables, including Lauren diffuse-type tumors; poorly or undifferentiated tumors or extensive nodal disease (N3) tumors had higher FGF2 levels than others ($p = 0.017$, 0.042, or 0.046, respectively). Finally, higher levels of HGF were found in patients with advanced TNM stage ($p = 0.012$).

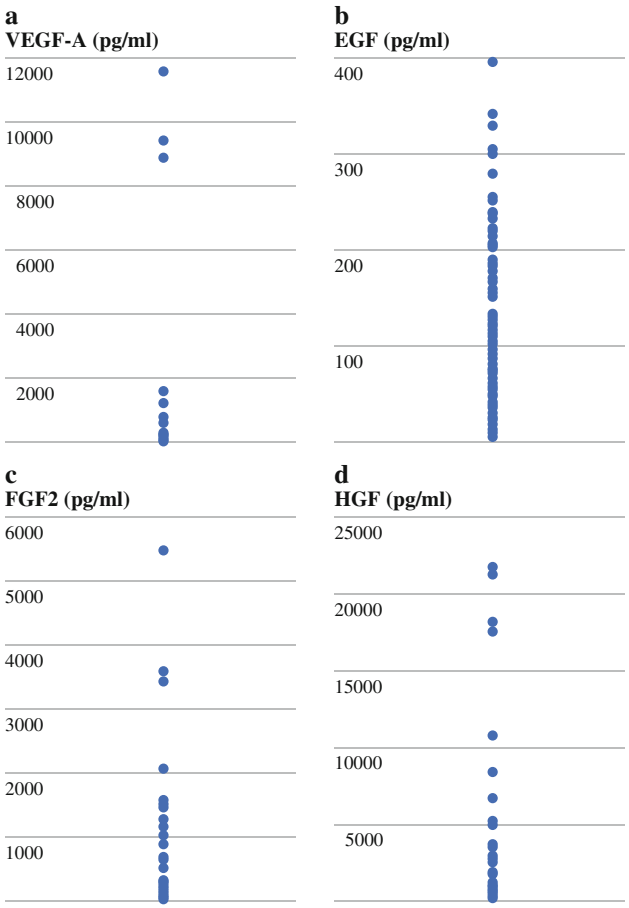


**FIG. 1** Scatter plots of circulating levels of VEGF-A (**a**), EGF (**b**), FGF2 (**c**), and HGF (**d**) in 147 patients with gastric and gastroesophageal junction cancers

### *Correlation between Factors and Overall Survival*

The median follow-up period was 35.3 months. At last follow-up, 101 were alive and 46 patients were dead. The overall survival of patients with higher VEGF-A levels or HGF levels was significantly worse that patients with lower VEGF-A or HGF levels, respectively (Fig. 2a, b). The 5-year overall survival rates of patients with a high versus low VEGF-A level were 49.6 and 76 % ($p = 0.009$), and the 5-year overall survival rates of patients with a high versus low HGF level were 49.6 and 71.7 % ($p = 0.005$).

Patients with higher EGF or FGF2 levels showed poorer overall survival, but these differences did not reach statistical significant (Fig. 3a, b). An ATV for all four factors

**TABLE 1** Patient characteristics/treatment and factor levels

| | *n* | % | VEGF-A (pg/ml) | | EGF (pg/ml) | | FGF2 (pg/ml) | | HGF (pg/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | median ± SD | *p* value | Median ± SD | *p* value | Median ± SD | *p* value | Median ± SD | *p* value |
| Gender | | | | | | | | | | |
| M | 86 | 58.5 | 77.4 ± 1,249.7 | 0.906 | 113 ± 972.6 | 0.756 | 74.4 ± 612.7 | 0.412 | 468.7 ± 3,510.6 | 0.423 |
| F | 61 | 41.5 | 72.3 ± 1,633 | | 103.2 ± 80.8 | | 115 ± 769.6 | | 412.2 ± 3,257.2 | |
| Age (year) | | | | | | | | | | |
| ≤67.0 | 77 | 52.4 | 64.9 ± 1,070.2 | 0.179 | 113.7 ± 1,027.2 | 0.804 | 111 ± 397.5 | 0.233 | 396.3 ± 3,206.3 | 0.059 |
| >67.0 | 70 | 47.6 | 80.7 ± 1,723.1 | | 106.4 ± 82.2 | | 67.7 ± 894.1 | | 469.8 ± 3,609.4 | |
| Race | | | | | | | | | | |
| White | 110 | 74.8 | 81.3 ± 1,632.2 | 0.241 | 113.5 ± 75 | 0.626 | 75.8 ± 743.8 | 0.760 | 469.3 ± 3,427.9 | 0.262 |
| Black | 6 | 4.1 | 31.2 ± 48.3 | | 110.9 ± 26.5 | | 116.3 ± 122.3 | | 396.8 ± 243.7 | |
| Hispanic | 10 | 6.8 | 41.2 ± 239.2 | | 80.8 ± 88.2 | | 84.5 ± 112 | | 492 ± 1,009 | |
| Asian | 19 | 12.9 | 67.7 ± 139.1 | | 121.7 ± 2,061.7 | | 112.5 ± 592.9 | | 291.9 ± 4,564.4 | |
| Unknown | 2 | 1.4 | | | | | | | | |
| Type of resection | | | | | | | | | | |
| Distal gastrectomy | 66 | 44.9 | 55.6 ± 1,090.6 | 0.071 | 109.1 ± 81.2 | 0.791 | 62.3 ± 712.9 | 0.095 | 428.5 ± 1,809.4 | 0.373 |
| Proximal gastrectomy | 4 | 2.7 | 35.7 ± 25.8 | | 87.1 ± 42.8 | | 60.1 ± 48.2 | | 290.8 ± 167.1 | |
| Total gastrectomy | 52 | 35.4 | 93.6 ± 2,034.6 | | 110.2 ± 1,248.3 | | 160 ± 782.7 | | 465.4 ± 5,211 | |
| Esophagogastrectomy | 25 | 17 | 92.8 ± 249.6 | | 119.1 ± 63.6 | | 89.7 ± 118.4 | | 409.7 ± 399.2 | |
| Lymphadenectomy | | | | | | | | | | |
| D1 | 7 | 4.8 | 31.2 ± 57.8 | 0.381 | 104.5 ± 45.8 | 0.751 | 50 ± 48.5 | 0.138 | 580.1 ± 1,146.9 | 0.812 |
| D1+ | 4 | 2.7 | 29.4 ± 132.1 | | 118.5 ± 44.6 | | 45.9 ± 81 | | 453.6 ± 87.4 | |
| D2 | 136 | 92.5 | 77.6 ± 1,472.2 | | 110.2 ± 774.7 | | 100.2 ± 703.4 | | 424 ± 3,518.7 | |
| Neoadjuvant treatment | | | | | | | | | | |
| None | 111 | 75.5 | 72.3 ± 1,220.5 | 0.389 | 112.3 ± 856.7 | 0.865 | 73.8 ± 642.3 | 0.328 | 429.5 ± 2,978.3 | 0.906 |
| Chemotherapy | 28 | 19.0 | 64.5 ± 2,174.9 | | 95.8 ± 66.1 | | 160.5 ± 889.7 | | 453.2 ± 5,068.1 | |
| Chemoradiation | 8 | 5.4 | 127.8 ± 50.4 | | 98.6 ± 78.2 | | 84 ± 142.1 | | 462.4 ± 395.7 | |
| Adjuvant treatment | | | | | | | | | | |
| None | 100 | 68 | 79 ± 1,479.6 | 0.788 | 108.8 ± 902.3 | 0.687 | 66.7 ± 480.6 | 0.102 | 411.7 ± 3,617.9 | 0.309 |
| Chemotherapy | 40 | 27.2 | 63.4 ± 1,397.6 | | 102.8 ± 73.2 | | 152.3 ± 1,040.2 | | 478.5 ± 3,129.1 | |
| Chemoradiation | 7 | 4.8 | 64.2 ± 47.8 | | 128.6 ± 66.6 | | 76.8 ± 151.6 | | 589.6 ± 880.2 | |

*SD* standard deviation

was determined for each patient (see "Methods"). High ATV was significantly associated with poorer overall survival that low ATV (Fig. 3c). The 5-year overall survival rates of patients with a high versus low ATV were 53.9 and 84.1 %, respectively ($p = 0.005$).

### *Univariate and Multivariate Analysis of Prognostic Factors*

Prognostic factors for overall survival after curative surgery in gastric and gastroesophageal junction cancers were investigated (Table 3). On univariate analysis, margin status (R0 vs. R1), tumor size, vascular invasion, neural invasion, grade, T category, N category, VEGF level, HGF level, and ATV were all statistically significant prognostic factors for overall survival. On multivariate analysis using two different models, only T category, N category, VEGF-A level, and ATV were statistically significant independent prognostic factors for overall survival.

### *Analysis of the Subgroup of Patients Not Receiving Neoadjuvant Treatment*

Tumor staging may have been altered by neoadjuvant treatment. Thirty-six of the 147 patients in this study received neoadjuvant treatment (28 chemotherapy alone, 8 chemoradiation). Patients receiving neoadjuvant treatment did not have significantly different levels of the four circulating factors compared with patients not receiving neoadjuvant treatment (Table 1). Neoadjuvant treatment was not associated with improved overall survival on

**TABLE 2** Pathological characteristics and factor levels

| | *n* | % | VEGF-A (pg/ml) | | EGF (pg/ml) | | FGF2 (pg/ml) | | HGF (pg/ml) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Median ± SD | *p* value | Median ± SD | *p* value | Median ± SD | *p* value | Median ± SD | *p* value |
| Size (cm) | | | | | | | | | | |
| ≤3 | 75 | 51 | 58.4 ± 1,091.6 | 0.198 | 107.1 ± 73.5 | 0.604 | 89.7 ± 330.6 | 0.317 | 411.2 ± 2,606.8 | 0.227 |
| >3 | 72 | 49 | 81.3 ± 1,696.5 | | 113 ± 1,062 | | 84.8 ± 903 | | 462.7 ± 4,045.5 | |
| Location | | | | | | | | | | |
| Lower | 52 | 35.4 | 71.7 ± 1,226.3 | 0.087 | 107 ± 83.2 | 0.133 | 69.3 ± 780.6 | 0.077 | 431.5 ± 1,414.3 | 0.978 |
| Middle | 35 | 23.8 | 46.7 ± 70.4 | | 86.2 ± 64.9 | | 62.1 ± 353.5 | | 406.1 ± 1,885.5 | |
| Upper | 28 | 19 | 80.3 ± 1,767.4 | | 126 ± 1,696.9 | | 131.3 ± 827.7 | | 432.9 ± 5,928.9 | |
| Gastroesophageal junction | 28 | 19 | 106.4 ± 236.4 | | 118.9 ± 67.1 | | 94.8 ± 133 | | 482.3 ± 532.6 | |
| Whole stomach | 4 | 2.7 | 214.3 ± 5,722.9 | | 133.2 ± 114.9 | | 974 ± 1,425.8 | | 2,652 ± 9,963.1 | |
| Surgical margin | | | | | | | | | | |
| R0 | 136 | 92.5 | 66.3 ± 1,105.5 | **0.037** | 111.3 ± 774.5 | 0.763 | 74.4 ± 647 | 0.099 | 430.5 ± 3,060.4 | 0.675 |
| R1 | 11 | 7.5 | 180 ± 3,457.6 | | 96.1 ± 85.9 | | 111 ± 1,009.1 | | 482.1 ± 6,283 | |
| Pathological treatment effect | | | | | | | | | | |
| 0–25 % | 18 | 50 | 132.5 ± 2,705.5 | 0.772 | 120.9 ± 67.7 | 0.38 | 182.7 ± 1,085.9 | 0.597 | 539 ± 6,233.3 | 0.155 |
| 26–50 % | 9 | 25 | 67.7 ± 72.7 | | 81.3 ± 60.9 | | 169.8 ± 187.7 | | 373.7 ± 311.6 | |
| 51–75 % | 3 | 8.3 | 52.7 ± 91.8 | | 35.2 ± 54.4 | | 181.9 ± 114.1 | | 753.3 ± 474.6 | |
| 76–89 % | 3 | 8.3 | 147.7 ± 80.6 | | 201.4 ± 73.7 | | 68.2 ± 36 | | 256.5 ± 100.7 | |
| 90–99 % | 3 | 8.3 | 37.9 ± 49.6 | | 78.1 ± 94.3 | | 55.3 ± 119 | | 418.6 ± 366.5 | |
| Lauren classification | | | | | | | | | | |
| Intestinal | 70 | 47.6 | 80.3 ± 1,121.6 | 0.393 | 112 ± 73.8 | 0.537 | 56.5 ± 335.7 | **0.017** | 426.9 ± 2,599.5 | 0.631 |
| Diffuse | 47 | 32 | 51.3 ± 2,094.3 | | 104.5 ± 1,312.5 | | 170.2 ± 1,072.1 | | 431.4 ± 4,909.4 | |
| Mixed | 29 | 19.7 | 94 ± 130.7 | | 102.5 ± 61.9 | | 87.1 ± 318.8 | | 429.5 ± 1,222.8 | |
| Unknown | 1 | 0.7 | | | | | | | | |
| Grade | | | | | | | | | | |
| Well- or moderately differentiated | 51 | 34.7 | 62.4 ± 1,314.6 | 0.168 | 101 ± 65.5 | **0.020** | 55.3 ± 337.9 | **0.042** | 373.7 ± 3,015.3 | 0.075 |
| Poorly or undifferentiated | 96 | 65.3 | 85.1 ± 1,475.5 | | 119.2 ± 919.8 | | 114 ± 798.2 | | 441.8 ± 3,581.3 | |
| Vascular invasion | | | | | | | | | | |
| No | 62 | 42.2 | 55.2 ± 133.5 | 0.08 | 108.5 ± 1,144.8 | 0.959 | 103.8 ± 321.6 | 0.748 | 408.6 ± 2,301.5 | 0.165 |
| Yes | 85 | 57.8 | 92.8 ± 1,850.1 | | 112.3 ± 64.7 | | 76.8 ± 846.1 | | 463.7 ± 3,998.2 | |
| Neural invasion | | | | | | | | | | |
| No | 77 | 52.4 | 74.8 ± 1,460.4 | 0.581 | 99.5 ± 1,027.9 | 0.617 | 57.6 ± 706.2 | 0.085 | 429.5 ± 3,325 | 0.966 |
| Yes | 70 | 47.6 | 74 ± 1,377.2 | | 119.2 ± 64.8 | | 111.5 ± 654.4 | | 434.6 ± 3,497.1 | |
| T stage | | | | | | | | | | |
| 1 | 43 | 29.3 | 52.7 ± 1,436.1 | 0.605 | 100.4 ± 68.3 | 0.361 | 61.8 ± 252.8 | 0.053 | 378.5 ± 3,275.2 | 0.06 |
| 2 | 23 | 15.6 | 80.1 ± 63.7 | | 103.2 ± 78.5 | | 82.5 ± 266.1 | | 498.8 ± 502. | |
| 3 | 43 | 29.3 | 64.9 ± 149.5 | | 113.7 ± 1,374 | | 68.2 ± 418.3 | | 567.9 ± 2,787.6 | |
| 4 | 38 | 25.9 | 91.2 ± 2,318.3 | | 131.8 ± 75.8 | | 137.9 ± 1,168.3 | | 441.8 ± 4,760.7 | |
| N stage | | | | | | | | | | |
| 0 | 75 | 51 | 72.3 ± 1,091.3 | 0.943 | 110 ± 1041. | 0.737 | 89.7 ± 547.9 | **0.046** | 406.1 ± 3,906.6 | 0.199 |
| 1 | 37 | 25.2 | 74.8 ± 2,353.4 | | 113.7 ± 56.6 | | 62.1 ± 1,045.8 | | 474.9 ± 3,658.5 | |
| 2 | 19 | 12.9 | 78 ± 76.1 | | 92.1 ± 60.4 | | 87.1 ± 201.9 | | 395.8 ± 1,526.1 | |
| 3 | 16 | 10.9 | 86.1 ± 102.6 | | 124.4 ± 63.4 | | 210.8 ± 526.3 | | 539.2 ± 1,262.1 | |
| TNM stage | | | | | | | | | | |
| 1 | 50 | 34 | 65.1 ± 1,331.7 | 0.533 | 99.8 ± 73.7 | 0.307 | 76.8 ± 248.7 | 0.293 | 375.4 ± 3,048.6 | **0.012** |
| 2 | 51 | 34.7 | 80.1 ± 138.8 | | 124.9 ± 1,260.1 | | 73.8 ± 641.6 | | 552.6 ± 3,709.7 | |
| 3 | 46 | 31.3 | 91.1 ± 2,113 | | 107.2 ± 58.6 | | 111.5 ± 967.1 | | 460.5 ± 3,423.3 | |

univariate or multivariate analysis (Table 2). We repeated all analyses using only the 111 patients who did not receive neoadjuvant therapy (see Supplementary Tables 3–7). The ATV of all four factors remained a significant prognostic factor for overall survival on both univariate and multivariate analyses (Supplementary Table 7).

**TABLE 3** Univariate and multivariate analyses of prognostic factors for overall survival

| | Hazard ratio | 95 % CI | Univariate analysis *p* value | Multivariate analysis[a] *p* value | Multivariate analysis[b] *p* value |
|---|---|---|---|---|---|
| Age | 1.803 | 0.978–3.326 | 0.059 | | |
| Gender | 0.819 | 0.505–1.717 | 0.819 | | |
| Race | 1.105 | 0.907–1.347 | 0.323 | | |
| Location | 1.041 | 0.811–1.336 | 0.754 | | |
| Margin status | 3.716 | 1.642–8.411 | **0.002** | | 0.058 |
| Neoadjuvant treatment | 1.24 | 0.746–2.061 | 0.407 | | |
| Adjuvant treatment | 1 | 0.61–1.639 | 0.999 | | |
| Lauren classification | 0.985 | 0.677–1.432 | 0.935 | | |
| Tumor size | 2.786 | 1.484–5.231 | **0.001** | 0.078 | 0.163 |
| Vascular invasion | 2.228 | 1.16–4.28 | **0.016** | | |
| Neural invasion | 2.135 | 1.16–3.929 | **0.015** | | |
| Grade | 2.279 | 1.093–4.754 | **0.028** | | |
| T category | 1.909 | 1.417–2.573 | **<0.001** | **0.006** | **0.004** |
| N category | 1.686 | 1.305–2.177 | **<0.001** | **0.028** | **0.041** |
| Serum VEGF-A levels (low vs. high) | 2.298 | 1.214–4.35 | **0.011** | **0.028** | |
| Serum EGF levels (low vs. high) | 1.597 | 0.865–2.945 | 0.134 | | |
| Serum FGF2 levels (low vs. high) | 1.547 | 0.843–2.841 | 0.159 | | |
| Serum HGF levels (low vs. high) | 2.306 | 1.258–4.227 | **0.007** | **0.160** | |
| Adjusted total value (low vs. high) | 3.196 | 1.349–7.575 | **0.008** | | **0.013** |

[a] Model 1: tumor size, T category, N category, VEGF-A, and HGF were included in multivariate analysis

[b] Model 2: margin status, tumor size, T category, N category, and ATV were included in multivariate analysis

## DISCUSSION

In this study of 147 patients undergoing potentially curative resection of gastric or gastroesophageal adenocarcinoma, high VEGF-A levels correlated with R1 resection, high EGF levels correlated with poorly differentiated tumors, high FGF-2 levels correlated with diffuse type, poorly differentiated, or N3 category tumors, and high HGF levels correlated with advanced stage tumors. Despite all patients undergoing potentially curative surgery and the majority of patients receiving neoadjuvant or adjuvant chemotherapy and/or radiation, 46 patients (31 %) died after a median follow-up of 35.3 months. High VEGF and HGF levels were correlated with decreased overall survival ($p = 0.009$ and 0.005, respectively), and a preoperative ATV of all four factors stratified patients into good and poor prognosis groups ($p = 0.005$). By multivariate analysis, high VEGF-A and ATV were independent prognostic factor for overall survival. Thus, this study demonstrates the utility of measuring circulating angiogenic and growth factors before resection of gastric adenocarcinoma, and these preliminary results should be validated in a larger cohort of patients.

This study demonstrates that circulating angiogenic and growth factors may be helpful in determining the prognosis of gastric cancer patients undergoing surgical resection. Gastric cancer patients are most commonly staged using the American Joint Committee on Cancer (AJCC) TNM system.[6] In our previous analysis of the Surveillance, Epidemiology and End Results (SEER) database, we found the 7th AJCC staging system misclassifies or mispredicts the prognosis of over half of SEER patients.[7] There are several alternatives to TNM-based staging systems, and our group has developed a nomogram which incorporates additional prognostic clinical factors, including site of age, sex, tumor size, site of primary tumor, and Lauren subtype. This nomogram has been validated at other Western institutions.[8] However, as has been demonstrated most prominently in breast cancer patients, the addition of biological biomarkers to traditional clinical parameters can greatly improve the ability to determine prognosis and make treatment decision.[9] This study demonstrates that circulating levels of VEGF-A and HGF are statistically significant predictors of overall survival independent of T status or N category.

Levels of circulating factors not only correlated with overall survival but also with certain pathological characteristics of tumors. The median VEGF-A level in the 11 patients with positive resection margins was nearly three times higher than patients with negative margin resections, suggesting tumors with high VEGF-A expression may be more infiltrative.[10] Patients with Lauren diffuse type

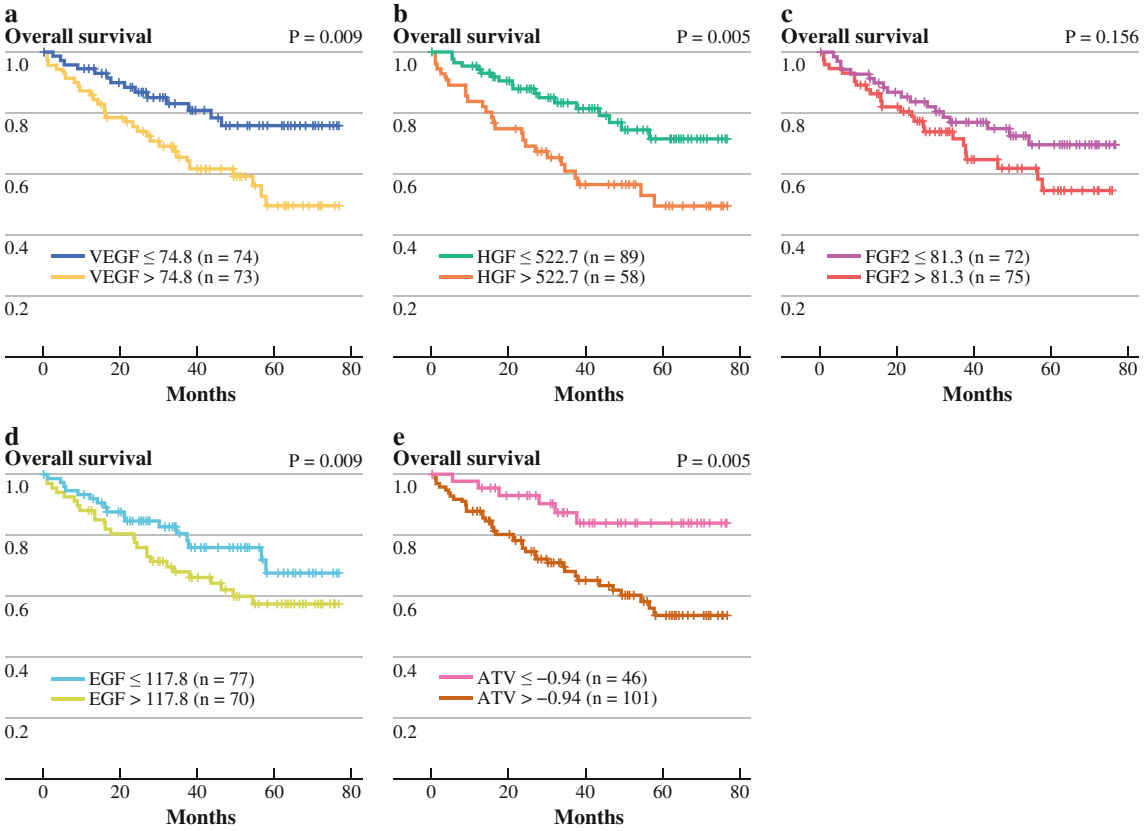


**FIG. 2** Kaplan–Meier overall survival curves based on serum VEGF-A levels (**a**), HGF levels (**b**), serum EGF levels (**c**), FGF2 levels (**d**), and adjusted total value (ATV) (**e**)

tumors had FGF2 levels over three times higher than patients Lauren intestinal type tumors, confirming prior reports of the FGF pathway being preferentially amplified in Lauren diffuse type tumors.[11] The HGF-Met signaling pathway in gastric and other cancers promotes invasiveness and metastasis, and we found increasing levels of HGF with increasing TNM stage.[12] Thus, this study confirms those of prior studies correlating specific pathways with certain biological characteristics of gastric cancers.

Analysis of circulating factors may not only help to determine prognosis but also may direct the use of targeted therapies. Approximately 10–30 % of gastric adenocarcinomas overexpress HER-2, and the addition of trastuzumab to chemotherapy for metastatic disease prolongs survival in these patients from 11 to 14 months in a randomized trial.[13] The addition of bevacizumab, an anti-VEGF-A antibody, to chemotherapy has been examined for metastatic gastric cancer patients in the phase III AVAGAST trial.[14] Median OS was not significantly improved in the bevacizumab group and compared to the placebo group, but the addition of bevacizumab was associated with significant increases in progression-free survival and response rate. Van Cutsem et al.[15] demonstrated that patients in the AVAGAST trial with higher circulating VEGF-A levels "showed a trend toward improved overall survival" suggesting that this trial may have been positive in terms of overall survival if patients were selected based on baseline VEGF-A level]. Similar to the AVAGAST trial, the EXPAND and REAL-2 trials found that adding cetuximab or panitumumab, both anti-EGFR antibodies, to chemotherapy for advanced gastroesophageal cancer patients did not improve progression-free survival and overall survival, respectively, and perhaps the use of a biomarker to select patients may have been worthwhile in these studies as well.[16,17]

There are several limitations to this study. First, Tables 1 and 2 demonstrate some correlations between circulating factor levels and patient/treatment/pathological variables. Given there is multiple testing, any significant associations should be considered hypothesis generating and not hypothesis proving. Second, there are 147 patients in this study, but a larger number of patients would have allowed for more definitive associations and conclusions. Third, 36 of 147 (24 %) patients in this study received neoadjuvant treatment, which may have affected levels of circulating factors and pathological staging. Thus, all analyses were repeated in the 111 patients not receiving neoadjuvant treatment and the primary conclusion

remained, namely that ATV predicted overall survival. Last, there are no correlations made in this study between levels of circulating factors and response to targeted therapies, but current studies are underway to address this issue.

In summary, to our knowledge this is the first study to examine a panel circulating angiogenic and growth factors (VEGF-A, EGF, FGF2, and HGF) representing targetable pathways in patients with gastric adenocarcinoma undergoing potentially curative resection. VEGF-A and HGF levels are inversely correlated with overall survival, and an ATV of all four factors is better than any single factor in discriminating overall survival. Levels of specific factors correlate with biological characteristics, such as histologic subtype, invasion, and metastasis. In the current era of targeted therapies for specific signaling pathways, the measurement of circulating ligands for these pathways may play an increasing role in determining which pathways to target and yield personalized information on tumor biology and patient prognosis.

**DISCLOSURES** None.

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