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Micropapillary Colorectal Carcinoma:

Clinical, Pathologic, and Molecular Properties, Including Evidence of Epithelial-Mesenchymal Transition

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Abstract

Aims—Colorectal carcinoma (CRC) with micropapillary (MP) features has only been recently described and is still being characterized.

Methods and Results—We reviewed the clinicopathologic and molecular features of 42 CRC with MP features. Twenty-nine cases were also evaluated for immunohistochemical evidence of epithelial-mesenchymal transition (EMT). The extent of MP features within our cohort ranged from 5% (13 cases) to 100% (1 case). Twenty-seven cases featured prominent cribriforming with dirty necrosis in the non-MP component; nine displayed mucinous features. Twenty-four of 29 cases (83%) demonstrated evidence of EMT. Thirty-six cases (86%) showed advanced T-category (pT3 or pT4), 31 (74%) had lymph node metastases, and 23 (55%) had distant metastases. Median overall follow-up was 36 months. Seventeen patients (40%) died of disease, with median survival of 23 months. Mutations were seen in 17 of 31 tested cases (55%), including 11 *KRAS* mutations and 4 *BRAF*V600E mutations. Microsatellite instability testing was performed on 21 cases; all were microsatellite-stable. Compared to a cohort of 972 conventional CRC, MP CRC was more likely to present as Stage IV disease ($P<0.001$), but patients with MP CRC showed no significant differences in overall survival after adjusting for stage.

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Conflicts of interest

The authors affirm they have no conflicts of interest arising from this manuscript.

Conclusions—MP features in CRC portend a high likelihood of advanced local disease and distant metastases. MP CRC is often associated with a cribriform pattern elsewhere in the tumour and cystic nodal metastases with prominent necrosis. They also show frequent mutations in *KRAS* and *BRAF*. Immunohistochemical evidence of EMT is common in MP CRC.

Keywords

Colorectal Carcinoma; Micropapillary; Epithelial-Mesenchymal Transition; Classification; Prognosis

Introduction

Morphologic subtyping of colorectal carcinoma (CRC) is a commonly accepted practice among gastrointestinal pathologists and often carries an impact on patient prognosis. The 2010 edition of the WHO Classification of Tumours of the Digestive System recognizes six subtypes of colorectal carcinoma: mucinous, signet ring cell, medullary, serrated, cribriform comedo-type, and micropapillary.¹ Literature on the latter three subtypes has only appeared in recent years, most notably for micropapillary (MP) colorectal carcinoma, a well-recognized subtype of adenocarcinoma in numerous other organ systems, including breast² and bladder.³

Beginning in 2005, several case series^{4–11} and case reports^{12–21} were published on MP CRC, elucidating its clinical behavior, genetic properties, and prognosis. In general, this subtype of colorectal adenocarcinoma presents as locally advanced disease, displays a tendency toward lymphovascular invasion and lymph node metastasis, and reportedly imparts a poor prognosis compared to conventional CRC.^{5–10} These findings are in keeping with micropapillary carcinomas reported in other organ systems.²² While these tumours have not been studied extensively on a genetic level, *TP53* and *KRAS* mutations appear to be frequent, and microsatellite instability (MSI) is very uncommon.⁸

Epithelial-mesenchymal transition (EMT) is a multistep dynamic cellular phenomenon in which epithelial cells lose their cell–cell adhesion and gain migratory and invasive traits that are typical of mesenchymal cells. EMT involves loss of membranous localization of the epithelial marker E-cadherin and an increase in levels of one or more mesenchymal markers, such as vimentin, N-cadherin, α -smooth muscle actin, or fibronectin, along with a loss of polarized function of epithelial cells.^{23–24} This phenomenon has been associated with increased rate of metastasis in many types of carcinoma²⁵ and has been implicated in the pathogenesis of CRC.^{26–29} Immunohistochemistry for certain proteins, including E-cadherin, vimentin, Snail, Zeb, and Twist, can indicate the occurrence of EMT.^{30–32} We have observed that the interface between tumoural epithelial cells and mesenchymal stromal cells often does not show a clear transition in MP CRC, suggesting the possibility of EMT. To our knowledge, this has only been investigated in one study to date.³³

To further our understanding of this putatively aggressive subtype of CRC, we report our experience analyzing them on a clinical, pathologic, immunohistochemical, and molecular basis.

Materials and Methods

With appropriate Institutional Research Board approval, slides from 1,003 CRC resections in the institution-restricted research files of the authors were reviewed for MP features anywhere within the tumours. The same criteria for diagnosis of micropapillary carcinoma in other organs were applied; specifically, MP features were defined as areas demonstrating small rounded clusters of malignant epithelial cells with reversed polarity (apical surfaces facing the periphery rather than the center of the tumour cluster), abundant eosinophilic cytoplasm, and retraction artefact creating lacunae-like spaces around the micropapillae (Figure 1A). Fibrovascular cores were absent. Micropapillae were distinguished from tumour budding using the following criteria: presence within the tumour bulk rather than just the leading edge, composition of more than five cells, well-formed nature (rather than small clusters of cells), reversed polarity, eosinophilia, and lacunae.³⁴ All included tumors were at least 5% micropapillary, as with previous series.^{5-8,10}

Thirty-five CRC patients with at least focal micropapillary features were identified, representing 3.5% of the CRC cases in file. Seven additional cases were identified prospectively or during review of colorectal cancer cases for separate research projects, bringing the total number of cases to 42, each from a different patient.

For each case, all available slides were reviewed, along with the original pathology report, to determine tumour size, depth of invasion, lymphovascular invasion, perineural invasion, peritumoral chronic inflammation, and spread to lymph nodes or distant sites. Patient sex, age, and date of resection were recorded, as were date of and vital status at last known follow-up. Tumours were staged using American Joint Committee on Cancer (AJCC) TNM 7th edition criteria.³⁵ The percentage of the tumour demonstrating micropapillary features was estimated, and the presence of other identifiable microscopic patterns was noted.

For immunohistochemistry, 4- μ m unstained sections from formalin-fixed, paraffin-embedded tissues were first deparaffinized by routine methods. For antigen retrieval, sections were heated to 104°C for 20 minutes in a pH-6.0 citrate buffer and then allowed to cool to room temperature. Afterward, tissue sections were quenched with 3% H₂O₂ in NaN₃ for 5 minutes at room temperature. Primary antibodies used included anti-SMAD4 (1:100 dilution; Santa Cruz Biotechnology, Dallas, TX), anti-vimentin (1:100 dilution; Dako, Carpinteria, CA), anti-AE1/AE3 (1:100 dilution; Dako), and anti-E-cadherin (1:800; BD Biosciences, San Jose, CA); these four markers were chosen due to our experience regarding their reliability in our laboratory. Immunolocalization was performed using the Dako EnVisionTM+ HRP-labeled polymer. Staining was visualized by 5-minute incubation with diaminobenzidine.

Mutational analysis for *KRAS*, *NRAS*, *BRAF*, *PIK3CA*, *AKT*, *PTEN*, and *SMAD4* was performed using a lab-developed assay.³⁶ Mutations analyzed included *AKT1* (codon E17), *BRAF* (codons G466, G469, D594, G596, V600), *KRAS* (codons G12, G13, Q61, A146, K117), *NRAS* (codons G12, Q61), *PIK3CA* (codons H1047, E542, E545, Q546, D549), *PTEN* (codons R233, R159, R267), and *SMAD4* (codons E330, D351, D355, R361). This

panel was chosen based on mutation frequency and relevance to potential targeted therapy in CRC. Results of microsatellite instability testing were available for some tumours as well.

A cohort of 972 patients with conventional (non-micropapillary) CRC was used as a comparison group. Categorical variables were compared using Fisher's exact test, and continuous variables were compared using Student's t-test with unequal variances. The prognostic significance of the micropapillary subtype on disease-specific survival was assessed using a Cox proportional hazards regression model, and risk of death relative to percentage of tumor with micropapillary features was analyzed using logistic regression. Other variables significant in univariate analysis were included with histologic type in a subsequent multivariate Cox regression model. All statistical tests were performed using Stata v13.1 (College Station, TX). All hypothesis tests were two-sided, with $\alpha=0.05$.

Results

Clinicopathologic findings are summarized in Table 1. The extent of each tumour composed of MP features averaged 27%; 13 cases demonstrated approximately 5% MP areas, while 10 were at least half MP, including one tumour that was entirely micropapillary. The micropapillary component was frequently associated with a dense hypercellular stromal reaction (Figure 1B).

The majority of MP cases (27/42; 64%) possessed a characteristic morphology elsewhere in the tumour, with numerous cribriform glands and large, dilated glands filled with dirty necrotic debris; micropapillary foci were often focally distributed around these areas (Figure 1C). Four cases (10%) demonstrated "pseudo-micropapillary" areas, wherein the nests of tumour cells were confluent rather than discrete, and retraction artefact did not completely surround the nests (Figure 1D); classic MP features were present elsewhere in these tumors. In five of the included cases (12%), the micropapillary nests floated in a background of mucin, rather than infiltrating stroma (Figure 1E); four other cases had separate, more traditional areas of mucinous adenocarcinoma separate from the micropapillary component. Other minor components seen in some tumours included signet ring areas (1 case) and poorly differentiated areas (2 cases). Lymphovascular invasion was present in 35 cases (83%).

Lymph node metastases were common in MP CRC (74% of cases). Typically, nodal metastases resembled either conventional CRC or appeared as large, cystic spaces filled with dirty necrosis and lined by malignant cells with eosinophilic cytoplasm similar to those seen in the MP component of the primary tumour. However, MP features were present in ten of the lymph node metastases (32%) (Figure 1F).

Twenty-three patients (55%) developed distant metastases (liver, n=13; omentum, n=5; peritoneum, n=5; lungs, n=2; ovaries, n=2; small intestine, n=2; cystic duct lymph node, n=1). Tissue was available from metastatic lesions in seventeen of these patients, with micropapillary areas present in seven (41%).

Molecular analysis targeting specific genetic mutations was performed in 31 of the 42 MP cases. While 14 showed no mutations in the analyzed codons, the other 17 (55%) harbored

at least one mutation. The most frequently mutated gene was *KRAS*, with mutations in 11 tumours (35%): G12A (n=1), G12D (n=2), G12S (n=2), G13C (n=1), G13D (n=4), and Q61H (n=1). Four cases showed a *BRAF* V600E mutation; of these, one also harbored a *SMAD4* R361H mutation, and one showed a *PIK3CA* H1047R mutation. The same *PIK3CA* mutation was evident in one of the cases with a *KRAS* G12S mutation. Finally, one case showed only a *SMAD4* R361H mutation, and one showed only an *NRAS* Q61L mutation. In three of the analyzed cases, the mutational analysis was performed on both cribriform and micropapillary areas separately, with no difference in mutational profile observed. Results of MSI testing were available on 21 cases, of which all were microsatellite-stable.

The possibility of EMT was examined in 29 cases by immunohistochemical expression of epithelial and mesenchymal markers and SMAD4 localization. Eighteen of 29 cases (62%) showed focal loss or decrease of AE1/AE3 (Figure 2A), and membranous E-cadherin was focally lost in 78% of cases (21/27; Figure 2B). Occasional vimentin-positive carcinoma cells were observed in 13 of 29 cases (45%) (Figure 2C), and 24 of 29 cases (83%) showed at least focal nuclear localization of SMAD4 (Figure 2D).

Overall median patient follow-up was 36 months (range: 1-133 months). Seventeen patients (40%) died of disease, all of whom had distant metastases (median length of survival: 23 months). An additional seven patients died of unknown or unrelated causes. The remaining 18 patients were alive at last follow-up, four of whom had distant metastatic disease.

As shown in Table 1, patients with MP CRC were younger than patients with conventional CRC and more likely to present with Stage IV disease. On univariate analysis, larger tumour size, lymphovascular invasion, perineural invasion, lack of peritumoral inflammation, and advanced stage (including advanced T-category, N-category, and M-category) were associated with shorter disease-specific survival (Table 2). On multivariate analysis, independent prognostic factors for shorter disease-specific survival included female sex, older age, larger tumour size, lymphovascular invasion, lack of peritumoral inflammation, and advanced stage. Stage for stage, MP patients fared similarly to patients with conventional CRC (Figure 3). Among MP patients, for every 10% increase in MP component within the tumor, the risk of disease-specific death decreased by 8.5%, but this was not statistically significant ($P=0.35$).

Discussion

Our findings generally agree with those observed in prior case series of MP CRC. Specifically, this CRC subtype tends to present as advanced disease with lymphovascular invasion and distant metastasis. Consequently, a high proportion of patients with this histologic subtype die of disease (5-year disease-specific survival of 54%, compared to 67% of conventional CRC in our cohorts).⁵⁻¹⁰

There were, however, some discrepancies between our findings and those previously reported. Whereas we found at least focal MP features in 35/1,003 cases of colorectal carcinoma (3.5%), other groups have reported prevalences ranging from 9%⁵ to 19%.⁶

(Akimoto et al observed MP features at the invasive front in 4.5% of their Stage III or IV CRCs.¹¹) These discordant results may be due to several factors, including population variance, different methods of tumour sampling from resection specimens, or possibly differences in diagnostic criteria.

In addition, while others have observed a tendency for even pT1 or pT2 micropapillary carcinomas to spread to lymph nodes,⁶⁻⁷ only one of our pT1 or pT2 cases had nodal metastasis. This may be due to the low numbers of cases in these categories (pT1, n=4; pT2, n=2).

Finally, we found that although patients with MP CRC typically present at advanced AJCC stage, they have similar outcomes compared to patients with conventional CRC. Thus, MP features have a limited impact on patient prognosis after adjusting for the confounding effect of advanced tumour stage.

Other studies comparing MP CRC to conventional CRC have reported somewhat incongruous results, including that patients with Stage I-II MP CRC fare similarly, if not somewhat worse, than those with Stage III-IV disease.⁷ While some authors report that MP features are an independent poor prognostic factor in multivariate analysis,¹⁰ others suggest that they are not.⁹ That MP CRC is more likely to present at an advanced stage seems clear, but given this variability among reported outcome studies, its overall significance with regard to patient prognosis remains somewhat uncertain.

While micropapillary architecture is rather striking and unique, it has a potential histologic mimic in tumour budding,^{5,8} a pattern that has come under increased scrutiny in the past few years. Described as “an isolated single cancer cell or a cluster composed of fewer than five cancer cells,”³⁴ tumour budding is usually present at the invading edge of a carcinoma but has also been reported intratumorally.³⁷ In situations where the budding tumour cells demonstrate eosinophilic cytoplasm or appear to fill lacunae-like spaces due to retraction artefact, the distinction is not always straightforward, as some published photographs of tumour budding illustrate.^{1,38} Moreover, micropapillary features are sometimes present at the leading edge of a tumour as well.¹¹ However, tumour buds by definition emerge from more well-established malignant glands and therefore should lie in close proximity to them, whereas micropapillary clusters are more widely infiltrative and can extend far beyond conventional glandular components in a tumour. Additionally, observation of reversed polarity is a helpful clue in identifying micropapillary features. One study found that MP CRC were more likely than conventional CRC to demonstrate tumour budding.⁷ Also of note is the so-called “poorly differentiated cluster,” a recently described finding in colorectal carcinoma³⁹ with a similar histologic appearance to micropapilla formation. This may in fact represent the same biological phenomenon.³³

Our study also identified a “background” morphology often seen in tumours with micropapillary features. Even well away from micropapillary areas, these tumours were composed of cribriforming malignant glands, often forming large cystic spaces filled with dirty necrotic debris. This morphology tended to persist in nodal and distant metastases, more so even than micropapillary morphology. Micropapillary features are almost always

seen in lymph node metastases from sites such as the breast,⁴⁰ but our findings and those of other researchers show this phenomenon is only sometimes seen in colon tumours.^{5,9} While cribriforming glands and large cystic glands with necrosis can certainly be seen in conventional CRC, the identification of these findings should heighten the search for micropapillary features elsewhere and, if seen on biopsy, may predict their presence on resection. Additionally, somewhat similar features have been reported in microsatellite-stable tumours with CpG island methylation (so-called “cribriform comedo-type adenocarcinomas”),¹ but micropapillary morphology was not mentioned in the original description of this CRC subtype.⁴¹

Given the characteristic morphology of these tumours, both in micropapillary areas and elsewhere, a unique molecular phenotype might be expected. One previous study⁸ analyzing molecular features of 60 MP CRC found a significant increase in *TP53* mutation and a trend toward increased *KRAS* and *BRAFV600E* mutation compared to conventional cases. Additionally, nearly all of their cases were microsatellite-stable or showed low microsatellite instability. The results of our molecular analysis were similar, with 35% of cases harboring *KRAS* mutation, 13% of cases harboring *BRAFV600E* mutation, and no cases showing microsatellite instability. In three tumours, we were able to analyze both micropapillary and cribriform areas, with no difference in molecular phenotype. Considering that *KRAS* and *BRAF* mutations are believed by some to impart a worse prognosis,^{42–43} and microsatellite instability a better prognosis,⁴⁴ the molecular findings in this subtype of CRC are not surprising. Given the current push toward subtyping CRC based on molecular properties in order to optimize therapy and manage prognostic expectations,⁴⁵ it seems likely that additional molecular characterization, potentially with unique defining genetic alterations, will be discovered in this carcinoma subtype in the future. Along these lines, Lee and colleagues¹⁰ observed increased expression of the stem cell markers SOX2 and NOTCH3 in micropapillary colorectal carcinoma.

While micropapillary carcinoma is associated with higher disease stage and increased rates of lymph node metastasis, an underlying mechanism for this behavior has not been identified. One possibility is EMT, which has been associated with increased metastatic potential in other carcinomas. When examined, most of our cases showed loss of epithelial markers (AE1/AE3 and E-cadherin) and gain of mesenchymal markers (vimentin). Most also showed nuclear localization of SMAD4; while this finding is not specific and has been reported in CRC previously,⁴⁶ it suggests the activation of transforming growth factor β signaling, which is known to be an important event promoting EMT.^{31,47–48} Our combined IHC findings indicate that EMT is characteristic of MP CRC and may play a role in its propensity to present with advanced, metastatic disease. Interestingly, EMT also plays a role in tumour budding, which can mimic MP features, as above.²⁴

Colorectal micropapillary carcinoma is an aggressive neoplasm with propensity toward metastasis. These tumours may harbor *KRAS* or *BRAF* mutation and are typically microsatellite-stable. Given the overall poor prognosis of this tumour due to presentation at advanced stage, its recognition is crucial. Further clinical and molecular studies will hopefully implicate additional genetic alterations and refine management of patients with this disease.

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RSG reviewed slides, compiled data, and wrote the manuscript. WJH interpreted immunohistochemical stains for EMT and edited the manuscript. JMMC performed statistical analysis and edited the manuscript. KW provided cases, reviewed selected cases, and edited the manuscript. RDB and RJC performed molecular analysis of some cases. CS initiated the project, reviewed selected cases, and edited the manuscript.

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Figure 1.

Colorectal carcinoma with micropapillary features. **A**, The tumour cells infiltrate as small nests surrounded by retraction artefact, simulating lymphovascular space involvement. The cells have eosinophilic cytoplasm and moderate nuclear atypia. **B**, The micropapillary component was often surrounded by a dense, cellular stromal reaction. **C**, Tumours with a micropapillary component often displayed a characteristic morphology elsewhere, with small and large malignant glands demonstrating cribriforming and prominent luminal dirty necrosis. **D**, Some tumours displayed areas of “pseudo-micropapillary” morphology, with confluence of nests and less retraction artefact. **E**, Some cases demonstrated micropapillae situated in mucin pools rather than in artefactual spaces. **F**, Lymph node metastases occasionally showed micropapillary areas, though they more characteristically displayed large, cystically dilated glands with prominent dirty necrosis; the malignant lining cells resembled micropapillary areas cytologically but not architecturally.

Figure 2. Evidence of epithelial-mesenchymal transition in micropapillary colorectal carcinoma. **A**, Immunohistochemical expression of AE1/AE3 is decreased in some clusters of micropapillary carcinoma cells. **B**, Loss of E-cadherin expression can be seen in some clusters of micropapillary carcinoma cells. **C**, Aberrant vimentin expression is seen in scattered micropapillary carcinoma cells (arrows). **D**, Micropapillary carcinoma cells show nuclear localization of SMAD4.

Figure 3.

Kaplan–Meier disease-specific survival curves for patients with micropapillary colorectal carcinoma and conventional colorectal carcinoma (NOS), by AJCC TNM stage.

Table 1

Clinicopathologic findings in 42 micropapillary colorectal adenocarcinomas and 972 conventional colorectal adenocarcinomas

	Micropapillary (n=42)	Conventional (n=972)	P-value
Patient demographics			
Age at diagnosis, years (mean; range)	60; 43-78	64; 10-93	0.004
Male:female ratio	26:16 (1.63:1)	502:470 (1:07:1)	0.19
Tumour characteristics			
Size of tumours (mean; range)	4.2 cm; 0.9-12.5 cm	4.2 cm; 0.1-18.5 cm	0.44
Lymphovascular invasion	35 (83%)	377/856 (44%)	<0.001
Perineural invasion	7 (17%)	77/855 (9%)	0.13
Peritumoral inflammation	19/41 (46%)	385/851 (45%)	0.89
Pathologic T-category of tumours			0.06
pT1	4 (10%)	101/895 (11%)	
pT2	2 (5%)	159/895 (18%)	
pT3	25 (59%)	397/895 (44%)	
pT4	11 (26%)	238/895 (27%)	
Pathologic N-category of tumours			<0.001
pN0	11 (26%)	558/951 (59%)	
pN1	21 (50%)	248/951 (26%)	
pN2	10 (24%)	145/951 (15%)	
Pathologic M-category of tumours			0.001
pM0	19 (45%)	694/972 (71%)	
pM1	23 (55%)	278/972 (29%)	
Molecular analysis			
<i>KRAS</i> mutation	11/31 (35%)	37/86 (43%)	0.53
<i>BRAF</i> V600E mutation	4/31 (13%)	17/80 (21%)	0.42
<i>SMAD4</i> R361H mutation	2/31 (6%)	None tested	
<i>PIK3CA</i> H1047R mutation	2/31 (6%)	None tested	
<i>NRAS</i> Q61L mutation	1/31 (3%)	None tested	
Microsatellite instability	0/21 (0%)	82/294 (28%)	0.003
Patient follow-up			
Follow-up length, months (median; range)	36; 1-133	47; 1-303	
Patients alive at last follow-up	18 (43%)	391/869 (45%)	
Patients dead of disease	17 (40%)	263/869 (30%)	
Patients dead of other/unknown causes	7 (17%)	215/869 (25%)	

Table 2

Prognostic factors among all cases of colorectal carcinoma

All cases (n=1014)	Univariate analysis		Multivariate analysis	
	Hazard ratio (95% CI)	P-value	Hazard ratio (95% CI)	P-value
Micropapillary subtype	1.43 (0.88-2.34)	0.15	0.71 (0.43-1.18)	0.19
Male sex	0.88 (0.69-1.11)	0.28	0.64 (0.49-0.82)	<0.001
Age	1.00 (0.99-1.00)	0.35	1.01 (1.00-1.03)	0.005
Size	1.12 (1.07-1.16)	<0.001	1.12 (1.07-1.18)	<0.001
Lymphovascular invasion	3.24 (2.51-4.17)	<0.001	1.77 (1.31-2.38)	<0.001
Perineural invasion	2.19 (1.57-3.06)	<0.001	1.12 (0.78-1.60)	0.54
Peritumoral inflammation	0.41 (0.31-0.53)	<0.001	0.65 (0.49-0.86)	0.003
AJCC T-category (reference: pT1)				
pT2	2.13 (1.05-5.09)	0.037	n/a	n/a
pT3	3.95 (1.92-8.11)	<0.001		
pT4	11.72 (5.73-23.94)	<0.001		
AJCC N-category (reference: pN0)				
pN1a	3.18 (2.22-4.54)	<0.001	n/a	n/a
pN1b	3.21 (2.27-4.54)	<0.001		
pN2a	6.45 (4.47-9.30)	<0.001		
pN2b	7.79 (5.35-11.34)	<0.001		
Distant metastases	17.73 (12.92-24.33)	<0.001	n/a	n/a
Overall AJCC stage (reference: stage I)				
Stage II	3.09 (0.85-11.24)	0.086	1.91 (0.51-7.11)	0.34
Stage III	12.05 (3.69-39.36)	<0.001	7.15 (2.14-23.86)	0.001
Stage IV	92.73 (29.65-290.03)	<0.001	57.10 (18.02-180.93)	<0.001

n/a indicates not applicable to the multivariable analysis.