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Clinicopathological Significance and Diagnostic Accuracy of c-MET Expression by Immunohistochemistry in Gastric Cancer: A Meta-Analysis

Jung-Soo Pyo*, Guhyun Kang[1]*, and Hyunjin Cho[2]

Department of Pathology, Kangbuk Samsung Hospital, Sungkyunkwan University School of Medicine, Departments of [1]Pathology and [2]Surgery, Inje University Sanggye Paik Hospital, Seoul, Korea

Purpose: The aim of the present study was to elucidate the clinicopathological significance and diagnostic accuracy of immunohistochemistry (IHC) for determining the mesenchymal epidermal transition (c-MET) expression in patients with gastric cancer (GC).

Materials and Methods: The present meta-analysis investigated the correlation between c-MET expression as determined by IHC and the clinicopathological parameters in 8,395 GC patients from 37 studies that satisfied the eligibility criteria. In addition, a concordance analysis was performed between c-MET expression as determined by IHC and *c-MET* amplification, and the diagnostic test accuracy was reviewed.

Results: The estimated rate of c-MET overexpression was 0.403 (95% confidence interval [CI], 0.327~0.484) and it was significantly correlated with male patients, poor differentiation, lymph node metastasis, higher TNM stage, and human epidermal growth factor receptor 2 (HER2) positivity in IHC analysis. There was a significant correlation between c-MET expression and worse overall survival rate (hazard ratio, 1.588; 95% CI, 1.266~1.992). The concordance rates between c-MET expression and *c-MET* amplification were 0.967 (95% CI, 0.916~0.987) and 0.270 (95% CI, 0.173~0.395) for cases with non-overexpressed and overexpressed *c-MET*, respectively. In the diagnostic test accuracy review, the pooled sensitivity and specificity were 0.56 (95% CI, 0.50~0.63) and 0.79 (95% CI, 0.77~0.81), respectively.

Conclusions: The c-MET overexpression as determined by IHC was significantly correlated with aggressive tumor behavior and positive IHC status for HER2 in patients with GC. In addition, the c-MET expression status could be useful in the screening of *c-MET* amplification in patients with GC.

Key Words: Stomach neoplasms; c-MET; Immunohistochemistry; Meta-analysis; Diagnostic test accuracy review

Introduction

Gastric cancer (GC) is one of the most common malignant cancers, and gastric tumors are biologically and genetically heterogeneous.[1-3] Several biological markers including human epidermal growth factor receptor 2 (HER2), p53, forkhead box O1A, E-cadherin, vascular epidermal growth factor receptor, and mesenchymal epidermal transition (c-MET) receptor, have been introduced.[4-9] Various therapies that use these biological markers have been developed, resulting in improved treatment outcomes.[9] Among these markers, the reported rates for *MET* overexpression and MET amplification are 4% to 98% and 1.5% to 59.0%, respectively.[9] These broad ranges could possibly be attributed to a variety of reasons. For example, variable antibody clones and evaluation criteria have been used to determine the expression of c-MET by immunohistochemistry (IHC) analysis

Correspondence to: Hyunjin Cho
Department of Surgery, Inje University Sanggye Paik Hospital, 1342 Dongil-ro, Nowon-gu, Seoul 01757, Korea
Tel: +82-2-950-1017, Fax: +82-2-951-9389
E-mail: yourdoc@paik.ac.kr
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*These authors contributed equally to this study as co-first authors.

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in different patient populations. In addition, various molecular tests, such as in situ hybridization (ISH), quantitative real-time polymerase chain reaction, and next generation sequencing, have been used to confirm the genetic alterations in *c-MET*, which are mostly in the form of *c-MET* gene amplification. The ligand for c-MET, which is a tyrosine kinase receptor, is the hepatocyte growth factor. Upon binding of its ligand, c-MET activates downstream signaling pathways such as the ras sarcoma/effector of ras/mitogen activated protein kinase and the phosphatidylinositol 3-kinase/AKT/mechanistic target of rapamycin pathways.[10,11] Consequently, the abnormal or aberrant activation of c-MET signaling results in tumor cell growth, survival, migration, invasion, and tumor angiogenesis.[10]

Accurate evaluation tests and well-defined detailed criteria are required for the appropriate selection of patients that can benefit from targeted molecular therapies. Although molecular tests, including ISH or quantitative real-time polymerase chain reaction, are more accurate and confirmatory methods for detecting gene alterations, they have several disadvantages including high costs, the requirement for multiple steps, and time consumption compared to IHC. In daily practice, cheap, simple, and popular methods, such as IHC, are ideal for screening tests. However, unlike HER2, the evaluation criteria for c-MET expression as determined by IHC have not been fully elucidated in patients with GC.[12]

We performed a systematic review and meta-analysis to elucidate the correlation between the overexpression of c-MET as determined by IHC and the clinicopathological parameters. In addition, the diagnostic accuracy of IHC was investigated using concordance analysis and performing a review of the diagnostic test accuracy.

Materials and Methods

1. Published study search and selection criteria

Articles relevant to the subject of the analysis were obtained by searching the PubMed and MEDLINE databases throughout January 31, 2016 using the following key words: 'MET' or 'mesenchymal epithelial transition' and 'IHC'. The titles and abstracts of all the searched articles were screened for exclusion. The review articles were also screened to identify additional eligible studies. Subsequently, the search results were reviewed and studies were included in the analyses if (1) the study was performed in human cases of GC and (2) information about the correlation between c-MET expression as determined by IHC and clinicopathological parameters, and the amplification of the *c-MET* gene was available. The articles were excluded if (1) they were case reports or non-original articles or (2) they were published in a language other than English.

2. Data extraction

The data from all eligible studies[13-49] were extracted by two independent authors and the extracted data were the first author's name, year of publication, study location, antibody clone and manufacturer, antibody dilution ratio, evaluation criteria, number of patients analyzed, and the data allowing the estimation of the impact of c-MET overexpression as determined by IHC on overall survival (OS). For the meta-analysis, we extracted all the data associated with the results of the IHC analyses.

3. Statistical analysis

For the meta-analysis, all data were analyzed using the Comprehensive Meta-Analysis software package (Biostat, Englewood, NJ, USA). We investigated the correlation between the overexpression of c-MET as determined by IHC and clinicopathological parameters such as sex, tumor differentiation, HER2 positivity by IHC, primary tumor (T) stage, regional lymph node (N) stage, and distant metastasis (M) stage. The concordance rates were determined according to the agreement rates between the expression of c-MET as determined by IHC and the mutation tests. For the quantitative aggregation of survival results, the correlation between the overexpression of c-MET as determined by IHC and OS was analyzed based on the hazard ratios (HRs) that were obtained using one of three available methods. For studies lacking information on the HR or its confidence interval (CI), these variables were calculated from the presented data using the HR point estimate, log-rank statistic or its P-value, and the O-E statistic (the difference between the number of observed and expected events) or its variance. If data on the HR values were not available, they were estimated using the total number of events, number of patients at risk in each group, and the log-rank statistic or its P-value. Finally, if useful data were provided only in the form of graphical illustrations of survival distributions and survival rates, these data were extracted at specified time points to reconstruct the HR estimate and its variance under the assumption that the patients were censored at a constant rate during the time intervals.[50] The published survival curves were read independently by two authors

Table 1. Main characteristics of the eligible studies

Author (year)	Location	Clone	Corporation	Criteria	c-MET by IHC		c-MET by GA		
					N	OE	N	GA	Method
Betts et al. (2014)[13]	UK	ND	Invitrogen	>10%, ≥mod	174	7			
Catenacci et al. (2011)[14]	USA	ND	Zymed	≥mod	35	15			
Choi et al. (2014)[15]	Korea	SP44	Ventana	HercepTest	535	128	521	72	SISH
Drebber et al. (2008)[16]	Germany	ND	Novacastra	>30%	114	84			
Fuse et al. (2016)[17]	Japan	SP44	Ventana	>50%	293	120			
Guo et al. (2013)[18]	China	ND	ND	>50%	98	58			
Ha et al. (2015)[19]	Korea	SP44	Ventana	HercepTest	1,588	150			
Han et al. (2005)[20]	Korea	C-28	Santa Cruz Biotech.	ND	50	39			
Heideman et al. (2001)[21]	Netherlands	ND	Upstate Biotech	Combination	43	30			
Huang et al. (2001)[22]	Taiwan	C-28	Santa Cruz Biotech.	>5%	45	32			
Janjigian et al. (2011)[23]	USA	C-12	Santa Cruz Biotech.	>25%, ≥mod	38	24	38	0	PCR
Kang et al. (2013)[24]	Korea	SP44	Ventana	>10%, ≥mod	159	56			
Kijima et al. (2002)[25]	Japan	C-28	Santa Cruz Biotech.	>5%	61	25	61	19	PCR
Koh et al. (2016)[26]	Korea	SP44	Ventana	>10%, ≥weak	331	175			
Kubicka et al. (2002)[27]	Germany	C-12	Santa Cruz Biotech.	>10%	42	11			
Kuboki et al. (2016)[28]	Japan	SP44	Ventana	≥strong	121	12	121	8	NGS
Kurokawa et al. (2014)[29]	Japan	SP44	Ventana	>50%, ≥mod	153	38			
Lee et al. (2011)[30]	Korea	24H2	Cell Signaling Tech	>5%	452	179	452	96	FISH
Lee et al. (2013)[31]	Korea	SP44	Ventana	>50%, ≥mod	495	9			
Li et al. (2012)[32]	China	ND	Santa Cruz Biotech.	Combination*	114	94			
Liu et al. (2014)[33]	China	SP44	Ventana	HercepTest	212	26	196	12	FISH
Ma et al. (2013)[34]	China	ND	Santa Cruz Biotech.	Combination*	436	191			
Metzger et al. (2016)[35]	Germany	SP44	Spring bioscience	Combination*	470	55	470	13	CISH
Nagatsuma et al. (2015)[36]	Japan	SP44	Ventana	>10%	713	237			
Nakajima et al. (1999)[37]	Japan	C-28	Santa Cruz Biotech.	>5%	128	59			
Paliga et al. (2015)[38]	Canada	3D4	Invitrogen	>10%, ≥mod	113	65			
Peng et al. (2015)[39]	China	MET4	Dako	>25%	137	53	113	8	FISH
Retterspitz et al. (2010)[40]	Germany	C-28	Santa Cruz Biotech.	>50%	94	47			
Sotoudeh et al. (2012)[41]	Iran	ND	Novacastra	≥strong	124	88			
Sun et al. (2005)[42]	China	ND	Novacastra	>30%	45	32			
Tang et al. (2004)[43]	China	C-28	Santa Cruz Biotech.	>10%	215	148			
Taniguchi et al. (1998)[44]	Japan	C-28	ND	ND	102	43			
Wu et al. (2014)[45]	China	D1C2	Cell Signaling Tech.	>5%	121	80			
Yun et al. (2015)[46]	China	ND	Santa Cruz Biotech.	>90%, ≥strong	161	30			
Zhang et al. (2014)[47]	China	SP44	Roche	>10%, ≥weak	154	68			
Zhao et al. (2011)[48]	China	ND	Beijing Zhongshan Goldenbridge Biotech	>1%, ≥weak	182	120			
Zhu et al. (2015)[49]	China	SP44	Ventana	>10%, ≥mod	47	13	47	4	FISH

IHC = immunohistochemistry; N = number of patients; OE = overexpression; GA = genetic alteration; ND = no description; mod = moderate intensity; SISH = silver in situ hybridization; PCR = polymerase chain reaction; weak = weak intensity; strong = strong intensity; NGS = next-generation sequencing; FISH = fluorescence in situ hybridization; CISH = chromogenic in situ hybridization. *The combination of intensity and fraction scores.

in order to reduce the interpretation bias. Subsequently, the HRs were combined into an overall HR using the Peto method.[51] Because eligible studies used various clones of the c-MET antibody and different evaluation criteria for various patient populations, a random-effects model was more suitable than a fixed-effects model. The heterogeneity between the studies was assessed using the Q and I^2 statistics, and the results were presented as P-values. Additionally, sensitivity analysis was performed to assess the heterogeneity of eligible studies and the impact of each study on the combined effect. In order to assess the publication bias, a Begg funnel plot and Egger test were used. If a significant publication bias was identified, the fail-safe N and trim-fill tests were performed to confirm the degree of publication bias. P<0.05 were considered to be statistically significant.

The review of diagnostic test accuracy was performed using the Meta-Disc program version 1.4 (Unit of Clinical Biostatics; the Ramon y Cajal Hospital, Madrid, Spain).[52] In order to calculate the pooled sensitivity and specificity, data were collected from each eligible study and forest plots were obtained. The summary receiver operating characteristic (SROC) curve was initially constructed by plotting 'sensitivity' and '1-specificity' of each study, and curve fitting was performed through linear regression using the Littenberg and Moses linear models.[53] Because the data were heterogeneous owing to differences in the evaluation criteria, the accuracy data were pooled by fitting a SROC curve and measuring the value of the area under the curve (AUC).[52] An AUC close to 1 would be considered a perfect fit and an AUC close to 0.5 would be considered a poor fit. In addition, the diagnostic odds ratio (OR) was calculated using the Meta-Disc program.

Results

1. Study selection and characteristics

In total, 3,010 reports were identified in the database search for this systematic review and meta-analysis. Among them, 77 were excluded owing to insufficiency or lack of information on the correlation between c-MET overexpression as determined by IHC and the clinicopathological parameters, and the amplification of *c-MET*. In addition, 2,876 reports were excluded because they were concerning other diseases, or they used animals or cell lines, and 20 were excluded because they were articles written in a language other than English or they were non-original articles. Finally, 37 studies were included in this systematic review and meta-analysis (Table 1, Fig. 1). The total number of patients from the 37 studies was 8,395. Table 1 shows the different clones of c-MET antibody and the evaluation criteria used in each study. The rate of c-MET overexpression as determined by IHC was 1.8% to 82.5% and the overall c-MET overexpression rate as determined by IHC was 31.5% (2,641 out of 8,395

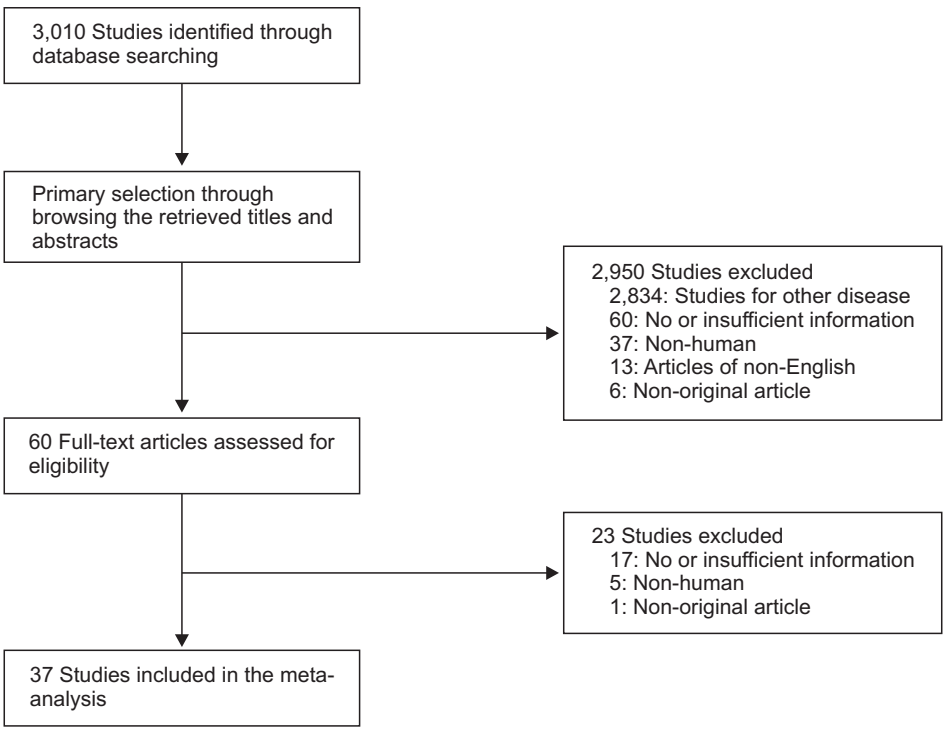

Fig. 1. Flowchart of the study search process and the selection methods.

patients) in the present study.

2. Clinicopathological significance of c-MET overexpression as determined by immunohistochemistry

The estimated c-MET overexpression rate as determined by IHC was 0.403 (95% CI, 0.327~0.484) (Table 2). The c-MET overexpression rates in male and female patients were 0.706 (95% CI, 0.668~0.741) and 0.491 (95% CI, 0.357~0.627), respectively. A significant correlation was identified between c-MET overexpression as determined by IHC and poor tumor differentiation. The c-MET overexpression rate was significantly higher in the HER2 positive GCs than in HER2 negative GCs (0.349; 95% CI, 0.183~0.563 vs. 0.148; 95% CI, 0.074~0.275). There was a significant correlation between c-MET overexpression and N stage and TNM stage; however, the T and M stages showed no correlation. In the assessment of publication bias, the Egger test and Begg funnel plots showed a significant publication bias for male patients and TNM III~IV stages (P=0.004 and P=0.021, respectively). To confirm the degree of publication bias for male patients and TNM III~IV stages, trim-fill and fail-safe N tests were conducted. In both groups, the publication biases were not large and the remaining groups did not show significant publication biases.

Next, we investigated the correlation between c-MET overexpression as determined by IHC and OS rate. The estimated HR was 1.588 (95% CI, 1.266~1.992) and the c-MET overexpression was significantly correlated with worse OS rate (Table 3). Because there was a significant heterogeneity (P<0.001), subgroup analysis would be needed to identify the cause of the heterogeneity. First, subgroup analysis was performed based on the year of publication divided by published year after 2012 and before 2011. The HRs calculated for studies after 2012 and before 2011 were 1.397 (95% CI, 1.067~1.829) and 2.111 (95%

Table 2. Meta-analysis of the c-MET overexpression as determined by immunohistochemistry in gastric carcinoma

Variable	No. of subset	Fixed effect (95% CI)	Heterogeneity test (P-value)	Random effect (95% CI)	Egger test
Overall overexpression rate	37	0.365 (0.353~0.377)	<0.001	0.403 (0.327~0.484)	0.171
Sex*					
Men	15	0.691 (0.666~0.716)	0.028	0.706 (0.668~0.741)	0.004
Women	15	0.440 (0.407~0.474)	<0.001	0.491 (0.357~0.627)	0.297
Tumor differentiation*					
Well/moderately	11	0.589 (0.543~0.633)	<0.001	0.545 (0.416~0.668)	0.297
Poorly/undifferentiated	11	0.557 (0.519~0.595)	<0.001	0.621 (0.501~0.728)	0.085
HER2 status*					
HER2 positive	7	0.318 (0.263~0.379)	<0.001	0.349 (0.183~0.563)	0.698
HER2 negative	6	0.126 (0.111~0.142)	<0.001	0.148 (0.074~0.275)	0.499
Tumor depth (T)†					
T1~2	14	0.463 (0.430~0.497)	<0.001	0.449 (0.341~0.561)	0.971
T3~4	14	0.508 (0.477~0.539)	<0.001	0.511 (0.360~0.660)	0.962
Regional lymph node (N)*,†					
N0	16	0.373 (0.343~0.403)	<0.001	0.420 (0.304~0.545)	0.249
≥N1	16	0.452 (0.426~0.479)	<0.001	0.539 (0.400~0.672)	0.053
Distant metastasis (M)†					
M0	4	0.701 (0.646~0.750)	<0.001	0.654 (0.452~0.812)	0.310
M1	4	0.685 (0.547~0.797)	0.022	0.695 (0.436~0.871)	0.769
Stage*,†					
I~II	12	0.385 (0.357~0.414)	<0.001	0.457 (0.319~0.601)	0.198
III~IV	12	0.436 (0.388~0.737)	<0.001	0.571 (0.388~0.737)	0.021

CI = confidence interval; HER2 = human epidermal growth factor receptor 2. *There was a significant difference between the two groups (P<0.05). †TNM stage was based on the American Joint Committee on Cancer staging system.

Table 3. Meta-analysis of the correlation between c-MET overexpression as determined by immunohistochemistry and the survival rate of patients with gastric cancer

Variable	No. of subset	Fixed effect (95% CI)	Heterogeneity test (P-value)	Random effect (95% CI)	Egger test
Overall survival	19	1.425 (1.289~1.575)	<0.001	1.588 (1.266~1.992)	0.059
After 2012	11	1.303 (1.166~1.456)	<0.001	1.397 (1.067~1.829)	0.291
Before 2011	7	2.104 (1.669~2.652)	0.328	2.111 (1.632~2.730)	0.376
Region group					
Asian	14	1.293 (1.155~1.447)	<0.001	1.488 (1.165~1.900)	0.015
Non-Asian	5	2.068 (1.660~2.577)	0.008	1.866 (1.196~2.912)	0.379
Criteria					
Low criteria (<25%)	11	1.408 (1.232~1.608)	<0.001	1.566 (1.073~2.284)	0.301
High criteria (≥25%)	4	1.349 (1.133~1.607)	0.202	1.416 (1.117~1.794)	0.096
Others	4	1.793 (1.323~2.429)	0.646	1.793 (1.323~2.429	0.037

CI = confidence interval.

Table 4. Analysis of the concordance between c-MET expression as determined by IHC and the confirmatory methods for *c-MET* amplification in patients with gastric cancer

Variable	No. of subset	Fixed effect (95% CI)	Heterogeneity test (P-value)	Random effect (95% CI)	Egger test
Overall	18	0.608 (0.572~0.643)	<0.001	0.739 (0.531~0.876)	0.624
Low criteria (<25%)	10	0.636 (0.598~0.673)	<0.001	0.761 (0.507~0.908)	0.670
High criteria (≥25%)	4	0.212 (0.121~0.346)	<0.001	0.623 (0.042~0.984)	0.380
Others	4	0.572 (0.456~0.680)	<0.001	0.816 (0.199~0.988)	0.570
Non-overexpression by IHC	9	0.872 (0.847~0.894)	<0.001	0.967 (0.916~0.987)	0.021
Low criteria (<25%)	5	0.858 (0.828~0.882)	<0.001	0.949 (0.850~0.984)	0.208
High criteria (≥25%)	2	0.984 (0.892~0.998)	0.480	0.984 (0.892~0.998)	-
Others	2	0.959 (0.914~0.981)	0.009	0.990 (0.684~1.000)	-
Overexpression by IHC	9	0.266 (0.227~0.309)	<0.001	0.270 (0.173~0.395)	0.937
Low criteria (<25%)	5	0.295 (0.248~0.347)	<0.001	0.367 (0.212~0.554)	0.362
High criteria (≥25%)	2	0.117 (0.061~0.213)	0.176	0.082 (0.016~0.334)	-
Others	2	0.225 (0.141~0.341)	0.602	0.225 (0.141~0.341)	-

IHC = immunohistochemistry; CI = confidence interval.

CI, 1.632~2.730), respectively. In addition, the subgroup analysis performed based on the study location showed that the HRs for the Asian and non-Asian subgroups were 1.488 (95% CI, 1.165~1.900) and 1.866 (95% CI, 1.196~2.912), respectively. The HRs for subgroups formed based on low (<25%) and high (≥ 25%) evaluation criteria for c-MET expression as determined by IHC were 1.566 (95% CI, 1.073~2.284) and 1.416 (95% CI, 1.117~1.794), respectively. In addition, there were no significant publication biases in the primary (Begg funnel plot and Egger test) and secondary (fail-safe N and trim-fill) tests.

3. Diagnostic accuracy of c-MET immunohistochemistry

In order to evaluate the diagnostic role of the *c-MET* in predicting c-MET amplification, a concordance analysis was performed. The overall concordance rates between c-MET expression level as determined by IHC and *c-MET* amplification was 0.739 (95% CI, 0.531~0.876; Table 4). In addition, the concordance rates of the non-overexpressed and overexpressed c-MET cases were 0.967 (95% CI, 0.916~0.987) and 0.270 (95% CI, 0.173~0.395), respectively. Subgroup analysis was performed based on the evaluation criteria for c-MET expression. The c-MET evaluation criteria were subdivided into low staining (<25%),

Table 5. The sensitivity, specificity, diagnostic OR, and AUC of the summary receiver operation characteristics curve according to the evaluation criteria used in the assessment of c-MET expression as determined by immunohistochemistry

Variable	Sensitivity (95% CI)	Specificity (95% CI)	Diagnostic OR (95% CI)	AUC
Overall	0.56 (0.50~0.63)	0.79 (0.77~0.81)	14.52 (3.43~61.38)	0.878
Low criteria	0.53 (0.46~0.60)	0.76 (0.73~0.78)	11.99 (2.04~70.47)	0.892
High and other criteria	0.79 (0.60~0.92)	0.85 (0.82~0.87)	22.08 (1.40~347.21)	0.899

OR = odds ratio; AUC = area under curve; CI = confidence interval.

high (≥25%) staining, and other. In cases showing c-MET non-overexpression, there were no significant differences between the subgroups of the evaluation criteria (low evaluation criteria: 0.949; 95% CI, 0.850~0.984 vs. high evaluation criteria: 0.984; 95% CI, 0.892~0.998 vs. other: 0.990, 95% CI, 0.684~1.000). However, in cases showing c-MET overexpression, the concordance rate of the high evaluation criteria subgroup (0.082; 95% CI, 0.016~0.334) was significantly lower than that of the other subgroups. There was no significant publication bias in the primary and secondary tests.

Next, a diagnostic accuracy test review was performed. In all cases, the pooled sensitivity and specificity were 0.56 (95% CI, 0.50~0.63) and 0.79 (95% CI, 0.77~0.81), respectively (Table 5). The sensitivity and specificity of the eligible studies were 0.25 to 1.00 and 0.53 to 0.91, respectively. The diagnostic OR and AUC on the SROC curve for all cases were 14.52 (95% CI, 3.43~61.38) and 0.878, respectively. According to the evaluation criteria, the subgroups were subdivided into low and high evaluation criteria and the remaining subgroups. In the subgroup showing low evaluation criteria, the pooled sensitivity and specificity were 0.53 (95% CI, 0.46~0.60) and 0.76 (95% CI, 0.73~0.78), respectively. The diagnostic OR and AUC on the SROC curve of the low evaluation criteria subgroup were 11.99 (95% CI, 2.04~70.47) and 0.892, respectively. In the others subgroup, all parameters of the diagnostic accuracy test review were higher than those of the low evaluation criteria subgroup. The pooled sensitivity and specificity, the diagnostic OR and AUC on the SROC curve of the other subgroup were 0.79 (95% CI, 0.60~0.92), 0.85 (95% CI, 0.82~0.87), 22.08 (95% CI, 1.40~347.21), and 0.899, respectively.

Discussion

Many preclinical and clinical studies have reported the effectiveness of various c-MET inhibitors in the treatment of GC. Although the effectiveness of c-MET was shown in preclinical studies, its effectiveness in clinical trials is controversial. Before the evaluation of the effectiveness of c-MET inhibitors, the confirmation of evaluation criteria for c-MET expression as determined by IHC expression and gene amplification were required. This study is the first meta-analysis to assess the clinicopathological significance and diagnostic accuracy of c-MET expression in patients with GC. The present study reported 4 major findings. First, the estimated overexpression rate of c-MET was 40.3% in patients with GC. Second, a higher overexpression rate of c-MET was significantly correlated with HER2 positivity, higher TNM stage, and worse OS rate. Third, the expression level of c-MET was in concordance with the *c-MET* gene amplification in c-MET non-overexpressed cases, but not in the c-MET overexpressed cases. Fourth, there was no difference between the diagnostic accuracy of IHC and molecular testing.

The assessment of the eligible studies showed that the rate of c-MET overexpression was 1.8% to 82.5%. The overall rate of overexpression for c-MET was 31.5% and the estimated overexpression rate was 40.3%. In the present meta-analysis, c-MET overexpression was significantly correlated with the male sex, poor differentiation, regional lymph node metastasis, and higher TNM stage. Nevertheless, there was a positive correlation between the overexpression of c-MET, and tumor depth and distant metastasis without statistical significance. However, previous studies have reported various correlations between the c-MET status and clinicopathological parameters; these studies used various processing protocols, antibody clones, and different evaluation criteria for c-MET.[13-49] These discrepancies could influence the clinicopathological significance of c-MET expression as determined by IHC. To obtain the confirmatory information for c-MET, systematic review and meta-analysis for pathological validation was required.

In the eligible studies, the rates of c-MET overexpression and *c-MET* amplification were 31.5% (2,641 out of 8,395 patients) and 11.5% (232 out of 2,019 patients), respectively. There

was a significant discrepancy between c-MET overexpression and *c-MET* amplification. There could be a variety of reasons that could explain this discrepancy. As described above, various antibody clones and evaluation criteria were used for the evaluation of c-MET expression. Indeed, the included patient population could have affected this discrepancy. In addition, the false positive c-MET expression could be one of the important causes of discrepancy. Therefore, concordance analysis and the review of the diagnostic test accuracy are required to confirm this discrepancy. In the present study, the overall concordance rate between c-MET overexpression and *c-MET* amplification was 0.739 (95% CI, 0.531~0.876). However, the concordance rate of non-expressed c-MET cases was significantly higher than that of overexpressed cases (0.967; 95% CI, 0.916~0.987), nearing 1. The discordance of positive rates between c-MET overexpression and *c-MET* amplification might be caused by false positive cases. According to our results, c-MET could be useful for the screening of *c-MET* amplification, similar to HER2 for stomach cancer.[12] However, further studies should be conducted to determine the accurate evaluation criteria to reduce the false positive rate.

Interestingly, in the non-overexpressed c-MET cases, the concordance rate of eligible studies with low staining was lower than that of eligible studies with high staining. In our unpublished data for non-small cell lung cancer, the concordance rate between c-MET expression and *c-MET* amplification was lower in the low expression group than in the high criteria group. In the present diagnostic test accuracy review, the estimates of the studies with low criteria for diagnostic accuracy were lower than that of studies with other criteria, as shown Table 5. In addition, in clinical trials with patients classified according to their c-MET expression status, rilotumumab showed a therapeutic effect for GC.[54] In that study, c-MET overexpression was evaluated at >25% of membrane staining and any intensity. Because the differences of evaluation criteria might have had an impact on the selected patients and the results for therapeutic effect, more detailed and accurate criteria for c-MET expression are required.

Previously, two studies reported a correlation between c-MET and survival rate through meta-analysis. However, the HRs between studies differed, 1.66 (95% CI, 1.17~2.36) and 2.42 (95% CI, 1.66~3.54), respectively.[55,56] Each meta-analysis included 9 eligible studies. Among eligible studies of two meta-analysis, 8 eligible studies that were identical. However, the estimated HRs of some studies differed between two meta-analyses and the largest difference reported was 1.55. However, the definitive difference between the extracting methods for survival data could not be found. The present meta-analysis included eligible studies from two previous meta-analyses. In the present meta-analysis, c-MET overexpression was significantly correlated with a poor OS rate (HR, 1.588; 95% CI, 1.266~1.992). The eligible studies differed in their follow-up periods. In the present meta-analysis, to avoid bias from the follow-up periods, survival data were extracted after a 60-month follow-up period. Although the follow-up period did not influence the correlation between c-MET overexpression and survival, the correlation between c-MET and survival differed from those in previous reports. Interestingly, the HRs of eligible studies before 2011 were higher than those of eligible studies after 2012 year. However, the reason for the differences associated with the study year could not be elucidated. Indeed, there were no differences between the c-MET overexpression, study location, and evaluation criteria.

The coexpression of HER2 and c-MET was found in 12% of the GCs.[27] Previous studies have reported that c-MET activation was associated with the resistance against molecular targeted inhibitory therapy for epidermal growth factor receptor.[57-59] Chen et al.[60] has reported that GC cells can evade lapatinib-induced growth inhibition through the activation of MET and reactivation of the downstream signaling pathways. However, a synergistic effect by the dual inhibition of HER2 and MET was not found in GC cells.[60] They concluded that dual inhibition is not required until the development of resistance.[60] In our meta-analysis, the overexpression rate of c-MET in HER2 positive cases was significantly higher than that of the HER2 negative cases (0.349; 95% CI, 0.183~0.563 vs. 0.148; 95% CI, 0.074~0.275, respectively). This result could be useful for elucidating the correlation between c-MET and HER2 and for appropriate patient selection for HER2 or MET monoclonal antibody therapy. In addition, further cumulative studies are required to confirm the detailed mechanism.

There were some limitations to the current meta-analysis. First, as described above, eligible studies used various antibody clones and evaluation criteria for evaluating the expression status of c-MET. Additional subgroup analysis based on antibody clones could not be performed due to insufficient information. However, in sensitivity analysis, individual studies had no effects on the pooled estimates. Second, as a confirmatory test for *c-MET* amplification, various molecular tests, such as fluorescence

ISH, silver ISH, chromogenic ISH, polymerase chain reaction, and next generation sequencing, were used. However, the diagnostic accuracy of c-MET IHC analysis according to the molecular test could not be evaluated due to insufficient information. Third, as described above, the concordance rate of low criteria group between c-MET expression and *c-MET* amplification was lower than that of other criteria groups. However, 2 of the eligible studies were using high and other criteria, respectively. Because the minimum number of included studies was 3 for the diagnostic test accuracy review, a subgroup analysis of the high and other criteria could not be performed. In order to evaluate the diagnostic accuracy of the low criteria for c-MET expression, we subdivided and compared the low and others subgroups.

In conclusion, this study showed that the overexpression of c-MET significantly correlated with HER2 positivity, higher TNM stage, and worse OS rate. The cases having non-overexpressed c-MET were in accordance with cases having *c-MET* gene amplification. The IHC analysis of c-MET expression could be useful for predicting prognosis and screening *c-MET* gene amplification in GCs.

Conflicts of Interest

No potential conflict of interest relevant to this article was reported.

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