Annals of Epidemiology 26 (2016) 500–503

Contents lists available at ScienceDirect

Annals of Epidemiology

journal homepage: www.annalsofepidemiology.org

Original article

Prognostic impact of tumor MET expression among patients with stage IV gastric cancer: a Danish cohort study

Rune Erichsen MD, PhD [a,*], Michael A. Kelsh PhD, MPH [b], Kelly S. Oliner PhD [c], Karsten B. Nielsen MSc, PhD [d], Trine Frøslev MSc [a], Anne-Vibeke Lænkholm MD [e], Mogens Vyberg MD [f], John Acquavella MD, PhD [a,b], Henrik Toft Sørensen MD, PhD, DrMedSci [a]

[a] Department of Clinical Epidemiology, Aarhus University Hospital, Aarhus N, Denmark
[b] Center for Observational Research, Amgen Inc., Thousand Oaks, CA
[c] Medical Sciences, Amgen Inc., Thousand Oaks, CA
[d] Dako Denmark A/S, Glostrup, Denmark
[e] Department of Pathology, Slagelse Hospital, Slagelse, Denmark
[f] Institute of Pathology, Aalborg University Hospital, Aalborg, Denmark

ARTICLE INFO

Article history:
Received 27 September 2015
Accepted 1 May 2016
Available online 12 May 2016

Keywords:
Cohort studies
Mortality
Prevalence
Proto-oncogene proteins c-met
Stomach neoplasia

ABSTRACT

Purpose: We aimed to investigate the prevalence and prognostic impact of tumor mesenchymal epithelial transition factor (MET) expression in stage IV gastric cancers in a real-world clinical setting because existing evidence is sparse.
Methods: The study included archived cancer specimens from 103 stage IV gastric cancer patients (2003–2010). We analyzed MET-protein expression by immunohistochemistry (MET-positive if ≥25% of tumor cells showed MET expression). We calculated overall survival using the Kaplan–Meier method and hazard ratios comparing mortality among MET-positive and MET-negative patients using Cox regression adjusted for age, gender, and comorbidity.
Results: We found that 62.1% (95% confidence interval, 52.0–71.5) of patients had MET-positive tumors. Median survival was lower among patients with MET-positive tumors (3.5 months) than among patients with MET-negative tumors (9.6 months), corresponding to an adjusted hazard ratio of 2.2 (95% confidence interval, 1.3–3.7).
Conclusions: Tumor MET expression is prevalent and has substantial prognostic impact in stage IV gastric cancer patients.

Introduction

Mesenchymal epithelial transition factor (*MET*) is a proto-oncogene that functions as a tyrosine kinase receptor [1]. The hepatocyte growth factor/scatter factor is the sole ligand of the MET receptor and initiates several biological responses that can result in invasive growth. Abnormal MET receptor activation may impair gastric cancer prognosis [2–8] by initiating several biological responses including proliferation, invasion, and antiapoptosis [9,10]. This knowledge highlights the potential of finding treatments that prevent the binding of hepatocyte growth factor/scatter factor to its receptor MET for patients with advanced stage gastric cancer [11] but also further insight into the prevalence and prognostic impact of MET expression in these patients. However, existing studies report rather large variation in prevalence of MET expression (26% to 82%) and its prognostic impact [12]. This variation is likely caused by different study populations with mixed-stage gastric cancers, varying methodologies for evaluating MET expression, and the application of different criteria to define MET expression. In addition, a newly optimized MET assay used in several clinical trial settings has not been evaluated previously in a population-based patient sample [11].

Given this background, we investigated the prevalence and prognostic impact of tumor MET expression in stage IV gastric cancers in a real-world clinical setting. We also explored the prognostic impact of changing the definitions of tumor MET expression.

Material and methods

We conducted this cohort study in the catchment area of Aalborg University Hospital in the North Denmark Region (600,000

* Corresponding author. Department of Clinical Epidemiology, Aarhus University Hospital, Olof Palmes Allé 43-45, 8200 Aarhus N, Denmark. Tel.: +45-87168231; fax: +45-87167215.
E-mail address: re@clin.au.dk (R. Erichsen).

http://dx.doi.org/10.1016/j.annepidem.2016.05.002
1047-2797/

people). The Danish National Health Service provides tax-funded medical care for all Danish residents. All Danes are assigned a unique 10-digit civil registration number by the Civil Registration System, which also maintains records on vital status, date of death or emigration, and residence of all Danish citizens [13,14].

Cohort definition and materials

We identified all patients aged 18 years or more diagnosed with incident stage IV gastric cancer in the study area between January 1, 2003 and December 31, 2010, based on the Danish Cancer Registry. This registry includes data on date of cancer diagnosis (ICD-10 code C16 for gastric cancer) and tumor spread at diagnosis for all incident cancers in Denmark [15].

Among this group of patients, we used data from the Danish Pathology Registry to identify those who had malignant gastric neoplasia specimens stored at the Aalborg Pathology Institute [16]. The Danish Pathology Registry has complete data on all pathology diagnoses since 1997. Pathology diagnoses are recorded according to the Danish modification of the SNOMED (Systematized Nomenclature of Medicine) coding system [16]. We identified 134 potential cases for the study comprising those with topography "stomach" (SNOMED code T63***) and morphology "malignant neoplasia" (SNOMED code M8***X, where X $\geq$ 3) and reviewed the pathology reports. We excluded nonadenocarcinomas (n = 4), autopsies (n = 1), patients for whom no tissue was left (n = 4), and patients whose pathology was reviewed for a second opinion in Aalborg but originated from a different geographical region (n = 19). For the remaining cases (n = 106), we selected tissue blocks from biopsies of the primary lesion and metastases, as well as blocks from surgical resection. The most suitable block was determined by a study pathologist (M.V.) and used for analyses.

Immunohistochemistry and FISH analyses

For immunohistochemistry (IHC), we used the optimized Dako assay which uses the monoclonal MET4 antibody and the Dako EnVision FLEX + visualization system [17]. This assay detects the extracellular domain of MET. *MET* gene amplification was analyzed by fluorescence *in situ* hybridization (FISH) using the Research Use Only *MET*/CEN-7 IQFISH Probe Mix assay. These assays were performed in the Dako Denmark laboratories with the assay analyst and pathologist blinded to patient outcome data. A patient's tumor MET expression was defined as MET positive when $\geq$25% of the tumor cells showed 1+, 2+, or 3+ staining in IHC analyses (i.e., overall membrane score $\geq$25%) and as *MET* amplified when FISH results showed an average *MET*:centromere 7 ratio of $\geq$2 [11]. IHC analyses failed in three patients, and FISH analyses failed in five patients, leaving a total of 103 and 101 patients with stage IV gastric cancer for IHC and FISH statistical analyses, respectively.

Covariates

Covariates are listed in Table 1. Using records from the Danish National Patient Registry, we extracted information on each subject's history of comorbid diseases in the five years before gastric cancer diagnosis. This registry records the patient's dates of admission and discharge, surgical procedures performed (codes KJC and KJD), chemotherapy (codes BWHA and BOHJ), radiation (codes BWG), and up to 20 discharge diagnoses coded by doctors according to the ICD (eighth revision until the end of 1993 and the 10th revision thereafter) [18]. We used the Charlson Comorbidity Index (CCI) to quantify the level of comorbidity. This scoring system assigns between one and six points to a range of diseases as the components of a summed, aggregate score [19]. Patients were classified in three groups according to their sum of points: 0 points (no comorbidity), 1–2 points (low comorbidity), and 3 or more points (high comorbidity).

Table 1
Characteristics of patients with stage IV gastric cancer according to MET status and MET-positive prevalence by patient characteristics

Characteristics	MET negative		MET positive		MET-positive prevalence
	No	%	No	%	%
Total	39	100.0	64	100.0	62.1
Median age (y)	65.1 (range, 29.1–89.4)		69.6 (range, 32.9–89.4)		N/A
Men	29	74.4	47	73.4	61.8
Women	10	25.6	17	26.6	62.1
Period of diagnosis					
2003–2006	18	46.2	22	34.4	55.0
2007–2010	21	53.8	42	65.6	66.6
Anatomic site of the cancer					
Cardia	14	35.9	29	45.3	67.4
Corpus	3	7.7	9	14.1	25.0
Antrum	4	10.3	2	3.1	33.3
Pyloric	1	2.6	1	1.6	50.0
Minor curvature	1	2.6	0	0	0
Several regions	5	12.8	4	6.3	44.4
Unspecified	11	28.2	19	29.7	63.3
Histology					
Neuroendocrine carcinoma	0	0	1	1.6	100
Low differentiation	15	38.5	28	43.8	65.1
Signet cell carcinoma	10	25.6	19	29.7	65.5
Tubular adenocarcinoma	14	35.9	16	25.0	53.3
Comorbidity (Charlson Comorbidity Index score)					
CCI score = 0	26	66.7	50	78.1	65.8
CCI score = 1–2	11	28.2	7	10.9	38.9
CCI score = 3+	2	5.1	7	10.9	77.8
Treatment					
Surgery	25	64.1	31	48.4	55.4
Chemotherapy	23	59.0	33	51.6	58.9
Radiation	2	5.1	12	18.8	85.7
Origin of lesions					
Metastasis	3	7.7	9	14.1	75.0
Primary biopsy	33	84.6	49	76.6	59.8
Surgical resection	3	7.7	6	9.4	66.7
MET gene amplification, FISH analysis (n = 101)	0	0	4	10.3	100.0

Statistics

Patients were followed from the date of gastric cancer diagnosis until the date of death, emigration, or end of follow-up. The Kaplan–Meier method was used to create survival curves and to estimate the median survival time. We used Cox proportional hazard regression to compare 0–1-year mortality of MET-positive patients to that of MET-negative patients, adjusting for age, gender, and CCI. We evaluated the impact of *MET* gene amplification on survival and/or mortality using the same methodology.

In a secondary analysis, we compared the prognostic impact of different cutoffs (Fig. 2) of MET-positivity (based on IHC data) using patients with a MET cutoff of <10% as the fixed reference.

The study was approved by the Danish Data Protection Agency (no. 2011-41-5912) and the Committee on Health Research Ethics (no. 1-10-72-290-12).

Results

Sixty-four of the 103 patients with a successful IHC analysis had MET-positive stage IV gastric cancer (62.1%, 95% confidence interval