Original Study

A Pilot Study Assessing the Incidence and Clinical Significance of Circulating Tumor Cells in Esophagogastric Cancers

Francesco Sclafani, Elizabeth Smyth, David Cunningham, Ian Chau, Andrea Turner, David Watkins

Abstract

This pilot study assessed the incidence and significance of circulating tumor cells (CTCs) in Western patients treated with first-line chemotherapy for advanced esophagogastric cancers. The CellSearch system was used. In 44% of patients, ≥ 2 CTCs were detected and appeared to be associated with lower response and shorter survival. CTCs in esophagogastric cancer are clinically relevant and worthy of further investigation.

Background: Circulating tumor cells (CTCs) have been found to be of clinical utility in predicting response to treatment and prognosis in several malignancies. Less is known of the prevalence and clinical relevance of CTCs in esophagogastric adenocarcinoma, with the available data arising from heterogeneous patient populations using varied detection methods. **Patients and Methods:** A pilot study was undertaken to assess the prevalence of CTCs in patients with advanced esophageal or gastric adenocarcinoma. Patients were eligible if they had advanced disease and either had received no prior therapy or had progressed after prior chemotherapy. Blood samples for CTC analysis were obtained at baseline and during the course of treatment. The CellSearch immunomagnetic CTC detection platform was used. **Results:** Twenty-two patients with metastatic esophageal or gastric adenocarcinoma were enrolled. Eighteen received first-line EOX (epirubicin/oxaliplatin/capecitabine) chemotherapy (± panitumumab) and had baseline samples suitable for CTC analysis. At baseline, ≥ 2 CTCs were detected in 8 patients (44%). Overall tumor response rate was 60% in patients with < 2 CTCs and 37.5% in patients with ≥ 2 CTCs. Median progression-free and overall survival were 6.1 and 10.5 months and 5.2 and 6.1 months in the groups of patients with < 2 CTCs and ≥ 2 CTCs, respectively. The study was prematurely discontinued, owing to the withdrawal of commercial support. **Conclusion:** The incidence of CTCs in locally advanced or metastatic esophagogastric cancer may be clinically relevant. Investigation of the potential clinical utility of CTCs is warranted in a larger cohort of patients with esophagogastric cancer.

Clinical Colorectal Cancer, Vol. 13, No. 2, 94-9
Keywords: Biomarkers, Chemotherapy, CTCs, Esophageal cancer, Gastric cancer

Introduction

The identification of reliable and reproducible indicators of tumor prognosis and treatment outcome is a recognized priority in cancer research. This is especially true given the development of novel therapies, which have significantly increased the available systemic treatment options.[1]

In current practice, baseline clinicopathologic characteristics continue to inform patient management in most cases, with computed tomography (CT) used to assess treatment response. CT response assessment is imperfect, being costly, inconvenient for patients, reliant on ionizing radiation, and open to subjective variation. Consequently, alternative methods to reliably assess treatment efficacy are increasingly sought. Serum tumor markers may aid in assessing response to treatment but frequently lack sensitivity and specificity.

The presence of circulating tumor cells (CTCs) in the bloodstream of patients with solid tumors was first recognized more than 50 years ago.[2] Since then, several studies have found that CTCs may represent a new diagnostic tool, providing useful information on tumor staging, prognosis, and treatment outcome.[3-7] In addition, the potential of CTCs to reflect changes in tumor phenotype and act as a dynamic resource for biomarker assessment has been investigated, with promising results.[8-11]

Department of Medicine, Royal Marsden NHS Foundation Trust, London and Surrey, United Kingdom

Submitted: Jun 17, 2013; Revised: Oct 10, 2013; Accepted: Nov 8, 2013; Epub: Nov 13, 2013

Address for correspondence: Francesco Sclafani, Department of Medicine, Royal Marsden Hospital, Downs Rd, Sutton, Surrey SM2 5PT, United Kingdom
E-mail contact: francesco.sclafani@rmh.nhs.uk

1533-0028/$ - see frontmatter
http://dx.doi.org/10.1016/j.clcc.2013.11.003

Several methods for the detection of CTCs have been developed, including immunocytochemistry, reverse transcription polymerase chain reaction, and flow cytometry.[12-14] However, these methods are predisposed to technical variation and have not been widely commercially available. More recently, technical developments in the methods of CTC capture and enumeration have led to the development of regulatory agency–approved CTC detection platforms such as the CellSearch system (Veridex LLC, Huntingdon Valley, PA). Through immunomagnetic cell separation, this system enables the consistent isolation and quantification of CTCs from the blood of patients with solid tumors.[15]

Studies evaluating the CellSearch platform have found wide variation in the detection of CTCs across different tumor types. A large study including patients with cancer, patients with nonmalignant diseases, and healthy volunteers found that the incidence of $\geq$ 2 CTCs in patients with cancer ranged from 11% to 57%.[16]

Historically, most data on the incidence and role of CTCs derive from studies conducted in breast, prostate, and colorectal cancer. Much less information is currently available on the role of CTCs in other malignancies. Studies in esophagogastric cancer suggest that CTCs may play a role in predicting tumor prognosis and response to treatment.[17-35] However, these studies used a variety of methods for CTC detection, and many of them were performed in East Asian populations. Significant differences in esophagogastric cancer epidemiology and etiology exist between Eastern and Western populations, manifesting as biologic heterogeneity, differences in clinical presentation, and occasionally differential response to therapeutic measures in these populations.[36,37] Thus investigation of the significance of CTCs in Western patients with esophagogastric disease is warranted.

Using the CellSearch immunomagnetic CTC detection platform, the authors undertook a small pilot study to assess the prevalence and role of CTCs in a prospective cohort of Western patients with esophageal or gastric adenocarcinoma.

Patients and Methods

This was a single-center prospective study. Eligible patients were $\geq$ 18 years old, with a diagnosis of adenocarcinoma of the esophagus or stomach. Patients must have had untreated disease or actively progressing disease after previous therapy (surgery, radiotherapy, or chemotherapy). Patients with active carcinomas other than esophagogastric cancer or who had received therapy for other carcinomas within the past 5 years (except cured nonmelanoma skin cancer and treated in situ cervical cancer) were excluded.

Baseline pretreatment blood samples for CTC analysis were obtained from all patients. In addition, for patients receiving chemotherapy, additional samples were obtained at the time of the second cycle of chemotherapy (before treatment), at the time of the third cycle of chemotherapy (before treatment), and at completion of chemotherapy or disease progression, whichever occurred first.

Eligible patients receiving chemotherapy underwent clinical and serum tumor marker assessment with each cycle of treatment. Tumor response was assessed every 12 weeks with CT scan and reported by a radiologist blinded to the results of CTC measurement. Tumor response was reported according to RECIST criteria (Response Evaluation Criteria in Solid Tumors) version 1.0. Responding patients received up to 6 months of chemotherapy. The study did not include a predefined therapeutic algorithm, and the choice of chemotherapy regimen was on an individual basis.

After the completion of the chemotherapy study, patients were followed up every 3 months. At each outpatient visit, patients had clinical and serum tumor marker assessment. A CT scan was also undertaken every 3 months to document disease status. After the radiologic evidence of progressive disease, all patients were followed up for survival.

The study was approved by the local ethics committee and performed in accordance with the ethical principles in the Declaration of Helsinki. Written consent was obtained from all patients.

CTC Analysis

Peripheral venous blood for analysis (7.5 mL from the peripheral vein) was collected in CellSave tubes (Veridex LLC). Blood samples were forwarded to Immunicon/Veridex LLC clinical laboratories (Enschede, The Netherlands) and analyzed within 72 hours after the draw, as previously described.[16]

The CellSearch system is a semiautomated system that enriches the sample for cells expressing the epithelial cell adhesion molecule (EpCAM), distinguishes epithelial cells from leukocytes, and allows for the identification and enumeration of CTCs (defined as nucleated cells lacking CD45 and expressing cytokeratin). Results are expressed as the number of cells per 7.5 mL of whole blood. As previously reported, detection of $\geq$ 2 CTCs was regarded as a positive result.[16]

Statistical Analysis

The primary objective of this study was to determine the number of CTCs detectable in patients with esophageal and gastric cancers. Secondary objectives were to assess the potential prognostic value of CTCs in these patients, to determine changes in CTC level during the early course of treatment, and to develop additional molecular markers for CTC characterization.

Initially the study was planned in 2 stages. Recruitment to the first stage was limited to patients with advanced disease (defined as metastatic or locally advanced disease that was not amenable to radical therapy). A sample size of 25 patients would allow the exclusion of a true incidence of $\geq$ 2 CTCs before the initiation of therapy in $\leq$ 5% of patients, with a .05 2-sided significance level and 90% power when the anticipated proportion of patients with $\geq$ 2 CTCs is 25%. If $\leq$ 4 of 25 patients were found to have CTCs $\geq$ 2 before the initiation of treatment, it would be concluded that the incidence of CTCs in patients is probably too low to be clinically relevant. If $\geq$ 5 of 25 patients were found to have $\geq$ 2 CTCs at baseline in the first stage of recruitment, then the study would proceed to a second stage.

During the second stage, planned recruitment was for a further 25 patients with advanced disease to increase the precision of the estimates of incidence and prognostic value of CTCs in this patient group. In addition, 25 patients with localized disease (defined as disease that is operable, amenable to radical radiotherapy, or both) would be recruited to assess the incidence of CTCs in this population.

The Kaplan-Meier method was used to calculate survival estimates, and comparison of the patient groups was carried out using a log-rank analysis. Hazard ratios and 95% confidence intervals were obtained with Cox regression.