Identification of clinical biomarkers for adjuvant chemotherapy in gastric cancer after D2 dissection by pooled analysis of individual patient data from three large randomized clinical trials

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Abstract
We aimed to identify prognostic and predictive markers among clinicopathological factors for efficacy of adjuvant chemotherapy in gastric cancer by pooled analysis from 3,521 patients on three large randomized trials: ACTS-GC, CLASSIC, and SAMIT. The primary endpoint was relapse-free survival, and the secondary endpoints were overall survival. The integrity of individual patient data was verified. Significant prognostic markers in surgery alone groups of ACTS-GC and CLASSIC were lower body mass index and advanced stage of disease.

Keywords: Gastric cancer, Adjuvant chemotherapy, Clinical biomarker, Meta-analysis

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Introduction
Gastric cancer is the fourth most common cancer worldwide and the second leading cause of cancer-related deaths due to its poor prognosis overall. Clinical guidelines generally recommend additional chemotherapy for advanced and curatively resected tumors, mostly stage II and III, based on data from randomized clinical trials (INT 0116 in USA, 2001¹; MAGIC in Europe, 2006²; ACTS-GC in Japan³,⁴; CLASSIC in Korea and China, 2012⁵; SAMIT in Japan, 2014⁶), although surgical procedures and regimens were different across trials. Recently, the West and East have collaborated to standardize cancer staging and surgical procedures to dissect lymph nodes (D2). D2 is systematic removal of lymph nodes around stomach and pancreas to prevent local and regional tumor regrowth. The three trials conducted in Asia recruited D2 received patients, although proportions of stage III were different, 55% in ACTS-GC, 50% in CLASSIC, and 60% in SAMIT, respectively.

Since comparing treatments by a new clinical trial takes long time, it is important to identify prognostic and predictive markers among clinicopathological factors for efficacy of adjuvant chemotherapy with the use of individual patient data from precious clinical trials. We therefore planned this pooled analysis of ACTS-GC, CLASSIC, and SAMIT.

Protocol digest of the study
Objective
We aimed to identify prognostic and predictive markers among clinicopathological factors for efficacy of adjuvant chemotherapy for patients who underwent curative
surgery for gastric cancer.

**Study Design**

We planned pooled analysis of individual patient data from ACTS-GC⁴, CLASSIC⁵, and SAMIT⁶ to explore prognostic markers for patients with gastric cancer curatively treated by D2 gastrectomy, to identify predictive marker for efficacy of adjuvant chemotherapy and in each regimen for those patients, and to develop postoperative survival prediction models for treatment selection. The statisticians had access to de-identify data of the primary analysis population of each trial; n = 1059 in ACTS-GC, n = 1035 in CLASSIC, and n = 1427 in SAMIT. The data-sharing agreement is effective among the primary investigators (AT, KY), a statistician (ST) and the sponsors of each trial. Written informed consent was obtained from all participants in each trial. The protocol of the pooled analysis was approved by the institutional review board.

**Statistical Analysis Plan**

**Preliminary analysis**

We verified the integrity of individual patient data from ACTS-GC, CLASSIC, and SAMIT by comparing their descriptive statistics against figures in literature. Relapse-free survival and overall survival according to treatment groups and trials were described by Kaplan-Meier method. Distributions of clinical and pathological factors common in the three trials were described as well and will be compared across trials by ANOVA or Fisher exact tests. Common clinical and pathological factors included age, sex, body mass index, times since surgery, T stage, N stage, histologic type (e.g. differentiated or undifferentiated), and pathological stage. For adjuvant groups, hazard ratios of capecitabine and oxaliplatin in CLASSIC vs. S-1 in ACTS-GC, and paclitaxel then S-1 in SAMIT vs. S-1 in ACTS-GC were estimated by Cox regression adjusted for clinical and pathological factors. Relapse-free survival and overall survival according to treatment groups and subgroups (defined based on the clinical and pathological factors) were described by Kaplan-Meier method. The hazard ratios, 95% confidence intervals, and p values of the treatment groups will be estimated by stratified Cox regression using trial as the stratified factor. Tests for treatment-subgroup interactions were examined by stratified Cox regression including a treatment-subgroup interaction as a covariate and trial as the stratified factor. The hazard ratios, 95% confidence intervals, p values and the baseline survival functions of the final models which include significant interactions were estimated and a postoperative survival prediction model were developed based on the estimates.

**Identification for predictive markers of efficacy of specific regimens**

If the hazard ratio of capecitabine and oxaliplatin in CLASSIC vs. S-1 in ACTS-GC, or paclitaxel then S-1 in SAMIT vs. S-1 in ACTS-GC were significant in preliminary analysis, we further explored predictive markers of efficacy of these regimens. Relapse-free survival and overall survival according to treatment groups and subgroups (defined based on the clinical and pathological factors) were described by Kaplan-Meier method. The hazard ratios, 95% confidence intervals, and p values of the treatment groups were estimated by Cox regression adjusted for clinical and pathological factors. Tests for treatment-subgroup interactions were examined by Cox regression including a treatment-subgroup interaction and clinical and pathological factors as covariates. The hazard ratios, 95% confidence intervals, p values and the baseline survival functions of the final models which include significant interactions were estimated and a postoperative survival prediction model were developed based on the estimates.

**Validation of prediction models**

We assessed the predictive accuracy of the prediction models using 10-fold cross-validation, i.e., we performed 10 rounds of cross-validation using different partitions. One round of cross-validation involved randomly partitioning data on the entire population into complementary subsets, fitting the final Cox regression models to one subset of 90% of patients, and validating the model on the remaining subset. Calibration, namely, how closely the prediction reflected observed events, was assessed for each event by Hosmer-Lemeshow test and the mean
of observed-to-predicted (O/P) ratios, which was calculated as the mean of ratios of the observed-to-expected events across the strata used in Hosmer-Lemeshow test. Discrimination, the ability to distinguish between those who experienced the event and those who did not, was evaluated using Harrell C statistics, the proportion of all patient pairs in which the predictions of the model and observed events were concordant.

**General**

All reported p values were two-tailed and p < 0.05 was chosen as the threshold for statistical significance. Academic statisticians (ST and JG) conducted all statistical analyses using SAS version 9.4 (SAS Institute, Cary, NC, USA). Missing data were substituted using the multiple imputation method.

**Results**

The integrity of individual patient data was verified. Table 1 shows preliminary results on prognostic markers among patients who did not receive adjuvant chemotherapy in ACTS-GC and CLASSIC trials. There was no significant difference in overall survival between the trials after adjustment for patient characteristics. In addition to stage of disease, low body mass index was a significant poor prognostic marker; and there was a trend for poor prognosis in older patients and male patients. The manuscript with the details of the findings and the interpretation are in preparation to be published elsewhere.

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