The Effect of the Change in Lymph Flow Following Gastrectomy for Initial Disease on the Prognosis of Remnant Gastric Cancer

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Research Article

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Abstract

Background: Remnant gastric cancer (RGC) has been increasing for various reasons such as longer life span, medical progress, and others. It generally has a poor prognosis, and its mechanism of occurrence is unknown. The purpose of this study was to evaluate the clinicopathological features of and clarify the prognostic factors of RGC.

Methods: Between January 2002 and January 2017, 39 patients with RGC following distal gastrectomy underwent curative surgical resection at the Okayama University Hospital; their medical records and immunohistochemically stained extracted specimens were used for retrospective analysis.

Results: On univariate analysis, initial gastric disease, pathological lymph node metastasis, and pathological stage were the significant factors associated with a poor overall survival (OS) ($p=0.0139$, 0.0061, and 0.0158, respectively). Multivariate analysis of these 3 factors showed that only initial gastric disease caused by malignant disease was an independent factor associated with a poor prognosis ($p=0.0141$, odds ratio [OR]:4.151, 95% confidence interval [CI]:1.333-12.93). In addition, the presence of a left gastric artery (LGA), and tumor-infiltrating $CD8^+$ T cell expression were higher in the benign disease group than in the malignant group ($p<0.0001$ and $p=0.0485$, respectively).

Conclusion: The lymph flow change caused by lymph node dissection for malignant disease in initial surgery might have an effect on the suppression of tumor immunity and the poor prognosis of RGC.

Background

Remnant gastric cancer (RGC) has been increasing with the evolution of medical technology, medical diagnostics, and longer life expectancy. RGC is defined as gastric cancer in the remnant stomach after partial gastrectomy for benign or malignant disease[1],[2]. The rate of RGC has generally been reported as 1-3%[3],[4], one of the less frequent cancers. However, especially in Eastern Asia, there is a high incidence of gastric cancer, and there is a possibility of a further increase in the future for the above reasons[2]. RGC is considered to have a different form of development from primary gastric cancer[5], and many details are unclear[6],[7],[8]. Moreover, it is difficult to perform a randomized, controlled trial because it is unknown when and who will develop the cancer after surgery. Therefore, there has been no improvement in prognosis over the past two decades, and the prognosis of RGC remains poor in comparison with that of primary gastric cancer[9],[10]. Thus, the goal was to clarify the characteristics of RGC based on its clinicopathological features and develop the treatment strategy for RGC based on the cases treated at our institution. Non-curative resection is excluded because it is well-known in the cancer treatment field that it does not improve the patients' prognosis.

Methods

Patients

In this study, RGC was defined in accordance with the Japanese Classification of Gastric Carcinoma (English edition, ver. 3) [2]. Between January 2002 and January 2017, 39 patients with RGC following distal gastrectomy underwent curative surgical treatment at the Department of Gastroenterological Surgery, Okayama University Hospital. This study included only curative treatment, not non-curative treatment. Medical records of all patients were obtained from the hospital database. Pre-treatment factors (age, sex, cause of initial gastric surgery, reconstruction methods in initial surgery, time from first to RGC surgery, and blood test results), treatment factors (presence of a left gastric artery [LGA] and tumor location), post-treatment factors (histopathological data, follow-up period, recurrence, and adjuvant therapy) were examined retrospectively. The cause of initial gastric surgery and reconstruction methods in primary surgery were categorized as benign and malignant disease, and Billroth-I and others, respectively. Tumor locations were categorized as anastomotic site and non-anastomotic site. Depth of invasion was categorized as T1 (mucosa, or submucosa) or T2/3/4 (muscularis propria, subserosa, serosa-exposed, or serosa-infiltrating). Lymph node metastasis was categorized as negative or positive. Pathological stage was categorized as stage I or stage II/III (there were no Stage IV cases). Histological types were categorized as differentiated type (well-differentiated, moderately differentiated, or papillary) or undifferentiated type (poorly differentiated, signet-ring cell carcinoma, or mucinous). Lymphatic invasion and venous invasion were categorized as negative or positive. All histopathological information was evaluated and determined in accordance with the International Union Against Cancer (UICC) TNM classification, 7th edition.

Postoperative follow-up

Patients were followed-up every 3-6 months with physical examinations and laboratory blood tests. Patients underwent computed tomography (CT) every 6 months and esophagogastroduodenoscopy every 1 year. The median follow-up period of the 39 patients was 48.1 months (IQR: 30.0-67.9 months).

Immunohistochemical staining and assessment

Formalin-fixed, paraffin-embedded tissue samples cut at a thickness of 2 μm were deparaffinized and soaked in 0.3% H_2O_2 for 10 min at room temperature to extinguish endogenous peroxidase activity. After antigen retrieval by heating in a sodium citrate buffer solution or EDTA using a microwave, the samples were incubated with primary antibodies against CD8 (eBioscience, San Diego, CA, USA) and CD4 (eBioscience) overnight at 4 °C, and then with peroxidase-linked secondary antibody for 30 min at room temperature. After washing, the samples were stained with 3,3’-diaminobenzidine (Dako, Glostrup, Denmark) for visualization, and counterstained with Meyer’s hematoxylin.

The stained area in the sample was measured using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Statistical analysis

All statistical analyses were performed using JMP software version 14.2 (SAS Institute, Cary, NC, USA). Pearson's chi-squared test or Fisher's exact test was used for categorical variables, and the Mann-Whitney *U* test was used for continuous variables. The Kaplan-Meier method was used to estimate overall survival (OS) in each group, and survival rates were compared using the log-rank test. A probability (*P*) value less than 0.05 was considered significant.

Results

Clinicopathological Features of RGC (Table 1)

The clinicopathological characteristics of the 39 patients are summarized in Table 1. Average age and PNI were 74.2±6.44 years and 45.4±12.1, respectively. Thirty-four (87.2%) patients were males, and 5 (12.8%) were females. The cause of initial gastric surgery was benign disease in 20 (51.3%) and malignant disease in 19 (48.7%). Reconstruction methods in initial surgery were Billroth-I reconstruction in 20 (51.3%) and others in 19 (48.7%). The mean interval from the initial gastric surgery to the RGC surgery was 28 years (IQR: 14.5-43.5 years). Of the treatment factors, tumor location was at the anastomotic site in 17 cases (43.6%), and the LGA was present in 16 cases (41.0%); both were slightly lower than half.

On histopathological examination, a large number were advanced cancers (61.6%), meaning that they were diagnosed as T2/3/4, but there were also many cases without lymph node metastasis (76.9%); as a result, pStage I (48.8%) accounted for approximately half of the cases. Differentiated type was the most common type (64.1%), and lymphatic and venous invasion-negative cases accounted for 30.8% and 33.3%, respectively.

Malignant initial gastric disease is independently associated with a poor prognosis for RGC (Table 2)

The 5-year OS rate was 60.7% (Supplementary File 1). The univariate analysis showed that initial gastric disease (malignant), pathological lymph node metastasis (+), and pathological stage (≥II) were significantly associated with a poor prognosis. Furthermore, the multivariate analysis including these 3 factors showed that malignant initial gastric disease (odds ratio [OR]:4.151, 95% confidence interval [CI]:1.333-12.93, p=0.0141) was independently associated with a poor prognosis; there was a difference of over 40 months in median OS between those with and without malignant initial disease (Figure 1).

Presence of the LGA and tumor-infiltrating $CD8^+$ T cell expression were significantly more common in benign

initial gastric disease

Table 3 shows the relationship between benign initial disease and each factor. Male sex and presence of the LGA were significantly higher in the benign initial disease group than in the malignant initial disease group (p=0.0471 and <0.001, respectively). Other background characteristics and tumor factors were not associated with initial disease.

Next, the relationship between the initial disease and T cell expression in RGC was examined. First, before the analysis, it was confirmed that staining by CD8 and CD4 was completely successful and that there was a difference in expression levels for each tumor tissue (Supplementary File 2, 3). The tumor-infiltrating $CD8^+$ T cell expression level in the RGC was significantly higher following benign initial disease than following malignant initial disease (p=0.0485) (Figure 2). However, tumor-infiltrating $CD4^+$ T cells did not show a significant difference (Supplementary File 4).

Discussion

RGC is often detected at an advanced stage, and the 5-year survival rate was reported to be poor in the previous studies[6],[11],[12],[13]. Part of the reason is that the resectability of RGC was low, the postoperative mortality was high, and surgeries were difficult[9],[14],[15]. In the initial surgery, the omental bursa and greater omentum, in addition to the lymph node dissection, were removed in some cases. Therefore, strong adhesions were observed around the remnant stomach. If any morbidity, such as pancreatic fistula, occurred at the initial operation, it was more complicated. The other change caused by the initial surgery is lymphatic flow. Some adhesions around the remnant stomach form a new lymphatic course[11],[16]. Previous studies identified differences in lymphatic flow between benign and malignant initial diseases[5],[17],[18]. In fact, the initial gastric disease was also a prognostic factor in the present study, which means that lymph node dissection at the initial surgery has some impact on the prognosis of RGC. With the change of lymph flow to the bloodstream, lymph node metastasis may develop more frequently than in the usual course and may easily metastasize beyond regional lymph nodes, to lymph nodes such as the paraaortic lymph nodes. It has already been reported that the frequency of lymph node metastasis in the splenic hilum and along the splenic artery is higher in RGC than in primary cancers of the upper third of the stomach[8, 9, 11],[19]. However, although there were some reports of changes in the lymph node metastasis site with the lymph flow change, there were no reports examining the change in lymph-related immunity, such as tumor-infiltrating lymphocytes (TILs).

Cancer tissue is first infiltrated by these TILs which represent the local immune response directed against tumor growth and metastasis; then invaded by the multiple immune cells: T cells, B cells, macrophages and natural killer (NK) cells. It is well known that $CD8^+$ T cells are an essential part of adaptive immunity, and $CD4^+$ T cells help the function of $CD8^+$ T cells[20]. In the present study, presence of the LGA was independently associated with initial gastric disease, and tumor-infiltrating $CD8^+$ T cells were significantly different by initial gastric disease, affecting OS. This result may suggest that lymph flow change with

lymph node dissection at initial surgery suppresses the induction of $CD8^+$ T cells. Therefore, tumor immunity suppression may be involved in the poor prognosis of RGC, and this seems to be the first report showing that $CD8^+$ T cells may be involved in RGC prognosis.

Although this study has provided some important information for clinical practice, it has several limitations. First, this was a single-center, retrospective study, and the first patient underwent RGC surgery 15 years ago, which is a very long time for a clinical study and may have decreased the study quality. Second, the sample size was small, involving only 39 patients.

Conclusion

The prognosis of patients with RGC was independently associated with initial gastric disease, and tumor-infiltrating $CD8^+$ T cells in RGC were expressed more in the benign initial gastric disease group than in the malignant initial gastric disease group. Generally, it is difficult to conduct a prospective, randomized trial in RGC; therefore, it is very meaningful to be able to predict the prognosis from preoperative or postoperative factors and, among them, TILs analysis may be very important.

Abbreviations

CI: Confidence interval

CT: Computed tomography

LGA: Left gastric artery

NK: Natural killer

OR: Odds ratio

OS: Overall survival

RGC: Remnant gastric cancer

TILs: Tumor-infiltrating lymphocytes

Declarations

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Contributions

YK collected the literatures, analyzed the data and wrote the manuscript. All authors read and approved the final manuscript.

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Ethics declarations

Ethics approval and consent to participate

This study was approved by the institutional review board (IRB) of Okayama University Hospital. The study was performed in accordance with the Declaration of Helsinki. The informed consent was obtained from the patients or their families according to the IRB.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare no competing interest.

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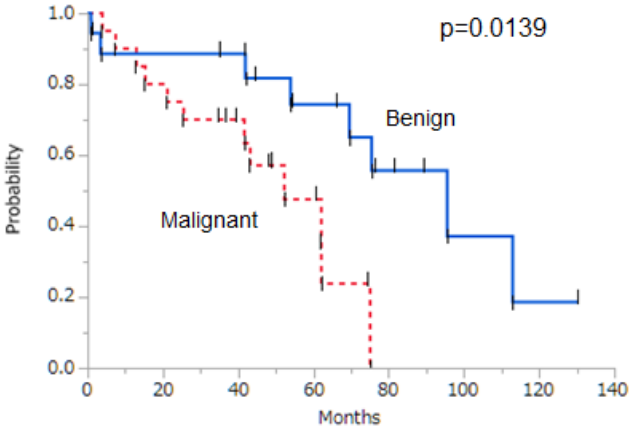
Tables

Due to technical limitations, tables are only available as a download in the Supplemental Files section.

Figures



	Initial disease	
	Malignant n=20	Benign n=19
OS median [95%CI], Months	52.2 [20.9, 75.1]	95.6 [53.9, NA]
Event, n(%)	12 (60.0)	8 (42.1)

Figure 1

Kaplan-Meier plots of OS according to initial disease.

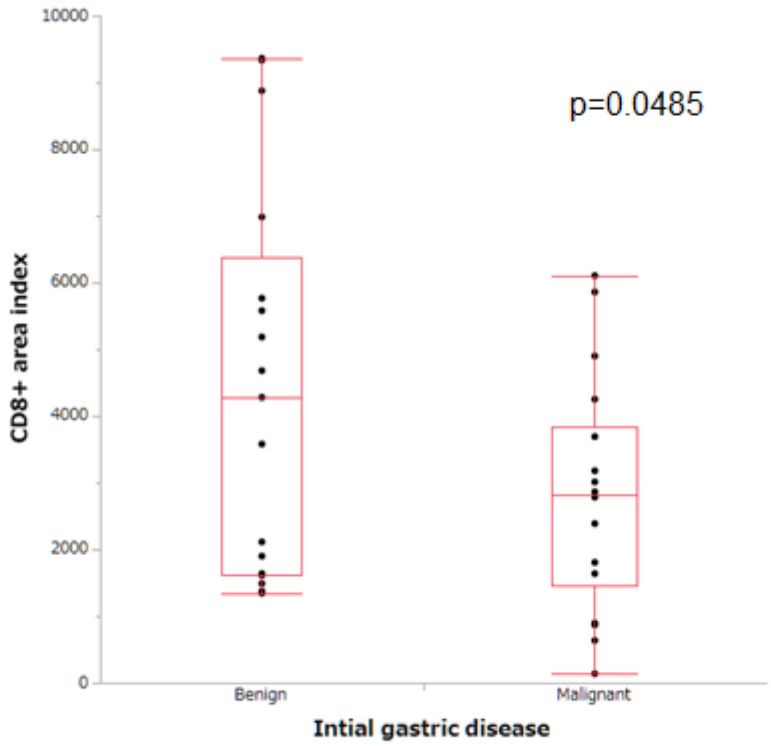


Figure 2

Comparison of tumor-infiltrating CD8+ T cell expression in benign and malignant initial diseases.

Supplementary Files

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