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Clinical Significance of Immunohistochemically-Identified Lymphatic and/or Blood Vessel Tumor Invasion in Gastric Cancer

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Background. **Tumor invasion and lymph node metastasis are significant prognostic factors for gastric cancer, and lymphatic and vascular tumor invasion are also significant risk factors for gastric cancer recurrence. Recently, the immunohistochemical detection of lymphatic and blood vessel tumor invasion (LBVI) has been shown to have a higher sensitivity and specificity than hematoxylin-eosin staining methods.**

Materials and Methods. **One hundred forty-nine gastric cancer patients who underwent curative resection at Korea University Hospital between November 2003 and December 2006 served as the study subjects. Lymphatic vessel invasion was evaluated by immunostaining with the new selective marker, D2-40, and blood vessel invasion was assessed with anti-CD31 antibody. Patients were divided according to the presence of LBVI, clinicopathologic factors were compared, and postoperative surgical outcomes were analyzed.**

Results. **LBVI was present in 66 patients (44.3%). LBVI was significantly correlated with depth of tumor invasion ($P < 0.001$), lymph node stage ($P < 0.001$), and lymph node micrometastasis ($P = 0.013$). Cancer recurrence was more common in the LBVI group ($P = 0.007$), and peritoneal seeding was the most prevalent type of recurrence ($P = 0.028$). Univariate analysis showed tumor size, depth of tumor invasion, lymph node stage, and LBVI to have a significant impact on survival. Based on multivariate analysis, however, depth of tumor invasion and lymph node stage were correlated with survival.**

Conclusion. **Immunohistochemical demonstration of LBVI is an additional prognostic marker, and provides useful information for planning treatment strategies in gastric cancer patients.**

Key Words: **immunohistochemistry; lymphatic invasion; vascular invasion; gastric cancer.**

INTRODUCTION

Even after curative resection, some types of recurrences remain the cause of cancer-related deaths in patients with gastric cancer [1]. Among several factors, tumor stage, such as depth of tumor invasion and lymph node metastasis, has generally been shown to be the main risk factor for recurrence in gastric cancer patients [2, 3]. In gastric cancer patients, surgical procedures, including gastrectomy and extensive lymphadenectomy, have been shown to be curative; critical methods to reduce the risk of recurrence have also been shown [3, 4]. Peritoneal and hematogenous metastases are the main causes of treatment failure because micrometastatic tumor foci cannot be removed with surgical management [1, 2]. Therefore, it is necessary to identify additional markers, which are readily available for detecting those patients at risk for recurrence among the different groups of patients with gastric cancer. These markers might also be of great clinical significance because they can be used in selecting candidates for further adjuvant or neoadjuvant therapies.

As the cancer stage becomes advanced, tumor cells invade blood vessels and lymphatic vessels near the tumor; lymphatic and/or blood vessel tumor invasion (LBVI) is the critical step in establishing tumor cell dissemination and metastasis in various types of cancers

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[5–7]. Several studies have shown that LBVI can be a useful marker to predict cancer recurrence and prognosis in gastric cancer patients [8–11]. D2-40 is a monoclonal antibody that is a specific lymphatic endothelial marker; staining for D2-40 has been utilized to evaluate lymphatic invasion in gastric cancers [9, 10]. Furthermore, CD31, a platelet/endothelial cell adhesion molecule, is a more sensitive marker for detecting endothelial cells that invade the microvessels around the main tumor in gastric cancer specimens [9, 12, 13]. In the present study, we determined the presence of LBVI in resected gastric cancer specimens obtained from patients who underwent curative surgery by immunostaining with D2-40 and CD31. We also determined the relationships between the presence of LBVI and clinical features, including the presence of lymph node macro- and micro-metastases, the prognostic effects, and cancer recurrence.

PATIENTS AND METHODS

This study included 149 patients with gastric cancer who underwent curative gastrectomy with standard lymph node dissection in the Department of Surgery of Korea University Hospital between 2003 and 2006. Standardized operative procedures were performed, such as total or subtotal gastrectomy, depending on the location of the gastric cancer, and D2 or more lymph node dissection according to the rules of the Japanese Research Society for Gastric Cancer. In all cases, with informed consent, serial 3 μm sections were cut, fixed with 10% formalin solution, and embedded in paraffin. All of the resected primary tumors and regional lymph nodes were examined histologically using hematoxylin-eosin (H-E) staining according to the Japanese Classification of Gastric Carcinoma. The following pathologic features were studied: location, macroscopic type, primary tumor size, and lymph node involvement.

With respect to the histologic type, the patients were classified into well-, moderate-, poorly-differentiated, and signet ring cell-type adenocarcinomas. Also, the cases were classified into intestinal-, diffuse-, and mixed-types by the Lauren classification.

Immunohistochemical staining was performed on 4 μm sections of formalin-fixed, paraffin-embedded tissues. Serial sections were deparaffinized in xylene, hydrated through a graded series of ethanol, and then immersed in 3% hydrogen peroxidase in 100% methanol for 30 min to inhibit endogenous peroxidase activity. To activate the antigens, the sections were boiled in 10 mM citrate buffer (pH 6.0) for 30 min. After rinsing in phosphate buffered saline (PBS), the sections were incubated with normal rabbit serum for 10 min, and then incubated overnight at 4°C in humid chambers with mouse D2-40 monoclonal antibody (1:100 dilution; Signet, Dedham, MA), which reacts with lymphatic endothelium. After washing with PBS, the sections were incubated with biotinylated anti-mouse immunoglobulin G (DAKO, Copenhagen, Denmark), and then incubated with a premixed avidin-biotin complex reagent (DAKO) for 20 min. The immunoreacted materials were visualized following a 1-min incubation with diaminobenzidine (DAKO) solution and hydrogen peroxidase. For the identification of blood vessels, an anti-CD31 monoclonal antibody (clone JC70A; DAKO) was used, whereas CAM5.2 (Becton Dickenson, San Jose, CA) was used for the detection of tumor cells and lymph node micrometastases.

The immunohistochemically-stained sections of all primary tumors were evaluated and LBVI was assessed by the same pathologist. LBVI was considered evident if at least one tumor cell cluster was clearly visible in the vascular space. In this study, we defined lymphatic vessel invasion (LVI) in immunohistochemically-stained sections as tumor cell nests in spaces and around the clump of tumor cell nests that were lined by flattened endothelium with no supporting smooth muscle or elastica, and/or were filled with lymphatic fluid (Fig. 1). Similarly, we defined blood vessel invasion (BVI) in immunohistochemically-stained sections as tumor cell nests in spaces and around the clump of tumor cell nests that were lined by endothelium, but not flattened, and/or were filled with red blood cells (Figs. 2 and 3).

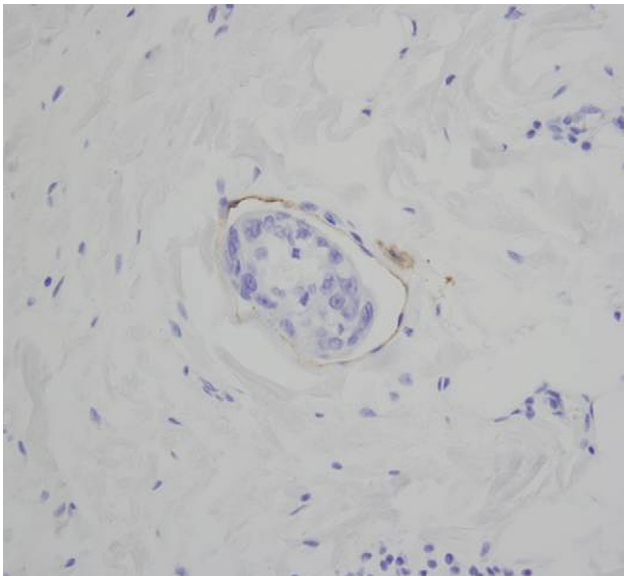

FIG. 1. Identification of lymphatic invasion after immunohistochemical staining with D2-40 (×400). (Color version of figure is available online.)

Vessels were considered to be lymphatics when the endothelium was stained with both D2-40 and CD31 antibodies. Vessels were classified as blood vessels when the endothelium was stained with CD31 monoclonal antibody and negative for D2-40 in consecutive sections. Vessels that were D2-40- and CD31-positive, and had red blood cells in the lumen, were considered to be lymphatic vessels; the red blood cells could be present in lymphatic-blood vessel capillary shunting or bleeding into lymphatic vessels. Vessels which were D2-40-positive but negative for CD31 antibody were considered to be lymphatic vessels.

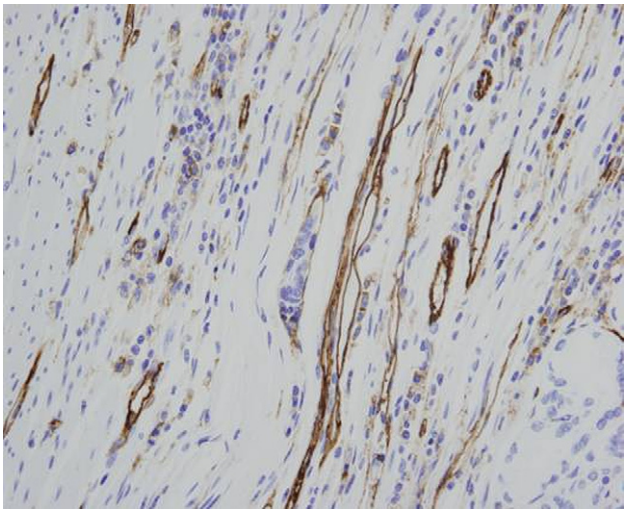

FIG. 2. Identification of vascular invasion after immunohistochemical staining with anti-CD31 (×400). (Color version of figure is available online.)