Introduction

The retrieval of lymph nodes after resection of colon cancer is an indispensable step for accurate tumor staging. Examination of a lower number of lymph nodes is associated with a poor prognosis in Stage II colon cancer, which could be attributable to stage migration from Stage III to Stage II. In contrast, an increase in the number of lymph nodes retrieved is associated with a decreased rate of adjuvant chemotherapy for Stage II colon cancer by reducing the number of patients identified as ‘high-risk’ Stage II.

In some countries, including Japan, a manual search is made for lymph nodes in the mesentery immediately after surgery without formaldehyde fixation. Although in selected institutes (i.e. those with the relevant expertise) this conventional method appropriately identifies lymph nodes, the number of lymph nodes retrieved is not always sufficient in procedures performed in non-specialized hospitals. To overcome this problem, a fat dissolution method has been proposed and its improved products are currently available commercially as Imofully (Sysmex, Kobe, Japan). However, as yet the efficacy of this new technique compared with the conventional method of lymph node retrieval has not been reported in the literature.

Patients and Methods

Ethics approval

This study will be conducted in two hospitals; Kochi Medical School Hospital and Hata Kenmin Hospital in Japan. The proposed study was approved by the institutional review board (IRB) of Kochi University (ID: 29-122). The trial has been registered with the University Hospital Medical Information Network (ID: UMIN000029448). An approval from IRB of Hata Kenmin Hospital will be obtained before participation. This study will be conducted in accordance with the Declaration of Helsinki and written informed consent will be obtained from all participants prior to their inclusion in the study.

Patients

Inclusion criteria

For subjects to be eligible for inclusion in the study, they must meet the following criteria: (1) a diagnosis of colorectal cancer, excluding Rb rectal cancer, confirmed by endoscopic and/or pathological examination; (2) the absence of distant metastases; (3) age ≥20 years; and (4) able to fully understand the study and provide written informed consent.

Exclusion criteria

Other than situations in which the investigator considers it inappropriate to include the patient in this study,
there are no exclusion criteria.

**Method for fat dissolution**

First, the mesentery is dissected from the large bowel. A commercially available fat dissolution liquid is prepared by adding 50 mL water to crystallized collagenase and lipase, according to the manufacturer’s instructions (Imofully, Sysmex Co, Kobe, Japan). The liquid is then injected into the fat tissue using an 18-G needle, followed by incubation of the tissue at 37°C in a water bath for 40–60 min. After the incubation, the tissue is transferred onto gauze to drain the liquid and blotted with the gauze, with pressure, several times to further push the liquid out. The lymph nodes exposed around blood vessels are then collected for pathological examination.

**Endpoints**

The primary endpoint of the proposed study is the number of lymph nodes retrieved using the conventional method versus the new method. The number of lymph nodes retrieved with the conventional method will be extracted from our database, which contains information for approximately 400 patients who have undergone colorectal surgery at Kochi Medical School Hospital between 2009 and 2014. Secondary endpoints include the number of lymph nodes retrieved in each institute, the microscopic observations reported for the retrieved lymph nodes, the stainability of the metastatic tumors by tumor-specific markers, and the diameter of metastasis-positive lymph nodes. Furthermore, in an explorative study, the remnant mesentery will be incubated for longer with the fat-dissolution liquid and the lymph nodes, if any, will be observed microscopically to clarify the effects of longer incubation at 40°C.

**Statistical considerations**

Data from Kochi Medical School hospital suggest that with the conventional manual method, a mean of 14 lymph nodes is retrieved, and data from a preliminary study suggested that fat-dissolution method yielded an additional seven lymph nodes. Thus, the sample size for the proposed trial was calculated based on the hypothesis that the fat-dissolution liquid could increase the mean number of lymph nodes retrieved from 14 to 21, with a standard deviation of 7.4. If data are collected prospectively from 40 patients, and if propensity score matching can extract the same number of patients from the database, statistical comparisons using t-tests could be performed with two-sided α = 0.05 and power >0.95. Thus, our aim is to prospectively collect data from 40 patients, in whom the new lymph node retrieval method will be conducted.

**Discussion**

The proposed study will reveal the efficacy of the fat-dissolution method in lymph node retrieval after colorectal resection. It is necessary to examine an appropriate number of lymph nodes for accurate diagnosis of node negativity. In addition, being able to discriminate between N1 and N2 status could be an important factor in determining the length of adjuvant chemotherapy in Stage III colon cancer. In this regard, the fat-dissolution method may contribute to treatment decisions regarding Stage II and III colon cancer.

**Support:** Imofully (Kit for fat dissociation) will be provided by Sysmex Co. (Kobe, Japan) according to the study contract.

**Conflict of interest:** None to declare for this study.

**References**

6) Shi Q, Sobrero AF, Shields AF, Yoshino T, Paul J, et al. Prospective pooled analysis of six Phase III trials investigating duration of adjuvant (adjuv) oxaliplatin-based therapy (3 vs 6 months) for patients (pts) with stage III colon cancer (CC): The IDEA (International Duration Evaluation of Adjuvant chemotherapy) collaboration. 2017 ASCO Annual meeting. Abstract#: LBA1.