gastric cancer in Japan began in the 1980s. Specifically, the development of the endoscopic submucosal dissection (ESD) method established endoscopy as a standard treatment for early gastric cancer, and the method has been gradually spreading, especially within Asia. The Japanese Ministry of Health, Labour and Welfare officially reported that 3251 early gastric neoplasms were resected throughout Japan in June 2012 alone.[3]

Post-ESD bleeding is one of the most common adverse events related to the ESD procedure, and it occurs in approximately 5% of patients even with perioperative administration of proton pump inhibitors.[4–9] Because performing a second-look endoscopy (SLE) after initial endoscopic haemostasis for peptic ulcer bleeding was suggested to reduce mortality and to improve clinical outcome,[10 11] SLE was similarly thought to be effective at reducing the incidence of post-ESD bleeding and was empirically performed after gastric ESD. However, because of recent improvements in management strategies for bleeding peptic ulcers, including endoscopic haemostasis with haemoclips or a combination of an injection of epinephrine with thermal therapy and pharmacological therapy using proton pump inhibitors, the most recent data do not support the use of routine SLE.[12 13] Accordingly, the international consensus recommendations published in 2010 do not endorse routine SLE for average-risk patients in clinical practice.[14]

According to our previous survey, SLE continues to be performed at most institutions,[8] and our previous retrospective analysis suggested that the incidence of post-ESD bleeding was not significantly different before and after performing SLE, although it was not a direct comparative study.[15] To address this controversy, we hypothesised that routine SLE would not be necessary after gastric ESD if bleeding and non-bleeding visible vessels were sufficiently treated during the ESD procedure and if perioperative proton pump inhibitors were administered. The aim of the current trial was to clarify the effectiveness of SLE at preventing bleeding after gastric ESD by performing a comparison between groups with and without SLE in clinical practice. Because SLE would not be performed on the day following ESD in the non-SLE group, treatment of the non-SLE group was less invasive than that in the SLE group. Therefore, the hypothesis that routine SLE would not be necessary would be proven by verifying the non-inferiority of the non-SLE group to the SLE group.

METHODS

Study design and participants

The current multicentre open-label prospective randomised controlled non-inferiority trial was undertaken in five referral institutions throughout Japan. Eligible patients were aged 20 years or older with adequate performance status (Eastern Cooperative Oncology Group 0–2) and normal major organ function; each patient also had histologically confirmed solitary adenocarcinoma or adenoma without lymph node and distant metastasis. The exclusion criteria were previous gastric surgery or gastric tube reconstruction, previous radiation therapy to the upper abdominal region, perforation and the administration of antithrombotic drugs, steroids or non-steroidal anti-inflammatory drugs (NSAIDs) during the perioperative period, which was defined as from 7 days before ESD until postoperative day (POD) 28.

The current trial complied with the Declaration of Helsinki, and the study protocol was approved by the institutional review board of each participating institution. All patients provided written informed consent prior to enrolment.

ESD procedures

ESD was performed according to the standard ESD procedure in both the SLE and non-SLE groups. Briefly, the procedure consisted of the following: (1) marking of a circumferential region around the lesion; (2) submucosal injection of solution outside of the marked region; (3) mucosal incision outside of the marked region; (4) additional injection into the submucosa underneath the lesion; (5) submucosal dissection with a cutting device; (6) haemostasis of active bleeding and prophylactic coagulation of visible vessels on the mucosal defect with haemostatic forceps in soft coagulation mode or with clips during both submucosal dissection and at the final step of ESD and (7) retrieval of the specimen. The following choices were made at the discretion of the surgeons: cutting devices: dual knife (KD-650L, Olympus Medical Systems, Co., Tokyo, Japan), insulated-tip knife-2 (KD-611L, Olympus Medical Systems, Co.), SAFE Knife V (DK2518DV1, Fujifilm Medical, Tokyo, Japan) or Clutch Cutter (DP2618DT, Fujifilm Medical); coagulating devices: Coagrasper (FD-410LR, Olympus Medical Systems), Radial Jaw hot biopsy forceps (Boston Scientific Japan, Tokyo, Japan) or Pentax high-frequency haemostatic forceps (HDB2422, Pentax Medical, Tokyo, Japan); electrosurgical generators: VIO300D (ERBE Elektromedizin, GmbH, Tübingen, Germany), ICC200 (ERBE Elektromedizin, GmbH) or ESG-100 (Olympus Medical Systems); clips: EZ clip (HX-610-090/HX-610-090S/HX-610-135, Olympus Medical Systems) and submucosal injection solution: 0.2%–0.4% sodium hyaluronate or normal saline with or without 0.1% epinephrine.

SLE and perioperative management

Patients in the SLE group underwent the standard SLE procedure, which was defined as a scheduled endoscopy performed 1 day after ESD without any suspicion of post-ESD bleeding. When adherent clots (Forest type IIb) were observed on the post-ESD ulcer during SLE, the surgeon carefully checked whether visible vessels (Forest type IIa) existed after removing the clots.[16] When visible vessels and/or active bleeding (Forest types Ia and Ib) were observed on the post-ESD ulcer, prophylactic coagulation or endoscopic haemostasis was performed with haemostatic forceps in soft coagulation mode or with clips until active bleeding and visible vessels were sufficiently treated. SLE was not performed in patients in the non-SLE group.

Patients consumed a liquid diet on POD 1 or POD 2; then, the diet changed daily to a soft meal by POD 5; and finally, patients without any complications were discharged from the hospital on POD 5 or POD 6. All patients took 10 mg of sodium rabeprazole once daily starting the day before ESD and for at least 4 weeks thereafter. All patients were observed at follow-up clinic visits for 4 weeks post-ESD. No follow-up endoscopy was performed during the 4-week follow-up period except for SLE in the SLE group and emergency endoscopy when post-ESD bleeding was suspected in both SLE and non-SLE groups.

Outcomes

The prespecified primary endpoint was the proportion of patients who experienced post-ESD bleeding, which was defined as haemorrhage with clinical symptoms and confirmed by emergency endoscopy from the time of the completion of ESD until POD 28. Clinical symptoms were defined as haematemesis, melaena or a decrease in haemoglobin of >2 g/dL since the patient's most recent laboratory test and emergency endoscopy was defined as endoscopy performed on a patient who had

clinical symptoms under suspicion of post-ESD bleeding. Confirmation by emergency endoscopy would rule out cases such as haematemesis or melaena associated with intraoperative bleeding during ESD or a decrease in haemoglobin caused by a dilution effect due to drip infusion. Patients who underwent haemostasis for subclinical bleeding without any suspicion of post-ESD bleeding during SLE were not included in the number of patients with post-ESD bleeding. Secondary endpoints were the proportion of patients with post-ESD bleeding after POD 1, the effectiveness of the prophylactic coagulation that was performed in patients in the SLE group, the subgroup analysis for post-ESD bleeding and the percentage of patients who required a blood transfusion. Curative resection was defined as complete tumour removal with tumour-free resection margins and a negligible risk of lymph node metastasis.[17]

During hospitalisation, adverse events were evaluated daily with patient interviews and physical examinations. A complete blood cell count was assessed on POD 1 or if there were symptoms of bleeding. All adverse events after discharge were verified by an interview with the patient at a consultation 4 weeks after ESD. The responsible clinicians reviewed each patient's medical records and input data into a web-based electronic case record form (University Hospital Clinical Trial Alliance Clinical Research Supporting System, Clinical Research Support Centre, The University of Tokyo).

Sample size

The current trial was powered for the assessment of non-inferiority of the non-SLE group compared with the SLE group with respect to the primary endpoint. On the basis of the previous large-scale clinical reports of more than 500 cases,[4–8] we assumed that the general incidence of post-ESD bleeding would be 5%. According to the multicentre survey, post-ESD bleeding occurred in up to 11.3% of patients post-ESD, even when SLE was performed.[8] Therefore, we assumed that the acceptable upper limit of the proportion of patients with post-ESD bleeding in the non-SLE group was 12%, and a non-inferiority margin in the difference in post-ESD bleeding between the SLE and non-SLE groups was set at 7%. With a one-sided type 1 error of 0.05, we calculated that 236 patients would yield a power of at least 80% to detect non-inferiority by the χ^2 test; that is, the upper limit of a two-sided 90% CI of difference in post-ESD bleeding risk between the groups (non-SLE minus SLE) included a 7% increase with no more than a 20% chance under the equivalence assumption. Assuming that approximately 5% of patients would be lost to follow-up, the required sample size was determined to be 250 patients.

Randomisation and masking

The responsible clinicians at each institution enrolled patients who met the inclusion and exclusion criteria before ESD. After the completion of ESD, participants were allocated to either the SLE or non-SLE group at a 1:1 ratio via minimisation method using the following four stratification factors: institution, tumour location (antrum or corpus), tumour size (≤20 mm or >20 mm) and ulceration finding (present or absent).[18] Briefly, our minimisation programme was provided via a web response system where the allocation sequence was computer-generated (University Hospital Clinical Trial Alliance Clinical Research Supporting System, Clinical Research Support Centre, The University of Tokyo). Through this minimisation programme, which could be accessed via internet by the responsible clinicians at each institution, the first patient was randomly allocated. Then, subsequent patient's allocation were sequentially

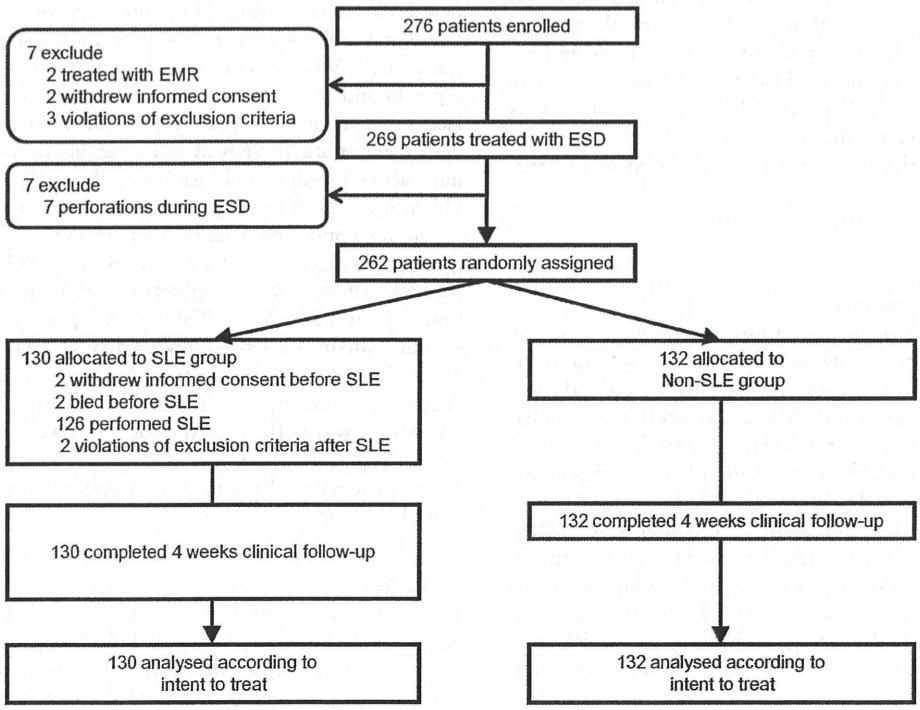


Figure 1 Flow diagram. In the SLE group, all 130 patients completed 4 weeks of clinical follow-up, including two patients who withdrew their informed consent, two patients who bled before SLE and two patients who violated the exclusion criteria (one by taking antithrombotic medication and the other by taking NSAIDs during the perioperative period). SLE, second-look endoscopy; EMR, endoscopic mucosal resection; ESD, endoscopic submucosal dissection; NSAIDs, non-steroidal anti-inflammatory drugs.

Table 1 Baseline characteristics

	SLE group n=130	Non-SLE group n=132
Age (years)	68.8 (8.6)	69.1 (9.0)
Men	104 (80.0%)	92 (69.7%)
Body mass index (kg/m^2)	23.5 (3.0)	23.6 (2.9)
Performance status		
0	128 (98.5%)	128 (97.0%)
1	2 (1.5%)	4 (3.0%)
History of gastric ulcer	15 (11.5%)	12 (9.1%)
Hypertension	59 (45.4%)	53 (40.2%)
Hyperlipidaemia	31 (23.8%)	21 (15.9%)
Diabetes mellitus	16 (12.3%)	12 (9.1%)
Longitudinal location*		
Body	80 (61.5%)	76 (57.6%)
Antrum	50 (38.5%)	56 (42.4%)
Cross-sectional location		
Anterior	27 (20.8%)	26 (20.0%)
Posterior	24 (18.5%)	24 (18.2%)
Lesser curvature	55 (42.3%)	56 (42.4%)
Greater curvature	24 (18.5%)	26 (20.0%)
Tumour size (mm)	14.7 (10.4)	14.5 (9.2)
Tumour size >20 mm*	18 (13.8%)	21 (15.9%)
Ulcerative finding*	15 (11.5%)	13 (9.8%)
Macroscopic type		
Protruding	33 (25.4%)	38 (28.8%)
Flat/depressed	85 (65.4%)	89 (67.4%)
Combined	12 (9.2%)	5 (3.8%)
Residual tumour	2 (1.5%)	2 (1.5%)
Histological depth		
Mucosal	112 (86.2%)	115 (87.1%)
Submucosal or deeper	18 (13.8%)	17 (12.9%)
Histological type		
Intestinal	120 (92.3%)	119 (90.2%)
Diffuse	5 (3.8%)	7 (5.3%)
Benign	5 (3.8%)	6 (4.5%)
Institution*		
A	30 (23.1%)	30 (22.7%)
B	42 (32.3%)	42 (31.8%)
C	36 (27.7%)	36 (27.3%)
D	12 (9.2%)	11 (8.3%)
E	10 (7.7%)	13 (9.8%)
Anaesthesia method		
Intravenous	128 (98.5%)	131 (99.2%)
General	2 (1.5%)	1 (0.8%)
Cutting device		
Needle-tip type†	31 (23.8%)	28 (21.2%)
Insulated-tip type‡	99 (76.2%)	104 (78.8%)
Submucosal injection solution		
With sodium hyaluronate	101 (77.7%)	99 (75.0%)
With epinephrine	97 (74.6%)	103 (78.0%)
Coagulating device		
Radial jaw hot biopsy forceps	68 (52.3%)	63 (47.7%)
Coagrasper	32 (24.6%)	40 (30.3%)
Pentax haemostatic forceps	30 (23.1%)	29 (22.0%)
Time of ESD (min)	86.3 (50.7)	81.9 (46.5)
Time of ESD >120 min	25 (19.2%)	16 (12.1%)
Time of prophylactic coagulation (min)	9.1 (5.3)	8.7 (4.8)
Time of prophylactic coagulation >9 min	59 (45.4%)	57 (43.2%)
Operator experience		
<30 cases	32 (24.6%)	35 (26.5%)
31–50 cases	18 (13.8%)	23 (17.4%)
51–100 cases	41 (31.5%)	36 (27.3%)
>100 cases	39 (30.0%)	38 (28.8%)
Specimen size (mm)	39.7 (16.0)	40.0 (14.5)
Specimen size >40 mm	50 (38.5%)	52 (39.4%)
En bloc resection	130 (100%)	132 (100%)
Curative resection	110 (84.6%)	113 (85.6%)

Data are the mean (SD) or number (%).
*For stratification factor.
†Including dual knife.
‡Including insulation-tipped knife-2, SAFE knife and clutch cutter.
ESD, endoscopic submucosal dissection; SLE, second-look endoscopy.

determined so as to minimise the imbalance of the four stratification factors between the groups: (1) for each stratification factor, the number of previously allocated patients who had the same value as the patient to be allocated was counted in each group; (2) the sum of the four numbers was calculated in each group and (3) the patient was allocated with high probability (from 50% to 99% according to the difference between the groups) to the group with the lower score.[19] The numbers in the algorithm were updated in the next allocation. We did not attempt to mask the patients or clinicians to the allocated treatment group.

Statistical analysis

The primary endpoint was analysed according to the intention-to-treat (ITT) principle. A Dunnett–Gent test was used to analyse the non-inferiority of the primary endpoint. We censored patients from the Kaplan–Meier plots when they reached the endpoint or when they were lost to follow-up. We also analysed the time to reach the endpoint according to the Kaplan–Meier method and applied the log-rank test to compare the incidence of the endpoint between the two groups. We assessed the continuous variables with Welch's t test and categorical variables with Fisher's exact test or the Wilcoxon signed-rank test, as appropriate. We used a one-sided p value of <0.025 to indicate the statistical significance of the non-inferiority of the primary endpoint (post-ESD bleeding) against the test hypothesis of 7% risk difference. For other endpoints, we conducted ordinary statistical tests against the null hypothesis of equivalence between groups; therefore, a two-sided p value of <0.05 was considered to indicate statistical significance. All CIs were set at the 95% confidence level at the intersection of the non-significant hypothesis set of the upper 2.5% tests and the lower 2.5% tests. JMP V.9.03 (SAS Institute, Cary, North Carolina, USA) was used for the analyses. Data processing and statistical analyses were conducted by an independent statistician. All authors had access to the study data and have reviewed and approved the final manuscript. The trial is registered with UMIN-Clinical Trials Registry, number UMIN-CTR 000007170.

RESULTS

Between February 2012 and February 2013, 276 patients were enrolled in the trial. Seven patients (2.5%) had perforations during ESD, two (0.7%) were treated with endoscopic mucosal resection, two (0.7%) withdrew informed consent and three (1.1%) violated the exclusion criteria before randomisation (figure 1). We randomly assigned 262 patients to either the SLE

Table 2 Major complications after ESD

	SLE group n=130	Non-SLE group n=132	p Value	$p_{non\text{-}inferiority}$ Value†
Post-ESD bleeding	7 (5.4%)	5 (3.8%)	0.570	<0.001**
Post-ESD bleeding requiring blood transfusion	0 (0%)	0 (0%)	–	–
Post-ESD bleeding requiring operation	0 (0%)	0 (0%)	–	–
Delayed perforation	0 (0%)	1 (0.8%)	1.000	–
Delayed perforation requiring operation	0 (0%)	0 (0%)	–	–
Post-ESD bleeding after POD 1	5 (3.9%)	3 (2.3%)	0.499	–
Post-ESD bleeding after POD 5	5 (3.9%)	1 (0.8%)	0.119	–

Data are numbers (%).
**p<0.025.
†Against the test hypothesis of a 7% risk difference between the SLE and non-SLE groups.
ESD, endoscopic submucosal dissection; POD, postoperative day; SLE, second-look endoscopy.

group (n=130) or the non-SLE group (n=132). In the SLE group, two patients withdrew informed consent, and post-ESD bleeding occurred in two patients before SLE; thus, 126 (96.9%) of the 130 patients in the SLE group underwent SLE, whereas no patient underwent SLE in the non-SLE group. An additional two patients in the SLE group violated the exclusion criteria after randomisation due to the use of an antithrombotic medication or NSAID during the perioperative period. All 262 patients were followed up for at least 4 weeks after ESD and were included in the ITT analysis of the primary endpoint.

Baseline characteristics of the patients, the lesions and the procedures are shown in table 1. Longitudinal location, tumour size, ulceration finding and institution, which were predefined as stratification factors, were well-balanced between the groups. A total of 47 operators (16 fellows and 31 trainees) were involved in this study. Operator experience and other parameters were well-balanced between the groups. All 262 patients underwent ESD in an *en bloc* manner, and curative resection was achieved in 110 (84.6%) and 113 (85.6%) patients in the SLE and non-SLE groups, respectively.

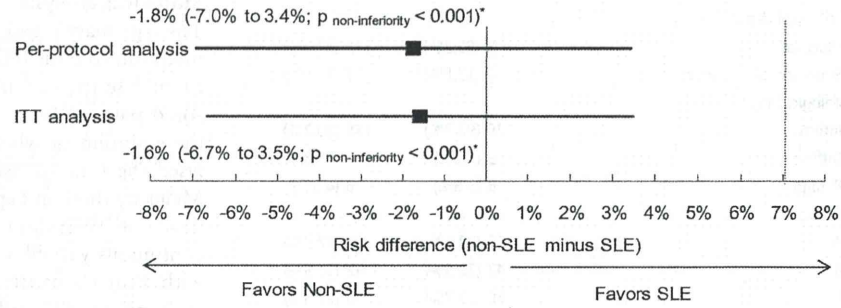


Figure 2 Clinical outcomes of the primary endpoint. *Risk difference (two-sided 95% CI; non-inferiority p value). ITT, intention-to-treat principle; SLE, second-look endoscopy.

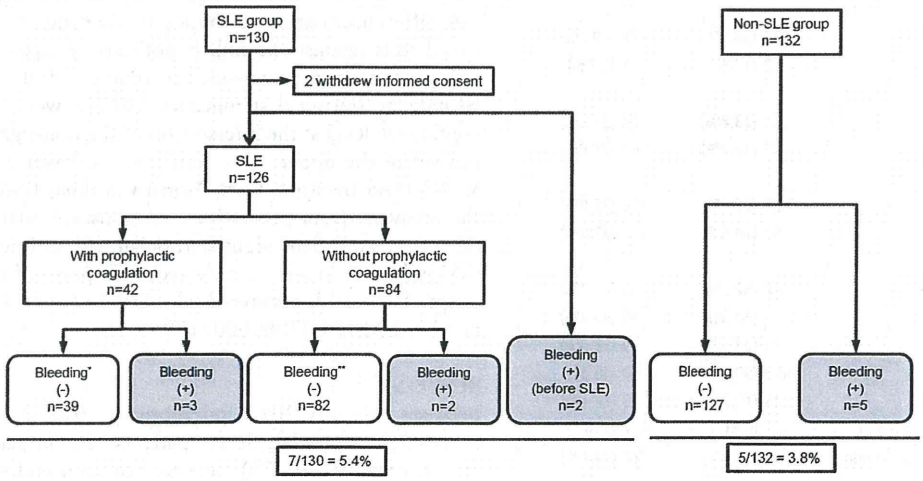
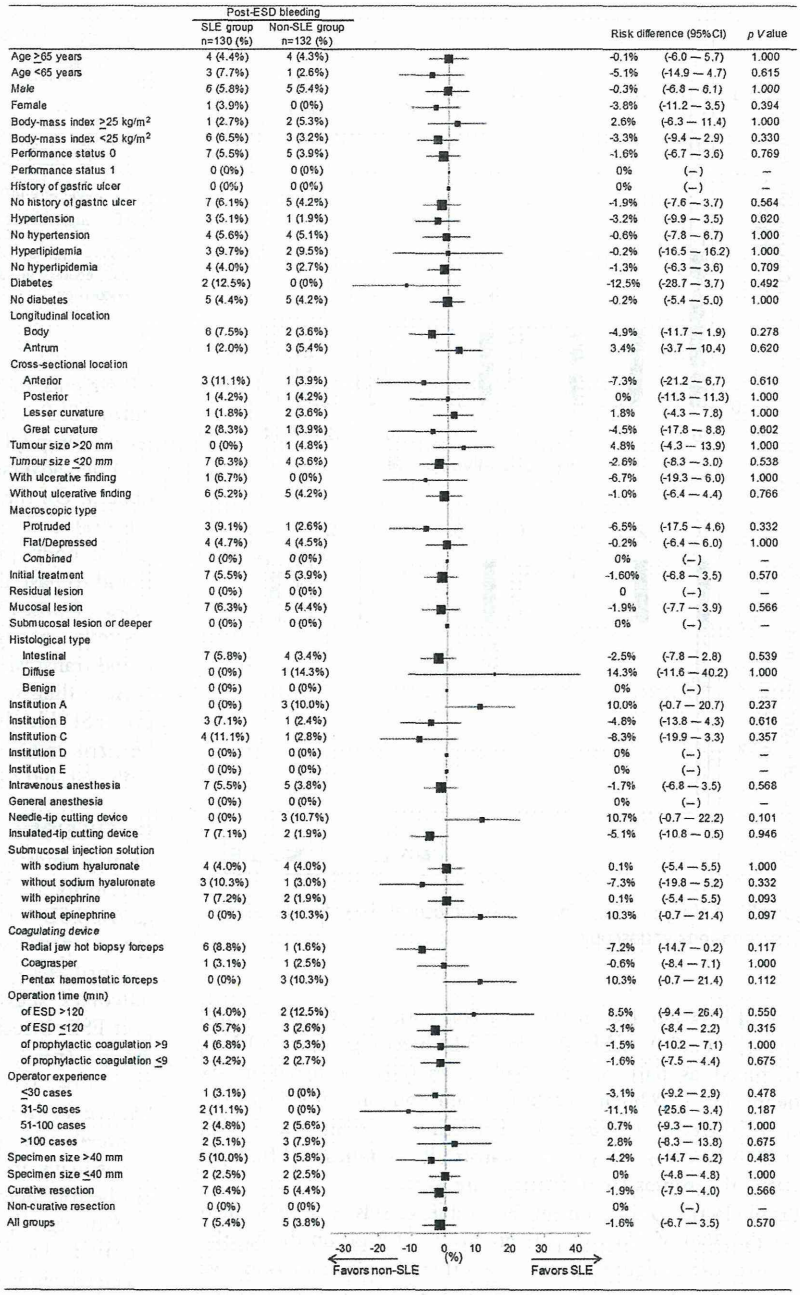


Figure 3 Clinical patient flow. *One patient violated the exclusion criteria by taking NSAIDs during the perioperative period. **One patient violated the exclusion criteria by taking antithrombotic medication during the perioperative period. SLE, second-look endoscopy; NSAIDs, non-steroidal anti-inflammatory drugs.



	Post-ESD bleeding				
	SLE group n=130 (%)	Non-SLE group n=132 (%)	Risk difference (95%CI)		p Value
Age ≥65 years	4 (4.4%)	4 (4.3%)	-0.1%	(-6.0 — 5.7)	1.000
Age <65 years	3 (7.7%)	1 (2.6%)	-5.1%	(-14.9 — 4.7)	0.615
Male	6 (5.8%)	5 (5.4%)	-0.3%	(-6.8 — 6.1)	1.000
Female	1 (3.9%)	0 (0%)	-3.8%	(-11.2 — 3.5)	0.394
Body-mass index ≥25 kg/m²	1 (2.7%)	2 (5.3%)	2.6%	(-6.3 — 11.4)	1.000
Body-mass index <25 kg/m²	6 (6.5%)	3 (3.2%)	-3.3%	(-9.4 — 2.9)	0.330
Performance status 0	7 (5.5%)	5 (3.9%)	-1.6%	(-6.7 — 3.6)	0.769
Performance status 1	0 (0%)	0 (0%)	0%	(—)	—
History of gastric ulcer	0 (0%)	0 (0%)	0%	(—)	—
No history of gastric ulcer	7 (6.1%)	5 (4.2%)	-1.9%	(-7.6 — 3.7)	0.564
Hypertension	3 (5.1%)	1 (1.9%)	-3.2%	(-9.9 — 3.5)	0.620
No hypertension	4 (5.6%)	4 (5.1%)	-0.6%	(-7.8 — 6.7)	1.000
Hyperlipidemia	3 (9.7%)	2 (9.5%)	-0.2%	(-16.5 — 16.2)	1.000
No hyperlipidemia	4 (4.0%)	3 (2.7%)	-1.3%	(-6.3 — 3.6)	0.709
Diabetes	2 (12.5%)	0 (0%)	-12.5%	(-28.7 — 3.7)	0.492
No diabetes	5 (4.4%)	5 (4.2%)	-0.2%	(-5.4 — 5.0)	1.000
Longitudinal location					
Body	6 (7.5%)	2 (3.6%)	-4.9%	(-11.7 — 1.9)	0.278
Antrum	1 (2.0%)	3 (5.4%)	3.4%	(-3.7 — 10.4)	0.620
Cross-sectional location					
Anterior	3 (11.1%)	1 (3.9%)	-7.3%	(-21.2 — 6.7)	0.610
Posterior	1 (4.2%)	1 (4.2%)	0%	(-11.3 — 11.3)	1.000
Lesser curvature	1 (1.8%)	2 (3.6%)	1.8%	(-4.3 — 7.8)	1.000
Great curvature	2 (8.3%)	1 (3.9%)	-4.5%	(-17.8 — 8.8)	0.602
Tumour size >20 mm	0 (0%)	1 (4.8%)	4.8%	(-4.3 — 13.9)	1.000
Tumour size ≤20 mm	7 (6.3%)	4 (3.6%)	-2.6%	(-8.3 — 3.0)	0.538
With ulcerative finding	1 (6.7%)	0 (0%)	-6.7%	(-19.3 — 6.0)	1.000
Without ulcerative finding	6 (5.2%)	5 (4.2%)	-1.0%	(-6.4 — 4.4)	0.766
Macroscopic type					
Protruded	3 (9.1%)	1 (2.6%)	-6.5%	(-17.5 — 4.6)	0.332
Flat/Depressed	4 (4.7%)	4 (4.5%)	-0.2%	(-6.4 — 6.0)	1.000
Combined	0 (0%)	0 (0%)	0%	(—)	—
Initial treatment	7 (5.5%)	5 (3.9%)	-1.60%	(-6.8 — 3.5)	0.570
Residual lesion	0 (0%)	0 (0%)	0	(—)	—
Mucosal lesion	7 (6.3%)	5 (4.4%)	-1.9%	(-7.7 — 3.9)	0.566
Submucosal lesion or deeper	0 (0%)	0 (0%)	0%	(—)	—
Histological type					
Intestinal	7 (5.8%)	4 (3.4%)	-2.5%	(-7.8 — 2.8)	0.539
Diffuse	0 (0%)	1 (14.3%)	14.3%	(-11.6 — 40.2)	1.000
Benign	0 (0%)	0 (0%)	0%	(—)	—
Institution A	0 (0%)	3 (10.0%)	10.0%	(-0.7 — 20.7)	0.237
Institution B	3 (7.1%)	1 (2.4%)	-4.8%	(-13.8 — 4.3)	0.616
Institution C	4 (11.1%)	1 (2.8%)	-8.3%	(-19.9 — 3.3)	0.357
Institution D	0 (0%)	0 (0%)	0%	(—)	—
Institution E	0 (0%)	0 (0%)	0%	(—)	—
Intravenous anesthesia	7 (5.5%)	5 (3.8%)	-1.7%	(-6.8 — 3.5)	0.568
General anesthesia	0 (0%)	0 (0%)	0%	(—)	—
Needle-tip cutting device	0 (0%)	3 (10.7%)	10.7%	(-0.7 — 22.2)	0.101
Insulated-tip cutting device	7 (7.1%)	2 (1.9%)	-5.1%	(-10.8 — 0.5)	0.946
Submucosal injection solution					
with sodium hyaluronate	4 (4.0%)	4 (4.0%)	0.1%	(-5.4 — 5.5)	1.000
without sodium hyaluronate	3 (10.3%)	1 (3.0%)	-7.3%	(-19.8 — 5.2)	0.332
with epinephrine	7 (7.2%)	2 (1.9%)	0.1%	(-5.4 — 5.5)	0.093
without epinephrine	0 (0%)	3 (10.3%)	10.3%	(-0.7 — 21.4)	0.097
Coagulating device					
Radial jaw hot biopsy forceps	6 (8.8%)	1 (1.6%)	-7.2%	(-14.7 — 0.2)	0.117
Coagrasper	1 (3.1%)	1 (2.5%)	-0.6%	(-8.4 — 7.1)	1.000
Pentax haemostatic forceps	0 (0%)	3 (10.3%)	10.3%	(-0.7 — 21.4)	0.112
Operation time (min)					
of ESD >120	1 (4.0%)	2 (12.5%)	8.5%	(-9.4 — 26.4)	0.550
of ESD ≤120	6 (5.7%)	3 (2.6%)	-3.1%	(-8.4 — 2.2)	0.315
of prophylactic coagulation >9	4 (6.8%)	3 (5.3%)	-1.5%	(-10.2 — 7.1)	1.000
of prophylactic coagulation ≤9	3 (4.2%)	2 (2.7%)	-1.6%	(-7.5 — 4.4)	0.675
Operator experience					
≤30 cases	1 (3.1%)	0 (0%)	-3.1%	(-9.2 — 2.9)	0.478
31-50 cases	2 (11.1%)	0 (0%)	-11.1%	(-25.6 — 3.4)	0.187
51-100 cases	2 (4.8%)	2 (5.6%)	0.7%	(-9.3 — 10.7)	1.000
>100 cases	2 (5.1%)	3 (7.9%)	2.8%	(-8.3 — 13.8)	0.675
Specimen size >40 mm	5 (10.0%)	3 (5.8%)	-4.2%	(-14.7 — 6.2)	0.483
Specimen size ≤40 mm	2 (2.5%)	2 (2.5%)	0%	(-4.8 — 4.8)	1.000
Curative resection	7 (6.4%)	5 (4.4%)	-1.9%	(-7.9 — 4.0)	0.566
Non-curative resection	0 (0%)	0 (0%)	0%	(—)	—
All groups	7 (5.4%)	5 (3.8%)	-1.6%	(-6.7 — 3.5)	0.570

Figure 4 Subgroup analysis for post-ESD bleeding. SLE, second-look endoscopy; ESD, endoscopic submucosal dissection.

A total of 12 (4.6%) patients—7 (5.4%) and 5 (3.8%) patients in the SLE and non-SLE groups, respectively (p=0.570)—reached the primary endpoint (table 2). Non-inferiority of the non-SLE group compared with the SLE group was confirmed with an absolute risk difference of −1.6% (two-sided 95% CI −6.7% to 3.5%, one-sided $p_{non\text{-}inferiority}$ <0.001) according to ITT analysis of the primary endpoint (figure 2). Moreover, with a strict non-inferiority margin of 4%, the result of non-inferiority of the non-SLE group compared with the SLE group was unchanged (one-sided $p_{non\text{-}inferiority}$=0.015). In the SLE group, two patients withdrew informed consent before SLE and two patients violated the exclusion criteria (figure 3). To exclude the possibility that the protocol-mandated SLE allocation may have influenced the outcome, we performed a per-protocol analysis (126 (96.9%) patients in the SLE group and 132 (100%) patients in the non-SLE group). Post-ESD bleeding occurred in 7 (5.6%) out of 126 patients in the SLE group and in 5 (3.8%) out of 132 patients in the non-SLE group, which resulted in the non-inferiority of the non-SLE group with an absolute risk difference of −1.8% (two-sided 95% CI −7.0% to 3.4%, one-sided $p_{non\text{-}inferiority}$<0.001); this finding was consistent with the result of the ITT analysis of the primary endpoint (figure 2). The findings regarding post-ESD bleeding were also consistent across the subgroups (figure 4).

The timing of post-ESD bleeding is shown in figure 5. All 12 cases of post-ESD bleeding occurred prior to POD 15, and 4 (33.3%) of the 12 cases occurred within 24 h post-ESD. Post-ESD bleeding after POD 1 occurred in five (3.8%) patients

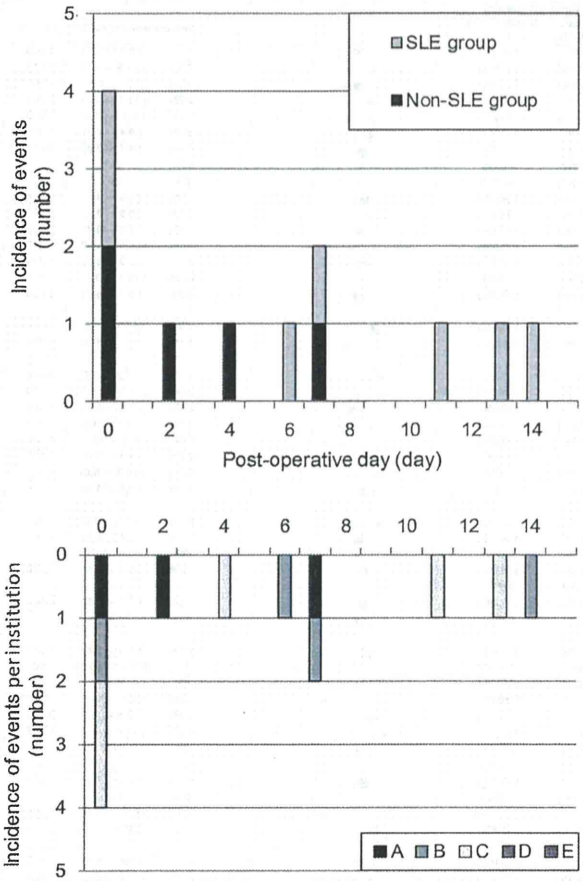


Figure 5 Timing of post endoscopic submucosal dissection bleeding. SLE, second-look endoscopy.

in the SLE group and in three (2.3%) patients in the non-SLE group (p=0.499) (table 2). Post-ESD bleeding after POD 5, determined as part of an ancillary analysis, occurred in six patients: five (3.8%) in the SLE group and one (0.8%) in the non-SLE group (p=0.119). The time-to-event curve for post-ESD bleeding showed no statistically significant difference between the groups (p=0.549) (figure 6).

Prophylactic coagulation in the visible vessels was performed in 42 (33.3%) of the 126 patients in the SLE group during the SLE procedure (figure 3). From another ancillary analysis, we determined that post-ESD bleeding occurred in 3 (7.1%) of the

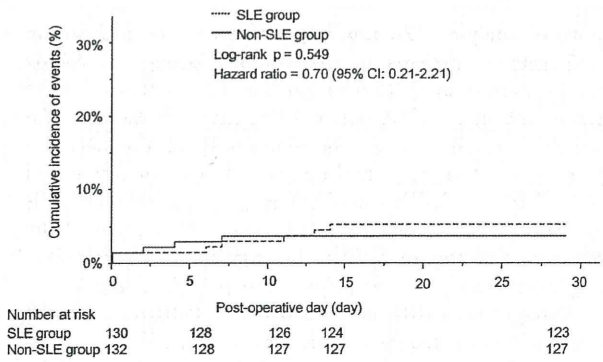


Figure 6 Time-to-event curve of post endoscopic submucosal dissection bleeding. SLE, second-look endoscopy.

Table 3 Comparison of the groups with and without prophylactic haemostasis

	Post-ESD bleeding after POD 1	p Value
SLE group with prophylactic haemostasis (n=42)	3 (7.1%)	0.332
SLE group without prophylactic haemostasis (n=84)	2 (2.4%)	

Data are numbers (%).
ESD, endoscopic submucosal dissection; POD, postoperative day; SLE, second-look endoscopy.

42 patients in whom prophylactic coagulation was performed during SLE and in 2 (2.4%) of the 84 patients who did not receive prophylactic coagulation (p=0.332) (table 3).

Other adverse events in each treatment group are shown in table 4. On the day after ESD, one patient with a 1.5 cm intramucosal early gastric neoplasm on the anterior wall of the gastric body presented with sudden epigastric pain and was found to have delayed perforation by an abdominal CT scan. All cases of post-ESD bleeding and delayed perforation were successfully managed with conservative treatment and without blood transfusions or surgery (table 2).

According to our supplementary analyses of risk factors for post-ESD bleeding, a resected specimen size >40 mm seemed to be a risk factor for post-ESD bleeding (see online supplementary tables S1 and S2).

DISCUSSION

In this multicentre, prospective, randomised controlled non-inferiority trial, the non-inferiority of omission of SLE after gastric ESD on the proportion of patients with post-ESD bleeding compared with the performance of SLE was demonstrated. In April 2013, a randomised clinical trial that evaluated similar outcomes suggested that no significant difference existed in post-ESD bleeding between patients in the SLE and non-SLE groups (p=0.66).[20] However, the small sample size in that study (n=155 from a single centre) did not indicate a 10% difference in the proportion of patients with post-ESD bleeding between the groups (two-sided α=0.05, power=0.38). Accordingly, we are convinced that SLE after gastric ESD should not be routinely performed based on the results of our study.

With regards to the timing of post-ESD bleeding, it has been reported that 25%–75% of the cases of post-ESD bleeding occurred within 24 h after ESD.[6 15] Similar to the previous reports, 4 (33%) of the 12 cases of post-ESD bleeding in the current trial occurred within 24 h post-ESD. Because SLE was performed on

Table 4 Other adverse events in the treatment groups

	CTCAE grade								
	SLE group (n=130)				Non-SLE group (n=132)				
	1	2	3	4	1	2	3	4	p Value
Fever	6	2	0	0	8	1	0	0	0.843
Abdominal pain	23	2	0	0	28	3	1*	0	0.307
Delayed perforation	–	0	0	0	–	1*	0	0	0.321

Data are numbers.
*Same patient.
CTCAE, Common Terminology Criteria for Adverse Events; SLE, second-look endoscopy.

the day after ESD, we analysed the incidence of post-ESD bleeding after POD 1 as a secondary endpoint with which to assess the potential effects of SLE; however, the difference between the groups was not statistically significant. Interestingly, the proportion of patients with post-ESD bleeding after POD 5 was rather high in the SLE group compared with the non-SLE group, although this difference was not statistically significant.

Prophylactic coagulation was performed in one-third of the patients during SLE; most patients, even those in the SLE group, did not require prophylactic coagulation. The proportion of patients with post-ESD bleeding was similar in the groups with and without prophylactic coagulation during SLE, even when prophylactic coagulation was performed. The reason why the delayed bleeding still occurred, particularly in patients who underwent prophylactic coagulation, is unclear. Unrecognised arteries that were not coagulated during SLE, thick arteries that were not coagulated completely during SLE, air insufflation and prophylactic coagulation that may have induced tissue damage or necrosis during SLE may have contributed to the exposure of arteries on the base of the ulcer, which in turn contributed to the delayed bleeding. From this perspective, prophylactic coagulation during SLE cannot prevent post-ESD bleeding and may even increase the incidence of post-ESD bleeding.

With regards to cost-effectiveness, the Japanese national insurance system has set the fee for a diagnostic oesophagogastroduodenoscopy at 11 400 yen, whereas that for endoscopic haemostasis or coagulation it is 46 000 yen. Approximately, 40 000 ESDs are performed each year in Japan, according to the official report by the Japanese Ministry of Health, Labour and Welfare.[3] Therefore, the total cost of SLE is estimated to be approximately 917 million yen per year, assuming that endoscopic haemostasis or coagulation is performed in one-third of all SLE cases. According to our findings, when unnecessary SLEs after gastric ESD are avoided, a substantial amount of money and human resources may be saved and the burden of endoscopy practices may be reserved for other purposes.

Our trial does have some limitations. First, we excluded patients with a high risk of bleeding, such as those who had chronic renal failure or liver cirrhosis and who continued using antithrombotic drugs, anticoagulants, steroids or NSAIDs during the perioperative period. However, SLE may not be effective in this population either and may even increase the incidence of post-ESD bleeding, as observed in the current study, because SLE may not reduce the bleeding of unrecognised or thick arteries, and air insufflation with over-coagulation may induce tissue damage or necrosis during SLE. Second, we only enrolled patients who were undergoing gastric ESD in advanced, high-volume institutions in Japan. Gastric ESD is technically demanding; thus, the technical aspects of ESD may affect the risk of post-ESD bleeding in low-volume centres within and outside of Japan, as it has been reported that longer procedure times may affect post-ESD bleeding.[7 21 22] Therefore, it is recommended that our findings be confirmed in other practical settings in future studies.

In conclusion, non-inferiority of the non-SLE group compared with the SLE group with respect to the incidence of post-ESD bleeding was demonstrated. When bleeding and non-bleeding visible vessels are sufficiently treated during the ESD procedure and perioperative proton pump inhibitors are administered, SLE after gastric ESD is not routinely recommended for patients without high bleeding risks because SLE does not contribute to the prevention of post-ESD bleeding. Drugs and prophylactic coagulation during SLE may even increase the incidence of post-ESD bleeding.

Author affiliations
[1]Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan
[2]Department of Gastrointestinal Oncology, Osaka Medical Centre for Cancer and Cardiovascular Diseases, Osaka, Japan
[3]Endoscopy Division, National Cancer Centre Hospital, Tokyo, Japan
[4]Department of Gastroenterology, Endoscopy division, National Cancer Centre Hospital East, Kashiwa, Japan
[5]Endoscopy Division, Gastrointestinal Centre, Cancer Institute Hospital, Tokyo, Japan
[6]Division of Research and Development for Minimally Invasive Treatment, Cancer Centre, School of Medicine, Keio University, Tokyo, Japan
[7]Department of Statistics and Information Management, Kameda Medical Centre Makuhari, Chiba, Japan
[8]Department of Endoscopy and Endoscopic Surgery, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan

Acknowledgements We thank Tomohiro Shinozaki (Department of Biostatistics, School of Public Health, Graduate School of Medicine, The University of Tokyo) for his insightful comments and advice for analyses in this study; we also thank Shigetaka Yoshinaga, Satoru Nonaka, Seiichiro Abe, Yasuhiro Ohno, Tomoyuki Odagaki, Atsushi Yagishita, Yusuke Yoda, Tomoji Kato, Satoshi Ono, Keiko Niimi, Yosuke Tsuji, Itsuko Asada-Hirayama, Maki Konno-Shimizu, Rie Matsuda, Chihiro Minatsuki, Yoshiki Sakaguchi, Yu Takahahi and Chiemi Nakayama for the enrolment of patients and data collection; and finally, we thank Kanako Yoshida for data entry.

Collaborators Tomohiro Shinozaki; Shigetaka Yoshinaga; Satoru Nonaka; Seiichiro Abe; Yasuhiro Ohno; Tomoyuki Odagaki; Atsushi Yagishita; Yusuke Yoda; Tomoji Kato; Satoshi Ono; Keiko Niimi; Yosuke Tsuji; Itsuko Asada-Hirayama; Maki Konno-Shimizu; Rie Matsuda; Chihiro Minatsuki; Yoshiki Sakaguchi; Yu Takahahi; Chiemi Nakayama; Kanako Yoshida.

Contributors MF, NU and SM were responsible for the concept of the trial and the formulation of the study design, which all authors subsequently accepted. MF and SM were responsible for the management of the trial and for the writing of the report. TS was responsible for data management and for the implementation of the statistical analysis. SK and NY contributed to the trial concept and design. The initial draft was written by MF and SM and was revised based on comments from all of the other authors. All authors contributed to the enrolment of patients and to the data collection, reviewed the final submitted report and agreed with its contents. The authors take full responsibility for the report.

Funding The current trial was self-funded.

Competing interests MF has received unrestricted grants from Astellas Pharmaceutical, Takeda Pharmaceutical, Zeria Pharmaceutical, Otsuka Pharmaceutical, AstraZeneca Pharmaceutical, Dainihon-Sumitomo Pharmaceutical, Taiho Pharmaceutical, Ajinomoto Pharmaceutical, Daiichi-Sankyo Pharmaceutical, Aska Pharmaceutical, and Eisai for his department; non-financial support from HOYA Pentax, Olympus Medical Systems, and Fujifilm for his department; and honoraria from Olympus Medical Systems, HOYA Pentax, Eisai, Daiichi-Sankyo Pharmaceutical, AstraZeneca Pharmaceutical, Aska Pharmaceutical, Taisho-Toyama Pharmaceutical, Otsuka Pharmaceutical, Zeria Pharmaceutical, Takeda Pharmaceutical, Astellas Pharmaceutical, Johnson & Johnson, Ajinomoto Pharmaceutical, Amco, Novartis Pharmaceutical, Boston Scientific, and Boehringer-Ingelheim outside the submitted work. The other authors declare that they have no conflicts of interest.

Ethics approval IRB of the participating institutions.

Provenance and peer review Not commissioned; externally peer reviewed.

REFERENCES

1 Ferlay J, Shin HR, Bray F, *et al*. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 1002;127:2893–917.
2 Isobe Y, Nashimoto A, Akazawa K, *et al*. Gastric cancer treatment in Japan: 2008 annual report of the JGCA nationwide registry. *Gastric Cancer* 2011;14:301–16.
3 http://www.e-stat.go.jp/SG1/estat/List.do?lid=000001111449. 7 December 2013.
4 Hirasawa K, Kokawa A, Oka H, *et al*. Risk assessment chart for curability of early gastric cancer with endoscopic submucosal dissection. *Gastrointest Endosc* 2011;74:1268–75.
5 Okada K, Yamamoto Y, Kasuga A, *et al*. Risk factors for delayed bleeding after endoscopic submucosal dissection for gastric neoplasm. *Surg Endosc* 2011;25:98–107.
6 Takizawa K, Oda I, Gotoda T, *et al*. Routine coagulation of visible vessels may prevent delayed bleeding after endoscopic submucosal dissection–an analysis of risk factors. *Endoscopy* 2008;40:179–83.
7 Toyokawa T, Inaba T, Omote S, *et al*. Risk factors for perforation and delayed bleeding associated with endoscopic submucosal dissection for early gastric neoplasms: analysis of 1123 lesions. *J Gastroenterol Hepatol* 2011;27:907–12.

8 Goto O, Fujishiro M, Oda I, *et al*. A multicenter survey of the management after gastric endoscopic submucosal dissection related to postoperative bleeding. *Dig Dis Sci* 2012;57:435–9.
9 Dinis-Ribeiro M, Pimentel-Nunes P, Afonso M, *et al*. A European case series of endoscopic submucosal dissection for gastric superficial lesions. *Gastrointest Endosc* 2009;69:350–5.
10 Marmo R, Rotondano G, Bianco MA, *et al*. Outcome of endoscopic treatment for peptic ulcer bleeding: Is a second look necessary? A meta-analysis. *Gastrointest Endosc* 2003;57:62–7.
11 Calvet X, Vergara M, Brullet E, *et al*. Addition of a second endoscopic treatment following epinephrine injection improves outcome in high-risk bleeding ulcers. *Gastroenterology* 2004;126:441–50.
12 El Ouali S, Barkun AN, Wyse J, *et al*. Is routine second-look endoscopy effective after endoscopic hemostasis in acute peptic ulcer bleeding? A meta-analysis. *Gastrointest Endosc* 2012;76:283–92.
13 Tsoi KK, Chiu PW, Sung JJ. Endoscopy for upper gastrointestinal bleeding: is routine second-look necessary? *Nat Rev Gastroenterol Hepatol* 2009;6:717–22.
14 Barkun AN, Bardou M, Kuipers EJ, *et al*. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010;152:101–13.
15 Goto O, Fujishiro M, Kodashima S, *et al*. A second-look endoscopy after endoscopic submucosal dissection for gastric epithelial neoplasm may be unnecessary: a retrospective analysis of postendoscopic submucosal dissection bleeding. *Gastrointest Endosc* 2010;71:241–8.
16 Heldwein W, Schreiner J, Pedrazzoli J, *et al*. Is the Forrest classification a useful tool for planning endoscopic therapy of bleeding peptic ulcers? *Endoscopy* 1989;21:258–62.
17 Association JGC. Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011;14:113–23.
18 Pocock SJ, Simon R. Sequential treatment assignment with balancing for prognostic factors in the controlled clinical trial. *Biometrics* 1975;31:103–15.
19 Altman DG, Bland JM. Treatment allocation by minimisation. *BMJ* 2005;330:843.
20 Ryu HY, Kim JW, Kim HS, *et al*. Second-look endoscopy is not associated with better clinical outcomes after gastric endoscopic submucosal dissection: a prospective, randomized, clinical trial analyzed on an as-treated basis. *Gastrointest Endosc* 2013;78:285–94.
21 Takeuchi T, Ota K, Harada S, *et al*. The postoperative bleeding rate and its risk factors in patients on antithrombotic therapy who undergo gastric endoscopic submucosal dissection. *BMC Gastroenterol* 2013;13:136.
22 Higashiyama M, Oka S, Tanaka S, *et al*. Risk factors for bleeding after endoscopic submucosal dissection of gastric epithelial neoplasm. *Dig Endosc* 2011;23:290–5.

J Gastroenterol (2014) 49:882–889
DOI 10.1007/s00535-013-0832-z

The Japanese Society of Gastroenterology

ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Helicobacter pylori infection is positively associated with gallstones: a large-scale cross-sectional study in Japan

Yu Takahashi · Nobutake Yamamichi · Takeshi Shimamoto · Satoshi Mochizuki · Mitsuhiro Fujishiro · Chihiro Takeuchi · Yoshiki Sakaguchi · Keiko Niimi · Satoshi Ono · Shinya Kodashima · Toru Mitsushima · Kazuhiko Koike

Received: 17 March 2013 / Accepted: 1 May 2013 / Published online: 5 June 2013

Abstract
Background Our aim is to elucidate causative factors for gallstones, especially focusing on *Helicobacter pylori (HP)* infection.
Methods We analyzed 15,551 Japanese adults who had no history of gastrectomy, cholecystectomy, *HP* eradication, and didn't use proton pump inhibitors, anti-diabetic drugs, or anti-cholesterol drugs. 1,057 subjects who previously had *HP* eradication were analyzed separately.
Results Gallstones were detected in 409 of 8,625 men (4.74 %) and 285 of 6,926 women (4.11 %) by ultrasonography. Among the 25 factors univariately analyzed, age, *HP* infection, alcohol intake, weight, body mass index (BMI), and 14 blood test values (AST, ALT, ALP, γ-GTP, T-Chol, HDL-Chol, LDL-Chol, TG, TP, Hb, HbA1c, pepsinogen I, pepsinogen II, and pepsinogen I/II ratio) displayed significant association with gallstones ($p < 0.05$), whereas gender, smoking, height, and three blood test values (Alb, T-Bil, MCV) did not. Multivariate analysis showed that age, gender, alcohol intake, BMI, γ-GTP, LDL-Chol, TP, and *HP* infection had significant association ($p < 0.05$). Successive multiple logistic regression analysis calculating odds ratio (OR) and standardized coefficients (β) showed that age (OR/β = 1.57/0.450), BMI (OR/β = 1.30/0.264), *HP* infection (OR/β = 1.51/0.206), lower alcohol intake (OR/β = 1.33/0.144), γ-GTP (OR/β = 1.15/0.139), and pepsinogen I/II ratio (OR/β = 1.08/0.038) have significant positive association with gallstones, whereas gender does not. The gallstone prevalence among *HP*-negative, *HP*-eradicated, and *HP*-positive subjects was 3.81, 4.73 and 6.08 %, respectively. The matched analysis controlling age, BMI, γ-GTP, alcohol intake, pepsinogen I/II ratio and gender

Yu Takahashi and Nobutake Yamamichi contributed equally to this work.

Y. Takahashi · N. Yamamichi (✉) · S. Mochizuki · M. Fujishiro · C. Takeuchi · Y. Sakaguchi · K. Niimi · S. Ono · S. Kodashima · K. Koike
Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan
e-mail: nyamamic-tky@umin.ac.jp

Y. Takahashi
e-mail: yutakahashi-tky@umin.ac.jp

S. Mochizuki
e-mail: satoshi-jp@umin.ac.jp

M. Fujishiro
e-mail: mtfujish-kkr@umin.ac.jp

C. Takeuchi
e-mail: ctakeuchi-tky@umin.ac.jp

Y. Sakaguchi
e-mail: ysakaguchi0116@yahoo.co.jp

K. Niimi
e-mail: k-niimi@umin.ac.jp

S. Ono
e-mail: satoshi-tky@umin.ac.jp

S. Kodashima
e-mail: kodashima-tky@umin.ac.jp

K. Koike
e-mail: kkoike-tky@umin.ac.jp

T. Shimamoto · T. Mitsushima
Kameda Medical Center Makuhari, CD-2, 1-3, Nakase, Mihama-ku, Chiba 261-8501, Japan
e-mail: tshimamoto@gmail.com

T. Mitsushima
e-mail: mitsushima@kameda.jp

also demonstrated that gallstone prevalence among *HP*-eradicated subjects was significantly lower compared with *HP*-positive subjects ($p < 0.05$).
Conclusions *HP* infection is positively associated with gallstones. *HP* eradication may lead to prevention of gallstones.

Keywords Gallstone · *Helicobacter pylori* · Cross-sectional study

Introduction

Gallstone is one of the most common digestive disorders worldwide [1]. The prevalence of gallstones varies widely; for example, 10 % of adult Americans [2], 50.9 % of North American Indians [3], 13.8 % of Italians [4], and 3.6 % of Japanese men [5] were reported to have gallstones. As can be predicted from the fact that gallstones are comprised of cholesterol, black pigment, brown pigment, or mixed stones, cholelithiasis is not a simple conceptual disorder [1]; a variety of risk factors have been reported. Female gender, family history and ethnicity (such as Pima Indians and Chilean Mapuche Indians) have been reported to be strongly associated factors of gallstone formation, suggesting that genetic background is a strong factor in gallstones [3, 6, 7]. Lifestyle also affects gallstone formation; for instance, it has been repeatedly reported that high alcohol consumption is a preventive factor for gallstone disease [5, 8, 9] whereas smoking shows no statistically significant association with cholelithiasis [6, 9]. In addition, some other internal disorders were thought to be related to the presence of gallstones such as aging, fertility, type 2 diabetes mellitus, obesity, hyperinsulinemia, etc. [1–4, 9–12]. It has also been reported that some blood test data show significant association with the presence of gallstones, such as serum lipids [2, 6, 13–15], several liver enzymes (AST, ALT, ALP, γ-GTP), total bilirubin [16], etc.

Despite the vast number of previous epidemiological studies analyzing background factors related to gallstones, there are few large-scale surveys with a cohort of more than ten thousand subjects. At present, three large-scale studies from Europe and North America are well known: MICOL study investigating 29,584 individuals (15,910 men and 13,674 women, 30–39 years old) from Italy [6], the third NHANES survey analyzing 14,238 Americans (6,688 men and 7,550 women) [7], and Swedish Twin Registry Studies investigating 43,141 or 58,402 twin pairs in Sweden [9, 17]. These three reports mainly address the ethnical properties of Caucasian population, but there have been no large-scale epidemiological studies in Asia. Therefore, one of the aims of our study is evaluating background factors associated with the presence of gallstones using healthy Japanese population, which should reflect the characteristics of East Asian.

Additionally, we aimed to clarify the influence of the gastroduodenal environment upon the presence of gallstones. In order to evaluate the adjacent alimentary canal, we evaluated the status of *Helicobacter pylori* (*HP*) infection, which is believed to be the strongest factor mediating the upper gastrointestinal environments. Among the several tests available for evaluating the presence of *HP*, the titers of serum anti-*HP* IgG antibody (*HP*-IgG) were measured in all the study participants. It has been well established that serological test for *HP*-IgG is one of the most reliable tests to judge actual *HP* infection; for instance, decrease in the titer of *HP*-IgG is often used to judge the eradication of *HP* [18, 19]. Furthermore, we also evaluated the state of gastric mucosal atrophy by measuring values of serum pepsinogen I (PG I, produced by chief and mucous neck cells in the fundic glands of stomach) and pepsinogen II (PG II, produced by chief and mucous neck cells in the gastric fundic glands, the cells in the pyloric glands of stomach, and the cells in duodenal Brunner's glands) [20, 21]. In proportion to the progression of gastric mucosal atrophy, the serum PG I level is known to gradually decrease, while the PG II level remains fairly constant [22]. Consequently, the decrease of PG I/II ratio (a ratio of PG I to PG II) is known to be a useful marker in assessing gastric mucosal atrophy progression [23].

By analyzing more than 15,000 healthy adults, the present study should help clarify characteristics and background factors for the presence of gallstones, unique to the Asian population. Analysis focusing on the association between *HP* and gallstones is the most essential feature of our study. We believe this large-scale study, accompanied by precise evaluation of *HP* infection, should shed light on the pathophysiology of gallstones.

Methods

Study subjects

Study subjects were participants in health examination programs held by Kameda Medical Center Makuhari (Chiba-shi, Chiba, Japan) in 2010. They underwent a variety of examinations such as upper gastrointestinal endoscopy, abdominal ultrasonography, blood chemistry tests, chest X-ray, physical examinations, etc. For subjects who had medical checkups twice in the year 2010, the former data was used. The protocol was approved by the ethics committees of the University of Tokyo, and informed consent was obtained from each subject according to the Declaration of Helsinki.