
Supplement Video. CAD using endocytoscopy in both NBI and stained modes. Real-time use of CAD, which was trained with 61,925 images of colorectal polyps, correctly predicted the pathology of 2 types of diminutive polyp: an adenoma (neoplastic) and a juvenile polyp (nonneoplastic). CAD = computer-aided diagnosis NBI = narrow-band imaging.

Supplement. Study Protocol

* This supplementary material was provided by the authors to give readers further details on their article. The material was reviewed but not copyedited.
Prospective study on clinical practice using ultra magnifying endoscope and artificial intelligence

Study protocol
(English-translated version of the Japanese-edition protocol)

Digestive Disease Center, Showa University Northern Yokohama Hospital
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Ethics Committee Approval:
This protocol was approval by the Ethics Committee of Showa University Northern Yokohama Hospital on 10 May 2017.

Clinical registration:
This trial was registered with UMIN clinical registration as UMIN000027360 on 16 May 2017.
0. Summary

0.1. Flow chart

<table>
<thead>
<tr>
<th>Eligible patients with consent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonoscopy with use of EndoBRAIN</td>
</tr>
<tr>
<td>Polyp(s) evaluated by EndoBRAIN with both NBI mode and staining mode and resected</td>
</tr>
<tr>
<td>Polyps eligible for analysis</td>
</tr>
<tr>
<td>(Primary endpoint 1)</td>
</tr>
<tr>
<td>=&lt;5mm polyps in rectosigmoid colon</td>
</tr>
</tbody>
</table>

| No polyp |
| IBD |
| All polyps left in situ |
| Both NBI mode or stained mode of EndoBRAIN were not used |
| No use of endocytoscope |

| SSA/Ps |
| Heterogenous histology |

0.2. Type of study
Single-center prospective trial
0.3. Purpose
To clarify the diagnostic performance of a computer-aided diagnostic system for endocytoscopy (EndoBRAIN, prototype; Cybernet Systems, Corp. Tokyo) for colorectal neoplasms and cancers and determine the image acquisition rate when using endocytoscopy combined with narrow band-imaging (NBI).

Primary endpoint: This trial is designed to simultaneously evaluate the following three primary endpoints

Primary endpoint 1: To determine whether EndoBRAIN combined with methylene blue staining has over 90% negative predictive value (NPV) in diagnosing <=5 mm diminutive adenomas located in the recto-sigmoid colon.

Primary endpoint 2: To determine whether trainees’ image acquisition rates are higher using endocytoscopy combined with NBI for <=5 mm polyps than using magnified endoscopy combined with NBI, the image acquisition rate being defined as the rate of lesions that the endoscopist keeps continuously in focus for more than 3 seconds.

Primary endpoint 3: To determine whether EndoBRAIN combined with methylene blue staining has over 90% positive predictive value (PPV) in diagnosing >=20 mm invasive colorectal cancers.

0.4. Participants
Inclusion criteria
· Age 18 years or older.
· Not receiving anticoagulants and therefore able to undergo resection of colorectal polyps.
· Ability to make decisions and provides agreement to participate in the trial.

Exclusion criteria (participants)
· History of inflammatory bowel disease
· History of chemotherapy or radiation therapy for colorectal lesions
· Refusal to give consent for enrollment

Exclusion criteria (lesions)
· Pathological diagnosis of heterogeneous lesion (e.g., adenoma with hyperplastic
polyp)  
・ Sessile serrated adenoma/polyp

0.5. Target number of patients and enrollment period
Target number of enrolled patients: 600
Enrollment period: between June 2017 and June 2019, enrollment to end earlier if a sufficient number of participants has been enrolled.

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1. Objectives

To clarify the diagnostic performance of a computer-aided diagnostic system for endocytoscopy (EndoBRAIN, prototype; Cybernet Systems, Cybernet Corp. Tokyo) for colorectal neoplasms and cancers and determine the image acquisition rate when using endocytoscopy combined with narrow band-imaging (NBI).

Primary endpoints: This trial is designed to simultaneously evaluate the following three primary endpoints. Required sample sizes were calculated for each endpoint, after which the largest sample size was selected for the final target sample size. In this trial, the calculated sample size required for primary endpoint 1 satisfies the numbers required for both primary endpoints 2 and 3.

Primary endpoint 1: To determine whether EndoBRAIN combined with methylene blue staining has over 90% NPV in diagnosing =<5 mm diminutive adenomas located in the recto-sigmoid colon.

Primary endpoint 2: To determine whether trainees’ image acquisition rate are higher using endocytoscopy combined with NBI for =<5 mm polyps than using magnified endoscopy combined with NBI, the image acquisition rate being defined as the rate of lesions that the endoscopist keeps continuously in focus for more than 3 seconds.

Primary endpoint 3: To determine whether EndoBRAIN combined with methylene blue staining has over 90% PPV in diagnosing >=20 mm invasive colorectal cancers.

Secondary endpoints
1. Accuracy of diagnosis of diminutive polyps using EndoBRAIN and rate of high confidence in accuracy of diagnosis in the sigmoid colon and rectum.
2. Accuracy of diagnosis of diminutive polyps using EC-NBI-EndoBRAIN and rate of high confidence in accuracy of diagnosis in the sigmoid colon and rectum.
3. Percentage of methylene blue staining or EndoBRAIN-NBI successfully performed for diminutive polyps less than 5 mm.
4. Diagnostic performance using EndoBRAIN for polyps of diameter 6–9 mm or ≥10 mm.
5. Discussion of differences between experts and trainees for each secondary outcome.
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9. Percentage by localization of lesion for which experts and trainees keep the lesion continuously in focus for more than 3 seconds.
10. Difference between EC-NBI and magnifying NBI in longest time of maintenance of focused state.
11. Sensitivity, specificity, rate of correct diagnosis, PPV, and NPV for invasive cancer for neoplastic colonic lesions of $\geq 20$ mm when experts and trainees have used EndoBRAIN.
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2. Background

2-1. Background of the study

According to a survey conducted by Japanese Society for Cancer of the Colon and Rectum, the five-year survival rate after curable resection of colon cancer was 90.4% for Stage I, 82.0% for Stage II, 69.2% for Stage III, and 15.9% for Stage IV. The corresponding survival rates for rectal cancer were 91.4%, 76.4%, 57.5%, and 14.6% respectively. These data suggest that early detection and treatment have a considerable positive effect on prognosis.

Currently, colonoscopic examination and treatment are widely performed in Japan; however, a method for making diagnoses by colonoscopy has not yet been standardized. For example, Japanese guidelines for treatment of colorectal cancers recommend considering surgery as a treatment option for cancers that invade the submucosal layers deeply; however, endoscopic diagnosis of depth of submucosal invasion requires expertise in observation of pit and vessel patterns using a magnifying endoscope, which is sometimes difficult, even for expert endoscopists. To address this issue, a computer-
Aided diagnostic system (EndoBRAIN) has been developed to enable standardization of establishing diagnoses of colonoscopy.\textsuperscript{1} EndoBRAIN is based on endoscopic images obtained by using an endocytoscope (EC) (CF-Y0058; Olympus, Tokyo, Japan), which enables \textit{in vivo} cellular observation with magnification power of 500-fold. Four pilot studies have shown the potential of the EndoBRAIN system in differentiating between neoplastic and non-neoplastic lesions and identifying invasive cancers.\textsuperscript{1-4} However, these were retrospective studies; thus, the efficacy of EndoBRAIN in a clinical setting has not yet been determined.

The diagnostic performance of EndoBRAIN for colorectal lesions in a clinical setting will therefore be evaluated in this prospective trial.

2-2. Trial design

This is a single center, prospective trial to be conducted in Showa University, Northern Yokohama Hospital, Japan. Individuals scheduled for screening, surveillance or diagnostic colonoscopy will be recruited as participants in this trial and undergo colonoscopy with real-time use of EndoBRAIN. Endoscopists are encouraged to assess polyps detected during the examination with real-time use of EndoBRAIN prior to polyp resection. The diagnostic ability (e.g., sensitivity, specificity, accuracy, PPV, and NPV) of EndoBRAIN for diminutive adenomas will be measured in both an intention-treat and per-protocol manner.

2-2-1: Basis for setting the endpoints

The American Society of Gastrointestinal Endoscopy has published a guideline for optical biopsy of diminutive colorectal polyps\textsuperscript{5}. In this guideline called “PIVI initiative”, diminutive hyperplastic polyps located in the recto-sigmoid colon are left \textit{in situ} if the NPVs of endoscopists’ diagnoses of these lesions as adenomatous polyps with high confidence are over 90%. The primary endpoint of the current trial has therefore been set as the NPV of diagnosis by EndoBRAIN of diminutive polyps located in the recto-sigmoid colon.

2-3: Benefits and risks (disadvantages) of trial participation

2-3-1: Predicted patient benefits

In the present trial, involved endoscopists are encouraged to resect all the detected polyps, which will potentially result in eradication of all of the participants’ adenomatous polyps. Eradication of colorectal adenomas is considered to strongly relate
to reduction of incidence of interval cancers and cancer mortality⁶. Therefore, the current trial offers possible benefits of reduction in cancer death rates.

2-3-2: Predicted disadvantages
There is a risk of prolonging the duration of colonoscopy when undergoing colonoscopy with use of an endocytoscope and EndoBRAIN compared with a standard colonoscopy.

2-3-3: Care after completion of research
In the present trial, assessments will only be made during the examination, not subsequently. Therefore, patients will be provided standard care after completion.

2-4: Significance of this trial
The present trial has a considerable significance for colonoscopy practice in that its results may accelerate implementation of optical biopsy into clinical practice, even for novice endoscopists. Currently, the diagnostic sensitivity of optical biopsy based on naked eye examination of small adenomas is considered to be limited to roughly 80%⁷, which is not sufficient to safely omit conventional biopsy or pathological assessment; however, optical biopsy can be allowed for any-level of endoscopist if EndoBRAIN provides sufficient ability to predict the pathological diagnoses of diminutive colorectal polyps. In addition, endoscopists will have the option to omit unnecessary biopsies if the PPV of EndoBRAIN for invasive cancers is sufficiently high.

3. Standard definitions used in this trial

3-1: Anatomical items
The following definitions will used, as reproduced from the Japanese Classification of Colorectal Carcinoma, 8th Edition.³⁶

3-1-1: Large intestines
The large intestines consist of the colon and rectum.

3-1-2: Segments of the large intestines
The large intestines are divided into the following eight regions.
C (Cecum): Saccular portion caudal to the superior lip of the ileocecal valve. The border with the ascending colon is the height of the superior lip of ileocecal valve.

A (Ascending colon): Portion that connects to the cecum and leads to the hepatic flexure.

T (Transverse colon): Portion between the hepatic and splenic flexures.

D (Descending colon): Portion fixed by the posterior peritoneum, which extends from the splenic flexure to the beginning of the sigmoid colon (at the approximate height of the iliac crest).

S (Sigmoid colon): Portion connecting to the descending colon, from the height of the iliac crest to the height of the sacral promontory.

RS (Recto-Sigmoid colon): Portion from the height of the sacral promontory to the height of the inferior border of the second sacral vertebra.

Ra (Upper rectum): Portion from the height of the inferior border of the second sacral vertebra to the height of the peritoneal reflection.

Rb (lower rectum): Portion from the peritoneal reflection to the superior border of the portion attached to the puborectal muscle.

3-2: Morphological types

0: Superficial lesions
I: Protruding lesions
   Ip: Pedunculated polyps
   Isp: Semi-pedunculated polyps
   Is: Sessile polyps
II: Flat lesions
   Ila: Flat elevation of the mucosa
   IIb: Flat mucosal changes
   IIc: Mucosal depression

Note 1: Endoscopic findings are priorities for macroscopic type. The shape of the
        lesion is to be captured as a whole picture without considering histogenesis
        or differences between neoplastic and non-neoplastic lesions.
Note 2: In tumors with two elements, the lesion with the larger surface area is to be
        indicated first and connect with a “+” to the second element.
Note 3: The term “laterally spreading tumor” (LST) describes superficially
        spreading tumors and is not included in macroscopic categories, but is to be
        noted in addition on the CRF if referring to a flat lesion with a diameter ≥10
        mm deemed appropriate to be so named by the examining physician.

Granular type (G): homogenous or nodular mixed type; Nongranular (NG) type: flat
        elevated or pseudo-depressed type]

3-3: Pathology
    The pathological diagnosis is based on the WHO classification.
    In this study, low/high grade adenomas and intra-mucosal cancers are regarded as
    “adenomas”. Carcinomas invading the submucosal layer (T1) or deeper are defined as
    “invasive cancers”.

4. Patient selection criteria
    Individuals who meet all the following eligibility criteria and have none of the
    exclusion criteria are deemed eligible for enrollment

4-1: Patient selection criteria
    Inclusion criteria
    · Age 18 years or older.
    · Not receiving anticoagulants and therefore able to undergo resection of colorectal
      polyps.
· Ability to make decisions and provides agreement to participate in the trial.

4-2: Exclusion criteria
Exclusion criteria (patients)
· History of inflammatory bowel disease
· History of chemotherapy or radiation therapy for colorectal lesions.
· Refusal to give consent for enrollment
Exclusion criteria (lesions)
· Pathologically heterogeneous lesion (e.g., adenoma with hyperplastic polyp)
· Sessile serrated adenoma/polyp

5. Enrollment

5-1: Enrollment procedures
The endoscopist in charge of the individual confirms that the participant meets all the eligibility criteria for enrollment and none of the exclusion criteria.

6. Endoscopy

6-1: Endoscopic system and artificial intelligence (AI) system used in the trial
The latest generation AI model used in this study provides a fully automated classification of colorectal polyps by analyzing images acquired with an EC. The EC (CF Y-0058-I; Olympus) is a prototype endoscope involving a contact light microscopy system (520-fold magnification with a focusing depth of 35 μm and a field of view of 570 × 500 μm) integrated into the distal tip of a normal colonoscope. The EC can provide both standard white light and EC images with 1.0% methylene blue and 0.05% crystal violet staining using a hand-operated lever and endoscopic treatment can be performed as with a traditional colonoscope. An EC enables in vivo observation of nuclei and gland duct lumens in the superficial layer, allowing precise prediction of lesion pathology with accuracies between 94% and 100% for identifying adenomas.

The algorithm used for development of original custom software for the latest generation model (EndoBRAIN®; Cybernet Systems) comprises three steps: (1) feature extraction, (2) classifier, and (3) diagnostic output.
Feature extraction
EndoBRAIN uses features from analysis of texture. These features are extracted by analyzing textures, which are characterized by differences in contrast expressed using a
binary system. The arrangement of both nuclei and gland duct lumens are comprehensively quantified on the basis of 288 variables.

Classifier
A support vector machine (SVM) classifies the image as non-neoplastic/neoplastic or as non-neoplastic/adenoma/SSA/P/invasive cancer based on the 288 extracted features. SVM is one of the best machine learning methods for optimally separating complex objects by drawing a nonlinear boundary called a “hyperplane”. Approximately 28000 EC images, none of which were used in the test set, will be used for machine learning when the trial is initiated.

Diagnostic output
The predicted pathologies are displayed based on either two categories (“non-neoplastic” and “neoplastic”) for small polyps or four categories (“non-neoplastic”, “adenoma”, “invasive cancer”, or “SSA/P”) for big polyps. The probability of diagnosis (0%–100%) calculated by the SVM is displayed with the diagnosis. If the probability exceeds 90%, the diagnosis is regarded as of high confidence, whereas diagnoses with a probability less than 90% are regarded as of low confidence. If the quality of the captured image is not appropriate for automated diagnosis (e.g., insufficient staining, multiple artifacts caused by mucus, or not an EC image such as a white light image or narrow-band image), EndoBRAIN automatically recognizes these irregularities, showing “Not a good sample” on the monitor, rather than providing a pathological classification.

The computer on which the EndoBRAIN is installed is directly connected to an endoscopy unit (EVIS LUCERA ELITE; Olympus). Thus, a fully automated diagnosis is available on acquisition of the endoscopic image by pushing the release button on the endoscope. The total time from pushing the endoscopy button to acquiring the diagnostic output is only 0.2 seconds (Video 1).

6-2: Endoscopist
In the present study, there are no inclusion criteria for endoscopists to perform EndoBRAIN.

6-3: Preparation
These are recommendations, not rules. A non-specified laxative is taken orally before
sleep.
Day before examination: Drinking is prohibited.
Day of the examination: Appropriate doses (2L) of polyethylene glycol solution are administered (additional preparation is undertaken depending on the condition of the bowel).
During examination: Antispasmodics scopolamine butylbromide or glucagon are used. Analgesics pethidine hydrochloride and sedatives midazolam or diazepam are used depending on the situation.

6-4: Assessed regions
Full colon and rectum (rectum to cecum)

6-5: Endoscopy
Endoscopists are requested to perform total colonoscopy using ECs to the cecum before resecting any polyps. During withdrawal from the cecum, they are encouraged to assess polyps by using EndoBRAIN and then resect them. However, the final decision on whether they use EndoBRAIN before the resection is made by the endoscopist. Basically resection of all detected polyps is recommended; however, the final decision is made by the endoscopist.

6-6: Flow of the trial examination.
The following procedures are followed in this trial.
1. Insertion to the cecum. As a general rule, a CO2 insufflation system will be used in all participants.
2. After reaching the cecum, video recording is initiated. If there is a stenosis in the colon, the video recording is started when the endoscope reaches the point of stenosis.
3. When a polyp is detected during withdrawal, that polyp is assessed using EndoBRAIN. First, the polyp is observed in a ultra-magnifying fashion in NBI mode, after which it stained with methylene blue and again observed. It is recommended that 10 or more endoscopic images of each lesion be taken.
4. After observation, the endoscopist resects the polyp or biopsies it. Alternatively, if the endoscopist diagnoses the polyp as invasive cancer, the patient is scheduled for surgery rather than undertaking endoscopic treatment on-site.
5. After completion of the trial, the outputs from EndoBRAIN and results of pathological examination of the resected specimens will be compared and subjected
to statistical analysis.

6-7: Flow chart of the examination procedures for this clinical trial

7. Predicted adverse events

The NCI-Common Terminology Criteria for Adverse Events v4.0 (CTCAE v4.0-JCOG)
will be used to assess adverse events in this trial. The adverse events will be graded Grade 0–4 according to which of these it most closely resembles. The Grade inscribed in the CRF must also be recorded in the patient file.

7-1: Predicted adverse events
Bleeding, perforation after endoscopic resection
Abdominal distention attributable to prolonged duration of procedure

8. Assessment items

8-1: Patient characteristics
① Age
② Sex
③ Past history of inflammatory bowel disease

8-2. Lesion characteristics
① Size
② Morphological type based on Paris Classification
③ Location
④ Pathological findings (type of histology, depth of invasion of cancer, lymphovascular invasion, lymph node metastasis)

8-3. Procedure characteristics
① Time required to obtain a focused image with magnifying NBI mode
② Time required to obtain a focused image with ultra-magnifying NBI mode
③ Time required to obtain endocytoscopic images after spraying methylene blue
④ Time required to obtain the first ten endocytoscopic images
⑤ Final diagnosis by EndoBRAIN (if multiple images have been obtained, the majority rule applies)
⑥ Name of endoscopists who performed the procedure
⑦ Insertion time to the cecum and type of the endoscope used
⑧ Complications related to the colonoscopy, if any.
⑨ The rejection box must be checked if the individual refuses to consent to participation in the trial

8-4: Method of treatment
The method of treatment is to be noted in the Endoscopy Report Form.
9. Data collection

9-1: Case report form (CRF)
   CRFs used in this trial
(1) Endoscopy Results Report for each polyp
(2) Pathology report for each polyp
(3) Excel data that is automatically created and stored by EndoBRAIN when it is used
to evaluate a target polyp. Information on outputs from EndoBRAIN for each polyp
is to be retrieved after the examination from this excel data

10. Definition of endpoints

10-1: Definition of endpoints
   The pathological diagnosis of a resected or biopsy specimen is the gold standard for
diagnostic performance of EndoBRAIN. Pathology predicted by EndoBRAIN is based
on the majority rule when multiple images of a polyp have been captured. Regarding
primary endpoint 1, data from participants who have diminutive polyps evaluated by
EndoBRAIN with both NBI and staining mode and subsequently resected are to be
included in the analysis.

10-2: Unanalyzable data from EndoBRAIN
   EndoBRAIN outputs “Not a good sample” when only low-quality images have been
captured. These outputs are defined as “Unanalyzable”. Thus, when multiple images have
been captured from a polyp and EndoBRAIN’s outputs are all “Not a good sample”, the
final diagnosis by EndoBRAIN for that polyp is to be defined as “Unanalyzable”.

10-3: Definition of experts and trainees
   Trainees are defined as endoscopists who have performed fewer than 200 colonoscopies
with use of endocytoscopy at the time of initiation of the study.
   Experts are defined as endoscopists who have performed more than 200 colonoscopies
with use of endocytoscopy.

11. Statistical analysis

11-1: Principal analyses and evaluation criteria
The three principal analyses in this trial are as follows, each to be conducted as both intention-to-treat and per-protocol analyses. The worst-case scenario will be used in the intention-to-treat analysis. Polyps that EndoBRAIN is unable to diagnose and the pathological specimens of which are lost are to be treated as misdiagnosed polyps in the intention-to-treat analysis, whereas such lesions are to be excluded from the per-protocol analysis.

Primary endpoint 1: To determine whether EndoBRAIN combined with methylene blue staining has over 90% NPV in diagnosing <=5 mm diminutive adenomas located in the recto-sigmoid colon.

Primary endpoint 2: To determine whether trainees' image acquisition rates are higher using endocytoscopy combined with NBI for <=5 mm polyps than using magnified endoscopy combined with NBI, the image acquisition rate being defined as the rate of lesions that the endoscopist keeps continuously in focus for more than 3 seconds.

Primary endpoint 3: To determine whether EndoBRAIN combined with methylene blue staining has over 90% PPV in diagnosing >=20 mm invasive colorectal cancers.

11-2: Scheduled number of participants

This trial is designed to simultaneously evaluate the following three primary endpoints. Required sample sizes were calculated for each endpoint, after which the largest sample size was selected for the final target sample size. In this trial, the calculated sample size required for primary endpoint 1 satisfies the numbers required for both primary endpoints 2 and 3.

(1)Primary endpoint 1

In their previous study (Mori et al. Endoscopy 2016), the NPV for 35 polyps was 0.9714. On the basis of this finding, the distribution of the NPV was predicted as an average of 0.9714 with a standard deviation (normal distribution) of 0.0282

\[
0.0282 = \sqrt{\frac{0.9714 \times (1 - 0.9714)}{35}}
\]

Therefore, with the coefficient of confidence set as 0.80, the NPV was predicted to be more than 0.9477 in the current trial. The 95% confidence interval (CI) of the NPV is expressed as (0.9477 - a, 0.9477 + a) (n = polyps that should be diagnosed as non-
neoplastic)

The lower limit of 95% CI should be over 0.90; that is, 0.9477 – a > 0.90.

Use of these two formulas yielded \( n > 83.54 \). Thus, at least 84 polyps that are diagnosed as non-neoplastic by EndoBRAIN are required.

In a previous prospective study with 1006 participants (Singh et al. World J Gastrointest Endosc 2015), 156 diminutive polyps in the rectosigmoid colon were diagnosed as non-neoplastic. On the basis of this rate, the estimated sample size required for determining the primary endpoint in the current study was calculated as 541. Allowing for loss of some participants for various reasons, enrollment of a cohort of 600 was considered necessary.

(2) Primary endpoint 2

According to previously unpublished data (n=13), the difference between magnifying NBI and ultra-magnifying NBI in time taken to acquire images is normally distributed. Therefore, Student’s \( t \)-test would be appropriate for comparing this endpoint. With that previously unpublished data (n=13), the difference between magnifying NBI and ultra-magnifying NBI in time taken to acquire images was 15.15 seconds (standard deviation 6.69 seconds), which yields an estimated sample size of four, given an \( \alpha \)-error of 0.05 and \( \beta \) error of 0.20. However, four is too few to use Student’s \( t \)-test, thus 20 diminutive polyps would be an appropriate sample size, this definitely being included in the sample size 600 required for primary endpoint 1.

(3) Primary endpoint 3

The formula for calculating the sample size required for primary endpoint 3 is the same as for primary endpoint 1. In previous unpublished research on a cohort of 434 images, the PPV was 0.9562. Substitution of this figure in the formula used to calculate the sample size for primary endpoint 1, the estimated sample size for primary endpoint 3 was 83 lesions. Given that 72% proportion of these \( \geq 20 \) mm lesions were invasive cancers and the diagnostic accuracy of EndoBRAIN for invasive cancers and adenomas is the same (unpublished local data), it was calculated that 115 such lesions (\( = 83/0.72 \)) are required for analysis. Because Showa University Northern Yokohama Hospital is a referral hospital with a very high proportion of symptomatic patients, it is considered that 115
such lesions would be detected in the 600 prospectively recruited patients.

12. Ethical considerations

12-1: Protection of patients
   All researchers are to conduct this trial in accordance with the Declaration of Helsinki.

12-2: Informed consent
   After discussion with the Ethics Committee of Showa University Northern Yokohama Hospital (No. 17H011; 10 May 2017), it was decided that informed consent would be obtained on an “opt-out” basis in the present trial because the intervention was considered to be minimally serious. With the “opt-out” method, absence of patients’ refusal to participate is defined as agreement to participate, provided sufficient information about the study has been provided to them both by distributing documents before colonoscopy and via publicly accessible channels such as the web site of the hospital’s home page. Information about the current trial and a means of informing staff of refusal to participate is presented on the home page of Showa University (www.showa-u.ac.jp).

12-3: Protection of privacy
   There is to be no exchange of data that allows third parties to directly identify an individual (e.g., patient name) between the participating site and the Data Center. Data generated through this clinical trial will be stored securely in a locked cabinet in the Data Center.

12-4: Handling inquiries from study participants and other persons involved
   The initial point of contact for inquiries from study participants and other persons involved is the contact written on the consent form (Principal Researcher at each site). If individual sites are not able to respond, the inquiry is to be made to the Study Office.

12-5: Reporting to the Study Institution Head
   An annual report on the status of the study is to be provided in writing to the Study Institution Head (format at the discretion of each site).

12-6: Use for other research
   Data obtained during this trial will not be used for other research. All data will be
destroyed after completion of the analysis.

12-7: Protocol compliance

Researchers participating in this trial are to comply with this protocol without compromising the human rights and safety of the participants.

12-8: Institution Review Board (IRB)

For participation in this trial, this protocol and patient manual must be approved by the Ethics Committee or IRB of Showa University Northern Yokohama Hospital. In the event that a revision is made to the protocol or patient manual during the trial, the revision must be approved by the Ethics Committee or IRB.

12-9: Clinical trial registration

To ensure transparency of the study, elements such as a summary, results, and status of progress are to be disclosed to the UMIN Clinical Trial Registry, UMIN000027360

13. Handling of research results and materials

13-1: Publication of research results

After completion of the trial, the outcomes will be compiled and presented at both domestic and overseas conferences, as well as published in English-language journals.

13-2: Storage and destruction of materials and data

Materials used in this research and the associated data will be stored until either 5 years after reporting of completion of the research or 3 years after the date of publishing the final results of the research, whichever is the later.

13-3: Secondary use of data

When the Principal Investigator determines that secondary use of data obtained through this trial in meta-analysis or similar is beneficial, secondary use of such data is to be implemented with utmost care to protect personal information. In such an instance, a new protocol will be created and newly approved by the Institutional Review Board/Ethics Committee as needed.

14. Conflict of interest

Yuichi Mori, Shin-ei Kudo, and Masahi Misawa hold the patent for EndoBRAIN. Yuichi Mori, Shin-ei Kudo, Masashi Misawa have received speaking honorariums from
Olympus Corp.

15. Principal Investigator and Research Organization

15-1: Principal Investigator
Yuichi Mori, Digestive Disease Center, Showa University Northern Yokohama Hospital

15-2: Data safety manager / data center
Kunihiko Wakamura, Digestive Disease Center, Showa University Northern Yokohama Hospital

15-3: Statistical analysis
Chiaki Nishimura, PhD, CN, Medical Research Corp.

15-4: Contacts
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16: Research grant:
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17. Acknowledgment
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18. References
19. History of revisions

The committee of the involved investigators evaluated the validity of the protocol and the progress of the trial every month after the approval of the first version protocol. All the required amendments to the protocol that were proposed in the committee were submitted to the ethical committee of Showa University Northern Yokohama Hospital periodically and approved.

1) Ver. 1: 24 April 2017 (first version)

2) Ver. 2: 2 May 2017
   i) Correction of a miss-spelling in the description of the secondary endpoints

3) Ver. 3: 16 May 2017
   i) Adding “biopsy” to the required measures to obtain the pathological specimen.

   Before: *The pathological diagnosis of a resected specimen is the gold standard for diagnostic performance of EndoBRAIN.*
   After: *The pathological diagnosis of a resected or biopsy specimen is the gold standard for diagnostic performance of EndoBRAIN.*

   ii) Correction of the estimated sample size from 423 polyps to 125 polyps. (It was revealed that the previous methodology for sample size calculation had been wrong)

   iii) Revision of the study period from “Between May 2017 and June 2019” to “Between June 2017 and June 2019”

4) Ver. 4: 6 November 2017
   i) Deleting the phrase “with high confidence” from the sentence of the primary endpoint 1.

   Before: *To determine whether EndoBRAIN combined with methylene blue staining has over 90% negative predictive value (NPV) in diagnosing <=5 mm diminutive adenomas with high confidence located in the recto-sigmoid colon.*
After: To determine whether EndoBRAIN combined with methylene blue staining has over 90% negative predictive value (NPV) in diagnosing $\leq 5$ mm diminutive adenomas located in the recto-sigmoid colon.

ii) Correction of misspellings in the description of the secondary endpoints

iii) Adding the following outcome measure to the secondary endpoints.

*Comparison of difference in time taken by experts and trainees to acquire 10 EC images after acquiring first EC image.*

iv) Adding detailed explanation regarding the definition of the final diagnosis of EndoBRAIN.

Before: *Final diagnosis by EndoBRAIN*
After: *Final diagnosis by EndoBRAIN (if multiple images have been obtained, the majority rule applies)*

v) Adding the specific description regarding the analysis procedures.

Before: *The three principal analyses in this trial are as follows*
After: *The three principal analyses in this trial are as follows, each to be conducted as both intention-to-treat and per-protocol analyses.*

5) Ver. 5: 14 November 2017 (after the initiation of the trial)

i) Adding the following outcome measure to the secondary endpoints.

*Diagnostic performance using EndoBRAIN for polyps of diameter 6–9 mm or $\geq 10$ mm.*

ii) Correction of the estimated sample size from 125 polyps to 600 patients. (It was revealed that the previous methodology for sample size calculation had been wrong)

iii) Adding the specific description regarding the analysis procedures.
Before: *The three principal analyses in this trial are as follows, each to be conducted as both intention-to-treat and per-protocol analyses.*

After: *The three principal analyses in this trial are as follows, each to be conducted as both intention-to-treat and per-protocol analyses. The worst-case scenario will be used in the intention-to-treat analysis. Polyps that EndoBRAIN is unable to diagnose and the pathological specimens of which are lost are to be treated as misdiagnosed polyps in the intention-to-treat analysis, whereas such lesions are to be excluded from the per-protocol analysis.*

iv) Adding the exclusion criteria for the resected pathological specimens.

*Exclusion criteria (lesions)*
- Pathological diagnosis of heterogeneous lesion (e.g., adenoma with hyperplastic polyp)
- Sessile serrated adenoma/polyp

6) Ver. 6: 8 December 2017
   i) Minor correction of the expressions for sample size calculation.

7) English-translated-version: 15 December 2017
   i) English-translated version of the protocol was completed based on the ver. 6 protocol written in Japanese.

8) English-translated-version-2: 12 June 2018
   i) Misdescriptions (mis-“copy-and-paste” from the other protocol [J-FUSE STUDY] which was used for a reference during making the English-translated-version protocol) were deleted from the sections “12-3: Protection of privacy” and “12-6: Use for other research”.
