STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation
Title and abstract	1	*(*a) Indicate the study's design with a commonly used term in the title or the abstract PAGE 2 **AIM:** To characterize esophageal endoluminal landmarks to permit radial and longitudinal esophageal orientation and accurate lesions location.
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found PAGE 2, 3. **METHODS:** Distance from the incisors and radial orientation was estimated for the main left bronchus and the left atrium landmarks in 207 consecutive patients using white light examination. A sub-study was additionally performed using white light followed by endoscopic ultrasound (EUS) on 25 consecutive patients to confirm the findings. The scope orientation throughout the exam was maintained at natural axis, where the left esophageal quadrant corresponds to the area between 6 and 9 o'clock. Once an anatomical landmark was identified, it was recorded with a photograph and its quadrant orientation and distance from the incisors were obtained. The reference points to obtain the distances and radial orientation were: the midpoint of the left main bronchus and the most intense pulsatile zone of the left atrium. With the video processor system set to moderate insufflation, measurements were obtained at the end of the patients' air expiration. **RESULTS:** The left main bronchus and left atrium esophageal landmarks were identified using white light in 99% and 100% of subjects at a mean distance of 25.8 (SD 2.3), and 31.4 cm (SD 2.4) from the incisors, respectively. The left main bronchus landmark was found as a tubular, concave, non-pulsatile, esophageal external compression, occupying approximately 1/4 of the circumference. Meanwhile, the left atrium landmark was identified as a round, convexe, pulsatile, esophageal external compression, occupying approximately 1/4 of the circumference. Both landmarks were identified using white light on the anterior esophageal quadrant. In the sub-study, the left main bronchus was identified in 24 (92%) of patients at 25.4 (SD 2.1) and 26.7 cm (SD 1.9) from the incisors, by white light and EUS respectively. The left atrium was recognized in all patients at 30.5 (SD 1.9), and 31.6 cm (SD 2.3) from the incisors, by both white light and EUS respectively. EUS confirmed the landmarks corresponded to these two structures respectively, and that they were located on the anterior esophageal wall. The Bland-Altman plot demonstrated a high agreement among the white light and EUS measurements.
Introduction		

Background/rationale	2	Explain the scientific background and rationale for the investigation being reported PAGE 3 **Introduction** Esophageal lesions are traditionally described according to the distance from the incisors[1,2]. This measure, while helpful to roughly describe large lesions, lacks radial orientation and is inaccurate for precise location of dysplastic lesions and small flat tumors. Endoscopic resection/ablation procedures as well as newer advanced procedures, such as per-oral endoscopic myotomy (POEM), are facilitated by accurate longitudinal and radial anatomic orientation[3,4]. Furthermore, commonly used esophageal divisions comprising cervical, thoracic, and abdominal segments are unrecognized during an upper GI examination, making this surgical division meaningless for endoscopists[5,6]. Since esophageal endoluminal anatomy has been poorly studied, current endoscopy practice lacks these essentials; offering vague lesion's identification to a second intervening endoscopist. Anatomical and cross-sectional radiological studies, however, have identified two esophageal landmarks, the left main bronchus and the left atrium, and revealed their anterior location to the esophagus[7,8]. Although we recently postulated these landmarks can be used to divide the esophageal length in three thirds as part of the systematic alphanumeric coded endoscopic approach[9,10], an endoscopic study characterizing these landmarks has never been reported.
Objectives	3	State specific objectives, including any prespecified hypotheses PAGES 3,4 The aim of this study is to determine these landmarks frequency, distance from the incisors and their quadrant orientation using white light endoscopy, and to confirm these findings by endoscopic ultrasound (EUS).
Methods		
Study design	4	Present key elements of study design early in the paper PAGE 3 Both landmarks were identified using white light on the anterior esophageal quadrant. In the sub-study, the left main bronchus was identified in 24 (92%) of patients at 25.4 (SD 2.1) and 26.7 cm (SD 1.9) from the incisors, by white light and EUS respectively. The left atrium was recognized in all patients at 30.5 (SD 1.9), and 31.6 cm (SD 2.3) from the incisors, by both white light and EUS respectively. EUS confirmed the landmarks corresponded to these two structures respectively, and that they were located on the anterior esophageal wall. The Bland-Altman plot demonstrated a high agreement among the white light and EUS measurements.
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection PAGE 4 Two hundred and thirty-four patients were enrolled in the main study from March to December 2012 at EmuraCenter LatinoAmerica, Bogotá DC, Colombia. Endoscopy was indicated for both dyspeptic patients and screening purposes. In the sub-study performed to confirm that the left main bronchus and left atrial indentations, truly represented those structures, 25 consecutive patients scheduled for upper EUS were enrolled between June and July 2016 at the Mayo Clinic, Jacksonville, USA.

Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case *Observational Studies* PAGE 4 Two hundred and thirty-four patients were enrolled in the main study from March to December 2012 at EmuraCenter LatinoAmerica, Bogotá DC, Colombia. Endoscopy was indicated for both dyspeptic patients and screening purposes. Exclusion criteria consisted of patients with previous history of esophageal strictures, scleroderma, achalasia, Barrett's esophagus, cardiac or esophageal motility disorders, and esophageal or gastric surgery. Those patients endoscopically diagnosed with peptic esophagitis, esophageal candidiasis, esophageal varices, and neoplastic lesions were also excluded from analysis. In the sub-study performed to confirm that the left main bronchus and left atrial indentations, truly represented those structures, 25 consecutive patients scheduled for upper EUS were enrolled between June and July 2015 at the Mayo Clinic, Jacksonville, USA.

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable PAGES 6,7 **RESULTS** Two hundred and thirty-four patients were examined in the main study using white light endoscopy; 21 patients with peptic esophagitis, 3 with esophageal varices, 2 with incomplete data, and 1 with squamous cell carcinoma were excluded. Finally, a total of 207 subjects were analyzed (Figure 2). Meanwhile, according to a calculated sample size of twenty-four for the sub-study, twenty-five subjects were enrolled and analyzed. **Morphometric Measurements** In the main study, **t**he mean age was 54 years and the ratio female:male was 1.4:1. The mean of the weight, height and BMI was 162 cm, 66 Kg and 25, respectively. As for the sub-study, the mean age was 64 years and the ratio female:male was 1.8:1. The mean of weight, height, and BMI was 170 cm, 79 Kg and 27.3, respectively. Patients' morphometric characteristics are shown in Table 1. There were no statistical differences among the groups. **Description of Endoscopic Findings** The left main bronchus and left atrium esophageal landmarks were endoscopically identified using white light in 205 (99%) and 100% of patients at a mean distance of 25.8 (SD 2.3), and 31.4 cm (SD 2.4) from the incisors, respectively. The left main bronchus landmark was found as a tubular convexe, non-pulsatile esophageal external compression, occupying approximately 1/4 of the circumference. The left atrium landmark was identified as a rounded concave, pulsatile esophageal external compression, occupying also approximately 1/4 of the circumference. In all subjects, the radial orientation of both landmarks was identified on the anterior esophageal quadrant (Figure 3). In the sub-study, the left main bronchus was identified in twenty-three (92%) of patients at 25.4 (SD 2.1) and in twenty-four (96%) of patients at 26.7 cm (SD 1.9) from the incisors, by white light and EUS respectively. On the other hand, the left atrium was recognized in all patients at 30.5 (SD 1.9), and 31.6 cm (SD 2.3) from the incisors, by both white light and EUS respectively. Both landmarks were also identified on the anterior esophageal quadrant. EUS confirmed the landmarks corresponded to these two structures respectively, and that they were located on the anterior esophageal wall (Figure 4). There were no significant differences between the distances measured by the methods (Table 2). **Plot of Differences Between White Light and EUS Measurements** The Bland-Altman limits of agreement plot showed a mean difference of -1.1 and -1.4 cm for the left main bronchus and the left atrium when using white light and EUS, respectively (Figure 5).

Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group PAGES 6,7 The left main bronchus and left atrium esophageal landmarks were endoscopically identified using white light in 205 (99%) and 100% of patients at a mean distance of 25.8 (SD 2.3), and 31.4 cm (SD 2.4) from the incisors, respectively. The left main bronchus landmark was found as a tubular convexe, non-pulsatile esophageal external compression, occupying approximately 1/4 of the circumference. The left atrium landmark was identified as a rounded concave, pulsatile esophageal external compression, occupying also approximately 1/4 of the circumference. In all subjects, the radial orientation of both landmarks was identified on the anterior esophageal quadrant (Figure 3). In the sub-study, the left main bronchus was identified in twenty-three (92%) of patients at 25.4 (SD 2.1) and in twenty-four (96%) of patients at 26.7 cm (SD 1.9) from the incisors, by white light and EUS respectively. On the other hand, the left atrium was recognized in all patients at 30.5 (SD 1.9), and 31.6 cm (SD 2.3) from the incisors, by both white light and EUS respectively. Both landmarks were also identified on the anterior esophageal quadrant. EUS confirmed the landmarks corresponded to these two structures respectively, and that they were located on the anterior esophageal wall (Figure 4). There were no significant differences between the distances measured by the methods (Table 2).

Bias	9	Describe any efforts to address potential sources of bias PAGES 5,6 **Protocol for Measurement of Distances** Once an anatomical landmark was identified, the natural axis position of the endoscope was confirmed. Then, it was recorded with a photograph, and its quadrant orientation was identified. The reference points to obtain the distance from the incisors were: the midpoint of the left main bronchus and the most protruded pulsatile zone of the left atrium. After identifying the radial orientation of the landmark, the distance from the incisors was calculated as follows: first, the endoscope tip was positioned immediately at the reference point, then, the distance was estimated using the standard demarcation of the endoscope located every 5 cm as a primary reference, and finally, for precise estimation, a flexible ruler marked in millimeters was positioned between the incisors and the closest endoscope's mark. Each measurement was determined at the end of the patients' air expiration. **Statistical Analysis** Data were analyzed using the IBM-SPSS version 20 (IBM Corporation, Armonk, New York, USA). The results are presented as absolute numbers and proportions for qualitative variables and means ± standard deviations (SDs) for continuous variables. The coefficient of variation was used as an additional statistic of measurements' precision, and calculated as: standard deviation/mean. Differences between groups were analyzed using the chi-square test for categorical variables, and the one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables. A two-tailed P value < 0.05 was considered significant. The Bland-Altman plot was used to asses agreement between white light and EUS measurements (CI 95%). The statistical methods of this study were reviewed by a statistician (LG) of Universidad de La Sabana. **Sub-Study Sample Size** The sub-study sample size was calculated to detect differences ≥ 5% between the subjects with 95% confidence level, 80% power, 1:4 ratio and 2.5 cm SD.
Study size	10	Explain how the study size was arrived at PAGE 6 Two hundred and thirty-four patients were examined in the main study using white light endoscopy; 21 patients with peptic esophagitis, 3 with esophageal varices, 2 with incomplete data, and 1 with squamous cell carcinoma were excluded. Finally, a total of 207 subjects were analyzed (Figure 2). Meanwhile, according to a calculated sample size of twenty-four for the sub-study, twenty-five subjects were enrolled and analyzed.
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why

Statistical methods	12	(a) Describe all statistical methods, including those used to control for confounding PAGE 6 Data were analyzed using the IBM-SPSS version 20 (IBM Corporation, Armonk, New York, USA). The results are presented as absolute numbers and proportions for qualitative variables and means ± standard deviations (SDs) for continuous variables. The coefficient of variation was used as an additional statistic of measurements' precision, and calculated as: standard deviation/mean. Differences between groups were analyzed using the chi-square test for categorical variables, and the one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables. A two-tailed *P* value < 0.05 was considered significant. The Bland-Altman plot was used to asses agreement between white light and EUS measurements (CI 95%). The statistical methods of this study were reviewed by a statistician (LG) of Universidad de La Sabana.
		(*b*) Describe any methods used to examine subgroups and interactions PAGE 6 Differences between groups were analyzed using the chi-square test for categorical variables, and the one-way analysis of variance (ANOVA) or the Kruskal-Wallis test for continuous variables. A two-tailed *P* value < 0.05 was considered significant. The Bland-Altman plot was used to asses agreement between white light and EUS measurements (CI 95%).
		(*c*) Explain how missing data were addressed
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy
		(*e*) Describe any sensitivity analyses

Continued on next page

Results

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed PAGE 6 Two hundred and thirty-four patients were examined in the main study using white light endoscopy; 21 patients with peptic esophagitis, 3 with esophageal varices, 2 with incomplete data, and 1 with squamous cell carcinoma were excluded. Finally, a total of 207 subjects were analyzed (Figure 2). Meanwhile, according to a calculated sample size of twenty-four for the sub-study, twenty-five subjects were enrolled and analyzed.
		(b) Give reasons for non-participation at each stage PAGE 6 21 patients with peptic esophagitis, 3 with esophageal varices, 2 with incomplete data, and 1 with squamous cell carcinoma were excluded. Finally, a total of 207 subjects were analyzed
		(c) Consider use of a flow diagram

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders PAGE 6 In the main study, the mean age was 54 years and the ratio female:male was 1.4:1. The mean of the weight, height and BMI was 162 cm, 66 Kg and 25, respectively. As for the sub-study, the mean age was 64 years and the ratio female:male was 1.8:1. The mean of weight, height, and BMI was 170 cm, 79 Kg and 27.3, respectively. Patients' morphometric characteristics are shown in Table 1. There were no statistical differences among the groups.
		(b) Indicate number of participants with missing data for each variable of interest 0
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time
		Case-control study—Report numbers in each exposure category, or summary measures of exposure
		Cross-sectional study—Report numbers of outcome events or summary measures PAGE 6 The left main bronchus and left atrium esophageal landmarks were endoscopically identified using white light in 205 (99%) and 100% of patients at a mean distance of 25.8 (SD 2.3), and 31.4 cm (SD 2.4) from the incisors, respectively. The left main bronchus landmark was found as a tubular convexe, non-pulsatile esophageal external compression, occupying approximately 1/4 of the circumference. The left atrium landmark was identified as a rounded concave, pulsatile esophageal external compression, occupying also approximately 1/4 of the circumference. In all subjects, the radial orientation of both landmarks was identified on the anterior esophageal quadrant (Figure 3). In the sub-study, the left main bronchus was identified in twenty-three (92%) of patients at 25.4 (SD 2.1) and in twenty-four (96%) of patients at 26.7 cm (SD 1.9) from the incisors, by white light and EUS respectively. On the other hand, the left atrium was recognized in all patients at 30.5 (SD 1.9), and 31.6 cm (SD 2.3) from the incisors, by both white light and EUS respectively. Both landmarks were also identified on the anterior esophageal quadrant. EUS confirmed the landmarks corresponded to these two structures respectively, and that they were located on the anterior esophageal wall (Figure 4). There were no significant differences between the distances measured by the methods (Table 2).
Main results	16	(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included PAGE 7 The Bland-Altman limits of agreement plot showed a mean difference of -1.1 and -1.4 cm for the left main bronchus and the left atrium when using white light and EUS, respectively (Figure 5).
		(*b*) Report category boundaries when continuous variables were categorized
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses PAGE 7 The Bland-Altman limits of agreement plot showed a mean difference of -1.1 and -1.4 cm for the left main bronchus and the left atrium when using white light and EUS, respectively
Discussion		
Key results	18	Summarise key results with reference to study objectives PAGES 9,10 The two landmarks herein characterized, can be potentially used to overcome the mentioned limitations. First, when taken together along with the cricopharyngeus narrowing and the esophagogastric (EG) junction landmarks reported at a distance of 15.7 ± 1.4 and 40.9 ± 2.8 cm from the incisors respectively[14,15], constitutes the fundaments for the systematic alphanumeric endoscopic proposal to evaluate the esophagus[9,10], and can be used, as reported by us, to improve longitudinal orientation by dividing the esophagus into three non-equal but practical endoscopic thirds: the upper third, located between the cricopharyngeus narrowing and the left main bronchus; the middle third, located between the left main bronchus and the left atrium; and the distal third, extending from the left atrium downward to the EG junction[9] (Figure 6). The potential usefulness of this endoscopic classification must be further studied, but it currently provides clinically relevant data to fulfill published guidelines by major gastroenterology societies that recommend accurate photo documentation of endoscopic landmarks and a careful description of the location of a lesion to allow subsequent therapeutic applications and future surveillance[16,17]. Furthermore, an appropriate recognition of the main left bronchus and/or the left atrium landmarks and their anterior location to the esophagus, may significantly improve radial quadrant orientation. Along with the identification of the left esophageal quadrant as herein described, the landmarks can be potentially used to properly distinguish the 4-quadrants in any esophageal portion including the EG junction. Once this proper radial orientation is achieved, a clock face-distribution can be used to precisely locate any abnormality in the esophageal circumference.
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence PAGES 7,8 The distance measurements from the incisors were consistent among the three cohort of patients with similar coefficients of variation to those found as in any other anatomical study. Although some anatomical variations might explain the non-observation of the left main bronchus landmark in two patients using white light in the main study, and in one patient using EUS, additional studies in larger subpopulations are warranted to further explain these findings. As for the 2 patients in which the left main bronchus was not observed using white light but seen during the subsequent EUS exam, a probable explanation can be credited to an unrecognized light extrinsic compression over the esophageal wall. Further studies however, are also necessary to explain these observations.

Generalisability	21	Discuss the generalisability (external validity) of the study results PAGE These observations when externally validated, may fulfill in part, the lack of current reliable performance measures to gauge the quality of an esophageal endoscopy examination. Recognition of these landmarks can improve radial orientation for both standardization of anterior or posterior approaches in POEMs procedures[3,4], and localization of Barrett's esophagus with dysplasia and small squamous cell carcinomas, increasing opportunities to diagnose and treat cancer in early stages [22,25]. In summary, this study provides an endoscopic identification of endoluminal esophageal landmarks corresponding to the left main bronchus and left atrium to permit radial and longitudinal orientation and accurate lesions location.
Other information		
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based PAGE 2 Supported in part by a grant in aid from the Emura Foundation for the Promotion of Cancer Research, ID No. 01221

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.