Endoscopic Submucosal Dissection Skills Transfer to Clinical Practice after Hands-On Workshops: An International Survey

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Keywords
Endoscopic submucosal dissection · Gastrointestinal endoscopy · Endoscopic skills · Hands-on workshops · Animal models · Simulation · Training

Abstract

Background: Endoscopic submucosal dissection (ESD) is a complex procedure, requiring enhanced technical skills. Translation into clinical practice of ESD training programs has not been documented. Our aim was to assess ESD training pathways of endoscopists participating in dedicated workshops and its clinical impact on ESD outcomes.

Methods: Participants of live porcine models ESD workshops, from 2013 to 2019, were included. They were invited to complete a survey focusing on human ESD performance after training, prior skills/competencies, complete learning pathway, and clinical outcomes.

Results: From 118 invited participants, 40 (34%) completed the questionnaire. Nineteen (47%) endoscopists performed human ESD after the workshop, predominantly male (89%). At the beginning of human ESD, endoscopists had a mean of 7.7 (standard deviation (SD) 4.1) years of endoscopic experience and were all performing endoscopic mucosal resection (and emergency endoscopy. Before ESD practice, 100% of the participants were trained with live animal models and 68% with ex vivo models. The majority started clinical ESD in the lower third of the stomach or rectum (90%), with lesions ≤30 mm (89%). Each endoscopist performed a median of 19 (interquartile range 8–32) cumulative ESDs, over a mean of 3.9 (SD 2.0) years. Total en bloc resection rate was 92%, R0 resection rate 88%, and curative resection rate 86%, whereas adverse events remained <10%. Endoscopists with >10 human ESD procedures achieve clinical competence thresholds.

Conclusions: Participants of ESD workshops are adequately skilled prior to clinical ESD, complying with recommendations for training and properly implementing the technique. Transfer to clinical practice, of prior ESD skills obtained in hands-on training courses, was documented. Structured training programs achieve clinical outcomes exceeding established standards, namely in the very initial clinical phase.

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Introduction

Gastrointestinal cancers represent a clinical heavy burden worldwide. Esophageal, gastric, and colorectal cancers combined represent 18.7% of total cancer incidence and 22.7% of cancer-related mortality [1]. Technology development as well as related skills and training present an increasing challenge for diagnosis and treatment of these diseases.

Endoscopic submucosal dissection (ESD) is currently used for treating superficial gastrointestinal neoplasms. The advantages over conventional endoscopic mucosal resection (EMR) are the ability of en bloc, R0 resection of lesions >20 mm, lowering the risk of local recurrence and allowing reliable histologic evaluation [2, 3]. When compared to surgical treatment in selected patients, ESD is associated with a lower rate of adverse events, shorter operative and hospitalization time, lower costs, and increased quality of life, with similar oncological outcomes [4, 5].

However, ESD is a complex procedure, requiring enhanced technical skills, carrying considerable risks of adverse events and has a prolonged learning curve [6]. Hence, ESD training programs [3, 7–11] have been developed. These typically include baseline endoscopic experience, theoretical knowledge, observing/assisting ESD procedures, hands-on training in animal models, and starting clinical practice, under the supervision of an expert. Their widespread implementation has been heterogenous [12–14] and clinical benefits have not been demonstrated.

Training in animal models plays an important role in the preclinical early learning phase, leading to an improvement in en bloc resection rates and ESD speed as well as a decrease in adverse events [15, 16]. Nevertheless, translation into clinical practice of a structured training program has not been yet documented. Our aim was to assess ESD training pathways of endoscopists participating in dedicated workshops and its clinical impact on ESD outcomes.

Methods

Study Design and Participants

Participants of EMR and ESD workshops with hands-on training with live porcine models from 2013 to 2019 were invited by email to answer an online survey. The workshops were co-organized by the European Association for Gastroenterology, Endoscopy and Nutrition (EAGEN) as well as the European Society of Gastrointestinal Endoscopy (ESGE) and were supported by a grant from United European Gastroenterology (UEG), to develop the workshop during the first 3 years and make it accessible for endoscopists from all socioeconomic regions in Europe.

The questionnaire was built in Google Forms (Google USA) and sent from July to November 2020 (a maximum of 3 requests, in case of nonresponse). The study protocol was approved by the Institutional Review Board of the Institute of Biomedical Sciences Abel Salazar, University of Porto (Ref No. SD/HCC/79).

Invites were informed that data would be analyzed and that completing the questionnaire was voluntary. Identity protection and confidentiality of the collected data was guaranteed according to the General Data Protection Regulation. Accordingly and in agreement with the Ethics Committee for Health of the Institute of Biomedical Sciences Abel Salazar, University of Porto, completing the questionnaire implied the participant’s implicit acceptance/consent.

Workshops

Workshops had a duration of 2 days and included theoretical lectures and hands-on practice in live porcine models. They were held in the training centers of the Erasmus School of Endoscopy at the Erasmus University Medical Center in Rotterdam, The Netherlands; Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; and Experimental-Research Center ELPEN, Athens, Greece.

The use of live porcine models for training purposes in the workshop was approved by local Ethical Committees for the welfare of animals in medical training. Procedures were conducted in accordance with the “Animal Research: Reporting of In Vivo Experiments” (ARRIVE) Guidelines [17].

Live pigs (Sus scrofa domesticus) weighing between 30 and 40 kg were used. Animals were given a liquid diet for 3 days and fasted for 8 h before the procedures. General anesthesia with endotracheal intubation and mechanical ventilation was performed, according to local protocol, with the support of the veterinary staff throughout the course.

Fully equipped interventional endoscopy workstations were used under the supervision of international faculty experts. Conventional flexible endoscopes, endoscopic ancillary devices, and electrosurgical units were used. The training modules consisted of esophageal multiband EMR, esophageal ESD, gastric ESD, as well as adverse event management, as previously described [18].

Survey Questionnaire

The questionnaire was divided into 7 parts. Demographic data were initially collected. Then, the participants were inquired if they were performing human ESD after attending the workshops. If they were not, reasons for that were assessed. Endoscopists practicing clinical ESD (human ESD in the endoscopist’s hospital) were asked to continue the survey. Further domains focused on skills/competence prior to starting clinical ESD, training methods employed, clinical ESD performance with outcomes (characterized according to the European Guidelines definitions [3]), and appreciation of the learning pathway (in a 10-point Likert scale).

Statistical Analysis

The IBM® Statistical Package for Social Sciences (SPSS®, Version 26; SPSS Inc, Chicago, IL, USA) software was used to store and analyze data. Descriptive statistics were determined for all measures according to type of variables. For quantitative variables, means (with SD) were described when data assumed a normal distribution according to the Shapiro-Wilk test, Kolmogorov-Smirnov and visual inspection of histograms, and normal Q-Q plots. Otherwise, medians (with interquartile range (IQR) 25–75) were employed. Paramet-
ric and nonparametric tests were used to assess statistical differences between groups (t test or Mann-Whitney U test). Proportions were reported for categorical variables and comparisons performed with χ² test. Significance level was defined as p < 0.05.

**Results**

**Participants**

From 118 invited participants, 40 (34%) completed the questionnaire. The mean global age was 43.9 (standard deviation [SD] 7.7) years, 75% were male, 95% gastroenterologists, 80% were working in Europe and 65% in academic or tertiary centers (Table 1). The mean follow-up after the workshop was 3.5 (SD 2.3) years. Nineteen (47%) endoscopists performed human ESD after the workshop. From these, there was a predominance of male gender (89%) as well as working in academic or tertiary centers (79%). In total, 20% (2/10) of the female participants started clinical ESD, while 56.7% (17/30) of males did so (p = 0.047). Major reasons not to initiate human ESD were inadequate endoscopic unit resources (38%) and limited local human cases suitable for the technique (24%) (Fig. 1).

![Fig. 1. Reasons for not starting ESD in humans.](image)

**Table 1. Participants’ characterization**

|                                | Global | Performing ESD in humans | Not performing ESD in humans | p value |
|--------------------------------|--------|---------------------------|-------------------------------|---------|
| Participants, n (%)            | 40 (100)| 19 (47)                   | 21 (53)                       |         |
| Female (%)                     | 10 (25) | 2 (11)                    | 8 (38)                        | 0.044   |
| Age in years, mean (SD)        | 43.9 (7.7)| 43.9 (6.9)              | 44.0 (8.7)                    | 0.982   |
| Origin, n (%)                  |        |                           |                               |         |
| Europe                         | 32 (80) | 14 (74)                   | 18 (86)                       | 0.307   |
| Asia                           | 5 (12)  | 2 (10.5)                  | 3 (14)                        |         |
| South America                  | 2 (5)   | 2 (10.5)                  | 0                             |         |
| Oceania                        | 1 (3)   | 1 (5)                     | 0                             |         |
| Background formation, n (%)    |        |                           |                               |         |
| Gastroenterology               | 38 (95)| 18 (95)                   | 20 (95)                       | 0.942   |
| Surgery                        | 2 (5)   | 1 (5)                     | 1 (5)                         |         |
| Working place, n (%)           |        |                           |                               |         |
| Academic or tertiary center    | 26 (65)| 15 (79)                   | 11 (52)                       | 0.178   |
| Regional hospital              | 13 (32)| 4 (21)                    | 9 (43)                        |         |
| Private clinic                 | 1 (3)   | 0                         | 1 (5)                         |         |
| Follow-up after workshop, in years, mean (SD) | 3.5 (2.3) | 3.9 (2.0) | 3.2 (2.5) | 0.318 |
**Table 2. Skills/competence when starting clinical ESD and training**

| n = 19 |
|--------|
| Familiarity with endoscopic classifications, n (%) | 19 (100) |
| Endoscopic experience (after formal residency program) | 7.7 (4.1) |
| Performance of | |
| Upper GI EMR, n (%) | 19 (100) |
| Lower GI EMR, n (%) | 19 (100) |
| Inject and snare EMR, n (%) | 19 (100) |
| Band and snare EMR, n (%) | 14 (74) |
| Cap and snare EMR, n (%) | 12 (63) |
| Bleeding control (emergency procedures), n (%) | 19 (100) |
| ERCP, n (%) | 11 (58) |
| EUS, n (%) | 9 (47) |

Before starting clinical ESD

- "ESD theory literature," n (%) | 18 (95) |
- ESD meetings, symposiums, conferences, and live demonstrations, n (%) | 18 (95) |

After starting clinical ESD

- ESD meetings, symposiums, conferences, and live demonstrations, n (%) | 19 (100) |
- On site observation of human ESD performed by experts, n (%) | 18 (95) |
- How many, median (IQR) | 3 (1–4) |
- Assistance in human ESD, n (%) | 9 (47) |
- How many, median (IQR) | 2 (1–3.5) |
- Confirmation of knowledge and skills, by an ESD expert, before clinical ESD, n (%) | 10 (53) |

Before starting clinical ESD

- Courses with ex vivo animal models, n (%) | 13 (68) |
- How many courses, median (IQR) | 2 (1.5–3) |
- How many ESD procedures (total), median (IQR) | 10 (4.5–20) |
- Supervision by trainers, n (%) | 13 (100) |

After starting clinical ESD

- Courses with ex vivo animal models, n (%) | 6 (35) |
- How many, median (IQR) | 2 (1–4) |
- Courses with live animal models, n (%) | 9 (60) |
- How many, median (IQR) | 1 (1–2.5) |
- Time from first animal model course to first human ESD, months, median (IQR) | 18 (6–36) |
- Time from last animal model course to first human ESD, months, median (IQR) | 3 (1–6) |

**ERCP, endoscopic retrograde cholangiopancreatography; EUS, endoscopic ultrasonography.**

**Skills/Competence when Starting Clinical ESD Practice**

At the beginning of human ESD practice, endoscopists had a mean of 7.7 (SD 4.1) years of endoscopic experience (after formal residency program; Table 2). All the participants were familiar with and made use of morphologic and chromendoscopic classifications. They were all performing upper and lower GI EMR and endoscopic bleeding control procedures.

**Training**

Before starting human ESD, 95% had accessed “ESD theory literature” resources (books, articles, DVDs, internet, and including videos) and had attended meetings, symposiums, conferences, and live demonstrations (Table 2). Likewise, 95% had observed, on site, human ESD procedures performed by experts (median of 3 [IQR 1–4]) and 47% had assisted in human ESD procedures by manipulation of endoscopic accessories, patient monitoring, drug administration, etc. (median of 2 [IQR 1–3.5]). Confirmation of knowledge and skills...
by an ESD expert, before starting clinical ESD was possible in 53%.

Prior to clinical ESD, 68% trained with supervised ex vivo animal models, attending a median of 2 (IQR 1.5–3) courses and performing a median of 10 (IQR 4.5–20) ESD procedures in total. Regarding supervised live animal models, attendance of courses was 100%, with a median of 3 (IQR 1–4) courses and 10 (IQR 5–20) ESD procedures in total.

After beginning clinical ESD, 35% continued training with ex vivo animal models, attending a median of 2 (IQR 1–4) courses. Training with live animal models occurred in 60%, with a median of 1 (IQR 1–2.5) course.

Time from first and last animal model course to first human ESD was 18 months (IQR 6–36) and 3 months (IQR 1–6), respectively.

**Initial Clinical ESD**

Clinical ESD procedures were prospectively registered by 58% of participants (Table 3). All had access to multidisciplinary management of patients.

The first human ESD was performed under the supervision of a proficient endoscopist in 53% and was conducted in the lower third of the stomach or rectum in 90% of cases. The correspondent lesion size was ≤30 mm in 89%.

**Clinical ESD Outcomes**

More than 80% of participants had experience in gastric or rectal ESD (89% and 84%, respectively), and these were the primary organs for which the technique was used (47% and 42%, respectively; Table 4).
Each endoscopist performed a median of 19 (IQR 8–32) cumulative ESDs and a median of 10 (IQR 4–24) ESDs in the last year. Total en bloc resection rate was 92%, R0 resection rate 88%, and curative resection rate 86%, whereas adverse events remained <10%.

A higher number of gastric ESDs were performed (293 in total; median of 10 (IQR 4.5–17.5) per endoscopist) followed by rectal ESDs (147 in total; median of 5.5 (3.25–12.75) per endoscopist).

En bloc resection rates were higher for esophageal (97%) and gastric (97%) lesions and lower for rectal (87%) and colonic lesions (78%). Similarly, R0 resection rates were higher for esophageal and gastric lesions (93% and 92%, respectively) and lower for rectal (82%) and colonic lesions (70%).

Regarding adverse events, globally, intra-procedural perforation and bleeding occurred in 5% and <1%, respectively. Delayed bleeding was reported in 3% and surgery due to an adverse event in 1%. These events were more frequent in the colon with 13% of intra-procedural perforation and 10% for surgery due to an adverse event compared to 3% and 1%, respectively, in gastric procedures.

Endoscopists performing ≤10 human achieved en bloc, R0, and curative resection rates of 84%, 81%, and 75%, compared to 93%, 89%, 87%, respectively, for endoscopists with >10 procedures (Table 5). Differences were not statistically significant with the exception of surgery due to noncurative resection (15.6% vs. 5%, \( p = 0.0154 \)).
En bloc, R0, and curative resection rates were above 90%, 85%, and 80%, respectively, for endoscopists performing 11–20, 21–30, and >30 (Fig. 2).

**Appreciation of the Learning Pathway**

When evaluating the satisfaction with the training pathway of each endoscopist, a median grade of 7 out of 10 (IQR 7–9) was scored (Table 6). The usefulness of courses with ex vivo animal models was appreciated with a grade of 7 (IQR 5–10) and in 63% they were considered to be a prerequisite prior to human ESD. The usefulness of courses with live animal models was rated with a grade of 10 (IQR 9–10) and they were considered to be a prerequisite by 89%.

Courses with live animal models (32%), human ESD under direct supervision (21%) and observing human ESDs performed by experts (19%) were the most valued learning methods. Endoscopists elected centers for observing/assisting human ESDs performed by experts (39%) and additional ESD courses with live animal models (34%) as the main methods lacking for better ESD training.

**Discussion**

To the best of our knowledge, this is the first study to describe the transfer to clinical practice, of prior ESD skills obtained in hands-on training courses. Furthermore, structured training programs achieve clinical outcomes exceeding established standards, namely, in the very initial clinical phase.

Endoscopists participating in EMR/ESD workshops are adequately skilled prior to human ESD initiation, complying with most of the pre- and post-clinical steps recommended for training and appropriately implementing the technique in clinical practice. Slightly more than half of the included endoscopists (53%) did not start human ESD after attendance of the workshops. We might infer that their training plan was not adequately organized and perhaps not every step of the program was timely arranged. Nevertheless, the majority of the invoked reasons can be locally addressed like adjusting endoscopic unit resources and increasing detection/referral of patients with lesions suitable for ESD.

From the endoscopists that started clinical ESD, most were male endoscopists, as in other reports [12]. Familiarity with endoscopic classifications, prior endoscopic therapeutic skills as well as literature and meetings resources were undertaken, in accordance with main recommendations [3, 9, 19, 20]. We should take into account that, for instance, the recommendations for ESD training by the ESGE [9] were only issued in 2019 and that many endoscopists had already started clinical ESD by that time.

We noted a current trend to decreasing the endoscopic experience when starting ESD compared to previous Western ESD reports (mean of 7.7 years in the current study vs. median of 15 years in an international survey [14]). However, for ESD learning endoscopists, this expe-
rience is still not as low as in Japan where, in 1 study, a mean of 5 years of postgraduation was reported [8] and in Korea where 66% were second year fellows or have finished the fellowship <5 years [21].

Animal models are considered an important tool in ESD training [18, 22], and the ESGE recommends performing at least 20 procedures before human practice [9]. Accordingly, in our study, a median of 5 courses and 20 procedures were accomplished before clinical ESD. Of note, courses were always supervised as recommended [9]. In the Western setting, these models are a frequently used resource, as manifested in an Italian survey, in which 93% of endoscopists performing ESD trained with ex vivo and 76% with live animal models [12]. On the other hand, in Korea, only 60% of the endoscopists used animal training [21].

In our study, even after starting human ESD, 60% still used live animal model training. This allows endoscopists with limited initial human cases to maintain continuous exposure to ESD practice, as previously demonstrated [23] and advocated by the ESGE [9]. Such a strategy underlines the importance of assuring additional and ongoing ESD hands-on courses.

Observation/assistance of experts performing ESD and performing the first human procedures with such supervision is usually suggested [3, 9–11]. One of the difficulties in implementing ESD in the West is having local available endoscopists. Nevertheless, in this study, 95% had the opportunity to observe experts and nearly half assisted in human ESD procedures. Also, 53% were able to start human ESD under supervision, in contrast to only 35.5% in an Italian survey [12], but still not universally as in Asian countries [6, 8, 19–21]. As recommended, the first procedures were performed in the lower third of the stomach or rectum in approximately 90% of the cases and none in the colon [3, 9, 19, 20].

The higher number of procedures performed in the stomach and rectum reflect the prevalence of neoplastic lesions in each endoscopist’s country but also the intention to work in the safest and most recommended locations in the beginning of the human learning curve.

The number of ESDs performed in the last year (median of 10) is below 25 per year as suggested by the ESGE [9]. This may be explained by the follow-up inferior to 1 year for some endoscopists and because ESD implementation is a progressive, stepwise process. Accordingly, if we consider only endoscopists who performed more than 30 procedures, the median ESD procedures in the last year increase to 24.

Global clinical outcomes in our study, with resection rates of en bloc 92%, R0 88%, and curative 86%, achieved the required thresholds advocated by the ESGE [9] (en bloc >90%, R0 >80–85%, and curative resections >75%). En bloc, R0, and curative resection rates were higher for esophageal (97%, 93%, and 93%, respectively) and gastric lesions (97%, 92%, and 89%, respectively), intermediate for rectal lesions (87%, 84%, and 82%, respectively), and lower for colonic lesions (78%, 73%, and 70%, respectively). Combined colorectal ESD, attained an en bloc resection rate of 85%, R0 of 82%, and curative of 79%. These results are comparable to or better than other Western series [12, 13, 24]. Adverse events were kept under the 10% considered for early competence [10] and below other national reports (29–14%) [13]. The global perforation rate of 5% in our study, although lower than the 18–8% [13] or 6% [12] of other surveys, was superior to the 3% suggested by the ESGE [9], mainly due to colonic ESD.

Beginning ESD training in humans is strongly discouraged [3, 9, 19, 20]. In a study with nonexperienced endoscopists without supervision, the perforation rate was 34% and the en bloc resection rate 52% in the initial human rectal ESD practice (first 25 cases) [25]. In our report, despite of a low median number of rectal ESD procedures per endoscopist of 5.5, the perforation rate was 7% and en bloc resection rate 87%.

En bloc, R0, and curative resection rates (84%, 81%, and 75%, respectively) for endoscopists performing ≤10 human ESDs were below, or at the ESGE-defined competency thresholds [9]. Even so, these outcomes exceed the en bloc resection defined for the early competence (>80%) [10], but of course it is not possible to exclude that the initial procedures were less complex. When we address endoscopists with >10 procedures the en bloc, R0, and curative resection rates of 93%, 89%, 87%, respectively, surpass the established clinical standards [9] and the results of other Western series [12, 13, 24]. Even acknowledging the limitation of number thresholds in endoscopic learning curves [26], assessment of colonoscopy competency is only recommended after 200–300 cases, which represent the average number of procedures to achieve a cecal intubation rate of 90% [27–29]. As a trainee should not be allowed to practice colonoscopy independently before reaching that threshold (besides other criteria), likewise an ESD trainee should have expert supervision at least in the first 10 human procedures, which is in line with the ESGE recommendations [9].

Therefore, applying a structured training program, including animal model simulation, allows an effective and safe initial human learning curve. Major benefits seem to occur in the early clinical practice, as with validated virtual reality simulators for flexible endoscopy [30, 31]. Other sources of evidence that support the relevance of training, such as prospective comparative studies with or
without animal model, do not seem appropriate, lacking common sense and ethical reasoning.

In a Korean survey, only 43% of the participants were satisfied with their training program [21], whereas a median of 7 out 10 was the score attributed in our study. Although the preferred ESD learning methods are common, a greater focus is placed on ESD under supervision/ESD observation in the Asian study [21], while in ours, an emphasis was given to training with animal models. On the other hand, endoscopists in our study look for places in which they can observe/assist ESDs that are scarce in Europe due to the limited number of ESD expertise. In this learning pathway, a clear challenge arises regarding narrowing the existing gap between Asian endoscopist’s preferences and European endoscopist’s desires (which is the opportunity to observe/assist experts performing human ESD and to start human ESD under supervision) and the European ability to provide such training.

Regarding the strengths of this study, we highlight the worldwide origin of the participants which broadens the impact of our results. Additionally, data are related to a continuum period, addressing different modalities of training, the implementation of the technique, and then the initial human learning curve (where the impact of training program may have a greater impact).

Concerning the limitations, we acknowledge the modest number of respondents (but still satisfactory for this type of study), the low number of procedures performed, and the reliance on self-reported data. In the near future, availability of centers for observation/assisting and starting clinical ESD under expert supervision must be addressed as they are considered scarce, relevant, and desired training methods.

In conclusion, participants of ESD workshops are adequately skilled prior to clinical ESD, complying with recommendations for training and properly implementing the technique. Structured training programs achieve clinical outcomes exceeding established standards, namely, in the very initial clinical phase.

Acknowledgments

We would like to thank all respondents for completing the survey.

Statement of Ethics

The use of the live porcine model for training purposes in the workshops was approved by local ethical committees for the welfare of animals in medical training. Procedures were conducted in accordance with the “Animal Research: Reporting of In Vivo Experiments” (ARRIVE) Guidelines. The study protocol was approved by the Institutional Review Board of the Institute of Biomedical Sciences Abel Salazar, University of Porto (Ref No. SD/HCC/79). This was not a research conducted on humans (but based on online questionnaires answered by humans). Only the ones who wished to do so responded willingly and all had the possibility of not doing it if they wished. Invitees were informed that data would be analyzed and that completing the questionnaire was voluntary. Identity protection and confidentiality of the collected data was guaranteed according to the General Data Protection Regulation. Accordingly and in agreement with the Ethics Committee for Health of the Institute of Biomedical Sciences Abel Salazar, University of Porto, completing the questionnaire implied the participant’s implicit acceptance/consent. Therefore, the requirement for written informed consent was waived.

Conflict of Interest Statement

Ricardo Küttner-Magalhães, Ricardo Marcos-Pinto, and Carla Rolanda have no conflicts of interest or financial ties to disclose; Mário Dinis-Ribeiro reports research grants from Olympus and Fujifilm and Medtronic consultancy; and Arjun D. Koch reports speaker fees from Cook Medical, ERBE, Pentax, and Boston Scientific.

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Author Contributions

All authors have contributed and agreed on the content of the manuscript. Ricardo Küttner-Magalhães contributed to the study conception, study design, data acquisition, data analysis, data interpretation, manuscript writing, and critical revision. Mário Dinis-Ribeiro contributed to the study conception, study design, study supervision, data analysis, data interpretation, and critical revision of the manuscript. Ricardo Marcos-Pinto and Carla Rolanda contributed to the study conception and design, data interpretation, and critical revision of the manuscript. Arjun D. Koch contributed to the study conception and design, study supervision, data analysis, data interpretation, and critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.
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