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Long-term results of laparoscopic gastric sleeve: the importance of follow-up adherence

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Abstract. – OBJECTIVE: This study was conducted to assess the long-term results of the Laparoscopic Sleeve Gastrectomy (LSG) in patients not compliant with follow-up, and in patients who completed a postoperative follow-up program.

PATIENTS AND METHODS: The data concerning LSG patients operated from February 2011 to December 2013 were retrospectively reviewed basing on a single center database. The patients with complete long-term follow-up were scheduled in Group A, while patients who failed to attend controls for more than two years were scheduled in Group B. Long-term results (weight loss, comorbidity improvement and late complications) were compared between the two groups.

RESULTS: The study population consisted of 285 patients. Of these, 101 had a complete follow-up with a mean duration of 71 ± 7.6 months (Group A). The remaining 184 patients (Group B) were not compliant with follow-up and, consequently, the mean duration of follow-up was 5.5 ± 7.3 months ($p < 0.00001$). A higher number of patients with insufficient weight loss was recorded in Group B with respect to Group A (78 *vs.* 23; $p = 0.001$). The number of patients with results below 25% EWL was significantly higher in Group B than in Group A (24 *vs.* 5; $p = 0.04$). In the long-term, the rate of patients with symptomatic reflux requiring medical treatment was two-fold higher in Group B than in Group A.

CONCLUSIONS: The adherence to a long-term follow-up plan after LSG seems to decrease the number of patients experiencing insufficient weight loss and those at risk for developing a gastro-esophageal reflux disease.

Key Words:
Laparoscopic sleeve gastrectomy, Follow-up, Long-term results, Failure.

Introduction

Obesity is becoming a global health emergency, and the treatment of this chronic disease and its comorbidities represents one of the most important items in the health budgets of Western countries[1-3]. Currently, the only long-term treatment proven to be effective for morbid obesity is the bariatric surgery. Over the last decade, LSG has become the most frequently performed bariatric procedure worldwide. The success of LSG in the bariatric community is due not only to its proven effectiveness in treating obesity and related comorbidities in the short- and long-term, but also because it is considered less technically demanding when compared to other malabsorptive procedures[4]. Despite these undeniable advantages, like all other bariatric procedures, LSG is not exempt from a certain percentage of long-term weight-loss failures. Rates of weight-loss failures following LSG are reported in eight heterogeneous studies as 5.7% at two years up to 75.6% at six years[5-14]. Therefore, even if performed with the best standards and following the rules of good practice, surgery is not sufficient to protect the patient from long-term failures. This is due to the fact that obesity is a multifactorial disease and lifestyle change, improved eating habits and psychological support play a role as important as surgery. Therefore, surgery is only the first step in a process that should ideally last for the patient's entire life. Several studies[15-17] have analyzed the various factors responsible for long-term failures of bariatric surgery, identifying the patient's lack of success in attending the follow-up program as one of the main reasons. Unfortunately, the num-

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ber of patients lost to follow-up increases with the increase in the length of follow-up, and, as a consequence, for a large number of patients the effects of surgery in terms of either weight loss or resolution of comorbidities are unknown.

The purpose of this study was therefore to analyze the long-term results of LGS in patients not compliant with follow-up, and as well to assess whether these results are comparable with those of patients who have always followed a postoperative follow-up program.

Patients and Methods

We reviewed in a retrospective way our prospectively collected data on consecutive morbid patients with obesity receiving LSG from February 2011 to December 2013. All data pertaining to each patient, including demographic data, preoperative and postoperative clinical data, duration of follow-up, were collected. In order to determine the quality of the patient's follow-up, the type and number of visits were analyzed. The patients who completed the follow-up were scheduled in the follow-up compliant group (Group A). The patients who failed to attend their clinic appointments for more than two years were considered not compliant with the follow-up (Group B). The patients not compliant with the follow-up were contacted by telephone and invited to re-schedule for the follow-up appointments. Those who refused to attend the visit were asked to answer a telephone questionnaire regarding their weight loss, clinical evolution, complications post-LSG, and reasons for not adhering to the postoperative follow-up program. If the patients were followed by another bariatric surgeon or a general practitioner, they were considered to be compliant with the follow-up. The patients who were not reached by telephone despite several attempts were considered definitively lost to follow-up and excluded from the study. The endpoint of the follow-up was January 2019.

A multidisciplinary team consisting of a surgeon, a physician/endocrinologist, a psychiatrist, and a clinical nutritionist evaluated all patients preoperatively. All patients met the following classical criteria for bariatric surgery: body mass index (BMI) of >40 kg/m^2, or >35 kg/m^2 with a comorbidity related to the obesity. All patients underwent LSG by the same surgeon (M.D.), and the technique remained constant over the period analyzed in the study. All patients were followed up regularly over the entire period on an outpatient basis. The postoperative assessments were conducted by the same surgeon monthly during the first year, every three months during the second and third years, and annually from the fourth year onwards. Furthermore, all patients were evaluated from the nutritional-dietary point of view every three months in the first year and annually in the following years. The psychological and physical-sport evaluations were planned for all patients in the first postoperative year, and only if deemed necessary in the subsequent years. Weight-loss results were expressed as the change in BMI, percentage of excess weight loss (%EWL) and total weight loss (TWL). The %EWL was calculated as follows: *preoperative weight – follow-up weight / preoperative weight – ideal weight*100*, where the ideal weight was considered as that equivalent to a BMI of 25 kg/m^2. According to Reinhold's classification[18], insufficient weight loss after LSG was defined as <50% EWL, while excellent results and poor results were considered when the % EWL was >75% and <25%, respectively. We used the following definitions for comorbidities: hyper-tension (systolic blood pressure ≥140 and/or diastolic blood pressure ≥90 mmHg, or anti-hyper-tensive drug therapy), Type 2 Diabetes mellitus (T2D, fasting plasma glucose ≥126 mg/dL or 2-hour plasma glucose ≥200 mg/dL during oral glucose tolerance test, or anti-diabetic drug with, or without insulin therapy), Obstructive Sleep Apnea (OSA, repeated upper airway occlusions during sleep with or without sleepiness and high apnea/hypopnea index and the need for continuous positive airway pressure during sleep). The remission of any comorbidity was defined as normal blood pressure and lab values without drug therapies. For T2D, the remission was defined as normal fasting glucose without drug therapies for 1 year, and a glycosylated hemoglobin (A1C) of <6%. The improvement was defined as: a change from insulin to oral anti-diabetic drugs, a lower dose or number of drugs needed, or an improvement in A1C with the same treatment. According to the Montreal definition, Post-sleeve Gastro-Esophageal Reflux Disease (GERD) was considered for patients presenting with intense heartburn, epigastric pain, nausea, vomiting, regurgitation, ascent of gastric fluid into the mouth when in a lying position, continuous dry cough, hoarseness and sore throat, and for those being treated with Proton Pump Inhibitors (PPIs)[19].

The main purpose of the study was to evaluate any differences in the long term LSG results in relation to weight loss and comorbidity resolution between patients not compliant with follow-up and those who had a complete follow-up. The study was approved by the Institutional Internal Reviewer Board. The informed consent was obtained from all participants included in the study.

Operative Technique

All operations were performed with a 5-port technique. The greater curvature of the stomach was dissected free by dividing the short gastric vessels, starting opposite the Crow's foot (approximately 5 cm proximal to the pylorus) and reaching the angle of His (AOH). The calibration was obtained by passing a 36-Fr gastric bougie, pushed toward and along the lesser curvature, and the stomach was transected with sequential firings of 60-mm linear purple GIA reloads (Medtronic, Minneapolis, MN, USA). No reinforced staple line was used. No abdominal drains or gastric probes were left at the end of the operation. The resected stomach was extracted from the abdomen in a plastic bag.

Statistical Analysis

The continuous demographic variables are expressed as the mean ± standard deviation and the range. The categorical variables, in addition to complications, are reported as numbers and percentages. The continuous outcome variables are generally reported as the mean ± standard deviation. The Fisher's exact test and the Chi-squared test were used to investigate the relationships between and the categorical variables. The comparison of continuous outcomes between the two groups was carried out by means of non-parametric testing, as appropriate (Mann-Whitney U test). A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using the Statistical Package for Social Sciences, version 17 (SPSS, Chicago, IL).

Results

In the study period considered, 351 patients underwent LSG. Of these, 66 patients were excluded from the study because, despite multiple attempts, it was not possible to contact them. The demographic data of the remaining patients are shown in Table I. The preoperative mean weight was 110.9 ± 18.9 kg (69-228.5) and the preoperative mean BMI was 42.4 ± 7.8 kg/m^2 (35-75.4). Furthermore, almost half of patients (139; 48.7%) had a history of gastric banding. The average follow-up duration for the entire patient population was 27.7 ± 31.9 months. Of these, 101 patients had a complete follow-up with a mean duration of 71 ± 7.6 months (Group A). The remaining 184 patients (Group B) were not compliant with follow-up and consequently the mean duration of follow-up was 5.5 ± 7.3 months ($p < 0.00001$). Similarly, the difference in terms of frequency of visits between Groups A and B was highly significant (14.2 ± 7.1 *vs.* 6.9 ± 4.3; $p < 0.00001$).

Weight Loss

The average BMI, TWL, and %EWL achieved by the entire patient cohort was 29.1 ± 5.2, 31.6 ± 15.7, and 63.3 ± 25.1, respectively. No significant difference was found between patients of Groups A and B regarding initial BMI (43 ± 6.5 *vs.* 40.5 ± 4.5; $p = 0.8$), final BMI (29.2 ± 5.3 *vs.* 27.2 ± 3.27; $p = 0.6$), TWL (33 ± 16.6 *vs.* 30.9 ± 15.3; $p = 0.4$), or %EWL (64.6 ± 24.8 *vs.* 62.6 ± 25.2; $p = 0.6$).

Table I. Preoperative patient characteristics.

	Total (n. 285)	Group A (n. 101)	Group B (n. 184)	p
Age (years)	42.2 ± 13.1	38.4 ± 12.4	44.1 ± 13.14	0.2
Sex (F/M)	247/38	91/10	156/28	0.3
Preop. weight (Kg)	110.9 ± 18.9	111.3 ± 18.3	110.6 ± 19.3	0.7
Preop. BMI (Kg/m^2)	42.4 ± 7.8	43 ± 6.5	40.5 ± 4.5	0.8
Previous GB (n. %)	139; 48.7%	45; 44.5%	94; 51.1%	0.3
T2D (n. %)	45; 15.8%	15; 14.8%	30; 16.3%	0.9
Hypertension (n. %)	60; 21%	20; 19.8%	40; 21.7%	0.8
OSA (n. %)	67; 23.5%	22; 21.7%	45; 24.4%	0.7

Group A: patients with a complete follow-up; Group B: patients not compliant with follow-up; F: female; M: male; Preop.: preoperative; Kg: kilogram; m: meter; BMI: body mass index; n.: number; OSAS: obstructive sleep apnea syndrome; T2D: Type 2 Diabetes; GB: gastric banding.

Table II. Postoperative follow-up and weight loss results.

	Total (285)	Group A (n.101)	Group B (n.184)	p
Follow-up (months)	27.7 ± 31.9	71 ± 7.6	5.5 ± 7.3	< 0.00001
N. of medical examinations	9.4 ± 6.4	14.2 ± 7.1	6.9 ± 4.3	< 0.00001
Postop. BMI (Kg/m^2)	28.6 ± 5.2	29.2 ± 5.3	27.2 ± 3.27	0.6
TWL (Kg)	31.6 ± 15.7	33 ± 16.6	30.9 ± 15.3	0.1
% EWL	63.3 ± 25.1	64.6 ± 24.8	62.6 ± 25.2	0.6
< 50% EWL (n. %)	111; 38.9%	23; 22.7%	78; 42.4%	0.001
< 25% EWL (n. %)	29; 10.1%	5; 5.9%	24; 13.9%	0.04
> 75% EWL (n. %)	95; 33.3%	35; 34.6%	60; 32.6%	0.8

Group A: patients with a complete follow-up; Group B: patients not compliant with follow-up N.: number; postop.: postoperative; BMI: body mass index; Kg: kilogram; m: meter; TWL: total weight loss; EWL: excess weight loss.

The difference between the two groups became significant when the results were analyzed based on Reynolds criteria. In fact, a higher number of insufficient results (<50%EWL) was recorded in the group of patients who did not complete the follow-up with compared to the group that did it (78 *vs.* 23; p = 0.001). Furthermore, among the latter patients, the number of those with results below 25%EWL was also significantly higher in Group B than in Group A (24 *vs.* 5; p = 0.04). On the other hand, this difference disappeared when the patients with excellent results were taken into consideration (35 patients in Group A *vs.* 60 in Group B; p = 0.8) (Table II).

Regarding the antecedents of the bariatric surgery, the previous gastric banding did not seem to be correlated with an insufficient result in terms of weight loss, independent of the quality of follow-up. Indeed, in Group A, among the 23 patients with <50%EWL, only 13 had a previous gastric banding, while 38 of 78 patients in Group B had a past medical history of gastric banding (p = 0.63). The analysis of the population with a previous gastric banding shows that, even if the percentage is greater in Group B, the difference between the two is not significant (13/45 Group A *vs.* 38/94 Group B; p= 0.38). On the other hand, this difference remains statistically significant if we only consider those patients without an antecedent gastric banding (10/56 Group A *vs.* 40/90 Group B; p=0.024) (Table III).

Comorbidities

In Group A, 15 patients had T2D, 20 had hypertension, and 22 had OSA. The percentages of comorbidities resolved or improved were: hypertension 65% (13 cases), T2D 53.3% (8 cases), and OSA 81.8% (18 cases). In Group B, 30 patients had T2D, 40 had hypertension, and 45 OSA. The percentages of comorbidities resolved or improved were: hypertension 52.5% (21 cases), T2D 40% (12 cases), and OSA 60% (27 cases). Although the percentages of comorbidities resolved were lower in Group B than in Group A, we did not record any statistically significant differences between the two groups (hypertension p = 0.4, T2D p = 0.5, OSA p = 0.1) (Table IV).

Table III. Postoperative outcomes of patients having primary LSG versus LSG after gastric banding (secondary LSG).

	Primary Group A (n. 56)	LSG Group B (n. 90)	p	Secondary Group A (n. 45)	LSG Group B (n. 94)	p
BMI (Kg/m^2)	28.8 ± 5.1	27.6 ± 2.5	0.7	29.3 ± 5.4	28.5 ± 4.5	0.6
TWL (Kg)	32.6 ± 16.5	31.1 ± 14.7	0.8	33.4 ± 16.8	30.7 ± 15.9	0.8
% EWL	64.5 ± 22.9	66.8 ± 24	0.5	64.7 ± 27	58.2 ± 25.9	0.2
< 50% EWL (n. %)	10 (18%)	40 (44.4%)	0.002	13 (28.8%)	38 (40.4%)	0.2
< 25% EWL (n. %)	2 (3.6%)	9 (10%)	0.2	3 (6.6%)	15 (16%)	0.4
> 75% EWL (n. %)	18 (32.1%)	31 (34.4%)	0.4	17 (37.7%)	29 (30.8%)	0.4

Group A: patients with a complete follow-up; Group B: patients not compliant with follow-up; n.: number; LSG: laparoscopic sleeve gastrectomy; BMI: body mass index; Kg: kilogram; m: meter; TWL: total weight loss; EWL: excess weight loss.

Table IV. Postoperative comorbidities results, long-term complications and reoperations.

	Total	Group A	Group B	*p*
Hypertension (n. %)	34/60; 56.6%	13/20; 65%	21/40; 52.5%	0.4
T2D (n.; %)	20/46; 43.5%	8/15; 53.3%	12/30; 40%	0.5
OSA (n.; %)	45/67; 67.1%	18/22; 81.8%	27/45; 60%	0.1
Postop. Reflux	49; 17.2%	10; 10.1%	39; 21.2%	0.02
Reintervention	27; 9.5%	14; 13.8%	13; 7.1%	0.09

Group A: patients with a complete follow-up; Group B: patients not compliant with follow-up n.: number; OSAS: obstructive sleep apnea; T2D: Type 2 Diabetes; postop.: postoperative.

Long-term complications and Revisional Surgery

In Group A, 14 patients (13.8%) had revisional surgery for weight regain (7 cases) or for severe reflux (7 cases). Of these, two patients underwent re-sleeve gastrectomy, while three underwent conversion to a One Anastomosis Gastric Bypass (OAGB), and nine to a Laparoscopic Roux-en-Y Gastric Bypass (LRYGB), of which two for weight regain and seven for reflux. According to Montreal criteria, 10 patients presented with a severe GERD post-sleeve and, of these, seven were converted to LRYGB. In Group B, five patients underwent surgery due to insufficient weight loss or weight regain and only eight were converted to LRYGB for reflux. On the other hand, 39 (21.2%) patients had severe reflux and took high dosages of IPPs. Therefore, there was a statistically significant difference between the two groups in terms of rate of post-operative GERD ($p = 0.02$), but not in terms of conversion to LRYGB ($p = 0.26$) (Table IV).

Discussion

The present study confirms LSG to be a valid option for the long-term treatment of obesity, as shown by an average %EWL of 63% at a minimum of five years' follow-up after surgery.

This very encouraging result actually conceals the fact that more than a third of the population studied achieved insufficient weight loss, with a %EWL of <50% and in some cases <25%. Given the spread of LSG worldwide, the reason why the sleeve fails has become a very common question for bariatric professionals. Several factors have been considered to be the cause of these failures. In a systematic review, Lauti et al[16] identified the initial sleeve size (technical factors), the sleeve dilatation, the follow-up support, and the lifestyle behaviors as the main causes of weight regain. Analogously, Karmali et al[15] performed a systematic review of weight regain following bariatric surgery and identified five principal etiologies: nutritional non-compliance, hormonal/metabolic imbalance, mental health, physical inactivity and anatomical/surgical factors. Regarding the latter factor, Deguines et al[20] demonstrated a correlation between residual gastric volume and LSG success as defined by % EWL >50%, BAROS >3, BMI <35 kg/m^2, and/or the Biron criteria. All the patients in this study were operated by the same surgeon with long experience in bariatric surgery, so that even if it was not possible to exclude single technical errors that may have caused poor weight loss in some patients, it seems difficult to imagine that such errors were repeated for 34% of patients. On the other hand, a certain degree of sleeve dilatation in the years after surgery is very common, but not all patients regain weight or have poor results, as also demonstrated by Braghetto et al[21] These authors showed a doubling of the volume of the sleeve at 2-3 years after the operation, with no correlation with the patient outcomes. Similarly, Disse et al[22], evaluating the stomach volume by computed tomography with gas (3D gastric CT) at 3 and 12 months after LSG, found that 61% of patients showed stomach dilation, with a 25% increase in total gastric volume between the two tests. Despite this evidence, dilation was not related to an increase in daily caloric intake and insufficient weight loss during the first 18 months after surgery. Two other interrelated factors that play a very important role in the success of the sleeve gastrectomy seem to be long-term follow-up and lifestyle modifications. Theoretically, in order to obtain a lasting result, the follow-up should last for the lifetime of the patient. In this respect, Lauti et al[23] pointed out that the feeling experi-

enced by the patients who had regained weight was that they had had a too short postoperative follow-up and that they would need longer-term psychological and dietary surveillance. In their series, Himpens et al[24] highlighted that weight regain coincided with discharge from the three-year follow-up. Karen et al[25] found a highly significant difference in terms of the percentage of excess BMI loss (%EBMIL) between patients who had actively joined the postoperative follow-up program and those who did not participate in this program but returned at the 30-month visit. Furthermore, following these patients for five years after surgery, the authors pointed out that the success in losing weight was significantly linked to a lifestyle modification, such as healthy eating and the practice of physical activity[26]. Also, in the present study we compared the results of patients who carefully followed the postoperative follow-up program with those of patients who, in contrast, disappeared, in some cases very quickly, after the operation. Many patients in the second group were contacted and returned for a visit or answered our questions by telephone. As already demonstrated for gastric banding, even in the case of LSG, poor adherence to the follow-up program was directly linked to the failure rate of the procedure evaluated as a % EWL of <50%. Although there was no difference in terms of average %EWL between the two groups of patients at five years after surgery, only 20% of patients in the regular follow-up group had attained an insufficient %EWL, compared with 36% of the patients not compliant with follow-up. In our opinion, this difference between the two groups underlines the importance of a continuous accompaniment of the patient in determining the final result of a bariatric procedure. During scheduled visits, even years after surgery, the surgeon assesses not only the onset of long-term complications but can lead the patient to a more extensive nutritional and psychological assessment if deemed appropriate. By doing so, we can theoretically reverse an initial weight regain and reduce the rate of redo surgery.

In order to reduce the number of patients that fail to adhere to postoperative visits and consequently improve participation in the follow-up, it is important to understand the reasons why patients abandon their postoperative follow-up program. The reasons are various, as previously demonstrated in several retrospective studies. In a study of 263 patients, Vidal et al[27] identified work-related problems, family-related issues, and having moved outside the city or country as the main causes for nonadherence to scheduled postoperative visits. On the other hand, Gold et al[28] and Harper et al[29] performed two studies involving patients operated on using Roux-en-Y gastric bypass and identified insurance coverage (economical issue) and weight regain as the main causes, respectively. It is likely that the different reasons are also linked to the geographical area and the health system of the country in which the patient is operated and followed-up. In the present study, the majority of our patients justified their non-adherence to the follow-up program by their distance from the hospital, frequent home moves, and secondarily economic problems. In our experience, weight regain is not a major issue that reduces compliance to follow-up programs. In our opinion, this is due to the fact that the bariatric surgery center serves a large area with important tourist presences at certain times of the year, and consequently long-term car trips are needed. Recently, to overcome these problems, we have put into practice the possibility of online visits (telemedicine) and the use of connected devices that could allow the movement of distant patients to be reduced, consequently increasing participation in the follow-up program while maintaining a high level of safety for the patients[30].

The main long-term side effect of LSG seems to be the onset of severe GERD. Recent studies[31-34] have suggested that 20-60% of patients who receive a gastric sleeve will present symptoms related to gastric reflux following the intervention. In addition to contributing to a poor quality of life, severe GERD, if not properly treated, can lead to severe esophagitis which, in turn, can develop into a precancerous lesion, such as Barrett's esophagus[35,36]. For these reasons, in the case of failure to control the reflux with medical treatment based on PPIs, GERD has become an important indication for the conversion of sleeve gastrectomy into LRYGB. In the present study, we did not record a significant difference between the two groups in terms of conversion of LSG into LRYGB for severe GERD. On the other hand, the rate of patients with symptomatic reflux requiring IPP was two-fold higher in the group of patients not compliant with follow-up compared to those with regular follow-up. This difference between the two groups is probably linked once again to the lack of adequate postoperative support. A certain type of diet rich in fats, with the too frequent intake of spicy foods, and the excessive consumption of tea, coffee, alcohol and tobacco, have

been associated with the onset or aggravation of GERD[37-41]. Furthermore, normal physical activity appears to have a protective effect against GERD, as demonstrated by Nilsson et al[42] on a large population of patients. In fact, subjects with typical GERD symptoms seem to be physically less active than those without. These poor dietary and lifestyle behaviors can be corrected if the patient follows a well-structured postoperative follow-up program. The difference in terms of the number of symptomatic patients between the two groups is probably justified by the fact that the patients of Group A, having benefited for a longer time from a medical-nutrition support, were able to put into practice those measures necessary to control the onset of GERD earlier on.

Besides its retrospective nature, the present study has several limitations. Although most of the patients not compliant with follow-up visited the clinic, some data were difficult to extrapolate. In fact, it would have been interesting to evaluate the difference between patients in the two groups who experienced either insufficient weight loss or weight regain. To do this, however, it would have been necessary to know the minimum weight reached by the patient. Unfortunately, this type of data has often been given in an inaccurate way, making the results unreliable. Moreover, GERD was evaluated above all in terms of clinical symptoms and dosage of IPP drugs. A gastroscopy to determine the presence of esophagitis and pH-metry to quantify reflux were often carried out in the group of patients who participated to the follow-up, but only rarely in Group B, especially among patients that answered only the telephone questionnaire. For this reason, we cannot completely exclude the bias linked to the patients' personal interpretation of symptoms.

Conclusions

Sleeve gastrectomy has proved to be an effective technique for treating patients suffering from obesity, even though it presents a long-term failure rate of between 20 and 36%. The causes of these failures are probably multifactorial, even if a very important role seems to have been played by the adherence to a well-structured postoperative follow-up. Actually, the adherence to a long-term follow-up seems to decrease the number of patients experiencing insufficient weight loss and/or risking developing a gastro-esophageal reflux disease after surgery.

Conflict of Interest
The Authors declare that they have no conflict of interests.

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Ethics Approval
The study was approved by the Institutional Internal Reviewer Board of the University of L'Aquila.

Informed Consent
The informed consent was obtained from all participants included in the study.

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Availability of Data and Material
The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' Contribution
Conceptualization, S.C. and F.S.; methodology, A.A., F.S. and S.C.; validation, M.C. and R.P.; formal analysis, F.S.; investigation, S.C., M.N., V.Z., A.A. and A.B.; data curation, FC; writing—original draft preparation, F.S. and S.C.; writing—review and editing, F.S. and M.C.; supervision F.S., R.P.; project administration, F.S. and M.C. All authors have read and agreed to the published version of the manuscript.

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