Influence of Preoperative Weight Loss on Outcomes of Bariatric Surgery for Patients Under the Enhanced Recovery After Surgery Protocol

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Abstract

Introduction The enhanced recovery after surgery (ERAS) protocol, which emphasizes preoperative interventions, is safely implemented in patients undergoing bariatric surgery. Patients are additionally encouraged to achieve weight loss preoperatively. We aimed to identify factors contributing to preoperative weight loss and assess their influence on outcomes of bariatric surgery among patients under the ERAS protocol.

Materials and Methods We reviewed a prospectively created database in two bariatric centers with 909 bariatric patients treated in accordance with ERAS principles. The database included demographic characteristics, factors related to the surgery or perioperative period, and short-term outcomes. Our endpoints included analyses of (1) factors potentially contributing to preoperative weight loss and (2) the influence of preoperative weight loss on short-term outcomes of bariatric treatment.

Results Diabetes mellitus (p = 0.007), obstructive sleep apnea (p < 0.001), and previous surgery (p = 0.012) were identified as predictors of preoperative weight loss. Steatohepatitis (p < 0.001) and respiratory disorder (p = 0.004) decreased the chance of achieving satisfactory preoperative body mass reduction. Except for operative time, early outcomes of bariatric surgery were not influenced by preoperative weight loss. Patients who achieved preoperative weight loss were less likely to be lost to follow-up (p = 0.023). Postoperative weight loss was better in patients who could lose ≥ 5% total weight preoperatively (p = 0.009).

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Conclusion Unsatisfactory preoperative weight loss among patients treated under ERAS principles is not associated with increased risk of complications. Satisfactory preoperative weight loss predicts superior postoperative weight loss and follow-up participation.

Keywords Obesity · Bariatric surgery · Preoperative weight loss · Perioperative care · ERAS · Sleeve gastrectomy · Gastric bypass · Outcomes

Introduction

Laparoscopic sleeve gastrectomy (LSG) and laparoscopic Roux-en-Y gastric bypass (LRYGB) are currently the most commonly performed bariatric procedures [1–3]. Improvement in the outcomes may be expected with implementation of a structured system of modern perioperative care. The enhanced recovery after surgery (ERAS) protocol or other fast-track approaches have been safely implemented in many bariatric centers [4]. The ERAS protocol designed for bariatric surgery emphasizes extensive preoperative interventions, including medical, educational, dietary, and psychological [5, 6]. The structured program of perioperative care allows discharge from the hospital as soon as patients achieve functional recovery, thereby reducing the length of hospital stay with no influence on postoperative morbidity [7]. The overall compliance rate with components of the ERAS protocol is an essential factor contributing to the beneficial impact on surgical outcomes [8, 9].

Even though weight loss is not a classic requirement of the ERAS protocol, bariatric patients are usually encouraged to achieve at least some reduction in their body mass before surgery because it is believed to influence results [10]. Data concerning the role of preoperative weight loss as an independent factor for bariatric patients (undergoing LSG or LRYGB) are so far inconclusive. Moreover, we were unable to find any study that had evaluated this factor in patients whose perioperative care was based on ERAS principles.

Our objective was to identify potential factors contributing to preoperative weight loss. We also aimed to investigate the influence of preoperative weight loss on short-term outcomes of LSG and LRYGB among patients under the ERAS protocol.

Materials and Methods

Study Design

We reviewed a prospectively created database. Data concerning patients operated on in two tertiary referral bariatric centers between 2012 and 2017 were collected by the authors directly involved in the treatment. Qualification for bariatric surgery was based on recommendations of the Metabolic and Bariatric Surgery Chapter of the Polish Surgical Society. Inclusion criteria for this study were informed consent to participate in the study, meeting the eligibility criteria for bariatric treatment [body mass index (BMI) ≥ 35 kg/m² with obesity-related comorbidities or BMI ≥ 40 kg/m²], and qualification for LSG or LRYGB. We excluded patients with insufficient data on coexisting ailments and those who had undergone revision surgery. Patients were not excluded from the study group, if they did not achieve a certain preoperative weight loss. The study was designed, and the results described, according to all STROBE checklist points for observational studies.

The database included demographic characteristics, factors related to the surgery or perioperative period, and short-term outcomes. Patients’ baseline characteristics included preoperative weight loss, age, sex, maximum preoperative BMI, BMI on the day of operation, American Society of Anesthesiologists physical status class, type of bariatric operation (LSG or LRYGB), comorbidities (diabetes mellitus, steatohepatitis, hypertension, dyslipidemia, cardiological disorders, respiratory disorders (asthma and chronic obstructive pulmonary disease), obstructive sleep apnea), and previous surgeries. Perioperative variables included the LSG or LRYGB operative time, intraoperative adverse events, conversions, postoperative complications (according to the Clavien–Dindo classification)—paying special attention to gastrointestinal leakage, gastrointestinal stricture, rhabdomyolysis, postoperative hemorrhage, wound infection, marginal ulcers, port-site hernia, abscess—reoperations, length of hospital stay (LOS), and readmissions. Outcomes of bariatric treatment were evaluated 6 months after the surgery, with the follow-up examination including measurement of total weight loss (%TWL), excess weight loss (%EWL), and excess BMI decrease (%EBMIL). Those parameters were calculated using patients maximal weight or maximal BMI measured during the qualification for surgery. Patients were divided into two groups: group 1 (preoperative weight loss < 5% total body weight) and group 2 (preoperative weight loss ≥ 5% total body weight). The cutoff point for preoperative weight loss of 5% was based on a report by Giordano and Victorzon, who defined it as (1) achievable by a high rate of patients; (2) associated with reduced operative time, hospital stays, and overall morbidity; and (3) associated with evidence of positive bariatric surgery results [11]. We also analyzed the correlation of preoperative weight loss as a continuous variable with bariatric surgery-associated postoperative weight loss.
An intraoperative adverse event was defined as any iatrogenic harmful event not derived from the standard LSG or LRYGB technique. Postoperative complications were defined as adverse events occurring within 30 days after the procedure and were categorized (according to the Clavien–Dindo classification) as minor (grade I, II) or major (III, IV, V). Rhabdomyolysis was defined as elevated creatinine phosphokinase (> 1000 IU/l) with coexisting increased myoglobin. Gastrointestinal leakage was diagnosed clinically and confirmed radiologically. Postoperative hemorrhage was defined as a significant drop in hemoglobin that required reoperation or transfusion with packed red blood cells.

**Treatment Protocol and Surgical Techniques**

To minimize bias, patients were treated in accordance with the ERAS pathway—i.e., preoperatively, intraoperatively, postoperatively (Supplement 1). The perioperative care protocol is described in detail in our previous publications [4–6]. The LSG and LRYGB surgical techniques used at our centers were also thoroughly described previously [7, 12].

**Measured Outcomes**

The primary endpoints comprised analysis of potential factors contributing to the preoperative weight loss:

- Age
- Sex
- Maximal BMI
- Comorbidities (diabetes mellitus, steatohepatitis, hypertension, dyslipidemia, cardiovascular disorders, respiratory disorders, obstructive sleep apnea)
- Previous surgeries

The secondary endpoints derived from the analysis of the association between preoperative weight loss and short-term outcomes of LSG and LRYGB, including:

- Operative time
- Intraoperative adverse events
- Postoperative complications
- Reoperations
- LOS
- Readmissions
- Percentage of patients lost to follow-up
- Absolute weight loss
- Absolute BMI loss
- %TWL
- %EWL
- %EBMIL

**Statistical Analysis**

Statistical data were calculated using a spreadsheet and StatSoft STATISTICA version 12 (StatSoft Inc., Tulsa, OK, USA). For testing categorical variables, the $\chi^2$ test of independence was applied. The Shapiro–Wilk test was used to test the normal distribution of data. The results are presented as medians and interquartile range (IQR) for non-normally distributed values. A non-parametric Mann–Whitney U test was used to compare non-normally distributed data. Univariate and multivariate logistic regression analysis for preoperative weight loss $\geq$ 5% was performed to assess the influence of selected baseline characteristics. We also addressed the influence of preoperative weight loss $\geq$ 5% on intraoperative adverse events, postoperative complications, gastrointestinal leakage, gastrointestinal stricture, rhabdomyolysis, postoperative hemorrhage, wound infection, port-site hernia, abscess formation, reoperation, and readmission using univariate logistic regression models. Pearson’s test was used to verify the correlation between preoperative weight loss and %TWL, %EWL, and %EBMIL. Results were considered statistically significant at $p < 0.05$.

**Results**

The average preoperative weight loss in our study group of 909 patients was 4.89%. Overall, 560 patients (61.61%) were included in group 1 and 349 patients (38.39%) in group 2. There were more men than women in group 1 (35.54% vs. 28.03%, $p = 0.019$). Although the median maximum BMI was comparable in groups 1 and 2 (45.65 vs. 46.06, $p = 0.263$), the median preoperative BMI was significantly higher in group 1 (45.17 vs. 41.14, $p < 0.001$). Diabetes mellitus and obstructive sleep apnea were more likely to be diagnosed in group 2 patients (29.11% vs. 36.1%, $p = 0.027$ and 5.36% vs. 10.03%, $p = 0.007$, respectively), whereas steatohepatitis was more commonly diagnosed in group 1 patients (64.82% vs. 50.16%, $p < 0.001$). Patients who underwent previous surgery were more common in group 2 (45.54% vs. 56.16%, $p = 0.002$). Additional preoperative study group characteristics are presented in Table 1. Patients’ flow throughout the study is presented in Fig. 1.

**Primary Endpoints**

Univariate logistic regression analysis revealed that high maximum BMI (OR 1.02, 95% CI 1.00–1.04, $p = 0.048$), diabetes mellitus (OR 1.38, 95% CI 1.04–1.83, $p = 0.027$), obstructive sleep apnea (OR 1.97, 95% CI 1.19–3.27, $p = 0.009$), and previous surgery (OR 1.53, 95% CI 1.17–2.01, $p = 0.002$) were related to increased chance of achieving preoperative weight loss of $\geq 5\%$, whereas male sex [odds ratio (OR)
Table 1  Patient baseline characteristics

| Parameter                        | Group 1                      | Group 2                      | \( p \) value |
|----------------------------------|------------------------------|------------------------------|---------------|
| Patients, \( n \)                | 560                          | 349                          |               |
| Mean preoperative weight loss, %TWL SD | 1.10% (0–2.87%)              | 8.62% (6.67–12.62%)         |               |
| Median age, years IQR            | 43 (35–51)                   | 42 (35–50)                   | 0.343         |
| Sex/number of males, \( n \)    | 199                          | 98                           | 0.019         |
| Median maximal BMI, kg/m\(^2\) IQR | 45.65 (41.99–50.34)         | 46.06 (42.02–51.72)         | 0.263         |
| Median preoperative BMI, kg/m\(^2\) IQR | 45.17 (41.43–49.64)       | 41.14 (37.48–45.73)         | < 0.001       |
| ASA score, \( n \)              | I 1.81%                      | 3.13%                       | 0.454         |
|                                 | II 73.49%                    | 73.96%                      |               |
|                                 | III 24.70%                   | 22.92%                      |               |
| Surgery/number of LSG, \( n \)  | 183                          | 102                         | 0.274         |
| Comorbidities, \( n \)          | 479                          | 288                         | 0.226         |
| Diabetes mellitus, \( n \)      | 163                          | 126                         | 0.027         |
| Steatohepatitis, \( n \)        | 363                          | 175                         | < 0.001       |
| Hypertension, \( n \)           | 361                          | 226                         | 0.839         |
| Dyslipidemia, \( n \)           | 313                          | 187                         | 0.488         |
| Cardiovascular disorders, \( n \) | 77                            | 57                          | 0.291         |
| Respiratory disorders, \( n \)  | 88                            | 43                          | 0.162         |
| Obstructive sleep apnea, \( n \) | 30                            | 35                          | 0.007         |
| Previous surgeries, \( n \)     | 255                          | 196                         | 0.002         |

Italic data statistically significant result

Fig. 1  Flowchart
Secondary Endpoints

The median LSG and LRYGB operative times were both significantly longer in group 1 than in group 2 (90 vs 75 min, p < 0.001 and 120 min vs. 100 min, p = 0.010, respectively). The incidences of intraoperative adverse events, postoperative complications in general, and specific postoperative complications were not significantly influenced by preoperative weight loss. Comparison of postoperative complications (Clavien–Dindo classification) between groups did not reveal significant differences. The groups were also comparable in terms of reoperation rates, LOS, and readmission rates (Tables 3 and 4).

It seems that we observed a trend among patients in group 1 of a slightly less frequent participation in follow-up examinations (65.0% vs. 72.2%, p = 0.023). The median %TWL was higher in group 2 (29.96% vs. 32.41%, p = 0.009). The preoperative weight loss correlated positively with both %TWL (R = 0.211, p < 0.001) and %EBMIL (R = 0.1, p = 0.015) (Tables 5 and 6).

Discussion

Our multi-center study is one of the first attempts to investigate the role of weight loss prior to bariatric treatment (LSG, LRYGB) as part of the ERAS protocol. Patients suffering from diabetes mellitus or obstructive sleep apnea and those with

Table 2 Factors contributing to achieving the preoperative weight loss of at least 5%—logistic regression analysis

| Parameter                  | OR   | 95% CI     | p value |
|----------------------------|------|------------|---------|
| Univariate                 |      |            |         |
| Median age                 | 0.99 | 0.98–1.01  | 0.474   |
| Sex/number of males        | 0.71 | 0.53–0.95  | 0.020   |
| Median maximal BMI         | 1.02 | 1.00–1.04  | 0.048   |
| Comorbidities              | 0.80 | 0.56–1.15  | 0.224   |
| Diabetes mellitus          | 1.38 | 1.04–1.83  | 0.027   |
| Steatohepatitis            | 0.55 | 0.42–0.72  | < 0.001 |
| Hypertension               | 1.03 | 0.79–1.36  | 0.839   |
| Dyslipidemia               | 0.91 | 0.70–1.19  | 0.488   |
| Cardiovascular disorders   | 1.22 | 0.84–1.77  | 0.291   |
| Respiratory disorders      | 0.76 | 0.51–1.12  | 0.162   |
| Obstructive sleep apnea    | 1.97 | 1.19–3.27  | 0.009   |
| Previous surgeries         | 1.53 | 1.17–2.01  | 0.002   |
| Multivariate               |      |            |         |
| Median age                 | 0.99 | 0.97–1.00  | 0.128   |
| Sex/number of males        | 0.74 | 0.47–1.17  | 0.203   |
| Median maximal BMI         | 1.03 | 0.98–1.07  | 0.189   |
| Comorbidities              | 1.26 | 0.72–2.20  | 0.415   |
| Diabetes mellitus          | 1.61 | 1.14–2.26  | 0.007   |
| Steatohepatitis            | 0.40 | 0.28–0.59  | < 0.001 |
| Hypertension               | 0.90 | 0.60–1.34  | 0.587   |
| Dyslipidemia               | 1.29 | 0.89–1.87  | 0.183   |
| Cardiovascular disorders   | 1.55 | 0.99–2.42  | 0.415   |
| Respiratory disorders      | 0.49 | 0.30–0.80  | 0.004   |
| Obstructive sleep apnea    | 4.02 | 2.12–7.63  | < 0.001 |
| Previous surgeries         | 1.49 | 1.09–2.02  | 0.012   |

Italic data statistically significant result

Table 3 Impact of the PWL on perioperative outcomes of bariatric surgery group 2 vs. group 1

| Parameter                          | Group 1 | Group 2 | p value |
|------------------------------------|---------|---------|---------|
| Median LSG time, min IQR           | 90 (70–120) | 75 (55–100) | < 0.001 |
| Median LRYGB time, min IQR         | 120 (95–170) | 100 (90–150) | 0.010 |
| Intraoperative adverse events, n    | 34      | 21      | 0.973   |
| Conversions, n                      | 0       | 0       | –       |
| Postoperative complications, n      | 38      | 25      | 0.839   |
| Gastrointestinal stricture, n       | 3       | 1       | 0.579   |
| Gastrointestinal stricture, n       | 3       | 1       | 0.579   |
| Rhabdomyolysis, n                   | 7       | 6       | 0.567   |
| Postoperative hemorrhage, n         | 10      | 8       | 0.599   |
| Wound infection, n                  | 1       | 0       | 0.429   |
| Port-site hernia, n                 | 1       | 0       | 0.429   |
| Abscess, n                          | 2       | 1       | 0.854   |
| Clavien–Dindo I–II, n               | 22      | 14      | 0.959   |
| Clavien–Dindo III–V, n              | 16      | 11      | 0.806   |
| Reoperation, n                      | 10      | 4       | 0.433   |
| Median LOS, days IQR               | 3 (2–5) | 4 2–5  | 0.884   |
| Readmission, n                      | 30      | 21      | 0.694   |

Italic data statistically significant result
previous surgery in their medical history were more likely to lose weight preoperatively. Although preoperative weight loss did not influence the perioperative course, it was associated with superior short-term bariatric surgery-related weight loss. Currently available studies investigating the role of preoperative weight loss in bariatric surgery often reported inconsistent results, which indicated the need for further research analyzing its influence under various circumstances [13].

The number of bariatric procedures performed yearly is steadily increasing [2]. This constantly growing demand for bariatric procedures yields a need to improve each aspect of bariatric treatment, including perioperative care [14]. The present study concentrates on preoperative weight loss. We aimed to verify whether it is a critical factor in achieving superior postoperative outcomes in patients undergoing perioperative care conducted in accordance with the ERAS protocol.

In our opinion, the ERAS protocol includes all interventions aimed at improving the effects of surgical treatment. In the case of bariatric procedures, it is executed during both preoperative and perioperative periods. Guidelines of ERAS Society for bariatric surgery include the preoperative weight loss with the “strong” grade of recommendation [15]. The ERAS protocol for bariatric patients implemented in our department includes a recommendation for weight reduction during the preparation for surgery as well (Supplement 1). In our experience, it is an important element of the protocol, but particularly difficult to execute; therefore, it is often overlooked. Nevertheless, implementing other components included in the ERAS pathway allows to diminish the negative impact of omitting the preoperative weight loss, by creating a clinical setting, which is different from traditional perioperative care.

In our opinion, identifying factors that influence the degree of preoperative weight loss could provide new insight into the difficulty of achieving it during implementation of the multi-step perioperative care protocol (ERAS). Moreover, defining factors affecting this component of the protocol could allow for better preparation of the patients for the procedure, by identifying ones who require more attention and more involvement during implementation of preoperative recommendations. In our study, groups 1 and 2 had mostly comparable preoperative characteristics. Male patients, however, were less likely to lose ≥5% total body weight prior to surgery, which is surprising, as most studies report that men generally lose more weight than women when enrolled in a weight-loss intervention. They also maintain their weight and continue to lose more after the intervention [16].

Diabetes mellitus and obstructive sleep apnea were more commonly diagnosed among patients who achieved preoperative weight loss of ≥5%. Steatohepatitis was more frequent in group 1, which is consistent with the results of Dudekula et al., who showed that, among patients with non-alcoholic fatty liver disease, achieving weight loss is largely unsuccessful in the clinical setting [17]. Bergh et al. reported that a high weight-loss goal, frequent self-weighing, and being close to

### Table 4 Impact of the PWL on OR of perioperative outcomes of bariatric surgery group 2 vs. group 1

| Parameter                        | OR   | 95% CI      | p value |
|----------------------------------|------|-------------|---------|
| Intraoperative adverse events    | 0.99 | 0.57–1.74   | 0.973   |
| Postoperative complications      | 1.06 | 0.63–1.79   | 0.827   |
| Gastrointestinal leakage         | 0.96 | 0.62–4.05   | 0.960   |
| Gastrointestinal stricture       | 0.53 | 0.60–5.15   | 0.587   |
| Rhabdomyolysis                   | 1.38 | 0.46–4.15   | 0.564   |
| Postoperative hemorrhage         | 1.29 | 0.50–3.30   | 0.595   |
| Wound infection                  | 0.53 | 0.02–13.14  | 0.701   |
| Port-site hernia                 | 0.53 | 0.02–13.14  | 0.701   |
| Abscess                          | 0.80 | 0.07–8.88   | 0.857   |
| Reoperation                      | 0.64 | 0.20–2.05   | 0.450   |
| Readmission                      | 1.13 | 0.64–2.01   | 0.674   |

### Table 5 Impact of the PWL on short-term weight-loss outcomes of bariatric surgery

| Parameter                        | Group 1 | Group 2 | p value |
|----------------------------------|---------|---------|---------|
| Follow-up, n                     | 364     | 252     | 0.023   |
| 65.00%                           | 72.21%  |         |
| Median absolute weight loss, kg  | 39.00 (30.00–48.00) | 44.50 (29.00–55.00) | 0.013   |
| IQR                              | 13.71 (10.73–16.94) | 15.24 (11.00–19.07) | 0.001   |
| Median %TWL, IQR                 | 29.96% (24.00–35.89%) | 32.41% (23.81–39.13%) | 0.009   |
| Median %EWL IQR                  | 56.13% (45.13–70.90%) | 53.20% (40.60–69.27%) | 0.158   |
| Median %EBMIL IQR                | 66.77% (52.60–81.65%) | 71.15% (55.00–82.47%) | 0.092   |

Italic data statistically significant result
(or at) the subject’s highest lifetime weight when applying for surgery were identified as predictors of effective preoperative weight loss [18]. According to Altieri et al., neither the patient’s sex, insurance, psychiatric history, comorbidities, referral status, nor type of counseling had a significant effect on weight loss before bariatric surgery [19].

Perioperative outcomes of bariatric surgery were not influenced by preoperative weight loss, except for the operative times (both LSG and LRYGB), which were higher in group 1. This difference may be associated with higher median preoperative BMIs in this group. Our results seem to correlate with previously published data, suggesting that the operative time for LRYGB could be reduced by preoperative weight loss [20]. Patients undergoing LSG do not seem to receive a similar benefit [21].

Previously published research shows mixed results regarding complication rates being reduced by preoperative weight loss [22–25]. We did not observe an increase in the incidence of postoperative complications among patients who did not achieve significant preoperative weight loss. This difference may have resulted from the beneficial effects of the perioperative care conducted in accordance with ERAS principles. Previously published studies reporting higher rates of postoperative complications among patients with unsatisfactory preoperative weight loss included patients undergoing perioperative care not based on the ERAS protocol [22, 23]. The LOS in our study was not influenced by preoperative weight loss, whereas Still et al. suggested that possible candidates for bariatric surgery who achieved at least 5% preoperative weight loss of body weight had a higher probability of a shorter LOS [26].

Patients exhibiting high compliance with the ERAS protocol and preoperative weight loss of ≥5% in the present study were more likely to continue to attend follow-up examinations. It seems that compliance with both ERAS components and follow-up examinations results in better short-term postoperative weight-loss outcomes [27]. The most common causes of non-adherence to the follow-up schedule after bariatric surgery reported by patients included work-related problems, family-related problems, or moving from their city or country of residence [28]. Our results show a correlation between preoperative and weight loss associated with bariatric treatment (measured as %TWL and %EBMIL). Most available studies offer results consistent with ours, which show significant improvement in weight-loss effect for highly compliant patients who were able to lose weight preoperatively [20, 29]. Most recent studies also report a direct correlation between preoperative and postoperative weight loss [30, 31]. Patients who had undergone previous surgery achieved superior preoperative weight loss. This finding may be explained by the benefit of previous experience with surgical care, which might indicate that better cooperation with the doctor and compliance with the recommendations improve the course of postoperative care.

This study has several limitations. First, it was a non-randomized study on a relatively small number of patients. Hence, because of the lack of necessary data, we were not able to compare participation in preoperative dietary and psychological interventions between groups 1 and 2. Potential bias could result from the lack of uniformity in terms of sex category between groups. During the preparation of the manuscript, we conducted a comprehensive literature search, which did not reveal evidence stating that sex category could significantly influence the operative times of bariatric procedures. However, we do not have evidence-based sources to support that statement. We were also not able to analyze postoperative complications occurring after discharge if patients were not treated in the center performing the initial surgery. Second, the influence of preoperative weight loss on alleviation of obesity-related comorbidities after bariatric treatment was not analyzed. However, we believe that the data on the correlation between preoperative and postoperative weight loss remains useful. Further randomized clinical trials are required to assess the benefit of the ERAS protocol and preoperative weight loss among bariatric patients, especially those undergoing LSG.

**Conclusion**

Unsatisfactory preoperative weight loss among patients treated in accordance with the ERAS protocol principles is not associated with an increased risk of complications. Patients who achieve good preoperative weight loss are more likely to be motivated to attend follow-up examinations. Achieving preoperative weight loss of ≥5% allows the prediction of superior postoperative weight loss.

**Compliance with Ethical Standards**

**Conflict of Interest** The authors declare that they have no conflict of interest.

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Due to the retrospective nature of the study, it did not require Bioethics Committee approval.

**Informed Consent** Informed consent was obtained from all individual participants included in the study.
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