Study Protocol

Version 1

26 June 2025

Title:

Efficacy of repeat dosing of liposomal bupivacaine on postoperative pain in patients with anal fistula: a multicenter, randomized, open-Label, controlled study

Design:

A multicenter, randomized, open-Label, controlled study.

Settings:

The Sixth Affiliated Hospital of Sun Yat-sen University; The First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine; Maoming People's Hospital; Nanjing Hospital of C. M.; Dongguan TCM Hospital; Xingyi People's Hospital; People's Hospital of Xinjiang Uygur Autonomous Region.

Patients:

Patients diagnosed with anal fistulas and undergoing surgical treatment.

Intervention:

Liposomal Bupivacaine Repeat Dosing Group: A single dose of liposomal bupivacaine is administered at the conclusion of surgery without adjunctive analgesics, followed by a supplemental dose 72 hours postoperatively.

Liposomal Bupivacaine Single-Injection Group: A single dose of liposomal bupivacaine is administered at the conclusion of surgery without adjunctive analgesics.

Primary Outcome Measure:

Area Under the Curve (AUC) of the Numerical Rating Scale (NRS) for pain within 7 Days postoperatively (NRS-AUC0-168h)

Secondary Outcome Measures:

1. Numerical Rating Scale (NRS) for pain during wound dressing changes within 7 days postoperatively.

2. Area Under the Curve (AUC) of the Numerical Rating Scale (NRS) for pain from 96 to 168 hours postoperatively (NRS-AUC96h-168h).

3. Number of breakthrough pain episodes and corresponding Numerical Rating Scale (NRS) scores during episodes.

4. Postoperative 7-day cumulative consumption of all analgesic drugs (calculated as morphine equivalents).

5. Postoperative 7-day cumulative consumption of rescue analgesic drugs (calculated as morphine equivalents).

6. Safety (including incidence of postoperative adverse events and serious adverse events): Adverse events and serious adverse events occurring within 14 days after medication were assessed.

7. Postoperative heart rate and blood pressure monitoring: Heart rate, systolic blood pressure, and diastolic blood pressure at 24h, 48h, 72h, 96h, 120h, 144h, and 168h postoperatively.

8. SF-12 scores at preoperative, 1 week, 1 month, and 2 months postoperatively.

9. Postoperative 8-week wound healing outcomes: Healing rate, healing time, edema score, granulation tissue color score, presence of infection, presence of bleeding, and other relevant parameters.

10. Preoperative, 14-day postoperative, and 1-month postoperative Wexner Incontinence Scores.

11. Analgesia satisfaction: Postoperative analgesic satisfaction was assessed using a 5-point categorical scale at 24h, 96h, and 168h postoperatively.

Study Participants:

Patients diagnosed with anal fistulas and undergoing surgical treatment were recruited and screened through a multi-center collaboration involving the following institutions: the Department of Colorectal Surgery at the Sixth Affiliated Hospital of Sun Yat-sen University, the Department of Proctology at the First Affiliated Hospital of Guizhou University of Traditional Chinese Medicine, the Department of Traditional Chinese Medicine Proctology at Maoming People's Hospital, the Department of Proctology at Nanjing Hospital of C. M., the Department of Proctology at Dongguan TCM Hospital, the Department of Colorectal Surgery at Xingyi People's Hospital of Guizhou Province, and the Department of Colorectal Surgery at People's Hospital of Xinjiang Uygur Autonomous Region.

Inclusion Criteria:

1. Diagnosed with anal fistula and scheduled to undergo anal fistula surgery;
2. Aged between 18 and 60 years;
3. ASA (American Society of Anesthesiologists) physical status class I-II;
4. Patient provides written informed consent after understanding the study protocol.

Exclusion Criteria:

1. Anal fistula caused by specific etiologies (e.g., tuberculosis);
2. Concurrent acute perianal skin infection;
3. Poorly controlled diabetes (HbA1c >9%);
4. Chronic use of corticosteroids;
5. History of radiotherapy or chemotherapy within the past 2 weeks;
6. Pregnancy or lactation;
7. Hypersensitivity to local anesthetics or any component of the investigational drug;
8. History of substance abuse, illicit drug use, or alcohol abuse;
9. Use or planned use of non-opioid/opioid analgesics within 12 hours before/during surgery;

10. Concurrent treatment with anticonvulsants, MAO inhibitors, antidepressants, neuromuscular blockers, or anticholinergics within 2 weeks prior to surgery;

11. Severe hepatic/renal impairment, cardiopulmonary dysfunction, coagulation disorders, or comorbidities contraindicating surgery;

12. History of severe psychiatric disorders or cognitive impairment;

13. Sensory disorders (e.g., hyperalgesia) or preexisting pain interfering with postoperative pain assessment;

14. Contraindications to amide-type local anesthetics, opioids, or propofol;

15. Participation in investigational drug trials within 90 days prior to enrollment;

16. Other clinical/laboratory conditions deemed by investigators to preclude trial participation.

Study Intervention And Post-Intervention Care:

The Liposomal Bupivacaine Repeat Dosing Group: a single 20 mL (266 mg) undiluted liposomal bupivacaine injection was administered at surgical conclusion without adjunctive analgesics. The solution was divided into six equal aliquots (≈3.3 mL each) and infiltrated circumferentially around the incision using a 25-gauge or larger needle, targeting supra- and subfascial planes as well as subcutaneous tissue, with frequent aspiration to minimize intravascular risk. At 72 hours postoperatively, topical lidocaine cream was applied peri-incisionally for ≥20 minutes followed by repeat administration of 20 mL (266 mg) undiluted liposomal bupivacaine via identical infiltration technique (both provided at no cost).

The Liposomal Bupivacaine Single-Dose Group: received identical intraoperative administration (20 mL undiluted solution, six-point infiltration, fascial/subcutaneous injection with aspiration) without postoperative bupivacaine re-dosing. At 72 hours, these patients underwent lidocaine cream application only prior to subsequent procedures.

Postoperative management followed standard anal fistula protocols, including

prophylactic antibiotics, liquid diet initiation, hemostatic measures, and continuous monitoring of vital signs (e.g., blood pressure, heart rate). For breakthrough pain (defined as sudden, transient severe pain with Numerical Rating Scale [NRS] ≥4 occurring despite stable background pain control), a stepwise rescue analgesia protocol was implemented: 1) Oral acetaminophen or NSAIDs were administered as needed; 2) If insufficient, tramadol (50-100 mg intramuscularly/orally) was added, with a maximum daily cumulative dose of 400 mg; 3) For tramadol-refractory pain, meperidine intramuscular injection was administered. All breakthrough pain episodes were documented with NRS scores and duration.

A standardized follow-up protocol was implemented for 8 weeks post-discharge, with biweekly assessments. Evaluations included wound healing status, adverse events (AEs), and serious adverse events (SAEs). Wound healing was defined as complete mucosal coverage with full re-epithelialization and absence of discharge, assessed by an independent blinded observer (unaffiliated with the surgical team) during clinic visits. The observer recorded wound parameters using a numerical scoring system at each visit, continuing for up to 8 weeks postoperatively or until complete healing (whichever occurred first). Specific metrics included: 1) Healing rate: Calculated as [(Original area - Current area)/Original area] × 100%, with area determined by maximal length × width measurements (baseline established on postoperative day 1); 2) Healing time: Days from initial dressing change to complete epithelialization; 3) Edema score: 0 (none), 1 (mild), 2 (moderate; requiring dressing intervention), or 3 (severe; requiring surgical excision); 4) Granulation tissue color score: 1 (bright red), 2 (pale red), or 3 (purplish); 5) Infection presence; and 6) Bleeding severity (none, oozing, active bleeding). A comprehensive electronic database was maintained by dedicated personnel, supplemented by remote tracking via telephone, mailed questionnaires, and email between scheduled visits.

Sample Size Justification:

This randomized controlled trial (RCT) was designed to detect a projected 56-unit difference in the area under the curve (AUC) of Numerical Rating Scale (NRS) scores

(0–168 hours postoperatively) between the liposomal bupivacaine repeat-dosing and single-dosing groups, with an estimated pooled standard deviation (SD) of 160 based on prior studies. Using a two-sample t-test module in NCSS PASS 21 software (LLC. Kaysville, Utah, USA; ncss.com/software/pass) with a two-sided significance level (α) of 0.05, a minimum of 173 subjects per group was required to achieve 90% power (1-β). Accounting for an anticipated 15% dropout rate, the final enrollment target was set at 204 subjects per group (total N=408).

Statistical Analysis:

Primary Efficacy Analysis:

All enrolled subjects underwent an 8-week follow-up period. The primary endpoint, NRS-AUC_{0-168h} over the initial postoperative week, was calculated using the trapezoidal rule. Treatment effects were evaluated through an analysis of covariance (ANCOVA) model, providing least-squares means with standard errors and 95% confidence intervals. This model adjusted for baseline NRS scores and randomization stratification factors to enhance precision.

Missing Data Handling for Primary Endpoint:

Missing resting NRS scores occurring during sleep periods were imputed using the last non-missing presleep value if below 3; otherwise, a conservative score of 3 was assigned. For other missing NRS assessments: 1) Baseline or critical timepoint (e.g., 72h redosing) missingness was addressed through group-level median imputation; 2) Intermittent missing values underwent linear interpolation; 3) Terminal missing observations utilized last observation carried forward (LOCF). All AUC calculations relied on scheduled assessment times when actual recording times were unavailable.

Secondary Endpoint Categorization and Analysis:

Secondary outcomes were classified into three domains. Continuous variables included late-phase NRS-AUC_{96-168h}, analgesia consumption metrics, healing duration, and serial Wexner incontinence scores. Categorical measures encompassed adverse event incidence, cardiorespiratory symptom rates, and binary healing outcomes (infection/bleeding status). Longitudinal parameters featured vital

signs, quality-of-life (SF-12) trajectories, wound assessment scores, and analgesia satisfaction surveys.

Statistical Testing Framework:

Continuous secondary endpoints underwent ANOVA or ANCOVA (covariate-adjusted) for between-group comparisons. Categorical outcomes were analyzed through frequency distributions with Clopper-Pearson 95% CIs, rate differences with corresponding CIs, and chi-square/Fisher's exact tests. Repeated measures employed mixed-effects modeling (MMRM) to account for temporal correlations. All inferential analyses were conducted using SAS® with two-sided testing at $\alpha=0.05$.