RESEARCH PROTOCOL

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Sequence-Dependent Analgesic Efficacy of Intraoperative Ketamine and Magnesium Sulfate After Radical Nephrectomy:

A Randomized, Double-Blind, Placebo-Controlled Trial

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Study Site	University Clinical Center of Serbia, Belgrade, Republic of Serbia
Study Period	August 2017 – January 2026
ISRCTN Submission Ref.	49194 (registration number pending payment and validation)
Ethics Approval	Institutional Ethics Committee, University Clinical Center of Serbia; Approval No. 361/15; Date: 13 July 2017
Protocol Version	1.0 – March 2025
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I. Background and Rationale

1.1 Disease Background

Renal cell carcinoma (RCC) is the most common malignant tumor of the renal parenchyma in adults, accounting for approximately 2.4% of all cancer diagnoses worldwide. The 2020 GLOBOCAN data recorded approximately 400,000 new cases and 180,000 deaths attributable to RCC annually, with the

highest incidence in developed countries. More recent 2022 GLOBOCAN estimates report 434,840 new renal cancer cases, with projections suggesting a 72% increase to nearly 746,000 annual cases by 2050, driven by population aging and modifiable risk factors including hypertension, obesity, and smoking. In the majority of patients with localized or locally advanced disease, radical nephrectomy – performed via open or laparoscopic approach – remains the cornerstone of curative treatment.

1.2 Postoperative Pain After Radical Nephrectomy

Postoperative pain after radical nephrectomy is clinically significant and can be moderate to severe, particularly in the early postoperative period. Peak pain intensity typically occurs within the first 30–60 minutes after surgery, with high rates of analgesic demand in both open and laparoscopic approaches. Approximately 11–16% of patients develop chronic postsurgical pain (CPSP) at two months after nephrectomy. Inadequate analgesic management is associated with prolonged hospital stay, impaired functional recovery, and increased risk of CPSP.

Opioids have traditionally formed the backbone of postoperative pain management, but their use is associated with significant adverse effects including respiratory depression, nausea, vomiting, excessive sedation, ileus, and the risk of dependency. This has driven increasing interest in multimodal, opioid-sparing analgesic strategies.

1.3 NMDA Receptor Antagonism and Perioperative Analgesia

Central sensitization – a state of neuronal hyperexcitability in the spinal dorsal horn induced by sustained nociceptive input – is critically dependent on activation of N-methyl-D-aspartate (NMDA) receptors, as established by the foundational work of Woolf and Thompson (1991). NMDA receptor antagonists interrupt this sensitization process perioperatively, reducing postoperative pain intensity, decreasing opioid consumption, and potentially preventing chronic pain states.

Two NMDA receptor antagonists have attracted particular interest for perioperative use: ketamine and magnesium sulfate ($MgSO_4$). Ketamine is a non-competitive open-channel blocker that inhibits NMDA receptors during nociceptive neuronal activation. Magnesium sulfate is an endogenous voltage-dependent channel blocker that occupies the receptor pore at resting membrane potentials. In vitro studies (Liu et al., 2001) have demonstrated super-additive (synergistic) interaction between these two agents at the molecular level.

1.4 Preclinical Evidence for Sequence-Dependent Pharmacodynamics

Preclinical data from Savić Vujović et al. (2015, 2017) have revealed that the direction of the pharmacodynamic interaction between ketamine and magnesium is critically dependent on the sequence of administration. In the rat tail-immersion model, the combination of ketamine followed by magnesium (K→Mg) produced synergistic antinociception, whereas the reversed sequence (Mg→K) produced an antagonistic interaction, requiring approximately 1.6-fold higher doses of ketamine to achieve the same analgesic effect. No prior prospective clinical trial has tested this sequence-dependent hypothesis in humans.

1.5 Rationale for the Present Study

Despite compelling preclinical evidence, the sequence-dependent interaction between ketamine and magnesium has never been directly tested in a prospective clinical trial. Published studies have either administered the agents simultaneously or used unspecified sequences. Moreover, no prior study has investigated this combination in patients undergoing radical nephrectomy. The present study was therefore designed to test the sequence-dependent analgesic hypothesis in a randomized, double-blind, placebo-controlled framework.

II. Research Objectives

2.1 Primary Objective

To evaluate the sequence-dependent analgesic efficacy of intraoperative ketamine and magnesium sulfate ($MgSO_4$) in patients undergoing elective open radical nephrectomy, comparing the K→Mg sequence (ketamine first, $MgSO_4$ second) with the reversed Mg→K sequence and with placebo.

2.2 Primary Hypotheses

- The K→Mg sequence will produce significantly lower postoperative pain scores (NRS 0–10) than placebo (Pl→Pl) at multiple time points over 48 hours.
- The Mg→K sequence will not differ significantly from placebo at any time point, based on the preclinical evidence of antagonistic interaction when magnesium precedes ketamine.

2.3 Secondary Objectives

- To compare cumulative postoperative morphine consumption and time to first analgesic request across treatment groups.
- To assess the safety profile of subanesthetic ketamine (0.2 mg/kg) in this population, specifically regarding psychomimetic adverse effects (hallucinations, dissociation, emergence excitation) and sedation.
- To explore the independent analgesic contribution of $MgSO_4$ as a pharmacological class by pooled analysis.
- To examine the convergent validity of NRS and VRS pain assessment scales across all time points.

III. Subject Selection and Recruitment

3.1 Study Population

Adult patients (≥18 years of age) with a unilateral renal tumor for whom open radical nephrectomy had been indicated by the responsible urologist, scheduled for elective surgery at the University Clinical Center of Serbia.

3.2 Inclusion Criteria

- Age ≥18 years
- ASA physical status classification I–III
- Unilateral renal tumor scheduled for elective open radical nephrectomy
- Written informed consent obtained prior to enrollment

3.3 Exclusion Criteria

- Resting bradycardia or atrioventricular (AV) block
- New York Heart Association (NYHA) functional class >2
- Glomerular filtration rate (GFR) <50 ml/min
- Severe or poorly controlled chronic obstructive pulmonary disease (COPD) or asthma
- Poorly controlled arterial hypertension
- Severe hepatic impairment
- Elevated intraocular or intracranial pressure (IOP or ICP)
- Significant psychiatric comorbidity (excluding mild anxiety or mild depression)
- Known allergy to any of the study medications
- Pre-existing chronic pain syndrome

- Regular use of analgesic medications in the month preceding surgery

3.4 Recruitment

Patients were recruited consecutively from the surgical list of the Department of Urology, University Clinical Center of Serbia, during the study period (August 2017 – January 2026). The study was explained to eligible patients during the preoperative assessment visit. Written informed consent was obtained from all participants prior to enrollment and prior to any study-related procedures.

3.5 Total Enrollment

A total of 208 patients were enrolled and randomized across nine treatment groups (range: 17–37 patients per group).

IV. Study Design

Design: Randomized, double-blind, placebo-controlled, parallel-group trial with a nine-arm factorial design

Setting: Single-centre; Department of Urology and Centre for Anesthesiology and Resuscitation, University Clinical Center of Serbia, Belgrade

Study period: August 2017 – January 2026

Follow-up duration: 48 hours post-surgery per participant

4.1 Treatment Groups

Patients were randomized into one of nine study groups defined by the identity and sequence of two sequential intraoperative bolus injections (Drug A administered immediately after induction of anesthesia; Drug B administered 10 minutes later, at least 10 minutes before surgical incision). Each of three possible agents – ketamine 0.2 mg/kg, $MgSO_4$ 15 mg/kg, or placebo (0.9% NaCl) – could occupy either the Drug A or Drug B position, yielding nine subgroups:

Group	Drug A	Drug B	Role	n
K→Mg	Ketamine 0.2 mg/kg	$MgSO_4$ 15 mg/kg	Primary comparison	32
Mg→K	$MgSO_4$ 15 mg/kg	Ketamine 0.2 mg/kg	Primary comparison	19
Pl→Pl	Placebo (NaCl 0.9%)	Placebo (NaCl 0.9%)	Primary comparison	37
K→Pl	Ketamine 0.2 mg/kg	Placebo (NaCl 0.9%)	Control	20
Pl→K	Placebo (NaCl 0.9%)	Ketamine 0.2 mg/kg	Control	17
Mg→Pl	$MgSO_4$ 15 mg/kg	Placebo (NaCl 0.9%)	Control	19
Pl→Mg	Placebo (NaCl 0.9%)	$MgSO_4$ 15 mg/kg	Control	24
K→K	Ketamine 0.2 mg/kg	Ketamine 0.2 mg/kg	Control	20
Mg→Mg	$MgSO_4$ 15 mg/kg	$MgSO_4$ 15 mg/kg	Control	20

Drug A was administered immediately after induction of anesthesia. Drug B was administered 10 minutes after Drug A, and at least 10 minutes before surgical incision. Total active treatment duration per participant: approximately 10–20 minutes. All study solutions were prepared as identical-appearing, colorless, transparent solutions in syringes of equal volume.

V. Randomization and Blinding

5.1 Randomization

The randomization sequence was generated prior to study commencement using a computer-based random number generator with block randomization. The sequence was maintained by a designated independent coordinator not involved in patient care or outcome assessment. Allocation concealment was ensured by preparing all study solutions in identical syringes by an anesthesia nurse not involved in patient care or data collection.

5.2 Blinding

The study was double-blind. The attending anesthesiologist, the surgical team, all postoperative assessors, and participants were blinded to group allocation throughout the study. All three study solutions (ketamine, $MgSO_4$, placebo) were visually indistinguishable (colorless, transparent, equal volume). Unblinding was pre-specified to occur only in the event of a serious adverse event requiring knowledge of treatment allocation for clinical management.

VI. Interventions

6.1 Study Drugs

- Ketamine: 0.2 mg/kg intravenous bolus (subanesthetic dose)
- Magnesium sulfate ($MgSO_4$): 15 mg/kg intravenous bolus
- Placebo: 0.9% sodium chloride (NaCl), volume-matched intravenous bolus

6.2 Standardized Anesthetic Protocol

All patients received a standardized premedication intramuscularly approximately 30 minutes before transfer to the operating room: midazolam 0.04 mg/kg and atropine 0.5 mg. General anesthesia was induced identically in all participants with intravenous fentanyl 2 µg/kg, propofol 2 mg/kg, and rocuronium 0.6 mg/kg. Tracheal intubation was performed in all cases. Anesthesia was maintained with sevoflurane (MAC = 1.0) in a mixture of 40% oxygen and 60% air, supplemented with intraoperative fentanyl and rocuronium as required.

All patients received intravenous paracetamol 1 g administered 30 minutes before emergence from anesthesia.

6.3 Postoperative Analgesic Protocol

On admission to the postoperative care unit (PACU) or intensive care unit (ICU), all patients received a standardized multimodal analgesic regimen. Paracetamol 1 g intravenously was administered every 8 hours as scheduled baseline analgesia. Rescue analgesia was provided with intravenous morphine in 1 mg incremental boluses, repeatable every 10 minutes, until the patient reported pain relief to NRS ≤3 at rest or NRS <5 on movement/deep breathing/cough.

6.4 Duration of Treatment and Follow-up

Active treatment duration: Approximately 10–20 minutes per participant (two sequential IV bolus injections within the induction period)

Postoperative follow-up: 48 hours from PACU/ICU admission

Assessment time points: 14 standardized assessments: 0 (arrival), 30 min, 1, 2, 3, 4, 6, 8, 12, 16, 20, 24, 32, and 48 hours post-surgery

VII. Outcome Measures

7.1 Primary Outcome Measures

- Postoperative pain intensity at rest measured using the Numerical Rating Scale (NRS, 0–10) at 14 standardized time points over 48 hours post-surgery
- Postoperative pain intensity on movement measured using the NRS (0–10) at 14 standardized time points over 48 hours post-surgery
- Cumulative postoperative morphine consumption (total number of rescue boluses) measured over 48 hours post-surgery

7.2 Secondary Outcome Measures

- Postoperative pain intensity at rest and on movement measured using the Verbal Rating Scale (VRS, 0–5) at 14 standardized time points over 48 hours post-surgery
- Time to first analgesic request (minutes from end of surgery/PACU admission) measured once per participant
- Nausea severity (0–4 scale) assessed at 14 standardized time points over 48 hours post-surgery
- Vomiting (presence/absence) and metoclopramide rescue requirement assessed at 14 standardized time points
- Presence of hallucinations (yes/no) assessed at each time point over 12 hours post-surgery
- Level of sedation (5-point scale: 0 = alert, 4 = unresponsive) assessed at 14 standardized time points over 48 hours post-surgery
- Postoperative bleeding (presence/extent) assessed at 14 standardized time points over 48 hours

VIII. Sample Size

Sample size was calculated a priori based on the primary outcome of postoperative pain intensity (NRS) at 1 hour after surgery. Assuming a clinically meaningful difference of 1.5 NRS points between groups, a standard deviation of 2.5, a two-sided significance level of $\alpha = 0.05$, and a desired statistical power of 80%, a minimum of 17 patients per group was required. To account for potential dropouts and to increase statistical power across the nine-group factorial design, enrollment was extended to 208 patients distributed across 9 groups (range: 17–37 per group).

IX. Statistical Analysis Plan

9.1 Data Presentation

- Continuous normally distributed variables: mean ± standard deviation (Mean ± SD)
- Continuous non-normally distributed variables (all pain scores, morphine data): median with interquartile range (Median, IQR: Q1–Q3)
- Categorical variables: absolute frequencies and percentages, n (%)
- Normality assessed using the Shapiro-Wilk test

9.2 Primary Analysis

Overall between-group differences at each time point assessed using the Kruskal-Wallis test. Pre-specified pairwise comparisons between the three key groups of interest – K→Mg (n=32), Mg→K (n=19), and Pl→Pl (n=37) – performed using the Mann-Whitney U test (two-sided). These were pre-specified based on the study hypothesis regarding sequence-dependent drug effects and constitute the primary inferential analysis.

9.3 Secondary Analyses

- Total morphine consumption and time to first analgesic request: Kruskal-Wallis test across all nine groups; Mann-Whitney U for pairwise comparisons
- Adverse events (nausea, vomiting, sedation, hallucinations): Chi-square test or Fisher's exact test as appropriate
- Exploratory pooled analysis: all $MgSO_4$-containing groups (n=95) vs. non-Mg groups (n=113) – Mann-Whitney U at selected time points
- Spearman's rank correlation: patient characteristics vs. postoperative pain at 1 hour; NRS vs. VRS convergent validity

9.4 Multiple Comparisons

Given the exploratory nature of pairwise comparisons across nine groups and multiple time points, no formal correction for multiple comparisons (e.g., Bonferroni) was applied, in accordance with current practice for pilot and exploratory clinical trials. All p-values are unadjusted. Findings should be interpreted in the context of the overall pattern of results.

9.5 Software

All analyses performed using IBM SPSS Statistics version 26.0. A two-sided p-value of <0.05 was considered statistically significant throughout.

X. Data Collection and Management

10.1 Preoperative Data

The following data were collected prior to surgery from all participants: sex, age, body weight, body height, BMI; smoking status; current comorbidities; history of prior renal or other surgical procedures; laterality of tumor (left/right kidney); preoperative pain expectation assessed via NRS.

10.2 Intraoperative Data

Duration of surgery (minutes); intraoperative blood loss (ml); total intraoperative fentanyl dose (ml; 1 ml = 0.05 mg); intraoperative events (pleural injury, rib resection).

10.3 Postoperative Data

NRS and VRS pain scores at rest and on movement; nausea severity; vomiting; hallucinations; sedation level; postoperative bleeding – all recorded at 14 standardized time points. Total morphine consumption (boluses) and time to first analgesic request recorded in case report form (CRF).

10.4 Data Storage

Data were recorded in individual case report forms (CRFs) by trained study personnel. Data are available on request from the corresponding author.

XI. Data and Safety Monitoring

This study did not employ a formal independent Data Safety Monitoring Board (DSMB), as it involved short-duration single-dose interventions using established agents with well-characterized safety profiles at subanesthetic doses. Safety was monitored continuously by the attending anesthesiology team intraoperatively and by trained postoperative care personnel at all assessment time points. Any serious adverse event would have triggered unblinding as per pre-specified protocol.

No interim analyses were conducted. The study was completed as planned. A total of zero hallucinations, zero psychomimetic excitation events, and no unexpected serious adverse events were observed across all 208 participants.

XII. Ethical Considerations

12.1 Ethics Approval

Ethics Committee: Institutional Ethics Committee, University Clinical Center of Serbia

Approval Number: 361/15

Approval Date: 13 July 2017

Regulatory Framework: Declaration of Helsinki (current revision)

12.2 Informed Consent

Written informed consent was obtained from all participants prior to enrollment and prior to any study-related procedures. Participants were fully informed about the study purpose, procedures, potential risks and benefits, voluntary nature of participation, and the right to withdraw at any time without consequence to their care. The consent process was conducted by the attending anesthesiologist during the preoperative assessment.

12.3 Ethical Issues and Risk Minimization

- Ketamine was administered at a subanesthetic dose (0.2 mg/kg), well below the threshold associated with psychomimetic adverse effects (typically >0.5 mg/kg)
- All exclusion criteria were designed to minimize cardiovascular, renal, and neuropsychiatric risks
- All patients had equal, unrestricted access to rescue analgesia regardless of group allocation
- The study introduced no additional procedural burden beyond standard clinical care
- Confidentiality of participant data was maintained throughout; data are anonymized in all study records

12.4 Benefits

Participants in the K→Mg group received an evidence-based analgesic combination with demonstrated reduction in postoperative pain intensity. All participants benefited from a standardized, optimized multimodal analgesic protocol.

XIII. Study Timeline

Milestone	Date
Ethics approval	13 July 2017

Milestone	Date
Study commencement / first enrollment	August 2017
Last participant enrolled	January 2026
Last participant follow-up completed	January 2026
Statistical analysis completed	February 2026
Manuscript submitted	March 2026
ISRCTN registration submitted	March 2026

Note: This study was completed prior to ISRCTN registration. Registration is retrospective and is being submitted to comply with journal reporting requirements.

XIV. Study Team and Responsibilities

Investigator	Role/Specialty	Contribution
Nebojša Lađević	PI, Anesthesiologist	Conceptualization, methodology, supervision, writing – review and editing
Nikola N. Lađević	Anesthesiologist	Conceptualization, methodology, investigation, software, formal analysis, writing – original draft
Zoran Džamić	Urologist	Conceptualization, methodology, writing – review and editing
Vesna D. Jovanović	Anesthesiologist	Investigation
Nataša Đ. Petrović	Anesthesiologist	Investigation, software
Svetlana D. Srećković	Anesthesiologist	Investigation
Miloš M. Lazić	Anesthesiologist	Investigation, software
Branka Terzić	Anesthesiologist	Investigation
Ivana Likić Lađević	Gynecologist	Investigation

XV. Dissemination Policy

Results of this study will be submitted for publication in a peer-reviewed international journal (currently under review at Medicina, MDPI). All authors listed have read and agreed to the published version of the manuscript. Authorship follows the ICMJE criteria. Results will be disseminated to the scientific community via peer-reviewed publication and conference presentations. Summary results will be uploaded to the ISRCTN registry upon study publication, in accordance with WHO transparency requirements.

XVI. Funding and Conflicts of Interest

Funding: No external funding was received for this study. All investigational agents (ketamine, magnesium sulfate, sodium chloride) are generic medications available as standard institutional stock.

Conflicts of Interest: The authors declare no conflicts of interest.

XVII. Key References

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