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ORIGINAL ARTICLE

Serum interleukin-6 is not associated with postoperative intra-abdominal adhesion severity in a rat model

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ABSTRACT

Aim To assess whether serum interleukin-6 (IL-6) measured on postoperative day 14 reflects adhesion severity and to compare intraperitoneal alteplase, N-acetylcysteine (NAC), and combined treatment.

Methods In this exploratory randomised study, 40 male Wistar rats were allocated to untreated adhesion, alteplase, NAC, alteplase plus NAC, or sham groups (n = 8 per group). Serum IL-6 and macroscopic adhesion grade were assessed on day 14. Group differences were analysed using the Kruskal-Wallis tests followed by Benjamini-Hochberg-adjusted Dunn comparisons; associations were assessed using Spearman correlation.

Results Serum IL-6 differed among groups (H = 18.08; p = 0.001). Median serum IL-6 concentrations were 14.52 pg/mL (IQR 9.91–19.13) in untreated animals, 30.70 pg/mL (IQR 27.31–34.09) after alteplase (p = 0.003), 24.95 pg/mL (IQR 17.48–32.42) after NAC (p = 0.037), 30.76 pg/mL (IQR 24.16–37.36) after combined treatment (p = 0.007), and 18.36 pg/mL (IQR 11.49–25.23) in sham animals (p = 0.320). Adhesion grades differed (H = 20.80; p < 0.001) and were lower after alteplase and combined treatment (both p = 0.041); the NAC comparison was not significant (p = 0.054). Serum IL-6 was not correlated with adhesion grade (ρ = -0.131; 95% CI -0.425 to 0.189; p = 0.422).

Conclusion A single serum IL-6 measurement on day 14 did not reflect adhesion severity. Serial serum measurements and concurrent peritoneal or tissue biomarkers may be more informative.

Keywords: fibrinolytic agents, interleukin-6, N-acetylcysteine, rats, Wistar, tissue adhesions

INTRODUCTION

Postoperative intra-abdominal adhesions are common after abdominal surgery and contribute to small-bowel obstruction, chronic pain, infertility, and technically difficult reoperations (1). Peritoneal injury initiates inflammation, fibrin deposition, impaired fibrinolysis, and fibrovascular organisation; persistent fibrin provides a scaffold for mature adhesions (2–4).

Interleukin-6 (IL-6) is rapidly produced after tissue injury and participates in inflammatory and fibrotic signalling. Experimental blockade of IL-6 signalling reduces adhesion formation, supporting its biological involvement (5). However, serum IL-6 may not reflect the peritoneal microenvironment, and circulating and peritoneal concentrations can be discordant in compartmentalised disease (6). Its value as a single late systemic biomarker of established adhesions is therefore uncertain.

Alteplase, a recombinant tissue plasminogen activator, and NAC have been evaluated as anti-adhesion interventions in experimental models (7,8). By modifying adhesion formation and inflammatory signalling through distinct pathways, these interventions may complicate the interpretation of serum IL-6 as a marker of local adhesion severity.

The primary aim of this study was to assess the association between serum IL-6 concentration on postoperative day 14 and the severity of postoperative intra-abdominal adhesions in a rat model. The secondary aim was to compare adhesion severity and serum IL-6 among animals treated with intraperitoneal alteplase, NAC, their combination, and control conditions.

MATERIAL AND METHODS

Subjects and study design

This exploratory, prospective randomised controlled animal study was conducted from 8 to 25 July 2025. No a priori sample-size calculation was performed. Forty healthy adult male Wistar rats weighing 200–250 g were allocated in a 1:1:1:1:1 ratio using a computer-generated random sequence to untreated adhesion, alteplase 3 mg, NAC 30 mg, alteplase 3 mg plus NAC 30 mg, or

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sham groups (n = 8 per group). The randomisation sequence was generated before the experiment, and coded group assignments were retained until outcome assessment was completed.

Animals were obtained from the Faculty of Veterinary Medicine, University of Sarajevo, Bosnia and Herzegovina at approximately 8–10 weeks of age and acclimatised for seven days. They were housed three to five per cage under controlled temperature (24 ± 2 °C), relative humidity 50 ± 10%, and a 12-hour light–dark cycle, with unrestricted access to standard chow and water.

Eligible animals were healthy adult male Wistar rats within the specified weight range and without previous procedures. Animals were excluded for pre-existing illness, infection, anatomical abnormality, protocol-deviating intraoperative complications, death before postoperative day 14, or an inadequate serum sample.

The study was approved by the Ethics Committee of the University of Sarajevo Faculty of Medicine (No. 02-3-4-AK-3434/25; 30 May 2025) and the University of Sarajevo Faculty of Veterinary Medicine (No. 07-03-328-3/25; 8 May 2025). All procedures complied with the Law on Protection and Welfare of Animals of Bosnia and Herzegovina.

Methods

Animals were anaesthetised with intramuscular ketamine 80 mg/kg. Following median laparotomy, adhesions were induced by 10–12 standardised passes of sterile gauze over the small bowel and parietal peritoneum. Sham animals underwent identical anaesthesia and laparotomy without peritoneal abrasion. The assigned intraperitoneal treatment was administered before closure, and the abdominal wall was closed in layers with 5/0 polypropylene sutures.

Alteplase and NAC were freshly dissolved in 0.9% sodium chloride solution at concentrations of 3 mg/mL and 30 mg/mL, respectively, and administered in a final intraperitoneal volume of 1 mL. The combination group received 3 mg alteplase plus 30 mg NAC in the same final volume. The untreated adhesion and sham groups did not receive intraperitoneal vehicle.

Postoperative analgesia was not administered. Animals were examined daily for pain or distress, wound complications, mobility, food and water intake, and general condition. Prespecified humane endpoints included persistent severe distress, inability to eat or drink, marked immobility, wound dehiscence or infection, or loss of more than 20% of baseline body weight.

On postoperative day 14, animals were deeply anaesthetised and underwent relaparotomy. One millilitre of blood was obtained by terminal aortic puncture, after which euthanasia was completed by exsanguination under deep anaesthesia. Samples were centrifuged at 4000 rpm for 10 minutes, and serum was stored at -80 °C until analysis. Serum IL-6 was measured using a rat sandwich enzyme-linked immunosorbent assay kit (ELK1158; ELK Biotechnology) according to the manufacturer's instructions. Samples were identified only by coded labels, and the investigator performing the IL-6 assay was blinded to treatment allocation. Values below the assay detection limit of 7.82 pg/mL were replaced with half the detection limit (3.91 pg/mL).

Adhesion severity was assessed at relaparotomy on postoperative day 14 using a macroscopic scale from 0 to 4: grade 0, no adhesions; grade 1, filmy and easily separable adhesions; grade 2, moderate adhesions requiring dissection; grade 3, dense vascularised adhesions requiring sharp dissection; and grade 4, extensive dense adhesions involving multiple sites (9). A single investigator blinded to treatment allocation assigned all scores.

Statistical analysis

Normality of serum IL-6 was assessed using the Shapiro–Wilk test. Because normality was not consistently met across groups, serum IL-6 and adhesion grade were summarised as median with interquartile range (IQR). Overall group differences were assessed using the Kruskal-Wallis test. Prespecified Dunn comparisons of each intervention and the sham group against the untreated group were adjusted using the Benjamini-Hochberg false discovery rate. The primary association between serum IL-6 and adhesion grade was assessed across all 40 animals using Spearman rank correlation with a 95% confidence interval (CI) calculated by Fisher z transformation. Exploratory Spearman correlations were also calculated separately within each of the four groups in which adhesions were induced. Serum IL-6 was compared between animals with absent or mild adhesions (grades 0–1) and those with moderate or severe adhesions (grades 2–4) using the Mann-Whitney U test. Values below the assay detection limit were analysed as 3.91 pg/mL. All tests were two-sided, and $p < 0.05$ was considered statistically significant. Analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA).

RESULTS

All 40 animals completed the protocol and were included in the analysis. Serum IL-6 differed among groups (Kruskal-Wallis H = 18.08; p = 0.001). Median serum IL-6 concentrations were 14.52 pg/mL (IQR 9.91–19.13) in the untreated group, 30.70 pg/mL (IQR 27.31–34.09) in the alteplase group (FDR-adjusted p = 0.003), 24.95 pg/mL (IQR 17.48–32.42) in the NAC group (p = 0.037), 30.76 pg/mL (IQR 24.16–37.36) in the alteplase plus NAC group (p = 0.007), and 18.36 pg/mL (IQR 11.49–25.23) in the sham group (p = 0.320) (Table 1).

Adhesion grades also differed among groups (H = 20.80; p < 0.001). The untreated group had the highest score, with a median of 3.0 (IQR 3.0–4.0), whereas the sham group had no adhesions. Compared with untreated animals, adhesion scores were lower after alteplase, median 1.0 (IQR 0.75–2.25; FDR-adjusted p = 0.041), and alteplase plus NAC, median 1.5 (IQR 0.75–2.0; p = 0.041). The difference after NAC, median 1.5 (IQR 1.0–2.0), did not reach statistical significance (p = 0.054) (Table 1).

Across all animals, serum IL-6 was not correlated with adhesion grade (Spearman ρ = -0.131; 95% CI -0.425 to 0.189; p = 0.422). Serum IL-6 was also comparable between animals with absent or mild adhesions, median 26.95 pg/mL (n = 21), and those with moderate or severe adhesions, median 20.77 pg/mL (n = 19; Mann-Whitney U = 263; p = 0.088). Exploratory within-group analyses showed no statistically significant correlation in the untreated adhesion group (ρ = 0.262; p = 0.530), alteplase group (ρ = -0.617; p = 0.103), NAC group (ρ = -0.125; p = 0.768), or alteplase plus NAC group (ρ = 0.259; p = 0.535).

DISCUSSION

This study showed that serum IL-6 measured on postoperative day 14 was not associated with macroscopic adhesion severity. In addition, groups receiving alteplase, NAC, or combined treatment had higher systemic IL-6 concentrations than untreated

Table 1. Serum interleukin-6 (IL-6) concentrations and adhesion grades by experimental group

Group	IL-6 (pg/mL) Median (IQR)	FDR-adjusted p*	Adhesion grade Median (IQR)	FDR-adjusted p*
Untreated adhesion	14.52 (9.91–19.13)	—	3.0 (3.0–4.0)	—
Alteplase	30.70 (27.31–34.09)	0.003	1.0 (0.75–2.25)	0.041
NAC	24.95 (17.48–32.42)	0.037	1.5 (1.0–2.0)	0.054
Alteplase + NAC	30.76 (24.16–37.36)	0.007	1.5 (0.75–2.0)	0.041
Sham	18.36 (11.49–25.23)	0.320	0.0 (0.0–0.0)	<0.001

* Dunn comparison with the untreated adhesion group; p values were adjusted using the Benjamini–Hochberg procedure. Overall Kruskal–Wallis results were $H = 18.08$, $p = 0.001$ for IL-6 and $H = 20.80$, $p < 0.001$ for adhesion grade.
FDR, false discovery rate; IL-6, interleukin-6; IQR, interquartile range; NAC, N-acetylcysteine.

animals despite generally lower adhesion scores. Thus, a single late serum measurement did not reflect the extent of local adhesion formation in this experimental setting.

Timing is a plausible explanation. IL-6 is produced promptly after tissue injury and changes substantially during the first 6–48 postoperative hours in rats undergoing abdominal surgery (10,11), whereas mature adhesions were assessed 14 days after surgery. In another rat model of peritoneal adhesions, hypoxia-preconditioned mesenchymal stem cells reduced prolonged IL-6 release during the proliferative phase and prevented adhesion formation (12). Recent prospective human data also showed that serum IL-6 measured 48 hours after hysteroscopic myomectomy was associated with subsequent intrauterine adhesion severity (13), reinforcing the importance of sampling time. Serial sampling during the first postoperative hours and days would better characterise the temporal relationship between IL-6 and adhesion development.

Compartmentalisation is also relevant. Adhesion formation occurs within the peritoneal cavity, and circulating IL-6 does not necessarily parallel concentrations in peritoneal fluid (6). Recent postoperative data showed that serum and drainage-fluid IL-6 provided different diagnostic information, with local IL-6 being more informative for intra-abdominal complications (14). Because this study measured serum only, it cannot determine whether local IL-6 was associated with adhesion severity.

The intervention design further limits the interpretation of the pooled correlation. Alteplase and NAC have demonstrated anti-adhesion effects in experimental models (7,8), and recent evidence indicates that NAC can also modify systemic IL-6 concentrations while reducing peritoneal adhesion burden (15). Treatment assignment may therefore alter both the biomarker and the outcome. The overall correlation should be interpreted as an association within this experimental model rather than as a formal validation of serum IL-6 as a diagnostic biomarker. The exploratory within-group coefficients varied in direction and magnitude, but none reached statistical significance; these estimates were based on only eight animals per group and were therefore imprecise. Alteplase and combined treatment reduced adhesion scores after false-discovery-rate adjustment, whereas the NAC comparison was borderline; these secondary findings require confirmation.

This study has several limitations. Each group contained only eight animals, no a priori sample-size calculation was performed, and the study should therefore be considered exploratory. IL-6 was measured at one late time point, peritoneal fluid and tissue IL-6 were not assessed, and adhesion severity was evaluated macroscopically without histological confirmation. The pooled correlation combined several active-treatment groups and a sham group with no adhesion variability, whereas the within-group analyses were markedly underpowered. The untreated adhesion and sham groups did not receive an equivalent volume of intraperitoneal vehicle; therefore, a potential effect of saline administration cannot be excluded. Replacement of values below the detection limit may also have influenced group estimates. Finally, scoring by one blinded investigator reduced observer bias but did not permit evaluation of inter-rater reliability. Larger studies with serial sampling, local biomarker assessment, and quantitative histopathological evaluation are warranted.

CONCLUSION

Serum IL-6 measured on postoperative day 14 was not associated with the severity of postoperative intra-abdominal adhesions in this rat model. The findings caution against using a single late systemic IL-6 value as an isolated surrogate for a local, time-dependent process. Serial measurements combined with peritoneal and tissue biomarkers may provide a more informative assessment.

FUNDING

No funding was received for this study.

CONFLICTS OF INTEREST

None to declare.

ETHICS STATEMENT

The study was approved by the Ethics Committee of the University of Sarajevo Faculty of Medicine (No. 02-3-4-AK-3434/25; 30 May 2025) and the University of Sarajevo Faculty of Veterinary Medicine (No. 07-03-328-3/25; 8 May 2025). All procedures complied with the Law on Protection and Welfare of Animals of Bosnia and Herzegovina.

DATA AVAILABILITY STATEMENT

The data generated and analysed during this study are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS (CRediT)

Conceptualization: A.G., A.V.; **Data curation:** A.D., L.A.; **Formal analysis:** A.D.; **Investigation:** A.G., A.D., L.A., S.I., A.K.; **Methodology:** A.G., A.V.; **Project administration:** A.G.; **Supervision:** A.V.; **Writing – original draft:** A.G.; **Writing – review & editing:** A.G., A.V., S.I., A.K., E.L. All authors have read and agreed to the published version of the manuscript.

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