



# Continuous midazolam infusion can minimize the pro-inflammatory response to anesthesia and surgery for pediatric patients with intra-abdominal infection: Comparative study versus continuous propofol infusion

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## ABSTRACT

**Objectives:** Comparison of serum levels of interleukin (IL)-1 $\beta$ , IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) estimated before and after propofol or midazolam infusion during emergency surgery for children had intraabdominal infection (IAI)

**Patients & Methods:** 140 children were allocated into Group P: included patients received propofol at the rate of 11 mg/kg/hr and Group M included patients received midazolam infusion at the rate of 0.3 mg/kg/hr. Anesthesia was induced with intravenous (IV) thiopentone (5 mg/kg) and maintained with 50% air in oxygen and an end-tidal concentration of 2–3% sevoflurane with IV rocuronium (0.6 mg/kg) and IV injection of paracetamol (10 mg/kg) as intraoperative analgesia. Study infusions were started before skin incision and stopped after skin closure. Blood samples were obtained before the start and at end of infusion for ELISA estimation of serum IL-1 $\beta$ , IL-6 and TNF- $\alpha$  levels. Study outcome is the effect of the study drugs on estimated serum cytokines' levels.

**Results:** At the end of propofol infusion, serum cytokines' levels were significantly higher in comparison to levels estimated before start of infusion. while, at the end of midazolam infusion, serum cytokines' levels were significantly decreased in comparison to levels estimated before the start of infusion.

**Conclusion:** Midazolam infusion not propofol infusion decreased serum cytokines' levels and could modulate the preexisting proinflammatory status of pediatric patients with IAI and prevent the immune stress of anesthesia and surgery.

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## 1. Introduction

Acute abdominal pain in pediatric patients is mostly secondary to acute appendicitis, which is one of the most frequent emergencies in pediatric surgery [1]. Intussusception is the most common cause of acute abdominal pain due to small bowel obstruction in children under 4 years [2]. Surgery remains the mainstay in treating intussusception especially when non-operative reduction is contraindicated or unsuccessful [3] and may involve manual reduction only or bowel resection for bowel necrosis, perforation or irreducibility [4]. Meckel's diverticulum is a congenital malformation that can be complicated with intestinal obstruction, diverticulitis or perforation [5]. In general, intra-abdominal sepsis causes disruption of gut microcirculation with subsequent tissue hypoxia, and the damaged gut acts as reservoir for inflammatory mediators and provides a continual source of these mediators to the systemic circulation [6].

Anesthetic drugs have discrepant effects on patients' immune milieu, desflurane alone or with NO<sub>2</sub> cause significant increase in systemic interleukin

(IL)-6 one day after surgery [7]. On contrary, sevoflurane can attenuate the inflammatory response during cardiopulmonary bypass [8] and dexmedetomidine, in ischemia-perfusion animal model, decreased levels of IL-1 $\beta$ , IL-6 and tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) [9].

Propofol is an intravenous general anesthetic widely used for general anesthesia and for sedation in ICU [10]. Propofol acts by binding to and modulating several neuronal ion channels and its effects are thought to occur through an impact on the ligand-gated channels including the GABA<sub>A</sub> receptor [11].

Midazolam is a benzodiazepine drug that is commonly used in procedural sedation and general anesthesia [12] by several routes including oral, intravenous, intranasal and intramuscular [13]. Midazolam increases the efficiency of the brain GABA receptors and reduces neuronal excitability with concomitant reduction of anxiety and sedation [14].

## 2. Design

Prospective comparative study

### 3. Setting

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### 4. Hypothesis

Anesthetic manipulation during surgery for children had intraabdominal infection (IAI) may minimize the impact of surgery on immune milieu of these patients.

### 5. Objectives

Comparison of serum levels of proinflammatory cytokines estimated before and after propofol or midazolam infusion during surgeries for children had IAI

### 6. Patients & methods

The study protocol was approved by the Local Ethical Committee (RC: 9-2-19) to include all children presenting with clinical manifestations of IAI for evaluation for inclusion and exclusion criteria. Inclusion criteria included age range of 3–11 years, ASA grade I or II, emergency surgical interference for IAI, operative duration of <60 min, absence of exclusion criteria. Exclusion criteria included predicted duration of surgery of >60 min, presence of history of allergy to anesthetics or study drugs, genetic disorders that may alter the immune response, diabetes mellitus, endocrinopathy, liver or kidney diseases, maintenance on immunosuppressive therapy for any indication, refusal of parents to sign the consent for study participation. All patients were clinically evaluated and underwent routine laboratory investigations and abdominal ultrasonography for the assurance of diagnosis.

### 7. Randomization & grouping

Patients were randomly allocated into two groups using dark colored envelopes containing cards carrying group label that were prepared by an assistant who was blinded about the significance of the label. Both propofol and midazolam infusions were not given as anesthetic drugs but as study drugs to evaluate the study hypothesis; to evaluate their effect on the levels of pro-inflammatory cytokines. Patients were grouped according to the study drug used as:

- (1) Group P: included patients received propofol infusion in a study dose of 11 mg/kg/h, which is the median of the dose used for McFarlan continuous propofol infusion regimen for propofol anesthesia [15,16].

- (2) Group M which included patients received midazolam hydrochloride (Dormicum, Roche Laboratories, France) infusion at a rate of 0.3 mg/kg/h, which is the median of the dose used by Memiş et al. [17] as anesthetic infusion.

### 8. Anesthetic procedure

All patients were premedicated with intramuscular atropine 0.01 mg/kg and were non-invasively continuously monitored for heart rate, blood pressure, pulse oximetry and end-tidal CO<sub>2</sub> during operation. Anesthesia was induced with intravenous thiopentone (5 mg/kg) and maintained with 50% air in oxygen and an end-tidal concentration of 2–3% sevoflurane. Intravenous rocuronium in a dose of 0.6 mg/kg was used to facilitate tracheal intubation and re-administered according to surgical requirements. Study infusions were started before skin incision and dropped after skin closure. Intraoperative analgesia was provided as intravenous injection of paracetamol (Perfalgan®; UPSA Laboratories, Agen, France) in a dose of 10 mg/kg.

### 9. Blood sampling and investigations

Two blood samples were obtained; before the start and at the end of infusion of the study drug. Blood samples were obtained under complete aseptic condition by a lab assistant who was blinded about diagnosis. Blood samples were allowed to clot in a warm water bath at temp of 37°C for 5 min and then centrifuged at 5000 rpm for 2 min to separate serum, which were collected in sterile Eppendorff tube and stored at –80°C till be assayed for serum interleukin (IL)-1β, IL-6 and tumor necrosis factor-α (TNF-α).

### 10. Methodology

Serum IL-1β, IL-6 and TNF-α were estimated using enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions and were read using a 96 well microplate ELISA reader (Dynatech. MR 7000) for estimation of:

- (1) Human IL-1β was measured with the enzyme linked immunoassay (ELISA) kit (catalogue no. ab46052, abcam Inc., San Francisco, USA) by quantitative sandwich enzyme immunoassay technique [18].
- (2) IL-6 with the enzyme-linked immunoassay (ELISA) kit (catalogue no. ab46042, abcam Inc., Cambridge, USA) by quantitative sandwich enzyme immunoassay technique [19].
- (3) Human TNF-α was measured with the enzyme-linked immunoassay (ELISA) kit (catalogue no. ab179886, abcam Inc., Cambridge, USA) by

quantitative sandwich enzyme immunoassay technique [20].

## 11. Study outcome

Study outcome is the effect of the study drugs on serum pro-inflammatory markers of IAI patients undergoing emergency surgery.

## 12. Sample size calculation

Previously, **Helmy & Al-Attiyah** [21] compared the effect of propofol and midazolam infusions on cytokines' levels in 20 pediatric patients admitted to surgical ICU and reported decreased levels with midazolam by 19–21% and **Lu et al** [22] reported significant differences between serum cytokines' levels before and after midazolam or propofol infusions given to 56 pediatric patients required postoperative sedation. Considering IAI is associated with increased levels of proinflammatory cytokines [6], and to obtain significant difference between levels estimated before and after infusion, sample size was calculated to include >60 patients per group to get a study power of 80% with a cutoff point of 0.05 and  $\beta$  cutoff point of 0.2.

## 13. Statistical analysis

Data are presented as mean, standard deviation (SD), numbers, percentages, median and interquartile range (IQR). Parametric results were analyzed using unpaired t-test for inter-group comparisons, paired t-test for comparisons of estimated cytokines' levels before and after study infusions and non-parametric results were analysed using Chi-square test and Mann-Whitney test. Statistical analysis was performed using SPSS software package (IBM Co, 2015) and p value was considered significant if was <0.05.

## 14. Results

The study included 161 patients eligible for evaluation; 21 patients were excluded for not fulfilling the inclusion criteria and 140 patients were randomly divided into two study groups (Figure 1).

There was non-significant difference between both groups regarding enrolment data. All surgeries were conducted uneventfully, with non-significant difference of operative time between both groups (Table 1).

Serum levels of IL-1 $\beta$ , TNF- $\alpha$  and IL-6 estimated before the start of infusion of the study drugs showed non-significant differences between patients of both groups (Table 2). At the end of propofol infusion, serum levels of the estimated cytokines were significantly higher in comparison to levels estimated before start of infusion. On contrary, at the end of midazolam infusion, serum levels of the three cytokines were significantly decreased in comparison to levels estimated before start of infusion.

## 15. Discussion

Estimated serum levels of IL-1 $\beta$ , IL-6, and TNF- $\alpha$  before induction of anesthesia were significantly higher in all patients in comparison to controls of cross-matched age and free of intra-abdominal infection (IAI). This finding indicated the burden of IAI on immune system, which was exaggerated with increased severity of infection. Similarly, **Peeters et al.** [23] detected higher serum levels of IL-6 and –8 and TNF- $\alpha$  in pediatric patients with acute appendicitis than in healthy controls and found serum IL-6 levels were significantly elevated at time of presentation, and serum levels of the three cytokines were higher in complicated than in non-complicated appendicitis. The detected high levels of serum pro-inflammatory cytokines could be attributed to the findings of **Xiao et al.** [24] who reported that IL-6 promotes the epithelial-to-mesenchymal transition process of peritoneum,

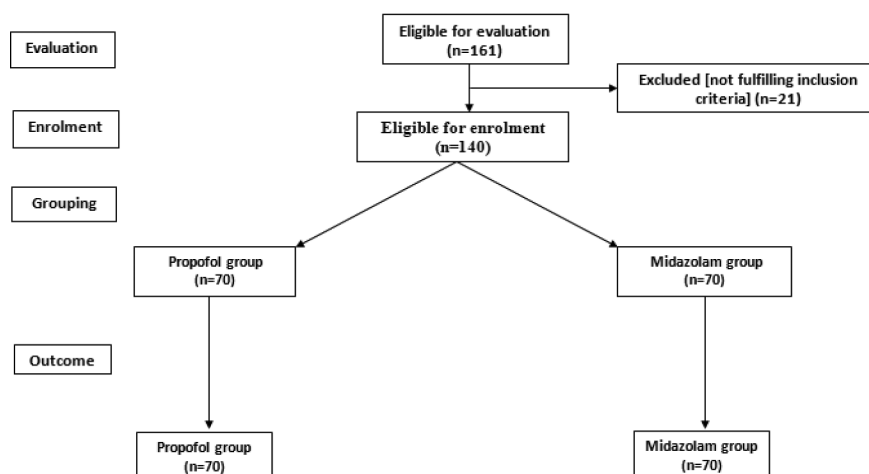


Figure 1. Consort flow sheet.

**Table 1.** At admission demographic and clinical data of patients of both groups.

Group Variables		Propofol	Midazolam	P
Age (years)	Categories	≤6	22(31.4%)	0.346
		7–11	48 (68.6%)	
	Mean (SD)	7.5 (2.6)	7.9 (2.1)	0.316
	Range	3–11	3.5–11	
Sex	Males	45 (64.3%)	51 (72.9%)	0.275
	Females	25 (35.7%)	19 (27.1%)	
Body weight (kg)	Categories	<15	1 (1.4%)	0.236
		15–25	28 (40%)	
		>25	41 (58.6%)	
	Mean (SD)	25.3 (7)	27.2 (6)	0.085
	Range	12.5–42	13–39	
ASA grade	ASA-I	57 (81.4%)	54 (77.1%)	0.532
	ASA-II	13 (18.6%)	16 (22.9%)	
Indication of surgery	Acute appendicitis	32 (45.7%)	28 (40%)	0.855
	intussusception	18 (25.7%)	22 (31.4%)	
	Irreducible hernia	15 (21.4%)	13 (18.6%)	
	Meckel's diverticulum	3 (4.3%)	4 (5.7%)	
	Volvulus	2 (2.9%)	3 (4.3%)	
Operative time (min)	Categories	≤30	5 (7.1%)	0.259
		>30-60	65 (92.9%)	
	Mean (SD)	44.5 (6.7)	46 (8.8)	
	Range	25–55	25–60	

Data are shown as mean, standard deviation, numbers, percentages, median & interquartile range [IQR]; p value indicates significance of difference between both groups; p < 0.05 indicates significant difference; p value >0.05 indicates non-significant difference.

**Table 2.** Serum levels of studied cytokines estimated before and after infusion of studied drugs.

Group Variables		Propofol	Midazolam	P1
IL-1β (ng/ml)	Before	5.17 (1.82)	5.71 (1.9)	0.088
	After	6 (1.86)	4.35 (1.92)	
	P2	0.008	<0.0001	
	% Of change	Median	32.71	
		IQR	14.3–67.57	
TNF-α (ng/ml)	Before	9.54 (3.08)	10.58 (4.85)	0.131
	After	10.88 (2.87)	8.08 (3.72)	
	P2	0.0089	0.0008	
	% Of change	Median	32.2	
		IQR	16.67–43.42	
IL-6 (ng/ml)	Before	156.1 (37)	154.7 (41.1)	0.833
	After	168.9 (34.2)	127 (37.2)	
	P2	0.0353	0.00005	
	% Of change	Median	19.93	
		IQR	15.13–28.81	

Data are shown as mean, standard deviation, median & interquartile range [IQR]; P1: indicates the significance of difference between both groups; P2: indicates the significance of difference between levels estimated before and after infusion; p < 0.05 indicates significant difference; p value >0.05 indicates non-significant difference.

possibly by activating the JAK2/STAT3 signaling pathway. Also, **Catar et al.** [25] attributed neutrophil recruitment and retention during peritonitis to the stimulation of human peritoneal mesothelial cells with IL-17, which causes increased C-X-C motif chemokine ligand-1 production and increased transmesothelial migration of neutrophils and found that such effects were amplified by TNF-α

After the end of the study infusion, estimated serum levels of IL-1β, IL-6, and TNF-α were significantly lower in patients received midazolam infusion in comparison to their levels estimated at the start of infusion and to levels estimated after the end of propofol infusion, which were significantly higher in comparison to their respective levels estimated before the induction of anesthesia. These data point to an

immunomodulatory effect of midazolam toward the anti-inflammatory side and of propofol toward the pro-inflammatory side.

These findings supported the results of the early study performed by **Helmy & Al-Attiyah** [21] who found that after 48 h of propofol or midazolam infusion for critically ill surgical patients, midazolam caused significant decreases in IL-1β, IL-6, and TNF-α, while propofol caused significant increases in the serum levels of these cytokines. Experimentally, **Xiao et al.** [26] using animal model of septic peritonitis found midazolam and/or fentanyl significantly reduced serum pro-inflammatory cytokines and mortality in comparison to model animals and found leucocytic count in peritoneal cavity lavage fluid was significantly reduced in the medication-treated groups

than in the model group. Clinically, **Lu et al.** [22] reported that in pediatric patients who required sedation for >48 hr after surgery, the levels of IL-1 $\beta$ , IL-8, and TNF- $\alpha$  measured before midazolam infusion were decreased significantly after the end of infusion. Also, **Tong et al.** [27] found that in critically ill children with multiple trauma who were maintained on midazolam or dexmedetomidine infusion, serum levels of IL-1 $\beta$ , IL-8, and TNF- $\alpha$  measured at 24-h were decreased, while serum level of IL-10 was increased with significant difference in favor of dexmedetomidine, while at 48-h serum level of inflammatory cytokines started to increase in patients receiving dexmedetomidine, while remained stationary with midazolam, and serum IL-10 started to increase with midazolam, but decreased with dexmedetomidine and these changes had progressed till 72-h after the start of infusion.

The reported immunomodulatory effect of midazolam may be explained depending on the results of previous experimental studies; wherein midazolam was found to inhibit the expression of IL-6 mRNA in human peripheral blood mononuclear cells [28] and inhibits the lipopolysaccharide (LPS)-induced release of nitric oxide and TNF- $\alpha$  in rat microglial cells via the peripheral-type benzodiazepine receptors (PBR) [29]. Also, it was found that high levels of brain IL-1 $\beta$  can significantly induce the microglia and astrocytes to produce TNF- $\alpha$  [30] and IL-6 [31], but midazolam can inhibit this stimulatory effect of IL-1 $\beta$  through binding to the central-type (CBR) benzodiazepine receptors which are coupled with the GABA<sub>A</sub> inotropic receptors, and are expressed on astrocytes [32], and through PBRs, which are increased in astrocytes and microglial cells upon glial activation in inflammation [33]. Thereafter, **Lu et al.** [26] showed that midazolam can significantly inhibit IL-1 $\beta$ -induced release of IL-6 in rat C6 glioma cells in a concentration-dependent fashion up to inhibition by 43.58% with 3  $\mu$ M midazolam concentration, while propofol elicited no significant effect on IL-1 $\beta$ -induced the release of IL-6. Recently, **Horiguchi et al** [34] and **Feng et al** [35] experimentally reported that midazolam pretreatment suppressed LPS-induced the upregulation of the costimulatory molecule CD80 and the release of IL-6 and NO in peripheral monocyte-derived macrophages with the suppression of the activation of NF- $\kappa$ B and mitogen-activated protein kinase in human cells.

## 16. Conclusion

Midazolam infusion not propofol infusion decreased serum cytokines' levels and could modulate the pre-existing proinflammatory status of pediatric patients with IAI and prevent the immune stress of anesthesia and surgery.

## 17. Recommendations

The outcome of the same sedative procedures needs to be evaluated in adult patients and during anesthesia for long-duration surgeries. Also, the same procedures were recommended to be evaluated in patients undergoing elective surgeries free of infection to evaluate the outcome on surgery and anesthesia-induced neuropathic pain.

## Disclosure statement

No potential conflict of interest was reported by the author(s).

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