

# Serum cytokine levels as markers of paralytic ileus following robotic radical prostatectomy at different pneumoperitoneum pressures

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## Abstract

**Background:** To evaluate intraoperative and postoperative cytokines in patients who underwent robotic prostatectomy (RP) at a pressure of 12 or 15 mmHg, and the risk of postoperative ileus.

**Materials and methods:** We presented the first series evaluating intraoperative and postoperative cytokines in patients undergoing RP at a pressure of 12 or 15 mmHg by a single surgeon. Changes in cytokine concentrations were shown to correlate with surgical outcomes and pathological states. The study investigated the changes in cytokine concentrations (interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , interleukin-1 $\beta$  [IL-1 $\beta$ ], IL-2, IL-4, IL-6, IL-12, and IL-17) at different pneumoperitoneum pressures and their potential role in the development of postoperative ileus.

**Results:** The data on 10 consecutive patients confirmed that a lower pneumoperitoneum pressure was associated with lower cytokine levels and a lower risk of ileus. There were increased levels of postoperative interferon- $\gamma$ , tumor necrosis factor- $\alpha$ , IL-12p70, IL-1 $\beta$ , IL-2, IL-4, and IL-17a at 15 mmHg when compared to 12 mmHg.

**Conclusions:** The data indicated that lower pressure RP reduced intra-/postoperative cytokine levels confirming our hypothesis. Larger patient numbers are required to further validate this but the implications of this data will benefit not only urological patients but also other speciality patients undergoing minimally invasive surgery.

**Keywords:** Cytokines; Ileus; Oncology; Pneumoperitoneum pressure; Prostate cancer; Robotic prostatectomy; Robotic surgery

## 1. Introduction

Postoperative ileus (POI) is a complication defined as a temporary nonmechanical inhibition of bowel motility preventing oral intake and presenting with decreased passage of flatus/stool and absence of bowel sounds.<sup>[1]</sup> This is a common complication affecting between 5% and 25% of patients undergoing trans-abdominal surgery, and it results in patient discomfort, prolonged admission, and delayed recovery.<sup>[2]</sup>

In urology, minimally invasive robotically assisted laparoscopic surgery has resulted in a dramatic reduction in postoperative complications, length of stay, transfusion rates, and an increase in better oncological outcomes. This has resulted in 74% of all prostatectomies performed in the UK being robotically assisted, a figure which was anticipated to reach 80% by 2020.<sup>[3,4]</sup> Despite the improvement in many areas of

postoperative recovery, robotic surgery still has POI as one of its main complications.<sup>[2]</sup>

Operative factors such as a steep Trendelenburg position (which is required to reflect the bowel away from the pelvic organs), occasional peritoneal adhesiolysis, and high intra-abdominal pressures with CO<sub>2</sub> insufflation of 15–20 mmHg that is necessary for the creation of the pneumoperitoneum predisposes robotic urological surgery patients to POI. Rohloff et al. published a retrospective study in October 2019, which showed that POI rates could be significantly reduced by lowering intraoperative pneumoperitoneum from 15 to 12 mmHg.<sup>[5]</sup> This finding supports the pathophysiology proposed by Luo et al. that intraabdominal pneumoperitoneum pressure could lead to an ischemia-reperfusion injury of the bowel due to changes in splanchnic blood flow during insufflation/desufflation.<sup>[6]</sup> This pathophysiology could indicate a potential for a predictive biomarker should a suitable measurable factor be identified. An animal study investigating bowel manipulation found a significant increase in activated macrophages that also resulted in reduced gut motility.<sup>[7]</sup> The inflammatory response triggered by these macrophages is cytokine mediated with tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), C-C motif chemokine ligand 2, IL-1 $\beta$ , and IL-10 having been previously highlighted.<sup>[8,9]</sup>

We hypothesized that cytokine levels, whether individual or in combination, may indicate a risk or predictive value of POI. A prospective pilot study was proposed to investigate the changes in

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cytokine profiles during robotic prostatectomy (RP) at differing insufflation pressures and the relationship to the incidence of POI.

## 2. Methods

Local and national ethical approval for the described protocol was granted by the Health Resource Authority (NHS England). Ten patients undergoing RP were prospectively enrolled in the study. The positioning, preoperative care, and surgical technique were identical in all patients. Five patients underwent RP at a pneumoperitoneum pressure of 12 mmHg and the other 5 at a pressure of 15 mmHg. A single surgeon at a single institute (NV) performed all the procedures using a standardized transperitoneal robotic assisted laparoscopic technique with a Da Vinci Si<sup>®</sup> robotic system. Pneumoperitoneum was maintained throughout the procedure by using the AirSeal<sup>®</sup> intelligent flow system. All patients received a standardized general anesthetic consisting of fentanyl 100 mg, midazolam 2 mg, and propofol induction and intubation facilitated by atracurium. In addition, paracetamol 1 g, ketorolac 30 mg, ondansetron 4 mg, and dexamethasone 6.6 mg were given. Anesthesia was maintained with oxygen, air, and desflurane through a circular system and positive pressure ventilation. Muscle relaxation was maintained with atracurium infusion. The caudal block administered contained 40 mL 0.25% bupivacaine, 150 mg clonidine, and 100 mg fentanyl. Bupivacaine 0.5% 20 mL was infiltrated into the skin wounds at the end of the operation. Regular paracetamol was prescribed postoperatively and ketorolac 30 mg im, oramorph 20 mg, and cyclizine were available on an as required basis. As per ERAS enhanced recovery recommendations, patients were encouraged to mobilize immediately after surgery, and clear fluids progressing to a soft diet was advised on day one as tolerated. Intravenous fluids are not routinely prescribed postoperatively as oral intake was encouraged. Patients were discharged within 28 days and subcutaneous low molecular weight heparin was stopped and the urethral catheter was removed at day 14 postoperatively.

Five blood samples were taken for cytokine analysis for each patient in the peri- and postoperative period as per the following protocol:

Sample A (preinduction): Taken prior to anesthesia, and served as an initial patient specific baseline and as second preoperative baseline prior to insufflation to account for any post procedural changes that may occur.

Sample B: Postinduction and preinsufflation.

Sample C: Taken immediately postoperatively and postextubation.

Sample D: Taken 2 hours postoperatively.

Sample E: Taken 14 days postoperatively at the time of trial without catheter.

Venous blood samples were collected in EDTA vials and were kept at 2–8°C while being handled and were immediately processed. Samples underwent centrifugation in a refrigerated centrifuge at 1000 g at 4°C for 15 minutes. The plasma was aliquoted and stored at –80°C until analysis.

The following 9 cytokines were included for assay analysis: IL-6, IL-10, interferon- $\gamma$  (IFN- $\gamma$ ), IL-12p70, IL-1 $\beta$ , IL-2, IL-4, TNF- $\alpha$ , and IL-17a. Analysis of plasma samples was performed as per manufacturer's instructions using a High Sensitivity 9-Plex Human ProcartaPlex<sup>™</sup> Panel kit (LCP0010) from Thermo Fisher Scientific. Cytokine levels were measured on a Luminex MAGPIX (Luminex Corp.), and analysed using the MILLIPLEX<sup>®</sup> Analyst software version 5.1 (Merck Millipore).

The raw data was measured as mean fluorescent intensity and the concentration of each cytokine in each sample was calculated using a 5-parameter logistic fit curve generated for each analyte from standards. Concentrations of cytokines were compared for differing pneumoperitoneum pressures.

Secondary data were collected on age, body mass index, renal function, intraoperative blood loss, operative/console time, on-table complications, ileus rates, postoperative complications, and length of stay. POI was defined as the inability to tolerate oral intake with decreased passage of flatus/stool and absence of bowel sounds with or without confirmatory radiological imaging in the absence of mechanical obstruction in the 2-week period following RP.

## 3. Results

### 3.1. Patient demographics

Ten patients were recruited to the study and successfully underwent RP, 5 at a pneumoperitoneum pressure of 12 mmHg and 5 at a pressure of 15 mmHg as per protocol. As shown in Table 1, the mean age was 65 and 62 years, respectively, and the average body mass index was 25.9 and 30.2 kg/m<sup>2</sup>, respectively. Preoperative prostate specific antigen levels were similar, with means of 10.2  $\mu$ g/L for the 12 mmHg group and 8.1  $\mu$ g/L for the 15 mmHg group. All preoperative Gleason scores were between 3+3=6 and 4+3=7.

### 3.2. Primary outcomes

There were 2 episodes of POI, both of which occurred in patients in the 15 mmHg group resulting in a POI rate of 40% (2/5) for this group but due to the small group sizes this was not significant ( $p=0.2235$ ) and cytokine levels specifically for the POI patients were also not significant.

Samples A and B, the samples taken preinduction and preinsufflation, were used to create baseline cytokine levels for each group, to which we could compare the postoperative levels. Table 2 shows the results of plasma concentrations for each cytokine for both 12 and 15 mmHg groups.

Analysis of cytokines in plasma samples collected preinduction (samples A and B), immediately postoperatively and postextubation (sample C), 2 hours postoperatively (sample D), and 14 days postoperative (sample E) revealed the trends shown in Table 2. There were large sample variations within groups because of the small numbers used in this pilot study. As a result, there were not major statistical differences between conditions. Despite this, there were evident trends between samples collected from the 12 mmHg group compared to the 15 mmHg group. For instance, all cytokines, with the exception of IL-6 and IL-10, were detected at much higher levels in the 15 mmHg group. IL-10 was found in higher concentrations in the 12 mmHg pressure group

**Table 1**  
Patient demographic characteristics.

	Pneumoperitoneum pressure 12 mmHg (n=5)	Pneumoperitoneum pressure 15 mmHg (n=5)
Mean age, years	65 $\pm$ 6.3	62 $\pm$ 5.2
Body mass index, kg/m <sup>2</sup>	25.9 $\pm$ 3.6	30.2 $\pm$ 3.2
Prostate specific antigen, $\mu$ g/L	10.2 $\pm$ 2.8	8.1 $\pm$ 2.8
Operative time, min	170 $\pm$ 14	165 $\pm$ 5

**Table 2**

**Cytokine plasma concentrations.**

Cytokine	Plasma concentration (pg/mL)							
	Pneumoperitoneum pressure 12 mmHg				Pneumoperitoneum pressure 15 mmHg			
	Baseline B	Sample C	Sample D	Sample E	Baseline B	Sample C	Sample D	Sample E
IFN- $\gamma$	23.2 $\pm$ 7.2	20.9 $\pm$ 6.4	17.8 $\pm$ 6.3	12.3 $\pm$ 7.8	42.8 $\pm$ 24.9	30.8 $\pm$ 18.3	30.7 $\pm$ 18.9	14.1 $\pm$ 2.2
TNF- $\alpha$	5.6 $\pm$ 2.8	4.1 $\pm$ 1.90	4.6 $\pm$ 1.8	2.2 $\pm$ 0.8	7.9 $\pm$ 3.0	6.2 $\pm$ 2.1	5.2 $\pm$ 2.1	5.5 $\pm$ 0.3
IL-12p70	6.0 $\pm$ 3.2	4.3 $\pm$ 2.3	4.1 $\pm$ 1.5	2.8 $\pm$ 0.9	9.7 $\pm$ 4.4	6.6 $\pm$ 2.2	5.2 $\pm$ 1.9	5.7 $\pm$ 0.8
IL-1 $\beta$	3.4 $\pm$ 1.0	3.1 $\pm$ 0.9	2.1 $\pm$ 0.9	2.1 $\pm$ 1.1	7.9 $\pm$ 3.8	5.6 $\pm$ 3.0	5.1 $\pm$ 3.1	3.0 $\pm$ 0.3
IL-2	3.0 $\pm$ 1.4	2.9 $\pm$ 1.7	2.9 $\pm$ 1.3	1.4 $\pm$ 0.4	5.0 $\pm$ 1.7	3.8 $\pm$ 1.1	2.7 $\pm$ 1.	3.9 $\pm$ 0.3
IL-4	8.8 $\pm$ 3.9	7.4 $\pm$ 2.7	7.4 $\pm$ 2.3	4.9 $\pm$ 1.6	17.6 $\pm$ 8.7	16.0 $\pm$ 7.2	13.1 $\pm$ 6.6	10.2 $\pm$ 1.4
IL-6	36.6 $\pm$ 13.0	110.4 $\pm$ 38.4	106.5 $\pm$ 36.1	27.1 $\pm$ 7.7	58.4 $\pm$ 31.7	84.0 $\pm$ 28.3	106.5 $\pm$ 37.7	18.5 $\pm$ 5.2
IL-10	2.2 $\pm$ 0.5	16.8 $\pm$ 9.1	27.8 $\pm$ 10.2	3.1 $\pm$ 1.5	2.2 $\pm$ 0.3	10.92 $\pm$ 2.90	9.20 $\pm$ 3.28	2.34 $\pm$ 0.19
IL-17a	2.02 $\pm$ 0.71	1.75 $\pm$ 0.68	1.68 $\pm$ 0.51	1.18 $\pm$ 0.26	3.12 $\pm$ 0.9	2.2 $\pm$ 0.5	1.8 $\pm$ 0.5	2.6 $\pm$ 0.2

but only immediately postoperative and postextubation (sample C), and also 2 hours postoperative (sample D). IL-6 also appeared enhanced following these procedures but the levels in samples D did not differ between the 12 and 15 mmHg groups. What is interesting and worth noting from our pilot data is the fact that IFN- $\gamma$ , TNF- $\alpha$ , IL-12p70, IL-1 $\beta$ , IL-2, IL-4, and IL-17a remained elevated in the 15 mmHg group even 14 days postprocedure at the time of trial without a catheter.

**3.3. Secondary outcomes**

Most of the secondary outcomes were unaffected by the change in pneumoperitoneum pressures with length of stay, operative/console time, and postoperative Hb and renal function being found to be not significantly different between the two groups ( $p > 0.05$ ). There was a clinically significant increase in intraoperative blood loss in the 12 mmHg group compared to the 15 mmHg group ( $p < 0.05$ ). With the exception of the 2 episodes of POI, no postoperative complications were recorded in either group (Table 3).

**4. Discussion**

While our data correlates with the findings of Rohloff et al.<sup>[5]</sup> that higher pneumoperitoneum pressures increases POI risk, with 40% of patients with higher insufflation pressures developing POI, unfortunately, due to the small sample size no statistical significance was reached in our study.

Luo et al. theorised that prolonged pneumoperitoneum pressure resulted in decreased splanchnic blood flow and proved that there was increased oxidative stress secondary to gut

hypoperfusion-reperfusion injury.<sup>[6]</sup> In their study, Malondialdehyde was used to assess levels of oxidative stress along with intramucosal pH, while incredibly useful to investigate the pathophysiology. However, this method required arterial blood samples and invasive procedures (NG tube), and thus was not ideal for a monitoring/screening test.

Many markers of oxidative stress have been proposed, including but not limited to, gastric intramucosal pH, histological tissue analysis, malondialdehyde, F2-isoprostanes and cytokines.<sup>[10]</sup>

Our pilot study investigated 9 frequently studied pro-inflammatory, anti-inflammatory, and regulatory cytokines, and assessed the changes in concentration in relation to different insufflation pressures. There were increased levels of postoperative IFN- $\gamma$ , TNF- $\alpha$ , IL-12p70, IL-1 $\beta$ , IL-2, IL-4, and IL-17a at 15 mmHg when compared to 12 mmHg. These findings are comparable to the work performed by Zhu et al.<sup>[11]</sup> which found elevated postoperative levels of abdominal exudates TNF- $\alpha$ , IL-1 $\beta$ , and IL-6 following laparoscopic colorectal surgery and showed that these changes were associated with poor surgical outcome, principally the development of POI. In their study, it was shown that 88%–100% of the patients that developed POI had elevated levels of TNF- $\alpha$ , IL-1 $\beta$ , or IL-6 at day 5 compared to elevated levels in only 14%–16% of the patients that did not develop POI.

We also investigated the ratio between IL-6, a pro-inflammatory mediator which regulates systemic inflammatory responses, and IL-10, an anti-inflammatory cytokine involved in the negative feedback loops of other cytokine production pathways.<sup>[12]</sup> Plasma levels of IL-6 were raised in both groups but in the higher pressure group these levels were reduced at a much slower rate. The IL-10 concentration decreased in the 15 mmHg group, potentially indicating a prolonged injury and larger inflammatory response.

This ratio between IL-6 and IL-10 has previously been linked to systemic inflammatory response related mortality and higher injury severity scores.<sup>[13]</sup> This ratio was useful in accurately differentiating between the 12 and 15 mmHg groups in our studies. Unfortunately, due to the small sample size and even smaller number of cases of POI, there was not sufficient data to give clinical relevance.

**5. Conclusion**

Current research into specific cytokine pathophysiology has not yet reached the level of detail required to be used as a biomarker.

**Table 3**

**Secondary outcome results.**

	Pneumoperitoneum pressure 12 mmHg (n=5)	Pneumoperitoneum pressure 15 mmHg (n=5)	p
Intraoperative blood loss, mL	93 $\pm$ 69	56 $\pm$ 9	<0.05
Length of stay, days	1.6 $\pm$ 0.5	2 $\pm$ 0	0.910
Operative time, min	170 $\pm$ 14	165 $\pm$ 5	0.578
Console time, min	143 $\pm$ 19	140 $\pm$ 14	0.553
Postoperative Hb, g/L	117.2 $\pm$ 6.4	120.4 $\pm$ 4.3	0.941
Postoperative creatinine, $\mu$ mol/L	87.6 $\pm$ 17.3	87.2 $\pm$ 4.4	0.076

The majority of the published literature focused on research into the initial stages of trying to identify a correlation between pathological states and variations in cytokine concentrations, whereas studies establishing diagnostic cut-offs were in the minority. Research into baseline cytokine concentrations in pathological states is rarely published and limits potential future study designs. It is clear that there is huge potential for cytokine research to be used as prognostic and diagnostic biomarkers for many conditions and postoperative complications but further research, especially with a focus on diagnostic ranges and significant cut-off levels is the key to unlocking this potential.

This study was built on existing work such as Rohloff et al.<sup>[5]</sup> and Zhu et al.,<sup>[11]</sup> and confirmed that plasma concentrations of cytokines IFN- $\gamma$ , TNF- $\alpha$ , IL-12p70, IL-1 $\beta$ , IL-2, IL-4 and IL-17a, and IL-17a are increased in higher pressures of pneumoperitoneum insufflation. Further research is required to identify a statistically significant cut-off value that will allow cytokine profiles to be used as predictor biomarkers of POI. This requires larger sample sizes and therefore higher number of POI cases, and a larger variability of patients.

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None.

### Statement of ethics

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

### Conflict of interest statement

The authors report no conflicts of interest.

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### Author contributions

None.

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