

## **Laparoscopic versus open appendectomy on serum levels of cytokines in children with perforated appendices, peritonitis and sepsis.**

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### **Abstract**

**Purpose:** To examine the effect of Laparoscopic (LA) and Open Appendectomy (OA) on serum levels of inflammatory factors Interleukin-6 (IL-6) and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ) and anti-inflammatory factor, Interleukin-10 (IL-10), in children with perforated appendices, peritonitis and sepsis.

**Methods:** 36 children received LA and 31 children received OA were included. Serum IL-6, TNF- $\alpha$  and IL-10 were determined at different time points before and after surgery with double-antibody sandwich ELISA.

**Results:** Preoperative serum IL-6, TNF- $\alpha$  and IL-10 were not significantly different between LA group and OA group ( $P>0.05$ ). However, serum IL-6 and TNF- $\alpha$  in LA group were significantly lower than those in OA group on postoperative days 1, 3 and 7 (all  $P<0.05$ ). Serum IL-10 in LA group was initially higher than that in OA group on postoperative days 1 and 3 ( $P<0.05$ ), but became significantly lower than that in OA group on postoperative day 7 ( $P<0.05$ ).

**Conclusion:** Decreased inflammatory response and enhanced anti-inflammatory response were evident postoperatively in children received LA reflected by reduced serum IL-6 and TNF- $\alpha$ , and increased serum IL-10, suggesting LA is a better choice over OA.

**Keywords:** Perforated appendix, Laparoscopic appendectomy, Open appendectomy, Inflammatory factor, Children.

### **List of Abbreviations:**

LA: Laparoscopic; OA: Open appendectomy; IL-6: Interleukin-6; TNF- $\alpha$ : Tumor necrosis factor- $\alpha$ ; IL-10: Interleukin-10; ELISA: Enzyme-linked immunosorbent assay.

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### **Introduction**

With technical advancements, clinical application of laparoscopic technique has become increasingly broadened for treating various infectious abdominal diseases including acute cholecystitis, acute appendicitis, gastrointestinal tract perforation and so on [1-4]. However, whether laparoscopy and carbon dioxide pneumoperitoneum (CO<sub>2</sub>PP) can be used in treating peritonitis remains controversial.

A major concern is that increased intra-abdominal pressure during laparoscopic surgery may increase risks of bacteraemia that can lead to systemic inflammatory response. Studies on the effect of pneumoperitoneum on systemic inflammation during sepsis remain contradictory [5,6]. Some studies reported that

CO<sub>2</sub>PP is associated with positive-end expiratory pressure and has no impact on systemic expansion of intra-abdominal *Escherichia coli* infection, but other studies showed that CO<sub>2</sub>PP can influence septic development by increasing bacterial (*E. coli*) translocation in rats [7,8]. More recent studies have shown that CO<sub>2</sub>PP can alleviate peritonitis and systemic inflammatory response in experimental models of *E. coli*-peritonitis and sepsis, and extracellular acidic pH environments after CO<sub>2</sub> insufflation during laparoscopic surgery can promote macrophage activation and function [9,10]. Perforated appendix is one of the common causes for acute abdominal diseases in children and is usually accompanied by severe peritonitis and septicopyemia. So far, only early effects of pneumoperitoneum during peritonitis have

been assessed, and the comparison between conventional and laparoscopic surgery has been only conducted in animal models which is not yet in human patients [11,12].

In current study, we aimed to compare the effect of laparoscope and conventional open appendectomy on postoperative infection to explore a better clinical approach.

## Subjects and Methods

### Subjects

67 children admitted to the Surgery Department of the Children's Hospital of Wuhan and surgically diagnosed with perforated appendices and peritonitis accompanied with sepsis were enrolled in this study.

Diagnoses were further confirmed by pathological examination. 36 children including 15 females and 21 males (2.8-14 y old and 15-42 kg) were included in LA group. 31 children including 12 females and 19 males (2.5-16 y old and 13-48 kg) were included in OA group.

Children were randomly grouped based on option of respective guardians. Operations were executed by same surgical team and in the same premises. No subjects had any recent history of infection or chronic disease. Subjects with immune diseases were excluded.

No statistical differences were noted between the two groups with respect to gender, age, weight, time of symptom onset, anesthesia, operative time or time of anesthesia ( $X^2=1.251, 1.637$ ;  $Z=0.396\sim 1.782$ ; all  $P>0.05$ ).

All manipulations were approved by the Ethic Committee of the Children's Hospital of Wuhan. All patients were well informed and signed written consents.

### Methods

**Sample collection:** Two hours before and 1, 3 and 7 d after operation, 2 ml venous blood was drawn from each subject. The samples were centrifuged at 5000 rpm for 10 min. Serum was collected and stored at  $-20^{\circ}\text{C}$  until use.

**Enzyme-Linked Immunosorbent Assay (ELISA):** Double-antibody sandwich ELISA was used to determine serum levels of IL-6, TNF- $\alpha$  and IL-10. ELISA kits were purchased from Shenzhen Dakewe Biotech Co., Ltd. (Shenzhen, China). All procedures were conducted following manufacturer's instructions.

### Statistics

SPSS 17.0 software was used for data analysis. Data were shown as mean  $\pm$  standard deviation ( $\bar{x} \pm s$ ).

Comparison between two groups was made by t-test or rank sum test. Enumeration data were analyzed using  $X^2$  test. Significance was accepted if  $P<0.05$ .

## Results

### Dynamics of serum IL-6 before and after operation

Preoperative serum IL-6 in the two groups was comparable ( $P>0.05$ ). Serum IL-6 significantly increased on postoperative day 1 in both groups ( $P<0.05$ ), but gradually declined thereafter.

On postoperative day 3, IL-6 level in the LA group had significantly decreased compared to postoperative day 1 and even its preoperative level ( $P<0.05$ ). Such a rapid decrement was not observed in the OA group ( $P>0.05$ ).

On all three postoperative time points, IL-6 level in the LA group remained significantly lower than that in the OA group ( $P<0.05$ ), as shown in Table 1 and Figure 1.

### Dynamics of serum TNF- $\alpha$ before and after operation

There was no statistical difference in preoperative serum TNF- $\alpha$  between LA and OA groups ( $P>0.05$ ). After operation, serum TNF- $\alpha$  were first significantly increased compared to that preoperatively (both  $P<0.05$ ), and then declined in both groups.

On postoperative day 3, TNF- $\alpha$  in the LA group was significantly decreased than postoperative day 1 ( $P<0.05$ ), whereas no statistical difference was observed in the OA group ( $P>0.05$ ).

Moreover, serum TNF- $\alpha$  in the LA group remained significantly lower than that in the OA group on postoperative days 1, 3 and 7 (all  $P<0.05$ ), as shown in Table 2 and Figure 2.

### Dynamics of serum IL-10 before and after operation

The preoperative serum IL-10 were comparable between LA and OA groups ( $P>0.05$ ). After operation, serum IL-10 increased in both groups.

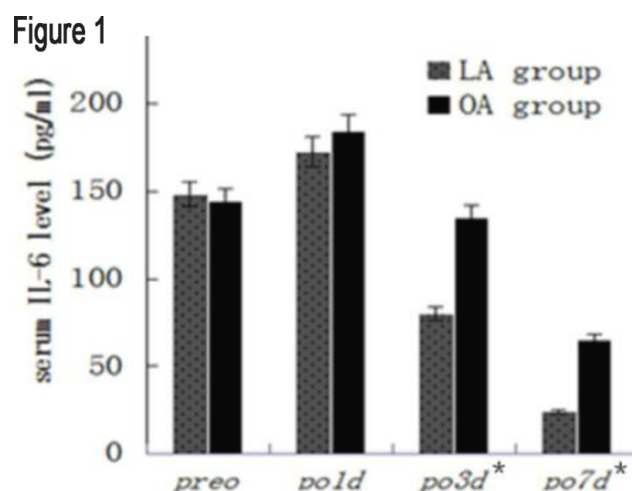
On postoperative days 1, 3 and 7, serum level of IL-10 in the LA group remained significantly higher than that the OA group ( $P<0.05$ ).

On postoperative day 7, serum level of IL-10 decreased rapidly ( $P<0.05$ ), but it remained in the OA group, as shown in Table 3 and Figure 3.

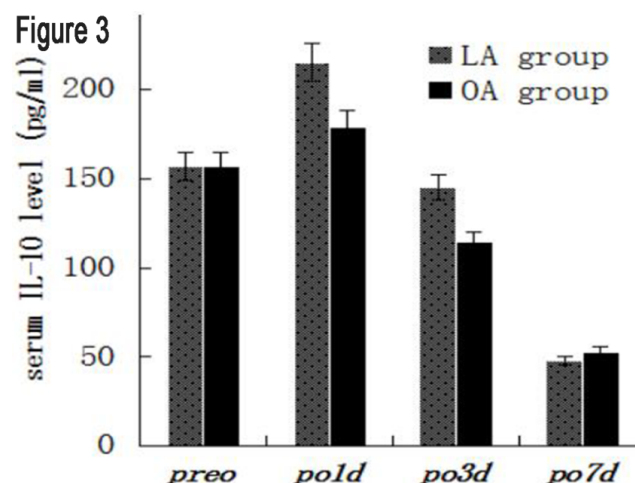
### Dynamics of IL-10/TNF- $\alpha$ ratio

There was no statistical difference IL-10/TNF- $\alpha$  ratio before operation between the two groups ( $P>0.05$ ).

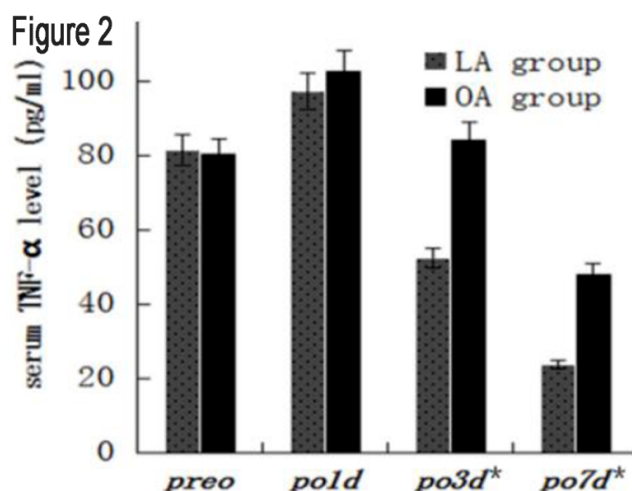
IL-10/TNF- $\alpha$  ratio in the LA group constantly remained higher than that in the OA group on postoperative days 1, 3, and 7 (all  $P<0.05$ ), as shown in Table 4 and Figure 4.



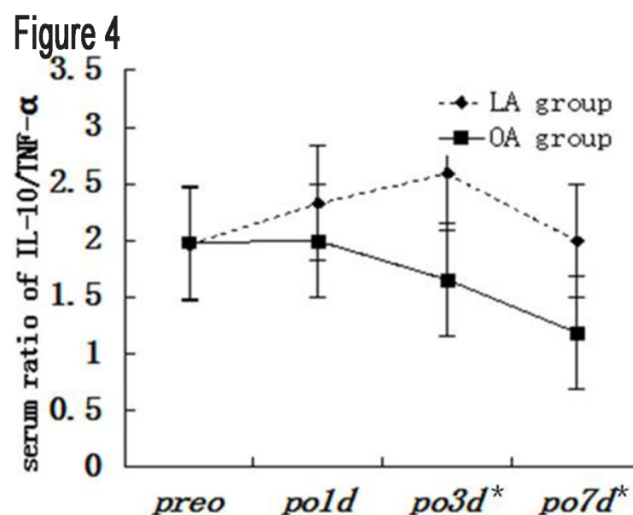
**Figure 1.** Dynamics of serum level of IL-6 before and after operation (\*indicates significant difference among two bars).



**Figure 3.** Dynamics of serum level of IL-10 before and after operation.



**Figure 2.** Dynamics of serum level of TNF-α before and after operation (\*indicates significant difference among two bars).



**Figure 4.** Dynamics of IL-10/TNF-α ratio before and after operation (\* indicates significant difference among two groups).

**Table 1.** Dynamics of serum level of IL-6 before and after operation ( $x \pm s$ , pg/ml).

Group	Pre-operative	Post-operative 1 d	Post-operative 3 d	Post-operative 7 d
LA	148.3 ± 19.0	172.7 ± 23.2 <sup>a*</sup>	80.1 ± 11.3 <sup>b*</sup>	23.9 ± 4.9 <sup>c*</sup>
OA	144.2 ± 22.1	184.5 ± 26.8 <sup>a*</sup>	135.3 ± 19.2 <sup>b</sup>	65.2 ± 16.4 <sup>c*</sup>
t	0.82	2.321	14.556	14.404
p	0.415	0.023 <sup>*</sup>	0.000 <sup>*</sup>	0.000 <sup>*</sup>

<sup>a</sup>indicates comparison with pre-operative level, tLA group=5.429, pLA group=0.000, tOA group=7.119, pOA group=0.000; <sup>b</sup>indicates comparison with pre-operative level, tLA group=18.486, pLA group=0.000, tOA group=1.679, pOA group=0.098; <sup>c</sup>indicates comparison with pre-operative level, tLA group=38.027, pLA group=0.000, tOA group=16.013, pOA group=0.000.

**Table 2.** Dynamics of serum level of TNF-α before and after operation ( $x \pm s$ , pg/ml).

Group	Pre-operative	Post-operative 1 d	Post-operative 3 d	Post-operative 7 d
LA	81.4 ± 10.7	97.3 ± 12.7 <sup>a*</sup>	52.4 ± 9.3 <sup>b*</sup>	23.7 ± 6.8 <sup>c*</sup>

OA	80.5 ± 8.9	102.8 ± 11.4 <sup>a*</sup>	84.5 ± 11.7 <sup>b</sup>	48.3 ± 9.5 <sup>c*</sup>
t	0.405	2.41	16.678	17.152
P	0.687	0.019 <sup>*</sup>	0.000 <sup>*</sup>	0.000 <sup>*</sup>

<sup>a</sup>indicates comparison with pre-operative level, tLA group=6.282, pLA group=0.000, tOA group=8.404, pOA group=0.000; <sup>b</sup>indicates comparison with pre-operative level, tLA group=12.266, pLA group=0.000; tOA group=0.926, pOA group=0.358; <sup>c</sup>indicates comparison with pre-operative level, tLA group=28.640, pLA group=0.000; tOA group=10.423, pOA group=0.000.

**Table 3.** Dynamics of serum level of IL-10 before and after operation ( $x \pm s$ , pg/ml).

Group	Pre-operative	Post-operative 1 d	Post-operative 3 d	Post-operative 7 d
LA	156.9 ± 39.5	215.3 ± 57.9 <sup>a*</sup>	145.3 ± 32.6 <sup>b*</sup>	47.8 ± 8.4 <sup>c*</sup>
OA	156.8 ± 42.3	179.1 ± 48.9 <sup>a*</sup>	114.4 ± 26.7 <sup>b*</sup>	52.7 ± 12.3 <sup>c*</sup>
t	0.008	7.981	7.344	1.907
p	0.994	0.000 <sup>*</sup>	0.000 <sup>*</sup>	0.061

<sup>a</sup>indicates comparison with pre-operative level, tLA group=13.662, pLA group=0.000, tOA group=3.754, pOA group=0.000; <sup>b</sup>indicates comparison with pre-operative level, tLA group=2.772, pLA group=0.007; tOA group=8.993, pOA group=0.000; <sup>c</sup>indicates comparison with pre-operative level, tLA group=39.601, pLA group=0.000; tOA group=12.055, pOA group=0.000.

**Table 4.** Dynamics of IL-10/TNF- $\alpha$  ratio before and after operation.

Group	Pre-operative	Post-operative 1 d	Post-operative 3 d	Post-operative 7 d
LA	1.96 ± 0.32	2.33 ± 0.54 <sup>a*</sup>	2.59 ± 0.59 <sup>b*</sup>	1.99 ± 0.65 <sup>c</sup>
OA	1.98 ± 0.38	1.99 ± 0.41 <sup>a</sup>	1.65 ± 0.43 <sup>b</sup>	1.18 ± 0.44 <sup>c*</sup>
t	0.247	2.823	7.322	5.889
p	0.805	0.006 <sup>*</sup>	0.000 <sup>*</sup>	0.000 <sup>*</sup>

<sup>a</sup>indicates comparison with pre-operative level, tLA group=3.511, pLA group=0.001, tOA group=0.714, pOA group=0.478; <sup>b</sup>indicates comparison with pre-operative level, tLA group=5.671, pLA group=0.000, tOA group=1.917, pOA group=0.060; <sup>c</sup>indicates comparison with pre-operative level, tLA group=0.264, pLA group=0.793; tOA group=4.852, pOA group=0.000.

## Discussion

In the current study, we found inflammatory response were lower and anti-inflammatory response was higher in postoperative children received LA reflected by reduced serum IL-6 and TNF- $\alpha$ , and increased serum IL-10, suggesting LA is a better choice over OA.

IL-6 indicates early tissue injury and its increments is proportional to surgical trauma and accompanying damage, therefore, it has been proven to be a major index in evaluating the severity of traumatic stress reactions and serves as marker for traumatic prognosis [13]. In present, we found IL-6 were greatly increased after operation and significantly lower in the LA group than in the OA group postoperatively (all  $P < 0.05$ ), indicating children received LA developed had less postoperative stress and immune reactions, which are consistent with studies in mice and women [14,15]. The possible mechanism whereby CO<sub>2</sub>PP reduces serum IL-6 has been suggested as: 1. Curbing and killing bacteria and inhibiting the growth of *Escherichia coli* and *Staphylococcus*; 2. Inducing peritoneum cell acidification, thereby suppressing release of multiple inflammatory mediators and cytokines by macrophages; 3. Inhibiting release of superoxide anion by

macrophages and mitochondrial function, thereby alleviating inflammatory reactions [16,17].

On the other hand, serum IL-6 in LA group increased more slowly but declined more rapidly. IL-6 in the LA group began to decline 3 d postoperatively and was significantly lower compared with its pre-operative level. IL-6 in OA group 7 d after operation differed significantly from pre-operative level ( $P < 0.05$ ), indicating that both LA and OA can lead to postoperative stress reactions in affected children, whereas the stress reactions provoked by LA are milder and recovered more rapidly than OA, which may underlie shorter hospital stay of patients received LA [18].

TNF- $\alpha$  is a key pro-inflammatory cytokine after inflammatory stimulation that can activate cytokine cascade reactions during inflammation and directly reflects the severity of an inflammatory response [19]. Early detection of TNF- $\alpha$  is of great significance for evaluating the severity of serious abdominal inflammation [20]. In this study, no statistical significance was observed in serum TNF- $\alpha$  between the two groups before surgery. However, serum TNF- $\alpha$  in LA group were significantly lower on postoperative days 1, 3, and 7 than that in OA group. Moreover, serum TNF- $\alpha$  in LA group

increased more slowly but decreased more rapidly than that in OA group. Alleviation of inflammatory response under LA may be also due to CO<sub>2</sub>PP as reported in rat [21,22].

IL-10 is a key anti-inflammatory cytokine produced by Th2 cells that can inhibit the release of pro-inflammatory cytokines, such as IL-6 and TNF- $\alpha$  [23], thereby preventing deterioration of sepsis and alleviating the damage to distal viscera *via* which CO<sub>2</sub>PP may enhance anti-inflammatory cytokine's releasing and alleviates inflammatory reactions as suggested [24]. Furthermore, IL-10 mainly alleviates hepatic inflammatory injury, delays liver regeneration and reduces TNF- $\alpha$  by decreasing formation of collagen and collagenase. A moderate release of inflammatory mediators in patients with sepsis may contribute to inhibition of inflammatory responses and enhance resistance against inflammation [25]. As an anti-inflammatory factor, IL-10 not only inhibits releasing of proinflammatory cytokines, but also limits injuries resulted from excessive inflammatory responses mediated by pro-inflammatory cytokines. However, a prolonged increase in IL-10 may suppress immune responses and aggravate the severity of disease. Therefore, IL-10/TNF- $\alpha$  ratio should be maintained within proper level from 1.3-1.9 in order to have an anti-inflammatory effect [26,27]. Our results revealed that IL-10/TNF- $\alpha$  ratio in LA group ranged from 1.99-2.59 on postoperative days 1, 3 and 7, and is significantly higher than that in OA group (1.2~1.99), suggesting children received LA had stronger anti-inflammatory potential at all-time points after operation. In the OA group, IL-10/TNF- $\alpha$  ratio on postoperative day 3 was normal and on postoperative day 7 it fell below the normal threshold, indicating that children received OA had less anti-inflammatory potential. These results demonstrated CO<sub>2</sub>PP contributes to physical clearance of bacteria and toxins and provides protection of normal immune function, which are consistent with previous study [28-30].

## Conclusion

LA reduces inflammatory responses in children with perforated appendices, peritonitis and sepsis and enhances the anti-inflammatory responses. This study provides evidence of advantages of LA to OA in treating perforated appendices. However, such effects resulted from CO<sub>2</sub>PP and within LA group, the effect of pressure, gas flow amount, operative time and anesthesia time on postoperative inflammatory responses and even long-term prognosis remains to be studied in future [31].

## Declarations

### *Ethics approval and consent to participate*

All manipulations were approved by the Ethic Committee of the Children's Hospital of Wuhan. All patients were well informed and signed written consents.

### **Consent to publish**

Not applicable.

### **Availability of data and materials**

All information supporting the conclusions of this manuscript is included within the article. Any additional information can be obtained upon request.

### **Competing Interests**

The authors declare that they have no competing interests.

### **Funding**

None.

### **Authors Contribution**

CSF, YGB, YJ and YXQ carried out the clinical, participated in clinical analysis. DXF, YXQ, BHQ and YJ performed statistical analysis and ELISA. YGG, XQ and ZSQ participated in experimental studies and analyzed data. DXF drafted the paper with inputs from BHQ, ZSQ and YGG. DXF, ZSQ and YGG conceived of the study, and participated in its design and coordination. All authors read and approved the final manuscript.

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### **Conflict of Interest**

None.

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