Mahmut BAŞOĞLU[1]
Ahmet KIZILTUNÇ[2]
Fatih AKÇAY[2]
Sait KELEŞ[2]
Cemal GÜNDOĞDU[3]
Durkaya ÖREN[1]

Tumor Necrosis Factor-α and Interleukin-6 in Peritoneal Adhesion Formation

Abstract: This study was carried out to assess the role of tumor necrosis factor (TNF-α) and interleukin-6 (IL-6) in plasma and peritoneal fluid in higher rates of adhesion formation following standard bowel injury. Forty-five albino rabbits were divided into three equal groups. Blood was obtained from all the rabbits preoperatively. All the rabbits were subjected to a laparotomy. In group 1(control group), the peritoneal cavity was irrigated with normal saline. In group 2 the cecal serosa was abraded, while the rabbits in group 3 had a resection in small bowel of 2 cm. A peritoneal catheter was placed in all the rabbits prior to closure. Blood samples were obtained at the 30th, 90th, and 150th min following the injury. Peritoneal exudate (PE) was collected and the catheter removed at the end of the 150th min: Blood samples and peritoneal exudate were processed and levels of TNF-a and IL-6 were determined.

The severity of adhesions was graded at the 3rd postoperative week using a score of 0-1 to 4.

The plasma levels of TNF-α and IL-6 correlated significantly with grades of adhesion. TNF-α and IL-6 values also correlated significantly in peritoneal fluid. Rabbits in group 1 had significantly lower adhesion grades when compared with groups 2 and 3 which had higher postoperative plasma and PE TNF-α and IL-6 levels than group 1.

These correlations suggest that monitoring of the plasma and peritoneal exudate TNF-α and IL-6 levels might be helpful biological markers for postoperative intra-abdominal adhesion formation.

Key Words: Peritoneal adhesion, Tumor necrosis factor-α, Interleukin-6

Departments of[1] General Surgery,
[2]Biochemistry and [3]Pathology Faculty of
Medicine, Atatürk University Erzurum-Turkey

Introduction

Peritoneal adhesion formation is clinically significant and a leading cause of postoperative morbidity, including small bowel obstruction. Knowledge of the pathogenic mechanism of peritoneal adhesion formation is required before prevention can approach clinical applicability. The cause of higher rates of peritoneal adhesion formation in some individuals remains unknown. Different immunological responses mounted by different individuals, following standard of injury, may be a contributing factor. Immunomodulatory and inflammatory mediators, secreted following bowel injury, potentiate collagen deposition and the transformation of fibrinous adhesions into thick and dense ones (1).

Cytokines, proteins produced by various cell types, are important mediators of immunoinflammatory reactions (2). Several of these peptides are considered to play a role in the pathogenesis of inflammatory bowel disease (3). Among the cytokines, TNF-α and IL-6 both have key roles in the pathophysiology of septic shock and multiple organ failure, and correlate with mortality in both meningococcal sepsis and septic shock (4). IL-6 has diverse biological functions, including the ability to induce immunoglobulin production in proliferating B cells, to enhance the development of multipotential hemopoietic progenitor cells, and to participate in the development of cytotoxic T cell (5). IL-6 is increasingly recognized as an almost ubiquitous participant in many types of inflammatory processes (6). TNF-α and IL-1 are associated with increases in body temperature, and hemodynamic and metabolic changes, and have been correlated significantly with the severity of illness in septic rats and mice (7,8). One regulating cytokine is TNF-α, which exerts multiple stimulatory effects on T cells by binding to specific receptors, increasing the expression of human leucocyte antigens (HLA) and high-affinity IL-2 receptors, and is postulated as a first messenger for priming immune cells (9). TNF-α is a macrophage-derived cytokine with chemotactic potency,

Table 1.   Adhesive Bands

Grade	Description of Adhesive Bands	Remarks
0	Complete absence of adhesions	Inbustantial adhesions
I	Single band of adhesions, between viscera, or from one viscus to abdominal wall	
II	Two bands, either between viscera or from viscera to abdominal wall	
III	More than two bands, between viscera, or viscera to abdominal wall, or whole of intestines forming a mass without being adherent to abdominal wall	Substantial adhesions
IV	Viscera directly adherent to abdominal wall, irrespective of number and extent of adhesive bands	

which has been implicated in the acute phase reaction under various inflammatory conditions(10).

This study investigated the relationship between plasma and PE TNF-α and IL-6 levels with of peritoneal adhesion formation following standard bowel injury.

Materials and Methods

Forty-five albino rabbits (2560 to 2950 g) were divided into three equal groups. These rabbits were fasted overnight with free access to water. The following morning, the animals were anesthetized with inhalation methoxyflurane and intramuscular ketamine (100 mg/kg body wt). Using the internal jugular vein, 0.5 ml of blood was obtained from all rabbits preoperatively. The peritoneal cavity was entered througha 5 cm midline laparotomy. In group 1 (control group), the peritoneal cavity was opened and irrigated with 10 ml of 0.9 % normal saline. In group 2, the cecum was identified and the cecal serosa was abraded. The rabbits in group 3 had a resection in the small bowel of 2 cm, approximately 7-8 cm from the ileocecal junction. Cultures were obtained before and after the injury and a peritoneal catheter was placed in all the rabbits. Rabbits with positive cultures were excluded from the study. Blood samples were obtained at the 30th, 90th, and 150th min following the injury. PE was collected and the catheter was removed 150 min after the injury. PE and blood samples (about 0.3 ml) were taken for the determination of TNF-α and IL-6 levels using heparinized syringes. Both TNF-α and IL-6 were measured using an enzyme-linked immunosorbent assay (Quantikine[TM], Human TNF-α immunoassay, CN DTA 50; Quantikine[TM], Human IL-6 immunoassay, CN D 6050). TNF-α and IL-6 values in the experimental samples were calculated using the standard curve. All the animals were put down at the end of the 3rd postoperative week. Intra-abdominal adhesions were graded blindly by

Table 2.   Adhesion Grades in the Three Study Groups.

Groups	Grade 0-I	Grade II	Grade III	Grade IV
1(n=15)	15	0	0	0
2(n=15)	4	7	2	2
3(n=15)	3	5	3	4

Table 3.   Correlation values between IL-6 and TNF-α in plasma.

Time(min)	G1	G2	G3
30	0.65	0.67	0.69
90	0.77	0.81	0.61
150	0.70	0.75	0.74

two surgeons according to the method of Nair et al. [11] (Table 1). Biopsies of the adhesions were stained with haematoxylin and eosin (H&E) and Masson's trichrome for collagen deposition. The amounts of collagen and fibroblasts were graded using light microscopy.

The data were expressed as mean ± SD. The results were analyzed using the to Student's t test and linear regression. The x^2 test was used to determine the grades of adhesion, and a p value of less than 0.05 was considered to be significant.

Results

The animals were further grouped based on their grades of adhesion (22 rabbits with grade 0-1, 12 with grade 2 , 5 with grade 3, and 6 with grade 4; Table 2). The rabbits in group 1 had significantly lower adhesion grades when compared with groups 2 and 3 (p<0.001)
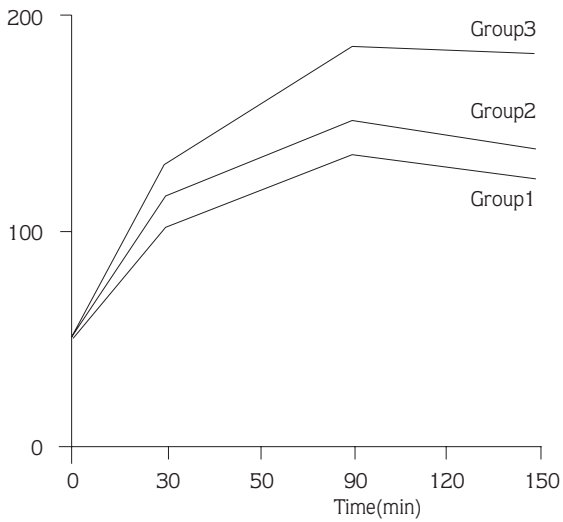
Figure 1.   Plasma TNF-α in the three study groups.
at 30th min: G1 vs G2 and G1 vs G3, p<0.0001
at 90th min: G1 vs G2, p<0.05; G1 vs G3, p<0.001
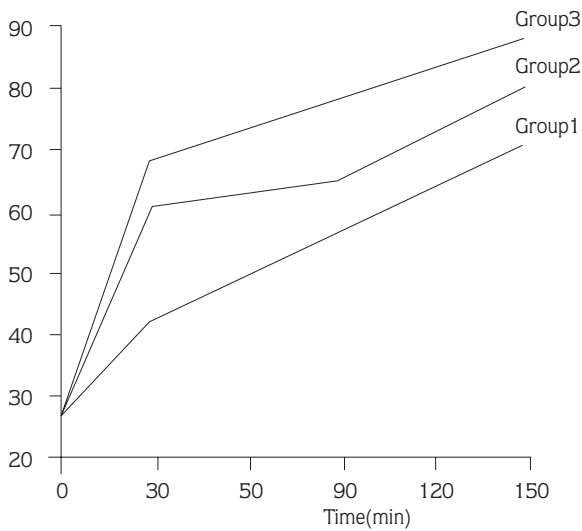at 150th min: G1 vs G2, p<0.001; G1 vs G3, p<0.0001.

Figure 2.   Plasma IL-6 in the three study groups.
at 30th min: G2 and G3 vs G1, p<0.001
at 90th min: G2 and G3 vs G1, p<0.001
at 150th min: G2 vs G1, p<0.05 and G3 vs G1, p<0.001.

Figure 3.   Plasma TNF-α and grades of adhesion.
Grades II, III, IV vs 0-I at 30th min: p<0.0001, p<0.01, p<0.001, respectively;
Grades II, III, IV vs 0-I at 90th min, p<0.01, p<0.001, p<0.0001, respectively;
Grades II, III, IV vs 0-I at 150th min, p<0.001, p<0.0001, p<0.0001, respectively.
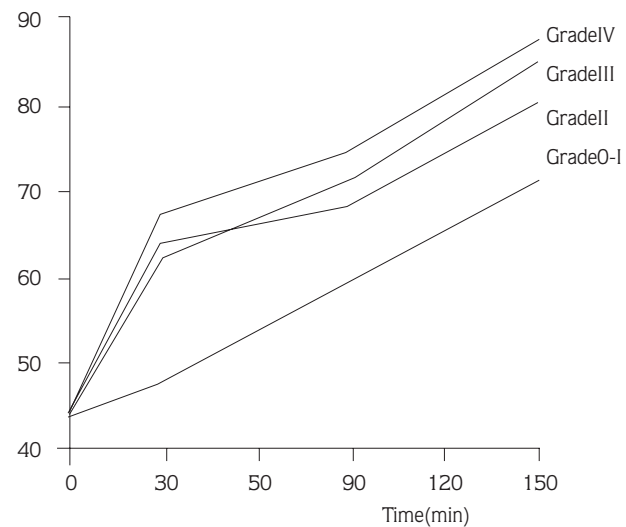
Figure 4.   Plasma IL-6 and grades of adhesion.
Grades II, III, IV vs 0-I at 30th min: p<0.0001, p<0.05, p<0.001, respectively;
Grades II, III, IV vs 0-I at 90th min, p<0.05, p<0.05, p<0.01, respectively;
Grades II, III, IV vs 0-I at 150th min, p<0.01, p<0.01, p<0.001, respectively.

(Table 2). The levels of TNF-α and IL-6 in 30th, 90th and 150th minutes were evaluated in groups 1, 2, and 3 (Figs 1 and 2). Significant correlations were found between groups in terms of plasma IL-6 and TNF-α values (Table 3).

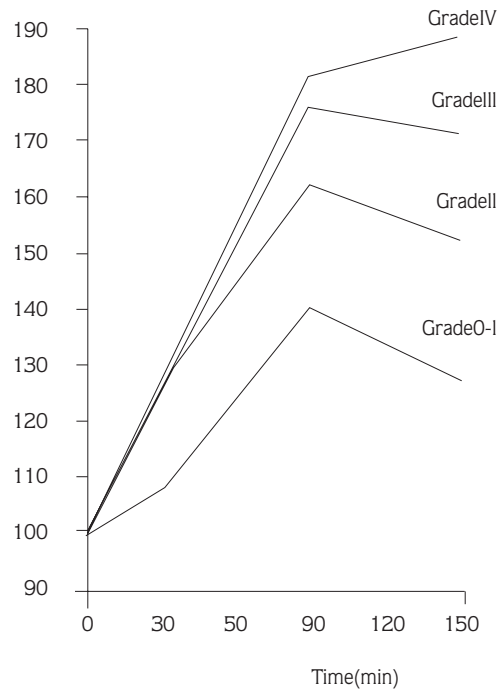Both TNF-α and IL-6 values in plasma at the 30th, 90th and 150th min following the operation increased gradu-
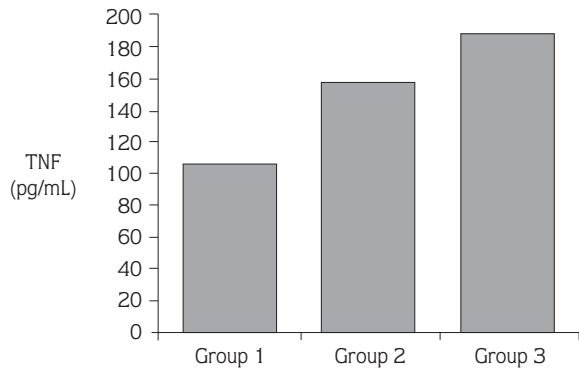
255

Figure 5. Peritoneal exudate TNF-α concentration in the three study groups. G1 vs G2, and G1 vs G3, and G2 vs G3, p<0.0001.

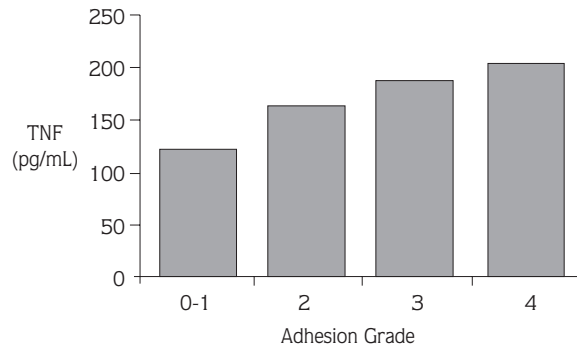Figure 7. Peritoneal exudate TNF-α and grades of adhesion. Grades o-I vs II, III, IV: p<0.0001; Grades II vs III, p<0.01; and II vs IV, p<0.001, Grades III vs IV, p<0.05.

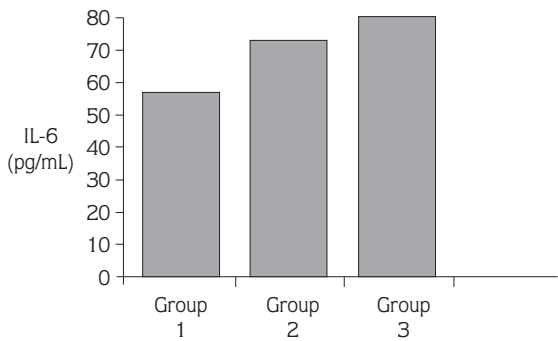Figure 6. Peritoneal exudate IL-6 concentration in the three study groups.
G1 vs G2, and G1 vs G3, p<0.0001; and G2 vs G3, p<0.001.
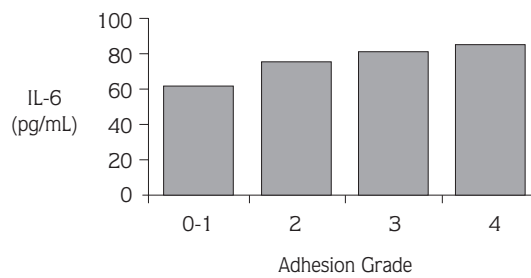

Figure 8. Peritoneal exudate IL-6 and grades of adhesion.
Grades 0-I vs II, III, IV, p<0.0001; Grades II vs III and IV, p<0.05, p<0.00001, respectively; Grades III vs IV, p<0.05.

ally as the adhesion grade number increased (Figs 3,4). Histopathologic findings were similar to the adhesion grades. Groups 2 and 3 had significantly higher PE TNF-α and IL-6 levels when compared with group 1 (Figs 5,6). Further, correlations existed between higher grades of intra-abdominal adhesions and higher PE TNF-α and IL-6 Levels postoperatively (Figs 7,8). There were also significant correlations between PE TNF-α and IL-6 levels (r=0.73 in group 1, r=0.80 in group 2, r= 0.69 in group 3)

Discussion

Intra-abdominal adhesions are among the leading causes of postoperative morbidity following abdominal surgery, frequently leading to small bowel obstruction. Various data have been reported regarding the incidence of postoperative intraperitoneal adhesions and its complications, varying from 1.9 % to 91.1 % (1). However, the cause of higher rates of adhesion formation in some individuals remains unknown.

TNF-α is an important cytokine that exerts multiple stimulatory effects on T cells by binding to a specific TNF-α receptor, increasing the expression of HLA, and is well-known to mediate allograft rejection (12). TNF-α plays a role in immunological reactions by stimulating the release of such inflammatory mediators as IL-1, IL-6, and platelet activating factor (13). By stimulating production of adhesion molecules, TNF-a promotes polymorphonuclear leukocyte-endothelial adhesion (14). High levels of TNF-α have been linked to multiple disease processes. TNF-α is believed to play a central role in the development of sepsis (15), transplant rejections (16) and ischemic colitis (17).

IL-6, synthesized by various cell types such as monocytes/macrophages, endothelial cells, and fibroblasts, exhibits multiple biological effects, including differentiation of B cells, induction of acute-phase protein synthesis by hepatocytes and T-cell activation and proliferation (18). IL-6 is major marker of tissue injury. A surgical intervention induces a systemic IL-6 response related to the magnitude of the operation.

Plasma TNF-α and IL-6 concentrations are well correlated with the septic state in the initial phase of intra-abdominal infection, but there are remarkable differences in cytokine concentrations of septic patients compared with those in patients with intra-abdominal infection without sepsis. The concentrations of both cytokines correlate well with the outcome of acute meningococcal septicaemia (19), but intra-abdominal infection is a less acute disease and is accompanied by lower concentrations of cytokines. TNF-α is toxic and causes circulatory, respiratory, and renal failure, but IL-6 may be the "alarm" cytokine in the infection. IL-6 induces fever but not shock, activates B and T lymphocytes, and mediates the hepatic acute phase response (20), so any therapeutic conclusions drawn from these data should concentrate on TNF-α.

In this study, plasma TNF-α and IL-6 were found to be increased when compared to the basal levels and were well correlated with the grading. This rise was progressive and highest in the rabbits with the most extensive bowel injuries, possibly caused by increased adhesion. The preliminary result of adhesion is that PE TNF-α and IL-6 increase together with both increased adhesion and grading. The release of IL-6 by the vascular edothelium in response to mediators elaborated in areas of tissue inflammation or damage may contribute significantly to the inflammatory response and to the levels of circulating IL-6 observed in various states, such as extensive burns (21). According to Starnes et al. (22), TNF-α is an early mediator released primarily by monocytes/macrophages, capable of inducing many of the metabolic and physiologic responses characteristic of septic. There was a significant correlation between plasma TNF-α and IL-6 in all groups. It has been profposed that IL-6 is influenced by some stimulating factors such as TNF-α, IL-6 and eukotrienes. The induction of IL-6 by TNF-α raised the possibility that IL-6 might modulate the effects of other factors on endothelial cell function (21). Ohzato et al. (23) found a close correlation between serum IL-6 and the stress of an operation. Tsuka et al. (24) also reported correlations between peritoneal concentrations of TNF-α, IL-1β and IL-6 after abdominal operations. Our results confirm these reports.

In conclusion, TNF-α and IL-6 appear to be good quantitative and biological markers for postoperative peritoneal adhesion formation.

References

1. Kaidi AA, Nazzal M, Gurchumelidze T, Ali MA, Dawe EJ, Silva YJ. Preoperative administration of antibodies against tumor necrosis factor-alpha (TNF-α) and interleukin-1 (IL-1) and their impact on peritoneal adhesion formation. Am Surg 61: 569-72,1995.

2. Rees RC. Cytokines as biological response modifiers. J Clin Pathol 45: 93-8,1992.

3. Gionchetti P, Campieri M, Belluzzi A, Brignola C, Bertinelli E, Ferretti M, Brignola C, Miglioli M, Barbara L. Mucosal concentrations of interleukin-1β, interleukin-6, interleukin-8, and tumor necrosis factor-α in pelvic ileal pouches. Dig Dis Sci 39: 1525-31,1994.

4. Függer R, Zadrobilek E, Götzinger P, Klinmann S, Rogy M, Winkler S, Andel H, Metelbock M, Roth E, Schulz F. Perioperative TNF-α and IL-6 concentrations correlate with septic state, organ function, and apache 2 scores in intra-abdominal infection. Eur J Surg 159: 525-9,1993.

5. McCurry KR, Campbell DA, Scales WE, Warren JS, Remick DG. Tumor necrosis factor, interleukin 6, and the acute phase response following hepatic ischemia/reperfusion. J Surg Res 55: 49-54,1993.

6. Gauldie J, Richards C, Hapaish D, Lansdorp P, Baumann H. Interferon B-2/B-cell stimulating factor type 2 share identity with monocyte derived heptocyte stimulating factor and regulates the major acute phase protein response in liver cells. Proct Natl Acad Sci 84: 7251-5,1987.

7. Bemelmans MHA, Gouma DJ, Greve JW, Buurman WA. Cytokines tumor necrosis factor and interleukin-6 in experimental biliary obstruction in mice. Hepatology 15: 1132-6,1992.

8. Fearon KCD, Mcmillan DC, Pretson T, Winstanley FP, Chruickshank AM, Shenkin A. Elevated circulating interleukin-6 is associated with acute-phase response but reduced fixed hepatic protein synthesis in patients with cancer. Ann Surg 213: 26-31,1991.

9. Lin H, Wei RQ, Bolling SF. Tumor necrosis factor-a and interferon-γ modulating of nitric oxide and allograft survival. J Surg Res 59: 103-10,1995.

10. Ming WJ, Bersani L, Mantovani A. Tumor necrosis factor is chemotactic for monocytes and polymorphonuclear leukocytes. J Immunol 138: 1469-72,1987.

11. Nair SK, Bhat IK, Aurora AL. Role of proteolytic enzyme in the prevention of postoperative intraperitoneal adhesions. Arch Surg 108: 849-53,1974.

12. Lin H, Chensue SW, Streiter RM, Remick DG, Gallagher KP, Bolling S, Kunkel SL. Antibodies against tumor necrosis factor prolong cardiac allograft survival in the rat. J Heart Lung Transplant 11: 330-4,1992.

13. Shalaby MR, Waage A, Aarden L. Endotoxin, tumor necrosis factor-alpha and interleukin 6 production in vivo. Clin Immunol Immunopathol 53: 488-98,1989.

14. Gamble JR, Harlan JM, Klebanoff SJ. Stimulation of the adherance of neutrophils to umblical vein endothelium by human recombinant tumor necrosis factor. Proct Natl Acad Sci USA 82: 2069-73,1985.

15. Bone RC. A critical evaluation of new agents for the treatment of sepsis. J Am Med Assoc 266: 1686-91,1991.

16. Arbustini E, Grasso M, Diegoli M. Expression of tumor necrosis factor in human acute cardiac rejection. Am J Pathol 4: 709-15,1991.

17. Sun X, Hsueh W. Bowel necrosis induced by tumor necrosis factor in rats is mediated by platelet-activating factor. J Clin Invest 81: 1328-31,1988.

18. Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: Il-6 and related molecules (IL-1 and TNF). Faseb J 4: 2860-7,1990.

19. Waage A, Brandtzaeg P, Halstensen a, Kierulf P, Espevik T. The complex pattern of cytokines in serum from patients with meningococcal septic shock. J Exp Med 169: 333-8,1989.

20. Dinarello CA. The proinflammatory cytokines interleukin-1 and tumor necrosis factor and treatment of the septic shock syndrome. J Infect Dis 163: 1177-84,1991.

21. Jirik FR, Podor TJ, Hirano T, Kishimoto T, Loskutoff DJ, Carson D,Lotz M. Bacterial lipopolysaccaharide and inflammatory mediators augment IL-6 secretion by human endothelial cells. J Immunol 142: 144-7,1989.

22. Starnes HF, Warren RS, Jeevanandam M. Tumor necrosis factor and the acute metabolic response to tissue injury in man. J Clin Invest 82: 1321-5,1988.

23. Ohzato H, Yoshizaki K, Nishimoto N. Interleukin-6 as a new indicator of inflammatory status: Detection of serum levels of interleukin-6 and C-reactive protein after surgery. Surgery 111: 201-9,1992.

24. Tsukada K, Katoh H, Shiojima M, Suzuki T, Takenoshita S, Nagamachi Y. Concentrations of cytokines in peritoneal fluid after abdominal surgery. Eur J Surg 159: 475-9,1993.