

Diagnostic value of plasma cytokine levels in acute mesenteric ischemia: an experimental study

Hasan Karaağaç (*), Nazif Zeybek (*), Yusuf Peker (*), Gökhan Yağcı (*), Ali Şengül (**), Ömer Günhan (***) , Turgut Tufan (*)

Summary

Frequency of mortality in patients with superior mesenteric artery occlusion is still high due to difficulties in current diagnosis. The initial symptoms of acute intestinal ischemia is non-specific and indistinguishable from acute abdomen. Regardless of the etiology, the time interval between diagnosis and therapy determines the prognosis of acute intestinal ischemia. Our purpose in this study was to compare serum leukocyte levels and cytokine gradients to evaluate their prognostic values in the early diagnosis of acute intestinal ischemia. In this study 80 rats were divided into 4 groups as control, laparotomy, strangulation, and superior mesenteric artery occlusion groups. Serum IL-1 β , IL-6 and TNF α and blood leukocyte levels were measured and the resected intestinal specimens were histopathologically evaluated for intestinal ischemia and tissue necrosis. The results showed an increase in cytokine levels in parallel with tissue ischemia. We believe that, although not specific for intestinal ischemia, serum IL-1 β , IL-6 and TNF α levels may be used as an early indicator of intestinal ischemia if rapid analysis techniques are developed.

Key words: Cytokine levels, intestinal strangulation, mesenteric ischemia

Özet

Akut mezenterik iskemide plazma sitokin düzeylerinin tanısal değeri: deneysel bir çalışma

Süperiyor mezenterik arter oklüzyonlu hastalardaki mortalite oranları, mevcut tanı yöntemlerindeki zorluğa bağlı

olarak hala yüksektir. Akut intestinal iskeminin başlangıç bulguları özgün değildir ve akut abdomenden ayırmak zordur. Etiyolojiden bağımsız olarak, tanı ve tedavi arasındaki süre akut intestinal iskeminin prognozunu belirler. Bu çalışmada amacımız serum lökosit düzeyleri ile sitokin düzeylerini karşılaştırarak, bunların akut intestinal iskeminin erken tanısındaki tanısal önemini değerlendirmektir. Çalışmada 80 rat; kontrol, laparotomi, strangülasyon ve süperiyor mezenterik arter oklüzyonu grupları olarak 4 gruba ayrıldı. Serum IL-1 β , IL-6, TNF α ve kan lökosit düzeyleri ölçüldü ve rezeke edilen barsaklar intestinal iske mi ve doku nekrozu açısından histopatolojik olarak değerlendirildi. Sonuçlar doku iskemisine paralel olarak sitokin değerlerinde yükselme gösterdi. İntestinal iskemiy e spesifik olmamakla birlikte, serum IL-1 β , IL-6 ve TNF α değerlerinin, daha hızlı analiz yöntemleri geliştirilirse, intestinal iskeminin erken göstergesi olarak kullanılabileceğini düşünüyoruz.

Anahtar kelimeler: Sitokin düzeyleri, intestinal strangülasyon, mezenterik iske mi

Introduction

Acute intestinal ischemia resulting from impaired blood flow within the mesenteric vascular system is a catastrophic event, inevitably leading to death if not diagnosed and treated promptly. Despite the progress in the art of medicine, diagnosis and treatment of this relatively infrequent disease, still continues to challenge physicians (1). There is an incidence of 8.6/100.000 in the population and 1-2 cases per 1000 hospital admissions (2), with reported mortality rates ranging from 44% to 91% depending on etiology and treatment (3). Although some studies have reported better survival rates compared to the past decade, there is disagreement among experts on this issue (4). The improvement, which still remains to be verified is promising but minimal, has been attributed to advances in supportive patient care and interventional procedures (2). But early

* Department of General Surgery, Gülhane Military Medical Faculty

** Department of Immunology, Gülhane Military Medical Faculty

***Department of Pathology, Gülhane Military Medical Faculty

Reprint request: Dr. Nazif Zeybek, Department of General Surgery, Gülhane Military Medical Faculty, Etlik-06018, Ankara
E-mail: nzeybek@hotmail.com

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diagnosis is still the most important factor affecting patient outcome (4). The non-specific nature of symptoms, lack of specific markers and the need for advanced diagnostic procedures such as angiography for accurate diagnosis are the major causes of delay in treatment, contributing to morbidity and mortality (6). Endogenous substances such as creatinin phosphokinase, alkaline phosphatase, ileal peptide and serum phosphate levels have been investigated on the search for a possible early marker of intestinal ischemia. Unfortunately due to lack of specificity and sensitivity, these potential markers gave positive results usually after the occurrence of irreversible intestinal damage (7-10).

Although the exact mechanism has not been clarified, intestinal ischemia is known to result in gut barrier function failure, initiating systemic immunoinflammatory response (11). Also ischemia has been found to cause cytokine production by the intestine, Payer's patches and mesenteric lymph nodes (12). Of these cytokines, IL-1 β and TNF α have been documented to play an important role in multiple organ failure in acute intestinal ischemia (13,14).

This study was designed to investigate if serum IL-1 β , IL-6 and TNF α levels could be used as a potential indicator of intestinal ischemia.

Material and Methods

Eighty male, 2-month-old Sprague-Dawley strain rats weighing 200 \pm 20 grams were divided into 4 groups consisting of 20 rats in each group. The animals were provided by the Clinical Sciences Research Department Division of Experimental Animals. The animals were grouped as follows; Group 1 (Non-operated control group): Only anesthesia was induced and no surgical procedure was performed, Group 2 (Laparotomy group): Midline incision laparotomy was performed and the abdomen was closed without any further surgical intervention. Group 3 (Segmental ischemia group-Strangulation model): A 10 cm segment of ileum including the mesentery was ligated 5 cm proximal to the ileocecal valve to form a strangulation ischemia model. Group 4 (Superior mesenteric artery ligation group): The superior mesenteric artery was ligated 0.5 cm distal to the abdominal aorta following careful dissection of the intestinal mesentery to form total mesenteric ischemia model.

Anesthesia was induced by Sevoflurane and maintained by Sevoflurane 40% N₂O and 60% O₂ inhalation. Blood samples were collected at 5 minutes, 2 hours and 4 hours after operation in all groups to determine the levels of IL-1 β , IL-6 and TNF α . Blood samples at 4

hours were drawn from the heart following thoracotomy.

Relaparotomy was performed at 4 hours post-operative. Ischemic segments in Groups 3 and 4 and corresponding intestinal segments in Groups 1 and 2 were resected and placed in 10% formalin for histopathological examination. Later on the rats were sacrificed.

Histopathological evaluation was made at the Department of Pathology. The statistical analysis was made by the Department of Biostatistics using SPSS 10.0 (SPSS Inc. Chicago, IL, USA). Differences between the groups were evaluated by Friedman and Bonferroni adjusted Wilcoxon signed ranks tests.

Results

Biochemical and immunological data: The blood leukocyte levels were measured at the Clinical Sciences Research Department and serum cytokine levels were measured at the Department of Immunology. The Rat IL-1 β and IL-6 kits were provided from Assay Designs Inc. AnnArbor Michigan USA and Rat TNF α kits were provided from CytImmune Sciences Inc. College Park Maryland USA.

Serum IL-1 β levels measured are given in Table I and Figure 1.

Table I. Median serum levels of IL-1 β , IL-6 and TNF α by time

Groups	Time*	IL-1 β	IL-6	TNF α
Group 1 (CG)	5. min	26.9	36.6	18.9
	2. hr	27.2	20.0	
	4. hr	28.5	20.2	
	p value	>0.05	>0.05	>0.05
Group 2 (L)	5. min	26.7	35.9	19.5
	2. hr	74.6	47.6	
	4. hr	206.3	175.9	
	p value	<0.001	<0.001	<0.001
Group 3 (SIG)	5. min	27.3	35.2	19.5
	2. hr	173.6	180.3	
	4. hr	300.8	383.8	
	p value	<0.01	<0.01	<0.01
Group 4 (SMIG)	5. min	26.9	36.0	20.9
	2. hr	365.8	435.4	
	4. hr	708.5	882.6	
	p value	<0.01	<0.01	<0.01

*: Time is duration of ischemia. CG: Control group, L: Laparotomy group, SIG: Strangulation ischemia group, SMIG: Superior mesenteric ischemia group

The median values of serum IL-1 β levels in Group 1 showed no statistical significant difference. The results of IL-1 β levels in all other groups showed statistically significant difference (p<0.01).

The values of Group 2 (Laparotomy) showed significant increase by time reaching 206 pg/ml by 4 hours.

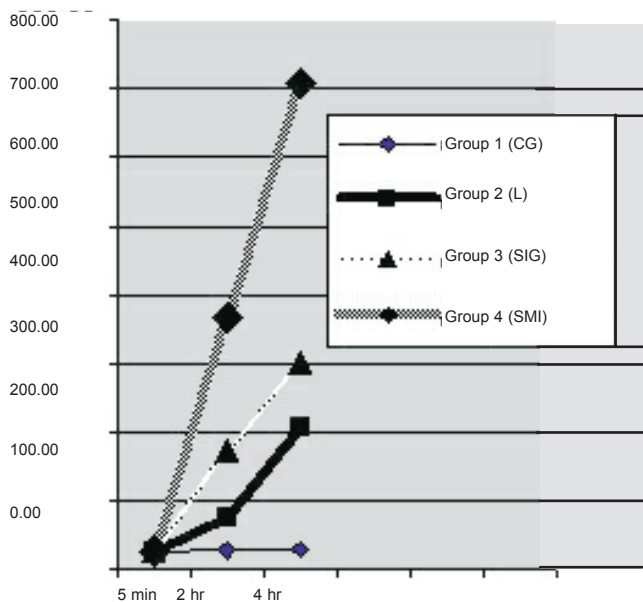


Figure 1. Median serum IL-1 β levels (pg/ml)

Both 2nd hour and 4th hour results were significantly different from the 5th minute results ($p < 0.01$) and control group IL-1 levels ($p < 0.01$).

IL-1 β levels in Group 3 (Strangulation ischemia) increased to 173.6 pg/ml by 2nd hour and 300.8 pg/ml by 4th hour showing a statistically significant change from 5 minute and laparotomy group results ($p < 0.01$).

The increase in Group 4 (Superior mesenteric ischemia) was most significant increasing to 365.8 pg/ml by the 2nd hour and dramatically increasing to 708.5 pg/ml by the 4th hour. The changes were significantly different from the other groups and 5th minute results ($p < 0.01$).

The median values of IL-6 levels are shown in Table I and Figure 2. Changes in the control group were not found to be significant by time ($p > 0.05$). Fifth minute values did not show significant difference among all the groups ($p > 0.05$).

The results of Group 2 showed a significant increase by time ($p < 0.01$) and the results were also significantly higher than those of Group 1 ($p < 0.01$).

Serum IL-6 values of Group 3 increased significantly ($p < 0.01$) and was apparently higher than those of Group 2 ($p < 0.01$).

Serum levels of IL-6 most prominently increased in Group 4, reaching 758.2 pg/ml at 4th hour, and this increase was found to be significantly higher than those of the other groups ($p < 0.01$).

The results of serum TNF α levels are shown in Table I and Figure 3. The change of TNF α levels in Group 1 was not found to be significant ($p > 0.05$). The differ-

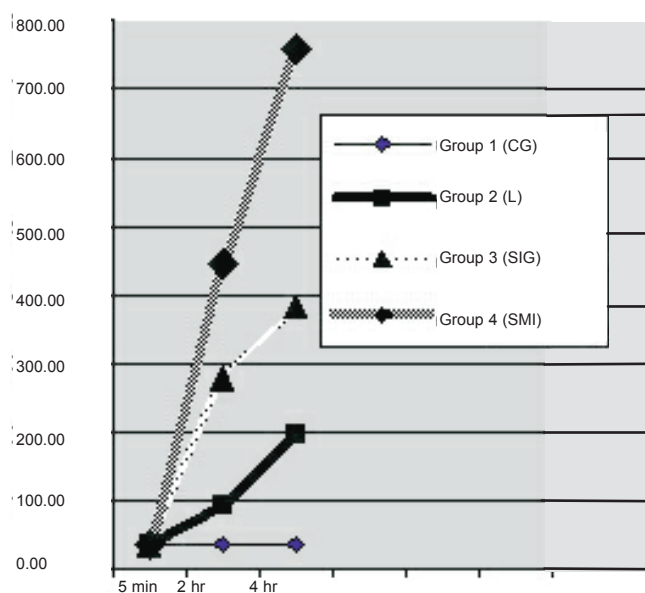


Figure 2. Serum IL-6 levels (pg/ml)

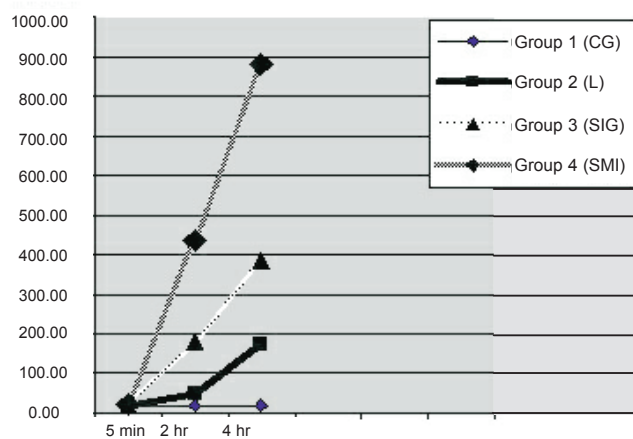


Figure 3. Serum TNF α levels (pg/ml)

ence of 5th minute samples was also not found to be statistically significant among the groups ($p < 0.01$).

The increase in TNF α levels of Group 2 was significant at 2nd and 4th hour samples ($p < 0.01$) when compared with 5th minute and Group 1 results.

TNF α levels increased significantly by time in Group 3 and was found to be significantly higher by time ($p < 0.01$) and than the results of Group 1 and 2 ($p < 0.01$).

TNF α levels of Group 4 showed the highest increase. The 2nd hour and 4th hour results were significantly higher than the results of the other groups ($p < 0.01$) and 5th minute samples ($p < 0.01$).

The standard leukocyte count range of rats was set as 5000-13.000/mm³ as published previously (15). The median results of leukocyte counts of groups are shown in Table I and Figure 4.

The median leukocyte counts of Groups 1 and 2 did

not show significant difference by time ($p>0.05$). The results of Group 1 and 2 were not found to be statistically different ($p>0.05$).

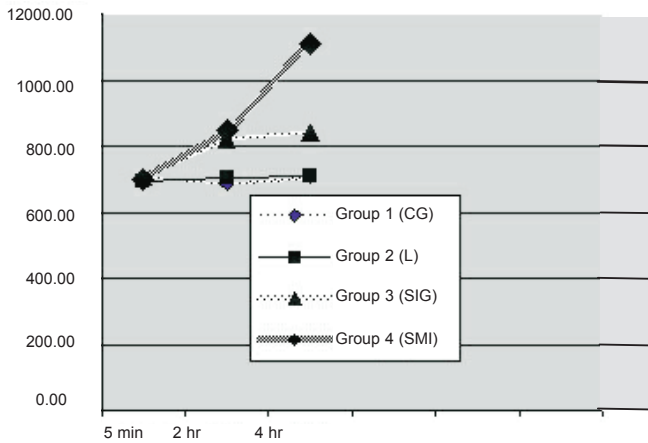


Figure 4. Blood leukocyte counts

The second hour leukocyte levels of rats in Group 3 showed a statistically significant increase when compared to fifth minute results ($p<0.05$), the levels increased by the fourth hour but the change was not found to be significant ($p>0.05$). However when the results were compared with Groups 1 and 2, both second hour ($p<0.01$) and fourth hour ($p<0.05$) results were found to be significantly different.

The leukocyte count results of Group 4 showed statistically significant increase by the second hour ($p<0.05$). The change was most apparent by the fourth hour ($p<0.05$). These results were higher compared to Group 3, but the difference was not found to be significant. The change at the 4th hour samples were statistically higher than the 4th hour results of Group 3 ($p<0.05$).

The intestinal segments were evaluated and graded on a scale from 1 to 8 for acute intestinal damage as described by Chiu et al. (16). The groups were evaluated by cross tab variance analysis. The histopathological abnormality in all rats in Groups 1 and 2 were reported as grade 0 (Figure 1). Ten rats (50%) in Group 3 developed grade 7 damage (Figure 2) and the remaining 10 rats developed grade 8 damage. When the tissues of Group 4 were examined 70% (14 rats) of the animals suffered grade 8 damage while 30% (6 rats) had grade 7 damage. The differences between the groups were found to be significant ($p<0.01$) (Figures 1,2,3).

Discussion

Acute mesenteric ischemia is a life threatening emergency causing significant morbidity and mortality if not diagnosed and treated before irreversible intestinal da-

mage has occurred. Results from studies with limited number of patients reported a mortality rate of 53% to 93% in this relatively infrequent disease (3). Most of the survivors of this catastrophic event become total parenteral nutrition dependent and have a reported estimated 5 year survival rate of 18% (17). When diagnosed prior to irreversible damage, these rates may be significantly reduced. In a recent study by Bingol et al. 24 patients were diagnosed with acute mesenteric ischemia while hospitalized for cardiac diseases (18). Twelve patients who were operated within 6 hours survived while 2 of 9 the patients operated within 12 hours died and all of the 3 patients operated after 12 hours died. These numbers point out to the importance of diagnosis on time which is the determinant of patient outcome for this notorious illness.

Angiography is the gold standard diagnostic technique in evaluation of the cause and diagnosis of acute intestinal ischemia (19), however it may be time consuming and access to a qualified interventional radiologist may not be possible in every center. The same is true with MR angiography and MR spectroscopy capable of giving excellent view of vasculature, but may be performed in tertiary care centers (20,21). Doppler USG may be useful in evaluating the mesenteric flow but the paralytic ileus and the intestinal distention associated with ischemia may prevent efficient evaluation of flow, and therefore it may be unreliable in some cases.

In our study we tried to investigate serum IL-1 β , IL-6 and TNF α levels as a cost effective, simple, potential indicator of intestinal ischemia.

Although the exact mechanism of release is unknown, severe mechanical trauma and thermal damage have been documented to cause an increase in cytokine levels and especially in IL-6 (22). IL-1 β , TNF α , endotoxins and free oxygen metabolites are some of the factors which may be related to the increment on IL-6 levels (23-26). There are reports on intestinal ischemia contributing to the intestine becoming a cytokine producing organ. Significant increase in mRNA coding for immunoregulatory and proinflammatory cytokines have been demonstrated in the Peyer's patches and mesenteric lymph nodes following intestinal ischemia (11,12).

Intestinal obstruction results with elevation in bacterial content (27), and thereby an increase in IL-6 and TNF α levels has also been shown following the exposure of enterocytes to E.coli, which may occur following mucosal damage due to ischemia (28). Several authors have reported increase in plasma cytokine levels and endotoxemia following intestinal ischemia in their experiment with cat models (29,30). Moore et al.

have also demonstrated the high levels of cytokines in blood samples drawn from the mesenteric vessels 60-120 minutes following intestinal ischemia and hypothesized that high cytokine levels and endotoxemia are early predictors of intestinal ischemia (31).

In our models, we compared intestinal strangulation, superior mesenteric artery occlusion (SMAO) with laparotomy and anesthesia groups for the levels of IL-1 β , IL-6 and TNF α which began to rise 60 minutes after the onset of ischemia and reached peak points at 4 hours.

In our study fifth minute levels of IL-1 β , IL-6 and TNF α did not show significant differences in all the groups. The results in all groups except for anesthesia (control) group including laparotomy groups showed an increase at 2 and 4 hours, but the increase was much more prominent in the strangulation and SMAO groups. The increase was also significant in strangulation and SMAO groups when compared to laparotomy and control groups ($p < 0.01$). We also observed higher cytokine levels in fourth hour samples compared to second hour samples in strangulation and SMAO groups ($p < 0.01$).

Regardless of the etiology, the ultimate goal for the operation is to preserve the viability of the intestine. Time is the most important factor determining prognosis. Once irreversible damage has occurred, regardless of the operation chosen whether vascular by pass, embolectomy or resection, mortality is high, leaving the patient TPN dependent with only intestinal transplantation as a hope for a cure (32).

This study has been designed in an effort to find an easily applicable marker of intestinal ischemia to provide guidance to the hesitating or unsuspecting physician to initiate advanced, invasive and expensive procedures in the diagnosis of this disease before the point of no return has been reached.

Current tests available for the determination of cytokine levels are still time consuming. The procedures we have used in our study have taken average 3.5 hours for the results which are still behind the optimal time range. We would definitely benefit from the development of faster analysis techniques in this manner. We believe that serum cytokine levels may be utilized as an aid for diagnosis if rapid and sensitive ways of measurement become available.

References

1. Karaağaç H. Akut İntestinal İskemi Erken Tanısında Serum IL-1 β , IL-6, TNF α ve Lökosit ölçümlerinin değerlendirilmesi. Uzmanlık Tezi, Ankara 2002.
2. Acosta S, Ogren M, Sternby NH, Bergqvist D, Bjorck M.

- Incidence of acute thromboembolic occlusion of the superior mesenteric artery--a population-based study. *Eur J Vasc Endovasc Surg* 2004; 27: 145-150.
3. Schoots IG, Koffeman GI, Legemate DA, Levi M, van Gulik TM. Systematic review of survival after acute mesenteric ischaemia according to disease aetiology. *Br J Surg* 2004; 91: 17-27.
4. Brandt LJ, Boley SJ. AGA technical review on intestinal ischemia. *American Gastrointestinal Association. Gastroenterology* 2000; 118: 954-968.
5. Sreenarasimhaiah J. Diagnosis and management of intestinal ischaemic disorders. *Br Med J* 2003; 326: 1372-1376.
6. Cleveland TJ, Nawaz S, Gaines PA. Mesenteric arterial ischaemia: diagnosis and therapeutic options. *Vasc Med* 2002; 7: 311-321.
7. Graeber GM, Cafferty PJ, Wolf RE, Harmon JW. An analysis of creatine phosphokinase in the mucosa and the muscularis of the gastrointestinal tract. *J Surg Res* 1984; 37: 376-382.
8. Graeber GM, Wolf RE, Harmon JW. Serum creatine kinase and alkaline phosphatase in experimental small bowel infarction. *J Surg Res* 1984; 37: 25-32.
9. Marks WH, Salvino C, Newell K, Wider M, Marks C. Circulating concentrations of porcine ileal peptide but not hexosaminidase are elevated following 1 hr of mesenteric ischemia. *J Surg Res* 1988; 45: 134-139.
10. Thompson JS, Bragg LE, West WW. Serum enzyme levels during intestinal ischemia. *Ann Surg* 1990; 211: 369-373.
11. Shenkar R, Abraham E. Effects of hemorrhage on cytokine gene transcription. *Lymphokine Cytokine Res* 1993; 12: 237-247.
12. Shenkar R, Chang YH, Abraham E. Cytokine expression in Peyer's patches following hemorrhage and resuscitation. *Shock* 1994; 1: 25-30.
13. Caty MG, Guice KS, Oldham KT, Remick DG, Kunkel SI. Evidence for tumor necrosis factor-induced pulmonary microvascular injury after intestinal ischemia-reperfusion injury. *Ann Surg* 1990; 212: 694-700.
14. Mester M, Tompkins RG, Gelfand JA, Dinarello CA, Burke JF, Clark BD. Intestinal production of interleukin-1 alpha during endotoxemia in the mouse. *J Surg Res* 1993; 54: 584-591.
15. Olfert ED, Cross MB, William A. Hematology median values and ranges. *Canadian Council on Animal Care, Guide to the Care and Use of Experimental Animals. Ontario, Canada: 2nd ed. Vol 1. 1993.*
16. Chiu CJ, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; 101: 478-483.
17. Edwards MS, Cherr GS, Craven TE, et al. Acute occlusive mesenteric ischemia: surgical management and outcomes. *Ann Vasc Surg* 2003; 17: 72-79.
18. Bingol H, Zeybek N, Cingoz F, Yilmaz AT, Tatar H, Sen D. Surgical therapy for acute superior mesenteric artery embolism. *Am J Surg* 2004; 188: 68-70.

19. Lefkovitz Z, Cappell MS, Lookstein R, Mitty HA, Gerard PS. Radiologic diagnosis and treatment of gastrointestinal hemorrhage and ischemia. *Med Clin North Am* 2002; 86: 1357-1399.
20. Greenwald DA, Brandt LJ, Reinus JF. Ischemic bowel disease in the elderly. *Gastroenterol Clin North Am* 2001; 30: 445-473.
21. Hunter GC, Guernsey JM. Mesenteric ischemia. *Med Clin North Am* 1988; 72: 1091-1115.
22. Pullicino EA, Carli F, Poole S, Rafferty B, Malik ST, Elia M. The relationship between the circulating concentrations of interleukin 6 (IL-6), tumor necrosis factor (TNF) and the acute phase response to elective surgery and accidental injury. *Lymphokine Res* 1990; 9: 231-238.
23. Ala Y, Palluy O, Favero J, Bonne C, Modat G, Dornand J. Hypoxia/reoxygenation stimulates endothelial cells to promote interleukin-1 and interleukin-6 production. Effects of free radical scavengers. *Agents Actions* 1992; 37: 134-139.
24. Cicco NA, Lindemann A, Content J, et al. Inducible production of interleukin-6 by human polymorphonuclear neutrophils: role of granulocyte-macrophage colony-stimulating factor and tumor necrosis factor-alpha. *Blood* 1990; 75: 2049-2052.
25. Meyer TA, Noguchi Y, Ogle CK, et al. Endotoxin stimulates interleukin-6 production in intestinal epithelial cells. A synergistic effect with prostaglandin E2. *Arch Surg* 1994; 129: 1290-1294.
26. Van Damme J, Opdenakker G, Simpson RJ, et al. Identification of the human 26-kD protein, interferon beta 2 (IFN-beta 2), as a B cell hybridoma/plasmacytoma growth factor induced by interleukin 1 and tumor necrosis factor. *J Exp Med* 1987; 165: 914-919.
27. Bishop RF, Allcock EA. Bacterial flora of the small intestine in acute intestinal obstruction. *Br Med J* 1960; 5175: 766-770.
28. Michalsky MP, Deitch EA, Ding J, Lu Q, Huang Q. Interleukin-6 and tumor necrosis factor production in an enterocyte cell model (Caco-2) during exposure to *Escherichia coli*. *Shock* 1997; 7: 139-146.
29. Gaffin SL, Gathiram P, Wells MT, Brock-Utne JG. Effect of corticosteroid prophylaxis on lipopolysaccharide levels associated with intestinal ischemia in cats. *Crit Care Med* 1986; 14: 889-891.
30. Roscher R, Oettinger W, Beger HG. Bacterial microflora, endogenous endotoxin, and prostaglandins in small bowel obstruction. *Am J Surg* 1988; 155: 348-355.
31. Moore JN, White NA, Berg JN, Trim CM, Garner HE. Endotoxemia following experimental intestinal strangulation obstruction in ponies. *Can J Comp Med* 1981; 45: 330-332.
32. Oldenburg WA, Lau LL, Rodenberg TJ, Edmonds HJ, Burger CD. Acute mesenteric ischemia: a clinical review. *Arch Intern Med* 2004; 164: 1054-1062.