

Nusret AKYÜREK¹
Mustafa KEREK¹
Osman YÜKSEL¹
Türkan PATIROĞLU²

The Role of Immunological Factors in Wound Infections (A Prospective Clinical Study)*

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Departments of ¹General Surgery and
²Immunology, Faculty of Medicine, Erciyes
University 38039, Kayseri-TURKEY

Abstract: Wound infections are among the major problems complicating surgical procedures. The aim of this study was to investigate the relationship between the immunological status of the patient and wound infections. Wounds were classified as clean, clean-contaminated, contaminated and dirty. T-lymphocyte(CD3+), B-lymphocyte (CD19+) and total lymphocyte(CD45+) counts, and IgG, IgM, IgA levels were measured at the beginning of treatment, and on the fifth and tenth days. The levels of CD3+, CD19+, CD45+, IgM, IgA were not different in the groups, while in the dirty group, IgG levels were lower than in the other groups, at the beginning of treatment (P<0.05). On the fifth and tenth days of the

treatment, IgG levels increased and CD19+ level decreased in the dirty group (P>0.05). There was no change in the levels of CD3+, CD19+, CD45+, IgG, IgM or IgA in the clean, clean-contaminated and contaminated groups on the tenth day of the treatment. In conclusion, low preoperative IgG levels and a progressive decrease in CD19+ levels throughout the postoperative period indicate a high risk for the development of wound infections. The value of the supportive administration of IgG and CD19+ for these patients in an attempt to prevent the development of infections should be further investigated.

Key Words: wound infection, lymphocytes, immunoglobulins.

Introduction

Wound infections are among the major problems complicating surgical procedures. Wound infections result in prolongation of the hospitalization, increment in the treatment costs and loss of working days (1). Postoperative wound infections are influenced by factors associated with the microorganism (such as virulence and microbial load), the actual status of the patient's immune system, environmental factors and the surgical technique. Among the factors increasing the risk of wound infections are also those associated with the patient (radiation therapy, immunosuppressive therapy, nutritional disorders and disease resulting in immunosuppression), the duration of the operation, blood transfusions during the postoperative period, overuse of electrocauterization, deviations from the surgical technique and the use of irrelevant prosthetic materials. In addition, supportive treatment administered to the patient in the presence of wound infection (nutritional support, immunotherapy,

administration of antibiotics) and the actual immunological parameters of the patient have an effects as well. In general, infections result in 1.5 to 4% of clean wounds and 28 to 40% of contaminated wounds (2).

The aim of this study was to evaluate the correlation between the actual immunological status of the patient immediately prior to the surgical procedure and the development of infections among various groups of wounds.

Patients and Methods

From November 1997 through March 1998, 60 successive patients undergoing surgery at the Department of General Surgery in Erciyes University Faculty of Medicine were enrolled in the study. The design of the study was approved by local ethics committees. The patients were categorized into wound groups of clean, clean-contaminated, contaminated and dirty (or infected)

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(2). Each group consisted of 15 patients. The clean wounds group included patients with cholelithiasis and hernias undergoing elective surgery, the clean-contaminated wounds group included patients with uncomplicated acute appendicitis, acute cholecystitis and common bile duct explorations, the contaminated wounds group included patients with advanced nonperforated appendicitis, necrotizing incarcerated hernias and necrotizing sigmoid volvulus and the dirty wounds group included patients with periappendicular abscess, delayed perforated ulcers and colonic perforations. Wound infection was described as those presenting with local tenderness, local warmth and purulent discharge within 30 days of the postoperative period. Those patients with negative cultures of the samples from infected wounds were not excluded from the study. Samples of the incisions were obtained for cultures in all patients postoperatively. Samples of the primary site of disease were obtained for cultures as well for clean and clean-contaminated wounds. Antibiotics were not administered for clean wounds. Prophylactic antibiotics were administered for clean-contaminated wounds. Duration of prophylaxis was one day. Contaminated wounds and therapeutic antibiotics were administered for dirty wounds. Duration of therapy was 5 to 10 days (mean 7.8 days). Prophylactic and therapeutic antibiotics administered were third generation cephalosporins. Bipolar electrocauterization was employed for all patients during the surgical procedure. The duration of the operation was evaluated among patient groups. Patients treated with radiation therapy and chemotherapy, and patients receiving blood transfusions prior to the surgical procedure were excluded from the study. Monoclonal antibody levels for T lymphocytes (CD 3+) (n:60-80%), B lymphocytes (CD 19+) (n:12-250%) and total lymphocytes (CD 45+) (n:more than 85%) and IgA (n:7-250 mg/dL), IgG (n:700-1500 mg/dL) and IgM (n:70-400 mg/dL) levels were determined on the day of the operation and on days 5 and 10. CD3+, CD19+ and CD45+ levels were measured using immunophenotyping with the Dako kit on a flow cytometer (Coulter Epicx XL). Separate tubes were prepared for lymphocytes. Into each tube was placed 100 mL of blood with EDTA and the respective antibodies. Tubes were incubated for 30 min at +4 °C. Following incubation in darkness, the tubes underwent a sequence of procedures (Q-prep workstation) and measurement on the flow cytometer. Lymphocyte counts were obtained on a cell counter analyzer (CELL-DYN 1700) using the Abbott kit and lytic agent, detergent and diluent solutions with 1 to 2 mL of blood in standard small tubes containing K3 EDTA.

Immunoglobulins were measured using nephelometry (Beckman 360 Array System) in 2 mL blood in standard biochemistry tubes.

Statistical Analysis

Comparisons within groups were made using ANOVA for repeated measures test and comparisons between groups were made using the factorial ANOVA test. $P < 0.05$ was considered statistically significant.

Results

Each group consisted of 15 patients. Distribution of patients between groups is presented in Table 1. There was no difference between groups in terms of demographic characteristics (Table 1). The mean duration of hospitalization was longer for the contaminated and dirty wounds groups than for the other groups. Wound infection rates were 1/15 (6.6%), 3/15 (19.8%), 4/15 (26.6%) and 6/15 (40%), in the clean, clean, clean-contaminated, contaminated and dirty wounds groups respectively. Cultures of samples obtained from the site of the operation revealed no bacterial overgrowth in the clean wounds group. Two patients each in the clean-contaminated and contaminated wound groups had growth of *E. coli* and *E. coli* was isolated in three patients and enterococcus sp in one patient in the dirty wounds group. Cultures of samples obtained from the incision following the operation revealed *E. coli* in one patient in the dirty wounds group. Staphylococcus aureus, *E. coli* and Pseudomonas aureginosa were isolated in cultures of infected wounds mostly in the contaminated and dirty wounds groups. Bipolar electrocauterization was employed for all patients during the operations. Coexistent diseases included chronic obstructive pulmonary disease in two patients in the clean wounds group and in one patient in the clean-contaminated wounds group and atherosclerotic heart disease in two patients in the dirty wounds group. There was no difference between groups other than the dirty wound groups for preoperative CD3+, CD19+, CD45+, IgA and IgM levels (Figures 1-2). For the dirty wounds group, the preoperative IgG level was found to be lower than in the other groups ($p < 0.05$). IgG level at 10 days postoperatively was significantly elevated the in dirty wounds group (Figure 2). A progressive decrease in CD19+ level was observed in the dirty wounds group at 10 days postoperatively ($p < 0.05$) (Figure 1). Two patients in the dirty wounds group died. In these patients, a progressive decrease in CD19+ and IgG levels was

Table 1. Demographic characteristics, duration of hospitalization and results of culture.

	Clean n: 15	Clean-contaminated n: 15	Contaminated n: 15	Dirty n: 15
Demographic characteristics				
Age (mean years)	39	42	45	44
Sex				
Female	8	6	10	4
Male	7	9	5	11
Wound infection	1 6.6%	3 19.8%	4 26.6%	6 40%
Duration of hospitalization (Day)	5	7	11	18
Samples from the primary site of disease				
<i>E. coli</i>	–	2	2	3
<i>Enterococ</i>	–	–	–	1
Samples from the incisions (Postoperatively)				
<i>E. coli</i>	–	–	–	1
Samples from infected wounds				
<i>Staph. aureus</i>	–	1	1	1
<i>Pseudomonas</i>	1	1	2	3
<i>aureginosa</i>				
<i>E. coli</i>				
Use of the antibiotics	–	Prophylaxy	Prophylaxy	Therapy

observed at 5 and 10 days postoperatively when compared with preoperative levels. No difference in CD3+, CD19+, CD45+, IgA, IgG and IgM levels was observed for the clean, clean-contaminated and contaminated wounds groups at 5 and 10 days postoperatively when compared with preoperative levels (Figures 1-2).

Discussion

Wound infections are among the significant problems associated with surgery. Wound infection rates range from 1.5 to 40%, depending on the type of wound (1). In our study, wound infection rates ranged from 6.6 to 40%. Our wound infection rate for the clean wounds group was higher than the rates reported in the literature. In addition, the duration of hospitalization is prolonged in case of wound infections. In our series, the duration of hospitalization was longer for the dirty

wounds group with a higher wound infection rate than in the clean wounds group.

Following trauma, changes in lymphocyte sub-populations might result in deterioration of the immune functions of T lymphocytes. Lymphocyte proliferation decreases following surgical procedures, trauma, burns and bleeding (2,3). These changes disappear within a few days of the injury. Experimental studies have shown that the decrease in proliferative response by T lymphocytes correlates with the increase in the infection rate and death (3,4,5). In a study on rats, an increase in T lymphocytes around the wound has been histopathologically demonstrated at 5 days following the injury, with a peak at 7 days (6). In our study, CD3+ levels were measured in peripheral blood and significant numerical changes were not observed.

There are studies on some components of T lymphocytes such as CD4+, CD8+ and CD4/CD8 ratios

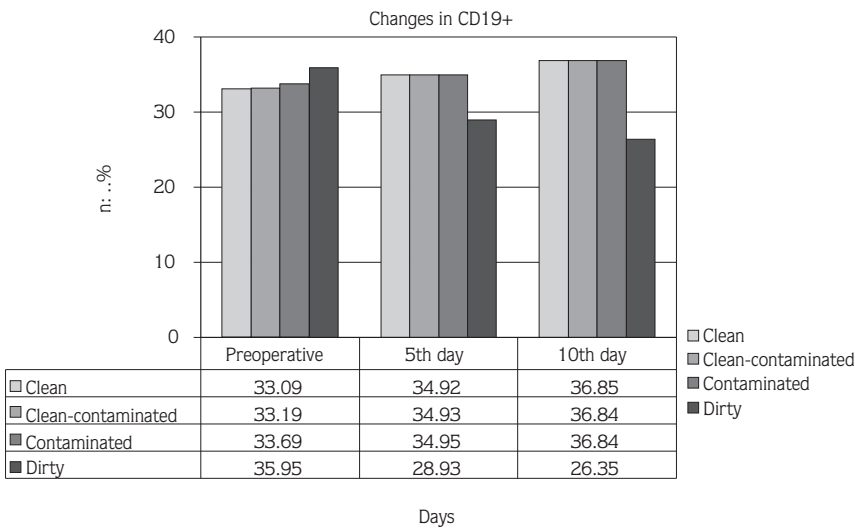


Figure 1. CD19+ levels in the dirty wounds group at 10 days postoperatively demonstrated a progressive decrease when compared with preoperative levels ($p < 0.05$).

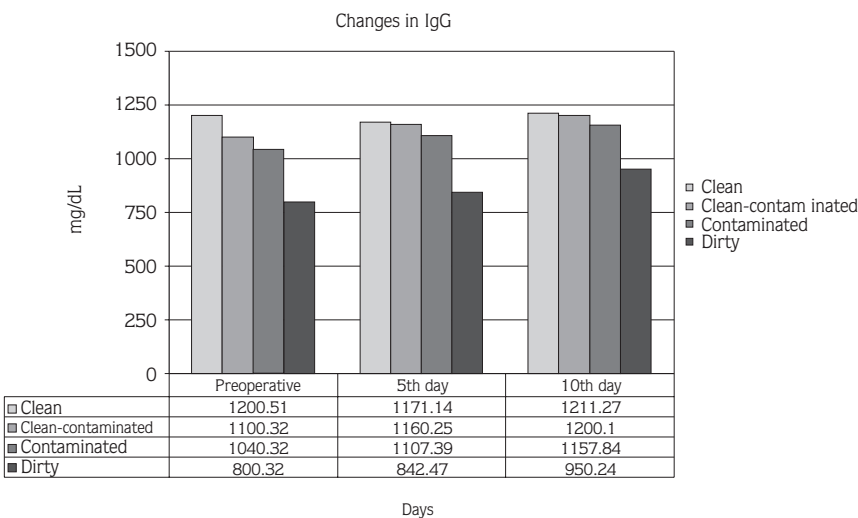


Figure 2. Preoperative IgG levels in the dirty wounds group were lower than in the other groups ($p < 0.05$) ($f = 4.97$).

(7). However, we have not come across any study concerning CD3+. Immunological changes following thermal injury and the decrease in the number of lymphocytes at distant sites might be the result of the difference in the distribution of the lymphocyte pool. The total number of lymphocytes decreases following trauma and burns. The decrease is more pronounced with CD4+ component when than CD8+ component. Feeney et al. have reported a decrease in the number of CD4+ and CD8+ in patients admitted to the Intensive Care Unit for medical and surgical indications (8). In our study, a statistically non-significant increase in CD3+ levels in peripheral blood samples was observed at 5 and 10 days postoperatively in the dirty wounds group while no difference was observed in the other groups.

B lymphocytes comprise the humoral component of specific immune response. B lymphocytes make up less than 1% of total plasma cells. An increase in this ratio follows systemic infections and might become as high as 10%. Antibodies are produced, protecting the host from infections through neutralization of the pathogen, opsonization and complement activation. B lymphocyte response by the host against the antigen should be supported with T lymphocytes(9). A gradual decrease is observed in B lymphocyte levels following major or minor trauma or stress conditions, maximizing at 2 hours. These levels are normalized within 24 hours in cases of major or minor trauma (10). In our study, there was no difference in CD19+ levels between groups preoperatively. A statistically non-significant increase in the CD19+ level was observed in the dirty wound groups

when compared with the other groups. However, the maximal decrease and the course of normalization could not been demonstrated since a control evaluation of the levels at 2 and 24 hours postoperatively was not performed. A non-significant increase was observed in CD19+ levels at 5 and 10 days postoperatively in the clean, clean-contaminated and contaminated wounds groups when compared with preoperative levels whereas a significant decrease was observed, in contrast, in the dirty wounds group (Figure 1). An important finding in our study is the progressive decrease in CD19+ levels postoperatively for the groups with wound infections.

In our study, preoperative CD45+ levels were found to be lower in the dirty wounds group than in the other groups whereas a non-significant increase in CD45+ levels was observed for all groups at 5 and 10 days postoperatively when compared with preoperative levels.

IgG makes up 75% of total serum immunoglobulins. IgG has four subgroups, IgG1 being the commonest. It has control over the opsonization system. In B lymphocyte cultures isolated from the peripheral blood of patients following trauma an increase (11) or no alteration (12) in IgG production has been reported whereas a decrease (12) or no alteration (11) in IgM production has been reported. The reason for the decreased B lymphocyte function following trauma is suggested to be the numerical insufficiency of helper T lymphocytes (12). In cases of bleeding, a decrease in serum immunoglobulin levels has been reported as a result of the decrease in the number of antibody producing B lymphocytes (13). A decrease in serum IgG level also occurs following thermal injury (14). In our study, preoperative IgG levels were found to be significantly lower in the dirty wounds group than in the other groups. An increase in IgG levels was observed in all groups postoperatively, yet IgG levels in the dirty wounds group on 5 and 10 days postoperatively were well below those in the other groups. Observation of such a course in IgG levels in patients developing wound infections is another important finding of this study.

IgA is a predominant factor in the mucosal immune system. IgA makes up 10% of all immunoglobulins.

Primary immune response against certain local infections is generated at IgA level. IgA and IgM levels are unaffected by thermal injury. Experimental and clinical studies have shown intravenous use of 5S, 7S immunoglobulin, IgG, IgM, IgA and Pseudomonas immunoglobulin preparations to have a protective effect against various infections (15-17). There is limited data on the role of secretory IgA in wound infections and generalized infections other than the mucosal defense role. In our study, preoperative IgA levels were not significantly different between the groups. In all groups, a non-significant increase was observed in IgA levels on 5 and 10 days postoperatively when compared with preoperative levels.

IgM makes up 10% of all immunoglobulins. IgM plays a role in the early response against a variety of antigens. In our study, preoperative IgM levels were found to be non-significantly lower in the dirty wounds group than in the other groups. Furthermore, an increase in IgM levels on 5 and 10 days postoperatively was observed in the dirty wounds group whereas no change was observed in the other groups.

In summary, low preoperative IgG levels and a progressive decrease in CD19+ lymphocytes throughout the postoperative period indicate a high risk for the development of wound infections (wound infection rate was 63% for patients with low IgG levels and 33% for patients with a progressive decrease in CD19+ levels). This might have important therapeutic and/or prophylactic implications in patients with low preoperative IgG levels and CD19+ lymphocytes. In addition, a further topic of investigation might be the value of the supportive administration of IgG and CD19+ for these patients in an attempt to prevent the development of infections.

Correspondence author:

Nusret AKYÜREK

Melikgazi Mah. Sivas Cad. Bademli Sok.

Buket Apt, 5/24,

38030 KAYSERİ

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