Lymphocyte Count at 4 Days Postoperatively and CRP Level at 7 Days Postoperatively: Reliable and Useful Markers for Surgical Site Infection Following Instrumented Spinal Fusion

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Study Design. Case-control study.

Objective. To identify biochemical markers for surgical site infection (SSI) in posterior instrumented spinal fusion that are not affected by operative circumstances and to determine diagnostic cutoffs for these markers

Summary of Background Data. Numerous biochemical markers may be used for early detection of SSI; however, these markers may be affected by operative factors.

Methods. We reviewed data on C-reactive protein level and total white blood cell count and differential count before instrumented spinal fusion and at 1, 4, and 7 days postoperatively. The 141 patients in our sample were divided into an SSI group (patients who developed deep SSI) and a no-SSI group. We determined which markers differed significantly between groups and identified those not affected by operative circumstances (operating time, intraoperative blood loss, number of fusion segments) in the no-SSI group. Then, we determined diagnostic cutoffs for these unaffected markers by using receiver operating characteristic curves.

Results. Three markers were selected: lymphocyte count at 4 days postoperatively (cutoff 1180/μL, sensitivity 90.9%, specificity 65.4%, area under the curve [AUC] 0.80), lymphocyte count of at 7 days postoperatively (cutoff <1090/μL, sensitivity 63.6%, specificity 78.5%, AUC 0.77), and C-reactive protein level at 7 days postoperatively (cutoff >4.4 mg/dL, sensitivity 90.9%, specificity 89.2%, AUC 0.95).

Conclusion. Lymphocyte count at 4 and 7 days postoperatively and C-reactive protein level at 7 days postoperatively are reliable markers for SSI following instrumented spinal fusion. Lymphocyte count at 4 days should be useful for screening because of its high sensitivity and because it can be measured early. C-reactive protein level 7 days should be useful for definitive diagnosis given its high sensitivity and specificity and large AUC.

Level of Evidence: 4

Key Words: Surgical site infection, laboratory data, laboratory marker, C-reactive protein, white blood cell, white blood cell differential, lymphocyte, neutrophil, instrumentation, sensitivity, specificity, screening test, diagnosis

INTRODUCTION

In recent years, spinal fusion with instrumentation has become more widely used because of ability to achieve strong fixation and correct deformities. However, this procedure involves a higher risk of complications than uninstrumented surgery. Surgical site infection (SSI) is one of the most serious potential complications.[1–5] Infection rates of 2.2% to 8.5% after instrumented spinal fusion have been reported.[6–13] Even a relatively small number of bacteria adhering to the surface of the implanted device may form a glycoprotein biofilm, resulting in infection; such biofilms are often formed by antibiotic-resistant bacteria, resulting in infection rates.[14] SSI may necessitate revision surgery, result in persistent pain or deformity, require additional hospitalization, prolong recovery time, and considerably increase treatment costs.[15–18] Preventing SSI should be prioritized, and when an infection does occur, early diagnosis and treatment are very important for preventing aggravation.[15,19–23] An SSI should be made based on a combination of systemic indicators of infection, such as fever and biochemical markers, and localized symptoms, such as tenderness, swelling, redness, and pus discharge.[19,23,24] Most tests for SSI rely on postoperative biochemical markers because of objectivity and convenience.[15,19–22,25] For instance, acute-phase-related C-reactive protein (CRP) and white blood cell (WBC) count and differential can be used to detect and monitor postoperative wound infections.[19,26,27] However, these markers might be affected by the circumstances of the operation, such as operating time, intraoperative blood loss volume, and

number of fusion segments. The aim of the present study is to identify which of the aforementioned markers are not affected by the circumstances of the operation and to determine appropriate diagnostic cutoffs for these markers using receiver operating characteristic (ROC) curves.

MATERIALS AND METHODS

After receiving approval from the institutional review boards of the participating institutions, we retrospectively reviewed the medical records of 221 patients who underwent instrumented posterior spinal fusion for degenerative spine disease at two hospitals between January 2009 and December 2014, and looked for evidence of deep SSI and for laboratory data. SSI was defined according to Centers for Disease Control and Prevention criteria.[28] We recorded patients as deep SSI patients if the attending surgeon diagnosed deep SSI and conducted debridement, performed a blood culture that was positive for infectious agents within four weeks, or drained the surgical wound. Patients were excluded if they had a trauma, tumor, or infection at the time of surgery or were under 20 years of age. We also excluded patients who did not undergo laboratory tests on day 1, 4, and 7 postoperatively. The tests were performed as a matter of routine and not only in cases of suspected infection. The final sample consisted of 141 patients and was divided into 11 patients who developed deep SSI and 130 who did not.

We collected data regarding CRP, WBC count, and neutrophil and lymphocyte percentages before surgery and 1, 4, and 7 days postoperatively. CRP was measured using the latex agglutination method, and an automatic cell counter was used to determine the WBC count. Neutrophil and lymphocyte counts were calculated from the WBC count and differential percentages. Operating time, intraoperative blood loss, and number of fusion segments were also recorded. All the patients remained hospitalized 7 days postoperatively.

We began our primary analysis by using Student's *t*-test to determine which markers exhibited statistically significant postoperative differences between the SSI and no-SSI groups. Next, we performed a test of noncorrelation (using Pearson's Correlation Coefficient) to determine which markers were not affected by any operative factor (operating time, intraoperative blood loss, number of fusion segments) in the no-SSI group. Finally, we determined appropriate diagnostic cutoffs of these selected markers using the ROC curve. In other analyses, differences in quantitative characteristics such as age, operating time, intraoperative blood loss, and number of fusion segments were analyzed with Mann-Whitney's *U*-test. Differences in qualitative characteristics such as sex were analyzed using Fisher's exact test. All statistical analyses were carried out using SPSS version 22.0 for Windows (IBM, Armonk, NY, USA). A p value <0.05 was considered statistically significant.

RESULTS

Demographics and Operative Circumstances

Three men and 8 women were included in the SSI group; while, 51 men and 79 women were in the no-SSI group. The median age at surgery was 73 years in the SSI group and 84 years in the no-SSI groups. Operational circumstances were as follows: median operating time, 315 min (range 143–552) for the SSI group, 234 min (range 80–849) for the non-SSI group; median intraoperative blood loss, 349 mL (range 100–600) for the SSI group, 273.5 mL (range 0–2440) for the non-SSI group; median number of fusion segments, 2 (range 1–7) for the SSI group, 1 (range 1–11) for the no-SSI groups. There were no significant differences in the age, sex, operating time, intraoperative blood loss, or number of fusion segments between the groups (Table 1).

Outcomes in the SSI Group

Of the 11 patients who developed deep SSI (3 men and 8 women), we conducted debridement in 7, 4 of whom had to have their instrumentation removed. The other 4 patients were treated with antibiotics. All patients recovered (Table 2).

Biochemical Markers

There were no significant differences between the SSI and no-SSI groups for all chemical markers before surgery and significant differences in CRP levels 1, 4, and 7 days postoperatively and in the neutrophil percentage, lymphocyte percentage, and lymphocyte

count 4 and 7 days postoperatively (Table 3). The test for noncorrelations found that the only markers unaffected by any operative circumstances in the no-SSI group were the lymphocyte count at 4 and 7 days post operation and CRP level at 7 days post operation (Table 4). We determined appropriate diagnostic cutoffs for these three markers using ROC curves, with the following results: lymphocyte count 4 days postoperatively, cutoff <1180/μL (sensitivity 90.9%, specificity 65.4%, area under the curve [AUC] 0.80; Figure 1); lymphocyte count 7 days postoperatively, cutoff <1090/μL (sensitivity 63.6%, specificity 78.5%, AUC 0.77; Figure 2); CRP level 7 days postoperatively, cutoff >4.4 mg/dL (sensitivity 90.9%, specificity 89.2%, AUC 0.95; Figure 3).

DISCUSSION

Treatment of SSI after instrumented spinal fusion should aim not only to resolve infection but also to maintain spinal stability. Ishii et al. reported that patients who developed SSI were more likely to be able to retain their implants if diagnosed early.[29] Early diagnosis of SSI may be made based on systemic indicators, such as fever and biochemical markers, in combination with localized symptoms such as tenderness, swelling, redness, heat sensation, and pus discharge.[15,19–21] However, moist healing, in which surgical wound healing is promoted by covering it with a wound-covering material, has become widespread in recent years, so it has become more difficult to monitor the wound directly, potentially increasing

the risk of delayed SSI diagnosis. Therefore, biochemical markers are very useful as indicators of SSI.

The most widely used biochemical markers of SSI are CRP levels, the erythrocyte sedimentation rate (ESR), and the WBC count and differential, which can be measured easily in most medical institutions. CRP was significantly superior to ESR as a marker of SSI in previous reports, where CRP had more reliable peaks and more stable values.[25,34,36] Hence, we did not select ESR as an SSI marker in the current study.

CRP is made in the liver in response to inflammation, infection, malignancy, and tissue damage, and CRP levels are characterized by a relatively high sensitivity and quick response.[30,31] After surgery, CRP levels tend to peak on postoperative day 3 and rapidly decrease to baseline between postoperative days 10 and 14.[21] Several studies have suggested that in cases of suspected SSI, it would be very useful to compare CRP levels on day 7 with those on day 3 or 4; an elevated level on day 7 would indicate possible infection.[20,21,25,30–33] However, factors other than infection, such as operative circumstances, have been reported to influence CRP level. The maximum postoperative CRP level depends on the region and type of surgery.[21,27,34] For example, Takahashi et al. reported that CRP levels were significantly higher after instrumented spinal fusion than after spinal surgery without instrumentation.[25]

Another frequently used marker is the WBC count and differential. Takahashi et al. reported that the WBC count and differential are useful for early detection of surgical wound

infection following instrumented lumbar spinal fusion.[19,25] Furthermore, changes in the WBC count, especially the neutrophil count, over time serve as useful markers of postoperative progress.[25] According to Takahashi et al., the renewed elevation of the WBC count, particularly the neutrophil count, after 4 to 7 postoperative days may be a critical sign of infection; the same may apply to a neutrophil percentage >75% after postoperative day 4.[19,25] On the other hand, lymphocytes, which are involved in nonspecific biophylaxis, often decrease after invasion, regardless of infection. The study found that in patients who developed infections, the percentage and number of lymphocytes had significantly decreased on day 4; this signified immune depression, making the patients more susceptible to infection, which may have been associated with a high concentration of anti-inflammatory cytokines and attendant compensatory anti-inflammatory reaction syndrome.[35,36] Thus, the authors consider postoperative lymphopenia (no more than 10% or 1000/μL) after 4 days to be indicative of possible surgical wound infection.[19,25]

We found three reliable biochemical markers that were not affected by operative circumstances: lymphocyte count 4 days postoperatively, lymphocyte count 7 days postoperatively, and CRP level 7 days postoperatively. In most previous studies, except those of Takahashi et al., CRP levels and WBC count were proposed as markers of infection if newly elevated 3–4 days postoperatively, but as these markers may be affected by operative circumstances, no specific values were recommended as diagnostic cutoffs.[21,20,25,30–33] Using

ROC curves, we were able to identify such cutoffs for the three unaffected markers: <1180/μL for lymphocyte count 4 days postoperatively, <1090/μL for lymphocyte count 7 days postoperatively, and >4.4 mg/dL for CRP level 7 days postoperatively. We believe that the lymphocyte count at 4 days will be more useful than that at 7 days because it can be measured earlier and has a larger AUC. CRP level at 7 days appears to be the most accurate of the three markers, with high sensitivity and specificity, and a large AUC.

Our study has several limitations. First, it was a retrospective study. As a result, there may have been an inherent bias associated with patient selection and missing patient information. Patients who did not fit the criteria for deep SSI were placed in the no-SSI group, which may reflect a significant underestimation of the actual number of SSI cases. Another limitation is the possibility that a type 2 error might have occurred because of the comparatively small number of SSI cases. A prospective study in a large cohort may eliminate these problems.

We believe that the role of laboratory markers lies in the initial diagnosis of SSI. Imaging methods such as enhanced CT and enhanced MRI allow for more accurate diagnosis, but such studies are expensive, and all patients cannot afford them. Laboratory markers are therefore very useful for initial diagnosis because of their convenience. In case of a lymphocyte count <1180/μL 4 days postoperatively in patients undergoing instrumented spinal fusion, clinicians should check the surgical wound more carefully. Then, if necessary,

more accurate diagnostic tools such as enhanced CT or enhanced MRI could be used. If the CRP level 7 days postoperatively is >4.4 mg/dL, the same diagnostic tools should be used as soon as possible. After a definite diagnosis, clinicians should perform debridement or administer antibiotics.

In conclusion, the lymphocyte count at 4 and 7 days post operation and CRP level at 7 days post operation are the most reliable biochemical markers for SSI following instrumented spinal fusion because they are not affected by operative circumstances. We believe that the lymphocyte count at 4 days post operation, with a cutoff of <1180/μL, would be useful in screening for infection because of its high sensitivity and because it can be measured early. For definitive diagnosis, we recommend evaluation of CRP level at 7 days post operation, with a cutoff of >4.4 mg/dL, as it shows high sensitivity and specificity, and a large AUC.

Key Points

- We reviewed laboratory data (C-reactive protein, total white blood cell count, and differential count) before instrumented spinal fusion and 1, 4, and 7 days postoperatively to identify reliable markers for surgical site infection that were not affected by operative circumstances and to determine diagnostic cutoffs for these markers.
- Lymphocyte count at 4 and 7 days postoperatively and C-reactive protein level at 7 days postoperatively were reliable markers for SSI that were not affected by operative factors.
- Lymphocyte count at 4 days postoperatively, with a cutoff of <1180/μL, should be useful for screening given that it is highly sensitive and can be measured early.
- CRP level at 7 days postoperatively, with a cutoff of > 4.4 mg/dL, should be useful for definitive diagnosis given its high sensitivity and specificity and large AUC.

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TABLE 1. Patient Data

	SSI group (n=11)	No-SSI group (n=130)	*P* value
Age, years (median [range])	73 [47-84]	68 [22-87]	0.444
Sex	male 3, female 8	male 51, female 79	0.330
Operating time, min (median [range])	315 [143-552]	234 [80-849]	0.317
Blood loss volume, mL (median [range])	349 [100-600]	273.5 [0-2440]	0.563
Number of fusion segments (median [range])	2 [1-7]	1 [1-11]	0.475

SSI, surgical site infection

TABLE 2. Patient Data in the SSI Group

Patient No.	Age (y)	Sex	Method of diagnosis	Time from surgery to diagnosis (days)	Culture	Method of treatment
1	71	F	Debridement	11	*Escherichia coli*	Debridement, implant removal
2	73	F	Debridement	4	Unknown	Debridement
3	73	F	Debridement	15	Unknown	Debridement
4	77	M	Wound drainage	7	MSSA	Antibiotic medication
5	57	M	Debridement	7	MRSA	Debridement, implant removal
6	78	F	Debridement	16	CNS	Debridement
7	84	F	Debridement	18	MRSA	Debridement, implant removal
8	47	F	Wound drainage	14	MRSA	Antibiotic medication
9	74	M	Wound drainage	9	MRSA	Implant removal
10	51	F	Blood culture	7	CNS	Antibiotic medication
11	70	F	Debridement	10	*Pseudomonas aeruginosa*	Debridement

SSI, surgical site infection; F, female; M, male; CNS, coagulase-negative *Staphylococcus aureus*; MSSA, methicillin-susceptible *Staphylococcus aureus*; MRSA, methicillin-resistant *Staphylococcus aureus*.

TABLE 3. Results of Statistical Analysis of Biochemical Markers Between The SSI and no-SSI groups

	Before surgery	1 day postoperatively	4 days postoperatively	7 days postoperatively
White blood cell count	0.063	0.077	0.665	0.740
Neutrophil percentage	0.928	0.573	0.001*	0.020*
Neutrophil count	0.166	0.194	0.405	0.399
Lymphocyte percentage	0.603	0.284	0.001*	0.005*
Lymphocyte count	0.069	0.063	0.001*	0.003*
C-reactive protein level	0.691	0.015*	< 0.001*	< 0.001*

*Statistically significant ($P < 0.05$). SSI, surgical site infection.

TABLE 4. Results of the Test for Non-correlation (using Pearson's Correlation Coefficient) in the No-SSI Group

	Operating time	Intraoperative blood loss	Number of fusion segments	Non-correlation for all factors
C-reactive protein level at 1 day postoperatively	<0.001*	0.175	< 0.001*	No
Neutrophil percentage at 4 days postoperatively	0.007*	0.028*	0.030*	No
Lymphocyte percentage at 4 days postoperatively	0.013*	0.090	0165	No
Lymphocyte count at 4 days postoperatively	0.200	0.307	0.626	Yes
C-reactive protein level at 4 days postoperatively	0.116	0.944	0.026*	No
Neutrophil percentage at 7 days postoperatively	0.139	0.196	0.001*	No
Lymphocyte percentage at 7 days postoperatively	0.125	0.159	0.009*	No
Lymphocyte count at 7 days postoperatively	0.776	0.387	0.610	Yes
C-reactive protein level at 7 days postoperatively	0.585	0.386	0.730	Yes

*Statistically significant ($P < 0.05$). SSI, surgical site infection.

Fig.1

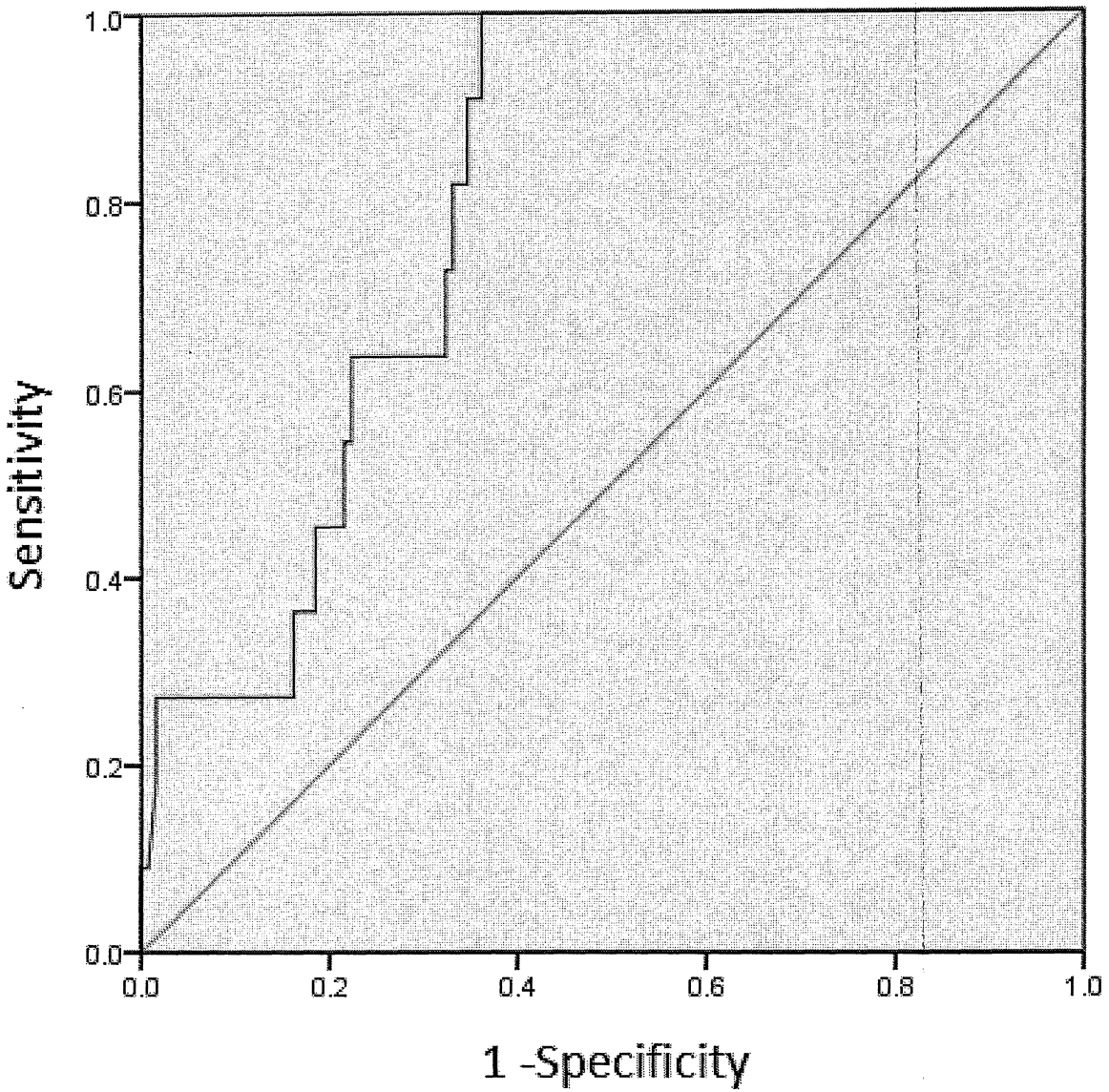

Fig.2

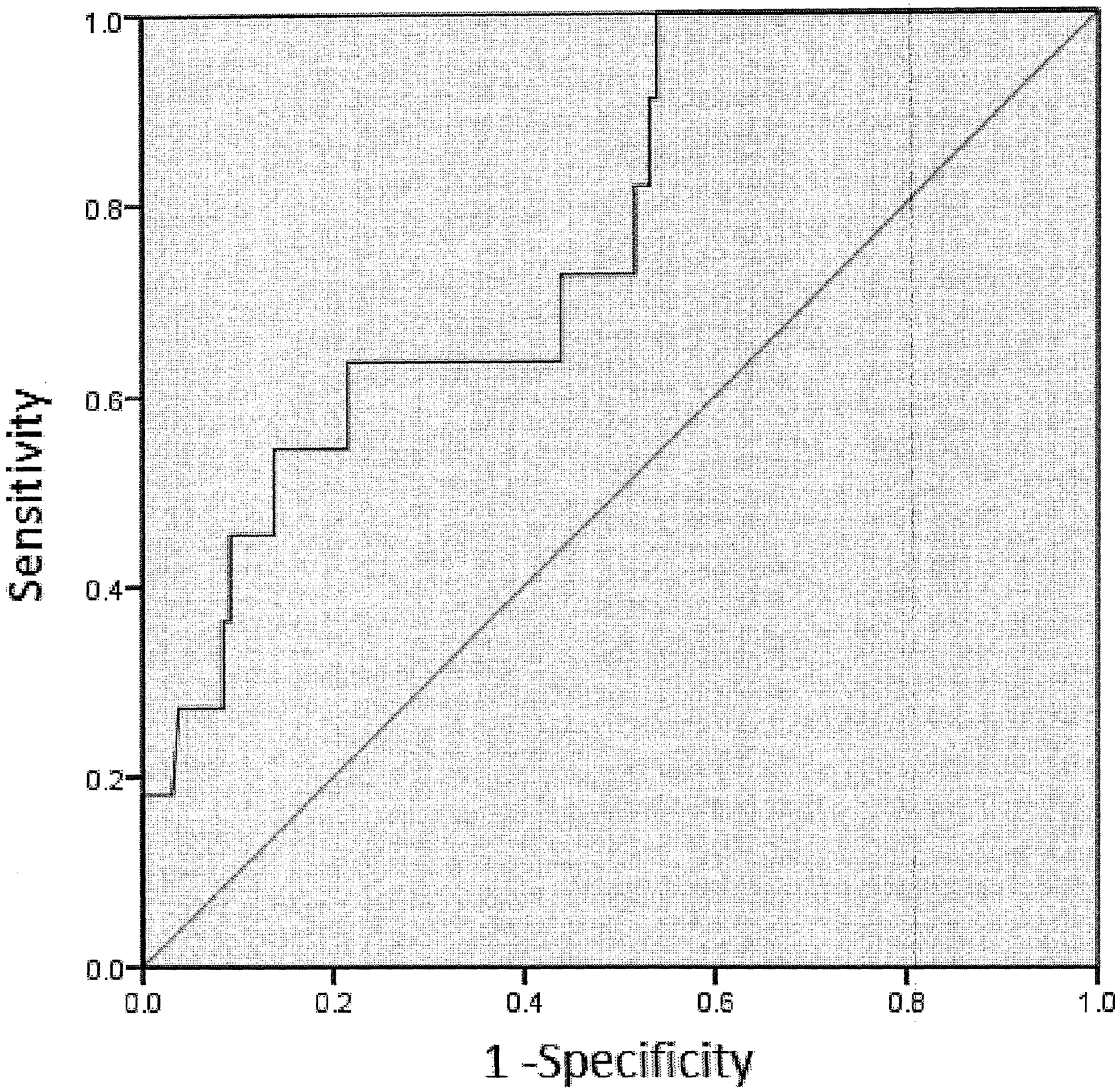

Fig.3

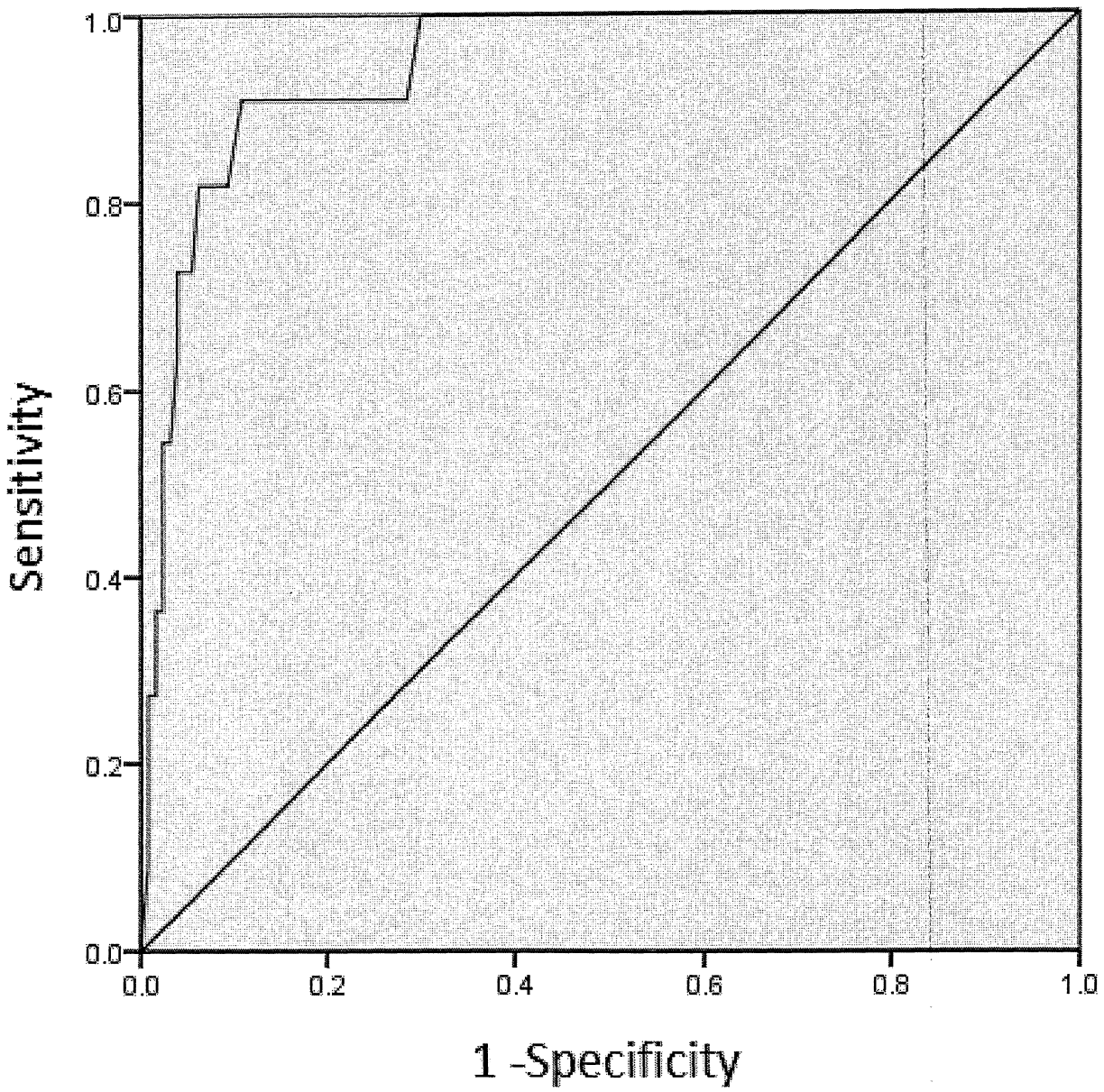

Figure 1. ROC curve used to calculate diagnostic cutoff for lymphocyte count at 4 days postoperatively. Cutoff: 1180/μL; sensitivity: 90.9%; specificity: 65.4%; AUC: 0.80.

Figure 2. ROC curve used to calculate diagnostic cutoff for lymphocyte count at 7 days postoperatively. Cutoff: 1090/μL; sensitivity: 63.6%; specificity: 78.5%; AUC: 0.77.

Figure 3. ROC curve used to calculate diagnostic cutoff for C-reactive protein level at 7 days postoperatively. Cutoff: 4.4 mg/dL; sensitivity: 90.9%; specificity: 89.2%; AUC: 0.95.