Elevated serum lactoferrin and neopterin are associated with postoperative infectious complications in patients with acute traumatic spinal cord injury

Ke Huang, Gang Du, Chengshou Wei, Song Gu, Jun Tang

Department of Orthopedics, Nanning Second People's Hospital (Guangxi Medical University Third Affiliated Hospital), Naning, P.R. China

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Corresponding author:
Ke Huang
Protomedicus
Department of Orthopedics
Nanning Second People's Hospital
13 Dancun Road
530031, Nanning, P.R. China
Phone/fax: 86 0771 2246352
E-mail:
Kehuang201107@163.com

Abstract

Introduction: Several studies have shown that lactoferrin (LF) and neopterin (NT) are correlated with infection. The aim of this study is to determine whether serum levels of LF and NT are associated with postoperative infectious complications in patients with acute traumatic spinal cord injury.

Material and methods: A total of 268 patients with acute traumatic spinal cord injury who underwent spinal surgery were enrolled in this study. Serum levels of LF, NT, and C-reactive protein (CRP), in addition to white blood cell count (WBC) and erythrocyte sedimentation rate (ESR), were measured preoperatively and 24 h postoperatively.

Results: In total, 22 of 268 patients (8.2%) developed postoperative infectious complications. The levels of serum LF, NT, and CRP were significantly higher in the infected patients than in the non-infected patients. No significant differences were observed in postoperative WBC count and ESR between the two groups. Multivariate logistic regression revealed that LF (OR: 1.004 (1.002–1.007)), NT (OR: 1.137 (1.054–1.227)), and CRP (OR: 1.023 (1.002–1.044)) were significantly associated with the presence of postoperative infectious complications. The area under receiver operating characteristic curves for LF, NT, and CRP was 0.709, 0.779, and 0.629, respectively.

Conclusions: Elevated serum concentrations of LF and NT are associated with early infection after surgery. Compared to CRP, elevated levels of LF and NT are better indicators for predicting postoperative infectious complications in patients with acute traumatic spinal cord injury.

Key words: lactoferrin, neopterin, acute traumatic spinal cord injury, postoperative infectious complications.

Introduction

Patients with acute traumatic spinal cord injury (SCI) are at a high risk for postoperative infection, with an incidence rate of nearly 10% [1, 2]. The infection may stem from either the initial trauma or corrective surgery, and is associated with neurological impairment. Once diagnosed, antibiotic therapy or reoperation is required to treat the infection and maintain stability. Therefore, early diagnosis of postoperative infection can prevent problems later.

Traditional inflammatory parameters such as erythrocyte sedimentation rate (ESR), white blood cell (WBC) count, and C-reactive protein (CRP) concentration are still used to predict the risk of developing postopera-

tive infection in patients with SCI. However, these indicators were shown to be poor indicators of postoperative infection risk in SCI patients [3]. Indeed, WBC counts, ERS, and CRP levels are influenced by many other factors and are also elevated in inflammation-related diseases such as coronary artery disease, or inflammation due to trauma and surgery. It appears, therefore, that these parameters are not specific enough to diagnose infection or to serve as prognostic indicators of postoperative infection in SCI patients, necessitating our search for more specific and predictive clinical markers.

Lactoferrin (LF) is an iron-binding glycoprotein and antibacterial agent released upon activation of neutrophils, a major component of the innate immune response during infection [4]. Increased concentrations of LF were observed during allograft rejection, urinary tract infections, and following thoracic surgery [5–7]. Furthermore, LF is a better clinical index for diagnosing bacterial meningitis than CRP [8].

Neopterin (NT) is a pyrimidine derivative secreted by interferon-γ responsive macrophages and released from activated T cells. Elevated serum NT occurs before the peak proliferation of T cells and is also a potential marker for predicting peripheral infections after surgery and central nervous system infection [9]. In fact, increased NT was more specific than CRP for diagnosing patients with septic complications [10, 11].

In the present study, we tested whether elevated levels of serum LF and NT were associated with complications from postoperative infection in SCI patients and if they could serve as markers to predict postoperative infection in an early postoperative stage.

Material and methods

Patients

Between April 2008 and July 2010, information was obtained on consecutive patients with acute traumatic SCI who underwent spinal surgery. The eligibility criteria were as follows: over 18 years old, more than one level of decompression or fusion, hospital stay over four days after surgery, and consented to participate. Patients with a history of infection or inflammatory disease, tumor, or liver diseases were excluded. This study was approved by the local ethics committee.

The clinical data obtained included age, gender, cause of SCI (traffic accident, fall, or violence), and region of SCI (cervical, thoracic, or lumbar). Operation information contained the American Spinal Injury Association Impairment Scale (AIS), type of operation (decompression or fusion), approach (posterior, anterior or combined), number of levels, operative estimated blood loss, time of operation, and hospital stay after surgery.

Postoperative infectious complications

Postoperative infectious complications included deep wound infection, urinary tract infection, and pneumonia, which were diagnosed according to the guidelines of the Centers for Disease Control and Prevention [12–15]. Deep wound infection was diagnosed by either (1) purulent exudates from deep incision, (2) deep incision dehiscence that was spontaneous or opened deliberately when the patient presented at least one infectious symptom (fever > 38°C, localized pain, or tenderness), unless the incision was culture negative, or (3) an abscess or other evidence of infection involving the deep incision found on direct examination, during reoperation, or by histopathologic or radiologic examination. Diagnosis of a urinary tract infection was based on (1) urine specimen with ≥ 10 WBC/mm^3, or (2) urine culture $\geq 10^5$ microorganisms/cm^3. Pneumonia was characterized by (1) at least one onset symptom of cough, dyspnea, purulent sputum, and auscultation crepitation or (2) new or progressive infiltrate, consolidation, or cavitation on chest radiographic examination and at least one infectious symptom (fever > 38°C, rigors, WBC > 10×10^9/l or WBC < 4×10^9/l).

Laboratory analysis

Laboratory tests included determination of LF, NT, CRP, WBC count, and ESR. Blood samples were obtained before surgery and 24 h after surgery, and were kept at –70°C until analysis. Serum LF levels were measured by an enzyme-linked immune-sorbent assay (ELISA; OXIS International, USA). Serum NT levels were also determined by ELISA (B.R.A.H.M.S. Diagnostica, Hennigsdorf, Germany). Detection limits for LF and NT were 1.0 ng/ml and 2.0 nmol/l respectively. Serum CRP was measured on a fully automated biochemical analyzer (Unicel DxC 800 Synchron Clinical System; Beckman Coulter, Shanghai, China) by immunoturbidimetric assay.

Statistical analysis

The median (interquartile range) or mean ± SD was used to describe quantitative data. Frequency and percent were used to describe qualitative data. Data from patients with or without postoperative infectious complications were compared with χ^2 test, *t*-test, or Mann-Whitney *U* test (non-normally distributed). Simple and multivariate binary logistic regression models were employed to estimate the association between postoperative infectious complications and elevated serum LF and NT. We used receiver operating characteristic (ROC) curves to evaluate the diagnostic value of LF and NT to differentiate infected patients from non-infected patients. The best cut-off value was identified by the maximum of Youden's index. Statistical analysis

was performed with SPSS 15.0 for Windows software (SPSS Inc, Chicago, IL, USA).

Results

Characteristics of patients

A total of 268 patients were included in our study (Table I). In all, 22 developed postoperative infectious complications, including 2 patients with deep incisional infections, 11 with urinary tract infections, and 9 patients with pneumonia. The infection rate was 8.2%. Of these 22 patients, 18 had a positive bacterial culture (8 Gram-positive bacteria, 9 Gram-negative bacteria, and 1 fungi).

Comparison of laboratory test results between patients with and without infection

During the preoperative period, there were no significant differences in laboratory results between patients who subsequently presented with postoperative infection and those who did not. Nevertheless, the levels of LF, NT, and CRP were significantly higher in the infected patients than in the non-infected patients 24 h after surgery (Figure 1). No significant differences were observed in postoperative WBC count or ESR between the two groups (Table II).

Associations of laboratory test results and postoperative infectious complications

Simple logistic regression revealed that LF, NT, and CRP were significantly associated with postoperative infectious complications. In the multivariate logistic regression, LF (OR: 1.004 (1.002–1.007)), NT (OR: 1.137 (1.054–1.227)), and CRP (OR: 1.023 (1.002–1.044)) remained significant. The adjusted ORs for WBC and ESR were 0.926 (0.683–1.255) and 1.147 (0.907–1.450) (Table III).

Table I. Characteristics of 268 patients with acute traumatic SCI

Characteristics	Infection (n = 22)	Non-infection (n = 246)	Value of p
Male, n (%)	18 (81.82)	209 (84.96)	0.934
Age, mean ± SD [years]	41.77 ±8.74	41.35 ±9.85	0.847
Cause of SCI, n (%)			
Traffic accident	8 (36.36)	104 (42.28)	0.253
Fall	6 (27.27)	90 (36.59)	
Violence	8 (36.36)	52 (21.14)	
Region of SCI, n (%)			
Cervical	6 (27.27)	95 (38.62)	0.508
Thoracic	10 (45.45)	103 (41.87)	
Lumbar	6 (27.27)	48 (19.51)	
AIS, n (%)			
A	8 (36.36)	86 (34.96)	0.929
B	7 (31.82)	71 (28.86)	
C	4 (18.18)	60 (24.39)	
D	3 (13.64)	29 (11.79)	
Type of operation, n (%)			
Decompression	4 (18.18)	36 (14.63)	0.892
Fusion	18 (81.82)	210 (85.37)	
Approach, n (%)			
Posterior	10 (45.45)	137 (55.69)	0.479
Anterior	8 (36.36)	83 (33.74)	
Combined	4 (18.18)	26 (10.57)	
Number of levels (n)	4.23 ±1.19	4.04 ±1.27	0.497
Operative estimated blood loss [ml]	904.55 ±297.97	850.81 ±343.79	0.479
Time of operation [min]	213.64 ±70.70	206.28 ±68.71	0.632
Hospitalization time after operation [days]	11.91 ±3.39	9.24 ±2.20	0.001

SCI – patients with acute traumatic spinal cord injury, AIS – American Spinal Injury Association Impairment Scale

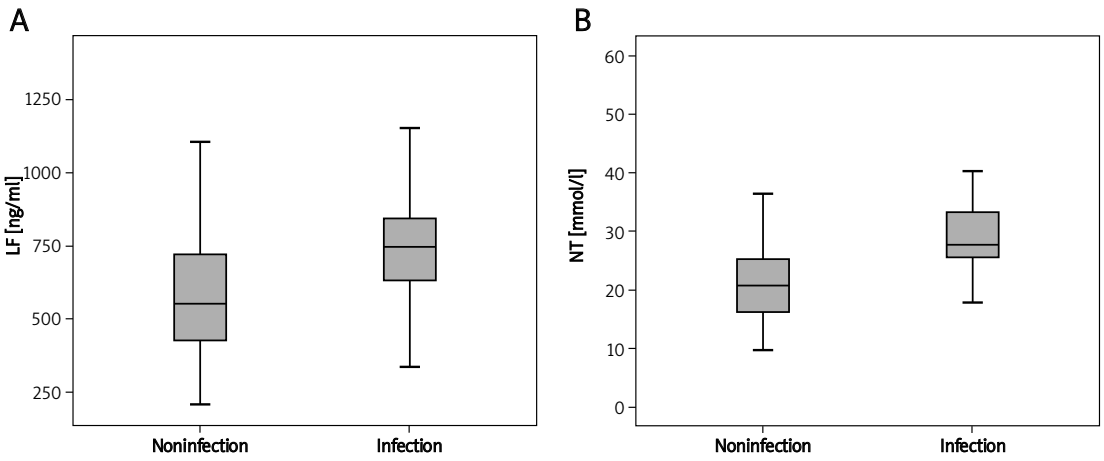

Figure 1. Postoperative serum levels of LF (**A**) and NT (**B**) in patients with and without infection. The concentrations of LF and NT were determined among infected and non-infected patients at 24 h after surgery. The levels of LF and NT were significantly higher in the infected patients than in the non-infected patients (Mann-Whitney *U* test, LF: $p = 0.001$; NT: $p < 0.001$), indicating that elevated LF and NT can be used to predict infection in the early post-operative stage

Table II. Comparison of laboratory test results between patients with and without infection

Laboratory tests	Infection ($n = 22$)	Non-infection ($n = 246$)	Value of p
Preoperative			
LF [ng/ml]	290.59 (257.32–384.53)	297.54 (250.06–378.36)	0.865
NT [nmol/l]	15.10 (12.13–19.78)	13.76 (10.20–18.06)	0.198
CRP [mg/l]	8.94 (5.67–31.38)	8.85 (5.77–18.56)	0.585
WBC [10^9/mm^3]	7.20 ±1.94	7.64 ±2.29	0.384
ESR [mm/h]	7.50 (5.00–10.00)	7.00 (4.00–9.00)	0.864
Postoperative			
LF [ng/ml]	730.55 (597.98–862.55)*	553.05 (426.69–721.80)*	0.001
NT [nmol/l]	27.19 (23.32–32.86)*	20.70 (16.08–25.26)*	< 0.001
CRP [mg/l]	54.00 (42.97–67.94)*	46.69 (28.24–58.80)*	0.046
WBC [10^9/mm^3]	8.04 ±2.23*	7.97 ±2.25*	0.885
ESR [mm/h]	8.00 (6.00–11.00)*	8.00 (6.00–9.00)*	0.321

**p < 0.05 compared with preoperative tests. LF – lactoferrin, NT – neopterin, CRP – C-reactive protein, WBC – white blood cell count, ESR – erythrocyte sedimentation rate*

ROC curve

The area under the curve (AUC) of LF (0.709, 95% CI: 0.599–0.820, $p = 0.001$) and NT (0.779, 95% CI: 0.683–0.875, $p = 0.000$) was greater than the AUC of CRP (0.629, 95% CI: 0.520–0.737, $p = 0.046$) (Figure 2). According to the maximum of Youden's index, the best cut-off values for LF, NT and CRP were 675 ng/ml, 25 nmol/l and 45 mg/l, respectively (Table IV).

Discussion

Postoperative infections in SCI patients can result in increased morbidity and mortality, prolonged hospitalization, and high costs to patients. As a result, it is critical to diagnose the infection through specific biomarkers in the early postoperative stage so that clinicians can choose appropriate antibiotic therapy. However, conventional laboratory CRP and ESR tests may be atypical in patients with postoperative infectious complications because these indicators are elevated in most patients after surgery or trauma, and so lack the specificity to predict potential postoperative infection [16–19]. Thus, it is necessary to find more specific indicators to detect postoperative infection.

It has been widely demonstrated that LF and NT are biomarkers for activation of the immune system stimulated by bacterial infection. Previous studies showed that serum LF and NT concentrations were reliable prognostic factors for detecting early infections [10, 20]. Timely and accurate diagnosis

Table III. Association of laboratory test results and postoperative infectious complications

Variables	Simple value of *p*	Multivariate value of *p*
Age	0.847	0.507
Gender	0.696	0.682
Cause of SCI	0.225	0.144
Region of SCI	0.249	0.092
AIS	0.864	0.502
Type of operation	0.655	0.913
Approach	0.723	0.093
Number of levels	0.496	0.638
Operative estimated blood loss	0.478	0.859
Time of operation	0.631	0.787
Postoperative		
LF [ng/ml]	0.001	0.001
NT [nmol/l]	< 0.001	0.001
CRP [mg/l]	0.036	0.003
WBC [10^9/mm^3]	0.884	0.620
ESR [mm/h]	0.193	0.251

SCI – patients with acute traumatic spinal cord injury, AIS – American Spinal Injury Association Impairment Scale, LF – lactoferrin, NT – neopterin, CRP – C-reactive protein, WBC – white blood cell count, ESR – erythrocyte sedimentation rate

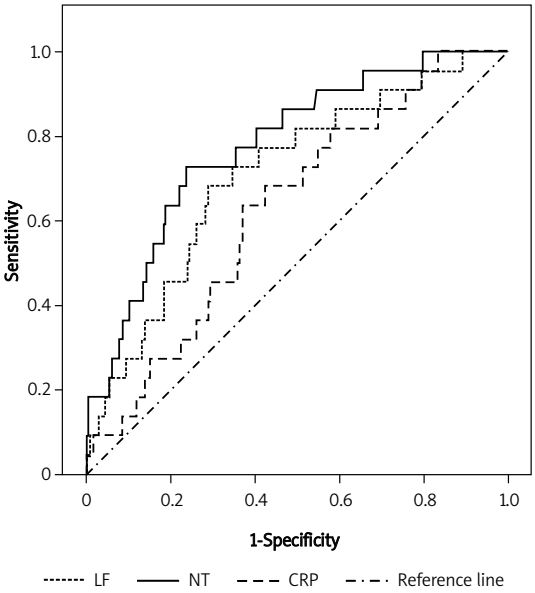

Figure 2. ROC curves for diagnosing postoperative infectious complications from LF (AUC = 0.709), NT (AUC = 0.779), and CRP (AUC = 0.629). Performance of LF, NT and CRP was compared with predictions made by chance. The AUCs of these indicators were significant in discriminating the infected and non-infected patients, and the performance of LF and NT was superior to that of CRP (ROC, LF, AUC = 0.709, 95% CI: 0.599–0.820, $p = 0.001$; NT: AUC = 0.779, 95% CI: 0.683–0.875, $p < 0.001$; CRP: AUC = 0.629, 95% CI: 0.520–0.737, $p = 0.046$)

of postoperative infectious complications determines whether and when to administer antibiotics. Indeed, elevations in LF and NT have been used to direct antibiotic therapy [11, 21], but the predictive value of LF and NT has not been explored in patients with SCI. In the present study, we addressed the issue by examining the serum LF and NT levels for diagnosing early postoperative infectious complications in a large cohort of SCI patients.

We found an incidence rate of postoperative infectious complications in SCI patients of 8.2%, consistent with a previous study [2]. There were no significant differences in WBC counts and ESR between the infected and non-infected patients. The concentrations of LF and NT were significantly higher in the infected patients within 24 h after surgery, indicating that elevated LF and NT can be used to predict infection in the early postoperative stage and to establish timely antibiotic therapy.

Lactoferrin is a multifunctional iron-binding glycoprotein of 80 kDa located in the secondary granules of neutrophils, a key cellular component of the

Table IV. Cut-off values of LF, NT and CRP for diagnosing postoperative infectious complications

Laboratory tests	Cut-off	Sensitivity [%]	Specificity [%]	Youden's Index
LF [ng/ml]	625	72.7	61.8	0.345
	675	68.2	71.1	0.393
	725	54.5	75.6	0.301
NT [nmol/l]	20	86.4	48.0	0.158
	25	72.7	73.2	0.459
	30	36.4	90.2	0.266
CRP [mg/l]	30	86.4	30.5	0.169
	45	72.7	52.8	0.199
	60	31.8	76.8	0.086

LF – lactoferrin, NT – neopterin, CRP – C-reactive protein

innate immune system against infection and inflammation [22]. Once activated, LF is released by neutrophil degranulation and exerts antimicrobial activity by sequestering iron necessary for bacterial survival and by inducing bacterial lysis through direct interaction with bacterial surfaces [23]. Previous studies found that LF can be used to diagnose infectious diseases such as sepsis or urinary tract infection [5, 24].

Neopterin, a metabolite of guanosine triphosphate, is an indicator associated with cell-mediated immunity. It is secreted by macrophages when stimulated by interferon-γ [25] and can generate reactive oxygen metabolites and trigger synthesis of nitric oxide [26]. Neopterin can be released as early as three days before peak proliferation of T cells. Increased serum NT is related to endothelium damage and septic complications, and has been observed in patients with tuberculosis and early bacterial infection [27, 28].

Serum CRP is a ubiquitous marker of inflammation. Although some studies have used CRP as an indicator of postoperative infection after spinal surgery [16, 17, 29], the predictive efficacy was not compared to LF and NT. In the present study, the larger AUCs of the ROC curves indicated that LF and NT were more reliable than CRP. At the best cut-off values (LF: 675 ng/ml, NT: 25 nmol/l, CRP: 45 mg/l), the specificity of LF (71.1%) and NT (73.2%) was also higher than that of CRP (52.8%). This indicates that during the early postoperative stage, the increase in CRP associated with trauma or surgery may mislead clinicians and result in the administration of unnecessary antibiotics. On the other hand, elevated LF and NT are specific enough to predict infection after surgery. Accurate diagnosis can lead to optimal and timely antibiotic therapy to prevent morbidity and mortality due to infection.

While the patient cohort was relatively large, the number of infected patients was relatively small. Thus, a larger scale prospective study is necessary to confirm the reliability of LF and NT. Furthermore, we measured biomarkers at only one time point after surgery, so further studies are necessary to assess the dynamics of LF and NT following surgery and during the course of different types of infection.

In conclusion, the present study suggests that serum LF and NT are timely and accurate biomarkers for the diagnosis of postoperative infectious complications. Elevated serum LF and NT in an early postoperative stage can be used to guide appropriate antibiotic therapy in infected patients and to reduce superfluous treatment for non-infected patients. Finally, postoperative infectious complication is a complex inflammatory state associated with bacterial infections, trauma, and the effects of surgery. Therefore, our conclusions on the prognostic value of LF and NT are preliminary and must be confirmed in larger patient samples.

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References

1. Blam OG, Vaccaro AR, Vanichkachorn JS, et al. Risk factors for surgical site infection in the patient with spinal injury. Spine (Phila Pa 1976) 2003; 28: 1475-80.
2. Pull ter Gunne AF, van Laarhoven CJ, Cohen DB. Incidence of surgical site infection following adult spinal deformity surgery: an analysis of patient risk. Eur Spine J 2010; 19: 982-8.
3. Codine P, Barbotte E, Denis-Laroque F, et al. C-reactive protein, leukocyte count and d-dimer monitoring after orthopedic surgery: early diagnosis of infectious or thromboembolic complications. Part two: does d-dimer measurement contribute to the diagnosis of postoperative venous thromboembolism?. Ann Readapt Med Phys 2005; 48: 598-602.
4. Valenti P, Berlutti F, Conte MP, et al. Lactoferrin functions: current status and perspectives. J Clin Gastroenterol 2004; 38: S127-9.
5. Pan Y, Sonn GA, Sin ML, et al. Electrochemical immunosensor detection of urinary lactoferrin in clinical samples for urinary tract infection diagnosis. Biosens Bioelectron 2010; 26: 649-54.
6. Pugh C, Hathwar V, Richards JH, et al. Disruption of iron homeostasis in the lungs of transplant patients. J Heart Lung Transplant 2005; 24: 1821-7.
7. Wanscher M, Tonnesen E, Antonsen S, et al. Increase in lactoferrin and elastase alpha-1-proteinase inhibitor complexes but lack of complement activation and IL-6 response following thoracic surgery. Acta Anaesthesiol Scand 1993; 37: 597-601.
8. Gutteberg TJ, Flaegstad T, Jorgensen T. Lactoferrin, C-reactive protein, alpha-1-antitrypsin and immunoglobulin ga in cerebrospinal fluid in meningitis. Acta Paediatr Scand 1986; 75: 569-72.
9. Millner MM, Franthal W, Thalhammer GH, et al. Neopterin concentrations in cerebrospinal fluid and serum as an aid in differentiating central nervous system and peripheral infections in children. Clin Chem 1998; 44: 161-7.
10. Ruokonen E, Ilkka L, Niskanen M, et al. Procalcitonin and neopterin as indicators of infection in critically ill patients. Acta Anaesthesiol Scand 2002; 46: 398-404.
11. Tasdelen Fisgin N, Aliyazicioglu Y, Tanyel E, et al. The value of neopterin and procalcitonin in patients with sepsis. South Med J 2010; 103: 216-9.
12. Garner JS, Jarvis WR, Emori TG, et al. CDC definitions for nosocomial infections 1988. Z Arztl Fortbild (Jena) 1991; 85: 818-27.
13. Ksycki MF, Namias N. Nosocomial urinary tract infection. Surg Clin North Am 2009; 89: 475-81.
14. Mok JM, Guillaume TJ, Talu U, et al. Clinical outcome of deep wound infection after instrumented posterior spinal fusion: a matched cohort analysis. Spine (Phila Pa 1976) 2009; 34: 578-83.

15. Morrow LE, Kollef MH. Recognition and prevention of nosocomial pneumonia in the intensive care unit and infection control in mechanical ventilation. Crit Care Med 2010; 38: S352-62.
16. Takahashi J, Ebara S, Kamimura M, et al. Early-phase enhanced inflammatory reaction after spinal instrumentation surgery. Spine (Phila Pa 1976) 2001; 26: 1698-704.
17. Chung YG, Won YS, Kwon YJ, et al. Comparison of serum crp and procalcitonin in patients after spine surgery. J Korean Neurosurg Soc 2011; 49: 43-8.
18. Takahashi J, Shono Y, Hirabayashi H, et al. Usefulness of white blood cell differential for early diagnosis of surgical wound infection following spinal instrumentation surgery. Spine (Phila Pa 1976) 2006; 31: 1020-5.
19. Dupont C, Rodenbach J, Flachaire E. The value of c-reactive protein for postoperative monitoring of lower limb arthroplasty. Ann Readapt Med Phys 2008; 51: 348-57.
20. Thuijls G, Derikx JP, Prakken FJ, et al. A pilot study on potential new plasma markers for diagnosis of acute appendicitis. Am J Emerg Med 2011; 29: 256-60.
21. van der Velden WJ, Blijlevens NM, Donnelly JP. The potential role of lactoferrin and derivatives in the management of infectious and inflammatory complications of hematology patients receiving a hematopoietic stem cell transplantation. Transpl Infect Dis 2008; 10: 80-9.
22. Ward PP, Uribe-Luna S, Conneely OM. Lactoferrin and host defense. Biochem Cell Biol 2002; 80: 95-102.
23. Gonzalez-Chavez SA, Arevalo-Gallegos S, Rascon-Cruz Q. Lactoferrin: structure, function and applications. Int J Antimicrob Agents 2009; 33: e301-8.
24. Barkhatova NA. The use of plasma lactoferrin in the diagnosis of pyonecrotic infections of soft tissues and sepsis. Klin Med (Mosk) 2008; 86: 36-8.
25. Hamerlinck FF. Neopterin: a review. Exp Dermatol 1999; 8: 167-76.
26. Pourakbari B, Mamishi S, Zafari J, et al. Evaluation of procalcitonin and neopterin level in serum of patients with acute bacterial infection. Braz J Infect Dis 2010; 14: 252-5.
27. Murr C, Widner B, Wirleitner B, et al. Neopterin as a marker for immune system activation. Curr Drug Metab 2002; 3: 175-87.
28. Ozdemir D, Cesur S, Annakkaya AN, et al. Serum neopterin concentrations in healthy healthcare workers compared with healthy controls and patients with pulmonary tuberculosis. Med Sci Monit 2006; 12: CR521-4.
29. Kang BU, Lee SH, Ahn Y, et al. Surgical site infection in spinal surgery: detection and management based on serial c-reactive protein measurements. J Neurosurg Spine 2010; 13: 158-64.