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

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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-
operative 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/mm³, or (2) urine culture ≥ 10⁷ microorganisms/cm³. 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 × 10⁹/l or WBC < 4 × 10⁹/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 Synchro 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 χ² 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
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).

**ROSC 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...
Elevated serum lactoferrin and neopterin are associated with postoperative infectious complications in patients with acute traumatic spinal cord injury 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 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, \( p = 0.046 \))

### 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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