Procalcitonin and C-reactive Protein Serum Levels After Hematopoietic Stem-Cell Transplant

Negar Azarpira, Mani Ramzi, Mahdokht Aghdaie, Masumeh Daraie

Abstract

Objectives: **Hematopoietic stem-cell transplant is a curative therapy for several malignant and nonmalignant disorders. The purpose of this study was to investigate the association of serum levels of high-sensitivity C-reactive protein and procalcitonin with complications such as acute graft-versus-host disease, veno-occlusive disease, and infection after hematopoietic stem-cell transplant.**

Materials and Methods: **Serum high-sensitivity C-reactive protein and procalcitonin levels were sequentially measured with an enzyme-linked immunosorbent assay and a semiquantitative immunochromatographic assay in 35 patients who had undergone hematopoietic stem-cell transplant.**

Results: **The high-sensitivity C-reactive protein serum level was increased in patients with acute graft-versus-host disease and in those with sepsis. Increased procalcitonin levels were associated only with bacterial infection. Only procalcitonin levels differentiated patients with infection from those with another transplant-related complication. Veno-occlusive disease did not alter C-reactive protein or procalcitonin levels.**

Conclusions: **Our results support theories that serum levels of high-sensitivity C-reactive protein and procalcitonin are biomarkers for transplant-related complications such as graft-versus-host disease or infection and that the procalcitonin level can differentiate patients with infection from those with graft-versus-host disease.**

Key words: *Biomarker, Bone marrow, Complication, Graft-versus-host disease, Infection*

In graft-versus-host disease, which is a serious complication after allogeneic hematopoietic stem-cell transplant, donor-derived T cells infiltrate recipient organs and cause severe tissue damage. Other complications (infection, veno-occlusive disease) that can develop after hematopoietic stem-cell transplant can cause similar symptoms. Therefore, identification of the sources of those complications is essential, and early, appropriate treatment is the only promising tool. Monitoring the serum levels of some biomarkers before and after hematopoietic stem-cell transplant may predict the occurrence of such complications (1).

C-reactive protein, which is produced by hepatocytes, is a useful biomarker for bacterial infections (2), but elevated C-reactive protein serum levels have also been noted during viral infections (3), in cancer patients with fever (4), and in patients who have undergone bone marrow engraftment (5). Procalcitonin, which is the propeptide of calcitonin, is devoid of hormonal activity and is usually produced in the C cells of the thyroid gland. Procalcitonin consists of 116 amino acids with a half-life of 25 to 30 hours, is produced in response to endotoxin, and is a sensitive indicator of bacterial sepsis (6). Levels of procalcitonin are undetectable (ie, $< 0.1\ \mu g/L$) in healthy individuals. In this study, we evaluated the effects of the above-mentioned biomarkers during pretransplant and posttransplant periods and assessed the association of those markers with acute graft-versus-host disease, veno-occlusive disease, and infection.

From the Organ Transplant Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

Acknowledgements: *Organ Transplantation Research Center affiliated to Shiraz University of Medical Sciences financially supported this study.*

Address reprint requests to: *Negar Nazarpira, MD, Shiraz University of Medical Sciences, Organ Transplant Research Center, Zand Street Shiraz 7193711351, Islamic Republic of Iran*
Phone*: +98 711 627 62 11* ***Fax****: +98 711 62 76 21* ***E-mail:*** *negarazarpira@yahoo.com*

Experimental and Clinical Transplantation (2009) 2: 115-118

Materials and Methods

This single-center study was performed in the Bone Marrow Transplantation Center of Shiraz University of Medical Sciences in Shiraz, Iran, to evaluate the clinical relevance of C-reactive protein and procalcitonin serum levels in patients who had undergone hematopoietic stem-cell transplant. No exclusion criteria were defined. Human leukocyte antigen-A and human leukocyte antigen-B typing were performed via serologic analyses or molecular typing. The study was conducted according to the guidelines of the Declaration of Helsinki 1975. The ethics committee of Shiraz University of Medical Sciences approved the study protocol before its initiation, and all patients provided written informed consent that permitted analyses of the clinical data and the testing described in this report. The clinical condition of the patients (eg, complications) was reported on the day of blood sampling. Uncomplicated hematopoietic stem-cell transplant was defined as *the absence of organ dysfunction and complications*. The commonly used conditioning regimen consisted of administration of busulfan, cyclophosphamide, and antithymocyte globulin. Conditioning regimens for thalassemic patients consisted of cyclophosphamide and busulfan. Graft-versus-host disease prophylaxis included cyclosporine/methotrexate 6 to 10 mg/m^2 on days 1, 3, and 6 after hematopoietic stem-cell transplant. Graft-versus-host disease was graded according to clinical and biochemical criteria (7). Hepatic veno-occlusive disease was diagnosed and classified according to the clinical syndrome of hepatomegaly and pain, fluid retention, weight gain, and a serum bilirubin concentration of more than 2 mg/dL (8).

Blood sampling and assessments

Blood sampling was performed on day zero (before transplant) and 1 and 3 weeks after transplant in 35 patients (11 women and 24 men; age range, 3-48 years) who had undergone hematopoietic stem-cell transplant. The demographic characteristics of the patients studied are shown in the Table 1. Sixteen patients had acute or chronic leukemia, 12 had thalassemia, and 7 had metastatic bone marrow lesions. The high-sensitivity C-reactive protein level was measured with an enzyme linked immunosorbent assay kit (MP Biomedicals. Diagnostics Division. Orangeburg, NY 10962-1294). The procalcitonin was determined by means of a semiquantitative immunochromatographic assay (B.R.A.H.M.S. Procalcitonin-Q, Aktiengesellschaft, Berlin, Germany) that required 200 μL of serum plasma per sample. According to information from the manufacturer of that assay, procalcitonin serum levels in healthy individuals are lower than 0.5 ng/mL, and levels higher than 10 ng/mL indicate a systemic bacterial infection.

Table 1. Transplant characteristic of the studied patients.

Parameter	Patient n (%)
Total	35
Sex,	
Male	24 (68.5)
Female	11 (31.4)
Age (Year, Range)	30 (13-50)
Underlying disease	
- Acute and Chronic leukemia	16 (45.7)
- Thalasemia	12 (34.2)
- Metastatic lesions	7 (20)
Outologus BMT	19 (54.3)
Allogenic BMT	16 (45.7)
Donor type in allogen, Sibling/ Unrelated	16/0
Donor sex in allogen , Male/Female	13/3
Donor age in allogen (Year, Range)	22.4 (25-30)
Graft source	
Bone marrow	3 (8.5)
Peripheral blood	32 (91.4)

Abbreviation: BMT, Bone marrow transplant

Statistical analysis

The Mann-Whitney U test, the Pearson chi-square test, and the Fisher exact test were used to perform the various statistical analyses. A *P* value of less than .05 was considered statistically significant. Analyses were performed with SPSS software (Statistical Product and Service Solutions, version 15, SSPS Inc, Chicago, IL, USA).

Results

Nineteen patients (54.3%) underwent autologous hematopoietic stem-cell transplant, and 16 (45.7%) underwent allogeneic hematopoietic stem-cell transplant. Acute graft-versus-host disease (range, grade I-III; mean, 17 days) developed in 11 patients (34.37%). Engraftment produced a neutrophil count of more than 0.5×10^9 /L after a mean of day 10 in patients without acute graft-versus-host disease (range, 8-18 days) and after a mean of day 13 in patients with acute graft-versus-host disease (range,

10-26 days). Engraftment was earlier in patients without acute graft-versus-host disease (mean of day 10 for neutrophils and day 12 for platelets > 20 000/μL) than in those with acute graft-versus-host disease (mean of day 13 for neutrophils and day 24 for platelets). We did not find a significant correlation between donor age, underlying disease, or sex, and acute graft-versus-host disease.

Serum levels of high-sensitivity C-reactive protein in patients with acute graft-versus-host disease were higher than those in patients without acute graft-versus-host disease, but that difference was not statistically significant (20 ± 2.5 mg/L vs 10.12 ± 5.2 mg/L, respectively) (P = .06). There was no significant difference in high-sensitivity C-reactive protein levels in patients with different grades of graft-versus-host disease. As acute graft-versus-host disease improved, high-sensitivity C-reactive protein levels decreased; this showed that those levels may be correlated with disease status.

The serum level of procalcitonin did not differ significantly on different days in subjects with acute graft-versus-host disease and those without that disease. The procalcitonin levels before hematopoietic stem-cell transplant did differ significantly on different days according to disease and drug regimen. Two patients exhibited fulminant sepsis with bacterial infection, and systemic mucormycosis developed in 1 patient. Only in the patients with bacterial sepsis was the procalcitonin serum level increased (ie, > 10 ng/mL). After hematopoietic stem-cell transplant, only elevated procalcitonin levels differentiated patients with infection from those with another transplant-related complication. No altered levels in high-sensitivity C-reactive protein and procalcitonin were noted in patients with veno-occlusive disease. There were no differences with regard to hematopoietic stem-cell transplant type (autologous or allogenic), underlying disease, age, or conditioning regimen and either high-sensitivity C-reactive protein or procalcitonin serum levels.

Discussion

Defining the pathogenesis of transplant-related complications (infection, graft-versus-host disease, veno-occlusive disease) and differentiating them is often difficult, because many of those disorders produce similar clinical symptoms. Early detection and prompt treatment of major transplant-related complications are critical to the recovery of the patient. Multiple cytokines have been shown to contribute to the development, severity, and persistence of those complications (9-12), the clinical significance of which remains controversial (12-14). We conducted this study in hematopoietic stem-cell recipients to evaluate the significance of high-sensitivity C-reactive protein and procalcitonin serum levels in the differentiation of transplant-related complications. Our major findings can be summarized as follows: The high-sensitivity C-reactive protein serum level was increased in patients with acute graft-versus-host disease and in those with sepsis. Increased procalcitonin levels were associated only with bacterial infection. Only procalcitonin levels could be used to differentiate infection from other transplant-related complications. Veno-occlusive disease did not alter serum levels of C-reactive protein or procalcitonin.

C-reactive protein has been shown to be a useful marker of bacterial infection (2); however, elevated C-reactive protein serum levels have also been noted in patients with a viral infection (3), in cancer patients with fever associated with the underlying malignancy (4), in patients who have undergone engraftment (5), in those with tissue necrosis (15), and in obese individuals (16). Conversely, the synthesis of C-reactive protein may be reduced in patients with liver failure and in those treated with a steroid (2, 17).

Pihusch and colleagues (13) reported that the C-reactive protein serum level was increased in patients with an infection. Those authors suggested that bacterial infections in particular correlate with high C-reactive protein serum levels and that C-reactive protein is not usually a biomarker for viral infections. They found (as we did) elevated serum levels of C-reactive protein in patients with either a bacterial infection or acute graft-versus-host disease. C-reactive protein seems to have a host-defensive mechanism, because it binds to the phosphocholine in microbial polysaccharides and activates the classic complement pathway. An elevated level of C-reactive protein is not a valuable clue for prediction in patients with an infection. However, it has also been reported that soon after allogeneic stem-cell transplant, serum C-reactive protein levels might display the graft-versus-leukemia effect (17).

Procalcitonin, a precursor protein of calcitonin, is produced in response to endotoxins and cytokines in the sepsis cascade (6). Procalcitonin has proven to be

a sensitive indicator of bacterial sepsis in immunocompetent and neutropenic patients (18,19). In septic patients, the prognostic value of procalcitonin has been higher than that of C-reactive protein (20, 21). An increased level of procalcitonin has also been noted after T-cell antibody infusion in adult renal transplant patients (22) and in adults and children receiving T-cell antibodies (5). Blijlevens and colleagues first reported single cases of graft-versus-host disease associated with moderately increased procalcitonin serum levels (14). However, in our study, neither acute graft-versus-host disease nor fungal infection increased procalcitonin levels. Only procalcitonin levels in patients with an infection were different from those in patients without a transplant-related complication; this finding is similar to that of Pihusch and colleagues (13).

According to recent research by Paczesny and colleagues (9), the biomarker panel of 4 proteins (interleukin 2 receptor alpha, tumor necrosis factor receptor 1, interleukin 8, and hepatocyte growth factor) can be used to differentiate patients with graft-versus-host disease from those without that disease. The technique used in our study is comparatively simple, inexpensive, and reproducible. Nevertheless, it has not achieved widespread clinical use because of limitations such as availability or cost.

Our study did not show any significant correlation between underlying disease and age or levels of inflammatory markers. One of the limitations of our study is its sample size. It is necessary to conduct a large prospective trial in allogeneic stem-cell recipients to validate the clinical significance of high-sensitivity C-reactive protein, procalcitonin serum levels, interleukin-8, and hepatocyte growth factor in the differentiation of transplant-related complications after hematopoietic stem-cell transplant.

References

1. Lunn RA, Sumar N, Bansal AS, Treleaven J. Cytokine profiles in stem cell transplantation: possible use as a predictor of graft-versus-host disease. Hematology. 2005;10(2):107-114.
2. Hansson LO, Carlsson I, Hansson E, et al. Measurement of C-reactive protein and the erythrocyte sedimentation rate in general practice. Scand J Prim Health Care. 1995;13(1):39-45
3. Toikka P, Irjala K, Juvén T, et al. Serum procalcitonin, C-reactive protein and interleukin-6 for distinguishing bacterial and viral pneumonia in children. Pediatr Infect Dis J. 2000;19(7):598-602
4. Legouffe E, Rodriguez C, Picot MC, et al. C-reactive protein serum level is a valuable and simple prognostic marker in non Hodgkin's lymphoma. Leuk Lymphoma. 1998;31(3-4):351-357.
5. Hambach L, Eder M, Dammann E, et al. Diagnostic value of procalcitonin serum levels in comparison with C-reactive protein in allogeneic stem cell transplantation. Haematologica. 2002;87(6):643-651.
6. Müller B, White JC, Nylén ES, Snider RH, Becker KL, Habener JF. Ubiquitous expression of the calcitonin-i gene in multiple tissues in response to sepsis. J Clin Endocrinol Metab. 2001;86(1):396-404.
7. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation. 1974;18(4):295-304.
8. Jones RJ, Lee KS, Beschorner WE, et al. Venoocclusive disease of the liver following bone marrow transplantation. Transplantation. 1987;44(6):778-783.
9. Paczesny S, Krijanovski OI, Braun TM, et al. A biomarker panel for acute graft-versus-host disease. Blood. 2009;113(2):273-278.
10. Dean RM, Fry T, Mackall C, et al. Association of serum interleukin-7 levels with the development of acute graft-versus-host disease. J Clin Oncol. 2008;26(35):5735-5741.
11. Shaiegan M, Iravani M, Babaee GR, Ghavamzadeh A. Effect of IL-18 and sIL2R on aGVHD occurrence after hematopoietic stem cell transplantation in some Iranian patients. Transpl Immunol. 2006;15(3):223-227.
12. Visentainer JE, Lieber SR, Persoli LB, et al. Serum cytokine levels and acute graft-versus-host disease after HLA-identical hematopoietic stem cell transplantation. Exp Hematol. 2003;31(11):1044-1050.
13. Pihusch M, Pihusch R, Fraunberger P, et al. Evaluation of C-reactive protein, interleukin-6, and procalcitonin levels in allogeneic hematopoietic stem cell recipients. Eur J Haematol. 2006;76(2):93-101.
14. Blijlevens NM, Donnelly JP, Meis JF, De Keizer MH, De Pauw BE. Procalcitonin does not discriminate infection from inflammation after allogeneic bone marrow transplantation. Clin Diagn Lab Immunol. 2000;7(6):889-892.
15. Yip HK, Hang CL, Fang CY, et al. Level of high-sensitivity C-reactive protein is predictive of 30-day outcomes in patients with acute myocardial infarction undergoing primary coronary intervention. Chest. 2005;127(3):803-808.
16. Saijo Y, Kiyota N, Kawasaki Y, et al. Relationship between C-reactive protein and visceral adipose tissue in healthy Japanese subjects. Diabetes Obes Metab. 2004;6(4):249-258.
17. Min CK, Kim SY, Eom KS, et al. Patterns of C-reactive protein release following allogeneic stem cell transplantation are correlated with leukemic relapse. Bone Marrow Transplant. 2006;37(5):493-498.
18. Christ-Crain M, Müller B. Procalcitonin in bacterial infections–hype, hope, more or less? Swiss Med Wkly. 2005;135(31-32):451-460.
19. Christ-Crain M, Jaccard-Stolz D, Bingisser R, et al. Effect of procalcitonin-guided treatment on antibiotic use and outcome in lower respiratory tract infections: cluster-randomised, single-blinded intervention trial. Lancet. 2004;363(9409):600-607.
20. Persson L, Söderquist B, Engervall P, Vikerfors T, Hansson LO, Tidefelt U. Assessment of systemic inflammation markers to differentiate a stable from a deteriorating clinical course in patients with febrile neutropenia. Eur J Haematol. 2005;74(4):297-303.
21. Simon L, Gauvin F, Amre DK, Saint-Louis P, Lacroix J. Serum procalcitonin and C-reactive protein levels as markers of bacterial infection: a systematic review and meta-analysis. Clin Infect Dis. 2004;39(2):206-217. Erratum in: Clin Infect Dis. 2005;40(9):1386-1388.
22. Sabat R, Höflich C, Döcke WD, et al. Massive elevation of procalcitonin plasma levels in the absence of infection in kidney transplant patients treated with pan-T-cell antibodies. Intensive Care Med. 2001;27(6):987-991.