

Neopterin and other inflammatory markers in the early period following allogeneic bone marrow transplantation: series of 3 cases

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KEY WORDS

allogeneic bone marrow transplantation, interleukin 18, neopterin, serum amyloid A

ABSTRACT

The 3 case reports presented here constitute a pilot study assessing the profile of changes in concentrations of selected inflammatory markers, including C-reactive protein, serum amyloid A, neopterin, and interleukin 18, in an early period after allogeneic stem cell transplantation.

INTRODUCTION Allogeneic hematopoietic stem cell transplantation (alloHSCT) is currently a recognized therapeutic option for malignant and nonmalignant diseases of the hematopoietic system. High-dose chemotherapy and transplantation-conditioning chemoradiotherapy entail organ and tissue damage and thus may cause inflammation and release of cytokines as well as inflammatory mediators (tumor necrosis factor- α [TNF- α], interleukin [IL]-1 β , IL-18).¹⁻³ The onset of acute graft versus host disease (aGVHD) coincides with the period of aplasia associated with the highest risk of infection and other complications resulting from the toxicity of conditioning therapy. Hence, the differential diagnosis of early posttransplant complications poses a major clinical challenge.^{1,2,4}

Acute phase proteins, especially C-reactive protein (CRP), are commonly used to monitor the activity of inflammatory response. Serum amyloid A protein (SAA) is synthesized in the liver in response to stress and inflammation, mainly under the influence of IL-1 β , IL-6, and TNF- α . SAA is characterized by the most dynamic increase in serum concentrations among all the known acute phase proteins, and its measurement is more useful than that of CRP levels in patients subjected to immunosuppression during bone marrow transplantation.⁵

Neopterin is regarded as a marker of cell-mediated immune response, and its deter-

mination is sometimes useful in evaluating the extent of the inflammatory response and its prognosis. Neopterin is mainly used for monitoring allogeneic graft recipients, so as to early diagnose complications such as bacterial and viral infections.⁶

CASE 1 The patient was a 19-year-old man with severe aplastic anemia. Cyclophosphamide (Cy200) was used in graft conditioning. The graft material was made up of stem cells harvested from human leukocyte antigen (HLA)-compatible bone marrow taken from the patient's brother (mononuclear cells [MNC] $5.95 \times 10^8/\text{kg}$ body weight, CD34(+) $3.15 \times 10^6/\text{kg}$ body weight). Cyclosporine A and 4 doses of methotrexate ($8 \text{ mg}/\text{m}^2$) were used for GVHD prophylaxis (TABLE). No complications occurred in the posttransplant period. Spontaneous hematopoietic regeneration (>500 neutrophils/ μl) was achieved on day 15. No aGVHD signs and symptoms were observed. The patient was discharged 28 days after grafting. We did not detect any symptoms of chronic GVHD during follow-up.

From day -7 (before alloHSCT), IL-18 concentrations started to fall within the value range determined in the population of healthy volunteers ($31.6\text{--}257.8 \text{ pg}/\text{ml}$), reaching maximum values of $235.9 \text{ pg}/\text{ml}$ on day +4 (after alloHSCT). The CRP and SAA concentrations remained close

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TABLE Clinical status of patients after alloHSCT with different grades of aGVHD

Patients	Sex (donor/bone recipient)	Disease progression by Tx	BMT/PBSCT	Conditioning	CMV status	aGVHD prophylaxis	CD34 10 ⁶ /kg body weight	MNC 10 ⁶ /kg body weight	aGVHD	Relapse	cGVHD	Death
case 1	M/M	CR1	BMT	Cy200	+/+	CSA + MTX	3.15	5.95	0	–	–	no
case 2	F/M	CR1	BMT	BuCy2	+/+	CSA + MTX	4.52	2.17	III	–	yes	yes
case 3	M/F	CR1	BMT	BuCy2	+/+	CSA + MTX	1.07	1.13	IV (+6)	–	–	yes

BuCy2 = busulfan 16 mg/kg + cyclophosphamide 120 mg/kg

CSA + MTX = cyclosporine A (3 mg/kg IV from day –1) + methotrexate IV (15 mg/m² on day +1; 10 mg/m² on day +3, +6, +11 [administration of methotrexate on day +11 depending on the patient's condition])

Cy200 = cyclophosphamide 200 mg/m²

Abbreviations: aGVHD – acute graft versus host disease, BMT – bone marrow transplantation, cGVHD – chronic GVHD, CR1 – first remission, CMV – cytomegalovirus, MNC – mononuclear cells, PBSCT – peripheral blood stem cell transplantation, Tx – transplantation

to the normal range, and during the whole peritransplant period did not exceed 7.2 mg/l for SAA, and 4.29 mg/l for CRP (day 0). Absence of an inflammatory process and activation of the immune system were confirmed by normal neopterin concentrations (0–10 nmol/l), which were maintained during the peritransplant period. The highest concentration (17.45 nmol/l) was observed on day 0.

CASE 2 The patient was a 38-year-old woman with acute myeloblastic leukemia (AML-M4 according to the French-American-British [FAB] classification), and a high risk of disease relapse due to complex cytogenetic changes. Chemotherapy using BuCy2 was applied for graft conditioning. Graft material was harvested from HLA-compatible peripheral blood obtained from the patient's brother after prior mobilization by granulocyte colony-stimulating factor (G-CSF), in doses of MNC 2.17×10^8 /kg body weight, and CD34(+) 4.52×10^6 /kg body weight (TABLE). Furthermore, there was major and minor ABO and Rh– incompatibility. Cyclosporine A and methotrexate were used for GVHD prophylaxis. During the period of aplasia, the patient was pyrexia (>38°C from day +7), and the results of microbiological tests were negative. Spontaneous regeneration of hematopoiesis (neutrophils >500/μl) was achieved on day +16. During follow-up, there were symptoms of aGVHD, manifest as a cutaneous rash covering 50% of the body surface area, cholestatic jaundice with deterioration of the patient's condition, classified as stage 3 and requiring systemic steroid therapy on day +17. Initially, there was a positive response to immunosuppressive therapy, but later infectious complications developed and increasing cytopenia occurred due to ABO incompatibility, among others. Eventually, aGVHD transformed into a chronic extensive form with a number of complications. The patient died on day 198 as a result of posttransplant complications.

From day –7, IL-18 concentrations were twice as high as in Case 1, thus exceeding normal values. An almost 2- to 3-fold increase in its concentration for 10 days after alloHSCT indicates activation of the immune system. Neopterin, whose concentrations between day –7 and +8 remained normal, is characterized by alternative dynamics. Starting from day +10, a more than 2-fold increase in concentration was noted (from 23.40 nmol/l on day +10, to 42.13 nmol/l on day +18). High neopterin concentrations, which confirmed constant inflammatory stimulation, were consistent with clinical symptoms of stage 3 aGVHD. In this case, the CRP measurement was useful. Starting from day +4, there was a significant increase in CRP, and the peak levels were reached on day +8. For SAA, however, an almost 3-fold increase was observed on day +2, with a maximum concentration of 293 mg/l on day +6.

CASE 3 The patient was a 43-year-old male with high-risk AML (AML-M1 according to the FAB classification). In this case, the course of pretransplant therapy caused posttransplant complications. Daunorubicine-cytarabine-cladribine induction therapy was complicated, during an 18-day agranulocytosis period, by hemorrhage originated from the descending portion of the duodenum, which resulted in hypovolemic shock. Initially, bleeding was treated endoscopically without success. Subsequently, laparotomy showed numerous ischemic foci on the wall of the small intestine, together with 2 perforations. On day +6, the patient was reoperated due to diffuse peritonitis, and segmental resection of the small intestine was performed. During the postoperative period, bleeding recurred and was treated conservatively. During histological examination of specimens from the intestinal wall, myeloblastic cells were identified. Hematological remission was achieved through chemotherapy. In total, 2 cycles of consolidation chemotherapy were administered, without complications.

The patient was scheduled for alloH SCT from his 41-year-old, HLA-compatible sister. Cytomegalovirus (CMV) serological status: recipient IgM+/donor IgM+, blood groups: recipient O RhD(+)/donor A RhD(+) (major ABO incompatibility). Graft conditioning was performed using the BuCy2 regimen. Graft material consisted of hematopoietic bone marrow cells in doses of MNC 1.13×10^8 /kg recipient body mass and CD34(+) 1.07×10^6 /kg recipient body mass (TABLE). Cyclosporine A and methotrexate were used for GVHD prophylaxis. From day +4, a gradual deterioration of the patient's condition was observed: pyrexia up to 39.5°C, abdominal pain, diarrhea (2000 ml/day), increasing jaundice, fluid retention, as well as symptoms of circulatory insufficiency. Conservative treatment was used including broad-spectrum antibiotic therapy, antifungal treatment, electrolyte supplementation, antithrombin III, diuretics, parenteral alimentation, transfusion of erythrocyte concentrate, platelet concentrates, and albumin, as well as modification of cyclosporine dosage. From day +11 there was a marked deterioration of the patient's condition: an increase in bilirubinemia from 151 to 330 $\mu\text{mol/l}$ on day +16, a gradual increase in the amount of loose stool excreted, as well as potentiation of the symptoms of renal insufficiency. Due to a lack of effective engraftment, additional transplantation of hematopoietic cells of the same donor's peripheral blood, after G-CSF stimulation, was planned for day +18. On the basis of the clinical manifestations and laboratory test results, aGVHD was diagnosed, and on day +13 methylprednisolone administration was initiated (2 mg/kg). An improvement in the patient's general condition was achieved. There was a reduction in diarrhea alongside deterioration of liver and kidney function. The patient had fever up to 39°C, and in microbiological tests, *Pseudomonas aeruginosa* infection of the central catheter

was diagnosed. Despite guided antibiotic therapy, there was further deterioration of the patient's condition, with dominant symptoms of circulatory and respiratory insufficiency, and episodes of pulmonary edema. Despite intensive therapy (mechanical ventilation), the patient died on day +16 as a result of multiple organ failure.

In the pre-alloH SCT period, we observed an almost 4-fold increase in IL-18 concentrations (720 pg/ml) compared with the healthy population, and the initial concentration in this patient was 2.5 times higher than in Case 2. The peracute course of aGVHD, which was finally graded as stage 6, is reflected in the dynamics of changes in IL-18 concentrations, whose lowest value, 496.45 pg/ml on day +6, exceeded the maximum value observed at this point in time in Case 2. The dynamics and pattern of changes in neopterin concentrations were different in Case 3 – no normal values were observed during the whole pretransplant period. Starting from day –7, neopterin concentration was almost twice as high as the values observed in stage 3 aGVHD (46.33 nmol/l in the fourth day after HSCT). Increased neopterin concentrations were sustained until day +16 (41.55 nmol/l), when the patient died. SAA concentrations showed more dynamic changes than CRP did. Its maximum concentration (666.0 mg/l) was achieved on day +4, while for CRP, it was observed until day +12 (260.0 mg/l).

DISCUSSION Acute GVHD often displays a chronic course, which reduces the quality of life of patients who have had transplantation.^{1,2,7} Immunosuppressive therapy initiated immediately after proper diagnosis determines the procedure's effectiveness and allows us to avoid steroid resistance, which is associated with the rate of fatal GVHD in almost 80% of patients.^{1,4}

Differential diagnosis of aGVHD poses a serious challenge. The similar mechanism of other complications indicates a clinical picture that is difficult to interpret unequivocally, and multiple GVHD risk factors result in further problems while establishing the definite diagnosis. Risk factors for aGVHD include the number of T lymphocytes in the graft, differences in minor histocompatibility antigens, the degree of compatibility in the HLA system, gender differences, donor alloimmunization (transfusion, partus), recipient age, the type and dose of conditioning chemotherapy/radiotherapy, CMV serological status, origin of hematopoietic cells, and the recipient's microbiological environment.^{4,7}

For many years researchers have sought laboratory markers, which would facilitate differential diagnosis of early posttransplant complications, with an emphasis on aGVHD. To date, most attention has been paid to proinflammatory cytokines, particularly TNF- α , IL-6, and IL-1 β . However, these cytokines only demonstrate activation of the immune system associated with toxicity of chemotherapy, and activation of bacteria, mainly present in the gastrointestinal

tract.⁴ Available studies provide discordant data on the use of other cytokines, such as IL-18, IL-7, and IL-15, among others.^{1,3}

According to a study by Fuchs et al.,⁶ in patients who have had bone marrow transplantation, a decrease in neopterin concentrations is often observed. However, if complications develop, its concentration increases. Neopterin determination between days 2 and 7 after alloHSCT is of potential prognostic value, and its increase during this time could suggest a highly probable occurrence of immunological complications. This, however, must be corroborated by further investigation.⁶ Therefore, increased neopterin concentrations, which were maintained before alloHSCT in Case 3, may be considered a marker of poor prognosis, and a useful supplementation to the diagnosis of the immune system activation.

The current results, like other reports, did not bring consistent conclusions. Despite evident changes in neopterin and SAA concentrations, apart from Case 1 (without evidence of aGVHD), they do not allow for the differentiation of aGVHD from other early complications of the posttransplant period. The SAA measurement may be used as a complementary test to CRP measurement.

This pilot study would be greatly improved by including additional patients. Nonetheless, our findings yield interesting new information which might facilitate the diagnosis of posttransplant complications.

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Neopteryna i inne markery zapalne we wczesnym okresie po allogenicznej transplantacji szpiku kostnego – seria 3 przypadków

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SŁOWA KLUCZOWE

allogeniczny
przeszczep szpiku
kostnego,
interleukina 18,
neopteryna,
surowiczy amyloid A

STRESZCZENIE

Przedstawiono opis 3 przypadków klinicznych stanowiący badanie pilotowe oceniające profil zmian stężeń wybranych parametrów zapalnych, w tym białka C-reaktywnego, surowiczego amyloidu A, neopteryny oraz interleukiny 18 we wczesnym okresie po allogenicznym przeszczepie komórek macierzystych.

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