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Peripheral Blood CD34+ Cell Counts in Patients With Severe Sepsis

Ağır Sepsisli Hastalarda Periferik Kan CD34+ Hücre Sayıları

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Objectives: This study aims to determine the impact of severe sepsis (SS) on CD34+ cell counts in the peripheral blood and to compare these counts with those of healthy subjects.

Patients and methods: CD34+ cell counts in the peripheral blood were measured in 20 patients with SS (12 males, 8 females; mean age 58.6±16.3 years; range 23 to 81 years) (group 1) and 32 healthy subjects (16 males, 16 females; mean age 51.8±8.6 years; range 36 to 64 years) (group 2). Blood samples were obtained from group 1 two times on second day (D2) within the first 24-48 hours and on seventh day (D7) after diagnosis and once from group 2 on their first day of admittance.

Results: In group 1, CD34+ cell counts on D2 were lower than those on D7, but the difference was not statistically significant. Neither on D2 nor on D7 CD34+ cell counts of group 1 were different than those in group 2. Although CD34+ cell counts in group 1 on D7 were higher than those of group 2, the difference was not statistically significant. CD34+ cell counts did not differ according to the presence of adrenal insufficiency (AI) or survival status.

Conclusion: Overall CD34+ cell counts of SS patients were not different from those in healthy subjects and not affected by the presence of AI or survival in the SS group.

Key words: CD34; healthy group; hematopoietic stem cell; severe sepsis.

Amaç: Bu çalışmada ağır sepsis (AS)'in periferik kandaki CD34+ hücre sayıları üzerine etkisi incelendi ve bu sayılar sağlıklı kontrollerinkiler ile karşılaştırıldı.

Hastalar ve yöntemler: Ağır sepsisli 20 hastanın (12 erkek, 8 kadın; ort. yaş 58.6±16.3 yıl; dağılım 23-81 yıl) (grup 1) ve 32 sağlıklı bireyin (16 erkek, 16 kadın; ort. yaş 51.8±8.6 yıl; dağılım 36-64 yıl) (grup 2) periferik kanlarındaki CD34+ hücre sayıları ölçüldü. Kan örnekleri grup 1'den tanı konduktan sonraki ilk 24-48 saat içerisinde ikinci gün (G2) ve yedinci gün (G7) olmak üzere iki defa, grup 2'den ise ilk başvurdukları gün, bir kez alındı.

Bulgular: Grup 1'de G2'deki CD34+ hücre sayıları G7'dekilerden daha düşük idi, fakat farklılık istatistiksel olarak anlamlı değil idi. Grup 1'deki CD34+ hücre sayıları ne G2'de ne de G7'de grup 2'dekinden farklı idi. Grup 1'de G7'deki CD34+ hücre sayıları grup 2'dekinden yüksek olsa da farklılık istatistiksel olarak anlamlı değil idi. CD34+ hücre sayıları adrenal yetmezlik (AY) varlığı veya sağkalım durumuna bağlı olarak farklılık göstermedi.

Sonuç: Ağır sepsisli hastaların toplam CD34+ hücre sayıları sağlıklı bireylerinkinden farklı değil idi ve AS'li grupta AY varlığı ve sağkalım durumundan etkilenmedi.

Anahtar sözcükler: CD34; sağlıklı grup; hematopoietik kök hücre; ağır sepsis.

Sepsis is defined as a systemic inflammatory response syndrome against an infection resulting in significant mortality. Proinflammatory cytokines such as tumor necrosis factor alpha, interleukin 6 and antiinflammatory cytokines all modulate the immune response to sepsis.[1,2] An infectious cause triggers releasing of cytokines from lipopolysaccharide-activated inflammatory cells[3,4] and stimulation of all these cytokines mobilize stem cells to peripheral blood from bone marrow in order to differentiate into various cell types.[5,6] Human stem cells (HSCs) have capacity of self renewal and differentiation. One of the important markers of HSCs is CD34. It is expressed in 0.5 to 5% of bone marrow stem cells and in 0.05% of peripheral blood nuclear cells.[7] Bone marrow

derived stem cells also express CD45 as well as CD34 on their surfaces.[8] Various causes such as infections, inflammation (due to burns, myocardial infarction, hypersensitivity reactions, collagen tissue diseases), stress, trauma, and acute bleeding may result in elevation of $CD34^+$ cell numbers.[9-11] Sepsis also increases $CD34^+$ cell numbers.[11] There are some studies showing that $CD34^+$ cell transfer to mice enhances immune clearance of late (chronic) bacterial infection and improves the outcome of late sepsis.[12,13] Thus, the authors suggested that $CD34^+$ cell adaptive transfer rebalances dysregulated immune responses associated with sepsis and endotoxic shock.[13] Human endothelial progenitor cells (EPCs) -subpopulation of $CD34^+$ cells- are defined as circulating cells that express a variety of cell surface markers [such as CD34, CD133 or vascular endothelial growth factor receptor (VEGFR)-2] which are similar to expressed by vascular endothelial cells, adhere to endothelium at sites of hypoxia/ischemia, and participate in new vessel formation.[14-16] It is also known that EPCs are increased during sepsis.[17-19] Endothelial progenitor cells are activated through hematopoietic mechanisms in order to replace monocytes and lymphocytes which go through apoptosis during septic process.[20,21] Some studies reported increased EPCs in patients suffering from sepsis and cell counts were found to be correlated with severity of the disease and survival.[17,18]

$CD34^+$ cells may increase in number in peripheral blood as an adoptive immune response in patients with sepsis or severe sepsis (SS). Therefore, in this study, we aimed to determine the impact of severe sepsis (SS) on $CD34^+$ cell counts in the peripheral blood and to compare these counts with those of healthy subjects.

PATIENTS AND METHODS

This study was performed on 20 patients (12 males, 8 females; mean age 58.6±16.3 years; range 23 to 81 years) (group 1) hospitalized in the Intensive Care Unit of the Department of Internal Medicine in Erciyes University Hospital, who met the American College of Chest Physicians/Society of Critical Care Medicine consensus definition of SS.[22] Also, 32 healthy subjects (16 males, 16 females; mean age 51.8±8.6 years; range 36 to 64 years) (group 2) were included as a control group. The protocol was prepared in accordance with the Helsinki Declaration and the study was approved by the Erciyes University Medical School Ethics Committee. Patients or their first-degree relatives were given information about the study, and those who agreed to participate were included in the study. Written informed consent was obtained from the patients and/or their first-degree relatives for publication of their individual details. Exclusion criteria included any hematologic malignancy or solid organ tumor, administration of granulocyte-colony stimulating factor (G-CSF) or granulocye-macrophage-colony stimulating factor (GM-CSF) within the last three months, neutropenia (≤500 neutrophils/mm^3), human immunodeficiency virus infection, and oral intake of corticosteroids at a dose equal to or higher than 1 mg/kg equivalent of prednisone during the previous month.

Blood samples were obtained from group 1 two times on second day (D2) within the first 24-48 hours and on seventh day (D7) after diagnosis and once from group 2 on their first day of admittance. A blood sample of 6 mL was collected into citrated blood tubes for CD34 determination from all patients and controls. The samples were analyzed at the Flow Cytometry Laboratory of Stem Cell Transplantation Hospital using a BD FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA). A single-platform, ProCOUNT method was used. Samples of peripheral blood placed in tubes with ethylenediaminetetraacetic acid were filtered in order to isolate particles with a diameter of 40 µm, and a sample of 100 µL was transferred into each tube and labeled with 10 µL CD34 peridinin chlorophyll protein tracers. Following incubation in a dark environment for 15 minutes, 1.5 mL BD FACS lysing solution was added into the tubes and vortexed rapidly. Subsequently, the tubes were washed with BD cell wash solution three times and centrifuged at 1500 rpm for five minutes. Cells in suspension were analyzed using a flow cytometry and their CD34 expression were measured. $CD34^+$ cell counts were reported as count per µL.

Hypothalamo-pituitary-adrenal axis was evaluated by random serum total cortisol (STC) measurements on D2 and D7. Patients, who were administered glucocorticoid therapy at the discretion of the treating physician, were excluded from the study. All the blood samples were stored at -80 °C and analyzed after completion of the study protocol. Patients were categorized as having adrenal insufficiency (AI) or not according to the basal STC cut-off level of 10 µg/dL for SS on D2 and D7.[23] STC levels were measured by radioimmunoassay method (Immunotech, Prague, Czech Republic). Sequential Organ Failure Assessment (SOFA) score was calculated for each patient on D2 and D7.[24]

Statistical analysis

All statistical analyses were performed using the Statistical Package for Social Sciences for Windows, version 15.0 (SPSS Inc., Chicago, IL, USA). Normal distribution of the data was tested by Shapiro-Wilk test. Since the data were not distributed normally, nonparametric tests were used for statistical analyses. The results were reported as median, minimum, and maximum levels. Comparisons between two groups of

TABLE 1

Sources of the infections of the patients with severe sepsis

Sources of infection	n	%
Lung (pneumonia)	8	40
Blood	2	10
Urine	4	20
Gastrointestinal tract	4	20
Skin and soft tissue	2	10

data were evaluated using the Mann-Whitney U test and the Wilcoxon signed-ranks test. Statistical significance was set at a p-value less than 0.05.

RESULTS

The ages and sex-distribution of two groups were similar. Infectious sources identified in group 1 are summarized in Table 1. In group 1, we could obtain blood samples from 12 patients on D7 because the other eight patients had died until that day.

In group 1, CD34+ cell counts on D2 were lower than those on D7, but the difference was not statistically significant. Neither on D2 nor on D7 CD34+ cell counts of group 1 were different than those in group 2 (Table 2).

Adrenal insufficiency was identified in eight patients on D2 and six patients on D7. CD34+ cell counts were found to be similar on D2 and D7 in patients with or without AI (Table 3 and Figure 1).

CD34+ cell counts (Table 4) and SOFA scores were similar in patients who survived or did not survive on D2 and D7. When patients were divided into two groups according to their SOFA scores as ≤ or >6 (less severe and more severe, respectively) on D2 and D7, no differences were detected in CD34+ cell counts between two groups (Table 5).

DISCUSSION

In the present study, CD34+ cell counts on D2 and D7 in SS patients were similar to healthy subjects. Although a slight increase in CD34+ cells was detected on D7, it was not statistically significant. There are a few studies investigating CD34+ cell counts in septic patients.[11,25] In one of these studies, C34/45+ cell numbers were found to be higher in patients with sepsis and SS compared to healthy controls.[11] Studies including subjects with SS are even rarer,[11,26,27] and, in most of these studies, EPC counts were investigated. As we mentioned before, EPCs are a subgroup of CD34+ cells. In a study, decreased absolute counts of EPCs were detected in a heterogeneous group of SS patients compared to healthy volunteers.[26] In contrast, Becchi et al.[17] reported increased number of EPCs in SS patients. That was a case-control study including 24 septic patients and they also reported increased percentage of EPCs within the first 72 hours after the onset of sepsis.[17] Being a marker of vascular repair, EPC counts can also indicate the severity of vascular damage in sepsis. However, in the present study, all CD34+ cells were counted. They were not differentiated according to whether or not they were EPCs. So, it is difficult to compare the present findings with previous studies. Still, whatever the origin of CD34+ cells is, they had a tendency to increase in number on D7.

TABLE 2

CD34+ cell counts in patients with severe sepsis and healthy individuals

Parameters	Severe sepsis group (n=32)			Healthy individuals (n=32)		
	D2 (n=20)	D7 (n=12)	p1	Mean	p2	p3
CD34+ cell count/µL	1.55 (0.13-14.41)	2.86 (1.23-9.17)	0.092	2.52 (0.54 D1-14.99)	0.168	0.530

D2: Second day; D7: Seventh day; p1: Comparison of C34 counts of the patients on D2 and D7; p2: Comparison of C34 counts of patients on D2 and healthy group; p3: Comparison of C34 counts of patients on D7 and healthy group; D1: First day of admittance.

TABLE 3

CD34+ cell counts of the patients in severe sepsis group on D2 and D7 who have adrenal insufficiency or not

Group	CD34+ cell count/µL				
	D2		D7		
	n	Mean	n	Mean	*p*
Adrenal insufficiency	8	1.66 (0.56-2.88)	6	1.91 (1.23-9.17)	0.273
Without adrenal insufficiency	12	1.43 (0.13-14.41)	6	6.10 (4.11-9.06)	0.208
p	0.672		0.100		

D2: Second day; D7: Seventh day.

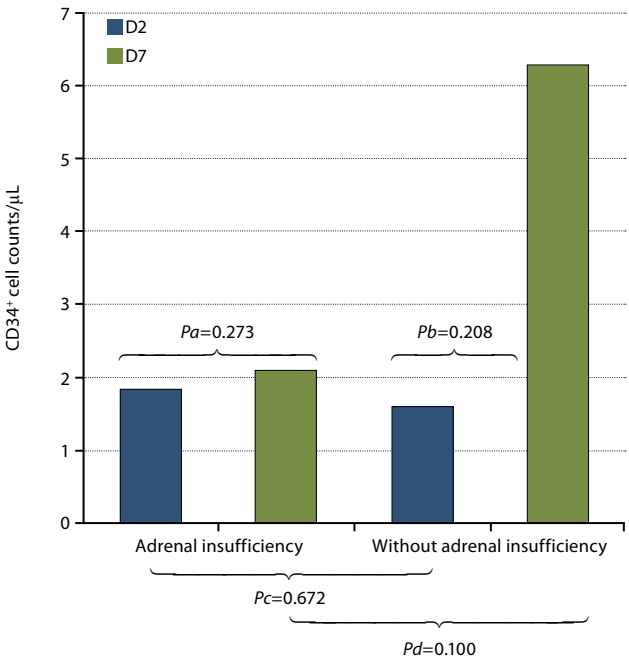

Figure 1. Median CD34⁺ cell counts of severe sepsis patients on second and seventh days with or without adrenal insufficiency.

D2: Second day, between 24-48 hours after severe sepsis; D7: Seventh day of severe sepsis.
pa: Comparison of CD34⁺ cell counts of patients on D2 and D7 with adrenal insufficiency.
pb: Comparison of CD34⁺ cell counts of patients on D2 and D7 without adrenal insufficiency.
pc: Comparison of CD34⁺ cell counts of patients on D2 with or without adrenal insufficiency.
pd: Comparison of CD34⁺ cell counts of patients on D7 with or without adrenal insufficiency.

Liu et al.[27] reported decreased EPC counts after traumatic brain injury (TBI) in a case-control study including 29 patients with TBI. They also reported a steady increase from day five to day seven with a peak on day seven.[27] A similar finding was also obtained in another study performed by the same researchers in a greater number of patients (n=84) with TBI.[28] In that study, they also found that EPC counts were lower in non-survivors compared to survivors.[28] These findings share some similarities with ours. In the present study, although it was not significant, CD34⁺ cell counts exhibited a tendency to be higher on D7 in comparison to D2. Thus, it was considered that an elevation of CD34⁺ cell count could be seen regardless of the type of injury.

CD34⁺ cell counts might differ according to organ dysfunction. In our study, there was no difference between SOFA scores of the patients on D2 and D7 according to survival status. It is possible to divide the patients into two groups according to their SOFA scores (≤6 or >6) on D2 and D7 and observed no difference between CD34⁺ cell counts of the groups in relation to the severity of illness.

We also hypothesized that there could be a relationship between CD34⁺ cell counts and AI in patients with SS. To our best knowledge, there are no studies investigating CD34⁺ cell counts in patients with sepsis-associated AI. CD34⁺ cell counts did not differ between patients with or without AI. In our study, the relationship between CD34⁺ cell counts of survivor and nonsurvivor SS patients was also investigated. In a study (n=32) by Rafat et al.,[18] lower percentage of EPCs was found in nonsurvivors. Their study also reported increased EPCs in patients suffering from sepsis, and cell counts were found to be

TABLE 4

CD34⁺ cell counts in patients with severe sepsis who have survived or not

	Severe sepsis				
	D2 (n=20)		D7 (n=12)		
Parameters	n	Mean	n	Mean	*p*
CD34⁺ cell count/µL					
Alive	12	1.26 (0.13-5.52)	9	1.91 (1.23-9.17)	0.407
Deceased	8	2.88 (1.06-14.41)	3	5.55 (4.11-9.06)	0.109
p		0.115		0.145	

D2: Second day; D7: Seventh day.

TABLE 5

CD34⁺ cell counts of the patients on D2 and D7 according to their SOFA scores ≤ or > 6

	CD34⁺ cell counts in patients with SOFA score ≤6		CD34⁺ cell counts in patients with SOFA score >6		
Day	n	Mean	n	Mean	*p*
D2	13	1.46 (0.24-5.57)	7	2.88 (2.16-5.59)	0.76
D7	9	2.69 (1.33-9.17)	3	4.11 (1.40-9.06)	0.86
p		0.22		0.36	

SOFA: Sequential Organ Failure Assessment; D2: Second day; D7: Seventh day.

correlated with survival.[18] However, in the present study, we did not find any difference between CD34+ cell counts of survivor and nonsurvivor patients with SS. Further studies with greater number of patients are needed to better determine the relationship between survival and CD34+ cell counts.

The major limitation of our study is that we could not use the other markers such as C133, KDR and VEGFR-2 to differentiate EPCs from all CD34+ cells. Furthermore, it is difficult to recruit an adequate number of subjects with SS. Because of that, the numbers of patients with SS enrolled in studies have been generally lower than the number of patients with sepsis. It was also difficult to construct a homogenous study group from SS patients with identical infectious sources. A clearer picture could be obtained if there were homogeneous study groups with larger sample sizes.

In conclusion, CD34+ cell counts of patients with SS did not differ from those of healthy subjects. Furthermore, CD34+ cell counts were not affected by the presence of AI or survival status.

Declaration of conflicting interests

The authors declared no conflicts of interest with respect to the authorship and/or publication of this article.

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