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Short communication

Peripheral blood natural killer cells in Crimean-Congo hemorrhagic fever

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Abstract

Background: The relationship of changes in the peripheral blood lymphocyte subgroups during Crimean-Congo hemorrhagic fever (CCHF) to prognosis has not been reported.
Objectives: To determine peripheral blood lymphocyte subgroups in CCHF patients at the time of diagnosis and relate these to clinical outcome.
Study Design: Peripheral blood samples were obtained from the patients treated at the Karadeniz Technical University Hospital for CCHF in 2004. Lymphocyte subgroups were analyzed by flow cytometry on these samples and their association with patients' risk group (severe vs. non-severe) and mortality was recorded.
Results: There were significantly more peripheral blood natural killer (NK) cells in severe risk CCHF patients than in non-severe risk group CCHF patients. A positive correlation was found between NK cell count and aspartate transferase (AST), alanine transferase (ALT) and activated partial thromboplastin times (aPTT). In addition, NK cell counts were observed to be higher in two patients who died.
Conclusion: Elevated NK cell counts may be a prognostic marker in CCHF patients.

Keywords: Crimean-Congo hemorrhagic fever; Mortality; Natural killer cell

1. Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne life-threatening infection endemic in Africa, the Middle East and Eastern Europe, which is caused by Nairovirus, a genus in the family of Bunyaviridae. The clinical course is predominated by acute onset of fever, myalgia, headache and nausea during the prehemorrhagic period (Ergonul et al., 2006). The mortality rates are relatively variable. While the mortality rate was 10% for the initial epidemic detected in Crimea (Hoogstraal, 1979), it was reported to be 21% for the 260 cases observed in China between 1965 and 1994 (Papa et al., 2002), and 64% during the epidemics observed in Iraq between 1979 and 1980 (Al-Tikriti et al., 1981). The first descriptions of CCHF in Turkey were in 2004 (Bakir et al., 2005; Ergonul et al., 2004; Karti et al., 2004).

Anemia, thrombocytopenia and leucopenia are common in CCHF. Although leucopenia is observed in a considerable portion of cases, there is little data on the changes in the lymphocyte counts and lymphocyte subgroups, and their association with the disease severity and mortality. The present study investigated the association of changes in the peripheral blood lymphocyte subgroups in CCHF patients.

2. Patients and methods

Twenty-six patients who had similar and typical clinical and laboratory findings, including malaise, fever, petechiae, headache, abdominal pain, nausea, vomiting, liver enzyme elevations, thrombocytopenia, leucopenia, and bleeding, were admitted to the Karadeniz Technical University Hospital during the spring and summer of 2004.

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3. Confirmation of the diagnosis

All of the patients had positive IgM and/or PCR results for CCHF virus in blood samples (18 patients were IgM-positive, 2 patients were IgM- and PCR-positive, 6 patients were PCR-positive).

4. Determining the factors influencing the risk groups

Twenty-six patients were divided into two groups based on the risk scale of Swanepoel et al. The presence of any of the following findings defined 'severe' disease: white blood cell $\geq$10,000 μL^{-1}; platelet count (PLT) $\leq$20,000 μL^{-1}; aspartate transferase (AST) levels $\geq$200 U/L; alanine transferase (ALT) levels $\geq$150 U/L; activated partial thromboplastin times (aPTT) $\geq$60 s; or fibrinogen levels $\leq$110 mg/dL, when these occurred during the first 5 days of the disease (Swanepoel et al., 1989).

5. Lymphocyte count

Using the peripheral blood samples obtained from the patients on admission, complete blood count (including differential leukocyte) were analyzed by using a Coulter Micro Diff II automated analyzer. A drop of peripheral blood was smeared on a slide and prepared with Wright's stain. A minimum of 200 leukocytes were counted on each slide.

6. Assessment of lymphocyte subtypes by flow cytometry

Surface phenotyping of lymphocytes in EDTA-anticoagulated peripheral blood was performed by means of Beckman-Coulter EPICS XL-MCL (Beckman Coulter, Nyon, Switzerland). CD3-positive (T lymphocytes), CD3/CD4-positive (inducer/helper T cells), CD3/CD8-positive (cytotoxic/suppressor T cells), CD19-positive (B lymphocytes) and CD3 negative/CD16 + 56-positive cells (natural killer lymphocytes: NK) were determined, and peripheral blood lymphocyte subgroup counts were calculated based on the rates obtained by flow cytometry.

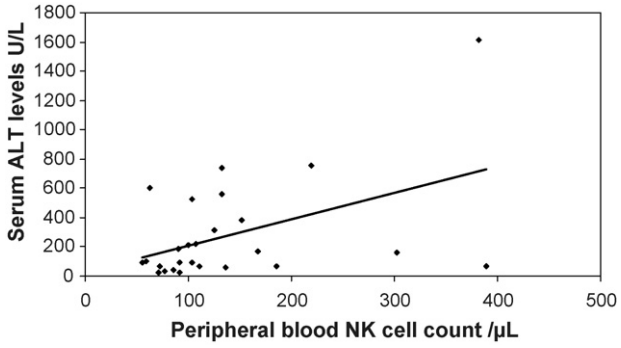

Fig. 1. The correlation of peripheral blood NK cell count and serum ALT levels in the CCHF patients. There is a positive correlation between NK cell count and serum ALT levels (r: 0.465; $p < 0.05$).

7. Statistical analysis

Statistical analyses were performed using SPSS 10.0. The Mann–Whitney U-test was used to analyze differences among the groups. The correlation analysis in the patient groups between NK cell count and other parameters was performed by Pearson correlation analysis. All tests were two tailed and P-values <0.05 were considered to be significant.

8. Results

Leucopenia (leukocyte count <4500 μL^{-1}), anemia (hemoglobin <12 g/dL for woman, <13 g/dL for man) and thrombocytopenia (thrombocyte count <150,000 μL^{-1}) was detected in 96% (25/26), 23% (6/26) and 92% (24/26) of patients, respectively. There were no significant difference between the risk groups (as defined in Section 4) with respect to any peripheral lymphocyte subgroup except NK cell count, which was significantly higher in the severe risk group (Table 1). The NK cell counts were higher in two patients who died compared to survivors (389, 382 μL^{-1} vs. 118 $\pm$ 56 μL^{-1}). There were positive correlations between NK cell count and AST, ALT and aPTT in CCHF patients (Figs. 1 and 2).

Table 1
The distribution of the leucocyte, lymphocyte counts and leucocyte subgroups in Crimean-Congo hemorrhagic fever patients with non-severe and severe risk groups

Peripheral blood cell type	Non-severe cases, $n = 12$ (μL^{-1})	Severe cases, $n = 14$ (μL^{-1})	P-value
White blood cell count	2791 $\pm$ 898	2707 $\pm$ 1888	NS
Lymphocyte count	898 $\pm$ 267	1008 $\pm$ 341	NS
T lymphocyte count	582 $\pm$ 179	630 $\pm$ 218	NS
T inducer/helper cells	367 $\pm$ 121	398 $\pm$ 154	NS
T cytotoxic/supressor cells	221 $\pm$ 64	251 $\pm$ 110	NS
B lymphocyte count	137 $\pm$ 60	241 $\pm$ 218	NS
Natural killer cell count	95 $\pm$ 36	176 $\pm$ 106	<0.05

Values are expressed mean $\pm$ S.D. NS: non-significant.

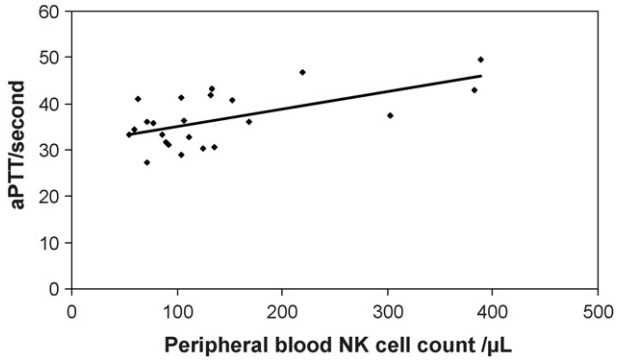

Fig. 2. The correlation of peripheral blood NK cell count and aPTT value in the CCHF patients. There is a positive correlation between NK cell count and aPTT values (r: 0.609; $p < 0.005$).

9. Discussion

In CCHF, some clinical and laboratory parameters may help predict fatal outcome. Swanepoel et al. (1989) reported that white blood cell count $\geq$10,000 μL^{-1}; platelet count $\leq$20,000 μL^{-1}; AST levels $\geq$200 U/L; ALT levels $\geq$150 U/L; aPTT $\geq$60 s; or fibrinogen levels $\leq$110 mg/dL in the first 5 days of illness were >90% predictive of fatal outcome. Bakir et al. (2005) also reported that INR, AST, lactate dehydrogenase, and creatine kinase levels were higher in the patients who died. Ozkurt et al. (2006) reported that confusion and neck stiffness and signs of central nervous system involvement were highly significant for poor prognosis. Papa et al. (2006) reported that high levels of some cytokines, especially tumor necrosis factor-α, were associated with the severe CCHF. Although leucopenia is observed in a considerable portion of the CCHF cases, there is little data on the association of disease severity and death within the lymphocyte counts and lymphocyte subgroups.

NK cells function to detect and lyse cells infected by an intracellular pathogen or tumor cells. NK cells are often part of the innate defenses against viral infections (French and Yokoyama, 2003). Our study demonstrated that higher NK cell counts were present in severe risk patients at the time of admission compared to non-severe risk patients. In addition, we observed that the highest NK cell counts occurred in the two patients who died. Since the standard severe risk factors are correlated with high NK cell counts in our study, NK cell counts may be considered in the clinical classification and approach to patients as a severity parameter. At least two mechanisms may explain these high NK cell counts: first, they represent a strong innate immune response to the higher viral load present in these patients; secondly, they may be caused by unusually extensive release of cytokines that cause NK cell proliferation. In one study serum interleukin-6, interleukin-10, tumor necrosis factor-α, and soluble tumor necrosis factor receptor levels were reported to be high in a fatal CCHF patients (Papa et al., 2006). In addition, interleukin-15 (IL-15) is known to cause proliferation of NK cells (Gosselin et al., 1999). Azeredo et al. (2006) shows that IL-15 was significantly elevated in the plasma of most dengue patients during the early acute phase as compared to healthy donors, and that IL-15 levels directly correlated with NK cell percentages (Azeredo et al., 2006). Excessive IL-15 release may cause high numbers of peripheral blood NK cells in severe risk group and fatal CCHF patients and their role warrants further investigation in CCHF patients.

In conclusion, the present study suggested that relatively high NK cell counts may be a prognostic marker in CCHF patients.

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