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# Relationship Between Hematopoietic Growth Factors Levels and Hematological Parameters in Argentine Hemorrhagic Fever

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**Argentine hemorrhagic fever (AHF) is a viral disease caused by Junin virus and characterized by hematologic and neurological involvement. The main hematologic features are leukopenia, thrombocytopenia, and bone marrow hypoplasia. Hematopoietic growth factors serum levels were measured by ELISA technique in forty-eight patients with confirmed diagnosis of AHF. Patients were classified according to the clinical picture in 15 severe (SCF), 17 moderate (MoCF), and 16 mild (MiCF) cases. Erythropoietin levels were decreased in 28 of 45 patients and raised in 4 SCF patients. Twenty-four of 38 patients had high G-CSF levels at admittance in accordance with clinical picture severity, while IL-3, GM-CSF, and TGF-β were normal in most cases. A direct correlation was found between G-CSF and TNF-α levels. Thrombopoietin levels were found to be raised in 19 of 21 patients. In conclusion, the low levels of Epo may contribute to the severe bone marrow erythroblastopenia described in AHF patients, while G-CSF seems to be a marker of illness severity. Am. J. Hematol. 64:1–6, 2000.** 

**Key words: Epo; G-CSF; Tpo; Argentine hemorrhagic fever**

## INTRODUCTION

Junin virus, a member of the arenaviridae group, is the etiological agent of Argentine hemorrhagic fever (AHF). This disease mainly affects young farm workers from a fertile rural area in Argentina. The signs and symptoms of AHF are malaise, fever, hemorrhagic diathesis, and central nervous system involvement. The 20% mortality rate was reduced to less than 1% by infusion of immune plasma obtained from convalescent patients when administered within 8 days of fever onset [1]. In the last years, vaccination employing an attenuated type of Junin virus strain, CANDID 1, is being successfully carried out in the affected areas to control the disease [2].

The hematologic profile is characterized by leukopenia, thrombocytopenia, and impairment of the hemostatic system [3–8]. Bone marrow studies carried out in patients on admission have shown hematopoietic abnormalities, followed by a fast recovery within the second week of disease [9]. Decreased global cellularity with marked erythroid cell depletion and megaloblastic changes was found. Granulocyte precursors were diminished and presented signs of unbalanced maturation. On the contrary, megakaryocyte lineage was less affected [10–12]. Interestingly, these patients had normal red blood cell count, hematocrit, and hemoglobin level during the acute phase, with minor changes in 20% of cases followed up to 180 days [13]. These data suggest a transient maturation arrest of erythrocyte progenitor cells during the acute phase of illness, a process which does not significantly modify the peripheral values, probably due to the long red cell survival.

Cytokine profiles were largely studied in AHF pa-

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tients. High titers of circulating interferon-α (IFN-α) were found on admission, which abruptly returned to normal values after infusion of immune plasma, as shown by Levis et al. [14,15]. Moreover, we have also shown raised TNF-α, IL-6, IL-8, and IL-10 levels which were related to the severity of the disease [16,17].

The aim of this study was to measure the hematopoietic growth factors levels and compare them with the hematological data in patients with AHF in the acute phase and at recovery, as an approach to a better understanding of the physiopathological mechanisms underlying the hematological abnormalities in this illness. Hence, we measured the hematopoietic growth factors related to erythroid lineage, erythropoietin (Epo), granulocytic lineage, G-CSF, megakaryocytes, thrombopoietin (Tpo), to both granulocyte and megakaryocyte growth (IL-3 and GM-CSF) as well as transforming growth factor-β (TGF-β), a factor related to extracellular matrix proliferation and assumed to have an inhibitory effect on erythroid lineage [18,19].

## MATERIALS AND METHODS

### Patients

Forty-eight patients with AHF were included in the study. The diagnosis was confirmed by the appearance of anti-Junin-virus antibodies in serum or Junin virus isolation from patients who died. Patients were grouped according to the clinical picture and the final outcome into 16 mild (MiCF), 17 moderate (MoCF), and 15 severe cases (SCF). Patients with MiCF had fever during the first week of illness, while tongue tremor was the only manifestation of central nervous system (CNS) involvement; MoCF cases had fever up to the second week of disease, and they had definite signs of CNS involvement including hyporreflexia or arreflexia, mental confusion or drowsiness and ataxia. Severe cases had marked neurological signs and symptoms such as severe muscular hypotonia, arreflexia, ataxia, seizures, and coma. All patients presented minor hemorrhagic manifestations. Eleven patients died with terminal shock syndrome. The onset of symptoms was taken as the first day of disease, and patients were admitted to the hospital 6–13 days thereafter. Patients with MiCF and MoCF recovered within 2–3 weeks. The study protocol was approved by the local ethics committee, and written informed consent was obtained from all patients.

### Blood Collection

Blood samples were taken on admission before treatment with immune plasma and also, in most cases, on remission at day 30. Blood was collected without anticoagulant and sera was separated by centrifugation at 1,800*g* for 20 min at room temperature, then aliquoted and stored at −70°C until use. Blood anticoagulated with 129 mM trisodium citrate in the proportion 1:9 (v/v), was used to prepare platelet-poor plasma for TGF-β measurement.

### Epo, G-CSF, GM-CSF, IL-3, Tpo, and TGF-β Assays

These cytokines were assayed by ELISA technique (R&D Systems, Minneapolis, MN). Normal values from our healthy controls were similar to those described by manufacturers: Epo, 3.1–16.6 mIU/mL; G-CSF, <78.1 pg/mL; GM-CSF, <7.8 ng/mL; IL-3, <31.2 pg/mL; Tpo, <136 pg/mL. For the measurement of total TGF-β plasma level, the latent form was activated as indicated [20], the normal range being 4,800–10,700 pg/mL. The lower limits of detection were as follows: Epo, 0.6 mIU/mL; G-CSF, 10.9 pg/mL; GM-CSF, 2.8 pg/mL; IL-3, 7.4 pg/mL; Tpo, 15.0 pg/mL; TGF-β, 7.0 pg/mL.

### Hematological Parameters and TNF-α Levels

Leukocyte, red cell, and platelet counts were carried out at INEVH (Pergamino, Argentina). TNF-α measurements were carried out by ELISA technique (SIGMA Chemical Co., St Louis, MO) as already published [16]. Normal values were <15.6 pg/mL, while the lower limit of detection was 4.4 pg/mL.

### Statistical Analysis

Data are presented as median values and ranges. Spearman's rank test was used for correlation coefficient calculation among the different parameters. Kruskal–Wallis one-way nonparametric AOV was used for the calculation of differences between the three clinical forms. Rank sum test (Mann–Whithney–Wilcoxon) with α descent modification to compensate multiple comparations was applied to compare data from each clinical form to the other. Wilcoxon signed rank test was applied to compare a single variable in the acute phase and at recovery. Statistical significance was defined as $P < 0.05$.

## RESULTS

### Erythropoietin

As shown in Figure 1a, 28 of 45 patients had decreased levels of Epo, 6 with SCF, 0.6 mIU/mL (0.6–1.3); 12 with MoCF, 0.65 mIU/mL (0.6–2.4), and 10 with MiCF, 1.85 mIU/mL (0.6–3.0). Raised levels of Epo, 33.6 mIU/mL (28.2–198.4), were found in 4 SCF cases who did not survive. Nineteen patients evaluated at recovery showed significantly higher Epo levels than those found during the acute phase ($P = 0.001$), Figure 1b. No correlation was found between Epo levels and the hematocrit.

### Granulocyte-Colony Stimulating Factor

Twenty-four of 38 patients had high G-CSF serum levels at admittance: 11 of 12 with SCF, 765.7 pg/mL

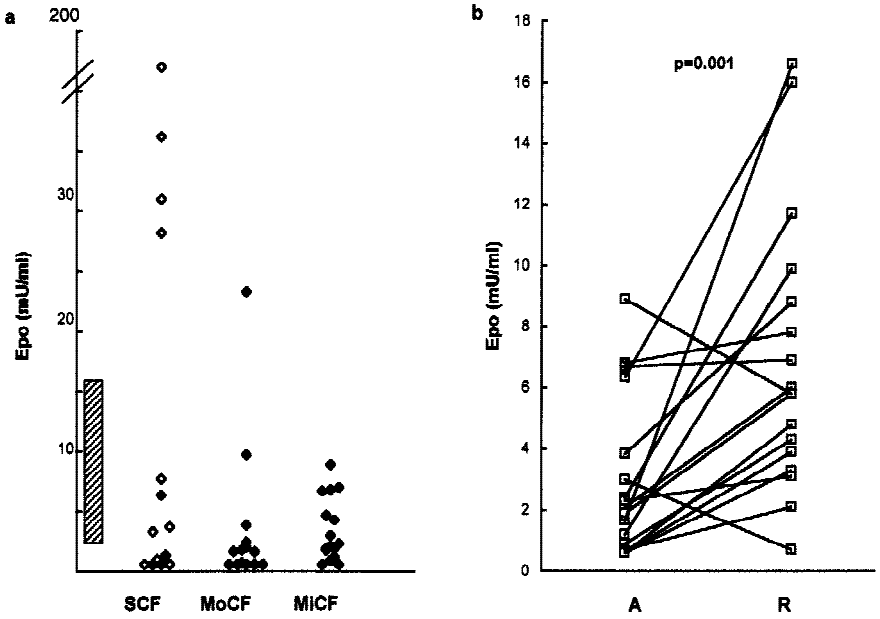


**Fig. 1. (a) Epo levels in serum samples obtained on admission from AHF patients grouped according to the clinical picture. SCF, severe clinical form; MoCF, moderate clinical form; and MiCF, mild clinical form. Open diamonds, fatal cases; filled diamonds, patients who recovered from the disease. Striped bar, normal range. (b) Evolution of Epo levels in sera from patients with AHF in the acute phase (A) and at recovery (R).**

(78.5–98,306); 6 of 11 with MoCF, 151.2 pg/mL (106.3–1,240); and 7 of 15 with MiCF, 121.2 pg/mL (84–177.3), Figure 2a. The values in SCF cases were higher than those obtained in MiCF, $P = 0.003$. Besides, the difference between levels from patients who died and those who survived was significantly different, $P = 0.0002$. G-CSF levels tested at recovery in 15 patients were statistically lower than those of the acute phase, $P = 0.002$ (Fig. 2b). We could not find correlation between G-CSF serum levels and both WBC count and PMN cell count.

### Thrombopoietin

All four patients with SCF evaluated had increased Tpo levels on admission: 797.9 pg/mL (278–3,788 pg/mL), as well as 6 of 7 patients with MoCF, 278.5 pg/mL (165.1–819.6 pg/mL); and 10 of 11 patients with MiCF, 503.9 pg/mL (239.3–960.7 pg/mL). Tpo levels were found normal in 10 of 11 cases at recovery, $P = 0.04$ (Fig. 3a,b). No correlation was found between Tpo levels and the platelet count.

### Interleukin-3

Values were within normal range in most patients. Raised IL-3 levels were found in 2 cases, one with SCF, 35.9 pg/mL and the other with MoCF, 94.8 pg/mL.

### Granulocyte-Macrophage-Colony Stimulating Factor

GM-CSF level was found increased in only one patient with SCF, 38.3 pg/mL.

### Transforming Growth Factor-β

TGF-β levels assayed in two SCF, six MoCF and six MiCF patients, were normal in all but one: 6,117 pg/mL (3,348–18,804). Only one patient had an elevated TGF-β level and died the same day of blood collection.

### Hematological Parameters and TNF-α Levels

Hematological data did not differ among patients during the acute phase of illness in the three clinical forms: hematocrit, 44.5% (38–56); white blood cell count, 2,400/μL (500–4,600); absolute neutrophil count, 1,452/μL (300–3,204); platelet count, 84,000/μL (36,000–155,000). As TNF-α was reported to be a modulatory factor on hematopoietic development [21], it was assayed in this cohort of patients. The values obtained were 24.2 pg/mL (4.4–265), similar to data published before [16]. It was found a positive correlation between G-CSF levels and those of TNF-α, $P < 0.001$.

## DISCUSSION

In the present study we measured the hematopoietic growth factors in sera from patients with AHF in order to find out the possible causes for the bone marrow and peripheral blood abnormalities described in this illness. The results presented here show predominantly decreased Epo levels in most patients in the acute phase as well as elevated G-CSF and Tpo levels while GM-CSF, IL-3, and TGF-β were within normal range in almost all patients.

Koury et al. [22] have shown that erythroid progenitor cells require Epo to arrest apoptosis. The finding that most patients with AHF had low Epo values suggests these levels could be insufficient to prevent apoptosis in bone marrow cells, hence contributing to the severe erythroblastopenia seen in this illness [9–11]. Epo level reduction in AHF could be due to an inhibitory effect of

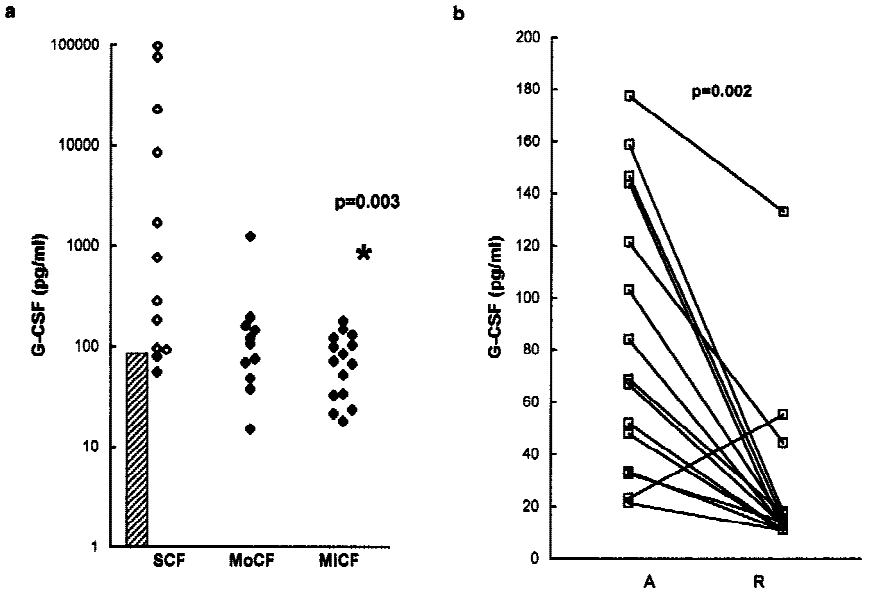


**Fig. 2. (a) Serum levels of G-CSF on admission. SCF, severe clinical form; MoCF, moderate clinical form; MiCF, mild clinical form. Open diamonds, fatal cases; filled diamonds, patients who recovered from the disease. Striped bar, normal range. SCF mean value was statistically different from MiCF, *P* = 0.003. (b) G-CSF levels in sera from patients with AHF in the acute phase (A) and at recovery (R).**

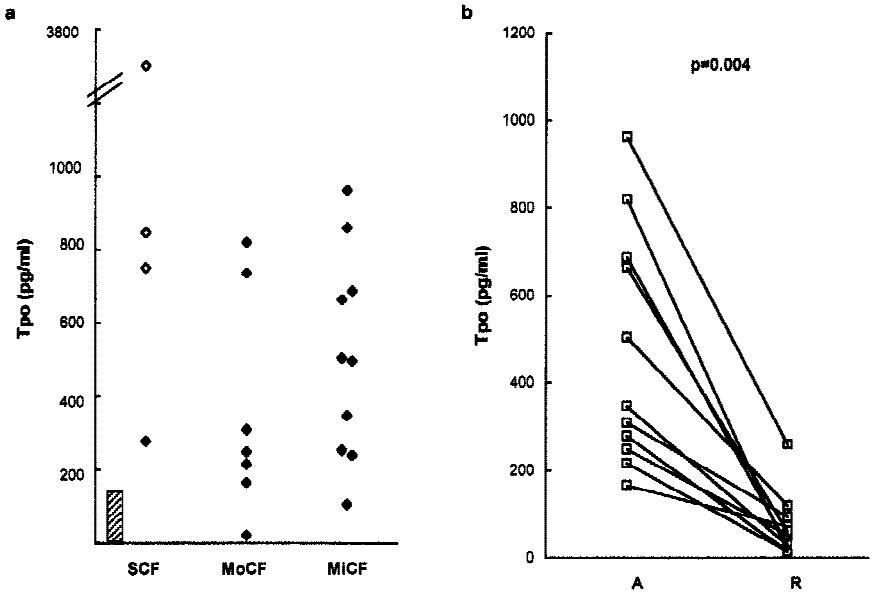


**Fig. 3. (a) Serum levels of Tpo at admittance. SCF, severe clinical form; MoCF, moderate clinical form; MiCF, mild clinical form. Open diamonds, fatal cases; filled diamonds, patients who recovered from the disease. Striped bar, normal range. (b) Tpo levels in sera from patients with AHF in the acute phase (A) and at recovery (R).**

high TNF-α levels as it has been shown on hepatocyte cell cultures [23] and in an animal model [24]. In addition, TGF-β was also evaluated, since it was considered as one of the factors that may lead to Epo level reduction [18,19]. Since TGF-β levels were normal in this cohort of patients, we believe that low Epo levels are more likely related to the high TNF-α levels found. In addition, the proven inhibitory effect of TNF-α on the growth of erythroid progenitor cells should be considered as another possible cause of erythroblastopenia [21]. In contrast to other viral infections such as dengue and parvovirus [25–27], Junin virus by itself does not seem to be responsible for the bone marrow hypoplasia since viral particles could not be found in bone marrow [9].

Patients in the acute phase of AHF resemble HIV infected cases [28,29] who have insufficient Epo production for their degree of anemia. AHF patients evolve to either a full recovery or to death in a few days. The low Epo levels in patients who recovered returned promptly to normal values. Hence, although bone marrow erythroid progenitor cells are markedly decreased, RBC count is not affected due to the long red cell survival. The raised Epo levels found in 4 patients with SCF who died could be due to hypoxia related to shock which is a common feature at end stages of the disease.

The high G-CSF levels we found in most patients with AHF has been described in other viral and bacterial infections [30]. G-CSF levels were related to the severity of illness as shown by the significantly different values found between patients with SCF and MiCF, as well as

by comparing cases who survived with those who died. In a previous work we have measured pro- and anti-inflammatory cytokines in the same cohort of patients presented here [17]. The direct significant correlation between G-CSF levels and those of TNF-α, and also with IL-6, IL-8, and IL-10 levels (data not shown) give support to consider G-CSF as a good marker of the degree of severity in AHF patients. The lack of correlation between G-CSF levels and WBC or granulocyte count is not unexpected because WBC count is regulated by a number of other cytokines and growth factors, the spleen function or cortisol levels. Besides, E-α 1AT plasma levels previously assayed in these patients [17] correlated with G-CSF measured here (data not shown). This statistical data would imply a role of this growth factor in PMN leukocyte activation in AHF similarly to the results found in healthy volunteers who had received G-CSF [31].

As it has been shown in patients with dengue hemorrhagic fever [32], Tpo levels in AHF patients were elevated in the acute phase of illness. It was postulated that Tpo serum levels depends on both the total number of bone marrow megakaryocytes and of circulating platelets [33–35]. Therefore, the absence of an inverse correlation between Tpo and the platelet count could be explained by the fact that bone marrow megakaryocytes are almost normal in AHF patients [9].

We believe the bone marrow erythroblastopenia found in AHF patients could be related to the low plasma Epo levels we are describing here. The high Tpo levels seen in the acute phase of illness, probably in response to thrombocytopenia, could stimulate megakaryocytopoiesis leading to a recovery of platelet count during the second week of disease. Finally, as G-CSF level is related to the severity of clinical picture, we believe it could be considered as a marker of disease severity.

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