# Plasma Erythropoietin Activity in Infants with Sepsis

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Moderate to severe anemia often presents as a classic hemorrhagic syndrome accompanying sepsis. Experimental and clinical studies show that signs of hemolysis are present in more than one half of subjects with pneumococcal, streptococcal, or staphylococcal sepsis [2], bartonellosis [7], or with septicemia caused by *H. influenzae* [7], *Clostridium* [2], or *Neisseria* [2] organisms; "septic anemia" has therefore been traditionally regarded as hemolytic anemia [2, 15]. An important role in the origin of anemia in sepsis may be played by impaired iron metabolism [8] and blood loss [13], but the major mechanisms by which anemia arises in sepsis have not been elucidated. Low levels of erythropoietin (EP), a hormone of glycoprotein nature produced in the kidneys [5, 9], have been observed in chronic inflammations [6, 8], whereas reduced EP activity in acute bacterial inflammatory infections (sepsis) has been reported in only one study [3].

The purpose of the present study was to examine erythropoietin activity in infants with sepsis.

## MATERIALS AND METHODS

Erythropoietin activity was measured in an *in vitro* bioassay [10] in Manakova and Setkov's modification, with minor alterations. As target cells for detecting this activity, nucleated cells obtained from spleens of anemic BALB/c mice weighing 20-25 g were used. The mice had been made anemic by being injected with phenylhydrazine hydrochloride intraperitoneally in a dose of 60 mg/kg twice on two successive days. Three days after the second injection, their spleen cells were suspended in a supplemented Eagle's minimum essential medium (alpha modification) (α-MEM). The complete medium contained 3 ml of α-MEM (Flow Laboratories), 4 ml of fetal calf serum (Flow Laboratories), 0.2 ml of L-glutamine (0.1 mmol/liter), 0.2 ml of 2-mercaptoethanol (0.1 mmol/liter), 0.4 ml of HEPES buffer (20 mmol/liter), and 0.2 ml of kanamycin sulfate in a concentration of 100 μg/ml. This medium with suspended murine cells ($4\times10^7$ cells/ml) was seeded into tissue-culture plates with 96 U-shaped or flat-bottomed wells (Linbro), 50 μl per well, adding patient's plasma or an EP standard in various dilutions. As the EP standard, Epoetin-Alfa (Epogen) (USA), a recombinant preparation having an activity of 20,000 mU/ml, was used. To calculate EP activity, 5-7 data points were considered, using 3 wells for each point. The cells were cultured for 20-22 h at 37°C and 100% humidity in the presence of 5% $CO_2$ in air, after which $^3$H-thymidine was added to the culture (1 μCi in 10 μl of α-MEM per well), and the culturing was continued for another 2 h. Thereafter the cells were transferred to nitrocellulose filters (Titertek) with a 12-well vacuum cell harvester (Millipore). The radioactivity of the samples was measured in a liquid scintillation counter (Contron). For the detection of a nonspecific inhibitor of EP activity in plasma samples from infants with sepsis during the acute and recovery periods, the EP standard (3 mU/ml) and/or 10% patient's plasma were added to the culture.

Erythropoietin (EP) activity was studied in 36 infants (age range 2 to 11 months) with sepsis of various origin; in 4 of the infants, the sepsis oc-

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**TABLE 1.** Comparison of Erythropoietin Activity and Hemoglobin Levels in Infants with Sepsis and in Those with Iron Deficiency Anemia (IDA) ($M \pm m$)

| Disease | № of infants | Erythropoietin activity, mU/ml | Hemoglobin concentration, g/liter |
|---|---|---|---|
| Sepsis, acute period | 19 | 12.6±3.1 (5.0–22.1) | 65.1±2.1 |
| | 17 | <5.0 | |
| Sepsis, recovery period | 21 | 28.5±3.2 (5.0–56.6) | 92.6±2.6 |
| | 7 | <5.0 | |
| IDA, mild | 17 | 59.9±4.1 (40.5–88.4) | 97.2±1.4 |
| IDA, severe | 9 | 619.6±20.9 (511.6–715.0) | 63.2±1.0 |
| Normal (control) infants | 45 | 12.9±1.1 (5.0–24.8) | 124.6±2.3 |

cured in the form of septicemic infection, while the remaining 32 had been diagnosed as having a septicopyemic form of sepsis.

The control group consisted of 45 healthy infants in the same age range, while the comparison group comprised of 38 infants with iron deficiency anemia (IDA) of varying severity diagnosed as recommended by Pizarro et al. [11]. The nonspecific plasma inhibitor of EP activity was determined in 20 infants with acute sepsis and in 8 convalescent infants.

## RESULTS

In the acute period of sepsis (fever, intoxication, spread of pyemic foci), EP activity in 19 of the 36 cases ranged from 5.0 to 22.1 mU/ml (means, 12.6±3.1 mU/ml) and virtually did not differ from that in the control group (12.9±1.1 mU/ml; range 5.0-24.8; $p<0.05$) (Table 1). EP activity in the plasma samples from the remaining 17 infants with acute sepsis was below the detection limit of the assay (5 mU/ml) [10].

The major stimulators of EP production are anemia and hypoxia [5], which were both at their height during the acute period of sepsis. Thus, hemoglobin ranged from 47 to 69 g/liter (mean, 65.1±2.1 g/liter) and oxygen partial pressure ($pO_2$) from 44.3 to 56.2 mm Hg (mean, 49.5±3.8 mm Hg) - values that significantly differed from those in the control group (124.6±2.3 g/liter and 68.4±2.8 mm Hg; $p<0.01$) and indicated the presence of severe anemia and hypoxia. For infants with acute sepsis, no correlation could be detected between EP activity and hemoglobin concentration ($r=-0.11$)

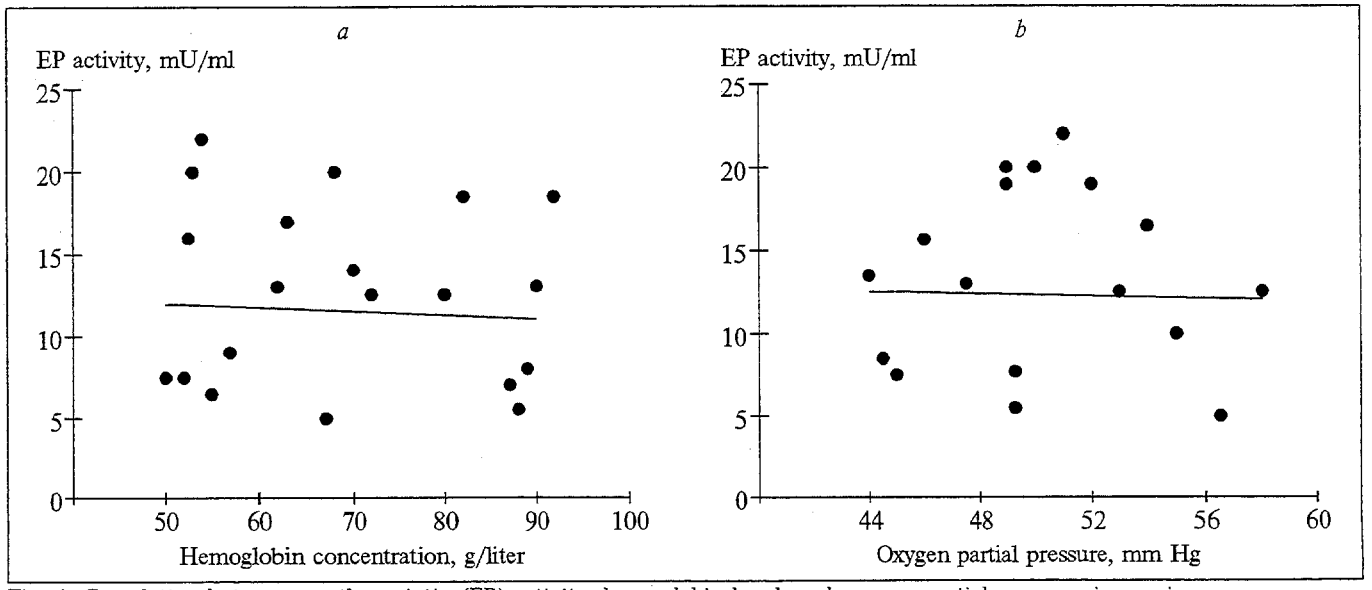


**Fig. 1.** Correlation between erythropoietin (EP) activity, hemoglobin level, and oxygen partial pressure in sepsis.

(Fig. 1, *a*), and a weak negative correlation was found between EP activity and $pO_2$ ($r$=-0.16) (Fig. 1, *b*).

During the recovery period, plasma samples which were taken from 28 of the 36 infants were found to have a higher EP activity than during the acute period in 21 cases (up to 56.6 mU/ml; mean, 28.5±3.2 mU/ml; $p<0.01$) (Table 1). Plasma samples from the remaining 7 infants had an EP activity below 5 mU/ml; all these infants were in a state of emaciation, having a weight-for-age deficit of more than 20%. EP activity during the recovery period was also low (mean, 9.4±2.2 mU/ml) in the plasma samples from 6 infants who had undergone repeated (on two or more occasions) transfusions of packed red blood cells. For convalescent infants, EP activity showed a weak correlation with hemoglobin concentration ($r$=-0.39).

In the comparison group (infants with IDA), EP activity varied widely from 40.5 to 715 mU/ml and correlated well with the severity of anemia, as was indicated by a high correlation coefficient between this activity and the hemoglobin concentration ($r$= -0.93). It was also found that plasma samples from infants with severe IDA had a 50-fold higher mean EP activity (619.6±20.9 mU/ml; range, 512.0 to 715.6 mU/ml) than those from "septic" infants with the same degree of anemia (Table 1).

For infants recovering from sepsis, plasma EP activity was compared to that determined for infants with mild IDA because the mean hemoglobin level in the former infants was 92.6±2.6 g/liter, indicating that the anemia was mild. This comparison showed that the infants recovering from sepsis had a mean EP activity only about half that in infants with IDA who had a mean EP activity of 59.9±4.1 mU/ml (range, 40.5 to 88.4 mU/ml) (Table 1).

Thus, our comparison of EP activity in plasma samples from normal infants, those with sepsis, and those with IDA demonstrated that septic infants had, in both the acute and recovery periods, a low EP activity that did correspond to the severity of anemia (Fig. 2).

TABLE 2. Erythropoietin Activity of Heat-Treated and Untreated Plasma Samples from Infants with Sepsis

| Plasma sample №. | Erythropoietin activity (mU/ml) | |
|---|---|---|
| | Before heat treatment | After heat treatment |
| 1 | 19.2 | 20.8 |
| 2 | 6.1 | 5.0 |
| 3 | 7.8 | 10.1 |
| 4 | 19.9 | 14.1 |
| 5 | 5.6 | 6.8 |
| 6 | 9.6 | 8.0 |
| 7 | 16.2 | 12.9 |
| 8 | 7.8 | 6.2 |
| 9 | 10.3 | 10.8 |
| 10 | 6.8 | 5.0 |
| 11 | 12.7 | 14.2 |
| $M\pm m$ | 11.1±1.6 | 10.4±1.5 |

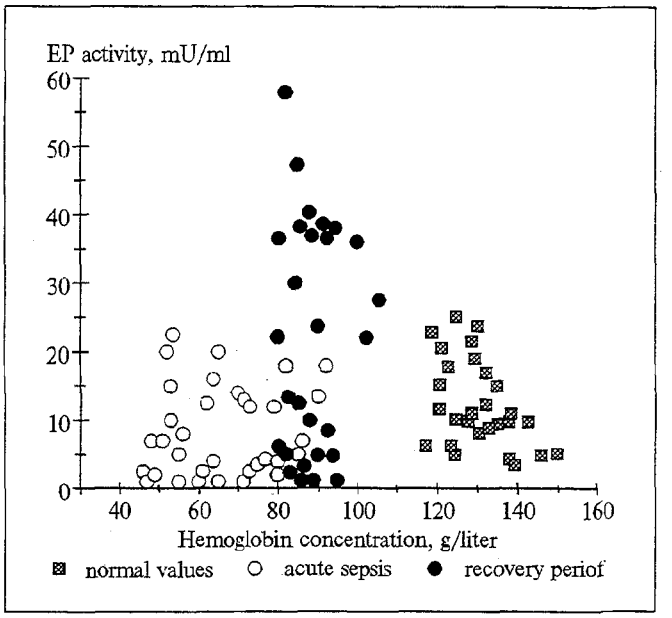


Fig. 2. Relationship between eythropoietin (EP) activity and severity of anemia in children with sepsis as compared to normal children.

One possible reason for the reduced plasma EP activity in sepsis and for the lack of its correlation with the severity of anemia and hypoxia may be the presence of a nonspecific inhibitor of EP in the plasma. Confronted with a similar problem (low EP activity that did not match the degree of anemia), many investigators arrived at the conclusion that the presence of EP inhibitor(s) is largely responsible for the reduced EP activity in the plasma of patients with rheumatoid arthritis, disseminated lung cancer [8], nephrogenic anemia [14], and partial red-cell aplasia [12]. It has been suggested that EP inhibitors in inflammatory states are most likely to be products of pathological iron metabolism, such as acid isoferritins and possibly also interleukin-1 [8], although both the nature and mechanism of action of these inhibitors remain unknown.

Thermal treatment of plasma samples (heating to 56°C for 30 min) was found to result in the effective removal of EP inhibitors of the IgM class in most instances [4]. In our study, however, such treatment of plasma samples from infants with sepsis did not alter EP activity to a significant degree (Table 2): the average difference in this activity between unheated and heated samples was only 6.5%, i.e., it lay well within the error range of the bioassay used [10] and so did not allow us to judge whether or not EP inhibitors of the IgM class were present in the plasma of septic patients. We then made an attempt to detect a nonspecific EP inhibitor in the *in vitro* bioassay system. To this end, 10% of plasma taken

TABLE 3. Inhibition of Target Cell Proliferation by Plasma Samples Taken from Patients with Sepsis in the Acute Phase or during Recovery (Convalescents) ($M \pm m$)

| Plasma sample № | $^3$H-Thymidine incorporation, cpm×10$^3$ | | % Inhibition |
|---|---|---|---|
| | Plasma from patients | Erythropoietin standard + plasma from patients | |
| 1 | 6.1±0.8 | 7.3±0.3 | 82.7±4.2* |
| 2 | 10.9±0.6 | 13.6±0.6 | 67.8±3.6* |
| 3 | 9.2±1.0 | 55.2±2.8 | |
| 4 | 3.8±0.4 | 48.0±2.2 | |
| 5 | 33.4±1.9 | 92.7±5.5 | |
| 6 | 9.5±0.5 | 16.6±2.0 | 60.7±3.1* |
| 7 | 4.4±0.2 | 8.1±0.6 | 80.8±4.2* |
| 8 | 6.4±0.3 | 58.6±3.0 | |
| 9 | 7.0±0.4 | 55.0±3.3 | |
| 10 | 16.4±1.2 | 34.3±2.1 | 18.6±1.4* |
| 11 | 6.8±0.3 | 18.8±0.9 | 55.4±3.1* |
| 12 | 6.3±0.4 | 10.8±0.6 | 74.4±2.9* |
| 13 | 6.7±0.5 | 68.2±3.2 | |
| 14 | 5.4±0.4 | 48.9±2.7 | |
| 15 | 5.8±0.2 | 56.1±1.3 | |
| 16 | 7.3±0.5 | 60.5±2.8 | |
| 17 | 12.3±0.7 | 56.9±2.0 | |
| 18 | 5.7±0.6 | 59.3±2.7 | |
| 19 | 11.3±0.5 | 19.4±1.3 | 54.0±2.8* |
| 20 | 28.6±1.4 | 78.5±3.2 | |
| 21** | 42.1±3.7 | 83.1±5.8 | |
| 22** | 36.1±1.4 | 79.5±3.6 | |
| 23** | 45.0±2.9 | 85.3±4.2 | |
| 24** | 42.3±2.0 | 77.4±3.9 | |
| 25** | 44.1±2.7 | 81.0±4.5 | |
| 26** | 46.2±2.0 | 90.6±5.2 | |
| 27** | 38.1±1.7 | 79.2±2.7 | |
| 28** | 36.7±4.1 | 93.3±4.6 | |

Note. *—% Inhibition of target cells was calculated by the formula $(C - P)/C \times 100\%$, where $C$ is $^3$H—thymidine incorporation into cells stimulated by erythropoietin standard (3 mU/ml, 42.2±3.3 cpm) and $P$ is $^3$H—thymidine incorporation into cells stimulated by erythropoietin standard enriched with the test plasma from a patient with sepsis. **Plasma from convalescent patients.

from infants with sepsis during the acute or recovery period was added to cultured target cells stimulated with the EP standard (3 mU/ml) (Table 3). In these tests, inhibition of $^3$H-thymidine incorporation by 17-86% was observed for 8 out of the 20 (40%) patients with acute sepsis but for none of the 8 convalescents. These findings led us to conclude that the failure of EP activity to correlate with the severity of anemia and hypoxia was largely due, in a substantial proportion of patients with acute sepsis, to the presence of an EP inhibitor or inhibitors in their plasma. Another likely reason for the lack of such correlation may be the presence in the plasma of cell breakdown products and of metabolic products of microorganisms.

In summary, infants with sepsis were found to show a low EP activity in their plasma and a lack of correlation between this activity and the severity of anemia and hypoxia. EP activity was lowest in plasma samples from emaciated infants and those who had undergone repeated blood transfusions, which agrees with the results of other experiments where starvation and emaciation [3] and repeated transfusions [6] were shown to be associated with low levels of EP production. Deficient production of this hormone in patients with sepsis appears to result from derangement of the mechanism regulating its biosynthesis. A further reason for the reduced plasma EP activity in patients with acute sepsis may be the presence of a nonspecific EP inhibitor or inhibitors in the plasma. The results of this study prompt us to recommend clinical trials of recombinant EP for correcting anemia in patients with sepsis.

## REFERENCES

1. T. E. Manakova and N. A. Setkov, *Byull. Eksp. Biol.*, **107**, № 3, 21-24 (1989).
2. T. H. Abshire and J. D. Reeves, *J. Pediat.*, **103**, 868-871 (1983).
3. J. Caro and A. J. Erslev, *Contrib. Nephrol.*, **66**, 54-62 (1988).
4. J. H. Elder, J. S. Shannon, and T. R. J. Lappin, *Blood Cells*, **11**, 409-416 (1986).
5. A. J. Erslev, J. Wilson, and J. Caro, *J. Lab. Clin. Med.*, **109**, 429-433 (1987).
6. A. J. Erslev, S. J. Shuster, and J. Caro, *Europ. J. Haemat.*, **43**, 367-373 (1989).
7. K. M. Kaplan and F. A. Oski, *Pediatrics*, **65**, 1101-1109, (1980)
8. A. Koijn and C. Hershko, *Iron in Immunity, Cancer and Inflammation* (M. de Sousa and J.H. Brock, eds), Chichester (1989), pp. 111-143.
9. S. B. Krantz, *Blood*, 77, 419-434 (1991).
10. G. Krystal, *Exp. Hemat.*, **11**, 649-660 (1983).
11. F. Pizarro, P. Yip, P. Dallman, *et al.*, *J. Pediat.*, **118**, 687-692 (1991).
12. M. Takanashi, K. Nikkuni, I. Tanaka, *et al.*, *Ann. Hemat.*, **63**, 9-14 (1991).
13. J. Tapley and P. Lawler, Anaesthesia, **45**, 395-398 (1990).
14. W. H. Whitcomb and S. S. Bottomley, *Kidney*, **10**, 19-24 (1977).
15. M. M. Wintrobe, *Clin. Hematol.*, **4**, 671-692 (1974).