岩手医科大学
審査学位論文
（博　士）

J Iwate Med Assoc Vol. 65, No. 5 (December 2013) pp. 333-341.

Assessment of hematopoietic factors in sepsis patients and in sepsis patients with disseminated intravascular coagulation

Koichi Hoshikawa[1], Masahiro Kojika[1], Gaku Takahashi[1], Naoya Matsumoto[1], Yasuhisa Fujino[1], Yasushi Suzuki[1], Yoshihiro Inoue[1], Hiroyuki Nitta[2], Go Wakabayashi[2] and Shigeatsu Endo[1]

[1] Department of Critical Care Medicine, School of Medicine, Iwate Medical University, Morioka, Japan

[2] Department of Surgery, School of Medicine, Iwate Medical University, Morioka, Japan

(Received on February 22, 2011 & Accepted on March 4, 2011)

Abstract

The number of sepsis patients has been tending to increase year by year. In the present study we conducted an analysis in relation to the outcome of sepsis patients centered on hematopoietic factors. The parameters assessed were the patient background factors, severity of illness, and plasma hematopoietic factor and cytokine values of 48 patients who were brought to our center and diagnosed with sepsis. Plasma thrombopoietin (TPO) was significantly higher in the group that died within 30 days (D30 group). Comparisons between the group that survived and the group that died (D group) showed that the leukemia inhibitory factor (LIF), interleukin-11 (IL-11), and interleukin 3 (IL-3) values were all significantly higher in the D group. Comparison between a group of sepsis patients diagnosed with disseminated intravascular coagulation (DIC) and a group not diagnosed with DIC on the basis of their DIC scores revealed that TPO, stem cell factor (SCF), and IL-11 values were significantly higher in the DIC group. TPO, LIF, IL-11, and IL-3 may be prognostic factors in sepsis patients. The results also suggested that TPO, SCF, and IL-11 may be important risk factors in DIC patients.

Key words : sepsis, hematopoietic factors, disseminated intravascular coagulation

I. Introduction

Sepsis is caused by a variety of diseases and pathological conditions, but pro-inflammatory cytokines are known to play important roles pathophysiologically. Cytokines are also known to be important factors in disseminated intravascular coagulation (DIC), which is closely associated with sepsis, and DIC often leads to fatal conditions, including multiple organ failure[1]. Tissue factors are released by cytokine-activated monocytes, macrophages, and vascular endothelial cells. Organ damage in DIC secondary to sepsis is thought to progress as a result of the production of thrombin, fibrin, FDP, and plasmin and the complement activation associated with it acting synergistically with neutrophil activation[2]. In addition, there have been several reports regarding

inflammatory sepsis mediators that are related to the pathogenesis of septic DIC[3-6]. According to some of the reports septic DIC is a pathological state accompanied by activation and consumption of a variety of blood cells and involvement of hematopoietic factors has been attracting attention, and we have also conducted a number of studies in that regard[5,9]. In the present study we assessed hematopoietic factors in sepsis and the pathological state of DIC, especially the values of factors in peripheral blood that regulate platelet production, i.e., interleukin 11 (IL-11), interleukin-3 (IL-3), stem cell factor (SCF), leukemia inhibitory factor (LIF), and thrombopoietin (TPO), and their relationships with the outcome.

II. Methods

The subjects of this study were the 48 patients diagnosed with sepsis among the patients brought to our emergency room and admitted during the period from 2001 to 2004. Sepsis was diagnosed based on the criteria of the American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference Committee[10]. The study was conducted by comparing three groups: a group of 39 patients who survived (survivor group: S group), a group of 6 patients who died within 30 days (death within 30 days group; D30 group), and a group of 3 patients who died after 30 days but within 60 days (death within 60 days group; D60 group), and also by comparing two groups: the S group and the group of patients that died (D group). Severity of illness on arrival at the hospital was evaluated by using Acute Physiology and Chronic Health Evaluation II (APACHE II) scores and Sequential Organ Failure Assessment (SOFA) scores, and disseminated intravascular coagulation (DIC) was evaluated on the basis of the DIC diagnostic criteria of the Special Diseases Designated by the Ministry of Health and Welfare Blood Coagulation Disorders Survey Research Group[11-13].

On arrival at the hospital a blood sample was collected in a heparinized endotoxin-free test tube. The sample was immediately centrifuged at 3000 × g for 40 sec at 4 °C, and the supernatant was collected and stored deep frozen at -80° C until analyzed. Plasma IL-3, IL-11, SCF, and LIF values were measured by enzyme-linked immunosorbent assays (ELISAs) (R&D System Inc., Minneapolis, MN, USA), and the lower limit of detection of each of the assays was 4 pg/ml. Plasma TPO values were measured by an ELISA (Immuno-Biological Laboratories Co., Ltd., Fujioka, Japan), and the lower limit of detection of the assay was 50 pg/ml. The measurement methods were the same as reported previously[5].

Statistical analysis

The statistics are reported as mean values ± standard deviation. Significant differences in all of the statistical data were considered to exist at p values<0.05. The SPSS® 2001 (SPSS Japan Inc., Tokyo, Japan) software program was used to perform the statistical analysis.

III. Results

The results of the analysis of the patients' background factors showed no significant differences in age or gender

Table 1. The clinical characteristics of patients with sepsis

	Survivor (n=39)	Death within 30day (n=6)	Death within 60days (n=3)	Statistical analysis
Gender (male/female)	24/15	5/1	1/2	
Age (years)	66.7 ± 13.0	76.0 ± 8.8	70.0 ± 24.2	NS
APACHE II score	26.4 ± 7.4	42.5 ± 7.7a	33.7 ± 0.6	p<0.01
SOFA score	9.6 ± 4.7	19.8 ± 3.5b	12.3 ± 2.3	p<0.01
DIC score	5.4 ± 1.3	7.7 ± 0.5c	7.0 ± 1.0	p<0.01

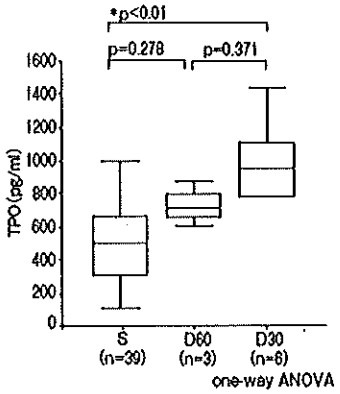

Fig. 1. The plasma thrombopoietin (TPO) values of the 48 sepsis patients divided into a group of 39 survivors (survivor group, S group), a group of 6 who died within 30 days (death within 30 days group; D30 group), and a group of 3 who died within 60 days (death within 60 days group, 60D group) are shown. The TPO values in the 30D group were significantly higher than in the S group. A significant difference was considered to exist at p values <0.05.

between the S group, D30 group, and D60 group. In the S group and D30 group the APACHE II scores were 26.4 ± 7.4 and 42.5 ± 7.7, respectively, the SOFA scores, 9.6 ± 4.7 and 19.8 ± 3.5, respectively, and the DIC scores, 5.4 ± 1.3 and. 7.7 ± 0.5, respectively, and all three values were significantly higher in the D30 group. However, there were no significant differences in any of these scores between the S group and D60 group or between the D60 group and D30 group (Table 1). The plasma TPO values in the D group were significantly higher than in the S group. The TPO values in the S group and D30 group were 482.5 ± 246.0pg/ml and 1001.7 ± 250.2pg/ml, respectively, and significantly higher in the D30 group. There were no significant differences in plasma TPO values between the S group and D60 group or between the D60 group and D30 group (Fig. 1). Comparisons between the S group and D group showed plasma IL-3 values of 0.41 ± 0.87pg/ml and 26.9 ± 57.4pg/ml, respectively, IL-11 values of 8.57 ± 10.3 pg/ml and 25.5 ± 17.8 pg/ml, respectively, and LIF values of 1.24 ± 2.43 pg/ml and 12.2 ± 16.4 pg/ml, respectively, and they were significantly higher in the D group. There were no statistically significant differences in SCF values between the groups, but the SCF values in the S group and D group were 3597.2 ± 2084.9pg/ml and 5000.0 ± 2711.9pg/ml, respectively, and they tended to be higher in the D group (Fig. 2). Significant correlations were found between the TPO values, IL-11 values, and LIF values, but the IL-3 values were not correlated with any of them (data not shown). Next, we assessed the results by dividing the 48 sepsis patients into a group of 17 patients who had been

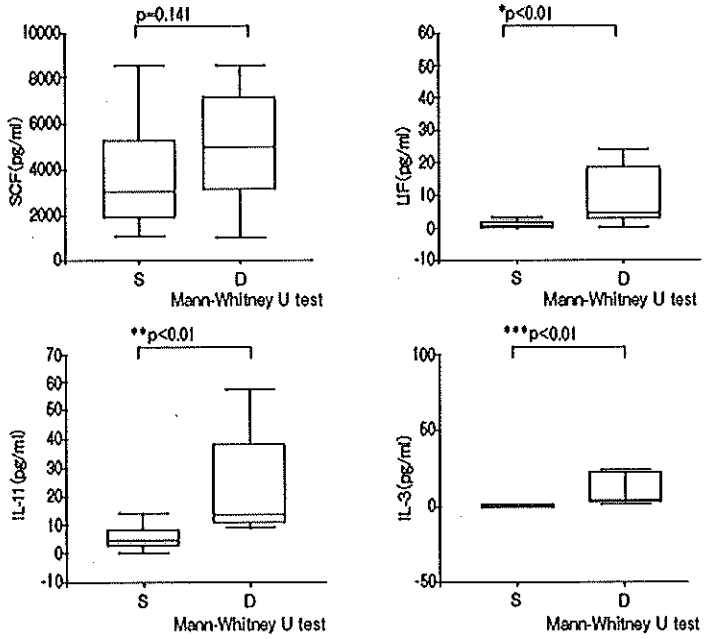

Fig. 2. The plasma stem cell factor (SCF), leukemia inhibitory factor (LIF), interleukin 11 (IL-11), and interleukin 3 (IL-3) values of the 48 sepsis patients divided into a group of 39 survivors (survivor group, S group) and a group of 9 patients who died (group who died; D group) are shown. The plasma LIF, IL-11, and IL-3 values were significantly higher in the D group than in the S group. A significant difference was considered to exist at p values <0.05.

diagnosed with DIC (DIC group) and a group of 31 patients who had not been diagnosed with DIC (non-DIC group), and compared them. The plasma TPO values in the DIC group and non-DIC group were 866.9 ± 203.5pg/ml and 396.4 ± 187.2pg/ml, respectively, and they were significantly higher in the DIC group (Fig. 3). In addition, the plasma SCF values in the DIC group and non-DIC group were 5205.9 ± 2447.4 and 3122.2 ± 1778.7, respectively, and their plasma IL-11 values were 13.4 ± 16.1 and 4.2 ± 8.7, respectively, and both were significantly higher in the DIC group (Fig. 4). The mean plasma LIF values and IL-3 values were both below the sensitivity of detection in both groups.

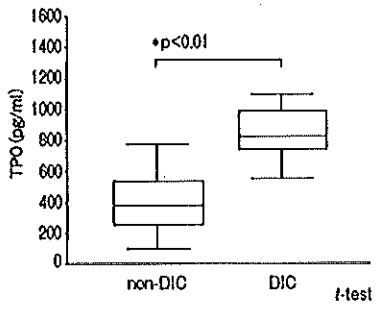

Fig. 3. The plasma thrombopoietin (TPO) values of the 48 sepsis patients divided into a group of 17 patients diagnosed with disseminated intravascular coagulation (DIC; DIC group) and a group of 31 patients not diagnosed with DIC (non-DIC group) are shown. The TPO values were significantly higher in the DIC group than in the non-DIC group. Even in the non-DIC group the TPO values were much higher than the normal value. A significant difference was considered to exist at p values <0.05.

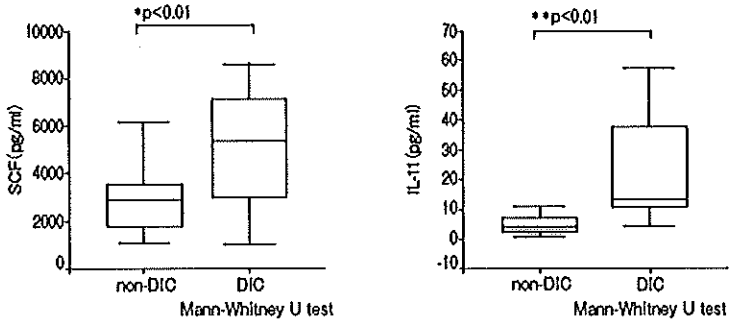

Fig. 4. The plasma stem cell factor (SCF) and interleukin 11 (IL-11) values of the 48 sepsis patients divided into a group of 17 patients diagnosed with disseminated intravascular coagulation (DIC; DIC group) and a group of 31 patients not diagnosed with DIC (non-DIC group) are shown. The plasma SCF and IL-11 values were both significantly higher in the DIC group than in the non-DIC group. Even in the non-DIC group the SCF values were much higher than the normal value. A significant difference was considered to exist at p values <0.05.

IV. Discussion

The major results of this study in the sepsis patients were: 1) the plasma TPO values were highest in the 30D group; 2) the plasma IL-3 values, IL-11 values, and LIF values were higher in the D group; and 3) the plasma TPO values, SCF values, and IL-11 values were higher in the patients with concomitant DIC.

TPO is a glycoprotein hormone that controls the number of platelets in peripheral blood by stimulating megakaryocyte proliferation and differentiation[14], and it is said that TPO values are strongly correlated with the severity of sepsis and that IL-6 is a possible trigger for TPO production[15].

Cytokine values, including TPO, IL-11, and SCF values, increase in pathological states in burn patients in which there is a decrease in platelets the same as the decrease in DIC, and, in particular, the values of these cytokines markedly increase in DIC accompanied by infection[5].

In a study of patients with DIC as a complication of generalized peritonitis, the values of pro-inflammatory cytokines, including TNF- α , IL-6, and IL-8, were found to be markedly elevated during septic DIC in comparison with DIC unaccompanied by infection. Moreover, the outcome is said to have been poorer when the plasma TPO and SCF values were high[6].

Leukemia inhibitory factor (LIF) is a substance that was purified as a rat leukemia cell line differentiation-inducing factor, and it has been reported to have a megakaryocyte-stimulating action similar to that of IL-6. LIF alone does not appear to have megakaryocyte colony stimulating factor (Meg-CSF) activity, but it has been reported to increase mouse megakaryocyte activity in the presence of IL-3 in vitro[16].

In the present study the plasma TPO values of the sepsis patients were found to be markedly elevated, the same as in previous reports (normal values: ~ 60 pg/ml), and, in particular, they were found

to be significantly higher in the D group than in the S group. Plasma IL-6 values and TNF-α values were also investigated, but the same as the TPO values, they were significantly higher in the D group (data not shown). Assessment of the cases with concomitant DIC showed significantly higher TPO values in the group with DIC. There was also a significant correlation between the TPO values and the IL-6 values ($r^2=0.755$, $p<0.001$). TPO is also regarded as an early response protein in sepsis[17], and it appeared to be related to its severity, and, in particular, to be capable of serving as an important indicator in the form of a prognostic factor. In addition, a significant elevation was seen in the cases associated with DIC, but the TPO values were also markedly elevated in the group not diagnosed with DIC (mean value: 396.4 ± 187.2pg/ml), suggesting that plasma TPO values may also be useful in the early diagnosis of septic DIC.

The assessment of plasma LIF values showed that only one patient in the S group of sepsis patients had a value at or above the sensitivity of the measurements (4 pg/ml), and that the LIF values were significantly higher in the D group (mean: 12.2 ± 16.4 pg/ml). More specifically, assessment of the results in the D60 group and D30 group showed that all of the plasma LIF values in the D60 group (n=3) were below the sensitivity of the measurements, and that the mean values (17.1 ± 18.5pg/ml) in the D30 group (n=6) were abnormally high. Although a statistical comparison would be meaningless because of the very small number of cases, plasma LIF values appear to be worth investigating as a prognostic factor in the future. IL-11 and IL-3 are said to exert an effect on megakaryocyte colony formation, and they were significantly elevated in the sepsis cases. IL-11 is said not to act alone, but to exert thrombopoietic activity in the presence of IL-3[18], and from the standpoint of increased numbers of platelets the above finding was not inconsistent with previous reports. However, significantly higher IL-11 values were observed in the DIC group in the comparison between the DIC group and the non-DIC group, and because the IL-3 values in both groups were below the detection sensitivity of the assay, it appears that further study will be needed in the future to determine whether or not these cytokines are produced in excess as thrombopoietic factors.

SCF is a hematopoietic factor that is expressed by stromal cells, but by itself it has weak differentiating and proliferating activity, and it is thought to reinforce the actions of hematopoietic factors and cytokines, including erythropoietin, IL-1, and IL-6[19]. Plasma SCF values are said to be significantly higher in patients with sepsis secondary to generalized peritonitis who do not survive[6]. Although the difference in plasma SCF values in the present study was not significant, they tended to be higher in the D group of sepsis patients, and they were significantly higher in the group diagnosed with concomitant DIC than in the non-DIC group. The plasma IL-6 values were 457.0 ± 184.6 pg/ml and 181.7 ± 98.5 pg/ml in the sepsis D group and S group, respectively, and 270.9 ± 163.9 pg/ml and 120.0 ± 68.2 pg/ml in the DIC group and non-DIC group, respectively, and they were

significantly higher in the D group and in the DIC group. There was also a significant correlation between the SCF values and IL-6 values. The plasma SCF values in the S group (n=9) and D group (n=8) of patients in the DIC group were 5178.8 ± 2263.9 and 5236.2 ± 2798.4, respectively (p = 0.964) , and almost the same. Although the SCF values did not govern the outcome of the sepsis patients, because they were abnormally high even in the non-DIC group, as were the TPO values, they appear to be useful in the early diagnosis of septic DIC.

V. Conclusion

We assessed hematopoietic factors in sepsis cases and in sepsis cases with concomitant DIC. The plasma TPO, LIF, IL-11, and IL-3 values were higher in the group of sepsis patients who died. The plasma TPO, SCF, and IL-11 values were higher in the group of sepsis with concomitant DIC. The results suggested that plasma TPO values and SCF values may be early diagnostic markers of DIC in patients with septic DIC.

Conflict of interest statement: Koichi Hoshikawa and other co-authors have no conflict of interest.

References

1) **Colman RW, Robboy SJ and Minna JD:** Disseminated intravascular coagulation (DIC) : An approach. Am J Med **52**, 679-689, 1974.
2) **Wada H, Minamikawa K, Wakita Y, et al.:** Increased vascular endothelial cell markers in patients with disseminated intravascular coagulation. Am J Hematol **44**, 85-88, 1993.
3) **Wada H, Tamaki S, Tanigawa M, et al.:** Plasma level of IL-1b in disseminated intravascular coagulation. Thromb Heamost **65**, 364-368, 1991.
4) **Wada H, Ohiwa M, Kaneko T, et al.:** Plasma level of tumor necrosis factor in disseminated intravascular coagulation. Am J Hematol **37**, 147-151, 1991.
5) **Minagawa Y, Yamada Y, Makabe H, et al.:** Examination of hematopoietic factors in burn patients with disseminated intravascular coagulation. J Iwate Med Assoc **61**, 305-312, 2009.
6) **Takahashi G, Sato N, Kojika M, et al.:** A study of hematopoietic factors in the presence of disseminated intravascular coagulation associated with diffuse peritonitis. Med Postgrad **44**, 61-65, 2006.
7) **Endo S, Inada K, Nakae H, et al.:** Blood levels of endothelin-1 and thrombomodulin in patients with disseminated intravascular coagulation and sepsis. Res Commun Molecul Phathol Pharmacol **90**, 277-288, 1995.
8) **Endo S, Inada K, Arakawa N, et al.:** Interleukin 11 levels in patients with disseminated intravascular coagulation. Res Commun Molecul Pathol Pharmacol **91**, 253-256, 1996.
9) **Endo S, Inada K, Sato N, et al.:** Cytokines in surgical stress. Jpn J Surg **97**, 708-715, 1996.
10) American college of chest physicians/society of critical care medicine consensus conference committee: Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med **20**, 864-874, 1992.
11) **Knaus WA, Draper EA, Wagner DP, et al.:** APACHE II: a severity of disease classification system. Crit Care Med **13**, 818-829, 1985.
12) **Vincent JL, de Mendonça A, Cantraine F, et al.:** Use of the SOFA score to assess the incidence of organ dysfunction/failure in intensive care units: results of a multicenter, prospective study. Working group on "sepsis-related problems" of the European Society of Intensive Care Medicine. Crit Care Med **26**, 1793-1800, 1998.
13) **Aoki N and Hasegawa H:** Annual report of the research committee on coagulation disorder. Ministry of Health and Wekfare of Japan, pp. 37-41, Tokyo, 1988.
14) **Kaushansky K:** Thrombopoietin. Drug therapy. N Engl J Med **339**, 746-754, 1998.

15) **Spyros GZ, Spyros P, Thodoris T, et al.**: Sepsis severity is the major determinant of circulating thrombopoietin levels in septic patients. Crit Care Med **32**, 1004-1010, 2004.
16) **Metcalf D, Hilton D and Nicola NA**: Leukemia inhibitory factor can potentiate murine megakaryocyte production in vitro. Blood **77**, 2150-2153, 1991.
17) **Wolber EM and Jelkmann W**: Thrombopoietin: The novel hepatic hormone. News Physiol Sci **17**, 6-10, 2002.
18) **Musashi M, Yang YC, Paul SR, et al.**: Direct and synergistic effects of interleukin-11 on murine hemopoiesis in culture. Proc Natl Acad Sci **88**, 765-769, 1991.
19) **McNiece IK, Langley KE and Zsebo KM**: Recombinant human stem cell factor synergizes with GM-CSF, G-CSF, IL-3, and EPO to stimulate human progenitor cells of the myeloid and erythroid lineage. Exp Hematol **19**, 226-231, 1991.

岩手医誌 65 巻，5 号（平成 25 年 12 月）333-341 頁.

敗血症患者および disseminated intravascular coagulation 症例における造血因子の検討

星川浩一[1)]，小鹿雅博[1)]，高橋　学[1)]，
松本尚也[1)]，藤野靖久[1)]，鈴木　泰[1)]，井上義博[1)]，
新田浩幸[2)]，若林　剛[2)]，遠藤重厚[1)]

[1)]岩手医科大学医学部，救急医学講座
[2)]岩手医科大学医学部，外科学講座

(Received on February 22, 2011 & Accepted on March 4, 2011)

要旨

今回我々は，敗血症患者における予後に関して，造血因子を中心として解析を行った．敗血症と診断された患者 48 名の患者背景，重症度，血漿中の造血因子やサイトカインを検討項目とした．生存群と 30 日以内死亡群（D3 群）とを比較し，Thrombopoietin (TPO) 値では，D30 群で有意に上昇していた．また，生存群と死亡群で比較した場合，leukemia inhibitory factor(LIF)，interleukin-11(IL-11) と IL-3 は，死亡群で有意に上昇していた．さらに，disseminated intravascular coagulation (DIC) score で，DIC と診断された群と DIC と診断されない群との比較では，TPO，SCF と IL-11 が DIC 患者において有意に上昇していた．今回の検討から，TPO，LIF，IL-11 と IL-3 は敗血症患者において予後因子となる可能性がある．また DIC 患者において，TPO，SCF と IL-11 が重要な risk factor となる可能性が示唆された．