Journal of the Egyptian Society of Haematology & Research, Vol. 2, No. 2, September: 161-168, 2006

Study of Sepsis Related Changes in Platelet Adhesion, Aggregation and Angiogenic Growth Factor Release

SOMAYA E.T. SOLIMAN, M.D. and RANIA A. ZAYED, M.D*

The Departments of Radioisotopes, Nuclear Research Center, Atomic Energy Authority and Clinical Pathology, Faculty of Medicine, Cairo University.*

ABSTRACT

Background: Reassessment of the platelets' role in the various pathogenic mechanisms that have been recently implicated in sepsis-related coagulopathy has not yet been made. The aim of this study was to elucidate the effect of sepsis on platelet adhesiveness, aggregation, and growth factor release.

Setting: Radioisotopes department, Nuclear Research Center, in Collaboration with Clinical Pathology Department, Faculty of Medicine, Cairo University.

Patients and Methods: Platelets' count and function were investigated in 30 patients with sepsis. Agonist induced platelet aggregation was measured using three agonists (ADP, collagen, arachidonic acid [A.A]). Release of vascular endothelial growth factor (VEGF) and platelet derived growth factor (PDGF), were measured using immunoradiometric (IRMA) assay. Expression of platelet adhesion molecules was measured using flowcytometry (fibrinogen binding, glycoprotein V and p-selectin expression).

Results: All patients with sepsis exhibited thrombocytopenia that was related to the severity of illness and associated with bad outcome. Agonist-induced platelet aggregation was consistently reduced in patients, specially with AA.

Platelet aggregation decreased regardless of the platelet count or thrombin generation, rather, correlated to the severity of sepsis. Flowcytometric analysis, revealed significantly reduced fibrinogen binding, maintained CD42a, and significantly elevated CD62p. VEGF and PDGF release were significantly increased as compared to normal controls.

Conclusion: These results suggest that sepsis induces a complex regulation of platelets' function, with redistribution of platelets' role from heamostasis towards other functions including inflammatory response and vascular healing. These changes occur even when the platelet count is normal, independent of thrombin generation.

Key Words: *Platelet adhesion – Aggregation – PDGF – VEGF – DIC.*

INTRODUCTION

Platelet function can be seen as a succession of overlapping events involving adhesion, aggregation, secretion, and promotion of procoagulant activity. All patients with sepsis have an activated coagulation system, which may range from minor changes in highly sensitive molecular markers for hypercoagulability to full blown disseminated intravascular coagulation (DIC) with intravascular fibrin deposition and consumption of platelets and coagulation factors **[1,2]**. Ongoing systemic activation of coagulation does not only lead to microvascular failure with obstruction of the blood supply to various organs; thereby, contributing to organ failure, but also plays a central role in the inflammatory response to severe infection and sepsis **[3]**. The various mechanisms that are important for the derangement of coagulation in sepsis have been elucidated in the last 10-15 years. The focus has mainly been on plasma coagulation proteins, activated inflammatory cells expressing cytokines and tissue factor, and perturbed endothelial cells, which play central role in the impaired anticoagulant function, fibrinolysis, and potentially as a source of tissue factor expression **[4]**. Sepsis is a clear risk factor for thrombocytopenia in critically ill patients and the severity of sepsis, correlates with the decrease in platelet count **[5,6]**. Studies on platelet function in sepsis have yielded conflicting results. Many investigators reported endotoxin-induced platelet accumulation and enhanced platelet aggregation in animal models **[7-9]**. In

vitro, bacterial compounds such as lipopolysaccharide and staphylococcus aureus lipoteichoic acid can bind to platelets and endothelial cell membranes in patients with sepsis and inhibit platelet aggregation **[10]**. On the other hand, sepsis-generated cytokines did not seem to activate human platelets either directly or via thrombin **[11]**. Several clinical studies have reported decreased platelet aggregability during sepsis **[12,13]**, whereas, Gawaz et al. **[14]** reported increased platelet aggregability.

Platelet α-granules contain vascular growth factors, such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and platelet factor 4 (PF-4), which are released during aggregation. VEGF is a potent angiogenic factor regulating proliferation, differentiation, and survival of microvascular endothelium **[15]**. PDGF contributes to endothelial repair and angiogenesis **[16,17]** but little is known about their regulation during the septic response.

In the present study, we studied platelet aggregation, adhesion, and growth factor release in patients with sepsis in order better to define sepsis-related alterations in platelet functions and delineate platelets' position into the new insights in the pathogenesis of sepsis-related coagulopathy.

SUBJECTS AND METHODS

Subjects and study design:

Platelet aggregation, adhesion and growth-factor release were investigated in 30 patients with sepsis of recent onset (14 patients uncomplicated sepsis admitted to the medical, surgical, gynecological units of Cairo University hospitals, and 16 critically ill patients admitted to intensive care unit (ICU) of Cairo University hospital. Fifteen healthy volunteers matched for age and sex were included as normal controls. Healthy volunteers had no evidence of infection and had not received antibiotics or non steroidal anti-inflammatory drugs (NSAID) within the 10 days prior to the study.

Diagnosis of sepsis was established by having at least one positive blood culture and/or an identified focus of infection. Severity of the disease was assessed according to the Acute Physiology and Chronic Health Evaluation (APACHE) II score **[18]**, and the Sequential Organ Failure Assessment (SOFA) score **[19]**. Presence of at least one organ failure related to sepsis was considered as severe sepsis, presence of sustained sepsis-related hypotension necessitating the administration of vasopressor agents was defined as septic chock.

The criteria for DIC were those defined by the subcommittee on disseminated intravascular coagulation of the international society of thrombosis and haemostasis (based on platelet count, soluble fibrin monomers, prothrombin concentration, and fibrinogen concentration) score above 5 was considered as DIC **[20]**.

Exclusion criteria were; blood creatinine >2.5mg/dl at entry, administration of heparin or drugs affecting prostanoid synthesis. However, antibiotics could not be avoided, although, some of them may affect platelet functions.

Methods:

5ml of venous blood were collected in 3.8% sodium citrate tubes with the ratio of 9:1. Blood samples were withdrawn on the day of admission to the ICU from patients with severe sepsis, and at the onset of sepsis in patients who developed sepsis during hospitalization.

Whole blood (500ml) was used for flow-cytometry; platelet rich plasma (PRP) was immediately prepared from the remaining blood sample by centrifugation at 90xg for 10 min. Platelet poor plasma (PPP) was prepared from PRP by a second centrifugation at 2000xg for 15 min. PRP was used for assessment of platelet aggregation and growth factor release. PPP was frozen at -70ºC for use in the measurement of thrombin generation and growth factor release. PRP from healthy volunteers was sequentially diluted in their PPP to match the low platelet counts in PRP of the thrombocytopenic patients with sepsis.

* Thrombin generation assessment was done by enzyme-linked immunoassay (ELISA) method using 4 commercial kits to measure, thrombin-antithrombin (TAT) complex (Enzygnost TAT), prothrombin fragment 1+2 (Enzygnost F1+2) and soluble fibrin monomers (Enzygnost SF), all produced by Boehring®, Schwalbach, Germany. D-dimer was measured using Mini Vidas DDI, Biomereux®, Letoile, France Kit.

* Platelet aggregation was assessed according to the manufacturer's instructions using lumiaggregometer (Chrono-log Corp, Havertown, PA, USA). The agonists used were: 500 µg/ml arachidonic acid (Biol Data Corporation), 5µm ADP (Roche, Mannheim, Germany) and 2.5µg/ml collagen (Chrono-log corp. Havertown, PA, USA). Agonist induced aggregation was recorded graphically for 10 min. Results were given as a ratio of percentage aggregation of the patients' in relation to the healthy controls' with the closest platelets count after dilution.

**VEGF and PDGF measurement:*

To evaluate in vitro growth factor release, the supernatant of the aggregation induced by collagen was collected after centrifugation at 2000xg to remove aggregates and free remaining platelets and was kept at -70ºC for determination of VEGF and PDGF content by Immunradiometric assay (IRMA) technique using the kits (Human VEGF, IRMA, and Human PDGF-AB IRMA) produced by LINCO Research St-Charles Missouri 63304, USA. The sensitivity of the test for VEGF was 9pg/ml and for PDGF was 8.4pg/ml. The amount of growth factor released in vitro per million platelets was calculated as described by Yagouchi et al. **[13]** from the formula:

$$\text{G.F. released per } 10^6 \text{ platelets in pg} = \frac{\text{G.F in PRP} - \text{G.F in PPP}}{\text{No. of platelets in PRP sample}} \times 10^6$$

**Measurement of platelet adhesion by flow cytometry:*

- Avidity of GP IIb/IIIa for fibrinogen was studied with the use of directly fluorescein isothiocyanate (FITC) stained fibrinogen (Dako, Glostrup, Denmark).
- The adhesion molecule glycoprotein V involved in von-Willebrand factor rolling (CD42a), was studied using FITC-stained anti CD42a produced by Immunotech, Marseille, France.
- α-granule secretion was studied by detection of P-selectin expression with the use of FITC-stained monoclonal anti CD62p, produced by Becton Dickinson.

Platelets were stained with FITC-conjugated mouse monoclonal antibodies against CD42a, CD62p, fibrinogen and with the appropriate isotype matched control monoclonal antibody. After 15 min incubation at room temp, the platelets were fixed in 1ml 1% paraformaldhyde in edta phosphate-buffered saline. Data were acquired using FACScan flowcytometer (Coulter Epics, Elite). Platelets were defined as events fitting the platelet size and complexity in log scale forward and side scatter and expressing fluorescence in the FL3 channel. The median fluorescence intensity (MFI) was used to depict molecule expression on the platelet surface.

Statistical analysis:

All values were expressed as mean±SD (normal distribution) or median±SD (Scewed data). Data were analyzed by unpaired student t-test (normal distribution) or Mann-Whitney U-test. Pearson's correlation coefficient was used for correlations.

$p < 0.05$ was considered to be statistically significant.

RESULTS

Clinical diagnosis of the patients included in the study, isolated micro-organisms from blood culture, and antibiotic therapy used are shown in Table (1). Demographic data and clinical classification of the studied patients according to severity of illness and coagulation status are shown in Table (2). Of the 30 patients 14 had uncomplicated sepsis (47%) and 16 had severe sepsis or septic shock (53%). The healthy control group included 10 males and 5 females with mean age of 39±9 years, and median platelet count of $240x10^3 \pm 90x10^3/mm^3$ (range $180\text{-}380x10^3/mm^3$). Thrombocytopenia was evident in patients and correlated positively with severity of illness, 53% of patients had platelet count $<150x10^3/mm^3$, and 56.6% showed count $<100x10^3/mm^3$, as shown in Table (2).

Platelet aggregation assessment revealed significantly reduced aggregation ratio in all the patients compared to diluted matched-count PRP of the controls, regardless of the agonist used. Patients were divided into 3 groups according to their platelet counts, those with platelet count $>180x10^3/mm^3$ (8 sepsis and 2 complicated sepsis), those with platelet count between $180x10^3$ and $100x10^3/mm^3$ (3 sepsis and 9 complicated sepsis), and those with low platelet count $<100x10^3/mm^3$ (3 sepsis and 5

complicated sepsis). Results of platelet aggregation with different agonists in relation to severity of illness are presented in Table (3). Platelet aggregation was more severely reduced in patients with severe sepsis than in those with uncomplicated sepsis, the difference being particularly significant in patients with thrombocytopenia. The most sensitive pathway to sepsis-induced alteration in platelet aggregation was the cyclo-oxygenase pathway with arachidonic acid (AA) inducing 48% maximal aggregation in patients with normal platelet count and 12% in thrombocytopenic patients (Table 3). Platelet aggregation was similarly altered in patients with and without DIC, unless there was thrombocytopenia. (Table 4).

Thrombin generation assessment revealed that, there was neither correlation between soluble fibrin monomer, D-dimers, TAT and F1+2 and DIC status, nor, the aggregation ratio related to ADP, AA, and collagen in all patients. (Table 5).

Flowcytometric analysis revealed that the activated conformation of GP IIb/IIIa assessed by FITC-stained fibrinogen binding was significantly decreased in patients after platelet activation by collagen, whereas, the ex-vivo expression of the adhesion molecule CD42a involved in von-willebrand rolling revealed no significant change in patients as compared to controls. Results are shown in Table (6). Alpha-granule secretion as detected by the expression of P-selectin (CD62p) with collagen activation was significantly increased in patients compared to controls ($p<0.05$). The difference being markedly significant in patients with uncomplicated sepsis. (Table 6). VEGF release by platelets after collagen activation was significantly increased in all patients. (Table 7). PDGF release was also significantly increased in all patients. The increase in both VEGF and PDGF release was more significant in uncomplicated sepsis than in severe sepsis, and in patients without DIC than in those with DIC. Results are represented in Table (7). There was no correlation between thrombin generation and VEGF or PDGF release regardless of the patient's platelet count.

Table (1): Clinical diagnosis, isolated organisms, and therapy in patients.

	No of patients
Clinical diagnosis:	
• Purperal sepsis and septic abortions	5
• Appendicular abscess and abdominal sepsis	4
• Post-splenectomy pneumonia or overwhelming sepsis	5
• Diabetic foot	6
• Pyelitis and pyelonophritis	3
• Foreign body	3
• Major trauma and severe lacerations	4
Isolated organisms:	
• Escherichia coli	12
• Staphylococcus aureus	9
• Klebsiella	6
• Pseudomonas aeruginosa	3
Antibiotics, used:	
• Aminopenicillin	12
• Cephalosporins	27
• Aminoglycosides	16
• Vancomycin	10
Vasoactive agents in septic shock:	
• Dopamine	10
• Dopamine and norepinephrine	4
• Dobutamine	2

Table (2): Demographic data and clinical classification of the patients (n=30) according to severity of illness and the occurrence of DIC (mean ±SD).

	Severity of illness		Coagulation status	
	Sepsis	Sever sepsis/ septic shock	No DIC	DIC
Number of patients	14 (47%)	16 (53%)	13 (43.4%)	17 (56.6%)
Age (years)	40±10	50±9	40±13	56±4
Male: female	9:5	10:6	9:4	14:3
APACHE II score	9±4	15±4*	13±6	15±7
SOFA score	2±1	7±4*	4±3	8±4**
Platelet count $x10^3/mm^3$	210±90	140±100* (53.4%)	200±90	80±30** (56.6%)
Number of non survivals	0	14 (87.5%)	5 (39.3%)	17 (100%)

* $p < 0.05$ sepsis Vs. severe sepsis / septic shock, ** $p < 0.05$ no DIC Vs. DIC.

Table (3): Maximal aggregation (%) induced by different agonist in patients in relation to severity of illness (mean +SD).

Platelet count x10^3/mm^3		No of patients	5 µm ADP	2.5 µg/ml collagen	500 µg/ml AA
>180x10^3/mm^3	Sepsis	8	80±1	70±5	63±6
	Severe sepsis	2	71±6*	68±3*	58±3*
<180x10^3/mm^3	Sepsis	3	81±2	70±4	51±8
>100x10^3/mm^3	Severe sepsis	9	70±2*	65±1*	48±13*
<100x10^3/mm^3	Sepsis	3	69±3	65±1	43±2
	Severe sepsis	5	40±4**	43±19**	12±2**

* $p < 0.05$ sepsis Vs. severe sepsis, ** $p < 0.01$ sepsis Vs. severe sepsis.

Table (4): Maximal aggregation (%) induced by different agonist in patients with and without DIC (mean±SD).

Platelet count x 10^3/mm^3		No of patients	5 µm ADP	2.5 µg/ml collagen	500 µg/ml AA
>180 x 10^3/mm^3	No DIC	8	80±1	70±5	63±6
	DIC	0	–	–	–
<180 x 10^3/mm^3	No DIC	5	80±6	69±3	51±8
>100 x 10^3/mm^3	DIC	7	79±2	70±1	48±13
< 100 x10^3/mm^3	No DIC	0	–	–	–
	DIC	10	45±41*	43±16*	12±2*

$p < 0.05$ thrombocytopenia Vs. normal platelet count, otherwise, $p > 0.05$ for DIC Vs.

Table (5): Thrombin generation in the studied patients (median±SD).

	Coagulation status	N	Soluble fibrin monomer mg/L	D-dimers ng/ml	Thrombin-Anti -thrombin µg/L	Prothrombin fragment 1+2
Normal values			0-17	< 500	1.0–4.1	0–1.1
All patients		30	28.1±10.3	1890±1150	15.0±21.2	1.9±0.93
>180x10^3/mm^3	No DIC	8	26.9±6.5	1531±960	10.8±10.5	1.9±0.93
	DIC	0	–	–	–	–
<180x10^3/mm^3	No DIC	5	27.3±14.4	2010±1200	14.2±14.6	1.9±1.0
>100x10^3/mm^3	DIC	7	28±6.3	2080±1233	14.9±11.9	2±0.86
<100x10^3/mm^3	No DIC	0	–	–	–	–
	DIC	10	30.4±10.1	2210±1260	20±30.4	2.4±0.5

N: number of patients. Markers of thrombin generation did not correlate to DIC status ($p > 0.05$).

Table (6): Flowcytometric analysis for platelet adhesion molecules and P-selectin expression. (MFI±SD) (median fluorescence intensity on FL3-channel linear scale).

Collagen induced activation	Control (n=7)	Patients (n=30)	
		Sepsis (n=14)	Severe sepsis/septic shock (n=16)
Fibrinogen binding	740±25	415±150*	300±96**
CD42a	481±70	400±85	403±80
CD62p	420±130	608±120**	536±100*

* $p < 0.05$ Vs. control, ** $p < 0.01$ Vs. control.

Table (7): PDGF release and VEGF release induced by collagen in patients and controls in relation to severity of illness and DIC status (median±SD).

Collagen induced release	Control n=15	Patients (n=30)			
		Severity of illness		Coagulation status	
		Sepsis (n=14)	Severe sepsis/ shock (n=16)	No DIC (n=13)	DIC (n=17)
PDGF pg/10^6 platelets	12±6	28.9±10.4**	21.4±16*	28.1±13*	20.3±11*
VEGF pg/10^6 platelets	15±11	36±15.1**	28.6±13.9*	30.1±12**	28.5±10.6*

* $p < 0.05$ Vs. control, ** $p < 0.01$ Vs. control.

DISCUSSION

In the present study, all patients with sepsis had an activated coagulation system. We observed that sepsis was a clear risk factor for thrombocytopenia in critically ill patients (53.4% <$150x10^3/mm^3$ and 56.6% <$100x10^3$ / mm^3), and the severity of sepsis correlated with the decrease in platelet count. It was previously reported that, the incidence of thrombocytopenia (platelets <$150x10^3/mm^3$) in critically ill patients was 34-44%, whereas, platelet count of less than $100x10^3$ was seen in 20-25% of patients, and 12-15% of patients had platelet count <$50x10^3/mm^3$ **[21]**. Mavromattis et al. **[22]** and Akca et al. **[23]** reported that, the platelet count in patients with sepsis decreased during the first 4 days in the intensive care unit, this decrease was correlated to the severity of sepsis. The mechanism by which thrombocytopenia in sepsis occurs, however, is not completely clear. Impaired production of platelets from the bone marrow may seem contradictory to the high levels of platelet production-Stimulating pro-inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α) and interleukin (IL-6), and high concentration of circulating thrombopoietin in patients with sepsis. These cytokines and growth factors should theoretically stimulate megakaryopoiesis in the bone marrow **[24]**. However, in a substantial number of patients with sepsis marked hemophagocytosis by monocytes and macrophages may occur, hypothetically, due to stimulation with high levels of macrophage colony-stimulating factor (M-CSF) in sepsis **[25]**. Platelet consumption may also play an important role in patients with sepsis, due to ongoing thrombin generation (which is the most potent activator of platelets in vivo) **[26]**.

In the present study, we observed that platelet functions can be regulated separately in sepsis, with reduced aggregation, maintenance of adhesion molecule expression, increased α-granule secretion (↑ P-selectin expression) and modulation of growth factors with increased VEGF and PDGF release. The decrease in platelet aggregation was related to the severity of sepsis. Flowcytometry confirmed this aggregation defect, demonstrating concomitant reduction in fibrinogen binding on the platelets of septic patients. These changes were observed even in patients with normal platelet count or low DIC score, and were completely independent of thrombin generation. Similar results were reported by Yaguchi et al. **[13]**. At first sight, this result can be interpreted in two ways. It may be that there is decreased platelet aggregation in sepsis, but, a more likely explanation is that an increased platelet aggregation activity in patients with sepsis will lead to circulating platelets that are already activated and will not aggregate in an ex-vivo setting. The assembly of several coagulation factor complexes, such as the tenase or prothrombinase complexes, will be markedly facilitated if a suitable phospholipids surface is available, and occurs in vivo on the membrane of activated platelets **[27]**. In the setting of inflammation induced activation of coagulation, platelets can be activated directly by endotoxin **[28]** or by pro-inflammatory mediators, such as platelet activating factor **[29]**. Once thrombin is formed, this will activate additional platelets. In the present study, regardless of the severity of sepsis, platelets preserved normal expression of the adhesion molecule CD42a (involved in von-Willebrand binding), suggesting that the ability of platelets to adhere is probably preserved, even, when aggregation induced by this agonist was profoundly affected.

This finding evokes a defect at the intracellular transduction pathway rather than down regulation of surface receptors. The most affected pathway was found to be the cyclo-oxygenase pathway with arachidonic acid. In accordance with these findings, Lundahl et al. observed that platelets had decreased fibrinogen binding capability in response to AA, which was correlated with a poor outcome **[30]**. In contrast to aggregation, α-granule secretion function was enhanced with increased p-selectin (CD62P) expression on platelet surface. Similar results have been reported by Salat et al., who observed higher expression of CD62P in patients with sepsis, but with a low level of statistical significance **[31]**. However, Gawaz et al. **[14]** and Yaguchi et al. **[13]** reported unmodified expression of P-selectin on the platelet surface. Previous studies have shown that expression of P-selectin on the platelet membrane not only mediates the adherence of platelets to leukocytes and endothelial cells, but, also enhances the expression of tissue factor on monocytes **[29]**. The molecular mechanism of this effect relies on nuclear factor kappa-B (NFK-B) activation, induced by binding of activated platelets to neutrophils and mononuclear cells **[29]**. P-selectin can be relatively easily shed from the surface of the platelet membrane and soluble P-selectin levels have indeed been shown to be increased during systemic inflammation **[32]**. We also, observed, enhanced release of VEGF and PDGF in patients with sepsis compared to the control group. This increase occurred mainly in patients with uncomplicated sepsis, no DIC, low thrombin generation and normal platelet count. Since platelets have no nucleus, this observation suggests that changes in α-granule content may occur at the megakaryocyte level, probably, as a result of the inflammatory response **[33]**. Yaguchi et al., in their study reported differential release of growth factors from platelets in patients with sepsis, with increased VEGF and unchanged PDGF release **[13]**. Results of the present study, suggest that, platelet release of inflammatory mediators and growth factors may be another link between activation of coagulation and inflammation, moreover, it adds to previously accumulated knowledge on the role of platelets in sepsis, suggesting that, sepsis induces a redistribution of platelet function from haemostasis towards other functions, including vascular healing.

Conclusion:

Platelets seem to occupy one of the essential crossroads in the complex interaction between inflammation and coagulation, not only by facilitating and propagating thrombin generation (which in itself will further activate platelets) but also, by being an important mediator of growth factor and adhesion molecule activity. More research on the role of platelet activation in sepsis will undoubtedly be helpful in further unraveling the pathogenesis of sepsis and in understanding the tight crosstalk between inflammation and coagulation.

Acknowledgment:

The authors greatly appreciate Prof. Dr. Hesham Soliman, professor of vascular surgery, Cairo University; Prof. Dr. Mohammad Zaater, Professor of nephrology, Cairo University; and Prof. Mohammed Abdel Kader, professor of gynecology and obstetrics, Cairo University, for their great help to accomplish this work.

REFERENCES

1- Levi M, Keller T, Van Gorp E, Tencate H. Infection, inflammation and the coagulation system. Cardiovasc. Res. 2003, 60: 26-39.

2- Levi M, Tencat H. Disseminated intravascular coagulation. N Engl J Med. 1999, 341: 586.

3- Esmon CT, Fukudome K, Mather T, Bode W, Regan LM, Stecrns-Kurosawa DJ, Kurosawa S. Inflammation, sepsis, and coagulation. Haematologica. 1999, 84: 254-9.

4- Esmon CT. Are natural anticoagulants candidates for modulating the inflammatory response to endotoxin? Blood. 2000, 95: 1113-1116.

5- Vanderschweran S, DeWeendt A, Malbrain M, Vankerschaever D, France E, Wilmer A, Bobbaers H. Thrombocytopenia and prognosis in intensive care Crit. Care Med. 2000, 28: 1871-6.

6- Baughman RP, Lower EE, Flessa HC, Tollerud DJ. Thrombocytopenia in the intensive care. Chest. 1993, 104: 1243-7.

7- Itoh H, Cicala C, Douglas GJ, Page CP. Platelet accumulation induced by bacterial endotoxin in rats. Thromb Res. 1996, 83: 405-419.

8- Matera C, Falzaino C, Berrino L, Russi F. Effects of tetanus toxin, salmonella typhimurin porin, and bacterial lipopolysaccharide on platelet aggregation. J Med. 1992, 23: 327-38.

9- Salden HJ, Bas BM. Endotoxin binding to platelets in blood from patients with sepsis syndrome. Clin Chem. 1994, 40: 1575-9.

10- Sheu JR, Lee CR, Lin CH, Hsia OG, Ko WC, Chen YC, Yen MH. Mechanisms involved in the antiplatelet activity of staphylococcus aureus lipoteichoic acid in human platelets. Thromb Haemost. 2000, 83: 777-84.

11- Leytin V, Shakoor S, Mody M, Allen D, Gravey B, Ereedman J. Sepsis and endotoxemia-generated cytokines do not trigger activation of human platelets Crit Care Med. 2002, 30: 2771-3.

12- Boldt J, Muller M, Rothe A, Lenzen P, Hempelmann G. Does continuous heparinization influence platelet function in the intensive care patient? Intensive care Med. 1997, 23: 567-73.

13- Yaguchi A, Lobo FLM, Vincent JL, Pradier O. Platelet function in sepsis. J Thromb Haemost. 2004, 2: 2096-2102.

14- Gawaz M, Dickfeld T, Bonger C, Fateh-Moghadam S, Neumann FJ. Platelet function in septic multiple organ dysfunction syndrome. Intensive care Med. 1997, 23: 379-85.

15- Maloney JP, Silliman CC, AMbruso DR, Wang I, Tuder RM, Vaelkel NF. In vitro release of vascular endothelial growth factor during platelet aggregation. Am J Physiol. 1998, 275: H1054-H1067.

16- Linder BL, Sherroff A, Kaplan KL, Goodman DS. Release of platelet derived growth factor from human platelets by arachidonic acid. Proc Noth Acad Sci. USA 1979, 76: 4107-11.

17- Thomnen R, Human R, Misevis G, Pepper MS, Hahn AW, John M, Batergay EJ. PDGF-BB increases endothelial migration on cord movements during angiogenesis in vitro. J Cell Biochem. 1997, 64: 403-13.

18- Knaus WA, Draper EA, Wanger DP, Zimmerman JE. APACHE II. A severity of disease classification system. Crit care Med. 1985, 13: 818-29.

19- Vincent JL, Moreno R, Takala J, Willates S, deMendonce A, Bruining H, Reinhart CK, Sater PM, Trijs LG. TheSOFA (sepsis-related organ failure assessment score) to describe organ dysfunction/failure Intensive care Med. 1996, 22: 707-10.

20- Taylor FB, Toh CH, Hoots WK, Wada H, Levi M. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation Thromb Haemost. 2001, 86: 1327-30.

21- Strauss R, Wehluk, Kreutzer D, Koebrick C, Hahn EG. Thrombocytopenia in patients in the medical intensive care unit, bleeding prevelance, transfusion requirements and outcome. Crit care Med. 2002, 30: 1765-71.

22- Mavrommatis AC, Theodoridis T, Orfanidon A, Roussor C, Christopoulor V, Zakynthins S. Coagulation system and platelets are fully activated in uncomplicated sepsis. Crit care Med. 2000, 28: 451-7.

23- Akca S, Haji Michael P, de Medonca A, Sater PM, Levi M, Vincint JL. The time course of platelet counts in critically ill patients. Crit care Med. 2002, 30: 753-6.

24- Folman CC, Linthorst GE, Van Maurik J, Van Willigen G, de Jonge E, Levi M, de Haes M, dem Borne AE. Platelet release thrombopoietin (Tpo) upon activation, another regulatory loop in thrombopoiesis. Thromb Haemost. 2000, 83: 923-30.

25- Francois B, Trinoreau F, Vignon P, Fixe P, Praloran V, Gastirne H. Thrombocytopenia in the sepsis syndrome, role of hemophagocytosis. And macrophage colony stimulating factor Am J Med. 1997, 103: 114-20.

26- Wackentin TE, Aind WC, Rand JH. Platelet endothelial interactions, sepsis, HIT and antiphospholipid syndrome. Hematology (Am Soc Hematol Edue Program) 2003, 497-519.

27- Mann KG, Nesheim ME, Church WR, Holey P, Krishnaswamy S. Surface dependent reactions of the vitamin K dependent enzyme complexes. Blood. 1990, 76: 7-16.

28- Zielinski T, Wachowerz B, Saluk-Juscczak J, Kaca W. Polysaccharide part of proteus mirabilis lipopolysaccharide may be responsible for the stimulation of platelet adhesion to collagen. Platelets. 2002, 13: 419-24.

29- Zimmerman GA, Maintyre TM, Prescott SM, Stafforini DM. The platelet activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit care Med. 2002, 30: S295-S307.

30- Lundahl TH, Lindahl TL, Fagerberg JH, Egberg N, Bunesor A, Larsson A. Activated platelets and impaired platelet function in intensive care patients analyzed by flow cytometry. Blood Coagl Eibrinolysis. 1996, 7: 218-20.

31- Salat A, Bodingbauer G, Boehm D, Murabito M, Tochkow E, Sautoner T, Mueller MR, Fuegger R. Changes of platelet surface antigens in patients suffering from abdominal septic shock. Thromb. Res. 1999, 95: 289-94.

32- Shebuski RJ, Kilgore KS. Role of inflammatory mediators in thrombogensis. J Pharmacol Exp Ther. 2002, 300: 729-35.

33- Majka M, Janowska-Wrezorek A, Ratajoca K, Ehrenman K, Pietrzkowski Z, Kowalska MA, Gewirtz AM, Emelson SG, Ratjaezak MA. Numerous growth factors, cytokines, and chemokines are secreted by human CD34 +ve cells, myeloblasts, erythroblasts, and megakaryoblasts and regulate normal hematopoiesis in an autocrine/paracrine manner, Blood. 2001, 97: 3075-85.