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Review

A Three-phase Approach for the Early Identification of Acute Lung Injury Induced by Severe Sepsis

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Abstract. *A number of studies have reported that acute lung injury (ALI) and acute respiratory distress syndrome (ARDS) are independent risk factors for organ dysfunction and mortality in patients with sepsis. Although ALI/ARDS might be an essential therapeutic target during the management of sepsis, severe sepsis should be treated effectively and as soon as identified. We have classified three phases, ranging from sepsis to organ dysfunction, characterizing the interaction between neutrophils and platelets. The first phase is neutrophil extracellular trap (NET) formation and intravasated platelet aggregation. The next phase is extravasated platelet aggregation (EPA), promoted by NET-facilitated detachment of endothelial cells. The final phase is organ dysfunction, caused by pulmonary veno-occlusive disease (VOD), fibrosis, and immunoparalysis induced by EPA. Severe sepsis is characterized by a continuum of coagulopathy, with coagulation abnormalities often developing before the onset of clinical symptoms. The initial medical treatment for ALI/ARDS is inhibition of NET formation and intravasated platelet aggregation to prevent endothelial cell damage (Phase 1). Beraprost and silvestat, phosphodiesterase 3 (PDE3) inhibitors, are often administered in clinical practice. To determine hypercoagulopathy, plasma levels of thrombin–antithrombin complex and plasmin–plasmin inhibitor complex are continuously monitored in patients with suspected sepsis. Furthermore, the implementation of quality indicators for the early management of severe sepsis and septic shock is strongly associated with a reduced mortality. We conclude that pathophysiology of organ dysfunction from severe sepsis is caused by pulmonary VOD, fibrosis, and EPA-facilitated immunoparalysis. In order to prevent ALI/ARDS in patients with sepsis, countermeasures for NET and platelet aggregation should be pre-emptively employed and confirmed by several trials.*

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Injury and shock syndromes can potentially induce systemic inflammation and subsequent organ dysfunction. Acute lung injury (ALI), characterized by widespread inflammation and life-threatening hypoxemia, results from a discrepancy in ventilation and perfusion within the lung (1). Acute respiratory distress syndrome (ARDS), previously known as respiratory distress syndrome/adult respiratory distress syndrome/shock lung, may be triggered by traumatic injury or lung infection but is often the result of sepsis. Moreover, the risk of developing ARDS rises dramatically when multiple risk factors for acute lung injury are present (2).

ALI and ARDS describe clinical syndromes of acute respiratory failure with substantial morbidity and mortality (3). ALI/ARDS consists of acute inflammation and tissue injury of the lung, leading to decreased gas exchange of oxygen and carbon dioxide (4). ALI/ARDS is associated with several pathological changes: the release of inflammatory cytokines, the breakdown of endothelium lining the lung's blood vessels, the loss of surfactant (leading to decreased alveolar surface tension), the accumulation of fluid in the lung, and the formation of excessive fibrosis.

The highest incidence of ALI/ARDS occurs in patients with sepsis and those undergoing multiple emergency transfusions (5). Patients with multiple traumatic events are also at increased risk for developing ALI/ARDS (6). Patients with sepsis-related ARDS have a significantly higher 60-day mortality rate than patients with non-sepsis-related ARDS (38.2% *vs*. 22.6%, respectively) (7). The mortality rate for ALI/ARDS varies widely based on disease severity, patient age and the presence of other medical conditions (8, 9). ALI/ARDS can cause multiple organ dysfunction syndrome and death (10).

The combined activation of coagulation and inflammation play an important role in multiple organ dysfunction and poor outcomes following severe trauma (11). Elevated activation of the extrinsic coagulation pathway is common in patients with severe sepsis (12). Furthermore, disseminated intravascular coagulation (DIC) is a frequent complication of systemic inflammatory response syndrome (SIRS) and is substantially involved in the prognosis of conditions. These range from SIRS to sepsis, to severe sepsis, and eventually to septic shock (13, 14). In critically-ill patients with thrombocytopenia, coagulopathy and organ dysfunction progress with significant mutual correlation and are dependent on rising SIRS score. This leads to the concept of 'SIRS-associated coagulopathy', that may play a critical role in inducing DIC and multiple organ dysfunction syndrome in patients with SIRS (15).

Recent studies have shown that activation of platelets and leukocytes, mutually interacting, correlates with the severity of organ dysfunction in sepsis (16). Moreover, the systemic activation of inflammation and coagulation, associated with endothelial injury, holds prognostic value for the development of ALI/ARDS (11, 17).

We have classified three phases, from sepsis to organ dysfunction, characterizing the interaction between neutrophils and platelets.

The first phase is neutrophil extracellular trap (NET) formation and intravasated platelet aggregation. The next phase is extravasated platelet aggregation (EPA), promoted by NET-facilitated detachment of endothelial cells. The final phase is organ failure by pulmonary veno-occlusive disease (VOD), fibrosis and EPA-induced immunoparalysis (Figure 1).

We review the roles of platelet aggregation and leukocytes in the progression from sepsis to organ dysfunction, and outline possible preventative measures for ALI/ARDS.

Case Report

An 82-year-old female was admitted to our Institution for hepatocellular carcinoma with non-alcoholic steatohepatitis. Upon dynamic computed tomography, an enhanced tumor, 50 mm in size, was detected in the S4/8 area. She underwent partial hepatectomy. Approximately 26 days following surgery, the patient suddenly developed a high fever (up to 38.6˚C), dyspnea and hypoxemia. Laboratory findings 24 h following the high fever revealed a markedly increased total WBC count of 13,500/μl and CRP of 9.2 mg/dl, with a decreased platelet count of 109,000/μl (31% rate of decline/48 h). Forty-eight hours following the high fever, the CRP increased to 11.5 mg/dl, with WBCs and platelets at 5,840/μl and 92,000/μl, respectively. At that time, the P/F ratio decreased to 82 and the patient was intubated because she presented with shock and was diagnosed with ARDS. The thrombin-antithrombin complex (TAT) and plasmin-plasmin inhibitor complex (PIC) was 8.2 ng/ml and 0.5 μg/ml, respectively. This correlates to severe coagulation activation but mild fibrinolytic activation.

Hypotension persisted despite levofloxacin, gamma globulin administration and continuous infusion of dopamine. Simultaneously, we started continuous infusion of sivelestat sodium hydrate in order to reduce excessive neutrophil elastase release. The patient was followed-up for 2 days and recovered from shock. Six days following the high fever, methicillin-resistant *Staphylococcus aureus* was detected in the sputum culture. Given the patient's immunocompromised state, treatment was continued for persistent ARDS and pneumonia. However, she died 40 days later from complications due to DIC.

Possible Mechanism of Progression from Sepsis to Organ Dysfunction

Phase 1: NET and intravasated platelet aggregation. The prevalence of neutropenia during sepsis carries an independent risk for mortality (18). The concept of NETs, entailing a biophylactic mechanism for neutrophils targeting bacteria, is currently being explored (19). NETs, composed of neutrophil nuclear and granule constituents, are extracellular chromatin structures that entrap microbes. Activated neutrophils release anti-microbial granule proteins such as damage-associated molecular pattern (DAMP) proteins, elastase, myeloperoxidase, histones and high mobility group box 1, which form extracellular fibers and bind bacteria (Figure 2). NETs degrade virulence factors and eventually initiate the rupture of the cell (20). This self-sacrificing action of neutrophils against invading microbes, although beneficial with respect to entrapping microbes, when excessive causes cellular damage (21, 22).

Neutrophils and intravasated platelets are known to participate in the pathogenesis of severe sepsis. The sequestration of neutrophils in the lungs is necessary for the recruitment of platelets, suggesting that neutrophils function as a pro-adhesive surface for platelets (23). Clark *et al*. reported several cellular events that lead to the enhanced trapping of bacteria in blood vessels: Platelet toll-like receptor 4 (TLR4), activated by lipopolysaccharide, detects TLR4 ligands in blood and induces platelet binding to adherent neutrophils. This leads to robust neutrophil activation and the formation of NETs. Plasma from patients

with severe sepsis also induces TLR4-dependent platelet–neutrophil interactions, leading to the production of NETs. It was shown that NETs retain their integrity under flow conditions and ensnare bacteria within the vasculature. The entire event was found to occur primarily in liver sinusoids and pulmonary capillaries, where NETs have the greatest capacity for bacterial trapping (24, 25). Blocking NET formation reduces the focalization of circulating bacteria during sepsis, resulting in their increased dissemination to distant organs. Thus, NETs ensnare circulating bacteria and provide intravascular immunity that protects against bacterial dissemination to distal organs during septic infections (26).

In recent years, several studies have elucidated the crucial role of NETs in thrombosis (27, 28). Excessive microvascular thrombosis causes disorders of the microcirculation and leads to organ dysfunction. In addition to their protective role, NETs were noted to be associated with endothelial injury (24). Histones, released from NET-activated neutrophils, have recently been demonstrated to function as endogenous danger signals or DAMPs when they translocate from the nucleus to the extranuclear space (29). Extracellular histones released in response to inflammatory challenges contribute to endothelial damage, organ failure and death during sepsis (22).

Formation of NETs by neutrophil–intravasated platelet interaction results in both endothelial damage and thrombosis following ALI/ARDS. As such, NET formation represents a dynamic balance between bacterial focalization in order to prevent distant organ dissemination, and excessive endothelial injury to the host (30).

Phase 2: EPA. In ALI and ARDS, platelets and platelet–neutrophil complexes can be found within the pulmonary vasculature, airways, interstitial, and alveolar compartments (17). Endothelial damage and detachment after NET formation followed by EPA is the root cause of organ dysfunction in sepsis. We present the case of a patient with severe postoperative sepsis occurring after hepatectomy. Immunohistochemical analysis for the presence and localization of platelet aggregation in autopsy specimens are shown in Figure 3A. EPA was observed in airways, known as extravasated spaces.

Microvascular endothelial injury leads to an increase in capillary permeability. This alteration in permeability permits the exudation of protein-rich fluid, as well as platelets, into the peribronchovascular interstitium, ultimately crossing the epithelial barrier into the distal airspaces of the lung (31). Damage to the vascular endothelium by NETs can result in the denudation of the endothelium or the loss of fenestrations, allowing platelets to enter the extravasated space.

Platelets contain storage pools of peptide growth factors including platelet-derived growth factor, vascular endothelial growth factor (VEGF), transforming growth factor-β (TGFβ), and nonpeptide vasoactive compounds including serotonin (5-hydroxytryptamine: 5HT), thromboxane A2 (TXA2), norepinephrine, histamine, bradykinin, and platelet activating factor (32). Platelets also contain proteins such as thrombospondin-1 (TSP1), platelet factor 4 and CD40L(33).

Plasminogen activator inhibitor type 1 (PAI1), present in vascular smooth muscle cells, endothelial cells and platelets, is the primary inhibitor of tissue-type plasminogen activator and urokinase plasminogen activator. Moreover, PAI1 plays an integral role in the regulation of fibrinolysis. Elevated levels of PAI1 result in deficient plasminogen activation and are associated with a predisposition to thrombosis and veno-occlusive disease (VOD) following bone marrow transplantation (34). Platelets release potent pro-inflammatory chemokines and also modulate leukocyte function (35).

In phase 2, EPA may be stimulated by tissue injury, hypoxia, cytokines, endotoxin and endotoxemia. This ultimately leads to pulmonary VOD by EPA-derived TXA2 and 5HT, resulting in pulmonary hypertension. TXA2 is a strong vasoactive metabolite of arachidonic acid, with powerful pro-aggregatory and pro-inflammatory properties, inducing platelet aggregation and vasoconstriction (36). 5HT is an important mediator in both the enhancement of platelet aggregation and the induction of local vasoconstriction (37). Although VEGFA acts as a vasodilator under ordinary circumstances, it acts, paradoxically, as a vasoconstrictor in patients with endothelial failure (38).

Low platelet counts have long been recognized as an important prognostic factor in sepsis, based on the assumption of their role as a biomarker for sepsis severity (39). The consumption of platelets may be induced by intravasated platelet aggregation followed EPA.

Phase 3: Organ dysfunction, Immunoparalysis. The Scientific Subcommittee on Disseminated Intravascular Coagulation, of the International Society on Thrombosis and Haemostasis, defined DIC as "an acquired syndrome characterized by the intravascular activation of coagulation with loss of localization arising from different causes. It can originate from and cause damage to the microvasculature, which if sufficiently severe, can produce organ dysfunction" (40). DIC is characterized by the systemic activation of coagulation, which results in the generation and deposition of fibrin, and leads to microvascular thrombi in various organs – significantly contributing to multiple organ dysfunction syndrome (41). The crosstalk between inflammation and coagulation is essential since coagulation is activated by inflammation, as is observed in sepsis (42, 43).

PAI1 regulates the degree of fibrinolytic activation and is an important factor in characterizing DIC (43). Platelet-alpha granules contain large amounts of PAI1, which are released during vascular injury and assist in fibrin clot stability. Although the patient presented here died from DIC, intravasated thrombus was not observed (Figure 3B), indicating that the patient's mortality was not a direct result

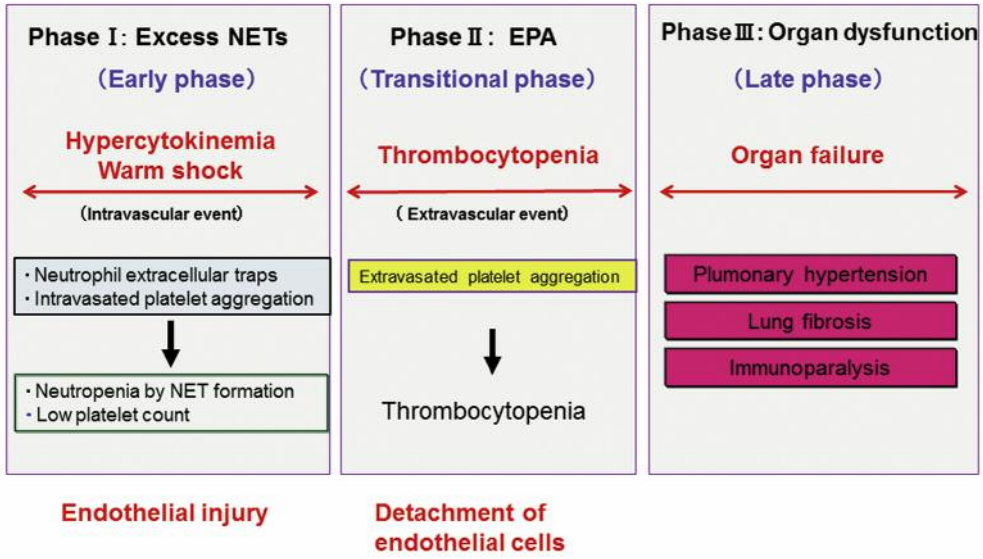

Figure 1. *Phases leading to organ dysfunction. We classified three phases, from sepsis to organ dysfunction, characterizing the interaction between neutrophils and platelets. Phase 1 is formation of neutrophil extracellular traps (NETs) and intravasated platelet aggregation. Phase 2 is extravasated platelet aggregation, promoted by NET-facilitated detachment of endothelial cells. Phase 3 is organ failure by pulmonary veno-occlusive disease, fibrosis and immunoparalysis.*

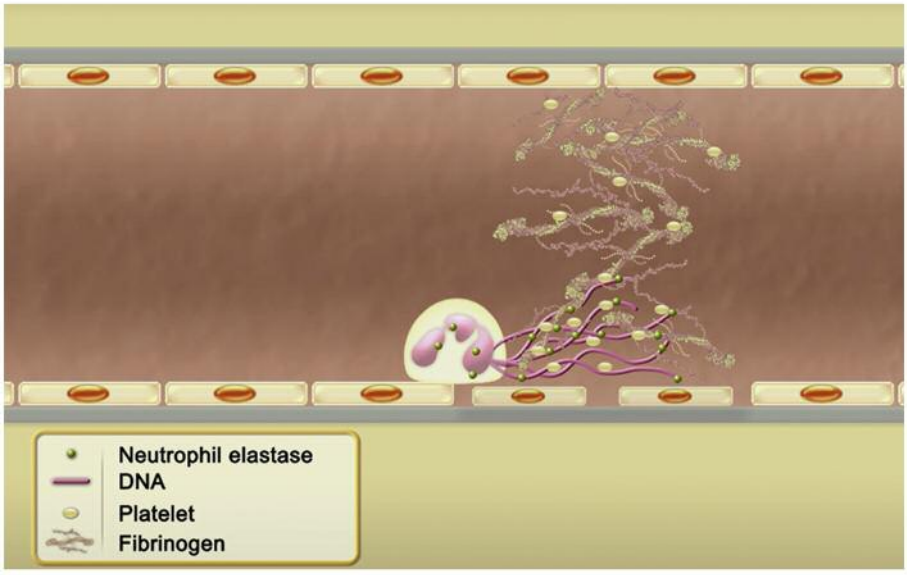

Figure 2. *Schematic representation of neutrophil extracellular trap (NET) formation. NETs are extracellular chromatin structures that entrap microbes. NETs release anti-microbial granule proteins such as damage-associated molecular pattern proteins, neutrophil elastase, myeloperoxidase, histones and high mobility group box 1. These function to form extracellular fibers and bind bacteria. NETs degrade virulence factors and eventually initiate rupture of the cell. This self-sacrificing mechanism of neutrophils against invading microbe although beneficial, in excess causes cellular and endothelial damage.*

of DIC. The organ dysfunction observed may have been caused by pulmonary VOD and EPA-facilitated pulmonary hypertension, not intravasated microvascular obstruction. Therefore, EPA-derived TXA2 and 5HT can induce pulmonary VOD and pulmonary hypertension *via* vasoconstriction. EPA-derived PAI1 and TGFβ induce pulmonary fibrosis, leading to ultimate organ dysfunction (Figure 4).

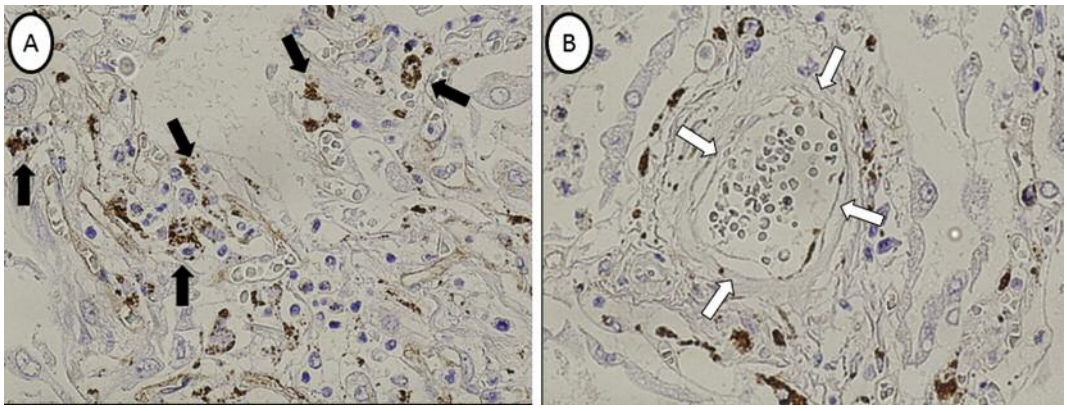

Figure 3. *Immunohistochemical analysis for the presence and localization of platelet aggregation in autopsy specimens from presented hepatectomy patient. A: CD42b expression is evident as dark particles, morphologically characterized as platelets. Extravasated platelet aggregation was observed in airways, known as extravasated spaces (black arrows). B. No intravasated thrombus was observed in the vessel (white arrows), indicating that the patient's mortality was not directly caused by DIC. Original magnifications: CD42b, ×400.*

Furthermore, platelet-derived TGFβ and PAI1 may also induce pulmonary fibrosis. PAI1 suppresses fibrinolysis and the progression to fibrosis in the tissue microenvironment (44). TGFβ is also important for the induction of fibrosis, often associated with chronic phases of inflammatory diseases (45).

Shock and multi-organ dysfunction occur following the intense inflammatory reaction to sepsis. Complications arise from sepsis-related immunoparalysis and contribute to morbidity and mortality from sepsis (46, 47).

It is well known that TGFβ and VEGFA are potent immunosuppressive factors that drive the expansion of regulatory T-cells and myeloid-derived suppressor cells (48). The CD40–CD40L co-stimulatory pathway has been shown to play a crucial role in the production of cytokines, including interleukin (IL)-10, which is a known immunosuppressive cytokine inhibiting macrophage-dependent antigen presentation, T-cell proliferation, and Th1 cytokine secretion of IL2, IFNγ, and TNFα (49). Among various functions, these cytokines, especially IL2, also modulate the activity and proliferation of T-lymphocytes (50). Thrombospondin-1 is a potent suppressor of T-cell activation *via* its receptor CD47 (51).

EPA-derived factors, including VEGFA, TSP, CD40L and TGFβ, may also directly contribute to immunoparalysis (Figure 4). Therefore, EPA plays an essential role in the progression of organ failure in cases of ALI/ARDS.

Future Therapies

Conventional treatments, including anti-inflammatory therapy and other experimental treatments have largely been unsuccessful (52, 53). Pulmonary hypertension is of serious clinical concern and a disease that eventually leads to lung or heart failure (54).

We assert that ALI and ARDS treament should be implemented in patients with pulmonary hypertension. Pre-emptive medical care for ALI/ARDS in its early stages is important in preventing progression to phases 2 and 3. As such, the key treatment for ALI/ARDS relies on the inhibition of NET formation and platelet aggregation while simultaneously preventing host endothelial damage. In clinical practice, we administer a phosphodiesterase (PDE) 3 inhibitor, beraprost and sivelestat.

PDE3 inhibitor. The isoform PDE3 comprises two subfamilies, PDE3A and PDE3B. Recently, it has been shown that PDE3A is the predominant subtype of PDE3 expressed in platelets (55). Milrinone, a specific PDE3A inhibitor, has been shown to reduce acute pulmonary hypertension and is an effective vasodilator (56, 57). Milrinone also inhibits arachidonic acid-induced change in platelet shape and adenosine diphosphate (ADP)-induced platelet aggregation (58). Milrinone induces an elevation of intraplatelet cyclic adenosine 3',5'-monophosphate in a dose-dependent manner, resulting in the inhibition of platelet aggregation (59). Cilostazol is a specific and potent inhibitor of PDE3 in platelets and smooth muscle cells, where it diminishes intracellular calcium, causing smooth muscle cell relaxation and the inhibition of platelet activation (60). Therefore, use of PDE3 inhibitors cilostazol and milrinone may be appropriate, owing to their antiplatelet properties, and ability to increase tolerance to ALI/ARDS injury.

Beraprost. There are many pathophysiological changes during severe sepsis and septic shock; one of the most striking is metabolic derangement (61). Among the metabolic changes, hyperglycemia is the most important (62).

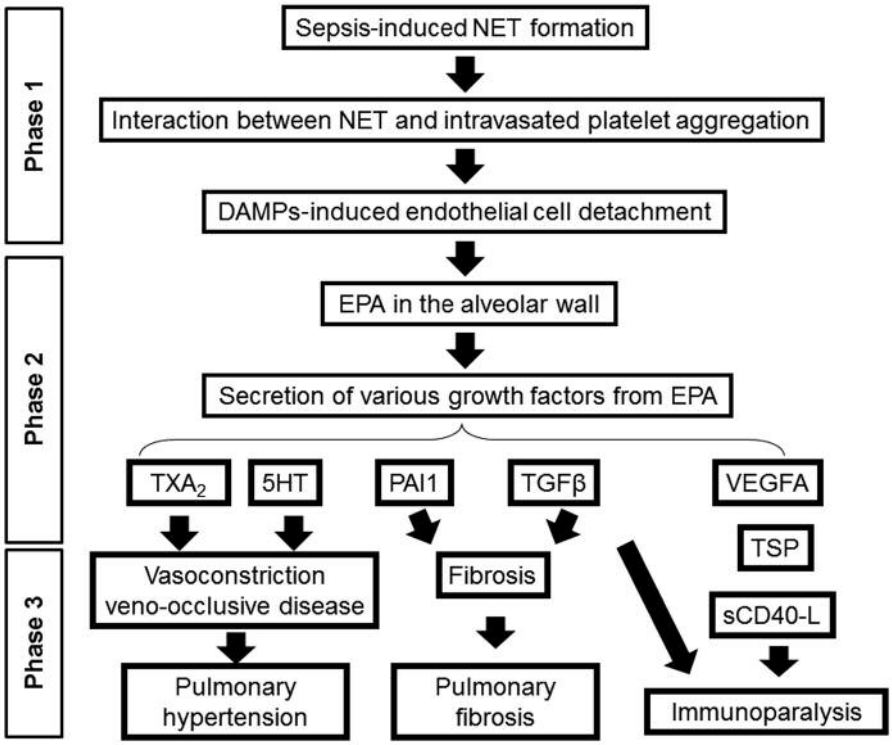

Figure 4. *Mechanism of progression from sepsis to organ dysfunction, characterized by neutrophil extracellular traps (NETs) and platelet aggregation. Extravasated platelet aggregation (EPA) in the airway space, initiated by damage to the endothelium, is induced by formation of NETs or intravasated platelet aggregation in sepsis. Various growth factors released by EPA, including thromboxane A_2 (TXA_2), 5-hydroxytryptamine (5HT), plasminogen activator inhibitor type 1 (PAI1) and transforming growth factor β (TGFβ), may induce pulmonary hypertension and promote the progression of lung fibrosis, as well as suppress lung regeneration, initiating acute lung injury and acute respiratory distress syndrome. Furthermore, EPA-derived vascular endothelial growth factor A (VEGFA), thrombospondin-1 (TSP1), sCD40L and TGFβ may also contribute to immunoparalysis. DAMP: Damage-associated molecular pattern.*

Hyperglycemia is a major risk factor for endothelial dysfunction and vascular complications. In recent years, significant advances have been made in understanding endothelial cell dysfunction triggered by high glucose concentration (63). Accordingly, the control of hyperglycemia in sepsis is considered to be a very effective therapeutic target (64). Additionally, insulin has an anti-inflammatory effect through the suppression of inflammatory cytokines (e.g., nuclear factor-kappa B) (65). In critically ill diabetic patients, insulin delivery and insulin-dependent glucose uptake by skeletal muscle are delayed and impaired. Therefore, it is pivotal to impair insulin resistance of endothelial cells (66). Kubota *et al*. demonstrated that impaired insulin signaling in endothelial cells, due to reduced insulin receptor substrate 2 expression and insulin-induced endothelial nitric-oxide synthase phosphorylation, causes attenuation of insulin-induced capillary recruitment and insulin delivery, reducing glucose uptake by skeletal muscle (67). The use of agents such as beraprost, a stable prostacyclin analog capable of improving insulin resistance and vascular endothelial function, may ultimately contribute to increasing the life expectancy of patients with peripheral artery disease (68). To improve insulin resistance, we administer beraprost sodium during the perioperative state. Beraprost also prevents platelet aggregation by increasing cAMP and reducing TXA2, which has coagulant properties and is produced by platelets. We also use a closed-loop glycemic control system with an artificial pancreas (STG-55, NIKKISO Co., ltd., Tokyo, Japan) to the intensive care unit for patients administered beraprost.

Sivelestat. Sivelestat is a selective inhibitor of neutrophil elastase (69). The perioperative administration of sivelestat sodium hydrate mitigated postoperative hypoxia, partially suppressed postoperative hypercytokinemia, shortened the duration of SIRS, and stabilized postoperative circulatory status after thoracoscopic esophagectomy (70). The administration of sivelestat was also shown to improve the

outcome for patients with sepsis with associated ARDS (71). Our previous study demonstrated that sivelestat inhibits the adhesion and migration of neutrophils to the vascular endothelium in hepatic ischemia-reperfusion injury, thereby suppressing liver injury (69). Sivelestat can limit the number of circulating activated neutrophils and improve pulmonary oxygenation in patients (72). Sivelestat inhibits the adhesion and migration of neutrophils to the vascular endothelium and may prevent from endothelial damage (69, 71).

We conclude that the administration of these bundle treatments by phase 2 could play a crucial role in preventing organ dysfunction in cases of ALI, ARDS and sepsis.

Useful Markers for Treatment

Asakura reported that suppressed-fibrinolytic-type DIC, in which coagulation activation is severe but fibrinolytic activation is mild, is typically seen in sepsis. It was described that PAI1, the fibrinolytic inhibitory factor, is markedly increased, fibrinolysis is strongly suppressed, and the dissolution of multiple microthrombi is more difficult. As a result of microcirculatory impairment, severe organ dysfunction may occur (43, 73).

PAI1 is supplied from EPA during phase 2. Thus, plasma levels of TAT and PIC should be continuously monitored in patients with suspected sepsis in order to detect hypercoagulopathy. The measurement of TAT and PIC activity can identify patients with ongoing severe coagulopathy during the early stages of sepsis. As such, the implementation of quality indicators for the early management of severe sepsis and septic shock is strongly associated with decreased mortality.

Conclusion

We propose that pulmonary VOD, fibrosis and immunoparalysis (initiated by NET and platelet aggregation), result in endothelial cell damage and may primarily contribute to ALI and ARDS in sepsis. Counter-measures for NET formation and anti-platelet treatments such as PDE3 inhibitors, beraprost and sivelestat may be advantageous treatments preventing ALI/ARDS in patients with sepsis.

Conflicts of Interest

The Authors declare that no financial or other conflicts of interest exist in relation to the content of this article.

References

1 Kuhlen R and Dembinski R: Mechanical ventilation of acute lung injury. Pneumologie *61(4)*: 249-255, 2007 (in German).

2 Niederman MS and Fein AM: Sepsis syndrome, the adult respiratory distress syndrome, and nosocomial pneumonia. A common clinical sequence. Clin Chest Med *11(4)*: 633-656, 1990.

3 Johnson ER and Matthay MA: Acute lung injury: Epidemiology, pathogenesis, and treatment. J Aerosol Med Pulm Drug Deliv *23(4)*: 243-252, 2010.

4 Proudfoot AG, McAuley DF, Griffiths MJ and Hind M: Human models of acute lung injury. Dis Model Mech *4(2)*: 145-153, 2011.

5 Hudson LD, Milberg JA, Anardi D and Maunder RJ: Clinical risks for development of the acute respiratory distress syndrome. Am J Respir Crit Care Med *151(2 Pt 1)*: 293-301, 1995.

6 Oliveira RH and Basille Filho A: Incidence of acute lung injury and acute respiratory distress syndrome in the intensive care unit of a university hospital: A prospective study. J Bras Pneumol *32(1)*: 35-42, 2006.

7 Sheu CC, Gong MN, Zhai R, Chen F, Bajwa EK, Clardy PF, Gallagher DC, Thompson BT and Christiani DC: Clinical characteristics and outcomes of sepsis-related *vs*. non-sepsis-related ards. Chest *138(3)*: 559-567, 2010.

8 Johnston CJ, Rubenfeld GD and Hudson LD: Effect of age on the development of ards in trauma patients. Chest *124(2)*: 653-659, 2003.

9 Daurat A, Millet I, Roustan JP, Maury C, Taourel P, Jaber S, Capdevila X and Charbit J: Thoracic trauma severity score on admission allows to determine the risk of delayed ARDS in trauma patients with pulmonary contusion. Injury *47(1)*: 147-153, 2016.

10 Khadaroo RG and Marshall JC: Ards and the multiple organ dysfunction syndrome. Common mechanisms of a common systemic process. Crit Care Clin *18(1)*: 127-141, 2002.

11 Gando S, Kameue T, Matsuda N, Hayakawa M, Ishitani T, Morimoto Y and Kemmotsu O: Combined activation of coagulation and inflammation has an important role in multiple organ dysfunction and poor outcome after severe trauma. Thromb Haemost *88(6)*: 943-949, 2002.

12 Gando S, Nanzaki S, Sasaki S, Aoi K and Kemmotsu O: Activation of the extrinsic coagulation pathway in patients with severe sepsis and septic shock. Crit Care Med *26(12)*: 2005-2009, 1998.

13 Gando S, Kameue T, Nanzaki S and Nakanishi Y: Disseminated intravascular coagulation is a frequent complication of systemic inflammatory response syndrome. Thromb Haemost *75(2)*: 224-228, 1996.

14 Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS and Wenzel RP: The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. JAMA *273(2)*: 117-123, 1995.

15 Ogura H, Gando S, Iba T, Eguchi Y, Ohtomo Y, Okamoto K, Koseki K, Mayumi T, Murata A, Ikeda T, Ishikura H, Ueyama M, Kushimoto S, Saitoh D, Endo S, Shimazaki S and Group JAfAMDICS: SIRS-associated coagulopathy and organ dysfunction in critically ill patients with thrombocytopenia. Shock *28(4)*: 411-417, 2007.

16 Russwurm S, Vickers J, Meier-Hellmann A, Spangenberg P, Bredle D, Reinhart K and Lösche W: Platelet and leukocyte activation correlate with the severity of septic organ dysfunction. Shock *17(4)*: 263-268, 2002.

17 Katz JN, Kolappa KP and Becker RC: Beyond thrombosis: The versatile platelet in critical illness. Chest *139(3)*: 658-668, 2011.

18 Leibovici L, Drucker M, Samra Z, Konisberger H and Pitlik SD: Prognostic significance of the neutrophil count in immunocompetent patients with bacteraemia. QJM *88(3)*: 181-189, 1995.

19 Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y and Zychlinsky A: Neutrophil extracellular traps kill bacteria. Science *303(5663)*: 1532-1535, 2004.

20 Brinkmann V and Zychlinsky A: Neutrophil extracellular traps: Is immunity the second function of chromatin? J Cell Biol *198(5)*: 773-783, 2012.

21 Yipp BG and Kubes P: Netosis: How vital is it? Blood *122(16)*: 2784-2794, 2013.

22 Xu J, Zhang X, Pelayo R, Monestier M, Ammollo CT, Semeraro F, Taylor FB, Esmon NL, Lupu F and Esmon CT: Extracellular histones are major mediators of death in sepsis. Nat Med *15(11)*: 1318-1321, 2009.

23 Andonegui G, Kerfoot SM, McNagny K, Ebbert KV, Patel KD and Kubes P: Platelets express functional toll-like receptor-4. Blood 106(7): 2417-2423, 2005.

24 Clark SR, Ma AC, Tavener SA, McDonald B, Goodarzi Z, Kelly MM, Patel KD, Chakrabarti S, McAvoy E, Sinclair GD, Keys EM, Allen-Vercoe E, Devinney R, Doig CJ, Green FH and Kubes P: Platelet TLR4 activates neutrophil extracellular traps to ensnare bacteria in septic blood. Nat Med *13(4)*: 463-469, 2007.

25 Lu YC, Yeh WC and Ohashi PS: Lps/tlr4 signal transduction pathway. Cytokine *42(2)*: 145-151, 2008.

26 McDonald B, Urrutia R, Yipp BG, Jenne CN and Kubes P: Intravascular neutrophil extracellular traps capture bacteria from the bloodstream during sepsis. Cell Host Microbe *12(3)*: 324-333, 2012.

27 Kambas K, Mitroulis I and Ritis K: The emerging role of neutrophils in thrombosis-the journey of TF through NETS. Front Immunol *3*: 385, 2012.

28 Martinod K and Wagner DD: Thrombosis: Tangled up in NETS. Blood *123(18)*: 2768-2776, 2014.

29 Chen R, Kang R, Fan XG and Tang D: Release and activity of histone in diseases. Cell Death Dis *5*: e1370, 2014.

30 Cheng OZ and Palaniyar N: Net balancing: A problem in inflammatory lung diseases. Front Immunol *4*: 1, 2013.

31 Pugin J, Verghese G, Widmer MC and Matthay MA: The alveolar space is the site of intense inflammatory and profibrotic reactions in the early phase of acute respiratory distress syndrome. Crit Care Med *27(2)*: 304-312, 1999.

32 Pakala R, Sheng WL and Benedict CR: Eicosapentaenoic acid and docosahexaenoic acid block serotonin-induced smooth muscle cell proliferation. Arterioscler Thromb Vasc Biol *19(10)*: 2316-2322, 1999.

33 Nurden AT: Platelets and tissue remodeling: Extending the role of the blood clotting system. Endocrinology *148(7)*: 3053-3055, 2007.

34 Lee JH, Lee KH, Kim S, Seol M, Park CJ, Chi HS, Kang W, Kim ST, Kim WK and Lee JS: Plasminogen activator inhibitor-1 is an independent diagnostic marker as well as severity predictor of hepatic veno-occlusive disease after allogeneic bone marrow transplantation in adults conditioned with busulphan and cyclophosphamide. Br J Haematol *118(4)*: 1087-1094, 2002.

35 Ghasemzadeh M and Hosseini E: Platelet-leukocyte crosstalk: Linking proinflammatory responses to procoagulant state. Thromb Res *131(3)*: 191-197, 2013.

36 Li P, Ferrario CM and Brosnihan KB: Losartan inhibits thromboxane a2-induced platelet aggregation and vascular constriction in spontaneously hypertensive rats. J Cardiovasc Pharmacol *32(2)*: 198-205, 1998.

37 Moerland M, Kemme M, Dijkmans A, Bergougnan L and Burggraaf J: Modulation of vasoactivity and platelet aggregation by selective 5-ht receptor antagonism in humans. J Cardiovasc Pharmacol *58(6)*: 575-580, 2011.

38 Parenti A, Brogelli L, Filippi S, Donnini S and Ledda F: Effect of hypoxia and endothelial loss on vascular smooth muscle cell responsiveness to VEGF-A: Role of FLT-1/VEGF-receptor-1. Cardiovasc Res *55(1)*: 201-212, 2002.

39 Fiusa MM, Carvalho-Filho MA, Annichino-Bizzacchi JM and De Paula EV: Causes and consequences of coagulation activation in sepsis: An evolutionary medicine perspective. BMC Med *13*: 105, 2015.

40 Taylor FB, Toh CH, Hoots WK, Wada H, Levi M and (ISTH) SSoDICDotISoTaH: Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. Thromb Haemost *86(5)*: 1327-1330, 2001.

41 Vincent JL and De Backer D: Does disseminated intravascular coagulation lead to multiple organ failure? Crit Care Clin *21(3)*: 469-477, 2005.

42 van der Poll T and Levi M: Crosstalk between inflammation and coagulation: The lessons of sepsis. Curr Vasc Pharmacol *10(5)*: 632-638, 2012.

43 Asakura H: Classifying types of disseminated intravascular coagulation: Clinical and animal models. J Intensive Care *2(1)*: 20, 2014.

44 Nakanuma S, Miyashita T, Hayashi H, Tajima H, Takamura H, Tsukada T, Okamoto K, Sakai S, Makino I, Kinoshita J, Nakamura K, Oyama K, Inokuchi M, Nakagawara H, Ninomiya I, Kitagawa H, Fushida S, Fujimura T and Ohta T: Extravasated platelet aggregation in liver zone 3 may correlate with the progression of sinusoidal obstruction syndrome following living donor liver transplantation: A case report. Exp Ther Med *9(4)*: 1119-1124, 2015.

45 Pohlers D, Brenmoehl J, Löffler I, Müller CK, Leipner C, Schultze-Mosgau S, Stallmach A, Kinne RW and Wolf G: TGF-beta and fibrosis in different organs – molecular pathway imprints. Biochim Biophys Acta *1792(8)*: 746-756, 2009.

46 Hotchkiss RS, Coopersmith CM, McDunn JE and Ferguson TA: The sepsis seesaw: Tilting toward immunosuppression. Nat Med *15(5)*: 496-497, 2009.

47 Sundar KM and Sires M: Sepsis induced immunosuppression: Implications for secondary infections and complications. Indian J Crit Care Med *17(3)*: 162-169, 2013.

48 Terme M, Tartour E and Taieb J: VEGFA/VEGFR2-targeted therapies prevent the VEGFA-induced proliferation of regulatory t cells in cancer. Oncoimmunology *2(8)*: e25156, 2013.

49 Santin AD, Hermonat PL, Ravaggi A, Bellone S, Pecorelli S, Roman JJ, Parham GP and Cannon MJ: Interleukin-10 increases TH1 cytokine production and cytotoxic potential in human papillomavirus-specific CD8(+) cytotoxic T lymphocytes. J Virol *74(10)*: 4729-4737, 2000.

50 Schlom J, Jochems C, Gulley JL and Huang J: The role of soluble CD40l in immunosuppression. Oncoimmunology *2(1)*: e22546, 2013.

51 Miller TW, Kaur S, Ivins-O'Keefe K and Roberts DD: Thrombospondin-1 is a CD47-dependent endogenous inhibitor of hydrogen sulfide signaling in T-cell activation. Matrix Biol *32(6)*: 316-324, 2013.

52 Fein AM and Calalang-Colucci MG: Acute lung injury and acute respiratory distress syndrome in sepsis and septic shock. Crit Care Clin *16(2)*: 289-317, 2000.

53 Zeni F, Freeman B and Natanson C: Anti-inflammatory therapies to treat sepsis and septic shock: A reassessment. Crit Care Med *25(7)*: 1095-1100, 1997.
54 Klok FA, Mos IC, van Kralingen KW, Vahl JE and Huisman MV: Chronic pulmonary embolism and pulmonary hypertension. Semin Respir Crit Care Med *33(2)*: 199-204, 2012.
55 Sun B, Li H, Shakur Y, Hensley J, Hockman S, Kambayashi J, Manganiello VC and Liu Y: Role of phosphodiesterase type 3a and 3b in regulating platelet and cardiac function using subtype-selective knockout mice. Cell Signal *19(8)*: 1765-1771, 2007.
56 Lobato EB, Beaver T, Muehlschlegel J, Kirby DS, Klodell C and Sidi A: Treatment with phosphodiesterase inhibitors type III and V: Milrinone and sildenafil is an effective combination during thromboxane-induced acute pulmonary hypertension. Br J Anaesth *96(3)*: 317-322, 2006.
57 Raghavendran K, Pryhuber GS, Chess PR, Davidson BA, Knight PR and Notter RH: Pharmacotherapy of acute lung injury and acute respiratory distress syndrome. Curr Med Chem *15(19)*: 1911-1924, 2008.
58 Barradas MA, Jagroop A, O'Donoghue S, Jeremy JY and Mikhailidis DP: Effect of milrinone in human platelet shape change, aggregation and thromboxane a2 synthesis: An *in vitro* study. Thromb Res *71(3)*: 227-236, 1993.
59 Manns JM, Brenna KJ, Colman RW and Sheth SB: Differential regulation of human platelet responses by CGMP inhibited and stimulated camp phosphodiesterases. Thromb Haemost *87(5)*: 873-879, 2002.
60 Schrör K: The pharmacology of cilostazol. Diabetes Obes Metab *4(Suppl 2)*: S14-19, 2002.
61 Mizock BA: Metabolic derangements in sepsis and septic shock. Crit Care Clin *16(2)*: 319-336, vii, 2000.
62 Taylor JH and Beilman GJ: Hyperglycemia in the intensive care unit: No longer just a marker of illness severity. Surg Infect (Larchmt) *6(2)*: 233-245, 2005.
63 Georgescu A, Popov D, Dragan E, Dragomir E and Badila E: Protective effects of nebivolol and reversal of endothelial dysfunction in diabetes associated with hypertension. Eur J Pharmacol *570(1-3)*: 149-158, 2007.
64 Hirasawa H, Oda S and Nakamura M: Blood glucose control in patients with severe sepsis and septic shock. World J Gastroenterol *15(33)*: 4132-4136, 2009.
65 Wang S, Moustaid-Moussa N, Chen L, Mo H, Shastri A, Su R, Bapat P, Kwun I and Shen CL: Novel insights of dietary polyphenols and obesity. J Nutr Biochem *25(1)*: 1-18, 2014.
66 Wieser V, Moschen AR and Tilg H: Inflammation, cytokines and insulin resistance: A clinical perspective. Arch Immunol Ther Exp (Warsz) *61(2)*: 119-125, 2013.
67 Kubota T, Kubota N, Kumagai H, Yamaguchi S, Kozono H, Takahashi T, Inoue M, Itoh S, Takamoto I, Sasako T, Kumagai K, Kawai T, Hashimoto S, Kobayashi T, Sato M, Tokuyama K, Nishimura S, Tsunoda M, Ide T, Murakami K, Yamazaki T, Ezaki O, Kawamura K, Masuda H, Moroi M, Sugi K, Oike Y, Shimokawa H, Yanagihara N, Tsutsui M, Terauchi Y, Tobe K, Nagai R, Kamata K, Inoue K, Kodama T, Ueki K and Kadowaki T: Impaired insulin signaling in endothelial cells reduces insulin-induced glucose uptake by skeletal muscle. Cell Metab *13(3)*: 294-307, 2011.
68 Utsunomiya K: Treatment strategy for type 2 diabetes from the perspective of systemic vascular protection and insulin resistance. Vasc Health Risk Manag *8*: 429-436, 2012.
69 Sakai S, Tajima H, Miyashita T, Nakanuma S, Makino I, Hayashi H, Nakagawara H, Kitagawa H, Fushida S, Fujimura T, Saito H, Munesue S, Yamamoto Y and Ohta T: Sivelestat sodium hydrate inhibits neutrophil migration to the vessel wall and suppresses hepatic ischemia-reperfusion injury. Dig Dis Sci *59(4)*: 787-794, 2014.
70 Kawahara Y, Ninomiya I, Fujimura T, Funaki H, Nakagawara H, Takamura H, Oyama K, Tajima H, Fushida S, Inaba H and Kayahara M: Prospective randomized controlled study on the effects of perioperative administration of a neutrophil elastase inhibitor to patients undergoing video-assisted thoracoscopic surgery for thoracic esophageal cancer. Dis Esophagus *23(4)*: 329-339, 2010.
71 Hayakawa M, Katabami K, Wada T, Sugano M, Hoshino H, Sawamura A and Gando S: Sivelestat (selective neutrophil elastase inhibitor) improves the mortality rate of sepsis associated with both acute respiratory distress syndrome and disseminated intravascular coagulation patients. Shock *33(1)*: 14-18, 2010.
72 Eguchi T, Yoshida K, Kondo R, Hamanaka K, Shiina T, Komatsu Y, Yamamoto H, Kubo K, Hasegawa J and Koizumi T: Sivelestat prevents cytoskeletal rearrangements in neutrophils resulting from lung re-expansion following one-lung ventilation during thoracic surgery. Inflammation *36(6)*: 1479-1484, 2013.
73 Asakura H, Jokaji H, Saito M, Uotani C, Kumabashiri I, Morishita E, Yamazaki M, Aoshima K and Matsuda T: Study of the balance between coagulation and fibrinolysis in disseminated intravascular coagulation using molecular markers. Blood Coagul Fibrinolysis *5(5)*: 829-832, 1994.

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