


Markers of Inflammation and Infection in Sepsis and Disseminated Intravascular Coagulation

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Abstract

Sepsis is a severe systemic inflammatory response to infection that manifests with widespread inflammation as well as endothelial and coagulation dysfunction that may lead to hypotension, organ failure, shock, and death. Disseminated intravascular coagulation (DIC) is a complication of sepsis involving systemic activation of the fibrinolytic and coagulation pathways that can lead to multi-organ dysfunction, thrombosis, and bleeding, with a 2-fold increase in mortality. This study demonstrates the diagnostic and prognostic value of profiling various biomarkers of inflammation and infection in patients with sepsis-associated DIC to assess the severity of illness. Deidentified samples were obtained from adult patients with sepsis and suspected DIC. Platelet count, prothrombin time, D-dimer, and fibrinogen levels were used to assign International Society of Thrombosis and Hemostasis DIC scores to plasma samples from 103 patients with sepsis and suspected DIC. Using commercially available enzyme-linked immunosorbent assay, chromogenic assay, and RANDOX Biochip methods, levels of procalcitonin (PCT), extracellular nucleosomes, interleukin (IL) 6, IL-8, IL-10, and tumor necrosis factor α (TNF α) were measured in patients with sepsis and DIC and compared to levels in healthy individuals. Elevated levels of PCT, IL-6, IL-8, IL-10, and TNF α were observed in most patients with sepsis and DIC. Additionally, the levels of these markers show significant positive correlations with each other and with DIC score. Currently, no single biomarker can effectively diagnose DIC in patients with sepsis. This study lays the groundwork for the development of a diagnostic algorithm using several markers of inflammation and infection and DIC score as parameters in assessing severity of sepsis-associated coagulopathy in a clinical setting.

Keywords

sepsis, disseminated intravascular coagulation, inflammation, infection

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Background

Despite the advances in the understanding of the pathophysiology of sepsis, it continues to be a leading cause of morbidity and mortality. According to the Centers for Disease Control, over 1.5 million people in the United States develop sepsis annually, of which 250 000 will ultimately die. Approximately 20% to 40% of all cases of sepsis are complicated by disseminated intravascular coagulation (DIC) which results in a 2-fold increase in mortality compared to patients without DIC.^{1,2}

Sepsis develops from the spread of a localized infection. It is characterized as a severe and often fatal clinical syndrome that involves a systemic inflammatory response to infection and manifests with microvascular dysfunction, derangements in coagulation, and ultimately multi-organ dysfunction. Sepsis

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is diagnostically defined as having a suspected infection plus at least one characteristic symptom such as temperature change, tachycardia, respiratory distress, altered mental status, hypotension, white blood cell count changes, presence of elevated biomarkers, or microvascular dysfunction. Sepsis can progressively worsen as organ dysfunction ensues and eventually culminate with septic shock where hypotension is refractory to fluids and vasopressors.³

The key pathophysiological mechanism of DIC, a complication of sepsis, involves systemic activation of both the coagulation and fibrinolytic systems ultimately leading to microvascular thrombosis and organ dysfunction.^{4,5} This process of consumptive coagulopathy eventually exhausts the body's supply of platelets and coagulation factors leading to increased bleeding risk.

Numerous biomarkers have been studied in both sepsis and DIC; however, no single biomarker alone can be used to diagnose DIC.⁶ As sepsis and DIC are highly heterogeneous disease processes, the identification of a specific biomarker of sepsis and DIC could be used as a tool for the diagnosis and staging of disease, prognosis, and response to intervention. Ultimately, early biomarkers that can detect the onset of endothelial and multi-organ dysfunction prior to clinical decompensation may aid in the lowering of mortality rates.⁷

Inflammation plays a key role in sepsis. Components of the bacterial cell wall, particularly lipopolysaccharide, induce an inflammatory response, including dramatic elevations in the interleukins (ILs) and tumor necrosis factor α (TNF α). These cytokines, particularly IL-6 and TNF α , induce the expression of tissue factor on the surface of cells, which leads to the activation of the coagulation cascade.⁸⁻¹²

In addition to generalized inflammation, the infection response may provide an additional link between sepsis and coagulopathy and therefore biomarkers of infection response may present another approach for evaluation or prediction of DIC in patients with sepsis. Procalcitonin (PCT) is normally rapidly cleaved into calcitonin and therefore plasma PCT levels are expected to be very low (<0.1 ng/mL) in healthy humans.^{13,14} However, during infections, PCT is produced ubiquitously and released at 1000-fold higher levels.¹⁵ Procalcitonin is thought to act as a "hormokine" inflammatory mediator through its role in inducing pro-inflammatory effects on leukocytes such as increasing expression of surface markers and increasing concentrations of pro-inflammatory cytokines.¹⁶ Numerous studies have shown that PCT is elevated in sepsis.¹⁷

Nucleosomes are another novel biomarker of infection that may be involved in the pathophysiology of DIC. Nucleosomes are complexes of chromosomal DNA wrapped around core histone proteins that are typically found intracellularly.¹⁸ Extracellular nucleosome levels are thought to be an indicator of degree of cell death since nucleosomes are found in the blood primarily after cells release their contents either due to necrosis or apoptosis.¹⁹ However, newer literature describes a second source of extracellular nucleosomes from the process of "NETosis" whereby neutrophils release extracellular traps (NETs) composed of extracellular fibers and nucleosomes in

order to disarm and kill bacteria extracellularly.^{20,21} When these NETs are ultimately degraded, the free nucleosomes play a procoagulant function by triggering the intrinsic pathway of the coagulation cascade. Nucleosomes have also been implicated as a mediator of thrombosis by promoting thrombin generation and inducing platelet activation.¹⁶ The individual components of nucleosomes, DNA, and histones have also been shown to have prothrombotic properties.²²⁻²⁴ Furthermore, nucleosomes activate both inflammation and cell death pathways through direct interaction with cell membranes and Toll-like receptors signaling pathways.²⁵ Therefore, the level of circulating nucleosomes may serve as an independent predictor of sepsis and DIC through the multiple roles it plays in infection, inflammation, and coagulopathy.

This study specifically evaluated various biomarkers of inflammation and infection such as IL-6, IL-8, and IL-10, TNF- α , PCT, and nucleosome levels. The aim of this study was to evaluate if there is a relationship between known markers of inflammation and infection and DIC score and to determine their prognostic role in assessing severity of disease.

Materials and Methods

Healthy Controls

Frozen, citrated plasma samples from healthy individuals (n = 50) were purchased from George King Biomedical (Overland, Kansas). These samples included 25 male and 25 female volunteers, ages 19 to 54 years, with a mean age of 32 years. All volunteers were nonsmokers, nonmedicated, and of geographically diverse origins. Plasma was aliquoted and stored at -80 C prior to use.

Plasma Samples in Patient Cohort

Deidentified serial plasma samples from adult patients with sepsis and suspected DIC (n = 137) were obtained between 2008 and 2012 from the University of Utah Medical Center under an institutional review board-approved protocol. The plasma samples were collected from patients at University of Utah Hospital intensive care unit (ICU) or an associated community hospital ICU upon admission and prior to receiving any treatment.

Enrollment criteria for this study required patients to meet the clinical criteria for systemic inflammatory response syndrome and have an identified source of infection. Systemic inflammatory response syndrome was defined as the presence of 2 or more of the following: (1) temperature <36 or >38, (2) heart rate >90 beats per minute, (3) respiratory rate >20 breaths per minute or PaCO₂ < 32 mm Hg, or (4) white blood cell count $\geq 12\,000$ cells/mm³ or ≤ 4000 cells/mm³ or >10% bands.²⁶

Patients were excluded from the study if they had received a blood transfusion within the past 4 months, platelet transfusion within the past 14 days, or platelet count of <20 000/ μ L. Patients were also excluded if they had a preexisting disorder affecting platelet number or function such as idiopathic thrombocytopenic purpura, thrombotic thrombocytopenic purpura,

Table 1. The ISTH Scoring System for DIC.

Variable	Value	Points
Platelets (K/ μ L)	>100	0
	50-100	1
	<50	2
INR	<1.3	0
	1.3-1.7	1
	>1.7	2
D-Dimer (ng/mL)	<400	0
	400-4000	2
	>4000	3
Fibrinogen (mg/dL)	>100	0
	<100	1

Abbreviations: DIC, disseminated intravascular coagulation; INR, international normalized ratio; ISTH, International Society of Thrombosis and Hemostasis.

hemolytic uremic syndrome, end-stage liver disease, myeloproliferative disorders, multiple myeloma, Waldenstrom macroglobulinemia, end-stage renal disease requiring hemodialysis, or inherited platelet disorders such as gray platelet syndrome, Bernard-Soulier syndrome, May-Hegglin anomaly, Wiskott-Aldrich syndrome, Glanzmann thrombasthenia, Chediak-Higashi syndrome, or Hermansky-Pudlak syndrome.

Disseminated Intravascular Coagulation Scoring

The International Society of Thrombosis and Hemostasis (ISTH) scoring algorithm, shown in Table 1, was used to stratify patients with sepsis into 3 categories based on the severity of coagulopathy. Using this scoring system, points are assigned based on patient values for platelets, international normalized ratio (INR), D-Dimer, and fibrinogen. Patients with a score of 0 to 2 were categorized as sepsis without DIC, patients with a score of 3 to 4 were categorized as sepsis with nonovert DIC, and patients with a score of ≥ 5 were categorized as sepsis with overt DIC. Disseminated intravascular coagulation is thought to occur in 2 phases. Nonovert DIC describes an intermediate phenotype where the hemostatic system has only mild dysfunction, while overt DIC describes a decompensated hemostatic system with more severe coagulopathy.^{6,27}

Coagulation Testing

Prothrombin time/INR and fibrinogen were measured using the ACL-ELITE coagulation analyzer (Instrumentation Laboratory, Bedford, Massachusetts).

Enzyme-Linked Immunosorbent Assays

Markers of infection were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits, including PCT (Abcam, Cambridge, United Kingdom) and nucleosomes (Roche Diagnostics, Indianapolis, Indiana). d-Dimer levels were measured using Hyphen BioMed ELISA kit (Neuville-Sur-Oise, France). Markers of inflammation

Table 2. Disseminated Intravascular Coagulation Score Distribution.

	All Patients	Sepsis + No DIC	Sepsis + Nonovert DIC	Sepsis + Overt DIC
DIC score	Any	0-2	3-4	≥ 5
Day 0 (n)	103	20	59	24

Abbreviation: DIC, disseminated intravascular coagulation.

including IL-6, IL-8, IL-10, and TNF α were measured using the Biochip Array (Randox, Antrim, United Kingdom).

Statistical Analysis

Data are shown as mean \pm standard error of the mean or mean (standard deviation) as individually specified. For comparing the multiple groups (healthy controls, sepsis without DIC, sepsis + nonovert DIC, and sepsis + overt DIC), analysis of variance (ANOVA) with Kruskal-Wallis ANOVA test was used. A *P* value of $<.05$ was used as the cutoff for statistical significance throughout this study. Correlations were evaluated by Spearman rank method. All data were tabulated within Microsoft Excel 2013 and statistical analysis was performed using GraphPad Prism v7.

Results

The cohort of patients with sepsis included 103 adults with sepsis diagnosed according to the previously described criteria. The baseline characteristics of this patient cohort are typical and appropriate for patients with sepsis. Comorbidities analyzed in this patient cohort include conditions that may affect coagulation status and thus the development of DIC, particularly active cancer and cirrhosis. Both of these conditions occurred with low frequency (5.8%) in this patient population. The most prevalent recorded comorbidity in this cohort was hypertension, reported in 45.6% of patients.

As shown in Table 2, the majority of patients (57.2%) had nonovert DIC at baseline. The remaining patients were split between sepsis without DIC (19.4%) and overt DIC (23.3%). The division of patients into these 3 categories based on the DIC score provides a means to assess whether the levels of the measured inflammatory factors are associated not only with the inflammatory state of sepsis but also with the development of coagulopathy. As shown in Figure 1, the levels of each measured factor were compared between patients with sepsis without DIC, patients with sepsis and nonovert DIC, and patients with sepsis and overt DIC, as well as with a group of healthy controls.

Procalcitonin and Nucleosomes

Procalcitonin levels were significantly elevated in all patients with sepsis or DIC as compared to healthy controls, as shown in Figure 1A. Procalcitonin is well established as a biomarker of infection and is used clinically to distinguish infectious sepsis from similar conditions of noninfectious origin. Notably,

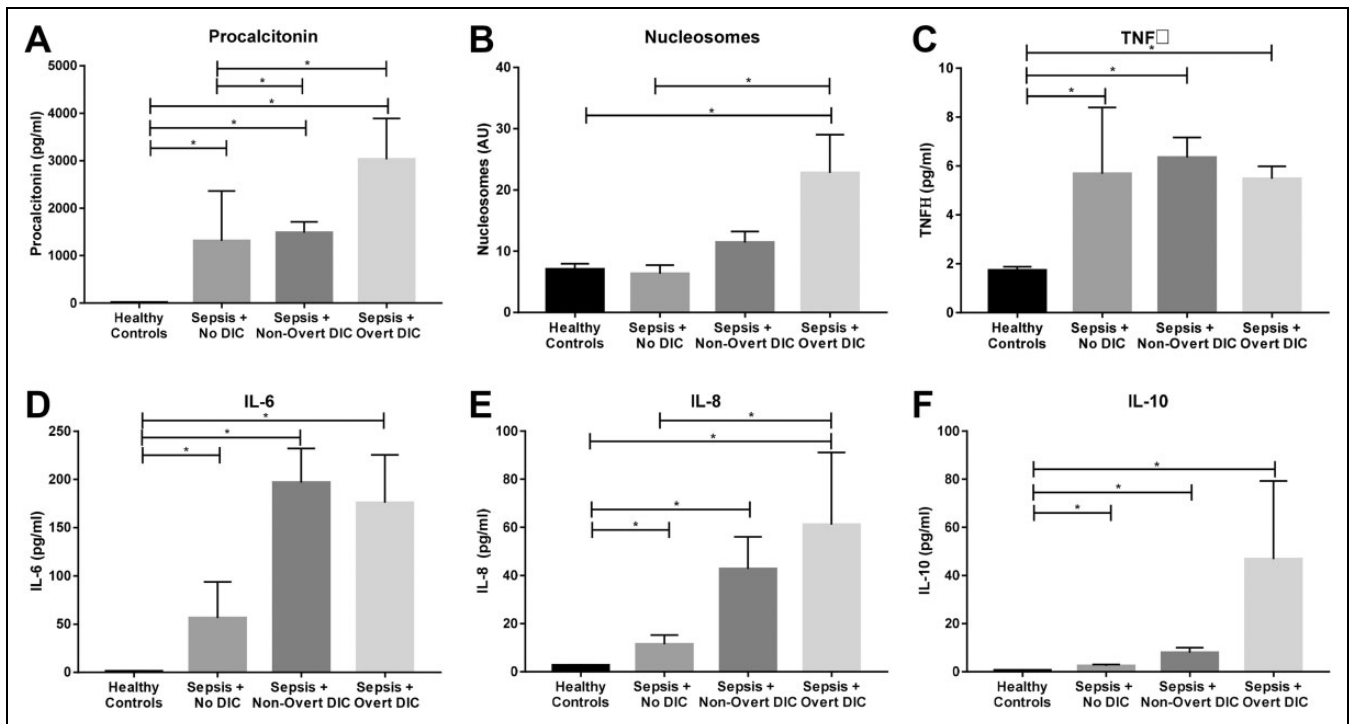


Figure 1. Levels of biomarkers of inflammation and infection in patients with sepsis stratified by DIC score and in healthy controls. Data are shown as mean \pm SEM. Groups were compared using the Kruskal-Wallis 1-way ANOVA and Dunn multiple comparison test, with $P < .05$ as the cutoff for significance (indicated by *). ANOVA indicates analysis of variance; DIC, disseminated intravascular coagulation; SEM, standard error of the mean.

further elevations in PCT were observed in both the nonovert DIC and overt DIC group as compared to the patients with sepsis without DIC. This demonstrates that levels of PCT, representative of the intensity of ongoing infection, may be useful for assessment of the severity of DIC in patients with sepsis and that infection-related processes may contribute to the development of coagulation dysfunction.

In contrast to the other measured biomarkers, no significant elevations in nucleosomes were observed in patients with sepsis and no DIC or sepsis and nonovert DIC compared to healthy controls, as shown in Figure 1B. However, a significant elevation in nucleosomes was observed in patients with sepsis and overt DIC compared to both the healthy controls and sepsis without DIC groups.

Inflammatory Cytokines

Levels of the inflammatory cytokines TNF α , IL-6, IL-8, and IL-10, shown in Figure 1C to F, were significantly elevated in all sepsis patient groups as compared to the healthy controls. However, no significant differences in levels of IL-6, IL-10, or TNF α were present between patient groups divided on the basis of DIC score. The only variation between sepsis groups was observed for IL-8 where patients with sepsis + overt DIC had significantly higher levels of IL-8 than patients with sepsis without DIC.

Correlations among biomarkers, including the inflammatory and infection biomarkers and the components of the DIC score

as well as the DIC score itself, were assessed using Spearman correlation coefficients with $P < .05$ as the cutoff for significance. The correlation matrix comparing the relationship between individual markers and DIC scores is shown in Table 3. All significant correlations with $P < .05$ are highlighted in gray and the significant correlations with an $r > 0.4$ are bolded.

Disseminated intravascular coagulation score correlated strongly with 3 of the 4 hemostatic markers included in its calculation; however, no significant correlation was observed between DIC score and fibrinogen. Disseminated intravascular coagulation score also correlated strongly with PCT as well as significantly but less strongly with nucleosomes, IL-6, IL-8, IL-10, and TNF α . Disseminated intravascular coagulation score had the strongest correlation with the biomarkers PCT and IL-6 ($r = 0.4$).

Nucleosomes, which have the potential to provide a mechanistic link between infection response and thrombosis, correlated moderately with d-Dimer, PCT, IL-8, and IL-10. Procalcitonin is an established marker of infection and correlated moderately with platelets, nucleosomes, IL-6, and IL-8 and strongly with D-Dimer, IL-10, and TNF α . Strong correlations were noted among the inflammatory cytokines IL-6, IL-8, IL-10, and TNF α .

Discussion

The purpose of this study was to determine the relationship between biomarkers of infection and inflammation that are

Table 3. Correlations Among Biomarker Levels in Patients With Sepsis.^a

	DIC Score	D-Dimer	INR	Platelets	Fibrinogen	Nucleosomes	Procalcitonin	IL-6	IL-8	IL-10	TNF α
DIC score		0.63	0.56	-0.53	-0.18	0.33	0.47	0.37	0.38	0.39	0.29
D-Dimer	0.63		0.11	-0.27	0.03	0.34	0.47	0.34	0.34	0.36	0.31
INR	0.56	0.11		-0.30	-0.11	0.17	0.24	0.33	0.32	0.17	0.16
Platelets	-0.53	-0.27	-0.30		0.26	-0.14	-0.35	-0.03	-0.16	-0.21	-0.28
Fibrinogen	-0.18	0.03	-0.11	0.26		0.04	0.02	0.21	0.06	-0.09	-0.02
Nucleosomes	0.33	0.34	0.17	-0.14	0.04		0.31	0.19	0.24	0.18	0.03
Procalcitonin	0.47	0.47	0.24	-0.35	0.02	0.31		0.41	0.41	0.45	0.52
IL-6	0.37	0.34	0.33	-0.03	0.21	0.19	0.41		0.61	0.50	0.38
IL-8	0.38	0.34	0.32	-0.16	0.06	0.24	0.41	0.61		0.57	0.49
IL-10	0.39	0.36	0.17	-0.21	-0.09	0.18	0.45	0.50	0.57		0.57
TNF α	0.29	0.31	0.16	-0.28	-0.02	0.03	0.52	0.38	0.49	0.57	

Abbreviations: DIC, disseminated intravascular coagulation; IL, interleukin; INR, international normalized ratio; TNF α , tumor necrosis factor α .

^aSpearman correlation coefficients are shown. Significant correlations ($P < .05$) are highlighted in gray. Significant correlations with a Spearman correlation ≥ 0.40 as a cutoff are bolded.

known to be elevated in sepsis with ISTH DIC scores in order to assess their role in predicting severity of coagulopathy. This study found that the infection biomarkers PCT and nucleosomes in addition to the inflammatory cytokine IL-8 related to the pathogenesis of both sepsis and the severity of coagulopathy involved in DIC.

Although numerous studies have demonstrated that PCT levels are elevated in patients with sepsis and serve as a valuable biomarker of ongoing infection, fewer studies have explored if this also translates to the development of DIC. In this study, PCT levels were found to be significantly elevated in patients with sepsis and DIC compared to healthy controls. Interestingly, PCT levels were also significantly higher in the patients with DIC as compared to the patients with sepsis without substantial coagulopathy. This suggests that plasma PCT levels may be a useful prognostic marker in detecting not only sepsis but also the progression of onset to DIC. Additionally, PCT may have a potential role in early risk stratification and prediction of overall morbidity and mortality. Further studies are warranted to examine the relationship of PCT levels to clinical outcomes in the sepsis and DIC patient populations. In addition to a relationship with the aggregate DIC score, PCT correlated strongly with D-Dimer levels and platelet count, further emphasizing the largely unexplored relationship of PCT with hemostatic dysfunction.

Nucleosome levels were significantly elevated in patients with overt DIC compared to healthy controls and in patients with sepsis without DIC; however, no difference in nucleosome levels was present between healthy controls and patients with sepsis without DIC. This specific elevation of nucleosomes in patients with severe coagulopathy suggests that nucleosome level may be useful as a tool to identify patients with sepsis having overt DIC from patients with sepsis without coagulopathy. There were moderate correlations observed between concentrations of nucleosomes, PCT, and inflammatory mediators such as IL-8 and IL-10, suggesting that nucleosomes are implicated in both the infectious and inflammatory pathways of sepsis and DIC. The moderate correlation between

nucleosomes and DIC score also supports their role in the pathogenesis of DIC through the known mechanism of promoting thrombin generation and microvascular thrombosis.

Although there is currently no single biomarker that can effectively diagnose DIC in patients with sepsis, this study lays the groundwork for developing a diagnostic algorithm that can incorporate several known markers of inflammation and infection in order to better predict the onset of DIC as well as assess severity of coagulopathy. Procalcitonin and IL-6 demonstrated the strongest correlation of the measured markers with DIC score. Not only does this support that PCT should be added to the profile of biomarkers indicative of DIC but also suggests that PCT may actually be more useful than some of the other less specific inflammatory markers such as IL-8, IL-10, and TNF α . A combination of biomarkers of inflammation and infection may better distinguish the degree of DIC severity and clinical outcome in this patient group than use of DIC score alone. Further studies are warranted to explore if other markers in addition to PCT and nucleosomes can be incorporated into a more comprehensive biomarker profile for earlier risk stratification and prediction of overall morbidity and mortality in DIC.

Authors' Note

Ethical approval to report this case was obtained from the Loyola University Chicago IRB (LU Number 207958). Written informed consent was obtained from the patient(s) for their anonymized information to be published in this article.

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References

- Ishikura H, Nishida T, Murai A, et al. New diagnostic strategy for sepsis-induced disseminated intravascular coagulation: a prospective single-center observational study. *Crit Care*. 2014;18(1):1-9. doi:10.1186/cc13700.
- Gando S, Levi M, Toh CH. Disseminated intravascular coagulation. *Nat Rev Dis Prim*. 2016;2:16037. doi:10.1038/nrdp.2016.37.
- Finfer SR, Vincent JL, Angus DC, Van Der Poll T. Critical care medicine severe sepsis and septic shock. *N Engl J Med*. 2013; 9369(29):840-851. doi:10.1056/NEJMra1208623.
- Hoppensteadt D, Tsuruta K, Hirman J, Kaul I, Osawa Y, Fareed J. Dysregulation of inflammatory and hemostatic markers in sepsis and suspected disseminated intravascular coagulation. *Clin Appl Thromb*. 2015;21(2):120-127. doi:10.1177/1076029613509476.
- Levi M. The coagulant response in sepsis and inflammation. *Hamostaseologie*. 2010;30(1):10-16. doi:10.1016/j.ccm.2008.06.006.
- Wada H, Matsumoto T, Yamashita Y. Diagnosis and treatment of disseminated intravascular coagulation (DIC) according to four DIC guidelines. *J Intensive Care*. 2014;2(1):15. Published 2014 Feb 20. doi:10.1186/2052-0492-2-15.
- Biron BM, Ayala A, Lomas-Neira JL. Biomarkers for sepsis: what is and what might be? *Biomark Insights*. 2015;10(suppl 4):7-17. doi:10.4137/BMI.S29519.
- Levi M, van der Poll T, Buller HR. Bidirectional relation between inflammation and coagulation. *Circulation*. 2004;109(22): 2698-2704. doi:10.1161/01.CIR.0000131660.51520.9A.
- Abraham E. Coagulation abnormalities in acute lung injury and sepsis. *Am J Respir Cell Mol Bio*. 2000;22(4):401-404. doi:10.1165/ajrcmb.22.4.f184.
- Bevilacqua MP, Pober JS, Majeau GR, Fiers W, Cotran RS, Gimbrone MA Jr. Recombinant tumor necrosis factor induces procoagulant activity in cultured human vascular endothelium: characterization and comparison with the actions of interleukin 1. *Proc Natl Acad Sci*. 1986;83(12):4533-4537. doi:10.1073/pnas.83.12.4533.
- Esmon CT. The impact of the inflammatory response on coagulation. *Thromb Res*. 2004;114(5-6):321-327. doi:10.1016/j.thromres.2004.06.028.
- Hezi-Yamit A, Wong PW, Bien-Ly N, et al. Synergistic induction of tissue factor by coagulation factor Xa and TNF: evidence for involvement of negative regulatory signaling cascades. *Proc Natl Acad Sci*. 2005;102(34):12077-12082. doi:10.1073/pnas.0504526102.
- Riedel S. Procalcitonin and the role of biomarkers in the diagnosis and management of sepsis. *Diagn Microbiol Infect Dis*. 2012; 73(3):221-227. doi:10.1016/j.diagmicrobio.2012.05.002.
- Kobeissi ZA, Zanotti-Cavazzoni SL. Biomarkers of sepsis. *Yearb Crit Care Med*. 2010;2010(7):227-228. doi:10.1016/S0734-3299(10)79402-8.
- Müller B, Becker KL, Schächinger H, et al. Calcitonin precursors are reliable markers of sepsis in a medical intensive care unit. *Crit Care Med*. 2000;28(4):977-983.
- Becker KL, Snider R, Nylen ES. Review Procalcitonin in sepsis and systemic inflammation: a harmful biomarker and a therapeutic target. *Br J Pharmacol*. 2010;159(2): 253-264. doi:10.1111/j.1476-5381.2009.00433.x.
- Chen Q, Ye L, Jin Y, et al. Circulating nucleosomes as a predictor of sepsis and organ dysfunction in critically ill patients. *Int J Infect Dis*. 2012;16(7):e558-e564. doi:10.1016/j.ijid.2012.03.007.
- Prkno A, Wacker C, Brunkhorst FM, Schlattmann P. Procalcitonin-guided therapy in intensive care unit patients with severe sepsis and septic shock—a systematic review and meta-analysis. *Critical Care*. 2013;17(6):R291. doi:10.1186/cc13157.
- Rhodes A, Wort SJ, Thomas H, Collinson P, David ED. Plasma DNA concentration as a predictor of mortality and sepsis in critically ill patients. *Crit Care*. 2006;10(2):1-7. doi:10.1186/cc4894.
- Xu J, Zhang X, Monestier M, Esmon NL, Esmon CT. Extracellular histones are mediators of death through TLR2 and TLR4 in mouse fatal liver injury. *J Immunol*. 2011;187(5):2626-2631. doi:10.4049/jimmunol.1003930.
- Delabranche X, Stiel L, Severac F, et al. Evidence of netosis in septic shock-induced disseminated intravascular coagulation. *Shock*. 2017;47(3):313-317. doi:10.1097/SHK.0000000000000719.
- Ammollo C, Semeraro F, Xu J, Esmon NL, Esmon CT. Extracellular histones increase plasma thrombin generation by impairing thrombomodulin-dependent protein C activation. *J Thromb Haemost*. 2011;9(9):1795-1803. doi:10.1111/j.1538-7836.2011.04422.x.
- Semeraro F, Ammollo CT, Morrissey JH, et al. Extracellular histones promote thrombin generation through platelet-dependent mechanisms: involvement of platelet TLR2 and TLR4. *Blood*. 2011;118(7):1952-1961. doi:10.1182/blood-2011-03-343061.
- Yang X, Li L, Liu J, Lv B, Chen F. Extracellular histones induce tissue factor expression in vascular endothelial cells via TLR and activation of NF- κ B and AP-1. *Thromb Res*. 2016;137:211-218. doi:10.1016/j.thromres.2015.10.012.
- Gould T, Lysov Z, Liaw P. Extracellular DNA and histones: double-edged swords in immunothrombosis. *J Thromb Haemost*. 2015;13(suppl 1):S82-S91. doi:10.1111/jth.12977.
- Marik PE, Taeb AM. SIRS, qSOFA and new sepsis definition. *J Thorac Dis*. 2017;9(4):943-945. doi:10.21037/jtd.2017.03.125.
- Taylor FJ, Toh C, Hoots W, Wada H, Levi M. Scientific Subcommittee on Disseminated Intravascular Coagulation (DIC) of the International Society on Thrombosis and Hemostasis. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation. *Thromb Haemost*. 2001;86(5):1327-1330. doi:10.1111/j.1538-7836.2007.02313.x.