Coagulation in Sepsis

All patients with sepsis have some degree of coagulation activation

These abnormalities range from:

➜ subtle activation of coagulation (only detected by sensitive markers for coagulation factor activation)

➜ to mild thrombocytopenia and subclinical prolongation of clotting times

➜ to disseminated intravascular coagulation (DIC) characterized by simultaneous widespread microvascular thrombosis and profuse bleeding

Sepsis-associated coagulopathy and DIC

Systemic activation of coagulation ➜ intravascular **fibrin deposition ➜ organ failure**
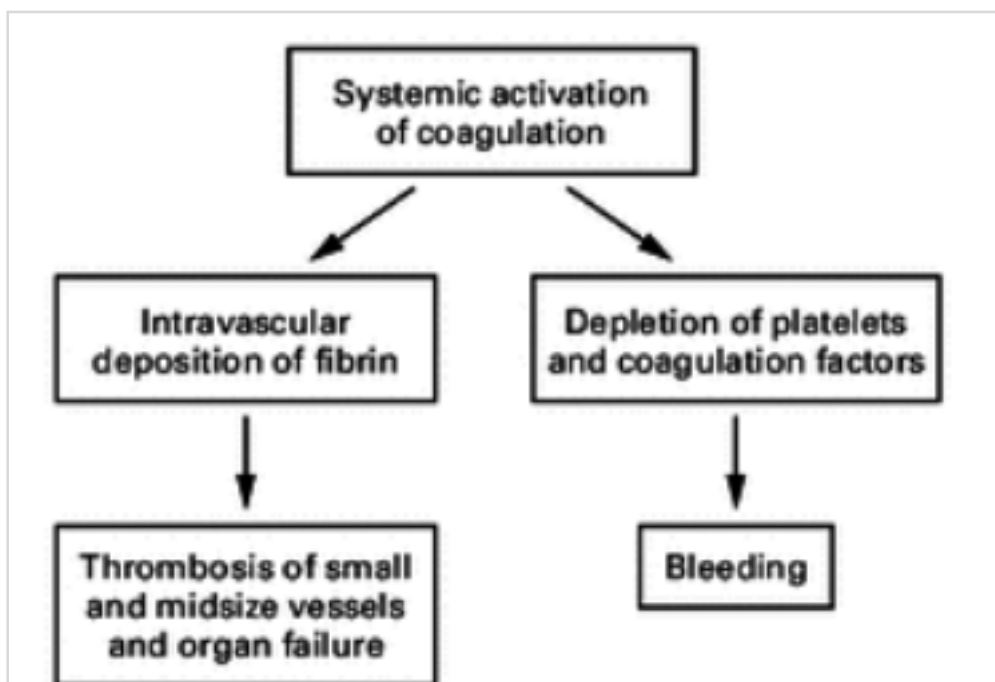**Consumption** of platelets and coagulation factors ➜ **bleeding**

Figure 1. The Mechanism of Disseminated Intravascular Coagulation.
Systemic activation of coagulation leads to widespread intravascular deposition of fibrin and depletion of platelets and coagulation factors. As a result, thrombosis of small and midsize vessels may occur, contributing to organ failure, and there may be severe bleeding.

- Autopsy will show diffuse bleeding at various sites, hemmorhagic necrosis of tissue,

micro-thrombi in small blood vessels, thrombi in midsize vessels and arteries
- Presence of intravascular thrombi is specifically related to the dysfunction of the organ

50-70% of septic patients will have clinically relevant hemostatic changes
35% of septic patients will meet the criteria for DIC

Coagulation abnormalities are an important determinant of clinical outcome.
Fournier F, Chopin C, Gourmand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest 1992;101(3):816–823
- "The mortality rate reached 77 percent in group DIC + vs 32 percent in DIC- (p<0.001) …. DIC is a strong predictor of death and multiple organ failure in patients with septic shock."

Sepsis-Associated Coagulopathy Severity Predicts Hospital Mortality*

Patrick G. Lyons, MD[1]; Scott T. Micek, PharmD[2]; Nicholas Hampton, PharmD[3]; Marin H. Kollef, MD[1]

Critical Care Medicine. 2018;46(5):736-742.
- Retrospective cohort study
- 6,148 patients hospitalized between 2010-2015
- Categorized patients as mild, moderate or severe sepsis-associated coagulopathy
  - Mild: INR >1.2 and <1.4; platelet count >100k and <150k
  - Moderate: INR >1.4 and <1.6; platelet count >80k and <100k
  - Severe: INR >1.6; platelet count <80k
- Results
  - Hospital mortality increased progressively from 25.4% in patients without sepsis-associated coagulopathy to 56.4% in patients with severe sepsis-associated coagulopathy
  - Duration of hospitalization and ICU similarly increases as sepsis-associated coagulopathy severity worsened
    - controlled for patient characteristics, hospitalization variables, and severity of illness
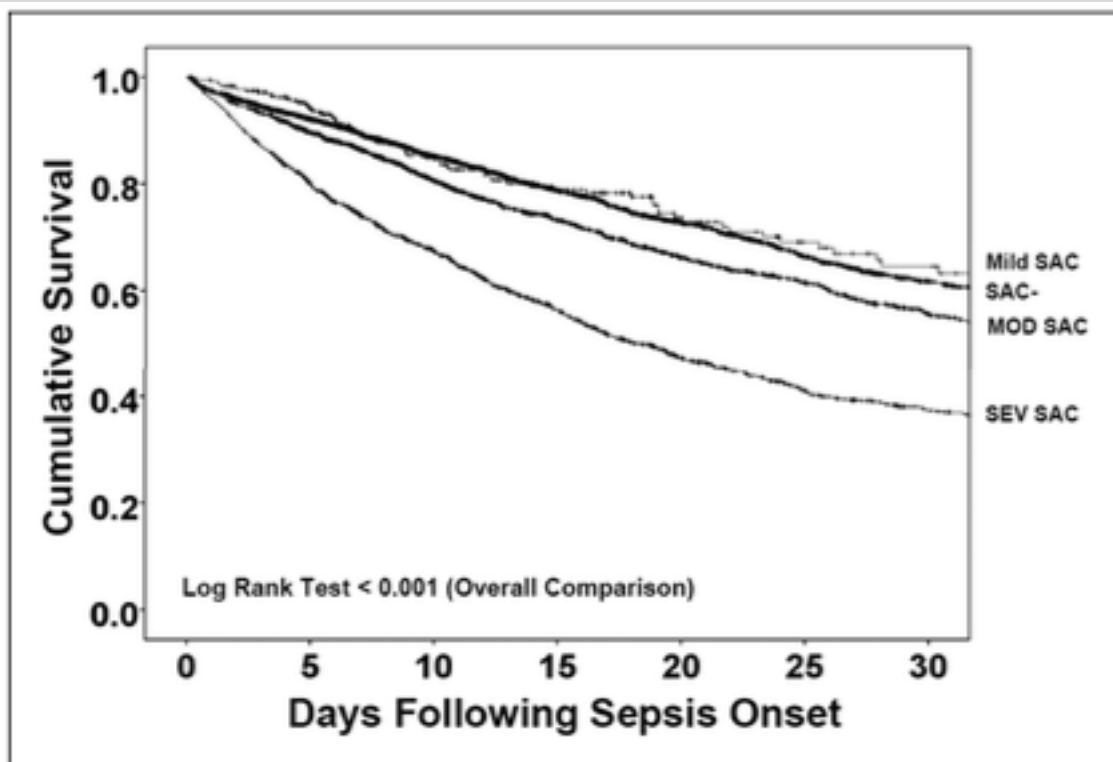
Figure 2. Kaplan-Meier curves for cumulative survival according to sepsis-associated coagulopathy (SAC) severity. Statistical comparisons by log-rank test: overall comparison, $p < 0.001$; SAC negative (SAC−) versus mild SAC, $p = 0.153$; SAC− versus moderate (MOD) SAC, $p < 0.001$; SAC− versus severe (SEV) SAC, $p < 0.001$; mild SAC versus MOD SAC, $p = 0.002$; mild SAC versus SEV SAC, $p < 0.001$; MOD SAC versus SEV SAC, $p < 0.001$.

Pathways leading to coagulation abnormalities in sepsis

1. **Excess fibrin** deposition (driven through activation of tissue factor)
2. **Impaired anticoagulant** mechanisms (including protein C system, antithrombin, and tissue factor pathway inhibitor)
3. Compromised fibrin removal owing to **depression of the fibrinolytic system** (enhanced release of plasminogen activator type I (PAI-1))
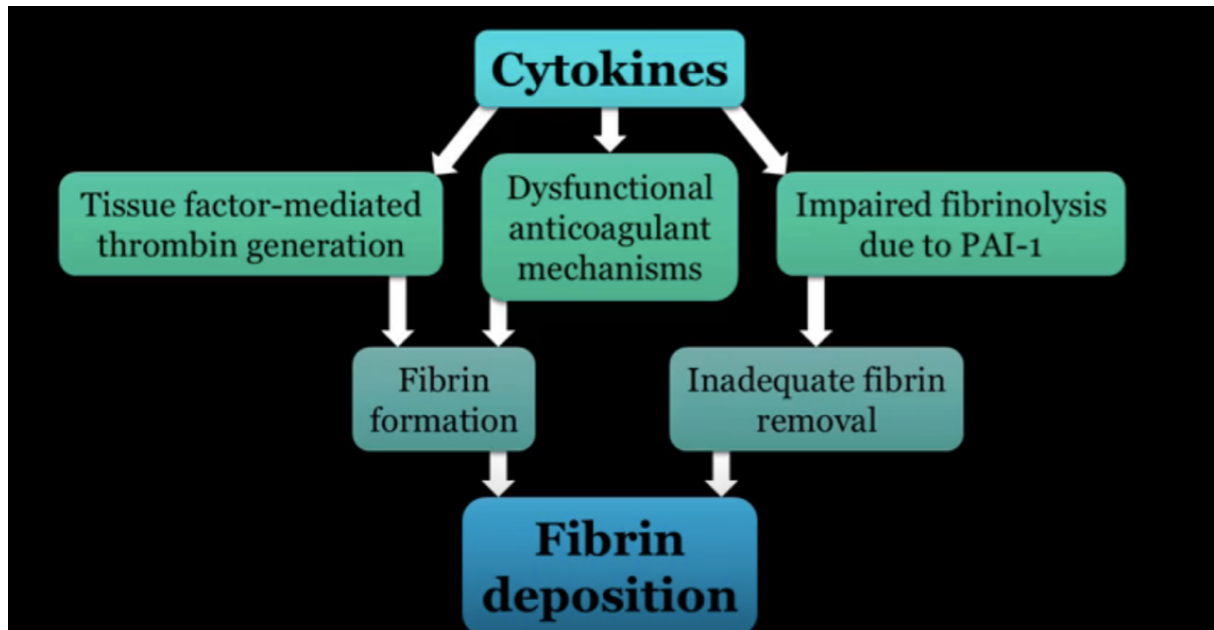
(1) Excess Fibrin Deposition

Most important initiator of thrombin generation in sepsis is **tissue factor**

Tissue factor exists as a transmembrane glycoprotein with both cytoplasmic and extracellular domains on mononuclear cells (extravascular monocytes, macrophages, fibroblasts). It is found in high levels in close proximity to the vascular space, where it can activate coagulation in response to an interruption in vascular integrity

- Tissue factor is also found circulating on microparticles and cell membrane fragments; however this TF is generally considered inactive

TF-FVIIa complex activates FX ➜ FXa, which converts prothrombin to thrombin, and ultimately the conversion of fibrinogen to fibrin. Simultaneously, the TF-FVIIa complex activates FIX. FIXa with it's cofactor FVIIIa, allows for additional activation of FX, leading to conversion of prothrombin to thrombin. An additional amplification loop is the activation of FXI by thrombin, that results in additional FIX and thus additional FX.
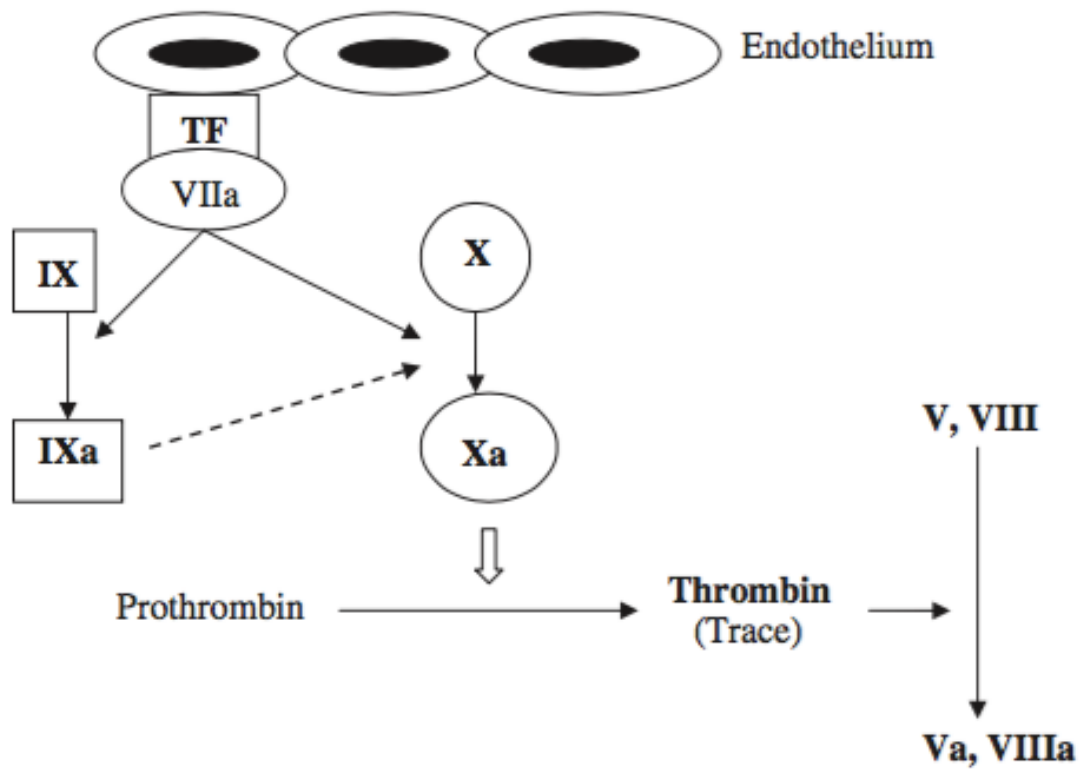
Figure 2: Initiation phase of the tissue factor pathway leading to the generation of trace levels of thrombin and activation of factors V, VIII, IX and X.
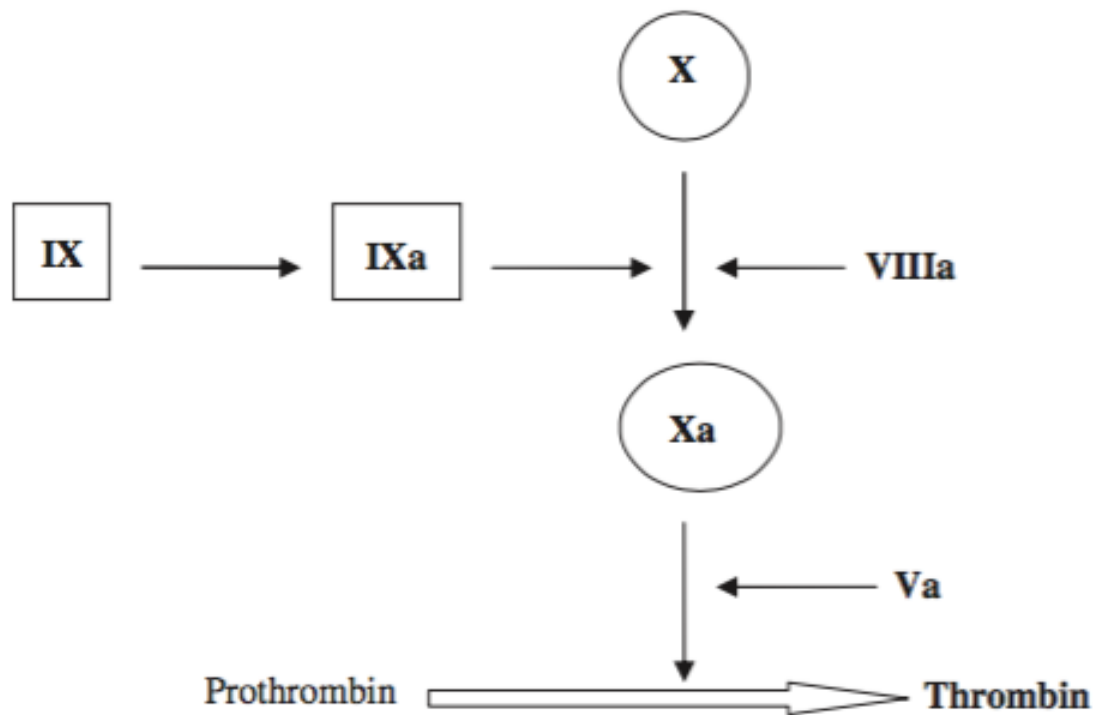
Figure 3: The propagation phase of the tissue factor pathway as a consequence of activated factors V, VIII, IX and X, resulting in the generation of thrombin.

Induction of intravascular TF expression is a major mechanism of inflammation-induced coagulation activation

Pro-inflammatory mediators, such as endotoxin, TNF-α, lipoproteins and growth factor stimulate TF expression on endothelial cells and circulating monocytes.

- *Monoclonal antibodies directed against tissue factor or factor VIIa result in complete inhibition of thrombin generation in endotoxin-challenged chimps and prevent the occurrence of DIC and mortality in baboons infused with E Coli*
- *Experimental low-dose endotoxemia in healthy humans results in 125-fold increase in tissue factor mRNA levels in blood monocytes*
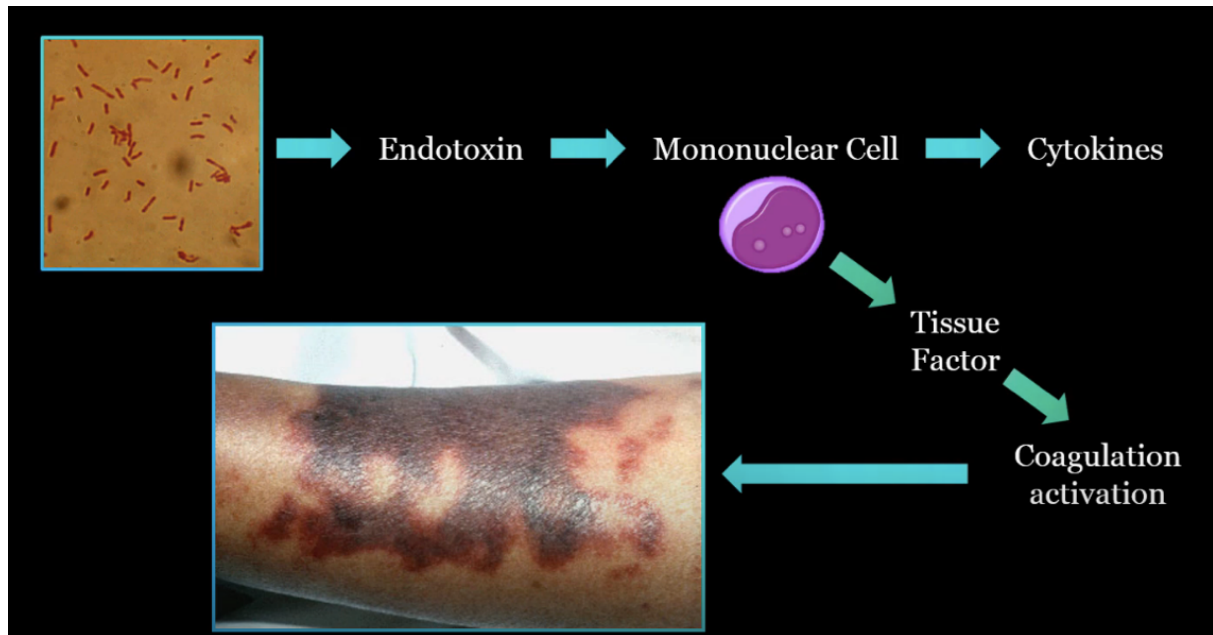
(2) Impaired anticoagulant mechanisms

3 major anticoagulant pathways: Antithrombin, Protein C, TFPI

1. **Antithrombin**:
   - Serine protease inhibitor
   - Main inhibitor of **thrombin** and **factor Xa**
   - Has heparin-binding domain that when bound (by heparin sulfated proteoglycans), there is a conformational change in AT, that will potentiate its' activity by more than 1000x
   - In sepsis, there are **reduced AT levels** due to:
     1. consumption from ongoing thrombin generation
     2. impaired synthesis as a result of a negative acute phase response
     3. Degradation by neutrophil elastase
   - **Reduced AT function** in sepsis due to:
     - heparin sulfated proteoglycans less available on the endothelial surface due to glycocalyx shedding in severe inflammation
   - Magnitude of AT reduction has been correlated with the severity of illness and predicts survival

2. **Activated Protein C**
   - vitamin K dependent complex; synthesized in the liver
   - inactivates **cofactors Va and VIIIa**
     - FVa and FVIIIa are vital in the propagation phase of TF-mediated coagulation
   - activated by **thrombin-thrombomodulin** complex

- Thrombomodulin: membrane protein; when thrombin binds there is an 100-fold increase in activation of protein C and blocking of thrombin-mediated conversion of fibrinogen to fibrin
  - Thrombomodulin expression is drastically reduced by TNFα, IL-1, and IL-6
- **also has *anti-inflammatory activity**:
  - inhibits TNF release, NFκβ activation, leukocyte adhesion and TF expression
  - The signalling pathways for these actions are undetermined
- **Improves fibrinolysis** by inhibiting Plasminogen activator inhibitor (PAI)
- **Decreased levels of aPC** in sepsis due to:
  1. Impaired hepatic synthesis (vitamin K dependent) during severe inflammation
  2. Degradation by neutrophil elastase
  3. Consumption by pro-coagulant processes
- **Down regulation of thrombomodulin** at the endothelial cell surface in sepsis due to:
  - presence of pro inflammatory cytokines TNF-α and IL-1β
- Protein C is consistently depleted in sepsis and is correlated with mortality in humans (several studies have documented this)
  - *Fournier F, Chopin C, Gourmand J, et al. Septic shock, multiple organ failure, and disseminated intravascular coagulation. Compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest 1992;101(3):816–823*

Hemostatic Changes in Dogs with Naturally Occurring Sepsis

Armelle M. de Laforcade, Lisa M. Freeman, Scott P. Shaw, Marjory B. Brooks, Elizabeth A. Rozanski, and John E. Rush

- Case-control study
- 20 dogs with naturally occurring sepsis (heterogenous population)
- Blood collected within 24 hours of admission: platelet count, PT, aPTT, FDPs, D-dimers, total protein C, antithrombin
- Results
  - 10/20 dogs survived
  - **AT and Protein C activity was significantly lower in septic dogs**
  - no relationship with outcome
  - Septic dogs also had significantly higher PT, aPTT, FDPs, and D-dimers
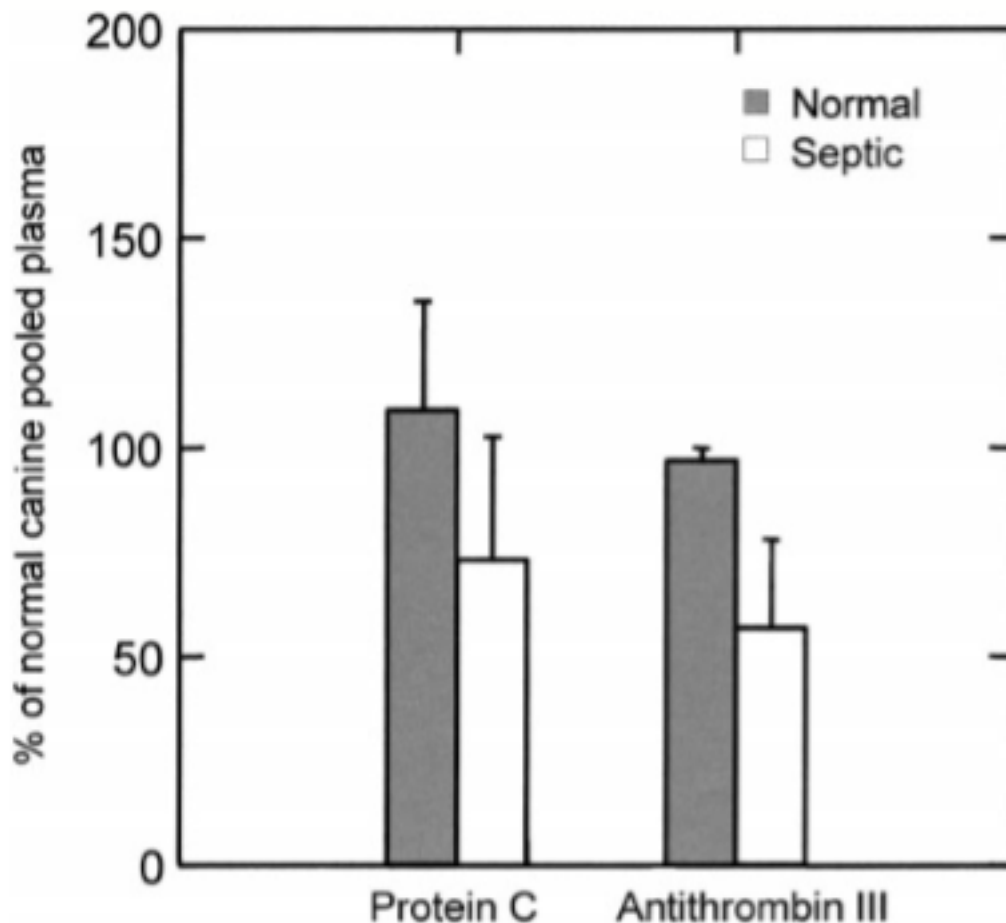 - Platelet counts not significantly different

Fig 1. Protein C (shaded bars) and antithrombin (open bars) activities in normal dogs (n = 28) and in dogs with naturally occurring sepsis (n = 20) (mean ± SD).

Alterations in the hemostatic profiles of dogs with naturally occurring septic peritonitis

Adrienne M. Bentley, DVM, DACVS; Philipp D. Mayhew, BVMS, DACVS; William T. N. Culp, VMD, DACVS and Cynthia M. Otto, DVM, PhD, DACVECC

- Prospective, observational study
- 27 dogs with naturally occurring septic peritonitis
- Blood collected pre-operatively, day 1 and day 3 post-op: platelets count, PT, aPTT, D-dimer, fibrinogen concentrations, total protein C, antithrombin, and TEG
- Results
  - 16/27 dogs survived
  - Mean AT activity was below the reference interval for both survivors and non-survivors

- Mean PC activity was below the reference interval for non survivors; 10 of 11 (91%) nonsurvivors and only 2 of 15 (13%) survivors had preoperative PC activity below the reference interval.
- **Non survivors had lower mean preoperative PC** (98 ± 24% versus 49 ± 26%; P< 0.001) **and AT activities** (53 ± 9% versus 32 ± 16%; P< 0.001)
- PC and AT decreased from pre-op to day 1; and in survivors, these values recovered on day 3
- The K value was significantly lower (more hypercoaguable) among survivors, although the median value for both groups was below the reference interval.
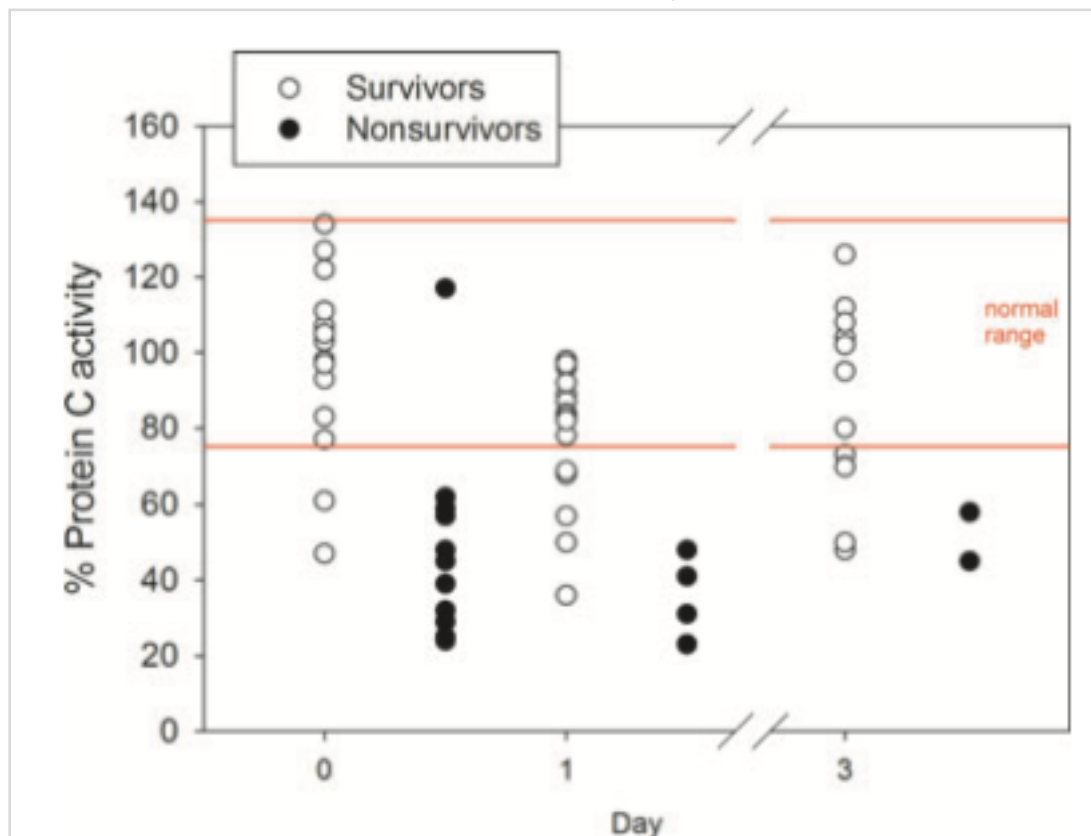
Figure 1: Serial data for protein C activity among dogs with naturally occurring septic peritonitis. Dot plot of protein C activity in plasma of survivors (open circles) and nonsurvivors (closed circles) prior to surgery for septic peritonitis (day 0; $n = 15$ survivors, $n = 11$ nonsurvivors), the day after surgery (day 1; $n = 16$ survivors, $n = 4$ nonsurvivors), and 3 days after surgery (day 3; $n = 11$ survivors, $n = 2$ nonsurvivors).

3. **Tissue factor pathway inhibitor (TFPI)**
   - Complex multi domain protease inhibitor
   - Main inhibitor of the **TF-FVIIa complex**
     - "extrinsic pathway inhibitor"
   - Also inhibits **factor Xa**

- Plasma levels of TFPI have been reported to be low, normal or even elevated in septic patients
 - Low levels of TFPI lead to insufficient down regulation of TF-FVIIa complex activity
  - The specific role of TFPI in the development of DIC is unknown

(3) Compromised fibrinolysis

- During activation of coagulation in sepsis, endogenous fibrinolysis is essentially turned off
  - Contributes to widespread microvascular thrombosis by preventing thrombin removal
- Typically, plasminogen activators (**tissue plasminogen activator (t-PA)** and **urokinase-type plasminogen activator (u-PA)**) cleave plasminogen into plasmin
  - Activated contact group coagulation factors can also activate plasminogen ➜ plasmin
  - Plasmin binds to fibrin and degrades cross-linked fibrin
- In sepsis, there is an increase in:
  1. **plasminogen activator inhibitor type 1 (PAI-1)**
     - PAI-1 inhibits t-PA and u-PA, thus plasminogen is not activated to plasmin
  2. **thrombin-activatable fibrinolysis inhibitor (TAFI)**
     - plasma protein activated by high levels of thrombin or thrombin-TM complexes that removes plasminogen binding sites from partially degraded fibrin
  3. **Cell-free DNA** (cfDNA)
     - released upon cell activation or by neutrophils as part of neutrophil extracellular traps (NETs)
     - incorporated into fibrin, and prevents plasmin-mediated fibrin degradation

Fibrinolytic response in endotoxemia:
At onset of endotoxemia, there is a fast and significant increase in fibrinolysis due to release of t-PA and u-PA, and then there is a immediate decrease in fibrinolysis due to a sustained increase in PAI-1
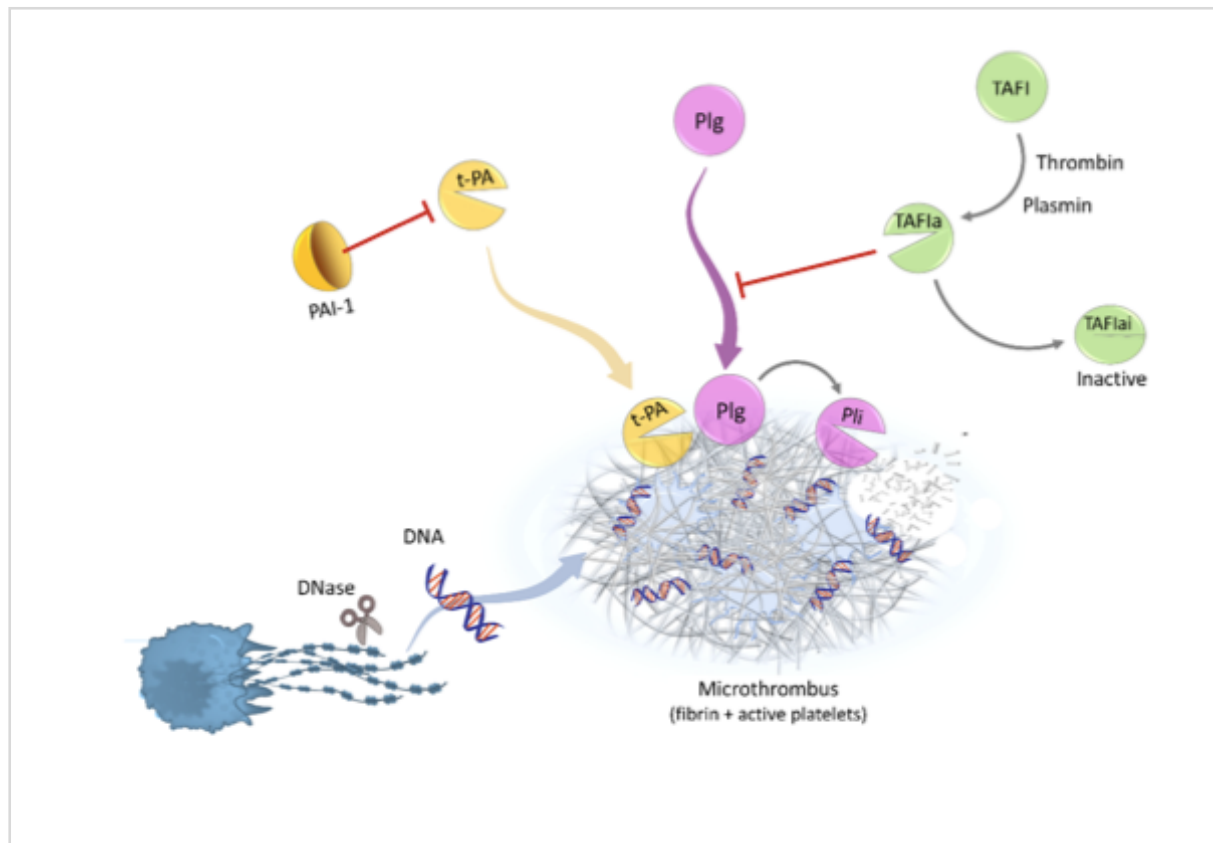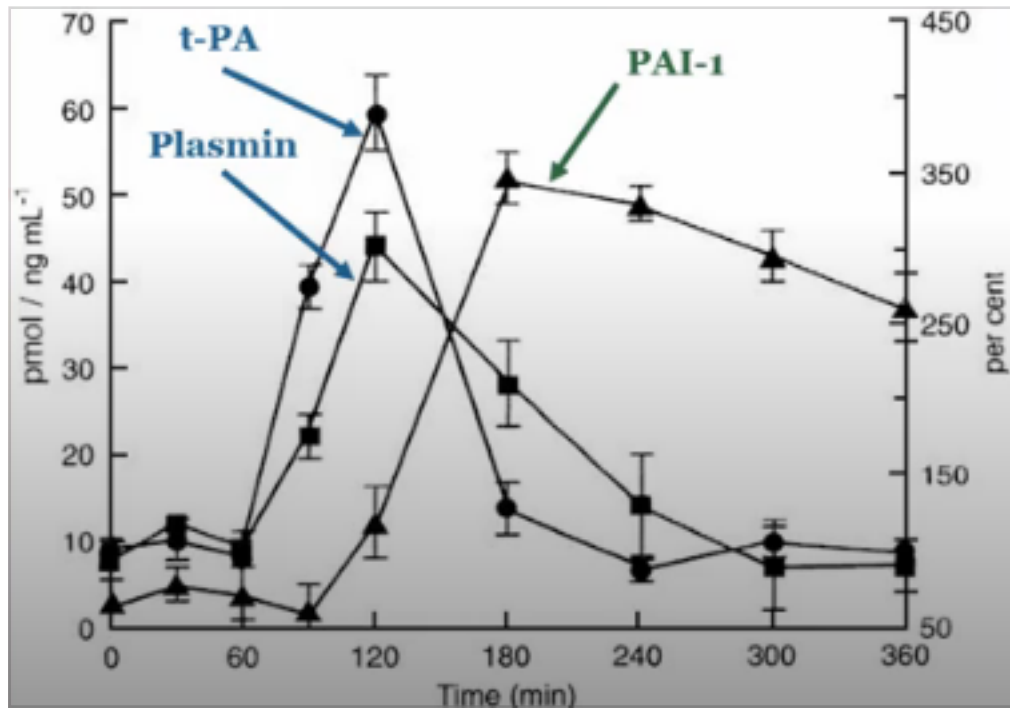
Supplemental Figure S1. Mechanisms of fibrinolysis inhibition in sepsis. Plasminogen (Plg) is activated to plasmin (Pli) by t-PA on the fibrin surface where both t-PA and plasminogen bind through specific interactions. Plasmin degrades fibrin, releasing small degradation products, including D-dimer (DD). During sepsis, fibrinolysis is inhibited in different ways. 1) PAI-1 binds and neutralizes t-PA. 2) TAFI, once activated by thrombin or plasmin (TAFIa), inhibits plasminogen binding to fibrin (by removing the plasminogen binding sites from fibrin). TAFIa is unstable and is rapidly converted to an inactive derivative (TAFIai). 3) DNA, shed by activated or damaged cells, is incorporated into nascent fibrin and impairs plasmin-mediated fibrin degradation.

Table 2: Summary table; components of the hemostatic system and their general role in coagulation and inflammation.

Name	Coagulation effects	Inflammatory effects	Sepsis-associated changes
TF, Tissue factor	Pro-coagulant Initiation of coagulation	Pro-inflammatory	Elevated
TFPI, Tissue factor pathway inhibitor	Anti-coagulant Inhibition of FXa and TF–VIIa complex	Anti-inflammatory	Variable
AT, Anti-thrombin	Anti-coagulant Inhibition of thrombin, Xa, VIIa, IXa, XIa, XIIa	Anti-inflammatory	Reduced
PC, Protein C	Anti-coagulant Inhibition of FVa, FVIIIa Reduced fibrinolysis	Anti-inflammatory	Reduced
TM, Thrombomodulin	Anti-coagulant Inhibits thrombin, activation of protein C and activates TAFI	Anti-inflammatory	Reduced
EPCR, Endothelial cell protein C receptor	Anti-coagulant activation of protein C	Anti-inflammatory	Reduced
PAI-1, Plasminogen activator inhibitor 1	Inhibits fibrinolysis Inhibits plasminogen	No known role	Elevated
TAFI, Thrombin activatable fibrinolysis inhibitor	Inhibits fibrinolysis Reduces plasminogen activity	No known role	Variable

Platelets and Sepsis

- Thrombocytopenia is one of the most common hemostatic disorders in sepsis (prevalence ~50%)
- patients with platelet count <50 x 10^9/L have a four to fivefold higher risk of bleeding
- Platelet count (initially or development during hospitalization) correlates with severity of sepsis and is an independent predictor of ICU mortality
  - *Vanderschueren S, De Weerdt A, Malarian M, et al. Thrombocytopenia and prognosis in intensive care. Brit Care Med 2000;28(6): 1871–1876*
  - *Thiery-Antier N, Banquet C, Vinault S, et al; Epidemiology of Septic Shock Group: Is thrombocytopenia an early prognostic marker in sep- tic shock? Crit Care Med/ 2016; 44:764-772*
  - *Moreau D, Tilsit JF, Vesin A, et al: Platelet count decline: An early prognostic marker in critically ill patients with prolonged ICU stays. Chest/2007; 131:1735–1741*

Why is platelet count decreased in sepsis?
1. **Decreased platelet production:**
   - There are high levels of platelet production-stimulating pro-inflammatory mediators (TNF-α, IL-6, thrombopoietin) which should stimulate megakaryopoiesis. However, in sepsis, there is substantial **hemophagocytosis of platelet precursors** (and other hematopoietic cells) by mononuclear cells -

presumably caused by elevated concentrations of macrophage colony stimulating factor (M-CSF)

2. **Enhanced platelet consumption**
   - Continuous production of thrombin resulting in continuous platelet activation, leading to consumption and destruction
3. **Sequestration in the spleen**

<u>Platelet activation in sepsis</u>
- Increased platelet-vessel wall interaction leads to microthrombosis and subsequent hemolysis (red cells mechanically lysed) and organ failure
  - increased platelet-vessel wall interaction is due to the abundance of ultra-large von Willebrand factor multimers
- Platelet-activating factor
  - directly activates platelets
  - released in response to inflammation
- Other inducers of platelet activation: thrombin, ADP, TXA2
- P-selectin:
  - expressed on platelet membranes
  - mediates the adherence of platelets to leukocytes and endothelial cells
  - enhances expression of tissue factor on monocytes

Platelet Drop and Fibrinolytic Shutdown in Patients With Sepsis

Fabrizio Semeraro, MD, PhD[1]; Mario Colucci, DSc[1]; Pietro Caironi, MD[2,3]; Serge Masson, PhD[4]; Concetta T. Ammollo, PhD[1]; Roberto Teli, MS[4]; Nicola Semeraro, MD, PhD[1]; Michela Magnoli, MS[4]; Giovanni Salati, MD[5]; Michele Isetta, MD[6]; Mauro Panigada, MD[7]; Tommaso Tonetti, MD[8]; Gianni Tognoni, MD[4]; Roberto Latini, MD[4]; Antonio Pesenti, MD[7,9]; Luciano Gattinoni, MD[8]

- Nested study from multi centre RCT on albumin replacement in sepsis
- 3 groups of patients: (1) patients with platelet count <50 x 10^9/L at study entry (n=85); (2) baseline platelet count > 100 x 10^9/L who then developed thrombocytopenia (n=100); (3) patients with platelet count >100 x 10^9/L who never developed thrombocytopenia
- Results
  - Patients with early or late thrombocytopenia compared to patients with normal platelet counts had:
    - higher illness severity and higher mortality
    - markedly depressed fibrinolysis, reflected by increases in:

- plasminogen activator inhibitor 1 (PAI-1)
- thrombin activatable fibrinolysis inhibitor (TAFI)
- cell-free DNA (cfDNA)
- Changes reflecting impaired fibrinolysis may proceed thrombocytopenia

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Inflammation leads to activation of coagulation and coagulation leads to inflammation

Cytokines
Various mechanisms of the coagulation system act simultaneously to produce a prohemostatic state, primarily mediated by cytokines.

Prominent pro-inflammatory mediators: TNF-α, IL-6, IL-1

TNF-α
- first mediator that become detectable
- has potent procoagulant effects, however several trials have shown that blocking TNF activity does not affect activation of coagulation

IL-6
- Demonstrated to be important mediator for cytokine-induced coagulation activation
- Strategies to block IL-6 resulted in complete inhibition of endotoxin-induced activation of coagulation
- Infusion of recombinant IL-6 results in marked thrombin generation

IL-1
- Potent agonist of tissue factor expression in vitro, it's role has not been fully elucidated in vivo

Protease-activated receptors (PARs) and TLR-4
- PARs form the link between coagulation and inflammation
- transmembrane G-protein coupled receptors
- 4 different types have been recognized: PAR1, PAR2, PAR3 and PAR4
- PAR1 is particularly implicated in sepsis
  - activated by thrombin, TF-factor VIIa complex, and factor Xa
- TLR-4 that binds to fibrinogen

Tissue factor mediated activation of coagulation leads to generation of thrombin and subsequent fibrinogen conversion to fibrin, this will lead to the generation of coagulation

proteases (Xa, thrombin, VIIa) that can bind to specific PARs on inflammatory cells, or fibrinogen that binds to TLR-4. Binding to PARs results in release of additional pro-inflammatory cytokine and chemokine (TNFα, IL-1β, IL-6), that then further augment the activation of coagulation.

Adapted from: Levi et al., Circulation, 2004

Figure 1. The role of protease-activated receptors (PARs) in the crosstalk between coagulation and inflammation. Tissue factor (TF) expression within the vasculature leads to activation of the coagulation proteases factor VIIa, factor Xa, and thrombin (IIa). Thrombin activates platelets, cleaves fibrinogen, and, when bound to thrombomodulin (TM) in association with the endothelial cell protein C receptor (E), activates protein C to generate activated protein C (APC). Thrombin also activates PAR-1 and PAR-4. The TF–factor VIIa–factor Xa complex activates PAR-1 and PAR-2.

Tissue factor, coagulation proteases, and protease-activated receptors in endotoxemia and sepsis. Crit Care Med 2008

Neutrophil Extracellular Traps (NETs)
- Extracellular DNA traps that are released from dying neutrophils
- webs of entangling neutrophils and cationic proteins (including histones)
- important mediators of thrombus formation

Diagnostics

Tests to actually measure systemic activation of coagulation are not available on a routine basis. What we actually measure, is a secondary effect of DIC (consumption of platelets and coagulation factors)

Soluble Fibrin, measurement of fibrin monomers, markers of thrombin generation - direct measurement of activation of coagulation
- Generally high diagnostic accuracy for DIC but not readily excessible

- Thrombocytopenia
- Prolonged PT or aPTT in 15-30% of septic patients
- High fibrin degradation productions (FDPs) in >95% of patients with sepsis, D-dimers, etc
- Low levels of AT and protein C in >90% of septic patients
- Fibrinogen: often high (acute phase response) or normal, uncommonly low representing consumption (you can consume a lot of fibrinogen before it will measure low)

TEG or ROTEM - to be in more detail later

Treatment of thrombotic complications in sepsis

Treatment of underlying cause, i.e. sepsis, with appropriate antimicrobials and control of the infectious source

Treatment of overt thrombotic conditions is not any different from non-septic patients, except that the bleeding risk may be substantially higher

Surviving sepsis campaign does not recommend any particular anti-coagulant treatment

Transfusions
- i.e. plasma, fibrinogen, cryoprecipitate, platelets
- Should not be instituted just based on laboratory changes (thrombocytopenia, prolonged coagulation times)
- Indicated in patients with active bleeding or in those that require invasive procedure

Heparin
- Blocks thrombin and Xa
- Heparin can at least partially inhibit the activation of coagulation in sepsis, but the improvement in clinical outcome using heparin to treat DIC has not been demonstrated
- May not be safe in patients prone to bleeding
- Indicated in patients who have clinically overt thromboembolism

Antithrombin concentrates
- beneficial effect in improvement of laboratory parameters, shortening duration of DIC, improvement of organ function
    - *Taylor FB Jr., Emerson TE, Jordan R Jr., et al. Antithrombin-III prevents the lethal effects of Escherichia coli infusion in baboons. Circa Shock 1988; 26(3):227–235.*
- Large RCT showed no significant reduction in mortality
    - *L. Warren, A. Eid, P. Singer, S.S. Pillar, P. Carl, I. Novak, et al., Caring for the critically ill*

patient. High-dose antithrombin III in severe sepsis: a randomized controlled trial, JAMA 286 (15) (2001) 1869–1878.

Recombinant human APC

- conflicting results
- PROWESS clinical trial: Bernard GR, Vincent J-L, Laterre P-F, et al. Efficacy and Safety of Recombinant Human Activated Protein C for Severe Sepsis. 2009;344(10):699-709.
  - Large RCT
  - Septic patients received either intravenous infusion of drotrecogin alfa activated for 96 hours, or placebo
  - Mortality benefit: 30.8% mortality in placebo, 24.7% in APC group
  - Incidence of serious bleeding was higher in the treatment group (3.5% versus 2.0%)

  Dhainaut J-F, Yan SB, Joyce DE, et al. Treatment effects of drotrecogin alfa (activated) in patients with severe sepsis with or without overt disseminated intravascular coagulation. J Thromb Haemostat. 2004;2(11):1924-1933.
  - analysis of the PROWESS database stratifying patients into those with and those without DIC
  - At baseline, 29% (454/1568) of patients had overt DIC. Overt DIC was a strong predictor of mortality, independent of APACHE II score and age.
  - Placebo-treated patients with overt DIC had higher mortality than patients without (43 vs. 27%)
  - DrotAA-treated patients with overt DIC had a trend towards greater relative risk reduction in mortality than patients without (29 vs. 18%, P=0.261) but both groups had greater relative risk reduction than placebo-treated patients.
- *Ranieri VM, Thompson BT, Barie PS, et al; PROWESS-SHOCK Study Group: Drotrecogin alfa (activated) in adults with septic shock. N Engl J Med 2012; 366:2055–2064*
  - Large RCT
  - Results from previous study not repeatable
    - At 28 days, 223 of 846 patients (26.4%) in the DrotAA group and 202 of 834 (24.2%) in the placebo group had died (relative risk in the DrotAA group, 1.09; 95% confidence interval [CI], 0.92 to 1.28; P=0.31).
    - Minority of patients in this study had moderate or severe sepsis-associated coagulopathy
- Potentially still a role for this in sicker patients, i.e. those with DIC
- Human recombinant aPC is very species specific and requires a 15-20 fold higher dose in dogs to achieve a similar level of anti-coagulation; risk of anaphylaxis

Recombinant Thrombomodulin

- improves bleeding manifestations compared to heparin

- no survival benefit
  - *iK. Yamakawa, M. Amhara, H. Ogura, H. Yuhara, T. Yamasaki, T. Shimizu, Recombicant human soluble thrombomodulin in severe sepsis: a systematic review and meta-analysis, J. Thromb. Haemostat. 13 (4) (2015) 508–519.*
  - *Vincent JL, Ramesh MK, Ernest D, LaRosa SP, Pachl J, Aikawa N, Hoste E, Levy H, Hirman J, Levi M, Daga M, Kutsogiannis DJ, Crowther M, Bernard GR, Devriendt J, Puigserver JV, Blanzaco DU, Eamon CT, Carrillo JE, Guzzi L, Henderson SJ, Pothirat C, Mehta P, Fareed J, Talwar D, Tsuruta K, Gorelick KJ, Osawa Y, Kaul I (2013) A randomized, double-blind, placebo-controlled, phase 2b study to evaluate the safety and efficacy of recombinant human soluble thrombomodulin, ART-123, in patients with sepsis and suspected disseminated intravascular coagulation. Crit Care Med 41:2069–2079*
    - most benefit was seen in patients with at least one organ system dysfunction and an international normalized ratio greater than 1.4 at baseline

Recombinant TFPI
- large human clinical trial did not show a survival benefit and risk of bleeding events was higher in the treated patients
  - *Abraham E, Reinhart K, Opal S, et al. Efficacy and safety of tifacogin (recombinant tissue factor pathway inhibitor) in severe sepsis: a randomized controlled trial. J Am Med Assoc 2003; 290(2):238–247*

New Targets
- Novel interventions aimed at restoration of the **glycocalyx**
  - endothelial glycosaminoglycans present in the glycocalyx are down regulated by pro inflammatory cytokines, thereby impairing the functions of antithrombin (AT), tissue factor pathway inhibitor (TFPI), leukocyte adhesion, and leukocyte transmigration
- Non-anticoagulant heparin: inhibits expression and function of adhesion molecules, such as P-selectin and L-selectin , and targets pro-inflammatory mediators such as NF-κβ, cytokines and endothelial cell dysfunction

Prevention of thrombosis in sepsis

Surviving Sepsis campaign recommends all patients with sepsis should receive anticoagulant prophylaxis to reduce the risk of DVT and venous thromboembolism

Unfractionated or LMW Heparin
- Lowers the risk of deep vein thrombosis by more than 50% in ICU patients

Rivaroxiban

- factor Xa inhibitor
- non-inferior for the prevention of VTE in critically ill patients

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Questions

1. What are the three main 'pathways'/ changes that lead to coagulation abnormalities in sepsis?

2. What substance is primarily responsible for the down-regulation of fibrinolysis seen in septic patients?
   A) PAI-1
   B) TFPI
   C) Thrombomodulin
   D) TNF-α

3. Name 3 cytokines that are most prominent in mediating activation of coagulation in sepsis:

   _____

4. Why are antithrombin levels decreased in states of severe inflammation?

Answers

1. What are the three main 'pathways'/ changes that lead to coagulation abnormalities in sepsis?
   1. Excess fibrin deposition
   2. Impaired anticoagulant mechanisms
   3. Depression of the fibrinolytic system

2. What substance is primarily responsible for the down-regulation of fibrinolysis seen in septic patients?
   A) **PAI-1**
   B) TFPI
   C) Thrombomodulin
   D) TNF-α

3. Name 3 cytokines that are most prominent in mediating activation of coagulation in sepsis:
   TNF, IL-6, IL-1

4. Why are antithrombin levels decreased in states of severe inflammation?
    1. Consumption from ongoing thrombin generation
    2. Impaired synthesis as a result of a negative acute phase response
    3. Degradation by neutrophil elastase

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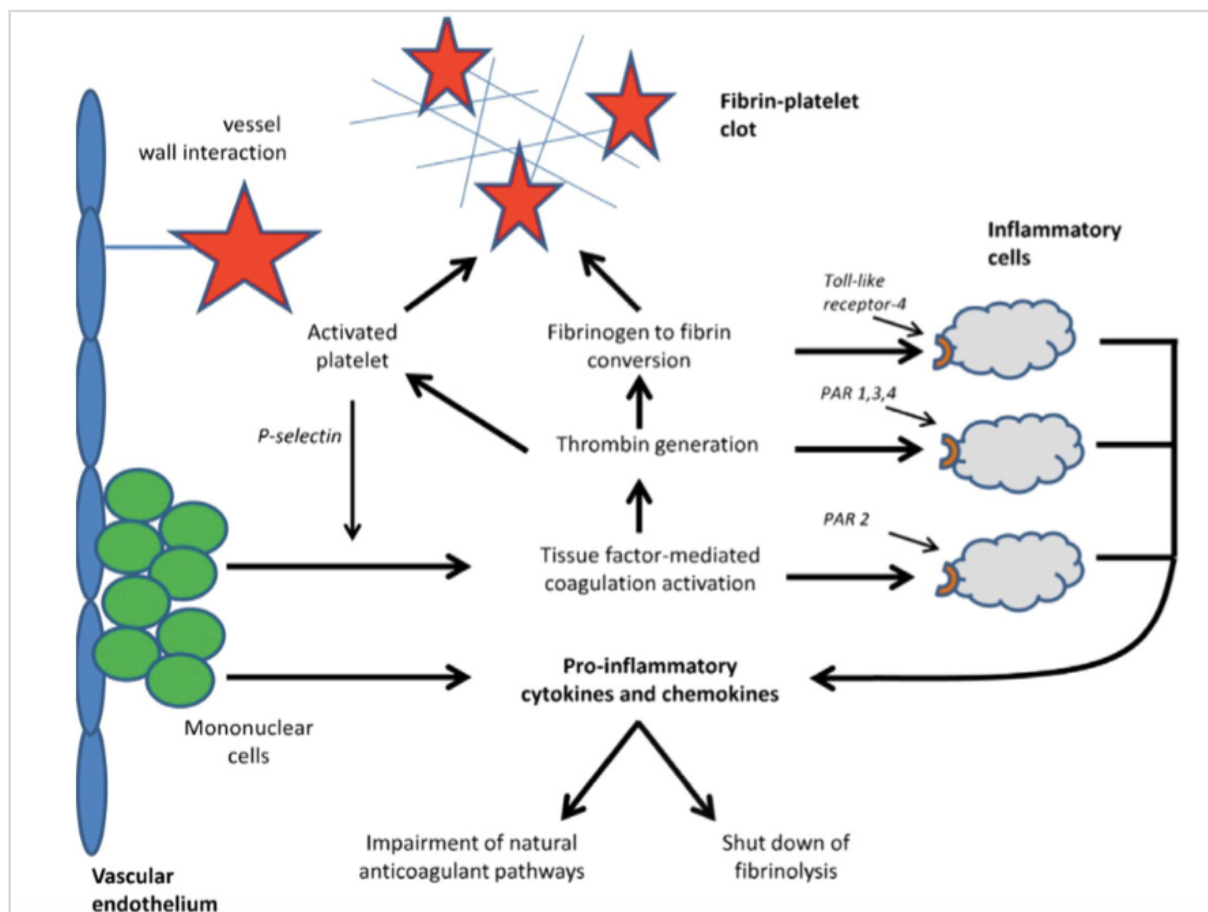
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Fig. 1. Interaction between inflammation and coagulation in sepsis. Expression of tissue factor in mononuclear cells and subsequent exposure to blood results in thrombin generation followed by fibrinogen to fibrin conversion. Simultaneously, platelet vessel wall interaction and activation of platelets contribute to (micro)vascular clot formation. Platelet-derived P-selectin further enhances tissue factor expression. Binding of tissue factor, thrombin, and other activated coagulation proteases to specific protease activated receptors (PAR's) and binding of fibrin to Toll-like receptor 4 on inflammatory cells affects inflammation through the consequent release of pro-inflammatory cytokines and chemokines, which further modulate coagulation and fibrinolysis.
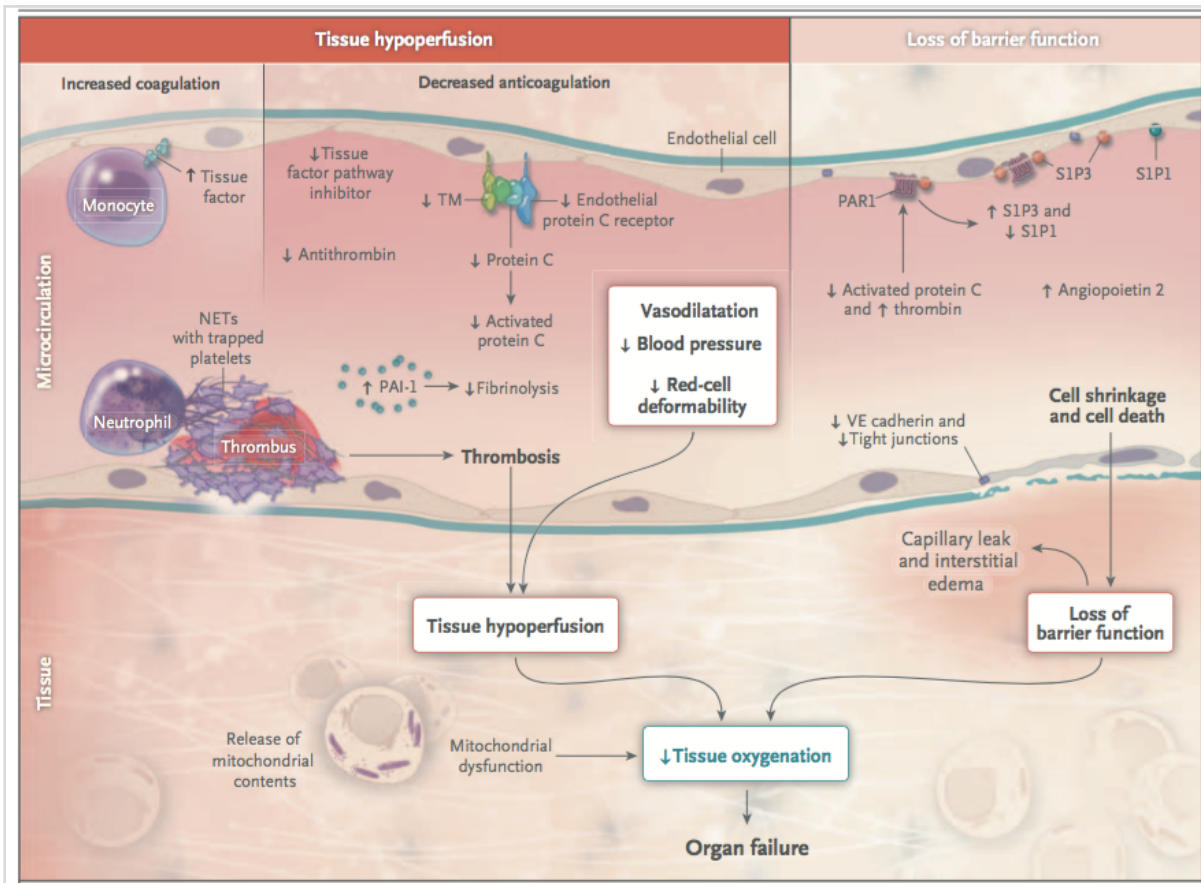
Figure 2. Organ Failure in Severe Sepsis and Dysfunction of the Vascular Endothelium and Mitochondria.

Sepsis is associated with microvascular thrombosis caused by concurrent activation of coagulation (mediated by tissue factor) and impairment of anticoagulant mechanisms as a consequence of reduced activity of endogenous anticoagulant pathways (mediated by activated protein C, antithrombin, and tissue factor pathway inhibitor), plus impaired fibrinolysis owing to enhanced release of plasminogen activator inhibitor type 1 (PAI-1). The capacity to generate activated protein C is impaired at least in part by reduced expression of two endothelial receptors: thrombomodulin (TM) and the endothelial protein C receptor. Thrombus formation is further facilitated by neutrophil extracellular traps (NETs) released from dying neutrophils. Thrombus formation results in tissue hypoperfusion, which is aggravated by vasodilatation, hypotension, and reduced red-cell deformability. Tissue oxygenation is further impaired by the loss of barrier function of the endothelium owing to a loss of function of vascular endothelial (VE) cadherin, alterations in endothelial cell-to-cell tight junctions, high levels of angiopoietin 2, and a disturbed balance between sphingosine-1 phosphate receptor 1 (S1P1) and S1P3 within the vascular wall, which is at least in part due to preferential induction of S1P3 through protease activated receptor 1 (PAR1) as a result of a reduced ratio of activated protein C to thrombin. Oxygen use is impaired at the subcellular level because of damage to mitochondria from oxidative stress.