The Fritsma Factor
YOUR INTERACTIVE HEMOSTASIS RESOURCE

COVID-Coag Controversies and Confusion

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The Fritsma Factor
Your Interactive Hemostasis Reference
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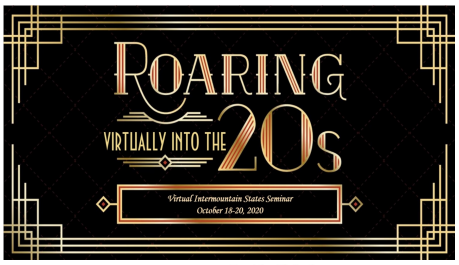
Intermountain States Seminar



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Bottom Line at the Start [BLAST] Keep it Simple and Short [KISS—no hope]

The participant…

- Proposes the pathophysiology and comorbidities associated with COVID infections.
- Applies relevant COVID diagnostic and prognostic laboratory assays results.
- Aligns COVID lab results and pathophysiology with disease progress.

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32 YO Male With Shortness of Breath

A biker who attended the Sturgis, SD rally appears at his local ED experiencing a sore throat, cough, fever, and shortness of breath, O_2 sat. 88%. The physician suspects COVID-19. A lab scientist collects a nasopharyngeal specimen and employs a Cepheid GeneXpert® POC RT-PCR, which detects the SARS-CoV-2 virus in 30". The patient is placed in isolation.

As of 8-1-20 there were 82 FDA-EUA-approved RT-PCR platforms

Smithgal, MC, Dowlatshahi M, Spitalnik SL, Hod EA, Rai AJ. Types of assays for SARS-CoV-2 testing: a review. Lab Med 2020 DOI: 10.1093/labmed/lmaa039

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Symptoms Specified in Vaccine Clinical Trials to Define COVID Infection

- Pfizer, BioNTec [fast track] Moderna, Astra Zeneca, J&J, Merck
- No minimum duration: fever, dyspnea, shortness of breath
- Minimum 2 days duration: Chills, cough, fatigue, muscle or body aches, headache, loss of taste and/or smell, sore throat, congestion, runny nose, nausea, vomiting, diarrhea

Smithgal, MC, Dowlatshahi M, Spitalnik SL, Hod EA, Rai AJ. Types of assays for SARS-CoV-2 testing: a review. Lab Med 2020 DOI: 10.1093/labmed/lmaa039

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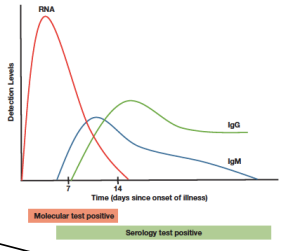
COVID Diagnosis

- Batchwise RT-PCR: look for throughput and FN rate <2%
  - Biological false positives
  - Do not use to confirm recovery
- RT-PCR POC: FN rates 2–15%
- Antigen assay: 16% FN rate, confirm with RT-PCR
- Antibody assay: IgM & IgG recovery documentation



As of 8-1-20 there were 20 FDA-EUA approved antibody assays

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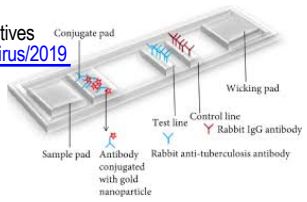
SARS-CoV-2 Diagnostic Assays

- Molecular—NP or nasal swab, saliva: definitive assays https://www.aphl.org/programs/preparedness/Crisis-Management/COVID-19-Response/Pages/Lab-resources.aspx
- Antibody—serum or plasma https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antibody-tests-guidelines.html
- Antigen tests—POC whole blood lateral flow immunoassays detect ~84% of RT-PCR positives https://www.cdc.gov/coronavirus/2019-ncov/lab/resources/antigen-tests-guidelines.html



Courtesy of Andrew J. Goodwin, MD

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LOD and Clinical Sensitivity for Six RT-PCR Kits

Following CLSI EP17-A guidelines the lowest RNA concentration giving a detection rate of 95% was taken as the limit of detection [LOD] for each.

Mean LODs of four kits was 484 copies/mL, whereas the LOD of BioGerm was 968 copies/mL and the LOD of GeneoDx was 7744 copies/mL.

The GeneoDx LOD may be attributable to unreasonable primer design, primer or probe impurities, reagent instability, or inappropriate reagent ratios.

GeneoDx may fail to identify many COVID-19 patients who consequently would be unlikely to receive appropriate treatment in time.

Xueling W, Hangpping Y, Xu X,et al. Limits of detection of six approved RT–PCR kits for the novel SARS-coronavirus-2 (SARS-CoV-2). Am J Clin Pathol.2020 doi/10.1093/clinchem/hvaa099/5819547

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Cleveland Clinic Senstivities of 5 PCR Methods

239 specimens meeting inclusion criteria, LOD 20 copies/µL

Method	Sensitivity	Comment
CDC nCoV RT-PCR	100%	Full extraction, freeze-thaw, high throughput, 3 target genes
TIB MOLBIOL/Roche z 400	96.5%	
Cepheid Xpert Xpress SARS-CoV-2	97.6%	POC direct specimen, two target genes
DiaSorin Simplexa COVID-19 Direct	88.1%	
Abbott ID-NOW; COVIS-19	83.3%	POC direct, single target

"Today, the FDA is alerting the public to data that suggest potential inaccurate results from the Abbott ID NOW point-of-care test to diagnose COVID-19. Specifically, the test may return false negative results." *May 14, 2020 FDA News Release: COVID-19 Update: FDA Informs Public About Possible Accuracy Concerns with Abbott ID NOW Point-of-Care Test*

Procop GW, Brock JE, Erineks EZ. A comparison of five SARS-CoV-2 molecular assays with clinical correlations. Am J Clin Pathol 2020: DOI: 10.1093/AJCP/AQAA181

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Four Antibody Assays

PCR-positive Pts >14 d after onset, increasingly positive over time

Method	Sensitivity	Specificity	Comment
EUROIMMUNE	93.1–96.6%	86.3%–96.4%	IgG: S1 target EIA
Roche	98.3%	100%	IgG/M N target CLIA
Siemens	96.6%	100%	IgG/M RBD target CLIA
DiaSorin	87.7%	96.1–97%	IgG: S1 & S2 targets CLIA

S1, S2=spike proteins; N=nucleocapsid protein; RBD=receptor binding domain of S1. The specificity and sensitivity achieved by the Roche and Siemens assays are acceptable for testing in lower-prevalence regions.

Manthei DM, Whalen JF, Schroeder EF, et al. Differences in performance characteristics among four high-throughput assays for the detection of antibodies against SARS-CoV-2 using a common set of patient samples. Am J Clin Pathol 2020: DOI: 10.1093/AJCP/AQAA200

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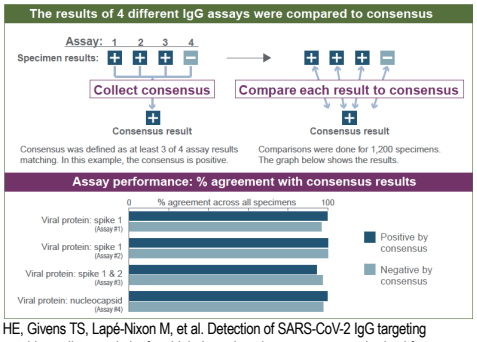
Antibody Testing



Prince HE, Givens TS, Lapé-Nixon M, et al. Detection of SARS-CoV-2 IgG targeting nucleocapsid or spike protein by four high throughput immunoassays authorized for emergency use. J Clin Microbiol. 2020 Aug 18;JCM.01742-20. doi:10.1128/JCM.01742-20

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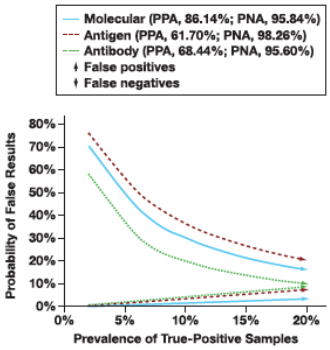
Impact of prevalence on false results, with documented percent positive agreement (PPA) and percent negative agreement (PNA)



Brooks ZC, Das S. Impact of prevalence, sensitivity, and specificity on patient risk and cost. Am J Clin Pathol 2020. DOI: 10.1093/AJCP/AQAA141

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COVID Progression Overview

A hemostasis abnormality unmistakably associates with COVID infection progression, but what is the abnormality? VTE? DIC? LAC? HIT? TMA? PLT activation? Arterial thrombosis? Sepsis? Hypofibrinolysis? We apply routine and specialized hemostasis laboratory assay results to attempt a model and to develop COVID antithrombotic therapy.

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COVID Infection Progression Assays at Onset

- CBC: platelet count
- PT and PTT
- Fibrinogen
- D-dimer
- FDPs

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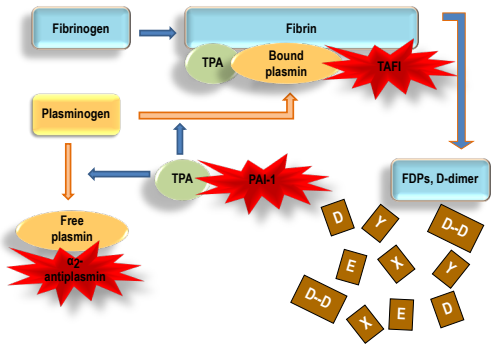
Fibrinolysis



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32 YO Biker with Shortness of Breath
Onset Labs

- D-dimer: 854 ng/mL
- FIB: 405 mg/dL
- PT: 12.5 s
- PTT; 31 s
- PLT: 170,000/uL

Mean coag parameters of 94 COVID patients Vs 40 healthy controls			
Assay	**Patients**	**Control**	**p-value**
PTT	29.01 s	28.65 s	0.518
PT	12.43 s	12.08 s	0.678
TT	18.00 s	18.34 s	0.137
D-dimer	1036 ng/mL	260 ng/mL	<0.001
FDP	31.81 mg/L	1.55 mg/L	<0.001
Fibrinogen	502 mg/dL	290 mg/dL	<0.001
AT	85.46%	98.83%	<0.001

Han H et al. Clin Chem Lab Med. 2020;58:1116–20. Courtesy of Andrew J. Goodwin, MD.

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D-dimer Correlated to Severity

Assay	Control	Onset	Intermediate	Severe
D-dimer [ng/mL]	260 ng/mL	2140	19,110	20,040
FDP [mg/L]	1.55 mg/L	7.93	60.01	69.15

Courtesy of Andrew J. Goodwin, MD

- FDP used in China—automated, not in North America
- D-dimer units [DDUs]
  - Normal limit per manufacturer <240 ng/mL or 0.24 mg/L or ug/mL
- Fibrinogen equivalent units [FEUs]
  - Normal limit per manufacturer <500 ng/mL or 0.5 mg/L or ug/mL
- Most research reports fail to specify FEUs or DDUs; units

- Favaloro EJ, Thachil J. Reporting of D-dimer data in COVID-19: some confusion and potential for misinformation. Clin Chem Lab Med 2020; 58: 1191–9.
- Han H et al. Clin Chem Lab Med. 2020;58:1116–20.

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The D-dimer Assay

- "Promiscuous"
  - Screening is ineffective
  - Baseline necessary when there are indications
  - R/O DVT or PE when there is low pre-test probability
- Normals are age-adjusted
  - If over 50, multiply age by 10 ng/mL FEUs
  - For example, age 60 = <600 ng/mL FEUs, age 70 = <700 ng/mL FEUs
- Marked elevation implies DIC
- Tang: "Recent studies described that severe COVID-19 is commonly complicated with coagulopathy, DIC may exist in the majority of deaths."

Tang N, Bai H, Chen X, et al. Anticoagulant treatment is associated with decreased mortality in severe coronavirus disease. JTH 2020 as doi: 10.1111/JTH.14817

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32 YO Biker: Is it DIC?

- Mild thrombocytopenia
- No schistocytes
- PT slightly prolonged
- PTT unaffected
- Coag factors normal
- No bleeding
- Is it hyperfibrinolysis?

ISTH Overt DIC Score		
Assay	DIC	Score
PLT	<50,000/uL	2
PLT	50–100,000/uL	1
PT	Prolonged >6 s	2
PT	Prolonged 3–6 s	1
D-dimer	Marked increase	2
D-dimer	Moderate increase	1
Fibrinogen	<100 mg/dL	1
Overt DIC when total = 5 or more		

Iba et al. J Thromb Haemost. 2019;17:1989–94.

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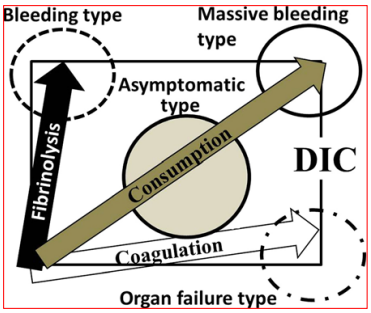
Coagulation in Sepsis: Bleeding



Wada et al. J Intensive Care. 2014, 2:15

Courtesy of Andrew J. Goodwin, MD

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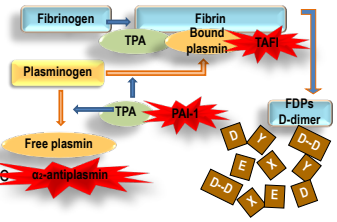
Hyperfibrinolysis? What to Assay?

- Elevated plasminogen?
- Reduced α_2-antiplasmin?
- Elevated tissue plasminogen activator [TPA from ECs]?
- Reduced plasminogen activator inhibitor-1 [PAI-1 from ECs]?
- Reduced thrombin-activatable fibrinolysis inhibitor [TAFI]?
- But can we measure these?
- If not, how can we measure fibrinolysis?



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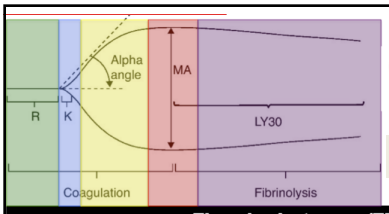

R.E.B.E.L.em

Thromboelastography

Thromboelastogram (TEG)

Components	Definition	Normal Values	Problem with...	Treatment
R Time	Time to start forming clot	5 – 10 minutes	Coagulation Factors	FFP
K Time	Time until clot reaches a fixed strength	1 – 3 minutes	Fibrinogen	Cryoprecipitate
Alpha angle	Speed of fibrin accumulation	53 – 72 degrees	Fibrinogen	Cryoprecipitate
Maximum Amplitude (MA)	Highest vertical amplitude of the TEG	50 – 70 mm	Platelets	Platelets and/or DDAVP
Lysis at 30 Minutes (LY30)	Percentage of amplitude reduction 30 minutes after maximum amplitude	0 – 8%	Excess Fibrinolysis	Tranexemic Acid and/or Aminocaproic Acid

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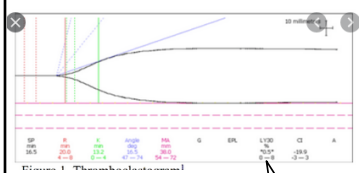
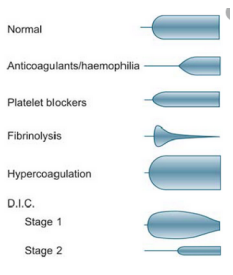
Representative TEG Tracings

Hypofibrinolysis





LY-30 < 8%

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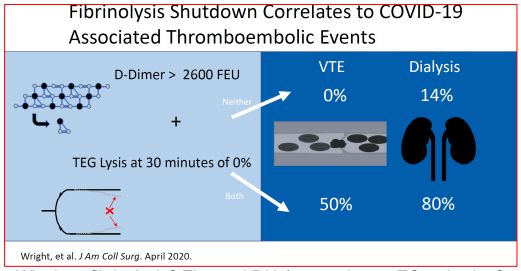
32 YO Biker: SARS-CoV-2 Tracing = Hypofibrinolysis



Courtesy of Andrew J. Goodwin, MD

Why hypofibrinolysis? Elevated PAI-1 secondary to EC activation? But also rise in MA, K, and R—like DIC but no coagulopathy

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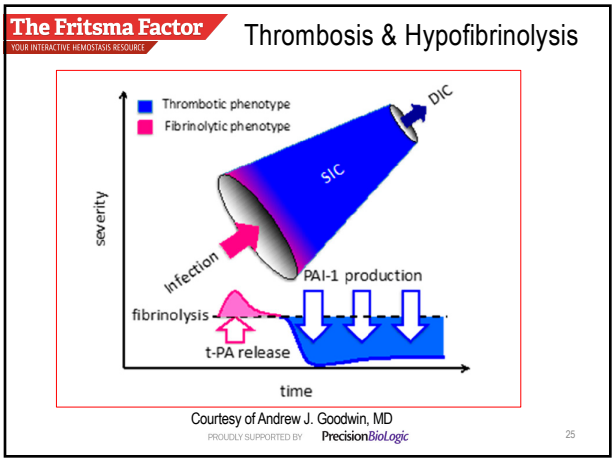

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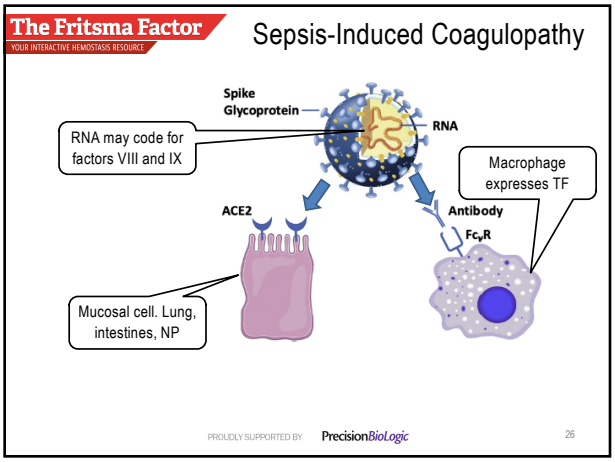

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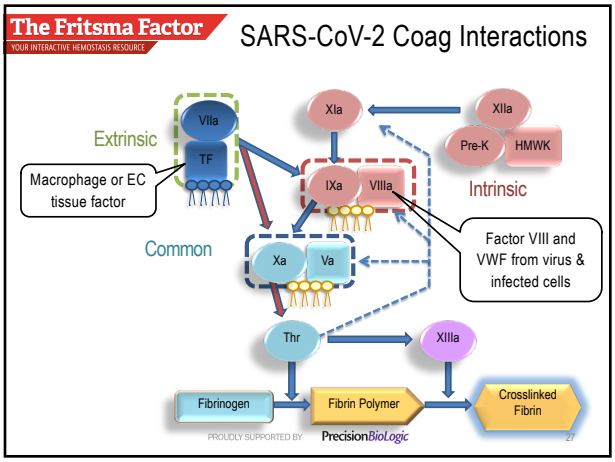

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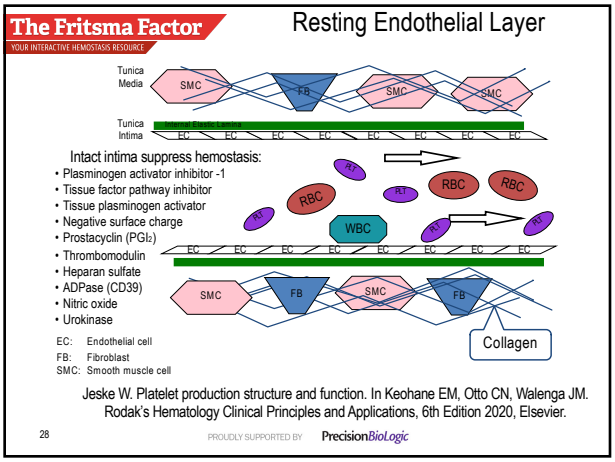

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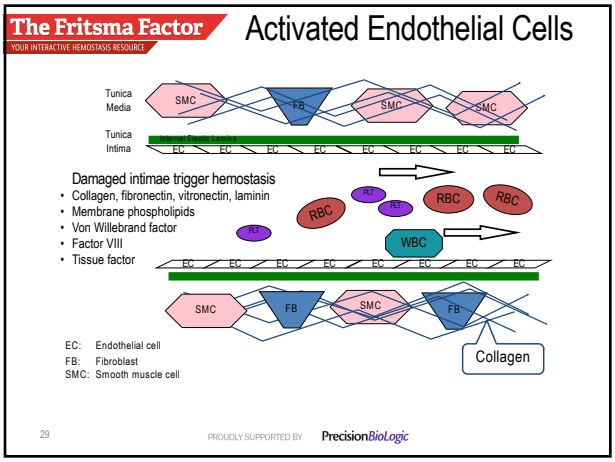

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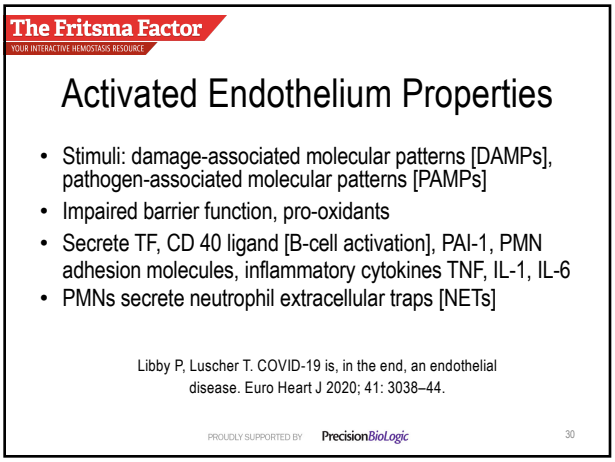

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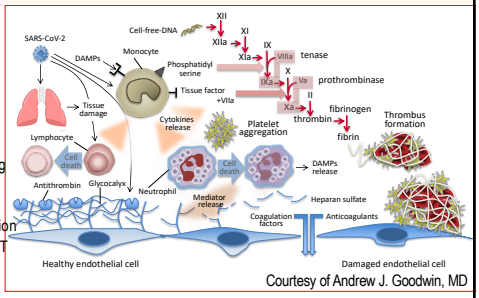
Sepsis-Induced Coagulopathy

1-COVID enters cell via ACE2 or Fc receptors, replicates
2-PAMP-induced TF expression on mac membranes
3-DAMP→NETs
4-NETs→TF creating polyanionic surface, activating coag
5-Glycocalyx disruption →EC damage →PLT activation and consumption



Courtesy of Andrew J. Goodwin, MD

PAMP: Pathogen-associated molecular patterns; DAMP: Damage-associated molecular patterns; NET: Neutrophil extracellular traps

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32 YO Biker, 5 Days: Is it SIC?

- Tpenia: 90,000/uL
- No schistocytes
- PT prolonged—16.2s
- PTT unaffected
- Coag factors normal
- Thrombosis
- Is it sepsis?

Iba et al. J Thromb Haemost. 2019;17:1989–94.

Assay	Score	DIC	SIC
PLT	2	<50,000/uL	<100,000/uL
PLT	1	50–100,000/uL	100–150,000/uL
PT/PT Ratio	2	> 6 s	> 1.4
PT/PT Ratio	1	3–6 s	1.2–1.4
D-dimer	2	Marked increase	–
D-dimer	1	Moderate increase	–
Fibrinogen	1	< 100 mg/dL	–
SOFA Score	2	–	2 or more
SOFA Score	1	–	1
Overt DIC when total = 5 or more			4 or more

- SIC = sepsis intravascular coagulation
- SOFA = sequential organ failure assessment; respiratory, cardiovascular, renal, hepatic

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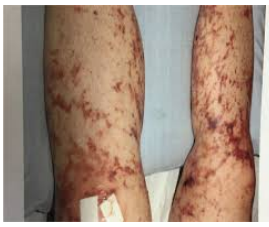
Sepsis: Infection Response



Endothelium normally releases cytokines to fight infection. Sepsis occurs when cytokine response is out of balance, damaging multiple organs. If sepsis progresses to shock, BP drops.

Organ failure accompanies severe sepsis. Livedo reticularis, anuria, thrombocytopenia, dyspnea, abnormal heart rhythm, chills, weakness, loss of consciousness.

Treat with fluids, antibiotics, norepinephrine and vasopressin. Target a mean arterial pressure of 65 mm Hg.

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32 YO Biker, Sepsis Lab Assays

Assay	Patient	Reference Interval
PMNs	750/uL	17–7500/uL
Pelgeroid	10%	–
Lymphs	900/uL	1000–11500/uL
Reactive	15%	–
PLTs	65,000/uL	150–450,000/uL
CRP	1425 ug/dL	< 820 ug/dL
Serum ferritin	850 ng/mL	40–400 ng/mL
IL-6	Elevated	–
Procalcitonin	1.6 ug/L	< 0.5 ug/L
Anti β-2-glycoprotein 1	Positive	Negative
Antithrombin, PC, PS	< 60%	>60%

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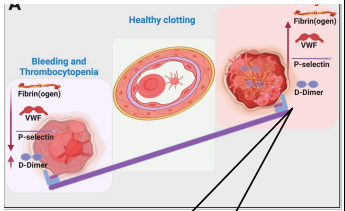
Platelets

- EC TF, VWF activate PLTs, triggers localized thrombi
- PLT activation marked by P-selectin [CD62] release
- Is it thrombotic microangiopathy like TTP?
- ADAMTS13 levels reduced to ~15% but no schistocytes
- Is it complement dysregulation like aHUS? Complement component assays?



Localized [organ-specific] "white clot" thrombi

Grobler C, Maphumulo SC, Grobbelaar M, et al. Covid-19: the rollercoaster of fibrin(ogen), D-dimer, von Willebrand factor, P-selectin and their interactions with endothelial cells, platelets and erythrocytes. Int. J. Mol. Sci. 2020, 21, 5168; doi:10.3390/ijms21145168

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Localized Inflammation--Lungs

- COVID: 15 inpatients, 15 outpatients, 8 controls
- COVID: elevated TF, reduced protein S, remains post-Rx
- Monitor long-term therapy with TF, PS
- But—also hepatic, renal, CNS, cardiac, and intestinal localization
- How to treat?

What do they have in common? Endothelium

University of Kentucky COVID-19 Unified Research Experts (CURE) Alliance, 9-17-20

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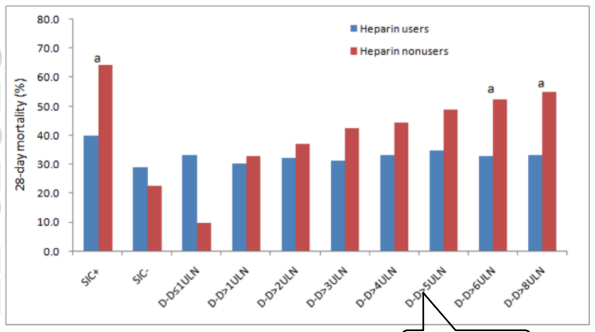
Heparin in SIC, DIC



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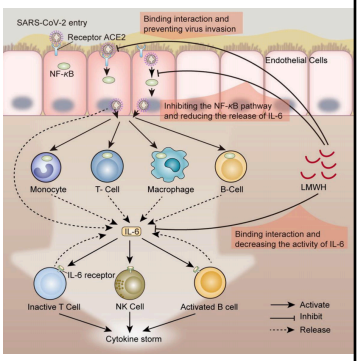
LMWH Anti-inflammatory

- Animal studies: Heparin reduces pulmonary microvascular EC barrier dysfunction via microtubule stabilization in a sepsis mouse model and in-vitro human ECs
- Retrospective study in COVID-19 patients: LMWH reduced nuclear factor κB reduces IL-6 & increases lymphocyte %
- Heparin reduces expression of VWF and fibrinogen in animal lung injury models



- Tang et al. J Thromb Haemost. 2020
- Mu et al. Respir Res. 2008
- Li et al. Zhonghua 2019.

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Heparin-induced Thrombocytopenia

- 88 COVID inpatients received unfractionated heparin > 5 d
  - 58 for COVID-19 coagulopathy
  - 15 for presumed thrombosis
  - 15 for non-valvular atrial fibrillation
- Cumulative incidence of HIT in COVID: 12% at 25 days
- In 5,415 non-COVID patients on UFH, HIT rate 0.76%
- Results suggest a high incidence of HIT in COVID-19 patients treated with UFH

Patel R, et al. Am J Hematol. 2020;1–2; Smythe MA et al. Chest 2007;131:1644–9; Ban-Hoefen M. et al. Thromb Res. 2009;124:189–92.

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Heparin-induced Thrombocytopenia

- Case reports of HIT in COVID; overdiagnosis?
  - Many fail to confirm HIT by serotonin release assay [SRA].
  - Potential for COVID-associated "spontaneous" HIT syndrome
  - In some patients, anti-heparin-PF4 IgG detected prior to UFH
- Recommendation
  - Employ the 4T clinical pre-test probability
  - Refer immunoassay positives for SRA
  - Refer to lab with PF4-dependent platelet activation assay or P-selectin expression assay

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Experimental Rx: Ab8

- U of Pittsburgh scientists isolated the smallest molecule to date that completely and specifically neutralizes the SARS-CoV-2 virus. This antibody component, 10X smaller than a full-sized antibody, has been used to construct a drug— Ab8—for use as a therapeutic and prophylactic against SARS-CoV-2.
- "The researchers report today [9-22-20] that Ab8 is highly effective in preventing and treating SARS-CoV-2 infection in mice and hamsters. Its tiny size not only increases its potential for diffusion in tissues to better neutralize the virus, but also makes it possible to administer the drug by alternative routes, including inhalation. It does not bind human cells—a sign that it may not have negative side-effects in people."

LI W, Schafer A, Kulkami SS, et al. High potency of a bivalent human VH domain in SARS-CoV-2 animal models. Cell 2020. DOI:https://doi.org/10.1016/j.cell.2020.09.007

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Ongoing COVID-Coag Challenges

- What assays detect COVID-coagulopathy?
- Is COVID-coagulopathy a form of DIC? Is it SIC?
- Why do most patients not progress to DIC?
- Is it platelet-triggered thrombotic microangiopathy?
- Should patients be treated with A/Cs for COVID-coagulopathy?
- Is monitoring UFH & LMWH required in COVID-coagulopathy?
- What is the target therapeutic level of anticoagulation?
- Does HIT occur in COVID-coagulopathy?
- Is COVID-coagulopathy linked to antiphospholipid antibody syndrome?
- Randomized control trials necessary.

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The Fritsma Factor
YOUR INTERACTIVE HEMOSTASIS RESOURCE

Bottom Line at the End [BLEAT]

The participant…

- Proposed the pathophysiology and comorbidities associated with COVID infections.
- Applied relevant COVID diagnostic and prognostic laboratory assays results.
- Aligned COVID lab results and pathophysiology with disease progress.

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