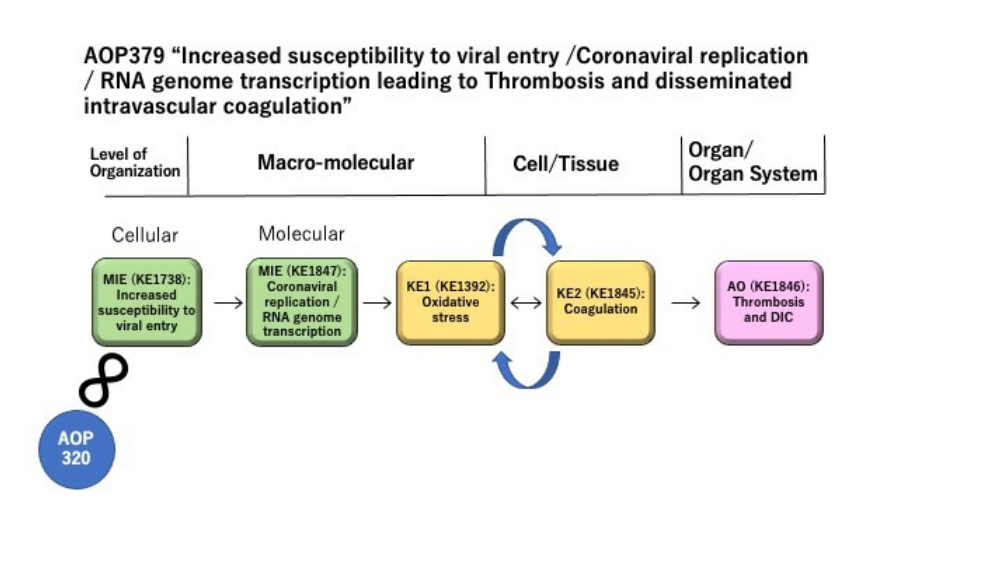
AOP ID and Title:

AOP 379: Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation
Short Title: SARS-CoV2 to thrombosis and DIC

Graphical Representation



Authors

Shihori Tanabe, Young-J Kim, Alicia Paini, Sally Mayasich, Maria J Amorim, Penny Nymark, Marvin Martens, Dan Jacobson, Felicity Gavins, Luigi Margiotta-Casaluci, Sabina Halappanavar, Natalia Reyero, Julija Filipovska, Steve Edwards, Rebecca Ram, Adrienne Layton, and CIAO members

Status

Author status	OECD status	OECD project	SAAOP status
Under development: Not open for comment. Do not cite			

Abstract

Coronavirus disease-19 (COVID-19) is circulating all over the world. To understand and find a way of the COVID-19 treatment, the therapeutic mechanism of COVID-19 is focused on in this Editorial. The pathogenesis of COVID-19 includes molecular networks such as the binding of the membrane proteins, signaling pathways, and RNA replication. The mechanism of infection and targets of the therapeutics are explored and summarized. The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is a new type of coronavirus causing COVID-19, infects the cells via the binding of the membrane proteins of human cells and is internalized by the cells. The viral genome is replicated by RNA-dependent RNA polymerase (RdRp), followed by the packaging and releasing of the viral particles. These steps can be the main targets for the therapeutics of COVID-19. This AOP379 "Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation" consists of the molecular initiating events (MIE) as "Increased susceptibility to viral entry" (KE1738) and "Coronaviral replication / RNA genome transcription" (KE1847), key events (KEs) as "Oxidative stress" (KE1: KE1392) and "Coagulation" (KE2: KE1845), and adverse outcome (AO) as "Thrombosis and Disseminated Intravascular Coagulation" (KE1846).

Summary of the AOP

Events

Molecular Initiating Events (MIE), Key Events (KE), Adverse Outcomes (AO)

Sequence	Type	Event ID	Title	Short name
1	MIE	1738	Increased susceptibility to viral entry	Increased susceptibility to viral entry
2	MIE	1847	Coronaviral replication / RNA genome transcription	RNA genome transcription

Sequence	Type	Event ID	Title	Short name
3	KE	1392	Oxidative Stress	Oxidative Stress
4	KE	1845	Coagulation	Coagulation
5	AO	1846	Thrombosis and Disseminated Intravascular Coagulation	Thrombosis and DIC

Key Event Relationships

Upstream Event	Relationship Type	Downstream Event	Evidence	Quantitative Understanding
Increased susceptibility to viral entry	adjacent	Coronaviral replication / RNA genome transcription	High	Moderate
Coronaviral replication / RNA genome transcription	adjacent	Oxidative Stress	Moderate	
Oxidative Stress	adjacent	Coagulation	Moderate	
Coagulation	adjacent	Oxidative Stress	Moderate	Not Specified
Coagulation	adjacent	Thrombosis and Disseminated Intravascular Coagulation	High	

Stressors

Name	Evidence
Sars-CoV-2	High

Overall Assessment of the AOP

Domain of Applicability

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Sex Applicability

Sex	Evidence
Unspecific	High

References

1. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic Coronaviruses. Nature Reviews Microbiology. 2019;17(3):181-192.

2. Chen B, Tian EK, He B, Tian L, Han R, Wang S, et al. Overview of lethal human Coronaviruses. Signal Transduction and Targeted Therapy, 2020;5(1):89.

3. Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini,E, et al. Immune-mediated approaches against COVID-19. Nature Nanotechnology. 2020:15(8):630-45.

4. Blanco Melo D, Nilsson-Payant BE, Liu WC, Uhl S, Hoagland D, Møller R, et al. Imbalanced Host Response to SARS-CoV-2 Drives Development of COVID-19. Cell. 181;(5):1036-1045.

5. Pizzorno A, Padey B, Julien T, Trouillet-Assant S, Traversier A, Errazuriz-Cerda E, et al. Characterization and Treatment of SARS-CoV-2 in Nasal and Bronchial Human Airway Epithelia. Cell Reports Medicine. 2020:1(4).

6. Riva L, Yuan S, Yin X, Martin-Sancho L, Matsunaga N, Pache L, et al. Discovery of SARS-CoV-2 antiviral drugs through large-scale compound repurposing. Nature. 2020.

7. Kowalewski J, Ray A. Predicting novel drugs for SARS-CoV-2 using machine learning from a & g 10 million chemical space. Heliyon. 2020;6(8).

6. Tanabe S. The Therapeutic Mechanism of COVID-19. J Clin Med Res. 2020;2(5):1-3. DOI: https://doi.org/10.37191/Mapsci-2582-4333-2(5)-048

Appendix 1

List of MIEs in this AOP

Event: 1738: Increased susceptibility to viral entry

Short Name: Increased susceptibility to viral entry

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:320 - Binding of viral S-glycoprotein to ACE2 receptor leading to acute respiratory distress associated mortality	KeyEvent
Aop:379 - Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	MolecularInitiatingEvent

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
cell

Organ term

Organ term
organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Homo sapiens

ACE 2 is highly expressed in gastrointestinal system such as small intestine and duodenum, as well as oral and nasal mucosa, lung, kidney and brain [6-8].

Key Event Description

Coronavirus, which is a nanoparticle, is sphere-shaped and its diameter is 80-120 nm on average, where it sometimes ranges from 50 nm to 200 nm [Masters PS. (2006)]. Spike protein (S protein), the so-called peplomer, on the surface of the particle binds to the receptor on the host cellular membrane, then internalized inside the cells. Viral RNA (plus strand) in the viral particles is replicated and translated into the viral structural protein in the host cells, which is followed by replication of new viral particles [Weiss SR, Navas-Martin S. (2005)]. Coronavirus is recognized by the binding of S protein on the viral surface and angiotensin I converting enzyme 2 (ACE2) receptor on the cellular membrane, then internalized into the cell via processing of S protein by transmembrane serine protease 2 (TMPRSS2) protease [Hoffmann M, et al. (2020)]. The inhibition of this internalization of the viral particle would theoretically prevent the viral infection and replication [Tanabe S. (2020)].

How it is Measured or Detected

SARS-CoV entry can be determined by quantitative RT-PCR specific to the subgenomic mRNA of the N transcript, following the infection of the 293T-hACE2 cells with SARS-CoV [Glowacka I, et al. (2011)].

For analyzing cell entry of S protein of SARS-CoV-2, vesicular stomatitis virus (VSV) particles expressing eGFP and firefly luciferase bearing SARS-2-S are cultured with cell lines, followed by determining luciferase activity in cell lysates [Hoffmann M, et al. (2020)].

References

1. Masters PS. (2006) The molecular biology of coronaviruses. Adv Virus Res. 66:193-292.
2. Weiss SR, Navas-Martin S. (2005) Coronavirus pathogenesis and the emerging pathogen severe acute respiratory syndrome coronavirus. Microbiol Mol Biol Rev. 69(4):635-64.
3. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, et al. (2020).SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 181(2):271-280.e278.
4. Tanabe S. (2020) Cellular Internalization and RNA Regulation of RNA Virus. Adv Clin Med Res. 1(1):1-3.
5. Glowacka I, Bertram S, Müller MA, Allen P, Soilleux E, Pfefferle S, et al. Evidence that TMPRSS2 Activates the Severe Acute Respiratory Syndrome Coronavirus Spike Protein for Membrane Fusion and Reduces Viral Control by the Humoral Immune Response. Journal of Virology. 2011;85(9):4122.
6. Fagerberg L, Hallström BM, Oksvold P, Kampf C. Djureinovic D, et al. (2014) Analysis of the human tissue- specific expression by genome-wide integration of transcriptomics and antibody-based proteomics. Mol Cell Proteomics. 13(2):397-406.
7. Farmer D, Gilbert M, Borman R, Clark KL. (2002) Quantitative mRNA expression profiling of ACE 2, a novel homologue of angiotensin converting enzyme. FEBS Lett. 532(1-2):107-110.
8. Xu H, Zhong L, Deng J, Peng J, Dan H, et al. (2020) High expression of ACE2 receptor of 2019-nCoV on the epithelial cells of oral mucosa. Int J Oral Science. 12(1):8.

Event: 1847: Coronaviral replication / RNA genome transcription

Short Name: RNA genome transcription

Key Event Component

Process	Object	Action
viral RNA genome replication	viral RNA-directed RNA polymerase complex	increased
positive stranded viral RNA replication	viral RNA-directed RNA polymerase complex	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:379 - Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	MolecularInitiatingEvent

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization
Molecular

Cell term

Cell term
cell

Organ term

Organ term
organ

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Homo sapiens

Key Event Description

Coronavirus is a class of viruses which have single-stranded positive-sense RNA genomes in their envelopes [Cui J, et al. Nature Reviews Microbiology. 2019;17(3):181-192.]. Infected virus particles release their genomic RNA inside the human cells, followed by RNA translation or genomic RNA replication by RNA-dependent RNA polymerase (RdRp). RNA viral genome is transcribed into messenger RNA by viral RdRps [Ahlquist, P. Science 2002, 296, 1270, Florindo HF, Nature Nanotechnology. 2020:15(8):630-45.]. Viral RdRps act in combination with other viral

and host factors involved in selecting template RNAs, elongating RNA synthesis, differentiating genomic RNA replication from mRNA transcription, modifying product RNAs with 5' caps or 3' polyadenylate [Ahlquist, P. Science 2002, 296, 1270]. Positive-sense (messenger-sense) RNA viruses replicate their genomes through negative-strand RNA intermediates [Schwartz, Michael et al. Molecular Cell. 2002;9(3):505-514]. Upon virus entry into host cells, genomic RNA serves as mRNA for the first open reading frame (ORF1), being thus translated into viral replicase polyproteins [Florindo HF, Nature Nanotechnology. 2020:15(8):630-45]. The cleaved-polyproteins assemble on double-membrane vesicles, where the RNA genome replication and subgenomic RNA transcription occur [Florindo HF, Nature Nanotechnology. 2020:15(8):630-45, Schwartz, Michael et al. Molecular Cell. 2002;9(3):505-514]. The RdRp complex uses the genome as a template to generate negative-sense subgenome and genome-length RNAs, which are in turn used as templates for synthesis of positive-sense full-length progeny genomes and subgenomic mRNAs [Hartenian E, et al. J Biol Chem. 2020;295(37):12910-12934].

How it is Measured or Detected

The mRNA transcripts are detected by the real-time reverse transcription-PCR (RT-PCR) assay. Several methods targeting the mRNA transcripts have been developed, which includes the RT-PCR assays targeting RdRp/helicase (Hel), spike (S) and nucleocapsid (N) genes of SARS-CoV-2 [Chan, Jasper Fuk-Woo et al. J Clin Microbiol. 2020:58(5)e00310-20]. RT-PCR assays detecting SARS-CoV-2 RNA in saliva include quantitative RT-PCR (RT-qPCR), direct RT-qPCR, reverse transcription-loop-mediated isothermal amplification (RT-LAMP) [Nagura-Ikeda M, Imai K, Tabata S, et al. J Clin Microbiol. 2020;58(9):e01438-20]. The viral mRNAs are reverse-transcribed with RT, followed by the amplification by PCR.

References

1. Cui J, Li F, Shi ZL. Origin and evolution of pathogenic Coronaviruses. Nature Reviews Microbiology. 2019;17(3):181-192.
2. Ahlquist, P. RNA-Dependent RNA Polymerases, Viruses, and RNA Silencing. Science 2002, 296, 1270 [DOI: 10.1126/science.1069132]
3. Florindo HF, Kleiner R, Vaskovich-Koubi D, Acúrcio RC, Carreira B, Yeini,E, et al. Immune-mediated approaches against COVID-19. Nature Nanotechnology. 2020:15(8):630-45.
4. Schwartz, Michael et al. A Positive-Strand RNA Virus Replication Complex Parallels Form and Function of Retrovirus Capsids. Molecular Cell. 2002;9(3):505-514.
5. Hartenian E, Nandakumar D, Lari A, Ly M, Tucker JM, Glaunsinger BA. The molecular virology of coronaviruses. J Biol Chem. 2020 Sep 11;295(37):12910-12934. doi: 10.1074/jbc.REV120.013930. Epub 2020 Jul 13. PMID: 32661197; PMCID: PMC7489918.
6. Chan, Jasper Fuk-Woo et al. Improved Molecular Diagnosis of COVID-19 by the Novel, Highly Sensitive and Specific COVID-19-RdRp/Hel Real-Time Reverse Transcription-PCR Assay Validated *In Vitro* and with Clinical Specimens. J Clin Microbiol. 2020:58(5)e00310-20. doi:10.1128/JCM.00310-20
7. Nagura-Ikeda M, Imai K, Tabata S, et al. Clinical Evaluation of Self-Collected Saliva by Quantitative Reverse Transcription-PCR (RT-qPCR), Direct RT-qPCR, Reverse Transcription-Loop-Mediated Isothermal Amplification, and a Rapid Antigen Test To Diagnose COVID-19. J Clin Microbiol. 2020;58(9):e01438-20. doi:10.1128/JCM.01438-20

List of Key Events in the AOP

Event: 1392: Oxidative Stress

Short Name: Oxidative Stress

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:220 - Cyp2E1 Activation Leading to Liver Cancer	KeyEvent
Aop:17 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress during brain development leads to impairment of learning and memory	KeyEvent
Aop:284 - Binding of electrophilic chemicals to SH(thiol)-group of proteins and /or to seleno-proteins involved in protection against oxidative stress leads to chronic kidney disease	KeyEvent
Aop:379 - Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	KeyEvent

Stressors

Name
Acetaminophen
Chloroform
furan

Name

Biological Context

Level of Biological Organization

Molecular

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
rodents	rodents	High	NCBI
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Mixed	High

Oxidative stress is produced in, and can occur in, any species from bacteria through to humans.

Key Event Description

Oxidative stress is defined as an imbalance in the production of reactive oxygen species (ROS) and antioxidant defenses. High levels of oxidizing free radicals can be very damaging to cells and molecules within the cell. As a result, the cell has important defense mechanisms to protect itself from ROS. For example, Nrf2 is a transcription factor and master regulator of the oxidative stress response. During periods of oxidative stress, Nrf2-dependent changes in gene expression are important in regaining cellular homeostasis (Nguyen, et al. 2009) and can be used as indicators of the presence of oxidative stress in the cell.

In addition to the directly damaging actions of ROS, cellular oxidative stress also changes cellular activities on a molecular level. Redox sensitive proteins have altered physiology in the presence and absence of ROS, which is caused by the oxidation of sulfhydryls to disulfides (2SH àSS) on neighboring amino acids (Antelmann and Helmann 2011). Importantly Keap1, the negative regulator of Nrf2, is regulated in this manner (Itoh, et al. 2010).

The brain possesses several key physiological features, such as high O2 utilization, high polyunsaturated fatty acids content, presence of autooxidable neurotransmitters, and low antioxidant defenses as compared to other organs, that make it highly susceptible to oxidative stress (Halliwell, 2006; Emerit and al., 2004; Frauenberger et al., 2016).

How it is Measured or Detected

Oxidative Stress. Direct measurement of ROS is difficult because ROS are unstable. The presence of ROS can be assayed indirectly by measurement of cellular antioxidants, or by ROS-dependent cellular damage:

- Detection of ROS by chemiluminescence (https://www.sciencedirect.com/science/article/abs/pii/S0165993606001683)
- Glutathione (GSH) depletion. GSH can be measured by assaying the ratio of reduced to oxidized glutathione (GSH:GSSG) using a commercially available kit (e.g., http://www.abcam.com/gshgssg-ratio-detection-assay-kit-fluorometric-green-ab138881.html).
- TBARS. Oxidative damage to lipids can be measured by assaying for lipid peroxidation using TBARS (thiobarbituric acid reactive substances) using a commercially available kit.
- 8-oxo-dG. Oxidative damage to nucleic acids can be assayed by measuring 8-oxo-dG adducts (for which there are a number of ELISA based commercially available kits),or HPLC, described in Chepelev et al. (Chepelev, et al. 2015).

Molecular Biology: Nrf2. Nrf2's transcriptional activity is controlled post-translationally by oxidation of Keap1. Assay for Nrf2 activity include:

- Immunohistochemistry for increases in Nrf2 protein levels and translocation into the nucleus;
- Western blot for increased Nrf2 protein levels;
- Western blot of cytoplasmic and nuclear fractions to observe translocation of Nrf2 protein from the cytoplasm to the nucleus;
- qPCR of Nrf2 target genes (e.g., Nqo1, Hmox-1, Gcl, Gst, Prx, TrxR, Srxn), or by commercially available pathway-based qPCR array (e.g., oxidative stress array from SABiosciences)
- Whole transcriptome profiling by microarray or RNA-seq followed by pathway analysis (in IPA, DAVID, metacore, etc.) for enrichment of the Nrf2 oxidative stress response pathway (e.g., Jackson et al. 2014).

References

Antelmann, H., Helmann, J.D., 2011. Thiol-based redox switches and gene regulation. Antioxid. Redox Signal. 14, 1049-1063.

Chepelev, N.L., Kennedy, D.A., Gagne, R., White, T., Long, A.S., Yauk, C.L., White, P.A., 2015. HPLC Measurement of the DNA Oxidation Biomarker, 8-oxo-7,8-dihydro-2'-deoxyguanosine, in Cultured Cells and Animal Tissues. J. Vis. Exp. (102):e52697. doi, e52697.

Emerit, J., Edeas, M., Bricaire, F., 2004. Neurodegenerative diseases and oxidative stress. Biomed. Pharmacotherapy. 58(1): 39-46.

Frauenberger, E.A., Scola, G., Laliberté, V.L.M., Duong, A., Andreazza, A.C., 2015. Redox modulations, Antioxidants, and Neuropsychitrica Disorders. Ox. Med. Cell. Longevity. Vol. 2016, Article ID 4729192.

Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97(6):1634-1658.

Itoh, K., Mimura, J., Yamamoto, M., 2010. Discovery of the negative regulator of Nrf2, Keap1: a historical overview. Antioxid. Redox Signal. 13, 1665-1678.

Jackson, A.F., Williams, A., Recio, L., Waters, M.D., Lambert, I.B., Yauk, C.L., 2014. Case study on the utility of hepatic global gene expression profiling in the risk assessment of the carcinogen furan. Toxicol. Applied Pharmacol.274, 63-77.

Nguyen, T., Nioi, P., Pickett, C.B., 2009. The Nrf2-antioxidant response element signaling pathway and its activation by oxidative stress. J. Biol. Chem. 284, 13291-13295.

Event: 1845: Coagulation

Short Name: Coagulation

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:379 - Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	KeyEvent

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization
Cellular

Cell term

Cell term
blood cell

Organ term

Organ term
blood plasma

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Moderate

Sex Applicability

Sex	Evidence
Unspecific	Moderate

Homo sapiens

Key Event Description

Coagulation is a process that responds to injury by the rapid formation of a clot. Activation of coagulation factor proteins are involved in coagulation. In the extrinsic pathway, platelets, upon the contact with collagen in the injured blood vessel wall, release thromboxane A2 (TXA2) and adenosine 2 phosphates (ADP), leading to the clot formation. Extravascular tissue factor (TF) binds to plasma factor VIIa (FVIIa) and promotes the activation of FXa. Activated FXa assembles with cofactors FVa and FVIIIa on the surface of aggregated platelets, which lead to generation of thrombin (FIIa). Thrombin catalyzes the production of fibrin (FG) which creates a clot.
The binding of prekallikrein and high-molecular weight kininogen activate FXIIa in the intrinsic pathway.
Many regulators are involved in coagulation system. Plasmin is one of the modulators required for dissolution of the fibrin clot. Plasmin is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activation (uPA). SERPINs inhibit thrombin, plasmin and tPA. For example, SERPINE1 or plasminogen activator inhibitor-1 (PAI-1) inhibits tPA/uPA and results in hypofibrinolysis [Bernard I,et al. Viruses. 2021; 13(1):29.]. In addition, SERPING1 inhibits FXII, and thus down-regulation of SERPING1 lifts suppression of FXII of the intrinsic coagulation cascade [Garvin et al. eLife 2020;9:e59177]. Protein C, protein S and thrombomodulin degrade FVa and FVIIIa. [Ref. IPA, Coagulation System, version60467501, release date: 2020-11-19]

How it is Measured or Detected

Coagulation and inflammatory parameters are measured in COVID-19 patients [Di Nisio et al. 2021]. Coagulation parameters include platelet count, prothrombin time, activated partial thromboplastin time, D-dimer, fibrinogen, antithrombin III [Di Nisio et al. 2021]. These parameters are measured in the blood.

In vitro systems

Whole human blood model for testing the activation of coagulation and complement system, as well as clot formation [Ekstrand-Hammarström, B. et al. Biomaterials 2015, 51, 58-68, Ekdahl, K.N., et al. Nanomedicine: Nanotechnology, Biology and Medicine 2018, 14, 735-744, Ekdahl, K.N., et al. Science and Technology of Advanced Materials, 20:1, 688-698,]

References

1. Bernard I, Limonta D, Mahal LK, Hobman TC. Endothelium Infection and Dysregulation by SARS-CoV-2: Evidence and Caveats in COVID-19. *Viruses*. 2021; 13(1):29. DOI: https://doi.org/10.3390/v13010029
2. Garvin et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. *eLife* 2020;9:e59177. DOI: https://doi.org/10.7554/eLife.59177
3. Di Nisio, Marcello et al. Interleukin-6 receptor blockade with subcutaneous tocilizumab improves coagulation activity in patients with COVID-19 European Journal of Internal Medicine, Volume 83, 34 - 38 DOI: https://doi.org/10.1016/j.ejim.2020.10.020
4. Ekstrand-Hammarström, B.; Hong, J.; Davoodpour, P.; Sandholm, K.; Ekdahl, K.N.; Bucht, A., Nilsson, B. TiO2 nanoparticles tested in a novel screening whole human blood model of toxicity trigger adverse activation of the kallikrein system at low concentrations. Biomaterials 2015, 51, 58-68 DOI:https://doi.org/10.1016/j.biomaterials.2015.01.031
5. Ekdahl, K.N.; Davoodpour, P.; Ekstrand-Hammarström, B.; Fromell, K.; Hamad, O.A.; Hong, J.; Bucht, A.; Mohlin, C.; Seisenbaeva, G.A.; Kessler, V.G., Nilsson, B. Contact (kallikrein/kinin) system activation in whole human blood induced by low concentrations of α-Fe2O3 nanoparticles. Nanomedicine: Nanotechnology, Biology and Medicine 2018, 14, 735-744 [DOI: https://doi.org/10.1016/j.nano.2017.12.008]
6. Kristina N Ekdahl, Karin Fromell, Camilla Mohlin, Yuji Teramura & Bo Nilsson (2019) A human whole-blood model to study the activation of innate immunity system triggered by nanoparticles as a demonstrator for toxicity, Science and Technology of Advanced Materials, 20:1, 688-698, DOI: 10.1080/14686996.2019.1625721

List of Adverse Outcomes in this AOP

Event: 1846: Thrombosis and Disseminated Intravascular Coagulation

Short Name: Thrombosis and DIC

Key Event Component

Process	Object	Action
Venous thrombosis	platelet	increased

AOPs Including This Key Event

AOP ID and Name	Event Type
Aop:379 - Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	AdverseOutcome

Stressors

Name
Sars-CoV-2

Biological Context

Level of Biological Organization
Organ

Organ term

Organ term
blood

Domain of Applicability

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	Moderate	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	Not Specified

Sex Applicability

Sex	Evidence
Unspecific	Not Specified

Homo sapiens

Key Event Description

Thrombosis is defined as the formation or presence of a thrombus. Clotting within a blood vessel may cause infarction of tissues supplied by the vessel. Extreme aggravation of blood coagulation induces multiple thrombi in the microvasculature, which leads to consumption coagulopathy followed by disseminated intravascular coagulation (DIC).

DIC is a pathological syndrome resulting from the formation of thrombin, subsequent activation and consumption of coagulation proteins, and the production of fibrin thrombi. The initial pathologic events are thrombotic in nature resulting in thrombotic vascular occlusions. The initial clinical events are usually hemorrhagic resulting in oozing from mucosa and massive gastrointestinal blood loss. The occlusive events occur as a result of fibrin microthrombi or platelet microthrombi that obstruct the microcirculation of organs. This obstruction can result in organ hypoperfusion and ischemia, infarction, and necrosis. All organs are potentially

vulnerable to the effects of thrombotic occlusions.

The renal effects of DIC are multifactorial and may be associated with hypovolemia or hypotension. If the hypotension is not corrected it may lead to renal failure due to acute tubular necrosis. Fibrin thrombi may also block glomerular capillaries causing ischemic, renal cortical necrosis (Colman, 1984).

The cerebral effects of DIC often result in nonspecific changes such as altered state of consciousness, convulsions, and coma. Major vascular occlusions, subarachnoid hemorrhage, multiple cortical and brain stem hemorrhages may occur following microvascular occlusions (Schwartzman RJ, 1982).

The pulmonary effects of DIC may be caused by interstitial hemorrhage resulting in a clinical effect resembling acute respiratory distress syndrome (Schwartzman RJ,1973; Shahl RL, 1984).

How it is Measured or Detected

Clinical laboratory tests are used to diagnose DIC.

Prothrombin time (**PT**) is a blood test that measures how long it takes blood to clot. PT measures the time required for fibrin clot formation after the addition of tissue thromboplastin and calcium. The average time range for blood to clot is about 10 to 13 seconds.

Activated partial prothrombin time (APTT). Platelet poor plasma [PPP] is incubated at 37°C then phospholipid (cephalin) and a contact activator (e.g. Kaolin, micronized silica, or ellagic acid) are added. This leads to the conversion of Factor XI [FXI] to FXIa. The remainder of the pathway is not activated as no calcium is present. The addition of calcium (pre-warmed to 37°C) initiates clotting. The APTT is the time taken from the addition of calcium to the formation of a fibrin clot. The clotting time for the APTT lies between 27-35 seconds.

Decreased fibrinogen concentrations

Diluted plasma is clotted with a high concentration of Thrombin. The tested plasma is diluted (usually 1:10 but this may vary if the Fibrinogen concentration is very low or very high) to minimize the effect of 'inhibitory substances' within the plasma e.g. heparin, elevated levels of FDPs. The use of a high concentration of Thrombin (typically 100 U/ml) ensures that the clotting times are independent of Thrombin concentration over a wide range of Fibrinogen levels.

The test requires a reference plasma with a known Fibrinogen concentration and that has been calibrated against a known international reference standard. A calibration curve is constructed using this reference plasma by preparing a series of dilutions (1:5 –1:40) in the buffer to give a range of Fibrinogen concentrations. The clotting time of each of these dilutions is established (using duplicate samples) and the results (clotting time(s)/Fibrinogen concentration (g/L) are plotted on Log-Log graph paper. The 1:10 concentration is considered to be 100% i.e. normal. There should be a linear correlation between clotting times in the region of 10-50 sec.

The test platelet-poor diluted plasma (diluted 1:10 in buffer) is incubated at 37°C, Thrombin [~100 U/mL] added (all pre-warmed to 37°C). The time taken for the clot to form is compared to the calibration curve and the Fibrinogen concentration deduced. Test samples whose clotting times fall out with the linear part of the calibration curve should be re-tested using different dilutions.

Most laboratories use an automated method in which clot formation is deemed to have occurred when the optical density of the mixture has exceeded a certain threshold.

Platelet Measurements-

A platelet count is the number of platelets a person has per microliter. The ideal platelet range is 150,000 – 400,000 per microliter in most healthy people.

Fibrinolysis measurements-

- d-dimer concentration ALERE TRIAGE® D-DIMER TEST

D-Dimer can be measured by a fluorescence immunoassay. To determine cross-linked fibrin degradation products containing D-dimer in EDTA anticoagulated whole blood and plasma specimens. The test is used as an aid in the assessment and evaluation of patients suspected of having disseminated intravascular coagulation or thromboembolic events including pulmonary embolism

Procedure:

Commercially available kits are available to measure d-dimer in whole blood or plasma. The kits contain all the reagents necessary for the quantification of cross-linked fibrin degradation products containing D-dimer in EDTA anticoagulated whole blood or plasma specimens.

Regulatory Significance of the AO

Thrombosis is one of the world's main concerns in terms of severe symptoms or adverse responses of the vaccine for COVID-19 which is caused by SARS-CoV-2. Excess thrombosis leads to DIC, which might be mortal. For safely developing the therapeutics and vaccines of COVID-19, it is regulatory significant to understand the cellular and molecular mechanisms in the pathogenesis of coronaviral infection, which may include thrombosis and DIC, AO1846.

References

Hemostasis and Thrombosis Basic Principles and Clinical Practices Robert W Colman, Jack Hirsh, Victor J. Marder, Edwin W. Salzman (ed) Philadelphia, 1994.

Schwartzman RJ, Hill JB: Neurologic complications of DIC. Neurology 32:791, 1982

Robboy SJ, Minna JD, Colman RW et.al. Pulmonary hemorrhage syndrome as a manifestation of DIC: Analysis of 10 cases. Chest 63:718, 1973.

Stahl RL, Javid JP, Lackner H: Unrecognized pulmonary embolism presenting as DIC. SM J Med 76:772, 1984.

Appendix 2

List of Key Event Relationships in the AOP

List of Adjacent Key Event Relationships

Relationship: 2310: Increased susceptibility to viral entry leads to RNA genome transcription

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	adjacent	High	Moderate

Relationship: 2291: RNA genome transcription leads to Oxidative Stress

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	adjacent	Moderate	

Relationship: 2289: Oxidative Stress leads to Coagulation

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	adjacent	Moderate	

Relationship: 2313: Coagulation leads to Oxidative Stress

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	adjacent	Moderate	Not Specified

Relationship: 2290: Coagulation leads to Thrombosis and DIC

AOPs Referencing Relationship

AOP Name	Adjacency	Weight of Evidence	Quantitative Understanding
Increased susceptibility to viral entry / Coronaviral replication / RNA genome transcription leading to thrombosis and disseminated intravascular coagulation	adjacent	High	

Evidence Supporting Applicability of this Relationship

Taxonomic Applicability

Term	Scientific Term	Evidence	Links
Homo sapiens	Homo sapiens	High	NCBI

Life Stage Applicability

Life Stage	Evidence
All life stages	High

Sex Applicability

Sex	Evidence
Unspecific	High

Key Event Relationship Description

Many regulators are involved in coagulation system. Plasmin is one of the modulators required for dissolution of the fibrin clot. Plasmin is activated by tissue plasminogen activator (tPA) and urokinase plasminogen activation (uPA). SERPINs inhibit thrombin, plasmin and tPA. For example, SERPINE1 or plasminogen activator inhibitor-1 (PAI-1) inhibits tPA/uPA and results in hypofibrinolysis [Bernard I,et al. Viruses. 2021; 13(1):29.]. In addition, SERPING1 inhibits FXII, and thus down-regulation of SERPING1 lifts suppression of FXII of the intrinsic coagulation cascade [Garvin et al. eLife 2020;9:e59177]. Protein C, protein S and thrombomodulin degrade FVa and FVIIIa. [Ref. IPA, Coagulation System, version60467501, release date: 2020-11-19]

Quantitative Understanding of the Linkage

Known Feedforward/Feedback loops influencing this KER

Decreased fibrinolysis is involved in coagulation system. Coagulopathy may also be involved in this KER. [Mast AE et al, Garvin MR et al.]

References

1. Bernard I, Limonta D, Mahal LK, Hobman TC. Endothelium Infection and Dysregulation by SARS-CoV-2: Evidence and Caveats in COVID-19. *Viruses*. 2021; 13(1):29. DOI: https://doi.org/10.3390/v13010029
2. Garvin et al. A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. *eLife* 2020;9:e59177. DOI: https://doi.org/10.7554/eLife.59177
3. Mast AE, Wolberg AS, Gailani D, Garvin MR, Alvarez C, Miller JI, Aronow B, Jacobson D (2021) SARS-CoV-2 suppresses anticoagulant and fibrinolytic gene expression in the lung. eLife 10:e64330. doi:10.7554/eLife.64330
4. Garvin MR, Alvarez C, Miller JI, Prates ET, Walker AM, Amos BK, Mast AE, Justice A, Aronow B, Jacobson D (2020) A mechanistic model and therapeutic interventions for COVID-19 involving a RAS-mediated bradykinin storm. eLife 9:e59177. doi:10.7554/eLife.59177