Welcome to HyTest Webinar Biomarkers of Inflammation: Interleukin-6 and Beyond

May 27th, 2021

1. Introductions: Company and Speaker *by Mr. Teppo Heimo*
2. Talk 45 min *by Dr. Vladimir Filatov*
3. Q&A 15 min *by Dr. Vladimir Filatov and Dr. Karina Seferian*

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HyTest Ltd.

One of the key raw material suppliers for the IVD industry

We develop and produce monoclonal antibodies and antigens that are mainly used as key components in laboratory tests.

HyTest was established in 1994.

- Headquarters in Finland, operations in China, Russia and North America
- Sales to over 50 countries
- Active participation in IFCC and AACC standardization committee work
- Operations ISO 9001:2015 compliant

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Comprehensive product line

PRODUCT CATEGORIES

- Monoclonal antibodies
- Polyclonal antibodies
- Antigens
- Plasma and serum
- Over 1,000 different reagents

KEY PRODUCT AREAS

Cardiac Markers | Metabolic Syndrome | Infectious Diseases | Inflammation | Veterinary

OTHER PRODUCT AREAS

Blood Coagulation and Anemia | Immunology and Serology | Fertility and Pregnancy | Hormones | Tumor Markers | Neuroscience

Gangliosides | Kidney diseases | Inflammation | Microbial and Plant Toxins | Biodefence

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Inflammation reagents from HyTest

- Interleukin-6
- Procalcitonin
- C-reactive protein
- Serum amyloid A
- Other cytokines

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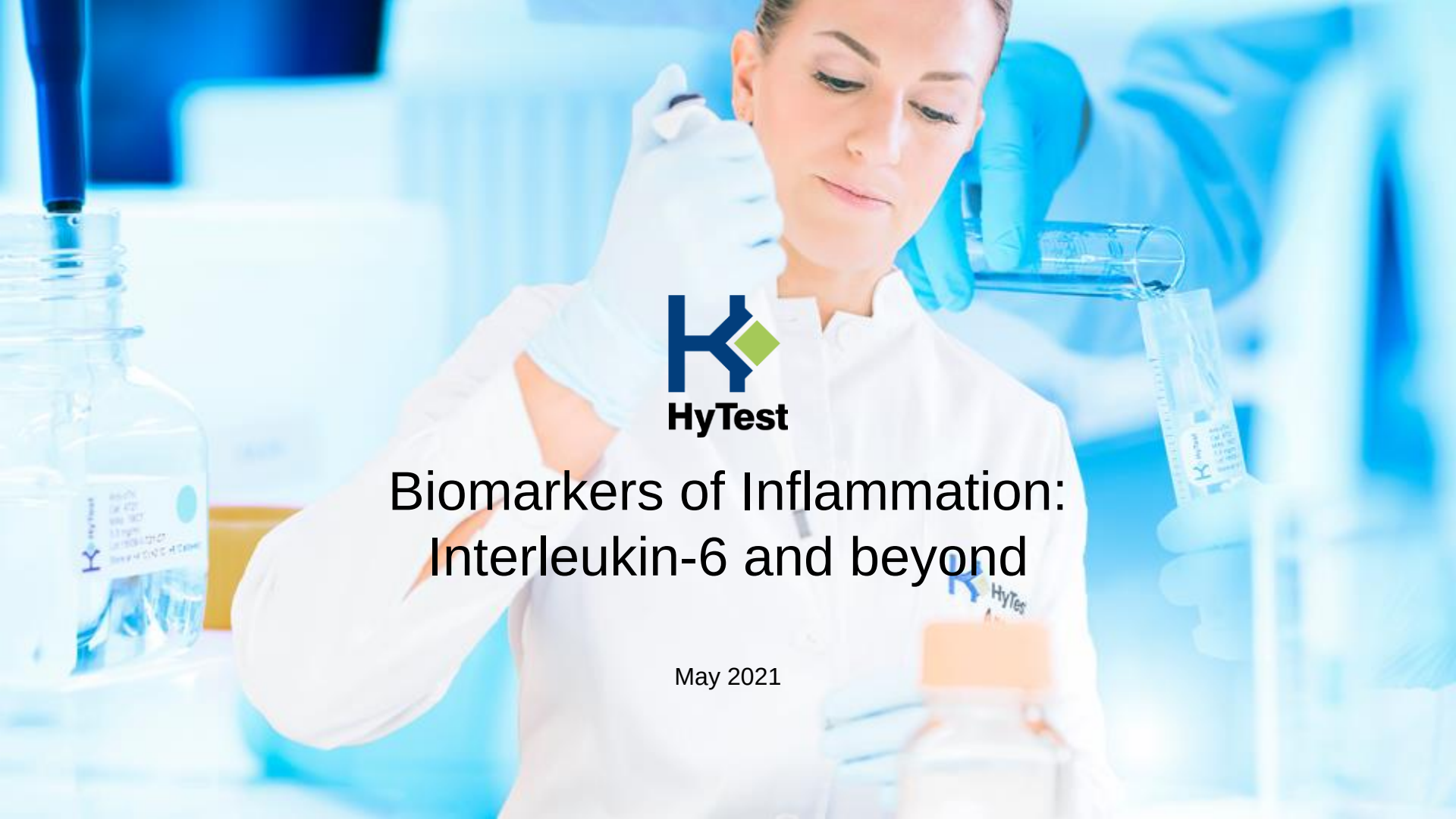
Today’s speaker: Dr. Vladimir Filatov

- MSc. and PhD degrees from the Moscow State University (MSU)
- Senior Scientist and Project Manager, joined HyTest R&D in 1998
- Involved in research projects focused on certain inflammatory biomarkers, in charge of developing antibodies and immunoassays specific to IL-6
- Co-author of about 20 publications and patents

Panelist Dr. Karina Seferian

- MSc and PhD degrees from the Moscow State University (MSU)
- Senior Scientist and Project Manager, joined HyTest R&D in 2003
- Involved in research projects related to the heart failure biomarker Brain Natriuretic Peptide (BNP). Ongoing research is focused on the inflammatory biomarker serum amyloid A (SAA)
- Co-author of about 10 scientific publications and patents



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Biomarkers of Inflammation: Interleukin-6 and beyond

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2. Interleukin-6 biochemistry and signaling
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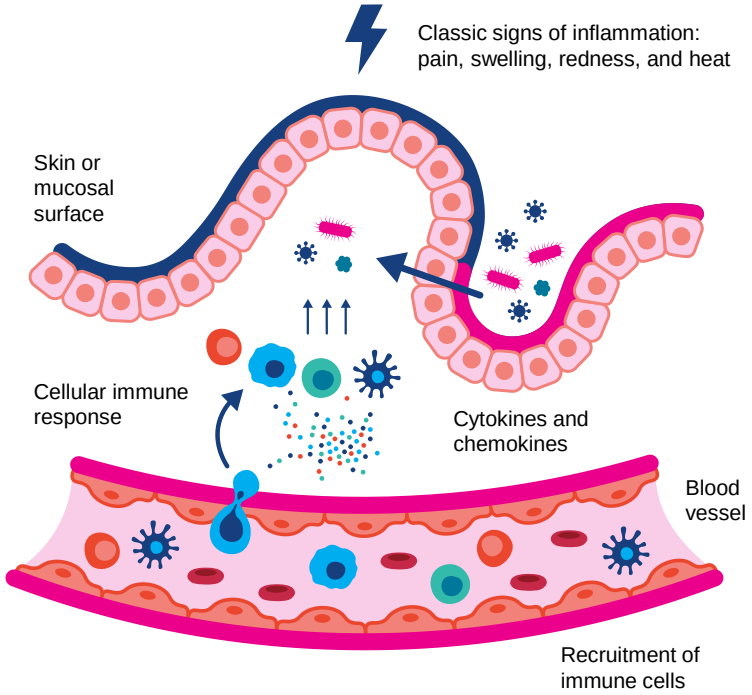
5. PCT in SARS-CoV-2
6. CRP in SARS-CoV-2
7. Immunothrombosis and hypercoagulability in COVID-19

1. Inflammation process definition



- Inflammation is a reaction of the organism to an invasion of an infectious agent or damage, physical or ischemic.
- Mechanism of inflammation represents a chain of organized, dynamic responses including both cellular and vascular events regulated by chemokines, cytokines and enzyme systems.

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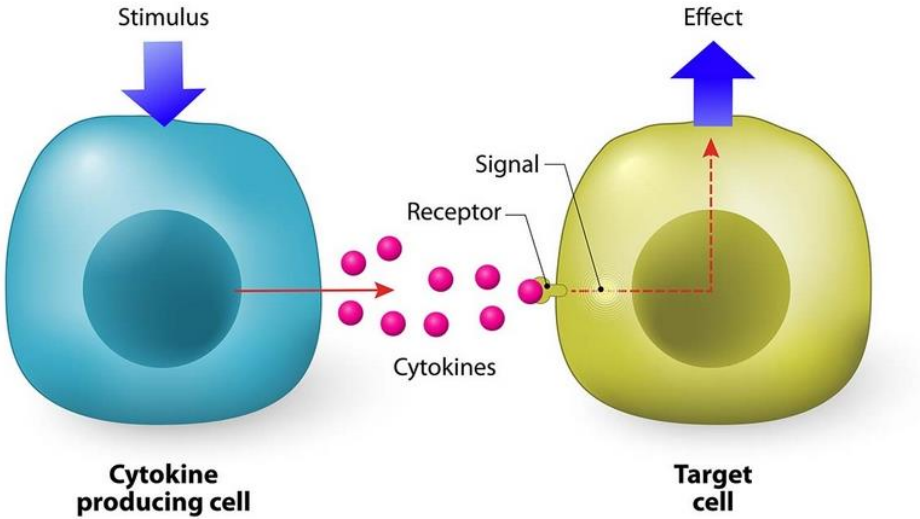
1. Chemical mediators of inflammation



- Variety of chemical mediators from circulation system, inflammatory cells, and injured tissue actively contribute to and adjust the inflammatory response.
- Cytokines are secreted by activated cells during inflammation. These cytokines trigger signal transduction cascade resulting in release of acute-phase proteins into the bloodstream.

From: https://www.bioagilytix.com/blog/2020/12/02/the-importance-of-cytokine-detection-and-analysis/

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1. Clinical importance

- Inflammation is a vital organism’ reaction and accompanies many pathologic conditions.
- It is very important for a clinician to have an ability to detect ongoing inflammation process for appropriate and timely diagnosis and treatment.

1. Biomarkers of inflammation

Cytokines and acute-phase proteins which are released into the bloodstream upon onset of inflammatory reaction, could be detected in the blood samples and therefore serve as a markers of inflammatory conditions.

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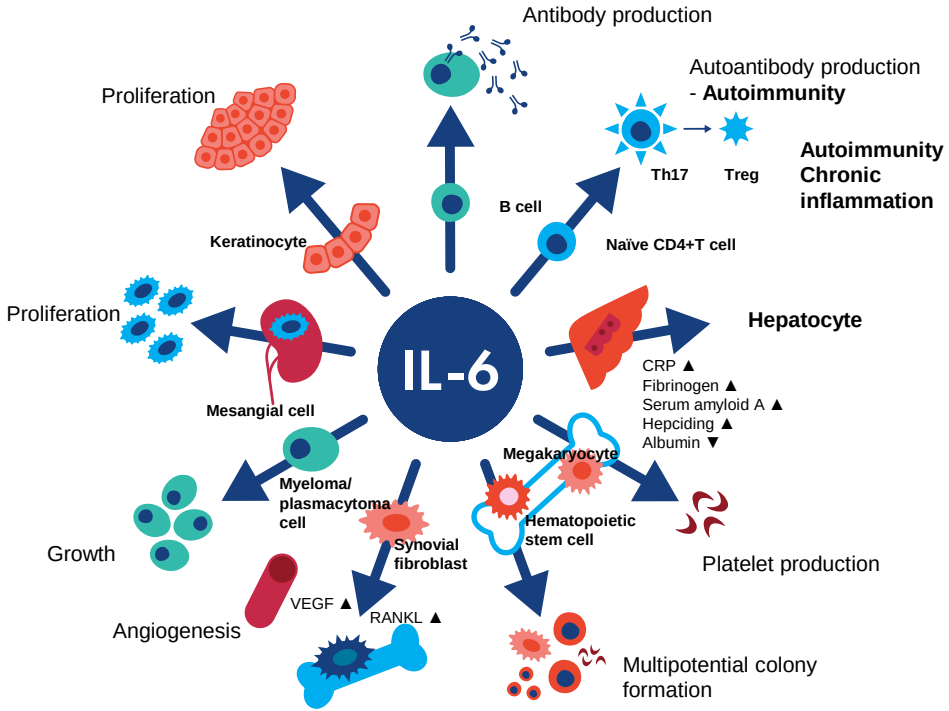
2.Interleukin-6



Interleukin-6 (IL-6) is a cytokine which is produced by macrophages and immune cells upon insult with adverse agent or tissue damage. IL-6 has pleiotropic effect on various organs and tissues in the human body

From: Tanaka T, Kishimoto T. Targeting Interleukin-6: All the Way to Treat Autoimmune and Inflammatory Diseases. Int J Biol Sci 2012; 8(9):1227-1236

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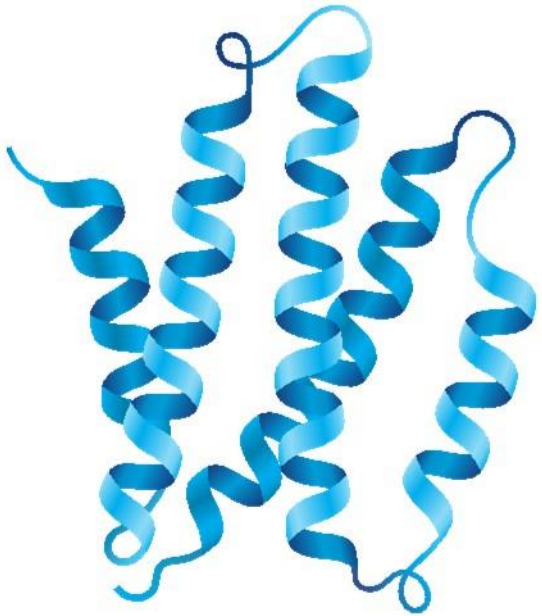
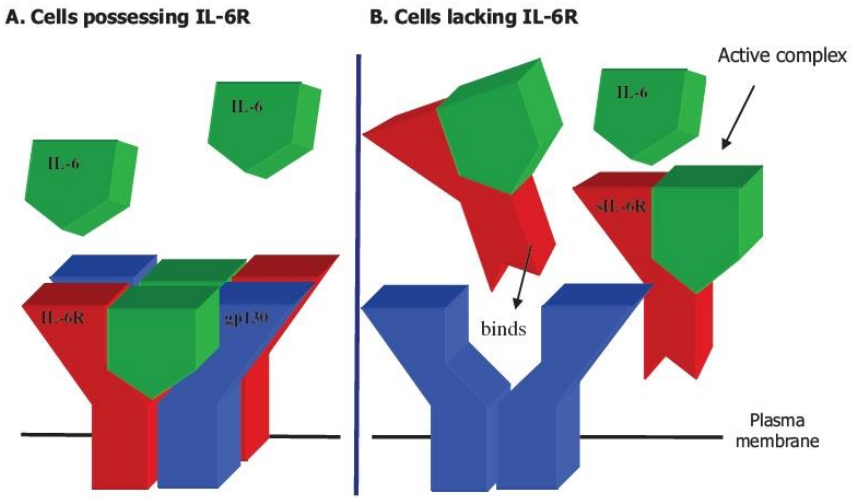
2. What is IL-6?



- Interleukin-6 (IL-6) is a cytokine, which plays important role in inflammation as well as immune system regulation.
- IL-6 is a protein 184 amino acid residues in length, glycosylated, phospholylated and highly structured.

From: Xu, G.Y., et al., Solution structure of recombinant human interleukin-6. 1997, J Mol Biol 268: 468-481

2. What are the forms of IL-6 in the bloodstream?



From:Robson-Ansley, p., et al., The effect of exercise on plasma soluble IL-6 receptor concentration: a dichotomous response. Exercise Immunology Review, 2010, 38-57.

IL-6 binds to IL-6 receptor but does not bind to gp130. Only complex of IL-6 with IL-6 receptor binds to gp130.

IL-6 receptor can be in two forms – membrane-bound and soluble.

So, there are several forms of IL-6 in the blood: 1) free IL-6, 2)complexed with soluble receptor and 3) complexed with soluble receptor and soluble gp130 simultaneously

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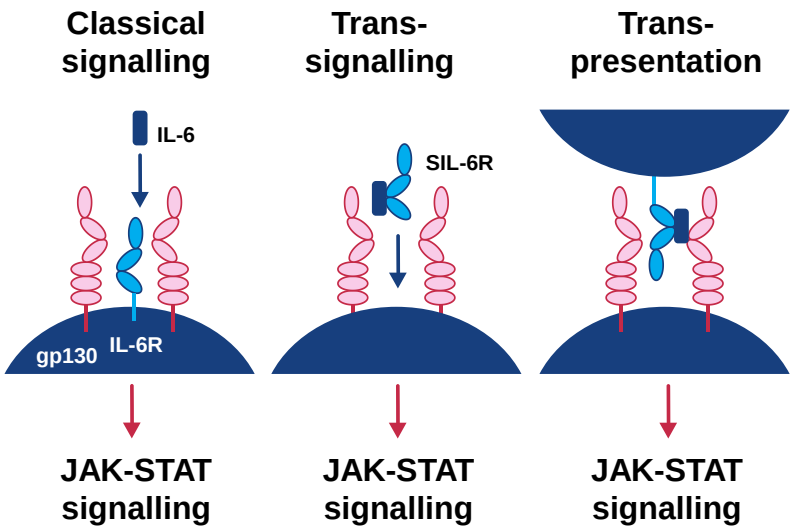
2. How IL-6 signalling mediate inflammation in healthy and non-healthy states ?



From: McElvaney et al., Interleukin-6: obstacles to targeting a complex cytokine in critical illness. Lancet Respir. Med. 2021, Apr 16:S2213-2600(21)00103-X

- IL-6 receptor (IL-6R) is expressed by only a few cell types (hepatocytes, immune cells)
- Gp 130 is expressed by all cell types.
- Healthy state – IL-6 provides signaling through its membrane-bound receptor on hepatocytes and immune cells. **Classic signaling**
- Over-inflammation – cleavage of IL-6R (by ADAM-17 in response to inflammation) with formation of IL-6-IL-6R soluble complex which is capable of transmitting the signal for all gp130-expressing cells. **Trans-signaling**
- T-cell activation with dendritic cell expressing membrane-bound IL-6R. **Trans-presentation**

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2. Clinical value of IL-6

In the blood of healthy individual, IL-6 level are very low (0,2-7,8 pg/ml), but could be increased up to 10 000 pg/ml during severe septic conditions.

Increased levels of IL-6 may be detected during various diseases which accompanied by inflammation, acute as well as chronic:

- Obesity
- Diabetes
- Cardiovascular diseases
- Allergy
- Rheumatoid arthritis

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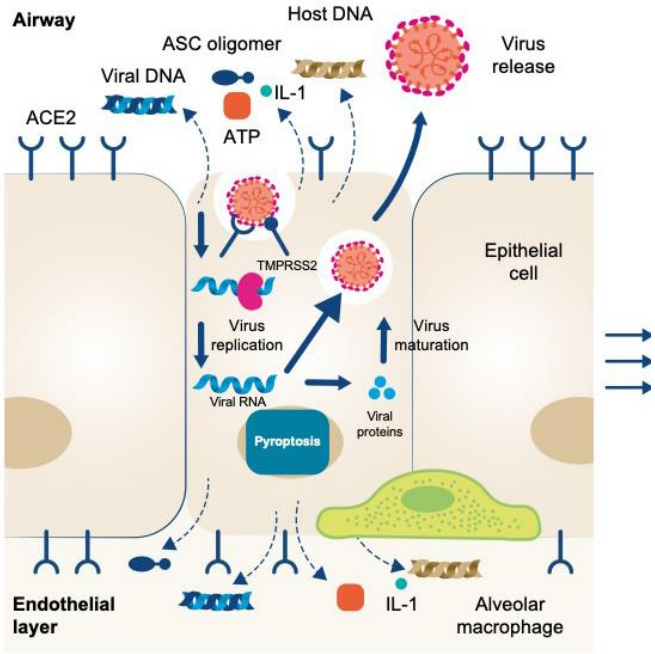
COVID-19 pandemic

- Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) appeared in China in December 2019 and now is global pandemic disease.
- >160 million cases worldwide, >3.3 million deaths
- Transmitted through close contact from person to person, airborne transmission

From: https://www.bbc.com/news/world-54337098



3. COVID-19 pathogenesis – initial events

- SARS-CoV-2 virus uses ACE2 receptor and TMPRSS2 receptor on the cell surface to enter the cell.
- Virus replicates and causes cell death via pyroptosis

From: *Tay et al., 2020, Nature Rev. Immunol., 20, 363-374*

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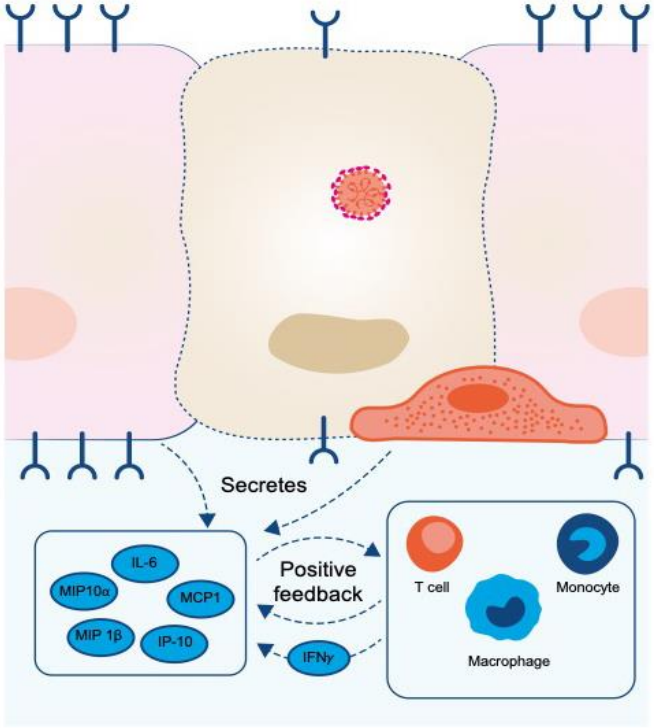

3. COVID-19 pathogenesis – initial events

- Damage-associated molecular patterns are released
- They are recognized by epithelial cells and macrophages, triggering release of IL-6, MIP-1, MCP1, IP-10.

From: *Tay et al., 2020, Nature Rev. Immunol., 20, 363-374*

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3. Inflammatory pathogenesis of COVID-19

- Cell death upon viral infection promotes local inflammatory response including recruitment of macrophages, cytokine release and immune cells (B-cells and T-cells) triggering.
- In some cases, this local response could further develop into generalized out-of-control immune response (“cytokine storm”) which can cause multi-organ damage.
- IL-6 plays crucial role in inflammatory reaction advancement

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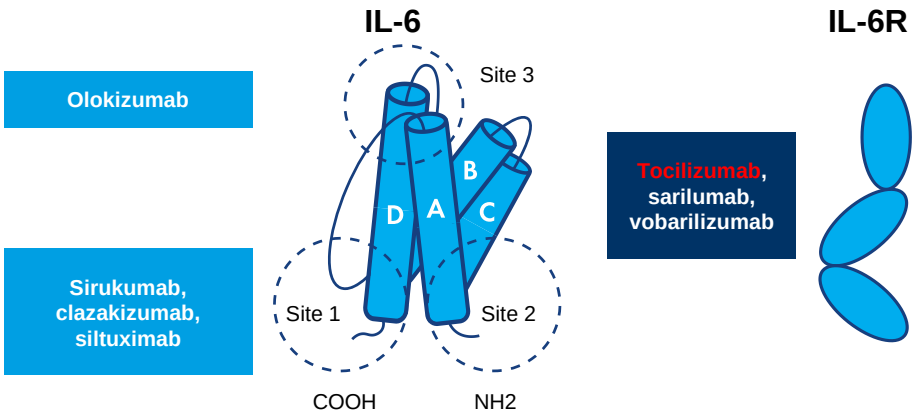
3. IL-6 and COVID-19

1. It is now well established fact that COVID-19 is often accompanied by highly inflammatory state -“cytokine storm” which could lead to multi-organ failure and life-threatening conditions.
2. Tocilizumab which is antibody targeting IL-6 receptor and disrupting IL-6 signalling cascade is being used in therapy of COVID-19 patients
3. IL-6 levels determination in COVID-19 patients can have prognostic effect and predict clinical outcome

4. IL-6 therapeutics in COVID-19 treatment

Since IL-6 plays central role in immunopathology of COVID-19 disease, there were attempts to inhibit it's biological activity and prevent severe complication such as multi-organ failure.



Therapeutic Mabs are available which block IL-6 as well as IL-6 receptor.
Tocilizumab is frequently used for alleviating inflammation during COVID-19

4. Current situation with anti-IL-6 therapy

COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 23.04.2021.

- The Panel recommends using **tocilizumab (anti-IL-6 receptor)** (single intravenous [IV] dose of tocilizumab 8 mg/kg actual body weight up to 800 mg) **in combination with dexamethasone** (6 mg daily for up to 10 days) in certain hospitalized patients who are exhibiting rapid respiratory decompensation due to COVID-19.
- The Panel **recommends against** the use of anti-IL-6 monoclonal antibody therapy (i.e., **siltuximab**) for the treatment of COVID-19

4. IL-6 and COVID-19

1. It is now well established fact that COVID-19 is often accompanied by “cytokine storm” which could lead to multi-organ failure and life-threatening conditions.
2. Tocilizumab which is antibody targeting IL-6 receptor and disrupting IL-6 signalling cascade is being used in therapy of COVID-19 patients
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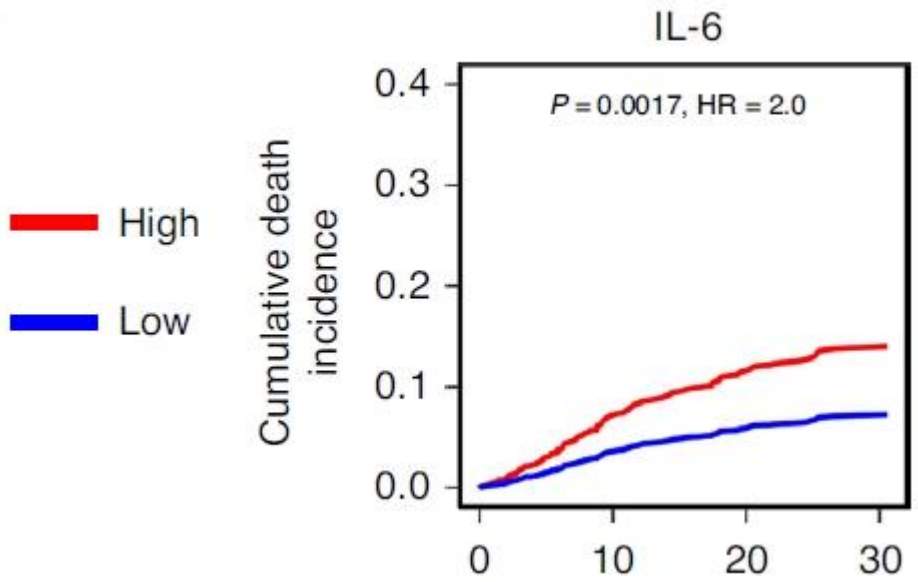
4. IL-6 predicts outcome in patients with COVID-19



IL-6 levels can predict survival in patients with COVID -19 after adjusting for demographic features and comorbidities

From: Del Valle et al., 2020, Nature Medicine, 26, 1636–1643

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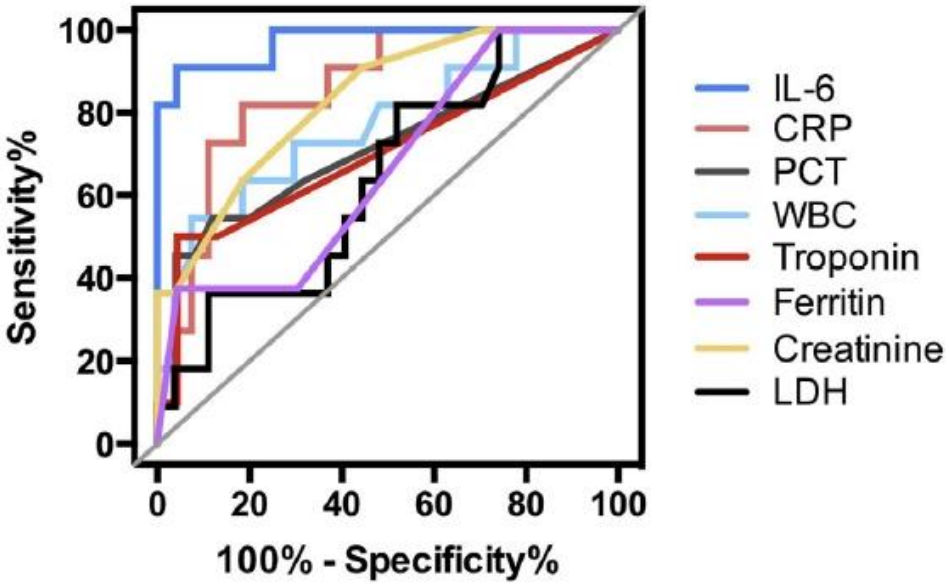
4. IL-6 levels could predict need for mechanical ventilation for patients with COVID-19



From: Herold et al. J Allergy Clin Immunol. 2020;146(1):128–36 e4.

In relatively small cohort of COVID-19 patients (N=40), IL-6 outperformed other biomarkers tested for prediction of disease worsening

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4. NIH COVID-19 treatment guidelines

Elevated IL-6 levels included in the clinical definition of multisystem inflammatory syndrome in adults (https://www.covid19treatmentguidelines.nih.gov/critical-care/general-considerations/)

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5. Procalcitonin (PCT)

PROCALCITONIN, 116 a.a.r.

APFRSALESSPADPATLSEDEARLLLAALVQDYVQMKASELEQEQEREGSSLDSPRS-KR-CGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP-GKKR-DMSSDLERDHRPHVSMPQNAN

Amino acid sequence of human procalcitonin.

Procalcitonin is a biomarker for early detection of (systemic) bacterial infections.

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5. Pathophysiology of Procalcitonin

Calcitonin, the 32-amino acid hormone responsible for serum calcium regulation, is formed after cleavage by the enzyme prohormone convertase. Normally, physiological conditions result in very low serum procalcitonin levels (less than 0.05 ng/mL).

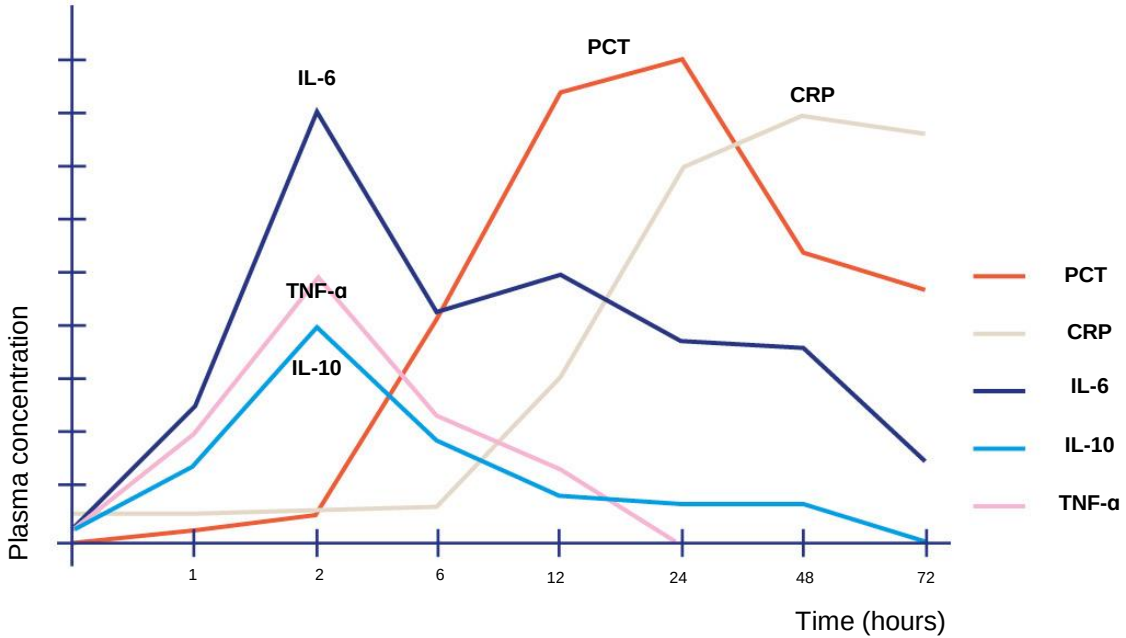
However, the synthesis of PCT can be increased (up to 100 to 1000 fold) as a result of cytokines stimulation (IL-6, tumor necrosis factor (TNF)-alpha) during bacterial infection.

Another cytokines, such as interferon (INF)-gamma, which get released following viral infection, lead to down-regulation of PCT therefore main clinical advantage of PCT is **differentiation between infections of bacterial and viral origins.**

5. Clinical value of procalcitonin

High serum levels of PCT correlate with bacterial infection and sepsis. PCT is not elevated in viral infections and serum levels of PCT decrease following administration of appropriate antibiotic therapies.

5. Time course of serum PCT and other markers of inflammation in sepsis



From: Brunkhorst, et al., Intens. Care Med., 1998, 24, 888-892.

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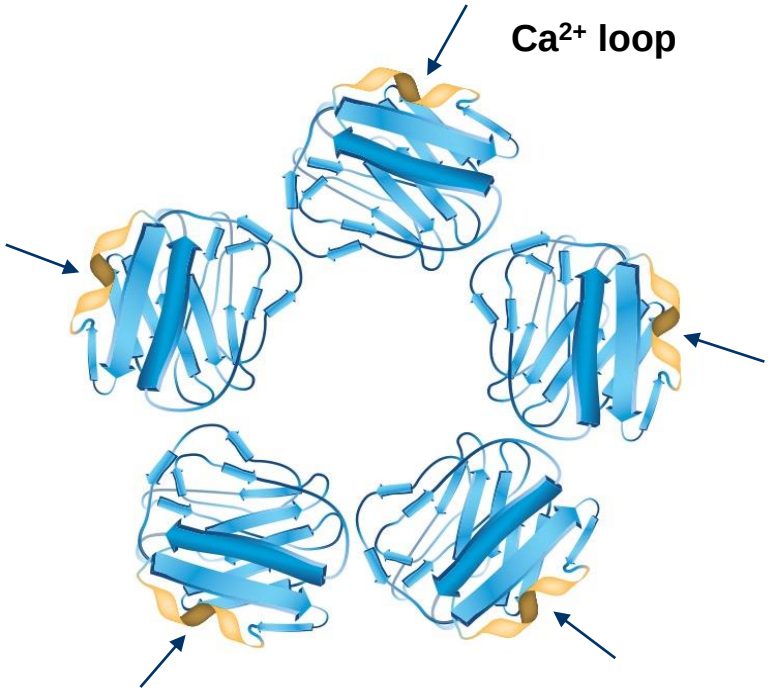
6. CRP – C-reactive protein



- Human CRP is a pentamer.
- Belongs to Acute phase proteins
- Can bind phosphatidylcholine, plasma lipoproteins, apoptotic cells, cell wall components of bacteria.
- Activates complement system
- Interacts with FcγI and FcγII immunoglobulin receptors

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6. CRP and inflammation

- CRP is a pattern recognition protein which role is to react with specific structures which get exposed upon cell death or exist on surface of pathogens
- CRP binds oxidized LDL and apoptotic cells by recognizing their common cognate epitope, oxidized phosphatidyl choline. Phosphatidylcholine (PC) is the major lipid component on membranes and lipoproteins, and most frequently susceptible to oxidative conversion under reactive oxygen species attack.

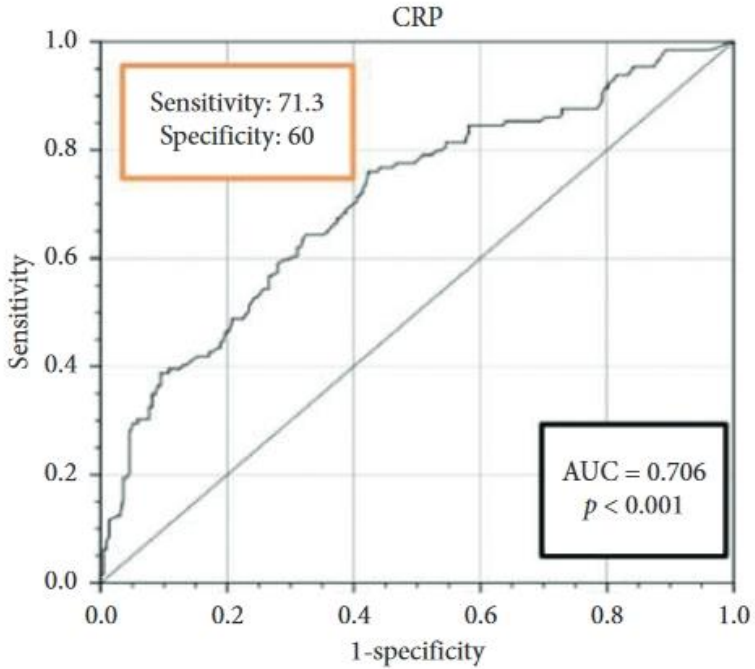
6. Clinical value of CRP

- Sensitive to inflammation
- Increased in all types of infection
- May precede clinical signs of infection by 24 hours
- Can be used to monitor antibiotic therapy as its levels decline with removal of pathogen and rises on recurrent infection

6. CRP levels in COVID-19

- Significantly higher levels of CRP along with IL-6 and PCT were demonstrated in patients with severe COVID-19 patients compared to the non-severe group.
- Similarly, the levels of CRP, PCT and IL-6, were significantly higher in non-survivors compared with survivors.
- Some retrospective studies demonstrate that patients treated with corticosteroids had a swift and marked reduction in CRP levels. Reduction in CRP by 50% or more within 72 hours of initiating corticosteroid therapy potentially predicts inpatient mortality. (Cui et al., Journal of Hospital Medicine2021;16:142-148.)

6. ROC curve of CRP for predicting COVID-19 severity



CRP levels > 64,75 mg/L correlate with poor prognosis in patients with non-severe COVID-19 (N=254) when compared to patients with severe COVID-19 (N=175)

From: Sadeghi-Haddad-Zavareh, et al., C-Reactive Protein as a Prognostic Indicator in COVID-19 Patients, 2021, Interdisciplinary Perspectives on Infectious Diseases. https://doi.org/10.1155/2021/5557582

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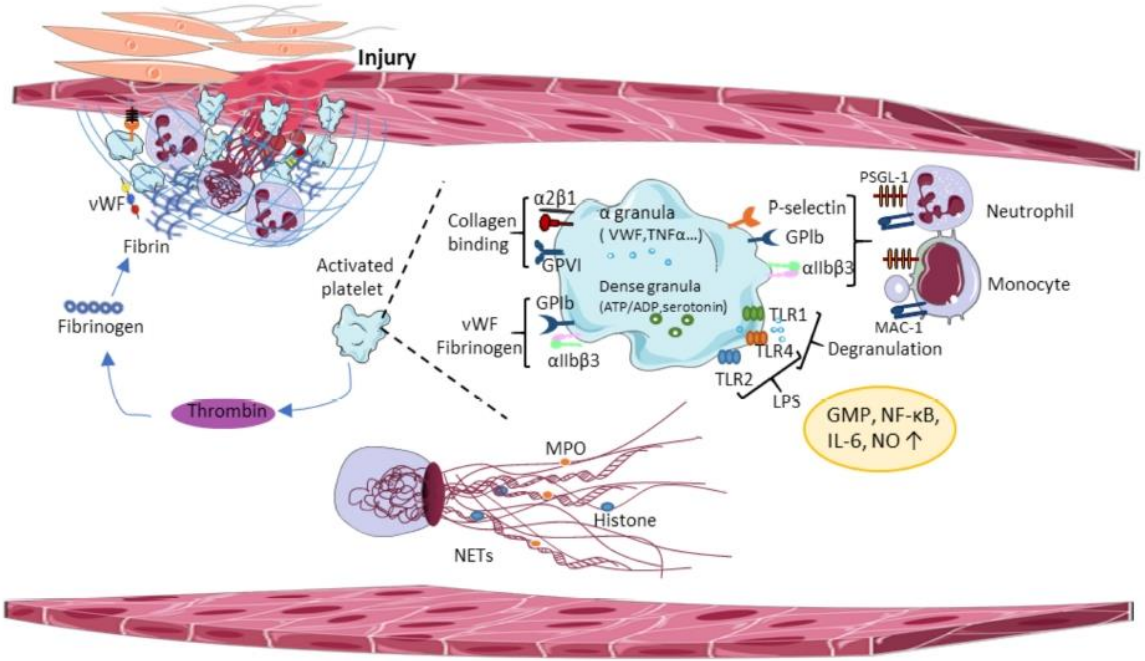
7. **Immunothrombosis and hypercoagulability in COVID-19**

Predisposition to thrombosis in patients with severe COVID-19

About 10% of all COVID-19 patients develop severe COVID-19 with compromised lung function

COVID-19 patients with acute respiratory distress syndrome (ARDS) develop significantly more thrombotic complications compared to non-COVID-19 ARDS patients (Helms et al., Intensive Care Medicine, 2020, 46(6), 1089-1098.)

7. Immunothrombosis as a link between inflammation and coagulopathy in COVID-19



After recognition of pathogen at the injury place, damaged endothelial cells present tissue factor on their surface causing platelets activation and aggregation.

Activated platelets promote thrombin formation and therefore fibrin formation. Activated platelets release proinflammatory cytokines from a-granules, which promotes platelet-neutrophil interaction and triggers
the release of the NETs.

From: Shi C, Yang L, Braun A and Anders H-J (2020) Extracellular DNA—A Danger Signal Triggering Immunothrombosis. Front. Immunol. 11:568513. doi: 10.3389/fimmu.2020.568513

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7. Imbalance of immunothrombosis and fibrinolysis

- Inflammation and vascular endothelial dysfunction leading to dysregulated immunothrombosis, mainly affect lungs resulting in pulmonary intravascular coagulopathy.
- Fibrinolysis is downregulated further contributing to prothrombotic state in severe COVID-19
- vWF and PAI-1 levels are increased in patients with severe COVID-19 compared to non-severe patients further corroborating the prothrombotic imbalance of coagulation system.

7. D-dimer levels in COVID-19

- Patients with severe COVID-19 have higher levels of D-dimer then those with non-severe disease (2.9±3.1 vs 0.8±0.8 mg/dL, Chaudhary et al., 2021, Mayo Clin Proc Inn Qual Out 2021;5(2):388-402).
- Patients with COVID-19 generally have several other coagulation (like prothrombin time, antithrombin levels) parameters at the baseline or slightly elevated (Bonaventura et al., Nat. Rev. Immunol., 2021, 21, 319-329) further confirming that coagulation process during SARS-CoV-2 infection differs from classical pathway.

Take-home messages

- Inflammatory biomarkers play an important role in disease monitoring and treatment and impact many components of patients care.
- COVID-19 disease is untypical viral respiratory disorder with pathology which is rather caused by dysregulated immune response and coagulation then direct viral damage to host cells.
- Inflammatory biomarkers (IL-6, PCT, CRP) bring additional clinical value for COVID-19 patients risk stratification and treatment.

Thank you for the participation!

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