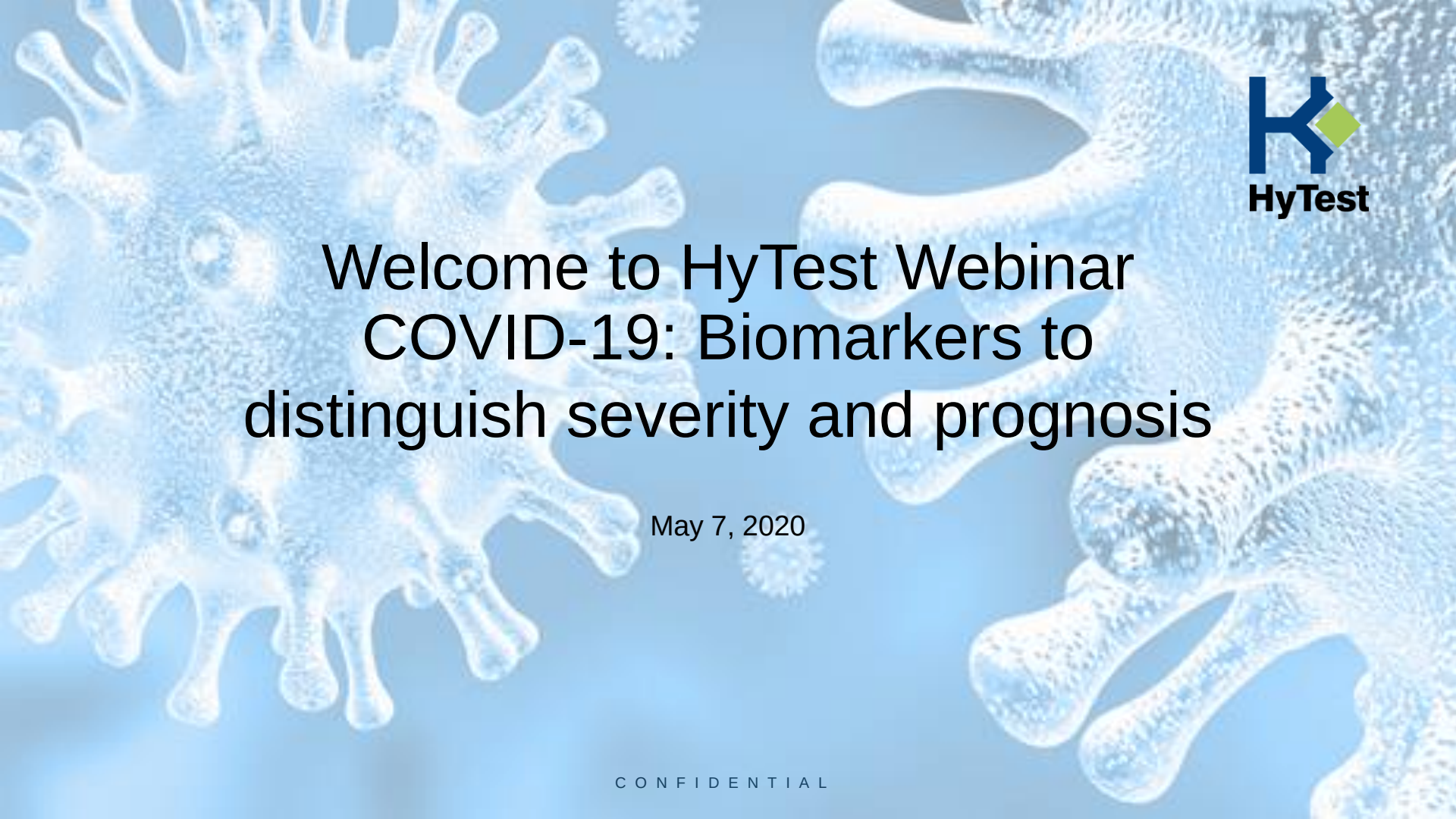

Welcome to HyTest Webinar COVID-19: Biomarkers to distinguish severity and prognosis

May 7, 2020

1. Introductions: Company and Speaker *by Dr. Netta Fatal*
2. Talk 45 min *by Dr. Alexander Semenov*
3. Q&A 15 min *by Dr. Alexander Semenov and Dr. Alexander Postnikov*

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

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 | Molecular Biology

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Today’s speaker: Dr. Alexander Semenov

- MSc. and PhD degrees from the Moscow State University (MSU)
- Senior Scientist and Project Manager, joined HyTest R&D in 2005
- Involved in research projects focused on BNP and NT-proBNP, in charge of developing antibodies and immunoassays specific to these heart failure biomarkers
- Co-author of about 20 publications and patents
- Member of The Joint Committee for Traceability in Laboratory Medicine (JCTLM) Proteins Review Team

Panelist Dr. Alexander Postnikov

- MSc and PhD degrees from the Moscow State University (MSU)
- Joined HyTest R&D in 2006, manages the group of development and production of recombinant antibodies
- Involved in several NPD projects including invention of novel biomarkers for cardiac risk assessment (IGFBP-4 fragments)
- Co-author of about 30 scientific publications and patents
- Member of the IFCC Working Group on PAPP-A Standardization

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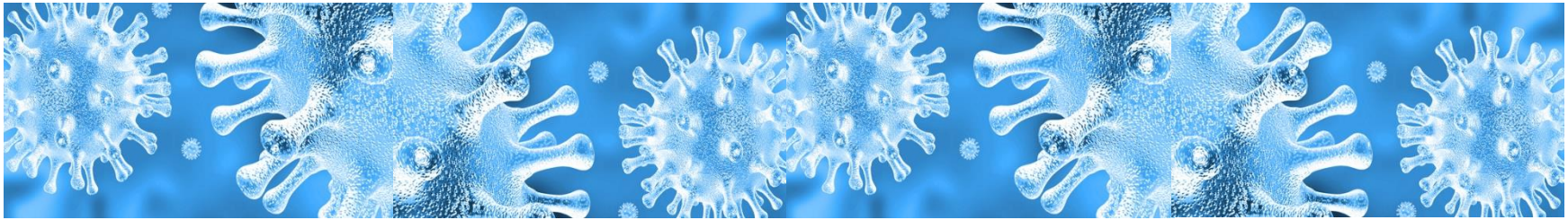
COVID-19: Biomarkers to distinguish severity and prognosis

Alexander G. Semenov, PhD

Coronavirus disease 2019 (COVID-19) pandemic

- In December 2019, a cluster of atypical pneumonia patients epidemiologically linked to a wholesale market in Wuhan (Hubei Province, China) was detected
- Initially, a novel coronavirus was called 2019-nCoV
- Later it was termed the SARS-CoV-2 virus as it is very similar to the one that caused the outbreak of severe respiratory disease (SARS) in 2003



Coronavirus disease 2019 (COVID-19) pandemic

- At the end of January the World Health Organization (WHO) declared the new infectious disease COVID-19 a global health emergency

- On 11 March 2020 the new infectious disease was recognized as a pandemic by the WHO

- In up to 15% of infected patients the clinical course may be complicated by the onset of a severe form of interstitial pneumonia, which may then progress towards acute respiratory distress syndrome and/or multi organ failure and death

1. SARS-CoV-2 virus
2. Nucleic acid testing and serological assays for SARS-CoV-2
3. Effects of SARS-CoV-2 on the cardiovascular and other systems
4. COVID-19 and cardiac biomarkers
5. COVID-19 and inflammatory biomarkers
6. COVID-19 and coagulation, kidney and muscle injury biomarkers

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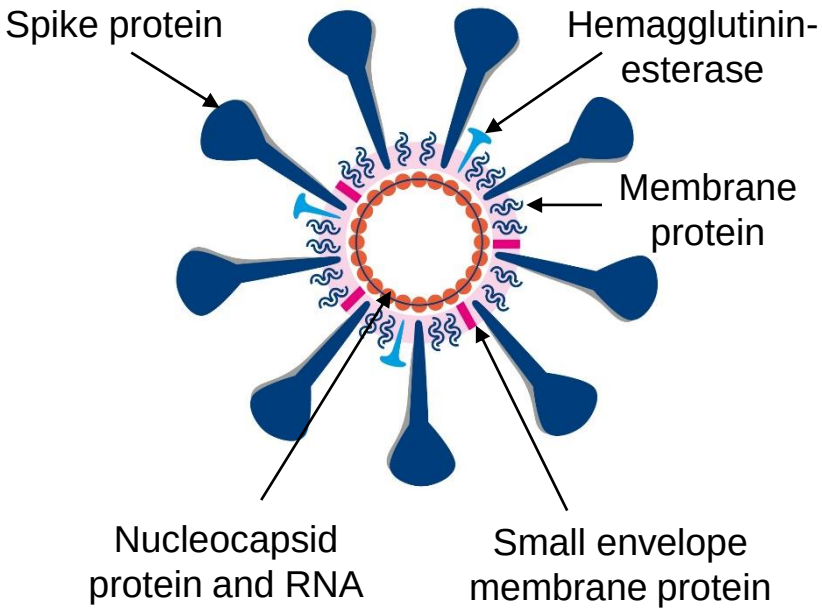
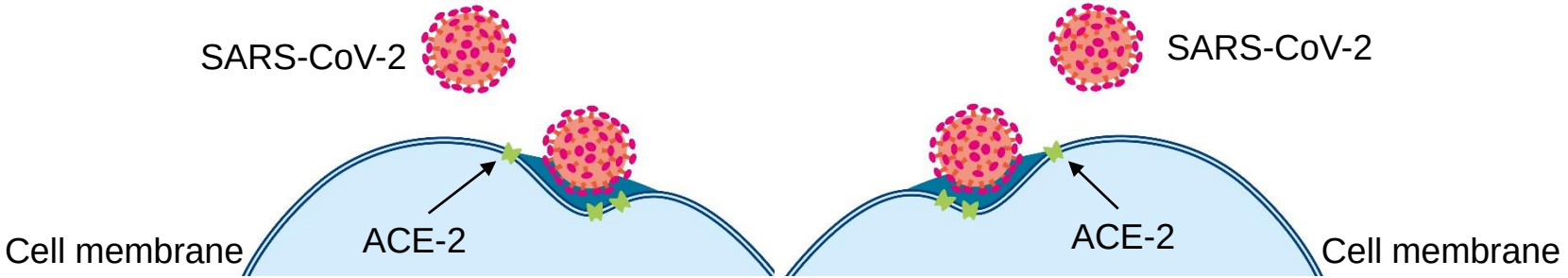
SARS-CoV-2 is a novel coronavirus of zoonotic origin

- SARS-CoV-2 belongs to a large family of single-stranded RNA viruses (+ssRNA)
- It has a crown-like appearance under an electron microscope (*coronam* is the Latin term for crown) due to the presence of spike glycoproteins on the envelope
- Beta-coronaviruses (like SARS-CoVs) can cross species barriers and can cause in humans illness ranging from the common cold to more severe diseases such as SARS (2003) and MERS (2012)



Mechanisms of entry of SARS-CoV-2

- SARS-CoV-2 enters the body through the nose and throat (e.g. when virus-laden droplets are inhaled)
- SARS-CoV-2 enters cells through an interaction with angiotensin-converting enzyme 2 (ACE-2)
- ACE-2 is a transmembrane peptidase that degrades angiotensin II to generate angiotensin 1-7 (vasodilator)



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Should inhibitors of the renin–angiotensin system be withdrawn in patients with COVID-19?

- ACE and ACE-2 belong to the same peptidase family, however, they have two very different physiological functions. ACE is a target for the treatment of hypertension.
- It’s hypothesized that ACE inhibitors (ACE-Is) could act as a potential risk factor for COVID-19 by up-regulating ACE-2
- Patients taking ACE-Is or ARBs (angiotensin-receptor blockers) may be more susceptible for viral infection and have higher mortality because they are older, more frequently hypertensive, diabetic, and/or having renal disease
- It’s suggested that ACE-I and ARB therapy should be maintained or initiated in patients with heart failure, hypertension, or myocardial infarction, according to current guidelines, irrespective of SARS-CoV-2

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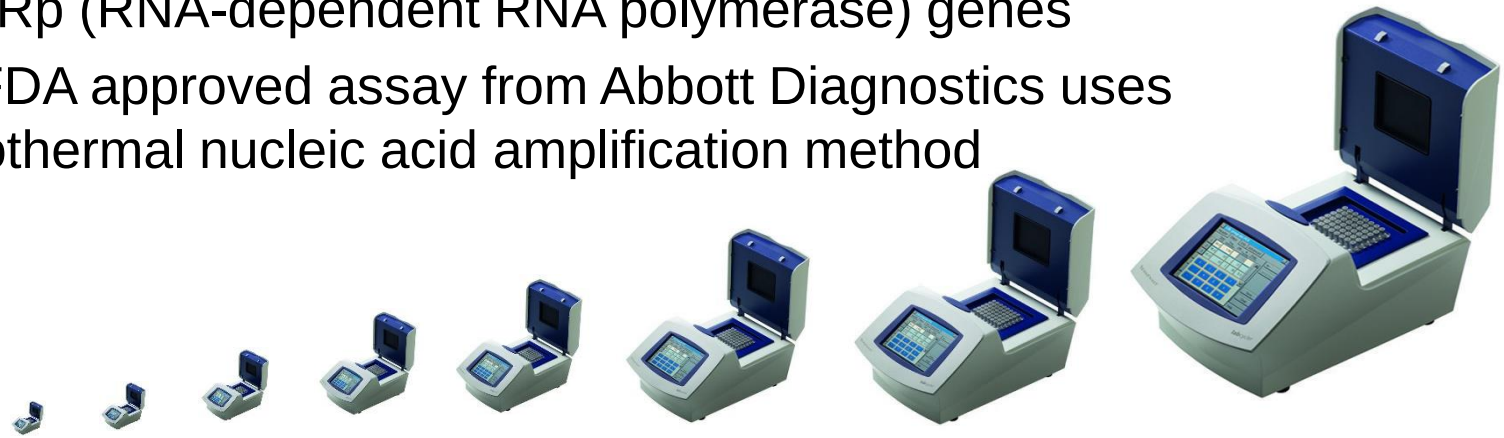
Assays available for the detection of SARS-CoV-2

- Nucleic acid testing:
  - The most sensitive method combined with high specificity and high efficiency
  - The limit of detection reaches 100 copies/mL
  - Stringent performance assessment is still an urgent need
- Serological testing:
  - Testing for specific IgM, IgG or viral antigens (ELISA, CLIA, etc. and rapid serological testing)
  - Help clinically discriminate among infections when the nucleic acid testing result is negative
  - Sensitive and specific serological assays are not as easily established as nucleic acid testing assays

Nucleic acid testing for the detection of SARS-CoV-2

- The assays used in many laboratories are real time PCR assays targeting two different amplification regions:
  - The E (envelope protein)
  - RdRp (RNA-dependent RNA polymerase) genes
- The FDA approved assay from Abbott Diagnostics uses an isothermal nucleic acid amplification method



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Nucleic acid testing for the detection of SARS-CoV-2

- Viral load peaks in the first week of disease onset
- Viral RNA can be detected in patients in the 2nd week of disease onset, but the viral load is low
- The optimal specimen type for SARS-CoV-2 detection is yet to be determined
- Saliva samples may be better than nasal/throat swabs, however, additional studies are needed

Limitations of nucleic acid testing for Covid-19

- Require high-quality viral RNA in sufficient amounts (amounts vary tremendously between patients)
- Viral RNA is sensitive to spoilage during collection, transport, and storage
- Despite high sensitivity, a negative nucleic acid testing is insufficient to exclude SARS-CoV-2 infection in patients with high clinical suspicion
- If a negative nucleic acid testing is observed at one or two time points, other approaches for testing should be considered, including specific IgM and IgG assays (serological assays)

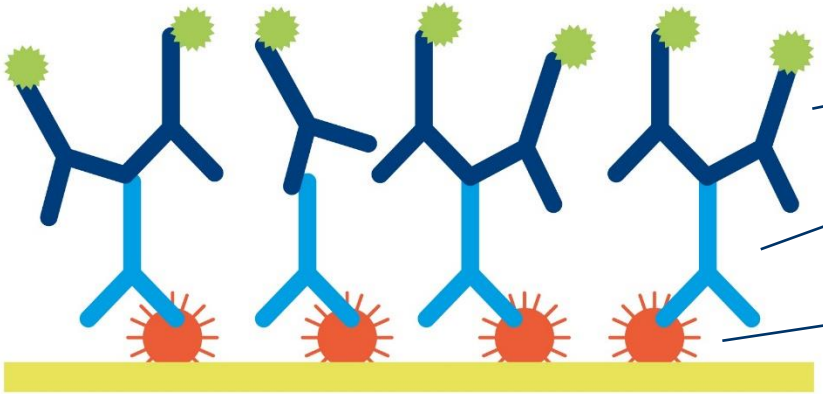
Serological testing of SARS-CoV-2

- Serology tests are used for the detection of antibodies in the blood in order to diagnose an active or previous infection

- These antibodies can be detected only several days after the exposure to the disease causing agent (e.g. SARS-CoV-2)

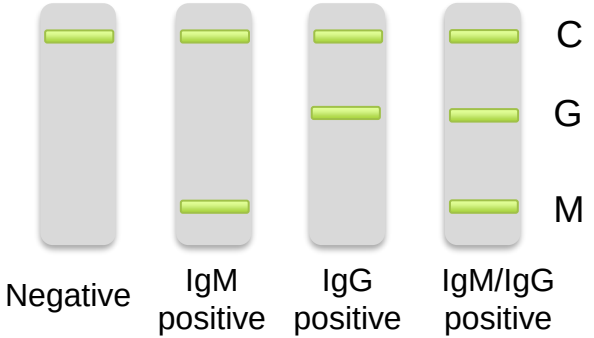
- Tests are suitable for assessing whether the person has been exposed to the virus at some point and developed immune response against it

Principle of serological testing of SARS-CoV-2



Secondary antibody (labelled)	Anti-IgM or Anti-IgG
Primary antibody	Antibody in serum (anti-SARS-Cov-2)
Antigen	Rec. antigen (e.g. Spike S1)

Detection of anti-SARS-CoV-2 antibodies in serum samples enables to confirm the immune response.



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Serological testing of SARS-CoV-2

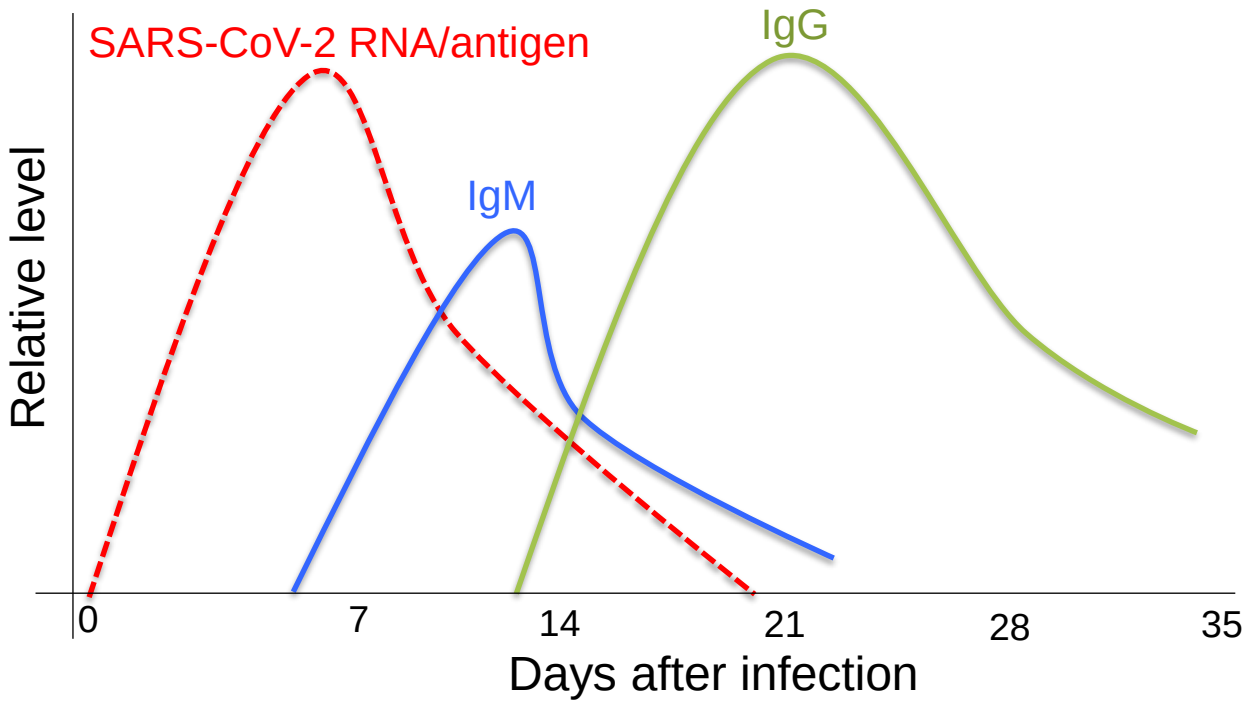
- In general, IgM antibodies can be detected earlier than IgG antibodies, however, studies related to SARS-Cov-2 show mixing results

- Serology tests are useful for:
  - Determining how widely a disease has spread within a community
  - Determining whether the person already has developed immune response to the disease
  - Determining who may donate their blood, which may serve as a possible treatment for those who are seriously ill from COVID-19
  - Monitoring the effectiveness of vaccine (ability to raise immune response)

Immunoassays to detect SARS-CoV-2 antigen

- Rapid antigen tests may provide the advantage of fast time to results and low-cost detection, but require a high viral count
- Immunoassays for SARS-CoV-2 antigen are still new to the market
- The viral spike protein can be used as an antigen
- Nucleocapsid protein is a promising target for clinical diagnostics:
  - Mutations are much less common than in spike protein
  - Produced at high levels within infected cells

Levels of SARS-CoV-2 RNA/antigen, IgM and IgG after infection



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Effects of SARS-CoV-2 on different organs

Strokes, seizures, confusion, and brain inflammation

Inflamed and damaged alveolus

Infection of gastrointestinal tract causes diarrhea

Conjunctivitis/ inflammation

Blood clots, heart attacks, and cardiac inflammation

Kidney damage

Liver disfunction

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Effects of SARS-CoV-2 on the cardiovascular system

- Acute respiratory infections are well-recognized triggers for cardiovascular diseases (CVD), and the underlying CVD is usually associated with comorbidities, which may increase the incidence and severity of infectious diseases.

- Advanced age (>60 years), male sex, and presence of comorbidities are known to be the major risk factors for COVID-19 mortality

- Presence of cardiac injury (defined by elevated troponin levels), myocarditis, and ARDS are other strong and independent factors associated with mortality.

Effects of SARS-CoV-2 on the cardiovascular system

- Heart damage and arrhythmias are common in patients hospitalized for COVID-19

- Abnormal blood clotting (causing pulmonary embolism or stroke) and blood vessel constriction have been reported

- How SARS-CoV-2 attacks the heart and blood vessels is unknown:
  - Heart and blood vessels are rich in ACE-2 (the target of SARS-CoV-2)
  - Lack of oxygen may damage blood vessels
  - Cytokine storm may cause damage to the heart

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Myocardial injury is a common comorbidity in patients with COVID-19

- Rise and/or fall of troponin indicating myocardial injury is common among patients with acute respiratory infections and correlated with disease severity

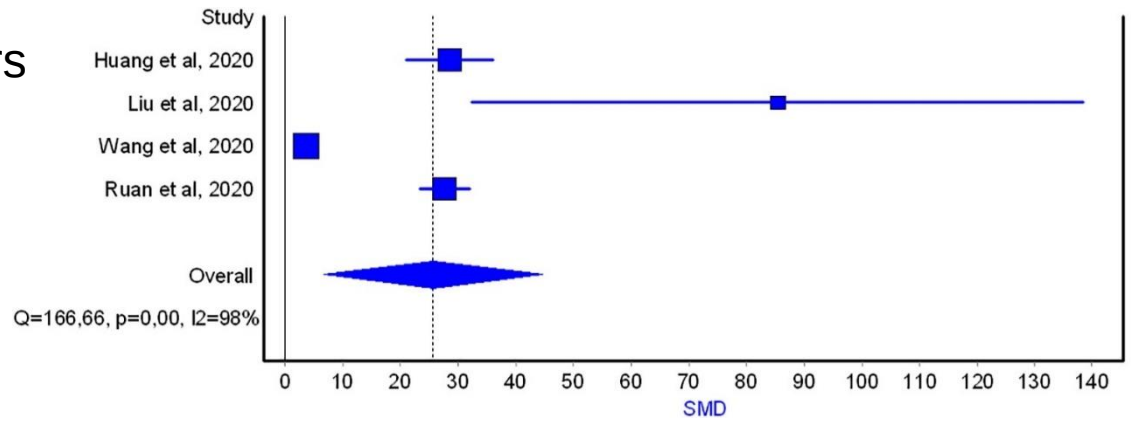
- Abnormal troponin values are common among those with COVID-19 infection particularly when testing with a high sensitivity cardiac troponin (hs-cTn) assay

- Detectable hs-cTnI was observed in most patients with COVID-19, and hs-cTnI was significantly elevated in more than half of the patients that died

Januzzi J. ACC, 2020.

Cardiac troponin I (cTnI) levels in patients with COVID-19

- cTnI values are significantly increased in patients with severe SARS-CoV-2 infection compared to those with milder forms of disease
- Adjunctive cardioprotective therapies may be advisable in patients with significant elevation of cardiac injury biomarkers



Meta-analysis by Lippi G. et al. Progress in Cardiovascular Diseases, 2020.

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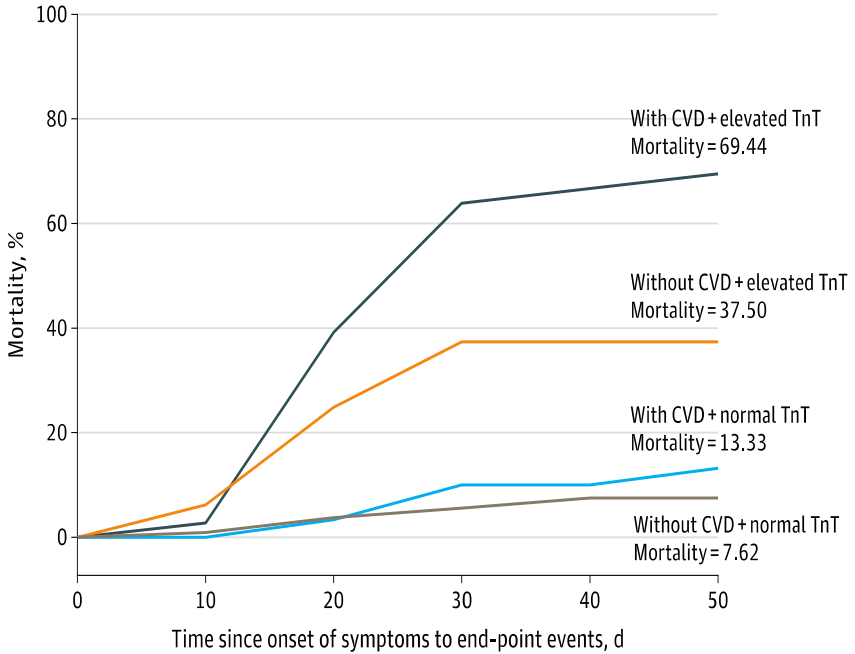
Cardiac troponin T (cTnT) testing in patients with COVID-19

- Myocardial injury was significantly associated with fatal outcome of COVID-19, while the prognosis of patients with underlying CVD, but without myocardial injury was relatively favorable
- Aggressive treatment may be considered for patients at high risk of myocardial injury



Guo T. et al. JAMA Cardiology, 2020.

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- *Data from Wuhan, China, confirm that cardiac injury is common in patients hospitalized with COVID-19 and, unsurprisingly, is tightly linked to higher mortality.*

- *Initial measurement of cardiac damage biomarkers immediately after hospitalization for SARS-CoV-2 infection may help identifying a subset of patients with possible cardiac injury.*

- *Monitoring of cTnI/cTnT during hospital stay may also be helpful to predict the progression of COVID-19 towards a worse clinical picture.*

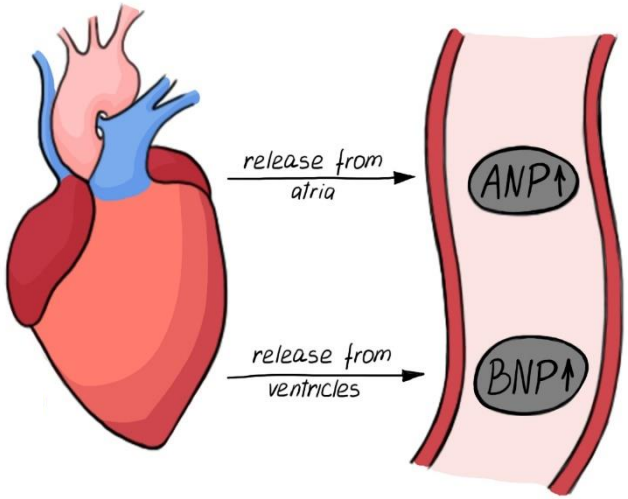
High-sensitivity cardiac troponin "could actually be an ally in the fight against COVID-19".

Troponin is a "crucial diagnostic and prognostic aid in what will become even more challenging times for healthcare provision worldwide".

Andrew R Chapman
Cardiologist in BHF Centre for Cardiovascular Science, Royal Infirmary of Edinburgh, Edinburgh, UK

Natriuretic peptide response in patients with COVID-19

- Natriuretic peptides are biomarkers of myocardial stress and are frequently elevated among patients with severe respiratory illnesses typically in the absence of elevated filling pressures or clinical heart failure
- Much like troponin, elevation of BNP or NT-proBNP is associated with an unfavorable course among patients with ARDS



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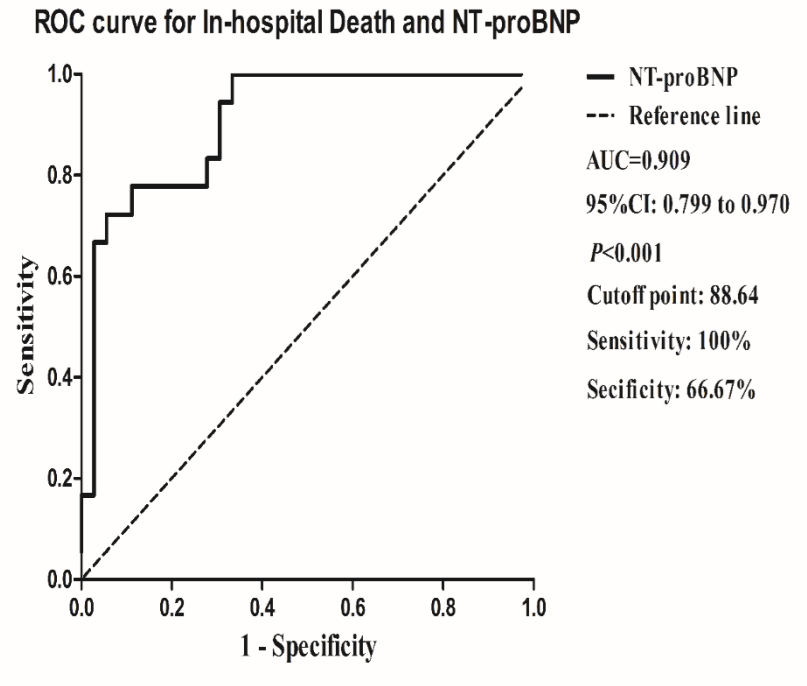
NT-proBNP levels in patients with COVID-19

- Patients with higher NT-proBNP (above 88.64 pg/mL) level had more risks of in-hospital death

- After adjusting for potential cofounders in separate modes, NT-proBNP presented as an independent risk factor of in-hospital death in patients with severe COVID-19



Gao L. et al. Medrxiv, 2020.

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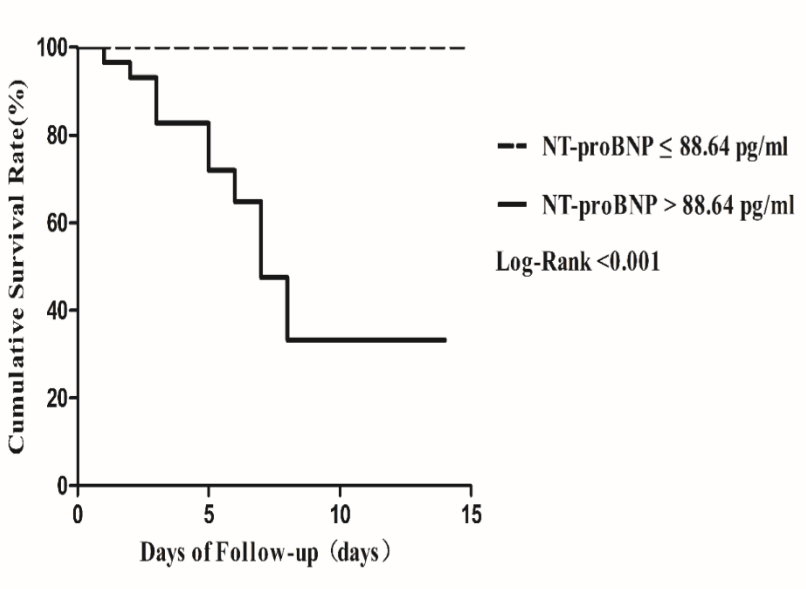
NT-proBNP levels in patients with COVID-19

- The cutoff value of NT-proBNP to predict the adverse outcome of severe COVID-19 patients was far lower than the threshold to diagnose heart failure
- ***The prognostic effect of NT-proBNP might indicate the extent of cardiac stress and inflammation***
- NT-proBNP level might be helpful to identifying patients with poor prognoses early



Gao L. et al. Medrxiv, 2020.

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BNP levels in patients with COVID-19

- Patients with COVID-19 often have abnormal BNP/NT-proBNP in plasma
- Compared with patients with normal BNP, patients with high BNP (>100 pg/mL) were more likely to develop severe pneumonia, invasive mechanical ventilation, continuous renal replacement therapy, and be admitted to the intensive care unit
- ***The level of BNP in plasma may reflect the severity of inflammation and stress.*** This may partly explain why patients with high plasma BNP levels had a bad outcomes

Liu Y. et al. Medrxiv, 2020.

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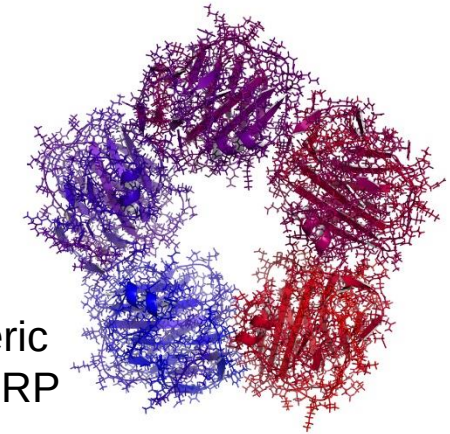
Inflammatory biomarkers

- C-reactive protein (CRP) is routinely used as a non-specific marker of inflammation
  - To be distinguished from high-sensitivity CRP (hsCRP) that used as a marker of increased risk for cardiac diseases
- Procalcitonin (PCT) is a marker of disorders that are accompanied by systemic inflammation and sepsis
- Serum amyloid A (SAA) proteins form a family of apolipoproteins. Some SAAs are expressed constitutively, while others are expressed in response to inflammation

C-reactive protein (CRP) testing in patients with COVID-19

- CRP does not normally elevate significantly in mild viral respiratory infections
- However, significant increase of CRP has also been reported in COVID-19 patients
- CRP testing may be useful in the initial evaluation of coronavirus patients
- CRP testing and complete blood count can be also used at the forefront for evaluation of infection and to direct patients further on the treatment path

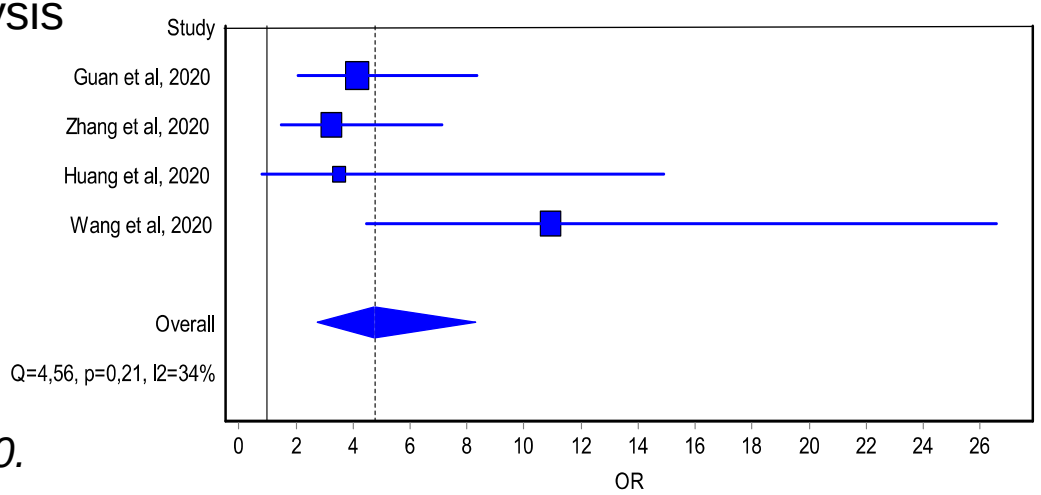


Pentameric plasma CRP

Zhang et al. Lancet Respir Med, 2020.

Procalcitonin (PCT) testing in patients with COVID-19

- PCT values are not substantially modified in patients with viral infections
- Serial PCT measurement in patients with or without severe COVID-19 may play a role for predicting evolution towards a more severe form of disease
- Evidence from a meta-analysis



Lippi G. et al. Clin Chem Acta, 2020.

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CRP, PCT and ferritin testing in patients with COVID-19

- The serum levels of CRP, PCT and ferritin are markedly increased in very severe compared with severe COVID-19

- Increased CRP, PCT and ferritin level might correlate to secondary bacterial infection and associated with poor clinical prognosis

Zhou B. et al. Research Square, 2020.

SAA is a biomarker to distinguish the severity and prognosis of COVID-19

- Patients with higher initial SAA are more likely to have poor CT imaging
- The level of SAA and CRP significantly increased in patients with COVID-19
- As disease progressed from mild to critically severe, SAA and CRP gradually increased
- SAA and lymphocytes are valuable in predicting the severity and distinguishing critically ill patients from mild ones

Li H. et al Journal of infection, 2020.

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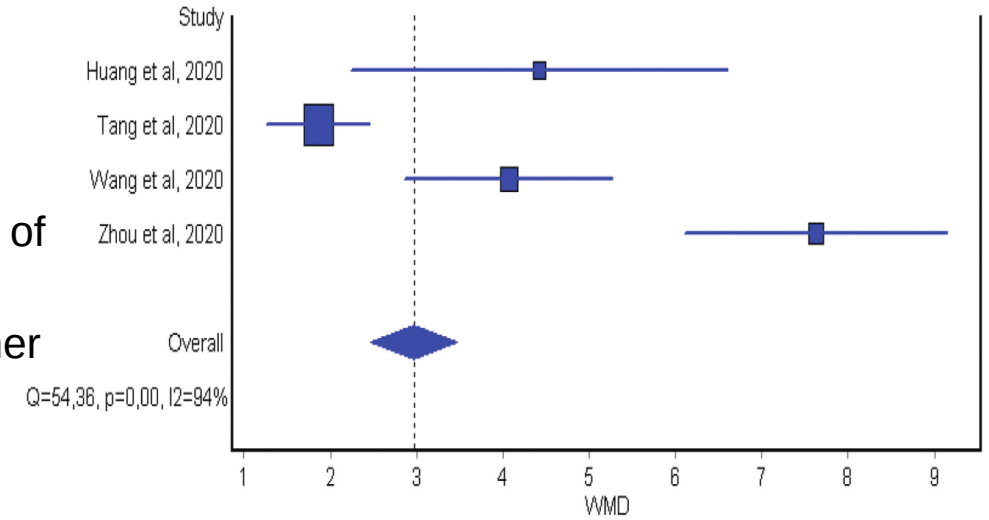
D-dimer levels in patients with COVID-19

- D-dimer is a biomarker of pathological coagulation that underlies the pathogenesis of most cardiovascular diseases
- D-dimer values are frequently enhanced in patients with COVID-19, being variably observed in 36 to 43% of positive cases
- D-dimer values are considerably higher in COVID-19 patients with severe disease than in those without the disease



Lippi G. et al. Thrombosis and Haemostasis, 2020.

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D-dimer levels in patients with COVID-19

- D-dimer measurement may be helpful in predicting evolution toward worse clinical picture in COVID-19 patients

- D-dimer measurement may help to define whether adjunctive antithrombotic therapies (e.g., anticoagulants, antithrombin or thrombomodulin) might be helpful in patients with severe COVID-19

Lippi G. et al. Thrombosis and Haemostasis, 2020.

“The more we look, the more likely it becomes that blood clots are a major player in the disease severity and mortality from COVID-19.”

Behnood Bikdeli
Cardiovascular medicine fellow at Columbia University Medical Center

Meredith W. et al. Science, 2020

Cystatin C levels in patients with COVID-19

- Patients infected with COVID-19 has renal failure is a frequent complication of acute respiratory distress syndrome

- Serum biomarkers, including urea, creatinin, cystatin C, which reflect glomerular filtration function, may be useful as potential indicators for the early diagnosis of severe COVID-19 and to distinguish it from mild COVID-19

Xiang J. et al. MedRxiv, 2020.

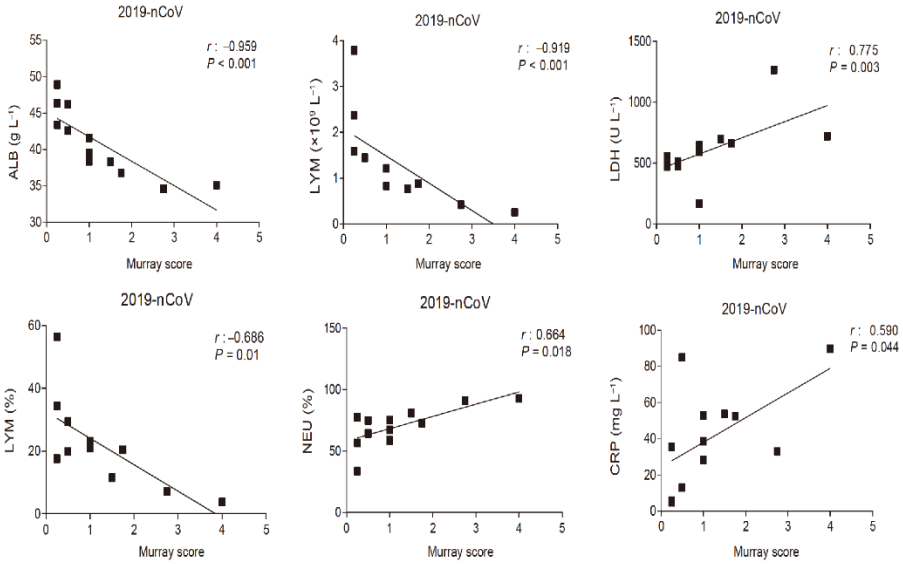
Elevated levels of myoglobin in association with COVID-19 severity

- Several studies have reported elevated levels of myoglobin in association with COVID-19 severity
- SARS-CoV-2 infection might induce a myositis similar to that observed in severe influenza infections
- Rapid clinical recognition of muscle injury in patients with COVID-19 can be lifesaving

Bangash M. et al. Lancet Gastroenterol Hepatol, 2020.
Jin M. et al. Emerging Infectious Diseases, 2020.

Albumin levels in patients with COVID-19

- Serum albumin (HSA) levels were associated with an increased risk of death
- Decreased level of HSA may be a medical sign of decreased production in the liver, increased loss in the gastrointestinal tract or kidneys, increased use in the body
- The combinations of the hypo-albuminemia, lymphopenia, and high concentrations of CRP and LDH in SARS-CoV-2 infected patients upon hospital admission may predict more severe acute lung injury



Liu Y. et al. Sci China Life Sci, 2020.

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COVID-19: biomarkers to distinguish severity and prognosis

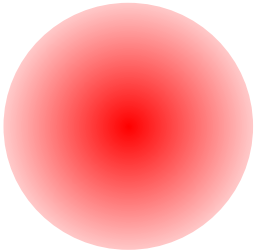
Heart

↑ Troponins
Natriuretic peptides

Coagulation

↑ D-dimer

Inflammation



↑ PCT
CRP
SAA

Kidney

↑ Cystatin C

Muscles

↑ Myoglobin

↓ Albumin

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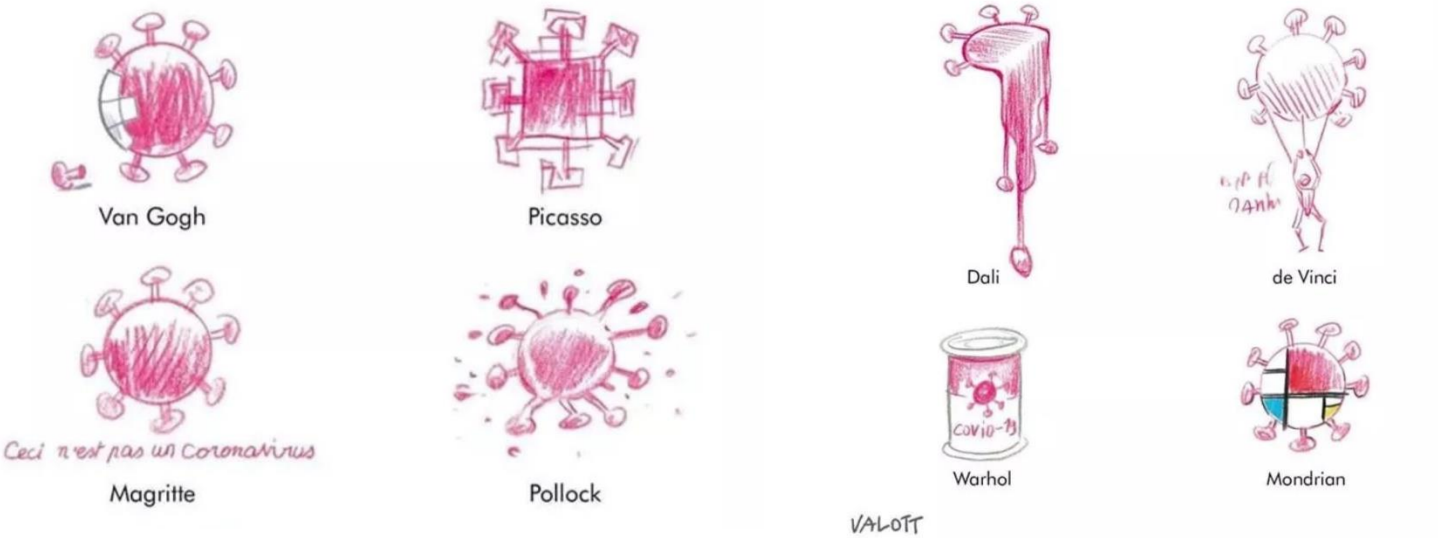
Coronavirus as if painted by famous artists



Similarly, the response to the virus is totally different in different individuals.

Biomarkers can be used as precise tools to distinguish individual differences and guide therapy accordingly.

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Concluding remarks

- Patients with comorbidities (like diabetes mellitus, hypertension, cardiovascular, chronic lung and chronic kidney disease) are particularly susceptible to COVID-19 infection and are likely to have more severe illness

- COVID-19 is associated with multiple direct and indirect cardiovascular complications including acute myocardial injury, myocarditis, arrhythmias and venous thromboembolism

- Future therapies for COVID-19 may have cardiovascular and other systemic side effects

Concluding remarks

- The key role of biomarkers may be to predict the progression of COVID-19 towards a worse clinical picture and provide a signal to manage patients differently
- They can help to triage patients to critical care, guide the use of supportive treatments, and facilitate targeted investigations in those most likely to benefit
- Careful monitoring of biomarkers is important in reducing the complications and mortality in COVID-19 patients

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Products available from HyTest

- For serology assays:
  - Antibodies specific to IgM and IgG
  - SARS-CoV-2 antigens **in development** (ask for availability)
- SARS-Cov-2 specific antibodies **in development**
- Antibodies and native/recombinant antigens for immunoassay development covering several clinical categories:
  - Cardiac markers
  - Inflammatory markers
  - Blood coagulation
  - Infectious diseases
  - Etc.

HyTest


From the science
in our hearts to
a healthier world.
Together with you.
Today and tomorrow.
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