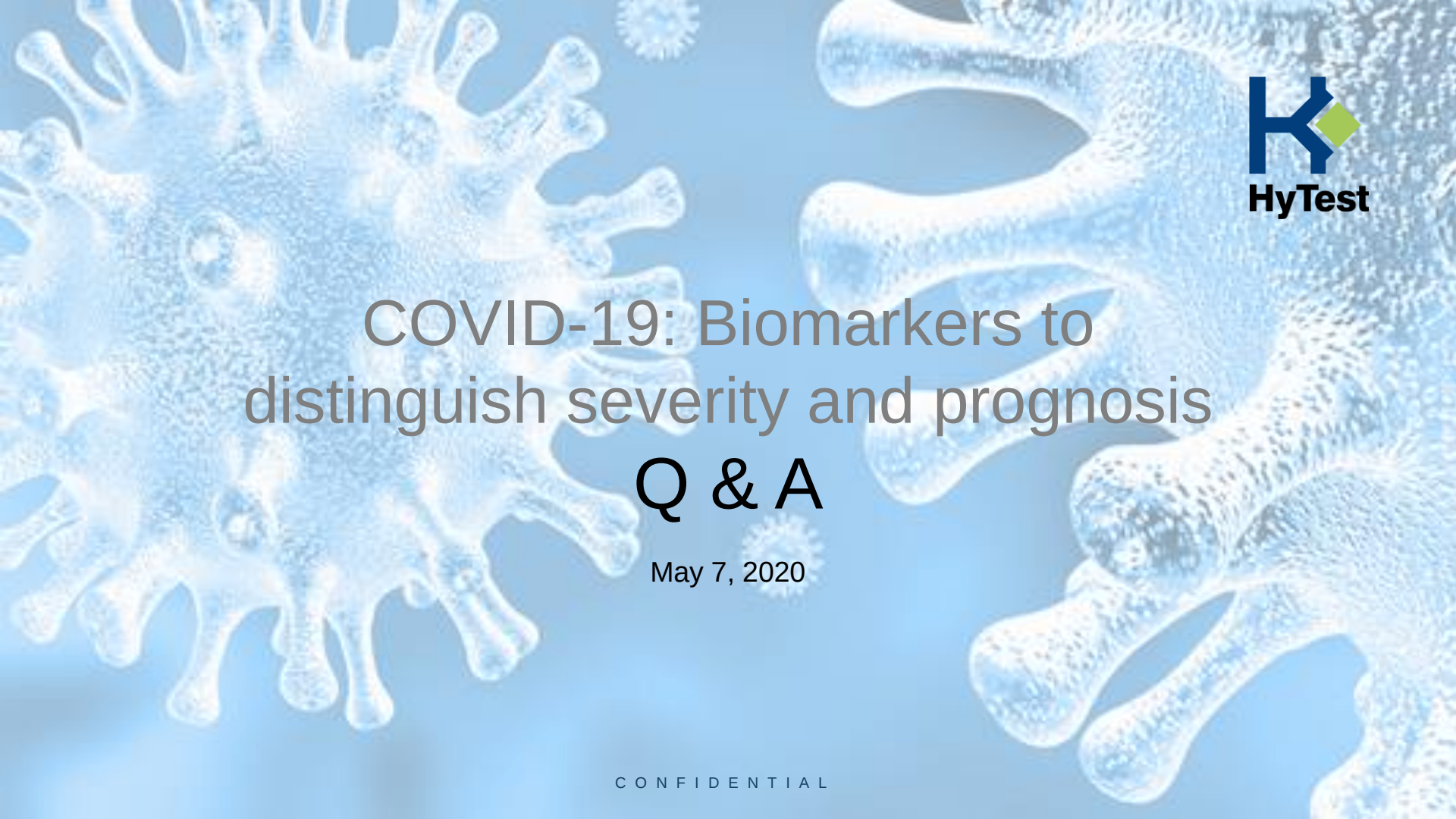

COVID-19: Biomarkers to distinguish severity and prognosis

Q & A

May 7, 2020

Questions & Answers

Q: What is the additional value of a serological IgA ELISA versus IgG?

A: Currently, IgA serology testing can be considered as an addition to IgG testing. For testing IgA, the same Spike antigen or its RBD fragment is usually used, which allows you to create combined assays. HyTest offers anti-human IgA monoclonal antibodies along with anti-human IgG and IgM.

Q: ELISA and real time PCR precision and kits?

A: Automated quantitative ELISA for serology are quite accurate tests. ELISA plate kits for serology are also quite accurate.

The biggest problem with real time PCR accuracy is the preanalytical stage, that typically includes throat swabs. The amount of material on such swabs can vary significantly.

The most inaccurate is rapid lateral flow tests due to non-standardized reaction conditions.

Questions & Answers

Q: How (and importantly, when) you do think clarity will be brought to the RDT serology discussion? Claims and counter-claims now.

A: The most important argument FOR serological testing, including rapid serology tests, is a strong pronounced immune response to SARS-CoV2 virus in humans. The humoral antibody response is quick and strong. The counter-argument is the lack of information about the stability of the immune response after infection.

It is important that the immune response is directed to the surface spike glycoprotein and to the RBD part, which is the most unique when compared with seasonal coronaviruses. This allows to develop serological tests of high specificity.

However, mix of Spike and Nucleoprotein for serology testing can also be recommended for monitoring of large groups when sensitivity of the tests is most important.

Only large clinical trials and solid clinical validation can clarify the usefulness of rapid diagnostic test. Within a few months, there will be enough of such data.

Questions & Answers

Q: Antibodies to differentiate the symptomatic and asymptomatic conditions in the COVID-19?

A: In monitoring serological studies in different countries, many patients were found who carried COVID-19 asymptomatically. No differences in the antibody profile of symptomatic and asymptomatic patients were detected. An antibody titer also does not correlate well with the severity of the disease or future complications. Specific biomarkers or CT scans in the early stages of the disease are more informative and useful for predicting future complications.

Q: Can we use whole antibody IgG H+L vs F(ab)2 for COVID19 Antibodies ELISA test kit?

A: The use of Fab or Fc-specific antibodies to human IgG, IgA, and IgM is preferred. Fc-specific antibodies are usually more specific and have less cross-reaction with antibodies of other isotypes.

Questions & Answers

Q: What are the kinetics (on rate) for HyTest's antibodies?

A: When we develop antibodies for rapid tests, we conduct on-rate selection. For example, we are currently developing antibodies for detecting SARS-CoV2 antigens and selecting antibodies with the best on-rate values.

Q: Does HyTest have COVID specific antibodies against an specific proteins discussed in this talk?

A: HyTest currently develops monoclonal antibodies to SARS-CoV2 spike and nucleoprotein.

Questions & Answers

Q: Do you manufacture spike S1 protein? Could this be purchased from HyTest?

A: HyTest currently develops recombinant SARS-CoV2 spike RBD and nucleoprotein.

Q: Is HyTest developing, or are already available for purchase from HyTest, antigens/antibodies for the detection of COVID-19? Which are the best antigens types (spike, transmembrane, nucleocapsid protein) for the detection of IgM/IgG antibodies?

A: HyTest currently develops recombinant SARS-CoV2 spike RBD and nucleoprotein as well as monoclonal antibodies to SARS-CoV2 spike and nucleoprotein. These two antigens seem the best for the detection of IgM/IgG.

Questions & Answers

Q: For your knowledge, is there a possibility of false positive results in serological tests of persons who own domestic animal or even who work with laboratory animals (e.x. rodents)? How close are animal's common betta-coronaviruses (e.x. feline coronavirus or murine hepatitis coronavirus)?

A: There is a negligible possibility that the persons who own domestic animals or contact with laboratory animals infected with animal betta-coronaviruses would have positive results in the COVID-19 serology test. However, there are several common seasonal coronaviruses circulating in the human population. It should be further investigated if a patient after seasonal coronavirus would have false-positive results in the COVID-19 serology test.

Questions & Answers

Q: Do you think there will be value in genotyping for SNPs or polymorphisms that may contribute to susceptibility?

A: Yes, definitely, I believe that there is a value in genotyping for SNPs to predict susceptibility to SARS-CoV-2, although currently available data are a bit controversial. As follows from the results of analysis of several large genomic datasets, some genetic variants of ACE2 are predicted to increase susceptibility to SARS-CoV-2, while other variants are putative protective variants predicted to show decreased binding to SARS-CoV-2 Spike protein. However, a recent study showed that there was no significant evidence that ACE2 levels and polymorphism are associated with disease severity/sex bias in the Italian population, however, TMPRSS2 (host protease involved in the priming of spike protein) levels and genetic variants proved to be possible candidate disease modulators. Another study found that susceptibility to SARS-CoV-2 was affected by human ACE1 deletion/insertion polymorphism.

Questions & Answers

Q: Is there any data on SARS-Cov-2 and dyslipidemia?

A: Yes, the data showing that lipid metabolism is affected by SARS-CoV-2 infection have been released. In a recent study it was shown that low-density lipoprotein can be a potential predictor of poor prognosis in patients with COVID-19. It was found that low-density lipoprotein levels inversely correlated to disease severities. The exact mechanisms how lipid metabolism is affected by the virus are currently unknown. It may result from a liver injury by SARS-CoV-2 infection or due to indirect effects of the virus on the organism.

Q: What is the relation between BNP and cardiac troponin I?

A: In COVID-19 patients, elevated levels of these cardiac biomarkers, BNP and cardiac troponin I, were shown to predict the severity of COVID-19 and have a prognostic role and are associated with an unfavorable course among patients with COVID-19, regardless of the lack of a link to myocardial infarction (for troponins) or heart failure (for BNP/NT-proBNP). Their prognostic value might indicate the extent of cardiac stress and inflammation.

Questions & Answers

Q: What will be reference for COVID-19 assay standardization? Defined human sera for serological assays?

A: It should be noted that standardization of testing for COVID-19, like any other infectious disease, is complex and will require detailed knowledge of the performance of the test kits used. If we are talking about immunoassays to detect SARS-CoV-2, one may expect that a well-characterized recombinant protein (either a fragment or full length protein) corresponding to the one used as a diagnostic target (e.g. spike protein or nucleoprotein) can be considered as a standard. As for serological assays, a defined pool of human sera or pooled human immunoglobulin (e.g. freeze-dried residue of human immunoglobulin diluted in saline) can be potentially used as SARS-CoV-2 standard. However, it should be stressed that the "measurand" in this kind of standard is a polyclonal mixture of antibodies, and is not exactly representative of the measurand in what is measured by SARS-CoV-2 serological assays. So, other approaches should be considered, including an option to report results qualitatively.

Questions & Answers

Q: Do you think there is a big role for hsCRP for the cardiac indications or Troponin is enough for diagnostic and prognostic info.

A: Recent papers show that higher serum hsCRP could be used to predict the risk of death in severe COVID-19 patients, similarly to troponins. However, the existing data are rather limited. And at the moment it's unclear weather hsCRP may have either different or similar prognostic value than troponins or rather both biomarkers should be monitored to get a better picture of the disease state in order to choose a treatment strategy in COVID-19 patients.

Q: Is there anyone in the US that is doing widespread biomarking?

A: We are not aware of how widely this approach is currently used in the US. It should be stressed that it’s still not fully clear whether the additional information provided by the multiple biomarkers strategy translates into better clinical outcomes. One may expect that this will be clarified soon, as the knowledge base for managing COVID-19 patients is shifting rapidly.

Questions & Answers

Q: Is Heart FABP as a cardiac marker used in the prediction of cardiac complication and severity prediction in COVID-19?

A: The available data regarding the use of heart-type FABP as a cardiac marker to predict cardiac complication and severity prediction in COVID-19 are rather limited and do not provide a consistent picture on the use of this biomarker in COVID-19 patients. As follows from the study by Yin L. et al. (published in PLOS ONE 2020), the elevation of FABP is closely related to the severity of the disease, potentially reflecting rapid development of mild into severe forms of COVID-19. One may expect that the utility of FABP will be further validated in future studies.

Questions & Answers

Q: You said that an increase of CRP levels is related with a severe state of COVID-19; This protein corresponds to a pentraxin, meaning that is conformed by 5 identical monomers. This would be related with that in a sandwich immunoassay the antibodies will bind to different portions of a monomer. My question is how could we be fully sure of the level of CRP if instead of having the entire pentraxin, CRP gets divided and the antibodies are binding to monomers?

A: Similarly to many other biomarkers that may exist in the blood as a mixture of forms, CRP levels detected by immunoassays depend on the specificity of utilized antibodies, and these levels are kind of “apparent” levels (as they reflect the amount “visible” for antibodies). Some CRP assays are able to detect only pentameric forms, almost with no cross-reaction to monomeric forms, as antibodies are specific to conformational epitopes, while other assays may detect both pentameric and monomeric forms of CRP (antibodies recognize linear epitopes). The data on cross-reactivity of immunoassays to different oligomeric and monomeric forms of CRP generally provided by the manufactures of the assays and can be found in assay specifications.

Questions & Answers

Q: Troponin I is said to be "an ally" for prognosis and severity but according to ACC the recommendations are only to measure it if the diagnosis of acute MI or heart failure are being considered on clinical grounds due to the frequency and non-specific nature of abnormal troponin. Will the recommendations of ACC will evolve or not?

A: As follows from a number of recent studies, elevated cardiac troponin in COVID-19 patients reflect illness severity and tightly linked to higher mortality. Elevation of troponin has many causes, including myocardial injury arising due to myocardial oxygen supply–demand imbalance, or it may be due to viral myocarditis or stress cardiomyopathy, or as a consequence of acute coronary syndromes. A key question is whether elevated troponin provides clinicians with a signal to manage patients differently. Currently the experts suggest clinicians should be cautious in how they proceed, as it’s not fully clear how elevated troponin really informs treatment. However, the knowledge base is shifting rapidly, as more papers are coming out rapidly. Considering this, one may expect that the recommendations of ACC will evolve accordingly.


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