Profiling Immune Response to SARS-CoV-2 for Diagnosis of COVID-19 and Characterization of Immunity

There is an urgent need for simple, inexpensive and massively scaleable testing for COVID-19 diagnosis and SARS-CoV-2 immunity. These can both be achieved by high sensitivity profiling the immune response to SARS-CoV-2 antigens across antibody isotypes.

It is well known that the immune response to SARA-CoV-2 causes antibodies to be raised to resist infection and that these antibodies seroconvert. The period of time from initial infection to seroconversion is a few days and varies among antibody isotypes. It is generally known that IgG's (the most abundant isotype) persist for years following the infection to protect against re-infection. Recent studies[1,2,3] show that the IgG's increase in abundance through the course of the infection and persist post infection at relatively high levels.

Recent studies show that the IgA isotype seroconverts quite early in the course of infection, peaks through the infection and falls back to low levels after the infection clears. In a recent study[1] that combined the signals from IgA and IgG, diagnostic sensitivity of 88% is demonstrated between 4 and 10 days after onset of infection, after that, sensitivity of nearly 100% is demonstrated. Diagnostic sensitivity is quite high, except for the earliest infection phase.

There is also evidence that the IgM isotype seroconverts early in the course of infection. It is widely accepted that IgM provides a first line of defense during microbial infections, prior to the generation of adaptive, high affinity IgG responses that are important for long-lived immunity and immunological memory[4]. In SARS-CoV

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[1] Huan Ma, et al. COVID-19 diagnosis and study of serum SARS-CoV-2 specific IgA, IgM and IgG by chemiluminescence immunoanalysis. medRxiv preprint doi: https://doi.org/10.1101/2020.04.17.20064907

[2] Christine Dahlke, et al. Distinct early IgA profile may determine severity of COVID-19 symptoms: an immunological case series. medRxiv preprint doi: https://doi.org/10.1101/2020.04.14.20059733

[3] Padoana A, Sciacovellib L, Bassoa D, Negrinia D, Zuina S, Cosmab C, Faggianb D, Matricardic P, Plebani M. gA-Ab response to spike glycoprotein of SARS-CoV-2 in patients with COVID-19: A longitudinal study. Clinica Chimica Acta 507 (2020) 164–166, https://doi.org/10.1016/j.cca.2020.04.026

[4] Racine R, Winslow G. IgM in Microbial Infections: Taken for Granted? Immunol Lett. 2009 August 15; 125(2): 79–85. doi:10.1016/j.imlet.2009.06.003

infections, the earliest time when the IgM could be detected was at 3 days after the onset and IgG in the convalescent sera could be firstly detected on 8 days of the course[5]. A recent study demonstrated 88.66% sensitivity and 90.63% specificity in the diagnosis of SARS-CoV-2 using a lateral flow immunoassay that combined IgG and IgM signals on 525 patients suspected of COVID-19[6].

The early seroconversion of IgA and IgM and the high long-lived affinity of IgG post the infection can, together, be very useful in characterizing SARS-CoV-2 during the infection and post the infection. In general, when IgA or IgM is positive, there is an active infection that is diagnostic for COVID-19. When IgA or IgM is positive and IgG is negative, the active infection is in its early phase. When IgA or IgM is positive and IgG is positive, the active infection is in it mid phase. When IgA or IgM is low and IgG is positive, the active infection is in its end phase. When IgA or IgM is negative and IgG is positive the course of the infection is complete and immunity is persisting.

This demonstrated sensitivity is based on reaction with recombinant SARS-CoV-2 spike protein expressed in HEK293. This sensitivity can likely be enhanced by including reaction with recombinant SARS-CoV-2 nucleocapsid protein expressed in HEK293. Very recent studies[7] suggest that COVID-19 fatal cases have dominant IgA reactivity with the nucleocapsids protein while recovery cases have dominant IgA reactivity with the spike protein. Thus, the inclusion of the nucleocapsid protein may enhance sensitivity in the early infection phase.

The diagnostic sensitivities demonstrated by combining IgG with either IgA or IgM were derived with ug/ml detection sensitivity. It may be that increasing the detection sensitivity to ng/ml with mass spectrometry can result in even better diagnostic sensitivity.

While multiple studies demonstrate high specificity to the reactions with the SARS-CoV-2 spike protein, there are fewer demonstrations of specificity with the SARS-CoV-2 nucleocapsid protein. There is high similarity between the SARS-CoV nucleocapsid protein and the SARS-CoV-2 nucleocapsid protein - about 90% of the

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[5] Zhuoyue W, Xin Z, Xinge Y. IFA in testing specific antibody of SARS coronavirus. South China Journal of Preventive Medicine, 31 Dec 2002, 29(3):36-37

[6] Li, et al. Development and clinical application of a rapid IgM-IgG combined antibody test for SARS-CoV-2 infection diagnosis. J Med Virol. 2020;1–7

[7] Galit Alter. SARS-CoV-2, & Glycans, Common Fund Glycoscience Program Workshop. May 1, 2020.

sequences are the same; but, since SARS-CoV has not circulated in human population since 2003 it seems that there is a low likelihood of cross-reactivity in current human samples.[8]

Early phase sensitivity is the challenge for direct virus detection tests like PCR. When viral load is low, the heterogeneous distribution of the virus reduces the probability that any given sample from an infected body will contain sufficient viral content to raise a positive signal. A recent study characterized PCR sensitivity[9] of 1070 specimens collected from 205 patients late enough in the infection course to be in hospital beds; bronchoalveolar lavage fluid samples showed the highest sensitivity (14 of 15; 93%), followed by sputum (72 of 104; 72%), nasal swabs (5 of 8; 63%), fibrobronchoscope brush biopsy (6 of 13; 46%), pharyngeal swabs (126 of 398; 32%), feces (44 of 153; 29%), and blood (3 of 307; 1%). PCR in even earlier phases of the infection would be expected to demonstrate lower sensitivities than reported in this study.

Testing of asymptomatic populations is an urgent need. It seems likely that PCR sensitivity will be much lower for asymptomatic populations; the low level of viral content heterogeneously distributed decreases the odds that samples collected from positive, but asymptomatic, persons will contain enough viral content for the PCR to raise a positive signal.

The analytical challenge of diagnosing COVID-19 and characterizing SARS-CoV-2 seems to be less about detector sensitivity (both PCR and mass spectrometry are sensitive and highly specific detectors) and more about adequate sample collection. With PCR, the challenge is about obtaining a sample with sufficient viral content to raise a positive signal when the virus is heterogeneously distributed throughout the body. This compares with mass spectrometry profiling of immune response that detects signals from the body reacting to the virus that are homogeneously distributed throughout the blood compartment. For SARS-CoV-2, these studies show that these indirect signals (antibody content) that are homogeneously distributed in the blood compartment outperform the direct signals (virals content) that are heterogeneously distributed throughout the body.

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[8] Okba N, et al. SARS-CoV-2 specific antibody responses in COVID-19 patients. medRxiv preprint doi: https://doi.org/10.1101/2020.03.18.20038059

[9] Wang W, Xu Y, Gao R. Detection of SARS-CoV-2 in Different Types of Clinical Specimens. JAMA. Published online March 11, 2020. doi:10.1001/jama.2020.3786

The path with the best chance of meeting the urgent needs of humanity may be to immediately commence widespread, high sensitivity, high granularity profiling of antibody reaction to the 1) SARS-CoV-2 spike protein expressed in HEK293 and 2) SARS-CoV-2 nucleocapsid protein expressed in HEK293. Characterize response by antibody isotype; IgA, IgD, IgE, IgG and IgM using high sensitivity (ng/ml) detection (10 measurements per sample). Combine antibody response with symptom interviews.

An Initial Model for characterizing this response profile can be derived based on existing studies and small scale specific studies that would appropriately characterize its initial error intervals. The model's inputs are the longitudinal data sets comprised of the response measurements and the symptom interviews. The model's outputs are 1) diagnosis of COVID-19 and 2) characterization of immunity to SARS-CoV-2; not present or present (with degree and type). The Initial Model can be usefully validated with appropriately characterized error intervals. As more and more data from response profiling accumulates, the model can be continuously improved to lower its error intervals and increase its precision.

The crude models used in recent studies[1,6] demonstrate diagnostic sensitivity of 88% and 88.6% respectively. These models can easily be used to derive the Initial Model. As such, this Initial Model should exceed the sensitivity of each sampling method in the recent PCR study[9] except for the bronchoalveolar lavage fluid samples.

This approach seeks to quickly scale testing to the population. As it scales, it uses the scaled data to optimize the model. It benefits by quickly scaling to population while shortening the period of time needed to completely characterize the diagnosis and immunity model; along with the benefit that the model precision increases as the data scales.

Segmented Flow/Electrospray Ionization/Mass Spectrometry (SF/ESI/MS) is particularly well suited for detecting this reaction profile. Segmented Flow enables very throughput (>1 sample per second). Mass spectrometry direct detection innately profiles the isotypic reaction profile at high sensitivity. With mass spectrometry direct detection, reagent supply chain issues are avoided as it scales to the population.