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Perspective

Emerging Biomolecular Testing to Assess the Risk of Mortality from COVID-19 Infection

Morteza Mahmoudi*

Cite This: https://dx.doi.org/10.1021/acs.molpharmaceut.0c00371

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ABSTRACT: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2 and COVID-19) has produced an unprecedented global pandemic. Though the death rate from COVID-19 infection is ~2%, many infected people recover at home. Among patients for whom COVID-19 is deadly are those with pre-existing comorbidities. Therefore, identification of populations at highest risk of COVID-19 mortality could significantly improve the capacity of healthcare providers to take early action and minimize the possibility of overwhelming care centers, which in turn would save many lives. Although several approaches have been used/developed (or are being developed/suggested) to diagnose COVID-19 infection, no approach is available/proposed for fast diagnosis of COVID-19 infections likely to be fatal. The central aim of this short perspective is to suggest a few possible nanobased technologies (i.e., protein corona sensor array and magnetic levitation) that could discriminate COVID-19-infected people while still in the early stages of infection who are at high risk of death. Such discrimination technologies would not only be useful in protecting health care centers from becoming overwhelmed but would also provide a powerful tool to better control possible future pandemics with a less social and economic burden.

KEYWORDS: *COVID-19, SARS-CoV-2, mortality, biomolecular corona, sensor array, magnetic levitation*

INTRODUCTION

COVID-19 was declared a pandemic a few months after first being reported in Wuhan, China.[1] As of May 19, 2020, over 4,941,300 cases had been confirmed with a total death count of ~321,800[2] (access date: May 19, 2020). COVID-19's 2% case fatality rate is the product of several mechanisms including complications of comorbidities (e.g., cardiovascular disorders[3−5]) or massive alveolar damage and progressive respiratory failure.[1,6,7] Although tight policies to control disease spread are still in place, the numbers of cases and deaths are still increasing, and there are statistical models forecasting of severe shortages in healthcare resources and death rates from COVID-19 (e.g., in the U.S.[8]).

In the absence of vaccines, one powerful approach to control the spread of disease would be fast, cheap, reliable, and portable means of diagnosing COVID-19 infection. Details on current diagnostic methods (e.g., nucleic acid and computed tomography testing) and possible new approaches (e.g., protein and point-of-care testing) based on nanotechnologies can be found in a recently published review paper.[9]

In addition to the detection of COVID-19 infection, we need a complementary approach for early identification of infected patients at high risk of death. Assessment of risk before the progression of the disease is of crucial importance to protect limited healthcare resources and to lower death rates. However, it is not possible to classify individuals as having a high-risk life-threatening condition or being asymptomatic carriers other than clinical observation; faster, more reliable strategies are needed.

Special Issue: Nanomedicines Beyond Cancer

Received: April 6, 2020
Revised: April 29, 2020
Accepted: May 7, 2020
Published: May 7, 2020

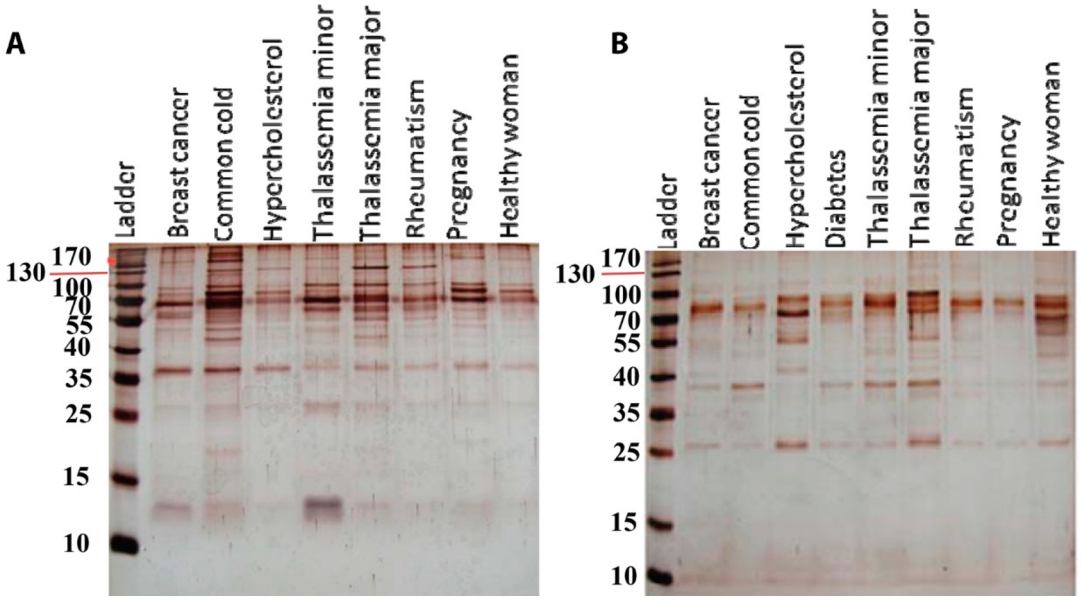

Figure 1. Protein corona patterns of (A) polystyrene and (B) silica nanoparticles after incubation with the human plasma of various diseases/conditions including common cold. Copyright 2014, Royal Society of Chemistry.[19]

This perspective describes possibilities for point-of-care diagnosis of COVID-19 patients at high risk of mortality. Such diagnostics for early stage identification of high-risk patients could (i) prevent severe shortages of healthcare resources, (ii) minimize death rates, and (iii) improve management of future epidemics/pandemics.

BIOMOLECULAR CORONA

Upon interactions with biological fluids such as human plasma, the surfaces of nanoparticles (NPs) are quickly covered by a layer of biomolecules to form the "protein/biomolecular corona".[10−12] One unique feature of the protein corona is that its constituent proteins rarely correspond to the most abundant proteins in plasma.[11,13−15] In other words, NPs offer a novel type of omics data (in terms of the number of biomolecules and their concentrations) that is totally different from similar data on plasma proteins. The main reason for the lack of correlation is the dynamic nature of the protein/biomolecular corona, which (i) absorbs proteins with higher affinities and/or lower absorption kinetics and (ii) recruit new proteins/biomolecules in corona composition through their favorable interactions with the proteins/biomolecules already incorporated into the corona.[11,16] For instance, precoating NPs with specific proteins can enhance the recruitment of similar proteins from plasma during corona formation.[16,17]

Over the past decade, our collaborative team has identified more than 10 previously overlooked factors required for successful nanomedicines,[18] including disease-specific NP protein coronas,[19−21] the effects of the NP protein corona on drug-release profiles,[22] cell shape,[23] cell division,[24−26] cell age,[27] cell sex,[28] incubating temperature,[29] and plasmonic activation of NPs,[30] that have been validated by others (summarized in our recent reviews[31,32]). These factors (as well as others) challenge and complicate the therapeutic efficacy of NPs, slowing their clinical translation. In 2014, our group introduced the novel concept of the "personalized" or "disease-specific" protein corona, in which we revealed that the composition of protein coronas on the surface of identical NPs differ dramatically depending on the type of disease(s) the plasma donors have had (see Figure 1 as an example of corona patterns of various diseases including the common cold, which is a viral infection).[19,20] The concept of disease-specific NP protein corona[19−21] opens the door to unique diagnostic applications. In other words, identical NPs may form different protein corona profiles in patients with various diseases, significantly affecting the safety and therapeutic efficacy of the NPs across patients; however, those very differences in the protein corona that constitute a huge shortcoming for therapeutic applications can be exploited for disease detection *ex vivo*.

Very recently, we published the proof-of-concept that disease-specific protein corona, in combination with advanced classifiers, can be used for early detection and discrimination of cancers (Figure 1A−F)[33] and some neurodegenerative disorders.[34] Other groups have demonstrated the unique capacity of the protein corona in identifying disease-specific biomarkers.[35−39] We have also shown that the sensitivity, specificity, and predictive accuracy of disease detection using protein corona techniques could be significantly improved by the addition of more NPs to create a "protein corona sensor array."[33] In other words, sampling the same human plasma using multiple NPs as sensor array elements can significantly increase the number and concentration range of identified plasma proteins that, in combination with supervised classifiers, may provide disease-specific information.

The protein corona sensor array platform could also identify and discriminate between selected cancers in cohort plasma samples of healthy individuals who developed cancers several years after plasma collection.[33] Therefore, the platform may have early diagnostic applications. The same approach could be used for accurate discrimination between fatal and nonfatal COVID-19 infection, as we previously demonstrated (Figure 1) that common cold can change the profile of protein corona at the surface of silica and polystyrene NPs. Protein corona sensor array technology may help us in defining the plasma protein/biomolecule patterns that indicate fatal COVID-19 infection at very early stages. It is noteworthy that, although the lion share of the biomolecular corona is occupied by proteins, other types of biomolecules (e.g., lipids, metabolomes, and nucleic acids) that

https://dx.doi.org/10.1021/acs.molpharmaceut.0c00371
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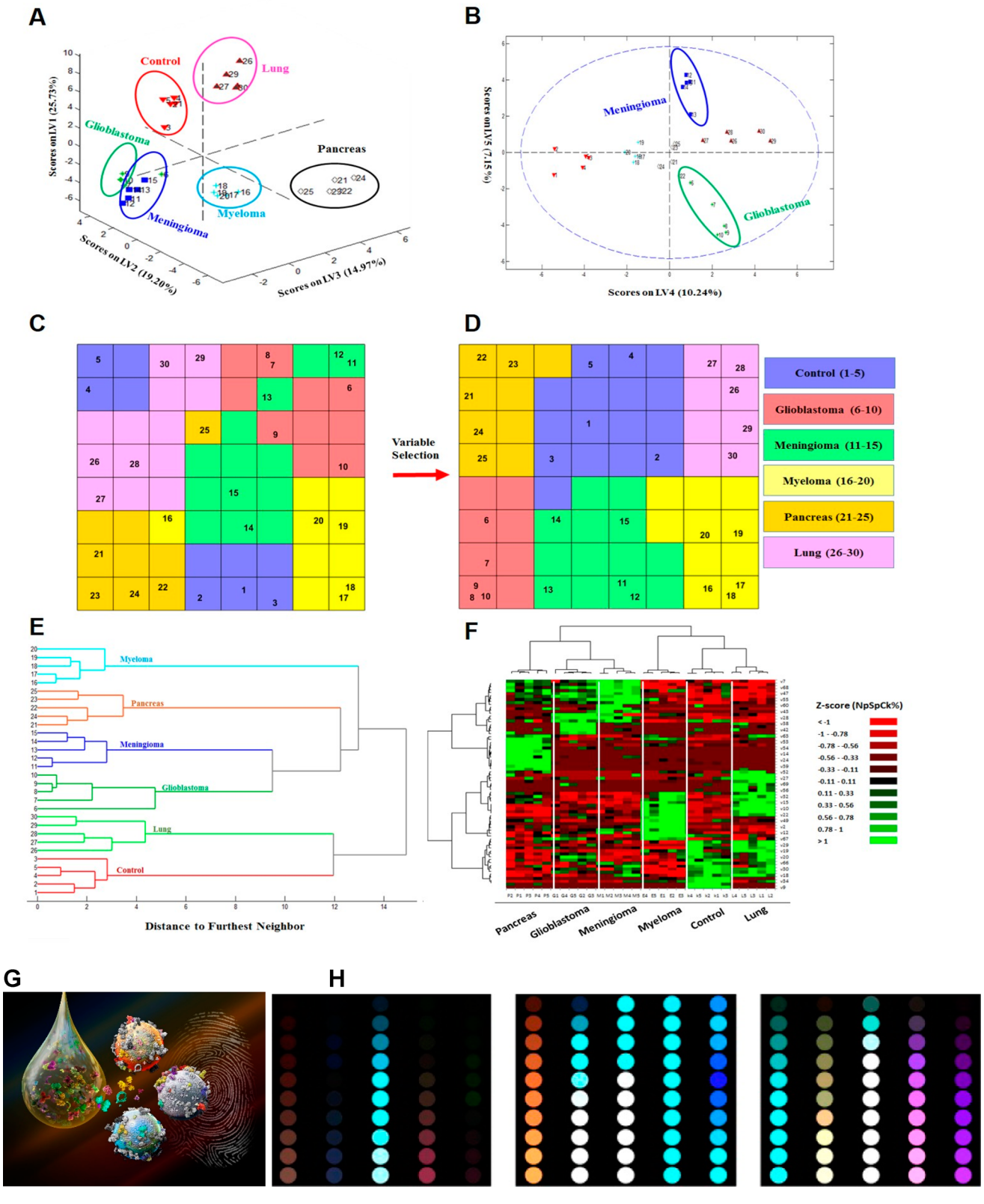

Figure 2. Development of protein corona sensor array for disease identification and discrimination. Use of two distinct supervised classifiers consisting of (A, B) partial least-squares discriminant analysis (PLS-DA) and (C, D) counter-propagation artificial neural network algorithm (CPANN) to analyze protein corona profiles for identification and separation of 5 different cancers from each other and from healthy controls. (E) The proteins identified as capable of distinguishing among the 5 different cancers and healthy controls using a Hierarchical clustering dendrogram and (F) heat map. (G) Scheme showing the formation of protein corona after incubation of multinanoparticles with biological fluids of COVID-19 infected patients to define "fingerprint" protein patterns of the at-risk population, using advanced classifiers/machine learning. (H) Development of optoelectronic nose for the detection of identified proteins in (G) for point-of-care discrimination of at-risk populations. (A–G) Copyright 2019, Royal Society of Chemistry.[33] (H) Copyright 2016, American Chemical Society.[50]

https://dx.doi.org/10.1021/acs.molpharmaceut.0c00371
Mol. Pharmaceutics XXXX, XXX, XXX−XXX

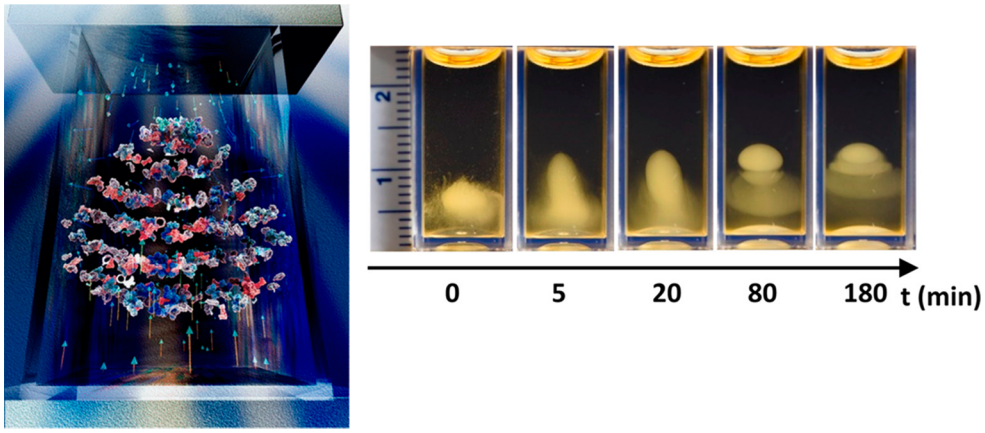

Figure 3. Superparamagnetic solution levitates proteins in the MagLev platform. Scheme and optical images show the progress of levitated plasma proteins/biomolecules which forms ellipsoidal biomolecular patterns over time in the MagLev platform. Copyright 2020, American Chemical Society.[58]

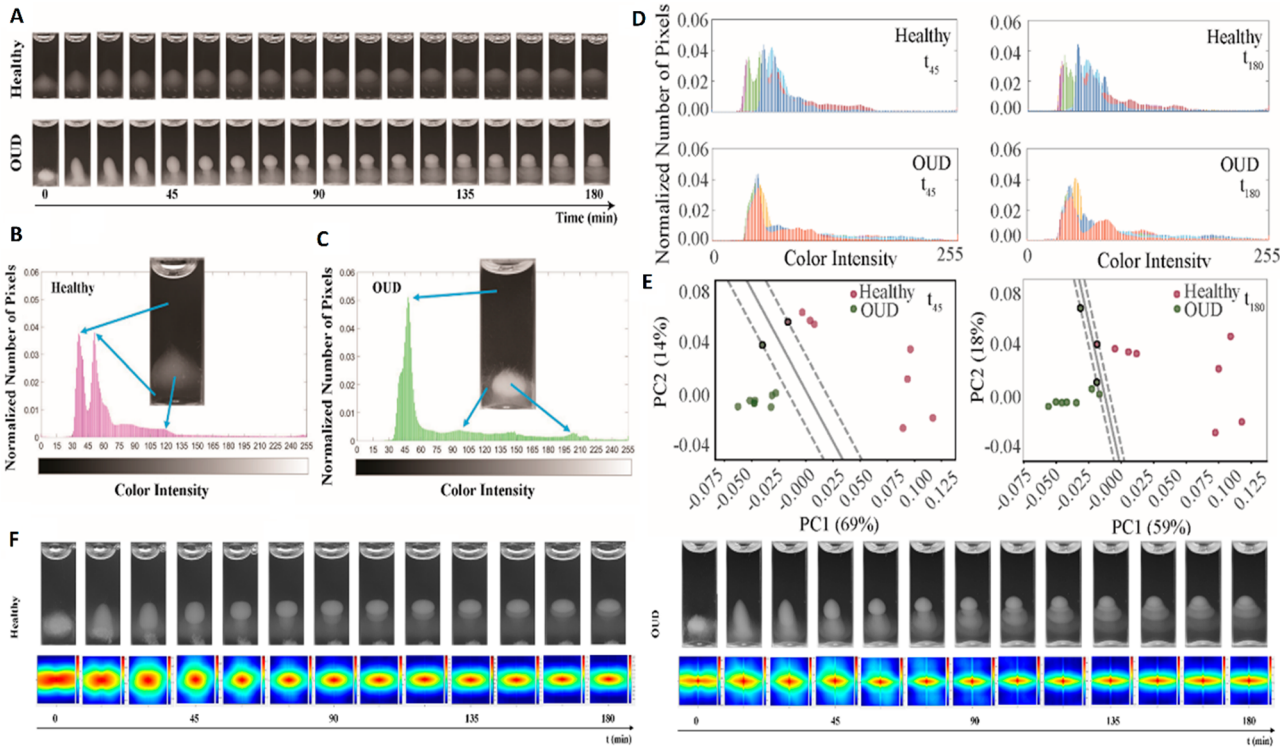

Figure 4. Development of a MagLev platform with point-of-care diagnostic capacity. (A) Gray-scale progressive levitation images of healthy and patients [with opioid use disorder (OUD)] plasmas. (B and C) Examples of the normalized intensity histograms of the optical images together with (D) intensity histograms of healthy and OUD plasmas at selected time frames (i.e., 45 and 180 min). (E) Principal component analysis (PCA) and support vector machine (SVM) results for the selected time frames showing the detection capacity of the MagLev optical images. (F) The initial time point of 45 min was chosen based on the observed stability of the levitations, identified via no major change in the processed heatmaps (using two-point statistics) of the MagLev images over time. Copyright 2020, Wiley.[59]

may have diagnostic capacities are available in the corona composition.

The use of biological fluids that can be collected noninvasively (e.g., tears,[40,41] saliva,[42,43] and urine[44,45]) may also be considered in the protein corona sensor array approach as they carry disease-related protein markers. Compared to human plasma, the use of simple-to-gather biological fluids in such a discrimination platform is the goal of creating a point-of-care device that does not require an expert healthcare provider to be present. The main disadvantage of nonplasma biological fluids over human plasma is that tears, saliva, and urine contain a dramatically smaller range of biomolecules, which may reduce their sensitivity, specificity, and prediction accuracy.

After identification of a protein pattern to serve as a "fingerprint" of COVID-19 infected patients at high risk of death, colorimetric nanotechnologies (including optoelectronic nose[46,47] and plasmonic nanoparticle[48,49] technologies) can be developed for point-of-care identification of these vulnerable patients (Figures 2G,H).

https://dx.doi.org/10.1021/acs.molpharmaceut.0c00371
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MAGNETIC LEVITATION

Magnetic levitation (MagLev) is a fast, easy, and powerful technique for levitating nonbiological and biological species, including different types of materials and human/animal cells incubated in paramagnetic fluid.[51−56] The conventional configuration of a MagLev device consists of two permanent magnets with two like-poles facing each other along the vector of gravity. It is noteworthy that the MagLev system is unrelated to conventional immunoadsorption using superparamagnetic particles.[57]

Proteins are not stable in conventional MagLev paramagnetic solutions; however, it was very recently shown that the substitution of conventional paramagnetic solutions with superparamagnetic iron oxide nanoparticles (SPION) can resolve this issue and levitate plasma proteins (Figure 3).[58] We recently showed the diagnostic value of disease detection via MagLev optical images of biological proteins.[59] Specifically, our supervised classification analysis demonstrated proof-of-concept that the movement of levitating proteins in MagLev has disease detection capacity, with potential use in a point-of-care device.[59] More specifically, we found that MagLev optic images of levitated proteins, subjected to machine-learning analysis, offer valuable information on the individual's health status (Figure 4).[59] In addition, the levitated ellipsoidal patterns of proteins can be separated and analyzed with proteomic approaches to learn more about the role of important proteins in disease development.[59]

Based on these results, the MagLev platform may have the capacity for rapid discrimination of patients at risk of fatal COVID-19 progressive disease (e.g., by exacerbating cardiovascular diseases[3−5]) and also accelerate the development of biomarker(s) to identify such patients. The use of nonplasma biological fluids (e.g., tear, saliva, and urine) is preferred but will require testing, for the reasons mentioned in the Biomolecular Corona section.

PROS AND CONS OF THE PROPOSED APPROACHES

The main advantage of the proposed approaches, compared to the conventional diagnostic tests, is their capacity of biomolecular pattern recognition among various types of biomolecules. The conventional tests, on the other hand, are focused on the identification of specific biomolecules to confirm or rule out the COVID-19 infection regardless of their fatal risk. The pattern recognition is essential for fast and accurate diagnosis of COVID-19 infections that likely to be fatal; this is mainly because many of the biomolecules may be associated with the personalized plasma variation and/or comorbidity. By the combination of advanced supervised classifiers with the proposed approaches, the patterns of biomolecules (including proteins, nucleic acids, metabolomes, and lipids) that may have fatal risk diagnostic capacity will be defined. In addition, as we found recently,[60,61] a variation of nonprotein biomolecules (e.g., metabolomes) can significantly alter the protein corona composition, which can further improve the diagnostic capacity of the corona sensor array system. Finally, the protein corona sensor array system has the ability to improve its diagnostic accuracy by using more sensor array elements.

The central disadvantage of the proposed approaches is the fact that, unlike conventional assays, we do not have specific biomarkers or nucleic acid to detect. Therefore, the very first step for developing such assays for identification of populations at the highest risk of COVID-19 mortality is to collect human plasma and other nonplasma biological fluids from a considerable number of COVID-19-infected patients at fatal and nonfatal stages; the collected biological fluids will then need to examined by MagLev and protein corona sensor array platforms and analyzed by omics techniques and machine learning to define the library of biomolecular patterns that have a high association with the highest risk of COVID-19 mortality.

CONCLUSIONS AND FUTURE PERSPECTIVE

This short perspective proposed a few possible complementary diagnostic approaches for the development of point-of-care devices for on-site early detection of COVID-19-infected patients at high risk of death for a range of reasons including the exacerbation of comorbidities. This is mainly due to the fact that different infection levels and/or disease type/stage can cause slight variations in the composition and characteristics of biological fluids such as tears, saliva, urine, and plasma; these, in turn, can be significantly magnified using our protein corona sensor array and magnetic levitation technologies.[33,19,20,58,59] The proposed approaches may eventually yield a sensitive, easy-to-use optical system to accurately identify COVID19-infected patients at high risk of death. For example, analyzing MagLev optical images via advanced machine-learning techniques might produce a "fingerprint" to distinguish COVID19-infected individuals who will recover on their own from those at risk of death from further complications. The proposed systems could also be combined with omic approaches in a unique approach to discover new biomarkers specific to complications of COVID-19 infection in that subset of patients at high risk of death. It is noteworthy that the multidisciplinary nature of the proposed approach requires the proactive contribution of many stakeholders[62] (access date: April 29, 2020). Such discrimination technologies could significantly improve both the management of health care resources (e.g., avoiding overwhelming hospitals) and improve our control of possible future pandemics without incurring such a large social and economic burden.

AUTHOR INFORMATION

Corresponding Author

Morteza Mahmoudi − *Precision Health Program and Department of Radiology, Michigan State University, East Lansing, Michigan 48824, United States;* orcid.org/0000-0002-2575-9684; Email: Mahmou22@msu.edu

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.molpharmaceut.0c00371

Notes

The author declares no competing financial interest.

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