Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Biosensors and Bioelectronics: X 13 (2023) 100307

Contents lists available at ScienceDirect

Biosensors and Bioelectronics: X

journal homepage: www.journals.elsevier.com/biosensors-and-bioelectronics-x

Multiplex sensing of IL-10 and CRP towards predicting critical illness in COVID-19 infections

Sasya Madhurantakam [a], Zachary J. Lee [a], Aliya Naqvi [a], Jayanth Babu Karnam [b], Sriram Muthukumar [b], Shalini Prasad [a,*]

[a] Department of Bioengineering, The University of Texas at Dallas, 75080, USA
[b] EnLiSense LLC, Allen, TX, USA

ARTICLE INFO

Keywords:
Biosensor
Electrochemical
Impedance
Inflammation
Biomarkers
COVID-19

ABSTRACT

Here we present a sensitive method for the detection and quantification of two (IL -10 and CRP) immunoresponsive biomarkers in various biofluids. The significance of these immune response biomarkers lies in them displaying elevated levels in critically ill COVID -19 patients. The developed electrochemical sensor contains a gold film electrode with ZnO nanoparticles deposited on its surface to increase the surface area of the working electrode while integrating antibody-antigen interactions into the detection system. This multiplex biosensor has a wide linear range from 0.01 μg/mL to 100 μg/mL and 0.1 pg/mL to 1000 pg/mL for CRP and IL10, respectively. The cross-reactivity of this multiplex sensor platform was evaluated between these two proteins and was <20%. Recovery studies were performed by spiking known concentrations in serum and urine samples. The recovery was calculated and ranged from 80% to 100%, confirming clinical applicability. This electrochemical sensing platform can aid in the early screening of COVID -19 patients to monitor for the development of more serious and potentially lethal symptoms.

1. Introduction

The clinical utility of biological markers that correlate with disease severity and mortality has long been explored. The interpretation of these biomarkers has shifted the clinical threshold for medical diagnosis. While there are a variety of sensing techniques that can be used for biomarker application, electrochemical sensors hold a strong appeal due to their electrical output that can be directly analyzed and interpreted. With the emergence of coronavirus disease in 2019 (COVID -19), electrochemical sensors may be key to early detection as well as tracking disease progression in patients (Ali, 2020). Though there are a variety of diagnostic measures for COVID -19, there is still a lack of severity prediction that can track or predict the evolution of a patient's symptoms. Currently, the international gold standard for detection is reverse transcription polymerase chain reaction (RT-PCR)(Benda et al., 2021). Real-time PCR RT-PCR or quantitative PCR (qPCR) is used to amplify and detect changes in amplicon concentration during sequencing, whereas conventional PCR assays detect these changes after sequencing. This assay method offers several advantages, expressed as high accuracy and sensitivity, low turnaround time, and small sample size required (Filchakova et al., 2022). While this diagnostic measure has impressive capabilities in diagnosing a patient with COVID -19, it lacks predictability in disease progression. To address this issue, biomarkers identification is an effective option in predicting disease progression. Associations between lymphopenia, thrombocytopenia, and elevated levels of C-reactive protein (CRP), PCT, LDH, and D-dimer have been studied with the severity of COVID-19 (Chilimuri et al., 2020; Malik et al., 2021; Santos et al., n.d.). In addition, major efforts have been made to identify the role of inflammatory cytokines in COVID-19, with increased production of IL-1, IL-2, IL-4, IL-6, IL-10, IL-12, IFN-γ, TNF-α, and other molecules, typically associated with lung tissue as a part of the COVID-19 cytokine signature (Costela-Ruiz et al., 2020). IL-6, IL-10, IFN-γ, and TNF-α have been specifically found to be potential COVID-19 severity predictors (del Valle et al., 2020; Han et al., 2020). These biomarkers have the potential to be used as early indicators of the development and severity of COVID-19 in a patient. This will allow the patients at high risk to be identified and allocated the necessary resources to as early as possible.

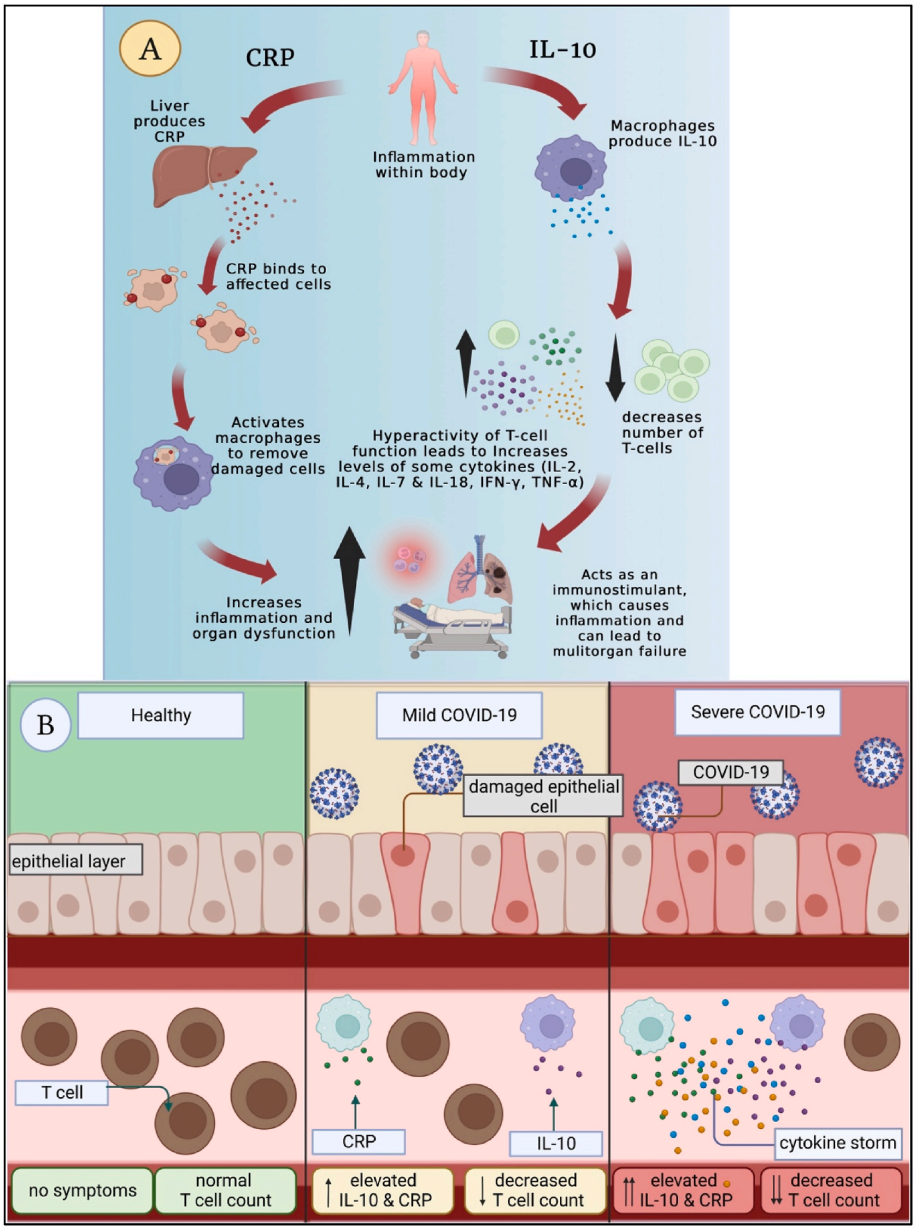
C-reactive protein (CRP) is an acute phase reactant (APR) that acts as a proinflammatory cytokine. CRP is synthesized mainly in liver

* Corresponding author.
E-mail addresses: sxp118230@utdallas.edu, shalini.prasad@utdallas.edu (S. Prasad).

https://doi.org/10.1016/j.biosx.2023.100307
Received 14 November 2022; Received in revised form 6 January 2023; Accepted 16 January 2023
Available online 26 January 2023

hepatocytes in response to infection and accompanying cytokines such as IL-6. During a host immune response, CRP enhances macrophage phagocytosis by binding to specific molecules (e.g. phosphocholines) often found on the surface of pathogens and other microorganisms (Marnell et al., 2005). CRP typically interacts with bacterial pathogens and damaged or dead cells which can trigger hyperaggressive inflammatory activity that results in damaged tissue as shown in Scheme. 1B. Specifically in COVID-19, CRP appears to be responsive to COVID-19 pneumonia leading it to react excessively to the large amount of damaged lung tissue cells in severe COVID-19 patients (Luan et al., 2021; Sheriff et al., 2021). CRP plays an important role in the activation of the C1q molecule which triggers several immune responses, most notably the increased synthesis of pro-inflammatory cytokines (Sproston and Ashworth, 2018). This increased level of cytokines results in an increase in inflammation, the excess of which can lead to organ dysfunction. Because of CRP's role in supplementing pro-inflammatory cytokines, CRP elevation can be used as an early indicator of inflammation(Ponti et al., 2020). An early indication of inflammation can predict the development of severe symptoms in admitted patients (Ali, 2020; Smilowitz et al., 2021). In addition to its ability to predict prognosis, CRP interpretation outperforms a physiological score of respiratory function (ROX) and CT scans in early prediction and indication of inflammatory development (Luan et al., 2021; Mueller et al., 2020).

Interleukin-10 (IL-10) belongs to group of inflammatory cytokines that have been presented as possible prognostic markers for COVID-19 disease severity (Dhar et al., 2021). While IL-10 typically regulates the host immune response to foreign pathogens (Couper et al., 2008; Saraiva et al., 2020; Subramanian Iyer and Cheng, 2012), there are conflicting observations in the current literature regarding its role as an immunosuppressant, implying its role within COVID-19 is pro-inflammatory (Islam et al., 2021). While its role as an immunostimulant in COVID-19 needs further investigation, several lines of evidence support this interpretation. First, increased levels of IL-10 have been associated with increases in pro-inflammatory cytokines (e.g., IFN-γ, IL-2, IL-4, IL-7, IL-18, TNF-α) (Lauw et al., 2000; Lu et al., 2021). Second, within COVID-19, there is a possibility of IL-10 induced hyperstimulation of



Scheme. 1. (A) Illustration of pathophysiology of IL-10 and CRP during COVID-19 symptom development. (B) Visual depiction of internal inflammatory processes caused by IL-10 and CRP during different COVID-19 developmental stages. Both images were created with biorender.com.

$CD8^+$ T cells (Wang et al., 2020). Hyperstimulation of $CD8^+$ T cells leads to both an increase in IFN-γ and other cytokine levels and T cell exhaustion(Rha and Shin, 2021). In addition, T-cell numbers have been shown to correlate inversely with the levels of IL-10, such that increased IL-10 levels could signify decreasing T cell numbers, a trend with prospective prognostic value (Moon, 2020). This combined increase in pro-inflammatory cytokines, triggered by high IL-10 levels results in increases inflammation that can lead to organ failure. Because of these factors, an increase in IL-10 and other cytokine levels may indicate the development of more severe disease and id an important prognostic measure for COVID-19.

The combination of IL-10 and CRP to monitor and predict the development of COVID-19 symptoms has great potential (Herold et al., 2020). With similar pathophysiological features (Scheme. 1A), IL-10 and CRP could unlock prognostic capabilities in COVID-19 that we do not currently have. Elevated levels of either may not be indicative of future symptom severity because the high incidence of CRP in viral infection is so abnormal, but COVID-19 patients who have high levels of both may be interpreted as an indicative of future development of severe symptoms. Nevertheless, caution is warranted because the combined biomarking abilities of IL-10 and CRP have not been fully explored.

Another respiratory infection that shows similar tendencies toward high IL-10 and CRP levels is pneumonia. Pneumonia plays a central role in the severity of COVID-19, and results in increased mortality in patients with COVID-19. Even in patients who had recovered or are recovering from COVID-19 pneumonia, long term respiratory complications often persist (George et al., 2020). Both IL-10 and CRP have been found in elevated levels in patients with pneumonia. IL-10 typically acts as a potential mediator during and after respiratory infection (van der Sluijs et al., 2004), while CRP indicates the severity of inflammation and potential respiratory complications (Karakioulaki and Stolz, 2019). Although pneumonia is not an infection strictly associated with COVID-19 cases, especially in critical condition, with COVID-19 to develop pneumonia (Scheme 1B).

Nanomaterials play a vital role in improving biosensor efficiency. Metal and metaloxide nanoparticles have been used in wide range of biosensing applications because of their chemical stability and high surface area(Madhurantakam et al., 2020). Among several metal-oxides, zinc oxide (ZnO) based biosensors have become the predominant material for sensing applications due to their remarkable features such as wider band gap, good electrical transport, and high exciton binding energy. ZnO is considered a good candidate for biosensor applications due to its chemical stability, non-toxic nature, high isoelectric point, cost effectiveness. Due to high isoelectric point, ZnO has higher uptake of biomolecules such as enzymes, proteins, antibodies and DNA or RNA through electrostatic interactions(Ahmadivand et al., 2021; Gwiazda et al., 2022; Sharma et al., 2021). ZnO has been considered as multifunctional nanomaterial because of its utilization in wider number of analytical detection methods such as optical, mass-based and electrochemical sensors. Among all these sensor types, electrochemical biosensors have attracted more attention in the field of healthcare due to their capability of rapid response and early diagnosis. Here, in this article we functionalized antibodies using a cross-linker molecule on ZnO deposited gold electrode. Deposition of ZnO on gold electrode will increase the surface area without drastically increasing the volume of the electrodes. This increase in surface area allows a greater amount of antibody to bind to the ZnO surface, resulting in a wider detection range. In addition, the nanoparticles enable an increase in the linear detection range, covering both low and high sensitivities. The ability to interpret biomarkers in both the high and low ranges provide a comprehensive understanding of a patient's condition which is necessary for appropriate treatment and medication. This sensor platform is the first step towards a multiplexed sensor that with capabilities to both detect and predict the development of COVID-19 in a patient. The inclusion of additional cytokines such as IL-6, IL-8, IFN-y, and many others would allow for greater accuracy and sensitivity in COVID-19 specific situations (Madhurantakam et al., 2022).

Industrial-standard diagnostics are currently available for the interpretation in COVID-19 patients, and their traditional clinical workflows are shown in (Scheme 1). In defining the performance of immunoassays, it can be quantified by their specificity (SP) and sensitivity (SE). Industry standards such as the Enzyme-Linked Immunosorbent Assay (ELISA) and LUMINEX multiplex bead array assays provide effective but inefficient means of biomarker detection. ELISA provides highly sensitive and specific quantitative measurement of proteins. While it can only detect a single analyte specifically and requires a large sample volume, it provides an incredibly sensitive measurement of the single target protein. Unfortunately, ELISA remains inefficient for COVID-19 testing due to its unbalanced performance and low-cost effectiveness (Santano et al., 2021). In contrast, LUMINEX assays were developed to provide a more cost-effective and time-efficient alternative. In addition, LUMINEX allows multiplex detection, meaning that one sample can be used to detect multiple targets. While this allows quantification of a larger number of molecules, it has shown lower sensitivity for the molecules measured. In addition, there is evidence that the manufacturer's protocol for sample volume can be reduced by half without compromising quantitative reliability (Mountjoy, 2021). Both have their strengths depending on different experiments, both have long response times before patients receive test results. LUMINEX and ELISA typically require 5–8 h, but however more specialized tests can take up to 2–3 days (Fig. 1).

In comparison, our multiplexed sensor platform presents a more cost-effective and time-efficient solution than either of the current standards. Optimized for use as a point-of-care diagnostic measure, our sensor allows a small sample (100 μL) to be applied to the surface with an incubation time of 10 min (Fig. 1). Depending on the levels of these biomarkers at the time of admission clinicians can assess the patient inflammation levels, which can help to provide time-efficient treatments. If CRP levels remain stable and do not increase within the first 48 h of hospitalization, then this indicates stable disease state (Mueller et al., 2020). If the levels of IL-10, remain stable within the first 50–100 h of admission, then this too predicts stable symptom. If rise in these two levels observed, then it indicates the severe disease progression. Currently, our multiplex system incorporates two inflammatory cytokines, but future development aims to utilize up to 16 different markers. The use of a wide range of inflammatory cytokines (IL-2, IL-6, IL-8, IFN-γ, TNF-α) and other biomarkers would allow more sensitive and specific prognostic COVID-19 severity diagnostics.

1.1. Materials & methods

Dithiobis(succinimidyl propionate) (DSP), Phosphate buffer saline (PBS), dimethyl sulfoxide (DMSO), and Tween-20 were purchased from Thermo Fisher Scientific, USA. IL-10 and CRP proteins and their respective antibodies were purchased from Abcam, USA. Healthy human pooled plasma was purchased from Innovative Research, Inc (USA). All of these proteins, antibodies, and pooled human serum and urine samples were stored according to their respective storage instructions until further use. All antibodies were diluted in PBS, and various antigen concentrations were added to pooled human serum to generate a calibrated dose-response curve and to perform other sensor property studies. To avoid protein denaturation, freeze-thaw cycles were limited to less than 3.

1.2. Immunoassay development

Our sensor surface was initially functionalized using 10 mM DSP which was dissolved in DSMO and incubated at room temperature away from light. Specific antibodies tailored towards IL-10 and CRP were subsequently functionalized onto the sensor surface as well. Superblock was used to prevent non-specific interactions with the DSP linkers. Superblock was used to cut-off the non-specific binding of target to DSP crosslinker. Calibrated dose response and spike and recovery studies

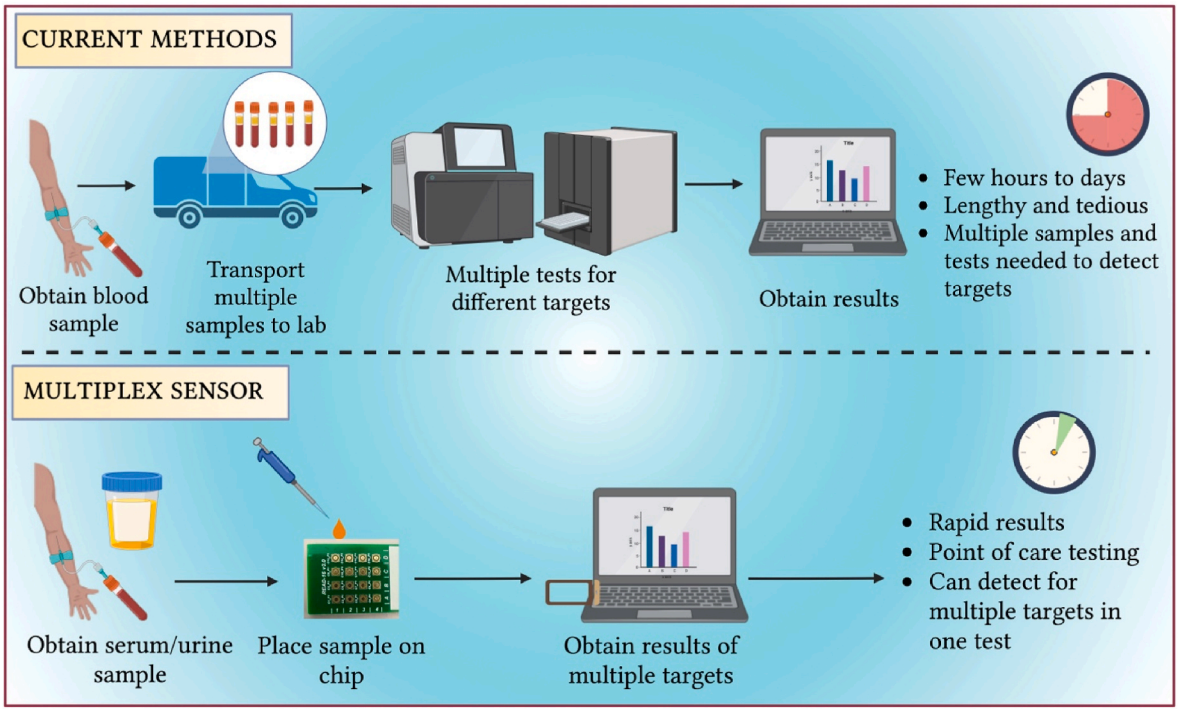


Fig. 1. Illustration depicting current clinical chronology vs. multiplex sensor timeline utilizes rapid bedside point-of-care approach as a disease severity prognostic. Image was created with biorender.com.

were then established and assessed against each other in both urine and serum samples. The response was quantified as a percent difference from an initially set baseline, typically a zero dose. Optimal sample size was established to be 100 μL for full 16-electrode coverage.

1.3. Experimental design for validating binding chemistry

To ensure successful immobilization and binding of antibodies with the crosslinker molecule (DSP) on the gold working electrode, attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was used to confirm the binding chemistry. Using this technique, the infrared spectra of the DSP crosslinker and CRP antibody and IL-10 antibody were obtained, and the characteristics peaks were identified to validate the binding chemistry. DSP crosslinker, CRP antibody bound to DSP and IL-10 antibody bound to DSP were studied.

1.4. Electrochemical studies

Electrochemical impedance spectroscopy was used to measure the response of the sensor. Electrode array consists of 16 gold electrodes individually surrounded by gold reference electrode. The impedance response is measured by the subtle changes between the antibody-antigen affinity interaction when a small input voltage (10 mV) in the frequency range of 1000–80Hz is applied to the sensor.

1.5. Statistical analysis

All the data presented in the manuscript as mean ± SEM, n = 3. Statistical representation followed as ns: not significant, *p 0.05, **p 0.01, ***p 0.001, ****p 0.0001. The calibrated dose response was analyzed by asymmetric nonlinear fitting (5 parameters). One-way ANNOVA was performed to measure the difference in significance between low and high range in calibrated dose responses. All statistical calculations were performed using GraphPad Prism.

2. Results and discussion

The characterization of ZnO nanostructures functionalized with DSP and immobilized antibodies for IL-10 and CRP. Fig. 2 shows the FTIR spectra of all combinations DSP-CRP antibody (A) conjugate, DSP-IL-10 antibody (B) conjugate and DSP crosslinker superimposed. All significant peaks used to validate successful bonding of the crosslinker, and antibody capture probes are annotated and presented in S. Table1. As shown in Fig. 2, the spectrum was recorded in the wavelength range of 500–4000 cm^{-1}. The functionalization of DSP on the ZnO surface was confirmed by the presence of thiol bonds around 2900-3100 cm^{-1}. The peak at 2930 cm^{-1} and 3010 cm^{-1} indicates the presence of saturated and unsaturated C–H stretches respectively. This confirms the binding of DSP to ZnO. The shift of the peak from 1677 to 1640 cm^{-1} and 1683 to 1650 cm^{-1} in IL10 and CRP was observed with increasing intensity, confirming the immobilization of the antibody on the ZnO surface by DSP through the formation of an amide bond.

Currently, there are no point-of-care prognostic devices for predicting COVID-19 inflammatory status and for follow-up. Because elevated levels of IL-10 and CRP are established early indicators of inflammation in COVID-19 patients, a sensing platform targeting these inflammatory markers would aid clinicians in COVID-19 development prediction. Consequently, the first prognostic devices used to assess disease development and management should have both high sensitivity and broad coverage for their target markers. A broad range is defined to include both healthy physiological levels and irregular elevations in the disease state of each biomarker.

Since, an impedance response generated by an antibody-antigen interaction is used to measure concentration, optimization of antibody concentration on the sensor surface is vital. An antibody saturation study was performed to determine the optimal antibody concentration and incubation time. Four different antibody concentrations ie; 25 μg/mL, 50 μg/mL, 100 μg/mL and 150 μg/mL for CRP and 1 ng/mL, 2.5 ng/mL, 5 ng/mL and 10 ng/mL for IL-10 were immobilized on sensor surface. As shown in Fig. 3 (A-B), the response was significantly higher when higher antibody concentrations (150 μg/mL and 10 ng/mL for

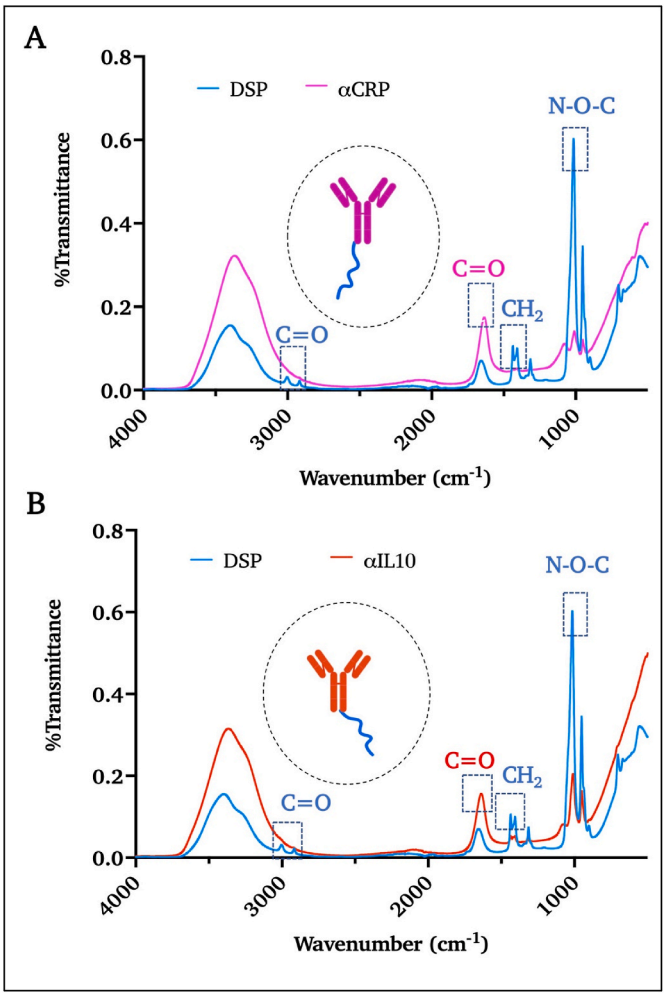


Fig. 2. FTIR characterization of functionalized DSP surface and antibody immobilized surface with CRP(A) and IL-10(B) antibodies.

CRP and IL-10 respectively) were used. In addition, the variation in impedance response between different antibody incubation times was evaluated and found that an incubation time of 2 h provided the most optimal response. The incubation time allows the antibody to interact with the DSP treated ZnO surface, and typically the longer the incubation time the greater volume of interactions will occur. However, the incubation time tests showed that there was negligible difference in response output between 2 h and longer incubation periods. This was the perfect incubation time as it both gave a high response and allowed the sensor to be used only after a couple hours.

With established antibody concentrations and incubation times found, elected to evaluated sensor functionality by incrementally increasing dose concentrations for our two analytes as a calibrated dose response (CDR) study. Calibrated dose concentrations study demonstrated a high sensitivity for both markers with a wide concentration range. This wide concentration range allows for more flexibility and stronger capability in monitoring and distinguishing healthy, moderate, and severely affected patients. Additionally, as different body fluids have utilized in establishing calibrated doses, this allows for sensor utilization in multiple body fluids. CDR in PBS buffer produced a wide range of detection for CRP and IL-10 finding 0.01 μg/mL to 100 μg/mL and 0.1 pg/mL to 1000 pg/mL respectively as shown in Fig. 2 (C-D). An increase in the percentage change with respect to zero dose was observed with increasing concentrations from low to high. A significant difference between low (0.1 pg/mL) and high (1000 pg/mL) concentration with p value of <0.0001 was observed for IL-10 with a regression co-efficient value of 0.96. Similarly for CRP, significant difference with p value of <0.05 was observed between 0.01 μg/mL and 100 μg/mL and a regression co-efficient of 0.92. This robust response demonstrates capable detection of both highly elevated and depressed levels of each biomarker providing full coverage of different disease states.

With the establishment of a reliable and sensitive response and range, the accuracy of our multiplex sensing platform was also evaluated. By spiking known concentrations onto the sensor, percentage response was calculated and used to measure the unknown concentration from the established calibrated dose responses. Spiked concentrations were compared with the recovered concentrations to evaluate the sensor accuracy. The accuracy of our platform was measured in both serum and urine. This flexibility between the two kinds of body fluids would allow for a potentially more flexible point-of-care system. While serum biomarkers have incredibly potency as disease prognostics, the ease of collection and utilization of serum has proven more difficult than that of urine. As a developed bedside point-of-care prognostic, flexibility is an essential trait for this device. As shown in Fig. 4 (A-B), the linear calibration curve provides visualization of the sensor response against spiked concentrations and calculated recovered concentrations. Recovered concentration of CRP in both serum and urine were (Fig. 4A and C) displayed a correlation with an R^2 value of 0.99 while the IL-10 in serum (Fig. 4B) urine (Fig. 4D) showed an R^2 value of 0.99 and 0.94 respectively.

Beyond established sensitivity and accuracy, specificity of each antibody to its own respective analyte must be evaluated as well. This was accomplished through two cross-reactivity studies that assessed each antibody respectively. Exemplified in Fig. 5, the response given by an antibody to differing quantities of specific and non-specific molecules was quantified. This incorporated low and high non-specific concentrations with a set specific concentration. In addition to these, the sensors were assessed with a concoction of a set specific concentration with low or high non-specific concentrations to validate the sensing platform specificity. Displayed in Fig. 5, the impedance response of both antibodies exemplified negligible cross-sensitivity between the two antigens. While there is a mild response to just the presence of non-specific molecules, the quantitative impact upon the response when specific molecules are present is insignificant.

In addition to accuracy and specificity, repeatability and reproducibility were assessed in our multiplexed platform. Single electrode sensors are typically assessed with repeatability and reproducibility being established as variation within the same sensor and variation between separate sensors respectively. However, due to the multiplexed and multi-electrode of our sensing platform, repeatability and reproducibility were established as within an electrode and between electrodes on the sensor surface respectively. The coefficient of variation (%CV) was calculated in both target markers for each medium to assess any variation within and between sensors. Typically, the lower concentration thresholds exemplified higher variability with all %CV exhibited between a range of ~1.5%–17.9%. As shown in Fig. 6, both markers exhibited a reproducibility (A&B) and repeatability (C&D) with a %CV $< 20\%$, sitting within the acceptable clinical threshold set by the Clinical and Laboratory Standards Institute (CLSI) guidelines (McEnroe et al., n. d.). These results demonstrate our multiplexed platform's accurate, repeatable, and reproducible detection within its range determined by our previously established CDR.

Finally, the quantification and analysis of the percent recovery was implemented as a supplement to the accuracy of the sensor. As shown in Fig. 7, CRP in serum showed a recovery percentage between 95% and 102% and in urine it ranged between 72% and 125%. Whereas IL-10 in urine showed a recovery range between 75 and 98% and in serum 81–112%. This demonstrates consistency and low variability regarding the calibrated response to IL-10 and CRP. Currently, within our knowledge, this is the first demonstrated multiplex biomarker sensor targeting CRP and IL-10 for point-of-care prognostic use with reliable sensor performance. Additionally, our sensing platform aims to explore

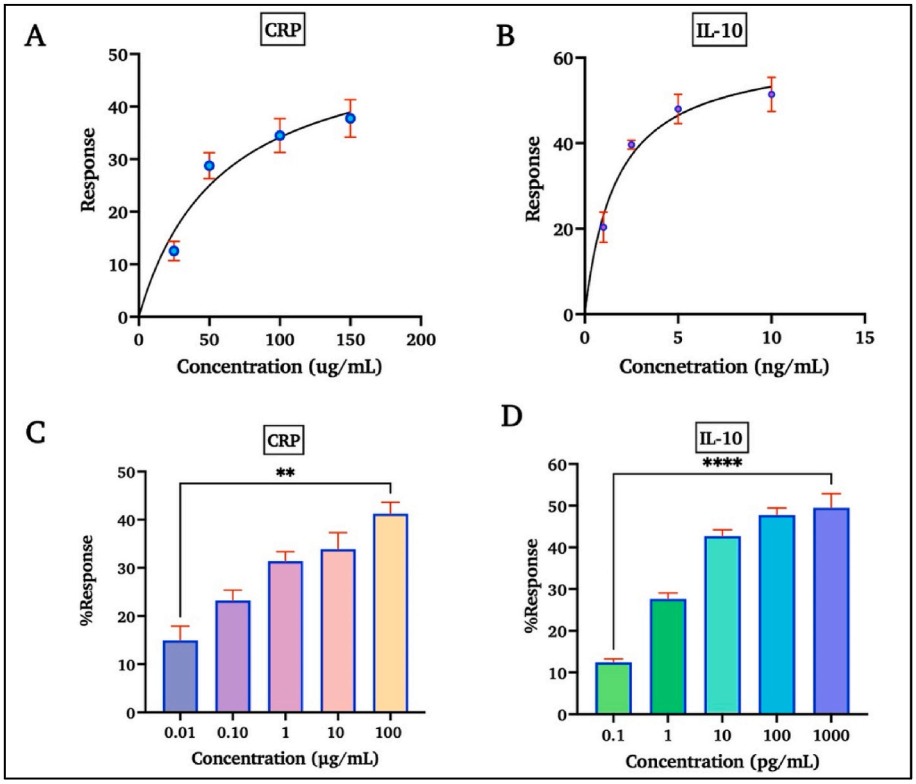


Fig. 3. (A–B) Antibody saturation displaying an impedance response curve in correlation to increasing antibody concentration. (C–D) Calibrated dose response for IL-10 and CRP at incrementally increased concentrations.

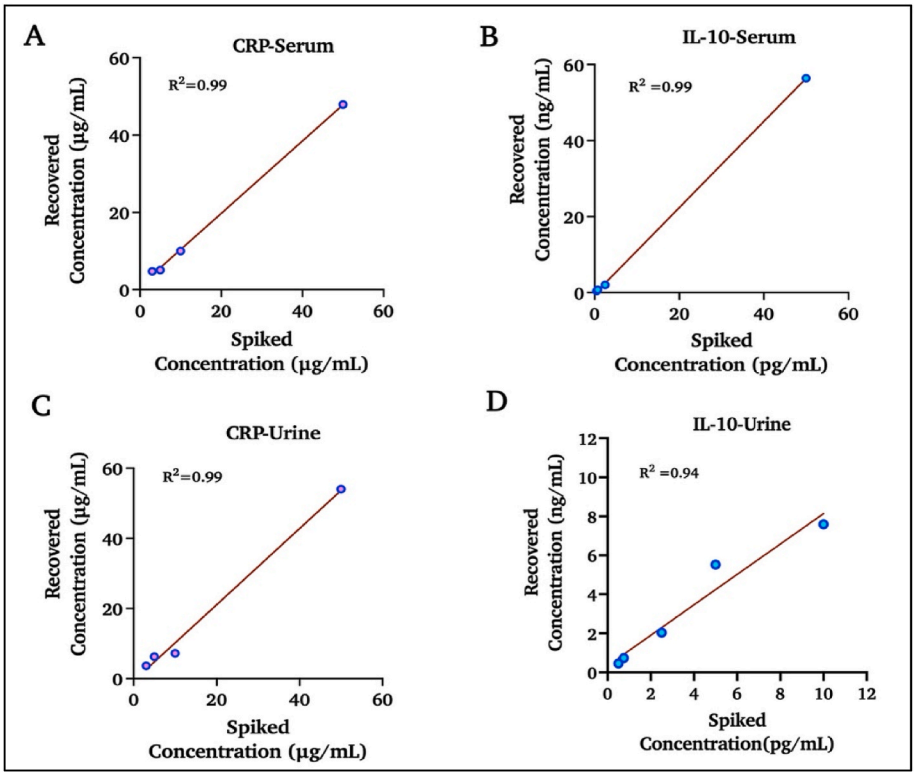


Fig. 4. Actual spiked concentrations plotted against measured concentrations in serum (A–B) and urine (C–D) as a correlation plot for both CRP and IL-10.

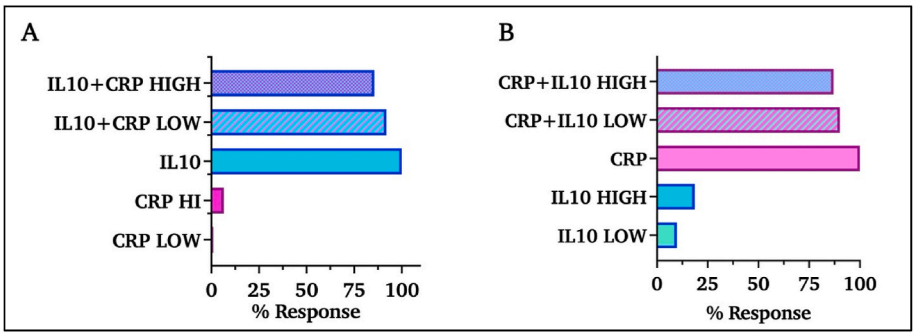


Fig. 5. Cross reactivity study utilized to assess specificity of multiplexed platform with high/low nonspecific, specific, and combined high/low nonspecific and specific biomarker mixtures. A) analysis of IL-10 specific antibody. B) analysis of CRP specific antibody.

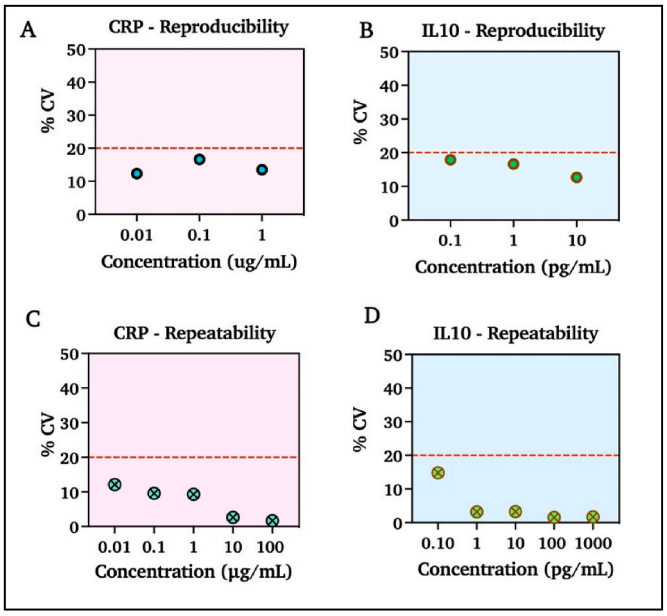


Fig. 6. Coefficient of variation plots examining the precision of both IL-10 and CRP. Dotted red line at 20% signifies the CLSI acceptable clinical limit. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

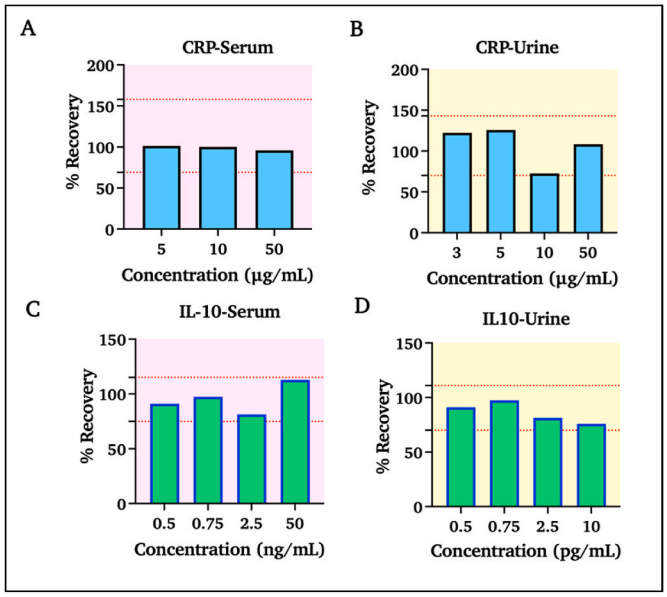


Fig. 7. Repeatability and reproducibility plots assessing the percent recovery of IL-10 and CRP in serum (A–B) and urine (C–D). Dotted red lines signify bounds of acceptable threshold. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

the potential prognostic capabilities of urine biomarker detection as its collection and analysis hold higher practicality for bedside patient monitoring.

3. Conclusions and future scope

With respect to the results provided in the manuscript, we achieved the uniform distribution of ZnO thin film on gold working electrode and functionalized the Au–ZnO sensor surface with crosslinker and antibodies successfully. The developed portable point-of-care sensing platform can specifically detect IL-10 and CRP proteins from blood matrices within a short time using <100 µL of sample volume. Wide linear detection ranges of 0.01–100 µg/mL for CRP and 0.1–1000 pg/mL for IL-10. Also, the developed biosensor exhibited good correlation in estimating unknown spiked concentrations in both buffers proving the biosensor efficiency in all biofluids. Moreover, multiplex detection of CRP and IL-10 provides comprehensive inflammatory status of individual for personalized treatment. Additionally, this combination of proteins helps to track the inflammation status not only in COVID-19 conditions, but also in several other infectious diseases. Patient stratification based on these inflammatory biomarkers is a key factor for proceeding to treatment modalities very quickly.

In future, continuous monitoring of these biomarkers can be achieved along with integration of this portable platform to internet of things, that can help to track patients' inflammation status in an effective manner(Chaudhary et al., 2023). More efforts are needed to develop multiplex sensing platform for detection more than two biomarkers across multiple biofluids. Implementation of the current developed biosensing platform as a base to detect other inflammatory biomarkers in biofluids could lead to an evolutionary state of point-of-care devices which can be used across many infectious and inflammatory diseased conditions.

CRediT authorship contribution statement

Sasya Madhurantakam: Conceptualization, Resources, Methodology, Validation, Data curation, Investigation, Writing – original draft. **Zachary J. Lee:** Writing – original draft, Data curation, Validation. **Aliya Naqvi:** Writing – original draft, Data curation, Validation. **Jayanth Babu Karnam:** Resources, Writing – review & editing. **Sriram Muthukumar:** Conceptualization, Resources, Formal analysis, Writing – review & editing. **Shalini Prasad:** Conceptualization, Resources, Methodology, Formal analysis, Writing – review & editing.

Declaration of competing interest

Shalini Prasad and Sriram Muthu Kumar have a significant interest in EnLiSense LLC, a company that may have a commercial interest in the results of this research and technology. The potential individual conflict of interest has been reviewed and managed by The University of Texas at Dallas and played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

Data availability

No data was used for the research described in the article.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biosx.2023.100307.

References

Ahmadivand, A., Gerislioglu, B., Ramezani, Z., Kaushik, A., Manickam, P., Ghoreishi, S. A., 2021. Functionalized terahertz plasmonic metasensors: femtomolar-level detection of SARS-CoV-2 spike proteins. Biosens. Bioelectron. 177 https://doi.org/10.1016/j.bios.2021.112971.

Ali, N., 2020. Elevated level of C-reactive protein may be an early marker to predict risk for severity of COVID-19. J. Med. Virol. https://doi.org/10.1002/jmv.26097.

Benda, A., Zerajic, L., Ankita, A., Cleary, E., Park, Y., Pandey, S., 2021. COVID-19 testing and diagnostics: a review of commercialized technologies for cost, convenience and quality of tests. Sensors. https://doi.org/10.3390/s21196581.

Chaudhary, V., Khanna, V., Ahmed Awan, H.T., Singh, K., Khalid, M., Mishra, Y.K., Bhansali, S., Li, C.Z., Kaushik, A., 2023. Towards hospital-on-chip supported by 2D MXenes-based 5th generation intelligent biosensors. Biosens. Bioelectron. https://doi.org/10.1016/j.bios.2022.114847.

Chilimuri, S., Sun, H., Alemam, A., Mantri, N., Shehi, E., Tejada, J., Yugay, A., Nayudu, S. K., 2020. Predictors of mortality in adults admitted with COVID-19: retrospective cohort study from New York City. West. J. Emerg. Med. 21, 779–784. https://doi.org/10.5811/westjem.2020.6.47919.

Costela-Ruiz, V.J., Illescas-Montes, R., Puerta-Puerta, J.M., Ruiz, C., Melguizo-Rodríguez, L., 2020. SARS-CoV-2 infection: the role of cytokines in COVID-19 disease. Cytokine Growth Factor Rev. https://doi.org/10.1016/j.cytogfr.2020.06.001.

Couper, K.N., Blount, D.G., Riley, E.M., 2008. IL-10: the master regulator of immunity to infection. J. Immunol. 180, 5771–5777. https://doi.org/10.4049/jimmunol.180.9.5771.

del Valle, D.M., Kim-Schulze, S., Huang, H.H., Beckmann, N.D., Nirenberg, S., Wang, B., Lavin, Y., Swartz, T.H., Madduri, D., Stock, A., Marron, T.U., Xie, H., Patel, M., Tuballes, K., van Oekelen, O., Rahman, A., Kovatch, P., Aberg, J.A., Schadt, E., Jagannath, S., Mazumdar, M., Charney, A.W., Firpo-Betancourt, A., Mendu, D.R., Jhang, J., Reich, D., Sigel, K., Cordon-Cardo, C., Feldmann, M., Parekh, S., Merad, M., Gnjatic, S., 2020. An inflammatory cytokine signature predicts COVID-19 severity and survival. Nat. Med. 26, 1636–1643. https://doi.org/10.1038/s41591-020-1051-9.

Dhar, S.K., K, V., Damodar, S., Gujar, S., Das, M., 2021. IL-6 and IL-10 as predictors of disease severity in COVID-19 patients: results from meta-analysis and regression. Heliyon 7. https://doi.org/10.1016/j.heliyon.2021.e06155.

Filchakova, O., Dossym, D., Ilyas, A., Kuanysheva, T., Abdizhamil, A., Bukasov, R., 2022. Review of COVID-19 testing and diagnostic methods. Talanta. https://doi.org/10.1016/j.talanta.2022.123409.

George, P.M., Barratt, S.L., Condliffe, R., Desai, S.R., Devaraj, A., Forrest, I., Gibbons, M. A., Hart, N., Jenkins, R.G., McAuley, D.F., Patel, B.v., Thwaite, E., Spencer, L.G., 2020. Respiratory follow-up of patients with COVID-19 pneumonia. Thorax 75, 1009–1016. https://doi.org/10.1136/thoraxjnl-2020-215314.

Gwiazda, M., Kaushik, A., Chlanda, A., Kijeńska-Gawrońska, E., Jagiełło, J., Kowiorski, K., Lipińska, L., Święszkowski, W., Bhardwaj, S.K., 2022. A flexible immunosensor based on the electrochemically rGO with Au SAM using half-antibody for collagen type I sensing. Appl. Surf. Sci. Adv. 9 https://doi.org/10.1016/j.apsadv.2022.100258.

Han, H., Ma, Q., Li, C., Liu, R., Zhao, L., Wang, W., Zhang, P., Liu, X., Gao, G., Liu, F., Jiang, Y., Cheng, X., Zhu, C., Xia, Y., 2020. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg. Microb. Infect. 9, 1123–1130. https://doi.org/10.1080/22221751.2020.1770129.

Herold, T., Jurinovic, V., Arnreich, C., Lipworth, B.J., Hellmuth, J.C., von Bergwelt-Baildon, M., Klein, M., Weinberger, T., 2020. Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. J. Allergy Clin. Immunol. 146, 128–136.e4. https://doi.org/10.1016/j.jaci.2020.05.008.

Islam, H., Chamberlain, T.C., Mui, A.L., Little, J.P., 2021. Elevated interleukin-10 levels in COVID-19: potentiation of pro-inflammatory responses or impaired anti-inflammatory action? Front. Immunol. 12 https://doi.org/10.3389/fimmu.2021.677008.

Karakioulaki, M., Stolz, D., 2019. Biomarkers in pneumonia-beyond procalcitonin. Int. J. Mol. Sci. https://doi.org/10.3390/ijms20082004.

Lauw, F.N., Pajkrt, D., Hack, C.E., Kurimoto, M., van Deventer, S.J.H., van der Poll, T., 2000. Proinflammatory effects of IL-10 during human endotoxemia. J. Immunol. 165, 2783–2789. https://doi.org/10.4049/jimmunol.165.5.2783.

Lu, L., Zhang, H., Dauphars, D.J., He, Y.W., 2021. A potential role of interleukin 10 in COVID-19 pathogenesis. Trends Immunol. https://doi.org/10.1016/j.it.2020.10.012.

Luan, Y.Y., Yin, C.H., Yao, Y.M., 2021. Update advances on C-reactive protein in COVID-19 and other viral infections. Front. Immunol. https://doi.org/10.3389/fimmu.2021.720363.

Madhurantakam, S., Karnam, J.B., Brabazon, D., Takai, M., Ahad, I.U., Balaguru Rayappan, J.B., Krishnan, U.M., 2020. Nano": an emerging avenue in electrochemical detection of neurotransmitters. ACS Chem. Neurosci. 11, 4024–4047. https://doi.org/10.1021/ACSCHEMNEURO.0C00355/ASSET/IMAGES/LARGE/CN0C00355_0009 (JPEG).

Madhurantakam, S., Muthukumar, S., Prasad, S., 2022. Emerging electrochemical biosensing trends for rapid diagnosis of COVID-19 biomarkers as point-of-care platforms: a critical review. https://doi.org/10.1021/acsomega.2c00638.

Malik, P., Patel, U., Mehta, D., Patel, N., Kelkar, R., Akrmah, M., Gabrilove, J.L., Sacks, H., 2021. Biomarkers and outcomes of COVID-19 hospitalisations: systematic review and meta-analysis. BMJ Evid Based Med. https://doi.org/10.1136/bmjebm-2020-111536.

Marnell, L., Mold, C., du Clos, T.W., 2005. C-reactive protein: ligands, receptors and role in inflammation. Clin. Immunol. https://doi.org/10.1016/j.clim.2005.08.004.

McEnroe, R.J., Paul Durham Marc Goldford Marina V Kondratovich, A.D., Lababidi, S., Magari, R., Guy Middle, J., Pierson-Perry Jeffrey E Vaks, J.F., n.d. Evaluation of Precision of Quantitative Measurement Procedures; Approved Guideline-third ed..

Moon, C., 2020. Fighting COVID-19 exhausts T cells. Nat. Rev. Immunol. 20, 277. https://doi.org/10.1038/s41577-020-0304-7.

Mountjoy, K.G., 2021. ELISA versus LUMINEX assay for measuring mouse metabolic hormones and cytokines: sharing the lessons I have learned. J. Immunoassay Immunochem. 42, 154–173. https://doi.org/10.1080/15321819.2020.1838924.

Mueller, A.A., Tamura, T., Crowley, C.P., DeGrado, J.R., Haider, H., Jezmir, J.L., Keras, G., Penn, E.H., Massaro, A.F., Kim, E.Y., 2020. Inflammatory biomarker trends predict respiratory decline in COVID-19 patients. Cell Rep Med 1. https://doi.org/10.1016/j.xcrm.2020.100144.

Ponti, G., Maccaferri, M., Ruini, C., Tomasi, A., Ozben, T., 2020. Biomarkers associated with COVID-19 disease progression. Crit. Rev. Clin. Lab Sci. https://doi.org/10.1080/10408363.2020.1770685.

Rha, M.S., Shin, E.C., 2021. Activation or exhaustion of CD8+ T cells in patients with COVID-19. Cell. Mol. Immunol. https://doi.org/10.1038/s41423-021-00750-4.

Santano, R., Barrios, D., Crispi, F., Crovetto, F., Vidal, M., Chi, J., Izquierdo, L., Gratacós, E., Moncunill, G., Dobaño, C., 2021. Agreement between commercially available ELISA and in-house Luminex SARS-CoV-2 antibody immunoassays. Sci. Rep. 11 https://doi.org/10.1038/s41598-021-98296-y.

Santos, C.S., Morales, C.M., Díez Álvarez, E., Castro, & C.Á., Robles, & A.L., Perez Sandoval, T., n.d. Determinants of COVID-19 disease severity in patients with underlying rheumatic disease. https://doi.org/10.1007/s10067-020-05301-2/(Published).

Saraiva, M., Vieira, P., O'Garra, A., 2020. Biology and therapeutic potential of interleukin-10. J. Exp. Med. 217 https://doi.org/10.1084/jem.20190418.

Sharma, P.K., Kim, E.S., Mishra, S., Ganbold, E., Seong, R.S., Kaushik, A.K., Kim, N.Y., 2021. Ultrasensitive and reusable graphene oxide-modified double-interdigitated capacitive (DIDC) sensing chip for detecting SARS-CoV-2. ACS Sens. https://doi.org/10.1021/acssensors.1c01437.

Sheriff, A., Kayser, S., Brunner, P., Vogt, B., 2021. C-reactive protein triggers cell death in ischemic cells. Front. Immunol. https://doi.org/10.3389/fimmu.2021.630430.

Smilowitz, N.R., Kunichoff, D., Garshick, M., Shah, B., Pillinger, M., Hochman, J.S., Berger, J.S., 2021. C-reactive protein and clinical outcomes in patients with COVID-19. Eur. Heart J. 42, 2270–2279. https://doi.org/10.1093/eurheartj/ehaa1103.

Sproston, N.R., Ashworth, J.J., 2018. Role of C-reactive protein at sites of inflammation and infection. Front. Immunol. https://doi.org/10.3389/fimmu.2018.00754.

Subramanian Iyer, S., Cheng, G., 2012. Role of Interleukin 10 Transcriptional Regulation in Inflammation and Autoimmune Disease.

van der Sluijs, K.F., van Elden, L.J.R., Nijhuis, M., Schuurman, R., Pater, J.M., Florquin, S., Goldman, M., Jansen, H.M., Lutter, R., van der Poll, T., 2004. IL-10 is an important mediator of the enhanced susceptibility to pneumococcal pneumonia after influenza infection. J. Immunol. 172, 7603–7609. https://doi.org/10.4049/jimmunol.172.12.7603.

Wang, F., Hou, H., Luo, Y., Tang, G., Wu, S., Huang, M., Liu, W., Zhu, Y., Lin, Q., Mao, L., Fang, M., Zhang, H., Sun, Z., 2020. The laboratory tests and host immunity of COVID-19 patients with different severity of illness. JCI Insight 5. https://doi.org/10.1172/JCI.INSIGHT.137799.