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# Advancement in biosensors for inflammatory biomarkers of SARS-CoV-2 during 2019–2020

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## ABSTRACT

COVID-19 pandemic has affected everyone throughout the world and has resulted in the loss of lives of many souls. Due to the restless efforts of the researchers working hard day and night, some success has been gained for the detection of virus. As on date, the traditional polymerized chain reactions (PCR), lateral flow devices (LFID) and enzyme linked immunosorbent assays (ELISA) are being adapted for the detection of this deadly virus. However, a more exciting avenue is the detection of certain biomarkers associated with this viral infection which can be done by simply re-purposing our existing infrastructure. SARS-CoV-2 viral infection triggers various inflammatory, biochemical and hematological biomarkers. Because of the infection route that the virus follows, it causes significant inflammatory response. As a result, various inflammatory markers have been reported to be closely associated with this infection such as C-reactive proteins, interleukin-6, procalcitonin and ferritin. Sensing of these biomarkers can simultaneously help in understanding the illness level of the affected patient. Also, by monitoring these biomarkers, we can predict the viral infections in those patients who have low SARS-CoV-2 RNA and hence are missed by traditional tests. This can give more targets to the researchers and scientists, working in the area of drug development and provide better prognosis. In this review, we propose to highlight the conventional as well as the non-conventional methods for the detection of these inflammatory biomarkers which can act as a single platform of knowledge for the researchers and scientists working for the treatment of COVID-19.

## 1. Introduction

COVID-19 outbreak has wreaked the havoc on humanity. The outbreak started with the first report of pneumonia of unknown origin in China on December 31, 2019, and thereafter the number of COVID-19 infection cases has gone well above 36 million (as on October 08, 2020) and are increasing day by day. Since its beginning, the terminology used to describe this viral infection by World Health Organization (WHO) has also seen a change. According to the information provided by World Health Organization on its website, the viral infection was referred to as Novel Coronavirus (2019-nCoV) from the beginning till February 11, 2020 (Situation report-22) but afterwards it is being referred to as Coronavirus disease 2019 (COVID-19) till date. It is also interesting to note that WHO used to publish Situation Report daily till August 16, 2020, but afterwards this has been changed to a weekly report (1 report in 3 days). Also, during this entire period, there

has been a tremendous change in the policies throughout the globe and many countries have observed lockdowns to prevent the spread of this contagious virus.

Basically, COVID-19 is caused by Severe Acute Respiratory Syndrome-Coronavirus 2 (SARS-CoV-2) virus particle whose sequences show a close resemblance to severe acute respiratory syndrome-related coronaviruses (SARS-CoV) and belongs to the Betacoronavirus genera. This is a single stranded RNA virus enclosed by a capsid made of proteins, which are attractive targets for the potential drugs (Kang et al., 2020). The spike glycoproteins present on the surface of the virus attacks the receptors on the target host and facilitate binding with those cells (Mousavizadeh and Ghasemi, 2020). The entry of the SARS-CoV-2 virus inside the host cell is a complex system and involves viral spike proteins, angiotensin-converting enzyme 2 (ACE2) and host proteases (Mahmoud et al., 2020). The spike proteins present on the virus surface binds to the ACE2 receptors present on the human cells (host). This is activated by

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human proteases which helps in the entry of virus into the host cells (Shang et al., 2020). Through study it has been shown that this virus not only affects the respiratory system but also causes damage to digestive, urogenital, circulatory and central nervous system (Zhang et al., 2020). Several hematological, inflammatory and biochemical biomarkers have been associated with the COVID-19 infections (Ponti et al., 2020) as is shown in Fig. 1. The figure highlights the conventional method such as ELISA and non-conventional methods such as electrochemical and optical methods for the detection of the mentioned inflammatory biomarkers.

Hematological biomarkers that have been found to be involved in SARS-CoV-2 disease are lymphocyte count, neutrophil count and the ratio of lymphocyte to neutrophil count. Various studies have shown that in case of critically ill COVID-19 patients, lymphopenia (low number of lymphocytes) is observed (Huang and Pranata, 2020). However, in case of mild patients, this condition is not that grave (Chen et al., 2020).

The biochemical biomarkers include D-dimer, troponin and creatine kinase. The levels of D-dimer are indicative of disease severity as is shown in a study by Yao et al., (2020). Significant evidence regarding the elevated levels of troponin in COVID-19 related infections is also available (Tersalvi et al., 2020). A work by Chan et al. states that the COVID-19 infected patients initially shows high creatine kinase levels and rhabdomyolysis. The work goes on to state that on admission of a patient in the hospital, a total creatine kinase tests should be done (Chan et al., 2020).

High levels of inflammatory biomarkers such as C-reactive protein (CRP), Interleukin-6 (IL-6), procalcitonin (PCT) and ferritin (FT) reflect the severity of the COVID-19 infections; the details for which will be discussed later in this review.

Since, its outbreak from China in December 2019, researchers across the globe have been working day and night to find a suitable cure for this novel coronavirus infection. Consequently, the efforts are going on throughout the world to develop vaccines and suitable drugs for the treatment of this viral infection. Many existing drugs have been repurposed and are being tried as potential candidates for the cure of infected patients. At the same time, multiple new vaccines are being developed and are in different stages of clinical trials, Table 1 & Fig. 2. As on September 8, 2020, a total of 34 candidate vaccines are in clinical evaluation (Source: World Health Organization Website). Fig. 3 shows

the various biosensing platforms for the different inflammatory biomarkers.

Research related to Severe Acute Respiratory Syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) states that the cytokine storm syndrome (inflammatory response) is a key player for the disease progression (Alosaimi et al., 2020; Coperchini et al., 2020; Mahallawi et al., 2018; Ragab et al., 2020; Ye et al., 2020). In cytokine storm syndrome, the excessive release of cytokines or inflammatory biomarkers is observed (Lingeswaran et al., 2020). The inflammatory biomarkers have been shown to be playing role in the COVID-19 infections also (Upadhyay et al., 2020). Meta-analysis of the available data reveals the involvement of inflammatory biomarkers such as C-reaction protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6) and ferritin (FT) (Feng et al., 2020; Vaseghi et al., 2020; Zeng et al., 2020) in COVID-19 infections. The relationship between inflammatory biomarkers and COVID-19 infections has been documented in many published reports. The work by Mahapatra et al. highlights the use of various diagnostic methods. These includes oligonucleotide based methods (PCR and CRISPR), POC immunodiagnostics, digital radiographical screening and nano biosensing platforms. This review enlists the various inflammatory biomarkers that are recommended in a laboratory test. It has been reported that biomarkers such as PCT, FT, CRP and IL-6 are found to be elevated in patients which is associated with COVID-19 progression (Mahapatra and Chandra, 2020). A work by Kermali et al. shows the association of various biomarkers with COVID-19 which can help in determining the progress of the viral infection (Kermali et al., 2020). Markers such as ferritin, CRP, IL-6, PCT and serum amyloid A indicate the severity of the viral infections (Zeng et al., 2020). Hence, the monitoring of these inflammatory biomarkers becomes a necessity. This will not only help in predicting the concerned infections progress but will also provide new targets for the prognosis. Though various detection methods for the COVID-19 such as RT-PCR and ELISA have been developed (Adams et al., 2020; van Kasteren et al., 2020), the major challenge remains the prediction of the progress of the infections. Various manufacturers such as Thermo Fisher Scientific, Abcam and Merck have ELISA kits for these inflammatory biomarkers that are commercially available. These are the gold standards for the clinical detection. However, to increase the sensitivity, reduce the complexity and obtain better response time, there is a need for some other alternatives. In a review by Morales-Narváez et al., the use of

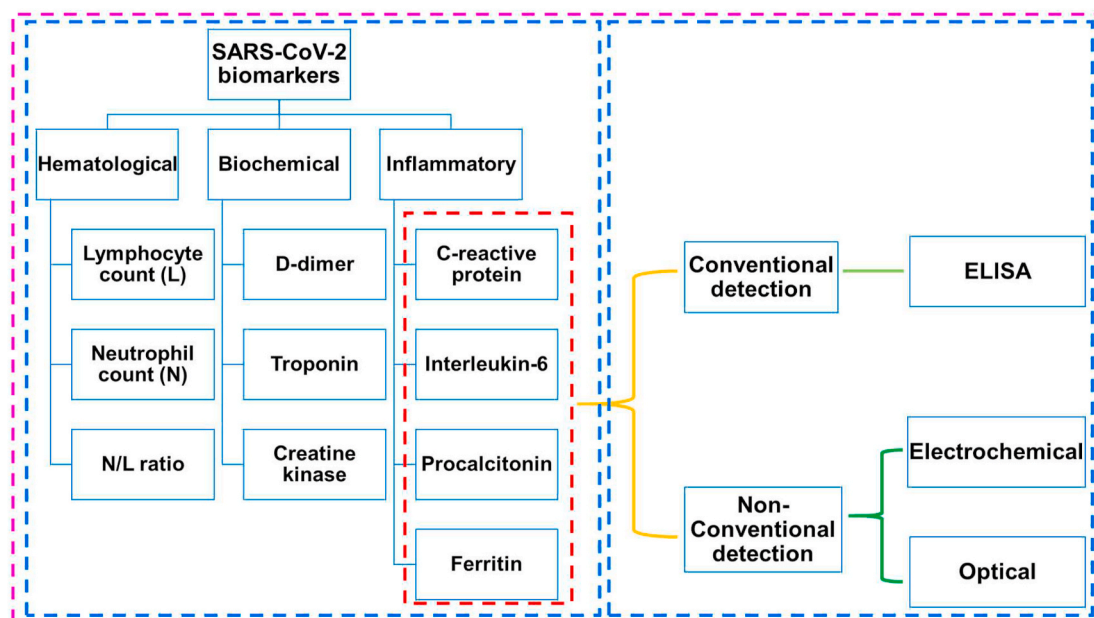


Fig. 1. Various biomarkers of the SARS-CoV-2 disease and the conventional and non-conventional detection methods for the inflammatory markers.

**Table 1**  
Status of various vaccines under development.

Vaccine developer/manufacturer	Vaccine platform	Clinical stage
University of Oxford/AstraZeneca	Non-Replicating Viral Vector	Phase 3
CanSino Biological Inc./Beijing Institute of Biotechnology	Non-Replicating Viral Vector	Phase 3
Gamaleya Research Institute	Non-Replicating Viral Vector	Phase 3
Janssen Pharmaceutical Companies	Non-Replicating Viral Vector	Phase 3
Sinovac	Inactivated	Phase 3
Wuhan Institute of Biological Products/Sinopharm	Inactivated	Phase 3
Beijing Institute of Biological Products/Sinopharm	Inactivated	Phase 3
Moderna/NIAID	RNA	Phase 3
BioNTech/Fosun Pharma/Pfizer	RNA	Phase 3
Novavax	Protein Subunit	Phase 2b
Anhui Zhifei Longcom Biopharmaceutical/Institute of Microbiology, Chinese Academy of Sciences	Protein Subunit	Phase 2
Curevac	RNA	Phase 2
Institute of Medical Biology, Chinese Academy of Medical Sciences	Inactivated	Phase 1/2
Research Institute for Biological Safety Problems, Rep of Kazakhstan	Inactivated	Phase 1/2
Inovio Pharmaceuticals/International Vaccine Institute	DNA	Phase 1/2
Osaka University/AnGes/Takara Bio	DNA	Phase 1/2
Cadila Healthcare Limited	DNA	Phase 1/2
Genexine Consortium	DNA	Phase 1/2
Bharat Biotech	Inactivated	Phase 1/2
Kentucky Bioprocessing, Inc	Protein Subunit	Phase 1/2
Sanofi Pasteur/GSK	Protein Subunit	Phase 1/2
Arcturus/Duke-NUS	RNA	Phase 1/2
ReiThera/LEUKOCARE/Univercells	Non-Replicating Viral Vector	Phase 1
Clover Biopharmaceuticals Inc./GSK/Dynavax	Protein Subunit	Phase 1
Vaxine Pty Ltd/Medytox	Protein Subunit	Phase 1
University of Queensland/CSL/Seqirus	Protein Subunit	Phase 1
Medigen Vaccine Biologics Corporation/NIAID/Dynavax	Protein Subunit	Phase 1
Instituto Finlay de Vacunas, Cuba	Protein Subunit	Phase 1
FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo	Protein Subunit	Phase 1
West China Hospital, Sichuan University	Protein Subunit	Phase 1
Institute Pasteur/Themis/Univ. Of Pittsburg CVR/Merck Sharp & Dohme	Replicating Viral Vector	Phase 1
Imperial College London	RNA	Phase 1
People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech.	RNA	Phase 1
Medicago Inc.	VLP	Phase 1

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), lateral flow assays, serological assays and biosensors are reported. CRISPR based systems allow the detection of nucleic acids in low resource settings, lateral flow kits can be used to identify herd immunity, serological assays allow detection of whole virus whereas the developed biosensors (for other applications) can be adapted for the detection of SARS-CoV-2 virus. The review highlighted the relevance of the inflammatory biomarkers like procalcitonin, C-reactive protein, D-dimer and Interleukins (IL-6 and IL-10) with COVID-19 infections (Moralés-Narváez and Dincer, 2020). Pokhrel et al. highlighted the use of nucleic acid based methods, immunoassays and chest computed tomography (CT) imaging for the detection of the coronavirus. The work shows that though chest CT imaging is a useful tool for the identification of the features of the COVID-19 infections, it is still not recommended by Centers for Disease Control, USA. The review reported the development of various RT-PCR kits developed by various manufacturers globally. The kits based on the various mechanisms of the immunoassay such as lateral flow assay, chemiluminescence immunoassay and colloidal gold assay have also been highlighted in detail in the work documented by

Pokhrel et al. The work also covered the use of lateral flow assays for nucleic acids and proteins and isothermal amplification of nucleic acids as emerging techniques to detect the SARS-CoV-2 virus (Pokhrel et al., 2020). Various point of care platforms of chips, paper, thread and film-based biosensors have been highlighted in a review by Choi, (2020). The work mentions in detail the advantages and limitations of these various point of care platforms. The chip, paper and thread based methods provide the advantages of low sample requirement, low cost, and user friendly, whereas the film-based method has advantage of withstanding temperature changes during a PCR cycle. Though these methods have attractive advantages, these come with some limitations also. For instance, the chip-based and film-based methods involve highly complex fabrication steps, whereas paper and thread-based methods suffer lack of quantification.

Biosensors are very promising option for many years now which easily fills the lacuna created by gold standard methods. The biosensors have the advantages such as high selectivity, sensitivity, quick turn-around time, cost-effective, easy to fabricate, user friendly, adaptable nature and ability to be miniaturized. This allows for the detection of the analytes directly at the site of the analyte generation which is particularly useful for the detection of minute levels of the inflammatory biomarkers produced inside the human body. Thus, the various steps such as separation and purification of the analyte prior to the detection can be avoided. These advantages can be of utmost importance while designing the biosensors for the various inflammatory biomarkers.

In the current review, the state of the art biosensors developed over the period (2019-till date) for the inflammatory biomarkers namely C-reaction protein (CRP), procalcitonin (PCT), interleukin-6 (IL-6) and ferritin are discussed. The most recent advances in the field of electrochemical, optical and microfluidic biosensors will be discussed in detail for these biomarkers.

## 2. Inflammatory biomarkers and their detection platforms

### 2.1. C-reactive protein (CRP)

C-reactive protein is a major biomarker present in the blood stream at the time of infection or inflammation and is produced by the liver cells in response to the inflammation (Seo, 2012). CRP levels below 0.3 mg/dL are considered normal in health adults (Nehring et al., 2020). The concentration in COVID patients is reported to be on higher side. A work by Ali et al. shows that high levels of CRP may be early indicators of how the COVID-19 disease will progress. In their work, they report that CRP levels in COVID-19 patients range from 20 to 50 mg/L which is significantly higher than the normal range for CRP (Ali, 2020). In case of infection or an inflammation, the levels of this protein increase about 1000 folds (Sproston and Ashworth, 2018). It is involved in cardiovascular, diabetes and neurodegenerative diseases (Luan and Yao, 2018). Hence, this biomarker becomes the “go to” in case of inflammation, which is commonly observed in the SARS-CoV-2 infections. Conventionally, ELISA based immunoassays have been used for the detection of this biomarker and many ELISA kits are available in the market by various manufacturers. However, the development of biosensors for this protein gives more advantages over the conventional methods in terms of covering the clinically prescribed ranges and portability for clinical use. ELISA though is a gold standard technique but the ELISA kits available by different manufacturers though show sensitivity in pg/mL range but do not focus on the upper limits of CRP detection which is well taken care of by a biosensor which has a broad linear range while simultaneously showing sensitive detection limits. Biosensors for CRP will be discussed in the coming sections.

#### 2.1.1. Electrochemical platforms

In the present review, we have covered the work published during 2019–2020 on inflammatory biomarkers. For CRP, an electrochemical sensor was reported by Boonkaew et al wherein graphene modified

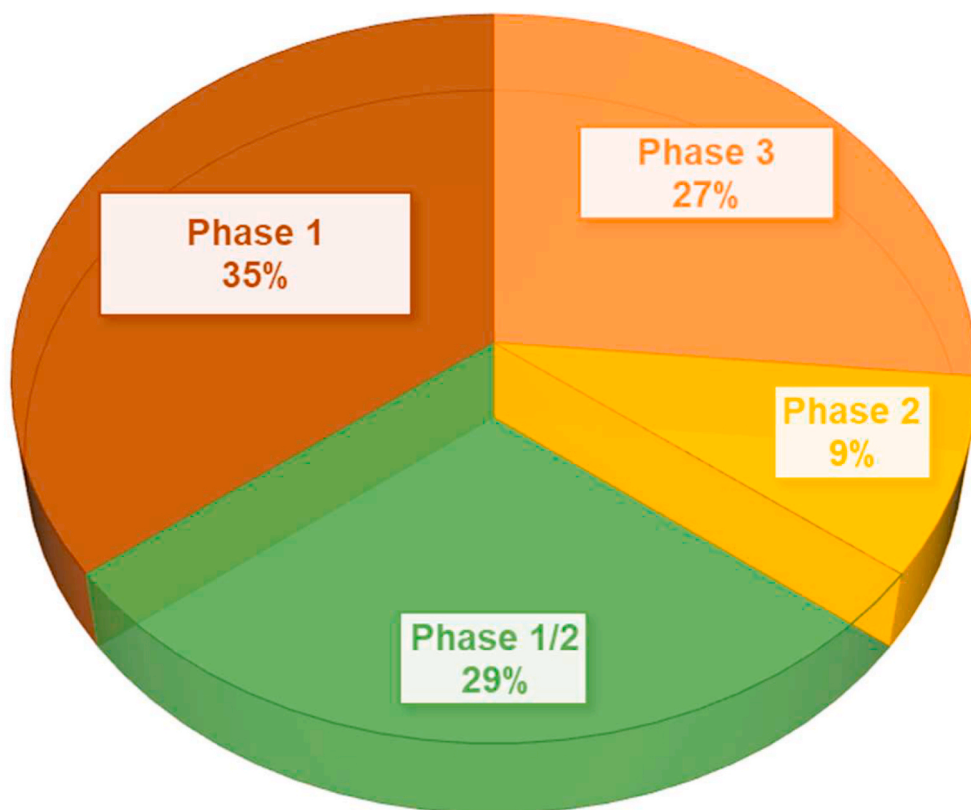


Fig. 2. Status of various vaccines under development in terms of the clinical stages [Note: Vaccine in Phase 2b is mentioned in the Phase 2 for the pie chart].

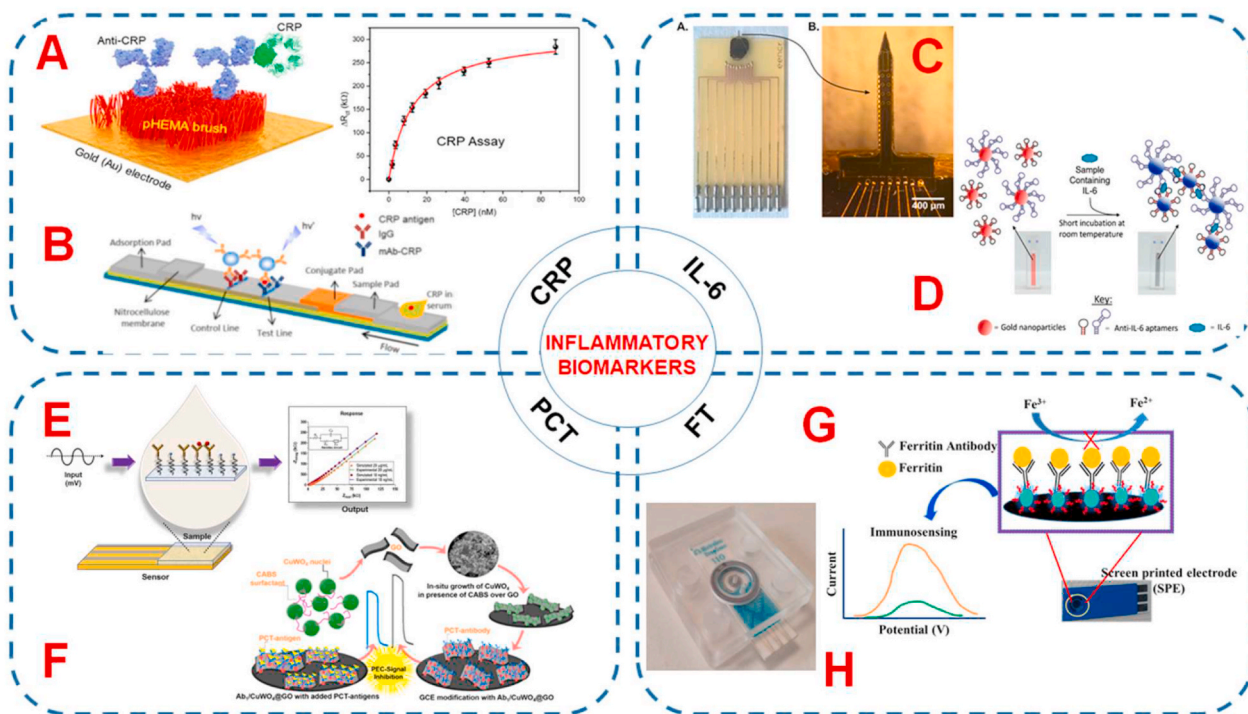


Fig. 3. Various biosensing platforms for the detection of various inflammatory biomarkers A: Impedometric detection of CRP using Poly (2-hydroxyethyl methacrylate) Brushes (Reprinted from (Kanyong et al., 2020)); B: LFA based detection of CRP (Reprinted from (Park et al., 2019)); C: Microelectrodes in the shape of needles for the detection of IL-6 (Reprinted from (Russell et al., 2019)); D: Colorimetric detection of IL-6 (Reprinted from (Giorgi-Coll et al., 2019)); E: Impedometric detection of PCT (Reprinted from (Tanak et al., 2019)); F: PEC based detection of PCT (Reprinted from (Abbas and Soomro, 2019)); G: Electrochemical immunosensing of ferritin (Reprinted from (Garg et al., 2020a)); H: Microfluidic chip based detection of ferritin (Reprinted from (Garg et al., 2020b)).

screen printed electrode (SPE) followed by electrodeposition of gold nanoparticles was used for the study. The electrode was modified with an additional layer of cysteine for easy immobilization of the antibodies. Electrochemical Impedance Spectroscopy (EIS) was used as the sensing technique and a linear range of 0.05–100 µg/mL with limit of detection (LOD) of 15 ng/mL was obtained (Boonkaew et al., 2019). However, an incubation time of 50 min was given for antibody-antigen bindings to take place which is a limitation for a proposed microfluidic device. In a bid to improve the LOD further, Zr (IV) based organic framework with 2, 5-thiophenedicarboxylate anion was deployed which provided an effective immobilization of the antibodies by virtue of which the electrochemical properties of the sensor also got enhanced (Dong et al., 2019). This system was able to achieve an LOD of 0.2 ng/mL, which is a significant improvement. In an effort to provide inexpensive biosensor for CRP, a disposable paper based biosensor was reported by Pinyorosphathum et al. In this work, a gold modified SPE functionalized with thiol-terminated poly (2-methacryloyloxyethylphosphorylcholine) was employed. This modified electrode not only provided a CRP detection in a very broad range of concentrations (5–5000 ng/mL) but also retained significantly low detection limit (1.6 ng/mL) (Pinyorosphathum et al., 2019). Dithiobis (succinimidyl propionate) modified interdigitated electrodes performed even better than the previously mentioned biosensors for CRP. In this referred work, an improvement was observed in terms of the linear range (0.01–10,000 ng/mL) and LOD (0.025 ng/mL) (Chinnadayala et al., 2019). Recently, for the very first time, a biosensing system for CRP has demonstrated the LOD in pg/mL. To achieve this feat, the authors used two systems; a conducting composite of gold nanoparticles and ionic liquid functionalized molybdenum disulfide (MoS<sub>2</sub>), which provided a large surface area for the reaction to occur. The second component of this biosensing system was the use of a composite of iridium nanoparticles and 1,5-diaminonaphthalene absorbed on the graphene oxide (GO). This component provided high electrocatalytic activity to reduce hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), which was used to generate signals. The sensor based on gold nanoparticles and ionic liquid functionalized MoS<sub>2</sub>/iridium nanoparticles and 1,5-diaminonaphthalene, absorbed on the graphene oxide (GO) showed satisfactory results in the serum samples (Ma et al., 2020). The latest reports for the electrochemical detection of CRP are published in March 2020. All these reports mainly focused on increasing the linear range of the sensor rather than a low detection limit. The biosensing platform in these reports were fabricated using poly (2-hydroxyethyl methacrylate) (pHEMA) brushes (Kanyong et al., 2020), nickel immersion gold (Sar-desai and Dhamu, 2020) and rGO/Ni/PtNPs (Molinero-Fernández et al., 2020a). Of these, the use of rGO/Ni/PtNPs based immunoassays was demonstrated for a microfluidic based method. The total assay time was 8 min with sample volume of only 10 µL.

### 2.1.2. Optical platforms

Several reports are available on the optical based detection of CRP. The gold nanoparticles remain to be a favorite choice, while designing and fabricating the optical sensors because of their proven excellent optical properties and ease of synthesis. In several reports, gold nanoparticles based sensors have been demonstrated for CRP estimation. Gold-silver alloy film was used to detect CRP via surface plasmon resonance (SPR) phenomenon. The motive of using silver in this alloy (gold-silver) was to incorporate the sharper angular resonance advantages of silver in SPR, which gives more sensitivity to the system. The sensor was able to detect CRP with an LOD of 5 pg/mL (Yi et al., 2019). Decrease in the fluorescent intensity of the CRP DNA aptamer modified CdSe/ZnS QDs was used as the signal for the detection of the target analyte (CRP). Though this method reports a relatively high value of LOD (45 pg/mL), but sensing system has several advantages such as easy to construct, requires only one set of recognition antibodies, and low cost of the sensor (Ghosh et al., 2019). Another aptasensor based on gold nanoparticles and magnetic beads reports the detection limit of 2.71 nM. The sensor works on the principle, that when the antigen is introduced in

the system, it binds to the aptamers, and gold nanoparticles gets released. This aptamer is simultaneously bound to magnetic beads also. This complex is imaged via dark field imaging and counted using MATLAB program (Zhao et al., 2019). Though this sensor works well, but not comparable to other optical based sensors in terms of linear range and LOD. CRP was used a model system to demonstrate the working of gold nanoparticles in a cuvette based localized surface plasmon resonance (LSPR) sensor chip also. The system was able to detect CRP but the linear range (0.01–10 µg/mL) and LOD of 0.01 µg/mL were not much impressive (Oh et al., 2019). Park et al. developed a fluorescent fullerene nanoparticle based sensor for the lateral flow immuno chromatographic assay of CRP. This was done to overcome the limitations of the gold based LFID assay such as less sensitivity. The fullerene based system was able to provide a quantitative aspect of CRP detected within 15 min, though its linear range was not at par with the earlier reports (Park et al., 2019). Xu et al. used single layer graphene instead of generally used gold for the SPR based CRP detection to reduce the cost. The fabricated sensor had better sensitivity and detection limit (5 pg/mL) in comparison to gold based system (Xu et al., 2020). Recently waveguide based sensor system for CRP are reported where aptamer and antibody are used as recognition elements (Walter et al., 2020). The system with aptamers as the recognition element had a wider linear range but the system with antibodies as the recognition element displayed a better detection limit (Ashiba et al., 2020).

### 2.2. Interleukin-6 (IL-6)

Interleukin-6 belongs to glycoprotein family and is involved in various immunomodulation and inflammatory processes. It is produced as a result of tissue injury and infections (Tanaka et al., 2014). It helps in the maturation of B cells (Matsuda and Kishimoto, 1998). IL-6 plays significant role both in acute phase as well as chronic inflammations (Gabay, 2006). Normal range for IL-6 is from 0 to 16.4 pg/mL (Khan and Ali, 2014). IL-6 is a known sepsis marker along with being a marker for SARS-CoV-2 viral infections. It has been reported that complicated COVID-19 patients have more than 3 times the IL-6 values than non-complicated diseases (Grifoni et al., 2020).

#### 2.2.1. Electrochemical platforms

In the past, numerous sensors have been developed for the detection of interleukin-6, which have been summarized by Khan et al. (Khan and Mujahid, 2020). The authors have discussed the electrochemical and optical sensors for IL-6. In the current work, we shall highlight only the recent developments in the detection of IL-6.

From an electrochemical platform point of view, the use of needle shaped microelectrode has been demonstrated for the detection of multi-analytes, using IL-6 as model biomarker to establish the proof of concept (Russell et al., 2019). The microelectrodes were modified with IL-6 specific antibodies and the system was able to detect the antigen within 2.5 min, which is a significant reduction in the detection time (usually 12–72 h) when compared with other method of culturing body fluids. The linear range for the detection using this sensor was found to be 20–100 pg/mL, though limit of detection was not calculated in this work as this was mainly a proof of concept like study. In contrast to the macro electrodes, which are bulky, these microelectrodes can be claimed to be potential candidates for point of care testing or inserted in the blood vessel for continuous monitoring. As mentioned above, IL-6 is a major biomarker for sepsis that is caused by the inflammatory response in response to a bacterial infection in the blood stream, an early monitoring of this biomarker can help us predict the onset of sepsis. Therefore, point of care testing of IL-6 can rapidly predict this disease rather than having to wait for almost 1–3 days via conventional methods of IL-6 detection. In another study, IL-6 is pointed out as a biomarker for screening colorectal cancer. Instead of using the microelectrodes, the authors of this study employed the conventional glassy carbon electrode (GCE) (Tertis et al., 2019). The electrode was modified with

p-aminobenzoic acid, p-aminothiophenol and gold nanoparticles, which was further used to bind thiol terminated aptamers specific for IL-6. Electrochemical Impedance Spectroscopy (EIS) was used as the sensing technique for the sensor, which performed well in terms of linear range (5 pg/mL to 100 ng/mL) and LOD (1.6 pg/mL) as compared to the microelectrode work of Russell et al. mentioned before (Russell et al., 2019). With an intention to continuously monitor the secreted IL-6 and Tumor Necrosis Factor- $\alpha$  (TNF- $\alpha$ ), a flow cell integrated with electrodes, modified with specific antibodies is also reported (Ortega et al., 2019). An amperometric detection of these biomarkers was carried out and the sensor was found to be highly sensitive for both the target analytes.

### 2.2.2. Optical platforms

Raman spectroscopy is a highly sensitive method for studying very fine details of the target molecules and has shown a great potential in the biomedical applications (Das et al., 2017; Krafft and Sergio, 2006; Pence and Mahadevan-Jansen, 2016; Popp et al., 2011). In this direction, a proof of concept was shown wherein the combination of drop-coating deposition, Raman spectroscopy and graphene-enhanced Raman spectroscopy was used for IL-6 detection. The study was able to detect IL-6 even in 1  $\mu$ L sample having IL-6 concentration of 1 pg/mL (de la O-Cuevas et al., 2019), however, the sensitivity of the sensor was found to be affected with the substrates used. In case of silicon (Si) substrate, the detection limit was found below 1 ng/mL, but with reduced graphene oxide/silicon substrate (rGO/Si), the detection limit was found even below 1.0 pg/mL, i.e. 1000 times better than that of Si substrate. Though this technique is very sensitive, but is not very suitable to be deployed as a biosensor. A more practical biosensor for IL-6 was developed by Eriksson et al., wherein, a geometric flow control lateral flow immunoassay device was fabricated to overcome the limitations of the conventional lateral flow devices (Eriksson et al., 2019). The conventional lateral flow immunoassay devices have limitations such as low resolution, unable to control the flow rate, requirement of large volumes of antibodies and limited bioassay chemistries. These were overcome by giving slight angles to the input and output in the microfluidic geometric lateral flow device. The authors compared the performance of their device with the conventional flow device and results demonstrated 10 times increased sensitivity as compared to the conventional one. The linear range of detection was between 0.1 and 10 ng/mL with an LOD of 29 pg/mL.

Further, to improve the performance parameters of a sensor and have some automation in the detection system, Dai et al. developed a colorimetric semi-automatic microfluidic immunoassay platform. (Dai et al., 2019). All the steps, which are usually involved in an ELISA such as reagent loading, washing and reading were performed in this microfluidic device with semi-automation. This semi-automatic platform could give 11 simultaneous readings for IL-6 in only 35 min requiring just 20  $\mu$ L sample volume, which is very less as compared to the time taken by conventional ELISA. The LOD (1.262 pg/mL) was also significantly better than the work reported by Eriksson et al. wherein, LOD achieved was 29 pg/mL.

The use of aggregation phenomenon of the gold nanoparticles (AuNPs) for developing visual color changes, is well tried and accepted method for colorimetric sensors. Giorgi-Coll also encashed this phenomenon of AuNPs to develop an aptamer based biosensor for IL-6 detection, (Giorgi-Coll et al., 2019), however the performance parameters of sensor were not at par with the reports discussed previously in this section. This system had a detection limit in the order of  $\mu$ g/mL, which is not very significant. Another recent report for the colorimetric detection of IL-6 involved the use of antibody modified gold nanoparticles (Sene Ingridi de Souza et al., 2020). Herein, a lateral flow immunoassay device was fabricated, which could demonstrate a detection limit of 0.38 ng/mL for IL-6. Though the LOD is not very good as compared to the other optical methods highlighted above yet it is the best performing color based system for IL-6 detection till date. In another recent work for the detection of IL-6, the nanophotonics based

detection was performed using cobalt ferrite and magnetite nanoparticles. (Khan et al., 2020). This composite material was embedded into a chitosan-gelatin hydrogel and was probed with visible light using reflectance spectroscopy. The obtained signal was directly proportional to the amount of antigen present in the hydrogel. LOD values were reported separately for the cobalt ferrite (0.52 pg/mL) and magnetite nanoparticles (0.08 pg/mL) and these values represented the most sensitive detection of IL-6 till date using this approach.

### 2.3. Procalcitonin (PCT)

PCT is a precursor for calcitonin (hormone), found in the cells as a result of infections or injury. It is a potential diagnostic marker for sepsis and its level below 0.15 ng/mL is considered as normal (According to an article in Medscape) (Vijayan et al., 2017). In a meta-analysis study done by Lippi et al. it was shown that when levels of PCT goes above 0.5  $\mu$ g/L, the risk of severe infection is more than 5 times in contrast to low PCT value patients (Lippi and Plebani, 2020). Though, IL-6 is also reported as a biomarker for sepsis, procalcitonin is reported as more specific as compared to other markers for the early detection of sepsis (Jin and Khan, 2010). PCT has been reported to be detected using various sensing systems like electrochemical, electro-chemiluminescent and photo-electrochemical immunosensors, as a major biomarker for sepsis (La, 2020).

#### 2.3.1. Electrochemical platforms

Yang et al. fabricated an electrochemiluminescence sensor for PCT (Yang et al., 2019). In this work, N-(Aminobutyl)-N-(ethylisoluminol) (ABEI) linked with ferritin acted as a donor molecule, whereas the single gold nanoparticle acted as the acceptor molecule. This donor-acceptor system was able to generate a linear range of 100 fg/mL to 50 ng/mL with 41 fg/mL LOD. Though the system is highly sensitive but the complex sensor fabrication and use of multiple reagents increased the overall cost and complexity of the sensor. To simplify the sensor construction, electrochemical methods are used. In one such method (Liu et al., 2019), molybdenum trioxide/gold on reduced graphene oxide (MoO<sub>3</sub>/Au@rGO) nanocomposites having excellent electrocatalytic activity towards hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) was used, which further generated the signal. This was indirect method. This system was able to demonstrate high and sensitive PCT detection with LOD of 2 fg/mL, which is better than obtained with electrochemiluminescence sensor mentioned previously. An impedometric sensor based on zinc oxide (ZnO) thin films modified gold interdigitated electrode is reported for PCT detection (Tanak et al., 2019). Since, the sensor fabrication involved only one set of antibodies, it is easy to fabricate with reduced cost. However, the sensor was not able to provide better performance parameters as compared to the work of Yang et al. and Liu et al.

Toluidine blue functionalized NiFe Prussian-blue analog nanocubes modified electrodes as sensing platforms displayed a highly sensitive detection of PCT with extremely low value of LOD. This is attributed to the nanocubes synthesized, which provided large surface area for antibody binding (Gao et al., 2020). To improve the sensing parameters further, Abbas et al. grew cupric tungstate (CuWO<sub>4</sub>) nanospheres in-situ over graphene oxide and used it as a photoelectrochemical sensor towards PCT sensing (Abbas and Soomro, 2019). The sensor had LOD of 0.15 pg/mL. The good performance of the sensor was attributed to the synergistic effects of both CuWO<sub>4</sub> nanospheres and GO, who formed a superb photo-active heterojunction, which is a main requirement in a photoelectrochemical sensing platform.

A new sensor was fabricated using glassy carbon electrode (GCE) modified with gold nanoparticles and delaminated sulfur doped MXene for PCT detection (Medetalibeyoglu et al., 2020). To the best of our knowledge, this sensor is the best in terms of the obtained LOD value (2 fg/mL), which is far better than already discussed. In an effort to improve the sensors performance parameters, iron sulfide (Fe<sub>3</sub>S<sub>4</sub>) loaded with Pd nanoparticles were used for PCT detection. Just like

previously mentioned MoO<sub>3</sub>/Au@rGO nanocomposites based sensor, which used H<sub>2</sub>O<sub>2</sub> for indirect signal generation, iron sulfide (Fe<sub>3</sub>S<sub>4</sub>) loaded system also had similar methodology. Though LOD value of only 130 fg/mL could be achieved, the linear range in this sensing system was found to be better (Qu et al., 2020). The most recent work on PCT detection compared the performance of a microfluidic device having integrated gold electrode and a screen printed carbon electrode (SPCE) based method for PCT detection (Molinero-Fernández et al., 2020b). In both the cases, a magnetic bead based immunoassay was performed. From the study, it was concluded that the microfluidic based method provided a low value of LOD as compared to the SPCE, whereas the SPCE method provided a wide working range as compared to the chip based system.

### 2.3.2. Optical platforms

In respect to the optical methods for PCT detection, only two methods are reported in 2019–2020 (Chiang et al., 2020; Nie et al., 2019). Nie et al. developed a chemi-luminescence based sensor which used biotin–streptavidin-mediated peroxidase nanocomplex for signal amplification (Nie et al., 2019). A drastic change in the chemi-luminescence was observed in the absence of a signal amplification. The capillary based method provided enhanced sensitivity because of high loading of the antibodies inside the capillary channels. A nano plasmonics based PCT biosensors used capture probe immobilized on the fiber core and a gold conjugated detection antibody was reported recently (Chiang et al., 2020). When the analyte comes in contact with the antibody, an immunocomplex sandwich is formed, which causes a change in the plasmon resonance, which is to be detected. The method achieved a detection limit of 0.095 pg/mL with a response time of 15 min.

## 2.4. Ferritin

Ferritin is an iron storage protein, which plays a significant role in regulating the iron content of the body. According to WHO, ferritin levels for males and females should be in the range of 15–200 ng/mL and 15–150 ng/mL, respectively. It plays a vital role in the oxidative stress and anemia also. More recently it has been shown to be playing significant role in SARS-CoV-2 viral infections as is mentioned in the introduction section. Severe COVID-19 patients have shown ferritin levels as high as 1000 ng/mL which is way more than the clinical range values for this protein (Zhou et al., 2020). Though this protein is widely researched for many years and many electrochemical and optical sensors exist for this, not much work has been done on this protein in the period of 2019–2020, making it a hot field to explore. We shall discuss the literature published during this period only.

### 2.4.1. Electrochemical platforms

In the month of March 2020, our group reported electrochemical detection of ferritin using biosurfactant functionalized quantum dots (QDs) (Garg et al., 2020a). The sensor exhibited a linear detection range from 10 to 1500 ng/mL, which covers prescribed clinical range. The sensor performance was evaluated with human serum samples also which showed satisfactory results. For electrochemical detection, differential pulse voltammetry (DPV) and cyclic voltammetry (CV) techniques were used for which two LOD values of 3.800 and 6.048 ng/mL were obtained for respective techniques. In an effort, to further improve both the linear range and LOD, white graphene QDs (hexagonal boron nitride QDs), were deployed for sensor functionalization and system was able to provide much wider linear range than our previous work. The linear range in this case was 10–2000 ng/mL and LOD value was calculated to be 1.306 ng/mL (Singh et al., 2020). More recently, a graphene modified paper based electrochemical sensor for ferritin was also reported (Boonkaew et al., 2020), in which the antibodies were attached to the electrode surface using the EDC/NHS chemistry. The linear range in this sensor is reported to be from 1.0 to 1000 ng/mL with

LOD of 0.19 ng/mL. Though the linear working range is less than reported by Garg et al. but an improvement in LOD is observed. In the most recent report for ferritin detection, an FET based sensor was fabricated. This was modified with graphene and further modified with 1-pyrenebutanoic acid, and succinimidyl ester for the attachment of anti-ferritin antibodies. The sensors linear range and LOD values were found to be less as compared to the previously mentioned platforms. The response time for this sensor was 1–10 s only, which is a big advantage of the biosensor (Oshin et al., 2020). Very recently, a microfluidic chip based system is reported by Garg et al. for the continuous electrochemical detection of ferritin. The system involved the use of a screen printed electrode modified with amine functionalized graphene oxide housed in a microfluidic chip. Anti-ferritin antibodies were immobilized on the electrode surface which was able to selectively detect ferritin. Though the system allowed a dynamic detection of ferritin, the linear range and limit of detection were not comparable to the previously mentioned work (Garg et al., 2020b).

### 2.4.2. Optical platforms

Not much literature is available for the optical detection of ferritin in 2019–2020. One study reported the use of Variable Angle Spectroscopic Ellipsometry (VASE) in-situ for the detection of ferritin. However, this was mainly a proof of concept and a concentration of 0.1 mg/mL was used which does not lie in the clinically recommended range (Tanovska et al., 2019).

In an effort to summarize the biosensors mentioned above for all the inflammatory biomarkers, the performance aspects of the biosensors are presented in Table 2 below. It can be observed from Table 2 that for all the discussed inflammatory biomarkers, the biosensors reported do cover the required clinical range of these biomarkers. Almost all the known electrochemical methods, and various optical based solutions have been employed for the development of the biosensing platforms.

EIS: Electrochemical Impedance Spectroscopy; DPV: Differential Pulse Voltammetry; SPR: Surface Plasmon Resonance; FL: Fluorescence; DFM: Dark Field Microscopy; LSPR: Localized Surface Plasmon Resonance; WMR: Waveguide Mode Resonance; O.D.: Optical Density; C.I.: Color Intensity; EL: Electrochemiluminescence; CL: Chemiluminescence; FOPPR: Fiber Optic Particle Plasmon Resonance; CV: Cyclic Voltammetry; I–V: Current-Voltage; VASE: Variable Angle Spectroscopic Ellipsometry.

The linear ranges of reported references cover both lower limits as well as upper limits and both are important depending upon the severity of infection.

The review of the biosensors reported for the mentioned inflammatory biomarkers indicate that significant work is done in the development of the biosensing platforms which are quite stable and robust. However, the true benefit to the society will happen only if such work can be extended for a point of care type device. This can help in the monitoring of early signs of COVID-19 related infections.

## 3. Conclusion

COVID-19, which is caused by SARS-CoV-2 virus has affected each and every individual on this planet in one or other way. To break the chain for its spread, many countries were or are still under some kind of lockdown. The closure of research institutions and universities across the globe has hit the research activities, which are required during this kind of pandemic. Despite the lockdown, the research institutes, which were allowed to function, worked day and night towards the development of kits for the detection of this deadly virus. Majorly antigen and antibody-based ELISA or lateral flow kits and Real Time- Polymerized Chain Reaction (RT-PCR) methods have been developed. These methods provide the screening and confirmation of the viral infections. It can now be said that the detection of the virus is not a bottleneck but the aftermaths of the infection is a challenge. The infection causes the biological functions to go haywire. The under-rated inflammatory markers



**Table 2**  
Comparison of Biosensor performance for inflammatory biomarkers.

Biosensor type	Technique	Linear range	Limit of detection	Reference
<b>C Reactive Protein</b>				
Electrochemical	EIS	0.05–100 µg/mL	15 ng/mL	Boonkaew et al. (2019)
Electrochemical	DPV	10–600 ng/mL	0.2 ng/mL	Dong et al. (2019)
Electrochemical	DPV	5–5000 ng/mL	1.6 ng/mL	Pinyorospatham et al. (2019)
Electrochemical	Impedimetry	0.01–10,000 ng/mL	0.025 ng/mL	Chinnadayala et al. (2019)
Electrochemical	Amperometry	0.01–100 ng/mL	3.3 pg/mL	Ma et al. (2020)
Electrochemical	Amperometry	1–100 µg/mL	0.54 µg/mL	Molinero-Fernández et al. (2020a)
Electrochemical	EIS	0–31,500 ng/mL	7.02 ng/L ± 1.57	Kanyong et al. (2020)
Electrochemical	Amperometry	1–10,000 ng/mL	100 ng/mL	Sardesai and Dhamu (2020)
Optical	SPR	5 pg/mL to 50 µg/mL	5 pg/mL	Yi et al. (2019)
Optical	FL	3 pM to 65 nM	45 pg/mL	Ghosh et al. (2019)
Optical	DFM	6.1–49 nM	2.71 nM	Zhao et al. (2019)
Optical	LSPR	0.01–10 µg/mL	0.01 µg/mL	Oh et al. (2019)
Optical	FL	0.1–10 ng/mL	–	Park et al. (2019)
Optical	SPR	5 pg/mL to 0.5 µg/mL	5 pg/mL	Xu et al. (2020)
Optical	SPR	0–100 nM	12.46 nM	Walter et al. (2020)
Optical	WMR	0–50 pM and 0–10 pM	10 pM	Ashiba et al. (2020)
<b>Interleukin-6</b>				
Electrochemical	DPV	20–100 pg/mL	–	Russell et al. (2019)
Electrochemical	EIS	5 pg/mL to 100 ng/mL	1.6 pg/mL	Tertis et al. (2019)
Electrochemical	Amperometry	0–1 µg/mL	8 ng/mL	Ortega et al. (2019)
Optical	Raman	–	below 1 pg/mL	de la O-Cuevas et al. (2019)
Optical	Color	0.1–10 ng/mL	29 pg/mL	Eriksson et al. (2019)
Optical	O.D.	4.7 pg/mL to 300 pg/mL	1.262 pg/mL	Dai et al. (2019)
Optical	O.D.	3.3–125 µg/mL	1.95 µg/mL	Giorgi-Coll et al. (2019)
Optical	C.I.	1.25–9000 ng/mL	0.38 ng/mL	Sene Ingridi de Souza et al. (2020)
Optical	Reflectance	50–350 pg/mL	0.52 and 0.08 pg/mL	Khan et al. (2020)
<b>Procalcitonin</b>				
Electro/optical	EL	100 fg/mL to 50 ng/mL	41 fg/mL	Yang et al. (2019)
Electrochemical	Amperometry	0.01 pg/mL to 10 ng/mL	0.002 pg/mL	Liu et al. (2019)
Electrochemical	Impedimetry	0.01–10 ng/mL	0.10 ng/mL	Tanak et al. (2019)
Electrochemical	DPV	0.001–25 ng/mL	3 × 10 <sup>-4</sup> ng/mL	Gao et al. (2020)
Electrochemical	Amperometry	10 pg/mL to 50 ng/mL	0.15 pg/mL	Abbas and Soomro (2019)
Electrochemical	DPV	0.01–1.0 pg/mL	2 fg/mL	Medetalibeyoglu et al. (2020)
Electrochemical	Amperometry	500 fg/mL to 50 ng/mL	130 fg/mL	Qu et al. (2020)
Electrochemical	Amperometry	0.05–100 ng/mL	0.02 ng/mL	Molinero-Fernández et al. (2020b)
Optical	CL	25000–80,000 pg/mL	0.5 pg/mL	Nie et al. (2019)
Optical	FOPPR	1 pg/mL to 100 ng/mL	95 fg/mL	Chiang et al. (2020)
<b>Ferritin</b>				
Electrochemical	CV & DPV	10–1500 ng/mL	3.800 ng/mL and 6.048 ng/mL	Garg et al. (2020a)
Electrochemical	EIS	10–2000 ng/mL	1.306 ng/mL	Singh et al. (2020)
Electrochemical	DPV	1–1000 ng/mL	0.19 ng/mL	Boonkaew et al. (2020)
Electrochemical	I-V	5.3 ng/L to ~0.5 µg/L	5.3 ng/L	Oshin et al. (2020)
Electrochemical	CV	7.81–500 ng/mL	0.413 ng/mL	Garg et al. (2020b)
Optical	VASE	0.1 mg/mL	–	Tanovska et al. (2019)

have been found to be closely related to the viral infections. The monitoring of these biomarkers can help the medical staff with the prognosis of the infections in the body and can give an idea of the severity of the viral infections. Contrary to the conventional detection platform, the non-conventional biosensors provide the required sensitivity and reliability apart from being inexpensive. These biosensors excel in the terms of selectivity, sensitivity, reproducibility and robustness to the real samples. The biosensor can be highly crucial in those patients, wherein they exhibit low SARS-CoV-2 RNA which has been missed by the traditional tests because of the limitations of those methods. However, these biosensors need to be developed further so that they can be used in the clinical settings. This can be accomplished by the development of various point-of-care testing devices such as chip-based, paper-based, thread-based and film-based just like for the detection of the SARS-CoV-2 virus mentioned in the introduction of this review. Considering the amount of burden experienced by the health-care facilities throughout the world, the need of the hour is cheap, user-friendly, sensitive and reliable detection platforms which can be used by users at the comforts of their homes. In this regards, the use of various microfluidic approaches towards multiplexed detection of the inflammatory biomarkers can be of much help. These simple point of care devices can allow for the sensitive detection of these important biomarkers for the rapid and accurate surveillance of the population in

question.

#### CRediT authorship contribution statement

**Mayank Garg:** Conceptualization, Writing - original draft, Funding acquisition. **Amit L. Sharma:** Conceptualization, Supervision, Writing - review & editing. **Suman Singh:** Conceptualization, Supervision, Writing - review & editing.

#### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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