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A Short Review on Recent Advances in Sensors for Cardiovascular Disease Markers in COVID-19 Management

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Abstract: ***Coronavirus 2019 (COVID-19) induced new cardiac pathologies and exacerbate underlying cardiovascular diseases. Cardiac biomarkers are frequently measured to provide guidance on the well-being of a patient in relation to cardiac health with many assays having been developed and widely utilised in clinical assessment. Effectively treating and managing cardiovascular disease (CVD) relies on swiftly responding to signs of cardiac symptoms, thus providing a basis for enhanced patient management and an overall better health outcome. Emerging biomarkers and corresponding sensors have the capacity to detect these markers along with design of nanomaterials has greatly assisted in the development of detection techniques that offer ultra-sensitive performances.***

Keywords: Point of care testing, Covid-19, Cardiovascular disease markers

1. Introduction

COVID-19 can precipitate conditions in affected patients with additional cardiovascular complications. Cardiovascular events surpassed all other causes of mortality, even exceeding superimposed pneumonia. In early clinical studies of patients admitted with COVID-19 in China, 32%-46% of patients had underlying diseases, including hypertension (15%-31%), cardiovascular disease (14.5%-15%), and diabetes (10%-20%) [1, 2].

The factors associated with mortality in COVID-19 patients include male sex, advanced age, and presence of hypertension, diabetes mellitus, cardiovascular and cerebrovascular diseases [3, 4, 5]. A high fatality rate was observed in patients with coronary heart disease[6, 7].

Heart condition assessment associated with COVID-19: Previous epidemics such as SARS and MERS have been linked to acute myocarditis, acute myocardial infarction, and rapid-onset heart failure[8, 9]. An autopsy case reported that patients had higher levels of troponin, myoglobin, C-reactive protein, serum ferritin, and interleukin-6 further suggestive of a high inflammatory burden in COVID-19 and a possible rise in myocarditis-related cardiac events[10]. Therefore, careful monitoring of the myocardial enzyme profiles is of great importance in reducing the complications and mortality in patients with COVID-19.

Han *et al* reported higher concentration in venous blood of CKMB, MYO, ultra- Troponin TnI, and NT-proBNP which were associated with the severity and CFR of COVID-19[11]. The natriuretic peptides, specifically B-type natriuretic peptide (BNP) and N-terminal prohormone of BNP (NT-proBNP) are considered the gold standard as they provide high diagnostic and prognostic relevancy and have high negative predictive value and are useful in the exclusion of heart failure (HF) as a cause of symptoms[12].

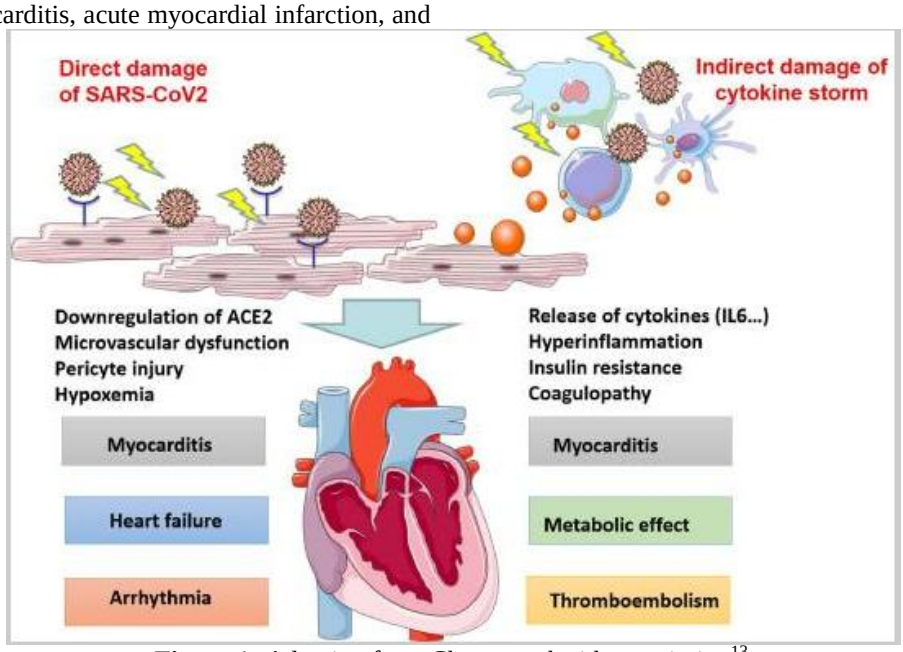

Figure 1: Adaption from Chang *et al* with permission[13]

The current healthcare system depends on centralised laboratories performing analysis of cardiac biomarkers with high turnaround times for such a critical organ. Point-of-care testing (POCT) involves conducting the diagnostic test in the presence of the patient, with a short turnaround time, requiring small sample volume with good sensitivity. Thus, formulation of ultra-sensitive assays and the design of biosensors will be critically along with focus on the feasibility of these techniques for POCT integration. The focus of this short review would be focus on recent advances in BNP and troponin detection using sensors.

Sensors:

Current BNP testing is confined to conventional immunoassay-based laboratory tests whose results require several hours or even days to be delivered. The other assays available are radioimmunoassay, Fluorescent-Based Immunoassays, ELISA and electro chemiluminescent immunoassay (ECLIA). The sensors for detecting BNP vary from Optical Immunosensors, Surface Plasmon Based Biosensors, Optical Intensity Based Biosensors and electrochemical sensors.

POCT:

An improved lateral flow immunoassay (LFIA) was developed which relied on an on-strip sandwich reaction between BNPs and their Abs. The formation of the complexes at the test line produced a qualitatively detectable signal that could be quantified based on the optical[14]. Using particles of diameter 35 nm and a concentration of 1.5 mg/mL for the Abs, detection limit of 0.1 ng/mL within 10 to 15 min, with an improved sensitivity (15-fold) was obtained.

Two biomarkers, BNP and ST2, were simultaneously quantified using dual-colour core-shell up conversion nanoparticles as probes. The lateral flow strip reached detection limits of 5 pg/mL and 1 ng/mL and detected minimal concentrations of 17.46 pg/mL and 29.92 ng/mL in clinical samples, for BNP and ST2, respectively[15]. Another up-converting phosphor technology-based lateral flow assay was developed to quantify NT-proBNP in human plasma and performed consistently with the Roche Elecsys assay, reaching a limit of detection of 116 ng/L with a coefficient of variation less than 15%[16].

A lateral flow immunoassay for NT-proBNP using antibody-labelled quantum dots was also developed and used monoclonal antibodies, engineered with high specificity to the tail of NT-proBNP and showed linear response range and repeatability with pooled variance of 8.8% across concentrations of NT-proBNP[17]. A handheld biosensor developed was able to test for BNP in a single drop of whole blood in 5 min. The system was highly sensitive, with a range of 0 to 1000 pg/mL.

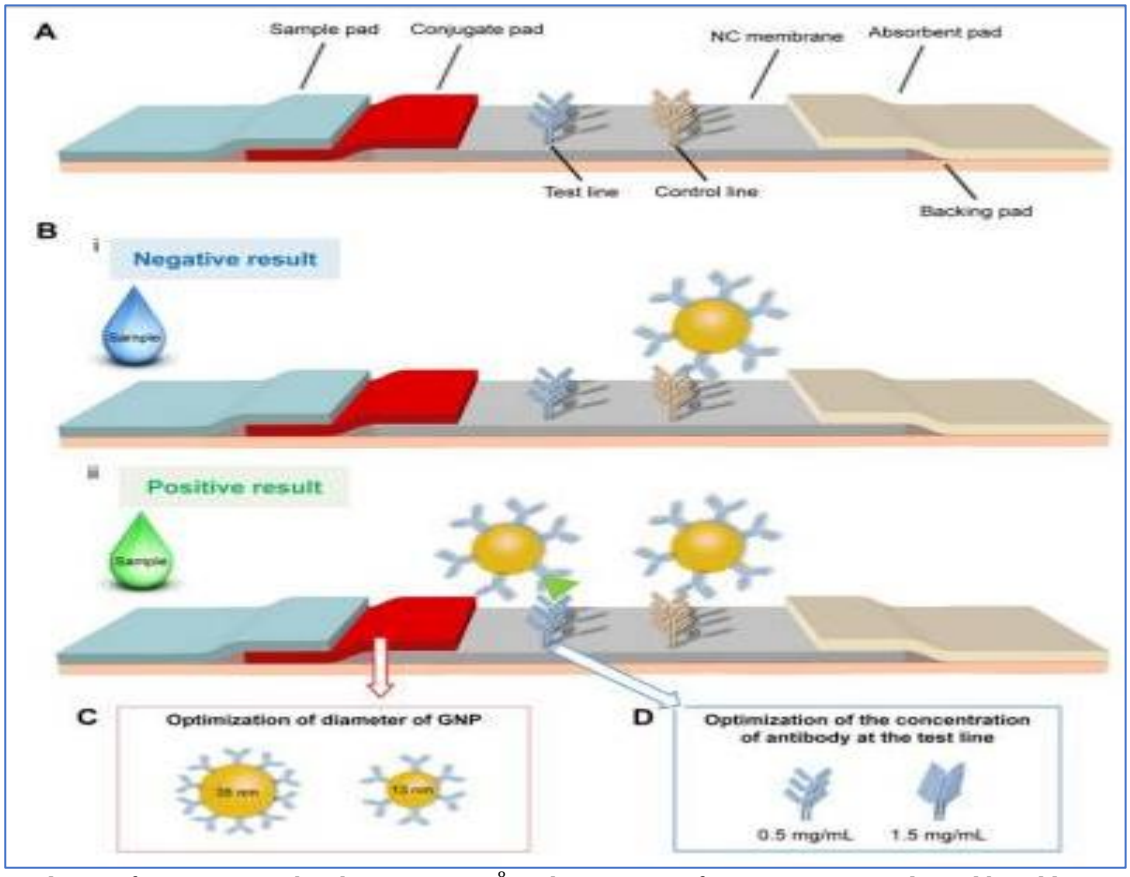

Figure 2: Adaption from Gong *et al* with permission.1[8]**A**-The structure of previous LFA is adjusted by adding a conjugate pad to form a new type of LFIA. **B**-The negative result **i** – positive result **ii**-the principle of LFIA. **C**-The diameter of GNP is adjusted from 13+3 to 35+3 nm. **D**-The concentration of test line is adjusted from 0.5 to 1.5 mg/ml.

A hand-held device was able to combine a lab-on-a-chip module with interdigitated circular capacitive electrodes for label-free detection of BNP. Due to the electron exchange between Ab and Ag, the change in the dielectric constant

creates a measurable change in the capacitance. The device was able to report results in less than 30 min human serum[19].

Troponin:

The cardiac troponins (cTn) are among the most utilised cardiac biomarkers, with cardiac troponin I (cTnI), a protein found only in the myocardium, often being considered as the gold standard for cardiac biomarkers[20]. The high sensitivities have to be achieved by assays by reducing the negative impact of the "troponin blind" period during which troponin levels are not detectable using the standard cTn assays though AMI symptoms may be present. We assess the latest research developments involving biosensors with a focus on evaluating their suitability towards integration into POC platforms.

Krupen *et al* developed the LRSPP biosensors for the detection of human cardiac troponin I (cTnI) protein using label-free detection sensor consist of 5-µm-wide 35-nm-thick gold stripes embedded in a low-index optical-grade fluoropolymer (CYTOP^{TM}) with fluidic channels etched to the Au surface of the stripes[21]. Direct and sandwich assays were developed and demonstrated over the concentration range from 1 to 1000 ng/mL, yielding detection limits of 430 pg/mL for the direct assay and 28 pg/mL for the sandwich assay (1 standard deviation), the latter being physiologically relevant to the early detection or onset of AMI.

Xu *et al* developed an ultrasensitive plasmonic biosensor that converts plasmonic absorption to electrical current in order to detect AFU and cTnI using whole human blood in a real-time and parallel fashion. The detection limit was calculated to be 0.016 U/L for AFU and 0.015 ng/mL for cTnI, respectively[22].

Cimen *et al* developed a surface plasmon resonance biosensor system to investigate kinetic properties for cardiac tropinin I. The limit of detection and limit of quantification were calculated as 0.00012 ng/mL and 0.00041 ng/mL, respectively[23]. Their study showed that surface plasmon resonance biosensor has high selectivity for cardiac troponin I. Elaine *et al* developed electrochemical biosensor using a combination of a novel monoclonal antibody, mAb20B3, and a novel Ir (III)-based metal complex was used for detection using faradaic electrochemical impedance spectroscopy. A limit of detection of 10 ag/mL was achieved, which was significantly lower than established assays[24].

Palladini*et al*an affinity-based biosensor for detection of troponin T (TnT), a preferred biomarker of AMI. This combined a stable and inexpensive molecularly imprinted polymer (MIP) based on polydopamine (PDA) with surface plasmon resonance (SPR) transduction[25].

Mehmet *et al* developed a novel imprinted biosensor approach based on boron nitride quantum dots (BNQDs) for cTnI detection in plasma samples[26]. cTnI imprinted electrode was developed in the presence of 100.0 mM pyrrole containing 25.0 mM cTnI. The sensor had 0.01-5.00 ng mL^{-1} and 0.0005 ng mL^{-1} linearity range and the detection limit (LOD) respectively in presence of other nonspecific and specific proteins including cardiac myoglobin (MYG), bovine serum albumin (BSA) and cardiac troponin T (cTnT), respectively.

POCT:

Singh *et al* developed a microfluidic system in conjunction with the detection method by fabricating a microfluidic biochip and nanoengineered a microporous manganese-reduced graphene oxide (Mn3O4-RGO) nanocomposite for the purpose of increasing the capture Ab loading capacity[27]. Kumar *et al*developed an electric double layer gated FET-based biosensing system that has achieved a LOD of 2.62 ng/L from an untreated 2 L blood sample within 5 min[28].

Indu *et al* developed a high sensitivity assay for the detection of cardiac troponin I using electrical double layer gated high field AlGaN/GaN HEMT biosensor[29]. The unique gating mechanism overcame the drawback of charge screening seen in traditional FET based biosensors, allowing detection of target proteins in physiological solutions without sample processing steps. The tests were carried out using purified protein solution and clinical serum samples depict high sensitivity, specificity and wide dynamic range (0.006-148ng/mL). No additional wash or sample pre-treatment steps were required, which greatly simplified the biosensor system. The miniaturized HEMT chip was packaged in a polymer substrate and easily integrated with a portable measurement unit, to carry out quantitative troponin I detection in serum samples with < 2µl sample volume in 5min.

Kim *et al* demonstrated highly sensitive and label-free detection of cardiac troponin I (cTnI), a biomarker for diagnosis of acute myocardial infarction, using silicon nanowire field-effect transistors[30]. Monoclonal antibodies for cTnI were covalently immobilized on the nanowire surface. The detection limit of the sensor was ~5 pg/mL, the lowest reported in the literature.

Multiplexing:

Multiplexed detection of cardiac markers has been shown to dramatically improve the predictive capabilities of a clinical assay. Zethelius *et al* has shown that the predictive risk of death due to cardiovascular causes is up to 10 times greater when taking into account the four biomarkers than when only measuring cTnI[31].

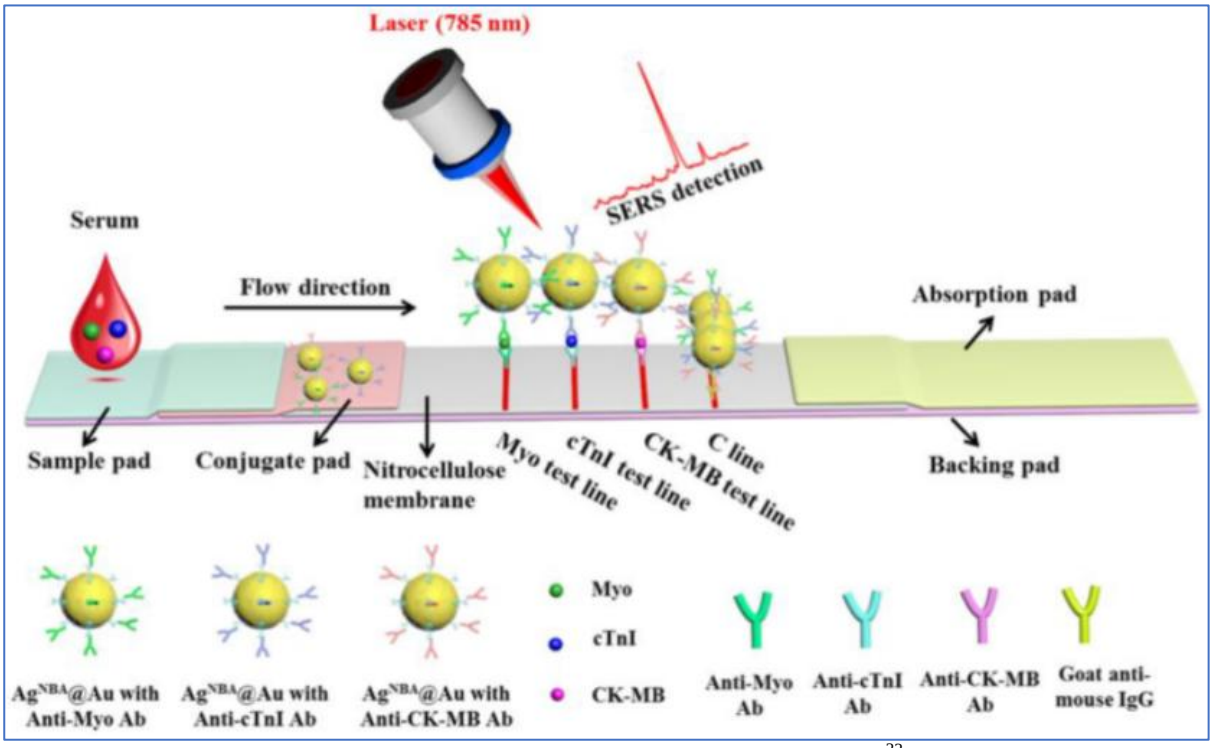

Figure 3: Adapted from Regan et al with permissions[32]

Shanmugam *et al* presented a multiplexed electrochemical immunoassay for the detection of cTnI and cTnT comprising of two sensors. They detected both troponins (I and T) at concentrations as low as 1 ng/L[33]. NT-proBNP is released in greater concentrations from the cardiac ventricles during cardiac stress and has a half-life six times that of BNP. This detection technique integrated 9G DNACHIP technology, a specific DNA microarray technique that uses an oligonucleotide with nine consecutive guanines on AMCA-1, 3-dialdehyde (AMCA) slides for specific orientation of the biorecognition element. The authors have followed the DNA-directed immobilisation (DDI) method and conjugated complementary DNA (cDNA) to the capture Abs and requires more iterations to reduce the detection time.

Cardiac Issues after mRNA vaccine administration

In December 2020, the Food and Drug Administration (FDA) issued Emergency Use Authorizations (EUAs) for the Pfizer-BioNTech COVID-19 (BNT162b2) vaccine and the Moderna COVID-19 (mRNA-1273) vaccine. According to the US Centres for Disease Control and Prevention, myocarditis/pericarditis rates are ≈12.6 cases per million doses of second-dose mRNA vaccine among individuals 12 to 39 years of age. In reported cases, patients with myocarditis invariably presented with chest pain, usually 2 to 3 days after a second dose of mRNA vaccination, and had elevated cardiac troponin levels[34]. Two-thirds of patients underwent cardiac magnetic resonance imaging, which revealed evidence of myocardial inflammation despite a lack of echocardiographic abnormalities[35]. Point of care devices for monitoring markers of cardiac injury for monitoring individuals after vaccinations could be handy tool useful for clinicians for quick remedial measures and allay fears in the community.

2. Conclusions

Devising cardiac markers detection POCT platforms enables clinicians through rapid implementation of a suitable management plan which ultimately leads to a better health outcome for the patient. POCT platforms in particular have the potential to reduce pressure on emergency departments (EDs) and enable the widespread distribution of affordable portable cardiac diagnostics. Designing a disease-specific standardised panel of biomarkers will provide clinicians with enhanced diagnostic capabilities and will increase the efficacy of cardiac diagnostics.

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