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Recent advances in developing biosensing based platforms for neonatal sepsis

Sapna Balayan [a], Nidhi Chauhan [a], Ramesh Chandra [b], Naresh K. Kuchhal [c], Utkarsh Jain [a,*]

[a] Amity Institute of Nanotechnology, Amity University, Noida, 201313, Uttar Pradesh, India
[b] Department of Chemistry, University of Delhi, Delhi, 110007, India
[c] Bio Diagnostic Laboratory, Rohini, Delhi, 110085, India

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ABSTRACT:

Neonatal sepsis is a bloodstream infection primarily caused by *Escherichia coli* (*E. coli*), Group B *Streptococcus* (GBS), *Listeria monocytogenes*, *Haemophilus influenzae, S. aureus, Klebsiella* spp. and non-typhoidal *Salmonella* bacteria. Neonatal Sepsis is referred as a critical response to the infection in the neonatal period that can lead to the failure of body organs and thereby causing damage to the tissues resulting in death of the neonates. Nearly 4 million deaths across the world are occurred due to neonatal sepsis infections. In order to prevent the bloodstream infections in the neonates, it is indispensable to diagnose the disease properly for appropriate treatment during the point of care. Numerous studies have been reported to identify major biomarkers associated with neonatal sepsis including Serum Amyloid A (SAA), C - reactive protein (CRP), Procalcitonin (PCT) and Lipopolysaccharide-binding protein (LBP). Distinct diagnostic platforms have also been developed detecting the presence of bloodstream infections including electrochemical, potentiometric, and impedimetric sensors. Recently, electrochemical biosensors with the integration of nanomaterials have emerged as a better platform for neonatal sepsis biomarkers detection. This review article summarizes the diverse screening platforms, evaluation parameters, and new advances based on implications of nanomaterials for the development of biosensors detecting neonatal sepsis infections. The review further elucidates the significance and future scope of distinctive platforms which are predominantly associated with detection of neonatal sepsis.

1. Vocabulary

Immunosensor: Immunosensor is a biosensing device principally based on the recognition of antigen and antibody binding events. Previously reported immunosensors are optical, microgravimetric, and electrochemical.

Biomarker: Biomarkers are biomolecules which facilitate the diagnosis of a disease. Their level is increased or decreased during the infection.

Electrochemical detection: This is a type of platform used for the detection of a biomolecule or an analyte through the electrochemical cell coupled with two or multiple electrode systems.

Sepsis: Sepsis is defined as the inflammation or infection in the body which may lead to organ damage and in severe cases cause death.

Biomedical devices: Biomedical devices refer to the machines helping in the detection, prevention, treatment, and rehabilitation of the disease or disorder.

2. Introduction

Major challenges in disease management and prevention are attributed towards rapid diagnosis of the disease. Early diagnosis of illness results into the effective clinical consequences, and thereby facilitates better treatment and mitigating physical and psychological suffering which may further proliferate the cost associated with the diagnosis, therapy, and treatment (Arruda et al., 2009). Currently, there are diverse screening methods available for identifying the presence of infections including blood detection, biopsy, and clinical imaging. However, these methods are not that robust for early detection since they are very costly and require combinations of tests to identify every microbial infection and are not accessible to all patients. For a better understanding of disease management, prevention, and biology, further diagnosis through evaluating biomarkers can appear to be a positive approach (Ludwig and Weinstein, 2005). Neonatal sepsis is considered to be the major cause of mortality and morbidity among preterm and

* Corresponding author.
E-mail address: ujain@amity.edu (U. Jain).

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newborn. It is referred as a critical response to the bacterial infection in blood during the neonatal period that may lead to the failure of the body organ, damage to the tissue and in result cause death in severe cases. Furthermore, being a clinical disorder of having a constitutive position with enormous irregularities in essential circulation in newborn babies, the indicating symptoms are not discretely specific making it difficult to diagnose the disease at an early stage. Neonatal sepsis is listed into two major categories depending upon the duration of emergence of the disease as – Early Onset Sepsis (EOS) which occurs within 72 h and afterwards, a Late-Onset Sepsis (LOS). Additionally, when the systemic inflammatory response syndrome (SIRS) transform into septic shock in adults, this results in respiratory distress syndrome (RDS), acute renal failure, and disseminated intravascular coagulation (DIC) (Rangel--Frausto et al., 1995). *Escherichia coli (E. coli), Aerobactin,* Group B *streptococcus (GBS), Listeria monocytogenes (L. monocytogenes),Haemophilus influenzae, S. aureus, Klebsiella* spp. and non-typhoidal *Salmonella* bacteria are considered to be the prime pathogens for neonatal sepsis (Chauhan et al., 2017). During infection, change in the level of proteins or certain biomarkers causing inflammation facilitates accurate diagnosis of neonatal sepsis. Diverse biomarkers have been reported for identifying the proper cause of infection in neonates. Among various biomarkers, procalcitonin (PCT), C-reactive protein (CRP), and Serum Amyloid A (SAA) are predominantly evaluated during infections. The levels of biomarkers in healthy and affected neonates are listed in Table 1.

2.1. Present state of diagnosis and major constraints of detection

Presently, the diagnosis of EOS and LOS require application of those techniques which exhibit higher sensitivity and specificity and therefore can be achieved by the combined computational study of Interleukin-6 (IL-6), Interleukin-8 (IL-8), and CRP (Benitz, 2010). Due to the uncertainty of IL-6 diagnostic window, which results in a shorter half-life of less than 20 min. After the onset of symptoms during the window of 24–48 h, CRP is considered to provide the optimal specificity and sensitivity. An Early observation of CRP for a period of 2–3 days post-infection leads to ensure the selection of the right type of antimicrobial therapy in neonates. Because of the non-availability of a specific biomarker for neonatal sepsis, combinations of biomarkers are examined for the identification of the presence of inflammation in the neonates' bloodstream based on their specificity (Fig. 1) and sensitivity (Fig. 2).

Numerous platforms have been developed for the detection of infections in the blood, urine, and cerebrospinal fluid of neonates. However, these methods have various drawbacks including expensiveness, time taken for test response, requirement of skilled personnel, and high sample volume. Incorporation of nanotechnology-based electrochemical sensing with integrated electronics can be introduced for overcoming these drawbacks. Recently, nano-electronics has facilitated the medical field into development of advanced sensing devices with home-based detection setup and therefore miniaturization of the medical devices will further give progression towards biosensor fabrication (Cui et al., 2001; Tian et al., 2012; Tian and Lieber, 2013). For the detection of biomarkers which are associated with the bacterial infection in neonates and their distinct sensing platforms which have been developed are depicted in Table 2.

Table 1
Biomarkers for neonatal sepsis and their cut-off value in healthy and infected neonates.

S. No.	Biomarkers	Normal level	Level in patient	References
1.	C-reactive protein (CRP)	CRP<10 mg/mL	CRP>10 mg/mL	Monneret et al. (1997)
2.	Procalcitonin (PCT)	PCT<1.0 ng/mL	PCT > 1.0 ng/mL	Chiesa et al. (1998)
3.	Serum Amyloid A (SAA)	10.2 ± 8.3 μg/mL	187.6 ± 78.3 μg/mL	Arnon et al. (2007)
4.	lipopolysaccharide-binding protein (LPBP)	0.6–17.4 l μg/mL	13.0–46.0 l g/mL	Behrendt et al. (2004)
5.	Tumor necrosis factor-alpha (TNF-α)	TNF< 6 ng/mL	TNF> 6 ng/mL	Messer et al. (1996)
6.	Interleukin-6 (IL-6)	IL-6< 100 pg/mL	IL-6> 100 pg/mL	Messer et al. (1996)
7.	Pentraxin 3	26.82 ± 7.52 g/L	43.06 ± 3.88 g/L	Fahmey and Mostafa (2019)
8.	Interleukin-8	IL-8 <60 pg/mL	IL-8 >60 pg/mL	Boskabadi and Maamouri (2010)
9.	Neopterin	N < 32.2 nmol/L	N > 32.2 nmol/L	Nemer et al. (2015)

2.2. Electronics to nano-electronics: playing a pivotal role towards progressing future in biomedical technology

Electronics has made a positive and valuable contribution to the medical domain. Knowledge in the application of electronics into medical technologies is progressive towards the development of medical electronic devices. In order to use advanced tools for the betterment of patient, the wide range of applications of electronics in medicine are incorporated for both diagnostics as well as therapy. The development of new medical electronic devices are based on miniaturization and integration of technologies that are resulting in the fabrication of wearable, portable, and unobtrusive devices with low cost (Zhang and Lieber, 2016).

The main aim of such devices is to measure the physiological changes due to imbalances that occurred in the body (Kong et al., 2000). With low-cost and easy fabrication techniques, the field of electrical detection is swiftly progressing (Song et al., 2010). Field-effect transistor (FET)--based electrochemical nanosensors integrated with various shapes of nanomaterials such as nanotubes, ribbons, and wires are developed. These sensors significantly evaluate a change in resistivity activated on the binding site through the functional molecule, providing a direct and easy mensuration in real-time with easy transportability (Zheng et al., 2005). The diverse shapes of nanomaterials provide higher sensitivity comparatively to the planar surface of the biosensor because of their maximum higher current flow over the cross-section area of nano-scale dimensions. Several studies have been carried out for the detection of neonatal sepsis biomarkers by incorporating various platforms integrated with electronics. Furthermore, in order to identify the neonatal sepsis infections at the early stages, the electrochemical biosensors have been fabricated by comprising nanomaterials in sensing and incorporating tools of nanotechnology which can serve as next-generation nanoelectronics.

2.3. Importance of nanotechnology in biomedical sensing

With the applications of nanotechnology in biomedical sensing through advancements in biosensing and thereby integrating with the other fields, it is possible to overcome the problems associated with the selectivity and sensitivity of biomolecular detections (Claridge, 2008; Kim et al., 2010). The nanosensors are implemented to fabricate devices for sensing biological, chemical, or mechanical forces detected at the nanoscale level caused by change in the target or analyte parameters. Nanomaterials are the building blocks of nanosensors that are interlinked with ligand and acts as a signal enhancer and assist in increased sensitivity. Moreover, nanosensors assist in improving specificity while detecting the particular biomarker of interest. Significant enhancements in detection are attributed to the fact that nanoparticles possess magnetic properties, electrical conductivity, high reactivity, and particular surface area-volume ratio (Mendes et al., 2011). Due to higher surface area to volume ratio of nanoparticles, biomarkers with much higher as well as much lower concentrations can be significantly detected from the samples. Nanosensors provide multi-parametric analysis with actual

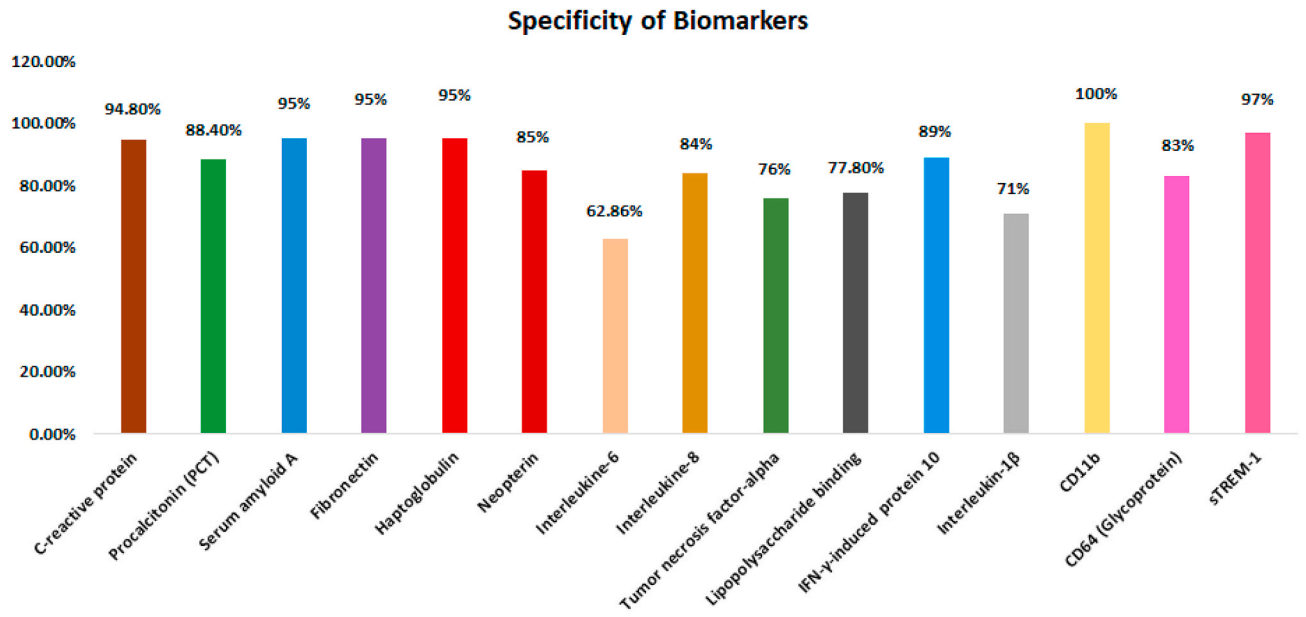


Fig. 1. Specificity of the biomarkers for the neonatal sepsis.

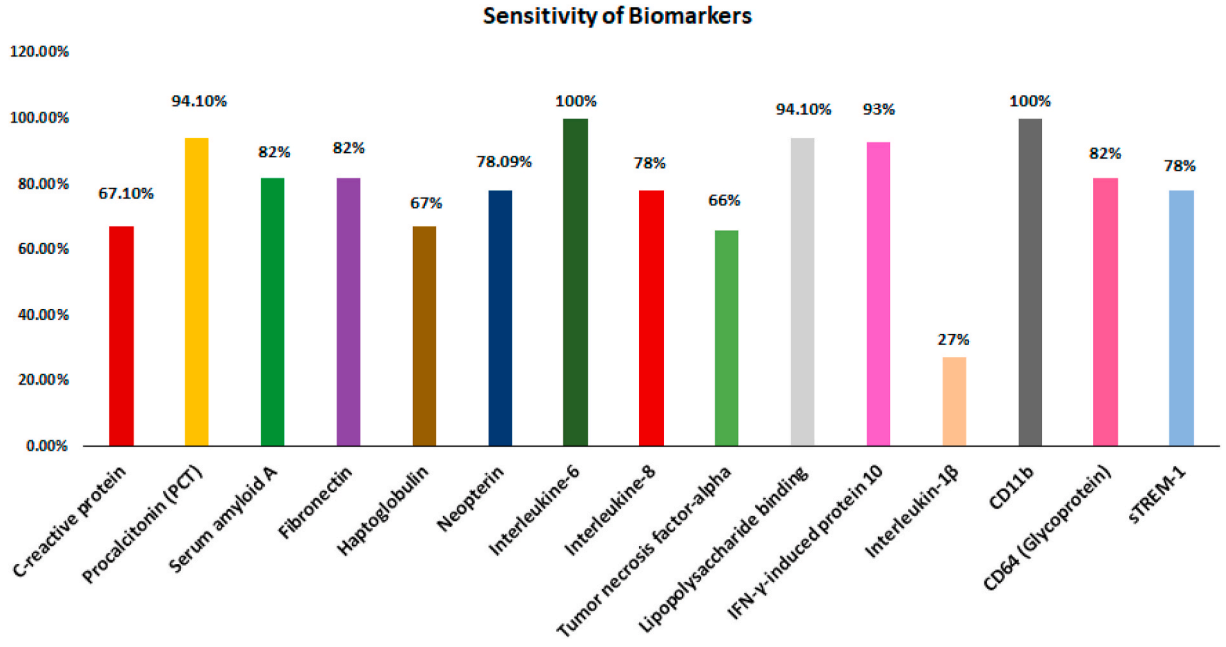


Fig. 2. Sensitivity of the biomarkers for the neonatal sepsis.

time and uninterrupted data output of the diagnosed setup. Furthermore, because of their characteristic properties of altering different shapes, nanowires, nanocantilevers, nanotubes, and thin films, nanoparticles provide multifaceted and high sensitivity diagnosis (Mendes et al., 2011).

2.3.1. Applications of nanotechnology in imaging

It is observed that the early diagnosis of the disease and better visualization of the lesion can be significantly improved by applications of nanotechnology in imaging. Implications of engineered nanomaterials (ENMs) in imaging considerably improve sensitivity and specificity. The European Medicines Agency (EMA) and the US Food and Drug Administration (USFDA) have already approved the iron oxide nanomaterials for Magnetic resonance imaging (MRI) (Leso et al., 2019).

2.3.2. Utilization of nano-sensors in biochemical and molecular sensing

The nanotechnology-based platforms for biochemical, molecular sensing, and diagnosis have furnished several advantages including high selectivity, low cost, home-based, and can be used as a point of care. Development of nano-robots once combined with biological aspects can lead to the advanced development in biomedical sensing devices especially in neurosurgical, haematological, cardiovascular, and dental practices (Leso et al., 2019).

2.3.3. Implementation of nanotechnology-based approaches in drug delivery and therapies

The nano-drug delivery approaches play an important role in therapy since nanomaterials increase solubility, stability, and target specificness; which can be easily accessed. Therefore, personalized treatment to the patient can be given (Leso et al., 2019). Studies on various drugs such as

Table 2
Different biomarkers associated with neonatal sepsis and their detection platform.

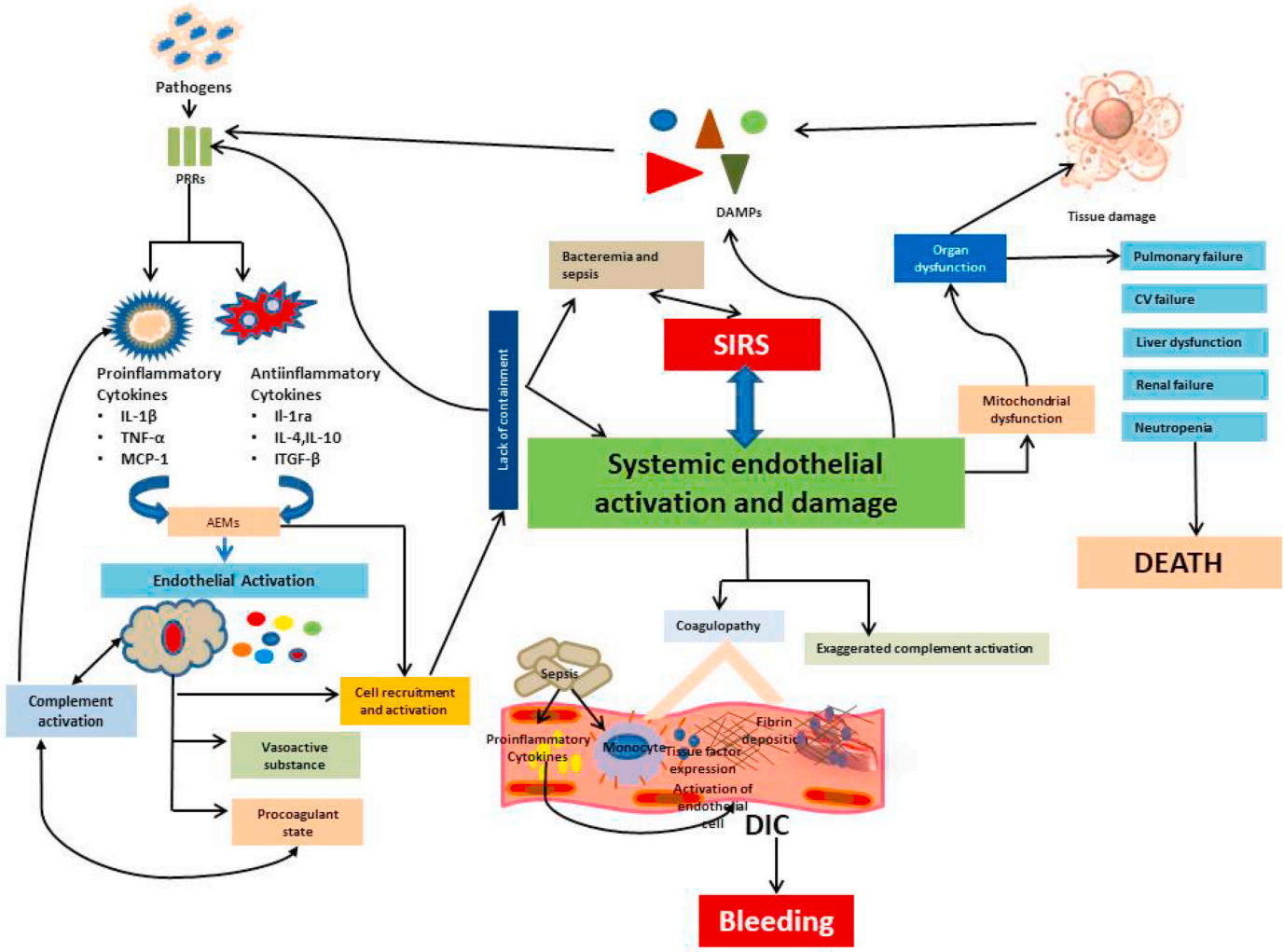
S. No.	Biomarker	Functionality	Detection Platforms	Detection limit/range	Detection time/ response time	Reference
1.	**C-reactive protein (CRP)**	Complement binding to foreign and damaged cells	Screen printed electrode)	0.15 nM - 17 ng/mL	30 s.	Thangamuthu et al. (2018)
			Sandwich immunoassay	0.1–100 ng/mL	20 min.	Kim et al. (2013)
			Field Effect Transistors	220-200 mg/L	30 min.	Magliulo et al. (2016)
			Electrochemical immunosensor	2.2 ng/mL	2 h	Fakanya and Tothill (2014)
			Electrochemical impedimetric	0.5–50 nM, LOD-176 pM	10–15 min.	Bryan et al. (2013)
			Magnetic biosensor	25 ng/mL to 2.5 mg/ mL	30 min.	Meyer et al. (2007)
			Electrochemical biosensors	(0.5–70 nM)	60 min.	(Bing and Wang, 2017)
			RNA aptamer-based capacitive biosensor	100–500 pg/mL	NA	Qureshi et al. (2010)
			DNA aptamer-based sensor	1 pM	30 min.	Jarczewska et al. (2018)
2.	**Procalcitonin (PCT)**	Calcium and phosphate metabolism	Sandwich DNA aptamer immunoassay	0.01–350 ng/mL LOD - 0.5 pg/mL	60 min.	Fang et al. (2014)
			Electrochemical biosensor	Binding constant (Kd) - 0.39 +_ 0.11 nM	NA	Lim et al. (2017)
			Electrochemical peptide sensor	12.5 ng/mL	NA	Lim et al. (2017)
3.	**Fibronectin**	Stimulate reticuloendothelial system clearance of immune complexes, damaged platelets etc.	Colorimetric Biosensor	NA	30 min.	Nekouian et al. (2014)
			Sandwich-type electrochemical immunosensor	15 ng/mL	NA	Tian et al. (2020)
4.	**Neopterin**	Antioxidant inducing or enhancing cytotoxicity and apoptosis	Screen printed electrode	1–12 ppb , 0.44 ppb (LOD)	20 min.	(Zhang et al., 2017)
			Electrochemical immunoassay, SPE	0.008 ng/mL	20 min.	Centi et al. (2015)
			Electrochemically synthesized MIP	5.6 μg/mL	NA	Sharma et al. (2016a)
5.	**Interleukine-6**	Regulates the immune response	Potentiostatic capacitance measurements	$5*10^{-16}$–$5*10^{-13}$ M	NA	Berggren et al. (1998)
6.	**Interleukine-8**	Neutrophils in inflammatory regions are attracted and activated	Electrochemical impedance-based sensor	900 fg/mL - 900 ng/ mL	15 min.	Sharma et al. (2016b)
			Gold nanowire-based biosensor	200 pM	120 min	Tien et al. (2015)
7.	**Tumor necrosis factor-alpha**	Control expression of cytokines	Electrochemical impedance spectroscopy (EIS)	1–100 pg/mL	30 min	Bellagambi et al. (2017)
			Electrochemical impedance spectroscopy	1–1000 pg/ml	15 min.	Kongsuphol et al. (2014)
8.	**Interferon Gamma-Induced Protein 10**	Chemo attractant for activated T lymphocytes	Electrochemical DNA aptamer-based biosensor	0.06 nM	15 min.	Liu et al. (2010)
9.	**Interleukin-1β**	Incorporated in various cellular activities and mediator of the inflammatory responses	Dual screen printed electrodes (DSPE), for detection of interleukin-1β and tumor necrosis factor-α, simultaneously in saliva and serum samples	0.38 pg/mL	2 h 30 min	Sanchez-Tirado et al. (2017)
10.	**TLR4**	Pathogen identification and stimulation of innate immunity	Electrochemical endotoxin sensor	0.0002 EU/mL	30 min	Yeo et al. (2011)

gentamicin (Tariq et al., 2019), ampicillin (Saha et al., 2010; Fan et al., 2019) and cloxacillin (Lacoma et al., 2020; Fuchs et al., 2016) for the treatment of neonatal sepsis were conducted through the integration of nanomaterials-based drug delivery system.

3. Developed sensors for the detection of various biomarkers of neonatal sepsis infections

In order to detect the level of various biomarkers for neonatal sepsis, distinct methods have been used including nephelometric and turbidimetric technologies, Enzyme-Linked Immunosorbent Assay (ELISA), and blood culturing. However, these methods possess limitations such as low sensitivity, high cost, and are time-consuming. Therefore, over the past few years, new techniques are being developed for the identification of biomarkers for instance Quartz crystal microbalance, Surface plasmon resonance (SPR), Piezoelectric microcantilevers, Electrochemistry and Microfluidics (Bryan et al., 2013).

Quartz crystal microbalance (QCM) is an analytical tool used for measuring the small mass change on the biosensor surface. They are highly suitable with molecular imprinting polymer (MIP) technology, the sensors developed exhibit high accuracy, reproducibility, stability, and sensitivity (Diltemiz et al., 2017; Kim et al., 2009). The phenomenon of SPR is based upon the immune reaction using optical sensing for monitoring the binding process between antigen-antibodies, proteins, and another biological complex. The SPR sensor provides detection within less than 1-h with up to 8 simultaneous measurements (Meyer et al., 2006). Piezoelectric microcantilevers measure the resistance variation caused by the biomolecule's specific binding. This technique provides simple, high throughput, and low-cost electrical detection as compared with optical detection (Wee et al., 2005). Electrochemical measurements are recorded with the change in resistance, potential or charge due to the movements of electrons through the biological



SIRS-Systemic inflammatory response syndrome; DIC-Disseminated intravascular coagulation, PRR-Pattern recognition receptors, CV-cardiovascular, DAMPs- Damage/Danger associated molecular patterns

Fig. 3. Pathophysiology of neonatal sepsis. SIRS-Systemic inflammatory response syndrome; DIC-Disseminated intravascular coagulation, PRR-Pattern recognition receptors, CV-cardiovascular, DAMPs- Damage/Danger associated molecular patterns.

complex on the surface of the sensor (Centi et al., 2009; Buch and Rishpon, 2008). Microfluidic systems are used for controlling the flow conditions providing a higher mixing rate for distinct reagents, throughput, and low sample requirement for the sensing platform (Lee et al., 2011; Bryan et al., 2013; Luka et al., 2015).

Among these methods, the electrochemical assays are the most accessible and accurate providing higher sensitivity, faster response time, point-of-care, economical, and user flexible (Bryan et al., 2013).

3.1. Acute phase reactants

3.1.1. C-reactive protein (CRP)

CRP is a circular calcium-dependent ligand-binding plasma protein, which is composed of similar five non-glycosylated polypeptide unit-section with closed-chain conformity. CRP production is mainly occurred in the liver, kidney, and atherosclerotic tissues. CRP is considered as a biomarker for cardiovascular diseases and their infections. The reported level of protein in apparently healthy persons was measured as 2.0 mg/L but during the infection period, the level increases up to 1000-folds. The level of CRP has been categorised into three categories: (i) low risk when concentration is below 1.0 mg/L, (ii) ranging between 1.0 and 3.0 mg/L represent the average probability for inflammation whereas (iii) concentration above 3.0 mg/L is considered as a higher risk for cardiovascular diseases (Bryan et al., 2013; Letchumanan et al., 2019; Hennessey et al., 2009). The pathophysiology of neonatal sepsis is specified in Fig. 3. Once binding to pattern recognition receptors (PRRs) pathogens are first recognized by sentinel cells through Danger Associated Molecular Patterns (DAMPs). The PRRs stimulation leads to the elevation in the level of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), Interleukin-1β (IL-1β), Interferon gamma (IFNγ), IL-6, Interleukin-18 (IL-18), IL-8, and Interleukin-12 (IL-12) triggering the activation of endothelial cells together with an increase in cell adhesion molecules (CAMs). During sepsis, the upregulation of CAMs facilitates rolling and extravascular migration of leukocytes. If the inflammatory homeostasis is not controlled, it can lead to SIRS causing death and failure of organs (Wynn and Wong, 2010).

In order to detect the level of CRP, various platforms with specific nanomaterials have been developed. An electrochemical impedimetric biosensor for identifying the CRP concentration was fabricated by immobilizing CRP polyclonal antibody on the surface of polycrystalline

gold (Au) electrode. The sensor has exhibited high sensitivity, effective reusability, amplified, and label-free detection. The lower limit of detection (LOD) of the specific sensor was reported as 176 pM (Bryan et al., 2013). Besides, a biosensor for CRP detection for the cardiac disease was fabricated on the carbon nanofiber grown with chemical vapour deposition (CVD). Further the sensor was evaluated with cyclic voltammogram (CV) and electrochemical impedance spectroscopy (EIS). The developed biosensor exhibits a lower LOD to be 90 pM or 11 ng/mL (Gupta et al., 2014). An electrochemical assay was fabricated with a monoclonal antibody, RNA aptamer and alkaline phosphatase (AP) at a surface modified with magnetic particles on the screen-printed carbon electrodes (SPCEs) (Centi et al., 2009) and an electrochemical impedimetric assay was developed with direct immobilization of DNA on microwire sensor for detection of CRP. In order to evaluate the sensor, EIS and CV measurements were recorded for each modification step (Songjaroen et al., 2016). Furthermore, a novel zinc oxide (ZnO) nanotube-based sensor was fabricated showing the development of electromotive force (EMF) for complex formation of CRP and monoclonal antibodies on ZnO nanotubes. The concentration of CRP for human plasma was determined by electrochemical potentiometric technique. The sensor shows good stability, excellent reproducibility, high selectivity, considerable repeatability, and a time response of less than 10 s for the developed sensor (Ibupoto et al., 2012). On the other hand, RNA aptamer-based aptasensor for the detection of CRP based on functionalization of silica microspheres using gold nanoparticles was developed. The square wave voltammetry (SWV) technique was adopted for the measurement of electrochemical response and the LOD of the sensor was reported as 0.0017 ng/mL (Signal/Noise = 3) (Wang et al., 2017). For determining the level of CRP in human serum samples, an immunosensor was also developed on bismuth citrate modified graphite screen printed electrode decorated with quantum dots (QD). The sensing mechanism was based upon physisorption of C-reactive protein captured by antibody and consecutively reactions with biotinylated CRP and CRP antibody were occurred. The reaction was then carried out with streptavidin-conjugated Phosphate-Buffered Saline (PBS) immersed QD. The LOD of the biosensor was recorded as 0.05 ng/mL and the sensor operates in linear range from 0.2 to 100 ng/mL (Kokkinos et al., 2015). In order to simultaneously detect high sensitive CRP (hsCRP) and soluble CD40 ligand ($sCD40_L$), an electrochemical immunosensor with reduced graphene oxide–tetraethylene pentamine (rGO-TEPA) was fabricated and has shown higher conductivity making it suitable for loading metal ions (Pb_{2+} and Cu_{2+}) enhancing the current signal which is detected by differential pulse voltammetry (DPV). The LOD of the biosensor was recorded as 16.7 pg/mL (S/N = 3) (Yuan et al., 2015). Another label-free electrochemical immunosensing platform was fabricated for the identification of CRP level in human serum using Molybdenum disulfide–polyaniline–gold nanoparticles (MoS_2–PANI–GNPs) composite providing enlarge surface area and excellent conductivity which have substantially improved the biosensor performance. The defined detection range for the biosensor was 0.2–80 ng/mL and LOD observed was 40 pg/mL (Zhang et al., 2016). In addition, copper nanoparticle (Cu NPs) based electrochemical immunosensing platform for CRP identification was developed. While the electrochemical response was recorded in PBS buffer with differential pulse voltammetry (DPV) technique, the determined concentration range of the biosensor was 1.0 fg/mL to 100 ng/mL and exhibited LOD of the system was 0.33 fg/mL (S/N = 3) (Zhang et al., 2017). Further, a disposable electrochemical biosensor for CRP identification was fabricated using magnetic beads functionalized with a carboxylic acid group, and electrochemical studies were performed with CV and EIS technique. The CRP detection range for the sensor was reported as 0.1–10 pg/mL (Chammem et al., 2015) as shown in Supplementary Table 1.

3.1.2. Procalcitonin (PCT)

PCT is a prohormone of calcitonin with a molecular weight of 14-kDa. PCT is composed of 116 amino acids and synthesized in the liver (Dong et al., 2019; Eyraud et al., 2008; Memar et al., 2019) along with IL-6 and TNF-α. It is reported that during the inflammation, the level of PCT increases rapidly within 2–4 h and once the infection is passed by 6–8 h, the PCT level is increased to the peak value. Unlike other biomarkers such as CRP, TNF-α, and IL-6, the PCT concentration in serum remains high when inflammation persists for 24 h. During the viral infection, the reported level of PCT is 1.5 ng/mL whereas, under bacterial infection, it is reported above 1000 ng/mL. In comparison with CRP, because of the abrupt increase in the PCT level during the onset of infection, it can be considered as a good biological marker for the early identification of inflammation in the neonates (Memar et al., 2019). For determining PCT level distinct platforms have been developed based on electrochemical sensing (Supplementary Table 2) which provides significant benefits over other approaches. Therefore, an electrochemical biosensor based on copper/manganese (Cu/Mn) Double-Doped CeO_2 nanocomposites was fabricated exhibiting an excellent catalytic activity of the nanocomposite by enhancing the generated signal. The developed biosensor has defined a broad detection range from 0.1 pg/mL to 36.0 ng/mL with LOD i.e. 0.03 pg/mL (Yang et al., 2017). Moreover, an electrochemical immunosensing platform was developed with the integration of graphene (G) and multiwalled carbon nanotubes (MWCNTs) with glutaraldehyde (GA) and chitosan (CHIT) for enhancing charge transfer and enlarging the surface area for capturing antibodies. The recorded LOD for the sensor was 0.5 pg/mL and the defined linear range was observed from 0.01 to 350 ng/mL. Besides, the biosensor has shown excellent specificity and stability, less expensive, and stable reproducibility (Fang et al., 2014). In addition, for PCT identification an immunosensor based on immobilization of the PtNP-Fc-C_{60} nanocomposites (Amino group-functionalized C_{60} nanoparticles, ferrocene carboxylic acid (Fc), platinum nanoparticles (PtNPs)) was fabricated. The biosensor shows a detection limit as 6 pg/mL (S/N = 3) with linear concentration range from 0.01 to 10 ng/mL (Li et al., 2015). Another electrochemical immunosensor incorporating cobalt phthalocyanine nanoparticles (nanoCoPc) functionalized with MWCNTs for the detection of PCT was also developed. The exclusive property (electric conductivity and large surface area) of MWCNT facilitated the enhancement of electrochemical signals. This improvement in analytical performance of the immunosensor provided low LOD 1.23 pg/mL and operates in the linear range from 0.01 to 100 ng/mL (Yang et al., 2016). Furthermore, an immunosensor comprising N,N-bis(ferrocenoyl)-diaminoethane/β-cyclodextrins/poly (amidoamine) dendrimer-encapsulated gold nanoparticles (Fc-Fc/β-CD/PAMAM-Au) was fabricated showing an excellent lower LOD as 0.36 pg/mL and the significant linear response was observed ranging in 1.80 pg/mL to 500 ng/mL (Shen et al., 2015). Besides, an ultrasensitive electrochemical immunosensor coated with reduced graphene oxide (rGO) and gold nanoparticles (Au NPs) - enhanced tyramide signal amplification (Au NPs-TSA) was developed. Nanocomposites used in this biosensor provided an enhancement in the signal and large surface area for trapping antibodies. For this sensor ultra-low LOD as 0.1 pg/mL was observed. The linear range of the biosensor was defined within 0.05 ng/mL to 100 ng/mL (Liu et al., 2019). On the other hand, an ultrasensitive immunosensor incorporating rGO-Au nano--composite film as a sensing platform was developed. The sensor was developed for enhancing the electrochemical signal of an immobilized antibody. Single-walled carbon nanohorns (SWCNHs) and hollow platinum chains (HPtCs) were further used for labeling procalcitonin antibodies as signal tags. The LOD of the sensor was recorded as 0.43 pg/mL and operating in a linear range from 1.00 pg/mL to $2.00*10^1$ ng/mL (Liu et al., 2014). Moreover, an immunosensor was developed using ordered mesoporous carbon-silica nano-composites functionalized with Zn NPs (OMCSi-Zn). The excess quantity of OMCSi-Zn material facilitated enhancing the signal for electrochemical detection of the biomarker. The sensors possess high sensitivity, stability, and high charge transfer due to the biocompatibility of graphene and CHIT. The determined LOD of the sensor was 0.013 pg/mL and the improved

concentration range for calibration was defined as 0.05 pg/mL to 80 ng/mL (Fang et al., 2018). A self-assembling immunoelectrode performance was measured for PCT quantification using monoclonal antibodies of procalcitonin on the surface of indium tin oxide (ITO) and conjugated with zinc sulfide capped Cadmium selenide QD. The QD provides a large surface area and excellent electron transfer kinetics improving the performance of the sensor. The developed sensor has detected the lower concentration of PCT about 0.21 ng/mL for a healthy person (Ghrera, 2019). An antibody-based impedimetric biosensor identifying the PCT level was also demonstrated. In this biosensor, highly oriented pyrolytic graphite (HOPG) is used as an electrode for the fabrication of the sensor and 1-pyrenebutanoic acid succinimidyl ester (PYSE) was used for PCT antibodies coupling. This biosensor exhibits an LOD of 3.70 ng/mL and the concentration range obtained for PCT detection for this specific biosensor was 10–100 ng/mL (Mahe et al., 2014).

3.1.3. Serum Amyloid A (SAA)

Since nonspecific symptoms of neonatal sepsis cause confusion among common infections (Çetinkaya et al., 2009), SAA, a polymorphic apo-lipoprotein with a molecular weight of 12–14 kDa is considered as one of the major biomarkers for sepsis in neonates. SAA is synthesized by liver, monocytes, and endothelial cells. The level of SAA increases significantly during the inflammation; therefore, it is considered as an acute-phase reactant. After the synthesis, it binds quickly with high-density lipoprotein and stimulates lymphocytes and phagocytes. Evaluating the level of SAA facilitates the diagnosis of other disorders including heart disease, traumatic, bacterial, and viral infections. It has been reported that the level of SAA depends upon the age of the patient indicating older patients have higher SAA levels whereas found lowest in umbilical cord blood. SAA is more accurate for diagnosis in EOS as compare to CRP because of its prompt increase during infection. During the onset of neonatal sepsis, the level of SAA increases rapidly and consistently decreases after some time (Arnon et al., 2007). Infected neonates have a high level of SAA than non-infected neonates. Moreover, SAA level was increasing faster than the level of CRP, making it as a better biological marker for the identification of neonatal sepsis (Arnon et al., 2007; Çetinkaya et al., 2009; Memar et al., 2019; Sharma et al., 2018). Numerous platforms have been developed for the detection of SAA. Spectrometric-based surface-enhanced laser desorption/ionization (SELDI), which provides rapid and reproducible results for SAA detection. This technique is used as a simple mode for analysis of inflammation in the serum (Tolson et al., 2004), ELISA, which is an assay based on antigen-antibody interaction immobilized on microplate and provide highly specific results (Timucin et al., 2012) and nanomaterial based immunosensor for detection of SAA. An immunosensor was developed with the glassy carbon electrode, further modified with MWCNTs and carboxy-endcapped polypyrrole (PPy-α-COOH). Furthermore, Ionic liquid and CHIT were applied as a support platform. The surface of PPy-α-COOH was modified with the immobilization of SAA antibodies. Because of the presence of nanomaterials, the electrochemical signal was enhanced providing a large surface area for capturing antibodies on the surface. This improves the performance of the biosensor with higher sensitivity, stability, and selectivity. The LOD of the biosensor for SAA was recorded as 0.3 pg/mL and the linear range for the biosensor was determined from 0.001 to 900 ng/mL (Xia et al., 2015).

3.1.4. Lipopolysaccharide-binding protein (LBP)

LBP is protein of molecular weight 50 kDa polypeptide which binds with lipopolysaccharide (LPS) to elicit immune response. LBP is a soluble pattern-recognition molecule and interacts with endotoxin during bacterial infection. LBP is synthesized in the liver, muscle, and epithelial cells. Once synthesized, it passes through bloodstream and glycosylated. LBP is responsible for the secretion of various proinflammatory mediators and binds with CD14. It has been reported that the concentration of LBP ranges between 2 and 10 mg/mL, whereas patients exposed to sepsis or disease show concentration nearly or greater than 100 mg/mL. The change in the level of LBP is only noticeable during bacterial or fungal infection and while no increase is observed during viral infections. The level of LBP rises rapidly once the new-born is exposed to the infection within first 6–8 h and continues for more than 24 h, thus LBP concentration facilitates in the early detection of sepsis (Memar et al., 2019; Sharma et al., 2018; Mussap et al., 2011). Furthermore, lipopolysaccharide-binding protein concentration has been evaluated in various studies where fabrication of sensors are used for providing several advantages e.g., piezoelectric crystal biosensor operates on the principle of change in oscillation due to mass attached on the surface of the piezoelectric crystal surface for developing the device which is used as piezoelectric quartz crystal (PQC) (Chang et al., 1997) Moreover, ELISA (De Haas et al., 2000) and an aptamer-based impedance biosensor were developed to determine the LBP concentration. In the aptamer-based sensor, single-stranded DNA (ssDNA) probe was used specifically for LBP detection while the Au electrode was used as a platform for the sensor and immobilized with 3-mercaptopropionic acid (MPA) that act as a linking agent between the probe and the electrode. The determined range of the specific sensor was reported as 0.001–1 ng/mL (Su et al., 2012). The fiber-optic biosensor for identifying LPS from *E. coli* at a lower concentration of 10 ng/mL in 30 s was developed (James et al., 1996). On the other hand, an impedimetric biosensor was developed for the identification of LBP in *E. coli, Salmonella enterica (S. enterica), Serratia marcescens (S. marcescens),* and *Klebsiella pneumonia* infections. The electrodes fabricated for determining the LPBP from these bacterial infections were modified with l-cysteine-gold nanoparticle (AuNpCys) and poly (vinyl chloride-vinyl acetate maleic acid) (PVM) with self-assembling of CramoLL lectin on the modified PVM–AuNpCys electrode. The electrochemical response of the sensor was observed with cyclic voltammogram and electrochemical impedance spectroscopy techniques (Oliveira et al., 2011). An electrochemical biosensor was developed with immobilization of synthetic peptide on thin films. For measurement of electrochemical signal the cyclic voltammetry technique was adopted. The LOD of the developed sensor was recorded to be 21.8 pg/mL (Zuzuarregui et al., 2015). Besides, an electrochemical biosensor was developed using a gold electrode as a platform and the surface was modified with Copper (Cu) and nitrilotriacetic acid (NTA) complex for determining the interaction between LPS and NT-Cu complex. For observing the charge transfer response of the biosensor, EIS technique was used. The sensor was calibrated in a linear range from 0.0001 to 0.1 ng/mL (Cho et al., 2012). Further, an electrochemical sensor developed with immobilization of Toll-Like Receptor-4 (TLR-4) protein on the surface of Au electrode. The sensor utilized surface-modified Self-Assembled Monolayer (SAM) for tethering LPS-probe, and TLR-4 to Au electrode. The EIS studies were performed for determining the electrons movement (Mayall et al., 2017).

3.1.5. Inter-alpha inhibitor proteins (IaIp)

IaIp are classified into a class of serine protease interception which are present in the human plasma. The heavy and light polypeptide subunits are used for the formation of the IaIp protein. These subunits form covalent bond with the glycosaminoglycan chain (Fries and Blom, 2000; Blom et al., 1999; Zhuo et al., 2004). IaIp is involved in distinct physiologic and pathologic activities including inflammation, stabilization of the extracellular matrix, tumor invasion, wound healing, and metastasis (Bost et al., 1998; Kobayashi et al., 2003; Fries and Kaczmarczyk, 2003). The concentration of IaIp doesn't change with the growing age of the person. However, the new-borns suffering from sepsis show a decrease in the level of Inter-alpha inhibitor proteins instead of a higher level as seen in healthy infants. Therefore, IaIp can be considered as a biomarker for neonatal sepsis, however studies are still being carried out for understanding its specific role in sepsis (Sharma et al., 2018; Memar et al., 2019; Chaaban et al., 2009).

3.1.6. Mannose-binding lectin (MBL)

MBL is a plasma protein belonging to the category of collectin. MBL binds with pathogens and shows inflammatory effects and facilitate phagocytosis through macrophages activation. It is produced by the liver. The lower level of MBL in neonates is a serious concern as it shows a higher risk of inflammation suggesting a high risk of early-stage sepsis. It has been reported that approximately 15 %–20 % of the white population is affected with a lower level of MBL. Studies are being carried out to examine the consideration of MBL as a biomarker for the diagnosis of neonatal sepsis (Sharma et al., 2018; De Benedetti et al., 2007).

3.1.7. Pentraxin-3 (PTX-3)

PTX-3 belongs to the family of pentraxin which acts as an acute phase glycoprotein. The production of PTX-3 is through endothelial and mononuclear phagocytes. It plays a pivotal role in defending pattern recognition receptors against microorganisms. The concentration of PTX increases abruptly during the phase of inflammation. In some studies, it has been reported that the PTX works as a significant biomarker for sepsis. However, the role of PTX in neonatal sepsis is still under evaluation (Sharma et al., 2018).

3.1.8. Fibronectin

Fibronectin is a glycoprotein in plasma with a Molecular Weight of 440 kDa and is generated by hepatocytes. It has been observed that the level of fibronectin decreases in adults or neonates when the patients are suffering from SIRS (Parlato and Cavaillon, 2015). Platforms developed for the detection of fibronectin are colorimetric biosensor (Nekouian et al., 2014) and sandwich-type electrochemical immunosensor (Tian et al., 2020). Recently, electrochemical biosensors are developed with high sensitivity and low cost for fibronectin detection.

3.1.9. Neopterin

Neopterin (2-amino-4-hydroxy-6-(d-erythro-1̓,2̓,3̓-trihydroxypropyl) pteridine) is a catabolite of purine nucleotide triphosphate (guanosine triphosphate (GTP)) having a molecular weight of 253 g/mol (Murr et al., 2002; Zhao et al., 2017). Neopterin facilitates the diagnosis of infection in neonates and adults (Ercan et al., 2016). Once the T lymphocytes are activated and release interferon-gamma, the human macrophages produce neopterin. Studies have shown the increase in the level of neopterin during the bacterial infection, therefore it can act as an early biomarker and provide high sensitivity and specificity. During inflammation, neopterin is responsible for the stimulation of cellular immune responses and therefore the increased level of neopterin leads to the risk of sepsis and endothelial cells damage (Nemer et al., 2015). Electrochemical biosensors (Supplementary Table 3) were developed for detecting the level of neopterin including an electrochemical immunoassay for neopterin determination was developed where the immobilization of antibodies was done on the protein modified magnetic beads. Electrochemical measurements were then taken with differential pulse voltammetry techniques. The biosensor showed a LOD of 0.008 ng/mL while average RSD % = 10 (Centi et al., 2015). On the other hand, a molecularly imprinted polymer-based biosensor for the identification of neopterin was fabricated. In order to fabricate the biosensor, the molecule was imprinted on poly (ethylene-*co*-vinyl alcohol) via solvent evaporation. The CV measurements were then performed for signal detection (Huang et al., 2011). Furthermore, an integrated microfluidic and potentiostatic biosensor based on molecular imprinting technology was developed. The target was imprinted on polydimethylsiloxane (PDMS) which was further used for making microfluidic channels. CV measurements were then taken for measuring electrochemical response (Hsieh et al., 2011).

3.2. Cytokines

3.2.1. Interleukin-6 (IL-6)

During the inflammation, IL-6 is processed and produced by B-lymphocytes, T-Lymphocytes, fibroblasts, monocytes, and the endothelial cells. IL-6 is the composition of 2 N-glycosylation 184 amino acid and 4-cysteine residues. IL-6 is considered as a biomarker for sepsis because of its rapid evaluation after the onset of pathogen before the change in the level of CRP is reflected. The half-life of IL-6 is within 24 h. The neonates which are affected with inflammation have a high concentration of IL-6 in the umbilical cord. The studies have reported that IL-6 can be beneficial in the early identification of sepsis since it provides results with better sensitivity. Recent studies suggested that IL-6 has responded as a significant biomarker for sepsis detection along with PCT (Memar et al., 2019; Sharma et al., 2018).

3.2.2. Interleukin-8 (IL-8)

IL-8 is reported as a pro-inflammatory cytokine generated by fibroblast, endothelial cell, monocytes, and macrophages. It has been recommended as one of the most suitable biomarkers for the early identification of inflammation in new-borns. The half-life of IL-8 is less than 4 h. In order to initiate recruitment, of immune cells at the site of inflammation, IL-8 activates leukocytes resulting in the proliferation of leukocytes within 1–3 h which is useful in the early diagnosis of sepsis. The level of IL-8 is higher in infected individuals while comparing with apparently healthy new-borns. Further studies are required to perform on the development of effective measurement techniques for IL-8 as a biomarker for neonatal sepsis (Memar et al., 2019; Sharma et al., 2018).

3.2.3. Tumor necrosis factor-α (TNF-α)

A pro-inflammatory cytokine, TNF-α, is a moderator of systemic inflammation that triggers an acute-phase reaction and therefore is elevated after the neonatal infections. The concentration rises within 2–4 h of the inflammation. An early rise in the concentration of TNF-α provides an early recognition of infection in the neonates. During the pathogenesis of sepsis, TNF-α level is investigated by ELISA to understand the type and cause of infection.

Besides, there are significantly reported biomarkers which contribute in the detection of neonatal sepsis including Interleukin-10 (IL-10), Visfatin, Resistin, Cell surface markers, Granulocyte colony stimulating factor (G-CSF), Neutrophil CD11b/Integrin alpha M, Interferon Gamma-Induced Protein 10, Soluble CD14 subtype presepsin (sCD14-ST), Interleukin-35 (IL-35), CD64 and CD62L. In order to strengthen our perspective which is related to neonatal sepsis, the role of these biomarkers is still being investigated (Memar et al., 2019; Sharma et al., 2018).

4. The commercial value and significance of the sensor

Presently, few monitoring systems for neonatal sepsis are commercially available such as HeRO monitoring (Heart rate observation). The system is based on the monitoring of the slight change in the heart rate of the neonates (Fairchild, 2013)facilitating the early detection of the inflammation. Furthermore, Neo-Bedside Monitoring Device for (iNICU) provides real-time data and is based on the Internet of Things (IoT) integrated with several electronic devices (Singh et al., 2018). These devices are used to monitor various parameters in the new-born, the data can be recorded manually at every hour and the data can be accessed from the remote areas (Supplementary Table 4).

In the present scenario, methods which are available in the market for identifying neonatal sepsis are ELISA based approaches or selective bacterial culture. These methods are highly tedious and require trained personnel to conduct the experiments. Therefore, the development of biosensors for detecting sepsis may be potentially beneficial providing rapid with accurate results, specific and selective performances, and portability with home-based approaches for diagnosis. Furthermore, a major significant advantage with biosensor is that it can facilitate early detection of the inflammation while taking a very less sample volume at the point of care condition associated with a new born. Commercially, there is no significant biosensor is available in the market which can directly detect neonatal sepsis. However, studies are still under progress

in developing biosensing based platform for specific biomarkers associated with neonatal sepsis.

5. Future scope in emerging technologies

The future research is focussed towards the development of early detection platforms of neonatal sepsis infections in neonates. Approaches towards nanomaterial-based biosensors integrated with the multi-microelectrode array, microfluidic devices, and spintronic detection techniques have emerged. These future sensors are capable of detection with portability, miniaturization, and point of care diagnosis. These devices facilitate the enhancement in analytical properties including, faster results, portability, highly efficient sensing with low power electronics. Furthermore, the research should be focused on less biological sample volume with high sensitivity and selectivity for detection. All these necessary steps will lead to the development of exploring the advanced detection platforms for early diagnosis of neonatal sepsis in clinical settings.

6. Conclusion

In this review, we have discussed about neonatal sepsis infections, a type of bloodstream infections which is a dominant cause for high morbidity and mortality in the new-borns. Major microorganisms causing neonatal sepsis are *E. coli, L. monocytogenes,* Group B *Streptococcus, Haemophilus influenzae, S. aureus*, *Klebsiella* spp. and non-typhoidal *Salmonella* bacteria. Despite numerous advancements have been made for identifying the cause of neonatal microbial infection, no appropriate platform for early detection has been developed. Various biomarkers including Serum Amyloid A (SAA), C - reactive protein (CRP), Procalcitonin (PCT) and Lipopolysaccharide-binding protein (LBP) are recommended for evaluating the possible source of infection. Diverse detection approaches have been already reported for the sensing of sepsis but not a particular biomarker provides significant information about the infection. Combinations of biomarkers are examined for the diagnosis of the infection with high specificity and selectivity. This review highlights the varieties of biosensors for biomarker detection. Development and fabrication of nanomaterial based electrochemical biosensors for the identification of different biomarkers at single platform incorporated with electronics may solve the problem. Electrochemical biosensors provide better platforms and can be adopted for the early diagnosis of sepsis in the neonates. These sensors possess numerous advantages including higher sensitivity, low-cost requirement, lesser time consumption, and high specificity. Applications of nanomaterials in electrochemical biosensors facilitate improvement in the analytical performance of the platform. Efforts are being made in the future for developing the multiplexed platform for distinct biomarkers with multiparameter screening of neonatal sepsis.

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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Appendix A. Supplementary data

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