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SERUM BIOMARKERS OF SEPSIS

Abstract: Sepsis is an innate immunological response of the systemic inflammatory pathway to infection, and is growing problem worldwide inn terms of mortality rates. Rapid molecular based tests have been developed to address this need but are not always accurate in this respect. The most commonly used biomarkers specially the serum markers are reviewed for their current uses and the diagnostic accuracies. Challenges that are faced during biomarker research lied in the lack of uniform protocol and methodology. define sepsis to be a systemic inflammatory response to a bacteria, virus or fungi. In clinical setting several other physiological symptoms must be presented to properly diagnose sepsis. The gold standard of sepsis diagnosis has been traditionally the use of microbial cultures to identify the source of illness. The major limitation of using cultures in the length of the time required to develop cultures to identifiable quantities to detect sepsis. The gold standard of sepsis diagnosis has been traditionally the use of microbial cultures to identify the source of illness. The major limitation of using cultures in the length of the time required to develop cultures to identifiable quantities to detect sepsis.

Biomarkers for the Infections caused by Bacteria: C-reactive protein is the general acute phase reactant protein, **Procalcitonin** is a precursor of the hormone calcitonin and is another potent biomarker for bacterial infections, **Serum Amyloid A** is an important apolipoprotein,

Biomarkers for the Infections caused by Fungi. Mannan and Antimannan antibodies are used to detect the fungal infections due to the presence of mannan in the cell walls of the invasive fungal pathogens. The main disadvantage Mannan and Antimannan antibodies test alone is the high rate of false positives and negatives.

Biomarkers for Viral Infections: Interferon Gamma inducible protein 10 (IP-10)

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IP-10 a proinflammatory chemokine is an important biomarker for diagnosing viral infections.

Other Biomarkers on Avenue*:* IL10, Lipopolysaccharide Binding Protein, Soluble triggering receptor, TLRs, Toll like Receptors 2 (TLR-2) and Neutrophil CD64 receptors, CD163, Micro RNAs, SNP, TNF alpha, IL-1.

Sepsis, as an increasing cause of mortality in the group of multiple injured and critically ill patients in the ICU, requires rapid diagnosis and treatment, and hence the importance of biomarkers is highly important.

Key words: sepsis, early diagnosis, biomarkers, morbidity, mortality

Introduction

Sepsis is an innate immunological response of the systemic inflammatory pathway to infection, and is growing problem worldwide inn terms of mortality rates. Immediate treatment is always required which necessitates the need of early and accurate diagnosis.[1] Rapid molecular based tests have been developed to address this need but are not always accurate in this respect. The most commonly used biomarkers specially the serum markers are reviewed for their current uses and the diagnostic accuracies including the c reactive proteins and the PCT levels and also serum amyloid and others.[2] It is clear that a single biomarker which is ideal though has not been identified but a series specific studies on individual pathogen mediated sepsis has been studied extensively. Challenges that are faced during biomarker research lied in the lack of uniform protocol and methodology, standardization of the assays and the detection levels to be fixed age wise from population to population. The reliable detection of Biomarkers can be useful to have an immense diagnostic and prognostic relevance in relation to the management of sepsis.[2]

Sepsis is considered as the most important cause of morbidity and mortality. By sepsis we mean infection of the blood and if it is caused by bacteria, we term it as bacteremia and if by virus it is termed viremia. As such the infection in the blood is not so dangerous but as blood perfuses the important tissues and organs infections can be carried by blood to the kidneys or lungs which can result in the end organ failures of a patient leading to death in severe conditions. The main issue is that sepsis often lacks clinical manifestations so if the specific and the sensitive indicators of the infections are collected and analyzed then it will create superior out comes in the management of the disease. Immunologically it can be considered as the dysregulation of the response of the innate immune system. Biomarkers are hence important to signal the presence and severity of sepsis in early conditions for effective prognostic values and importance.[1,2]

It is very important to note that sepsis is not a true disease and just a physiological manifestation of the systemic immune response in innate immunity majority of the studies define sepsis to be a systemic inflammatory response to a bacteria, virus or fungi. In clinical setting several other physiological symptoms must be presented to properly diagnose sepsis. The common treatments of patients suspected with sepsis aims to eradicate the bacteria in the blood or reduce its growth by using broad spectrum antimicrobials. The gold standard of sepsis diagnosis has been traditionally the use of microbial cultures to identify the source of illness. The major limitation of using cultures in the length of the time required to develop cultures to identifiable quantities to detect sepsis and the possible microbes to start the treatment with antimicrobials and this with Bactek culture takes around 48 hours and hence there is a need of early detection through the detection of serum biomarkers which thus becomes important. Even the cultures might be insensitive under several conditions including the slow growing and non cultivatable organisms present at very low concentration. So, in the light of this problem alternative diagnostic methods using molecular biology tool kits have been developed to address the situation for rapid and automated detection of sepsis. These tests are basically the ELISA tests, flow cytometric detection of the specific marker antigens, immunocolorimetric tests, FISH methodologies and PCR and amplified PCR techniques and the main ideology was to detect the marker proteins.[3]

In parallel to the development of biomarkers which provides a faster and more sensitive diagnostic methods for infectious microbes thus relies on the monitoring of the changes in specific serum protein biomarker concentrations.

Biomarker is best defined as a factor or feature that can be measured and evaluated objectively as an indicator of normal biological responses or pathological processes. This article will focus on the wide range of biomarkers that is being in use and are destined to be launched in the market on the basis of their diagnostic accuracy (DA) which takes into account parameters like sensitivity, specificity and the predictive values.

For the purpose of being clinically relevant the biomarkers should have a very high DA that is it must have a very high sensitivity, specificity and positive predictive values and at the same time having negative predictive values. Some studies also calculated the likelihood ratios to determine the results which were actual cause of the disease or just occurred due to random chance. These values helped to assign the cut off limits for the purpose of correct diagnosis. As sepsis is a condition caused by bacterial, fungal or viral infections and thus leading to the infection of blood, the biomarkers will also be discussed for the specific pathogens in relation to sepsis. The individual markers discussed below may not be an absolute and specific for a particular diseased microbe but can be used to specify the class of the microbe responsible for sepsis so as to design target methods for antibiotic initiation or other antimicrobials for the management of this life-threatening critical condition.[4]

Biomarkers for the Infections caused by Bacteria

C-reactive protein

This is the general acute phase reactant protein rises in concentration up to 1000 times in the blood in response to the inflammation and infection. The sensitivity values have been reported in the range of 30-97% with specificity values of 75-100% and PPV of 31-100%. The use of different assays caused the variation of the results, as because each assay had unique functional limits assigned. Serum CRP levels also varied with age and in healthy individuals. A CV of 10mg/l was used for elderly patients over 65 years of age, while a CV of 5mg/ml was used for adults and infants in the range of 3-7 days old. CRP levels are higher in older children than in younger ones.[3,5] Studies have indicated that CRP is highly sensitive and is highly specific for infection. This is the reason it can differentiate between gram positive and Gramm negative infections. It can also be used as a prognostic marker for community acquired pneumonia and suggesting a need for hospitalization with a CV value of 110mg/l. It also differentiates between bacterial and fungal infections where the levels above 100mg/l are indicated for bacterial infections and below that as fungal infections.CRP is however unreliable for viral infections.[6]

Procalcitonin

PCT is a precursor of the hormone calcitonin and is another potent biomarker for bacterial infections. It has been reported to be in low concentrations of 0.33ng/ml in the serum of healthy individuals and increases around 1000 times under the inflammatory conditions. It is reported to rise within 2 to 4 hours of infection and peak at 6-8hr. Persistent increased levels will indicate continuous presence of bacterial infections. The rapid upregulation and sustenance in the serum makes it a unique biomarker. And as this molecule his highly stable it remains stable in blood preparation methods and freezing procedures even after long term storage.[7]

PCT has high DA and the sensitivity values around 75-100% and specificity values of 70-100%. The PPV values are 55-100%. Many studies have CV of PCT in the range of 0,5ng/ml a range of CVs has been proposed. As a biomarker for the bacterial infections most studies find PCT to be accurate biomarker than other inflammatory markers. It has very high prognostic values. PCT is also distinguish fungal and viral infections. During the viral infections the PCT values remain low often like the values found in the normal individuals. PCT is also used to make difference between bacterial and viral meningitis. In contrast fungal infections tend to cause mild elevations in the PCT concentration compared with the levels seen in the bacterial infections. Studies on invasive Aspergillosis and invasive candidiasis has shown high levels of PCT values in patients with bacterial sepsis than with fungal sepsis.[5,8] PCT has also shown to be

a potential biomarker for pneumonia, abdominal infections, urinary tract infections, lower respiratory infections, myocardial infarctions and as a biomarker for the speculated therapy with antibiotics in patients with community acquired pneumonia.

Serum Amyloid A

Serum Amyloid A is an important apolipoprotein which is reported to have potential value for diagnosis of sepsis. SAA is expressed in levels around 1000times higher after 8-24 hours from the onset of sepsis. Compared to the levels of CRP, SAA levels are reported to rise faster and higher after the onset of sepsis and remain at higher relative elevations. Serum SAA levels of less than 15mg/l for the elderly over 65 years and les than 10mg/l for adults and newborns aged 3-7days has been indicated for fixing diagnostic criteria.

Though SAA is mainly used as markers for bacterial infections but it can be used to report for viral infections above the levels of 10mg/l among the infected patients. SAA might not be too sensitive and hence its clinical values may be overestimated.[8]

Biomarkers for the Infections caused by Fungi

Mannan and Antimannan antibodies are used to detect the fungal infections due to the presence of mannan in the cell walls of the invasive fungal pathogens. Bothe the levels of Mannan and Antimannan remain elevated high in conditions like aspergillosis and candidiasis making them useful markers in fungal sepsis. There are lot of variations in the DA, PPV, sensitivity and specificity values due to the variation of the assay protocols.[9]

The main disadvantage Mannan and Antimannan antibodies test alone is the high rate of false positives and negatives that the assays produce requiring these tests to be used as markers in conjunction with other diagnostic tests. Beta –D glucan tests have been used in combination with these tests because Beta-D glucan is highly sensitive and specific for invasive mycosis and are not species specific.[10]

Biomarkers for Viral Infections

Interferon Gamma inducible protein 10 (IP-10)

IP-10 a proinflammatory chemokine is an important biomarker for diagnosing viral infections due to its role in host response to viral infections. Study on bacterial infections at very low birth weight infants concluded that IP-10 is a good biomarker during initial measurements recording elevated levels in infected patients at both initial and 24-hour measurements with no overlap of the ranges. IP-10 levels can correlate also with the severity of sepsis. IP-10 is released in response to viruses like rhinovirus,

respiratory syncytial virus, hepatitis B and hepatitis C and H5N1 influenza virus. It has also shown to be a very good marker for prognostic treatment in hepatitis C patients.[11]

Other Biomarkers on Avenue

IL-10

IL-10 has been indicated as a useful biomarker for sepsis specially in the diagnostic values in early and late onset of sepsis amongst the neonates where CV values of greater than 17.3 pg/ml has been estimated as significant DA values. This marker is also sensitive and specific to the tune of 80%. The main problem is that in general circulating cytokines have very short half life thus resulting in false negatives hence limiting their DA values.[12]

Lipopolysaccharide Binding Protein

Lipopolysaccharide Binding Protein is also a useful biomarker specific to bacterial infections. Serum Lipopolysaccharide Binding Protein levels are known to increase during bacterial and fungal infections but not in viral infections.[13]

Soluble triggering receptor

Triggering receptor expressed on myeloid cells-1 TREM are a group of cell-surface receptors that belong to the immunoglobulin superfamily [26]. TREM-1 is expressed mainly on macrophages and neutrophils, and has been identified as an amplifier of the immune response that strongly enhances leukocyte activation in the presence of microbial products [26, 27]. The latter results suggest that sTREM-1 can be released by a broad spectrum of inflammatory stimuli. The first promising result of the use of sTREM-1 in plasma to diagnose sepsis in ICU patients indicated that sTREM-1 might be that perfect diagnostic sepsis biomarker that everybody had been looking for. Results are ranging from an accuracy of almost 100 % (a ROC-AUC of 0.97) for the diagnosis of sepsis in ICU patients.[14]

TLRs

The results obtained by our group show that TLR and other cellular surface receptors may be differently regulated on mononuclear cells and neutrophils, and that they are dynamically modulated across the stages of sepsis. Toll-like receptor signaling gene expression in mononuclear cells is decreased in more severe forms of the disease. In contrast, up-regulated genes are seen along the clinical spectrum of sepsis in neutrophils.[12] Expressed by the macrophages, dendritic cells, and other cells plays

an important role in the innate immune responses to response to infection by cross reacting and recognizing specific bacterial antigens.[15] TLR 4 is critical for recognition of LPS while the tLR-2 is useful to recognize gram positive bacterial components.[5,12]

Toll like Receptors 2 (TLR-2) and Neutrophil CD64 receptors

Toll like Receptors 2 (TLR-2) and Neutrophil CD64 receptors are two important biomarkers present on the cell surfaces, during infection both these biomarkers are upregulated on the surface of the monocytes and neutrophils[15]. These receptors are significantly upregulated during both bacterial and viral infections. They are increased in conditions like influenza A and B, respiratory syncytial virus, mumps, Herpes zoster virus, cytomegalovirus infections which indicates that TLR-2 could be potential biomarkers for viral infections[5,15]. Up to now, the CD64 is used for the marking of bacterial infections. CD64 expression is not affected by the underlying inflammatory diseases and is not differentially between systemic and local bacterial infections, Da values, sensitivity values and specificity values with PPV range from 71-100% justifying it as a significant biomarker.

CD163

CD163 is transmembrane molecule found on the membrane of mononuclear phagocytes. Being a specific scavenging receptor for hemoglobin heme inside the body it is very capable to identify hemoglobin-haptoglobin complex. CD163 regulates the expression of anti-inflammatory molecules like IL-10 and hemeoxigenase-1. Soluble CD163 comes from the molecules peeled from these phagocyte surfaces they becomes very good markers of sepsis and severe sepsis and was found to be superior than the PCT and CRP levels.

Micro RNAs

They are the type of endogenous non coding small RNAs about 22 nucleotides in length and play important roles by inhibiting the expression of the messenger RNAs. A significant amount of the miRNAs has been observed outside the cells within the various body fluids. These cell free miRNAs are stable under harsh conditions. miR-150 has been indicated as a biomarker for sepsis and the plasma ratio of miR-150/ interleukin 18 can be used to study sepsis severity.

SNP

C-reactive protein (CRP) is an important biomarker of sepsis. Several single-nucleotide polymorphisms (SNPs) in the CRP gene can determine plasma CRP levels and

also are risk factors in many diseases, such as cancer, arteritis, and diabetes. However, it is unknown whether polymorphisms in CRP are associated with susceptibility to and outcome of infantile sepsis.

TNF alpha

Tumor necrosis factor alpha a pleiotropic cytokine produced by the activated macrophages plays a key role in inflammatory response and in the progression of the autoimmune diseases. An association with sepsis and expression of TNF-2 genotype was found.[16]

IL-1

The interleukin 1 receptor associated kinase is important for the TLR2 and TLR4 induced activation of nuclear factor kappa beta which is a critical factor in the transcriptional regulation of many sepsis associated proinflammatory mediators.[16]

Sepsis, as an increasing cause of mortality in patients with infectious disease specially in the critically ill patients in the ICU requires rapid diagnosis and treatment, and hence the importance of biomarkers is highly important.

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