**Chapter: Host Biomarker-Based Diagnostics**

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# **Abstract**

Host biomarkers have been useful for the diagnosis of disease for many decades. Host biomarker-based diagnostics identify particular biological markers in a patient's body, such as proteins, metabolites, or nucleic acids that signify the existence, intensity, or kind of disease. Clinical diagnosis depends significantly on classifying patient diseases, managing cases, and guiding treatment and care plans. Therefore, there is always a requirement for rapid and point-of-care testing. These biomarkers can assist in the differentiation of infection amongst pathogens, such as bacteria and viruses, tracking disease advancement, and directing treatment choices. However, some candidate biomarkers have emerged from recent studies in infectious diseases, autoimmune diseases, and cancers. The promising biomarkers are given strong consideration in current diagnostic settings: C-reactive protein, Procalcitonin for sepsis, various cytokines for diagnosing infectious diseases such as tuberculosis (e.g., Interferon-γ release assays, C- reactive proteins, Interleukin-6, Interferon gamma induces protein-10, Interleukin-2), quick identification of sepsis (e. g., presepson, Interleukin-6,), differentiating between bacterial and viral infections (e. g., Procalcitonin, C-reactive protein), early identification of cancer (e. g., Cancer Antigen-125 for ovarian cancer, Prostate surface antigen, for prostate cancer), monitoring tumor progression (e. g., circulating tumor DNA), identifying autoimmune disorders (e. g., anti-nuclear antibodies for lupus), evaluating inflammatory responses (e. g., Tumor necrosis factor-α, Interleukin-1β), biomarkers for neurodegenerative diseases like Alzheimer’s (e. g., amyloid-beta, tau protein). Nonetheless, there is a restricted comprehension of biomarker effectiveness, and its consequences in diagnostic settings are prompting new advancements in this area. In this chapter, we reviewed the scientific bases and clinical uses of host biomarker-based diagnostics, examining their revolutionary effect on infection management in various healthcare settings.

# **Introduction**

Dominant global health threats, infectious diseases, continue to kill millions each year and stress healthcare systems across every level of society 1. Of these, bacterial and viral infections are the two major classes of etiological agents, each causing different but overlapping clinical syndromes, which complicate fast and correct diagnosis 2,3. The ability to correctly and promptly distinguish between bacterial and viral causes is essential since it affects the course of medical therapy—antibiotics for bacterial pathogens and supportive care or antiviral agents. Still, misuse of antibiotics in situations of diagnostic uncertainty has driven a subtle epidemic: the continuous increase of antimicrobial resistance (AMR). This phenomenon undermines the efficacy of prevailing treatments and makes it more difficult to cure infections globally 4,5..

Furthermore, exacerbating this problem is the restriction of modern diagnostic techniques that include blood culture, gram stain, and polymerase chain reaction (PCR). Though these techniques still establish gold standards for pathogen identification, they often call for much knowledge and resources, have limited sensitivity to certain pathogens, and require long turnaround times. Their performance also drops in instances where pathogen identification is incomplete due to low bacterial or viral counts, or with polymicrobial infections 6.

With these problems, host biomarker-based diagnostic tools present a revolutionary answer. Traditional exams focus on finding the causative pathogen; these fresh approaches use the host immune system complex interplays of the cell activation markers, acutely active agents, and inflammation cytokines, to expose the fundamental kind of disease. For example, biomarkers like C-reactive protein (CRP) and Procalcitonin (PCT) deliver surrogate measures of the general inflammatory state of the host, thus offering information on whether the immune reaction is caused by bacterial or viral triggers. Recent developments in multi-marker panels and artificial intelligence systems, together with these biomarkers, greatly enhance their diagnostic ability by enabling accurate, real-time differentiation previously not possible7,8.

This change in approach opens the door for focused antimicrobial stewardship initiatives that would lower the likelihood of AMR by limiting the unselective use of antibiotics in addition to enhancing clinical decision-making. This chapter investigates the scientific foundation and practical uses of host biomarker-based diagnostics, together with their revolutionizing effect on infection control in several kinds of medical settings.

**2.** **Host Biomarker-Based Diagnostics**:

Host biomarker-based tests use immune system-generated compounds to help identify diseases, inflammation, or illness. Important groups include—

## **2.1. Cytokines:**

Cytokines are crucial signaling molecules released by immune cells that control inflammation, immune activation, and tissue homeostasis 9. Their levels can fluctuate in response to infections, autoimmune diseases, and cancer, making them valuable biomarkers for diagnosing, prognosing, and monitoring diseases 10,11. Cytokines are secreted through different paracrine and autocrine or endocrine systems and are involved in various infections, influencing patients through both inflammatory and anti-inflammatory processes 12,13. There are different pro-inflammatory cytokines, including interferon-ϒ, interleukin-17, tumor necrosis factor-α (TNF-α), interleukin-β and interleukin-6, as well as immune suppressive cytokines like Interleukin-4, Interleukin-10, Interleukin-1ra14 16. Nonetheless, the difference between pro-inflammatory or anti-inflammatory pathways is well known—various factors such as pathway interactions greatly influence a patient, and the mixtures of multiple cytokines may either enhance or weaken the effects of other cytokines, and certain cytokines might play a dual role as anti-inflammatory or pro-inflammatory based on the immune background, like Interleukin-6—pro-inflammatory during acute infections but anti-inflammatory by promoting Interleukin-10 induction 17,18; TGF-β reduces inflammation but can also promote fibrosis and immune evasion during cancer progression and involved in the differentiation of Th17 molecules; IL-27 – Regulates both Th1 and Treg cell responses, maintaining a balance in inflammation19–22. (Ungefroren, 2019; Yang, 2010; Zhang, 2018; Pot et al., 2011). The function of Interleukin-27 in Th1 control is pivotal in the initial differentiation of Th1 cells by stimulating T-box transcription factor (T-bet, TBX21; responsible for the variation and function of Th1 cells) and increasing IFN-γ production 23,24. However, subsequently, it inhibits excessive Th1 responses, thereby averting tissue damage. IL-27 boosts the growth of Foxp3+ Tregs and stimulates the creation of Tr1 cells (regulatory T cells that induce Interleukin-10), assisting the limitation of immune-mediated disease 25,26. Simultaneously, Interleukin-27 suppresses Th2 and Th17 responses, decreasing the intensity of autoimmune and inflammatory disorders. It additionally decreases pro-inflammatory cytokines such as Tumor Necrosis Factor-α and Interleukin-6. The IL-27 also has antimicrobial and antitumor effects 25,27. Two of the primary effector pathways of cytokines are the NF-κB and JAK-STAT.

JAK-STAT Pathway- This pathway is initiated by a cytokine ligand and regulated by pro-inflammatory cytokines such as Interleukin-12, and promotes cytokine production. Cytokines and growth factors interact with specific receptors within the JAK-STAT pathway, causing the receptor to dimerize and leading to the activation of Janus Kinases (JAKs) linked with the receptor’s intracellular domain. The JAK proteins then phosphorylate, in turn, facilitating phosphorylation of the tyrosine. This establishes a lodging site for signal transducers and activators of transcription (STATs). The STATs are attracted to the receptor complex also phosphorylated on tyrosine by JAK; STATs detach from the receptor and produce either homodimers or heterodimers; the STAT dimers enter the nucleus, bind to DNA, and affect transcription. This allows the STATs to form a dimer and translocate to the nucleus, causing regulation of gene expression 28–30 (Figure 1).

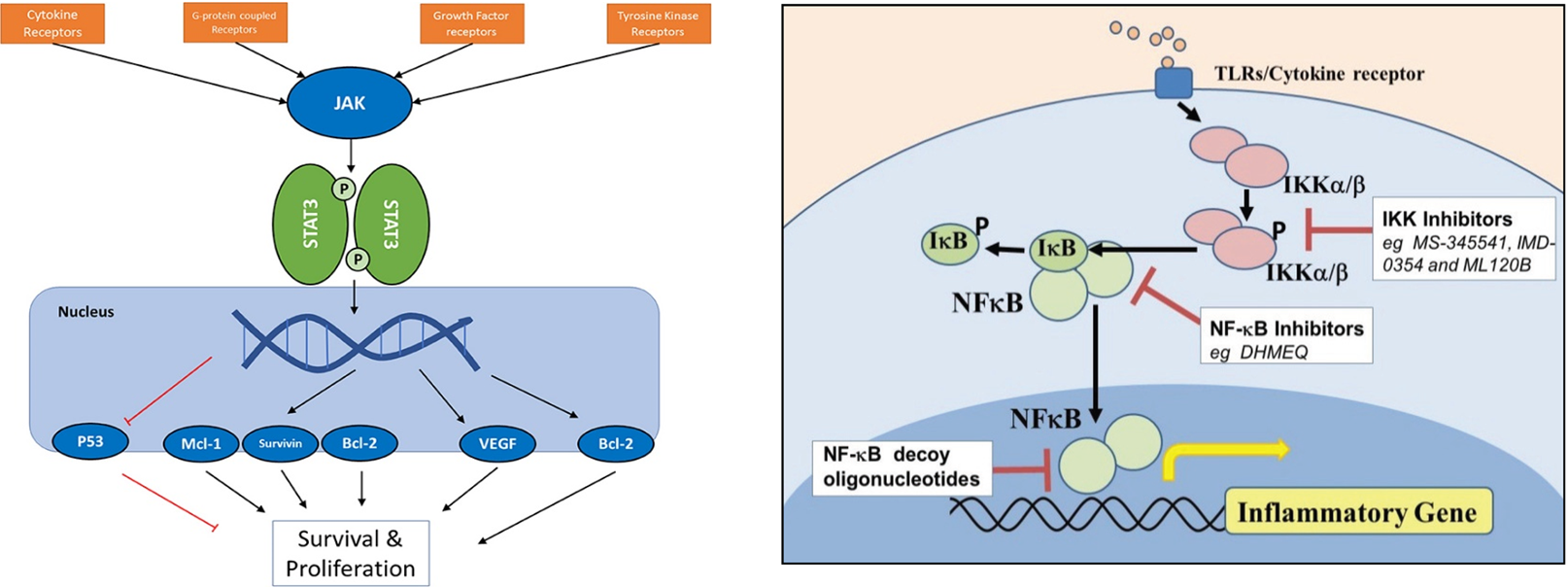


Figure 1: A diagrammatic overview of the JAK-STAT pathway NF-κB pathway. Several STAT proteins are not mentioned in this representation (Image Courtesy of Bousoik and Montazeri Aliabadi, 2018, 31,32 .

Nuclear Factor kappa-light-chain-enhancer of activated B cells (NF-κB pathways)-

The pathway involves IκB proteins, which obstruct the κB component in the lack of a ligand, avoiding κB from initiating the gene transcription associated with inflammatory and stress reactions. This pathway is often triggered by external stimuli, including pro-inflammatory mediators (such as Interleukin-1 and Tumor necrosis factor-α), pathogen-associated molecular patterns, or other stressors. These stimuli trigger the IκB kinase complex (IKK), which phosphorylates the IκB proteins that typically inhibit NF-κB dimers p65/p50. The phosphorylation of IκB leads to its degradation via the proteasome. This mechanism releases NF-κB dimers from the inhibitory complex. The released NF-κB dimers move into the nucleus to combine with κB sites in DNA to activate the target genes' transcription. The NF-κB regulates genes associated with immune responses, inflammation, cell survival, and programmed cell death. This pathway is crucial for activation of inflammation, which enables the ability of the immune system's response against infections and stressors 33,34.

These pathways possess various modes of action in conjunction with cytokines. For instance, in macrophages, when Interleukin-12 acts as a ligand of JAK-STAT pathways, pro-inflammatory cytokines are increased. Conversely, with the existence of Interleukin-10, the activities of Interleukin-12 are reduced, causing downregulation of pro-inflammatory pathways 35,36. Similarly, in mast cells, when TNF functions as a ligand for the NF-κB pathway, resulting in pro-inflammatory Signaling 37,38. Conversely, in the presence of IL-10, the NF-κB pathway diminishes pro-inflammatory responses 39,40. Another cytokine, IL-6, is likewise engaged in proinflammatory responses, and IL-6 promotes the IL-21 responses, leading to increased activity of CD4+ cells and inhibition of IFN-γ mediated T-cell differentiation. This facilitates the TGF-β-mediated CD4+ differentiation 41–43. This exemplifies an outdated instance of signaling pathways mediated by multiple cytokines.

Cytokines are essential in disease mechanisms; however, their usefulness for diagnosis is complicated by variations in expression levels, differences among individual patients, and the ever-changing nature of immune responses. Although there is still a lack of standardization, several studies have given certain cytokine thresholds for diseases, including sepsis, rheumatoid arthritis, and particular infections. Future research should focus on thorough validation work, study cytokine panels instead of single markers, and merge cytokine data with other biomarkers to improve diagnostic accuracy. However, cytokines have been evident as novel biomarkers in some extensively researched fields, including sepsis, autoimmune disease (e.g., SLE, rheumatoid arthritis), infectious illnesses (e.g., COVID-19, tuberculosis), cancer and neurological disorders (e.g., multiple sclerosis, Alzheimer's disease). For instance, cytokines are involved in the pathogenesis of sepsis, and measuring their levels can assist in diagnosis and prognosis. The important Cytokines as biomarkers assessed in various studies are stated below (Table 1).

Table 1: The important cytokines as host-biomarkers along with their disease/disorder 44–46**.**

|  |  |  |  |
| --- | --- | --- | --- |
| Cytokines | Host-Biomarkers | Disease / Disorder | Use in Diagnostic |
| IL-6 | Pro-inflammatory | Various infections ( Sepsis, COVID-19), autoimmune disorders and Cancer | Critical illness markers and cytokine storm indicators are useful in the Diagnosis of Cancer. |
| IFN-ϒ | Pro-inflammatory | Tuberculosis, viral infection ( Predicts severity in viral infections (COVID-19, dengue, RSV, the high level found in severe Zika Virus; monitors antiviral Th1 response) | Diagnostic in TB (IGRA tests), Biomarker for immune activation. |
| IFN-α, IFN-β | Pro-inflammatory | Early detection of infection in virus (e.g., COVID-19, influenza); cytokine storms in severe COVID-19 linked to deregulated IFN-α/β | Early antiviral defense is produced by infected cells marked by severe disease. |
| IFN-λ | Pro-inflammatory | Mucosal immunity; antiviral response at epithelial barriers (lung, gut) | Host-biomarkers for respiratory viruses (e.g., RSV, influenza); prospective prognostic marker in chronic HBV/HCV |
| IL1-β | Pro-inflammatory | Infections (Sepsis, Bacterial Pneumonia, COVID-19, Tuberculosis), autoimmune disease ( Rheumatoid Arthritis, Gout, SLE), Inflammatory Bowel Disease, Cancer | Inflammatory markers, associated with Inflammasome activation. |
| TNF-α | Pro-inflammatory | Infections (sepsis, tuberculosis, COVID-19, Malaria), Autoimmune disorders (Rheumatoid Arthritis (RA), Crohn’s Disease, Psoriasis, SLE), Cancer [Solid tumors (e.g., melanoma, colorectal cancer), Leukemia], Inflammatory Disease (IBD, asthma), Neurological disorders (Alzheimer’s Disease, Multiple Sclerosis) | Inflammation marker, Severity marker for autoimmune disease, response to anti-TNF. |
| IL-17 | Pro-inflammatory | Autoimmune diseases (e.g., psoriasis, RA), Helminthes and fungal infections | Marker for Th17 and association with chronic inflammation |
| GM-CSF | Pro-inflammatory | Autoimmune diseases, infections and cancer | Recruitment of myeloid cells, resolution of inflammation |
| IL-12 | Pro-inflammatory | Infections, autoimmune diseases, and cancer | Th1 polarization, an indicator of immunotherapy |
| IL-23 | Pro-inflammatory | Autoimmune diseases (e.g., psoriasis, IBD) | Th17 polarization, target for biologics |
| IL-18 | Pro-inflammatory | Infections, autoimmune diseases, and cancer | Inflammasome activation marker, disease severity predictor |
| IL-2 | Pro-inflammatory | Infections, cancer immunotherapy | T cell activation marker, response in immunotherapy |
| IL-4 | Anti-inflammatory (Th2 cytokine) | Allergies, parasitic infections, and asthma | An indicator of Th2 response, an allergy biomarker |
| TGF-β | Anti-inflammatory | Fibrosis, cancer, and autoimmune diseases | Marker of immunosuppression, tumor progression |
| IL-10 | Anti-inflammatory | Infections, autoimmune diseases, and cancer | Immunoregulatory marker, predictor of immunosuppression |
| IL-8 (CXCL8) | Chemokine | Bacterial infections, cancer, and inflammation | Neutrophil recruitment marker, cancer progression |
| Monocyte Chemoattractant Protein-1 (MCP-1) (CCL-2) | Chemokine | TB, HIV, Autoimmune Diseases | Monocytes, macrophages, fibroblasts, and endothelial cells |
| MIP-1α (Macrophage Inflammatory Protein-1 Alpha) (CCL-3) | Chemokine | It is critical in **immune cell recruitment,** TB, **granuloma formation,** HIV, and other infections. | Macrophages, Monocytes, Dendritic cells, Neutrophils, T Cells, Natural Killer Cells, Fibroblasts & Epithelial Cells. |
| MIP-1β  (Macrophage Inflammatory Protein-1 Beta) (CCL-5) | Chemokine | It is a critical **inflammatory chemokine** induced by infections, cytokines, and cellular stress. It is involved **in chemoattractant, granuloma formation, and disease pathogenesis** in TB, HIV, and other infectious diseases. | Macrophages, Monocytes, Dendritic cells, Neutrophils, T Cells, Natural Killer Cells, Fibroblasts & Epithelial Cells. |
| IP-10  Interferon gamma-induced protein 10, CXCL10 | Chemokine | Chemotaxis ( T cell, monocytes, NK cells) to the site of infections or inflammations, Immune cell activation (particularly Cytotoxic CD8+ cells), regulation of inflammation, and antiviral response. Elevated levels serve as biomarkers in conditions like tuberculosis, HIV, sepsis, and autoimmune diseases. | Activated endothelial cells, macrophages, monocytes, fibroblasts, dendritic cells, epithelial cells |

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### 2.1.1. Sepsis

A dysfunctional immune response to infection results in systemic organ failure and bloodstream infection, a potentially fatal illness. Biomarkers aid in prognosis, early diagnosis, and therapy response tracking. Combining these biomarkers can improve diagnostic precision. For example, the pairing of Interleukin-6 (greater than 24.65 pg/mL) and C-reactive protein (greater than 4.82 mg/L) resulted in a sensitivity of 53% and a specificity of 100% for diagnosing neonatal sepsis.

Note: Cutoff values may differ depending on patient populations, assay techniques, and clinical environments. Clinicians should treat these values as recommendations and consider individual patient considerations when making diagnostic choices. Rheumatoid arthritis (RA) identification and treatment depend on multiple biomarkers and clinical evaluations (Table 2).

Table 2: The important Biomarkers and cytokines along with their corresponding cutoff values for sepsis11,45,47,49

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Cutoff Value** | | **Diagnostic Performance** | | | |
| **Sensitivity** | **Specificity** | **Positive Predictive Value** | **Negative Predictive Value** |
| Procalcitonin | 1.1 ng/mL | 97% | 78% | 94% | 88% |
| Interleukin-6 (IL-6) | **Sepsis:**  52.60 pg/mL to distinguish sepsis from controls  **Septic Shock:** 348.92 pg/mL to identify septic shock | 97.0%  91.8% | 97.2%  63.2% |  |  |
| Interleukin-8 (IL-8) | 30 ng/mL | 63% | 78% | 90% | 39% |
| C-reactive protein (CRP) | 4.82 mg/L | 67% | 97% | 99% | 39% |

### 2.1.2. Rheumatoid arthritis

In rheumatoid arthritis, joints are damaged by a chronic inflammation underlying Rheumatoid arthritis (RA), a chronic autoimmune illness. Numerous biomarkers aid in disease activity monitoring, diagnosis, and prognosis (refer to Table 3).

Table 3: The important Biomarkers and cytokines along with their corresponding cutoff values for Rheumatoid arthritis 50–54

|  |  |  |  |
| --- | --- | --- | --- |
| **Cutoff Value** | **Diagnostic Performance** | | |
|  |  | **Sensitivity** | **Specificity** |
| 1. Rheumatoid Factor (RF): | Greater than 20 IU/mL  Autoantibody targeting the IgG Fc region.  **Interpretation:** Levels above 20 IU/mL may suggest RA, but elevated RF can also occur in other conditions. Approximately 20% of RA patients may have normal RF levels. | 70-80% | 70-80% |
| Anti-Cyclic Citrullinated Peptide  (Eg. Anti-CCP) Antibodies: | Greater than 20 Units  **Interpretation:** Levels above 20 Units suggest RA. Anti-CCP antibodies are more specific for RA than RF. | 70-80% | ~ 95% |
| Interleukin-6 (IL-6): | Greater than 8.75 pg/mL  **Interpretation:** Levels of IL-6 exceeding this threshold can reliably signify early RA. | 100% | 100% |
| Tumor Necrosis Factor-alpha (TNF-α): | Greater than 10.50 pg/mL  **Interpretation:** Increased TNF-α levels can assist in differentiating RA from healthy people. | 90% | 100% |
| Erythrocyte Sedimentation Rate (ESR): |  Men: 0–15 mm/hr.   Women: 0–20 mm/hr.  **Interpretation:** Elevated values suggest inflammation but are not exclusive to RA. | 60-80% | Low Elevated in many conditions. |
| C-Reactive Protein (CRP): | Greater than 1.0 mg/L  **Interpretation:**  Increased levels indicate inflammation; nonetheless, CRP is not exclusive to RA. | NA | NA |
| 14-3-3η Protein: | Less than 0.19 ng/mL  **Interpretation:** Increased levels are linked to the severity of RA and could indicate the progression of the disease. | 73% | 88% |

**Note: Anti-nuclear Antibodies (ANA) differentiation in RA-** Research indicates that around 37. 5% of RA patients demonstrate positive results for ANAs. This suggests that although a considerable proportion of RA patients show ANA positivity, it is not an all-encompassing finding in RA. Furthermore, in 2019, a study participants amongst 230 patients with RA revealed that 25. 2% were positive for ANAs. This positivity for ANAs was strongly linked to the occurrence of other autoantibodies, including anti-Ro/SSA, anti-La, and anti-Jo-1 50, 51.

2.1.3. Systemic Lupus Erythematosus (SLE)

A long-term autoimmune disorder whereby the immune system erroneously assaults healthy cells, causing major inflammation and tissue damage, is SLE. The condition may affect many parts, including the joints, lungs, heart, renal systems, brain, and skin.   
Women, especially those in the childbearing age range, are mostly impacted by SLE. Notably, people of Black ethnicity encounter an increased risk. Black individuals are eight times more likely to be hospitalized for lupus than white individuals57.

Elevated Type I interferon, as IFN-α release, is frequently found in SLE patients and is linked to disease activity. Additionally, IFN-β and IFN-λ: These interferons influence gene expression and the progression of the disease, contributing to the IFN gene signature observed in SLE. A new digital ELISA method has made it possible to identify IFN-α in SLE serum with higher sensitivity 58–61.

Interferon-inducible protein 10 (CXCL10) is raised in SLE patients, especially when renal involvement is present. Serum CXCL10 levels have been demonstrated to predict renal flares more effectively than more conventional markers such as complement levels or anti-dsDNA antibodies, 62–64.

The IL-1 receptor 4 is raised in patients with active SLE and corresponds to levels of anti-dsDNA and anti-C1q antibodies. Levels of sIL-1R4 are additionally linked to urinary protein levels in patients suffering from active nephritis 62,65.

Both B Cell Activating Factor (BAFF) and A Proliferation-Inducing Ligand (APRIL) are increased in SLE patients and have important functions in B-cell development and survival. Increased levels of these cytokines correlate with serological activity and may define subgroups of SLE cases, like those impacted by subclinical atherosclerosis 62,66,67.

IL-18 concentrations are raised in patients with SLE and are associated with disease progression and other markers such as anti-dsDNA and anti-C1q antibody. Active nephritis represents the primary disease manifestation linked to elevated IL-18 concentrations 62,68,69.

In SLE patients with comorbidities, nephritis, increased Interleukin-4 levels were reported. IL-4 is linked with other cytokines, including Interleukin-6, Interleukin-10, sCD40L, and Interleukin-8, indicating B cell participation in lupus nephritis 70.

ANA is found in most SLE patients, which makes it an essential screening tool. Nevertheless, a positive ANA test is not unique to SLE and may occur in other autoimmune disorders and even in healthy people, particularly as they grow older ANA is highly **Sensitive:** 95-98% (high for SLE) and specificity is low (~50-60%) due to its presence in other conditions in SLE. While ANA serves as a useful screening instrument for SLE because of its great sensitivity, it necessitates additional validation through particular autoantibody tests and clinical assessment for a precise diagnosis 70.

Increased concentrations of certain cytokines can assist in diagnosing SLE, particularly when standard autoantibody tests yield inconclusive results. Cytokines like Interleukin-4 and TNF-α represent possible therapeutics. For example, Interleukin-4 and TNF-α are examples of cytokines that could be targets for treatment. For instance, the efficiency of TNF-α inhibitors in treating SLE has been reported, and the function of IL-4 in B cell activation suggests that it may be a potential target for treatment 71.

### 2.1.4. COVID-19

Determining the level of the disease and guiding treatment depends much on host biomarkers for COVID-19. These markers belong in several categories: genetic components, inflammatory signs, organ malfunction signals, coagulation markers, and immune reaction indicators.

This part features some momentous immune system indicators and inflammatory markers. Inflammatory biomarkers are crucial for evaluating disease severity, predicting complications, and informing treatment decisions in COVID-19. Infection with COVID-19 triggers an excessive immune reaction, leading to a "cytokine storm" in severe cases. The key inflammatory biomarkers examined in various studies during the COVID-19 pandemic are presented below (Table 3).

Table 3: The important inflammatory biomarkers for COVID-19.

|  |  |  |
| --- | --- | --- |
| **Host-Biomarker** | **Role in COVID-19** | **Cutoff value for the level of COVID-19** |
| TNF-α | Endothelial damage and coagulopathy | Elevated 72–74 |
| IL-1 | Neutrophil activator, fever | Elevated 75,76 |
| IL-6 | Cytokine storm mediator | > 80 pg/ml 77 |
| CRP | General inflammation | > 100 mg/L 78 |
| IFN-ϒ | Antiviral response | Impaired in severe cases 79 |
| Ferritin | Hyper-inflammation (Macrophage activation Syndrome- MAS) | > 1000 ng/L 80,81 |
| Serum Amyloid A (SAA) | Acute inflammation | Persistent elevation 82 |
| Procalcitonin (PCT) | Bacterial co-infection | > 0.5 ng / ml83 |
| Neutrophil-to-Lymphocyte Ratio (NLR) | Immune response balance | > 6.584 |

As per this, many strains of COVID-19 have emerged as a result of the new virus variants, and each one has a distinct significance on the body's inflammatory response. Understanding these differences was essential to developing effective therapies and vaccinations. This intensified inflammatory response may lead to severe disease manifestations 85. In contrast to the Wuhan strain and Delta variant, the Omicron variant elicits a milder innate immune response with significantly lower levels of cytokines and chemokines. The variant's increased transmissibility and chance of reinfection may be linked to this decreased inflammatory response 85.

There are various immune response indicators were assessed during COVID-19 infections. One of the crucial indicators noted was Lymphopenia. Lymphopenia, defined by a reduced lymphocyte count, is a defining feature of severe COVID-19 and has been linked to heightened disease morbidity and mortality. Numerous studies have emphasized the importance of lymphocyte count in COVID-19 patients-

For instance, a previous research conducted in Brazil assessed lymphocyte counts, T-cell populations, and the neutrophil-to-lymphocyte ratio as preliminary indicators for the morbidity and effect of COVID-19. The results suggested that Lymphopenia is viewed as a criterion for determining disease severity and may serve as a sign of an unfavorable prognosis 86. Another study from hospitals in Portugal and Brazil examined Lymphopenia in 2020 to 2021 among unconscious patients with COVID-19. The findings highlighted lymphopenia as a unique characteristic noted in critically ill COVID-19 patients and allegedly acts as a conclusive marker of the illness 87. These investigations highlight the significance of tracking lymphocyte levels in COVID-19 patients, given that lymphopenia may act as an initial sign of disease progression and an indicator of negative clinical outcomes.

CD4+ and CD8+ cells of the adaptive immune system play a vital role in COVID-19 infection. A reduction in these T-cell populations in people with COVID-19 has been linked to high risk. Specifically, individuals with severe COVID-19 had markedly lower CD4+ and CD8+ cell counts. Additionally, research has shown that CD4+ and CD8+ cell counts can serve as predictive biomarkers for disease development 88. For instance, lower CD4+ cell counts have been linked with higher chances of ICU admission and mortality, whereas increased CD8+ cell populations were identified as a significant risk factor for ICU admission89. These studies indicate that tracking CD4+ and CD8+ cell counts in COVID-19 patients can provide critical insights regarding the severity of the illness and potential outcomes. A reduction in these T cell counts might indicate a compromised immune response, necessitating closer monitoring and possibly more aggressive treatment approaches.

SARS-CoV-2 has an association with the Human Leukocyte Antigen (HLA). Specific HLA genes are linked with differing levels of susceptibility to and severity of COVID-19. For example, the HLA-B 46:01 fragment of genes has been shown to exhibit a weak binding affinity for SARS-CoV-2 peptides. Individuals possessing this allele may have a reduced capacity to demonstrate viral antigens to T cells, resulting a compromised immune response linked to this lower affinity 90. The HLA-B 46:01 allele is linked with increased threat of severe COVID-19 cases. Patients containing this allele in their genome are associated with more severe health effects due to a lack of antigen presentation 90. Conversely, certain HLA alleles are correlated with a healthier immune response to COVID-19. In addition, The HLA-B 15:03 allele, for instance, is associated with a greater binding affinity for viral peptides, which may boost the immune response and provide protection against severe disease 91. The HLA-B22 serotype, which encompasses alleles such as B54:01, B55:01, B55:07, B55:12, and B\*56:01, has been shown as a predicators for COVID-19 infection. These alleles display lower binding affinity to COVID-19 infection peptides, which might influence susceptibility to the virus. Therefore, identifying individuals with these alleles could aid in forecasting susceptibility to infection and enabling targeted preventive measures. Examining HLA interactions with SARS-CoV-2 was a tactic for developing vaccines that elicit strong immune responses across different population groups.

### 2.1.5. Tuberculosis

Host biomarkers are progressively being investigated for their significance in diagnosing and evaluating tuberculosis (TB). These markers could be categorized as inflammatory, anti-inflammatory, and genetic biomarkers, which have immense potential to comprehend tuberculosis disease pathogenesis and vaccine design. One of the key cytokines, IFN-ϒ, shows a vital role in TB immunity and is crucially associated with macrophage activation. IFN-ϒ level is increased in LTBI (latent tuberculosis infection), and IFN-γ is induced by Natural killer cells, Th1 cells, and CD8⁺ cells. IFN-γ Activates macrophages, improving their capacity to phagocytose *Mycobacterium. tuberculosis (M. tb),* and generates oxidative free radicals and NO to eradicate the bacteria. The granulomas, which localize the infection and stop its spread, are maintained in part by IFN-γ. It promotes a robust cellular immune response that is necessary for M. tb regulation by influencing the equilibrium between Th1 and Th2 responses 92–96.

Interferon-Gamma Release Assays (IGRAs) are generally involved as QuantiFERON-TB Gold Plus (QFT-Plus) and T-SPOT assays. *M. tb* antigens stimulate the immune system to release interferon-gamma (IFN-γ), which is measured by TB blood tests. The investigation of tuberculosis infection has an immense role for these tests 97.

IFN-γ is highly induced in people with latent tuberculosis infection (LTBI) than in people without the illness, which may predict that it is an important biomarker for the containment of *M.tb.* IFN-γ levels are also higher in active TB cases than in LTBI cases, suggesting a stronger immune response during active disease 98.

A cytokine essential to T-cell propagation and the induced of memory reactions during Mycobacterium tuberculosis (*M.tb*) infection is interleukin-2 (IL-2). Rather than active tuberculosis sickness, raised levels of IL-2 have been related to latent tuberculosis infection (LTBI). To distinguish between distinct stages of tuberculosis infection, it has been investigated to combine IL-2 measures with interferon-gamma (IFN-γ) assays. A meta-analysis that assessed IL-2's diagnostic accuracy for LTBI found that it had an 81% sensitivity and a 95% specificity. High diagnostic performance was indicated by the area under the curve, which was 0.96. According to the study's findings, Interleukin-2 is a reliable marker for LTBI diagnosis and, when combined with IFN-γ release assays (IGRAs), can improve its diagnostic potential 99. To distinguish active TB from LTBI, a different meta-analysis evaluated several cytokines, such as Interleukin-2 and IFN-γ. With Interleukin-2 exhibiting the highest sensitivity among the assessed indicators, the results indicated that combining these cytokines could increase diagnostic accuracy 100.

The interleukin-6 (IL-6) greatly affects the acute phase response to diseases including, but not limited to, tuberculosis (TB). Active tuberculosis sufferers have been discovered to have increased IL-6, which are linked with disease progression and reaction to therapy. Patients suffering with active PTB have clearly higher serum levels of IL-6 than in those in good101. These findings show that IL-6 is a best biomarker of the extent of disease pathology since in TB sufferers, high IL-6 levels correspond with a high bacterial load and more severe radiologic abnormalities102. Raised starting IL-6 levels are related with poor TB treatment results including relapsing sickness, mortality, and treatment failure 103. Patients with higher starting IL-6 levels 98 are more prone to unfavourable results. Research on patients with active pulmonary tuberculosis (APTB) found that baseline to 6 months of anti-TB medication treatments evaluated serum inflammatory cytokines. The data indicate that baseline IL-6 levels exceed considerably those of average, healthy individuals. Treatment started and IL-6 levels fell rapidly and stabilized by the fourth month, hence indicating the drug was effective 104.. Proinflammatory cytokines in plasma were also quantified in pulmonary tuberculosis patients prior and after getting ordinary anti-TB medication in a different study. The findings suggested that proinflammatory cytokine levels—especially IL-6—dropped quite after successful treatment; therefore, measuring IL-6 levels could help to determine the treatment's success 105.

TNF-α is an important cytokine that controls the granuloma formation in the immune reaction to *M.tb*. Granuloma contains the bacterium by localizing *M. tb* infections and halting their spread with organized groups of Immune cells. TNF-α is a crucial cytokine which necessary for the host's defenses against TB 106, is essential for the development and monitoring of these granulomas. Elevated TNF-α levels, a sign of the body's effort to control the infection, have been observed in active TB cases. TNF-α is necessary for launching a successful immune response, but too much of it can cause immunopathology. An overactive TNF-α response may be a subsidizing factor to tissue injury in TB, as high TNF-α concentrations have been associated to tissue death and cachexia in experimental animal 107. Therefore, a balanced TNF-α response is necessary; too low levels can prevent granuloma formation and bacterial containment, while too high levels can damage tissue. This tight balance emphasizes TNF-α's dual role as a preventive factor against tuberculosis and a possible contribution to its development106.

One acute-phase protein in TB infection is serum amyloid A (SAA), which plays an important role in inflammation. Thus, elevated SAA levels have been associated with active TB cases. Thus, it may be used as a biomarker for tracking the effectiveness of treatment as well as the severity of the disease. Patients with extensive lung damage showed strong acute-phase reactions, which were characterized by extremely high SAA levels, according to a study that examined SAA levels in pulmonary TB patients. This implies that more severe disease pathology is associated with higher SAA concentrations108. Studies have demonstrated that SAA levels drop quickly following the start of anti-TB treatment, especially in individuals who do not have severe lung damage. This quick drop suggests that SAA might be a helpful indicator for tracking the effectiveness of treatment 109.

A regulatory cytokine called interleukin-10 is necessary to regulate *M.tb* infection. An important Th1 cytokine, interferon-gamma, is vital for activating macrophages to eliminate *M.tb.* However, the induction of IL-10 suppresses expression of IFN-γ. Interleukin-10 can subvert the immune reaction to a Th2 phenotype by inhibiting Th1 responses, which may make it more difficult for the host to contain the infection 110. The studies have reported that people with active TB, especially those with severe cases, have increased interleukin-10. This increase could lead to a weak immune response, which would enable the germs to continue and spread the illness111,112. Effective immunity against tuberculosis depends on the stability between T-helper 1 (Th1) and T-helper 2 (Th2) responses, which it modifies.

Research has indicated that people with active TB, especially those with severe cases, have higher levels of IL-10. Several chemokines are considered profound biomarkers in TB disease, such as an important chemokine induced by IFN-γ, involved in immune cell recruitment- IP-10 (CXCL10). IP-10 is noted to be elevated in active TB, LTBI, and subclinical TB infection. It has been proposed as an alternative to IFN-ϒ-IGRA with a higher sensitivity to children and immunocompromised individuals. It is noted to be induced at higher levels in HIV-TB co-infections. Research has shown, IP-10 levels are noticeably higher in those with active TB and LTBI. It is still difficult to distinguish between LTBI and active TB based solely on IP-10 values113. In IGRAs, IP-10 has been suggested as a substitute biomarker for interferon-gamma (IFN-γ), particularly in populations like children and immunocompromised people, where conventional IGRAs may be less sensitive. The strong IP-10 induction in reaction to TB antigens points to the possibility of its use in these populations 114.

Another important acute-phase protein, CRP found to be elevated in active TB cases and considered a general biomarker of inflammation. CRP is not a specific biomarker only for TB, but it is useful for tuberculosis disease progression. According to studies, people with active TB had much higher CRP levels than healthy controls. For instance, the average first CRP level amongst TB cases was 18.52 µg/ml, whereas in controls it was 2.77 µg/ml. This change a significant effect on the infection level, according to one study 115. More severe forms of TB, such as cavitary lesions and significant lung involvement, have been linked to elevated CRP levels. In additional study, TB participants with higher CRP levels had a higher frequency of cavitary lung lesions, indicating a association between the CRP and the level of the condition 116. Effective anti-TB treatment tends to lower CRP levels. In one trial, CRP levels decreased considerably with clinical improvement, from a mean of 18.52 µg/ml at diagnosis to 5.93 µg/ml after one month of treatment (p < 0.001)115. It is noted to have an increased CRP level in tuberculosis infection. Despite of CRP is an important biomarker in TB cases, it can also be elevated in some other ailments, including autoimmune diseases, bacterial infections, and other inflammatory disorders. Therefore, CRP should be utilized as part of a thorough review that also includes imaging studies, microbiological testing, and clinical evaluation to identify tuberculosis. A favorable treatment response has been linked to the gradual normalization of CRP levels.

Furthermore, neopterin is considered a marker for macrophage activation. It is elevated in active TB and TB-HIV co-infection. Neopterin has an association with TB disease activity. Research has shown that patients with active tuberculosis have substantially greater serum neopterin concentrations than healthy controls. For instance, in a study, pulmonary tuberculosis patients had average neopterin levels around 23.74 nmol/L, which was much greater than the 4.03 nmol/L found in healthy people 117. Moreover, hematological indicators, including hemoglobin levels and ESR, as well as clinical signs like hemoptysis and weight loss, are correlated with elevated neopterin levels, implicating an association of elevated neopterin concentration and the severity of TB disease 117. Remarkably, serum neopterin volumes are raised in clients with people living HIV associated TB infections than in patients with TB alone. According to one study, patients with HIV and TB had median neopterin concentrations of 27.2 nmol/L, which was greater than the 14.6 nmol/L seen in patients with TB alone 118. The higher neopterin levels were associated with decreased CD4 cell counts within the HIV-TB co-infected people, especially in individuals whose counts were less than 200/mm³. These people may have a worse prognosis and progressing HIV illness if their neopterin levels are consistently increased 118. Thus, neopterin is a promising biomarker for TB diagnosis and monitoring due to its correlation with immunological response and macrophage activation, particularly in resource-limited clinical settings, where lack of sophisticated diagnostic instruments. However, it is abundant in other inflammatory responses and needs to be interpreted carefully. To completely understand neopterin's function and usefulness in TB diagnosis and treatment, further investigation is necessary.

Leucine-Rich Alpha-2-Glycoprotein-1 (LRG-1) is considered a remarkable diagnostic marker for TB and is associated with immune signaling, as it is increased in active TB patients. It has been recommended that LRG-1 may be a biomarker for active tuberculosis. In reaction to inflammatory cytokines like interleukin-6, the liver produces the acute-phase protein LRG-1. The TNF-α, IL-1β, and IL-22 all have an impact on LRG-1 synthesis, in contrast to C-reactive protein, which is mostly generated by IL-6 119.

Certain strain lineages or combinations of lineages predominate in specific geographic areas, indicating that the genotypes of *M. tb* strains are not distributed uniformly around the world. This conclusion can be explained by the fact that evolutionary selection pressure results from long-term host-pathogen contact and is influenced by both host and bacterial genetics. Variability in both host and bacterial parameters is expected to affect the interaction between bacteria and the host immune system, which is likely to determine the outcome of the disease120. Several studies have already shown variation in inflammatory cytokine response amongst East-Asian (Beijing strains) strains of sub-lineages 3, 4 and 5, and it could be attributed to pks1-15 intact pgl-tb production *in vitro* macrophages and animal studies121–125. In mice's lungs, the lineage 4-outbreak strain (Euro-American), CDC1551, produced elevated TNF-α, IFN-ϒ, IL-12, IL-10, and IL-6 mRNA126. On the other hand, the Beijing lineage 2 outbreak strain, HN878, which killed mice early, produced a change from Th1 to Th2 cytokine production by inducing decreased levels of TNF-α, IL-6, IL-12, and IFN-ϒ mRNA in the lungs while considerably increasing levels of IL-10, IL-4, and IL-5 mRNA127,128.

In Leicester, UK, a TB outbreak among schoolchildren was brought on by the lineage 3 (CAS) strain CH. Within a year, 140 CH was linked to 77 occurrences of active pulmonary tuberculosis and 254 cases of latent tuberculosis infection. Compared to H37Rv, CH-infected MDMs (monocyte-derived macrophages) produced more anti-inflammatory IL-10 and less protective IL12p40. Additionally, CH caused MDMs to express more IL-10 mRNA. This immunological phenotype was partially attributed to an Rv1519 deletion. Since lineage 3 strains share the Rv1519 deletion, the immune-subversive phenotype121,129. Notably, variable macrophage responses in Tanzanian tuberculosis patients are dominated by bacterial diversity130. Regardless of the MTBC lineage that first infected the patients, the study discovered that Lineage-1 infections led to a substantially lower bacterial load and that the intracellular replication rate of Lineage-2 strains was significantly higher than that of the other MTBC lineages. Furthermore, compared to macrophages infected by all other strains, L4-infected macrophages generated noticeably higher levels of TNF-α, IL-6, IL-10, MIP-1β, and IL-1β. The pathogen strain and host race/ethnicity both affect the innate immune response. This discrepancy could affect the creation of novel TB treatment, immunodiagnostic, and vaccination technologies120,131.

## 2.2**. Acute-phase proteins**

In 1930, the discovery of C-reactive protein (CRP) as a response to the pneumococcal C-polysaccharide marked the beginning of recognizing acute phase proteins in different bacterial and autoimmune diseases 132. Pneumococcus contains several antigenic elements (e.g., capsular polysaccharides, cell wall proteins, and other surface molecules). The immune reaction to these various elements might change throughout the illness. Sera from patients severely ill with lobar pneumonia can trigger a non-protein somatic fraction produced from pneumococci (Fraction C) in high titers. Nevertheless, this precipitation reaction vanishes once the patient recovers from the crisis. Thus, the serological response here appears to be an immune reaction directed towards pneumococcal antigens. Pneumococci (*Streptococcus pneumoniae*) create different fractions, one of which is Fraction C, likely associated with the capsular polysaccharide or other structural elements of the bacterium. The vanishing of this response following a convalescence phase may imply that antibodies present during the early and acute phase are temporary, and their existence is linked with an ongoing infection. This observation could assist in gaining a clearer understanding of the immune dynamics triggered by infection. Certain antibodies might be associated with different phases of infection (acute versus convalescence) and could aid in diagnosis or potentially in evaluating the severity of the infection. Serological examinations of this nature could serve as diagnostic indicators for acute pneumococcal infection and to track the progression of the illness.

The precipitation of pneumococcus Fraction C is not solely associated with pneumonia induced by *Streptococcus pneumoniae* but has also been noted in infections caused by Streptococcus, infections caused by Staphylococcus, and acute rheumatic fever. This indicates that there is cross-reactivity between immune responses to various bacterial infections. Streptococci (including both *S. pneumoniae* and other species) and *Staphylococcus aureus* may have analogous or homologous somatic antigens that can activate an immune response. Acute rheumatic fever is an additional condition linked to streptococcal infections, and it is recognized for involving autoimmune reactions where the body system erroneously hurt the tissues, which may also generate antibodies that cross-react with bacterial antigens. The precipitation of pneumococcal Fraction C is not limited to pneumococcal infections; it can also be observed in infections caused by Streptococcus, Staphylococcus, and in cases of acute rheumatic fever. CRP is generated as a reaction to inflammation and can be increased in various infections, not solely pneumococcal infection. This includes staphylococcal and streptococcal infections, which could explain why Fraction C precipitation is seen in these other situations. Serological tests may yield erroneous outcome due to immune response cross-reactivity.

As example, CRP levels could also increase in streptococcal or staphylococcal infections or even in situations like rheumatic fever, potentially resulting in comparable immune reactions, including the production of immunoglobulin’s that false-react with pneumococcal antigens.   
In these instances, heightened CRP levels would signify general inflammation, whereas Fraction C precipitation may serve as a more specific yet possibly cross-reactive indicator for bacterial infections overall. CRP serves as a nonspecific indicator of acute inflammation and infection. Its levels increase in reaction to pneumococcal infection (along with other bacterial infections), and they decrease as the infection subsides. Fraction C precipitation specifically evaluates an immune response to pneumococcal antigens, and identifying this reaction can assist in diagnosing pneumococcal pneumonia. CRP levels are linked with the early phase of infection, while the antibody response (which includes reactions to Fraction C) contributes to both diagnosis and long-term immunity. Cross-reactivity within the immune system (for example, among pneumococci, streptococci, staphylococci, and conditions such as acute rheumatic fever) can result in comparable immune responses, causing CRP to serve as a broad biomarker of inflammation in multiple situations. In clinical settings, CRP levels may be evaluated to determine the existence of infection or inflammation, whereas tests for Fraction C antibodies might assist in delivering more detailed information regarding pneumococcal infection 133–135.

Despite their presence in many acute and chronic disorders, many systemic changes are mentioned to as the acute phase response 136. Acute-phase changes may be classified based on the level of many plasma proteins with the differences in various behavioural, physiological, nutritional and biochemical states. Acute-phase proteins are categorized into two types: positive acute-phase proteins, which get an increase in their plasma concentration, and negative acute-phase proteins, which experience a lower in their plasma concentration of at least 25% during inflammatory disease state 137.

### 2.2.1. Positive Acute-phase proteins

These proteins are found in high concentrations during the inflammatory state. They mainly serve to control the immune reactions, tissue repair and combating infections 138,139.

These kinds of proteins are a types of proteins that exhibit significant raised in their plasma concentrations when there is inflammation, infection, injury, or trauma. These proteins are components of the acute-phase response, a quick immune reaction triggered by the liver in response to cytokines like IL-1, IL-6, and TNF-α (Table 4).

Table 4: The important positive acute phase proteins as inflammatory biomarkers.

|  |  |
| --- | --- |
| **Positive acute phase proteins** | **Roles** |
| C-reactive proteins | Opsonization, complement activation, and inflammation marker (Pepys and Hirschfield, 2003) |
| Fibrinogen | Formation of blood clots and wound curing 140 |
| Serum amyloid A (SAA) | Immune cell employment to inflammation 141. |
| Ferritin | Iron sequestration to limit microbial growth 142 |
| Heparin Cofactor II | Anticoagulant 143 |
| Plasminogen | Fibrinolysis 144 |
| Complement (C3, C4, C9) | Opsonisation of phagocytes and immunity 145 |
| Alpha-1 antitrypsin | Protease inhibitors, protect tissue from enzymatic damage146 |
| Ceruloplasmin | Cu transport and antioxidant 147 |
| Haptoglobin | Bind to hemoglobin and reduce oxidative stress 148. |
| Procalcitonin (PCT) | Biomarkers for bacterial infection and Sepsis 149 |

Various cytokines that promote inflammation influence the production of acute-phase proteins. For instance, Interlukin-6 is stimulated by macrophages, T-cells, endothelial cells, and fibroblasts. It serves as a crucial signaling molecule that instructs the liver to boost the synthesis of positive acute-phase proteins like C-reactive protein, Serum amyloid A, Fibrinogen, Haptoglobin, and Alpha-1 antitrypsin. Most importantly, IL-6 level rises in various infections (especially in bacterial infections), trauma and autoimmune disorders. High IL-6 was an indicator in sepsis, COVID-19, 19 and autoimmune disorders as rheumatic arthritis. Similarly, TNF-α involves a vital role in the induction of acute-phase proteins. The TNF-α is induced by macrophages, monocytes, T-cells and natural killer cells. TNF- -α plays the first cytokine-induced and involves a major role in infection, trauma, and tissue injury and activates endothelial cells (increase vascular permeability), neutrophils (recruited in the site injury), liver cells (induces CRP, SAA, fibrinogen etc.) and febrile response (acts on hypothalamus). Elevated TNF-α indicates sepsis can result in septic shock (extensive inflammation, decrease in blood pressure, multi-organ failure). TNF-α   
Chronic Inflammation indicates increased TNF-α, and it is observed in rheumatoid arthritis, IBD, and psoriasis (this is the reason anti-TNF therapies such as infliximab are utilized for treatment). Cytokine storm is indicated in critical infections (such as COVID-19), and unregulated TNF-α release contributes to hyper-inflammatory conditions. TNF-α is induced in the early onset of inflammation and triggers acute-phase proteins indirectly by stimulating Interlukin-6 and the liver.

Interleukin-1 is a type of pro-inflammatory cytokine majorly induced by macrophages, monocytes, dendritic cells, and epithelial cells. There are forms of IL-1: IL-1β, which plays a crucial function in inflammation, and IL-1α, which is more involved in tissue injury. It acts directly on the liver to promote the production of acute-phase proteins, working together with IL-6 and TNF-α to enhance the acute inflammatory response and initiate fever by influencing the hypothalamus (pyrogenic effect).

#### 2.2.1.1. Important Positive Acute Phase Proteins

**C-reactive protein (CRP):** is another acute-phase protein generated largely from the liver. This protein is a positive acute-phase marker making it a valuable tool for clinical diagnostics. It rapid and significant rise in inflammation allows healthcare providers to assess various acute infections to chronic inflammatory states. CRP is a component of the innate immune response. It binds to the surface of dead or dying cells, as well as some bacteria, activating the complement system—a collection of proteins that aid in the removal of pathogens and injured cells. When there is inflammation in the body, the blood's CRP levels rise. As a result, it is a valuable biomarker for identifying and tracking inflammatory diseases. Acute infections, chronic inflammatory conditions or even tissue damage may be indicated by elevated CRP levels. The doctor often measures the CRP levels to check how a patient is responding to treatment for inflammatory disorders. High-sensitivity CRP (hs-CRP) assays assess the risk of cardiovascular disease. An increased risk of heart attacks and strokes can be linked to even modest levels of inflammation as measured by hs-CRP 116,133. The following are two regularly used sets of CRP cutoff values: one for assessing cardiovascular risk using high-sensitivity CRP (hs-CRP), and another for measuring acute inflammation (Table 5). High-sensitivity CRP (hs-CRP) assays assess the risk of cardiovascular disease. An elevated risk of heart problems and strokes can be linked to even modest levels of inflammation as measured by hs-CRP 133,150. The following are two regularly used sets of CRP cutoff values: one for assessing cardiovascular risk using high-sensitivity CRP (hs-CRP), and another for measuring acute inflammation (Table 5).

Table 5: The level of CRP in high sensitivity (hs-CRP) in cardiovascular risk 150 and standard acute inflammation 133.

|  |  |
| --- | --- |
| **CRP level (mg/L)** | **Risk category- cardiovascular** |
| < 1 | Low risk |
| 1-3 | Intermediate risk |
| >3 | High risk |
| **CRP level (mg/L)** | **Risk Category- acute inflammation** |
| < 10 | Normal / No significant inflammation |
| 10-40 | Mild inflammation |
| 40-200 | Moderate inflammation |
| > 200 | Severe inflammation |

The latter part of this chapter has covered the specifics of CRP's ability to distinguish between viral and bacterial infections in host biomarkers.

**Serum Amyloid A (SAA):**

The liver produces SAA, a helpful acute-phase protein, mainly against inflammation or infection. TNF-α, IL-1, and IL-6 enhance its synthesis. There are around four families of SAA: SAA1 and SAA2, the two main acute-phase proteins, drastically increase during inflammation; SAA3, a human pseudo gene, is non-functional; and SAA4, a constitutive isoform, shows baseline synthesis even when inflammation is not present.

SAA's primary roles include attracting neutrophils, monocytes, and T cells to inflammatory areas, enhancing phagocytosis through opsonisation, which helps immune cells identify pathogens, playing a crucial role in reverse cholesterol transport, specifically by removing cholesterol from tissues, controlling inflammation by interacting with the Toll-like receptor (TLR) and intensifying the inflammatory response, and activating matrix metalloproteinase by assisting in tissue remodeling during inflammation. Serum SAA levels are typically very low, less than 3 mg/L. Acute inflammation causes it to increase 1000-fold to over 1000 mg/L in 24 to 48 hours. IL-1, IL-6, and TNF-α stimulate the production of SAA during bacterial and viral infections; tissue damage from burns, trauma, and surgery; immune-pathological problems like rheumatoid arthritis, systemic lupus erythematosus (SLE), and cancers.

The SAA level is continuously high in chronic inflammation. Misfolded SAA builds up as amyloid fibrils in organs (most notably the kidneys, liver, and spleen) in secondary amyloidosis (AA amyloidosis), which is caused by persistently elevated SAA levels. The SAA is seen in inflammatory illnesses, including Crohn's disease and rheumatoid arthritis, as well as persistent infections like tuberculosis. Acute bacterial infections resulting in a significant rise in SAA levels; exacerbations of autoimmune diseases result in very high SAA levels; persistent infections maintain chronically elevated SAA levels; extended exposure to consistently high SAA levels may result in AA amyloidosis; and certain cancer types exhibit moderately high SAA levels 141.

**Fibrinogen:** Fibrinogen, known as Factor I in the coagulation cascade, is a ~340 kDa glycoprotein synthesized in the liver. The usual range of fibrinogen is around 2-4 g/L in plasma. The half-life of fibrinogen is nearly 3-5 days. Fibrinogen is a vital acute-phase protein that significantly rises during acute inflammation, tissue injury, infections, and chronic inflammatory conditions. It acts as a precursor for clot formation, establishes a framework for wound healing and cell movement, and affects leukocyte adherence and cytokine release. Elevated fibrinogen levels enhance red blood cell aggregation, leading to an increased erythrocyte sedimentation rate (ESR), which is recognized as a common indicator of inflammation. The fibrin network also restricts the physical dissemination of pathogens at infection locations. Fibrinogen is controlled by IL-6, IL-1β, and TNF-α. The level of fibrinogen quickly rises within 24-48 hours during acute infections. It remains consistently high during chronic inflammatory conditions. Thrombosis, atherosclerosis, and venous thromboembolism (VTE) are cardiovascular diseases that are more likely to occur when fibrinogen levels are high. It mainly rises in sepsis, but due to consumption, it may decrease in the later phases of DIC. Low fibrinogen levels also indicate serious hepatic dysfunction caused by defective synthesis. As an acute-phase reactant, fibrinogen increases during systemic inflammation. Being a component of the coagulation system, it has an essential function in thrombus development, and excessive fibrinogen adds to pro-thrombotic conditions observed in chronic inflammation (e.g. metabolic syndrome) 140.

**Haptoglobin:** Haptoglobin is also generated during a certain extra-hepatic tissues, including the skin and lungs. The standard range in adults is: 30-200 mg/dL. However, it varies slightly depending on the population and assay. The half-life is approximately 5 days (though it is significantly shorter when bound to hemoglobin, about 20 minutes). It is composed from glycoprotein as α and β chains, creating Hp1-1, Hp2-1, or Hp2-2 phenotypes, which are determined by genetically. Haptoglobin is significantly increased during inflammation, infection, and tissue damage. It’s most distinctive and essential function is to bind free hemoglobin (Hb) released during intravascular hemolysis. It is a hemoglobin scavenger that binds free Hb, forming stable **Hp-Hb complexes.** Free Hb is a potent oxidant, causing tissue injury as Hp neutralizes this. Moreover, Hp-Hb complexes are removed through CD163 receptors on macrophages, facilitating the efficient recycling of iron. Haptoglobin prevents Hb-mediated free radical generation, it protects tissues from **oxidative stress.** In addition, it crucial role in immune modulation during infection. Haptoglobin is driven by cytokines such as IL-6, IL -1β and TNF- α. It is observed to rise significantly as positive APP during acute infection. Nevertheless, it diminishes quickly as it attaches to free Hb because severe hemolysis can lead to undetectable Hb in intravascular hemolysis (e.g., hemolytic anemia). In cases of extravascular hemolysis, it is typically normal since Hb is removed by the spleen and not found in the plasma. It is noted that there is a decrease in severe liver dysfunction due to decreased synthesis. Haptoglobin is identified to be in low concentration in urine in nephrotic syndrome. However, it remains consistently elevated in rheumatoid arthritis, IBD, and similar conditions. Low levels of Haptoglobin indicate hemolysis in particularly intravascular. It may also decrease in cases of severe liver disease or nephrotic syndrome. Conversely, an elevated level of Haptoglobin signifies acute inflammation, infection, or a chronic inflammatory disease 148.

**Procalcitonin (PCT):** Procalcitonin is an acute phase protein and precursor to hormones. Under typical circumstances, it is mainly synthesized by the thyroid gland as a precursor to calcitonin. During systemic bacterial infections and sepsis, PCT is significantly generated by parenchymal tissues such as lungs, liver, intestines, etc., establishing its importance as a primary biomarker for bacterial infection. The standard range is less than 0.05 ng/mL in healthy individuals with a 24-hour half-life of PCT. Interestingly, PCT is highly specific for bacterial infections. In viral infections, PCT levels usually remain low, which helps differentiate between bacterial vs. viral infections. PCT is induced systemically in reaction to bacterial toxins and cytokines, especially Interleukin-1β 1β, Interleukin-6, and Tumour necrosis factor-α, and it is a marker for bacterial infections. It promotes inflammation in bacterial infection by interacting with immune cells. It is normally converted to calcitonin in the thyroid as this pathway is suppressed during systemic inflammation, Sepsis 149,151. However, it is contradictory as it acts as an immune modulator, and it may act to **limit excessive immune responses** during severe infections. PCT levels are observed to show a significant rise (frequently >2 ng/mL in sepsis) during bacterial infections and sepsis. In the scenario of a viral infection, the PCT levels show little or no elevation (staying at 2 0 ng/mL), with extremely high levels in cases of critical bacterial sepsis exceeding 10 ng/mL. Also, it is observed that PCT level was somewhat elevated in trauma, surgery, and severe burns, but generally remains <2 ng/mL. The diagnostic threshold of PCT is regarded as in standard infection or absence of infection as <0.05 ng/ml. The diagnostic cut-off of low-risk bacterial infection is defined as 0. 05 - 0. 5 ng/ml, while a possible bacterial infection, as local or mild systemic, is considered to be 0. 5 - 2. 0 ng/ml. In the case of autoimmune disorders rarely cause PCT rarely rises, and in the case of fungal infections, PCT may rise, but it is lower than in bacterial infections. For instance, a patient arrives with fever, tachycardia, and hypotension, PCT = 8. 5 ng/mL. This provides strong evidence for severe bacterial sepsis instead of viral infections or non-infectious reasons. The final part of this chapter has addressed the details of PCT's capability to distinguish among bacterial and viral infections as host biomarker152–154.

**Alpha-1 Antitrypsin (AAT):** AAT is produced by the **liver,** including **monocytes, macrophages and lung epithelial cells.** AAT's normal range is **~100-200 mg/dL** at a half-life of 4-5 days. AAT is increased during times of inflammation, infection, and tissue damage. Its main function is to block proteases, particularly neutrophil elastase, which is released in response to inflammation and tissue damage. This serves to safeguard lung tissues from excessive proteolytic harm. The primary function of AAT involves the hindrance of neutrophil elastase, trypsin, and other serine proteases. AAT prevents the excessive degradation of extracellular matrix during inflammation, particularly in the lungs, where it serves an anti-inflammatory function by decreasing tissue damage induced by excessive immune activation. Additionally, it has been observed to increase during infections, trauma, malignancies, and autoimmune disorders. Most importantly, it is revealed to be discreetly raised in infections with bacteria or viruses. Moreover, it is noted to prevent tissue damage, such as trauma and burns, as its level rises to protect against proteases. Also, it is stated to be consistently augmented in chronic inflammation, such as inflammatory bowel disease and rheumatoid arthritis.

There exists a genetic array in the AAT gene located on chromosome 14 referred to as the SERPINA1 gene polymorphism. The standard allele is PiM, while there are two deficient alleles - PiZ regarded as the most severe deficiency and PiS, classified as the milder deficiency. PiMM (Normal) indicates a normal serum AAT level along with a low disease risk. Moreover, PiMZ (Carrier) signifies a mildly low AAT level and is associated with a slightly increased risk of COPD among smokers. On the other hand, PiZZ, a Severe deficiency, was observed to be remarkably low (<50 mg/dL) AAT in serum associated with a high risk of early emphysema and liver cirrhosis as AAT polymer buildup in hepatocytes. In clinical settings, serum AAT indicates further screening for deficiency and monitoring of the acute phase response. The phenotyping test with isoelectric focusing determines the **AAT variant** (Pi type). However, genotyping detection of SERPINA1 mutation analysis is marked as a hereditary deficiency. Importantly, a liver biopsy is considered in suspected liver disease due to AAT accumulation (PAS+ globules). In general, serum AAT level is increased in inflammation. However, a deficiency of AAT indicates lung and liver disease risk.

### 2.2.2. Negative acute phase proteins

A group of negative acute-phase proteins experience a drop in concentration in response to tissue damage, infection, or inflammation. Positive acute phase proteins, on the other hand, rise under these conditions. It is believed that the positive acute-phase proteins are found to be raised in these conditions, and lowering these negative acute-phase proteins aids in rerouting amino acids and metabolic resources to produce positive acute-phase proteins, which are critical for tissue repair and the immune response155.

During extreme inflammation, the liver's synthetic emphasis changes to create good acute phase proteins like haptoglobin, fibrinogen and C-reactive protein (CRP)—they directly support wound healing, coagulation and host protection 138. The quick phase response supports tissue regeneration, infection control, and prevention of further damage as part of the body's natural immune system defense mechanism 155. The body down-regulates negative acute-phase proteins, such as albumin, transferrin, and transthyretin, to preserve metabolic resources and give priority to the synthesis of these protective proteins. (Gabay and Kushner 1999; Cray et al. 2009). When these unfavourable acute phase proteins are lowered since they help to guide metabolic substrates and amino acids toward good acute phase proteins production, the body's ability to produce a successful inflammatory and immune response is enhanced 138 (Table 5).

Table 5: The important negative acute phase proteins as inflammatory biomarkers 155.

|  |  |
| --- | --- |
| **Negative acute phase proteins** | **Roles** |
| Transferrin | Iron carriage in blood |
| Transthyretin (Prealbumin) | Supply thyroxin (T4) and retinol (A4) |
| Albumin | Regulation of oncotic environment and carriage of fatty acids, hormones and drugs |
| Retinol binding proteins | Vitamin A carrier |
| Anti-thrombin | Control blood coagulation by preventing thrombin |

The most prevalent plasma protein, albumin, is a negative acute phase protein, which means that inflammation, infection, trauma, or chronic sickness causes its concentration to drop 138. Conditions including sepsis, burns, major surgery, cancer, and chronic inflammatory disorders like rheumatoid arthritis and inflammatory bowel disease are frequently associated with this hypo-albuminemia 156. The decrease in albumin levels is evidence of a shift in liver protein synthesis since the liver first makes positive acute phase proteins that are directly related to immune defense, coagulation, and wound healing: C-reactive protein (CRP), fibrinogen, and haptoglobin 138. Low albumin is a non-specific but clinically relevant sign of systemic sickness because it is not only a measure of inflammation but also depends on nutritional condition 156. Serum albumin is frequently employed as part of prognostic scoring systems because of its high predictive value in hospitalized patients, particularly in critical care, cancer, and chronic illness management 156.

Being a negative acute phase protein transferrin, both acute and chronic inflammation, infection, trauma, and cancer cause a decrease of transferrin in its plasma levels 138. The main role of transferrin is to transport iron through the bloodstream and control its distribution to various tissues, particularly those involved in erythropoiesis 157. The liver lowers transferrin production during the acute phase response, which is believed to restrict the amount of iron available to invasive pathogens and support the body's nutritional immunity strategy 138. Although total body iron reserves may be normal or even elevated, this decrease in the transferrin is a characteristic of anemia of inflammation, also known as anemia of chronic illness, in which iron is sequestered in tissues and iron transport is impeded 158. Although transferrin is a measure of nutritional status, inflammation also has a significant impact on its levels, making it difficult to interpret in patients who are severely ill or have chronic inflammation 156. As a result, transferrin serves as a biomarker of both nutritional evaluation and inflammatory response, together with other acute-phase proteins and iron indicators 157.

Previously referred to as prealbumin, transthyretin (TTR) is a negative acute phase protein whose concentration falls with chronic sickness, infection, inflammation, and trauma 159. According to Ritchie et al. (1999), transthyretin is essential for the movement of retinol-binding protein (RBP), which transports vitamin A, and thyroxin (T4)160. Transthyretin is a sensitive indicator of protein-energy deficiency due to its quick half-life and high rate of synthesis 159. However, as the liver switches from producing proteins involved in nutrient transport to those directly related to host defense and tissue repair, like C-reactive protein and fibrinogen, its levels are greatly impacted by inflammation, making it a component of the negative acute phase response (Gabay and Kushner, 1999a). In individuals with acute or chronic inflammatory disorders, this inflammation-driven suppression lessens its value as a stand-alone nutritional marker 156. To differentiate between inflammatory downregulation and nutritional insufficiency, contemporaneous inflammatory indicators like CRP must frequently be taken into account when interpreting transthyretin levels in clinical practice 159.

# **Antigenic biomarkers**

**Prostate Cancer Biomarker-**

One well-known biomarker that is mostly utilized for prostate cancer screening, diagnosis, and surveillance is prostate-specific antigen (PSA). Both healthy and malignant prostate epithelial cells release the protein known as PSA. The KLK3 gene encodes PSA, a member of the serine protease KLK family of protein-cleaving enzymes found in a healthy prostate. Prostate cancer, benign prostatic hyperplasia (BPH), or inflammation of the prostate (prostatitis) can all be indicated by elevated blood levels of PSA, a protein generated by the prostate gland. The screening tool is a blood test that is frequently used to evaluate PSA levels to screen for prostate cancer. It is not conclusive, though, as other illnesses, including prostatitis or BPH can also cause elevated PSA levels. Diagnostic markers to confirm prostate cancer, and other diagnostic procedures, including biopsies may be carried out if PSA levels are elevated. PSA is a monitoring tool because increased PSA levels can signal recurrence. PSA is also used to track the progression of prostate cancer, particularly following therapies like radiation or surgery 161. Normal PSA is usually regarded as 0–4 ng/mL, while age-related variations may occur. In BPH, PSA levels are normally between 4 and 10 ng/mL, although in extreme situations, they may be higher. The prostate cancer grey zone is 4–10 ng/mL, which may indicate malignancy or BPH. It could be necessary to do additional tests (such as an MRI, biopsy, or free PSA ratio). >10 ng/mL: Prostate cancer risk is elevated.>20 ng/mL: indicates a greater risk of metastatic or advanced illness 162. Because BPH causes an increase in prostate volume, it might gradually raise PSA.

When compared to normal prostate tissue, prostate cancer cells exhibit a substantial overexpression of PCA3, a prostate-specific, non-coding mRNA biomarker. It is employed as a diagnostic tool, specifically to decrease needless biopsies and increase the sensitivity of prostate cancer detection. Prostate cancer antigen 3 (PCA3) is the most well-known biomarker for prostate cancer that is becoming available as a non-PSA-based diagnostic test. A major difference from blood PSA is that PCA3, a long noncoding RNA (lncRNA), has been demonstrated to be raised in >90% of prostate cancer tissues but not in normal or BPH cells 163. Extremely Specific to prostate cancer, in contrast to PSA, benign diseases such as prostatitis or BPH do not cause an increase in PCA3. Measured in Urine - A urine sample obtained following a digital rectal exam (DRE), which releases prostate cells into the urine, is used for PCA3 testing. The PCA3 Score is measured by dividing the urine's PCA3 mRNA by PSA mRNA. Prostate cancer risk is decreased if the ratio of PSA/ PCA3 is <25, and increased risk if the ratio of ≥25. In this case, prostate cancer requires additional testing (biopsy, for example). PCA3 aids in differentiating between true cancer and increased PSA brought on by BPH164.

## **2.3. Genetic Markers as Host biomarkers**

Genetic markers differ in gene sequences and that can influence phenotypical characteristics of an individual. These may be associated with single nucleotide polymorphisms (SNPs), gene expression signatures, epigenetic modifications, or copy number variations (CNVs), resulting in the advancement of disease and therapeutic effects 165–167.

### 2.3.1. Single Nucleotide Polymorphisms (SNPs)

* SNPs may be associated with modifications to protein-coding sequences, regulatory areas, and miRNA (microRNA) binding sites, resulting in changes in gene expression and elevated risk of cancer
* **Association of SNPs in the regulatory region and Cancer susceptibility –**

SNPs in enhancers, promoters, and transcription factor binding sites may change the gene expression profile of a particular gene. For instance, the 8q24 locus, rs6983267, is linked to breast, prostate, and colorectal cancer. It affects the expression of the MYC oncogene, which causes unchecked cell division 168. The chromosomal location of this locus is 8q24.21, which is located within a gene desert (non-coding gene sequences). This is approximately 335 bp upstream of Myc, a master regulator for the growth of cells, differentiation and apoptosis. Since rs6983267 is found in a non-coding region, regulatory mechanisms rather than direct protein changes mediate its impact on cancer risk. The 8q24 area contains an enhancer element that regulates MYC expression. rs6983267, which changes the ability of this enhancer to bind transcription factors (TFs), is also included. Greater MYC expression comes from increased enhancer activity, which is related to the risk allele (G). TCF4 (T-cell factor 4), a key transcription factor in the Wnt/β-catenin signaling pathway, preferably binds to the risk allele (G).

**Rs6983267 SNP association in Colorectal Cancer-**

Colorectal cancer frequently shows downregulation of the Wnt/β-catenin pathway. Improved by the risk allele (G), which raises MYC expression and drives unlimited intestinal epithelial cell proliferation, TCF4 binding is boosted. According to genome-wide association studies (GWAS), GG or GT genotype carriers are more prone to suffer from colorectal cancer than TT genotype carriers, according to epidemiological research. The rs6983267 risk allele increases Wnt activity that is necessary for colorectal cancer (CRC) development, leading to uncontrolled cell growth 169,170.

* **Rs6983267 association in** **Prostate Cancer:**

According to Genome-Wide Analysis Studies, the 8q24 region is the most potent genetic risk factor for prostate cancer. By interacting with androgen receptor (AR) signaling, rs6983267 may have a role in the advancement of prostate cancer 171. The link between rs6983267 and the risk of prostate cancer was examined in a thorough meta-analysis. The study concluded that there is a substantial correlation between the G allele of rs6983267 with an elevated risk of prostate cancer172. Although there is little direct evidence connecting rs6983267 to the androgen receptor (AR) signaling in prostate cancer, possible interactions are suggested by the 8q24 region's proximity to the MYC oncogene. Variations in this region, such as rs6983267, may impact MYC expression, which in turn may impact the progression of prostate cancer, as MYC is known to be impacted by AR signaling 173.

* **Rs6983267 association in Breast Cancer**

MYC-driven carcinogenesis is linked to rs6983267 in breast cancer. The impact of this SNP on the expression of MYC in breast tissue may be modulated by estrogen receptor (ER) signaling. By up-regulating oncogenes such as MYC, estrogen promotes the growth of ER-positive breast cancer cells. According to one study, ER-positive breast cancer cell lines treated with estradiol showed a sharp rise in c-myc mRNA levels, indicating that MYC is a direct target of estrogen signaling 174. Variants in the estrogen receptor genes (ESR1 and ESR2) have been linked to an increased risk of breast cancer. For example, a study revealed that certain polymorphisms in these genes were associated with a higher risk of breast cancer in Chinese women, underscoring the role that ER genetic variants play in the development of breast cancer175.

Therefore, rs6983267 genotyping is a biomarker for cancer risk prediction that may be used to identify people who are more likely to develop prostate and colorectal cancer. Targeting MYC expression in treatments that block MYC or its enhancers (such as BET inhibitors) may be helpful because MYC is a major driver in several malignancies. Personalized medicine could facilitate more thorough cancer screening methods may be advantageous for those with the GG genotype.

* **Association of SNPs in microRNA (miRNA) Sequences in Cancer:**

Small non-coding RNAs known as miRNAs control the expression of genes by attaching to the 3' UTR (untranslated region) of mRNA and causing translation to be inhibited or degraded. Oncogenes or tumor suppressors may become dysregulated as a result of SNPs in miRNA genes that alter their biosynthesis, stability, or function. For example, the NF-κB pathway is impacted by miR-146a (rs2910164), which increases inflammation and accelerates the growth of cancer. linked to thyroid, breast, and stomach cancers 176. The expression of HOX genes, which control cell differentiation, is impacted by miR-196a2 (rs11614913) connected to gastrointestinal, breast, and lung malignancies 177.

* **Association of SNPs in Target Genes of miRNA Binding Sites**

Target gene 3' UTR SNPs can generate or interfere with miRNA binding sites, which can change how oncogenes or tumor suppressors are regulated. For example, increased KRAS expression results from changes in the binding of tumour-suppressing let-7 miRNA caused by the KRAS gene (rs61764370 in the let-7 binding site) connected to ovarian and lung malignancies. The TP53 gene (rs1042522 in the miR-125b binding region), which is associated with breast and colorectal cancer, influences the activity of the p53 tumor suppressor. This results in decreased apoptosis and increased tumor development178.

* **Association of SNPs' Functions in Cancer Development**

Oncogene Activation and Overexpression of oncogenes (e.g., KRAS, MYC) can result from SNPs in miRNA binding sites that lessen miRNA repression of these genes. Tumor Suppressor Inactivation is mediated when miRNA binding is disrupted, and tumor suppressors (like TP53) may express less. Therapeutic Resistance may facilitate SNPs in genes linked to miRNA and can change how well a patient responds to targeted treatments, including chemotherapy 179,180.

* **Association of SNPs in Gene expression variation**

Single-nucleotide polymorphisms (SNPs) in promoters, enhancers, or untranslated regions (UTRs) can affect transcription factor binding, RNA stability, and translation efficiency. For example, the IL-6 (-174 G/C SNP) alters the expression of the IL-6 cytokine, which affects inflammation and the risk of sepsis. Additionally, the SNPs in IFNL3 (formerly IL-28B) affect the interferon response and the clearance of the hepatitis C virus (HCV)181.

* **Association of SNPs in structural mechanisms and protein functions**

Exonic SNPs can lead to missense mutations that alter the amino acid composition or nonsense mutations that create early stop codons in coding areas. Sickle cell anemia (HBB; rs334) is an example of a condition brought on by a single SNP (A→T) that causes changes in Hemoglobin S, leading to the disease. Similarly, CYP2C19\*2 (rs4244285) decreases enzyme activity, affecting the metabolism of clopidogrel and cardiovascular risk182.

* **Association of SNPs in immune functions**

SNPs in genes linked to immunity can alter immune responses as well as host-pathogen interactions. For example, TLR4 (rs4986790, rs4986791) lowers Toll-like receptor activity, which increases the risk of Gram-negative infections by making it more difficult for the immune system to detect pathogens 183. An elevated pro-inflammatory tumor necrosis factor (TNF)-α response to lipopolysaccharide (LPS) stimulation has been linked to the Asp299Gly polymorphism. In contrast, there is no discernible change in the cytokine response between the wild-type TLR4 and the combination Asp299Gly/Thr399Ile haplotype 183. A meta-analysis evaluating the association between TLR4 polymorphisms and neonatal sepsis susceptibility indicated that both rs4986790 and rs4986791 variations could influence sepsis risk in neonates184.

* **Association of SNPs in epigenetic modifications**

Long-term alterations in gene expression can result from SNPs' effects on histone modifications or DNA methylation. For instance, MTHFR (C677T, rs1801133) -> modifies DNA methylation and influences folate metabolism, raising the risk of cardiovascular disease or neural tube abnormalities. The enzyme methylenetetrahydrofolate reductase, which is essential for changing 5,10-methylene tetrahydrofolate into 5-methyltetrahydrofolate—a critical methyl donor in DNA methylation processes—is encoded by the MTHFR gene. The alanine-to-valine mutation caused by the C677T SNP makes the enzyme less active and thermo labile. Because folate metabolism is hampered by this decline, homocysteine levels rise and DNA methylation patterns change. Global DNA methylation is frequently lower in people homozygous for the T allele (TT genotype) than in people with the CC genotype 185,186.

* **Association of SNPs in Infectious Disease**

**Tuberculosis-** Genetic variations in host immunological genes have an impact on tuberculosis (TB), which is brought on by *Mycobacterium tuberculosis* (*M.tb*). NRAMP1 (SLC11A1, rs17235409, rs34448891) is one major SNP, that encodes a transporter that is essential for the antibacterial action of macrophages. SNPs in SLC11A1 affect metal ion transport, which is necessary for pathogen management, and are linked to an increased risk of tuberculosis187. TLR4 (rs4986790, rs4986791) & TLR2 (rs3804100, rs5743708). TLRs, or toll-like receptors, identify *M.tb* components and initiate immunological reactions. SNPs in TLR2 and TLR4 enhance susceptibility to tuberculosis and decrease cytokine production 188. IFNG (rs2430561) interferon-gamma, which is essential for Th1 immune responses, is encoded. The T/A variation, SNP rs2430561, is associated with elevated susceptibility to tuberculosis and decreased IFN-γ levels 189. VDR (Rs2228570, rs731236, Vitamin D Receptor) Vitamin D-dependent antimicrobial responses are modulated by VDR polymorphisms. SNPs that impact immunological function and impact TB susceptibility include rs2228570 (FokI) and rs731236 (TaqI)190,191.

**HIV-** SNPs in immune response and viral restriction genes affect HIV progression and treatment results. CCR5 (rs333, Δ32 mutation) - HIV enters CD4+ cells via the co-receptor CCR5. HIV cannot use CCR5 due to the Δ32 deletion (rs333), which makes it resistant to infection192. HLA-B\*57 (rs9264942, rs2395029). Good control of HIV (low viral load without treatment) is linked to a protective allele. The HLA-B region's SNP rs2395029 improves immune-mediated viral suppression 193. SNPs in immune response and viral restriction genes affect HIV progression and treatment results. CCR5 (rs333, Δ32 mutation) HIV enters CD4+ cells via the co-receptor CCR5. (rs8177832, rs5757463) APOBEC3G encodes an antiviral enzyme that causes HIV to undergo hypermutation 194. SNPs change the efficiency of HIV replication by influencing APOBEC3G activity. IFNL3 (rs12979860, rs8099917, IL28B) impacts the immune system's reaction to HIV and HCV co-infection. Better management of HCV/HIV co-infection is associated with the rs12979860 CC genotype 195,196.

**Malaria-** Sickle Cell Anemia (rs334): Sickle cell disease is caused by mutations in the HBB gene, which codes for hemoglobin. One such mutation is the rs334 SNP. Because sickle-shaped red blood cells are less hospitable to Plasmodium falciparum, heterozygous carriers of the sickle cell trait (AS genotype) are more resistant to malaria 197.G6PD Deficiency (rs1050828): An enzyme involved in the metabolism of red blood cells is encoded by the G6PD gene. Hemolysis under oxidative stress can result from an enzyme deficiency brought on by different SNPs in the G6PD gene, which may provide some protection against malaria, particularly *P. falciparum*. However, exposure to specific triggers, such as fava beans or specific drugs, increases the risk of hemolytic anemia. One of the most researched SNPs in G6PD deficiency is rs1050828, which can affect a person's susceptibility to malaria 198. Thalassemia carriers with alpha or beta thalassemia exhibit higher resistance to malaria, much as those with sickle cell disease. This is probably because the changed structure of red blood cells makes it more difficult for the parasite to infect the cells 198.*Plasmodium vivax* enters red blood cells using a receptor encoded by the DARC gene, which results in the Duffy Negative Phenotype (rs2814778). People who have a mutation in the DARC gene (rs2814778) are immune to *P. vivax* malaria because their red blood cells do not have the receptor. Due to the prevalence of the Duffy-negative gene, this condition is typical in sub-Saharan Africa199,200. HLA Class I and II SNPs: Malaria susceptibility has been linked to variations in HLA genes, which are implicated in immunological responses. Certain SNPs in HLA alleles, like HLA-B\*53 and HLA-DRB1, alter how well the immune system reacts to Plasmodium antigens, which in turn affects how severe the illness is. According to studies, some HLA genotypes enhance vulnerability to malaria, while others provide protection 201.The cytokine encoded by the TNF-α gene (rs1800629) is implicated in the immunological response. Because TNF-α contributes to the inflammatory response to malaria infection, the rs1800629 SNP has been linked to malaria susceptibility. Severe malaria, particularly in children, has been associated with elevated TNF-α levels 202.MCP-1 Gene (rs1024611): This gene produces a chemokine that helps draw monocytes to infection areas. SNPs in MCP-1 have been linked to both susceptibility and resistance to *P. falciparum* and can affect the immunological response to malaria 203. The receptor encoded by the CD36 gene (rs3211938) aids in the malaria parasites' attachment to the host's red blood cells. Because they impact the parasite's capacity to infiltrate and cause serious illness, certain CD36 gene variations are linked to malaria resistance204.

* **Association of SNPs in Auto-immune disease**

**SLE-** The complicated autoimmune disease known as systemic lupus erythematous (SLE) has a significant hereditary component. A higher risk of getting SLE has been linked to several single nucleotide polymorphisms (SNPs). These SNPs are frequently discovered in genes related to inflammatory pathways, apoptosis, and immunological modulation.

Interferon Regulatory Factor 5, or IRF5 a higher risk of SLE is associated with SNPs in this gene, including rs2004640. A major factor in the pathophysiology of SLE is the type I interferon pathway, which is mediated by IRF5205,206. Variants such as rs5029939 in TNFAIP3 (A20) are linked to heightened vulnerability to SLE. By regulating the NF-κB pathway, which is essential for immunological responses, this gene controls inflammation 207.

STAT4 (Signal Transducer and Activator of Transcription 4) SNPs like rs7574865 in STAT4 have been strongly associated with SLE. STAT4 is involved in the activation of T-helper cells, which are central to the immune dysregulation in SLE208. CLEC16A (C-type lectin domain family 16, member A): SNPs in CLEC16A, such as rs12708716, have been associated with SLE and other autoimmune diseases. CLEC16A is involved in the regulation of autophagy and immune responses 209,210. PTPN22 (Protein Tyrosine Phosphatase, Non-Receptor Type 22): The SNP rs2476601 in PTPN22 is a well-known risk factor for several autoimmune diseases, including SLE. This gene encodes a tyrosine phosphatase that regulates immune cell signaling 211,212. SNPs in BLK (B lymphoid kinase), such as rs13277113, are linked to a higher chance of developing SLE. B lymphocytes play a key role in the pathophysiology of SLE and BLK is implicated in their signaling 213,214. SLE has been connected to the SNP rs1801274 in FCGR2A (Fc Gamma Receptor IIa). FCGR2A is involved in both inflammatory reactions and the removal of immunological complexes215. SLE-associated risk loci in genome-wide association studies (GWAS) have also revealed other loci linked to SLE susceptibility, including those close to the genes encoding IRF7, CX3CR1, and TNF216.

**Multiple Sclerosis-** Numerous single-nucleotide polymorphisms (SNPs) have been linked to an increased risk of developing multiple sclerosis (MS), a complex autoimmune illness with a significant genetic component. Key SNPs and loci associated with MS include: Human Leukocyte Antigen, Class II, DR Beta 1 (HLA-DRB1) - the most closely linked genetic risk factor for multiple sclerosis is SNPs in the HLA-DRB1 gene. Certain alleles, such as HLA-DRB1 1501, raise the risk of multiple sclerosis, although other alleles may have a protective impact 217. Further, MS has been linked to SNPs like rs6897932 in the IL7R gene. This gene contributes to T cell signaling, which is essential for the immunological response in multiple sclerosis 218. Also, vitamin D is activated by CYP27B1, and several SNPs in this gene, including rs10877012, are linked to an increased risk of developing multiple sclerosis. It has been proposed that vitamin D influences the development of MS through immunomodulatory effects219.A higher risk of MS has been associated with SNPs in TREM2, including rs2234253. Variants of TREM2, which is involved in microglial activity, can affect the central nervous system's inflammatory response 220. One important cytokine implicated in inflammation is TNF. Because TNF contributes to the inflammatory processes in MS, SNPs in the TNF gene, such as rs1799964, have been linked to an increased risk of developing MS 221. Different alleles at the HLA-DRB1 locus (e.g., DRB1\*1501) are known to be strongly related to multiple sclerosis (MS), and this locus is crucial to MS genetic susceptibility. Antigen presentation and immune response are impacted by this locus. MS has been associated with variations of the CLEC16A gene, including rs12708716. The immunological response in multiple sclerosis may be influenced by the SNPs of CLEC16A, which is implicated in immune regulation and autophagy 222.

### 2.3.2. Gene Expression Signature

The use of gene expression profiles as biomarkers for infectious illness diagnosis, tracking, and pathophysiology understanding is becoming more widely accepted. By aiding in the differentiation of various pathogens and predicting the seriousness or progression of a disease, these profiles provide understanding of the host's immune reaction to infections. Some of these are mentioned previously. As potential biomarkers for neurodegenerative conditions such as Parkinson's disease, Alzheimer's disease, and others, gene expression profiles have garnered significant attention in this area. Insights into the fundamental mechanisms of the disease can be gained from these signatures, which show the molecular alterations in cells and tissues brought on by disease processes. Key information on gene expression signatures as biomarkers in neurodegenerative illnesses is as follows:

**Alzheimer's disease –** Gene expression profiles for Alzheimer's disease are used to find treatment targets, forecast the illness's course, and identify early stages. Inflammation Signature is increased expression of inflammatory genes, including TREM2, TLR4, and IL6, has been linked to Alzheimer's disease. The neuroinflammatory response that is essential to the pathophysiology of AD is reflected in these genes 223. Others related biomarkers are discussed in later section of this chapter.

**Parkinson's disease -** To find biomarkers for diagnosis and the advancement of the disease, gene expression profiles are also being investigated in Parkinson's disease. Genes Associated with Dopamine Dysregulation: Parkinson's disease alters the expression of genes related to dopamine signaling, including TH (tyrosine hydroxylase), SLC6A3 (dopamine transporter), and COMT (catechol-O-methyl transferase)224. Further, Increased expression of immune-related genes, including IL1B, TNFα, and CXCL8, which suggest neuroinflammation in the substantia nigra, is linked to Parkinson's disease225. Parkinson's disease alters the expression of genes linked to mitochondrial function, including PINK1, DJ-1, and LRRK2. One of the main causes of neuronal damage in Parkinson's disease is mitochondrial malfunction 226. The molecular mechanisms behind motor neuron degeneration are being understood through the use of gene expression markers in ALS. Factors associated with excitotoxicity are genes linked to glutamate signaling and excitotoxicity, including GLT-1 (glutamate transporter) and GRIA1 (AMPA receptor), which are expressed differently in ALS 227. In addition, Oxidative stress-related genes, including glutathione peroxidase (GPX1) and superoxide dismutase 1 (SOD1), exhibit changed expression patterns in ALS, indicating the part oxidative damage plays in motor neuron degeneration 228. As biomarkers for diagnosis and progression, TARDBP gene expression alterations and C9ORF72 mutations are prevalent in FTD. TDP-43 and Genes Associated with C9ORF72. FTD is associated with altered expression of TARDBP (TAR DNA-binding protein) and C9ORF72 229. Blood Transcriptomics in the Periphery, a non-invasive technique for finding biomarkers for neurodegenerative illnesses, is a blood-based gene expression profiling. Patients with AD, PD, ALS, and other neurodegenerative disorders frequently have dysregulated blood levels of genes linked to inflammation, oxidative stress, and neuronal damage230.

**Biomarkers for Alzheimer's disease -**To diagnose neurodegenerative disorders like Alzheimer's disease, track their progression, and create treatments, biomarkers are essential. Several important indicators of Alzheimer's disease (AD) are frequently employed in clinical and research settings:

One of the defining pathological characteristics of Alzheimer's disease is amyloid-beta plaques. Amyloid-beta buildup in the brain causes plaque to develop, which damages neurons and interferes with cell-to-cell communication. Biomarkers for Cerebrospinal Fluid (CSF) levels of amyloid plaque buildup in the brain are linked to lower CSF levels of amyloid-beta (Aβ1-42). PET Imaging by using tracers such as florbetapir or florbetaben, PET imaging can visualize amyloid plaques in vivo, aiding in diagnosis and tracking the course of the disease 231. In neurons, tau is a microtubule-associated protein that keeps microtubules stable. Tau gets hyperphosphorylated and tangles inside neurons in Alzheimer's disease, which impairs cellular processes and causes neurodegeneration. Increased levels of tau and phosphorylated tau, or p-tau, in the cerebrospinal fluid (CSF), are linked to neurodegeneration and may be a sign of Alzheimer's disease. It is believed that tau tangles in the brain are correlated with p-tau in particular. In vivo imaging of tau disease is made possible by the ability of PET tracers, like flortaucipir, to attach to tau tangles in the brain. 232. Further, Neurons include a structural protein called neurofilament light chain (NfL). When neurons are injured, it is released into the blood and CSF. Increased NfL levels have been connected to a number of neurodegenerative illnesses, including Alzheimer's, and can act as a sensitive biomarker for neurodegeneration. Blood/CSF NfL: Elevated blood or CSF NfL levels have been linked to Alzheimer's disease and can be used to track the development of the disease and neurodegeneration233. Moreover, one of the main characteristics of Alzheimer's disease is neuroinflammation. The potential of a number of inflammatory indicators as biomarkers has been investigated. Cytokines in Alzheimer's patients' CSF and blood have been found to contain higher concentrations of pro-inflammatory cytokines, including IL-6, TNF-α, and IL-1β. Microglial Activation in Neuroinflammation *in vivo* can be evaluated by imaging microglial activation with PET tracers such as PBR28, which binds to the TSPO receptor 234. Acetylcholine, a neurotransmitter involved in memory and learning, is broken down by the enzyme acetyl cholinesterase. Reduced acetyl cholinesterase activity is frequently seen in Alzheimer's disease, especially in memory-related brain areas. PET Imaging of AChE Activity shows Acetyl cholinesterase activity distribution in the brain can be assessed by PET scans using tracers such as [11C] donepezil 235. A molecule called brain-derived neurotropic factor (BDNF) promotes the survival and flexibility of neurons. Alzheimer's disease patients have been found to have lower levels of BDNF, which could be an indication of neuronal malfunction or damage. Reduced levels of BDNF in the plasma or serum have been associated with cognitive decline and may aid in the early detection of Alzheimer's disease 236. A higher risk of Alzheimer's disease is linked to specific genetic markers, especially the APOE ε4 allele. It has been demonstrated that the APOE ε4 allele increases the buildup of amyloid plaque in the brain and is implicated in lipid metabolism. APOE Genotyping: When combined with other biomarkers, the identification of the APOE ε4 allele through genetic testing can aid in determining genetic risk for Alzheimer's disease 237.

### 2.3.3. Epigenetic changes and copy number variants (CNVs)

In the diagnosis, prognosis, and therapy of a variety of illnesses, such as cancer, autoimmune disorders, neurological diseases, and infectious diseases, epigenetic changes and copy number variants (CNVs) have become crucial indicators. These biomarkers help track the course of the disease or its response to treatment, uncover new therapeutic targets, and offer crucial insights into the mechanisms underlying the disease.

**Epigenetic changes-** Changes in the regulation of gene expression without alterations to the underlying DNA sequence are known as epigenetic modifications. These changes include histone modifications, DNA methylation, and the control of non-coding RNAs (like miRNAs)238. They are crucial to the onset of many illnesses, particularly autoimmune diseases, neurological conditions, and cancer. For example, Breast, colorectal, and lung cancers are among the many malignancies that commonly exhibit hyper methylation of tumor suppressor genes (e.g., p16INK4a, MGMT). These alterations can function as indicators for early diagnosis, prognosis, and therapy response 239. Many types of cancer frequently exhibit dysregulation of histone deacetylases (HDACs) 240. For instance, solid tumors usually overexpress HDAC1, and blocking it may be therapeutic. Numerous malignancies have either elevated or downregulated noncoding sequences as expression levels of miRNAs, including miR-21, miR-155, and miR-34. These expression levels can be used as biomarkers for diagnosis, prognosis, and response to treatment 241.

**Copy number variants-** Copy number variations (CNVs) are structural changes in the genome that impact how many times a particular gene or genomic region is present. CNVs242. are associated with a variety of disorders, such as cancer, neurological disorders, and developmental disabilities. CNVs significantly influence cancer biology by changing gene dosage, turning on oncogenes, or removing tumor suppressor genes. Oncogene Amplification: Chromosomal instability in cancer can lead to copy number variations (CNVs) that impact critical regions, such as those harboring oncogenes (MYC, CCND1) or tumor suppressor genes (TP53, CDKN2A)243. CNVs like HER2 amplification in breast cancer and EGFR amplification in non-small cell lung cancer (NSCLC) are examples of CNVs that are employed as diagnostic biomarkers and therapeutic targets244. CNVs are associated with many neurodegenerative diseases as well as neurodevelopmental disorders like intellectual disabilities, schizophrenia, and autism spectrum disorder (ASD). Certain copy number variations (CNVs), like deletions or duplications in 16p11.2 and 1q21.1, are strongly associated with ASD. These CNVs contribute to the pathophysiology of autism by affecting the expression of genes involved in neural development. CNVs, such as those found in 22q11.2, are associated with an elevated risk of schizophrenia245. The genetic vulnerability of at-risk individuals can be assessed using these CNVs. CNVs in genes like APP (associated with Alzheimer's disease) and MAPT (related to tauopathies) are being studied as potential biomarkers for neurodegenerative disorders246. CNVs in immune-related genes, such as FCGR3B, which encodes an IgG receptor, have been associated with SLE. Certain CNVs exacerbate the abnormal immune responses that characterize autoimmune diseases.

# **Techniques for detecting host biomarkers (e.g. CRP, Procalcitonin)**

Biomarkers play an important role in clinical diagnostics. Here important biomarker detection techniques are described below for Procalcitonin (PCT) and C-reactive protein (CRP). PCT and CRP can be detected using a variety of methods, each having advantages based on available resources, sensitivity, and specificity.

## **Enzyme-linked immunosorbent Assay or ELISA**

O **CRP:** CRP can be measured in serum or plasma samples using ELISA. CRP-specific antibodies conjugate with CRP antigen in the specimens, which is proportional to the colorimetric signal in ELISA.  
O **Procalcitonin:** Similarly, PCT can be measured in ELISA using polyclonal or monoclonal antibodies. It is a highly sensitive method to identify even low concentrations of CRP and PCT.

* 1. **Chemiluminescent Immunoassay (CLIA)**
* **CRP and PCT** are tested using a chemiluminescent substrate. Antigen-antibody complexes are formed, which are measured by a chemiluminescent light signal.

This procedure is often used in clinical laboratories as CLIA is faster and sensitive than ELISA.

* 1. **Latex Agglutination Test**
* CRP: In this test, latex beads are used to detect CRP using anti-CRP antibodies coated on beads.
* A positive agglutination test is indicated in an affirmative latex agglutination test.
* Procalcitonin: PCT could be visualized using an agglutination test mediated by latex-coated anti-PCT antibodies.

## **Immunoturbidimetry**

* This technique involves turbidometric analysis of CRP or PCT conjugation with antibodies in the specimens. The concentration of the biomarkers associated with the turbidity of the test, which is often useful the clinical laboratories.

## **Radioimmunoassay (RIA)**

* In this method, a radiolabeled antibody is useful to measure CRP or PCT in the specimen. This method is restricted in clinical practices due to radio logical hazards.

## **Western blotting**

* Western blotting comprises three techniques: SDS-PAGE and blotting, and gel electrophoresis. PCT and CRP could be isolated in western blot analysis, which could be developed using detection antibodies. This technique is highly useful in research laboratories rather than the diagnostics.

## **Mass Spectrometry**

* Mass Spectrometry is a complex technique with high sensitivity and specificity, which could be useful for the detection of CRP and PCT in the research setting. However, its use is restricted in diagnostic settings due to its high cost-effectiveness

## **Point-of-Care Testing (POCT)**

* There are several quick assays as pregnancy tests, dot-blot ELISA with immunochromatographic strips to use in point of care testing (POCT) in poor clinical settings. These tests are faster and reliable with often used as qualitative test in public health facilities.

Generally, ELISA, CLIA, Immunoturbidimetry, and latex agglutination are regularly employed techniques for CRP. ELISA, CLIA, and fast point-of-care tests are commonly used for Procalcitonin, with Immunoturbidimetry being used to some extent.

# **Biomarkers differentiating bacterial vs viral infections**

By reflecting the ongoing interaction of the immune system and invading pathogens, host biomarkers offer a view of the source of the infection. These biomarkers mirror small changes in the response of the host immune system, including the release of cytokines, acute-phase proteins, and cellular activation markers that vary widely from bacterial to viral infections 247–251. Bacterial infections, for instance, generally elicit a strong neutrophil reaction together with increased levels of pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α)248 252. By contrast, viral diseases usually cause lymphocytic activation and interferon production, which activate genes essential for anti-viral defense systems 253,254. Whether singly or as part of multi-marker panels, the measurement of such biomarkers offers a strong lens via which doctors may more quickly and accurately detect the cause of infection255,256. In environments where usual clinical techniques are restricted by time, sensitivity, or pathogen variability, this dynamic profile is particularly useful. Several biomarkers have shown practicality for telling viral from bacterial infections250.

## **Procalcitonin (PCT)**

Bacterial infections elicit Procalcitonin, a peptide precursor of calcitonin produced by parenchymal cells. Since high PCT levels are associated with bacterial infections, it is a trustworthy biomarker for distinguishing viral diseases. Studies show that PCT-guided treatment reduces antibiotic use without damaging patient safety. PCT is synthesized in response to bacterial endotoxins and systemic inflammatory cytokines, particularly interleukin-1β and TNF-α 257,258. Unlike other biomarkers, PCT production is suppressed during viral infections due to the activity of interferon-gamma, providing a clear distinction in etiology259,260. Clinical studies show that in situations like sepsis, PCT levels correspond with the severity of bacterial infections and help to gauge treatment response 261. PCT has also shown potential in distinguishing bacterial from fungal co-infections 242 and in spotting bloodstream disorders, lower respiratory tract infections, and even different kinds of disease. Its rapid kinetics and stability are particularly useful for guiding early treatments in infant and children populations. High-sensitivity tests have been used in point-of-care testing and international integration in antimicrobial consumption plans 262.

## **C-reactive protein (CRP)**

Among the most often used biomarkers for diagnosing infection and inflammation is C-reactive protein (CRP), a widely known acute-phase reactant. Generated in great part by hepatocytes in reaction to interleukin-6 (IL-6) stimulation, CRP levels can soar sharply within six to twelve hours after a wound or infection starts, peaking within 48 hours. Rising in both bacterial and viral diseases, CRP levels are higher and more persistent, notably in severe bacterial pneumonia or sepsis 133,248. The main drawback of CRP, on the other hand, is its lack of specificity; it can be elevated in many non-infectious inflammatory diseases, including surgery, trauma, and autoimmune conditions 263. To enhance diagnostic accuracy, CRP is being more and more utilized in multi-biomarker panels, together with sometimes, markers TRAIL (tumor necrosis factor-related apoptosis-inducing ligand) and IP-10 (interferon gamma-induced protein 10), showing more infection-specific responses250. Furthermore, by allowing more precise measurement at lower levels and thereby expanding their applicability in subclinical inflammation surveillance, high-sensitivity CRP (hs-CRP) tests have raised their usefulness in infection risk classification 263. CRP's general availability, fast turnaround, and low cost, which assure that it remains a staple of infection diagnosis in both resource-rich and resource-poor environments, offer stability.

One of the intriguing advances in CRP-based diagnostics is the estimation of CRP velocity (eCRPv), which is calculated on the time lapse since the onset of infection, to estimate the inflammatory kinetics associated with bacterial infections. A value of eCRPv less than 0.25 mg/L/h is more commonly associated with viral infections, while greater than 1.1 mg/L/h strongly suggests a bacterial origin. Notably, eCRPv is particularly operational at distinguishing infections in individuals with intermediate CRP levels (100–150 mg/L), a range that often causes diagnostic ambiguity. One of the most promising improvements in CRP-based diagnostics is the use of estimated CRP velocity (eCRPv), which assesses the rate of CRP growth use of estimated CRP velocity (eCRPv), which assesses the rate of CRP increase about time after symptom onset.

High eCRPv is found in bacterial infections than in viral infections 264,265. High levels of eCRPv (>1.1 mg/L per hour) suggest bacterial infections, while low levels (<0.25 mg/L per hour) suggest viral infections or self-limiting inflammatory conditions 248. This method is especially useful in individuals with intermediate CRP levels (100-150 mg/L), a diagnostic gray zone where discriminating between bacterial and viral illnesses is most difficult 266. Combining eCRPv with clinical context and other biomarkers, such as Procalcitonin (PCT) or host gene expression profiles, shows potential for precision diagnosis in acute febrile illness.

Additionally, CRP is crucial for understanding the body's inflammatory response during serious infections. Its ability to bind to phosphorylcholine on bacterial surfaces and activate the complement system emphasizes its dual role as both a diagnostic and prognostic tool 133. High CRP levels are a crucial marker for evaluating treatment response because of their relationship to disease severity and their link to bacterial infections 267.

Notably, new research combined CRP measurements with machine learning algorithms, which further enhances the specificity and sensitivity of CRP data by combining it with other clinical and laboratory information7. This advancement in CRP measurements aids in diagnosing patients at the point of care in real time in poor clinical and diagnostic settings268.

Although bacterial infections typically result in a greater increase but CRP levels are increased during both viral and bacterial infections. However, the diagnostic utility of CRP measurements has been enhanced by advancements in CRP measurement, such as high-sensitivity assays.

CRP continues to be among the most commonly utilized biomarkers for differentiating bacterial infections from viral ones. However, CRP levels may be raised in bacterial and viral infections, but bacterial infections result in high levels of CRP269. The advancement in high-sensitivity CRP (hsCRP) test has readily enabled the detection and identification of early inflammatory reactions263.

## **Interferon-Stimulated Genes (ISGs)**

Viral infection induces interferon, which activates a cascade of interferon-stimulated genes (ISGs) essential for the host's antiviral defense. In this context, important biomarkers: IP-10 and TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) play a pivotal role in immune modulation and entry of the pathogen270. An increase in IP-10 is highly associated with virus-mediated infections by stimulating the Th1 immune response. On the other hand, TRAIL facilitates apoptosis in the infected cells270–272. These biomarkers are included in the diagnostic panel, enabling their utilization in the differentiation of viral and bacterial infections

Additionally, specific viral pathogens such as influenza and coronaviruses have been identified using ISGs like MX1 (myxovirus resistance protein 1) and OAS1 (2'-5'-oligoadenylate synthetase 1). Several recent studies have shown that combining conventional inflammatory markers with ISG biomarkers greatly enhances diagnostic precision. The vibrant profiling of these biomarkers facilitates readily diagnostic information and supports prognostic evaluations, including predicting the seriousness of viral illnesses like COVID-19, suggesting a framework for differentiating infections while enhancing bacterial-focused indicators such as PCT and CRP 273,274.

For the proper administration of antibiotics and patient care, it is essential to accurately distinguish between bacterial and viral infections. Key biomarkers utilized in clinical practice and research are summarized in the table below (Table 6):

Table 6: The important biomarkers Differentiating Bacterial vs. Viral Infections 275–277

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Biomarker** | **Functions** | **Bacterial Infection** | **Viral infection** | **Clinical importance** |
| Procalcitonin (PCT) | Immune cells' release in reaction to bacterial toxins | High | Normal or mild | Highly specific for bacterial infection, facilitates antibiotic treatment |
| C-reactive proteins | Increases in inflammation, acute phase protein | Moderate to high | Mild to Moderate | Raised in both infections; highly induced in bacterial infections |
| Serum Amyloid A ( SAA) | Acute phase protein; inflammation | High | Mild | Higher in bacterial infections; not entirely specific |
| Interferon-Gamma-Induced Protein 10 (IP-10/CXCL10) | Chemokines, associated with infection | Normal or mild | High | Robust biomarkers for viral infections. |
| Interleukin-6 (IL-6) | Pro-inflammatory Cytokine | High | Mild to Moderate | Early infection recognition, raised in both infections |
| Tumor Necrosis Factor-α (TNF-α) | Pro-inflammatory Cytokine | High | Mild | Elevated in bacterial infections, not specific |
| Soluble CD14 (Presepsin) | Biomarker for Monocyte activation | High | Normal | Highly specific for bacterial sepsis |
| Myxovirus Resistance Protein A (MxA) | Interferon-stimulated protein against viruses | Normal | High | Highly Specific for viral infections |
| Neutrophil-to-Lymphocyte Ratio (NLR) | Immune cell ratio | High (Neutrophilia, Lymphopenia) | Low or Normal | Bacterial infections originate from neutrophilia, while viral infections cause lymphocytosis |
| Lipocalin-2 (LCN2) | Iron transport and immune modulation | High | Normal or mild | More pronounced in bacterial infections |
| TRAIL (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand) | cytokine uniquely upregulated in response to viral infections | Low levels or unchanged in bacterial infection | Elevated levels in viral infections, especially in respiratory viruses like influenza and COVID-19 | Used for distinguishing bacterial from viral respiratory infections, especially in febrile illnesses  Key biomarker for viral infections, such as influenza, COVID-19, and other viral respiratory illnesses |

Recent, antibiotic treatment is followed by PCT and CRP measurement. On the other hand, MxA and IP-10 are specific to viral infections, leading to a reduction in the misuse of antibiotics. NLR may serve as a swift and cost-effective method, especially in poor clinical and diagnostic settings.

## TRAIL (Tumor Necrosis Factor-Related Apoptosis-Inducing Ligand)

TRAIL is a cytokine that is particularly elevated in response to viral infections, making it an accurate biomarker for distinguishing between viral and bacterial origins. TRAIL, showing more than 85% sensitivity and specificity, has been incorporated into diagnostic panels together with biomarkers such as CRP. This integrated approach significantly improves the diagnostic accuracy for febrile illnesses, particularly in cases with ambiguous clinical signs278. This combined strategy greatly enhances the diagnostic precision for febrile diseases, especially in instances with unclear clinical indicators 278.  
  
Since it slows viral replication, assists in disease resolution by inducing apoptosis in infected cells, and thus helps to manage viral copy, TRAIL is therefore critical for the immune system. Essentially, it has been discovered to be a major biological marker for differentiating viral respiratory diseases, from flu and COVID-19, from those of bacterial origin. Among numerous patient categories, including tiny children and those with weakened immune systems, TRAIL also increases the sensitivity of ImmunoXperts™ in a multi-marker system279.  
  
The future of TRAIL-based diagnostics depends on its integration with cutting edge tools including multiplex assays and machine learning. Real-time point-of-care testing will let these developments totally unfold, therefore allowing doctors to quickly produce evidence-based judgments that optimize patient results and antibacterial management280.

## **IP-10**

A chemokine, IP-10, plays an important role in the immune response, as a potent chemoattractant for immune cells and facilitates the recruitment of these cells to the site of infection. This chemokine is induced by interferon-gamma (IFN-γ) in response to viral infection. This chemokine significantly increased in such an infection, making this chemokine as a biomarker for viral infection. This chemokine is seen as a viral disease marker since its levels are significantly elevated in viral infections281. .

Based on thorough studies and verification in both adult and pediatric populations, IP-10 is said to be useful for differentiating viral respiratory disorders from bacterial or other non-viral ones. Another term for IP-10 (Interferon gamma-induced protein 10) is CXCL10; it is a compound essential for immune system operation. One of the main players is interferon-gamma (IFN-γ), a cytokine manufactured in response to viral infections. Particularly T cells, IP-10 enables immune cell migration to sites of infection by acting as a strong chemoattractant 282.

## **Myxovirus Resistance Protein A**

MxA (Myxovirus resistance protein A) is an interferon-induced protein that plays a critical role in the body's defense against viral infections. It is specifically upregulated in response to type I and type II interferons, which are released during viral exposure. MxA functions as an antiviral protein by inhibiting the replication of various viruses, including influenza and other RNA viruses, by interacting with viral components and preventing their ability to replicate within host cells283.

Due to its strong association with viral infections, MxA has become a valuable biomarker for distinguishing viral from bacterial infections. It is a key component of the FebriDx assay, a diagnostic test that combines MxA and C-reactive protein (CRP) to rapidly differentiate between viral and bacterial respiratory infections. The FebriDx assay leverages the elevated levels of MxA during viral infections and the inflammatory response indicated by CRP to provide a quick and accurate distinction, aiding clinicians in making more informed decisions regarding treatment 284.

# **Application in distinguishing between bacterial and viral infections**

## **RNA Transcriptome Profiling**

Emerging diagnostic techniques utilizing RNA Transcriptome profiles have shown remarkable potential in differentiating bacterial and viral infections. These approaches leverage host RNA signatures that reflect the distinct transcriptional responses elicited by bacterial and viral pathogens. Notably, two-transcript RNA signatures have demonstrated approximately 94% sensitivity and specificity in identifying bacterial infections, making them a highly reliable diagnostic tool.

A prominent example is the use of blood transcriptomics in febrile children, which has consistently provided accurate identification of infection types, reducing diagnostic uncertainty in pediatric populations. Furthermore, studies employing RNA and protein biomarker profiling in adults with lower respiratory tract infections (LRTIs) have revealed significant advancements in optimizing treatment strategies and minimizing unnecessary antibiotic usage.

These RNA-based approaches not only complement traditional diagnostics but also open new avenues for integrating transcriptomics into real-time, point-of-care diagnostics. The precision and speed offered by RNA transcriptome profiling hold promise for revolutionizing infection management and advancing personalized medicine 285.

## Proteomics

Proteomics has emerged as a powerful tool in the quest to enhance diagnostic precision for bacterial and viral infections. By leveraging multiplex protein assays, proteomics enables the simultaneous analysis of multiple biomarkers, increasing the sensitivity and specificity of diagnostics. For instance, combining well-established markers such as procalcitonin (PCT) with novel proteins like lactoferrin has demonstrated significant improvements in diagnostic accuracy.

Studies have highlighted those proteomic approaches achieve approximately 87% sensitivity and 91% specificity in distinguishing bacterial from viral infections. One notable example is the application of proteomic profiling in patients with sepsis, where unique protein signatures are identified to guide targeted interventions. Additionally, the use of these techniques in lower respiratory tract infections (LRTIs) has shown promise in optimizing treatment strategies and minimizing unnecessary antibiotic use.

Advancements in mass spectrometry and bioinformatics have further enhanced the potential of proteomics. These technologies allow the identification of pathogen-specific protein signatures and host-response patterns, providing a comprehensive view of the infection landscape. As proteomics continues to evolve, its integration with other omics approaches, such as transcriptomics and metabolomics, promises to revolutionize infection diagnostics and support personalized medicine strategies 286,287.

## **Multi-Marker Approaches**

Single biomarkers, while valuable in providing insights into disease processes, often lack the specificity or sensitivity needed for a definitive diagnosis. A single biomarker may be elevated in multiple conditions, leading to potential misdiagnosis or limitations in distinguishing between different disease etiologies. Furthermore, some biomarkers may not provide sufficient sensitivity in early-stage or mild infections, resulting in false negatives. These challenges highlight the need for more comprehensive diagnostic approaches.

Multi-marker panels combine complementary biomarkers, each providing unique information about different aspects of disease pathology. By evaluating multiple biomarkers simultaneously, multi-marker panels enhance diagnostic accuracy, improving the ability to differentiate between disease states, identify the underlying cause, and even assess disease severity. This approach can be particularly useful in complex clinical situations, such as differentiating between bacterial and viral infections, where the clinical presentation may overlap. For example, the combination of biomarkers like MxA and CRP in the FebriDx assay allows for rapid and reliable differentiation between viral and bacterial respiratory infections. Such panels not only increase the precision of diagnostic testing but also enable more personalized treatment strategies, helping clinicians make well-informed decisions and reducing the unnecessary use of antibiotics or other treatments 288,289.

# **Future Directions**

The future of host biomarker-based diagnostics lies in integration and innovation. Emerging trends include:

## **Artificial Intelligence (AI) Integration**

The integration of machine learning (ML) algorithms into diagnostic frameworks is revolutionizing the way biomarker data is analyzed, particularly in the context of differentiating bacterial and viral infections. Machine learning models are increasingly being developed to process complex, multi-dimensional biomarker data, such as levels of MxA, CRP, and other inflammatory markers, enabling enhanced diagnostic accuracy and providing actionable insights that were previously unattainable through traditional methods. These algorithms can identify patterns and correlations that may not be immediately apparent to human clinicians, improving both the sensitivity and specificity of diagnostic tests.

One of the most promising applications of AI in clinical diagnostics is its potential to integrate multiple blood parameters in a way that provides a comprehensive profile of a patient's immune response. This integration allows for more precise differentiation between bacterial and viral infections, conditions that often present with similar clinical symptoms but require vastly different treatment approaches. By leveraging machine learning to analyze not just single biomarkers, but a complex array of parameters, AI models can provide real-time, data-driven predictions that guide clinical decision-making. This not only reduces diagnostic errors but also aids in minimizing unnecessary antibiotic prescriptions, a crucial step in combating the growing threat of antimicrobial resistance (AMR).

The promise of AI in this domain is vast, but there are still significant hurdles to overcome. Future research should focus on validating these AI models in diverse patient populations, taking into account variations in age, comorbidities, and geographic differences in pathogen prevalence. Additionally, further exploration of additional biomarkers, such as cytokines, interferons, and other immune modulators, could refine these models, enabling even more accurate differentiation between bacterial and viral infections. By continuously improving these AI systems, the medical community can achieve faster, more accurate diagnoses, reduce unnecessary healthcare costs, and make substantial progress in the fight against AMR. The potential for AI to transform diagnostic practices is vast, but it requires rigorous, ongoing research and validation to realize its full promise.

## Combining proteomics, transcriptomics, and metabolomics with host biomarker data

The fusion of proteomics, transcriptomics, and metabolomics with host biomarker data holds the promise of unlocking an entirely new dimension of diagnostic power, especially in the nuanced task of distinguishing between bacterial and viral infections. These "omics" technologies provide multifaceted views into the biological processes occurring at various levels within the host. Proteomics, for instance, doesn’t just identify proteins; it illuminates the complex interplay of immune responses and cellular signaling pathways activated during infection. Transcriptomics, by analyzing gene expression, takes a deep dive into the genetic signatures, revealing how cells are responding, not just to the pathogen, but to the shifting dynamics of the infection. Meanwhile, metabolomics offers a glimpse into the metabolic reprogramming that occurs as a result of the infection—changes in the metabolites can reflect critical shifts in energy production, nutrient usage, and waste management, all of which may differ markedly between bacterial and viral invaders.

Now, imagine combining these three powerhouses with traditional host biomarkers, such as cytokines and chemokines. What you get is a comprehensive map of the host’s immune response, metabolic activity, and genetic adaptation, all of which are intertwined in ways that traditional diagnostic methods cannot capture. This confluence of data offers unprecedented opportunities to identify novel biomarkers—ones that are potentially more accurate or sensitive than those currently used in routine clinical practice. For example, proteomics might reveal previously unknown viral or bacterial proteins that exhibit specific expression patterns during infection, while transcriptomics may uncover hidden signatures in gene expression that mark the host's immune response to viral or bacterial stimuli. Meanwhile, metabolomics could highlight subtle but critical shifts in the host’s metabolic pathways—alterations that could decisively point to the infection type.

By weaving these omics together, we can refine existing diagnostic assays, creating a powerful, multi-dimensional diagnostic tool that doesn’t just detect infection, but understands the finer details of the host-pathogen interaction. This promises to reduce diagnostic ambiguity, accelerate the time to accurate diagnosis, and help clinicians make better-informed treatment decisions, particularly in the context of differentiating between viral and bacterial infections. What’s more, this approach could pave the way for precision medicine, allowing for individualized treatment strategies that target specific biomarker signatures or metabolic pathways unique to the infection at hand. Shortly, combining proteomics, transcriptomics, and metabolomics with host biomarker data could become the gold standard for infectious disease diagnostics—more accurate, faster, and more personalized than anything we've seen before.

# **Advantages and Limitations**

## **Advantages**

* Host biomarker detection offers numerous benefits across various sectors, particularly in diagnostics, disease management, and personalised treatment approaches.
* Early disease detection can be improved by biomarkers that help identify conditions at their initial stages, often before symptoms appear. This is crucial for illnesses such as cancer, infections, or autoimmune disorders, where prompt intervention can significantly enhance outcomes.
* Non-invasive testing facilitates the detection of various biomarkers, which can be identified in bodily fluids like blood, urine, or saliva, making the process less invasive compared to traditional tissue biopsies or imaging techniques. This improves patient comfort and adherence.
* Personalised medicine can be advanced through biomarkers, providing insights into an individual's specific disease characteristics, thus enabling customised treatments that are more effective and have fewer side effects. This aspect is particularly important in cancer treatment, where genetic biomarkers can guide the selection of targeted therapies.
* Monitoring disease progression and treatment response involves tracking biomarker levels over time to assess the course of a disease or a patient’s response to treatment. This helps medical professionals adjust therapies as needed.
* Recognising disease subtypes is critical, as certain conditions have distinct subtypes that may respond differently to therapy. Biomarker identification can aid in pinpointing these subtypes, which further informs more accurate treatment choices.
* Additionally, disease outcome prediction is enhanced by biomarkers, as they can indicate how a patient may respond to therapy or how a disease is likely to progress. This supports decision-making and prognostication, including identifying the necessity for more intensive therapy.
* Monitoring for recurrence or relapse involves biomarkers that can detect the return of diseases, such as cancer, well before imaging or clinical signs reveal it, allowing for earlier intervention.
* Improved diagnostics in complex disease identification are offered by host biomarkers, which provide a more comprehensive insight into the disease process and help differentiate between related symptoms in intricate or multifactorial diseases (such as sepsis and autoimmune disorders).
* Cost-effectiveness is another benefit; compared to more invasive or resource-intensive diagnostic techniques like imaging, biopsies, or extended hospital stays, biomarker detection may often be less costly.

## **Limitations**

* Deficiency of Specificity: It's possible that some biomarkers aren't disease-specific. A biomarker's elevated levels can be observed in a variety of ailments, which might result in false positives and make it challenging to differentiate between various illnesses.
* Problems with Sensitivity: A lot of biomarkers might not be sensitive enough to identify diseases at low concentrations or in their early stages, which could result in false negative results. For diseases like cancer, where early detection is essential, this can be very difficult.
* Variability across Populations: Individuals' levels of biomarkers may differ according to comorbidities, age, sex, and ethnicity. It may be challenging to set uniform thresholds for disease monitoring or diagnosis because of this diversity.
* Complexity of Interpretation: Biomarker data interpretation may not always be simple and may call for highly sophisticated equipment and knowledge. Although their levels do not necessarily correspond with clinical results, several biomarkers might alter in response to treatment.
* Ethical and confidentiality: Because biomarkers frequently incorporate genetic data, there are ethical issues with consent, privacy, and possible abuse of private health data. Particularly, genetic indicators may give rise to privacy or discrimination concerns.
* Population Variability: The levels of biomarkers can differ. High Costs: Although biomarker detection can occasionally be economical, the infrastructure and technology needed for detection can be costly. In environments with limited resources, testing may not be possible due to the need for specialized tools, chemicals, and skilled workers.
* Limited Standardization: Biomarker detection techniques are frequently not standardized, which might produce variable results in various labs or medical settings. This restricts the data' repeatability and makes it more difficult for biomarker-based diagnostics to be widely used.
* Regulatory and Validation Difficulties: A large number of biomarkers are still in the research stage and have not received full validation or clinical use approval. Implementing biomarker detection in standard clinical practice can be delayed by the drawn-out and complicated regulatory approval procedure.
* False Positives and False Negatives: Biomarkers are not flawless, just like any other diagnostic instrument. False negatives (failing to identify disease when it exists) and false positives (indicating disease when none exists) can happen and may result in incorrect diagnoses, ineffective treatments, or lost chances for early intervention.

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