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Significance of inflammatory biomarkers in clinical diagnostics: Erythrocyte sedimentation rate versus other inflammatory biomarkers: A review

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Abstract

Inflammation is the immune system's response to various stimuli such as cellular injury, microbial infections, physical and chemical insults, tissue damage, and autoimmune or hypersensitivity reactions. Biomarkers derived from serum, plasma, or blood proteins provide valuable diagnostic and prognostic information regarding these inflammatory conditions. Inflammatory biomarkers, crucial for assessing the presence, severity, and progression of inflammation, include the erythrocyte sedimentation rate (ESR) which remains a key laboratory measure for disease activity, useful in diagnosing and monitoring diseases like polymyalgia, rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple myeloma, Hodgkin's disease, septic arthritis, and osteomyelitis, thus, commonly measured in clinical practice. ESR is influenced by factors including technical aspects such as room temperature, time from specimen collection, tube orientation, and use of anticoagulants; lifestyle factors like physical activity; as well as serum protein concentrations, age, and gender, prompting the development of more advanced biomarkers which offer improved sensitivity, specificity, and predictive value. Acute-phase proteins like C-reactive protein, serum amyloid A protein, and cytokines such as interleukin-6 (IL-6) and tumour necrosis factor-alpha (TNF-α), are crucial for understanding disease pathophysiology and improving patient care. Measuring these biomarkers aids in early disease identification, accurate diagnoses, and effective treatments, ultimately enhancing patient outcomes and optimising healthcare spending. While ESR remains widely used, newer biomarkers offer greater sensitivity and specificity, improving decision-making and therapeutic monitoring.

Keywords: Inflammation; ESR; Biomarkers; Cytokines; C-reactive protein

1. Introduction

Inflammation serves as the immune system's reaction to various stimuli, including cellular injury, microbial infections (caused by bacteria, viruses, or parasites), physical and chemical insults (like burns or radiation), tissue deterioration (necrosis), as well as autoimmune or hypersensitivity reactions. When encountering these stimuli, the immune system triggers a series of molecular responses aimed at containing, neutralising, and repairing the affected tissue [1-4]. This process involves a complex interplay of vascular and cellular responses, orchestrated by chemical signals derived from cells or plasma. Remarkably, even injured or deceased tissues contribute to the release of these mediators [2-4].

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According to the Biomarkers Definition Working Group [5], a biomarker is defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic responses to an intervention. More significantly, a biomarker points out a change in the state or expression of proteins, peptides, genes, and other factors that are associated with the progression or risk of disease, initial diagnosis, drug response of the patient to a given treatment, drug target identification, or disease intervention [6, 7]. Based on their application in various disease states, biomarkers are classified into five categories: prognostic biomarkers which predict disease outcomes, recurrence, therapy responses, and treatment effectiveness; antecedent biomarkers which identify the risk of developing diseases; screening biomarkers which are used to detect sub-clinical disorders; diagnostic biomarkers which recognise evident disorders; staging biomarkers which are used to assess the severity of diseases; and diagnostic biomarkers which recognise evident disorders [8].

Biomarkers of inflammation are commonly derived from serum, plasma, and blood-derived proteins or enzymes that offer valuable diagnostic and prognostic insights into underlying disease states, including the presence of cytokines and acute phase proteins [9]. Serum or plasma levels of these proteins and enzymes may either increase (positive acute phase reactants) or decrease (negative acute phase reactants) in response to inflammation [10]. These biomarkers serve as quantifiable indicators of normal or aberrant biological processes, enabling disease screening, diagnosis, activity monitoring, prognosis prediction, and treatment response assessment [5, 11].

The integration of biomarkers into clinical research and practice has revolutionised patient risk assessment [5] [11]. In the ever-evolving landscape of clinical diagnostics, the identification and monitoring of inflammatory processes stand as pivotal tasks in the management of various diseases. Central to this are inflammatory biomarkers which serve as indispensable tools for assessing the presence, severity, and progression of inflammatory conditions. Among these biomarkers, erythrocyte sedimentation rate (ESR) is one of the most commonly measured markers of inflammation or tissue injury in clinical practice [12]. Although it was first described more than a century ago [13], its clinical utility remains unaltered [14]. However, with advancements in technology, a wide array of modern inflammatory biomarkers including c-reactive proteins, serum amyloid A, fibrinogen, and cytokines, has emerged, promising enhanced sensitivity, specificity, and prognostic value.

2. Key Features of an Effective Biomarker

Regardless of the intended use of a biomarker, several crucial properties determine its clinical usefulness. A biomarker must be specific in identifying the disease state [15]. A useful biomarker directly and uniquely identifies a disease-causing agent, is easy to detect in various settings, and indicates whether a disease is currently active. Its clinical value depends on the strength and consistency of the association between the marker and the disease outcome [16, 17]. To ensure high sensitivity and specificity across diverse patient populations, this association should be confirmed through multiple studies, as reliable biomarkers provide accurate measurements across a range of patients [18]. Robustness to variations in patient demographics and sample handling before testing is an essential quality of effective biomarkers [19]. According to Menzel et al. [9], an ideal biomarker should ideally be measurable through minimally invasive procedures. Hence, body fluids are preferred mediums. Furthermore, the assay should be simple, reproducible and robust [9, 20].

For measuring disease progression, biomarkers should have relatively short half-lives and concentrations proportional to disease severity [21]. The accessibility of the assay, ease of analysis, straightforward interpretation, and reasonable cost significantly enhance the biomarker's value [22]. A biomarker is useful if it aids clinicians in managing patients and improving patients' outcomes. A transformative biomarker offers important new information that either adds to or improves upon existing tests [17]. Ideally, an effective biomarker would also identify asymptomatic and early disease states, reducing the time between disease onset and treatment; such biomarkers can decrease adverse outcomes [23] and disease transmission [24].

3. Role of Inflammatory Biomarkers in Diagnosis

In inflammatory processes, inflammatory biomarkers include cellular factors such as lymphocytes or molecular factors such as cytokines, either present in the circulation or localised to tissues [25]. Circulating inflammatory biomarkers might reflect homeostatic tissue remodelling, defence against infection, wound repair or prolonged low-level repair in response to a continuous insult [25]. Inflammatory biomarkers have the potential to allow the prediction of patient and health outcomes on the basis of physical functions and symptoms, particularly in the elderly population [25].

Biomarkers provide a powerful and dynamic tool to grasp the spectrum of inflammatory diseases with usage in observational and analytic epidemiology, clinical trials in populations, and screening with diagnosis and prognosis. Biomarkers can also reflect the entire steps of a disease from the earliest symptoms/screening to the terminal stages. Notably, individual biomarkers might reflect either a propensity to develop a disease state or the degree of underlying disease [25]. Biomarkers used to monitor inflammation must be valid: they must reflect the inflammatory process under study and they must be predictive of the future health status of patients [25].

4. Erythrocyte Sedimentation Rate (ESR): Mechanism and Measurement

Erythrocyte sedimentation rate (ESR) is an inexpensive and simple test for evaluating inflammatory or acute response. It was discovered by the Polish physician Edmund Biernacki in 1897, but his discovery remained unknown for many years. The test was rediscovered and introduced to the scientific world in 1918 by the Swedish haematologist and pathologist Robert Fahraeus who initially used the ESR as a pregnancy test [26]. The ESR is the most widely used laboratory measure of disease activity in clinical medicine and remains a useful tool for diagnosis, monitoring, and follow-up of several diseases such as polymyalgia, rheumatoid arthritis, systemic lupus erythematosus (SLE), multiple myeloma, Hodgkin's disease, septic arthritis, and osteomyelitis [27-29]. Increased ESR is used in the clinic to determine whether the disease is present, monitor the known disease's course, and evaluate response to treatment [26, 27].

The reference method for measuring the ESR, proposed by the International Committee for Standardization in Haematology (ICSH), is rooted in the findings described by Westergren a century ago [30]. Over many decades, numerous methods have evolved to perform this test. In 2001, the landscape of ESR testing saw the emergence of several new methods, some of which were automated or semi-automated. These innovations aimed to enhance existing procedures, offering advantages such as shorter testing times and reduced biohazards by aspirating samples from closed tubes [31]. These methods, which employ techniques like centrifugation or photometric rheology, measure ESR differently, focusing on either the sedimentation rate or final length [32]. Despite the introduction of automated machines for ESR analysis, the Westergren method was reaffirmed as the gold standard in 2011 by the ICSH and the Clinical and Laboratory Standards Institute (CLSI) [33]. While these alternative methods may yield varying results compared to the Westergren method and amongst themselves, they can be deemed acceptable if they undergo appropriate validation and are compared against the gold standard [32].

The ESR measures the rate (mm/hr) at which red blood cells form aggregates (or rouleaux) that sediment when anticoagulated fresh blood is left in a vertical tube at room temperature [34]. The ESR has three phases: rouleaux formation, rapid fall and slow fall. During rouleaux formation, RBCs are pulled together weakly by van der Waal forces (first 10 min). Over the next 40 min, the interaction with other plasma macromolecules stimulates greater agglutination, and RBC falls rapidly. During the last stage, the rate decreases as RBC accumulate at the bottom of the tube [35].

ESR ranges in adults from 2 to 20 mm/hour [36]. Normal values of ESR depend on age and gender [37]. It is typically higher in females than males and increases gradually with age [37]. An extreme elevation of ESR (defined as a rate ≥ 100 mm/hour) is often associated with serious diseases and calls for clinical interventions [38].

The ESR is an estimator of overall inflammation because it depends on the concentration of acute-phase proteins circulating in the blood. There is an increase in the number of proteins including immunoglobulins and acute phase proteins (prothrombin, plasminogen, fibrinogen, C-reactive protein, alpha-1 antitrypsin, haptoglobin, complement proteins) that are present in several inflammatory conditions [10]. These proteins increase the dielectric constant in the blood and neutralise the negative charges on the surface of red blood cells, which repel one another and physiologically oppose aggregation [39]. The ESR rises within 24–48 hours of the onset of inflammation and when it falls, it lags behind the resolution of inflammation [28]. Despite its limitations and the introduction of more specific inflammation markers, the ESR is still widely used for diagnosis and monitoring of a variety of conditions, particularly infections and rheumatic diseases [14]. Erythrocyte sedimentation rate sensitivity and specificity are not high but the test has the advantages of familiarity, simplicity, speed and low cost [29].

5. Factors Affecting ESR

The most apparent explanation for elevated ESR levels is the presence of inflammation. However, various health conditions and factors are effective in the reduction or increase of ESR levels [40].

- ***Technical Factors***: The ESR is affected by several technical factors including seasonal variations in room temperature, time from specimen collection, tube orientation and inclination, and vibration, and also use of

excessive anticoagulant during blood sampling [28, 41]. According to Hu et al. [42], the measurement of ESR depends on the duration and the temperature at which samples are stored. There is a decreased stability of ESR at 4°C and 23 ± 2 °C [42]. The ESR increases as the temperature increases. Direct sunlight or a higher room temperature decreases blood viscosity and may increase the ESR [41]. However, it can be falsely decreased if blood is refrigerated rather than maintained at room temperature. Samples kept in the refrigerator need to be tested within eight hours, and before testing, samples should be brought to room temperature as rapidly as possible. Otherwise, the rise in plasma viscosity at lower temperatures will cause an ESR decrease [42]. ESR stability degrades over time due to changes in the shape of the red blood cells, which become spherical [42]. Rouleaux formation is impeded by the difficulty of aggregating these spherical cells. Furthermore, there is a modification in the charge interaction between the plasma proteins and the red blood cell membrane surface [42]. The tube, rack, and table orientation are crucial since a tilted tube will increase the ESR [28, 41]. On the other hand, it can be lowered by a short tube or by vibration of the test apparatus. Using ESR tubes with inconsistent internal boreholes can lead to RBC clumping and may cause variations in the ESR results [29]. Patient medications can falsely lower or raise the ESR compared to its value in the individual or the presence of the underlying disease [41].

- ***Red Blood Cell Size, Concentration, and Shape***: Sedimentation may be impacted by the intrinsic characteristics of red blood cells including the shape, mass and rigidity of the red blood cells [41, 43, 44]. According to Taşkın et al. [45], the main determinant of ESR is haematocrit. The ESR is elevated when haematocrit is low [45], this may be because there is less slowing of rouleaux descent by cells piling at the bottom of the tube [46] or because rouleaux formation increases when red blood cells are less dense [47]. On the other hand, the rouleaux are less compact in polycythaemia, hyperviscosity, and severe leucocytosis, and the ESR may be falsely decreased [48]. Larger RBCs (macrocytes) cause higher ESR [45, 49], as they have a lower surface/volume ratio and sediment quickly. Abnormal red cell shapes and reduced deformability (such as sickle cells and spherocytes) can affect the sedimentation rate by preventing RBC aggregation and lowering the ESR [50]. Anisocytosis, poikilocytosis, and spherocytes also prevent erythrocytes from stacking, which lowers the ESR [51].

- ***Lifestyle Factors:*** Lower ESR has been reported with physical activity [37, 52]. High and moderate regular physical exercise was associated with lower ESR [37]. Previous studies have also shown that smoking increases ESR in females [53] and in selected samples of patients with arthritis [54]. Heavy drinking has pro-inflammatory effects; in fact, alcoholic liver disease is a paradigm of inflammatory disorder [55]. Hence, the ESR is increased in patients with complications of alcohol abuse and those with alcoholic hepatitis [55]. However, moderate alcohol consumption is associated with lower ESR values in selected samples of patients with inflammatory diseases, both high-grade (chronic arthritis) [54] and low-grade (coronary disease) [56]. ESR is increased with a higher body mass index (BMI) [57, 58].

- ***Serum Protein Concentrations:*** In addition to being affected by RBC characteristics, the rate of settling is affected by plasma proteins [33]. The number of immunoglobulins and acute phase proteins (prothrombin, plasminogen, fibrinogen, C-reactive protein, alpha-1 antitrypsin, haptoglobin, complement proteins) present in various inflammatory situations influences the formation of rouleaux (and consequently, the ESR) [10, 33]. Serum proteins, such as fibrinogen and globulins, are positively charged and serve to overcome the negative repulsion between RBC by neutralising the negative zeta potential charges on RBC, and hence greatly contribute to rouleaux formation resulting in high ESR. Of these, fibrinogen is the largest contributor to agglutination, followed by γ globulins, which have a lower capacity to induce agglutination [59]. Hypofibrinogenaemia of the newborn or congenital hypofibrinogenaemia leads to decreased ESR [59]. Albumin is thought to break up rouleaux and slow down red cell aggregation, resulting in a lower ESR [60]. Hypoalbuminemia on the other hand (e.g., associated with nephrotic syndrome) elevates the ESR. ESR rate is also increased by immunoglobulins. Furthermore, immunological disorders like multiple myeloma led to elevated ESR [38].

- ***Age and Gender:*** The ESR increases with age in adults because the acute phase reactants tend to increase with age [45, 61-63], and is higher in females than in males [61, 64]. Sex has an important role in defining normal values for the ESR [45, 61].

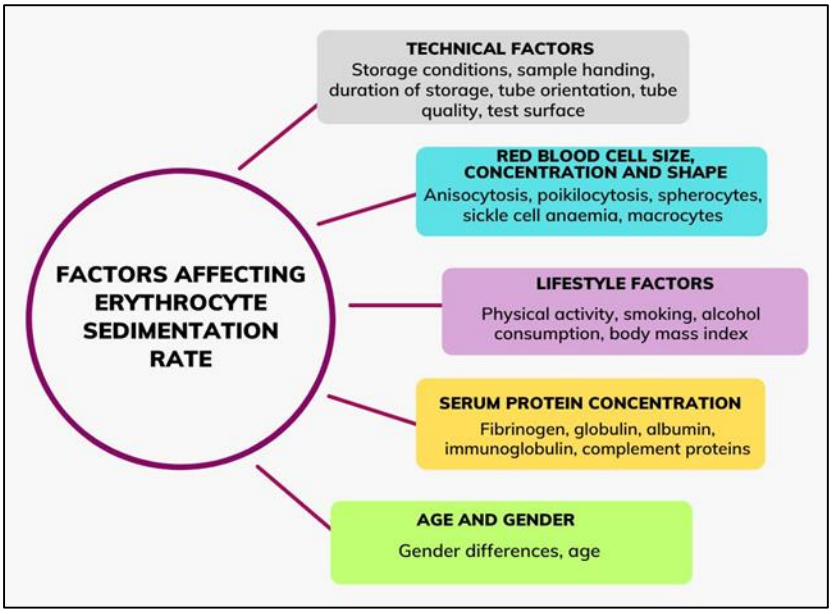

Figure 1 Factors affecting erythrocyte sedimentation rate can be either technical factors, factors due to red blood cell size, concentration and shape, lifestyle factors, serum protein concentration and factors associated with age and gender

6. Other Inflammatory Biomarkers

In recent years, there have been significant advancements in understanding and diagnosing inflammation, a key component of many chronic diseases. While the ESR remains a valuable biomarker for detecting inflammation, its accuracy and reliability can be influenced by a variety of factors. These factors have led to the development or search for other markers for an inflammatory response that can be more specific, overcoming some of these challenges. Some of these biomarkers are specific acute-phase proteins and are more commonly measured now. Markers of the acute phase reaction are C-reactive protein, serum amyloid A protein, and fibrinogen. They increase 100-fold or more in patients with acute or chronic inflammatory processes [65]. Other inflammatory markers include interleukin-6 (IL-6), and tumour necrosis factor-alpha (TNF-α) which are both cytokines [66]. These biomarkers provide important information on the pathophysiology of diseases and have the potential to enhance patient care.

6.1. C-reactive Protein (CRP)

C-reactive protein is an acute-phase protein that is produced predominantly in the liver in response to a variety of acute and chronic inflammatory conditions. It is an important component of the innate immune system [67-69]. CRP is produced mainly by hepatocytes in response to stimulation by interleukin (IL)-6 and, to a lesser extent, in response to TNF-α and IL-1b, which are produced at the site of inflammation [67, 68]. The name CRP arose because it was first identified as a substance in the serum of patients with acute inflammation that reacted with the "c" carbohydrate antigen of the capsule of pneumococcus [70]. Assay for CRP is automated on commonly used chemistry analysers. The most frequently used detection methods include turbidimetric assays, lateral flow assays, sandwich immunoassays, fluorescence assays, chemiluminescence assays, electrochemical assays, and innovative lab-on-a-chip based immunoassays [71].

CRP has both proinflammatory and anti-inflammatory properties. It plays a role in the recognition and clearance of foreign pathogens and damaged cells by binding to phosphocholine, phospholipids, histone, chromatin, and fibronectin [70]. Serum CRP concentrations are elevated in a variety of inflammatory disorders of infectious and noninfectious causes, as well as in certain malignancies [72]. CRP is considered a serum biomarker in patients undergoing acute inflammatory response [73]. The elevation in baseline CRP level was shown to be useful for gauging chronic inflammation and tissue damage resulting from excessive inflammation or failure of the initial inflammatory response [74]. Furthermore, some chronic inflammatory diseases, such as haemorrhagic stroke, Alzheimer's disease (AD), and Parkinson's disease (PD), are also associated with CRP formation [75-77]. Higher CRP concentration over time, rather than spikes in CRP, may result in cardiovascular diseases (CVDs) and problems leading to atherosclerosis [78].

CRP is an excellent biomarker of chronic inflammation and a participant in the pathological process [79]. The differentiation between the physiological and pathophysiological CRP levels may allow better management of inflammation-related diseases. As a nonspecific marker of inflammation, CRP plays a vital role in the monitoring of bacterial infection, inflammation, neurodegeneration, tissue injury, and recovery as it responds quickly to the inflammatory process [80, 81].

It is an easy and reliably measured biomarker across diagnostic laboratories and has a short plasma half-life of 19 hours [69]. Within 24–48 hours, the increase in CRP levels may be 500–1000 times higher than under basal circumstances. Inversely, with its increase, the reduction of CRP may be similarly rapid, as the acute-phase response subsides, with a fall from peak with a half-time of 48 hours [67, 68]. Once the stimulus disappears, CRP concentrations quickly decrease due to CRP's short half-life; hence, there is usually no need for further CRP measurements.

Measurements of CRP concentration and ESR are frequently conducted jointly in clinical practice and are intended to detect and monitor systemic inflammation; however, their results do not exactly correlate, and discordances can be present [82]. As compared to the ESR, which is an indirect test for inflammation, the levels of CRP rise and fall quickly with the onset and removal of the inflammatory stimulus, respectively, making it a better marker of the acute phase reaction than the ESR [82-84]. Also, CRP levels are less affected by patient age [85]. CRP is considered a better marker of disease activity in autoimmune diseases such as polymyalgia rheumatica and giant cell arteritis, despite the ESR also being elevated in most of these conditions [86]. Patients with rheumatoid arthritis show considerable variation in ESR and CRP elevations during times of increased disease activity. According to Harrison [65], a prudent approach may be to measure both initially to identify the best marker to use. Patients with raised CRP and a normal ESR usually have an infection, but some have other tissue damage (e.g., myocardial infarction or venous thromboembolism). These discrepancies may be due to timing, with the rise in CRP manifesting itself before the sedimentation rate elevates, or simply because the sedimentation rate does not change with minor inflammation [85].

However, there are two instances in which the sedimentation rate may be a more accurate indicator of an inflammatory process: first, in certain low-grade infections of the bones and joints (such as infections of joint prostheses caused by low-level pathogens like coagulase-negative staphylococci); and second, in autoimmune diseases, specifically in certain cases of systemic lupus erythematosus [65]. Despite severe tissue damage and inflammation, a patient with systemic lupus erythematosus may have a normal CRP; type 1 interferon, which prevents hepatocytes from producing C-reactive protein, maybe the cause of this. An elevated CRP test is still helpful because it can reveal concurrent bacterial infections, active serositis, and persistent synovitis [65].

To a minor extent, CRP is negatively influenced by light-to-moderate alcohol consumption [87]. Also, higher CRP concentrations are observed in smokers [88, 89]. Studies by Ahmadi-Abhar et al. [88], García-Lorda et al. [90] and Alende-Castro et al. [91] reported that sex has no significant influence on serum CRP concentrations. In contrast, the ESR is highly influenced by sex (being higher in women) and age [37]. Weight, lipid levels, and blood pressure can also alter baseline CRP levels [92].

6.2. Cytokines

The inflammatory process produces cytokines that stimulate organs, especially the liver, to produce reactants including fibrinogen and CRP, raising the ESR [93]. Under these circumstances, it is possible to regard CRP and ESR as second- and third-hand indicators of inflammation, respectively. Despite this and the fact that they are more than a century old, these tests are still widely employed [94]. However, direct measurement of serum cytokines is gaining attention as routine, first-hand markers of inflammation [91].

Cytokines are proteins, glycoproteins or signalling peptides with potent biological functions at a picomolar concentration [95]. They include interleukins, chemokines, interferons, and tumour necrosis factors, which have a wide range of pleiotropic effects in different organs [96]. They are released via several paracrine, autocrine, or endocrine pathways and have been implicated in a variety of infections and immune system-affecting disorders by both proinflammatory and anti-inflammatory mechanisms. Cytokines that have pro-inflammatory effects include interferon- (IFN-) γ, interleukin- (IL-) 17, IL-1β, and tumour necrosis factor- (TNF-) α [66], while cytokines with anti-inflammatory effects include IL-10, IL-4, and IL-1ra [66]. Distinguishing between the pro- and anti-inflammatory effects of cytokines is often challenging. This complexity arises because cytokines can exhibit both types of effects and influence the regulation of other cytokines, either upregulating or downregulating them [97]. Overproduction of cytokines can present as an organ- or tissue-specific chronic inflammatory disease that can be acute, delayed, or persistent [98].

Cytokines are disrupted in various disease states and are considered biomarkers due to their significant role in assessing physiological and pathological processes [99, 100]. Variations in cytokine levels in biological fluids such as serum, blood, stool, saliva, and sweat provide valuable diagnostic, staging, and prognostic information for various diseases. Abnormal or elevated cytokine production, such as during a cytokine storm, can lead to organ failure and death [101]. Consequently, the detection and quantification of cytokine levels have become increasingly important in clinical laboratory medicine for assessing many immunologic and inflammatory disorders, as well as infectious diseases [102-104]. This information is crucial for evaluating the immune status of the host and adjusting therapies for different inflammatory conditions [105].

There are various approaches to measuring cytokine levels [106]. The most commonly used methods for cytokine quantification are enzyme-linked immunosorbent assay (ELISA) [107] and polymerase chain reaction (PCR) [108]. While these methods are reliable, they are also time-consuming and require expensive laboratory instruments, trained personnel, lengthy sample preparation times (over 6 hours), and complex sample handling procedures. Additionally, some methods do not allow for the simultaneous measurement of multiple cytokines in real-time. Consequently, there is a significant demand for the development of sensitive, selective, and rapid real-time cytokine analysis platforms [106, 109].

Cytokine quantification in human circulation has been a significant challenge for both researchers and clinicians [110]. Accurate detection is difficult due to the trace amounts of cytokines (in the picomolar range) and their dynamic secretion processes [109]. Furthermore, cytokines exert biological effects at low pharmacological doses, and their circulating concentrations often fall below the detection limits of commercially available assay kits. Many have low to undetectable levels in naïve animals, which can create challenges for the design of species-specific assays [110, 111]. Their short half-lives and significant variability—affected by factors such as diurnal rhythms, blood handling, processing, storage, and assay methods—add to the complexity [110, 111]. They may also be undetectable if their antagonistic molecules are present in high amounts.

Evaluating the diagnostic ability of cytokines is particularly challenging due to the difficulty in establishing “normal” versus “abnormal” levels [112]. In comparison to routine serum biomarkers, i.e., the clinical chemistry panel, there is often a lack of assay standardization for cytokines [113]. Cytokine levels vary greatly among individuals and can be influenced by activating signals, specific cell targets, and physiological factors such as stress, fitness level, and feeding state [114].

Additionally, cytokine levels can differ in various physiological locations and environments, necessitating those studies comparing abnormal and normal circumstances only do so within the same type of biological fluid (e.g., serum, amniotic fluid, pleural fluid) [115]. Moreover, there is a paucity of studies investigating cytokine levels in healthy subjects and those that do often consider only a limited number of variables when examining healthy cytokine profiles [116-118]. This gap in research further complicates the ability to use cytokine levels reliably as diagnostic tools.

6.3. Serum Amyloid A (SAA)

Serum amyloid A (SAA) is a highly conserved family of acute-phase response proteins released in response to inflammation or infection, with its pathophysiology studied for over 60 years [119]. Although SAAs are primarily produced in the liver, they are also expressed in other areas such as the stomach and intestines [120-122]. The production of acute-phase SAA (A-SAA) is triggered by proinflammatory cytokines like interleukin-6 (IL-6), IL-1, tumour necrosis factor (TNF), interferon-γ, and transforming growth factor-β (TGF-β). SAA plays a role in inflammation by inducing cytokines and chemokines, demonstrating chemotactic activity, and activating the NLRP3 inflammasome [122, 123].

High levels of SAA are associated with various chronic inflammatory diseases, including atherosclerosis, rheumatoid arthritis, psoriasis, Alzheimer's disease, and Crohn's disease, and may also serve as a potential biomarker for several malignancies [124-128]. In healthy individuals, plasma SAA levels are typically very low, usually below 3 mg/L. However, the concentration of SAA increases dramatically during acute inflammation, trauma, and viral infections, reaching levels 1000 times greater than normal within 5-6 hours [122, 123, 129]. This is followed by a rapid decrease, suggesting an exceptional feedback regulation [130]. Consequently, SAA is believed to have various functions during the acute phase of the inflammatory response. Recent studies have shown that SAA participates in immune regulation, particularly in T-cell immunity [131, 132].

Serum amyloid A is not only an acute-phase protein but also an apolipoprotein involved in cholesterol metabolism. Under normal conditions, SAA circulates at low levels bound to high-density lipoprotein (HDL). However, during

inflammation, SAA can constitute up to 80% of the HDL apolipoprotein (apo) composition, surpassing apo-A1 in quantity and impairing HDL's protective functions. Additionally, SAA may increase the oxidation of low-density lipoprotein (LDL), potentially linking it to cardiovascular disease (CVD) and atherogenesis [133, 134].

SAA has been shown to correlate more closely with disease activity than other inflammatory markers in conditions such as ankylosing spondylitis and juvenile idiopathic arthritis, and it outperforms C-reactive protein (CRP) as an activity indicator in Crohn's disease and ulcerative colitis [128, 135]. In viral infections, SAA levels rise more significantly than CRP and CRP levels return to baseline more quickly than SAA levels [136]. It has also been shown to be superior to CRP and ESR in sensitivity and specificity in rheumatic disorders [137, 138]. These may suggest that the SAA test may be particularly useful in conditions where CRP shows a limited response. However, serum levels of SSA depend on a larger number of inflammatory cytokines, such as IL-1 and IL-6, than those of CRP [139, 140].

SAA is easily measurable, and various assay methods have been employed by research laboratories, including radioimmunoassay, radial immunodiffusion, and enzyme-linked immunosorbent assay (ELISA) [140]. Additionally, some assays are used in routine clinical laboratories to provide rapid results through fully or partially automated methods, such as automated latex agglutination immunoassay and kinetic photometry of anti-SAA-coated latex particles [140]. These assays vary in sensitivity, with some highly sensitive methods capable of detecting SAA levels as low as 100 ng/L. However, for SAA concentrations exceeding 10 mg/L, highly sensitive assays are unnecessary [141]. Among the available methods, ELISA is noted for its high sensitivity and ability to measure low levels of SAA. Nonetheless, no widely accepted cutoff values have been established for some commercially available immunoassays [142].

SAA is less popular primarily due to technical challenges associated with large-scale purification, stable production of high-titer antibodies, assay development, and standardization [130]. Various methods have been used for SAA quantification. Radioimmunoassay (RIA), radial immunodiffusion, and enzyme-linked immunosorbent assay (ELISA) are highly sensitive (with detection limits as low as 0.2 μg/L) but are time-consuming and inconvenient for clinical use. In contrast, immunonephelometric and immunoturbidimetric assays are rapid and fully automated but have relatively low sensitivity (with detection limits greater than 3 mg/L). Additionally, commercially available SAA kits often use polyclonal antibodies, which lack isotype specificity [130].

Hepatic function and host nutritional status can alter SSA levels [143]. Additionally, the SAA genotype may influence baseline SAA levels [144-146], highlighting the importance of an individualized approach when utilizing SAA plasma levels to assess disease activity. Notably, there are significant variations in the absolute values of SAA among different research groups. Even physiological SAA concentrations vary substantially across studies (ranging from 0.1 to 10 mg/L), potentially leading to critical errors, as in some cases, even a 100-fold increase may not be detected [130].

Table 1 Comparison between Features of Erythrocyte Sedimentation Rate (ESR), C-reactive Protein (CRP), Cytokines and Serum Amyloid A (SAA)

Features	**ESR**	**CRP**	**Cytokines**	**Serum Amyloid A**
Mechanism/ Measurement Method	Measures the rate of red blood cell sedimentation due to rouleaux formation influenced by acute-phase proteins [10].	An acute-phase protein produced by the liver in response to cytokines [68, 69]	Small proteins involved in cell signalling; are produced by various cells in response to inflammation [66]	An Acute-phase protein produced by the liver, involved in the immune response [119]
Response time	Rises within 24-48 hours of inflammation onset, slow to return to normal [28]	Rapid increase within 24-48 hours, falls quickly (short half-life of 19 hours) [67, 68]	Rapid response; varies by cytokine. [109, 110]	Rapid response within 24 hours of inflammation and rapid decline [122, 130]
Factors Influencing Levels	Age, gender, room temperature, tube orientation, sample storage, serum protein concentration, RBC characteristics,	Less affected by age and gender; influenced by smoking, alcohol consumption, and comorbidities [87, 88, 91], Weight, lipid	Highly variable; influenced by numerous factors including activating signals, specific cell targets, stress,	Baseline levels may be affected by the SAA genotype [145, 146]. Levels depend largely on several pro-inflammatory cytokines [139]

	lifestyle, and medications [29, 33, 37, 41, 42, 45]	levels, and blood pressure [92]	fitness, and diurnal rhythms [113, 114]	
Advantages	Simple, inexpensive, widely used, and familiar in clinical practice [29]	Better marker of acute inflammation, helpful in monitoring infections and chronic inflammation, reliable, and easily measured [69, 82, 84]	Direct measure of inflammation [91], detailed information on immune response, potential for targeted therapy [105]	Easily measurable, rapid response to inflammation, useful in both acute and chronic conditions [128] [122]
Limitations	Low specificity, affected by multiple non-inflammatory factors [29, 40, 41]	Nonspecific [80, 81], affected by comorbidities, not always elevated in all inflammatory conditions [65, 85]	Complex measurement, variability in levels, expensive, require specialized equipment and trained personnel, and low detection limits [106, 108-110, 113-115]	Technical challenges in large-scale purification, production of high-titer antibodies, assay development, and standardisation. Varying concentrations among different populations [130]. No widely accepted cut-off values for some commercially available immunoassays [142]
Specificity and Sensitivity	Low specificity [29, 40, 41]	Higher than ESR, especially for acute inflammation [82, 84]	High specificity for certain conditions; varies by cytokine [112]	Highly sensitive [137, 138, 141]

7. Clinical Implementation of Biomarkers

Biomarkers are allowing early identification of disease, improved diagnoses, and safer and more efficacious treatments leading to better patient outcomes and efficient and cost-effectiveness in public expenditure on health. Promising results from initial uses of biomarkers demonstrate that under the right conditions, their integration into evidence-based medicine may transform our approach to chronic disease and other serious diseases, changing the way disease is diagnosed and treated [147].

Developing a biomarker involves several iterative steps, starting with the discovery of healthy and diseased samples. The process includes phases such as pre-analytical and analytical validation, clinical validation, regulatory approval, and demonstration of clinical utility [148-150]. In the pre-analytical phase, indicators are standardised and quality factors like process, storage, and sample collection are analysed. The analytical validation phase ensures that the biomarker test is repeatable, reliable, and has appropriate specificity and sensitivity [150]. For a biomarker to succeed, it must pass through these validation stages, which depend on its intended application [151]. The final stage involves assessing the biomarker's clinical validity and utility through a clinically validated assay used in clinical trials, with various design options based on the test's intended use and specimen availability from previous trials [149]. Validation criteria are defined by the specific question the biomarker aims to address [150]. Population studies must confirm the biomarker's association with a disease state. This proof, along with other necessary information, must be shared with stakeholders to obtain regulatory approval for the biomarker-based test and its clinical application [147].

The process for the development of a biomarker assay spans from discovery to validation to implementation. These markers provide a medium for uniform classification of a disease with its risk factors and can be extended in understanding the basic underlying pathophysiology of the disease [152]. An analytical assessment of the validity of biomarkers is required to correlate the stage of the disease. Variability in the measurement of biomarkers ranges from individual error in laboratory technicians, machine dysfunction, improper storage of body fluid, and other bias and confounding issues [152].

8. Conclusion

Inflammation is the immune system's response to various harmful stimuli, and biomarkers play a crucial role in detecting and monitoring this process. As relatively novel and ideal clinical tools, biomarkers are invaluable for diagnosing, prognosing, and treating various diseases including inflammatory conditions. They are particularly effective because they can be measured through minimally invasive methods and exhibit stability and robustness under different conditions. The integration of biomarkers into clinical practice has revolutionised patient risk assessment, especially for inflammatory diseases. Among these, the erythrocyte sedimentation rate (ESR) remains a widely used marker, although advancements in technology have introduced newer biomarkers with enhanced capabilities. These modern biomarkers are expected to provide tests with greater sensitivity and specificity, improve the decision-making process, and monitor the potential effects of therapeutic interventions. While the promise of biomarkers is clear, significant challenges remain to be overcome to achieve widespread adoption of biomarkers within the practice of personalised medicine. Overcoming these challenges will be essential for fully realising the potential of biomarkers to improve clinical outcomes and advance medical practice.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declare no conflict of interest.

Author's Contribution

This work was carried out in collaboration with all authors. All authors read and approved the final manuscript.

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