

Biomarker Development in Chronic Inflammatory Diseases

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Abstract Chronic inflammatory diseases, such as inflammatory bowel disease—namely, Crohn’s disease and ulcerative colitis—psoriasis, multiple sclerosis, rheumatoid arthritis, and many others affect millions of people worldwide, causing a high burden of disease, socioeconomic impact, and healthcare cost. These diseases have common features including autoimmune pathogenesis and frequent co morbidity.

The treatment of these chronic inflammatory diseases usually requires long-term immunosuppressive therapies with undesirable side effects. The future of chronic inflammatory disease prevention, detection, and treatment will be greatly influenced by the use of more effective biomarkers with enhanced performance. Given the practical issues of collecting tissue samples in inflammatory diseases, biomarkers derived from body fluids have great potential for optimized patient management through the circumvention of the abovementioned limitations.

In this chapter, peripheral blood, urine, and cerebrospinal fluid biomarkers used in chronic inflammatory conditions are reviewed. In detail, this chapter reviews biomarkers to fore used or emerging to be used in patients with chronic inflammatory conditions. Those include inflammatory bowel diseases, chronic inflammatory

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conditions of the liver, biliary tract, pancreas, psoriasis, atopic disease, inflammatory skin diseases, rheumatic diseases, demyelination, and also the chronic inflammatory component of various other diseases in general medicine—including diabetes, cardiovascular disease, renal disease, and chronic obstructive pulmonary disease.

Development of personalized medicine is closely linked to biomarkers, which may serve as the basis for diagnosis, drug discovery, and monitoring of diseases.

Keywords Biomarkers • Chronic inflammation • Inflammatory bowel diseases • Neurology • Dermatology • Rheumatology • Diabetes • Liver • Pancreas • Medicine

Introduction

As personalized medicine is becoming an integral component of modern healthcare, the development of biomarker-based clinical tests emerges as a key challenge. In the conventional setting, chronic inflammatory diseases are assessed by clinical activity indices that measure clinical symptoms or by radiologic or endoscopic indices that measure tissue inflammation. Disease subtypes are often defined on the basis of medical history, physical findings, imaging parameters, serum markers, as well as endoscopic and histopathological characteristics of the disease. Noninvasive or minimally invasive diagnostic procedures present less burden and risk to patients than, for example, invasive biopsy sampling. They may thus support clinical decision-making by providing information on disease status and prognosis. The requirements of these applications cannot always be met by a single biomarker class.

A comprehensive biomarker approach is needed, given its ultimate significance for clinical application. This strategy could lead to an optimized management of chronic inflammatory diseases with existing drugs and may facilitate the development of novel, more effective therapies. Chronic inflammation is a component of many disorders that are discussed as per the system involved. Major disorders with chronic inflammation involve many diseases including those of the digestive tract, liver, and cardiovascular and nervous systems. Diseases such as diabetes and rheumatoid arthritis are also characterized by inflammation. In addition, rejection of allografts also involves chronic inflammatory immune mechanisms, and there is an interest in finding predictive biomarkers of organ rejection after transplantation.

This chapter provides an overview of the field of biomarkers in the peripheral blood, urine, or cerebrospinal fluid (CSF) that are currently used or can be easily used in combination with other clinical facilities to diagnose, treat, and tailor therapy in patients with chronic inflammatory diseases.

Biomarkers in Inflammatory Bowel Diseases

For patients diagnosed with inflammatory bowel diseases (IBD) including Crohn’s disease (CD) or ulcerative colitis (UC), several laboratory markers have been investigated for diagnosis and differential diagnosis of IBD as well as for assessment of disease activity and risk of complications, prediction of relapse, and monitoring the effect of therapy [1, 2].

We can differentiate the biomarkers in three major categories: serological, fecal, and other biomarkers. Each category includes other subcategories, for instance, serological biomarkers are differentiated in acute-phase reactants, cytokines, and others [Table 1].

Serological biomarkers are measurable substances in body fluids (blood), whose application is cheaper, less laborious, less invasive, and more objective compared to the endoscopy-/biopsy-based approach [3]. Serological acute-phase proteins are those whose plasma concentration increases (positive acute-phase proteins) or decreases (negative acute-phase proteins) by at least 25% during inflammatory disorders [1]. It is widely accepted that the concentrations of multiple components of the acute-phase response do not increase uniformly in all patients with the same illness, although the serological acute-phase proteins commonly increase together.

As serum markers can be elevated in a variety of conditions, fecal markers of inflammation would be more specific for IBD, in absence of enteric infection [4]. Fecal biomarkers are valuable in their specificity to the gastrointestinal tract. They include a heterogeneous group of substances that either leak from or are generated by the inflamed intestinal mucosa [1]. In the setting of mucosal inflammation in patients with IBD, inflammatory proteins (protein cytokines and markers C of neutrophil activation), leukocyte products, and leukocytes themselves leak from a permeable mucosa. Fecal microbiota appear to be attractive biomarkers in IBD, since they provide a noninvasive way to directly monitor changes in the intestinal environment associated with mucosal inflammation. However, they do not necessarily play a causative role in disease. The ability of the organism to compete in the altered intestinal environment is associated with the appearance or disappearance of a microbial group in disease [5].

Table 1 Routine biomarkers used in inflammatory bowel disease

Serological	Acute phase reactants	CRP, ESR, platelets, orosomucoid, ASCA, pANCA, ALCA, ACCA, AMCA, anti-OmpC, anti-flagellin antibodies, anti-I2	
	Cytokines	Interleukins	Others
		IL-1, IL-2, IL-6, IL-10, IL-17, IL-23	TNF-a
Fecal	Serum proteins	Calprotectin, lactoferrin	

Serological Biomarkers of Acute Phase Response

C-reactive Protein

C-reactive protein (CRP), an acute-phase protein, is produced as an acute-phase reactant predominantly in the liver, in response to a variety of acute and chronic inflammatory conditions, and is an important component of the innate immune system [1, 4, 6]. 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 [1, 4].

CRP is an easy and reliably measured biomarker across diagnostic laboratories and has a short plasma half-life of 19 h [6]. CRP production is rapidly upregulated, in the presence of an acute-phase stimulus [4]. Within 24–48 h the increase may be 500 to 1000-fold higher than under basal circumstances. Inversely, to 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 h [1]. Once the stimulus disappears, CRP concentrations quickly decrease due to CRP's short half-life. Hence, this makes CRP a valuable marker to detect the activity of IBD [4]. CRP is an objective marker of inflammation correlating well with disease activity in CD. Increased CRP levels are associated with better response rates, whereas normal CRP levels predict high placebo response rates in clinical trials [2]. The same increasing trend can be observed in UC, although CRP levels are generally lower than in CD [4]. Several studies have identified increased levels of CRP in nearly 100% of patients with CD and approximately 50% of those with UC [7].

CRP is the most sensitive biomarker compared to other biomarkers of inflammation in adult population for the detection of IBD and is also used in screening patients with gastrointestinal symptoms. The sensitivity of CRP in discriminating CD from irritable bowel syndrome ranges between 70 and 100% and the sensitivity in UC between 50 and 60% [1]. Hence, CRP is a valuable biomarker in sorting out active CD from functional bowel disorders [6]. Moreover, since CRP levels tend to be higher in CD than in UC, CRP might be used to differentiate both types of IBD [1]. However, the relatively low sensitivity of the CRP test in detection of UC prohibits the use of this marker alone to identify patients with symptoms compatible with IBD, without further evaluation [7].

CRP distinguishes between active and quiescent IBD and trends with mucosal healing [6]. Various studies have been conducted, regarding CRP levels and their correlation to different clinical courses [4]. A retrospective analysis of Mayo Clinic data has associated moderate to greater clinical disease severity, active disease on colonoscopy, and histological severe inflammation with an elevated CRP, while 51% of UC patients with active disease also had an elevated CRP. In a follow-up investigation, among patients with symptoms of active CD and an elevated CRP, 86% exhibited evidence of inflammation at colonoscopy [4, 6]. This suggests that CRP has the ability to predict active mucosal inflammation. Across studies, however, it has been showed that CRP (in both UC and CD patients), when compared to

calprotectin and lactoferrin, is less sensitive and has a lower correlation with mucosal inflammation as shown by endoscopy [6]. Studies in patients with CD, with clinically inactive disease and elevated CRP, have demonstrated that the latter had higher chance of relapse in the following 2 years than those with normal CRP. In UC patients, CRP elevation was significantly correlated with severe clinical activity and active disease by means of colonoscopy but not with histological inflammation [4]. Yet, some patients have had persistent, normal levels of CRP despite active disease [7]. These patients often exhibit exclusive ileal disease and low body mass index [4]. For these patients, CRP will not be a useful marker for differentiation of quiescent from active disease [7]. Generally, the value of CRP as a predictor of relapse is controversial, with some studies considering it an accurate predictor and others not. A combination of CRP and erythrocyte sedimentation rate (ESR) has also been used to predict relapse in patients with CD [4].

Erythrocyte Sedimentation Rate

ESR is the rate at which red blood cells (RBC) migrate through the plasma over the period of 1 h [6]. The ESR determination is a commonly performed laboratory test and reflects the changes in the various acute-phase proteins [1]. The test measures the distance that erythrocytes have fallen after 1 h in a vertical column of anticoagulated blood under the influence of gravity [1]. ESR is not rapidly responsive to changes in clinical status, as the concentrations of many serum proteins vary and some have long half-lives [4]. ESR determinations have been shown to be satisfactory monitors of acute-phase response to disease after the first 24 h [1]. Therefore, ESR is less suited to detect changes in disease activity, and it fails to exhibit the real clinical course, as it may take several days to decrease even when rapid clinical improvement occurs. Hence, ESR is an indirect, crude, but fairly rapid assessment of the general acute-phase response [1, 4, 6].

Except from erythrocytes morphology and plasma constituents, such as immunoglobulins, ESR is influenced by several factors including age, gender, anemia, blood dyscrasias, and pregnancy [4, 6]. Although it is still widely used, especially as a biomarker of IBD activity, its usefulness has decreased as new methods of evaluating disease have developed [1, 6]. In UC there is a good correlation between ESR and disease activity. In CD the ESR appears to be a less accurate measure of disease activity [4]. The increase of ESR in increasing disease activity correlates more with colonic disease and does not reflect the disease activity in the small bowel [4]. Compared with CRP, during the first 24 h of inflammation, ESR will peak less rapidly, and may take days to decrease, even if the inflammation has subsided. However, CRP tests are costly, frequently unavailable, and more time-consuming to perform than the ESR [1].

Platelets

Besides their function, which is to stop bleeding, platelets also have a recognized role in inflammatory processes [6]. Therefore, platelet count correlates with disease activity in IBD [4]. The high platelet number correlates well with disease severity and may persist even after bowel resection in IBD patients [1]. Increased platelet concentration in the blood circulation of IBD patients is not yet understood, but is associated with inflammation, as a nonspecific response [1]. Thus, their relationship to IBD pathophysiology demands further investigation [6].

Platelet count is a potential method for the distinction of IBD from infectious diarrhea [1]. In active IBD, the platelet count may be elevated, while the mean platelet volume is low [6]. Causes of this occurrence are unknown, but there might be an association with the thrombopoiesis disturbance often observed in the early stages of systemic inflammatory processes, as occurs in CD and UC [1]. According to studies, in more than 30% of IBD patients, spontaneous platelet aggregation takes place independently of disease severity [1]. While the normal level varies considerably, in practice, the platelet count is routinely available in patients and, if elevated, may alert the clinician about ongoing inflammation [6].

Serological Markers with Restricted Clinical Use in IBD

Orosomucoid

Orosomucoid or alpha-1-acid glycoprotein is an acute-phase plasma glycoprotein, which is synthesized primarily in hepatocytes [6]. The levels of circulating orosomucoid have been shown to correlate with disease activity of IBD as assessed by standard disease activity indices [4, 6]. However, the long half-life (5 days) diminishes its usefulness in practice [6]. Therefore, orosomucoid does not appear to be a useful marker for screening health populations or for distinguishing between patients with inflammatory versus functional disorders [1].

Anti-Saccharomyces cerevisiae Antibodies

The most prominent member of anti-glycan antibodies is *Anti-Saccharomyces cerevisiae antibodies* (ASCA) [8], which are formed both as IgG and IgA antibodies [9]. They are believed to interact with mannose residues on mannan in the cell walls of *S. cerevisiae*. More specifically, the major antigen targeted with ASCA antibodies is a mannan, a cell wall glycoprotein, the 200 kDa phosphopeptidomannan (PPM), of the common baker's or brewer's yeast *Saccharomyces cerevisiae* [1, 7, 8]. In particular, the Su1 strain of *S. cerevisiae* used in beer brewing and mannotetraose has been labeled as the most vital polysaccharide epitope within PPM [8].

The production of these antibodies is poorly understood [9]. Therefore, three theories have been proposed regarding the widespread distribution of oligomannosides: The first theory is that dietary yeasts or yeasts that colonize the digestive tract activate the production of ASCA antibodies. The second theory concerns the epitopes shared by other microorganisms (*Mycobacterium* species). The third one assumes that there are structural homologies between *S. cerevisiae* oligomannosides and oligomannosides expressed on human glycoconjugates as autoantigens or neo-autoantigens [8].

ASCA are widely used as biomarkers for the discrimination among patients with CD versus those with UC [7, 8]. Increased titers of ASCA were reported to identify CD with high levels of specificity (96–100%) but low sensitivity (approximately 50%), while both IgA and IgG antibodies are formed [7, 8]. Indirect immunofluorescence (IIF) and standardized enzyme-linked immunosorbent assays (ELISA) are the two main methods used for the detection of these antibodies [8]. Moreover, the titers of these antibodies do not seem to correlate with disease activity, and ASCA levels appear to be stable for long periods. In fact, patients with CD have been reported to have still high rates of ASCA, though their last outbreak of CD was 20 years ago and their markings on gastroscopy, colonoscopy, and histology were normal [9]. Interestingly, the results vary between different populations, as the sensitivity of ASCA IgA is lower in Japanese and Chinese CD patients when compared to Caucasian CD patients [1]. This observation suggests that several distinct genetic determinants and/or environmental risk factors may influence the ASCA response [1].

Yet the question still remains, do high rates of ASCA antibodies indicate a predisposition to IBD and are they genetic markers or not? Numerous studies have been performed; in one study, ASCA antibodies were detected in 20–25% of healthy first-degree relatives of patients with CD. Another study showed detectable levels of ASCA in 31% of patients long before they were diagnosed with CD. For them the maximum frequency of antibodies was recorded 36 months before diagnosis [9]. These studies indicate that ASCA growth happens before or during early stages of disease. Hence, we assume that ASCA are likely produced in the context of early development of the disease, rather than as genetic markers in childhood. Further study needs to be done in order to conclude whether ASCA antibodies serve as indicators of future disease. For now, the initial event leading to IFPE is still unclear, but it is of great interest [9].

Anti-neutrophil Cytoplasmic Antibodies

Anti-neutrophil cytoplasmic antibodies (ANCA) are a group of autoantibodies, mainly of the IgG type, against antigens in the cytoplasm of neutrophil granulocytes (the most common type of white blood cell) and monocytes. They are detected during a blood test in a number of autoimmune disorders but are classically associated with small-vessel systemic vasculitis, so called ANCA-associated vasculitides, such as Wegener granulomatosis, Churg-Strauss syndrome, microscopic polyangiitis,

and its renal-limited variant (pauci-immune necrotizing and crescentic glomerulonephritis) [6, 8, 10]. In addition, ANCA are found in other chronic inflammatory disorders, most notably in rheumatoid arthritis and in UC [1]. Serum levels of ANCA are used for the purposes of diagnosis and prognosis and in monitoring of inflammatory activity [1, 8].

In vasculitis, ANCA antibodies target different proteins, usually located in the lysosomes of monocytes and in the azurophilic granules of neutrophils. The most commonly employed method in use as a screening method for ANCA is indirect immunofluorescence (IIF) on normal peripheral blood neutrophils [8]. But for diagnostic purposes, [enzyme-linked immunosorbent assay](#) (ELISA) is used in laboratories to detect ANCA [8, 10]. According to IIF, there are two types of fluorescence pattern: cytoplasmic granular with central interlobular accentuation (cANCA) and fine homogenous, rim-like staining of the perinuclear cytoplasm (or rim-accentuated fluorescence of the nuclei) designated as the pANCA pattern. Various target antigens of atypical pANCA have been intensively studied in IBD patients, and so far results have been conflicting regarding their potential use in clinical practice as their sensitivity and specificity are less than 60% [8]. It is likely that the target antigen for UC-related atypical pANCA is a complex conformational epitope which comprises the nuclear proteins histone H1, HMG-1, and HMG-2. Nevertheless, since the target antigen for UC-associated pANCA is yet unrecognized, sensitive and specific solid-phase methods cannot be developed [8]. The sensitivity of the IIF assay for UC reaches the 87.2% [8].

Anti-glycan Antibodies

ACCA, ALCA, and AMCA (ACCA, anti-chitobioside carbohydrate IgA antibody; ALCA, anti-laminaribioside carbohydrate IgG antibody; AMCA, anti-mannobioside carbohydrate IgG antibody) are novel anti-glycan antibodies to sugars on the surface of microorganisms. Various studies have taken place that have associated ALCA and ACCA with CD [6]. Interestingly, in a study with CD patients, a rate of 17–28% ALCA and ACCA was found, while the sensitivity ranged between 34 and 44% in CD patients who were ASCA negative [6, 7]. ASMA antibodies also seemed to be positive in 24% of patients with CD who were negative for ASCA and had a lower sensitivity but higher specificity when compared with ASCA. Moreover, ACCA and ALCA antibodies have similarly exhibited low sensitivity but relatively high specificity in CD patients, compared to patients with UC [6, 7]. Other studies have shown that the combination of gASCA, pANCA, and ALCA is more accurate than each individual test separately in distinguishing individuals with IBD (UC or CD) from healthy controls [7].

Antibody to Outer Membrane Porin (Anti-OmpC)

OmpC is a major outer-membrane protein, porin C isolated from *Escherichia coli* [1, 8]. Adherent-invasive *E. coli* has been found in ileal CD lesions, and OmpC is necessary for these organisms to thrive in the gastrointestinal tract [6]. An excessive secretion of OmpC antibodies has been recently reported, with ELISA assay, mainly in CD patients (55%), while it was insignificant in UC patients and in healthy subjects (5–11% and 5%, respectively) [1, 8]. Originally, this protein was identified as a pANCA cross-reactive antigen using the library of colonic bacteria [8]. Anti-OmpC may also be of value in aiding diagnosis of ASCA negative CD patients [1].

Anti-flagellin Antibodies

Flagellin is a protein that arranges itself in a hollow cylinder to form the filament in bacterial flagellum. It is the principal substituent of bacterial flagellum and is present in large amounts on nearly all flagellated bacteria. Flagellin is a common bacterial antigen which plays a vital role in mucosal immune responses, as it is present in most motile bacteria in the gastrointestinal tract and is highly antigenic [8]. Anti-flagellin antibodies can be directed against flagellin (Anti-Cbir1), *Pseudomonas fluorescens*-associated sequence I-2 (Anti-I2), and flagellins A4-Fla2 and Fla-X [6].

Anti-Cbir1

Cbir1 has been identified as an immunodominant colitogenic antigen which was initially identified in the enteric flora of mice and has the ability to induce colitis in immunodeficient mice [6, 8]. Cbir1 is closely related to flagellin from *Butyrivibrio*, *Roseburia*, *Thermagota*, and *Clostridium* species and appears in the *Clostridium subphylum* cluster XIVa of Gram-positive bacteria [1]. Cbir1 has been measured in human sera with the ELISA assay and has shown a high anti-CBir1 IgG reactivity (50%) in CD patients while has only exhibited minor reactivity in UC patients (5–11%) or other inflammatory GIT diseases [6, 8].

Antibody to *Pseudomonas fluorescens*: Associated Sequence I2 (Anti-I2)

The sequence I2 was discovered in 2000, and this DNA was homologous to the ptxR and tetR bacterial transcription factor family, which was isolated from CD colonic lesional mucosa [6, 8]. This suggests that the microorganism expressing the I2 gene product might be related to CD pathogenesis. This sequence derives from *Pseudomonas fluorescens* [8]. Studies using ELISA assays have shown 30–50% IgA seroreactivity against I2 in CD, 10% in UC 36–42% in indeterminate colitis, 19% in patients with other inflammatory gastrointestinal diseases, and 5% in healthy

controls [1, 6, 8]. Thus, the presence of anti-I2 seems to correlate with small bowel perforating disease [1].

Antibodies to Flagellins A4-Fla2 and Fla-X

Flagellins A4-Fla2 and Fla-X are newly identified flagellins. According to data some CD patients are seropositive to them. A 2-year study of 252 patients with CD was carried out which indicated that 76% of these patients had small bowel CD and 59% had antibodies to A4-Fla2 and 57% to Fla-X. Incidentally in a cross-sectional study, antibodies to flagellin A4-Fla2 and Fla-X were found in 29% and 26% of IBS patients, respectively [6]. Anti-flagellin antibodies need further refinement before they can be used in IBD clinical practice and routine diagnostics.

Emerging Serological Markers in IBD: Cytokines

The cytokines are intercellular signaling polypeptides produced by activated cells. In patients with active IBD, the expression of proinflammatory cytokines is markedly increased in the intestinal mucosa, but this increase is not always translated in higher serum concentrations. Some cytokines used as biomarkers are various interleukins (IL-6, IL-10, IL-17, IL-23) and tumor necrosis factor (TNF) and its receptor [1, 4].

Interleukins

Interleukins are detected in blood serum, and the most common noninvasive method of detecting them is ELISA (enzyme-linked immunosorbent assay), a widespread form of biochemical analytical test which uses a subtype and a heterogeneous solid-phase enzyme immunoassay for the detection of a substance, typically an antigen, in a liquid sample [11].

Some of the emerging interleukins, their sources, and biological activity are presented below [Table 2].

TNF- α and TNF α Receptors

Tumor necrosis factor α (TNF α) is produced by activated macrophages and monocytes. Serum levels of TNF α are usually increased in patients with active IBD, though they are not consistently elevated [4]. Hence, TNF α determination is of limited utility as a marker of disease activity in IBD patients [4]. Nowadays, biological therapies targeting TNF α have significantly improved the management of IBD refractory to conventional therapies, thus, turning TNF α into a crucial mediator of

Table 2 Emerging cytokine that could be used as biomarkers in IBD

Interleukin	Sources	Biological activity
Interleukin 1 (IL-1) Interleukin 1α (IL-1α) Interleukin 1β (IL-1β)	Activated mononuclear macrophages, fibroblasts, dendritic cells, B lymphocytes, NK cells, and epithelial cells	Possess strongly proinflammatory effect. Mediates the inflammatory responses of the host. Stimulates the production of chemokines and acute-phase proteins and activates fever
Interleukin 2 (IL-2) 55–75 kDa	Activated from antigen T cells	Stimulates proliferation of T cells, participates in apoptosis of T cells
Interleukin 6 (IL-6) 26 kDa. Stimulatory factor 2 of the B cells (BSF-2), hybridoma/plasmacytoma growth factor (HPGF), hepatocyte stimulating factor (HSF)	T cells, B cells, several nonlymphocytes including macrophages, stromal cells of the bone marrow, fibroblasts, endothelial cells, and astrocytes	Sets B and T cell functions. Effect in vivo in the process of hematopoiesis. Induction of acute-phase response
Interleukin 10 (IL-10) 35–40 kDa. Inhibitor of cytokine synthesis (CSIF)	Activated subpopulations of CD4+ and CD8+ T cells	Stimulates or enhances the proliferation of B cells, mast cells and thymocytes. In collaboration with the TGF- β stimulates the synthesis and secretion of IgA by B cells of humans. Antagonizes the creation of TH1 subset of T helper cells
Interleukin 17 (IL-17) 28–31 kDa. CTLA-8 (8 antigen associated with cytotoxic lymphocytes)	Mainly CD4+ T cells	Helps hematopoiesis indirectly, stimulating cytokine production by epithelial, endothelial, and fibroblast cell layers. Enhances expression of ICAM-1, thus giving the cells more adhesive ability
Interleukin 23 (IL-23) heterodimer comprising the p40 subunit of IL-12 (35–40 kDa) and the p19 subunit (18.7 kDa)	Activated dendritic cells	An important factor inducing differentiation of TH1 subset of T helper cells. Also induces interferon production by T cells and NK cells and enhances NK activity

this abnormal immune response [11]. The most widely used anti-TNF α agents are infliximab and adalimumab. Infliximab is the best studied anti-TNF α agent and is currently approved in the European Union for adults and children with CD and adults with UC [11]. In CD and UC, infliximab has confirmed efficacy in adults with benefits observed in both clinical remission and mucosal healing. It has also shown similar effectiveness in children with CD [11]. Evidence suggests that early treatment with infliximab may improve the natural course of the disease [11]. Other

cytokines except TNFalpha have been extensively studied, but drug development based on other cytokines is still under way.

Fecal Biomarkers in IBD

Fecal Calprotectin

Calprotectin is a 36 kDa calcium- and zinc-binding protein that represents 50–60% of cytosolic proteins in granulocytes [6, 7]. It has antimicrobial effects and is stable in feces for 1 week [7]. Calprotectin is measured by enzyme-linked immunosorbent assays (ELISA), and its concentration is an indirect measure of neutrophil infiltrate in the bowel mucosa [6, 7]. It is released with cell death or activation, making it a sensitive marker of inflammation [6]. Various studies identify calprotectin as a sensitive marker of activity in CD, which also correlates well with endoscopic and histological activity in UC [4, 7]. Increased levels of fecal calprotectin had a positive predictive value of 81% and a negative-predictive value of 90% for relapse of UC. In patients with CD, the positive predictive value was 87%, and the negative-predictive value was 43% [6, 7]. Its sensitivity and specificity of relapse in both CD and UC ranges approximately at 90% and 83%, respectively. Additionally, the sensitivity and specificity for identification of IBD in adults were 93% and 96%, while, in children, the test confirmed 92% and only 76%, respectively [4, 6, 7]. Despite calprotectin's high sensitivity and specificity, it ought not to be considered as a specific marker of inflammation, since increased levels are also found in neoplasia, polyps, microscopic colitis, allergic colitis, active celiac disease, infections, nonsteroidal anti-inflammatory enteropathy, increasing age, etc. [1, 6]. Relatively high levels of calprotectin are noticed in the stools of normal individuals, and this data is compatible with the hypothesis that in normal individuals most circulating neutrophils migrate through the mucosal membrane of the gastrointestinal tract wall and thereby terminate their circulating life [1].

Fecal Lactoferrin

Another fecal biomarker of inflammation is lactoferrin, an iron-binding glycoprotein found in neutrophil granules, which possesses antimicrobial properties [4, 6]. Fecal lactoferrin is easily quantified using an ELISA specific for human lactoferrin; it is resistant to freeze-thaw cycles and degradation, facilitating its use as a laboratory test [1]. Multiple studies estimated that the lactoferrin test identified patients with IBD with a mean sensitivity of 80% and specificity of 82% [7]. Furthermore, the protein's concentration increases in active IBD, compared to inactive IBD with specificity between 85 and 90%. Fecal lactoferrin levels may rise significantly prior to a clinically evident relapse and thus may be a good marker to predict subsequent IBD flares [4].

Biomarkers in Chronic Inflammatory Diseases of Hepatology

Over the past decade, there has been a renewed enthusiasm for developing noninvasive serum markers or tests to assess the presence and severity of chronic inflammation and fibrosis in chronic liver disease. Although a single marker or test has lacked the necessary accuracy to predict fibrosis, different combinations of these markers or tests have shown encouraging results [12].

Biomarkers of Chronic Viral Infections Affecting the Liver

For hepatitis B virus, the level of HBV DNA in serum or plasma probably reflects the replicative activity of HBV. Various techniques for detection of HBV DNA have been developed, including hybridization assays and PCR. For hepatitis C virus, the introduction of the approved immunoassay EIA has reduced the incidence of HCV transmission via blood transfusion. Another test available for HCV is an immunoblot assay (RIBA-2). Both methods are of limited use, however, because a period of several weeks separates infection and seroconversion. Recently, a genetic polymorphism near the IL28B gene, encoding IFN-lambda-3, has been reported to be associated with an approximately twofold change in response to treatment [12].

Biomarkers of Nonalcoholic Fatty Liver Disease

Serum proteomics are of importance for biomarker research in nonalcoholic fatty liver disease (NAFLD) but have not been implemented in clinical practice [13]. Recently, 4-hydroxynonenal-protein adducts have been suggested as a reliable biomarker of lipid oxidation in liver diseases [14].

Biomarkers of Liver Fibrosis and Cirrhosis

The current “gold standard” for liver cirrhosis detection is an invasive, costly, often painful liver biopsy. Therefore, there is a need for biomarkers that could obviate biopsy in cirrhosis patients. Among the noninvasive alternatives to liver biopsy, several studies have demonstrated the predictive value and a better benefit-to-risk ratio than biopsy of five combinations of simple serum biochemical markers i.e., FibroMAX in patients at risk of chronic liver diseases such as patients with chronic hepatitis B or C; FibroTest for the quantitative assessment of fibrosis in patients with chronic hepatitis or drug-induced liver damage; SteatoTest for the quantitative assessment of steatosis in fatty liver disease; ActiTest for the quantitative

assessment of necroinflammatory activity in chronic viral hepatitis C and B and Nash Test for the categorical diagnosis of nonalcoholic steatohepatitis; and AshTest for the quantitative assessment of alcoholic steatohepatitis (known in the USA as HCV FibroSURE, HBV FibroSURE, ASH FibroSURE, and NASH FibroSURE) [15].

Emerging Biomarkers in Chronic Liver Diseases

Activin

Activin is a cytokine, which belongs to the transforming growth factor- β superfamily and is released rapidly into the circulation during inflammation [16, 17]. Studies suggest an involvement of activin A not only in fibrosis but also in lipid accumulation [16]. Therefore it is used as a diagnostic marker of clinical inflammation and probably plays a therapeutic role [17].

Follistatin

Follistatin is an activin-binding protein. Although elevated follistatin can be detected both in tumor tissue and in the peripheral blood, it has no benefit as surveillance biomarker for HCC development in patients with alcoholic and nonalcoholic liver disease because of its already elevated levels in the underlying liver pathologies [16].

Thymosin β 4 (T β 4)

Elevated serum concentrations) of T β 4 might be a defense mechanism counteracting ongoing inflammation and fibrogenesis [18, 19].

C-reactive Protein

CRP is an acute-phase protein produced mainly by hepatocytes. It is produced as an acute-phase reactant predominantly in the liver, in response to a variety of acute and chronic inflammatory conditions, and is an important component of the innate immune system [1, 4, 6]. The hepatic inflammatory response of CRP to injury and the production of proinflammatory cytokines are the main factors driving stellate

cell activation and fibrogenesis [20]. Serum high-sensitivity CRP (hs-CRP) is measured by immunoturbidimetry or by multiplex enzyme-linked immunosorbent assay (ELISA)-based assays [20, 21].

IL-6

Interleukin-6 (IL-6) is a proinflammatory cytokine derived from the adipose tissue. IL-6 induces secretion of CRP in the liver and, hence, may contribute to hepatocarcinogenesis [22].

TNF- α

Tumor necrosis factor-alpha (TNF- α) is a proinflammatory cytokine, and emerging data leads to the conclusion that serum TNF- α levels could be used as a sensitive predictor of liver inflammation and even hepatic malfunctions [23, 24].

Adiponectin

Adiponectin is a protein, secreted from adipose tissue. High concentrations of adiponectin were associated with higher risk of hepatocellular carcinoma [25].

All these biomarkers are not used in the routine clinical practice but are expected to be integrated in future algorithms of diagnosing and treating patients with cirrhosis.

Biomarkers of Chronic Inflammatory Conditions in Neurology

Regarding biomarkers of chronic inflammatory conditions in neurology, most studies have concentrated on the discovery, characterization, and validation of several highly promising individual biomarkers, but their impact on different disease stages has hardly been extensively investigated. One of the primary goals of future studies on biomarkers should therefore be the evaluation and validation of given markers according to their impact on diagnosis of subjects at risk, differential diagnosis at early clinical stages, and predication and description of disease course. Future research should also focus on the development and validation of cost-effective and broadly available high-throughput technologies for biomarker quantification, as this

seems the only way to address the need for highly accurate diagnosis and sufficient supervision of therapeutic strategies.

Biomarker Development in Chronic Inflammatory Demyelination

Biomarkers in Central Nervous System Chronic Inflammation

Multiple sclerosis (MS) is the most important chronic inflammatory disease of the central nervous system (CNS) with a complex pathophysiological course that includes inflammation, demyelination, axonal damage, and repairing [26]. MS is characterized by heterogenous genetic backgrounds and immunopathogenetic subtypes, which are reflected in variable clinical disease courses and unpredictable therapeutic effects. Therefore it seems more credible that a panel of different biological markers, rather than a single antibody or other biological marker, should have to be discovered to reflect the various stages of inflammation, demyelination, axonal degeneration, and remyelination [27].

Despite the progress in new technologies (such as DNA microarrays, real-time polymerase chain reaction (PCR), multicolor flow cytometry) and the advances in the knowledge of the MS pathogenesis, the body of scientific evidence obtained so far cannot support the existence of reliable biological markers. Most of the peripheral blood markers under consideration are of little reproducibility, while biological markers in the cerebrospinal fluid (CSF) are of little utility due to inability of repeated sampling [28]. Biomarkers of disease activity in MS could help in predicting the disease course and treatment response, thus providing relapse monitoring, treatment guidance, and long-term outcome improvement.

Unseparated blood is used both for flow cytometry analysis of cellular subpopulations and for PCR studies. After coagulation, serum can be used for the measurement of soluble markers, such as antibodies and cytokines, while after separation procedures of uncoagulated samples, different cellular populations can be used for functional studies [29]. Numerous cytokines (TNF α , IL-12, IL-17, IL-23, INF- γ), cell surface markers, adhesion and migration markers, antibodies, and other markers of tissue damage have been explored as serum or CSF biomarkers for disease activity, but none of them has so far the necessary validated reliability for widespread clinical use [30, 31]. This could probably be attributed to the fact that MS is not a systemic disease and therefore serum molecules might be not able to depict the immunological changes that take place in the CNS.

Autoantibodies against myelin (anti-MOG, anti-MBP, anti-PLP, anti-GAGA4) have been thoroughly studied, but none of them has so far demonstrated convincingly a MS-specific antibody response to a certain CNS target antigen. Moreover, most of the antibodies, detected in MS, are also found in other disease conditions and, to a lower extent, in healthy controls. Among the aforementioned antibodies,

myelin oligodendrocyte glycoprotein (MOG) appears to be the most promising marker. MOG was initially identified as a dominant target antigen for demyelinating antibodies in experimental autoimmune encephalomyelitis, and its role in the pathogenesis of MS still remains unresolved and controversial [32].

To date, 24S-hydroxycholesterol is the only promising biomarker related to neuronal damage in peripheral blood. 24S-hydroxycholesterol is a cholesterol metabolite specific to the brain, formed by the catalytic activity of the cytochrome P450 enzyme, and thus could serve as a marker for changes in brain cholesterol turnover caused by demyelination or neurodegeneration [33]. A moderate correlation was found between the numbers of apolipoprotein e4 (ApoE4) alleles and MS disease progression, while the concentration of serum ApoE was not found to be consistently abnormal in all groups of MS patients. Apart from lipid transport and cholesterol homeostasis, evidence suggests that ApoE is also involved in the blood–brain barrier maintenance and in oxidative stress protection [33].

Serum fluctuations of β 2 microglobulin (β 2-MG), the 12 kDa light chain of the class I major histocompatibility complex (MHC-I) on the surface of many cells, and neopterin, a low molecular mass molecule that is synthesized from guanosine triphosphate, have been found to be good indicators of treatment effect but not of disease activity in MS. During the natural course of MS, β 2-MG was found to be stable over time, and although urinary excretion of neopterin was found to be higher during clinical relapses, neopterin blood levels were not found to correlate with clinical and MRI measurements. Furthermore, both neopterin and β 2-MG are unstable and rapidly eliminated by the kidneys, and thus these molecules can be easily detected in urine specimens [34]. Urine collection is a simple and noninvasive process; however, disabled multiple sclerosis patients often have chronic urinary tract infections or asymptomatic bacterial bladder colonization—due to bladder instability—which both can negatively affect the urine biomarker results. Currently, measurable urine biomarkers include neopterin, nitrate/nitrite, prostaglandin metabolites, β 2-MG, immunoglobulin (Ig) light chains, interleukins (IL-1, IL-2, sIL-2R, IL-6, IL-8), and myelin basic protein (MBP)-like material [29].

Finally, possible biomarkers in cerebrospinal fluid (CSF) that reflect key pathological processes of MS in inflammation, immune Th1 dysfunction, demyelination, oxidative stress, remyelination, and neuroaxonal damage are being investigated [35]. Currently, the main role of CSF examination in MS is to support the diagnosis of MS and to exclude other diagnoses. The most useful markers are currently the presence of two or more IgG oligoclonal bands (OCBs), as well as an elevated IgG index. However, numerous other CSF biomarkers are under investigation, including kappa free light chains (KFLC), anti-myelin antibodies, sVCAM-1 (soluble vascular cell adhesion molecule-1), and 24S-hydroxycholesterol [36]. At present none of them, except for the OCBs, fulfills the criteria of applicability in clinical practice and should therefore be further investigated and validated in future studies [37]. As for CSF oligoclonal banding test, it is estimated to have sensitivities between 69 and 91% with specificities between 59 and 94% for the diagnosis of MS, and when combined with MRI studies, both sensitivity (56–100%) and specificity (53–96%) are enhanced [38]. Other biomarkers that are currently used in clinical practice in the diagnosis or treatment of MS are mentioned in Table 3.

Table 3 Biomarker development in chronic inflammatory central nervous system demyelination

Biomarker	Biomarker abbreviation	Origin	Technique	Prevalence	Remarks
IgG oligoclonal bands	OCBs	Serum, CSF	Isoelectric focusing combined with immunoblotting	>95% of MS patients	Can be found in patients with demyelinating disease
IgG index	–	Serum, CSF	(CSF/serum IgG)/ (CSF/serum albumin)	Increased in 70% of MS patients	Can be found in patients with demyelinating disease
Anti-aquaporin-4 antibodies	<i>anti-AQP4</i>	Serum, CSF	Various assays	Almost absent in MS patients	75–90% of patients with neuromyelitis optica
Neutralizing antibodies	Nab	Serum	Various assays	2–45% of patients treated with INFb	If present, a switch to a non-INFb treatment should be considered
Anti-natalizumab antibodies	–	Serum	ELISA	4.1–6.1% of patients treated with natalizumab	If persistently positive, treatment with natalizumab should be discontinued
Anti-JC virus antibodies	Anti-JVC ab	Serum	ELISA	50–60% of MS patients	Estimation of the patient's risk for PML if treated with natalizumab
Anti-VZV antibodies	Anti-VZV ab	Serum	ELISA	90–95% of MS patients	Seronegative patients should be vaccinated at least 1 month before start of fingolimod

CSF cerebrospinal fluid, MS multiple sclerosis, *INFb* interferon b, *ELISA* enzyme-linked immunosorbent assay, *JC* John Cunningham, *PML* progressive multifocal leukoencephalopathy, *VZV* varicella-zoster virus

Biomarkers in Peripheral Nervous System Chronic Inflammation

Chronic inflammatory demyelinating polyneuropathy (CIDP) is an autoimmune demyelinating disease of the peripheral nervous system. Due to the high clinical heterogeneity and lack of a specific confirmatory biomarker, at least 14 different sets of diagnostic criteria (with varying sensitivities) have been proposed so far. Ancillary diagnostic tests include the measurement of cerebrospinal fluid protein levels, nerve biopsy, electrodiagnostic testing, and treatment response [39, 40].

High titers of IgM antineural antibodies to myelin-associated glycoprotein (MAG), sulfatide, and gangliosides (GM1, GM2, GD1a, GD1b) are highly predictive of a chronic immune-mediated neuropathy; however, they are not specific and thus cannot be strictly associated with a definite clinical syndrome [41]. Transthyretin (TTR)—a haptoglobin isoform—was highlighted in a small pilot study of CSF proteome analysis in patients with CIDP as a promising marker that warrants further evaluation [42].

The heterogeneity in therapeutic responses to immunoglobulin (IVIg), steroids, or plasmapheresis necessitates the need for identifying biomarkers to determine the most suitable therapy and to monitor the therapeutic response in patients with CIDP [43]. Potential biomarkers that may help to guide therapy and treatment response with IVIG include the alterations in transient axonal glycoprotein-1 (TAG-1) and measurement of Fcγ RIIB density on B cells, following infusion of IVIG [40].

Biomarkers of Chronic Inflammatory Diseases of the Skin

Scleroderma

Skin involvement is of major prognostic value in systemic sclerosis (SSc) and often the primary outcome in clinical trials. Nevertheless, an objective, validated biomarker of skin fibrosis is lacking. Optical coherence tomography (OCT) is an imaging technology providing high-contrast images with 4 μm resolution, comparable with microscopy (“virtual biopsy”) [44]. It has been also suggested that levels of adiponectin, a marker for PPAR-γ activity, correlate with skin fibrosis in systemic sclerosis [45].

Dermatitis Herpetiformis

The deamidated gliadin peptides (DGP) cross-linked to human tissue transglutaminase (tTg) comprise a novel neo-epitope structure (Neo-tTg) for serological screening of celiac disease (CD). Neo-epitope tissue transglutaminase autoantibodies have been suggested as a useful biomarker of the gluten-sensitive skin disease called dermatitis herpetiformis [46].

Psoriasis

Oxidative stress was implicated in the psoriasis disease development and may damage DNA leading to keratinocytes cell death. According to an interesting study, serum 8-OHdG levels could be used as good biomarker for the early diagnosis of psoriasis and its management [47]. Other serum biomarkers for psoriasis are YKL-40 (chitinase 3-like-1), which has been suggested as a biomarker for psoriasis vulgaris and pustular psoriasis, and beta-defensin-2 protein as a serum biomarker for disease activity in psoriasis which reaches biologically relevant concentrations in lesional skin [48].

Of interest, plasma levels of transforming growth factor (TGF)-beta1, tissue inhibitors of metalloproteinases (TIMP)-1, matrix metalloproteinase (MMP)-1, and interleukin(IL)-18, when analyzed separately, demonstrate an association with psoriasis severity and treatment efficacy [49]. Finally, soluble tumor necrosis factor-alpha receptor type 1 has been suggested as a biomarker of response to phototherapy in patients with psoriasis [50].

Atopic Dermatitis

Several cytokines/chemokines, especially in breast milk, are potential biomarkers for development of atopic dermatitis in early infancy [51]. Recently, urinary eosinophil protein X has been suggested as a novel inflammatory biomarker that identifies children at risk of developing atopic disease [52].

Graft-Versus-Host Disease of the Skin

Graft-versus-host disease (GVHD), the major complication of allogeneic bone marrow transplantation, affects the skin, liver, and gastrointestinal tract. There are no plasma biomarkers specific for any acute GVHD target organ [53]. Recently serum elafin has been suggested as a valuable biomarker of graft-versus-host disease of the skin [54].

Dermatomyositis

In patients with polymyositis and dermatomyositis, KL-6, which is a mucin-like high-molecular-weight glycoprotein, has been suggested as a promising biomarker for use in clinical practice to assess clinical response to treatment [55].

Biomarkers of Chronic Autoimmune Rheumatic Diseases

Autoimmune rheumatic diseases (ARDs) comprise a wide variety of chronic inflammatory disorders in which innate and adaptive immune responses lead to autoimmune-mediated tissue damage. While the etiology of ARDs remains unclear, multiple genetic, epigenetic, hormonal, and environmental influences appear to play a role in disease pathogenesis. The spectrum of their clinical manifestations is characterized by great diversity in terms of disease severity and the extent of organ involvement. Most ARDs run a relapsing-remitting course, yet the pattern of continuously active disease is described in a considerable proportion of patients diagnosed with these chronic inflammatory illnesses. Overall, ARDs affect approximately 5% of the population and result in substantial morbidity and increased mortality. Furthermore, these diseases place a significant burden on public health with high financial costs [56, 57].

The identification of biomarkers that measure the underlying biologic processes in ARDs reliably and reproducibly has been recognized as a growing need in rheumatology, but given the complex pathogenesis of these disorders, heterogeneous clinical manifestations, and varying rates of disease progression among patients, it is reasonable to assume that a particular biomarker may reflect only one specific aspect, rather than all aspects of the disease course at any given time. Therefore, some biomarkers provide prognostic information regarding severity of the disease, whereas others predict response to therapy optimizing risk/benefit assessment in individual patients. Likewise, biomarkers might predict or quantify the risk of ARDs in individuals or populations, and others could establish or confirm the diagnosis of a specific ARD.

This section will only focus on biomarkers detected through blood tests that have established clinical utility in major ARDs.

Rheumatoid Arthritis

Rheumatoid arthritis (RA) is an ARD that primarily affects the joints and is associated with progressive functional disability, systemic complications, high socioeconomic costs, and a guarded prognosis. While the exact prevalence across the entire population is unknown, available data suggest that it affects about 1% of the population, making it one of the most common ARDs [58]. RA involves a complex interaction among genotype and environmental triggers, leading to a breakdown of immune tolerance with autoantibody production and synovial inflammation in a characteristic symmetric pattern. Distinct mechanisms regulate inflammation and matrix destruction, including damage to bone and cartilage [59].

The diagnosis of RA remains clinical and is based on several criteria, including physical symptoms, joint radiographs, and serological tests [60]. Recently, emerging data suggest that a preclinical period precedes the onset of clinically apparent

RA, which is characterized by the presence of abnormalities in disease-related biomarkers. During this period many genetic and environmental risk factors are likely to act to initiate or propagate autoimmunity [61].

The main clinically useful biomarkers in patients with RA are rheumatoid factors (RF) and antibodies to citrullinated peptides (ACPA) for diagnosis and prediction of functional and radiographic outcomes. These autoantibodies, when appearing during the preclinical period, may predict the disease onset [61]. Other useful laboratory biomarkers are erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP).

Genetic Biomarkers in RA

Susceptibility to RA is clearly defined by a pattern of inherited genes, with the human leukocyte antigen (*HLA*)-DRB1 locus being the most important biomarker, particularly in patients who are RF or ACPA positive. However, other risk alleles that consistently aggregate functionally with immune regulation, including cytokine promoters and genes involved in T-cell stimulation, activation, and functional differentiation, contribute to susceptibility and disease severity [59].

Rheumatoid Factors

RF is an antibody directed against the constant (Fc) portion of IgG. The key pathogenic markers in RA are IgM and IgA RFs. They are found in 75–80% of RA patients (Table 4) at some time during the disease course. High titer IgM RF is relatively specific for the diagnosis of RA in the context of a chronic symmetric polyarthritis and was, for decades, the only serologic criterion widely used in the diagnosis of RA. Nevertheless, RFs may also be present in other ARDs and chronic infections (Table 4). RFs in RA patients are distinguished from RFs in healthy individuals in that they exhibit affinity maturation [62]. RF has little predictive value in the general population, because the disease prevalence is relatively low.

RF may have some prognostic value with regard to disease manifestations and activity, as well as the severity of joint erosions. Seropositive RA (i.e., disease associated with a positive RF test) is often correlated with more aggressive joint disease and is more commonly complicated by extra-articular manifestations than seronegative RA [62]. Rheumatoid nodules and vasculitis occur almost exclusively in seropositive patients. A positive RF test at the initial evaluation is associated with more destructive joint pathology. It has also been proposed that the rate of formation of new erosions among seropositive patients correlates with the RF titer [62]. The presence of RF increases the likelihood of a clinically significant response to rituximab which is a monoclonal antibody against the CD20 antigen found on the surface of B lymphocytes after failure of tumor necrosis factor (TNF)-alpha inhibitor therapy. Finally, the presence of RF, particularly at high titers, may antedate the clinical development of RA [61, 63].

Table 4 Biomarkers in major autoimmune rheumatic diseases

Biomarker	Biomarker abbreviation	Origin	Technique	Sensitivity/specificity	Remarks
Rheumatoid factor	RF	Serum	Agglutination of IgG-sensitized sheep red cells or bentonite or latex particles coated with human IgG; radioimmunoassay; enzyme-linked immunosorbent assay; nephelometry	75–80%/85% (for rheumatoid arthritis)	Can be found positive before rheumatoid arthritis diagnosis
Anti-citrullinated peptide antibodies	ACPA	Serum	Enzyme-linked immunosorbent assay (ELISA)	50–75%/95–97% (for rheumatoid arthritis)	Can be found positive before rheumatoid arthritis diagnosis
Antinuclear antibody	ANA	Serum	Indirect immunofluorescence assay; enzyme-linked immunosorbent assay (ELISA)	98% (for systemic lupus erythematosus)/may be positive in other rheumatic disease, organ-specific autoimmune diseases, chronic infections, and lymphoproliferative diseases	Can be found positive before systemic lupus erythematosus diagnosis
Anti-double-stranded DNA antibody	Anti-dsDNA	Serum	Farr radioimmunoassay; <i>Critithidia</i> immunofluorescence assay; enzyme-linked immunosorbent assay (ELISA)	70%/95% (for systemic lupus erythematosus)	Can be found positive before systemic lupus erythematosus diagnosis
Anti-Smith antibody	Anti-Sm	Serum	Immunodiffusion assay; enzyme-linked immunosorbent assay (ELISA)	20–30%/99% (for systemic lupus erythematosus)	Can be found positive before systemic lupus erythematosus diagnosis

(continued)

Table 4 (continued)

Biomarker	Biomarker abbreviation	Origin	Technique	Sensitivity/specificity	Remarks
Anti-Ro/SSA	Anti-Ro/SSA	Serum	Enzyme-linked immunosorbent assay (ELISA); immunoblot assay; immunodiffusion assay	30% (for systemic lupus erythematosus)/may be positive in Sjogren's syndrome	Can be found positive before systemic lupus erythematosus diagnosis
Anti-La/SSB	Anti-La/SSB	Serum	Enzyme-linked immunosorbent assay (ELISA); immunoblot assay; immunodiffusion assay	15–20% (for systemic lupus erythematosus)/may be positive in Sjogren's syndrome	Can be found positive before systemic lupus erythematosus diagnosis
Anti-ribonucleoprotein antibody	Anti-RNP	Serum	Immunodiffusion assay; enzyme-linked immunosorbent assay (ELISA)	30% (for systemic lupus erythematosus)/may be positive in mixed connective tissue disease and scleroderma	Can be found positive before systemic lupus erythematosus diagnosis
Anti-neutrophil cytoplasmic antibodies	C-ANCA	Serum	Immunofluorescence assay; enzyme-linked immunosorbent assay (ELISA) for proteinase-3 (PR3)	60–90% (for Wegener granulomatosis)/both a C-ANCA pattern on immunofluorescence and PR3-ANCA positive by ELISA are highly specific for Wegener granulomatosis	C-ANCA gives positive immunofluorescence result with cytoplasmic staining pattern
Anti-neutrophil cytoplasmic antibodies	P-ANCA	Serum	Immunofluorescence assay; enzyme-linked immunosorbent assay (ELISA) for myeloperoxidase (MPO)	70% (for microscopic polyangiitis)/both a P-ANCA pattern on immunofluorescence and MPO-ANCA positive by ELISA are highly specific for ANCA-associated vasculitis mostly microscopic polyangiitis	P-ANCA gives positive immunofluorescence result with perinuclear staining pattern

Anti-citrullinated Peptide Antibodies

ACPA are directed against citrullinated protein epitopes and are detected by use of ELISA for antibodies against synthetic cyclic citrullinated peptides. The sensitivity of ACPA assays for RA varies from about 50 to 75%, depending upon the assay and study population, while specificity of ACPA for RA is relatively high, usually >90% (Table 4). ACPA-positive patients with early RA are at increased risk of progressive joint damage, while ACPA testing may predict erosive disease more effectively than RF. The presence of ACPA is also predictive of more rapid radiographic progression [62]. Positive ACPA testing also appears to predict an increased risk for radiographic progression in patients with early oligo- or polyarthritis who are IgM RF negative. A decrease in ACPA titers can be seen in patients treated effectively, particularly if treated early with nonbiologic or biologic disease-modifying antirheumatic drugs (DMARDs), but is less frequent and of a lesser magnitude than the decrease in IgM RF. ACPA may be detectable months or even years before the development of RA, and the proportion of individuals who are seropositive for ACPA increases progressively until clinical onset of disease [61, 63]. Finally, this biomarker is useful in the differential diagnosis of early polyarthritis, because of the relatively high specificity for RA of these autoantibodies. The presence of ACPA is highly predictive of future development of RA with positive predictive values of >90% in most studies [61, 63].

Erythrocyte Sedimentation Rate

The rate at which erythrocytes fall through plasma, the ESR, depends largely upon the plasma concentration of fibrinogen and can be influenced by the size, shape, and number of red cells, as well as by other plasma constituents. Thus, results may be imprecise and sometimes misleading. Despite these shortcomings, an elevated ESR in patients with early RA is predictive of greater radiographic joint damage in subsequent years despite treatment with conventional DMARDs. ESR values tend to correlate with disease activity in RA as well as disease severity and may be useful for monitoring therapeutic response.

C-reactive Protein

CRP has been recommended as an objective measure of disease activity in RA. Unlike the ESR, CRP can be measured using stored serum samples; it is independent of the hemoglobin concentration and can be performed in automated serum analyzers. Radiographic damage is significantly more likely to progress when CRP and ESR are elevated, irrespective of the presence or absence of RF and irrespective of therapeutic intervention. Elevations of both ESR and CRP are stronger indications of radiologic progression than CRP alone. However, a wide variation in the relationship between the degree of radiographic change and cumulative CRP was noted in several studies between patients, particularly those with low CRP levels.

Other Investigational Biomarkers in RA

Cytokines are small proteins that regulate the immune system and participate in intercellular communications. There is more information on the role of cytokines in RA than in any other ARD which has led to clinical trials with several novel agents designed to interrupt the cytokine network of this disease. Anti-cytokine therapy has shown clear evidence of clinical efficacy, especially with TNF-alpha-directed therapeutic approaches. Overexpression of certain cytokines, such as IL-2, IL-6, IL-8, IL-17, IL-21, TNF- α and granulocyte-macrophage colony-stimulating factor have been reported in RA. However, discrepancy in the results of several studies, differential cytokine levels in sera and synovial fluid, analytical variability among immunoassays, and certain limitations of cytokine measurements [64] preclude their use as reliable biomarkers in everyday clinical practice.

Systemic Lupus Erythematosus

Systemic lupus erythematosus (SLE) is the most heterogeneous ARD with more than 100 autoantibodies found in patients and a disease spectrum ranging from mild symptoms to life-threatening multi-organ manifestations. The hallmark characteristics of SLE, including production of autoantibodies, deposition of immune complexes in tissues, and excessive complement activation, are generally thought to be consequences of immune dysregulation. Owing to its complex etiology and pathogenesis, diverse clinical presentation, and unpredictable course, SLE remains one of the greatest challenges in the field of rheumatology [65].

Genetic Biomarkers in SLE

The genome-wide association study (GWAS) approach has accelerated the discovery of genetic variations contributing to SLE. Over 50 loci associated with SLE susceptibility have been identified, and most encode gene products participating in the key pathways relevant to SLE pathogenesis, including those encoding the early components of the complement system, cytokines, chemokines, loci that mediate signaling transduction in B and T cells, as well as variants implicated in immune complex clearance. Yet, dysregulation of type I interferon (IFN) appears as a central driver of SLE pathogenesis [66, 67]. Variants of *FCGR2A* also represent significant risk factors for the disease, whereas the *FCG3A-V/F158* polymorphism has a substantial impact on the development of lupus nephritis [68, 69].

Biomarkers for Preclinical Disease Stage and for SLE Diagnosis

Individuals who develop SLE appear to have an initial preclinical stage of “benign autoimmunity” which is characterized by antinuclear antibody (ANA) positivity and detection of several autoantibodies (anti-Ro/SSA, anti-La/SSB, anti-phospholipid). This phase develops into a more aggressive stage of “pathogenic autoimmunity” characterized by the presence of antibodies targeting double-stranded DNA (dsDNA), the Smith (Sm) antigen, and ribonucleoproteins (RNP) that in turn rapidly evolves into clinically apparent disease and tissue inflammation [63]. While certain biomarkers such as anti-dsDNA and anti-Sm are elevated relatively shortly before SLE onset, the relationship with preclinical autoimmunity remains uncertain for ANAs which have been shown to be present at titers of $>1/40$ in up to 27% of subjects, most of whom will never develop SLE or other ARD [63].

Although the American College of Rheumatology (ACR) developed classification criteria for SLE (published in 1982 and revised in 1997), these criteria are often cited to support a lupus diagnosis; yet this approach is problematic in clinical practice. Traditionally, determination of autoantibodies (Table 4) such as ANA, anti-extractable nuclear antigen antibodies (anti-Ro/SSA, anti-La/SSB, anti-RNP, and anti-Sm), and anti-dsDNA is used in diagnosing and monitoring SLE. ANAs are present in virtually all SLE patients, whereas anti-dsDNA and anti-Sm are highly specific for the disease (Table 4). Nevertheless, there are considerable drawbacks to the use of these immunologic markers [65]. Recently, cell-bound complement activation products have been proposed as more sensitive biomarkers for SLE diagnosis compared to other autoantibodies [67], but larger studies are needed to provide a more definite confirmation of their validity.

Biomarkers for SLE Disease Activity

Currently, disease activity in SLE is frequently assessed using composite indices which include a variety of clinical and laboratory parameters. Laboratory measures of complement and autoantibodies are components of most disease indices. Several studies have been conducted to identify the associations of various autoantibodies, particularly anti-dsDNA and complement proteins (including C3 and C4 as well as activation products) with SLE disease activity. The results, however, are inconsistent, and such uncertainty may also confound the assessment of disease activity with the widely used disease indices. Therefore, the value of conventional tests measuring serum complement and autoantibodies, as biomarkers for SLE disease activity, is being revisited. Several candidate biomarkers have emerged recently; alteration of the B cell subpopulation (namely, the increase in CD27^{high} plasma cells), cell-bound complement activation products, IFN-inducible genes expression and/or serum levels of IFN-inducible chemokines, serum levels of B lymphocyte stimulator, cytokines (IL-6, IL-10, IL-16, and IL-18), and anti-nucleosome antibodies may be valuable biomarkers for monitoring disease activity [67].

Lupus Biomarkers for Specific Organ Involvement

SLE can affect practically any tissue and organ. Nevertheless, not all organs are affected simultaneously, and involvement of a specific organ will not necessarily be manifested in the same manner in all patients. Lupus patient care would benefit immensely from biomarkers that could determine and/or predict organ-specific disease.

Among the numerous manifestations of SLE, renal involvement is one of the most common, and it continues to cause significant morbidity and mortality. Laboratory biomarkers such as creatinine clearance, proteinuria, urine sediment, serum C3 and C4, and anti-dsDNA have for decades been used to follow the onset, course, and severity of lupus nephritis [65], yet, it is generally recognized that these measurements are inadequate. Current efforts are focused on identification of more sensitive and specific biomarkers to diagnose and monitor renal disease in SLE. Anti-nucleosome antibodies may be more sensitive and offer better diagnostic performance than anti-dsDNA for active disease, especially nephritis, in SLE patients. Other recent studies demonstrate a strong correlation between the presence of anti-C1q antibodies and lupus nephritis and suggest that anti-C1q determination may serve as a biomarker to monitor renal involvement and/or predict renal flares. Other promising biomarkers include the serum neutrophil gelatinase-associated lipocalin which might predict renal relapses, yet longitudinal studies of adult lupus are needed [67].

Many patients with SLE experience a wide range of neuropsychiatric (NP) manifestations that result predominantly from immune-mediated damage of the central nervous system (CNS). While NPSLE is a common manifestation, its diagnosis is extremely difficult due to lack of reliable biomarkers. Because autoantibodies are clearly involved in tissue damage in other organs, autoantibodies reactive to CNS antigens naturally become the focus of the investigation of NPSLE pathogenesis and the pursuit for reliable biomarkers. Stroke in patients with SLE is likely to reflect thrombosis due to antiphospholipid antibodies. In addition, recent studies provide convincing evidence that a subset of anti-dsDNA antibodies cross-react with a pentapeptide consensus sequence that is present in the extracellular domain of the *N*-methyl-D-aspartate (NMDA) receptor. NMDA receptors bind the neurotransmitter glutamate expressed by neurons throughout the forebrain and particularly at the hippocampus. If such cross-reacting autoantibodies enter the brain upon transient breach of the blood–brain barrier, they may bind to antigens expressed in different regions of the brain and induce non-inflammatory neuronal injury resulting in various neurologic and psychological changes [67]. Antibodies to ribosomal P proteins (anti-P) were initially considered extremely promising biomarkers for the diagnosis of lupus psychosis and of mood disorders. Yet, it was demonstrated that anti-P antibody testing has negligible diagnostic utility for NPSLE overall or for these particular manifestations [70]. Testing for anti-P antibody is not useful in excluding disease-mediated psychosis or mood disorder with enough certainty, since more than 60% of cases are false negative. Also, a false-positive rate of ~20% militates against the dependence on this laboratory test for diagnosing psychiatric disorders in lupus patients [70].

Vasculitis

Vasculitis comprises a heterogeneous group of ARDs that is characterized by inflammatory destruction of blood vessels which become liable to occlude or rupture. Depending on the size, distribution, and severity of the affected vessels, vasculitis can result in clinical syndromes that vary in severity from a minor self-limited rash to a life-threatening multisystem disorder.

The most important primary systemic vasculitis syndromes are granulomatosis with polyangiitis (GPA, formerly known as Wegener's granulomatosis) and microscopic polyangiitis (MPA) which are associated with circulating autoantibodies to neutrophil cytoplasmic antigens (ANCA). It has been suggested that they be grouped together as ANCA-associated vasculitis because of their histologic similarities, the absence of immune deposits in involved tissues, the potential contribution of ANCA to their pathogenesis, and their similar responses to immunosuppressive therapy. Renal involvement occurs in 70% of affected patients and is manifested as rapidly progressive glomerulonephritis; it results in either death or end-stage renal failure within 2 years in more than 40% of patients. MPA and GPA were once considered life-threatening ARDs, but immunosuppressive therapy has substantially improved the survival of affected patients [71, 72].

Biomarkers for GPA and MPA

The most useful biomarkers for diagnosis of ANCA-associated vasculitis include antibodies to proteinase-3 (PR3) or myeloperoxidase (MPO) which are highly specific for GPA and MPA, respectively (Table 4). Inflammatory markers such as ESR and CRP exhibit only modest sensitivity for active early vasculitis in untreated patients [71, 72].

All of these biomarkers are problematic for use in assessing disease activity in patients with established diagnoses. Many relapses of GPA or MPA are accompanied by increases in anti-PR3 or anti-MPO titers; yet these markers do not appear to have the same predictive value that they do before treatment, making their use controversial.

Laboratory biomarkers such as creatinine clearance, proteinuria, and urine sediment are thought to possess very high sensitivity for active glomerulonephritis, that is, a normal urinalysis is regarded as ruling out glomerulonephritis. Similarly, the presence of red blood cell casts is regarded as having high specificity, albeit low sensitivity for glomerulonephritis. Nevertheless, once renal damage has occurred due to glomerulonephritis, proteinuria, hematuria, and even red blood cell, casts may persist without evidence of progressive kidney disease.

Among many markers of inflammation, angiogenesis, tissue damage, and repair that were measured in GPA and MPA patients, the most promising include MMP-3, tissue inhibitor of metalloproteinase-1, and CXCL13 (B-cell attracting chemokine 1) [71, 72].

Novel Technologies for Biomarker Development

Metabolomics is a novel and powerful technique for studying biological systems that allows for the analysis of the specific response of organisms to environmental stimuli. This new approach belongs, along with genomics, proteomics, and transcriptomics, to the family of “-omics” sciences. It is based on non-hypothesis-driven scanning platforms for identifying biomarkers and profiling the patients. Thus, the fundamental rationale in metabolomics is that perturbations caused by a disease in a biological system will lead to correlated changes in concentrations of certain metabolites. This procedure has become feasible only recently with the advent of new high-throughput technologies, including mass spectrometry and nuclear magnetic resonance. Comparison of patients with active RA and those in remission provided different baseline metabolic profiles, suggesting that efficacious treatment may affect biological changes for improved metabolic profiles. In SLE, the disease influences the metabolite profile and particularly energy, amino acid, lipid, and purine metabolism [73].

Proteomic approaches include gel-based methods such as two-dimensional difference gel electrophoresis (2D DIGE) and modern mass spectrometric techniques to identify encoded proteins that may better reflect cell function and disease. Most profiling studies in ARDs have been conducted in RA. Using high-throughput mass spectrometric techniques, these studies have uncovered about 33 different proteins that are differentially expressed in RA, of which 3 (serum amyloid A, superoxide dismutase, and triose phosphate isomerase) are of particular interest as their elevations in plasma have been reported in multiple studies. Proteomics can also yield insights into the molecular pathways impacted by therapy. A recent example is the identification of the nuclear factor-kappa B pathway as being differentially expressed in RA patients treated with anti-TNF-alpha agents [74].

Transcriptomic profiling using DNA microarrays has been applied to the study of several ARDs. The analysis of antibody repertoires with antigen microarrays found that those RA patients who had antibodies to citrullinated peptides in which peptidylarginine has been converted to peptidylcitrulline were more prone to develop severe disease. In another SLE study, the investigators developed antigen microarrays including several SLE-related antigens, and they found clusters of antibody reactivity associated to glomerulonephritis and overall disease activity [75–77].

Biomarker discovery and development is a rapidly growing field in rheumatology, which is nevertheless fraught with several limitations. Important amendments to the translational research enterprise are necessary to address these challenges. Hence, imperative alterations should include rigorous design for studies of biomarkers that would allow valid interpretation of their clinical utility. In particular, for studies of biomarkers for diagnosis, greater attention to potential confounders would help ensure accuracy. When examining biomarkers of disease activity, emphasis should be placed on evaluating these markers longitudinally. In research regarding biomarkers of prognosis, longitudinal design and the use of validated outcome measures would provide reliable results in order to enable use in clinical

decision-making process of novel biomarkers that outperforms currently available tests. Finally, the development of novel high-throughput assays might hold tremendous potential for shaping how ARDs are diagnosed, prognosticated, and managed clinically over the coming years.

Future Trends

Personalized medicine is the best way to integrate new biotechnologies into medicine for improving the understanding of pathogenesis of diseases and management of patients. Development of personalized medicine is closely linked to biomarkers, which may serve as the basis for diagnosis, drug discovery, and monitoring of diseases.

Biomarker discovery is an ongoing process, with translation being tested *de novo* in every single study, providing us with the opportunity to revise our knowledge of the complex scheme of human physiology and pathophysiology.

An ideal biomarker must be specifically associated with a particular disease or disease state and be able to differentiate between similar physiological conditions. A rapid, simple, accurate, and inexpensive detection of the relevant marker should be available, together with a measurable and standard baseline as a reference point.

In the active search for new biomarkers, many potential candidates can be considered side by side, allowing many failures but a few winners. The traditional identification of biomarkers as an observational side product of clinical practice is increasingly turning into an industrialized process of biomarker discovery, supported by standardized paradigms of biomarker validation and translation from bench to bedside.

In recent years, significant advances in genomics increasingly impact disease detection, prognosis, prediction, and efficient patient stratification. Moreover, genomic biomarkers greatly influence the development of personalized medicine by providing treatments adapted to the genetic characteristics of the individual patient's disease.

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