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Use of Biomarkers in the Management of Children with Lupus

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Abstract Childhood systemic lupus erythematosus (SLE) is known to have a worse prognosis than adult-onset disease, and monitoring and treatment of the disease are still a challenge. Thus, there is an urgent need for highly reliable, non-invasive biomarkers for early detection of relapses, to avoid long-term complications and to optimize the management of children with LN. Recent studies of pediatric patients have yielded novel specific biomarkers for SLE diagnosis which can be used for monitoring disease activity and response to treatment. The most promising biomarkers in juvenile-onset SLE include cell-bound complement activation products, some genomic profiles, and urinary proteins such as neutrophil gelatinase-associated lipocalin, monocyte chemoattractant protein-1, and alpha-1-acid glycoprotein. None of these might be suitable for use as a single SLE-biomarker. More likely a combination of novel biomarkers with traditionally used data, including autoantibodies and complement, might help to enhance sensitivity and specificity for early diagnosis, disease monitoring, and prediction of relapses.cp

Keywords Juvenile-onset systemic lupus erythematosus · Biomarker · Autoantibodies · Complement · Cell-bound complement activation products · SLE-nephritis · Urinary biomarkers · Neutrophil gelatinase-associated lipocalin · Monocyte chemoattractant protein-1 · α-1-Acid glycoprotein

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Introduction

Juvenile-onset systemic lupus erythematosus (JSLE) is a rare, severe, multi-systemic, chronic but often episodic, autoimmune disease, characterized by autoantibodies directed against nuclear antigens [1] and immune complex deposition in organs [2]. Although the etiology of SLE is not entirely understood, numerous lines of evidence support the conclusion that genetic, hormonal, and environmental factors are clearly involved [3]. Several immunological abnormalities, including dysregulation of B and T lymphocytes and aberrant interaction among immune cells are believed to be involved in pathogenesis. Clinical manifestations of SLE are likely to be the consequence of a multifaceted, patient-unique immune-inflammatory process which evolved over the course of the disease [4]. JSLE has an estimated annual incidence of 0.36–0.9 per 100,000 children. For approximately 20 %, SLE is diagnosed in childhood [5, 6]. Compared with adult onset, childhood disease is associated with more serious organ involvement and a more aggressive disease course requiring the use of more immunosuppressive therapy. JSLE has a 2.5-fold higher mortality risk than adult-onset disease, with 10-year survival now approaching 90 % and a significantly lower life expectancy than the general population [7]. Currently, diagnosis of SLE is based on clinical and laboratory findings. The American College of Rheumatology (ACR) has formulated SLE classification criteria for adult and pediatric cases [6]. There is no laboratory test for reliable early identification or for prediction of relapses or remission [8]. Disease activity in SLE can be assessed by use of disease activity indices, for example the SLE activity index (SLEDAI), systemic lupus activity measure (SLAM), and the British Isles Lupus Assessment Group (BILAG) [9]. Early diagnosis of lupus, especially reactivation or remission, can be difficult because of the complex etiopathogenesis, heterogeneous presentation, and unpredictable course. In addition, initial

symptoms can include common signs or symptoms of non-lupus origin. Early detection and treatment, however, significantly improves the prognosis [10] and may spare patients from severe side effects. Thus, there is much interest in the identification of biomarkers that can quantify the susceptibility of SLE, the risk of organ involvement, and the association of their changes with disease activity [11].

Biomarkers

A biomarker can be defined as a measurement, including—but not limited to—a genetic, biological, biochemical, molecular, or imaging event whose alterations correlate with disease pathogenesis and/or manifestations [3]. A biomarker can be used to recognize or monitor an abnormal biological process. It should have diagnostic or prognostic utility, and can be evaluated qualitatively and/or quantitatively in laboratories [8, 12]. An effective biomarker is biologically and pathophysiologically relevant, simple for routine use, and responds accurately and sensitively to changes in disease activity [13]. It must measure an underlying biological process reliably and reproducibly. So far, no single biomarker has been universally accepted as "the lupus biomarker". Some biomarkers may be used for early diagnosis of SLE. Biomarkers could be useful in identifying the effect of susceptibility genes and/or environmental initiators, because it is known that genetic predisposition and environmental factors may be involved in initiation of systemic lupus erythematosus. Therefore, some markers may help to identify individuals prone to develop SLE and patients at risk of a severe course of the disease. Others may be useful for monitoring or predicting disease progression and the involvement of specific organ systems. In addition, biomarkers may aid evaluation of the effectiveness of therapy and could be used to optimize risk assessment for individual patients. Diagnosis, assessment of disease activity, and evaluation of treatment for this complex disease therefore require several biomarkers [5, 8]. The attempt to discover useful biomarkers for SLE has traditionally been conducted on the basis of hypothesis-driven approaches. A small number of factors, for example specific genes, autoantibodies, and cytokines, that are believed to be involved in the underlying pathogenesis of the disease were investigated. Although many studies on lupus biomarkers have been published during the past decade, most of these have either been with small patient numbers or the data were obtained by cross-sectional observations.

Autoantibodies

Traditionally, autoantibodies, for example antinuclear antibodies (ANA), anti-extractable nuclear antigen antibodies (anti-Ro/SSA, anti-La/SSB, anti-snRNP, anti-Smith(Sm)), and anti-double stranded DNA (anti-ds-DNA) antibodies, are frequently used in the diagnosis and monitoring of SLE. Although a large number of autoantibodies have been described in SLE, only anti-ds-DNA, anti-Sm, and phospholipid autoantibodies are part of the classification criteria outlined by the ACR [14]. In a recent study, two major autoantibody clusters, an Sm/RNP cluster and a Ro/La cluster, could be found in adult SLE patients, with over 90 % of the patients belonging to either the Sm/RNP or Ro/La cluster [14]. Although an association between the presence of anti-Sm autoantibodies and the clinical symptoms of serositis has previously been described for pediatric lupus patients [15], no such association could be observed in adult patients. In a study that included adolescent lupus patients anti-chromatin antibodies (anti-NCS) were shown to be highly correlated with disease activity, especially among patients who tested negative for anti-ds-DNA antibodies [16]. Anti-NCS were strongly associated with renal damage and anti-ds-DNA antibodies, especially in the initial stage of the disease [16]. They may serve as a sensitive marker for renal involvement, especially in the absence of anti-ds-DNA. More than 99 % of patients have an elevated ANA titer [6], however ANA tests may be positive in as many as 33 % of healthy children [17]. In a cohort study of 110 children with positive ANA titers it was shown that ANA titers as high as 1:1080 had positive predictive value for SLE whereas titers of 1:360 or less make diagnosis of SLE unlikely, particularly if obtained from children younger than 13 years [18].

Anti-C1q antibodies can be detected in 30–50 % of patients with JSLE [19]. Elevated anti-C1q titers are associated with renal involvement in lupus and are therefore used for disease monitoring. In adult patients they are known to correlate strongly with renal disease activity, with sensitivity of 44–100 % and specificity of 70–92 % [20]. Because the sensitivity of anti-ds-DNA for detection of renal flares is 50 %, a combination of anti-C1q and anti-ds-DNA antibody as biomarkers was tested to achieve stronger predictive value for renal flares [5].

Complement

The complement system has been linked more intimately to SLE than to any other disease [21•]. Measures of complement C3 and C4 have historically been viewed as the best laboratory test for SLE. Reduced serum complement levels have been used as a marker of active lupus disease relapses for decades [5] and reduction of C3 and C4 at diagnosis has been linked to higher mortality. C5b-9 was shown to correlate most strongly with disease activity scores in an adult patient cohort [22]. No associated difference in overall renal survival with reduced serum C3 and C4 levels could be found [23]. Plasma levels of complement reflect a dynamic state of complement

synthesis and consumption—both being increased during inflammation—and therefore have low sensitivity and specificity. In addition, given the higher proportion of JSLE patients who have inherited complement deficiencies, measurement of complement plasma levels may be of limited value for childhood-specific disease [24].

Complement Activation Products

Investigators have investigated the potential of measurement of soluble complement activation products, for example C3a, C3d, C5a, and C4d, to serve as possible biomarkers. Although correlation with disease activity has been widely described, so far they have not been able to replace measurement of native C3 and C4 in daily clinical practice, probably because their short-lived nature requires special sample handling. Soluble complement activation products may also become attached to a variety of circulating cells, altering their physiological function. Thus, they have recently been proposed as more reliable biomarkers for SLE [22].

Cell-Bound Complement Activation Products

Abnormal levels of erythrocyte, thrombocyte, and lymphocyte-bound complement activation product C4d (E-C4d, T-C4d, P-C4d, and B-C4d) have been found to be possible diagnostic biomarkers for SLE [25–27], whereas levels of C4d bound to reticulocytes (R-C4d) may effectively and precisely predict disease activity in an SLE patient [21•]. In a very extensive longitudinal cohort study that compared E-C3d and E-C4d with C3 and C4 as biomarkers for disease monitoring, E-C4d and E-C3d were significantly associated with SLE activity [10]—even though E-Cd4 was reported to be of limited value for monitoring disease activity in SLE patients with hemolytic anemia [28]. As far as we are aware, no significant SLE research dealing with cell-bound complement proteins has yet been performed for children.

B and T Cells

B Cells

Lymphocyte counts are known to be reduced in SLE. Lymphocytopaenia is often the first obvious laboratory abnormality [29]. Central involvement of B cells in the pathogenesis of SLE has become increasingly obvious. Alteration of peripheral B cell homeostasis may be of central importance in SLE and may therefore serve as a valuable biomarker for monitoring lupus disease. An increase of CD27 plasma cells [3], and increased expression of co-stimulatory molecules CD80 and CD86 [30] have previously been described in SLE patients. Although these studies mainly involved adult patients, subsets of B cells resembling plasma cell precursors have been detected in the peripheral circulation of pediatric patients [31]. A study involving 68 children with JSLE revealed that these patients suffer profound B cell lymphopenia because of a dramatic decrease in naive and memory B cells (CD20 + CD38), whereas oligoclonal plasma cell precursors are expanded threefold. Those findings did not correlate with disease activity, anti-ds-DNA, or complement titers [29]. In contrast, a subset of CD27−, IgD−, and CD95+ memory B cells [32], and CD19+ and CD86+ B cells [32] were identified as correlating with disease activity and serologic abnormalities.

BLys

B lymphocyte stimulator (BLys) is expressed on monocytes, macrophages, and monocyte-derived dendritic cells and is critical for B cell growth and survival [3]. Approximately 30 % of SLE patients have elevated BLys levels. Although the association of BLys with disease activity is controversial, a few smaller studies have shown a correlation with, particularly, anti-ds-DNA antibodies [3, 33].

T Cells

Centrally involved in the immune dysregulation of the pathogenesis of SLE, T lymphocytes seem to serve as a target for monitoring disease activity. CD8+, DR+ T cells were found to be increased before relapse [34]. NKG2D is a receptor on T cells that regulates natural killer cells. In humans, it includes the MHC class 1-related chain A, which is expressed in some autoimmune disease target tissues. Thus, NKg2D may worsen autoimmune disease progression. NKG2D co-stimulates the expansion of otherwise rare NKG2D+ CD4+ T cells, and may therefore dampen chronic immune activation. In this regard, a study of patients with juvenile onset lupus was performed and showed that increased frequencies of NKG2D+ CD+ T cells are inversely correlated with disease activity [35].

Genes

Genetic factors are clearly of major importance in the susceptibility of SLE. It is known that multiple genes are involved in regulating the thresholds of auto-reactivity and disease onset [36] and no single causative gene could be identified that causes SLE [37]. An association of specific alleles of the major histocompatibility complex (MHC) and SLE has been found [3].

Complement Genes

Amongst the so-called “lupus genes” are those encoding for complement components. Complement deficiency of the

early components of the classical complement system, for example C1q, C1r, C1s, C4, particularly C4A deficiency, or C2 have been shown to be associated with an increased risk of lupus susceptibility in different studies [38]. Inherited C1q deficiency is the strongest single genetic factor identified as predisposing to early-onset JSLE that usually has a more severe disease course and a predominance of cutaneous and renal manifestations [39].

IFN-Responsive Genes

The type 1 interferon (IFN) pathway is dysregulated in SLE. A group of type 1 IFN responsive genes has been identified as being up-regulated in the peripheral blood of most pediatric SLE patients. This IFN gene signature correlates with disease activity and severe complications including renal, central nervous system, and immunologic disease [11]. In both adult and pediatric patients, increased expression of IFN-inducible genes and/or serum levels of IFN-inducible chemokines correlated with the presence of autoantibodies specific for dsDNA and RNA-binding proteins such as Ro, U1-RNP, and Sm [40]. Because high levels of interferon alpha (IFN-alpha) have long been detected in patients suffering from SLE it is not surprising that single-nucleotide polymorphisms (SNPs) in interferon regulatory factor 5 (IRF5) genes were found to be a possible genetic risk factor for SLE [41].

T and B Cell Regulatory Genes

Aberrant T and B cell function is a characteristic of the immune alterations in SLE. Genes encoding for protein tyrosine phosphatase N22, which is involved in regulation of signaling in T-cells, were strongly associated with SLE [42].

The list of hypothetical candidate genes correlating with lupus susceptibility is long, and includes genes encoding for mannose-binding lectin, the cytokines IL-6, IL-10, IL-21, TNF-alpha, and chemokines [3].

Biomarkers in Urine Samples

SLE-Nephritis

JSLE typically has not only a more severe disease course but also more renal involvement than disease presenting in adulthood. Although lupus is a rare cause of glomerulonephritis—accounting for only 1–2 % of all causes of end-stage renal disease [5]—renal involvement, as shown by abnormal urinanalysis and/or renal function, is present in as many as 75 % of lupus cases [43]. Irreversible renal damage is one of the most common long-term consequences seen in JSLE, and the presence of renal involvement in patients with JSLE is independently associated with worse disease morbidity [44]. The symptoms are often not evident early in the course of the disease; therefore clinical findings underestimate the true prevalence of renal involvement [45]. Clinical symptoms vary from mild urine abnormalities to nephritic or nephrotic syndrome and may flare at any point over the patient's lifetime [46]. To confirm diagnosis and classify the lupus nephritis, renal biopsy is the recommended method. Lupus nephritis is classified according to the ISN/RPS classification into classes I–VI [47] ranging from minimum mesangial lupus nephritis to advanced sclerosing lupus nephritis in which more than 90 % of the glomeruli are sclerosed with no residual functional ability [5]. Approximately 50 % of children with LN have class IV glomerulonephritis, carrying the worst renal prognosis [48]. Despite its diagnostic strengths, the renal biopsy is an invasive procedure—for children often conducted under general anesthesia—with potential complications [49]; it is, therefore, not routinely conducted in childhood. Urinary, non-invasive biomarkers are, therefore, particularly attractive in pediatric medicine.

Proteinuria and GFR

Currently, proteinuria—most accurately measured in a 24-h urine sample or as the ratio of protein to creatinine in the urine—is the principal urinary biomarker [5]. Although it has been proved to correlate with eventual renal outcome [50], it is not necessarily related with the active or acute histological changes seen in LN and is, therefore, a poor indicator of disease severity or activity [5]. Glomerular filtration rate (GFR), estimated by use of the Schwartz formula, is a standardized indicator for defining renal function. A decrease is known to be associated with a worse renal outcome [51]. Urine sediment is incorporated in the SLEDAI and BILAG scores and has an accepted role as a useful measure of disease activity [5]. Because each of those standard markers is of very limited value for early detection or prediction of renal flares, there is a need for novel urinary biomarkers which predict flares before urine sediment or proteinuria can be measured. Amongst these, promising studies have been performed for urine neutrophil gelatinase-associated lipocalin (NGAL) and urinary monocyte chemoattractant protein 1 (MCP1), especially.

Neutrophil Gelatinase-Associated Lipocalin (NGAL)

NGAL, a constituent of neutrophil granules, is responsible for the growth and differentiation of epithelial cells. It is constitutively expressed at low levels in the kidneys [52] and is up-regulated in response to renal damage [53]. It is speculated that urinary NGAL in childhood-onset SLE nephritis is produced principally by the injured tubule cells, in direct proportion to the extent and severity of the disease [54]. NGAL is believed to induce apoptosis in mesangial

cells, facilitating recruitment of inflammatory cells to the kidney. Urinary NGAL has been shown to have high sensitivity and moderate specificity for prediction of future renal flares, outperforming anti-ds-DNA antibody titers, and to be an excellent biomarker for distinguishing between SLE patients with and without nephritis [55]. In a pediatric cohort significant increases in urinary and plasma NGAL were detected up to three months before worsening of lupus nephritis [55]. Urinary NGAL, in contrast with plasma NAGL, is more closely related to renal disease outcomes in childhood-onset SLE than it is to disease activity and damage of extra renal organ systems [54, 55]. So far, NGAL is the only single biomarker investigated in longitudinal studies in pediatric cohorts [55]. In addition, in a recent study by Brunner et al., urinary levels of NGAL and MCP-1, with creatinine clearance, were shown to be an excellent diagnostic test for LN chronicity [56•].

Monocyte Chemoattractant Protein-1

Urinary monocyte chemoattractant protein-1 (MCP-1) has been investigated in at least two cross-sectional pediatric cohorts. MCP-1 is expressed by mesangial, podocyte, and monocyte cells and is a leukocyte chemotactic factor that is involved in mediating inflammation and injury in LN [20, 57, 58]. Urinary concentrations of MCP-1 were shown to be significantly higher in patients with JSLE active renal disease than in those with non-active renal disease [49]. The presence of MCP-1 in the glomerulus has been shown to correlate with a poor renal prognosis in childhood LN [59], with histological findings in lupus glomerulonephritis, and with the BILAG disease activity score [60, 61].

Alpha-1-Acid Glycoprotein

A recent study identified alpha-1-acid glycoprotein (ACG), an acute inflammatory protein released from the liver, in significantly higher concentrations in urine from pediatric patients with active renal disease than in urine from those with non-active renal disease. UMCP-1 and ACG were, furthermore, related with urine albumin-to-creatinine ratio but did not correlate with C3, C4, dsDNA, creatinine, eGFR, leucocytes, and lymphocyte count [49].

Others

Different from findings in adult studies, urinary interferon-inducible protein 10 (IP-10) failed to cause a significant increase in renal active disease, although it was positively correlated to MCP-1 and AGP [62]. Another cross-sectional study of 32 children with LN revealed reduced plasma levels but increased urinary excretion of transforming growth factor-beta in those with active disease [63]. The tubular markers urinary *N*-acetyl-beta-D-glucosaminidase and retinol-binding protein were shown to enable distinction between JSLE patients with and without nephritis but they are also increased in vesicoureteric reflux and urinary tract infections [63]. By means of proteomic studies Suzuki et al. isolated a group of urinary proteins from pediatric patients that were significantly higher in patients with LN and strongly correlated with disease activity [64, 65]. Four of these were albumin or albumin fractional products and the others were transferrin, ACG, ceruloplasmin, and lipocalin-type prostaglandin D-synthetase.

Combinations of Urinary Markers

To investigate the relationship of several recently identified urinary markers, including NGAL, MCP-1, ceruloplasmin (CP) transferrin (TF), alpha-1-acid glycoprotein (AAG), and lipocalin-like prostaglandin D synthase (L-PGDS), to traditional measures of LN, and to test whether some combinations of these biomarkers are diagnostic for specific histological findings in LN, Brunner et al. recently conducted a study on 76 SLE patients, including 26 pediatric patients. The combination of MCP-1, AAG, CP, and protein-to-creatinine ratio was an excellent indicator of the activity of LN. NGAL and MCP-1, with creatinine clearance, proved an excellent means of diagnosis of LN chronicity [56]. Together the findings indicate that non-invasive evaluation of LN activity and chronicity will be possible in the near future.

Conclusions

Because of its complex pathogenesis, multifaceted phenotype, and unpredictable complications, JSLE is still a diagnostic and therapeutic challenge. The course of the disease in a given patient is characterized by unpredictable flares and remission [7], and is usually more severe than adult-onset disease. Serious complications could be the result of relapses that are either unrecognized or not treated sufficiently or early enough, or because of the use of aggressive immunosuppression. Therefore, identification of reliable biomarkers is critical to enhance diagnostic accuracy, to predict prognosis and disease severity, and to enable the early detection of relapse or remission. For children, in particular, invasive procedures, for example kidney biopsy that requires sedation [49], are not conducted routinely to monitor disease activity. Biomarkers that enable noninvasive evaluation and management of children with SLE are therefore in great demand. Recent studies suggest urinary markers are the most promising biomarkers for JSLE, in particular to anticipate impending renal flares. A Lupus Nephritis Renal Panel consisting of monocyte chemotactic protein 1 (MCP1), neutrophil gelatinase-associated lipocalin (NGAL), hepcidin-20 and hepcidin-25, lipocalin-like prostaglandin D synthetase (L-PGDS), alpha-1-acid glycoprotein

(AAG), ceruloplasmin, and transferrin (TF), has been developed to help measure the extent of active inflammation in the kidneys [55]. With regard to extra-renal involvement, especially, the presence of cell-bound complement activation products can enable monitoring of disease activity [10, 25–27]. Besides urinary markers and cell-bound complement factors, a genetic disease activity score developed by Chaussabel et al. has great promise for quantifying current disease activity and predicting future flares of SLE [66].

Most JSLE patients have to be treated with immunosuppressive medications and glucocorticoids [7]. Because side effects of therapeutic agents, for example growth retardation, in addition to long-term complications of the disease itself are of major importance in pediatric and adolescent patients, biomarkers should also enable monitoring of the efficiency of therapy.

Appropriate use of biomarkers in daily clinical practice requires a validated, standardized laboratory test that is available in centers where lupus patients are treated. Further, standard values for pediatric patients must be established, because these usually differ from those for adult patients.

Successes in biomarker discovery over the past five years will, hopefully, also facilitate management of pediatric SLE patients in the years to come [67]. Although a large number of novel biomarkers have been studied, few have been investigated for children or validated in large-scale longitudinal studies. It seems that the three types of novel biomarker that are closest to being available in daily routine are urinary biomarkers and cell-bound and genomic biomarkers in the blood [67]. It is unlikely any of the biomarkers described will have the power to stand alone for diagnosis or monitoring of the disease. Because of the multifactorial pathogenesis of the disease, biomarker profiles, rather than single biomarkers will meet the objectives of early diagnosis and appropriate management in daily clinical practice. It is likely that a combination of novel biomarker profiles and conventional clinical and laboratory data will help to enhance sensitivity and specificity in monitoring of disease progress and to enable prediction of relapses.

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