Juvenile idiopathic arthritis (JIA) is the most common, chronic rheumatic disease in children, with the current classification for JIA distinguishing various subtypes of disease. The disease often takes a remitting course, requires immunosuppression for many years, and has a long-term outcome that is not easy to predict. Physicians therefore have to balance the risk of doing too little versus the risk of doing too much (and consequently accepting the risk of adverse effects). Clinical tools for monitoring disease activity and the quality of daily life have been validated, thus enabling better evaluation of clinical interventions. However, current clinical, laboratory, or radiological parameters cannot accurately diagnose or predict disease outcomes. Biomarkers that help in making the diagnosis within a suitable time-frame and that can predict outcome – and thus guide therapeutic strategies – would improve the clinical management of JIA. Our knowledge about the immunological disturbances related to JIA pathogenesis is increasing. This review summarizes approaches to translate knowledge of the biology of JIA into tools for diagnosis, prognosis, patient classification, and, ultimately, into the stratification of individual therapeutic approaches for JIA patients. Int J Adv Rheumatol 2011;9(1):8–16.

The basis of juvenile idiopathic arthritis

Juvenile idiopathic arthritis (JIA) shares the feature of being a potentially disabling, chronic inflammation of the joint with rheumatoid arthritis (RA); however, it is a far more heterogeneous disease with different subtypes that are defined based on the number of joints affected from onset to 6 months, as well as the extra-articular involvement, according to the International League of Associations for Rheumatology (ILAR) classification [1]. The subtypes include oligoarthritis (<5 joints affected), polyarthritis (≥5 joints affected), systemic JIA (arthritis, fever, rash, and inflammation of other organs), enthesitis-related arthritis, and psoriatic arthritis (PsA). JIA is an example for a multi-faceted disease with certain genetic associations conferring overall susceptibility to the disease [2]. A variety of environmental triggers challenging a genetically susceptible person may ultimately cause the disease.

Following activation of both innate and adaptive immunity, various effector mechanisms contribute to the ongoing inflammation. These mechanisms consist not only of immune cells, but also of soluble factors that can act as mediators of the immune response. Two types of soluble factors are identified to be of importance in JIA, namely cytokines and damage-associated molecular pattern (DAMP) molecules, which serve as immune amplifiers and have an impact on both innate and adaptive immune responses [3,4]. Among the markers reviewed in the remainder of this article, the factors specifically linked to disease mechanisms are particularly interesting as biomarkers as they provide more disease-specific information than the commonly used pan-inflammation markers such as acute-phase reactants (Figure 1).

The usefulness of biomarkers

In recent years, patient outcomes have improved dramatically with the availability of effective treatments for the management...
of JIA. However, JIA is a heterogeneous disease with variable disease progression and treatment response. While some patients respond to a single disease-modifying antirheumatic drug (DMARD), others require more intensive treatment strategies. Better biomarkers for this heterogeneity are needed in all aspects of patient care (Table 1). Assessing disease severity at diagnosis and monitoring disease activity on an individual level could allow tailoring of therapy, ensuring optimal treatment for those at greatest risk of disease progression, long-term disability, and joint damage; thus, avoiding unnecessary overtreatment with potential side effects. Assessment of disease activity and severity is currently based on a combination of clinical and conventional laboratory parameters that aid treatment decisions. The use of biomarkers may provide a more accurate means of objectively assessing the disease.

To date, no simple diagnostic tests for monitoring inflammation are available. Commonly used laboratory parameters, such as C-reactive protein (CRP) levels or erythrocyte sedimentation rate (ESR), are measurements of acute-phase reactants that correlate poorly with disease activity. These markers reflect a summation of systemic host responses rather than being specific for inflammation, and they have no predictive value for the further course of the disease [5]. Ideal markers to be used in the clinical follow-up would be “noninvasive”, easy to perform, reproducible, inexpensive, and reflect disease activity with high sensitivity and specificity (Table 2) [6].

**Type 1 biomarkers: disease markers of JIA**

A “type 1” biomarker could be used to make the correct diagnosis when facing a patient with suspected JIA (a “type 1a” biomarker). In such a scenario, it would be important to exclude other possible causes of the complaints. A biomarker at this stage should be as noninvasive as possible; therefore, analyzing synovial fluid will be the exception rather than the rule. Analyzing markers in peripheral blood is presently routinely performed, and acute-phase reactants or antibodies are frequently measured.
A type 1 biomarker could also help in assessing prognosis or predicting potential complications typically linked to a specific phenotypic pattern of manifestations, which further distinguishes groups of patients with certain features that are different from others despite the same diagnosis (a “type 1b” biomarker). Analyses of such markers are not routinely performed for this purpose at present, but have recently been suggested [7].

**Antibody profiles**

Rheumatoid factor (RF) is a marker defining a distinct JIA category: RF-positive polyarticular JIA. Thus, positivity for RF is detected in only a minority of patients [8]. Patients with this subtype are more frequently female with a later onset of the disease. RF-positive polyarticular JIA is the subtype of JIA that most closely resembles adult RA. It is possible that this category has an overlap with juvenile-onset RA, with disease progression into adulthood likely [8].

It is well known that many patients, especially those with early-onset oligoarticular JIA, are positive for antinuclear antibodies (ANA) [9]. Published data support the hypothesis that patients with similar characteristics are currently classified into different JIA categories. Compared with ANA-positive patients, ANA-negative patients are older at disease presentation and have a reduced female prevalence, a lower frequency of iridocyclitis and asymmetric arthritis, a greater number of affected joints over time, and a different pattern of arthritis [10–12].

Anti-cyclic citrullinated peptide (anti-CCP) antibodies are less prevalent in JIA than in adult RA but are detectable in a significant proportion of RF-positive patients with polyarticular-onset JIA. There may be a relationship between anti-CCP-positivity and erosive joint disease in JIA [13,14]. Anti-CCP antibodies are not relevant for other subgroups of JIA; therefore, anti-CCP antibodies should not be investigated routinely in patients with JIA [15,16].

Taken together, these findings indicate that autoantibodies point to certain subtypes of the disease; thus, these autoantibodies could serve as biomarkers that help in assessing prognosis or predicting potential complications typically linked to a specific phenotypic pattern of manifestations. It should be noted that autoantibodies other than those mentioned above have additionally been found in JIA, but these are of limited value as biomarkers.

**Genomics**

Over the past few years, there has been a significant improvement in our insight into the genetic basis of rheumatic diseases. While it is clear that – as for other rheumatic diseases – JIA is not a monogenetic disorder, several associations with certain genetic alterations have been described. Thus, a specific genetic background could confer susceptibility to development of the disease or a predisposition to a more severe course of...
that JIA is a complex genetic disorder. Each disease pathogenesis and the various presenting phenotypes in JIA are difficult because of the complex nature of the disease. Among the possible genetic factors are human leukocyte antigen-DR4 (HLA-DR4) and HLA-DRB-1, and non-HLA single nucleotide polymorphisms in genes encoding a variety of proteins, including macrophage migration inhibitory factor (MIF), Toll-like-receptor 4 (TLR4), cytotoxic T lymphocyte antigen 4 (CTLA-4), protein tyrosine phosphatase non-receptor type 22 (PTPN22), and matrix metalloproteinase 3 (MMP3) [17–19]. As in other autoimmune diseases, genetic studies in JIA are difficult because of the complex nature of the disease pathogenesis and the various presenting phenotypes that are likely to involve different susceptibility genes. Each novel association identified adds further weight to the concept that JIA is a complex genetic disorder.

Transcriptomics

While associations of genetic polymorphisms with JIA are generally only weak, gene-expression profiling can still be informative as it provides insight into the transcriptome that reflects relevant disease processes in JIA. Gene-expression profiles in RA have revealed a number of upregulated genes that are particularly relevant for innate immune mechanisms [20]. The genes upregulated in peripheral blood in RA include CD14, defensins, major histocompatibility complex (MHC) class II molecules, neutrophil cytosolic factor 4, S100A8, and S100A12. It appears striking that the products of these genes point to monocyte and neutrophil activation processes. In JIA, peripheral blood mononuclear cell (PBMC) gene expression analysis revealed biological differences between patients with early- and late-onset JIA, independent of classification based on the number of joints involved [7,21]. Published data suggest that age at onset may be an important parameter to consider in JIA classification. Furthermore, pathological mechanisms may vary with age at onset, and understanding these processes may lead to improved treatment of JIA [21]. Griffin et al. demonstrated PBMC gene-expression signatures in a large population of children with recent-onset polyarticular JIA that correlated with differences in disease characteristics [22]. The findings support a subclassification of polyarticular JIA, with signatures identifying both RF-positive and RF-negative patients with disease similar to that of adult RA, a signature identifying a “less inflammatory” disease subset, and a group of patients with ANA-positive, early-onset disease expressing neither signature. Thus, such signatures offer a molecular classification of polyarticular JIA and may prove to be valuable tools for forecasting long-term outcomes [22].

In a more limited set of patients and analyzing neutrophil gene expression instead of PBMC gene expression, Jarvis et al. found that neutrophil gene abnormalities persisted in children with polyarticular JIA even after disease remission was achieved. This demonstrates that transcriptome profiles depend upon the material that is analyzed [23].

PBMC gene-expression profiles have proven to be particularly informative in systemic JIA. This is not very surprising considering the systemic nature of the disease, with alterations in peripheral blood leukocytes and elevation of acute-phase reactants and cytokines well recognized. An interleukin-1β (IL-1β)-related gene expression profile has been described, pointing to a key role of this cytokine in systemic JIA [24]. Later studies demonstrated that IL-1β is in a positive feedback loop with an S100A8/S100A9 protein complex released from phagocytes, thus leading to an unbalanced amplification of innate immunity likely triggered by either infection or tissue damage [25]. This provides an insight into the pathophysiology of systemic JIA, which is much more akin to an autoinflammatory syndrome than to a joint disease. As transcriptome profiling is rather complex and IL-1β is difficult to detect in patient samples, the link with S100A8/S100A9 provides a potential disease marker differentiating systemic JIA from other JIA forms, and also from other differential diagnoses (Figure 2). The same applies to a related neutrophil-derived protein, S100A12. This is important as systemic JIA initially often presents as fever of unknown origin and must be differentiated from infections or malignancies (S100A12 is not produced in infections or malignant cells) [3,26].

Proteomics

Cytokines and soluble inflammatory mediators could also be targets for proteomics approaches. Surprisingly, proteomic profiling by antibody-based multiplex assays was not able to confirm elevation of IL-1β in systemic JIA [27]. However, the authors found that patients with JIA had, irrespective of their subclassification, significantly higher levels of tumor necrosis factor (TNF), MIF, and the chemokines CCL2, CCL3, CCL11, CCL22, and CXCL9 in plasma than did control subjects. In paired plasma and synovial fluid samples of patients with JIA, significantly higher levels of IL-6, IL-15, CCL2, CCL3, CXCL8, CXCL9, and CXCL10 were present in synovial fluid. Cluster
analysis in all patients with JIA revealed a predominant pro-inflammatory cytokine cluster during active disease and a regulatory/anti-inflammatory-related cytokine cluster during remission [27]. Non-antibody-based proteomics approaches may be very powerful for unbiased discovery of novel biomarkers. Interestingly, studies using the sophisticated surface-enhanced laser desorption/ionization time-of-flight mass spectrometry (SELDI-TOF MS) ultimately came up with little more than a target linked to innate immunity, which was mentioned above: S100A8 [28]. In recent studies, plasma protein signatures of systemic JIA have been described, also revealing S100A8 and S100A9 as important factors associated with the disease [29]. In addition, a 17-urine-peptide biomarker panel was identified that could effectively discriminate systemic JIA patients at active, quiescent, and remission disease states. Targeted sequencing of these peptides revealed that they represent degradation products of seven different proteins (three types of collagens as well as four acute phase proteins including fibrinogen α and β, α-1 antitrypsin, and uromodulin), suggesting disease-specific proteolytic activities. Antibody array plasma profiling identified a systemic JIA flare signature consisting of tissue inhibitor of metalloproteinase-1 (TIMP-1), IL-18, CCL5 (RANTES), P-selectin, MMP-9, and L-selectin [30].

**Synovial markers**

Just as peripheral blood cytokine-related transcriptome and proteome analyses may be informative in systemic JIA, the same analyses may be of particular interest when performed in synovial tissue or fluid in those JIA subtypes that are typically restricted to only a few joints (and thereby do not evoke any systemic alterations). In oligoarticular JIA, biomarkers in peripheral blood are particularly problematic; however, since joint injections are frequently used in these patients, synovial fluid analyses can be easily performed. As oligoarticular JIA is very heterogeneous with many patients developing extended disease later on, identification of markers defining the subgroup of patients prone to extension would be useful (Figure 3).

Hunter et al. identified biomarkers in the first synovial fluid aspirate obtained from children with oligoarticular JIA that could be used to identify individuals whose disease was likely to extend to a more severe phenotype [31]. Synovial CCL5 levels were higher in children whose disease extended to a more severe phenotype. The CD4:CD8 ratio in the synovial fluid was significantly lower in patients who went on to have extended oligoarticular JIA. Gene-expression profiling revealed that 344 genes were >1.5-fold differentially expressed between outcome groups. These included genes associated with inflammation and macrophage differentiation that showed increased levels in patients with extended disease at 1 year, and genes associated with immune regulation that were present at increased levels in patients with persistent disease at 1 year [31].

Matched synovial fluid and plasma samples have also been analyzed by 2D-gel electrophoresis [32]. Forty-three protein spots, overexpressed in the joint, were identified in a study by Gibson et al. [33]. Synovial fluid from children with single-event knee joint inflammation was then compared with that from a group with recurrent knee disease. Nine synovial-specific proteins were significantly differentially expressed in the recurrent group. Proteolytic fragments of collagen X, fibrin β-chain, and T cell receptor α-region were identified among this protein cluster. These proteins may play a significant role determining the pathological state within the chronically inflamed joint, and influence disease progression in JIA [33].

**Type 2 biomarkers: surrogate markers of inflammatory activity**

A surrogate marker of inflammatory activity could help in determining the extent of (joint) inflammation in an easy way, that...
is, without the need for more invasive or complicated procedures such as imaging or even synovial biopsies. Despite being noninvasive in nature, such a marker should be more sensitive than clinical investigation. The latter is especially difficult in joints that are already damaged and thus always present as abnormal. Eventually, “type 2” biomarkers will help in monitoring the response to therapies or predicting flares (Figure 4).

**Biomarkers reflecting inflammation for monitoring response to therapies**

It is not surprising that, during active phases of an inflammatory disease such as JIA, there is usually nonspecific laboratory evidence of inflammation. Classical laboratory markers of inflammation are acute-phase reactants. CRP and ESR have been included into definitions used for active versus inactive disease [34]. These markers indicate the acute-phase reaction dominated by an upregulation of proteins in the liver. As such, acute-phase reactants are very nonspecific and give little information that is relevant for the disease mechanisms involved in arthritis. In addition, these markers can only indicate systemic inflammatory activity that may not be present in cases of synovial inflammation. Therefore, better markers would be those that originate from local sites of inflammation (Figure 1). Soluble factors involved in the disease processes (such as cytokines, chemokines, enzymes, or DAMPs) are promising candidates. Most biomarkers of JIA activity are initially identified as a disease or subtype marker (type 1a or type 1b biomarkers), proving a link to pathophysiology. These may subsequently be tested for their correlation with disease activity.

“The S100 proteins are currently the most promising potential biomarkers of disease activity and response to therapy in JIA”

Of the markers tested in this regard, the studies on S100A8 and S100A9 (also known as myeloid-related proteins 8 [MRP8] and MRP14, respectively) are the most advanced to date. Studies...
combining basic research with clinical studies have pointed to an important role for these DAMP proteins as biomarkers. These calcium-binding proteins are expressed in granulocytes, monocytes, and macrophages during early stages of differentiation, and can form complexes. They have clear pro-inflammatory effects on other cells, such as phagocytes and endothelial cells, and can even act as endogenous activators of Toll-like receptor 4 [35]. These proteins are complex and are secreted after activation of phagocytes through an alternative pathway, forming positive amplification loops with IL-1β. It transpires that these proteins can act as excellent biomarkers in a variety of autoimmune and inflammatory diseases [36]. Furthermore, they can be used for monitoring disease activity, as their levels decrease with successful treatment [25]. Another calcium-binding molecule more specific for neutrophils, S100A12, is also strongly elevated in the serum of patients with systemic JIA, and seems to be a more reliable marker than various conventional parameters [26]. It can also be used for monitoring inflammatory activity in several rheumatic diseases including JIA. Interestingly, the pro-inflammatory S100 proteins originate from inflammatory cells within the inflamed synovium and thus correlate closely with local joint disease even in cases without systemic involvement [29,37–39].

A recent study showed that S100A8/S100A9 analyses in plasma may not be as valuable as analyses in serum samples. Using 2D gels and a proteomics approach, S100A8/S100A9 was among the highly discriminating spots derived from 15 proteins that constituted a robust, systemic JIA flare signature. Measurement of S100A8/S100A9 in plasma samples still proved informative, but the performance of this marker in plasma was no better than that of CRP or ESR in the differentiation of patients with a flare of systemic JIA versus those with quiescent disease [29]. It is well known that the presence of complex forms of these proteins influences the measurement of these calcium-binding S100 proteins, and that the formation of these complexes depends on the calcium ion concentrations. Therefore, the plasma sampling method (e.g. with additives such as ethylenediaminetetraacetic acid [EDTA]) will have differed substantially from serum sampling, which was used for other studies on S100 proteins in JIA [40–42].

Interestingly, as mentioned above, a urine peptide biomarker panel was identified by peptidomic approaches that could effectively discriminate systemic JIA patients at active, quiescent, and remission disease states [30]. Further studies will be necessary to prove that the proteomics approaches used in studies such as this can be translated into noninvasive urine peptide analyses that may be useful for monitoring inflammation.

During active disease, cytokine concentrations in the plasma of patients with JIA increase significantly. This is even more prominent in synovial fluid, in which high levels of IL-6, IL-15, CCL2, CCL3, CXCL8, CXCL9, and CXCL10 have been detected [27]. However, analyzing these markers routinely is somewhat impractical, as cytokines are very unstable and cannot be easily shipped or analyzed in the routine clinical setting. Therefore, robust DAMP molecules such as the S100 proteins represent more practical markers of inflammation.

**Biomarkers detecting subclinical inflammation for predicting relapses**

Disease markers or surrogate markers of inflammation are used to confirm a suspected diagnosis. They provide additional confidence in situations in which a clinical impression already fits disease criteria. If a surrogate marker correlates with disease activity, this generally means that it shows something that is also evident during clinical evaluation, for example an improvement in disease activity in response to successful therapies. These markers thus add to the findings of other assessments, and, as a whole, will help clinicians with the complete evaluation of their patients’ condition. Even more important, however, are markers that provide information on inflammatory activity that is not detectable by any other means. This is a potentially crucial function, especially when considering the ultimate challenge in patient care in JIA – how to maintain clinical remission. At present, it is difficult to predict who will stay in remission and who is prone to disease flare [5].

In the case of JIA, methotrexate is the most widely used disease-modifying drug. Remission can be induced in most patients continuing on medication with combined anti-inflammatory treatment (also referred to as “remission on medication”), and up to 50% of such patients reach a continuous status of remission after discontinuing medication (referred to as “remission off medication”) [43]. However, approximately half of the patients have flares after withdrawing or tapering methotrexate [44,45]. Clinical scores and routine laboratory markers currently in use cannot detect residual inflammation that likely influences the risk of flares when stopping treatment; hence, clinicians and patients would benefit from the availability of improved molecular biomarkers of inflammation. Reliable molecular markers should allow stratification of patients: those with a high risk of relapse (with subclinical disease activity) should remain on therapy or even receive intensified therapy, while in those with a low risk of relapse, therapy could be withdrawn.

The best evidence for a predictive marker exists for S100A8/S100A9. In JIA, it has been shown to be a marker of subclinical disease activity that is not detectable by clinical investigation or other laboratory tests. It is a marker of local disease activity, and analysis of S100A8/S100A9 levels detects subclinical inflammation that would exclude immunological remission [46,47]. A controlled trial evaluating methotrexate withdrawal revealed that the flare risk could be significantly reduced either if therapy was continued or if therapy was only withdrawn in patients with low S100A8/S100A9 levels [48]. In the clinical setting, there appears to be a clear benefit for the patient of using S100A8/S100A9 as a biomarker of inflammation, as low levels make subclinical disease activity at the time the test is performed unlikely (negative predictive value 98%) [48].
Conclusion
Taken together, the research progress from studies elucidating disease mechanisms in JIA have led to the identification of novel markers associated with these mechanisms. These biomarkers are particularly interesting as they provide more disease-specific information than the nonspecific markers of inflammation. Understanding the basis for the JIA disease heterogeneity remains a major task for the future. Molecular markers of inflammation such as DAMPs also enable us to detect subclinical activity – a fact that may prove very important in therapeutic patient stratification, which is the ultimate goal in personalized medicine.

Disclosures
The authors declare that they have no conflict of interests.

References