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PREDICTING DISEASE OUTCOMES IN JUVNILE IDIOPATHIC ARTHRITIS: CHALLENGES, CURRENT EVIDENCE AND NEW DIRECTIONS

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Search Strategy

We searched PubMed, Medline and Embase for full-text manuscripts in English language published between January 1972 and November 2018 with terms relating to juvenile arthritis in combination with terms relating to predicting outcome, including the use of biological markers, biomarkers, and with outcome terms relating to disease remission, treatment response, or relapse . We also searched the reference lists of articles identified by this search strategy.

Key points:

1. It is currently challenging to predict outcomes in JIA using clinical factors alone and there are no validated biomarkers with which to predict treatment response

2. Conflicting evidence exists on how and when to withdraw therapies in disease remission to avoid flare.

3. Biological markers, particularly S100 proteins and single nucleotide polymorphism data, can add value to clinical models in predicting disease outcomes

4. Further standardisation and validation studies for biological markers are required

5. Collaborations will allow for streamlining and analysis of larger datasets with biomarker data, such as the CLUSTER consortium (UK) and the Understanding Childhood Arthritis Network UCAN (worldwide). These new networks will facilitate the development of clinical/biomarker panels to aid prediction of outcome in JIA.

ABSTRACT /SUMMARY

The aims of treating juvenile idiopathic arthritis (JIA) are to elicit treatment response toward remission, whilst preventing future flare. Understanding patient and disease characteristics that predispose young people with JIA to these outcomes would allow the forecasting of disease process and the tailoring of therapies. Currently, the strongest predictor of remission is disease category, particularly oligoarthritis, although a few additional clinical predictors of treatment response have been identified. More novel evidence using biomarkers, such as S100 proteins and novel single nucleotide polymorphism data, may add value to clinical models. Future directions for personalised medicine in JIA will be aided with international collaborations, allowing for the analysis of larger datasets with novel biomarker data. In a complex disease such as JIA, it is likely that a combined clinical and biomarker panel will be required for predicting outcome.

JUVENILE IDIOPATHIC ARTHRITIS

Juvenile idiopathic arthritis (JIA) is the most common inflammatory rheumatological condition affecting children and young people (CYP), with a prevalence of approximately 1 in 1000 CYP internationally [1]. It presents as chronic arthritis of unknown aetiology with onset before the 16th birthday[2]. JIA is a heterogeneous condition currently classified into seven distinct categories by the International League of Associations for Rheumatology (ILAR) [2]. The majority of CYP in Western countries present with oligoarthritis, a diagnosis distinct from adults, with the central feature of arthritis in fewer than five joints at diagnosis [2]. In regions such as southeast Asia, other ILAR categories such as enthesitis-related arthritis are more common [3]. In recent years, the classification of JIA has been revisited, with greater weighting placed on existing biomarkers, and to distinguish forms of the disease purely observed with childhood-onset rather than across the age spectrum [4].

DISEASE OUTCOMES IN JUVENILE IDIOPATHIC ARTHRITIS

Arthritis and other extra-articular manifestations of JIA result in pain, loss of functional ability and joint destruction [5]. Therefore, most current pharmaceutical therapies for JIA are targeted at the underlying inflammation of disease with the aim of treating both inflammation and the symptoms of disease, such as pain. Crucial to measuring and predicting both treatment response and outcomes in JIA is the ability to measure these disease states in a systematic and consistent fashion. In recent years, we have seen a wide introduction of standardised composite outcomes in JIA.

In 2004, the Preliminary Criteria for Inactive Disease and Clinical Remission in JIA were published [6] and later refined as the ACR Provisional Criteria for Defining Clinically Inactive Disease (CID) [7], a composite of physical exam and laboratory measures. These criteria further define 2 states: clinical remission on medication (6 months of CID on medication) and clinical remission off medication (12 months of CID off medication). As these measures aimed to define clinically inactive disease as opposed to inactive disease, they do not include patient reported outcomes. The Juvenile Arthritis Disease Activity Score (JADAS) [8] combines active joint count, ESR, and a physician and parent global assessment of disease into a single measure which captures both physician and patient assessment of disease. Subsequently, with the recognition that ESR may not be routinely captured in clinical practice, the clinical JADAS, which removes the ESR, has also been proposed [9]. These scores can give a measure of disease activity at any point in time and cut-offs have been proposed to represent various states of inactive disease and remission [10;11]. Although all of these measures aim to capture similar disease states of inactive disease or remission, they are not directly interchangeable [12;13], in most cases due to the inclusion of different components or different thresholds for defining inactive disease.

The use of common outcome measures aids the identification of clinical and biological predictors of these states. There has been an increased uptake of these composite outcomes in clinical research in JIA over recent years, whereas previously a wide range of study-defined definitions of inactive disease and remission had been used [14]. With time, it is envisioned that these existing measures will be incorporated into even more observational research, thus setting the stage for more harmonious outcomes research.

The main measure of treatment response is the JIA ACR response criteria. These criteria classify response or non-response based on percent improvement in a subset of the JIA core set

without >30% worsening in others [15]. Additional outcomes are often included in research settings to capture other extra-articular manifestations of disease, such as systemic features in systemic JIA [16]. The JIA ACR response criteria have been used primarily in clinical trials of new therapies of polyarticular JIA and increasingly in observational research. They do have certain limitations, such as their utility in CYP with oligoarthritis, for whom the score is difficult to apply mathematically. In addition, as it is based on a collection of core set variables but does not require improvement across all six variables, 'response' as currently defined, is a heterogeneous outcome. This may also limit the identification and validation of predictors and biomarkers of response.

PREDICTING TREATMENT RESPONSE AND REMISSION

Clinical Predictors of Treatment Response and Remission

A wide range of studies have attempted to identify clinical predictors of outcome in JIA, although overall few predictors have been identified. Despite differences in the outcome definitions previously discussed, study populations and study follow-up lengths, at a population level the strongest predictor of good outcomes is ILAR category. Specifically, oligoarthritis has been consistently associated with the highest achievement of clinically inactive disease and remission, with the lowest achievement in RF-positive polyarthritis [17;18]. The association between ILAR category and treatment response in less evident [19-21] compared to longer term disease outcomes. Conflicting evidence also exists for systemic, enthesitis-related, psoriatic and undifferentiated JIA [21-24]. However, when extra-articular manifestations are included in remission criteria, fewer CYP are classified in remission compared with criteria which do not include these features [13]. Demographic factors such as age at onset and gender have been associated with clinically inactive disease, remission [5;25;26] and treatment response [27;28] in univariable analyses. However, oligoarticular JIA is associated with younger age and female gender [29] and after statistical adjustment for ILAR category, associations between these demographic factors and remission and treatment response are rarely evident [18].

The length of time between symptom onset and either diagnosis or treatment has been shown to play a role in outcome. This has largely been driven by the 'window of opportunity' theory whereby optimal outcomes can be gained through treatment within a short period following disease onset [30]. At present, most CYP diagnosed with polyarthritis follow a step-wise

treatment pathway [31;32], usually with initial methotrexate. Unfortunately, on this pathway, 30-50% of children do not achieve remission with methotrexate [33;34] and/or experience adverse drug reactions, necessitating a switch to or addition of biologic therapies. A longer delay between symptom onset and treatment has been reported to be associated with lower treatment response with methotrexate [35;36] and etanercept [19] and lower remission rates in general [28;37]. However, the majority of evidence exists in studies of biologic therapies, where most shows no association between delay to treatment and treatment response [38-41], although in most studies, biologic therapies were initiated later in disease, suggesting the so-called window of opportunity may have passed.

In polyarthritis, three trials have demonstrated high response and clinically inactive disease using early aggressive treatment strategies including biologics [42-44], with an approximate 90% CID achievement during a two year extension period [38]. Early biologic use in systemic JIA has also been associated with an 85% ACR90 response within three months and 90% remission rate within three years [45]. Within a treat-to-target trial of quickly escalating methotrexate and methotrexate/etanercept combination therapy in CYP with recent-onset JIA (median 7.5 months), median time to CID was 9 months with 47 to 62% CYP in CID after one year [46]. However, with some exceptions such as sJIA or ERA, patients must generally be refractory or intolerant to methotrexate before they can receive biologic therapies in most healthcare systems currently, limiting the applicability of early aggressive therapy strategies that include first-line biologics [31;47].

There is limited evidence that other clinical factors measured early in disease can predict longer-term outcomes in JIA [19;34]. There is some evidence that involvement of certain joints may influence outcome. Arthritis in the knee is associated with greater odds of remission and arthritis in the wrist, ankle and hip have been associated with poorer outcome [27;40;48;49]. Disease activity may be more predictive of outcomes later in disease, with lower overall disease activity, longer time spent in clinically inactive disease and degree of previous improvement associated with higher odds of better outcomes [17;34;39].

The lack of strong, consistent clinical predictors of outcome in JIA means that few prediction models for outcome using clinical factors alone exist and they generally don't perform well [35;39;49-54]. At initial JIA diagnosis, ILAR remained the strongest predictor of a non-remitting disease course, with other clinical factors adding little to a prediction model from the ReACCh-

Out cohort [51]. Later in disease, further factors are predictive of outcome, with a model from the Nordic JIA group able to predict remission from six months following diagnosis [49;52]. Modelling treatment response following specific therapies in more specific subgroups of JIA has also proved challenging [35;39;53]. Even in these more homogenous populations, models generally have poor specificities and thus cannot accurately identify CYP who will not response to treatment; however, as previously discussed, treatment response is a heterogeneous outcome. Guzman et al., attempted to define strata of disease states over time rather than dichotomise outcome [50]. Fairly high prediction accuracy for controlled versus persisting disease course was gained (c-index: 0.87). However, the outcome strata were defined largely using patient-reported outcomes such as quality of life and patient-reported medication side-effect. These outcomes may not correlate directly with inflammation [55;56], and introduces a tension common in stratified medicines research in complex chronic conditions such as JIA, in which the manifestations of disease may be driven by multiple complex pathways, many of which may not be targets for current therapies.

Biomarkers to Aid the Prediction of Treatment Response in JIA

Alongside the use of clinical markers, biological markers of disease (biomarkers) may detect subclinical inflammation and aid the prediction of treatment response. Biomarkers are components typically measured in patient material, such as blood or urine. This review considers biomarkers that can i) facilitate selection of the most appropriate drug to induce disease remission and ii) guide decisions to discontinue treatment in patients in remission [57]. Ideally, these markers should be accessed via relatively non-invasive approaches (particularly in the paediatric population), be stable within the sampled patient material and generate reproducible and accurate results, at a reasonable cost [57].

In analysis of a large clinical trial of MTX in polyarticular course JIA, ANA positivity correlated with good response to MTX [58] and evidence suggests that high baseline ESR is associated with good response to MTX [59]. To date, several studies (low sample size cohorts) have investigated other biomarkers for treatment response in JIA (Table 1). Protein biomarkers for treatment response may be gained from serum and synovial fluid. S100A12 and S100A8/9 (also known as calprotectin or MRP8/14) have demonstrated sensitivity to change in JIA, following anti-rheumatic therapies, including intra-articular steroids, synthetic and biological DMARD therapies in smaller cohorts (all n<100) [60-63]. In a small multi-centre cohort of 22

patients with oligoarticular JIA, higher S100A12 levels prior to intra-articular steroids were associated with poor treatment response [63]. However, higher levels of either S100A8/9 or S100A12 prior to methotrexate and biologic therapies, in larger cohorts consisting of multiple ILAR categories, were associated with higher treatment response [59;62]. Predictive ability for initial S100A8/9 serum concentration to predict anti-TNF treatment response was moderate to high, with an area under the curve of 0.68 [59] and 0.76 [62]. In multivariable models, these S100 proteins added value in predicting outcome beyond factors such as baseline JADAS and active joint count [59;62]. However, it is unclear whether S100A8/9 is a more sensitive measure of disease activity than clinical measures, and whether, even in CYP who have low disease activity, S100A8/9 would be a useful biomarker for outcome in JIA. Further protein biomarkers for future study include MMP-3 and the TNFα/ETN complex, with increased levels of both associated with higher treatment response in multiple ILAR categories [64;65]. These associations require corroboration in larger cohorts.

Genetic biomarkers for JIA can be measured through blood or other tissue sampling (Table 1). In candidate gene analyses, several single-nucleotide polymorphisms (SNPs) have been associated with treatment response in JIA, with ABCB1 and ABCC3 associated with good response to methotrexate in a single-centre cohort of 287 CYP across multiple ILAR categories [66].Good response has also been predicted by the presence of three SNPs within the SLC16A7 region, with a single SNP *rs3763980* in this region and another in the ATIC region associated with non-response in both a UK and US validation cohort [67;68]. Non-response to methotrexate has also been associated with one SNP within the SLC19A1 region [66]. At a genome wide level in a study analysing almost 700 JIA cases [69], several genetic regions were implicated as associating with response to methotrexate in JIA but await validation in replication cohorts.

There is clearly more work needed to validate these findings and explore genetic predictors of response to additional medications, including biologic therapies. However, the potential utility of a set of validated biomarkers could revolutionise management of the disease. If a validated set of biomarkers could predict which children are unlikely to respond to methotrexate, the use of early biological agents could be justified. This would reduce exposure to drug side effects and reduce time to disease remission (thus prevent long-term joint damage and allow better growth catch-up).

PREDICTING FLARE UPON TAPERING OR WITHDRAWAL OF THERAPY IN JIA

Once therapies have proven successful and a low disease state or remission has been reached in JIA, it is currently unclear when to discontinue therapies, or whether to discontinue at all [60]. Since JIA affects CYP who have decades of life ahead of them, indefinitely continuing medications in the presence of remission may present an unnecessary risk of long-term adverse effects [60]. In a move to reduce the burden, risk and cost of long-term therapies in with JIA, there is a move toward tapering or discontinuing therapies after a state of disease remission has been reached [70]. The concern is that a withdrawal of therapy may cease ongoing suppression of inflammatory activity, resulting in a disease flare [70]. Certainly, relapses following the withdrawal of methotrexate and/or anti-TNF biologic therapies are a common occurrence, approximating 30 to 50% for methotrexate [60;71;72] and commonly exceeding 50% for biologic therapies within 18 months of withdrawal across multiple cohorts. At present, it is difficult to differentiate between those who will remain in remission and those who will relapse off treatment [73].

Clinical Predictors of Flare in JIA

Few studies have investigated predictors of flare. The majority of studies have reported that demographic factors, such as gender and age at onset, are not associated with the risk of flare [74-76]. Methods proposed to reduce these high flare rates include waiting for more time in remission to pass before discontinuing and gradual tapering rather than sudden discontinuation of therapies. Some data exist to corroborate these theories, with maintenance of remission for at least one [77] or two years [78] associated with lower flare rates in two multicentre studies of biologic therapies. In addition, tapered versus immediate discontinuation following remission on medication has been associated with lower flare rates in a small study of 19 CYP on anti-TNF [79] and an open-label study of etanercept tapering observed low flare rates of just 13% after 12 months of tapering to a very low dose in 31 patients with polyarticular or extended oligoarticular JIA [75]. However, greater evidence exists countering these theories, with similar flare rates irrespective of treatment or remission duration in a retrospective study of 25 patients [60], a clinical trial of early versus late methotrexate withdrawal [73], two larger retrospective studies of 136 and 110 patients discontinuing anti-TNF therapies [76;80] . In addition, no additional benefit has been observed of tapering versus abrupt discontinuation for patients using methotrexate and/or using etanercept therapies, including a larger cohort of 215 patients

in CID [81;82]. Also, a large prospective inception cohort study [83] showed that there was a 42.5% risk of flares (defined by recurrence of any feature that meant CID was no longer present) in the year after reaching CID while on stable treatment, and that the polyarticular RF+ve group were most likely to require treatment intensification for such flares.

There is increasing interest that in the presence of subclinical disease, tapering therapies may be contraindicated, although evidence for this is still limited. Commonly used technologies used to gain insights into subclinical JIA activity include ultrasound or power-doppler technologies to capture subclinical synovitis. Currently, the evidence in JIA is sparse and conflicting, since a proportion of patients across cohorts have evidence of subclinical disease and continue to remain in a remission-like disease state [74;84;85]. The use of ultrasound has produced mixed results, with subclinical synovitis predicting higher risk of flare in 88 patients after a minimum of 3 month CID duration [85] but in 39 patients in CID for the same length of time, there was no difference in risk of relapse between patients without evidence of subclinical activity, such as synovial hyperplasia, joint effusion or tenosynovitis [84]. Ultrasound with power-doppler technology has yielded some early promising results, with a five-fold risk of flare for CYP with subclinical synovitis and positive power-doppler signals compared with CYP with neither of these events [74]. Greater work is needed to define 'normal' imaging ranges for subclinical disease that may not lead to relapse in this population. In addition, there may be specific mechanisms or combinative risk factors that lead certain CYP with subclinical disease to relapse and others to maintain remission-like disease. The further exploration of these factors would aid guidelines as to which CYP to screen using these imaging technologies, and which could benefit from specific treatment strategies in light of these results.

Biological Markers Predicting Flare in JIA

As an alternative to imaging, protein biomarkers identifying children with subclinical inflammation might prevent inappropriate drug withdrawal in those likely to relapse and prompt drug withdrawal in those likely to achieve drug-free remission [86]. The most commonly studied are, similar to those predicting treatment response, the S100 proteins. A multicentre randomised controlled trial of 364 children with oligoarticular, polyarticular, systemic, enthesitis related arthritis and psoriatic subtypes of JIA demonstrated that higher serum levels of S100A8/9 prior to stopping methotrexate was associated with relapse [73]. Findings from several cohort studies with smaller sample sizes (Table 1) are in line with the results of this

randomised controlled trial. However, there is conflicting evidence on the ability of S100 proteins to predict disease flare. A multicentre prospective study of 130 children with extended oligoarticular and polyarticular JIA revealed no correlation between serum levels of S100A8/9 prior to discontinuation of anti-TNF therapy and future flare, and weak correlation between serum S100A12 and future flare [87]. These conflicting conclusions between studies might be due to discontinuation of different drugs, use of different assays or selection of different outcome measures. This highlights the complexity of using biomarkers in identifying subclinical disease activity, and reiterates the need for further standardisation and validation studies.

Further sparse evidence is available for VEGF and IL-18 biomarkers (Table 1), with higher levels associated with later flare in small single-centre cohorts [45;88].

New Developments

To date, small patient numbers, disease heterogeneity and multiple outcome definitions have hampered validation of biomarkers in JIA. However, a number of collaborations have been established to facilitate and streamline international clinical data collection (many linked with biobanks) in order to build statistical power. In the UK, the recently launched MRC-funded UK-wide CLUSTER consortium provides a unique opportunity to explore, discover and validate biomarkers of treatment response or flare, and also define strata of childhood arthritis based upon treatment trajectories. Internationally, the consortium Understanding Childhood Arthritis Network (UCAN) has developed a worldwide network of translational researchers, standardising procedures for collecting, processing and accessing data. Similarly, Paediatric Rheumatology Collaborative Study Group (PRCSG), Childhood Arthritis and Rheumatology Research Alliance (CARRA) and Paediatric Rheumatology International Trials Organisation (PRINTO) are established, successful international research networks which have facilitated many multicentre studies, including clinical trials. Due to the efforts of these groups, we have seen the development of standardised outcome measures in JIA, the advancement of trial design, and in conjunction with the EMA and FDA, the development of legislation around drug testing and licensing in children. These have led to paediatric trials for many new biologic agents for JIA, a significant increase in the number of tested and licensed products for JIA and significant progress in treatment options. With this revolution and expanding choice in

treatment, the need for better biomarkers is even more critical. A majority of research has focused on predicting response to methotrexate or a first biologic, but over time we are seeing an increasing number of CYP treated with multiple biologics [89], leading to a need for more evidence to better inform our treatment pathways.

Before individual or panels of biomarkers can be adopted in routine clinical practice, it is crucial that further research is carried out to establish biological marker cut-off levels [86]. Furthermore, although there is also biomarker research on similar therapies in rheumatoid arthritis (RA), it might not be appropriate to use evidence from studies on RA for application in JIA. JIA and RA have distinct pathogenesis and differing disease evolution affected by growth and development. Biomarkers might also have age-dependent normal ranges [90;91].

We also need more evidence to inform longer-term maintenance and discontinuation of therapy. PREVENT JIA is an ongoing study recruiting patients from the United States and a number of countries in Europe to examine stratification of medication withdrawal in JIA using novel biomarkers such as S100A12. Decisions to discontinue treatment are further complicated by the unique circumstance of each CYP, which may include coinciding major life events (exams, transition to secondary school or university) that can trigger disease flare, traumatic experiences of previous active disease, or intolerable drug side-effects [86]. A predictive model, comprising clinical and biological markers, could aid clinicians, CYP and carers during their complex decision-making process [86].

Many treatment registries, such as the international Pharmachild study, the UK Biologics for Children with Rheumatic Diseases and the German BIKER/JUMBO registers are also providing further insight into the longer-term safety of treatments [92]. The paediatric rheumatology community is now working within a rich environment of clinical and biological data. This sets the JIA research community up for future collaborative projects to overcome current challenges and more accurately predict disease outcomes.

Conclusions

In a heterogeneous, complex disease such as JIA, it is likely that a panel of clinical and biomarker data are needed to provide the most accurate prediction of treatment response and flare post withdrawal of drugs. To date, studies have demonstrated that predictive values are

improved when combining the protein biomarkers S100A12 and high sensitive C-reactive protein [93], combining genetic markers MDR-1/ABCB1, MRP-1/ABCC1 and PCFT [94], and adding MRP8/14 to a model using demographic, clinical and patient-reported outcomes [63].

In the future, the establishment of larger datasets alongside the advent of genome- and proteome-scanning tools, the application of advanced statistical models as well as machine learning techniques, will accelerate discovery of novel biomarkers. As international registries collate more linked biological and clinical data, these opportunities to build complex models to stratify CYP will expand.

Contributions

SSW , RD and QW did the literature search and drafted the manuscript; all authors read and commented on the manuscript at each version; KH and LW co led the work, and approved the final submitted manuscript.

Declaration of interests

KH reports consultancy fees paid to the Institution and grants from Pfizer and Bristol Myers Squibb, outside the remit of this work; LW reports speaker fees from Pfizer, paid to the Institution and research grant from Abbvie Inc, outside the remit of this work. The other authors declared no conflicts of interest.

Table 1: Biological markers of treatment response and relapse after discontinuing treatment

Biomarker	Source	Stability		Findings	Evidence level
Protein					
S100A8/9 (MRP8/14)	Serum	Cold storage + rapid processing not required	Rx response	Higher levels prior to starting MTX associated with good response [59] *oligo, poly, ERA, ps*	Single centre cohort, 87 children
				Reduction of levels in response to MTX [60] *oligo, poly*	Single centre cohort, 22 children
				Reduction of levels in response to MTX [61] *sys*	Multi centre cohort, 12 children
				Higher levels prior to starting ETN/ADA associated with good response [62] *poly, oligo, ps, ERA*	Multi centre cohort, 88 children
				Reduction of levels in response to ETN/ADA [62] *poly, oligo, ps, ERA*	Multi centre cohort, 43 children
				Reduction of levels in response to ETN/ANA [61] *sys*	Multi centre cohort, 12 children
			Relapse	Higher levels prior stopping MTX associated with relapse [73] *oligo, poly, sys, ERA, ps*	Multi centre RCT, 364 children
				Levels prior to stopping ADA/ETN/IFX not predictive of relapse [87] *oligo, poly*	Multi centre cohort, 130 children
				Higher levels in relapse [61] *sys*	Multi centre cohort, 13 children
				Lower levels at 3mo prior to tapering ANA had a trend towards association with remission post ANA discontinuation [45] *sys*	Single centre cohort, 15 children
				Higher levels prior stopping ETN associated with relapse [62] *poly, oligo, ps, ERA*	Multi centre cohort, 26 children
				Lower levels prior to stopping MTX associated with remission [60] *oligo, poly*	Single centre cohort, 22 children
S100A12	Serum	Cold storage + rapid processing not required	Rx response	Higher levels prior to IA CS associated with poor response [63] *oligo*	Multi centre cohort, 22 children
				Reduction in levels in response to IA CS [63]	Multi centre cohort, 22 children

				oligo	
				Reduction in levels in response to MTX [63] *poly, oligo*	Multi centre cohort, 20 children
				Reduction in levels in response to ETN [63] *poly, oligo*	Multi centre cohort, 21 children
			Relapse	Higher levels associated with earlier relapse after MTX withdrawal [93] *oligo, poly, sys, ERA, ps*	Secondary analysis of multicentre RCT, 188 children
				Higher levels associated with earlier relapse after stopping ADA/ETN/IFX [87] *oligo, poly*	Multi centre cohort, 130 children
				Higher levels up to 6mo prior to clinical relapse [63] *oligo, poly, sys*	Multi centre cohort, 45 children
				Lower levels at 3mo prior to tapering ANAK associated with remission post ANAK discontinuation [45] *sys*	Single centre cohort, 15 children
				Higher levels on MTX and/or biologic associated with relapse off medication [88] *oligo, poly*	Single centre cohort, 22 children
TNFα/ETN complex	Serum	Rapid processing + storage required	**Rx response**	Increase in level at 6wk post starting ETN associated with 5yr ETN efficacy [64] *oligo, poly, ERA, undifferentiated*	Single centre cohort, 41 children
MMP-3	Synovial fluid	Rapid processing + storage required	**Rx response**	Higher levels prior to IA CS associated with response [65] *oligo, poly, ERA, Ps, UC-related, Turner-associated, TRAPS, lyme*	Single centre cohort, 26 children
VEGF	Serum	Cold storage + rapid processing required	**Relapse**	Higher levels on MTX and/or biologic associated with relapse off medication [88] *oligo, poly*	Single centre cohort, 22 children
hsCRP	Serum	Stable but delayed processing > 24 hours impairs accurate measurement [95]	**Relapse**	Levels did not differ between children with relapse and remission after MTX withdrawal [93] *oligo, poly, sys, ERA, ps*	Secondary analysis of multicentre RCT, 188 children

IL-18	Plasma	Diurnal variation, cold storage + rapid required	**Relapse**	Lower levels at 3mo prior to tapering ANAK had trend towards association with remission post ANAK discontinuation [45] *sys*	Single centre cohort, 15 children

Genetic					
ATIC	Blood	Cold storage + rapid processing not required	**Rx response**	2 SNPs associated with poor response + 1 SNP trend towards association of good response to MTX [68] *oligo, poly, ERA, ps, sys*	Multi centre cohort, 197 children
SLC16A7				3 SNPs associated with good response to MTX + 1 SNP associated with poor response to MTX [67] *oligo, poly, ERA, ps, sys*	Multi centre cohort, 197 children
ITPA				SNP associated with poor response to MTX [68] *oligo, poly, ERA, ps, sys*	Multi centre cohort, 197 children
ABCB1				1 SNP associated with good response to MTX [66] *poly, oligo, ERA, ps, sys*	Single centre cohort, 287 children
ABCC3				1 SNP associated with good response to MTX [66] *poly, oligo, ERA, ps, sys*	Single centre cohort, 287 children
SLC19A1				1 SNP associated with poor response to MTX [66] *poly, oligo, ERA, ps, sys*	Single centre cohort, 287 children
CACNA1I				1 SNP associated with response to MTX [69] *poly, oligo, ERA, ps, sys*	Multi centre cohort, 694 children
ZMIZ1				4 SNP associated with response to MTX [69] *poly, oligo, ERA, ps, sys*	Multi centre cohort, 694 children
TGIF1				2 SNP associated with response to MTX [69] *poly, oligo, ERA, ps, sys*	Multi centre cohort, 694 children
CFTR				3 SNP associated with response to MTX [69] *poly, oligo, ERA, ps, sys*	Multi centre cohort, 694 children

MRP: myeloid-related protein; TNF: tumour necrosis factor; VEGF: vascular endothelial growth factor; hsCRP: high-sensitivity C reactive protein; IL: interleukin; Rx: treatment; MTX: methotrexate; ANAK: anakinra; ETN: etanercept; ADA: adalimumab; IFX: infliximab; IA CS: intraarticular corticosteroid; oligo: oligoarticular juvenile idiopathic arthritis; poly: polyarticular juvenile idiopathic arthritis; ERA: enthesitis-related juvenile idiopathic arthritis; ps: psoriatic juvenile idiopathic arthritis; sys: systemic juvenile idiopathic arthritis; UC: ulcerative colitis; TRAPS: tumour necrosis factor receptor associated periodic syndrome; SNP: single nucleotide polymorphism; ATIC: 5-aminoimidazole-4-carboxamide ribonucleotide transformylase; SLC16A7: solute carrier family 16 member 7; ITPA: inosine triphosphate pyrophosphatase gene; ABC: adenosine triphosphate-binding cassette transporter; SLC19A1: solute carrier 19A1; CACNA1I; calcium voltage-gated channel subunit alpha1 I; ZMIZ1: zinc finger MIZ-type containing 1; TGIF1: TGFB induced factor homeobox 1; CFTR: cystic fibrosis transmembrane conductance regulator; RCT: randomised controlled trial

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