Predicting duration of beneficial effect of joint injection among children with chronic arthritis by measuring biomarkers concentration in synovial fluid at the time of injection

M. Cattalini[1], V. Maduskuie[2], P.T. Fawcett[3], A.M. Brescia[4], C.D. Rosé[4]

[1]Department of Pediatric Immunology and Rheumatology, Pediatric Clinic, University of Brescia, Brescia, Italy; [2]Alfred I. DuPont Hospital for Children, Wilmington, Delaware, USA; [3]Division of Immunology, Departments of Research, and [4]Division of Rheumatology, Department of Pediatrics, Thomas Jefferson University, Philadelphia, Pennsylvania, USA.

Abstract

Objectives

Intra-articular corticosteroids injection (IAC) is a mainstay for the treatment of children with chronic arthritis; nonetheless its efficacy showed variability among published studies and it is still not possible to predict the outcome in a single patient. Our objective was to study the profile of biomarkers in the synovial fluid (SF) obtained at the time of injection and establish if such profile predicts duration of effect.

Methods

SF obtained from patients who underwent knee arthrocentesis and injection was procured and stored for cytokine analysis. Records of those patients who had at least 6 months of follow-up from the injection were reviewed. Time to flare was recorded. Levels of IL-6, IL-1α, TNF-α, IL-2sR, MMP-3, IL-10 and TGF-β1 were measured by ELISA. For primary analysis each patient was utilized once. For secondary analysis each injected knee was considered a single event.

Results

60 samples from 33 patients were obtained. In the primary analysis we found a correlation between MMP-3 synovial fluid levels and outcome at 6 months (p=0,02; p=0,03 for different quartiles). In the secondary analysis we found that IL-6 and IL-10 levels predicted outcome at six and at 12 months (IL-6: p=0.01; p=0.02 respectively) (IL-10: p=0.017; p=0.01 respectively), with higher levels of IL-6 predicting shorter time to relapse and higher levels of IL-10 longer duration of corticosteroids effect.

Conclusions

Our study identified MMP-3 and possibly IL-6 and IL-10 as candidates for the development of a set of biomarkers to predict response to IAC among children with chronic arthritis at the time of injection.

Key words

Chronic arthritis, intra-articular injection, synovial fluid, biomarkers, treatment outcome.

Marco Cattalini, MD, Pediatrician
Victoria Maduskuie, BS, MT(ASCP)
Paul T. Fawcett, PhD, Ass. Prof. Pediatrics
AnneMarie Brescia, MD, Staff Rheumatologist
Carlos D. Rosé, MD, CIP, Prof. Pediatrics

Please address correspondence and reprint requests to:
Dr. Marco Cattalini,
Clinica Pediatrica,
Spedali Civili di Brescia,
Piazzale Spedali Civili 1,
25123 Brescia, Italy.
E-mail: marco.cattalini@gmail.com

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Introduction

Whereas intra-articular corticosteroids injection (IAC) has been in use for adult rheumatoid arthritis since 1951, paediatric rheumatologists only incorporated this treatment modality more recently. The first report dates from the early 80s (1). IAC is nowadays a mainstay for the treatment of children with chronic arthritis. In one survey conducted in 1999 among North American rheumatologists, the use of IAC was second only to NSAIDs for oligoarticular JIA (2).

The efficacy of this procedure, measured as duration of beneficial effect, showed variability among published studies (1, 3-16). Different clinical and laboratory parameters have been proposed to predict the response to IAC, however it is still not possible to predict the outcome in a single patient (1, 4, 5, 7-12, 15). This estimation will be of much interest to rheumatologists when considering IAC. Moreover, the identification of a biomarker which can predict the response to steroid injection could possibly provide clues for the understanding of disease mechanism. Among the clinical variables investigated, joint injected, type of arthritis (1, 8), age at onset (1, 15), duration of disease (1, 10) and drug used (11, 12, 15, 16) are more consistently found to have an effect on outcome in different studies, while for other variables results are more contradictory (9, 10),

To identify such a predictor of outcome we studied the profile of biomarkers in the synovial fluid obtained at the time of injection among paediatric patients with a variety of rheumatic disorders who needed intra-articular injection for treatment and who agreed to allow us to store remnant fluid obtained during arthrocentesis.

Material and methods

Study design

All patients with chronic arthritis who underwent knee arthrocentesis and injection, at the A.I. duPont Hospital for Children between 2001 and 2006, were invited to participate in a prospective IRB-approved study whereby remnant synovial fluid was procured and stored for analysis.

Only knee injections were considered for this study, and no other joints were injected simultaneously to the knee/s in the patients recruited.

Records of those patients who had at least 6 months of follow-up from the procedure were reviewed. Patient demographics were collected, including age, sex, diagnosis, age at diagnosis, number of joints involved, systemic therapy administered. In addition, time of injection, time of detection of arthritis in the specific joint since injection, knee injected, quantity of fluid aspirated, drug used, number of injections for each joint and time since previous injections, if applicable, were carefully recorded.

Joint findings were recorded at the time of the procedure, one week, six months and one year after injection, or before if required by a flare of arthritis. For the intensity of swelling we used a three-grade scale (S1: mild, meaning effusion without distortion of landmarks: S2: moderate, meaning effusion with distortion of the landmarks; S3: severe, meaning effusion without detectable landmarks). Time to flare was recorded as the number of days from injection to documentation of mild swelling or worse. For those who still showed no evidence of synovitis at the time of review the last available visit was used as a surrogate for flare.

Physical examination and joint injections were all performed by two experienced pediatric rheumatologists (A.M.B. and C.D.R.). Triamcinolone hexacetonide was used as the drug of choice, given at a dose of 1 mg/kg. During a shortage period of this preparation, triamcinolone acetonide was used instead, at the same dose.

Remnant synovial fluid from patients who underwent knee arthrocentesis and injection was stored at -80ºC until assayed. Levels of IL-1α, IL-10, IL-6, IL-2sR, TGF-β1, TNF-α, and MMP-3 were determined by quantitative ELISA (R&D Systems) according to manufacturer's instructions.

Statistical analysis

For the statistical approach we decided to analyze the obtained data through two different approaches: in the first run, called "primary analysis" each

Table I. characteristics of the population analyzed.

patient	age (yrs)	sex	diagnosis	number of joints	age at diagnosis (yrs)	knee injected	time since arthritis (days)	time to flare (days)
1	8.91	M	JIA	extended	1.75	Lx	42	393
2	16.88	M	JIA	oligo	13.09	Rx	14	600
3	8.73	W	JIA	oligo	2.77	Lx	21	review
	9.19					Rx	74	21
4	10.44	M	ERA B 27+	oligo	9.96	Rx	90	review
5	13.79	W	JIA	oligo	10.34	Lx	84	review
6	21.44	W	JIA	poli	13.13	Lx	25	review
7	7.97	M	chronic Lyme	oligo	5.13	Lx	90	135
8	10.99	W	JIA	extended	6.00	Rx	180	review
9	12.26	W	JIA	oligo	3.17	Rx	45	201
	12.95					Rx	50	435
	14.10					Lx	8	180
	14.10					Rx	8	180
	14.76					Lx	30	180
	14.76					Rx	30	180
	16.05					Lx	50	192
	16.05					Rx	50	192
10	15.04	M	JIA	oligo	8.01	Lx	178	159
11	17.28	M	AS	oligo	13.28	Rx	11	110
12	16.09	M	JIA	oligo	11.92	Lx	120	110
	16.09					Rx	120	75
	16.52					Lx	83	109
13	15.03	W	psoriatic	oligo	5.34	Rx	21	review
14	13.96	W	JIA	extended	1.27	Rx	545	120
	13.96					Lx	545	120
	14.45					Rx	60	60
	14.45					Lx	60	review
15	2.86	M	JIA	oligo	2.65	Lx	92	28
16	14.84	W	JIA	oligo	1.13	Lx	58	30
	14.97					Lx	40	291
17	9.53	W	JIA	extended	2.69	Lx	600	1008
18	8.23	W	JIA	poli	3.00	Rx	390	646
	10.10					Lx	12	40
	10.31					Lx	42	126
19	12.85	W	Turner	poli	6.25	Rx	488	40
	13.98					Rx	99	293
	15.06					Rx	106	7
	16.62					Rx	22	180
20	14.52	W	JIA	oligo	13.94	Rx	63	48
	14.90					Rx	92	review
21	11.81	W	JIA	oligo	3.24	Lx	13	139
	12.40					Lx	58	68
22	13.36	W	chronic Lyme	oligo	11.75	Lx	227	35
23	9.53	W	JIA	oligo	4.17	Rx	62	114
	9.93					Rx	29	139
	10.47					Rx	64	585
	12.26					Rx	57	125
	12.68					Rx	31	445
24	19.61	W	JIA	oligo	4.00	Rx	15	review
25	10.03	W	JIA	extended	2.83	Rx	120	34
	10.66					Rx	11	review
26	15.89	M	psoriatic	oligo	15.65	Rx	NA	141
27	9.94	M	TRAPS	poli	5.35	Rx	3	616
28	17.58	M	JIA	oligo	15.55	Lx	837	7
29	7.57	W	JIA	oligo	5.87	Lx	660	286
30	11.25	W	ERA B27-	oligo	9.95	Rx	168	183
31	16.93	W	Ulcerative Colitis	oligo	12.05	Rx	366	13
32	12.80	M	chronic Lyme	oligo	12.39	Rx	150	278
33	15.19	W	psoriatic	oligo	14.53	Lx	322	615
	16.91					Lx	22	review

patient was utilized once. Hence, in patients who underwent more than one injection the first event only was used for primary analysis. For those who had initially both knees injected, one of the two was arbitrarily chosen for primary analysis.

For the second run, called "secondary analysis" each injected knee was considered a single event.

Pearson's test was utilized to analyze categorical data, while multiple linear regression and logistic regression model were applied to analyze cytokine levels. All the statistical studies were performed using Sigma Stat for Windows, version 3.5 (Systat Software, Inc.).

Results

Sixty samples from 33 patients were obtained, twenty one patients had oligo- or polyarticluar JIA, one arthritis related to ulcerative colitis, three psoriatic arthritis, one Turner-associated arthropathy, one TRAPS, three Chronic Lyme Disease, three spondyloarthropaty. Among patients with oligo- or polyarticular JIA two had polyarticular disease, 14 oligoarticular and 5 extended oligo-articular. Baseline demographics of the population analyzed are shown in Table I.

Of all the 33 patients, 24 had been on some systemic drug during the observation period. In particular 9 patients had been on methotrexate, 7 of them were put on methotrexate at a mean of 555 days before injection (range 21-2398), while in one patient methotrexate was added at the time of injection, and in one patient 27 days after. Systemic therapy and steroid injected for each patient are shown in Table II.

Primary analysis

Among the thirty-three patients who underwent knee injection seven patients had had a previous injection before the fluid collection was started, thus only twenty-six patients were considered for primary analysis.

Patients were injected at a mean time of 190,7 days, from the onset of arthritis in the injected joint and median time was 106 (range 3-837) days.

Mean time to flare was 361.5 (range 7-1530) days from injection, with a median of 171 days (range 7-1530). Fifty

Table II. Systemic therapy and steroid injected in the population analyzed.

Pt	sample	pre	TH	post	TX	Time since Inj	Steroid Injected
1	1	2398	MTX	cont	MTX		TH
2	2	804	Vioxx	0	0		TH
3	3	0	0	0	0		TH
3	4	21	Ibu	inj	MTX		TA
4	5	0		0	0		TH
5	6	84	ibu	0	0		TA
6	7	90	tolectin	0	0		TH
7	8	0	0	0	0		TA
8	9	60	napr	0	0		TH
9	10	0	0	0	0		TH
9	11	50	tolectin	0	0	160	TH
9	12	0	0	0	0		TA
9	13	0	0	0	0	515	TA
9	14	0	0	0	0	240	TA
9	15	0	0	0	0	240	TA
9	16	0	0	0	0	475	TH
9	17	0	0	0	0	475	TH
10	18	napr	178	0	0		TA
11	19	indo sulfa	489 806	see pre	see pre		TA
12	20	pred	11	MTX	27		TH
12	21	pred	11	MTX	27		TH
12	22	MTX	50	cont	MTX	158	TH
13	23	MTX	21	cont	MTX		TH
14	24	0	0	0	0		TA
14	25	0	0	0	0		TA
14	26	0	0	0	0	180	TA
14	27	0	0	0	0	180	TA
15	28	napr	62	0	0		TH
16	29	0	0	0	0		TA
16	30	40	napr	cont	napr	49	TH
17	31	573	MTX	cont	MTX	608	TH
18	32	887 1760	MTX napr	cont	MTX		TA
18	33	1573 12	MTX predn	cont	MTX		TH
18	34	1649 76	MTX napr	cont	MTX napr	76	TH
19	35	57	vioxx	cont	inj	1202	TH
19	36	380	tolectin	cont	tolectin	421	TH
19	37	779	tolectin	cont	tolectin	399	TA
19	38	1360	tolectin	cont	tolectin	581	TH
20	40	58	napr	0	0	154	TA
20	41	109	MTX napr	cont	MTX	140	TA
21	42	13	napr	cont	napr		TA
21	43	58	tolectin	cont	tolectin	219	TA
22	44	328	napr	cont	napr	238	TH
23	45	240	napr	cont	napr		TH
23	46	343	napr	cont	napr	143	TH
23	47	586	napr	0	0	203	TH
23	48	56	napr	cont	napr	664	TH
23	49	212	napr	0	0	156	TH
24	50	1070	relafen	0	0	293	TH
25	51	0	0	0	0	0	TA
25	52	195 124	MTX Napr	0	0	233	TH
26	53	85	napr	0	0	0	TH
27	54	275	enbrel	cont	enbrel	0	TH
28	55	0	0	0	0	0	TA
29	56	124	napr	0	0	0	TA
30	57	45	napr	cont	napr	0	TH
31	58	210	MTX	cont	MTX	0	TH
32	60	0	0	0	0	0	TH
33	61	64	tolectin	cont	tolectin	0	TH
33	62	701	tolectin	0	0	637	TA

Table III. synovial fluid cytokine concentrations (divided into quartiles) and outcome at 6 months: results from logistic regression analysis.

Variable	Coefficient	Standard Error	Z	*p*-value
IL-6 (1^{st}-2^{nd})	0.88	0.99	0.88	0.38
IL-6 (3^{rd}-4^{th})	-0.23	1.01	-0.23	0.82
IL-1α (1^{st}-2^{nd})	-0.96	0.98	-0.98	0.32
IL-1α (3^{rd}-4^{th})	-1.54	1.07	-1.42	0.153
TNF-α (1^{st}-2^{nd})	0.6	1.08	0.55	0.58
TNF-α (3^{rd}-4^{th})	0.01	0.98	0.02	0.99
MMP-3 (1^{st}-2^{nd})	-3.2	1.35	-2.37	0.02
MMP-3 (3^{rd}-4^{th})	-2.76	1.27	-2.16	0.03
IL-10 (1^{st}-2^{nd})	0.45	1.02	0.44	0.66
IL-10 (3^{rd}-4^{th})	-0.82	0.99	-0.83	0.41
TGF-β1 (1^{st}-2^{nd})	-1.85	1.11	-1.67	0.09
TGF-β1 (2^{nd}-3^{rd})	-0.37	0.99	-0.38	0.71

Table IV. synovial fluid cytokine concentrations (divided into quartiles) and outcome at one year: results from logistic regression analysis.

Variable	Coefficient	Standard Error	Z	*p*-value
IL-6 (1^{st}-2^{nd})	1.03	1.04	0.98	0.32
IL-6 (3^{rd}-4^{th})	0.29	0.99	0.29	0.77
IL-1α (1^{st}-2^{nd})	0.22	1.04	0.21	0.83
IL-1α (3^{rd}-4^{th})	-0.40	0.98	-0.41	0.68
TNF-α (1^{st}-2^{nd})	0.07	1.08	0.06	0.95
TNF-α (3^{rd}-4^{th})	-0.62	0.96	-0.65	0.51
MMP-3 (1^{st}-2^{nd})	-2.08	1.27	-1.63	0.10
MMP-3 (3^{rd}-4^{th})	-1.86	1.25	-1.48	0.14
IL-10 (1^{st}-2^{nd})	-0.74	1.08	-0.68	0.49
IL-10 (3^{rd}-4^{th})	-1.03	1.04	-0.98	0.32
TGF-β1 (1^{st}-2^{nd})	-1.25	1.07	-1.17	0.24
TGF-β1 (2^{nd}-3^{rd})	-0.56	1.07	-0.52	0.60

Table V. synovial fluid cytokine concentrations as predictors of outcome: multiple linear regression model results.

Variable	Coefficient	Std. Error	t	*p*-value
IL-6	-0.015600	0.007360	-2.119000	0.039000
IL-1α	166.167000	77.434000	2.146000	0.037000
TNF-α	-0.036600	0.199306	-1.277000	0.143750
IL-2sr	0.080556	0.090278	0.617361	0.262500
MMP-3	-0.702000	3.414000	-0.206000	0.581944
IL-10	5.865000	2.519000	2.328000	0.024000
TGF-β1	0.250000	0.377778	0.459028	0.354861

percent of patients had a good outcome six months after the injection. At twelve months, 34.5% of patients still did not show a flare in the injected joint.

Patients were then divided into two outcome groups depending on the status of the injected joint six months after the procedure: patients with no signs of flare six months after the procedure were defined as "good responders", while patients with a flare within 6 months from injection were defined as "bad responders". A Logistic Regression was utilized to analyze cytokine levels as predictors of outcome. MMP-3 showed an inverse correlation with outcome, with higher levels of MMP-3 predicting better outcome. Levels of IL-6, IL-1α, TNF-α, IL-10 and TGF-β1 showed no statistical correlation with outcome at six months (Table III).

We then divided the patients in "good responders" and "bad responders" according to the status of the injected joint one year after the injection. The logistic regression model failed to find any significant correlation between cytokine concentration and outcome, in particular the correlation between MMP-3 and outcome observed at six months of follow-up was not present at one year (Table IV).

Secondary analysis

All the samples obtained were utilized for secondary analysis.

Patients were injected at a mean time of 133 days from the detection of arthritis, with a median time of 187 days (range 3-837). The patients injected remained free from arthritis for a mean period of 367 days since injection; median time without a flare was 187.5 days (range 7-1530). At six months from injection, fifty-eight percent of patients did not have a flare in the injected joint, at one year still thirty-eight percent of patients maintained improvement.

Pearson analysis showed no correlation between the two main clinical variables investigated: time of detection of arthritis since injection and duration of corticosteroid effect (namely time to flare), nor there was a correlation between time of detection of arthritis since injection and cytokine levels.

A multiple linear regression model was utilized with time to flare in days as the dependent variable and cytokine concentrations as independent variables. We found that levels of IL-6; IL-1α and IL-10 predicted the selected outcome (Table V).

Patients were then segregated into outcome groups depending on the status of the injected joint at 6 and at 12 months from injection. A logistic regression was utilized to analyze cytokine levels as predictors of outcome. Levels of IL-6 and IL-10 predicted outcome at 6 months and at 12 months. In particular IL-6 showed an inverse relationship, with higher levels of IL-6 at the time of injection predicting shorter time to relapse, while IL-10 showed a direct relationship, with higher levels of IL-10 predicting longer duration of corticosteroids effect (Tables VI and VII).

Discussion

IAC injection is an important tool in the treatment of children with chronic arthritis, and JIA in particular (17).

The use of corticosteroids in an inflamed joint rapidly resolves synovitis, protects against deformity, resolves contractures and alleviates pain, thus allowing an early physiotherapy program (18). In addition IAC have been shown to prevent leg-length discrepancy in patients with oligoarthritis (19).

Usually the efficacy of steroids injection is measured by the period after the procedure elapsed without signs or symptoms of arthritis in the affected joint.

The percentage of good responders after six month from injection varies between 60% and 82% of children treated (1, 4, 7, 9-11, 16), while 16.1% to 63.6% of patients are reported to have a good response 2 years after the injection (1, 11, 13, 16). These discrepancies attest to the heterogeneity of the different studies, which is confirmed by the common experience that, while some patients show no signs or symptoms of arthritis more than one year after the procedure, others have a flare few weeks after the injection.

The efficacy of the injections observed in our population is slightly worse than what is observed in the literature: we obtained a rate from 50% to 58% (respectively for primary and secondary analysis) of good responders at 6 months, and a percentage from 34.5% to 38% at one year, while published studies reports that more than 60% of patients maintained remission six months after injection, and 30 to 80 percent one year after (11, 13, 16). Since drug used, dosage and technique adopted in this study are comparable to what described in the literature, poorer outcome may be related to patient selection. We should be reminded that those studies pertain to JIA only and the efficacy of treatment may vary in different diseases.

Different clinical and laboratory markers have been advocated for use in determining the outcome of the injection. Joint injected, type of arthritis, duration of disease and drug used are the variables more consistently found in correlation with injection outcome (1, 8, 10-12, 15, 16).

For other factors, more uncertainty exists: ANA, PMN fluid percentage and ESR correlate with injection outcome in some studies, but these observations are not universally confirmed: Lepore *et al*. (10) demonstrated that ANA negative patients have a longer remission after the injection, but other studies where ANA were considered as a marker of outcome did not show the same results (1, 4, 11). Honkanen *et al*. (5) found that the higher the PMN percentage in the fluid the shorter the time to relapse, but Padeh *et al*. (7) did not confirm this finding. Ravelli *et al*. (9) reported that patients with higher ESR at the time of injection are the one with a better response, but again this was not confirmed by others (4, 5, 7, 11). Finally, Vivarelli *et al*. found a single nucleotide polmorfism in the Macrophage Migration Inhibitor Factor (MIF) gene which correlate with duration of effect of steroid injection in children with oligoarticular-JIA (20).

Table VI. synovial fluid cytokine concentrations as predictors of outcome at 6 months: logistic regression model results.

Variable	Coefficient	Standard Error	*p*-value
IL-6	0.000290	0.000112	0.010000
IL-1α	-1.299000	0.606250	0.095139
TNF-α	0.000542	0.000986	0.404861
IL-2sr	-0.000985	0.000969	0.215278
MMP-3	-0.039200	0.189583	0.104861
IL-10	-0.055400	0.160417	0.017000
TGF-β1	-0.000615	0.000629	0.227778

Table VII. synovial fluid cytokine concentrations as predictors of outcome at one year: logistic regression model results.

Variable	Coefficient	Standard Error	*p*-value
Constant	6.814000	6.395000	0.199306
IL-6	0.000225	0.000096	0.020000
IL-1α	-0.593000	0.392361	0.204167
TNF-α	0.000364	0.000873	0.469444
IL-2sr	-0.000345	0.000795	0.461111
MMP-3	-0.018800	0.163194	0.293750
IL-10	-0.053500	0.144444	0.010000
TGF-β1	-0.000425	0.000426	0.221528

In the primary analysis of our study we found an association between MMP-3 fluid concentrations and injection outcome at 6 months, with higher levels of MMP-3 predicting more favorable response to steroid injection. MMP3 belongs to a large family of Zn dependent endoproteases (MMPs) that degrade most extracellular matrix components (21). The concomitant evaluation of MMP-3 in sera and SF showed a clear overexpression of this protease at the site of chronic inflammation, with higher levels of MMP-3 in the fluid samples from patients with active disease (22). Thus, MMP-3 was identified as candidate acute phase reactant in childhood arthritis, as postulated for RA (21, 23-25) To our knowledge, the possible predicting role of MMP-3 fluid levels on injection outcome in a pediatric population has not been noted before. Our study was not designed to investigate the mechanism through which joints with higher MMP-3 levels respond better to steroid injection. We can only speculate that higher MMP3-SF levels reflect a window in the course of knee arthritis more responsive to intra-articular injection of corticosteroids.

In the secondary analysis we found that IL-6 and IL-10 levels predicted outcome, with higher levels of IL-6 predicting shorter time to relapse and higher levels of IL-10 predicting longer duration of corticosteroids effect. This could have resulted from an enlarged sample size.

The use of the same patient more than once in this part of the analysis can be regarded as problematic. Conversely an episode of arthritis in a single patient may or may not represent the same

biological state. In fact, experienced clinicians know that the effect of a given injection varies widely in the same patients at different time points. The authors believe it is legitimate then to treat those injections as separate events for the clinical aim of the study (predicting response to IA injection).

For a similar reason, we decided to include in our study patients with different type of chronic arthritis, since – in our opinion – these conditions, although very different, may still share common downstream pathways of chronic inflammation, hence a common clinical biomarker is not only biologically plausible but also clinically practical. We do think that stratification of our results for each type of chronic arthritis would add strength to our study. Unfortunately the number of patients was too small for such an approach.

The use of methorexate in some of our patients could be seen as a source of bias in the evaluation of response to steroid injection, since this drug could add a 'protective' factor to the anti-inflammatory effect of IAC. However at the time of the injection all patients had acute inflammation, suggesting failure of methotrexate to control disease; a modulating effect however has to be considered. Still, an independent predicting biomarker should be useful across all possible real-life clinical scenarios, one of which is synovitis despite methotrexate therapy.

IL-6 and IL-10 are pro-inflammatory and anti-inflammatory cytokines respectively. Given our results from secondary analysis it can be argued that IL-10 high levels reflect a suppressive response to a state of active inflammation, to which the addition of a potent anti-inflammatory drug adds a synergistic anti-inflammatory effect (26-37).

Thus a combination of IL-6 and IL-10 levels, obtained at the time of injection, may be a valuable tool in predicting success of IAC.

In conclusion our study identified for the first time possible candidates (MMP-3, IL-6 and IL-10) for the development of a set of biomarkers to predict response to IAC among children with chronic arthritis at the time of injection. In addition, MMP-3 appears as an important actor in paediatric synovitis. We strongly believe that the levels of a single cytokine will be of little use in the task of predicting therapy outcome; therefore a prospective study for the development of a composite biomarker score involving a three-centre collaborative effort is currently underway. Development of such predictive score will probably involve clinical, serological, synovial fluid cytology and synovial fluid cytokine biomarkers as primary components.

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