Online supplement to S100A12 Is Associated with Response to Therapy in Juvenile Idiopathic Arthritis, *The Journal of Rheumatology*, doi:10.3899/jrheum.170438

Supplementary Table 1. Baseline demographics and characteristics in all responders and non-responders.

	MTX-treated patients (n=75)		**Anti-TNF-treated patients (n=88)**	
	Responders (n=57)	Non-responders (n=18)	Responders (n=66)	Non-responders (n= 22)
Baseline demographic				
Age at JIA onset in years, median (IQR)	6.1 (2.6-11.0)	4.5 (2.4-10.5)	10.0 (4.2-12.3)	9.4 (3.1-13.7)
Disease duration at therapy start in years, median (IQR)	1.3 (0.4-4.7)	2.2 (0.7-3.8)	2.4 (1.1-4.9)	2.3 (0.8-7.7)
Female, n (%)	39 (68)	13 (72)	48 (73)	18 (82)
Anti-TNF therapy,				
Etanercept, n (% of all Etanercept)	n/a	n/a	61 (75)	20 (25)
Adalimumab, n (% of all Adalimumab)	n/a	n/a	5 (71)	2 (29)
JIA Category at start, n (%)				
Oligoarticular persistent	7 (12)	6 (35)	2 (3)	3 (14)
Oligoarticular extended	15 (26)	2 (12)	16 (24)	8 (36)
Polyarticular RF-	23 (40)	6 (35)	26 (39)	7 (32)
Polyarticular RF+	5 (9)	1 (6)	11 (17)	2 (9)
Enthesitis-related arthritis	5 (9)	1 (6)	3 (5)	1 (5)
Psoriatic	2 (4)	1 (6)	8 (12)	1 (5)
Clinical variables at baseline				
Active joints, n	5 (2-10)	4 (2-5)*	11 (5-18)	8 (2-16)
CHAQ score (0-3)	1 (0.31-1.75)	0.81 (0.25-2.06)	1.49 (0.75-2.13)	1.35 (0.63-1.96)
ESR (mm/h)	25 (10-69)	19 (8-35)	16 (9-30)	12 (7-18)
JADAS-10 (0-40), median (IQR)	14 (8-23)	10 (7-14)	20 (14-23)	17 (11-22)
S100A12 at baseline, median (IQR), ng/ml	240 (125-615)	150 (87-233)*	308 (150-624)	151 (83-201)**

Abbreviations: **MTX** methotrexate, **anti-TNF** anti-tumour-necrosis factor therapy, **JIA** juvenile idiopathic arthritis, **CHAQ** Childhood Assessment Questionnaire, **ESR** erythrocyte sedimentation rate**, JADAS-10** Juvenile Arthritis Disease Activity

*/** indicates significance between responders and non responder within MTX treated patients, or within anti-TNF treated patients as follows: *p< 0.05, **p< 0.005 (Mann Whitney U).

Supplementary Table 2. S100A12 concentrations measured by commercial *CircuLex* ELISA.

Performance of in-house assay versus Circulex assay
MTX: S100A12 concentrations measured by the in house ELISA assay significantly correlate with *CircuLex* measured concentrations (Spearman's rho: 0.85, p< 0.001).
Anti-TNF: S100A12 concentrations measured by the in house ELISA assay significantly correlate with *CircuLex* measured concentrations (Spearman's rho: 0.687, p<0.001).
S100A12 levels at baseline and response to treatment
Baseline S100A12 serum levels were higher in responders (median 720 (IQR 320-1765) compared to non-responders (median 417, IQR 243-818, p=0.039) for MTX treated patients (Figure 1A).
For anti-TNF treated patients, baseline S100A12 serum levels were also higher in responders (median 407, IQR 212-710) compared to non-responders (median 239, IQR 150-436, p=0.020).
In a univariate logistic regression this resulted in an OR of 1.06 for MTX therapy (95%CI 1.004-1.115, and an OR of 1.14 (95% CI: 1.01-1.28) for achieving at least an ACRpedi 50 response per 50 units of S100A12 (*CircuLex*)(ng/ml) for anti-TNF therapy.
Prediction of response corrected for other variables
Baseline S100A12 serum levels were significantly associated with change in JADAS-10 in a univariate linear regression analysis (β = -0.149, 95% CI -0.298 to -0.0007, p=0.050 per 50 units change in S100A12 for anti-TNF treated patients. For MTX this was: β = -0.159 (95% CI -0.264 - -0.053) In the corrected multivariable analysis the corrected β was -0.089 per 50 units increase in ng/ml, 95% CI -0.212 to 0.034 for anti-TNF therapy. The change in explained variance was 1.4% (not significant). Multivariable analysis: corrected beta for MTX: -0.102 (95% CI: -0.139 - -0.039), the change in explained variance was 5.3% (p=0.002).
Multivariate models constructed with known predictors of response as shown in the method were performed to test their association of with JADAS-10 score for each treatment group. Without S100A12, the variables in the model explained 70 % (equal to that as measured by in-house ELISA) of the variance in change in JADAS-10 at follow-up for MTX-treated patients, and 50 % (also the same as with the in-house ELISA) for the anti-TNF group. Including S100A12 as a variable increased the models prediction by 5.3% (more than the 2% with the in-house ELISA) for MTX and 1.4% (vs 5% with the in-house ELISA for anti-TNF treated groups.

Use of S100A12 as a prognostic marker for response to treatment
The *CircuLex* ELISA was less accurate compared to the in-house ELISA for predicting response to anti-TNF treatment and MTX, shown in Supplementary Table 3.

Supplementary Table 3. Sensitivity, specificity and likelihood ratios for the determined cut-off of S100A12 predicting response to MTX anti-TNF treatment, *CircuLex* ELISA.

	CircuLex **ELISA: MTX**	*CircuLex* **ELISA: anti-TNF**
Cut-off level S100A12 (ng/ml)	846	508
Sensitivity	45.6	39.4
Specificity	83.3	86.4
Positive likelihood ratio	2.7	2.9
Negative likelihood ratio	0.7	0.7
Youden index	0.289	0.258
AUC	0.662 (0.532-0.791)	0.675 (0.550-0.800)

AUC= area under the curve.