Tryptophan metabolism in rheumatoid arthritis is associated with rheumatoid factor and predicts joint pathology evaluated by the Rheumatoid Arthritis MRI Score (RAMRIS)

G. Pongratz[1], T. Lowin[1], P. Sewerin[1], F. Zaucke[2], Z. Jenei-Lanzl[2], T. Pauly[3], B. Ostendorf[1], M. Schneider[1]

[1]Department of Rheumatology and Hiller Research Center for Rheumatology, University Hospital Düsseldorf, Germany; [2]Experimental Orthopaedics, Department of Orthopaedic Surgery Friedrichsheim, University Hospital Frankfurt, Germany; [3]Department of Orthopaedics, River Rhein Center for Rheumatology at St. Elisabeth Hospital, Meerbusch-Lank, Germany.

Abstract

Objective

Tryptophan and its metabolites have been suggested to play a role in inflammatory processes. However, studies in rheumatoid arthritis (RA) are scarce, which prompted us to investigate two cohorts of RA patients to better understand the importance of tryptophan metabolism in this disease.

Methods

Tryptophan and its metabolites were characterised by ELISA in a cross-sectional cohort 1 (81 RA, 55 OA) and a longitudinal cohort 2 (25 RA, 3 visits over 6 months) to investigate discriminatory power between diseases and predicitive value for radiologic outcome, respectively. Radiologic outcome was monitored by RA MRI Score (RAMRIS), including grading of synovitis, bone oedema and erosion, as well as delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) index assessing cartilage quality of the MCP II joint.

Results

RA patients showed higher levels of serum serotonin (RA: 206.8 ng/ml ± 156.7; OA: 81.2 ng/ml ± 63.6) and estimated indoleamine (2,3)-dioxygenase (IDO) activity (kynurenine / tryptophan ratio; RA: 0.065±0.067; OA: 0.021±0.010). IDO activity showed similar, or better discriminatory power between RA and OA (AUC 0.914) than anti-CCP antibody level (AUC 0.922) and rheumatoid factor (RF, AUC 0.783), respectively. In cohort 2, regression analysis revealed a predictive value of baseline serotonin levels and IDO activity for changes in RAMRIS score and erosions at month six, respectively.

Conclusion

This study supports the hypothesis that tryptophan and its metabolites can be used as biomarkers predicting radiologic outcome and discriminate between RA and OA patients. Overall, our results strengthen the notion that tryptophan metabolism is closely linked to RA disease mechanisms.

Key words

serotonin, IDO, kynurenine, tryptophan, rheumatoid arthritis, prognostic marker, rheumatoid factor, anit-CCP, MRI, RAMRIS, dGEMRIC

Georg Pongratz, Prof. Dr
Torsten Lowin, Dr rer nat
Philipp Sewerin, Dr med
Frank Zaucke, Prof. Dr
Zsuzsa Jenei-Lanzl, Dr rer nat
Thomas Pauly, PD Dr med
Benedikt Ostendorf, Prof. Dr
Matthias Schneider, Prof. Dr

Please address correspondence to:
Prof. Georg Pongratz,
Poliklinik, Funktionsbereich und Hiller Forschungszentrum für Rheumatologie,
UKD, HHUD Life Science Center,
Merowingerplatz 1A,
40225 Düsseldorf, Germany.
E-mail:
georg.pongratz@med.uni-duesseldorf.de

Received on March 9, 2018; accepted in revised form on July 23, 2018.

The datasets analysed during the current study are available from the corresponding author on reasonable request.

Funding: this work was supported by an unlimited grant by the Hiller Foundation and by a grant from the German "Bundesministerium für Bildung und Forschung" (BMBF, ArthroMark (01EC1009))

Competing interests: the authors received ELISA kits to determine tryptophan and its metabolites as a gift from Neuroimmun GmbH, Karlsruhe. However, the company had no influence over the data analysis and interpretation.

Introduction

Rheumatoid arthritis (RA) is a chronic inflammatory disease which is characterised by autoantibody production, joint destruction and disability (1). Healthy synovial tissue is comprised of synovial fibroblasts (SF) and macrophages, whereas lymphocytes migrate to the joint in the course of RA. Pro-inflammatory cytokines transform SF into a "tumor-like" phenotype with the capacity to degrade cartilage and bone (1, 2). One major goal in caring for patients with RA is to prevent joint destruction by immunosuppressive therapy (1). Easy to determine markers that allow predicting risk for joint destruction are useful for the management of RA patients since use of more stringent immunosuppressive regimens, *e.g.* biologics could be guided by such biomarkers. Sensitive imaging modalities, like magnetic resonance imaging (MRI), support evaluation of outcome and prognostication of disease course (3). Modern techniques even allow non-invasive fine assessment of finger joint cartilage degeneration in RA using Delayed Gd(DTPA)2-enhanced MRI of cartilage (dGEMRIC) (4, 5).

Already twenty years ago, it was reported that synovial fluid of RA patients shows highest indoleamine (2,3)-dioxygenase (IDO) activity and lowest tryptophan (TRP) as compared to gout, psoriatric arthritis (PsA), or osteoarthritis (OA) (6). IDO, which metabolises tryptophan to kynurenine, was clearly increased in lining layer of inflamed RA synovial tissue (7). In serum of treatment naïve RA patients TRP levels were decreased and kynurenine (KYN) was increased suggesting an increased activity of IDO (8). These changes are persistent even after treatment (8, 9) and associated with disease stage (10) consistent with a role of IDO activity in RA pathogenesis. Inhibiting IDO activity results in aggravated collagen-induced arthritis in mice (11). It has even been speculated that one mechanism by which shared epitope contributes to pathogenesis of RA involves inhibition of IDO (12, 13). However, in the K/BxN murine RA model which is more dependent on B cell antibody response, IDO inhibition results in amelioration of disease, possibly by paradoxically inhibiting B cell responses as opposed to innate and T cell responses (14).

TRP is a limiting and essential amino acid, and deprivation of TRP by IDO is one anti-inflammatory mechanism, as observed during pregnancy to achieve tolerance of the fetus (15) or in a fibroblast co-culture system showing Th1-inhibiting potential (16). Anti-inflammatory activity of IDO is also suggested by poor prognosis of several tumors which upregulate IDO to escape immune elimination (17). Besides degradation of TRP, anti-inflammation by IDO might be mediated via its product KYN which binds to the aryl hydrocarbon receptor (AHR) stimulating anti-inflammatory pathways (18) but also increasing bone resorption (19). Therefore, rather than suggesting a mere beneficial role for IDO in autoimmunity, IDO is more viewed as context-dependent modifier.

TRP levels are not only decreased by IDO activity, since it also serves as precursor of serotonin (SER) (20), a step initiated by tryptophanhydroxylase (TPH), which generates 5-hydroxytryptophan that gets further metabolised to SER. SER is linked to mood disorders linking IDO pathway to depression and fatigue (21). In models of arthritis, *i.a.* SER results in aggravation of synovial inflammation and a SER antagonist resulted in reduced local inflammation (22). In RA patients, SER serum levels are positively associated with erosions at the temporomandibular joints (23). A direct link between bone loss and SER was established in animal models showing a direct inhibitory function of SER on osteoblast activity (24), findings that correspond with reports showing negative association of bone mass with SER in postmenopausal RA patients (25). SER also has a direct effect on immune cells, which can be anti- or proinflammatory, depending on cell type, context, and receptors involved (26).

As presented above, multiple evidence points towards an involvement of TRP and its metabolites in several aspects of RA. Therefore, we investigated the potential of these metabolites to discrimi-

nate between RA and OA and predictive outcome in RA.

Materials and methods

Patient cohorts

Two cohorts of patients were analysed. Cross-sectional cohort 1 consisted of 55 patients with long-standing RA fulfilling ACR/EULAR revised criteria for RA (27) and 81 patients with long-standing OA who underwent elective joint replacement surgery.

Longitudinal cohort 2 comprised 25 patients with RA again classified according to the ACR/EULAR revised criteria for RA. This cohort was extracted from the arthromark study (28), where RA patients undergo high-field (3 Tesla) MRI (Magnetom Trio A Tim System; Siemens Healthcare, Erlangen, Germany) of the dominant hand, affected by RA at 0, 3, and 6 months using a 4-channel flex coil.

The MRI protocol includes a coronal short tau inversion recovery (STIR)-, T1-weighted turbo spin echo (TSE)-, and T1 weighted 3D fast low angle shot (T1w-3D-FLASH)-sequence for T1 mapping using a dual flip-angle approach. dGEMRIC was acquired with inversion recovery fast spin-echo sequences for 15 minutes. The application of the variable flip angle (VFA) method to dGEMRIC lead to shorter acquisition times and facilitated high resolution assessment of small joints (4, 5, 29). This dGEMRIC cartilage assessment is based on the fact that negatively charged glycosaminoglycan (GAG), a main component of hyaline cartilage, hinders the accumulation of Gd(DTPA)2-. Therefore, the T1-relaxation time of cartilage (dGEMRIC Index, [ms]) is a measure of GAG loss (30).

RAMRIS score was determined according to OMERACT guidelines, in consensus (one radiologist with at least six years' experience in musculoskeletal imaging and one rheumatologist with five years' experience in musculoskeletal imaging, both blinded to all relevant patient data) (31).

Patient characteristics are summarised in Table I.

ELISA to determine anti-CCP, tryptophan, serotonin and kynurenine in serum

Serum samples were taken at 0, 3 and 6 months before MRI readings from initially treatment naïve, active patients as described in (28), or at the time of elective surgery in a non-active state, and were stored at -80°C until further processing. L-tryptophan and L-kynurenine were detected by IDKs IDO activity ELISA kit (K7726, Neuroimmun GmbH, Karlsruhe, Germany), serotonin was detected by IDKs serotonin ELISA kit (K6880, Neuroimmun GmbH, Karlsruhe, Germany). Anti-CCP antibodies were detected using a commercially available assay (Anti-CCP hs (high sensitive)®, Orgentec diagnostic, Mainz, Germany). All assays were conducted according to the manufacturer's protocols.

Statistical analysis

Statistical analysis was performed with SPSS 24 (IBM, Armonk, USA). The general level of significance was $p<0.05$ and two-sided tests were performed. To minimise statistical error, non-normally distributed variables (Shapiro-Wilk test) were logarithmically transformed before parametric tests as indicated. To analyse discriminatory value of variables, ROC analysis was performed. To investigate the predictive value of baseline measurements for outcomes after six month, linear regression models were fitted.

Ethics approval and consent to participate

Both studies were approved by the Ethics Committee of the University of Düsseldorf cohort 1: no. 2018-88-KFogU; cohort 2: no. 3828) and in case of cohort 2 also by the ethics committee of

Table I. Patient characteristics of cross-sectional cohort 1 and longitudinal cohort 2.

	Cross-sectional cohort 1					longitudinal cohort 2	
	OA	range	RA	range	*p*-value (MWU)	RA	range
Number (n) / visit	81		55			25	
Female sex (%)	71.6		80			66.3	
median age (yr)	72	49 - 88	67	22 - 85	0.03	55	
median RF (U/ml)	5.8	0.2 - 25.3	29.3	1.2 - 567.2	< 0.001		
cDMARD	0		36				
one	0		31			25 (MTX)	
two	0		5				
bDMARD			15				
anti TNF	0		13				
anti IL-6R	0		2				
Prednisolon (mg/d)	0		5	2.5 - 8.0			
median CRP (mg/dl)						0.3	0.1 - 3.7
median DAS28						3.7	1.4 - 6.3
median RAMRIS						25	9 - 59
median dGEMRIC							
median no. of erosions						7	0 - 26
median dGEMRIC [ms]						360.1	189.5 – 529
Serotonin [ng/ml]						206.8	17 - 975
Tryptophan [µmol/l]						55.5	40 – 79.5
Kynurenine [µmol/l]						2.2	0 – 9.9

the Charité Berlin (EA1/193/10). The studies were conducted in accordance with the principles of the Declaration of Helsinki and the International Conference on Harmonisation Guidance for Good Clinical Practice. All patients were informed about the purpose of the study and gave written consent upfront.

Results

RA patients show increased serum tryptophan / kynurenine ratio (estimated IDO activity) as compared to OA patients and association of tryptophan metabolism with rheumatoid factor (RF), but not anti-CCP

Average SER was increased (Fig. 1A, $p<0.001$) whereas TRP levels were markedly decreased (Fig. 1B, $p<0.001$) in serum of patients with RA as compared to OA. KYN did show the least, but also significant difference, with increased serum levels in RA patients (Fig. 1C; $p=0.001$). As expected, IDO activity, estimated by TRP / KYN ratio, was also increased in RA patients as compared to OA (Fig. 1D).

Further analysis revealed a negative association between rheumatoid factor (RF) and TRP (-0.369, $p=0.006$), as well as a positive association between estimated IDO activity (KYN/TRP; r=0.385, $p=0.004$) and quantitative RF in RA patients Supplementary Table S1). In contrast, levels of KYN (r=-0.439, $p=0.001$) as well as estimated IDO activity (r=-0.276, $p=0.044$) showed negative association with RF in linear correlation analysis for OA patients (Table S1). These results might indicate a context-dependent involvement of IDO pathways in RF production. However, we could not detect any correlation of tryptophan metabolites and anti-CCP antibody levels.

To characterise the value of TRP and its metabolites to distinct between systemic inflammatory conditions, like RA and non-inflammatory OA, we performed ROC analysis. Estimated IDO activity (AUC 0.914, SD 0.860–0.969) showed better test characteristics than RF (AUC 0.783, SD 0.688–0.878) and even similar test characteristics to anti-CCP levels (AUC 0.922, SD 0.860–0.984) in discriminating RA from OA (Fig. 2). A cut-off value of 0.0253 for IDO activity (KYN/TRP ratio) discriminated patients with RA from OA with a specificity of 79.6% and a sensitivity of 96.3%.

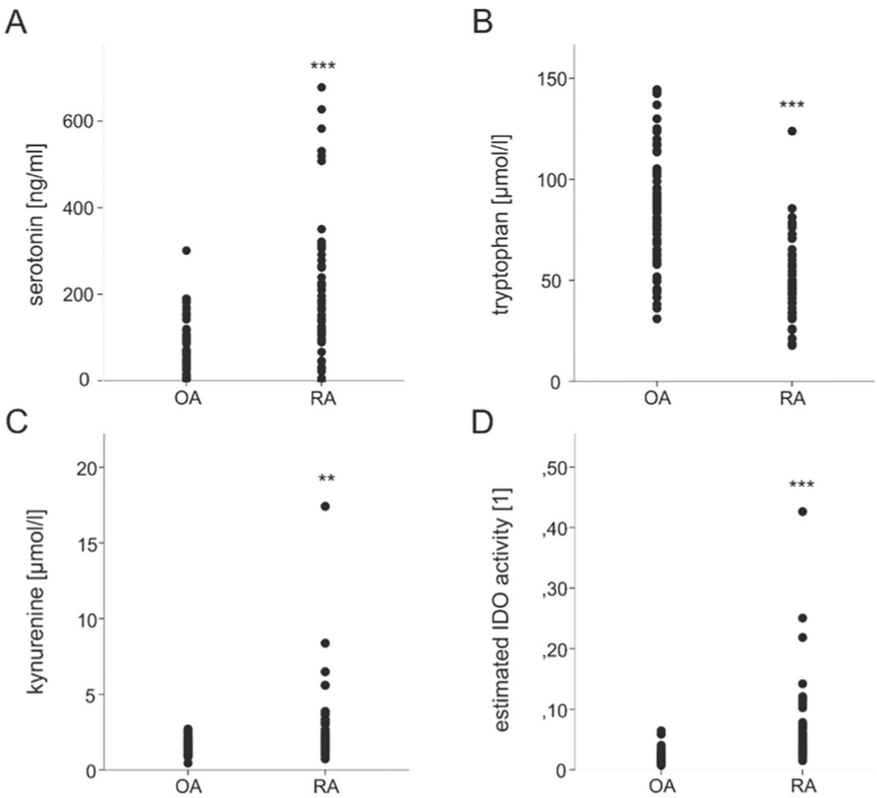

Fig. 1. Level of tryptophan and metabolites in serum of rheumatoid arthritis (RA) and osteoarthritis (OA) patients. Serum levels of serotonin (**A**), trypophan (**B**), kynurenine (**C**), and estimated indoleamine (2,3)-dioxygenase (IDO) activity (kynurenine / tryptophan ratio) (**D**), is depicted. Each dot corresponds to one individual patient. *p*-values were calculated by Mann-Whitney-U test. ***$p<0.0001$, **$p<0.001$.

In summary, these results confirm differences in RA *versus* OA regarding metabolites of the TRP metabolism with an increase in SER and IDO activity in RA patients, respectively. In contrast to anti-CCP serum levels, which did not show any correlation to tryptophan metabolites, the production of RF is positively associated with estimated IDO activity, whereas a negative association was found in OA patients.

Association of RA parameters with tryptophan metabolism is modulated by inflammatory status

Since levels of autoantibodies, like RF, are regarded as prognostic markers and therefore are directly related to joint destruction in RA, we further analysed association and predictive value of TRP metabolites in a second, radiologically well characterised patient cohort. These RA patients were characterised by CRP, DAS28, different radiologic scores and values (RAMRIS, dGEMRIC index) and number of radiologically detected erosions (Table I). We first performed simple correlation analysis to determine, if linear relationships exist between TRP metabolism and determined RA parameters.

Correlation analysis revealed that KYN (r=0.224, $p=0.046$), number of erosions (r=0.405, $p<0.001$), and RAMRIS (r=0.485, $p<0.001$) was associated with age and KYN (r=0.262, $p=0.020$) was also associated with sex. After controlling for age and sex by partial correlation analysis, no linear association of CRP with TRP or its metabolites was evident. However, only analysing patients with increased CRP (>0.3 mg/dl) values revealed a highly significant association with serum SER levels in partial correlation analysis (r=0.527, $p=0.006$, Fig. 3), whereas RA patients with normal CRP values (<0.3 mg/dl) did not show any association with CRP (r=-0.065, $p=0.7$). This points to an association that is only evident under in-

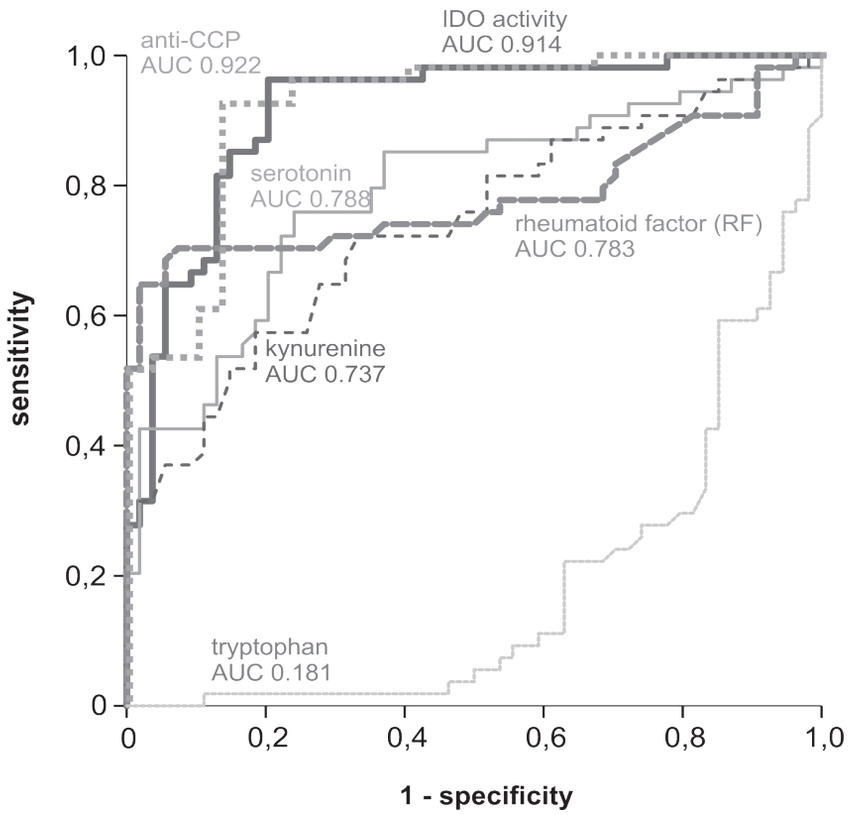

Fig. 2. Receiver operator characteristic (ROC) of tryptophan and metabolites to destinct between rheumatoid arthritis (RA) and osteoarthritis (OA). ROC of serotonin (thin line), kynurenine (thin dashed line), tryptophan (thin dotted line), estimated indoleamine (2,3)-dioxygenase (IDO) activity (thick line) are compared to rheumatoid factor (RF, thick dashed line) and anti-CCP antibody levels (anti-CCP, thick dotted line), respectively. Area under the curve (AUC) as a measure of the characteristic are given for each metabolite.

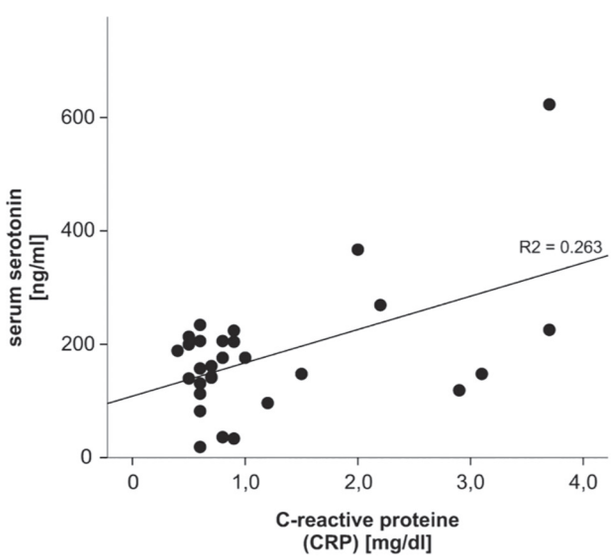

Fig. 3. Linear correlation of serum serotonin and C-reactive protein (CRP) in patients with increased CRP values. A dot plot of serum serotonin levels and CRP values is depicted. Each dot represents one patient. The straight line represents the linear regression of the data (r2 = 0.263).

flammatory conditions. For KYN and TRP such dependency on inflammation state was not deducible (data not shown).

DAS28 did show a weak linear relationship with KYN (r=0.237, *p*=0.055) and estimated IDO activity (r=0.218, *p*=0.079) in partial correlation controlled for age, sex and CRP. Relationship to radiological outcomes and parameters such as RAMRIS, dGEMRIC index, or the number of erosions could not be demonstrated in this first analysis (data not shown).

Predictive value of tryptophan meta-bolism for radiologic outcome in RA

In clinical practice, the most important question would be to determine outcome from one baseline measurement of TRP metabolites. Therefore, we investigated a possible predictive value for TRP metabolites measured at baseline regarding changes in RA outcomes at month 6 by linear regression modelling (Table S1). By backwards exclusion of predictors, serum SER level measured at baseline (standardised beta: 0.529, *p*=0.014) was shown to be the best sole predictor of percent change in RAMRIS at month six of follow-up. Controlling for age and sex weakened the role of SER in the model (standardised beta: 0.426, *p*=0.056) and the predictive value of this model was modest (corrected r2=0.245, *p*=0.074). However, since analysis above showed that associations were primarily observed in patients with increased CRP and in clinical practice this patient group would experience the highest risk of radiographic progression (32, 33), we analysed this subgroup of patients within the same model. This markedly improved model characteristics (Table II) and best predictive value for change in RAMRIS at month 6 was calculated for a model including TRP, KYN, and SER (corrected r2=0.529, *p*=0.01) with SER being the sole significant contributor (standardised beta: 0.676, *p*=0.004) even after controlling for sex and age (Table II). On the other hand, serum KYN levels at baseline were the sole significant factor in a model (corrected r2=0.228, *p*=0.048) predicting increase in erosions at month 6 (standardised beta: -0.536, *p*=0.048), which also remained significant after controlling for age and sex (Table S2). Using estimated IDO activity as a predictor for developing more erosions at month six further improved the model (Table III, standardised beta -0.609, *p*=0.021, corrected r2=0.318, *p*=0.021). A predictive model could not be fitted for baseline levels of TRP or its metabolites regarding changes in

Table II. Linear regression model predicting change in RAMRIS at month six in RA patients with increased CRP values.

Model#	model chracteristics	predictor	Std beta	T	*p*-value
1	r=0.800	constant		1.747	0.115
	r2=0.64	serotonin ng/ml	0.636	2.805	**0.021**
	corr. r2=0.44	tryptophan µmol/l	0.32	1.294	0.228
	ANOVA *p*=0.062	kyneurenine µmol/l	-0.198	-0.86	0.412
		age	-0.12	-0.486	0.638
		sex	0.02	0.084	0.935
2	r=0.800	constant		2.047	0.068
	r2=0.64	serotonin ng/ml	0.643	3.197	**0.01**
	corr. r2=0.496	tryptophan µmol/l	0.315	1.382	0.197
	ANOVA *p*=0.025	kyneurenine µmol/l	-0.204	-0.973	0.354
		age	-0.119	-0.51	0.621
3	r=0.794	constant		3.159	0.009
	r2=0.63	serotonin ng/ml	0.676	3.684	**0.004**
	corr. r2=0.529	tryptophan µmol/l	0.373	1.958	0.076
	ANOVA *p*=0.01	kyneurenine µmol/l	-0.239	-1.254	0.236
4	r=0.760	constant		2.848	0.015
	r2=0.577	serotonin ng/ml	0.685	3.651	**0.003**
	corr. r2=0.507	tryptophan µmol/l	0.308	1.641	0.127
	ANOVA *p*=0.006				
5	r=0.695	constant		9.32	0
	r2=0.483	serotonin ng/ml	0.695	3.482	**0.004**
	corr. r2=0.443				
	ANOVA *p*=0.004				

Table III. Linear regression model predicting change in erosions at month six in RA patients with increased CRP values.

Model#	model chracteristics	predictor	Std beta	T	*p*-value
1	r=0.779	constant		2.051	0.07
	r2=0.607	serotonin	0.476	2.052	0.07
	corr. r2=0.432	**IDO activity**	-0.831	-3.184	**0.011**
	ANOVA *p*=0.056	age	0.449	1.685	0.126
		sex	-0.186	-0.801	0.444
2	r=0.761	constant		1.952	0.079
	r2=0.579	serotonin	0.412	1.926	0.083
	corr. r2=0.452	**IDO activity**	-0.768	-3.143	**0.01**
	ANOVA *p*=0.029	age	0.393	1.557	0.151
3	r=0.690	constant		4.406	0.001
	r2=0.477	serotonin	0.329	1.495	0.163
	corr. r2=0.382	**IDO activity**	-0.566	-2.573	**0.026**
	ANOVA *p*=0.028				
4	r=0.609	constant		5.946	0
	r2=0.370	**IDO activity**	-0.609	-2.657	**0.021**
	corr. r2=0.318				
	ANOVA *p*=0.021				

DAS28 or dGEMRIC index at month 6 (data not shown).

In conclusion, for patients with increased CRP at baseline, a negative association of baseline KYN serum levels with increase in erosions after six month and a positive association of baseline SER serum levels with increases in RAMRIS at month six were detected.

Discussion

This study contributes to a better understanding of TRP and its metabolites in RA and OA patients. Basically, we found parameters of the TRP metabolism altered in RA as compared to OA, confirming already known differences described in the literature (8, 10, 34). However, to our knowledge, this is the first analysis to show a discriminatory power of IDO activity between OA and RA patients, which was even better, or similar than the established markers RF and anti-CCP, respectively. In addition, we could show that IDO is directly related to serum level of RF, pointing to a possible role of TRP metabolism in autoantibody production. This assumption is supported by reports showing a direct influence of TRP metabolites on B cell function, concluding that IDO2 activity is directly linked to autoantibody production in animal models of arthritis, and therefore proposing IDO2 as therapeutic target (14, 35, 36). Therefore, our report is the first to support such a mechanism in human RA, at least for RF, since we could not show such an association with anti-CCP antibody levels, possibly pointing to a different role of IDO in generation of RF *versus* anti-CCP antibodies. However, by creating mice deficient in IDO2, a specific defect in autoantibody production with decreased pathology in model of arthritis was demonstrated (36). Interestingly, this role for IDO2 seemed to be specific for pathologic autoantibody responses and was not observed in physiologic antibody responses (35, 36). In our study, we also provide support for this hypothesis in humans, since in patients suffering from OA, a non-systemic inflammatory arthritis, the production of RF was negatively associated with IDO activity, contrasting the result in RA patients and pointing to a different role of IDO activity depending on inflammatory state.

A similar dual role, depending on inflammatory state, was established for SER, since we observed an association of SER with CRP only in patients with increased CRP values, but not in non-inflamed patients. Therefore, in contrast to KYN and TRP, SER behaved like an acute phase reactant. SER has been shown to act proinflammatory via HT2A receptors in animal models of adjuvant induced arthritis in rats (37), however its effect on bone metabolism (38) and TNF mediated inflammation of arterial blood vessels (39) might be protective.

The measured total SER level depends more on release from thrombocytes than IDO or TPH activity and throm-

bocyte numbers are associated with inflammatory activity (40). Furthermore, platelets have been shown to contain less and release more SER under inflammatory conditions, like RA (41).

However, serum SER is a predictor of radiological outcome measured by RAMRIS in RA patients. RAMRIS is a MRI scoring system semiquantitatively assessing severity of synovitis, bone marrow oedema and erosions in hand and wrist joints, and was especially developed for evaluation of inflammatory and destructive changes in RA hands and wrists (31). It has been evaluated in many studies, including treatment-response trials, and a recent meta-analysis confirms validity for assessing extent of inflammatory changes and destruction in RA (42). Furthermore, subscores of the RAMRIS are directly linked to histopathological correlates, *e.g.* findings on MRI are linked to cartilage damage and reflect histological synovitis (29, 43). The predictive value of RAMRIS for RA outcome measured by DAS28 has been recently reported, and it has been suggested that RAMRIS might be a valuable parameter to predict treatment response (28). Therefore, prediction of RAMRIS by SER reflects the importance of SER as marker of inflammation, specifically for RA. In light of these findings it is not surprising that inhibitors of SER receptors were already successfully used systemically and intra-articularly in patients with RA (44).

It is unclear why cartilage integrity, measured by dGEMRIC is not associated with TRP or its metabolites in the predictive regression model. We expected that SER shows some association with dGEMRIC, since it reflects inflammation which itself is positively associated with production of metalloproteinases that are main contributors to cartilage degradation (45). However, dGEMRIC does not change considerable over time in our study within six month, although inflammation measured by CRP and DAS28 was decreased in our cohort over time (data not shown). Therefore, it is possible, that study period was too short to reflect changes in dGEMRIC due to changes in inflammation. Supporting this hypothesis is a study showing that during anti-TNF therapy, even a good response to therapy measured by clinical and laboratory parameters, did not halt further knee joint cartilage GAG loss as measured by dGEMRIC (46). Therefore, dGEMRIC seems not to reflect short term changes in inflammation and this might be the reason why it is not associated with serotonin in our study.

Interestingly, erosions itself are better predicted by a negative association with KYN or IDO activity, respectively, than SER serum baseline levels, suggesting that KYN might be particularly involved in bone pathology. Positive association of serum KYN with osteocalcin (OCN), a marker of bone resorption, is known (47). It is also known, that TRP metabolism and KYN, respectively, are positively linked to bone mineral density (48). *In vitro* experiments show that inhibiting IDO1, and therefore decreasing KYN, decreases osteoblastogenesis and increases osteoclast activity, leading to osteopenia (19). This could be a direct, mechanistic explanation for the negative association of KYN with erosions and points out the importance of IDO activity in RA bone pathology. SER on the other hand showed no predictive value for erosions in particular. In contrast to IDO activity and KYN, *in vitro* experiments using murine osteoclast- and osteoblast-differentiation assays clearly demonstrated a protective role for SER via HT2A receptors, acting via increasing osteoprotegerin and decreasing RANKL (38). In addition, protective HT2A receptors are downregulated in RA tissue (49). Therefore, it might well be that increased SER levels in RA patients, despite a possible systemic proinflammatory role, are a countermeasure against bone loss and not a cause of the latter. However, this needs to be determined in future mechanistic studies.

Conclusion

In summary, our study provides evidence for a role of SER serum level as prognostic marker of radiologic outcome in RA measured by composite RAMRIS, whereas IDO activity and KYN baseline levels predict developing erosions. Therefore, it is not surprising that IDO activity is an even better marker than RF, and equivalent to anti-CCP in discriminating between OA and RA patients. One important aspect is to take the inflammatory state of the patient into consideration while interpreting results concerning TRP metabolites. This might not only be relevant in RA, but also for other inflammatory diseases and could help solving some of the controversies regarding a pro- or anti-inflammatory role of TRP and its metabolites (50).

Key message

RA and OA patients can be discriminated by IDO activity better than RF, serotonin acts as marker of inflammation in RA and serotonin, as well as IDO activity have prognostic value for radiologic outcome.

Acknowledgement

We thank Ellen Bleck for her excellent technical support.

References

1. SMOLEN JS, ALETAHA D, MCINNES IB: Rheumatoid arthritis. *Lancet* 2016; 388: 2023-38.
2. BARTOK B, FIRESTEIN GS: Fibroblast-like synoviocytes: Key effector cells in rheumatoid arthritis. *Immunol Rev* 2010; 233: 233-55.
3. MCQUEEN FM, OSTENDORF B: What is mri bone oedema in rheumatoid arthritis and why does it matter? *Arthritis research & therapy* 2006; 8: 222.
4. MIESE FR, OSTENDORF B, WITTSACK HJ *et al.*: [cartilage quality in finger joints: Delayed gd(DTPA)(2)-enhanced MRI of the cartilage (dGEMRIC) at 3t]. *RoFo* 2010; 182: 873-8.
5. MIESE FR, OSTENDORF B, WITTSACK HJ *et al.*: Metacarpophalangeal joints in rheumatoid arthritis: Delayed gadolinium-enhanced mr imaging of cartilage--a feasibility study. *Radiology* 2010; 257: 441-7.
6. BERTAZZO A, PUNZI L, BERTAZZOLO N *et al.*: Tryptophan catabolism in synovial fluid of various arthropathies and its relationship with inflammatory cytokines. *Adv Exp Med Biol* 1999; 467: 565-70.
7. PARK MK, OH HJ, HEO YM *et al.*: Myeloid differentiation primary response protein 88 blockade upregulates indoleamine 2,3-dioxygenase expression in rheumatoid synovial fibroblasts. *Exp Mol Med* 2011; 43: 446-54.
8. FORREST CM, KENNEDY A, STONE TW, STOY N, DARLINGTON LG: Kynurenine and neopterin levels in patients with rheumatoid arthritis and osteoporosis during drug treatment. *Adv Exp Med Biol* 2003; 527: 287-95.
9. KURZ K, HEROLD M, WINKLER C, KLOTZ W, RUSSE E, FUCHS D: Effects of adalimumab therapy on disease activity and interferon-

gamma-mediated biochemical pathways in patients with rheumatoid arthritis. *Autoimmunity* 2011; 44: 235-42.
10. SCHROECKSNADEL K, WINKLER C, DUFTNER C, WIRLEITNER B, SCHIRMER M, FUCHS D: Tryptophan degradation increases with stage in patients with rheumatoid arthritis. *Clin Rheumatol* 2006; 25: 334-7.
11. SZANTO S, KORENY T, MIKECZ K, GLANT TT, SZEKANECZ Z, VARGA J: Inhibition of indoleamine 2,3-dioxygenase-mediated tryptophan catabolism accelerates collagen-induced arthritis in mice. *Arthritis Res Ther* 2007; 9: R50.
12. DE ALMEIDA DE, LING S, PI X, HARTMANN-SCRUGGS AM, PUMPENS P, HOLOSHITZ J: Immune dysregulation by the rheumatoid arthritis shared epitope. *J Immunol* 2010; 185: 1927-34.
13. HOLOSHITZ J, DE ALMEIDA DE, LING S: A role for calreticulin in the pathogenesis of rheumatoid arthritis. *Ann N Y Acad Sci* 2010; 1209: 91-8.
14. SCOTT GN, DUHADAWAY J, PIGOTT E *et al*.: The immunoregulatory enzyme ido paradoxically drives b cell-mediated autoimmunity. *J Immunol* 2009; 182: 7509-17.
15. BADAWY AA, NAMBOODIRI AM, MOFFETT JR: The end of the road for the tryptophan depletion concept in pregnancy and infection. *Clin Sci* (Lond) 2016; 130: 1327-33.
16. TYKOCINSKI LO, LAUFFER AM, BOHNEN A *et al*.: Synovial fibroblasts selectively suppress th1 cell responses through ido1-mediated tryptophan catabolism. *J Immunol* 2017; 198: 3109-17.
17. PRENDERGAST GC, CHANG MY, MANDIK-NAYAK L, METZ R, MULLER AJ: Indoleamine 2,3-dioxygenase as a modifier of pathogenic inflammation in cancer and other inflammation-associated diseases. *Curr Med Chem* 2011; 18: 2257-62.
18. OPITZ CA, LITZENBURGER UM, SAHM F *et al*.: An endogenous tumour-promoting ligand of the human aryl hydrocarbon receptor. *Nature* 2011; 478: 197-203.
19. VIDAL C, LI W, SANTNER-NANAN B *et al*.: The kynurenine pathway of tryptophan degradation is activated during osteoblastogenesis. *Stem Cells* 2015; 33: 111-21.
20. SWAMI T, WEBER HC: Updates on the biology of serotonin and tryptophan hydroxylase. *Curr Opin Endocrinol Diabetes Obes* 2018; 25: 12-21.
21. KIM S, MILLER BJ, STEFANEK ME, MILLER AH: Inflammation-induced activation of the indoleamine 2,3-dioxygenase pathway: Relevance to cancer-related fatigue. *Cancer* 2015; 121: 2129-36.
22. FAKHFOURI G, RAHIMIAN R, GHIA JE, KHAN WI, DEHPOUR AR: Impact of 5-ht(3) receptor antagonists on peripheral and central diseases. *Drug Discov Today* 2012; 17: 741-7.
23. VOOG U, ALSTERGREN P, ELIASSON S, LEIBUR E, KALLIKORM R, KOPP S: Progression of radiographic changes in the temporomandibular joints of patients with rheumatoid arthritis in relation to inflammatory markers and mediators in the blood. *Acta Odontol Scand* 2004; 62: 7-13.
24. CHABBI-ACHENGLI Y, COMAN T, COLLET C *et al*.: Serotonin is involved in autoimmune arthritis through th17 immunity and bone resorption. *Am J Pathol* 2016; 186: 927-37.
25. BERNARDES M, VIEIRA T, LUCAS R *et al*.: Serum serotonin levels and bone in rheumatoid arthritis patients. *Rheumatology Int* 2017; 37: 1891-8.
26. HERR N, BODE C, DUERSCHMIED D: The effects of serotonin in immune cells. *Front Cardiovasc Med* 2017; 4: 48.
27. ALETAHA D, NEOGI T, SILMAN AJ *et al*.: 2010 rheumatoid arthritis classification criteria: An american college of rheumatology/ european league against rheumatism collaborative initiative. *Arthritis Rheum* 2010; 62: 2569-81.
28. SEWERIN PL, VORDENBÄUMEN S, SCHLEICH C *et al*.: Rheumatoid arthritis magnetic resonance imaging score (RAMRIS) predicts das-28 therapy response in rheumatoid arthritis patients: Results of the German arthromark cohort. *J Rheumatol* 2018.
29. SCHLEICH C, MULLER-LUTZ A, SEWERIN P *et al*.: Intra-individual assessment of inflammatory severity and cartilage composition of finger joints in rheumatoid arthritis. *Skeletal Radiol* 2015; 44: 513-8.
30. BASHIR A, GRAY ML, BOUTIN RD, BURSTEIN D: Glycosaminoglycan in articular cartilage: In vivo assessment with delayed gd(DTPA) (2-)-enhanced MR imaging. *Radiology* 1997; 205: 551-8.
31. OSTERGAARD M, PETERFY C, CONAGHAN P *et al*.: Omeract rheumatoid arthritis magnetic resonance imaging studies. Core set of mri acquisitions, joint pathology definitions, and the omeract RA-MRI scoring system. *J Rheumatol* 2003; 30: 1385-6.
32. MIERAU R, GENTH E: Diagnosis and prognosis of early rheumatoid arthritis, with special emphasis on laboratory analysis. *Clin Chem Lab Med* 2006; 44: 138-43.
33. MARKATSELI TE, VOULGARI PV, ALAMANOS Y, DROSOS AA: Prognostic factors of radiological damage in rheumatoid arthritis: A 10-year retrospective study. *J Rheumatol* 2011; 38: 44-52.
34. CRAWFORD N: Some observations on the blood serotonin levels in rheumatoid arthritis with a study of platelet serotonin absorption. *Clin Chim Acta* 1969; 23: 139-46.
35. MERLO LMF, GRABLER S, DUHADAWAY JB *et al*.: Therapeutic antibody targeting of indoleamine-2,3-dioxygenase (ido2) inhibits autoimmune arthritis. *Clin Immunol* 2017; 179: 8-16.
36. MERLO LMF, PIGOTT E, DUHADAWAY JB *et al*.: Ido2 is a critical mediator of autoantibody production and inflammatory pathogenesis in a mouse model of autoimmune arthritis. *J Immunol* 2014; 192: 2082-90.
37. HARBUZ MS, MARTI O, LIGHTMAN SL, JESSOP DS: Alteration of central serotonin modifies onset and severity of adjuvant-induced arthritis in the rat. *Br J Rheumatol* 1998; 37: 1077-83.
38. GUSTAFSSON BI, THOMMESEN L, STUNES AK *et al*.: Serotonin and fluoxetine modulate bone cell function *in vitro*. *J Cell Biochem* 2006; 98: 139-51.
39. YU B, BECNEL J, ZERFAOUI M, ROHATGI R, BOULARES AH, NICHOLS CD: Serotonin 5-hydroxytryptamine(2a) receptor activation suppresses tumor necrosis factor-alpha-induced inflammation with extraordinary potency. *J Pharmacol Exp Ther* 2008; 327: 316-23.
40. SMITH AF, CASTOR CW: Connective tissue activation. Xii. Platelet abnormalities in patients with rheumatoid arthritis. *J Rheumatol* 1978; 5: 177-83.
41. ZELLER J, WEISSBARTH E, BARUTH B, MIELKE H, DEICHER H: Serotonin content of platelets in inflammatory rheumatic diseases. Correlation with clinical activity. *Arthritis Rheum* 1983; 26: 532-40.
42. WOODWORTH TG, MORGACHEVA O, PIMIENTA OL, TROUM OM, RANGANATH VK, FURST DE: Examining the validity of the rheumatoid arthritis magnetic resonance imaging score according to the omeract filter-a systematic literature review. *Rheumatology* (Oxford) 2017; 56: 1177-88.
43. VORDENBAUMEN S, SCHLEICH C, LOGTERS T *et al*.: Dynamic contrast-enhanced magnetic resonance imaging of metacarpophalangeal joints reflects histological signs of synovitis in rheumatoid arthritis. *Arthritis Res Ther* 2014; 16: 452.
44. SAMBORSKI W, STRATZ T, MACKIEWICZ S, MULLER W: Intra-articular treatment of arthritides and activated osteoarthritis with the 5-ht3 receptor antagonist tropisetron. A double-blind study compared with methylprednisolone. *Scand J Rheumatol Suppl* 2004; 119: 51-4.
45. GOLDRING MB, OTERO M, TSUCHIMOCHI K, IJIRI K, LI Y: Defining the roles of inflammatory and anabolic cytokines in cartilage metabolism. *Ann Rheum Dis* 2008; 67 (Suppl. 3): iii75-82.
46. TIDERIUS CJ, SANDIN J, SVENSSON J, DAHLBERG LE, JACOBSSON L: Knee cartilage quality assessed with dGEMRIC in rheumatoid arthritis patients before and after treatment with a TNF inhibitor. *Acta Radiol* 2010; 51: 1034-7.
47. ENTENMANN L, PIETZNER M, ARTATI A *et al*.: Comprehensive metabolic characterization of serum osteocalcin action in a large non-diabetic sample. *PloS one* 2017; 12: e0184721.
48. APALSET EM, GJESDAL CG, UELAND PM *et al*.: Interferon (ifn)-gamma-mediated inflammation and the kynurenine pathway in relation to bone mineral density: The hordaland health study. *Clin Exp Immunol* 2014; 176: 452-60.
49. KLING A, RANTAPAA-DAHLQVIST S, STENLUND H, MJORNDAL T: Decreased density of serotonin 5-ht2a receptors in rheumatoid arthritis. *Ann Rheum Dis* 2006; 65: 816-9.
50. FILIPPINI P, DEL PAPA N, SAMBATARO D, DEL BUFALO A, LOCATELLI F, RUTELLA S: Emerging concepts on inhibitors of indoleamine 2,3-dioxygenase in rheumatic diseases. *Curr Med Chem* 2012; 19: 5381-93.