Elevated kynurenine levels in diffuse cutaneous and anti-RNA polymerase III positive systemic sclerosis

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ABSTRACT

Systemic sclerosis (SSc) is a systemic disease characterized by vasculopathy, progressive fibrosis and autoimmune activation. Tryptophan (Trp) metabolism has been linked to altered immune cell function and to malignancy. We have investigated the role of Trp metabolic pathway in SSc measuring serum Trp, Kynurenine (Kyn) and Trp/Kyn ratio in a cohort of 97 SSc patients and 10 healthy controls. Association with disease characteristics was evaluated. We found that Trp levels in SSc patients were significantly lower compared to HCs. We also found that patients with diffuse cutaneous (dcSSc) had lower levels of Trp compared to limited cutaneous (lcSSc). These results were paralleled by higher levels of Kyn found in SSc patients compared to HCs and significantly lower levels in dcSSc compared to lcSSc. The autoantibody profile was also found to be significantly associated with Kyn and Trp levels as anti-RNA-polymerase III (ARA) positive patients were shown to have lower Trp levels and higher Kyn levels compared with anti-centromere and anti-topoisomerase I positive patients. Moreover, the highest Trp/Kyn was found in ARA+ patients with dcSSc, suggesting that an activation of the Kyn pathway, is more specifically associated with this subset of SSc patients. Stability over time makes these markers of Trp metabolism feasible for SSc stratification.

KEYWORDS

Scleroderma, tryptophan, kynurenine, cancer, anti-RNA-polymerase III, systemic sclerosis.

INTRODUCTION

Systemic sclerosis is a rare systemic disease characterized by chronic autoimmune activation, vasculopathy and progressive skin and internal organ fibrosis (1). The exact cause remains unknown, but it is thought to involve environmental factors in a genetically primed individual (2). SSc has been classically divided into two major subsets, limited cutaneous (lcSSc) and diffuse cutaneous (dcSSc) according to the degree of skin involvement (3). Autoantibody profile has also been shown to correlate with the extension of the disease involvement and with the prognosis of patients (4).

Autoimmunity and immune activation are evidently important in the pathogenesis of SSc. Thus factors that modulate immune activation, markers of this process and aspects of environmental exposure that may influence these processes are potentially important in SSc development and offer insight into aetiopathogenesis. One aspect that has not been fully explored is the altered amino acid metabolism that might reflect immune perturbation and have a direct functional role in the immune system. This is especially relevant for cases of SSc that may be associated with occult or undiagnosed malignancy as markers of amino acid metabolism have also been proposed as markers of malignant disease and used in screening strategies [5,6,7].

Tryptophan (Trp) metabolism has been linked to altered immune cell function and also to malignancy. There has been interest in the Trp metabolism in scleroderma spectrum diseases (8). A link was first suggested by a case series of patients developing SSc and SSc related diseases after Trp ingestion (9,10,11). Further studies of Trp metabolism demonstrated that IFNγ-induced indoleamine-2,3-dioxygenase-1 (IDO), expressed in epithelial cells, fibroblasts, brain microglia, macrophages and dendritic cells and tryptophan-2,3-dioxygenase (TDO) in the liver converted the essential amino acid Trp to Kynurenine (Kyn) (12,13). Kyn then initiates a pathway (KP) with powerful immunomodulatory effects through 4 major mechanisms: 1) depleting Trp via the induction of the stress response kinase and suppression of the mTOR1 pathway, which senses amino acid withdrawal and eventually inhibits effector T cell function and growth (14,15). 2) activating aryl hydrocarbon receptor (AhR) nuclear translocation that induces immune suppression (16). 3) inducing differentiation of CD4 T cells into Treg cells expressing CTLA-4 (17). 4) Kyn-mediated inhibition of IL-2 signaling (18). IDO is upregulated by inflammatory molecules, such as amyloid peptides and lipopolysaccharides, and by inflammatory cytokines, such as interleukin-1 (IL-1), interferon gamma (IFN-γ), and tumor necrosis factor alpha (TNF-α) (19,20). Moreover, IDO activity and Kyn have been linked to anti-fibrotic responses of dermal fibroblasts via formation of an aryl hydrocarbon receptor (AhR)-kynurenine ligand complex which mediates increased expression of metalloproteinase 1 in the extracellular matrix (21). Kyn to Trp ratio (Kyn/Trp) has been suggested to be a direct measure of IDO activity (22). In addition, IDO gene polymorphisms in SSc have been correlated with impaired suppressive activity of patient CD8+ Treg cells (23). In the present study we have further explored the role of the Trp metabolic pathway in SSc and explored clinical and autoantibody associations, taking advantage of a well-characterized cohort of SSc cases and healthy controls.

METHODS

We recruited 3 cohorts of patients, all meeting the ACR/EULAR criteria for SSc (24), according to their autoantibody profile: anti-centromere (ACA) positive patients, anti-topoisomerase-I (ATA) patients and anti-RNA-polymerase-III (ARA) patients. The study was conducted in compliance with the Declaration of Helsinki and all patients signed a written consent, approved by the ethics committee of Royal Free Hospital, London. Serum levels of Kyn and Trp were measured using HPLC (25). Kyn/Trp ratio was calculated in μmol/mmol.

Biomarker changes over time and their associations with demographic and clinical characteristics were assessed using mixed effects models, allowing for random intercept and slope. Restricted Maximum Likelihood was used to estimate model parameters. Multivariable models for associations between disease characteristics and biomarkers were developed using stepwise reduction, starting with a saturated model and removing initially interactions and then remaining covariates that did not demonstrate significant association with the outcome, as demonstrated by Wald test p-value. Models were compared using Akaike information criterion and Bayesian information criterion. For easier interpretation of results, time was centred at 12 months and estimates and fixed effect comparisons of biomarker levels were done for this time point of the disease.

RESULTS

The study cohort consisted of 97 SSc patients and 10 healthy controls (HC). Of the SSc patients, 16 (16.5%) were male and 58 (59.8%) had dcSSc. Mean age of SSc patients at first sample collection was 59 years. Mean age of HC was 51 years. Forty-seven (48.5%) of the patients were ARA positive, 25 (25.8%) were ACA positive and 25 (25.8%) were ATA positive. Clinical and demographic characteristics are summarised in **Table 1**.

Serial serum samples, collected on three separate occasions over the patients' follow-up were available for 40 of the SSc subjects, while the remaining 57 and the 10 HC had samples collected on only one occasion. The timing of the first available sample ranged between 2 and 186 moths from disease onset (median 27 and mean 40 months). Among the patients with multiple samples available, the time between samples ranged between 4 and 107 months (mean 32, median 28 months). There was no significant difference in terms of gender, subset or antibody specificities between the subjects who had multiple serum samples and those who had a single sample.

There was a strong association between disease subset and autoantibody profile, with all ACA+ patients having lcSSc, while of the ATA+ 60% (n=15) had dcSSc and of the ARA+ 91.5% (n=43) had dcSSc, $p<0.001$. Immunosuppressive and steroid treatments were more often used among dcSSc patients (69%, n=40 and 26%, n=15) compared to lcSSc ones (18%, n=7 and 8%, n=3; $p<0.001$ and $p=0.032$, respectively). Smoking history was more frequent in males (n=8, 50%) than females (n=8, 10%; $p=0.001$). Potential environmental exposures were greater in men (31.3%, n=5 v 1.2%, n=1 in females, $p<0.001$).

Kynurenine

Kyn levels ranged between 0.65 and 8.32 µmol/L. Among HC, mean Kyn level was 1.3 µmol/L (95%CI 1.01 - 1.7). For the cohort of SSc patients on average there was no significant change in Kyn levels over time. Kyn levels were significantly higher among SSc patients compared to HC and at 12 months from disease onset among lcSSc subjects estimated Kyn levels were on average 0.8 µmol/L higher (2.1 µmol/L, p=0.037) and among dcSSc those were 1.1 µmol/L higher (2.4 µmol/L, p=0.004) compared to HC, with no significant difference between the two subsets. Autoantibody specificity was also strongly associated with Kyn levels with ARA+ patients having significantly higher Kyn levels. At 12 months from disease onset among ARA+ patients average Kyn level was 2.6 µmol/L (95%CI 2.3 - 3.0) and Kyn levels among ACA+ and ATA+ patients were on average 0.5 µmol/L (p=0.043) and 0.8 µmol/L (p=0.002) lower (see Figure 1. Other explanatory variables that showed significant positive association with Kyn levels in the univariable analysis include age at onset, ESR, urate and NT pro-BNP, while Hb, Hct, eGFR showed negative associations (see Table 2). Both treatment with corticosteroids and immunosuppressive agents were associated with lower Kyn levels.

The results of multivariable analysis are summarised in **Table 5**. After controlling for other variables, autoantibodies remained a significant predictor of Kyn levels, with ARA+ patients having significantly higher levels compared to ACA+ and ATA+ ones. Treatment with steroids and immunosuppressive agents also associated with significantly lower Kyn levels when adjusting for other characteristics. Higher eGFR associated with reduced Kyn levels while higher ESR was positively associated with Kyn.

Tryptophan

Trp levels varied between 22.8 and 98.2 µmol/L and estimated mean Trp level in HCs was 59.4 µmol/L (95%CI 51.2 - 67.5). Compared to HC, SSc patients' Trp levels at 12 months from onset were significantly reduced - by 8.8 µmol/L in lcSSc (50.8 µmol/L, p=0.021) and by 13.6 µmol/L in dcSSc (45.8 µmol/L, p<0.001), with the difference between the two subsets being also significant (p=0.010). Trp levels were lowest among ARA+ patients (estimate at 12 months was 45.2 µmol/L), followed by ATA+ (48.4 µmol/L) and ACA+ (52.7 µmol/L), although the difference was significant only between the ACA+ and ARA+ patients (p=0.001), see **Figure 1**, Trp levels did not change significantly over time in SSc patients. Univariable analysis showed evidence for positive association between Hb, Hct and eGFR and Trp levels, while male gender, current and peak mRss, platelet count, inflammatory markers and NT pro-BNP demonstrated a negative association with Trp levels. Similar to Kyn, both steroid and immunosuppressive therapies were associated with lower Trp levels (Table 3).

The final multivariable model is shown in **Table 5**. When adjusting for other explanatory variables, there was evidence for a weak association between Trp and gender, with male patients tending to have lower Trp levels. Autoantibody specificity remained a strong predictor of Trp levels with ARA positivity predicting lower Trp levels compared to ACA or ATA. Finally, prednisolone, although not immunosuppressive treatments, was also associated with lower Trp levels.

Kynurenine/Tryptophan ratio

Kyn/Trp ratio varied between 13.5 and 225.1 µmol/mmol. The estimated mean Kyn/Trp ratio for HCs was 22.5 µmol/mmol (95%CI 17.9, 27.01) and this was significantly lower than in SSc patients. At 12 months from disease onset compared to HCs the Kyn/Trp ratio in lcSSc subjects was on average 21.4 µmol/mmol higher (43.9 µmol/mmol, p=0.059) and in dcSSc was 33.9 µmol/mmol higher (56.4 µmol/mmol, p=0.002) with a difference between the two subsets of 12.4 µmol/mmol (p=0.043). ARA+ patients had significantly higher ratio (62.2 µmol/mmol; 95%CI 53.3 - 71.2) compared to ACA+ and ATA+ patients (40.5 µmol/mmol, p=0.002 and 40.7 µmol/mmol, p=0.003 respectively), see **Figure 1**. The Kyn/Trp ratio did not change significantly over time. In the univariable analysis male gender, peak mRss, ESR, urate and NT pro-BNP showed evidence for positive association with Kyn/Trp ratio, while Hb, Hct and eGFR showed negative association. Immunosuppressive, but not steroid treatment, was also associated with lower levels of the Kyn/Trp ratio (see Table 4).

In the multivariable model (see **Table 5**), ARA positivity retained its significant positive association with Kyn/Trp ratio. Similarly, dcSSc subset also showed association with higher ratio, compared to lcSSc. Immunosuppresive treatment significantly reduced the Kyn/Trp ratio, when keeping other variables constant. Higher eGFR was associated with lower ratio, while increase in NT pro-BNP was associated with higher ratio.

DISCUSSION

A possible link between Trp and scleroderma spectrum diseases dates back the early 1960s when an outbreak of scleroderma (SSc) was identified among South African gold and iron miners (8) and associated with altered Trp metabolism and a possible environmental trigger. Subsequently, in the early 1990s an outbreak of a syndrome resembling SSc and called eosinophilia-myalgia syndrome (EMS) was associated with the use of tryptophan-containing products that were prescribed for insomnia, depression or obesity (6). In these patients it was found that the ingestion of Trp was associated with 3-4 fold higher levels of plasma Kyn compared to normal control subjects (9,10). Subsequent research has elucidated major links between the immune system and Trp metabolic pathway. Thus, activity of IDO and the production of Kyn have been associated with immunoregulatory functions in several autoimmune diseases (26). Patients with rheumatoid arthritis (RA) have been found to have lower levels of Trp and higher levels of Kyn compared to HCs (27,28,29). These results have been confirmed in a murine model of collagen-induced arthritis (CIA), where the upregulation of the Trp pathway was found to be crucial in the induction and resolution phase of the arthritis (30). The activation of this pathway in patients affected by autoimmune inflammatory diseases has been considered an attempt to regulate uncontrolled T cell response through the activation of Treg cells (31,32). Thus, IDO activation and the regulation of immunosuppressive Trp catabolism might represent a major mechanism of action of regulatory T cells (33,34). CTLA-4, which is constitutively expressed on the surface of Treg, is an inhibitory molecule that plays a key role in regulation T cell function (35,36). A major mechanism by which CTLA-4 exerts its regulatory function is through the induction of IDO and the resulting production of Kyn (37). In RA patients, it was found that Treg cell

function is compromised by CTLA-4 promoter methylation resulting in a failure to activate the IDO pathway, which in turn resulted in a failure to activate the immunomodulatory Kyn pathway (38). Similarly, in SSc patients it was found that although the levels of peripheral Treg cells are increased, their function is defective as the surface expression of CTLA-4 is significantly reduced (39). The Trp metabolic pathway could therefore be considered a physiological counteracting mechanism to increase the activity of regulatory T cells. The ability and the efficacy of the induction of this pathway could then be fundamental in autoimmune diseases.

In our cohort, we found that Trp levels in SSc patients were significantly lower compared to HCs. We also found that patients with dcSSc had lower levels of Trp compared to lcSSc. These results were paralleled by higher levels of Kyn found in SSc patients compared to HCs and significantly lower levels in dcSSc compared to lcSSc. The autoantibody profile was also found to be significantly associated with Kyn and Trp levels as ARA+ patients were shown to have lower Trp levels and higher Kyn levels compared with ACA+ and ATA+ patients. The metabolism of Trp is mediated mainly by IDO, whose activity can be indirectly measured through the Trp/Kyn ratio (22). Kyn/Trp as a better marker of activation of the ID pathways was proposed by Widner et al [25] and reveiwed by Schrocksnadel et al [29]. In our cohort the highest Trp/Kyn was found in ARA+ patients with dcSSC, suggesting that an upregulation of IDO, and therefore an activation of the Kyn pathway, is more specifically associated with this subset of SSc patients. These results suggest that in SSc patients the Trp metabolic pathway is activated but the degree of activation is different according to SSc subset and autoantibody profile. This may reflect specific prognostic and biological features of the different subsets of SSc patients. It is known indeed that dcSSc ARA+ patients, compared to dcSSc ATA+ patients tend to have not only a high degree of skin involvement in the early phase of their disease, but also tend to have a high rate of resolution of their skin thickening after 12 and 24 months (40). The difference in the reduction of the mRSS is significantly higher in this group compared to ATA +ve patients (40). The reason for this is still unknown, but it can be thought that these patients are more able to induce secondary regulatory pathways compared to the other subsets of patients. It is then interesting to notice that specifically ARA+ patients were demonstrated to have the higher levels of activity of IDO. The activation of IDO and the resulting production of Kyn could then be linked to this specific feature of having a higher degree of reversion of their skin involvement.

The role of Kyn in the resolution of the skin involvement can also find an explanation in the interaction of this molecule with metalloproteinases. Li et al. demonstrated that Kyn can induce the expression of metalloproteinase-1 (MMP-1) and metalloproteinase-3 (MMP-3) through the activation of the MEK-ERK1/2 signaling pathway in fibroblasts (21). A higher MMP-1 expression and lower collagen content than the controls have been demonstrated in wounds receiving Kyn (34). The mechanism by which Kyn can modulate MMP-1 and type-1 collagen, was found to be the activation of Aryl Hydrocarbon Receptor in dermal fibroblasts (42). It could then be speculated that the higher levels of Kyn in ARA+ dcSSc patients could be at least partially responsible for the skin thickening resolution. The association of ~~Trp/Kyn~~ Kyn/Trp in the univariate analysis, although not confirmed in multivariate analysis, with the peak mRSS could also suggest how this pathway is induced more pronouncedly in patients with a higher burden of disease. The use of immunosuppressive treatments, that we found in our multivariate analysis to be significantly associated with lower serum levels of Kyn and decreased IDO activity (29), could counteract

the stimulus required for the induction of this metabolic pathway. This could also explain why we found that lcSSc patients, as their disease is milder, have lower levels of induction of IDO and lower serum levels of Kyn. Interestingly, the levels of Kyn, Trp and Trp/Kyn ratio were stable over time in the samples analyzed prospectively. This may suggest that a specific upregulation of IDO and more generally of the Trp metabolism could be a specific signature of ARA+ dcSSc patients who are able to induce this pathway.

Trp metabolic pathway has also been studied in a small cohort of patients with idiopathic pulmonary arterial hypertension (IPAH) (43). In this study the authors found that IPAH patients had higher serum levels of Kyn compared to healthy controls. They also tested the effects of Kyn on human pulmonary arterial smooth muscle cells and found that Kyn acts in synergy with NO and exerts acute pulmonary vasodilatation in chronic PH models. They concluded then that Kyn may serve as a negative feedback mechanism in the event of a pulmonary vascular pressure increase. In our cohort, although the presence of PAH did not correlate with increased serum levels of Kyn, we found in univariate analysis that patients with higher serum levels of NT-proBNP and urate had higher serum levels of Kyn and increased IDO activity. In multivariate analysis though, only serum levels of NT-proBNP were confirmed to be significantly associated with increased IDO activity. It is known that serum levels of urate and NT-proBNP are markers of pulmonary hypertension in SSc patients (44). This might then suggest that, even in SSc patients, IDO activity is upregulated in those who might have increased pulmonary pressures. We cannot confirm the data as in our cohort not all the patients underwent right heart catheter to confirm the presence of elevated pulmonary pressures. The lack of correlation with PAH in our cohort could be due to the limited presence of patients with diagnosed PAH in our cohort (3%) and to the fact that patients with PAH were already on treatment for PAH when they were recruited for our study. The pharmacologically induced reduction in pulmonary pressures may have caused an indirect reduction in the serum levels of Kyn in our patients.

Trp and Kyn have also been studied in cancer immune-surveillance and tumor immune escape (45,46). In this context it was found that IDO is expressed by tumor cells and by myeloid cells surrounding the tumor to acquire immune tolerance (47). As it is known that ARA+ patients are more associated with cancer compared to other SSc patients (48), we investigated whether the increased levels of Kyn and IDO activity that we found in our ARA+ patients were due to a higher presence of patients with positive cancer history. We did not find such correlation in our cohort, but this could be due to the relatively small number of patients included. The theoretical case for a potential link is compelling as there is a large body of evidence pointing towards a role for altered amino acid metabolism in malignant disease (49,50). This may reflect the general metabolic reprogramming of cancer cells including a move to glycolytic metabolism. However, there is evidence supporting increased IDO activity and kynurenine levels in regulating immune cell function within the adaptive and innate compartments (51). This may be reflected in altered lymphocyte function as detailed above but also in changes in macrophage polarization that may lead to increased fibrogenic (M2) activity. It is notable that increased kynurenine pathway activity is associated with worse outcome in a number of tumours (52,53) Thus, it is possible that alterations in Trp metabolism are shared between cancer cells and fibrosis and this may have secondary relevance to stromal fibroblast function and other properties of cancer associated fibroblasts. Interestingly many recent studies point towards similarities between CAF biology and fibrosis, with implications for local

invasion and metastasis [54]. It is possible that links exist between cancer metabolism and fibrosis and this might be especially relevant in situations of concurrent SSc and malignancy. Thus, the association with ARA that we report is relevant considering the link between ARA and cancer in SSc that is now well established and outlined above. It is possible that further studies of kynurenine levels, IDO activity and Trp metabolism will lead to simple tests being developed to identify cases of paraneoplastic SSc, as has been proposed for other forms of malignant disease (55).

In conclusion, our data suggest that Trp metabolic pathway could be a regulatory pathway induced in patients with SSc, especially in those who have dcSSc and carry the anti-RNA-polymerase III autoantibody. Specific upregulation in this subset of patients may reflect differences between these cases and other diffuse SSc patients including association with SRC, malignancy or possible the average higher peak mRSS associated with ARA but also a great capacity to improve skin over time. These issues warrant further evaluation to determine whether Trp and its metabolites might be useful in classification or stratification of SSc. We cannot conclude whether there can also be an association between serum Kyn levels and IDO activity with increased pulmonary pressures or positive oncology history. Larger prospective studies are necessary to understand better the role of this metabolic pathway in SSc patients and the potential role of drugs targeting this pathway in this disease. The stability of these analytes over time may make these markers of Trp metabolism useful in SSc stratification.

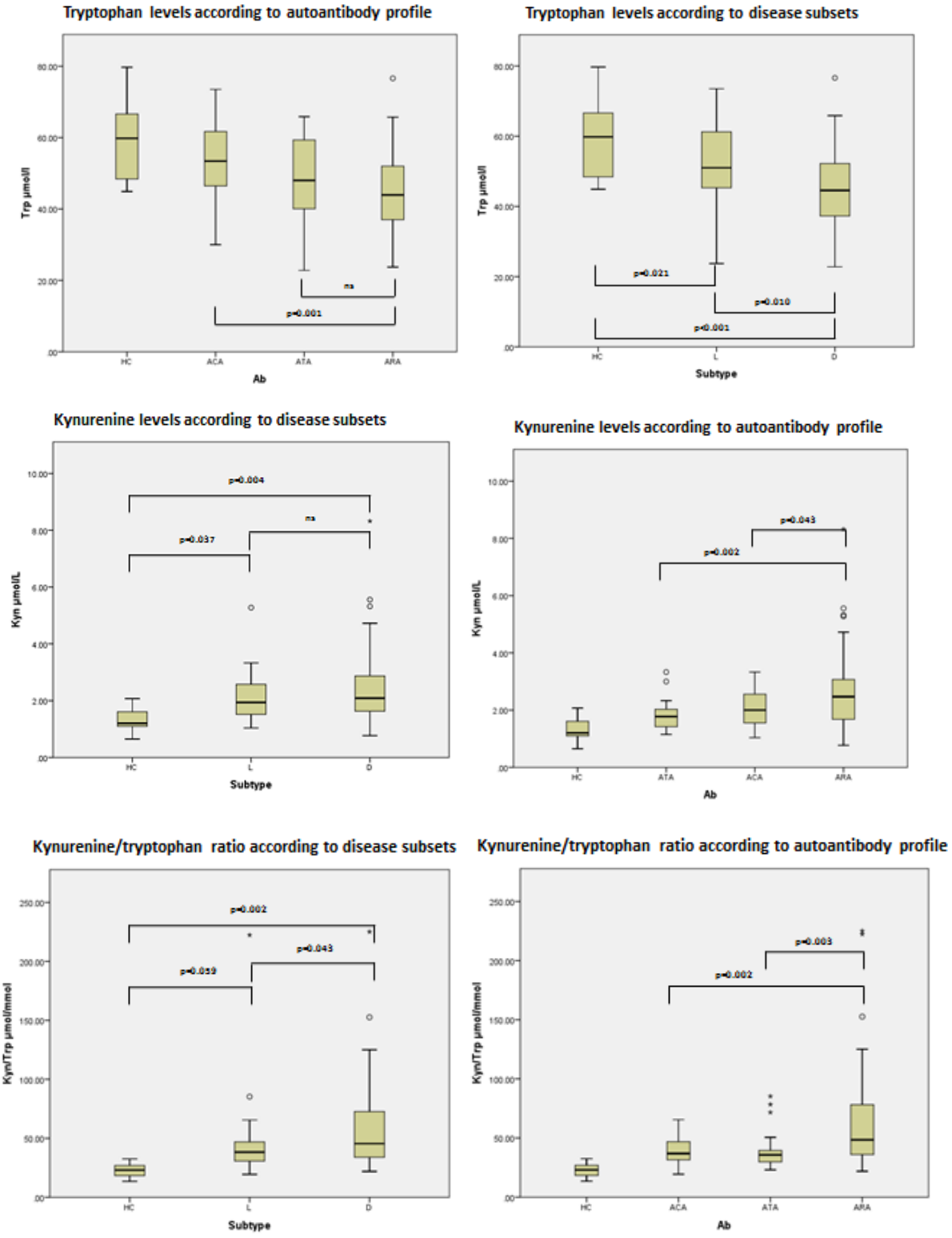

Figure legend:

Figure 1. Kynurenine, tryptophan and kynurenine/tryptophan ratio according to different disease subsets and autoantibody profile.

Box plots show median (horizontal bar) and interquartile range (box) and range (whisker; 1.5 quartile) for analytes in the distinct clinical and serological subgroups described in the manuscript. For Kyn the highest levels are observed in diffuse cutaneous SSc and those with ARA whilst levels in limited cutaneous SSc and healthy controls are much lower. Conversely Trp levels are reduced in diffuse and ARA positive subjects.

HC = healthy control. L=limited cutaneous SSc. D=diffuse cutaneous SSc. ATA=anti-topoisomerase. ACA=anti-centromere. ARA=anti-RNA-polymerase III

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Table 1. Clinical and demographic characteristics of patients according to their autoantibody (expressed in percentage unless specified otherwise)

Number of patients ()	**ATA (25)**	**ARA (47)**	**ACA (25)**
Sex (F)	80%	79%	96%
Age mean (years)	51	48	59
Smoking status	16%	21%	8%
Environmental exposure	8%	6%	0%
Diffuse	60%	91%	0%
Disease duration (months)	101	96	108
Lung fibrosis	64%	15%	8%
PAH	0%	2%	8%
Severe GI	0%	19%	12%
SRC	0%	23%	0%
Vasculopathy	72%	74%	68%
Cardiac	8%	2%	0%
Cancer Hx	12%	25%	8%
Steroid Therapy	28%	23%	0%
Immunosuppression	52%	68%	20%

Table 2. Kynurenine levels univariate analysis

Variables	Coefficient	p-value
Sex (male)	0,34614	0,259
Age of onset	0,01579	**0,057**
Diffuse vs limited	0,26153	0,265
ACA vs ARA	-0,54978	**0,043**
ATA vs ARA	-0,83474	**0,002**
mRSS	0,00199	0,831
Peak mRSS	0,01006	0,278
Immunosuppression	-0,50126	**<0.001**
Steroid therapy	-0,61006	**0,003**
Hemoglobin levels	-0,01262	**0,018**
Hematocrit	-3,44794	**0,038**
Platelets	-0,00110	0,257
ESR	0,02262	**<0.001**
CRP	0,00211	0,72
eGFR	-0,03879	**<0.001**
Urate	0,00481	**<0.001**
NT-proBNP	0,00290	**<0.001**

Table 3. Tryptophan levels univariate analysis

Variables	Coefficient	p-value
Sex (male)	-3,977263	0,088
Age of onset	0,0050488	0,939
Diffuse vs limited	-4,849887	0,009
ACA vs ARA	-4,319308	0,088
ATA vs ARA	-7,533541	0,001
mRSS	-0,4072257	<0.001
Peak mRSS	-0,3029741	<0.001
Immunosuppression	-2,702229	0,094
Steroid therapy	-7,231635	0,002
Hemoglobin levels	0,1949653	<0.001
Hematocrit	61,79502	0,001
Platelets	-0,0294065	0,003
ESR	-0,1188901	0,069
CRP	-0,1855313	0,027
eGFR	0,0924829	0,035
Urate	-0,0128347	0,331
NT-proBNP	-0,0124272	0,043

Table 4. Kynurenine/Tryptophan ratio univariate analysis

Variables	Coefficient	p-value
Sex (male)	18,75605	0,022
Age of onset	0,3226345	0,164
Diffuse vs limited	12,37955	0,043
ACA vs ARA	-21,69078	0,002
ATA vs ARA	-21,49784	0,003
mRSS	0,4182892	0,143
Peak mRSS	0,559979	0,026
Immunosuppression	-10,27917	0,013
Steroid therapy	-5,985879	0,337
Hemoglobin levels	-0,5917479	<0.001
Hematocrit	-147,7108	0,004
Platelets	-0,0079753	0,793
ESR	0,7233683	<0.001
CRP	0,2582023	0,204
eGFR	-1,101145	<0.001
Urate	0,1113823	<0.001
NT-proBNP	0,1225243	<0.001

Table 5 Multivariate analysis for kynurenine levels, tryptophan levels and kynurenine/tryptophan ratio

Variables	Coefficient	p-value
Kynurenine		
ACA vs ARA	-0,83514	<0.001
ATA vs ARA	-0,51149	0,002
Steroid therapy	-0,30021	0,05
Immunosuppression	-0,44905	<0.001
eGFR	-0,03055	<0.001
ESR	0,01205	0,002
Tryptophan		
Sex (male)	-4,85953	0,056
ACA vs ARA	7,265049	0,002
ATA vs ARA	3,296369	0,142
Steroid therapy	-6,630253	0,004
Kynurenine/Tryptophan ratio		
ACA vs ARA	-20,75013	0,004
ATA vs ARA	-12,13092	0,018
Diffuse vs limited	11,67699	0,044
Immunosuppression	-17,83405	<0.001
eGFR	-0,6160723	<0.001
NT-proBNP	0,0865195	<0.001