Serum Anti-PAD4 and Anti-PAD3/4XR Antibodies in Rheumatoid Arthritis Associated-Interstitial Lung Disease Are Associated with Better Lung Function

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Background

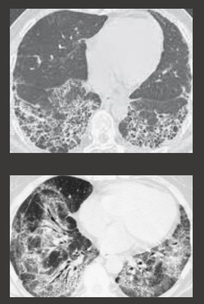
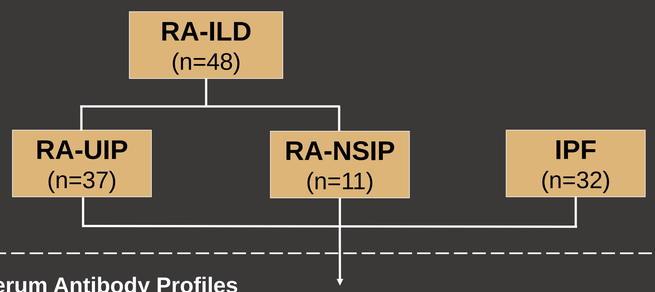


Figure 1. CT consistent with UIP (top) and NSIP (bottom) pattern

- Rheumatoid arthritis (RA) associated-interstitial lung disease (ILD) affects 5-10% of RA patients[1]. It is a leading cause of morbidity and mortality, which is more severe in the fibrotic subtype, RA-usual interstitial pneumonia (RA-UIP), compared to the cellular subtype, RA-nonspecific interstitial pneumonia (RA-NSIP) (Figure 1)[2].
- Serum antibodies to peptidylarginine deiminase type 4 (anti-PAD4), particularly a subset that cross-react with PAD3 (PAD3/4XR), have been associated with imaging evidence of ILD[3]. However, whether anti-PAD4 and anti-PAD3/4XR antibodies are associated with a particular phenotype of RA-ILD or clinically significant ILD is unknown.
- Importantly, idiopathic pulmonary fibrosis (IPF) shares similar risk factors and clinical features with RA-ILD, and a recently published study by our group found that anti-cyclic citrullinated peptide (anti-CCP) antibodies were present in 25% of IPF patients[4].
- **<u>In this study, we sought to identify the specificity and lung disease characteristics of anti-PAD4 and anti-PAD3/4XR antibodies in RA-ILD and IPF.</u>**

Methods

<u>Study subjects</u>



<u>Outcomes</u>

1. **Serum Antibody Profiles**
   - Anti-CCP (CCP3.1 IgG/IgA, Inova, ELISA)
   - Rheumatoid factor (RF-IgA and IgM, ELISA)
   - Anti-PAD4 and anti-PAD3/4XR (immunoprecipitation of radiolabeled target protein and gel electrophoresis, Darrah lab[3])
2. **Lung Disease Severity**
   - Pulmonary function tests (% predicted forced vital capacity [FVC]; % predicted diffusion capacity of carbon monoxide, [DLCO])
   - Data-driven textural analysis (DTA) score (quantifies total extent of lung fibrosis on high resolution CT (HRCT) scan including reticular abnormalities, honeycombing and traction bronchiectasis, expressed as a percentage of total lung volume)

<u>Analysis</u>

Clinical characteristics, antibody profiles and measures of lung disease severity were compared using Chi-square and t-tests as appropriate.

Results

Table 1. Characteristics, Pulmonary Function and Serum Antibody Testing in RA-ILD and IPF

					P value**			
	RA-ILD* (n=48)	RA-NSIP* (n=11)	RA-UIP* (n=37)	IPF* (n=31)	RA-ILD vs IPF	RA-NSIP vs IPF	RA-UIP vs IPF	RA-UIP vs NSIP
Age	62 ± 12	56 ± 13	64 ± 11	69 ± 8	<0.01	<0.01	0.03	0.05
Female	50%	55%	49%	19%	<0.01	0.05	0.02	1.0
Ever Smoker	53%	45%	56%	68%	0.24	0.21	0.33	0.73
Pack years	13 ± 18	10 ± 15	14 ± 19	23 ± 27	0.07	0.15	0.13	0.52
% predicted FVC	69 ± 20	68 ± 23	69 ± 19	67 ± 15	0.71	0.87	0.69	0.91
% predicted DLCO	47 ± 18	53 ± 22	45 ± 17	44 ± 16	0.48	0.15	0.81	0.19
Anti-CCP+	71%	73%	70%	32%	<0.01	0.03	<0.01	1.0
RF IgA+	63%	55%	65%	19%	<0.01	0.05	<0.01	0.72
RF IgM+	73%	55%	78%	0%	<0.01	<0.01	<0.01	0.14
Anti-PAD4+	19%	9%	22%	0%	0.01	0.26	<0.01	0.66
Anti-PAD3/4+	10%	9%	11%	0%	0.15	0.26	0.12	1.0

*Values displayed as mean ± SD or %.
**Based on Chi-square or t-test where appropriate, NS = not significant, P>0.05.
Clinical data was missing for: Ever smoking for 1 RA-UIP; pack years for 1 RA-NSIP, 4 RA-UIP, 3 IPF; FVC% for 2 RA-UIP, 5 IPF; DLCO % for 4 RA-UIP, 7 IPF.

Table 2. Differences in Characteristics and Pulmonary Function Based on Serum Anti-PAD4 and Anti-PAD3/4XR Antibody Positivity in RA-UIP

					P value**	
	PAD4(+)* (n=8)	PAD4(-)* (n=29)	PAD3/4XR(+)* (n=4)	PAD3/4XR(-)* (n=33)	PAD4(+) vs PAD4(-)	PAD 3/4XR(+) vs PAD3/4XR(-)
Age	62 ± 7	64 ± 12	60 ± 6	64 ± 11	0.69	0.51
Female	62%	44%	75%	45%	0.45	0.34
Ever smoker	75%	50%	100%	50%	0.26	0.11
Pack years	14 ± 15	14 ± 20	23 ± 14	13 ± 19	0.98	0.34
Anti-CCP+	75%	69%	100%	67%	1.0	0.30
% predicted FVC	82 ± 12	65 ± 20	87 ± 16	67 ± 19	0.02	0.05
% predicted DLCO	51 ± 15	43 ± 17	54 ± 19	44 ± 17	0.28	0.27
DTA fibrosis score	17 ± 8	40 ± 19	17 ± 7	36 ± 20	<0.01	0.07

*Values displayed as mean ± SD or %.
**Based on Chi-square or t-test where appropriate, NS = not significant, P>0.05.
Clinical data was missing for: Ever smoking for 1 PAD4-, 1 PAD3/4XR-; pack years for 1 PAD4+, 3 PAD4-, 4 PAD3/4XR-; FVC % for 2 PAD4-, 2 PAD3/4XR-; DLCO % 4 PAD4-, 4 PAD3/4XR-; DTA for 6 PAD4-, 6 PAD3/4XR-.

- Compared to IPF, subjects with RA-ILD were younger and more often female, but there were no differences in smoking history or baseline lung function.
- Anti-PAD4 and anti-PAD3/4XR antibodies were present in 9/48 (19%) and 5/48 (10%) subjects with RA-ILD, respectively, and in no subjects with IPF (Table 1).
- 8/9 (89%) anti-PAD4+ subjects with RA-ILD exhibited RA-UIP pattern.
- Within the RA-UIP group (Table 2), anti-PAD4 antibodies were associated with better lung function (Figure 2) and decreased fibrosis on quantitative CT (Figure 3)

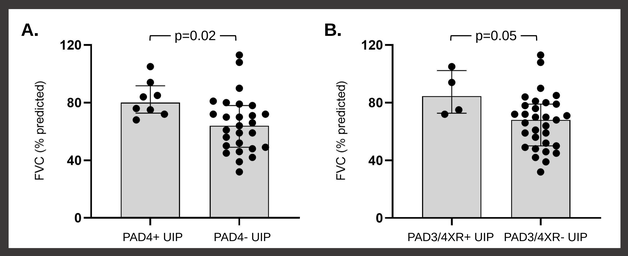


Figure 2. Forced vital capacity (FVC) in anti-PAD4+ (panel A) and anti-PAD3/4XR+ (panel B) RA-UIP.

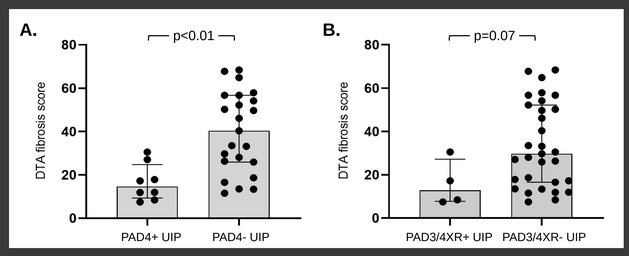


Figure 3. Quantitative data-driven textural analysis (DTA) HRCT fibrosis score in anti-PAD4+ (panel A) and anti-PAD3/4XR+ (panel B) RA-UIP.

Conclusion

- We demonstrate that serum anti-PAD4 and anti-PAD3/4XR antibodies are highly specific for RA-ILD and in RA-UIP are associated with better lung function and less lung fibrosis on quantitative CT.
- Because patients with RA-UIP have a worse prognosis than RA-NSIP patients, the identification of a biomarker that is associated with better lung function could provide new insights into RA-UIP pathogenesis and may identify a new prognostic biomarker for RA-UIP, although future longitudinal studies are needed.

References

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