Antinuclear antibodies in interstitial lung disease: Prevalence and clinical significance

Anticorps antinucléaires au cours des pneumopathies interstitielles diffuses : Prévalence et signification Clinique

Najla Ghrairi[1], Samira Aouadi[1], Youssef Zied Elhechmi[2], Soumaya Ben Saad[1], Imen Ben Ali[1], Sadok Yalaoui[1]

1-EPS abderrahmen Mami de l'Ariana / Faculté de médecine de Tunis,
2-EPS Habib Thameur / Faculté de médecine de Tunis,

RÉSUMÉ

Introduction : Le diagnostic étiologique des pneumopathies interstitielles diffuses (PID) nécessite la recherche d'une connectivite sous jacente. De ce fait une recherche des anticorps antinucléaires (AAN) est faite systématiquement chez ces patients. L'intérêt clinique de cette pratique n'a pas encore été prouvé.

Objectif : Déterminer la prévalence des AAN au cours des PID et de déterminer la signification clinque de la positivité des AAN chez ces patients.

Méthodes : L'étude était rétrospective ayant inclus 73 patients hospitalisés au service de pneumologie et chez qui une recherche des AAN a été effectuée au laboratoire d'immunologie du même hôpital.

Résultats : La prévalence des AAN au cours des PID était de 32%.La comparaison des données démographiques, des résultats des explorations fonctionnelles respiratoires et radiologiques n'a pas montré de différences statistiquement significatives entre les patients ayant des AAN positifs et les patients avec des AAN négatifs. En revanche, les patients avec des AAN positifs avaient plus de manifestations cutanés (p=0.011) et de syndrome de Raynauds (p=0.029). Le diagnostic de connectivite a été retenu chez 42% des patients avec des AAN positifs versus 8% des patients avec des AAN négatifs. Des AAN positifs à un titre >1/320 était un facteur prédictif de diagnostic de connectivite (OR=14.4, p<0.011).

Conclusion : La recherche des AAN chez les patients atteints de PID est un moyen diagnostique important de connectivite surtout chez les patients qui ont des manifestations extra pulmonaires orientant vers une maladie auto-immune.

Mots-clés

Pneumopathie interstitielle diffuse, anticorps antinucléaires, connectivite.

SUMMARY

Introduction: The diagnosis of interstitial lung disease (ILD) requires elimination of underlying connective tissue disease. Consequently, antinuclear antibodies (ANA) are routinely screened in patients with idiopathic interstitial pneumonia. However the clinical usefulness of this practice is not well clear.

Aim: In this study, we evaluated the frequency of ANA in ILD's patients and investigated the clinical significance of the ANA's presence in these patients.

Methods: We conducted a retrospective study of hospitalized patients diagnosed ILD at pulmonary department and for which ANA was performed in the immunology laboratory of our institution. Demographic features, clinical symptoms, biological and radiologic findings and CTD-ILD diagnoses were compared between patients with positive ANA versus negative ANA.

Results: We enrolled 73 patients. The ANA's prevalence was 32%. There were no significant differences in demographics, pulmonary function test values and radiologic findings between patients with and without ANA. Patients with positive ANA had more cutaneous manifestations (p=0.011) and Raynaud's phenomenon (p=0.029). The diagnosis of connective tissue disease was made in 42% of patients with positive ANA versus 8% with negative ANA (p=0.001). ANA's titer higher than 1/320 was predictive of CTD diagnosis (OR=14.4) (p<0.001).

Conclusions: The research of ANA in PID's patients is an important tool of CTD diagnosis specially in those with suggestive symptoms of autoimmune disease.

Key-words

Interstitial lung disease, antinuclear antibodies, connective tissue disease.

INTRODUCTION

Interstitial lung diseases (ILDs) are a heterogeneous group of diffuse parenchymal lung disorders of multiples etiology such as drug, environmental exposure and connective tissue diseases (CTDs)(1,2). In some cases, ILD may present as an only manifestation of an auto immune disease preceding extra pulmonary manifestations of CTD by years (3,4).Therefore, autoimmune serology testing are often routinely obtained as an initial diagnostic procedure of ILD with or without clinical features of CTD (5).The current international guidelines (6) for the diagnosis of ILD recommend testing for auto antibodies including ANA in all patients with or without clinical symptoms of CTD. However, the clinical relevance of ANA's presence remains unclear.

The aim of this study was to investigate the prevalence and the clinical significance of ANA's in ILDs patients.

METHODS

Study design:

It was a retrospective study. The records of 73 patients with ILD hospitalized at Abderrahmen Mami hospital, Tunis, Tunisia between January 2011 and December 2016 were reviewed. Patients were included if they were older than 18 years and if the serologic testing of ANA was performed. The diagnosis of ILD was made by pulmonologists based on clinical symptoms, chest high resolution computed tomography (HRCT) scan findings and available histopathological studies. Patients without ANA's testing were excluded.

For each patient, we collected clinical information, radiologic findings and laboratory data including demographic features of age and gender, clinical features of pulmonary and extrapulmonary manifestations at the time of diagnosis, smoking history and treatment regimen, white blood cells (WBC), erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), Antinuclear antibody (ANA), ENA, DNA (IIF), pulmonary function test (PFT) for forced vital capacity (FVC) and forced expiratory volume in one second (FEV1).

METHODS

Antinuclear antibody

ANA testing was done by indirect immunofluorescence (IIF) method using commercial slides of HEp-2 cells (BioSysytems®). If the serum tested positive at the initial dilution of 1/80, it was serially titrated. The serum titer is defined as the highest dilution showing a positive result. For each patient who had positive ANA we determinated titer and pattern.

Autoantibodies against extractable nuclear antigen (ENAs) was performed by commercial immunodot assay (AESKUBLOT ANA-17 Pro®): nucleosome, double stranded deoxyribonucleic acid (ds-DNA), histone, Smith antibody (SmD1), ribonucleiprotein (RNPU1), SSA Ro 52, SSA Ro60, Scl70, centromere B (CENP-B), antisynthetases antibodies Jo-1, Ku, Mi-2, Pm-scl and PCNA. A signal to cut off ratio greater than 1.0 was considered positive.

Anti-ds DNA antibodies:

Antibodies to double stranded DNA (anti-ds DNA) were tested in patients who has ANA positive as initial screening test. The method used in our laboratory to determine anti –ds DNA antibodies was the indirect immunofluorescence (IIF) using commercial slides of Crithidia lucilae (BioSysytems®) as substrate. Observation of specific fluorescent staining of the kinetoplast at the serum dilution of 1/10 was considered as positive result.

Statistical analysis:

For categorical variables, data were presented as frequencies and percentage. For continuous variables, results were expressed as mean and standard deviation (SD) or median and interquartile range (IQR). Differences between the two groups (with and without antinuclear antibody) were assessed with independent t tests, the Fisher exact test, or the Wilcoxon test, as appropriate. Two side p-value <0.05 was considered statistically significant. Epi info software was used for the statistical analysis. All data used in this study were anonymous.

This study was approved by local institutional ethics committee.

RESULTS

Patient characteristics

The mean age was 62 years (29-90). The majority of patients (71%) were female. The main symptoms observed were exertional dyspnea (87%) and cough (64%).

Prevalence of ANA

24 patients (32%) had positive ANA. The homogenous

Table 1 : Clinical and demographic characteristics

	Total Population (73)	Negative ANA (49)	Positive ANA (24)	p
Age	62 ± 12	63 ± 13	60 ± 12	0,287
Female	71%	83%	65%	0,11
Background				
COPD	3%	4%	0%	0,549
Asthma	3%	4%	0%	0,546
Bronchoctasies	1%	0%	4%	0,333
Pulmonary Tuberculosis	7%	6%	8%	1
High Blood Pressure	40%	43%	33%	0,435
Diabetes	25%	22%	29%	0,532
Hyperlipidemia	11%	14%	4%	0,258
Exposure Factors				
Smoking history	20%	22%	17%	0,76
Birds 'exposure	18%	15%	25%	0,336
Pulmonary symptoms				
Dyspnea	89%	90%	87%	0,704
Chest pain	21%	18%	26%	0,537
Hemoptysis	12%	18%	0%	0,05
sputum	39%	39%	39%	0,977
Extra-pulmonary symptoms				
Arthralgia	17%	13%	27%	0,177
Arthrite	3%	0%	9%	0,098
Raynauld's phenomenon	4%	0%	14%	0,029
Ocular symptoms	1%	2%	0%	1
Cutaneous symptoms	9%	2%	23%	0,011
Dry eyes	1%	2%	0%	0,319
Dry mouth	6%	4%	9%	0,59
Muscle weakness	1%	0%	4%	0,329
Non pruritic rash/ photosensibility	1%	0%	4%	0,319
General manifestations				
Fever	44%	46%	41%	0,748
Weight loss	41%	46%	31%	0,339
Clinical examination				
Polypnea	16%	18%	12%	0,739
crackling*	74%	75%	70%	0,594
Wheezing*	10%	10%	9%	1
Finger clubbing	16%	16%	14%	1
Articular mainfestations	8%	4%	15%	0,153
Cutaneous manifestations	12%	6%	25%	**0,046**
Gottron papules	1%	0%	5%	0,299
Skin ulcer	1%	0%	5%	0,299

Table 2 : Biological findings

	Total cohort (73)	Negative ANA (49)	Positive ANA (24)	p value
Biology				
Hemoglobin (g/dL)	12,1 [12,0-14,0]	13,0 [12,0-14,0]	12,0 [12,0-13,0]	0,558
WBC (10^3 e/mm^3)	7,7 [5,5-9,5]	8,0 [5,8-9,6]	6,8 [4,7-8,9]	0,219
Platelets (10^3 e/ mm^3)	251 ± 96	250 ± 91	255 ± 108	0,847
ERS (mm/hr)	49 ± 39	44 ± 37	73 ± 41	**0,025**
CPK (UI/L)	21 [0-58]	18 [0-58]	26 [0-76]	0,657
LDH (UI/L)	149 [0-216]	167 [0-220]	128 [0-173]	0,532
Creatinine (µmol/L)	65 [55-74]	66 [55-74]	62 [55-73]	0,546
Bronchoalveolar lavage				
Cellularity (10^3 e/ mm^3)	250 ± 96	237 ± 186	271 ± 132	0,536
Macrophages	63 ± 21	67 ± 23	58 ± 18	0,2
Neutrophils	13 [8-20]	11 [5-16]	14 [9-27]	0,198
Eosinophils	1 [0-5]	1 [1-6]	1 [0-3]	0,345
GOLD score	11 [0-38]	15 [0-43]	3 [0-20]	0,214
Spirometry				
FEV1	70 ± 20	68 ± 22	73 ± 15	0,277
FVC	62 ± 18	60 ± 19	66 ± 13	0,185
CTD-ILD	19%	8%	42%	**0,001**

WBC : White blood cells count ; ERS : Erythrocyte rate sedimentation ; CPK : creatine phosphokinase ; LDH : lactate dehydrogenase ; FVC: forced vital capacity

and the speckled were the most frequent patterns. ANA titer was ≥ 1/320 in 13 patients. SSA-Ro52 was the most common specificities. Ds-DNA was positive in only one case.

Comparisons of the clinical features of patients according to the presence of ANA

There were no significant differences in demographics, pulmonary function test values and radiologic findings between patients with and without ANA. Patients with positive ANA had more cutaneous manifestations (2% versus 23%, p=0.011) and Raynaud's phenomenon (0% versus 14% p=0.029). The diagnosis of connective tissue disease was made in 42% of patients with positive ANA versus 8% with negative ANA (**p= 0.001**). (Table 1, 2, 3).

ANA's titer higher than 1/320 was predictive of CTD diagnosis (OR= 14.4) (p<0.001). The CTD diagnosed in ANA positive group were: Rheumatoid Arthritis (3 cases), Sjögren's disease (3 cases), antisynthetase syndrome (2 cases), Systemic sclerosis (2 cases) and Systemic Lupus Erythematosus (3 cases). (Table 4,5)

In laboratory findings, patients with positive ANA had ERS≥70mm/hr more than patients with negative ANA (p= 0.02) (Table 2).

Table 3 : Radiologic findings

	Total population (73)	Negative ANA (49)	Positive ANA (24)	p
Alveolar Syndrome	19%	17%	22%	0,746
Nodules	14%	17%	9%	0,484
Interstitial syndrome	86%	83%	91%	0,488
Honey combing	9%	9%	9%	1
Pleural syndrome	4%	4%	4%	1
HRCT findings				
Ground glass opacities	62%	55%	80%	0,053
Honey combing	48%	50%	43%	0,585
Septal thickening and reticulation	41%	41%	43%	0,874
Condensations	31%	31%	32%	0,979
PBV* **reticulations**	56%	49%	71%	0,083
Micronodules	16%	15%	21%	0,492
Nodules	17%	19%	11%	0,489
Constrictive bronchiolitis	11%	9%	16%	0,401
Mediastinal lymphadenopathy	47%	41%	59%	0,153

* peribronchovascular

Table 4: Autoantibody profile of ILD subjects with and without CTD

	CTD-ILD	Non CTD-ILD	total
ANA (n=24)	13	11	24
Pattern			
Homogeneous	4	4	8
Speckled	5	3	8
Cytoplasmic	3	1	4
Nuclear	1	3	4
Titer			
1/80	1	1	3
1/160	2	6	8
1/320	4	2	6
1/640	3	1	4
1/1280	3	0	3
ENA			
SSA	6	2	8
SSB	1	0	1
Jo-1	1	0	1
Scl-70	2	0	2
PM-ScL	1	0	1
RNP	2	1	3
Sm	0	0	0
PCNA	0	1	1
Ribosomes	2	0	2
DNA (IIF)	0	1	1

Table5: Frequency of ANA in CTD-ILD patients

DISCUSSION

Our study showed that among ILD patients 32% had positive ANA. Cutaneous manifestations, Raynaud syndrome and ERS≥70mm/hr were more frequent in ILD positive ANA patients than negative ANA patients. The presence of ANA at titer ≥ 1/320 was predictive of connective tissue disease.

Reported prevalence of ANA among ILD patients has ranged from 12 to 54% (7,8–11). The discrepancies between study results could be explained by the difference between patients populations and the methods used for the assesment of ANA**.** Indirect immunofluorescence (IIF) on HEp-2 cells is the gold standard test for ANA investigation because of its high sensitivity (12–14). Neverthless, some specific autoantibodies (t-RNA synthetase, Ro-SSA) are not always detected because of the scarcity of antigen in HEp-2 cells and/or leaching and denaturation secondary to fixation procedure(15,16). Titer and pattern are two important parameters to consider. We found 11 patients with low ANA titer < 1/320. ANA is present at low title (1/40-1/160) in healthy individuals especially in the

elderly(17). Lee et al (7) found similar prevalence of ANA at low titer (≥1/40 and <1/320) between IPF patients and healthy individuals controls group. In our study, we found that homogenous and speckled patterns are the most frequent. Fisher et al (8) have reported that a nucleolar pattern is predominant in IIP with a frequent development of scleroderma. Mitoo et al (9) reported that a speckled pattern was predominant in these cases without any development of scleroderma.

In our study, SSA-Ro 52 was the most frequent ENA detected. The presence of anti Ro-52 antibody in patients with CTD was associated with ILD developpement in 71.4% (18). La Corte et al (19) reported that patients with antisynthetase syndrome who have positive SSA-Ro 52 antibody have more severe ILD. In our case, only one patient with SSA Ro52 had anti synthetase syndrome and 3 patients had a Sjogren's disease.

In our population, five patients presented with an established CTD and 13 patients were newly diagnosed as CTD. The overall frequency of CTD in our patients was 23%. Many reports found similar incidence of CTD in ILD patients: Homma et al (3) (19.1%) and Mittoo et al (9) (17.5%). Our study results showed that CTD diagnosis is closely associated with ANA. There was a high incidence of CTD in the ANA positive group (42%) than in the ANA negative group (8%) (p= 0.001). In our study we also found that the ANA titer ≥1/320 was a predictive factor for CTD diagnosis. This agreed with the findings of Mittoo et al (9). ANA titer >1/320 was proposed as a provisional criterion for lung dominant CTD (20).

The most common diagnosis found in the group of ILD's patients with positive ANA were connective tissue disease (16 patients), idiopathic pulmonary fibrosis (9patients) and sarcoidosis.

Rheumatoid arthritis (RA) was the most frequent CTD diagnosed in our study patients. According to the previous reports (9)(21) the most common types of subsequently diagnosed CTD are Rheumatoid arthritis (RA), inflammatory myositis and systemic sclerosis .

3 patients who had negative ANA were diagnosed RA. It has been well recognized that ANA are not positive in RA unless it is associated with another CTD like Sjögren's and SLE. For the diagnosis of RA, anti citrullinated cyclic peptide antibodies (anti CCP) has high specificities (22).

Our study has a few limitations. First the number of patients included may be more important to confirm our result. Second, the CTD diagnosis was made in 16 patients. In the remaining 54 patients, we do not know if these patients developed a CTD in follow up. Future researches should focus on prospective evaluation of patients with ILD to further investigate the significance of ANA in ILD and their relationship to prognosis and survival.

CONCLUSION

Our current findings show that ANA testing is an important tool of PID etiological diagnosis. The presence of ANA with a titer ≥1/320 was a predictive factor for CTD diagnosis. Interpretation of positive ANA test must take into consideration the presence of positive signs of autoimmune disease.

Conflicts of Interest

The authors have no financial conflicts of interest.

REFERENCES

1. Antoniou KM, Margaritopoulos GA, Tomassetti S, Bonella F, Costabel U, Poletti V. Interstitial lung disease. Eur Respir Rev Off J Eur Respir Soc 2014;23(131):40-54.
2. Cottin V, Capron F, Grenier P, Cordier J-F. [Diffuse idiopathic interstitial pneumonias. International multidisciplinary consensus classification by the American Thoracic Society and the European Respiratory Society, principal clinico-pathological entities, and diagnosis]. Rev Mal Respir.2004;21(2 Pt 1):299-318.
3. Homma Y, Ohtsuka Y, Tanimura K, Kusaka H, Munakata M, Kawakami Y, et al. Can interstitial pneumonia as the sole presentation of collagen vascular diseases be differentiated from idiopathic interstitial pneumonia? Respir Int Rev Thorac Dis. 1995;62(5):248-51.
4. Antin-Ozerkis D, Rubinowitz A, Evans J, Homer RJ, Matthay RA. Interstitial lung disease in the connective tissue diseases. Clin Chest Med.2012;33(1):123-49.
5. Bahmer T, Romagnoli M, Girelli F, Claussen M, Rabe KF. The use of auto-antibody testing in the evaluation of interstitial lung disease (ILD)--A practical approach for the pulmonologist. Respir Med. 2016;113:80-92.
6. Travis WD, Costabel U, Hansell DM, King TE, Lynch DA, Nicholson AG, et al. An Official American Thoracic Society/European Respiratory Society Statement: Update of the International Multidisciplinary Classification of the Idiopathic Interstitial Pneumonias. Am J Respir Crit Care Med.2013;188(6):733-48.
7. Lee JS, Kim EJ, Lynch KL, Elicker B, Ryerson CJ, Katsumoto TR, et al. Prevalence and clinical significance of circulating autoantibodies in idiopathic pulmonary fibrosis. Respir Med.2013;107(2):249-55.
8. Fischer A, Pfalzgraf FJ, Feghali-Bostwick CA, Wright TM, Curran-Everett D, West SG, et al. Anti-th/to-positivity in a cohort of patients with idiopathic pulmonary fibrosis. J Rheumatol.2006;33(8):1600-5.
9. Mittoo S, Gelber AC, Christopher-Stine L, Horton MR, Lechtzin N, Danoff

SK. Ascertainment of collagen vascular disease in patients presenting with interstitial lung disease. Respir Med.2009;103(8):1152-8.

10. Vij R, Noth I, Strek ME. Autoimmune-featured interstitial lung disease: a distinct entity. Chest. 2011;140(5):1292-9.
11. Kang BH, Park JK, Roh JH, Song JW, Lee CK, Kim M, et al. Clinical significance of serum autoantibodies in idiopathic interstitial pneumonia. J Korean Med Sci. 2013;28(5):731-7.
12. Chan EKL, Damoiseaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francescantonio PLC, et al. Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015. Front Immunol. 2015;6:412.
13. Voigt J, Krause C, Rohwäder E, Saschenbrecker S, Hahn M, Danckwardt M, et al. Automated Indirect Immunofluorescence Evaluation of Antinuclear Autoantibodies on HEp-2 Cells. Clin Dev Immunol [Internet]. 2012 [cité 15 mai 2018];2012. Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3502836/
14. Tebo AE. Recent Approaches To Optimize Laboratory Assessment of Antinuclear Antibodies. Clin Vaccine Immunol CVI [Internet]. 2017 [cité 15 mai 2018];24(12). Disponible sur: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5717181/
15. Kavanaugh A, Tomar R, Reveille J, Solomon DH, Homburger HA. Guidelines for clinical use of the antinuclear antibody test and tests for specific autoantibodies to nuclear antigens. American College of Pathologists. Arch Pathol Lab Med. 2000;124(1):71-81.
16. Tozzoli R, Bizzaro N, Tonutti E, Villalta D, Bassetti D, Manoni F, et al. Guidelines for the laboratory use of autoantibody tests in the diagnosis and monitoring of autoimmune rheumatic diseases. Am J Clin Pathol. 2002;117(2):316-24.
17. Brito F de A, Santos SME, Ferreira GA, Pedrosa W, Gradisse J, Costa LC, et al. Detection of anti-nuclear antibodies by indirect immunofluorescence on HEp-2 cells: setting the appropriate screening dilution for the diagnosis of autoimmune rheumatic diseases. Rev Bras Reumatol. 2014;54(1):13-20.
18. Ferreira JP, Almeida I, Marinho A, Cerveira C, Vasconcelos C. Anti-ro52 antibodies and interstitial lung disease in connective tissue diseases excluding scleroderma. ISRN Rheumatol. 2012;2012:415272.
19. La Corte R, Lo Mo Naco A, Locaputo A, Dolzani F, Trotta F. In patients with antisynthetase syndrome the occurrence of anti-Ro/SSA antibodies causes a more severe interstitial lung disease. Autoimmunity. 2006;39(3):249-53.
20. Fischer A, West SG, Swigris JJ, Brown KK, du Bois RM. Connective tissue disease-associated interstitial lung disease: a call for clarification. Chest. 2010;138(2):251-6.
21. Alsumrain M, De Giacomi F, Mirza S, Moua T. Utility of autoimmune serology testing in the assessment of uncharacterized interstitial lung disease: a large retrospective cohort review. Respir Res. 2017;18(1):161.
22. Whiting PF, Smidt N, Sterne JAC, Harbord R, Burton A, Burke M, et al. Systematic review: accuracy of anti-citrullinated Peptide antibodies for diagnosing rheumatoid arthritis. Ann Intern Med. 2010;152(7):456-64; W155-166.