Human Immunology 80 (2019) 523–532

Contents lists available at ScienceDirect

Human Immunology

journal homepage: www.elsevier.com/locate/humimm

Prevalence of autoimmune diseases and clinical significance of autoantibody profile: Data from National Institute of Hygiene in Rabat, Morocco

Hakima Missoum[a,b,*], Mohammed Alami[c], Fatema Bachir[d], Naima Arji[d], Abdelhakim Bouyahya[a], Mohammed Rhajaoui[d], Rajae El Aouad[e], Youssef Bakri[a]

[a] Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco
[b] Laboratory Autoimmunity, Department of Immunology, National Institute of Hygiene, Rabat, Morocco
[c] Laboratory of Microbiology and Molecular Biology, Faculty of Science, Mohammed V University, Rabat, Morocco
[d] National Institute of Hygiene, Rabat, Morocco
[e] Hassan II Academy of Science and Technology Rabat, Morocco

ARTICLE INFO

Keywords:
Systemic autoimmune rheumatic diseases
Organ specific autoimmune diseases, auto antibodies profile
Prevalence
Rabat

ABSTRACT

Aim: The objective of this study was to explore the prevalence of various autoimmune diseases (AIDs) in a large cohort of patients and to characterize the autoantibody profile in the patients with and without AIDs to confirm the diagnosis and to refine the Moroccan databases.

Patients and method: Retrospective study was conducted in the Laboratory of autoimmunity National Institute of Hygiene (NIH) of Rabat in Morocco. A total of 3182 consecutive Moroccan patients (2183 females and 999 males) whose sera were tested for 14 autoantibody profile between 2010 and 2016.

Results: Only 944 (29.7%) patients were diagnosed with AIDs of those suspected. The prevalence of systemic lupus erythematosus (SLE), intestinal malabsorption (IM) and arthritis polyarthralgia (AP) were the highest (4.2, 4.1 and 4%), subsequently followed by rheumatoid arthritis (RA) (2.8%), cholestatic syndrome (CS) (1.8%), interstitial lung disease (ILD) (1.6%).In females IM, AP and SLE also showed the highest prevalence (5.4%, 5.3% and 4.9% respectively), while of male, SLE showed the highest prevalence (1.9%). The prevalence of ANA was increased in most patients with systemic especially in neuropathy (NP), hemolytic anemia (HA), primary Sjogren's syndrome (pSS), dermatomyositis (DM), thrombocytopenia (Tb), systemic sclerosis (SSc), ANCA-associated vasculitis (AAV), AP, Renal impairment (RI), SLE, and mixed connective tissue disease (MCTD). Anti-dsDNA antibodies were higher in SLE and ENA showed the highest titers in MCTD. Others are relatively specific for certain disease, such as anti β2GP1 for thrombosis syndrome, anti ANCA for primary sclerosing cholangitis (PSC), AAV, ILD and RI, anti CCP2 for RA, ILD and AP. the prevalence of anti AMA was higher in primary biliary cirrhosis (PBC), followed in CS, also, ANA have been identified in up to 25% of patients with primary biliary cirrhosis. The prevalence of anti-SMA was higher in PBC, treated patients for Chronic hepatitis C (HCV), and autoimmune hepatitis (AIH) and anti-PCA was higher in biermer anemia patients with vitamin B12 deficiency (BA/Def vit B12). The prevalence of IgA EMA, IgA tTG and IgA AGA were higher in patients IM and celiac disease (CD). The prevalence of anti thyroperoxidase (TPO) was significantly increased in the autoimmune thyroiditis (AIT).

Conclusion: Our study shows the diagnostic value of auto antibodies in AIDs. It would be interesting to carry out prospective studies on each pathology separately, in order to fill the classic vagaries of the retrospective study and objectively estimate the prevalence in different AIDs. These data on the prevalence of each autoimmune disease are valuable for the public health system.

1. Introduction

Autoimmune diseases (AIDs) include a wide variety of illnesses targeting many sites in the body. These diseases translate the rupture of the immune tolerance against antigens of the self, which attack and damage the body's own tissues, organs and cells [1].To date, the

* Corresponding author at: Laboratory of Human Pathologies Biology, Department of Biology, Faculty of Sciences, and Genomic Center of Human Pathologies, Faculty of Medicine and Pharmacy, Mohammed V University in Rabat, Morocco.
E-mail address: hkmissoum@yahoo.fr (H. Missoum).

https://doi.org/10.1016/j.humimm.2019.02.012
Received 13 December 2018; Received in revised form 15 February 2019; Accepted 22 February 2019
Available online 23 February 2019

American autoimmune related disease association (AARDA) has classified more than 100 AIDs [2], making it the third most common type of disease in the United States [2]. And they represent an important health problem, affecting at least 5–10% of the world population [2–4], especially young adults, and women are more susceptible to these diseases than men [3–5]. Some of the AIDs are within the top 10 leading causes of death among women aged 65 and older [2,3].

Information on the prevalence of various diseases is insufficient, especially in countries out of Europe or North America [6]. There is epidemiological evidence of increased incidence and prevalence of certain AIDs in highly industrialized countries, which cannot be attributed to better diagnoses alone [3,4]. The incidence and prevalence of some AIDs appear to be increased in certain ethnic groups; for example, systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), type 1 diabetes, psoriasis and celiac diseases are increased in African American, certain Native American, and Caucasian populations, respectively [2].

According to the clinical manifestation, AIDs may be classified as systemic (e.g. systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), dermatomyositis (DM), mixed connective tissue disease (MCTD), etc) or as organ-specific (e.g. autoimmune thyroiditis (AIT), celiac disease (CD), etc). However, this clinically useful classification does not correspond to the underlying pathogenetic mechanisms [1]. Nevertheless, there is often overlap between these two groups. These diseases are characterized by the clinical appearance of inflammation, joint and muscle pain, fatigue, increased antibodies to the endocrine glands and other tissues, and connective tissue degradation. However, these AIDs are difficult to diagnose because they are not specifically symptomatic [7].The complexity of their spectrum is enormous and although their etiology is still uncertain, it has been shown that genetic and environmental factors are involved in triggering the pathological mechanism [2]. It is mentioned a theory according to which the level of hygiene and sanitary conditions (e.g. drinking water, food, housing conditions, etc.) in a given country might have an impact on the development of AIDs [2].

AIDs can only be defined on a set of specific clinical and biological criteria, among which the autoantibody titer is fundamental. The search for autoantibody is important and useful serological immunological markers widely used as laboratory tools for the diagnosis and monitoring of AIDs [8]. The classification of certain AIDs is based on the presence or absence of the corresponding antibodies. They can also be predictive of risk factors or be a criterion of therapeutic follow-up [9]. In clinical practice, most sera from patients suspected of having AIDs are required by clinicians to be tested for AIDs diagnosis confirmation. However, the prevalence of AIDs remained virtually unknown in subjects with clinically suspected AIDs manifestations, although it has been widely studied in the general population worldwide [10,11].

The aim of this study is to explore the prevalence of various AIDs in a large cohort of patients with AIDs diagnosed symptoms and to characterize the autoantibody profile in the patients with and without AIDs for helping to confirm or exclude the diagnosis of this pathology and increasing the Moroccan databases.

2. Materials and methods

2.1. Patients

A total of 3182 consecutive Moroccan patients (2183 females and 999 males) from Rabat- Morocco were received by the laboratory autoimmunity of National Institute of Hygiene (NIH), given that the laboratory was the first perform autoimmunity tests since 20 years, it still receive the major patients from different Moroccan cities, for auto antibodies testing, including antinuclear antibodies (ANA), anti- deoxyribonucleic acid antibodies (ds DNA), anti extractable nuclear antigens antibodies (ENA), anti-phospholipides antibodies (APL:β2 Glycoprotéine1), anti-peptides cycliques citrullinés antibodie (CCP2), anti- Anti-neutrophil cytoplasmic antibodies (ANCA), Anti-mitochondries antibodies (AMA), anti smooth muscle antibodies (SMA), anti parietal cell antibodies (PCA), anti liver kidney microsome antibodies (LKM1), anti liver cytosol antibodies (LC1) anti-endomysium antibodies IgA (EMA), anti-gliadine antibodies (AGA) IgA, anti-transglutaminase antibodies (tTG) IgA and anti-thyropéroxydase antibodies (TPO), between 2010 and 2016. Serum samples were referred from the departments of rheumatology, nephrology, gastroenterology, dermatology, general internal medicine, neurology and oncology from the hospitals of the city of Rabat and its large region for the diagnosis and follow-up of AIDs.

All registration forms were carefully checked and patients who had no positivity for autoimmune markers were excluded. However, 3182 patients (2183 females and 999 males) with positive antibodies were identified and included in the study (22%).

In this study, AIDs included systemic lupus erythemateux (SLE), rheumatoid arthritis (RA), mixed connective tissue disease (MCTD), dermatomyositis (DM), primary Sjogren's syndrome (pSS), ANCA-associated vasculitis (AAV), systemic sclerosis (SSc), arthritis polyarthralgia (AP), Interstitial lung disease (ILD), Renal impairment (RI), thrombocytopenia (Tb), hemolytic anemia (HA), Neuropathie (NP), celiac disease (CD), intestinal malabsorption (IM), Cholestatic syndrome (CS), autoimmune hepatitis (AIH), treated patients Chronic hepatitis C (HCV), primary biliary cholangitis (PBC), biermer anemia/vitamin B12 deficiency (AB/def vit B12), type 1 diabetes (Type1D) and autoimmune thyroiditis (AIT).

2.2. Immunological tests

This study was carried out by the laboratory of autoimmunity of NIH using two techniques with PhD Bio-Rad automate:

2.2.1. Indirect immunofluorescence (IIF)

Antibodies were detected by IIF using Hep-2 cells (Bio-Rad Laboratories, CA) for ANA as highly sensitive substrates and rodent tissue sections (triple tissue testing) for AMA, SMA, PCA, LKM1 and LC1 (Bio-Rad Laboratories, CA),on crithidia luciliae-coated slides (Bio-Rad Laboratories, CA) for anti dsDNA antibody, on polynuclear neutophil Cytoplasmic (Bio-Rad Laboratories, CA) for ANCA, on 1/3 oesophage of monkey (Bio-Rad Laboratories, CA) for EMA IgA.

2.2.2. Enzyme –linked immunosorbet assay (ELISA)

The anti ENA antibodies profile was performed by ELISA (Bio-Rad Laboratories, CA), such as anti smith antibody (Sm), anti-ribonucleoprotein antibody RNP, anti- Ro antibody (SSA), anti-la antibody (SSB), anti- Jo1 antibody and anti- Scl-70 antibody and also tests for anti APL:β2 Glycoprotéine1, anti-CCP2, anti AGA IgA, anti tTG IgA and anti-TPO were done by ELISA (Bio-Rad Laboratories, CA).

Serum samples were processed in initial dilution of 1:160, 1:5, 1:20, 1:40, 1:10 and 1:101 for the detection of anti-ANA, anti-dsDNA, anti-ANCA, anti tissue (AMA, MSA, PCA, LKM1, LC1), anti-EMA IgA and anti-ENA, anti B2GP1, anti CCP2, anti-AGA IgA, anti-tTG IgA and anti-TPO antibodies respectively.

2.3. Statistical analysis

The different parameters of descriptive statistics and the structure analysis were performed using the data of diseases and corresponding auto-antibodies. The indices and standard deviations were calculated using XLSTAT Software [12]. Also, the correlation between Auto-Antibodies and Diseases was detected by 4 connection analyzes, the distribution of auto-antibodies to each disease, the principal component analysis (PCA), the factorial analysis of the correspondences (FAC) and finally, the correspondence factor analysis (CFA). These analyzes were also realized using XLSTAT Software [12].

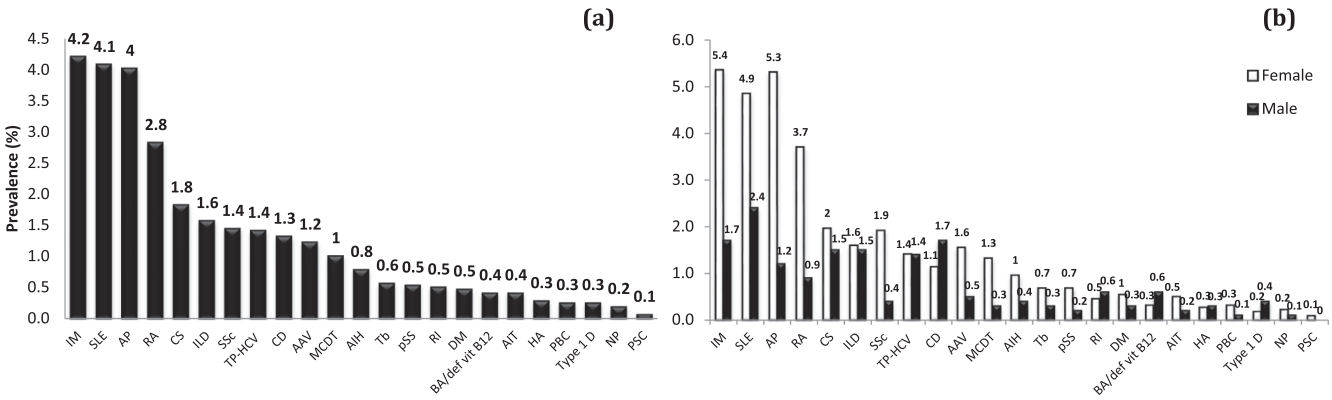

Fig. 1. Prevalence of various AIDs in patient cohort with suspected AIDs (a) and arrayed for sex (b).

3. Results

3.1. Prevalence of various AIDs

Of 3182 consecutive patients with 'suspected' AIDs, only 944 (29.7%) patients were diagnosed with AIDs and the remaining 2238 (70.3%) patients had non- AIDs. Of those with AIDs, the prevalence of SLE, IM and AP were highest (4.2, 4.1 and 4%), subsequently followed by RA (2.8%), CS (1.8%), ILD (1.6%), SSc and HCV (1.4%), CD (1.3%), AAV (1.2%), MCTD (1%), AIH (0.8%),Tb (0.6%), RI and DM (0.53%), BA/def Vit B12 and AIT (0.4%), HA, PBC and type 1 D (0.4%), NB (0.2%), CSP (0.1). Of female patients with AIDs, IM also showed the highest prevalence (5.4%), followed by AP (5.3%), SLE (4.9%), RA (3.7%), CS (2%), SSc (1.9%), ILD and AAV (1.6%), treated patients for HCV (1.4%), MCTD (1.3%), etc., while of male, SLE showed the highest prevalence (1.9%), followed by CD and IM (1.7%), ILD and CS (1.58%) (Fig. 1).

especially with regard to the age of patients with in particular as regards the age of patients with .Regarding the age of the patients with SLE, SSc, MCTD, Tb, RI, DM, HA, IM, CD, type 1 diabetes and AIH had the age groups mostly affected were of their age < 18 and 30 years (57%, 60%, 55%, 40%, 71%, 50%, 50%, 85%, 63% and 33.3% respectively) younger than patients with AP, RA, AAV, pSS, CS, HCV, whose the age groups mostly affected were of their age 41 and 50 years (27%, 20%, 25%, 33.3%, 54.5% and 50% respectively) for patients with ILD, the age groups mostly affected were of their age 50 and 60 years (50%) and patients in AIT. The age groups mostly affected were of their age > 60 years.

3.2. Immunological manifestations

In 3182 consecutive patients with 'suspected' AIDs, ANA were detected in 34.2% (1089/3182), anti-ds DNA in 17.2%, anti-ENA in 8.05%, anti APL: β2 Glycoprotéine1 and ANCA in 3%, anti CCP2 in 4.9%, AMA in 2.8%, SMA in 2.2%, PCA in 2.2%, anti LKM1 in 2.2%, anti-EmA IgA in 3.8%, anti AGA IgA in 5.4%, anti-tTG IgA in 9.8% and anti-TPO in 1.3%.

Furthermore, ANA were present in 100% of NP patients, 88.8% of AH patients, 80% of DM patients, 82% of pSS patients, 80% of DM patients, 78.3% of SSc patients, 77.8% of Tb patients, 75% of AP patients, 71% of ANCA patients, 68.8% of RI patients 63.% of SLE patients, 62% of MCTD and treated patients for HCV, 60% of AIH patients, 57% in RA patients, 50% of PSC patients, 48% of CS patients, 44% of ILD patients, 25% of CPB and type 1 diabetes patients and 1.7% of CD and AB/def vit B12.

For anti-dsDNA showed the highest prevalence 16.2% in SLE, subsequently followed by RI 6.3%, Tb 5.6%, MCTD 3.1%, AAV 2.6%, RA 2.2, CS 1.7% and AP 0.8%. Auto antibodies profile showed the highest prevalence in MCTD 28.1%, SSC 21.0%, Pss 17.6% and SLE 16.2%, subsequently followed by ILD 8%, RA 6.7%, R I 6.3%, AP 3.2%, CD 2.4% and, CS 1.7%. The frequency of the components ENA profile autoantibodies detected in our suspected SAIDs patients is presented in Table 2. Of the total of 65 patients studied, anti- SSA antibodies were found in 32 (49%) patients, anti-Sm antibodies were positive in 14 (22%), anti- Scl-70 in 10 (15%) patients; anti- SSB in 5 (8%) patients, anti-Sm/RNP and anti jo-1 antibodies in 2 (3%) patients.

Of anti-ENA antibodies anti-SSA and anti-Sm antibodies showed the highest specificities for SLE patients (52% and 48%). Anti-Scl-70 were also most commonly present in SSc patients (80%).Anti-Sm and anti SSA antibodies were also detected in very low frequencies of SSc patients (10% and 10%). Anti SSA antibodys were also most commonly found in AP (60%), in RA and ILD patients (50%). Anti SSB and anti Sm antibodies were low frequency in AP patients (20% and 20%). Anti-SSB, anti Scl-70 and jo-1 antibodies were Also low frequency in RA patients (17%, 17% and 17%). Anti-Sm and anti-Sm/RNP antibodies were also detected in ILD patients (25% and 25%). Anti-SSA and anti-SSB antibodies were most commonly present detected in DM patients (50% and 50%). Anti-SSA, anti-Sm, anti-SSB, anti- Sm/RNP, anti-Scl 70, and anti jo-1 antibodies were also most commonly present in MCTD patients (44%, 11%, 11%, 11%, 11% and 11%). Furthermore, anti- SSA and anti- SSB antibodies showed a high specificity for pSS patients (67% and 17%). we could not make an interpretation for these diseases RI, CD, Treated patients for HCV and HA because the number of patient was not enough for Anti-ENA antibodies.

Anti APL: β2 Glycoprotéine1was present in Tb, MCTD and SLE patients (16.7%, 6.3%, 4.3%), also detected in very low frequencies of RA, ILD and AP (2.2%, 2%, and 0.8%). ANCA were detected in 50% of CSP Patients, 25.6% of AAV, 22% of ILD and 18.8% of RI and were also detected in DM, AP, CS and, RA patients, but the prevalence was very low (< 10%). Anti CCP antibodies were most commonly present in RA, ILD and AP (28.9%, 24% 14.8%). Anti-liver antibodies, AMA antibodies were most commonly present in PBC and CS patients (50% and 17.2%), but the prevalence was low frequencies of BA/def vit B12 and AIH patients (7.7% and 4.4%). Anti SMA were also detected in PBC, treated patients for HCV and AIH patients (25%, 22.2% and 20%), they were also present in the prevalence low frequencies of CS, Type 1 D and CD patients (13.8%, 12.8% and 4.8%). Anti PCA was most commonly present in BA/def vit B12 patients (84.6%),also was detected in Type 1 D patient 12.3%, in CS, treated patients for HCV and AIH patients (8.9%, 8.6% and 8%), in AIT patients (7.7%) and in SLE and MCDT patients (0.8%). Anti LKM1 was detected in CS and treated patients for HCV patients (3.2% and 2.2%). Anti LC1 no patient was detected.

Anti EMA IgA antibodies were most commonly present in IM and Type 1 D patients (13.4%, 12.3%), in CD and AIH patients (8.0% and 7.1%), in CS patients (1.7%). Anti tTG IgA were also detected in IM and CD patients (52.2% and 50%), in Type 1 D patients (25%). Anti AGA IgA was detected in IM and CD (31.3% and 28.6%).

Anti TPO antibodies were most commonly present in AIT patients

Table 1
Comparisons of frequencies of various MAIs and serum autoantibody profile.

	N %		WOMEN %		ANA %		ds DNA %		ENA %		APL % (b2GP1)		C-ANCA %		P-ANCA %		CCP2 %
All	3182		2183		1089		549		256		95		93		1		155
	944	30%	774	82%	481	51%	29	3%	65	7%	13	1%	32	3%	1	0.11%	57
IM	134	4%	117	87%	0		0		0		0		0		0		0
SLE	130	4%	106	82%	82	63%	21	16%	21	16%	4	3%	0		0		0
AP	128	4%	116	91%	96	75%	1	1%	5	4%	1	1%	4	3%	0		19
RA	90	3%	81	90%	52	58%	2	2%	6	7%	2	2%	1	1%	0		26
CS	58	2%	43	74%	28	48%	1	2%	1	2%	0		2	3%	0		0
ILD	50	2%	35	70%	22	44%	0		4	8%	1	2%	10	20%	1	2%	12
SSc	46	1%	42	91%	36	78%	0		10	22%	0		0		0		0
TP-HCV	45	1%	31	69%	28	62%	1	2%	1	2%	0		0		0		0
CD	42	1%	25	60%	3	7%	0		1	2%	0		0		0		0
AAV	39	1%	34	87%	28	72%	1	3%	0		0		10	26%	0		0
MCTD	32	1%	29	91%	20	63%	1	3%	9	28%	2	6%	0		0		0
AIH	25	1%	21	84%	15	60%	0		0		0		0		0		0
Tb	18	1%	15	83%	14	78%	1	6%	0		3	17%	0		0		0
pSS	17	1%	15	88%	14	82%	0		3	18%	0		0		0		0
RI	16	1%	10	63%	11	69%	1	6%	1	6%	0		3	19%	0		0
DM	15	0.40%	12	80%	12	80%	0		2	13%	0		1	7%	0		0
BA/d.V. B_{12}	13	0.40%	7	54%	1	8%	0		0		0		0		0		0
AIT	13	0.40%	11	85%	0		0		0		0		0		0		0
HA	9	0.30%	6	67%	8	89%	0		1	11%	0		0		0		0
PCB	8	0.30%	7	88%	2	25%	0		0		0		0		0		0
D type 1	8	0.30%	4	50%	2	25%	0		0		0		0		0		0
NP	6	0.20%	5	83%	6	100%	0		0		0		0		0		0
PSC	2	0.10%	2	100%	1	50%	0		0		0		1	50%	0		0

	CCP2 %	AMA %		MSA %		PCA %		LKM 1%		LC1	EMA IgA %		tTG IgA %		AGA IgA %		TPO %	
All	155	89		71		69		70		0	120		311		172		42	
	6%	17	2%	28	3%	26	3%	3	0%	0	25	3%	97	10%	54	6%	16	2%
IM	0	0		0		0		0		0	18	13%	74	55%	42	31%	0	
SLE	0	0		0		1	1%	0		0	0		0		0		1	1%
AP	15%	0		0		1	1%	0		0	0		0		0		1	1%
RA	29%	1	1%	0		0		0		0	0		0		0		0	
CS	0	10	17%	8	14%	5	9%	2	3%	0	1	2%	0		0		0	
ILD	24%	0		0		0		0		0	0		0		0		0	
SSc	0	0		0		0		0		0	0		0		0		0	
TP-HCV	0	0		10	22%	4	9%	1	2%	0	0		0		0		0	
CD	0	0		2	5%	0		0		0	3	7%	21	50%	12	29%	0	
AAV	0	0		0		0		0		0	0		0		0		0	
MCTD	0	0		0		0		0		0	0		0		0		0	
AIH	0	1	4%	5	20%	2	8%	0		0	2	8%	0		0		0	
Tb	0	0		0		0		0		0	0		0		0		0	
pSS	0	0		0		0		0		0	0		0		0		0	
RI	0	0		0		0		0		0	0		0		0		0	
DM	0	0		0		0		0		0	0		0		0		0	
BA/d.V. B_{12}	0	1	8%	0		11	85%	0		0	0		0		0		0	
AIT	0	0		0		1	8%	0		0	0		0		0		12	92%
HA	0	0		0		0		0		0	0		0		0		0	

(*continued on next page*)

Table 1 (*continued*)

	CCP2 %	AMA %		MSA %		PCA %		LKM 1%	LC1	EMA IgA %		tTG IgA %		AGA IgA %		TPO %	
PCB	0	4	50%	2	25%	0		0	0	0		0		0		0	
D type 1	0	0		1	13%	1	13%	0	0	1	13%	2	25%	0		1	13%
NP	0	0		0		0		0	0	0		0		0		0	
PSC	0	0		0		0		0	0	0		0		0		0	

Abbreviation: ANA: antinuclear antibodies; ds DNA: deoxyribonucleic acid antibodies; ENA: extractable nuclear antigens antibodies; APL (β2 Glycoprotéine1): phospholipides antibodies; CCP2: peptides cycliques citrullines antibodies; ANCA: Anti-neutrophil cytoplasmic antibodie; AMA: Anti-mitochondriea antibodies; SMA: smooth muscle antibodies; PCA: parietal cell antibodies; LKM1: type 1 liver kedney microsome antibodies; LC1: Liver anti cytosol antibody; EMA: endomysium antibodies; AGA: anti gliadine antibodies. tTG: transglutaminase antibodies; TPO: thyropéroxydase antibodies; SLE: systemic lupus erythemateu; RA: rheumatoid arthritis; MCTD: mixed connective tissue disease; DM: dermatomyositis; pSS: primary Sjogren's syndrome; AAV: anti-neutrophil cytoplasmic antibodies associated vasculitis; SSc: systemic sclerosis; AP: arthritis polyarthralgie; ILD: Interstitial lung disease; RI: Renal impairment; Tb: thrombocytopenia; HA: hemolytic anemia; NP: Neuropathie; CD: celiac diseases; IM: intestinal malabsorption; CS: Cholestatic syndrome; AIH: Autoimmune hepatitis; TP-HCV: Treated patient for Chronic hepatitis C; PBC: primary biliary cholangitis; PSC: primary sclerosing cholangitis; BA/def vit B_{12}: biermer anemia/vitamin B_{12} deficiency; Type1 D: type 1 diabetes; AIT: autoimmune thyroiditis.

(92.3%), in Type 1 D patients (12.5%), but the prevalence was low frequencies of treated patients for HCV (2.2%), in SLE and AP patients (0.8%) (Table 1).

3.3. Statistical analysis

3.3.1. Descriptive statistics

Table 3 represents a descriptive statistic of the Auto-Antibodies tested in our analysis

3.3.2. Correlation between Auto-Antibodies and diseases

Principal Component Analysis (PCA) was performed with a correlation matrix according to the Pearson correlation coefficient (n).

The Auto-Antibody distribution revealed a relationship between them in three groups; the first group includes the AMA, MSA, PCA, type 1 LKM1 and Auto-Antibodies while the second group contains the 3 Auto-Antibodies: EmA, IgA tTG and IgA AGA. The last group contains the other Auto-Antibodies (ANA, ds DNA, ENA, APL (β2GP1), CCP2, C-ANCA and P-ANCA). TPO did not represent any distributions (Fig. 2). Also, LC1 was not part of our correlation analysis because it was not detected in any patient in our study.

Correlation between Auto-Antibodies (variables) and Diseases (factors) showed a strong binding between the first group of Auto-Antibodies (AMA, MSA, PCA, and LKM1) and Cholestatic Syndrome (CS) while the second group (EmA, tTG IgA and AGA IgA) shows strong binding with intestinal malabsorption (IM) disease. Also, systemic lupus erythematosus (SLE) disease exhibits binding to autoantibodies (ANA, ds DNA, ENA and APL (β2GP1)) (Fig. 3) [13].

Since the calculated p-value is below the significance level ($p < 0{,}0001$; alpha = 0.05), it can be concluded that there is a link between the auto-antibodies (variables) and the diseases (factors) which confirms the result of the Principal Component Analysis (PCA). Thus, the Correspondence Factor Analysis (CFA) result revealed a strong link between the thyroid autoimmune disease (AIT) and the TPO Autoantibody. This is also the case of the Principal Component Analysis (PCA), Auto-Antibody that has been linked to the diseases biermer anemia/deficiency vitamin B12 (BA/Def vit B12) and diabetes type 1 (D type 1) (Fig. 4).

4. Discussion

Autoimmune diseases are complex disorders caused by a combination of genetic susceptibility, level of hygiene and sanitary conditions and environmental factors (e.g. drinking water, food, housing conditions, etc.) [6,7,14–16].

This article contributes to the study of autoimmune diseases by defining the prevalence of the most common autoimmune diseases in a sample of the general population of Rabat area in Morocco. In fact, little is known about the prevalence of AIDs in Morocco.

Our retrospective study to the prevalence of suspected AIDs cases was evaluated to 29.7%, this percentage exceeds that of the literature which varies from 5% to 12.9% [4,6,13,14,17]. This high prevalence may be explained by a bias of AIDs clinical suspicion. Also, suggesting that suspected clinical manifestations of AIDs are confusing. In accordance with the literature [2], women from our series were more affected than men (82%).

Our series allowed to distinguish two clinical groups. The first group of patients with non-organ specific or systemic AIDs. Phenotype, the most frequent of these diseases were SLE, AP and RA, followed by, ILD, SSc, AAV, MCTD, Tb, pSS, RI, DM, HA and NB which range from 4.2% to 0.2% (Table 1). These frequencies were similar to those of the literature [4,13,18–20], however other studies showed lower frequencies [21]. Our study shows that the majority of systemic AIDs as occur in young patients aging 18–30 years old, with an exception for AP, RA, ILD, AAV and pSS where the patients were more than 30 years old. The prevalence of SLE is even higher in the juvenile suspected of systemic

Table 2
The components of anti-ENA antibodies in the patients with autoimmune diseases.

Autoantibodies tests	All	SLE	AP	RA	CS	ILD	SSc	Treated patients for HCV	CD	MCTD	pSS	RI	DM	HA
Anti-ENA test	256	83	20	24	4	16	39	4	4	35	12	4	8	4
Anti-ENA (+)	65	21	5	6	1	4	10	1	1	9	3	1	2	1
Anti-SM (+)	14	10	1			1	1			1				
Anti-SSA (+)	32	11	3	3	1	2	1	1	1	4	2	1	1	1
Anti-SSB (+)	5		1	1						1	1		1	
Anti-RNP/SM (+)	2					1				1				
Anti-SCL70 (+)	10			1			8			1				
Anti-Jo1 (+)	2			1						1				

ENA: extractable nuclear antigens antibodies; SLE: systemic lupus erythemateu; RA: rheumatoid arthritis; MCTD: mixed connective tissue disease; pSS: primary Sjogren's syndrome; SSc: systemic sclerosis; AP: arthritis polyarthralgie; ILD: Interstitial lung disease; RI: Renal impairment; HA: hemolytic anemia; CD: celiac diseases; CS: Cholestatic syndrome; TP-HCV: Treated patient for Chronic hepatitis C.

autoimmune rheumatic diseases (SARDs) than that in various adult groups compared to general population [20.23]. This result suggested that juvenile patients having clinical SLE manifestations need to be paid a closer attention than the adults. Our results are of an important help for the identification of a specific AIDs in certain age groups. Yet clinical features of this disease did not differ worldwide, their frequencies showed some discrepancies from a study to another [23]. This diversity could be explained by the geographic variation, environmental and socioeconomic factors which can likely play an important role in the manifestation of the disease [23], however a Chinese study showed similarities to our findings [24]. These estimates are probably due to signs and symptoms suggestive of systemic autoimmune disease as undifferentiated connective tissue diseases (UCTD) [25–27]. Rheumatologic signs also remains the predominant clinical manifestation in our present study. It occurs mainly in the form of arthritis polyarthralgia (AP) in 4% of our patients, a prevalence that is higher than reported in RA [24].

The second group of patients is that of those having organ specific autoimmune diseases. The most frequent of these diseases in our series were IM and CD (4.2% and 1.3% respectively). The CD patients showed atypical and uncomfortable symptoms in agreement with other reports [28]. This percentage exceeds that observed in the European and North American population (de 0,5–1%) [29–31], however similar frequencies were found in other population studies [29–32]. Despite considerable variations between the countries, celiac disease frequencies are high in Canada, Israel, Denmark and North African countries [33]. These observations point to an influence of environmental factors [33]. The reasons for this high frequency are not clear, but may be related to dietary and/or environmental changes and the diagnosis rate of celiac disease that has increased rapidly these last years. In fact, this phenomenon has been attributed to an increase in the prevalence of the disease and the development of non-invasive diagnostic tools [31]. Among the second group we have also noticed a high prevalence of autoimmune hepatopathies represented by CS (1.8%) and treated patients with HCV (1.4%), AIH (0.8%), BA/defVit B12, PBC and PSC were approximately similar to those reported in the literature [34]. Also in this group the prevalence of type 1 diabetes (type 1D) (0.4%), is similar to the values reported in the current literature for European countries [35] and Maghreb countries [36]. Similarly for prevalence AIT (0.4%), is slightly lower in other studies [37]. Regarding females, the prevalence of IM, AP, and SLE reaches 5.4%, 5.3% and 5% respectively (Fig. 1). In the order of the prevalence of various AIDs in this study, the most commonly seen in general population are RA and CD [19,22].These prevalences are comparable to those reported in other studies [4,13,38,39].

Our data on the prevalence of each autoimmune disease are useful for the public health system as they provide an accurate assessment of the burden of each disease. The particular genetic background of the studied population suggests careful conclusions in regards with generalizing these results. In all cases, these data provide an image of the relative burden of the diseases.

Regarding the immunological profiles in AIDs, the prevalence of ANA in our patients was high in most systemic AIDs (especially in NP, HA, pSS, DM, Tb, SSc, AAV, AP, RI, SLE, MCTD), these frequencies were similar to those reported in the literature [24,41,42]. This frequency of ANA could also be observed in organs specific AIDs like PSC, CS, ILD, AIH, PBC, but not in AIT and IM. The presence of ANA should lead to the search for antibodies of specificity defined according to the fluorescence detection and the disease suspicion [9,41,43].

Anti-DNA and antigen-specific antibodies (ENA profile) are considered as biomarkers of systemic active autoimmune disease, and their presence may reflect the severity of such pathology. For anti-dsDNA antibodies, we have found higher frequency in SLE than in the others AIDs. This could be explained by the specificity of autoantibodies for SLE however the presence of anti dsDNA antibodies varies considerably depending on the country and ethnicity [44,46]. This frequency is lower than found in literature (Kuwait, Dubai, Tunisia, China) [44]. It should be noted that the ENA profile has been mentioned in systemic AIDs because more specific antibodies confirm the suspected disease [24,42]. In our study, ENA profiles are relatively specific for certain diseases such as anti-Sm and anti-SSA antibodies for SLE, Anti-Scl 70 for SSc, Anti SSA for AP, RA, ILD and MCTD, Anti-SSA and anti-SSB for DM and Pss. however similar frequencies were found in other population studies [24,45,46]. Moreover, our results have made it possible to correct the diagnosis in 1/3 or 3/4 of cases, but the fact that it has been given the specificity of auto-antibodies. Of note, conventional ENA profile appears to be of no significance for RI, CD, Treated patients for HCV and HA, since ANA and any anti-ENA were rarely if ever seen in these AIDs in this study (Table 2).

Nevertheless, the autoantibody assessment has become an integral part of the differential diagnosis. The results of serologic tests for autoantibody, including tests for ANA, ENA and ds DNA, may hence be a cornerstone for the diagnostics of systemic rheumatic diseases, and ruling out misleading pathologies during differential and early diagnosis [40,44]. Others are relatively specific for certain disease, such as anti B2GP1 for thrombosis syndrome, anti ANCA for PSC, AAV, ILD and RI, anti CCP for RA, ILD and AP. In our study, the result shows a correlation between autoantibodies (ANA, dsDNA, ENA and APL (b2GP1) with systemic lupus erythematosus (SLE) (Fig. 2), these results are similar in the literature [13].

Regarding immunological markers in liver disease, our study shows that the prevalence of anti-SMA was higher in PBC, patients treated for HCV, and AIH, followed by CS. ANA have also been identified in this disease (Table 1), this percentage are similar in the literature [47,48]. ANA and/or anti-smooth muscle antibodies (SMA) are the common diagnostic markers of AIH type I [43]. The auto antibodies in autoimmune hepatitis (AIH) are extensively used for its diagnosis and classification. However, they are also useful for defining the prognosis

Table 3
Descriptive Analysis of Auto-Antibodies.

Variable	Observations	Minimum	Maximum	Average	Standard Deviation
ANA %	22	0.000	96.000	22.182	25.693
ds DNA %	22	0.000	21.000	1.409	4.426
ENA %	22	0.000	21.000	3.136	4.960
APL (β_2GP_1)%	22	0.000	4.000	0.591	1.141
C-ANCA %	22	0.000	10.000	1.455	2.972
P-ANCA %	22	0.000	1.000	0.045	0.213
CCP2 %	22	0.000	26.000	2.591	7.015
AMA %	22	0.000	10.000	0.727	2.251
MSA %	22	0.000	8.000	0.955	2.035
PCA %	22	0.000	5.000	0.500	1.144
LKM 1%	22	0.000	2.000	0.091	0.426
EMA IgA%	22	0.000	18.000	1.136	3.846
tTG IgA %	22	0.000	74.000	4.409	16.174
AGA IgA %	22	0.000	42.000	2.455	9.195
TPO %	22	0.000	12.000	0.818	2.594

Abbreviation: ANA: antinuclear antibodies; ds DNA: deoxyribonucleic acid antibodies; ENA: extractable nuclear antigens antibodies; APL (β2 Glycoprotéine1): phospholipides antibodies; CCP2: peptides cycliques citrullines antibodies; ANCA: Anti-neutrophil cytoplasmic antibodie; AMA: Anti-mitochondriea antibodies; SMA: smooth muscle antibodies; PCA: parietal cell antibodies; LKM1: type 1 liver kedney microsome antibodies; LC1: Liver anti cytosol antibody; EMA: endomysium antibodies; AGA: anti gliadine antibodies. tTG: transglutaminase antibodies; TPO: thyropéroxydase antibodies.

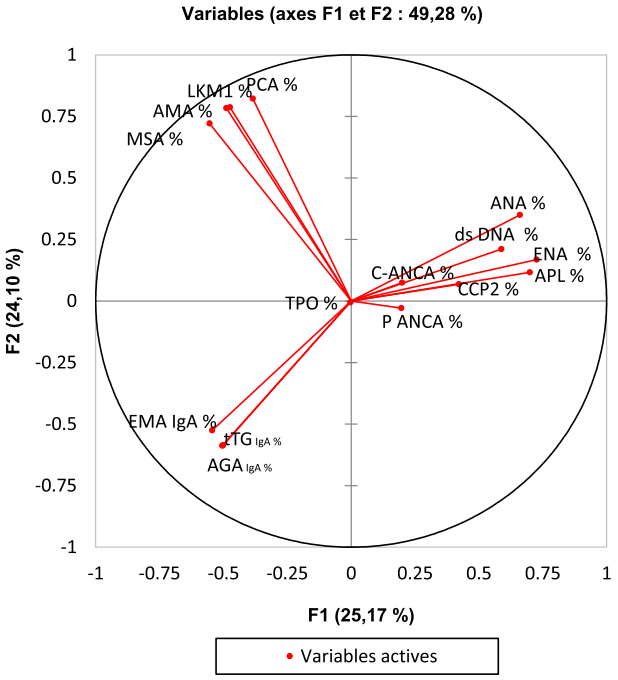

Fig. 2. Distribution of Auto-Antibodies.

and inferring clinical behavior [43]. Females (84.04%) accounted for the majority of patients with autoimmune liver diseases, wich is in accordance with the literature [49]. When AIH is suspected, the presence of one or a combination of anti-ANA, anti-SMA, anti-LKM-1 and pANCA antibodies is useful for the often difficulties in differential diagnosis between HAI and other liver disorders [43]. When these markers are absent, other secondary antibodies, such as anti-soluble liver/pancreas antigen (anti-SLA/LP) and liver cytosol type 1 (anti-LC1), should be used [45]. For anti AMA2 the frequency was high in PBC, followed by CS, also, ANA have been identified in up to 25% of patients with PBC (Table 1). This prevalence was lower as compared to the literature [51,52]. Moreover these auto antibodies are specific in the diagnosis of PBC. The characteristics of PBC can occur in the AIH, most often with overlapping syndromes [51]. In our series, PCA prevalence was high in biermer anemia patients with vitamin B12 deficiency (BA/Def vit B12) (84.6%). This prevalence is similar to the literature [52]. Nevertheless, anti PCA are predictors of autoimmune gastritis, as well as predictive markers of subsequent metabolic and hematologic manifestations [53]. Autoimmune atrophic gastritis may manifest as vitamin B12 deficiency, pernicious anemia or iron deficiency anemia [52]. In fact anti PCA markers are more common in people with anemia and the prevalence of Biermer disease accounts for more than 70% of vitamin B12 deficiencies [54] and 86% of B12 deficiencies in a Tunisian series [55]. Also we found anti PCA observed in type 1 D, PBC, AIH, CS and in AIT this is in line with the litterature [56,57].

In our study the association of these AIDs and anti PCA markers is not uncommon where the interests of its systematic screening in these diseases. Statistical analysis good correlation between AIT, D type 1 and PCA (Fig. 3). The prevalence of anti LKM1 was lower in CS and HCV treated patients, this frequency was in line with the literature [43].The detection of anti LKM1 in chronic hepatitis C seems to indicate an increased risk of exacerbation of the disease. In our study, auto-antibodies (PCA, LKM1, AMA and MSA) and Cholestatic Syndrome (CS) showed a high correlation (Fig. 3) these was also similarly reported in the literature [43].

Our results concerning the prevalence, we found that IgA EMA, IgA tTG and IgA AGA antibodies in IM and CD patients were very high as also reported in the different studies they constituted relatively frequent autoantibodies in CD and various symptoms of IM [58]. Moreover the prevalence of CD varies depending on the geography. In Europe the serological prevalence of tTG varies between 1.20 and 1.56% for EMA 0.78 et 1.88% [59]. In Nord Africa and occidental Sahara. the prevalence of EMA IgA varies between 0.64% et 5.66% and for tTG IgA was 2.21% [50,51]. These variations in the prevalence of CD as reported in different parts of the world may be explained by environmental factors between different populations and ethnicities and variable distribution of DQ alleles predisposing to CD. Therefore, we could propose that the high frequency of CD among the Moroccan population could be due to a high consumption of cereals (most staple foods in the Moroccan community contain wheat, rye or barley) and the frequent presence of predisposing HLA-haplotypes for CD, it has in fact been showed that HLA-DQ2 and DQ8 and conventional HLA-DQ have good associations with CD among Moroccans population [60]. The high frequency of homozygosity DQ2.5 (45.2%) observed in Moroccans with CD was significant compared to other populations (23% –32%) [61]. In addition, serological screening for CD in patients with symptoms or conditions closely associated with CD increases also the frequency of this disease [35,52]. One explanation is that in more recent years, the increasing use of serological tests to make the diagnosis of CD has resulted in milder cases being diagnosed who have reduced mortality risk. In our study, statistical analysis of the results showed a good correlation between auto-antibodies (tTG IgA, EMA IgA and AGA IgA) and CD (Fig. 3), these results are similar to those in the literature [62]. Also, we found that patients with AIH and CS (8.0% and 1.7%) were positive for EMA antibodies, this is in line with previous studies [63].Three of 47 patients with AIH (6.4%) were positive for IgA anti-tTG and EMA antibodies, and were subsequently confirmed to have CD and in Italy a prevalence of 4.9% [63]. For this reason, early serological screening for CD is strongly recommended for all patients with AIH. Also we found seroprevalence of anti-tTG IgA and EMA IgA of 25% and 12.5% respectively in diabetic patients of type 1. This situation is almost similar to data from a Moroccan study that shows a prevalence of 29% [64]. In Europe and America, it varies between 3.6% and 13.4% [65] and in the Maghreb countries the prevalence is significantly higher and ranges between 4 and 16% [66]. These variations are probably due to the use of serological screening tests that allowed the diagnosis of silent forms and symptomatic pauci.

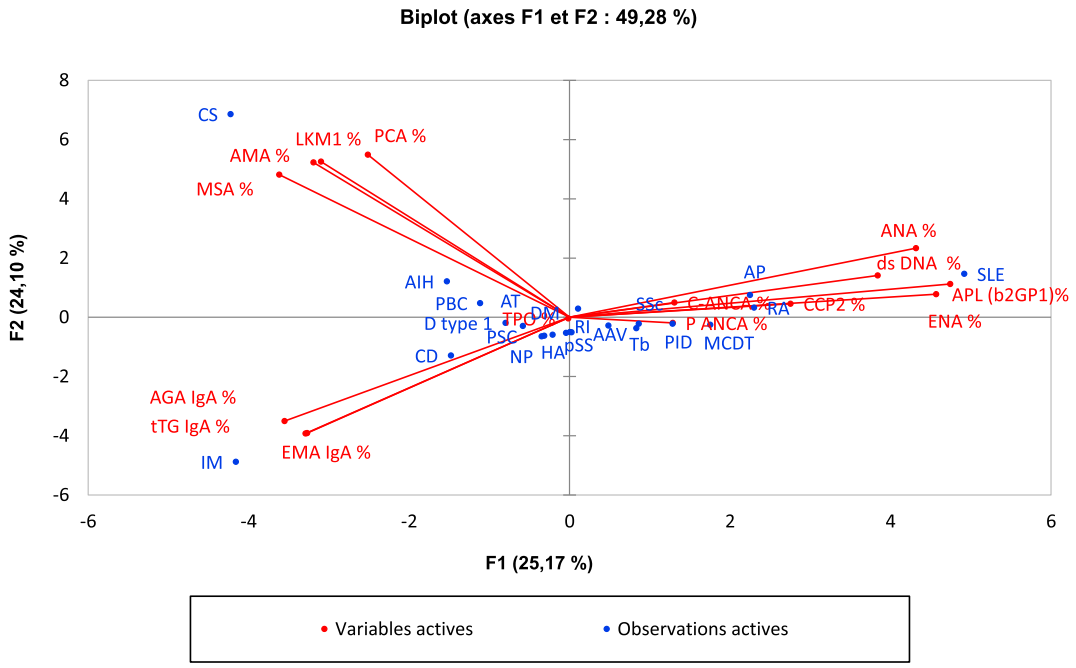

Fig. 3. Principal Component Analysis between different Auto-Antibodies and diseases.

In Morocco, we found no prevalence or multicenter studies. In our study, the association of type 1 diabetes with biological markers of CD is not uncommon, hence the value of its systematic screening in patients with type 1 diabetes. The diagnosis of this atypical and silent form of CD is important because of the risk of serious complications such as malabsorption and digestive cancers. During the autoimmune thyroid (AIT), the frequency of anti thyroperoxidase (TPO) was 92.3% which are considered to have an excellent diagnostic value [67], its presence constitutes a risk factor for developing such pathology or to develop hypothyroidism under interferon/interleukin 2 or lithium [67]. There is a strong correlation between this pathology, detected by anti-PCA antibodies, of 7.7% frequency. These two auto antibodies are known as predictors of this disease. It is also necessary to carry out the PCA search in the balance of an autoimmune thyroiditis to eliminate the different differential diagnoses [57].

5. Conclusion

The study is first analysis based on suspect clinical diagnosis supported by results of auto antibody testing, and on its basis, it is difficult to extrapolate the data of this regional study to the entire country. Further, population-based studies on epidemiology of AIDs, therefore, are urgently needed. It will not only improve our understanding of the prevalence and incidence of AID but also help in estimating their present medical and public health impact and an overall disease burden for the entire country.

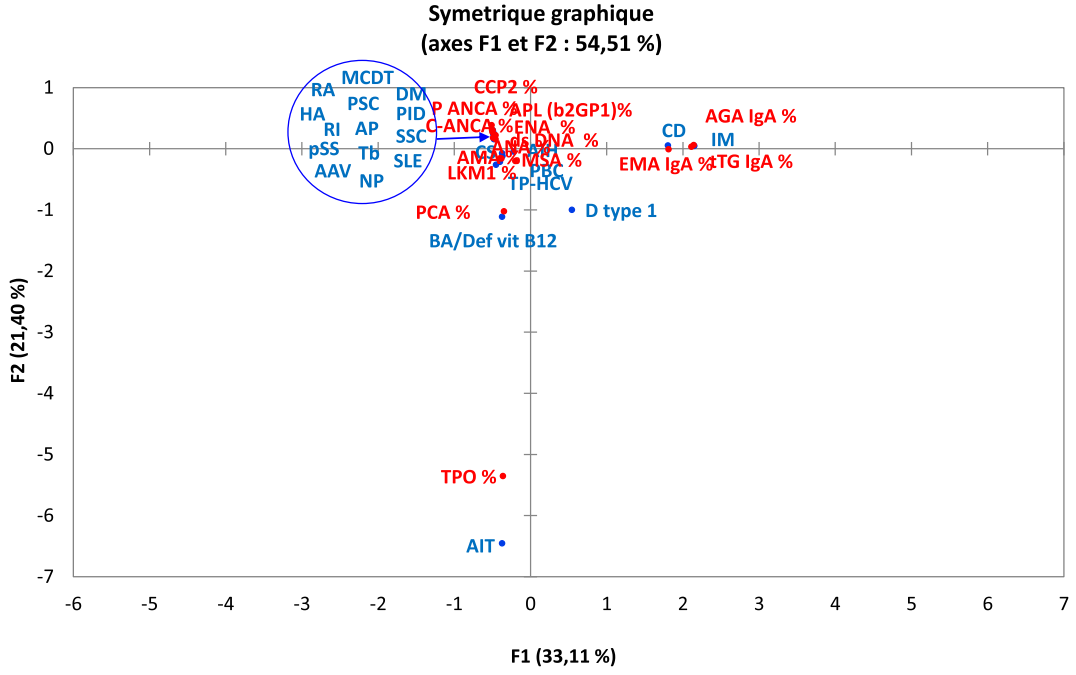

Fig. 4. Factorial Analysis of the Correspondences between the different Auto-Antibodies and diseases.

These data on the prevalence of each autoimmune disease are valuable for the public health system, because they may allow an accurate evaluation on the auto immune diseases. In any case, these data provide a picture of the relative burden of the studied diseases and make available an adequate control population for future clinical studies aimed to exploring the co- morbidity of autoimmune diseases.

This report helps the clinician to mak the correct diagnosis and, when necessary, adapts the antibiotic therapy at the earliest possible stage.

Our study also shows the diagnostic value of auto antibodies of organ specific AIDs and systemic autoimmune diseases (non organ specific). It would be interesting to carry out prospective studies for each pathology separately in order to fill the classic vagaries of the retrospective study and to objectively estimate the prevalence of different autoimmune diseases.

Acknowledgements

The authors would like to thank all patients for their participation. They also would like to thank all the health, laboratory professional and specially Asmae MIMOUNI that facilitate the realization of this work.

Ethics approval and consent to participate

Ethics approval and consent to participate Ethics approval and consent to participate was provided by all adults and legal guardians of minor individuals involved in this study. Statements of their signed consent are available on request. This study was approved by the ethics committee of the National Institute of Health in Rabat, Morocco.

Competing interests

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.humimm.2019.02.012.

References

[1] World Health Organization, Environmental Health Criteria 236 – Principles and methods for assessing autoimmunity associated with exposure to chemicals, World Health 4 (2006) 1–333 http://eutils.ncbi.nlm.nih.gov/entrez/eutils/elink.fcgi?dbfrom=pubmed&id=15522679&retmode=ref&cmd=prlinks.

[2] Matteo MASCOLO; Alicia MCNEILL;Meena FERNANDES, WORKSHOP Autoimmune Diseases – Modern Diseases Brussels, 25 September 2017, 2017.

[3] B.A. Scanlin, Autoimmune diseases: the growing impact, BioSupply Trends Q (2014) 44–47.

[4] G. Cooper, M. Bynum, E. Somers, Recent insight in the epidemiology of autoimmune disease: improved prevalence estimates and understanding of clustering of disease, J. Autoimmun. 33 (2009) 197–207, https://doi.org/10.1016/j.jaut.2009.09.008.Recent.

[5] N. Dragin, J. Bismuth, G. Cizeron-Clairac, M.G. Biferi, C. Berthault, A. Serraf, R. Nottin, D. Klatzmann, A. Cumano, M. Barkats, R. Le Panse, S. Berrih-Aknin, Estrogen-mediated downregulation of AIRE influences sexual dimorphism in autoimmune diseases, J. Clin. Invest. 126 (2016) 1525–1537, https://doi.org/10.1172/JCI81894.

[6] C. Sardu, E. Cocco, A. Mereu, R. Massa, A. Cuccu, M.G. Marrosu, P. Contu, Population based study of 12 autoimmune diseases in Sardinia, Italy: Prevalence and comorbidity, PLoS One 7 (2012), https://doi.org/10.1371/journal.pone.0032487.

[7] J.-F. Bach, The effects of infection on susceptibility to autoimmune and allergic diseases, N. Engl. J. Med. 347 (2002) 911–920, https://doi.org/10.1056/NEJMra020100.

[8] N. Agmon-Levin, J. Damoiseaux, C. Kallenberg, U. Sack, T. Witte, M. Herold, X. Bossuyt, L. Musset, R. Cervera, A. Plaza-Lopez, C. Dias, M. José Sousa, A. Radice, C. Eriksson, O. Hultgren, M. Viander, M. Khamashta, S. Regenass, L.E. Coelho Andrade, A. Wiik, A. Tincani, J. Rönnelid, D.B. Bloch, M.J. Fritzler, E.K.L. Chan, I. Garcia-De La Torre, K.N. Konstantinov, R. Lahita, M. Wilson, O. Vainio, N. Fabien, R.A. Sinico, P. Meroni, Y. Shoenfeld, International recommendations for the assessment of autoantibodies to cellular antigens referred to as anti-nuclear antibodies, Ann. Rheum. Dis. 73 (2014) 17–23, https://doi.org/10.1136/annrheumdis-2013-203863.

[9] N. Benseffaj, O. Atouf, S. Ouadghiri, C. Brick, M. Essakalli, Valeur diagnostique des auto-anticorps dans les maladies auto-immunes, Immuno-Analyse Biol. Spec. 27 (2012) 233–236, https://doi.org/10.1016/j.immbio.2012.06.009.

[10] T. Monjardino, R. Lucas, H. Barros, (n.d.).

[11] S.E. Gabriel, K. Michaud, Epidemiological studies in incidence, prevalence, mortality, and comorbidity of the rheumatic diseases, Arthritis Res. Ther. 11 (2009) 229, https://doi.org/10.1186/ar2669.

[12] XLSTAT, Data Analysis and Statistical Solution for Microsoft Excel, (2018).

[13] D.A. Mariama Sadjo Diallo, Babacar Mbengue, Abdoulaye Seck, Awa Cheikh Ndao, Maguette Sylla Niang, Yacouba Cissoko, Alassane Thiam, Gora Diop, Rokhaya Ndiaye Diallo, Moussa Diallo, Souhaibou Ndongo, Tandakha Ndiaye Dièye, Mohamed Cissé, Assane Kane, Evolution of autoantibodies profile in systemic lupus erythematosus according to age and clinical manifestations, Ann. Biol. Clin. (Paris) 72 (2014) 351–358, https://doi.org/10.1684/abc.2014.0963.

[14] A. Lerner, P. Jeremias, T. Matthias, The world incidence and prevalence of autoimmune diseases is increasing, Int. J. Celiac Dis. 3 (2016) 151–155, https://doi.org/10.12691/ijcd-3-4-8.

[15] O. M, C.F. Von, M.G. M, Interactions de la génétique et de l ' environnement sur le développement de la polyarthrite rhumatoïde, (2012) 305–313.

[16] S. Bernatsky, A. Smargiassi, C. Barnabe, L.W. Svenson, A. Brand, R.V. Martin, M. Hudson, A.E. Clarke, P.R. Fortin, A. van Donkelaar, S. Edworthy, P. Bélisle, L. Joseph, Fine particulate air pollution and systemic autoimmune rheumatic disease in two Canadian provinces, Environ. Res. 146 (2016) 85–91, https://doi.org/10.1016/j.envres.2015.12.021.

[17] C.A. Wagner, J. Sokolove, L.J. Lahey, C. Bengtsson, S. Saevarsdottir, L. Alfredsson, M. Delanoy, T.M. Lindstrom, R.P. Walker, R. Bromberg, P.E. Chandra, S.R. Binder, L. Klareskog, W.H. Robinson, Identification of anticitrullinated protein antibody reactivities in a subset of anti-CCP-negative rheumatoid arthritis: Association with cigarette smoking and HLA-DRB1 “shared epitope” alleles, Ann. Rheum. Dis. 74 (2015) 579–586, https://doi.org/10.1136/annrheumdis-2013-203915.

[18] E.C. Somers, S.L. Thomas, L. Smeeth, A.J. Hall, Autoimmune diseases co-occurring within individuals and within families, Epidemiology 17 (2006) 202–217, https://doi.org/10.1097/01.ede.0000193605.93416.df.

[19] L. Essaadouni, F.Z. Elhattab, Incidence du lupus à Marrakech Journées scientifique de Résidanat de Marrakech, Jrm (n.d.) (2013) 1–5.

[20] L. Broten, J.A. Aviña-Zubieta, D. Lacaille, L. Joseph, J.G. Hanly, L. Lix, S. O'Donnell, C. Barnabe, P.R. Fortin, M. Hudson, S. Jean, C. Peschken, S.M. Edworthy, L. Svenson, C.A. Pineau, A.E. Clarke, M. Smith, P. Bélisle, E.M. Badley, L. Bergeron, S. Bernatsky, Systemic autoimmune rheumatic disease prevalence in Canada: Updated analyses across 7 provinces, J. Rheumatol. 41 (2014) 673–679, https://doi.org/10.3899/jrheum.130667.

[21] E.I. Okoroiwu, I.L. Obeagu, E.I. Obeagu, G.U. Chikezie, C.C. Ezema, G.O. Obeagu, The prevalence of selected autoimmune diseases, Int. J. Adv. Multidiscip. Res. 3 (2016) 9–14 http://s-o-i.org/1.15/ijarm-2016-3-3-3.

[22] Z. Tazi-Mezalek, H. Harmouche, M. Adnaoui, A. Mohattane, M. Aouni, A. Maaouni, A. Berbich, Particularités du lupus érythémateux disséminé au Maroc. À propos de 166 observations, La Rev, Médecine Interne. 21 (2000) 465–466, https://doi.org/10.1016/S0248-8663(00)90021-3.

[23] B.P.T. Jennifer, M. Grossman, MD, Génétique et lupus érythémateux disséminé,, 2:13–18 ISSN 1523–3774 Curr, Rheumatol, Rep, 2000.

[24] Z. Yang, Y. Ren, D. Liu, F. Lin, Y. Liang, Prevalence of systemic autoimmune rheumatic diseases and clinical significance of ANA profile: data from a tertiary hospital in Shanghai, China, APMIS. 124 (2016) 805–811, https://doi.org/10.1111/apm.12564.

[25] M. Mosca, C. Tani, S. Bombardieri, Undifferentiated connective tissue diseases (UCTD): a new frontier for rheumatology, Best Pract. Res. Clin. Rheumatol. 21 (2007) 1011–1023, https://doi.org/10.1016/j.berh.2007.09.004.

[26] S.H.K. Murng, M. Thomas, Clinical associations of the positive anti Ro52 without Ro60 autoantibodies: Undifferentiated connective tissue diseases, J. Clin. Pathol. (2017) 1–8, https://doi.org/10.1136/jclinpath-2015-203587.

[27] M. Mosca, C. Tani, C. Neri, C. Baldini, S. Bombardieri, Undifferentiated connective tissue diseases (UCTD), Autoimmun. Rev. 6 (2006) 1–4, https://doi.org/10.1016/j.autrev.2006.03.004.

[28] P. Roujon, A. Sarrat, C. Contin-Bordes, I. Pellegrin, G. Guidicelli, J.L. Taupin, J.F. Moreau, P. Blanco, Diagnostic sérologique de la maladie cœliaque, Pathol. Biol. 61 (2013), https://doi.org/10.1016/j.patbio.2011.03.013.

[29] M. Leja, Z. Shums, L.N. Zake, M. Gavars, I. Kikuste, J. Milo, I. Daugule, J. Pahomova, V. Pirags, V. Dzerve, J. Klovins, A. Erglis, G.L. Norman, Prevalence estimation of celiac disease in the general adult population of latvia using serology and HLA genotyping, United Eur. Gastroenterol. J. 3 (2015) 190–199, https://doi.org/10.1177/2050640615569379.

[30] C. Catassi, L. Elli, B. Bonaz, G. Bouma, A. Carroccio, G. Castillejo, C. Cellier, F. Cristofori, L. de Magistris, J. Dolinsek, W. Dieterich, R. Francavilla, M. Hadjivassiliou, W. Holtmeier, U. Körner, D.A. Leffler, K.E.A. Lundin, G. Mazzarella, C.J. Mulder, N. Pellegrini, K. Rostami, D. Sanders, G.I. Skodje, D. Schuppan, R. Ullrich, U. Volta, M. Williams, V.F. Zevallos, Y. Zopf, A. Fasano, Diagnosis of non-celiac gluten sensitivity (NCGS): The salerno experts' criteria, Nutrients. 7 (2015) 4966–4977, https://doi.org/10.3390/nu7064966.

[31] N.E. Castillo, T.G. Theethira, D.A. Leffler, The present and the future in the diagnosis and management of celiac disease, Gastroenterol. Rep. 3 (2015) 3–11, https://doi.org/10.1093/gastro/gou065.

[32] D.A. Leffler, D. Schuppan, Update on serologic testing in celiac disease, Am. J. Gastroenterol. 105 (2010) 2520–2524, https://doi.org/10.1038/ajg.2010.276.

[33] L. Greco, Burden of celiac disease in the Mediterranean area, World J. Gastroenterol. 17 (2011) 4971, https://doi.org/10.3748/wjg.v17.i45.4971.

[34] A.H. Ali, E.J. Carey, K.D. Lindor, The management of autoimmunity in patients with cholestatic liver diseases, Expert Rev. Gastroenterol. Hepatol. 10 (2016) 73–91, https://doi.org/10.1586/17474124.2016.1095088.
[35] T. Tamayo, J. Rosenbauer, S.H. Wild, A.M.W. Spijkerman, C. Baan, N.G. Forouhi, C. Herder, W. Rathmann, Diabetes in Europe: an update, Diabetes Res. Clin. Pract. 103 (2014) 206–217, https://doi.org/10.1016/j.diabres.2013.11.007.
[36] L. Lamri, E. Gripiotis, A. Ferrario, Diabetes in Algeria and challenges for health policy: a literature review of prevalence, cost, management and outcomes of diabetes and its complications, Global. Health. 10 (2014) 11, https://doi.org/10.1186/1744-8603-10-11.
[37] G.J. Tobón, P. Youinou, A. Saraux, The environment, geo-epidemiology, and autoimmune disease: rheumatoid arthritis, J. Autoimmun. 35 (2010) 10–14, https://doi.org/10.1016/j.jaut.2009.12.009.
[38] D. Villalta, T. Imbastaro, S. Di Giovanni, C. Lauriti, M. Gabini, M.C. Turi, N. Bizzaro, Diagnostic accuracy and predictive value of extended autoantibody profile in systemic sclerosis, Autoimmun. Rev. 12 (2012) 114–120, https://doi.org/10.1016/j.autrev.2012.07.005.
[39] William W. Eatona, Noel R. Rose, Amanda Kalaydjiana, Marianne G. Pedersenc, P.B. Mortensenc, Epidemiology of autoimmune diseases in Denmark, J. Autoimmun. (2007) 1–9, https://doi.org/10.1016/j.jaut.2007.05.002.
[40] G.S. Cooper, M.L.K. Bynum, E.C. Somers, Recent insights in the epidemiology of autoimmune diseases: improved prevalence estimates and understanding of clustering of diseases, J. Autoimmun. 33 (2009) 197–207, https://doi.org/10.1016/j.jaut.2009.09.008.
[41] S. Feki, F. Frikha, Y. Ben Hadj Hmida, S. Abed, M. Ben Ayed, H. Turki, J. Hachicha, S. Baklouti, Z. Bahloul, H. Masmoudi, Prévalence et valeur diagnostique des anticorps antinucléaires de spécificité antigénique indéterminée: étude rétrospective à propos d'une série de 90 patients, La Rev, Méd. Interne 33 (2012) 475–481, https://doi.org/10.1016/j.revmed.2012.04.017.
[42] C. Bonaguri, A. Melegari, A. Ballabio, M. Parmeggiani, A. Russo, L. Battistelli, R. Aloe, T. Trenti, G. Lippi, Italian multicentre study for application of a diagnostic algorithm in autoantibody testing for autoimmune rheumatic disease: conclusive results, Autoimmun. Rev. 11 (2011) 1–5, https://doi.org/10.1016/j.autrev.2011.06.006.
[43] E.L.R. Cancado, C.P. Abrantes-Lemos, D.R.B. Terrabuio, The importance of autoantibody detection in autoimmune hepatitis, Front. Immunol. 6 (2015) 1–5, https://doi.org/10.3389/fimmu.2015.00222.
[44] J. AlSaleh, V. Jassim, M. ElSayed, N. Saleh, D. Harb, Clinical and immunological manifestations in 151 SLE patients living in Dubai, Lupus. 17 (2008) 62–66, https://doi.org/10.1177/0961203307084297.
[45] E. Osio-Salido, H. Manapat-Reyes, Epidemiology of systemic lupus erythematosus in Asia, Lupus. 19 (2010) 1365–1373, https://doi.org/10.1177/0961203310374305.
[46] C. Eriksson, H. Kokkonen, M. Johansson, G. Hallmans, G. Wadell, S. Rantapää-Dahlqvist, Autoantibodies predate the onset of systemic lupus erythematosus in northern Sweden, Arthritis Res. Ther. 13 (2011) R30, https://doi.org/10.1186/ar3258.
[47] L. Muratori, G. Deleonardi, C. Lalanne, E. Barbato, A. Tovoli, A. Libra, M. Lenzi, F. Cassani, P. Muratori, Autoantibodies in Autoimmune Hepatitis, Dig. Dis. 33 (2015) 65–69, https://doi.org/10.1159/000440748.
[48] Q.-X. Wang, L. Yan, X. Ma, Autoimmune hepatitis in the asia-pacific area, J. Clin. Transl. Hepatol. 6 (2018) 1–9, https://doi.org/10.14218/JCTH.2017.00032.
[49] B. Chang, B. Li, Y. Sun, G. Teng, A. Huang, J. Li, Z. Zou, Changes in etiologies of hospitalized patients with liver cirrhosis in Beijing 302 Hospital from 2002 to 2013, Mediators Inflamm. 2017 (2017), https://doi.org/10.1155/2017/5605981.
[50] C.P. Strassburg, M.P. Manns, Autoantibodies and autoantigens in autoimmune hepatitis, Semin. Liver Dis. 22 (2002) 339–351, https://doi.org/10.1055/s-2002-35704.
[51] C.J. Baños-Hernández, J.E. Navarro-Zarza, I. Parra-Rojas, M. Vázquez-Villamar, J. Ramón Padilla-Gutiérrez, Y. Valle, Z. Reyes-Castillo, N. Magdalena Torres-Carrillo, S. García-Arellano, L.M. Brennan-Bourdon, J.F. Muñoz-Valle, PADI4 polymorphisms and the functional haplotype are associated with increased rheumatoid arthritis susceptibility: a replication study in a Southern Mexican population, Hum. Immunol. 78 (2017) 553–558, https://doi.org/10.1016/j.humimm.2017.05.005.
[52] A. Belghith, S. Mahjoub, N. Ben Romdhane, L. Marsa, Causes of vitamin B12 deficiency, 93 (2015) 678–682.
[53] A. Belghith, S. Mahjoub, N. Ben Romdhane, L. Marsa, les étiologies des carences en vitamine B12 causes of vitamin B12 deficiency, 93 (2015) 678–682.
[54] D. Cattan, Anémies d'origine digestive, EMC – Gastro-Entérologie. 1 (2006) 1–18, https://doi.org/10.1016/S1155-1968(05)31205-3.
[55] C. Maktouf, Clinical spectrum of cobalamin deficiency in Tunisia, Abc. 65 (2007) 135–142, https://doi.org/10.1684/abc.2007.0041.
[56] S.R.R. Utiyama, R.S. De Bem, T.L. Skare, G.A. De Carvalho, L.M. Teixeira, M. Bertolazo, S.O. Ioshii, R. Nisihara, Anti-parietal cell antibodies in patients with autoimmune thyroid diseases, J. Endocrinol. Invest. 41 (2018) 523–529, https://doi.org/10.1007/s40618-017-0755-2.
[57] O. Twito, Y. Shapiro, A. Golan-cohen, Y. Dickstein, R. Ness-abramof, Anti-thyroid antibodies, parietal cell antibodies and tissue transglutaminase antibodies in patients with autoimmune thyroid disease, Arch Med Sci. (2016) 1–5, https://doi.org/10.5114/aoms.2016.58743.
[58] J.Y. Kang, A.H.Y. Kang, A. Green, K.A. Gwee, K.Y. Ho, Systematic review: Worldwide variation in the frequency of coeliac disease and changes over time, Aliment. Pharmacol. Ther. 38 (2013) 226–245, https://doi.org/10.1111/apt.12373.
[59] K. Mustalahti, C. Catassi, A. Reunanen, E. Fabiani, M. Heier, S. McMillan, L. Murray, M.H. Metzger, M. Gasparin, E. Bravi, M. Mki, The prevalence of celiac disease in Europe: results of a centralized, international mass screening project, Ann. Med. 42 (2010) 587–595, https://doi.org/10.3109/07853890.2010.505931.
[60] D. Piancatelli, I. Ben El Barhdadi, K. Oumhani, P. Sebastiani, A. Colanardi, A. Essaid, HLA typing and celiac disease in Moroccans, Med. Sci. 5 (2017) 2, https://doi.org/10.3390/medsci5010002.
[61] A.H.C. Alberto Rubio-Tapia, Ivor D. Hill, Ciarán P. Kelly, M. and M. and American College of gastroenterology clinical guideline : diagnosis and management of celiac, Am. J. Gastroenterol. 108 (2013) 656–676, https://doi.org/10.1038/ajg.2013.79.AMERICAN.
[62] M.O. Ma, S. Voida, Clinical and biological correlations in celiac disease in children: the prospective single experience of a romanian tertiary center, Medicine (Baltimore) (2017) 1–7.
[63] D. Villalta, D. Girolami, E. Bidoli, N. Bizzaro, M. Tampoia, M. Liguori, M. Pradella, E. Tonutti, R. Tozzoli, High prevalence of celiac disease in autoimmune hepatitis detected by anti-tissue tranglutaminase autoantibodies, J. Clin. Lab. Anal. 19 (2005) 6–10, https://doi.org/10.1002/jcla.20047.
[64] A.D. Bourhanbour, S. Ouadghiri, N. Benseffaj, M. Essakalli, Dépistage sérologique de la maladie cœliaque chez des patients marocains atteints de diabète type 1, Pan Afr. Med. J. 24 (2016) 1–6, https://doi.org/10.11604/pamj.2016.24.103.8555.
[65] S. Beltran, H. Bony-Trifunovic, V. Gouilleux-Gruart, D. Djeddi, I. Dadamessi, J.L. Dupas, B. Boudailliez, Prévalence des marqueurs biologiques de la maladie cœliaque dans une cohorte d'enfants et d'adolescents diabétiques de type I, Ann. Endocrinol. (Paris) 65 (2004) 131–135, https://doi.org/10.1016/S0003-4266(04)95661-2.
[66] A. Mankaï, H. Ben Hamouda, F. Amri, L. Ghedira-Besbes, A. Harbi, M. Tahar Sfar, A. Sahloul Essoussi, M. Jeddi, I. Ghedira, Screening by anti-endomysium antibodies for celiac disease in Tunisian children with type 1 diabetes mellitus, Gastroentérologie Clin. Biol. 31 (2007) 462–466, https://doi.org/10.1016/S0399-8320(07)89413-9.
[67] M.A. Chabchoub, G.M. Mnif, et al., Etude épidémiologique des maladies auto-immunes, thyroïdiennes dans le sud tunisien, Ann. d'endocrinol. 6 (2006) 591–595.