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Clinical Association of Antinuclear Antibodies (ANA) Anti-NuMA1 and Anti-NuMA2 (Anti-HsEg5) in Patients with Autoimmune and Cardiovascular Disease

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Abstract

Introduction: The prevalence of anti-NuMA1 and anti-NuMA2 antibodies in autoimmune, hepatic, infectious and renal inflammatory diseases is well known; however, its presence and possible relevance in cardiovascular diseases still needs to be elucidated.

Aim: To evaluate the prevalence of positive anti-NuMA1 and anti-NuMA2 antibody patterns in subjects with autoimmune, non-autoimmune and/or cardiac diseases.

Material and methods: This was an observational study run from January 2010 to January 2018. Individuals whose treating physician requested an antibody study and we detected anti NuMA1 and anti NuMA2 pattern in any patients. The files from these patients were reviewed to obtain general data, signs, symptoms, time of evolution of the disease, determination of specific antibodies and the established diagnoses.

Results: From a total of 7163 patient files 46 had NuMA1 pattern and out of them 24 (52%) had autoimmune disease (AD): 8 rheumatoid arthritis, 10 systemic lupus erythematosus (SLE), 2 antiphospholipid syndrome, 1 polymyositis, 1 fibromyalgia, 1 primary Sjogren and 1 Devic syndrome. In 15 patients (32%), cardiovascular diseases (CVD) were diagnosed and in only one of them there was an associated autoimmune disease. In three patients, there was a positive specificity for anti-SSA antigen, RNP in addition to the anti-NuMA. Rheumatoid factor, anti B2glicoprotein was present in 1 patient. Anti NuMA1 was found in 4 patients (9%); one of which had kidney disease and 3 had cardiopulmonary disease (7%). Eleven patients were positive to anti-NuMA2, five with autoimmune diseases (46%), one with cardiovascular disease (9%), two with cardiopulmonary diseases (18%) and three with renal disease (27%).

Conclusion: The prevalence of high levels of antinuclear antibodies with a NuMA1 and/or NuMA2 pattern is present in patients having cardiovascular disease without there being a coexisting autoimmune disease. This finding may indicate a specific form of autoimmunity.

Keywords: Anti-NuMA1 and anti-NuMA2 antibodies; Cardiovascular diseases; Prevalence of antibodies against the nuclear mitotic apparatus

Introduction

The proteins of the nuclear mitotic apparatus (NuMA), which weigh 238 kDa, are located in the nucleus during interphase and accumulate at the spindle poles during mitosis [1,2]. They belong to the BimC kinesin protein family which is distributed in the spindle during cellular division [3,4]. They are found in the spindle poles during metaphase and anaphase participating in the microtubule movement and stabilization of the mitotic spindle [5].

These proteins have specific functions during mitosis (spindle stabilization, centromere maturation, cytokinesis, and post-mitotic nuclear rearrangement). Five different types of antigens in the NuMA have been described: NuMA1 and NuMA2, centrosome (CE), middle body (MB) and F-centromere (CENP-F) [6].

Antibodies against NuMA1 and NuMA2 were found for the first time in 1981 [7] and differences regarding their antigenic orientation have been previously described [3]. The different antigenic characteristics give rise to specific immunofluorescent patterns. The anti-NuMA1 pattern is detected in cells undergoing interphase and at the poles of the mitotic spindle during metaphase and anaphase. The anti-NuMA2 pattern is only found at the poles during metaphase or anaphase [4].

Anti-NuMA1 and anti-NuMA2 antibodies are usually found in the serum of patients having clear clinical data of autoimmunity. In some patients these patterns are found together with other antibodies against

specific antigens. Therefore, the anti-NuMA patterns have been proposed as biomarkers for autoimmune ailments.

A clinical association between the anti-NuMA1 and anti-NuMA2 antibodies present in inflammatory connective tissue diseases [7-13], hepatic diseases [14] and infectious renal diseases [15] has also been found. Furthermore, the presence of anti-NuMA patterns has also been observed in subjects with cardiovascular and/or renal diseases that are associated with non-autoimmune ailments. Therefore, the epidemiologic conditions in which these patterns of antibodies are present render it difficult to determine their real prevalence and association to diseases. Moreover, the fact that an adequate training is needed for their recognition worsens this situation. Thus, there is interest in evaluating the real prevalence and clinical relevance of anti-NuMA1 and anti-NuMA2 antibodies.

The presence of these patterns of antibodies in isolated cardiovascular diseases or in cardiovascular diseases that coexist with autoimmune diseases has not been completely evaluated. Therefore, the goal of this paper was to evaluate the prevalence of the positive anti-NuMA1 and anti-NuMA2 antibody patterns in subjects with and without autoimmune ailments and cardiac disease

Material and Methods

This was an exploratory observational study done in patient files that were collected from January 2010 to January 2018. Files in which the treating physician had requested the study of antinuclear antibodies (ANA) by indirect immunofluorescence in HEp-2 cells because the presence of an autoimmune disease was suspected were selected. The presence of the antibodies was performed in the Immunology Laboratory of the National Institute of Cardiology "Ignacio Chávez".

Inclusion criteria

Files from individuals who showed anti NuMA1 and anti NuMA2 patterns during the laboratory study were included. There was no age or gender restriction. Files had to be complete so that a retrospective review and demographic data could be obtained that included age, gender, signs, symptoms, time of evolution of the disease, determination of specific antibodies and the final diagnosis(s) established by the attending physician. Files without complete information were excluded as well as files in which the NuMA pattern was not found.

Ethical standard

All procedures performed in studies involving human participants were done in accordance to the ethical standards of the institutional and/or national research committee agreements and tests followed the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This work was approved by the Research Ethics Committee, being registered with the number 15-924.

Antibody detection

The detection of antibodies was carried out using the indirect immunofluorescence technique. The binding of rabbit antibodies, conjugated with FITC to the Fc region of human antibodies that recognize antigens in HEp-2 cells, was determined, and staining patterns were identified by observing the samples in an epifluorescence microscope. The ELISA technique (Immunosorbent Analysis Linked to Enzymes) was used for the detection of specificities of antibodies. For the recognition of the specific antibodies present in the samples of the patients, we used an antibody directed against the human Fc region of any isotype (IgG, IgA or IgM) and even of any subclass (IgG1, IgG2, IgG3, IgG4, IgA1 or IgA2) of antibodies. Anti-Fc antibodies bind to enzymes such as peroxidase or alkaline phosphatase, so the antigens used in the ELISA plates were native and recombinant (complete antigen or epitope specific) or synthetic (epitope specific).

Interaction of the antibodies that were present in the patient samples with the antigen attached to the ELISA plate was allowed. Then, several washes were made to eliminate the nonspecific antibodies. The enzyme-bound human immunoglobulin antibody was added, to allow interaction during a determined time. Subsequently, the solution containing the chromogenic substrate specific for the enzyme (3, 30, 5, 50-Tetramethylbenzidine (TMB) for peroxidase or p-nitrophenyl phosphate for alkaline phosphatase), was added. This reaction produced a change in color that depended on the amount of antibodies conjugated with the enzyme which, in turn, correlate with the amount of the patient's antibodies that recognized the antigen stuck to the plate. Therefore, the intensity of the coloration was directly proportional to the amount of antibody from the patient bound to the antigen. The technique was quantitative and the amount of antibodies present in the samples could be calculated, by means of a specific curve of reactivity. The report of results from the following ELISA test was also examined: presence of specific antibodies to antigens anti SS-A/Ro, SS-B/La, RNP, Smith, Centromere, B2 Glycoprotein 1, Cardiolipin IgG and IgM. Results from Rheumatoid Factor (RF) and Cyclic Citrullinated peptide (CCP) were also taken into account. The kits for the ELISA tests examined had been obtained from (ORGENTEC Diagnostic GmbH, Mainz Germany. www.orgentec.com). The kits to determine Hep-2 ANA and DsDNA Crithidia Luciliae had been obtained from Inova Diagnostic, Inc (San Diego, CA USA www.inovadx.com).

Statistical analysis

Microsoft Excel and SPSS 19 were used. The nominal and dichotomous variables were reported in percentages and the quantitative variables with normal distribution with means and standard deviation. Spearman correlation analyses were made considering statistical significance when $p<0.05$.

Results

Out of a total of 7163 files from patients in which antibody determination was requested, 57 patients were positive for the anti-NuMA1 and NuMA2 pattern. Files included came from patients with a mean age of 45 ± 18 years. The median of years of evolution of the diseases was of 6 years (Min 1-Max 23). The demographic characteristics of the patients are shown in Table 1.

Variable	Total	Male	Female	p
	57 (100%)	n=14 (25%)	n=43 (75%)	
AGE X ± DE	45 ± 18	44 ± 19	46 ± 18	NS
BMI	26 ± 7	26 ± 3	26 ± 8	NS
Total Cholesterol	168 ± 42	151 ± 45	173 ± 39	NS
Triglycerides				

Median	120	103	132	0.02
(Min-Max)	(52-885)	(56-174)	(53-885)	
Co-morbidities n (%)				
Diabetes	5 (9)	2 (4)	3 (5)	NS
Arterial Hypertension	24 (42)	4 (7)	20 (35)	NS
Dyslipidaemia	14 (25)	2 (4)	12 (21)	NS
Smoking	9 (16)	5 (8)	4 (7)	NS
Place of origin n (%)				
Mexico City	19(33)	4 (7)	15 (26)	NS
State of Mexico	16(28)	4(7)	12 (21)	NS
Oaxaca	4 (7)	1(2)	3 (5)	NS
Michoacán	4 (7)	3(5)	1 (2)	NS
Hidalgo	3 (5)	1(2)	2 (3)	NS
Veracruz	3 (5)	0 (0)	3 (5)	NS
Morelos	2 (4)	0 (0)	2 (4)	NS
Puebla	2 (4)	1(2)	1 (2)	NS
Tabasco	1 (2)	0(0)	1 (2)	NS
Sinaloa	1 (2)	0 (0)	1 (2)	NS
Chile	1 (2)	0 (0)	1 (2)	NS

Table 1: General demographic characteristics of the population included in the study. Demographic characteristics divided by gender.

The prevalence of the type of antibody pattern detected in Hep2 cells found was as follows: (54%) was cytoplasmic, (48%) nuclear homogeneous pattern, 45% fine speckled nuclear pattern, (4.3%) centromeric cell cycle pattern, (2.3%) cycle nucleolar cell pattern, in 2.1% coarse nuclear mottled pattern, in 108 (1.5%) cytoplasmic cytoskeleton pattern and (0.7%) a NuMA pattern was present.

Demographic characteristics divided by gender

The prevalence of the type of antibody pattern detected in Hep2 cells found was as follows: in 3894 files (54%) the pattern was cytoplasmic, in 3452 files (48%) a nuclear homogeneous pattern was found, in 45% of files a fine speckled nuclear pattern was present, in 312 files (4.3%) a centromeric cell cycle pattern was observed, in 68 files (2.3%) a cycle nucleolar cell pattern was found, in 2.1% of files a coarse nuclear mottled pattern was observed, in 108 files (1.5%) a cytoplasmic cytoskeleton pattern was described and in 57 files (0.7%) a NuMA pattern was present. The frequency of the type of specificity of the ANAs in relation to the conditions presented by the patients is shown in Table 2.

Antibody type	**Total**	**Autoimmune**	**Cardiac**	**Nephropathy**	**Cardio-Neumopathy**
	57 (100)	28(49)	18 (32)	8 (14)	3 (5)
Anti-NuMA1	46 (80)	24 (42)	16 (28)	5 (8)	1 (2)
Anti-NuMA2	11 (19)	4 (7)	2 (3)	3 (5)	2 (3)
Rheumatoid Factor	17 (30)	12 (21)	4 (7)	1 (2)	-
CCP	10 (17)	10 (17)	-	-	-
Anti-SSA/Ro	10 (17)	8 (14)	2 (3)	-	-
Anti-SSB/La	9 (15)	7 (12)	-	-	-
Anti-Smith	2 (4)	2 (4)	-	-	-
Anti-RNP	7 (12)	4 (7)	3 (5)	-	-
Anti-centromere	2 (4)	1 (2)	1 (2)	-	-
Anti B2 glicoproteine1	7 (12)	6 (10)	1 (2)	-	-
Anticardiolipin IgM	4 (7)	4 (7)	-	-	-
CCP=Cyclic citrullinated peptide, Anti=Antibodies vs antigens: SSA SSB, RNP					

Table 2: Frequency of the anti NuMA1 and anti NuMA2 antibody pattern and specificity of antibodies in different ailments.

The pattern of antibodies observed in cases with autoimmune diseases is shown in Table 3. It can be observed that out of a total of nine samples from patients who had rheumatoid arthritis (RA) 8 (89%) had antibodies anti-NuMA1 and in one there was an anti-NuMA2 pattern. In addition, in 5 patients (55%) there was autoimmune overlap since in addition to RA, 3 patients had Sjögren's syndrome (SS), in another patient, besides RA and SS there was Scleroderma, in another patient there was pulmonary arterial hypertension and in another, autoimmunity in the thyroid was observed.

Autoimmune disease	NuMA1	NuMA2	Cytoplasmic	Homogeneous	Mitochondrial	Speckled	Golgi	Total
	24	4						
RA	3 (75)	1 (25)	3	-	1	-	-	4
RA+SS	2 (100)	-	1	-	-	-	-	2
RA+SS+Scl	1 (100)	-	-	-	-	-	-	1
RA+SS+PAH	1 (100)	-	1	-	-	-	-	1
RA+SS+HIPOT	1 (100)	-	-	-	-	-	-	1
DEVIC	1 (100)	-	-	-	-	1	-	1
FM	1 (100)	-	1	-	-	-	-	1
DL	1 (50)	1 (50)	1	-	-	-	-	1
SLE	5 (71)	2 (29)	4	1	1	-	-	7
SLE+IgAN	1 (100)	-	1	1	-	-	-	1
SLE+APS	1 (100)	-	-	1	-	-	-	1
SLE+APS+PAH	1 (100)	-	-	1	-	-	-	1
SLE+CNS	1 (100)	-	-	-	-	-	-	1
POLIMIOSITIS	1 (100)	-	-	-	-	-	-	1
APS	1 (50)	-	2	-	-	-	-	1
APS+PTE	1 (100)	-	-	-	-	1	-	1
Primary SS	1 (100)	-	-	-	-	1	-	1

NuMA=Nuclear Mitotic Apparatus, RA=Rheumatoid Arthritis, SS=Sjögren's Syndrome, ESC=Scleroderma, PAH=Pulmonary Arterial Hypertension, HIPOT=Hypothyroidism, DEVIC=Devic's Syndrome, FM=Fibromyalgia, DL=Discoid Lupus, SLE=Systemic Lupus Erythematosus, IgAN=IgA Nephropathy, APS=Antiphospholipid Syndrome, CNS=Central Nervous System, PTE=Pulmonary Thromboembolism.

Table 3: Type and Frequency of the antibody pattern in the different autoimmune diseases.

In seven patients the established diagnosis was Systemic Lupus Erythematosus (SLE) and in 5 of them (71%) an anti-NuMA pattern was present. The pattern of antibodies observed in non-autoimmune conditions is shown in Table 4.

Cardiac Ailments	NuMA1	NuMA2	Cytoplasmic	Homogeneous	Mitochondrial	Speckled	Golgi	Total
	15	2						
Pulmonary Atresia	1 (100)	-	-	-	-	-	-	1
CCC	2 (75)	1 (25)	1	-	-	-	-	3
CCA	1 (100)	-	-	-	-	-	-	1
CIA	-	-	-	-	1	-	-	1
CRI	1 (100)	-	-	-	-	-	-	1
DISNEA	1 (100)	-	-	-	-	-	-	1
HAS	3 (100)	-	2	-	-	-	-	3
HAS+ICC	1 (100)	-	1	-	-	-	-	1
ICC+VHC	1 (100)	-	1	-	-	-	-	1
Aortic Lession	1 (100)	-	1	-	-	-	-	1

MCD	1 (100)	-	1	-	-	-	-	1
Miocarditis	1 (100)	-	-	-	-	-	-	1
Intracardiac tumor	1 (100)	-	1	-	-	-	-	1
Cardiopulmonary and renal disease								
BAVC	0 (0)	1 (100)	-	-	-	1	-	1
HAP	2 (75)	1 (25)	1	-	-	-	-	3
TEP	1 (100)	-	1	-	-	-	-	1
ERC	3 (50)	3 (50)	3	-	-	-	1	6
NIgA	1 (100)	-	-	-	-	1	-	1

CCA=Cyanogenic congenital cardiopathy, CCA=Congenital Acyanogenic Cardiopathy, CIA=Interauricular Communication, CRI=Inactive Rheumatic Heart Disease, HAS=Systemic Arterial Hypertension, CCI=Chronic Heart Failure, HCV=Hepatitis C Virus MCD=Dilated cardiomyopathy BAVC=Atrioventricular Block, PAH=Pulmonary Arterial Hypertension, TEP=Pulmonary Thromboembolism CKD=Chronic Renal Disease, IgG=Immunoglobulin A Nephropathy

Table 4: Type and Frequency of antibody pattern in cardiac and renal diseases.

The frequency and description of the type of antibodies present against specific antigens and the type of dilution in autoimmune, cardiac and renal diseases are shown in Tables 5 and 6.

Autoimmune diseases	**Age (years)**	**NuMA**	**RF**	**CCP**	**SSA**	**SSB**	**RNP**	**Anti-Smith**	**Anti-Centromere**	**AntiB2Gp1**	**AcIgM**	**AcIgG**	**Total**
													100%
RA+SS+ESC	53	1:5120	-	1+	-	-	-	-	1+	-	-	-	1
RA+SS	59	1:5120	1+	1+	-	-	-	-	-	-	-	-	1
RA+SS+HIPOT	75	1:320	-	1+	-	-	-	-	-	-	1+	1+	1
RA+SS+PAH	57	1:160	1+	1+	-	-	-	-	-	-	-	-	1
RA+SS	51	01:40	1+	1+	-	-	-	-	-	-	-	-	1
primary SS	64	1:320	1+	1+	1+	1+	-	-	-	1+	-	-	1
RA	54	1:2560	1+	1+	-	-	-	-	-	-	-	-	1
RA	73	1:2560	1+	1+	-	-	-	-	-	1+	-	-	1
RA	46	1:320	1+	1+	1+	1+	-	-	-	-	-	-	1
FM	57	1:320	-	-	-	-	-	-	-	-	-	-	1
DL	87	1:5120	-	-	1+	1+	-	-	-	-	-	-	1
DL	51	1:320	1+	-	-	-	-	1+	-	-	-	-	1
SLE+CNS	28	1:5120	-	-	1+	1+	1+	-	-	-	-	-	1
SLE+IgA	44	1:1280	-	-	-	1+	-	-	-	-	-	-	1
SLE	28	1:1280	-	-	-	-	-	-	-	1+	-	-	1
SLE	49	1:640	1+	-	-	-	-	-	-	-	1+	-	1
SLE	48	1:640	-	-	-	-	-	-	-	-	1+	-	1
SLE	49	1:320	-	-	1+	1+	1+	-	-	-	-	-	1
SLE	35	1:320	-	-	-	-	1+	-	-	-	-	-	1

SLE	45	01:40	-	-	-	-	-	-	-	-	-	-	1
SLE	43	01:40	-	-	-	-	-	-	-	-	-	-	1
SLE+APS+HAP	63	1:320	1+	-	1+	-	-	-	-	1+	-	-	1
SLE+APS	58	1:160	-	-	-	-	-	-	-	1+	-	-	1
APS	43	1:160	-	-	-	-	-	-	-	-	-	-	1
APS+PTE	17	1:160	-	-	-	-	-	-	-	1+	-	1+	1
DEVIC Syndrome	34	1:160	-	-	1+	1+	1+	1+	-	1+	-	1+	1
POLIMIOSITIS	51	1:160	1+	-	1+	-	-	-	-	-	-	-	1

RA=Rheumatoid Arthritis, SS=Sjögren's Syndrome, ESC=Scleroderma, PAH=Pulmonary Arterial Hypertension, HIPOT=Hypothyroidism, DEVIC=Devic's Syndrome, FM=Fibromyalgia, DL=Discoid Lupus, SLE=Systemic Lupus Erythematosus, IgAN=Nephropathy IgA, APS=Antiphospholipid Syndrome, CNS=Central Nervous System, PTE=Pulmonary Thromboembolism

Table 5: Type of autoimmune disease, antibody dilution of the anti NuMA pattern and specificity of the related antibodies.

Cardiac diseases	**Age (years)**	**NuMA1**	**RF**	**CCP**	**SSA**	**SSB**	**RNP**	**Anti-Smith**	**Anti-Centromere**	**AntiB2Gp1**	**Ac IgM**	**AcIgG**	**Total**
			4	**0**	**2**	**2**	**3**	**0**	**1**	**1**	**0**	**0**	**100%**
MCD+PTE	33	1:5120	-	-	-	-	-	-	-	-	-	-	1
SAH+SS+ES	84	1:1280	1+	-	1+	1+	1+	-	1+	-	-	-	3
IRC+AVB	74	1:1280	1+	-	-	-	-	-	-	1+	-	-	1
ACC-PDA	29	1:640	-	-	-	-	-	-	-	-	-	-	1
CCI+HCV	50	1:640	-	-	-	1+	1+	-	1+	-	-	-	1
Myocarditis	23	1:320	-	-	-	-	-	-	-	-	-	-	1
Pericardial effusion	28	1:160	1+	-	-	-	-	-	-	-	-	-	3
ACC	24	1:160	-	-	-	-	-	-	-	1+	-	-	1
IAC	36	1:160	-	-	-	-	-	-	-	-	-	-	1
Dyspnea	22	1:160	1+	-	-	-	1+	-	-	-	-	-	1
SAH+ACC	32	1:160	-	-	-	-	-	-	-	-	-	-	1
Pulmonary Atresia	73	1:160	-	-	-	-	-	-	-	-	-	-	1
Intracardiac Tumor	20	1:160	-	-	-	-	-	-	-	-	-	-	1
Cardiopulmonary Diseases													
AVB	84	1:320	-	-	-	-	-	-	-	-	-	-	1
PAH+IgAN	44	1:160	-	-	-	-	-	-	-	-	-	-	1
PAH+CHF	51	1:160	-	-	-	-	-	-	-	-	-	-	1
PTE+Wegener (ANCA+)	73	1:320	1+	-	-	-	-	-	-	-	-	-	1
Renal diseases													
CKD+TRDC	24	1:160	-	-	-	-	-	-	-	-	-	-	1
CKD+TRDVR	25	1:320	-	-	-	-	-	-	-	-	-	-	1
CKD	58	1:320	-	-	-	-	-	-	-	-	-	-	1

CKD dense deposits	33	1:40	-	-	-	-	-	-	-	-	-	-	1
CKD+MD	65	1:320	-	-	-	-	-	-	-	-	-	-	1
CKD+SLE	48	1:40											
NIgA	37	1:160	-	-	-	-	-	-	-	-	-	-	1

CCA=Acianogen Congenital cardiopathy, PDA=persistent ductus arteriosus, IAC=Inter-auricular Communication, IRC=Inactive Rheumatic Cardiopathy, SAH=Systemic Arterial Hypertension, CCI=Chronic Heart Failure, HCV=Hepatitis C Virus, CHF=Chronic Heart Failure, CKD=Chronic Kidney Disease, IgAN=Immunoglobulin Nephropathy A, AVB=Atrioventricular Block, PAH=Pulmonary Arterial Hypertension, PTE=Pulmonary Thromboembolism, MCD=Cardiomyopathy dilata, TRDVR=Live donor transplant, TRDC=Corpse transplant, SLE=Systemic lupus erythematosus, MD=Mellitus diabetic, RF=Rheumatoid factor, CCP=Citrullinated cyclic peptide.

Table 6: Non-autoimmune disease, antibody dilution of the anti-NuMA pattern and specificity of the related antibodies

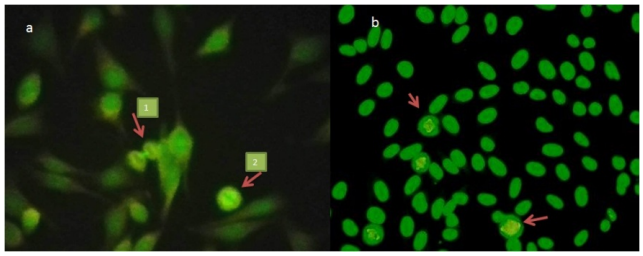

Figure 1: (a) Indirect immunofluorescence study in a 29-year-old man with benign auricular myxoma (left atrium tumor). Anti NuMA2 antibodies are shown at a dilution of 1: 320. An antigen in the mitotic poles, spindles and/or the intercellular bridge (1) Telophase (2) Metaphase is recognized. (b) Indirect immunofluorescence study in a 31-year-old woman diagnosed with SLE and nephrotic syndrome. Anti-NuMA1 antibodies at dilutions of 1: 2560 are present. Nuclear granular staining with staining of mitotic spindle fibres, intense marking in centrioles and spindle fibres are observed.

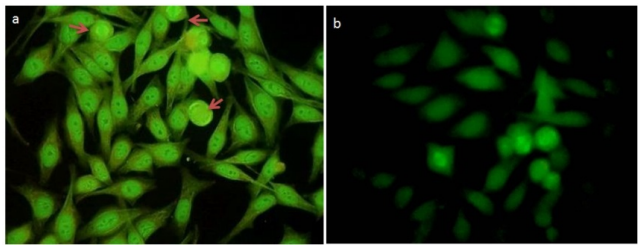

Figure 2: (a) Indirect immunofluorescence study in a 31-year-old man with dilated cardiomyopathy and hypothyroidism without a diagnosed autoimmune disease. There was pericardial effusion showing NuMA1 antibodies with intermediate filaments. (b) Indirect immunofluorescence study in a 25-year-old woman who arrived at the hospital in renal failure. A kidney transplant was performed, and autoimmune disease was not documented. Antibodies are shown staining the fibres of the chromatic spindle.

Antibody detection values found were: 150 U/ml for Rheumatoid Factor (FR). (Min 20-Max 667), for citrullinated cyclic peptide (PCC) 302 U/ml (145-345), β-2 IgG Glycoprotein 47 U/ml (22-72), β-2 IgG glycoprotein, 98.4 U/ml (26-204), anticardiolipin IgG 28 U/ml (13-54) and for anticardiolipin IgM the maximum concentration was 91.70 U/ml and the minimum concentration of 0 U/ml.

The type of NuMA pattern and clinical description of some cases with and without autoimmune disease is shown in Figures 1 and 2.

Discussion

There is a spatiotemporal distribution of the antigens of the mitotic apparatus (AMA) during the cell cycle. There is also a different expression during mitotic progression (prophase, metaphase, anaphase and telophase). The Centrosome and NuMA1 are present during interphase and mitotic cells, whereas NuMA2 and MB are present only in mitotic cells [16]. CENP-F is present in the mitotic prophase and throughout the cell cycle with the exception of the G1 stage. The method using indirect immunofluorescence (IIF) in the HEp-2 cells is the only routine method for the clinical detection of all types of anti-AMA antibodies; however, some antigens of the AMA have not been characterized in dividing cells by this technique [9].

Although there are antibodies directed against the AMA which are not always clearly identified, the pattern of antinuclear antibodies and their specificity renders them as key biomarkers in the evaluation of rheumatic diseases. The antibodies directed against NuMA are rare; however, their prevalence and their clinical significance have been previously reported in the literature [9]. The reported prevalence is of 1%; nevertheless, it is not known if this prevalence is the same in all populations. Moreover, the prevalence data may be biased since the presence of these antibodies is not usually reported in studies, thus prevalence depends on the laboratory experience [4]. The findings in this series of antibodies against antigens of the NuMA1 nuclear mitotic apparatus in autoimmune diseases are consistent with those reported in the literature [9].

The type of pattern of ANAs has been mostly related to autoimmune diseases where the frequency of anti-NuMA is known. This is the case in Sjögren's syndrome in which the prevalence is of 45%, undifferentiated connective tissue disease in which the prevalence is of 17%, and in non-autoimmune diseases where it is of 38% [10]. In relation to antibodies with anti-NuMA pattern it was found that out of a total of 68, 640 antibody tests requested in the Chinese population, only 180 had positive ANA and only 32 had antibodies against AMA, so the prevalence was 0.26%. Of these 32 samples, 50% corresponded to autoimmune diseases, and 19% to non-autoimmune diseases [17].

It is still unknown if the presence of antibodies is also associated with non-autoimmune diseases. Some antibodies are related with organ specific alterations and could be used as prognostic markers

[18]. The presence of specific antibodies against cellular components such as nuclear or cytoplasm molecules could also be specific for some diseases [19,20], while in some others, it might be completely unspecific [21,22]. There are reports of antibodies against the nuclear antigen of proliferative cells in patients with chronic B or C hepatitis. These antibodies have only been found in about 5% of patients with SLE. Antibodies have also been detected in polymyositis, systemic sclerosis and even in healthy individuals. However, their prevalence has not surpassed the 2% in any group [23]. The presence of other antibodies might depend upon a clinical characteristic of the disease, such as neuropsychiatric lupus, in which anti p ribosomal antibodies have a prevalence of 10% and are observed in 2% of all patients with SLE. In patients with scleroderma the prevalence is low and even below 1% in some series [24,25].

In the present series, out of the total samples which were positive for the NuMA1 pattern, 52% of them could be related to autoimmune diseases. The frequency of the diseases found was as follows: with RA of 33%, with LES 38%, with primary and secondary APS of 13% and in primary and secondary SS of 21%. The association of the NuMA1 pattern with other specificities such as FR PCC, SSA, SSB and anti-centromere was observed in patients with RA. In the same way, NuMA1 positive pattern had association with specific antigens against SSA and SSB in patients with SLE. For non-autoimmune conditions 32% of positive tests were associated with CVD, 12% with nephropathies and 5% with cardiopulmonary diseases. The prevalence of anti NuMA in patients with hepatitis corresponded to 21%. The findings in this series of antibodies against antigens of are consistent with those reported in the literature [9].

It has been suggested that in the presence of anti NuMA1 antibodies, it is important to first consider the possibility of conditions such as Sjögren syndrome within the differential diagnosis, while in the presence of anti-centrosome antibodies the suspicion must be of hepatitis infection.

The total number of cases with NuMA2 pattern in the present study was of 11, out of which three corresponded to patients with Lupus and 1 with RA, two corresponded to patients having cardiac diseases, two had cardio-pulmonary diseases and 3 patients had renal failure. At present, anti-NuMA2 antibodies have been demonstrated in association with conditions such as chronic idiopathic urticaria (CIU) and sensorineural hearing loss (SNHL) [26].

Here we report the presence of antibodies (particularly those with the anti-NuMA1 pattern) in patients having only a clearly defined cardiovascular disease without there being clinical data of autoimmune disease. There is evidence of the modulatory role of some specific antibodies in cardiovascular diseases (CVD); however, there is no consensus on the function this antibodies exert [27-32]. An association has been reported between myocarditis, and autoimmune diseases. In the present series, we found the coexistence of the anti-NuMA1 antibody pattern and autoimmune disease in one patient with myocarditis. There were two young women with high anti-NuMA antibodies and myocarditis, in one the dilution was of >320 and in the other of 5120 but without autoimmune disease. Dilutions greater than 620 were found in patients with hepatitis infection, and with kidney transplant from living donors or from corpses. Therefore, an explanation is still needed to recognize if certain CVD are the product of autoimmunity.

Although it is known that cardiac disease can coexist with autoimmune disease, the high percentage of anti NuMA antibodies found in the presence of cardiac disease and without autoimmune disease in this series leads us to consider that the presence of these antibodies can be indicative of autoimmunity.

The presence of anti-NuMA antibodies in subjects with autoimmune disease is explained by the theory of molecular mimicry. This theory is also fundamental for the understanding of autoimmune responses against cardiac antigens. It is worth mentioning that multiple infectious agents have been identified that have similarity with elements and epitopes of cardiac etiology [33]. In this context, infectious causes related to human myocarditis are known to be related with *Trypanosoma cruzi*, and molecular mimicry with cardiac antigens is proposed as a mechanism of damage [34]. However, there are other causative agents such as parvovirus B19, coxsackievirus [35] and *Borrelia* spp. [36].

In cases with cardiac damage, myosin appears to contain dominant epitopes that contain similar structural composition with antigens derived from pathogens. Rheumatic heart disease (RCE) provides an example of molecular mimicry in cardiomyopathies, and it is well known that repeated infections with *Streptococcus pyogenes*, [Group A *streptococcus* (GAS)] can cause rheumatic fever, rheumatic conditions, including polyarthritis, in addition to carditis [37]. The cross-reactivity between GAS and the components of cardiac proteins is currently accepted as a key ECR trigger [38].

The M proteins of GAS have similarity with cardiac myosin [39]. Other components of GAS such as the carbohydrate antigen and N-acetyl-β-d-glucosamine (GlcNAc) are similar to cardiac antigens [40,41] and in some others there is molecular mimicry with additional cardiac antigens, such as laminin [40] tropomyosin [41], endothelium [42-44]. The cell cycle-dependent distribution and function of NuMA is regulated by phosphorylation and dephosphorylation, this activity is important to the mitotic role of NuMA.

NuMA may represent a large group of proteins whose mitotic function is sequestered in the nucleus during interphase and plays diverse important roles in vertebrate cells.

It is an important component of the nuclear matrix in interphase cells, and is possibly involved in nuclear re-assembly after mitosis. In dividing cells, upon phosphorylation, NuMA disperses into the cytoplasm, associates with cytoplasmic dynein/dynactin to form a complex, and translocates along microtubules to the spindle poles where it organizes and others microtubules to spindle poles.

It is thought that the stable complex of NuMA/dynein/dynactin is needed to focus microtubule minus ends to the spindle poles. But, it has also been reported that NuMA can organize microtubules in the absence of centrosomes and dynein. It has been suggested that once localized to the spindle poles, spindle-associated NuMA's exchange with cytoplasmic soluble pools and its stable crosslinking with the microtubule fibres are independent of dynein/dyactin, NuMA's function in spindle microtubule organization [45].

Therefore antibodies against various antigens of the nuclear mitotic apparatus may lead to autoimmune or non-autoimmune disease and this may depend on the type of dysfunction generated by antibodies against a target antigen at the time of mitosis.

The detection of clinical data suggesting autoimmunity by the treating physician renders it obligatory to request laboratory tests to detect biomarkers such as antinuclear antibodies which aid confirm or exclude a specific diagnosis. This research has made it possible to detect timely ADs and the causal interconnections through time. There

is little research when the opposite situation in present in which autoimmunity parameters are detected, through the laboratory and then, clinical manifestation related to ADs are intentionally sought. This could be a factor not contemplated in the delay of diagnosis.

It is also known that patients who meet criteria for autoimmune disease can be sero-negative (antibodies not present) [46,47]. Recently, the explanation for ADs which can be seropositive or seronegative has been attributed to non-genetic factors and to a specific genetic architecture. The decision to treat sero-negative ADs continues to be debatable [48]. Although it is important to mention that in many cases sero-positivity for ANAs occurs later during the evolution of the disease.

The presence of antibodies in cardiovascular diseases in this study leaves open the question of whether the cardiovascular disease is the first manifestation of autoimmune disease or a consequence of it. However, in this series, the physicians who requested antinuclear antibodies in these patients with heart disease without having criteria for autoimmune disease in their vast majority reported at least one clinical data of autoimmunity. Furthermore, diseases are not mutually exclusive and non-autoimmune diseases can coexist with ADs. Thus, feedback between the laboratory and the treating physician are necessary, since in patients with suspected ADs, timely therapy, can improve the prognosis.

Limitations

The files studied were from a specialized center for the attention of cardiac problems and therefore the real prevalence is most probably increased by the type of medical attention that is offered in this center. The prevalence in centers that are not specialized in cardiological problems could not be evaluated.

Conclusion

The prevalence of anti-NuMA antibodies in cardiovascular disease without the coexistence of proven autoimmune disease was greater than 30%. The high levels in these patterns in heart disease found, suggest autoimmunity. The presence of anti NuMA patterns should be analyzed in relation to their relevance for the etiology of the condition.

Authors' Contributions

All of the authors have accepted responsibility for the entire content of this submitted manuscript and approved submission.

Competing Interests

The funding organization(s) played no role in the study design; in the collection, analysis, and interpretation of data; in the writing of the report; or in the decision to submit the report for publication.

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