Prevalence of non-organ-specific autoantibodies in a rural community from northeastern Brazil: a population-based study

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ABSTRACT

Non-organ-specific autoantibodies (NOSA) are well-recognized diagnostic markers of autoimmune hepatitis (AIH) and primary biliary cirrhosis (PBC), but can also be observed in patients with viral hepatitis as well as in healthy subjects. The aim of this study was to evaluate the prevalence of NOSA in subjects living in a rural community in Brazil and to correlate their occurrence with the presence of liver disease. Seven hundred twenty-five apparently healthy subjects were randomly selected for assessment of antinuclear (ANA), anti-smooth muscle (SMA), antimitochondrial (AMA), anti-liver/kidney microsome type 1, and anti-liver cytosol type 1 antibodies. Subjects with those NOSA were evaluated for the presence of AIH, PBC, and viral hepatitis. Reactivities for all NOSA, SMA, ANA, and AMA were detected, respectively, in 14, 10, 4, and 0.1% of subjects, with a mean titer of 1:40. NOSA-positive subjects were significantly older and more frequently females. No correlation was observed between the occurrence of NOSA and PBC, AIH, or viral hepatitis. The prevalence of NOSA in Brazilians was 14%. They were usually low titer. NOSA were more frequently observed in females and older subjects and their presence was not correlated with the presence of AIH, PBC, or viral hepatitis.

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1. Introduction

Non-organ-specifc autoantibodies (NOSA) may be observed in healthy subjects as part of the circulating repertoire of naturally occurring autoantibodies (Nabs). By contrast, several NOSA are also recognized as diagnostic markers of several autoimmune diseases [1]. In this regard, antinuclear (ANA), anti-smooth muscle (SMA), antimitochondrial (AMA), antisoluble liver antigen/liver-pancreas, anti-liver/kidney microsome type 1 (anti-LKM1), and anti-liver cytosol type 1 (anti-LC1) antibodies are employed, usually when titers are high, for the diagnosis of autoimmune liver diseases such as autoimmune hepatitis (AIH) [2] and primary biliary cirrhosis (PBC) [3]. However, they are also usually reported, particularly ANA and SMA, in 1–43% of apparently healthy subjects [4–8], in 14–66% [9] of patients with chronic hepatitis C, and in up to 90% of patients with autoimmune rheumatic diseases [10].

In contrast to disease-associated NOSA, Nabs were demonstrated to be germline-type antibodies, usually but not exclusively of immunoglobulin (Ig)-M isotype, and displaying multireactivity and low affinity for antigens [11–13]. They tend to cluster in females [7,14] and in the elderly [4,5,14–16]. By contrast, the occurrence of NOSA may be associated with an enhanced predisposition or even the presence of occult liver disease, as previously reported for AMA as well as for other NOSA, such as SMA, ANA, or anti-LKM1, respectively, for PBC [17,18], and for hepatitis C [9,19–26]. However, with the exception of AMA, data concerning the significance of those aforementioned NOSA as surrogate markers of autoimmune or viral diseases of the liver are lacking.

The purpose of the present study was to investigate the prevalence of NOSA in apparently healthy subjects from a rural community in the northeastern region of Brazil, to analyze their occurrence according to age and gender and the presence autoimmune liver diseases, as well as with evidence of past or present infection with hepatitis A (HAV), B (HBV), and C (HCV) virus.

2. Subjects and methods

2.1. Study population

This study was conducted in the district of Cavunge in Ipacaeta County in the semiarid region of Bahia, Brazil. The district had a population in 1999 of 2,049 subjects living in an area of 63.5 km² composed of 1 small village and several rural settlements scattered

N	725
% of the estimated population (*n* = 2,049)	35%
Female sex	369 (51%)
Age (years)	23 (0.2–91)
Age less than 16 years	39%
Place of residence	
Urban area	27%
Rural area	73%

[a]According to the 1999 census.[27]

in distinct farms. The demographic features and socioeconomic status of this population were previously described [27,28]. Briefly, its inhabitants were of highly admixed origin with differing percentages of Caucasoid, Negroid, and Amerindian ancestries. Most of the population lived in the rural settlements and were of poor socioeconomic status, with their main income derived from subsistence farming, relying on growing corn, beans, and cassava. Nearly half were illiterate.

The present study is part of a large project sponsored by the Brazilian National Health Foundation to perform a sentinel surveillance of HAV, HBV, and HCV infection in northeastern Brazil. According to the methodology employed, data collection was carried out in the Cavunge district at 2 different time periods.

In the first round of the project, carried out between 1999 and 2000, 1,800 subjects from Cavunge were enrolled to assess the prevalence of past or current infection with HAV, HBV, and HCV [28].

The second part of the project was carried out after 2004 to provide data collection for the prevalence of HAV, HBV, and HCV infection at least 5 years apart. During this time period, participants of this sentinel surveillance study were randomly invited by local agents from the Brazilian Family Health Program to be enrolled in the present investigation concerning the prevalence and significance of NOSA in the Cavunge District.

All subjects or their legal guardians who agreed to take part in the study had given their informed consent. Participants were required to state residence in the Cavunge District for more than 6 months.

Subjects who were unable to understand the principles of the study, such as those with chronic degenerative cerebrovascular disorders or psychiatric illness, were excluded from the current investigation, as well as all subjects less than 18 years of age without formal consent of their parents or legal guardians. Subjects who either required hospitalization or needed medical care in the previous 6 months were also excluded. Subjects less than 16 years of age were arbitrarily defined as children.

The study was approved by the Ethics Committee in Research of the Prof. Edgard Santos University Hospital and by the National Ethics Research Council.

Seven hundred twenty-five subjects were accepted to participate and underwent an interview to gather demographic data such as age, gender, and local address. Subsequently, blood samples were collected and processed into 1 to 4 aliquots of 1 mL of plasma, subsequently frozen, and stored at −80°C. Only a 1-mL aliquot was separated for NOSA determination.

2.2. Autoantibodies

All subjects were screened for SMA, ANA, AMA, anti-LKM1, and anti-LC1 antibodies by indirect immunofluorescence (IIF) at a screening dilution of 1:40 on rat liver, kidney, and stomach tissue sections according to international criteria [29]. SMA reactivity was further characterized as vessel (V), glomeruli (G), and tubule (T) patterns according to Bottazzo et al. [30]. All SMA[+] subjects had their sera subsequently tested for antiactin

(microfilament) reactivity by IIF at a screening dilution of 1:20 using the heat serum inactivation technique [31]. All sera were also tested for ANA on HEp-2 cells using a commercially available kit (code 44509, Biosystems, Barcelona, Spain). Reactivities for anti-LKM1 and/or anti-LC1 as well as for AMA, whenever present, were further confirmed by immunoblotting, respectively, using rat liver microsomal fractions [32,33], human liver cytosol [34], and submitochondrial fractions of beef heart as the source of antigens [35–37], as previously described.

2.3. Viral markers

Serologic markers of past or present hepatitis A, B, and C infection, including anti-HAV antibody (anti-HAV) IgG, hepatitis B surface antigen (HBsAg), anti-hepatitis B core antigen antibody (anti-HBC IgG), anti-hepatitis B surface antigen antibody (anti-HBs), and anti-HCV antibody (anti-HCV; ELISA III, Roche, Basel, Switzerland) were performed in the first round of the project. All anti-HCV[+] samples were subsequently examined for HCV-RNA quantification and genotyping.

2.4. Clinical follow-up

Subjects with reactivity to SMA, ANA, or anti-LKM1 at titers equal to or higher than 1:80 and to AMA at titers equal or higher than 1:40 were submitted to subsequent clinical and laboratory evaluation to assess the presence of AIH, PBC, and primary sclerosing cholangitis, as well as other viral and metabolic liver disorders. The titers were arbitrarily chosen as clinically relevant based on published guidelines for the diagnosis of AIH and PBC [2,3]. All patients with evidence of current infection with HBV or HCV were further investigated and treated whenever indicated according to established clinical, laboratory, and histologic criteria.

2.5. Statistical analysis

Clinical and laboratory data were compared using the χ^2 test with Yates' correction or the Mann–Whitney test when appropriate. A p value < 0.05 was considered significant. Clinical data are presented in the text and tables as median and range.

3. Results

Seven hundred twenty-five subjects (369 females, median age of 23 [0.2–91] years) from the rural community of Cavunge were evaluated. All were apparently healthy. Demographic data concerning those subjects are summarized in Table 1. Thirty-nine percent were less than 16 years of age and were considered children. Most subjects were living in the rural area of Cavunge County.

One hundred one subjects (14%) exhibited reactivity for NOSA. Positivity of SMA, ANA, and AMA was observed in 74 (10%), 32 (4%), and 1 (0.1%) subject, respectively. Six had concurrent reactivity for SMA and ANA and none of the subjects had either anti-LKM1 or anti-LC1 by IIF. Seventy-two (71%) subjects had NOSA at titers equal

Table 2
Frequency of non-organ-specific autoantibodies (NOSA) according to titers and immunofluorescence patterns in subjects from Cavunge County

	NOSA titers (%)		
	1:40	1:80	1:320
NOSA (*n* = 101)	72 (71)	28 (28)	1 (0.9)
SMA (*n* = 74)	51 (69)	23 (31)	0 (0)
V pattern (*n* = 59)	43 (58)	16 (22)	
G pattern (*n* = 15)	8 (11)	7 (9)	
ANA (*n* = 32)	26 (81)	5 (16)	1 (3)
Nuclear coarse speckled pattern (*n* = 27)	26 (81)	1 (3)	
Nucleolar homogeneous pattern (*n* = 2)		1 (3)	1 (3)
Fibrillar cytoplasmatic pattern (*n* = 2)		2 (6)	
Nuclear homogeneous pattern (*n* = 1)		1 (3)	
AMA (*n* = 1)		1 (100)	

Table 3
Comparison of demographic features of subjects from Cavunge County according to the occurrence of NOSA

	Subjects with NOSA			Subjects without NOSA
	Total (n = 101)	SMA+ (n = 74)	ANA+ (n = 32)	Total (n = 624)
Demographic features				
Age (years)	25 (0.5–91)[a]	27 (1–91)[b]	18 (0.5–81)	23 (0.2–85)
Female sex	72 (72)[c]	53 (72)[d]	23 (72)[e]	297 (48)
Place of residence				
Urban area	27%	23%	31%	26%
Rural area	73%	77%	69%	74%

Numbers in parentheses are percentages. Superscript letters indicate values different from apparently healthy subjects without NOSA at the level of [a] $p = 0.05$; [b] $p =$ NS; [c] $p = 0,00002$; [d] $p = 0,0003$, and [e] $p = 0.02$.

to 1:40, whereas 23 (31%) subjects with SMA and 6 (19%) with ANA had titers equal to or higher than 1:80 (Table 2).

Analysis of IIF patterns of those subjects with SMA revealed that 59 (80%) and 15 (20%) subjects had SMA-V and -G and none exhibited the SMA-T pattern. Antimicrofilament antibody at a titer of 1:20 was observed in only 1 patient with the SMA-G pattern at a titer of 1:80. All but 5 (84%) subjects with ANA exhibited the nuclear coarse speckled pattern. The other IIF patterns encountered were nucleolar homogeneous ($n = 2$), fibrillar cytoplasmic ($n = 2$), and nuclear homogeneous ($n = 1$). All but 1 subject with the nuclear coarse speckled pattern had titers equal to 1:40, whereas all subjects with other ANA patterns exhibited titers equal to or higher than 1:80 (Table 2).

The only female subject with reactivity for AMA had titers equal to 1:80 by IF, but the search for anti-M2 antibody by immunoblotting yielded negative results.

Analysis of demographic features of those subjects from Cavunge County according to the presence of NOSA revealed that subjects with NOSA (median age of 25 [0.5–91] years vs 23 [0.2–85] years in subjects without NOSA, $p = 0.05$) were older when compared with their counterparts without NOSA (Table 3), but the frequencies of NOSA were similar in children compared with adults (13% in children vs 16% in adults, $p =$ NS). In addition, subjects with NOSA, either SMA or ANA, were more frequently females (72% vs 48% of subjects without NOSA, $p = 0.00002$) when compared with their counterparts without NOSA (Table 3).

Serologic markers for HAV, HBV, and HCV were available for those 305 subjects who also took part in the first round of the project. Results for the remaining 420 subjects who were subsequently enrolled in this study were still pending at the time of this writing. The prevalence of positivity for all viral markers, IgG anti-HAV, AgHbs, IgG anti-HBC, and anti-HCV in those tested subjects was 90, 2.4, 8.4, and 0.3%, respectively. No correlation was disclosed between the occurrence of NOSA and serologic evidence of past or ongoing infection with HAV, HBV, or HCV (Table 4).

Twenty-four subjects (17 females, median age of 22 [2–86] years) had NOSA at clinically relevant titers equal to or higher than 1:80 for SMA and ANA or equal to or higher than 1:40 for AMA. All were subsequently evaluated for the presence of liver diseases, including AIH and PBC. None had any clinical evidence of rheumatoid or liver disease. Higher levels of aspartate aminotransferase (1.1 times the upper limit of normal) were disclosed in 1 SMA+ with a V pattern and a titer greater than 1:80. No other subject, including the AMA+ women, had abnormal liver enzymes.

4. Discussion

The present study demonstrated a frequency of NOSA of 14% in apparently healthy subjects, particularly SMA (10%) and ANA (4%). The occurrence of NOSA was more common in females and older subjects. Higher frequencies of ANA [8,14,38–43] and SMA [8,14–16,38] were previously disclosed in apparently healthy subjects in some but not all studies [4,5,7,19,39,44], leading altogether to a great variability in the reported frequency of NOSA in the literature, ranging from 1.5 to 43% [4,8]. This heterogeneity was probably caused by differences in the methodologies used in most of the reports. Several studies have employed blood donors [5,39,42,44,45] as apparently healthy subjects and most investigators have also used nonstandardized IIF techniques recognizing low titers of NOSA, different from those currently recommended by the International Autoimmune Hepatitis Group [29]. In addition, the striking differences observed in socioeconomic status, ethnicity, and demographics of the subjects included in the aforementioned studies may have influenced their results because they do play a role in the expression of Nabs.

Two previous population-based studies [4,7] also revealed a lower prevalence of NOSA in the general population. Njemini et al. [7] reported a frequency of either SMA or ANA of 9% of the patients in western Africa, whereas Hooper et al. [4] have disclosed a prevalence of 1.5 and 2% of SMA and ANA, respectively, in Busselton (Australia).

Similar to our findings, enhanced predisposition of NOSA in females was reported in some [7,14], but not all studies [15]. Conversely, older age was also correlated with the occurrence of NOSA in several reports [4,5,14,15].

Analysis of SMA patterns of reactivity revealed SMA-G and -V that are frequently encountered in the general population in all subjects. No subject had the tubular pattern and only 1 had low-titer antimicrofilament antibody, previously associated with a higher specificity for the diagnosis of AIH [29]. With regard to ANA, most subjects had the low-titer nuclear coarse speckled pattern that was previously demonstrated to be more common in patients with autoimmune rheumatoid disorders compared with controls [46–48], but it must be pointed out that patterns of IIF reactivities of ANA do overlap greatly between patients and apparently healthy controls [42,43,47–49]. These data altogether endorse the relevance of the proper interpretation of NOSA patterns for the diagnosis of autoimmune diseases.

Table 4
Analysis of results of viral markers for past or ongoing infection with hepatitis A, B, and C virus according to the occurrence of NOSA

Presence of viral markers	Subjects with NOSA			Subjects without NOSA
	Total (n = 80) (%)	SMA (n = 59) (%)	ANA (n = 25) (%)	Total (n = 225) (%)
IgG anti-HAV and/or HBsAg and/or total anti-HBc and/or anti-HBs and/or anti-HCV	94	92	96	88
IgG anti-HAV	75	75	72	78
HBsAg	2.2	1.6	3.3	0.3
Anti-HBc total	11	10	6	12
HBsAg and/or anti-HBc total and/or anti-HBs	18	16	15	19
Anti-HCV	1	2	0	0

HAV = hepatitis A virus; HBsAg = hepatitis B surface antigen; anti-HBc = anti-hepatitis B core antigen; anit-HBs = anti-hepatitis B surface antigen; HCV = hepatitis C virus.

The absence of anti-LKM1 and anti-LC1 in subjects from the present study was not unexpected because those autoantibodies are mostly observed only in patients with AIH and less often in hepatitis C patients [29,50]. By contrast, AMA was observed in only 1 female subject. Its reactivity was not confirmed by immunoblotting. These findings are in accordance with other studies, which have described AMA in less than 1% of the general population [51]. However, it must be emphasized that AMA, particularly when confirmed by the detection of anti-M2 using either enzyme-linked immunosorbent assay or immunoblotting, is specific for the diagnosis of PBC as well as enhanced predisposition for development of the disease in the long term [17]. Our AMA$^+$ subject had no evidence of liver disease and no reactivity for anti-M2 antibody.

The occurrence of NOSA in the present study was not associated with overt autoimmune rheumatoid disease or with the presence of liver disease, including PBC and AIH. Only 1 patient had a slight elevation of aspartate aminotransferase 1.1 times the upper limit of normal that was not correlated with any sign or symptom of liver disease and no laboratory evidence of viral, metabolic, or autoimmune liver disorders. The frequency of NOSA in cohorts of patients with HBV and HCV varies from 2 to 66% [9,20,21,22,23,26]. However, no association of NOSA with past or present HAV, HBV, or HCV infection was elicited in this investigation, suggesting, on the contrary, that the occurrence of NOSA is not related to undiagnosed or occult HBV or HCV infection or with past history of infection with hepatotropic virus. However, it should be stressed that the lack of association between NOSA and viral infection in the present study could be caused by the very low prevalence of current HBV and HCV infection in the study cohort.

In summary, NOSA, particularly SMA and ANA, can be observed in fewer than 10% of the apparently healthy population, whereas AMA, anti-LKM1, or anti-LC1 is either rare or absent in subjects without autoimmune liver diseases. In the general population, NOSA are more often encountered in females and in older subjects. They are usually in low titers and exhibit patterns of reactivity without specificity for rheumatoid or liver disorders. Their occurrence is not related to the presence of undiagnosed AIH or PBC and they are not surrogate markers for past or active infection of HAV, HBV, or HCV.

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