PREVALENCE OF ANTINUCLEAR AUTOANTIBODIES IN THE SERUM OF NORMAL BLOOD DONORS

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OBJECTIVE: To examine the presence of serum antinuclear autoantibodies in a healthy population.

METHODS: Serum of 500 normal blood donors between 18 and 60 years of age were tested for the presence of autoantibodies. Antinuclear antibodies were detected by indirect immunofluorescence technique using HEp-2 epithelial cells as the substrate. The presence of dnaN was detected by indirect immunofluorescence technique using *Critidia lucillae* as the substrate. Anti-SSA (RO), anti-SSB (LA), anti-Sm, and anti-RNP were determined by double radial immunodiffusion.

RESULTS: In the evaluation of the presence of serum antibodies, antinuclear antibodies were detected in 22.6% of the sera. The presence of other antibodies was not significant. The majority of the titers were 1:40.

CONCLUSION: The presence of autoantibodies is not necessarily pathologic and has to be related to the age group, gender, and clinical condition of the patient.

DESCRIPTORS: **Autoantibodies. Healthy population. Rheumatic diseases. Antinuclear antibodies. Autoimmunity.**

Immune humoral response in the presence of autoantibodies against intracellular antigens characteristically occurs in a majority of connective tissue diseases. This phenomenon is found in systemic lupus erythematous, systemic sclerosis, Sjögren syndrome, mixed connective tissue disease, polymyositis, and dermatomyositis, among others[1].

The detection of such autoantibodies is important not only for diagnosis but also for the prognosis of the diseases, in addition to allowing clinical follow-up and treatment evaluation in many cases[1].

It is relevant to point out that in healthy people and in patients with non-rheumatic conditions, such as chronic hepatic diseases, neoplasias, as well as active infections including tuberculosis, malaria, and subacute bacterial endocarditis, these autoantibodies may be present[1]. In general, they are found in lower titers than those detected in autoimmune diseases. It is believed that such titers may precede the appearance of diseases in normal and asymptomatic individuals for many years[2].

Previous studies have shown different prevalences of autoantibodies in healthy populations. Baig and Shere (1989), who investigated the serum of blood donors and patients without autoimmune diseases in Saudi Arabia, found antinuclear antibodies (ANA) present in 4.2%[3]. Vlam et al. (1993) detected ANA present in 13% of normal blood donors in a Belgian population[2]. Vazquez-Del Mercado et al. (1995) reported that 4.7% of a Mexican population tested positive for ANA[4].

The present study intends to evaluate the prevalence of autoantibodies in healthy people in relation to gender and age group. It is important to note that it is the first such study in Brazil, one that characterizes a specific population, that of blood donors of the Pro-Blood Foundation – Hemocenter of São Paulo, aiming to obtain parameters for comparisons with other populations.

All the samples were screened for the presence of ANA using the indirect

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immunofluorescence technique in HEp-2 cells. They were also tested for the identification of antibodies against antigens extracted from the nucleus such as anti-SSA/RO, anti-SSB/LA, anti-Sm, and anti-RNP by double radial immunodiffusion. They were still tested for dnaN using indirect immunofluorescence (IIF) technique with *Crithidia luciliae* as the substrate.

In the blood bank, samples are habitually tested for infectious diseases such as Chagas disease, syphilis, hepatitis B and C, HTLV-1 and HTVL-2, and HIV-1 and HIV-2, that may contraindicate the utilization of the blood taken.

This study was approved by the Institutional Ethical Committee. The volunteers who had significant titers (ANA>1:160) for the studied parameters were informed of that and were re-examined for any disease that could be clinically confirmed. They received follow-up evaluation and orientation when necessary.

PATIENTS AND METHODS

Blood samples collection

The blood samples were collected only after the volunteers had agreed to take part in the study and after they signed the consent form.. Five hundred consecutive blood samples of volunteer donors (182 women and 318 men), between 18 and 60 years of age, with no clinical symptoms of any disease, were analyzed (Fig. 1). They were obtained from the Pro-Blood Foundation – Hemocenter of São Paulo.

Autoantibody detection

After collection in plain red-top tubes, the blood samples were centrifuged (10 minutes at 2000 rpm), and the serum was separated into aliquots for performing the autoantibody-detection tests.

The indirect immunofluorescence (IIF) technique to detect antinuclear antibodies, described by Holbrow, Weir, and Jonhson in 1957, was used in slides covered with commercially obtained (Hemagen®) HEp-2 cells (epithelial cells of human larynx carcinoma). The usage of human cells guarantees the presence of nuclear antigens in such a concentration that enables the confirmation of the presence of antibodies in the serum.

At first, a screening test was carried out, in which serum samples were diluted in phosphate-buffered saline, pH 7.4 (PBS) to a titer of 1:40 to detect positive reaction and characterization of the fluorescence pattern. The positive samples were titered until testing negative.

The HEp-2 cells were incubated with the donor's serum samples in a humid and dark camera for 30 minutes and were then washed in PBS. Afterwards they were incubated with human anti-IgG antibody marked with fluorescein isothiocyanate. After being washed again, the slides were mounted with buffered glycerine pH 8. A Zeiss microscope equipped for epiluminescence was used for reading the slides.

In order to detect native anti-DNA autoantibodies (DNAn), the indirect immunofluorescence (IIF) technique was used in slides covered with *Crithidia lucillae* (a protozoan that is rich in kinetoplast DNA) obtained from the Department of Rheumatology of our Institution.

The slides were incubated with the samples diluted 1:20 in Tris-buffered saline, pH 7.5 (TBS) and were then washed and incubated with human anti-IgG antibody (SIGMA brand) marked with fluorescein isothiocyanate. A Zeiss microscope equipped for epiluminescence was used for reading the slides (immersion objective).

The double radial immunodiffusion technique, described by O. Ouchterlony in 1949, was used to investigate anti-SSA (RO), anti-SSB (LA), anti-Sm, and anti-RNP antibodies. The reaction was run on 0.6% agarose. The sources of antigens were extracted from the human spleen for SSA and rabbit thymus extract (Sigma brand) for anti-SSB, anti-Sm, and anti-RNP. The positive controls were commercially obtained (IMMCO brand).

Petri plates with agarose gel were perforated to restrain the samples (non-diluted serum), the controls, and the source of antigen. The plates were visually analyzed over a period of 24 to 48 hours for observation of the formation (positive) or non-formation (negative) of lines of immunoprecipitation between the samples and the controls.

Infectious diseases serology

The Pro-Blood Foundation – Hemocenter of São Paulo uses the following techniques to investigate infectious diseases in the blood of donors: ELISA and VDRL for syphilis; ELISA, and if positive, Western - Blot for HIV; ELISA for HTLV; ELISA, indirect hemaglutination and indirect immunofluorescence (IIF) for Chagas disease and ELISA for hepatitis B.

Statistical analysis

The statistical analysis was performed by the Department of Preventive Medicine of our Institution. The c2 test was used, adopting the value of *P* less than or equal to 5% as the indicator for statistical significance. The statistical analysis was carried out with the software STATA™ (Statistical/Data Analysis), version 7.0.

RESULTS

The study involved 318 men (63.6%) and 182 women (36.4%). The average age of the donors was 33.1

years (standard deviation = 9.7 years of age). No association was found between gender and age of the donors (*P* = 0.27) (Table 1; Fig.1). Out of the 500 donors included in the sample, 113 were positive for ANA, representing a prevalence of 22.6% ($CI_{95\%}$:18.9% to 26.3%) as shown in figure 2. Among the donors who presented ANA+, 73 (64.6%) had a titer of 1:40, 23 (20.4%) a titer of 1:80, 10 (8.8%) a titer of 1:160, and 7 (6.2%) a titer equal or higher than 1:320. Out of this last group, 1 donor presented a titer of 1:320, 2 donors 1:1280, 2 others 1:2560, 1 a titer of 1:5120, and another of 1:10240. Titers of ANA equal to or higher than 1:320 are considered pathological in the literature, and 7 of the 500 donors included in the study presented titers in this category, which corresponds to a prevalence of 1.4% ($CI_{95\%}$: 0.3 to 2.4). The prevalence of ANA among the donors included in the study according to the different titers found is shown in table 2. The prevalence of other autoantibodies was very low: 1 donor presented anti-SM+ (and did not present ANA+), and 2 other donors presented anti-RO+ (one with ANA+ 1:40 and another with ANA+ 1:10240). The association between gender and age group and the presence of ANA+ (in any titer) is shown in table 3. Female blood donors presented a higher risk of presenting ANA+ (PR =1.66; $CI_{95\%}$:1.20 to 2.28). As for the age group, donors under 40 years of age presented a tendency to a smaller prevalence of ANA+, with no statistical significance. The risk of presenting ANA+ according to gender did not present any significant alterations after the age adjustment (PR = 1.62; $CI_{95\%}$: 1.18 to 2.21), which means that age was not a confounding variable for this association. Only a few donors presented ANA titers equal to or higher than 1:320, and this hindered the investigation of the characteristics associated with such titers. There was a

Table 1 - Distribution by gender and age group of the volunteer blood donors included in the study (n=500).

Age group	MEN N	MEN %	WOMEN N	WOMEN %	TOTAL N	TOTAL %
Up to 30	149	46.9	84	46.1	233	46.6
31 to 40	109	34.3	52	28.6	161	32.2
41 to 50	38	11.9	32	17.6	70	14.0
51 to 60	22	6.9	14	7.7	36	7.2

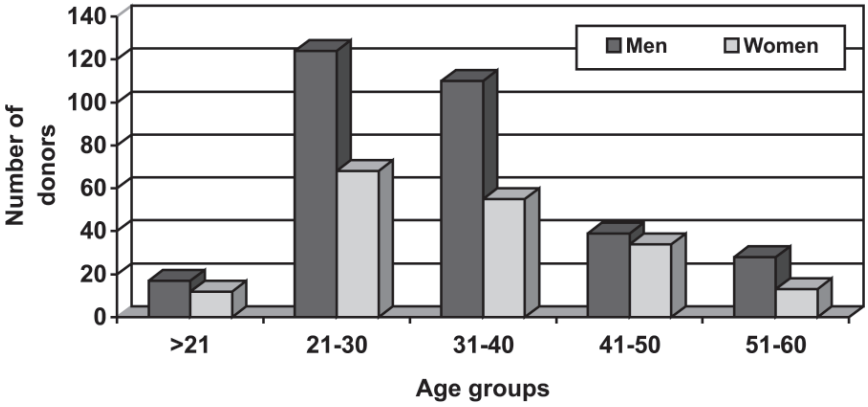

Figure 1 - Total number of blood donors, distributed according to gender and age group.

Table 2 - Prevalence of ANA+ of the volunteer blood donors included in the study, according to different titers (n=500).

ANA (titer)	N	Prevalence (%)	$CI_{95\%}$ (%)
1:40	73	14.6	11.5 to 17.7
1:80	23	4.6	2.8 to 6.4
1:160	10	2.0	0.8 to 3.2
1:320 or more	7	1.4	0.3 to 2.4
Any titer	113	22.6	18.9 to 26.3

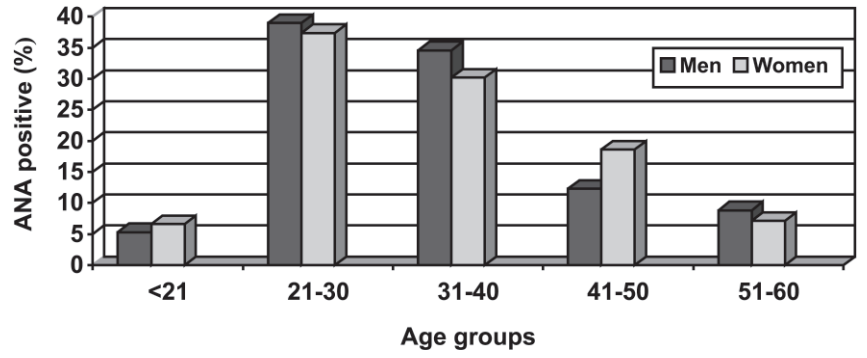

Figure 2 - Percentage of antinuclear antibodies positive in each age group, according to gender.

marginally significant tendency towards the presence of higher titers of ANA+ (PR = 4.4; $CI_{95\%}$: 0.9 to 22.3) among females, and no association was found between age group and titers of ANA higher than 1:320 (Table 4, Fig.3).

As for serologies for infectious diseases, only 2 donors presented positive serology for hepatitis B (anti-HBc) and were ANA positive with low titers (1:40).

Table 3 - Prevalence ratios of ANA+ in any titer, according to age group and gender.

Characteristic	Total	ANA+	%	PR	$CI_{95\%}$	*P*
Gender						
Male	318	58	18.2	1.0		
Female	182	55	30.2	1.66	1.20 to 2.28	0.002
Age group						
41 or older	106	30	28.3	1.0		
Up to 40	394	83	21.1	0.74	0.52 to 1.06	0.11

Table 4 - Prevalence ratios of antinuclear antibodies titers equal to or higher than 1:320, according to gender and age group.

Characteristic	Total	FAN+	%	PR	$CI_{95\%}$	*P*>1:320
Gender						
Male	318	2	0.6	1.0		
Female	182	5	2.7	4.4	0.9 to 22.3	0.06
Age group						
41 or older	106	1	0.9	1.0		
Up to 41	394	6	1.5	1.6	0.2 to 13.2	0.65

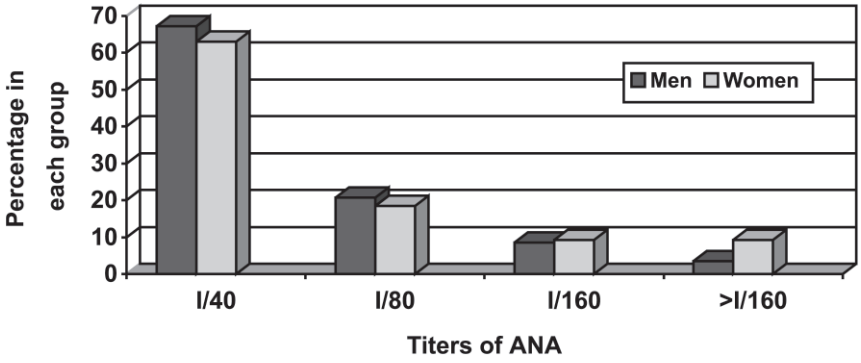

Figure 3 - Percentage of antinuclear antibodies positive in each titer group, according to gender.

DISCUSSION

Antinuclear antibodies (ANA) are present in some autoimmune diseases and in other non-autoimmune conditions, as mentioned before. In the autoimmune diseases, testing for ANA is considered a useful screening test due to its high sensitivity and low specificity. For instance, in case of systemic lupus erythematous, a positive test with titers higher than 1:40 and 1:80 presents a positive predictive value of 15% to 35%, indicating the need to complement the investigation with tests for other autoantibodies such as anti-DNAn, anti–RO, anti-Sm, and anti-LA[2]. In face of a positive test for ANA, in addition to the patient clinical profile, some other conditions have to be taken into consideration, such as the presence of positive serologies for hepatitis B and C, HIV, HTLV, Chagas disease, and syphilis, since patients with such diseases may test positive for ANA although in low titers[2]. In the present study, 2 out of the 112 ANA-positive samples presented positive serology for hepatitis B (ANA being found in low titers: 1:40), which may represent a false positive result due to the hepatitis. That is why it is always important to exclude the possibility of any infectious disease in face of a positive result for ANA in low titers.

In the present study, the prevalence of ANA in the general population was 22.6%, higher than the prevalences found in the literature, which vary from 4% to 13%[3,5,6,7]. The majority had low titers (83.9% with titers of 1:40 and 1:80), which is in accordance with the results obtained by other authors[2,8]. In 10 donors (8.9%), the ANA titer was 1:160, which is considered an intermediate value[2]. Since there are few studies of this kind in the literature and most of them deal with the European population, which does not have the same ethnic characteristics as ours, it is difficult to determine whether this incompatible result is due to any idiosyncrasy of the sample studied. In order to clarify this point, it would be preferable if this study were applied in other populations. Taking into consideration that 8 of the observed donors presented ANA>1:160, a titer considered significant for the diagnosis of collagenosis, it is advisable that such donors, although clinically healthy, should receive follow-up evaluation in order to detect any sign of the development of an autoimmune disease. Two of the observed donors presented positive anti-RO, one of them with an ANA titer of 1:160 and the other ANA

titer of 1:40. The positivity of such an antibody associated with ANA significantly increases the possibility of an autoimmune disease that is not clinically detectable at the present moment or that has not yet developed. Therefore, these 2 donors should be segregated from the ANA+ assuredly healthy population, reducing the positivity of such an autoantibody in the healthy individuals to 20.6%. Concerning gender, there was a higher positivity of ANA among females, which agrees with the available data in the literature[3,5,6,9]. As for ANA distribution among age groups, there was no statistical difference, which contradicts the observations reported in the literature of higher positivity of autoantibodies, including those of ANA, in the elderly as a result of the loss of autoregulation of the immune system due to senescence[3,5,10].

CONCLUSION

This paper reports on a study of a specific population of blood donors between 18 and 60 years of age. Although it does not cover all the age groups, it allows the following conclusions based on the ones observed:

1. ANA may be positive in low titers in the normal population, not necessarily being an indicator of collagenosis.
2. Infectious contagious diseases may lead to positivity for ANA although in low titers.
3. ANA positivity in healthy individuals was higher among females.
4. Positivity for other autoantibodies was low and not always related to the positivity of ANA.
5. The present study leads the way to population studies in Brazil so that we may have a chart of ANA and of other autoantibody positivity in different population groups in our country.

RESUMO

FERNANDEZ SAV e col. - Prevalência de auto-anticorpos antinucleares no soro de doadores de sangue normais. **Rev. Hosp. Clín. Fac. Med. S. Paulo 58**(6):315-319, 2003.

OBJETIVO: O objetivo deste trabalho foi detectar a presença de auto-anticorpos em pessoas sadias.

MÉTODOS: Foi estudado o soro de 500 doadores de sangue sadios, com idade entre 18 e 60 anos. Anticorpo antinuclear foi detectado por imunofluorescência indireta usando células Hep-2 como substrato. A pesquisa de anti-DNA-nativo (DNA-n) foi feita com a técnica de imunofluorescência indireta usando *Critidia lucillae* como substrato. A pesquisa de anti-SSA, anti-SSB, anti-Sm e anti-RNP foi feita utilizando a técnica de imunodifusão radial dupla.

REUSLTADOS: A presença de anticorpo antinuclear foi detectada em 22,6% das amostras estudadas. A maioria apresentou títulos 1/40. A presença de outros anticorpos não foi significativa.

CONCLUSÃO: A presença de auto-anticorpos não é necessariamente patológica e deve ser correlacionada à idade, sexo e condição clínica do paciente.

DESCRITORES: **Autoanticorpos. População Sadia. Doenças Reumáticas. Anticorpos Antinucleares. Autoimunidade.**

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