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Review

Serum antinuclear and extractable nuclear antigen antibody prevalence and associated morbidity and mortality in the general population over 15 years

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ABSTRACT

The prevalence of ANA and anti-ENA in the general population is not well established, especially their clinical significance in healthy subjects. We herein determined the prevalence and predictive value of serum ANA and anti-ENA for connective tissue diseases (CTD), cancer, and mortality. We took advantage of a randomly selected sample of the 1998 general population (Isola I) consisting of 2828 subjects (53% women, age 43 ± 13 years) from a well-defined Northern Italian area. Serum ANA and anti-ENA were tested on the 2690 samples available in 2012 (Isola II, 50% women, age 58 ± 13 years). Administrative databases were searched for CTD, cancer diagnosis, and death cases occurring between enrollment and December 31, 2013. The hazard ratio (HR) was calculated for incident cases. Serum ANA is positive in 18.1% for any titer and 6.1% for titers ≥1:160, 23% in subjects over 50 years and 13.1% and 6.1% for any titer and titers ≥1:160, respectively, in women. The HR for CTD development was significantly high for all ANA titers, with the highest for ANA ≥1:160 (HR 14.19, 95% CI 3.07–65.68). ANA positivity was not associated with cancer (HR 1.03; 95% CI 0.75–1.43), or with mortality (HR adjusted for age and sex 1.40; 95% CI 0.94–2.09). Serum anti-ENA is positive in a minority of subjects with highest figures for anti-nucleosome (1.9%), -histone (1.6%) and -PM/Scl (1.5%). In conclusion, serum ANA prevalence in the general population is highest in senior subjects and in women, while the female predominance is significantly lower compared to overt CTD. Serum ANA is associated with an increased probability of CTD development over time, but does not influence survival or cancer risk.

Contents

Abbreviations: ANA, Antinuclear antibody; ENA, extractable nuclear antigen; CTD, connective tissue disease; HR, hazard ratio; CI, confidence interval; IIF, indirect immunofluorescence; ELISA, enzyme-linked immunosorbent assay.

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1. Introduction

Serum antinuclear (ANA) and anti-extractable nuclear antigens (ENA) antibodies are widely used in clinical practice and are included in the diagnostic and classification criteria for connective tissue diseases (CTD) [1,2]. Nonetheless, ANA is frequently found in a considerable proportion of healthy subjects although studies are generally performed in selected populations such as blood donors or employees, while data on ANA prevalence [3–6], and clinical significance over time [7], in an unselected general population are limited. Further, serum ANA and anti-ENA are tested despite a low pre-test probability in the absence of a clinical suspicion for CTD and the significance of occasional positivity is unknown [8]. Lastly, an association between serum ANA and cancer development has been proposed, mainly in patients affected by systemic sclerosis and inflammatory myositis [9].

Our objective is to determine the prevalence and predictive value of ANA and anti-ENA for the development of CTD, cancer and mortality. We addressed these objectives by testing serum autoantibodies and evaluating the 15-year clinical outcomes in a randomly selected sample from the general population enrolled in 1998 from a well-defined Northern Italian area.

2. Materials and methods

2.1. Subjects and study design

The general population of a Northern Italian area called Isola, in the Lombardy region, was originally selected in 1998 to study the prevalence of viral hepatitis (Isola I study, *unpublished data*). The 1998 population of four cities (Bonate Sotto, Ponte San Pietro, Presezzo, Terno d'Isola) included 15907 subjects between the ages of 18 and 75, which were randomly selected 1:4 for participation (n = 3977). As a result, 71.1% of the randomly selected subjects (n = 2828) participated to the study (mean age 42 years, range 18–75; female/male 1.15) and underwent a blood draw. Of these, 2690 sera were available in 2012, and 2663 sera underwent ANA testing, while 237 sera anti-ENA testing (Isola II study). In 2014 we performed a retrospective analysis of administrative databases using ICD-9-codes from the copayment exemptions register (i.e. the Italian legal mechanism that allows subjects with a chronic condition to waive copayments for visits, medications, and blood tests, these are assigned by specialists usually at the diagnosis of chronic diseases) for cases of CTD (systemic lupus erythematosus 710.0, systemic sclerosis 710.1, Sjögren's syndrome 710.2, undifferentiated connective tissue disease 710.9, mixed connective tissue disease 710.8, dermato/polymyositis 710.3–.4) and cancer updated at December 31, 2013. Death cases were recorded using the administrative database of Lombardy region, as of December 31, 2013.

2.2. Serum ANA and anti-ENA tests

Serum samples were tested in 2012 by indirect immunofluorescence (IIF) for ANA and by ELISA for anti-ENA in ANA-positive sera (AESKU Diagnostics, Wendelsheim, Germany). Anti-ENA included Ro/SSA, La/SSB, Scl70, Sm, Cenp-B, nucleosome, dsDNA, Jo1, PM/Scl, RibP, and histone.

2.3. Statistical analysis

Determining the prevalence of ANA and anti-ENA autoantibodies in the general population was the aim of the primary analysis. As the number of subjects enrolled in the Isola I study differed from the number of samples tested for ANA and anti-ENA due to the deterioration of some samples over time we calculated prevalence rates taking into account the number of subjects enrolled as well as the number of tested samples. Confidence intervals (CI) for ANA positivity were ordinarily calculated at 95% confidence level. ANA positivity was compared between groups using the chi-square test.

The secondary analysis was performed to determine the hazard ratio (HR) of ANA and anti-ENA autoantibodies of developing a CTD over 15 years. We analyzed retrospectively the administrative database to detect disease specific copayment exemptions for CTD and cancer. Death cases were searched in the administrative databases. Subjects with a diagnosis of CTD predating the date of enrollment were excluded from the analysis. For ANA and anti-ENA, HRs were calculated for CTD, as well as for cancer. Finally, the association of autoantibodies with mortality risk was assessed using Cox proportional hazards models, both crude and adjusted by sex. Results are shown as HRs and 95% CIs, calculated using Cox regression test; when appropriate the analyses were adjusted for confounding variables. All statistical analyses were conducted with Stata 13.1 (StataCorp LP, version 13) and p-values <0.05 were considered statistically significant.

Table 1
Serum ANA prevalence in the Isola II cohort, and in previous studies for comparison. All studies used indirect immunofluorescence as method for serum ANA detection.

n	Women (%)	Age range	Any titer	% ≥1:80	% ≥1:160	Target population	Area	Reference
2690	1336 (54)	18–75	518	18.1	6.1	Voting (18–75years) population	Northern Italy	Present study
725	369 (51)	0.2–91	4	–	–	City residents	Brazil	[34]
2181	1409 (5)	20–91	26	–	9.5	City residents	Japan	[4]
918	634 (69)	18–66	12.9	7	1	Healthy individuals and patients with autoimmune rheumatic disease	Brazil	[3]
4754	2469 (52)	≥12	–	1.8	–	National Health and Nutrition Examination Survey US civilian, non-institutionalized population	USA	[7]
510	406 (80)	20–70	–	15.5	–	Blood donors	Israel	[35]