# Detection of antinuclear antibodies in HIV-infected individuals

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## Abstract

**Background:** The causes of autoimmune diseases (ADs) are still not clearly known, but it is believed that diseases are propelled by multiple factors including genetic, hormone and environmental factors. For the environmental factors that trigger ADs, infection is one of the important factor. In the present study, we seek to disclose whether HIV infection instigate autoimmune diseases.

**Objectives:** To investigate the proportion of antinuclear antibodies (ANA)-positive persons in HIV-infected patients.

**Materials and methods:** Blood samples were obtained from 76 HIV-infected patients and 100 healthy donors. Separated plasma from these samples were subjected to the ANA analysis by indirect immunofluorescent assay.

**Results:** A positive-ANA test was detected in 25% and 22% from HIV-infected patients and healthy blood donors, respectively. The ANA-positive result in female healthy donors tends to be higher than that of a male group, whereas the ANA-positive result in males seemed to be higher in HIV-infected patients, assuming that sex hormone may play some notable roles in altering an immune response to favor an autoimmunity. Moreover, high ANA-positive proportion was found in the oldest subject group and in HIV-infected patients the highest ANA-positive proportion was found in the group with the longest infection duration, assuming that age and chronic infection may also involve in ANA production.

**Conclusion:** Though the proportion of HIV-infected patients who had ANA positive results was not statistically significant different when compared to that of healthy blood donors, the tendency of the proportion seems to be higher in HIV-infected group. The highest proportion was found in the patients with the longest infection duration. Therefore, it could be suspected that chronic HIV infection might be associated with ANA production.

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**Keywords:** Autoimmune diseases, HIV infection, Antinuclear antibodies

## Introduction

The causes of autoimmune diseases (ADs) are still not clearly known but it is believed that diseases are motivated by multiple factors including genetic, hormone and environmental factors. For the environmental factors that trigger ADs, infection is one of the important factors.[1] HIV infection has been suggested as a possible factor to link with autoimmune diseases.[2]

In HIV infection, abnormalities were found in majority of lymphocyte due to chronic immune activation. Previous studies reported that the release of protein fragments from dying CD4+ T cells in HIV-infected patient may promote the formation of autoreactive CD8+ T cells.[3,4] Moreover, HIV infection is associated with several B cell defects including polyclonal B cell activation, poor antibody response to specific antigens and increase the production of autoantibodies.[5] There are the wide range frequencies of ADs in HIV patients including systemic lupus erythematosus, anti-phospholipid syndrome, vasculitis, primary biliary cirrhosis, polymyosits, Graves' disease, and idiopathic thrombocytopenic purpura.[6] There are several hypotheses regarding the development of ADs in HIV-infected patient. In illustration, the viral particles that may directly activate ADs, immune complex mediated diseases, dysregulation of the B/T lymphocyte interaction.[7] molecular mimicry.[8,9] or polyclonal B lymphocyte activation that might favor the synthesis of autoantibodies.[10] are among the appealing hypotheses. Nonetheless, the association between the immune dysfunction in HIV infected patient and the development of autoimmune diseases is still unclear. In this study, we determined the level of antinuclear antibodies (ANA) in HIV-infected patients in attempt to inspect whether chronic HIV infection engender the ADs.

## Materials and Methods

### Participants and sample collection

Heparinized blood samples (~10 mL) were obtained from 76 HIV infected patients. The sample size was calculated based on ANA prevalence in healthy individuals from the previous studies and was set at 100 per each group. However, due to some unavoidable circumstances, only 76 HIV patients could be present at the time of blood collection. These patients had been initiated combination antiretroviral therapy (cART) at Maeon Hospital, Chiang Mai, Thailand. They were selected based on the following criteria;

1) Between the ages of 18-60 years old
2) Received cARV drugs (first line drug regimen; TDF, 3TC, EFV or NVP)
3) Infected with HIV for at least one year
4) Viral loads less than 50 copies/mL (undetectable)
5) Contain CD4 number at any levels
6) No history of autoimmune diseases (based on medical records and the inflammation markers or other specific tests for ADs were not performed)
7) No history of opportunistic infections (OIs) and
8) Not pregnant.

In addition, heparinized blood samples (~10 mL) were also collected from 100 HIV-negative, age between 18 and 60 years old, no history of autoimmune disease donors from Maharaj Nakorn Chiang Mai Hospital. All participants had signed an inform consent before enrolling in this study and the ethical protocol was approved by the ethics committee at the Faculty of Medicine, Chiang Mai University.

### Antinuclear antibody (ANA) detection

For the detection of antinuclear antibodies, separated plasma from heparinized-blood samples were subjected to the ANA analysis by indirect immunofluorescent assay using the commercial cultured HEp-20-10/Liver (Monkey) slides (EUROIMMUN, Lübeck, Germany) according to manufacturer's protocol. This assay is designed exclusively for the in vitro determination of human antibodies in serum or plasma. Briefly, the cultured HEp-20-10/Liver (Monkey) slides were incubated with 30 µL diluted patient sample for 30 minutes at room temperature. After incubation, the slide was rinsed with a flush of PBS-Tween and immersed in a cuvette containing PBS-Tween for 5 minutes. Next, the slides were incubated with 25 µL of fluorescein-labelled antihuman immunoglobulin for 30 minutes in dark at room temperature. After incubation, the slide was rinsed with a flush of PBS-Tween and immersed in a cuvette containing PBS-Tween and Evans blue dye (for background staining) for 5 minutes. The embedding

medium was placed onto a cover glass. The slide was put onto a prepared cover glass and was ready to visualize for ANA titer and pattern by fluorescent microscope.

**Statistical analysis**

SPSS for window version 22.0 was used for statistical analysis. ANA positive prevalence in each group was shown in percentage. A nominal scale factor such as sex and ANA result was compared between group by Chi-square test. Descriptive statistic, frequency distribution was used for investigating the mode of age and infection duration groups. Parametric statistics, Independent t-test was performed for matched pairs. The statistical significant was considered at p-value of less than 0.05.

## Results

Samples from 76 HIV-infected and 100 healthy individuals were subjected to ANA determination using immunofluorescent technique. Of 76 HIV-infected patients, 29 (38.2%) were males and 47 (61.8%) were females. Averages of age, number of $CD4^+$ T cells and the duration of HIV infection of the patients were 45.2 years, 529.4 cells/μL and 12.6 years, respectively. Of 100 healthy donors, 67 (67%) were males and 32 (32%) were females. The average age of healthy volunteers was 32.7 years, which was significantly lower than that of HIV-infected participants ($p$<0.01) (Table 1 and Figure 1).

**Table 1** Participant characteristics

| Characteristics | Patients (n=76) | Healthy donors (n=100) |
|---|---|---|
| Age (yrs)* | 45.2±7.1 | 32.7±9.8 |
| Sex Male | 29 (38.2%) | 68 (68%) |
| Female | 47 (61.8%) | 32 (32%) |
| Infected duration (yrs)* | 12.6±5.5 | N/A |
| CD 4 (cells/μL) | 529.4±234.5 | N/A |
| %CD4* | 24.8±6.8 | N/A |
| Viral load (copies/mL) | <50 (undetectable) | N/A |

**the characteristics were presented as mean*±SD

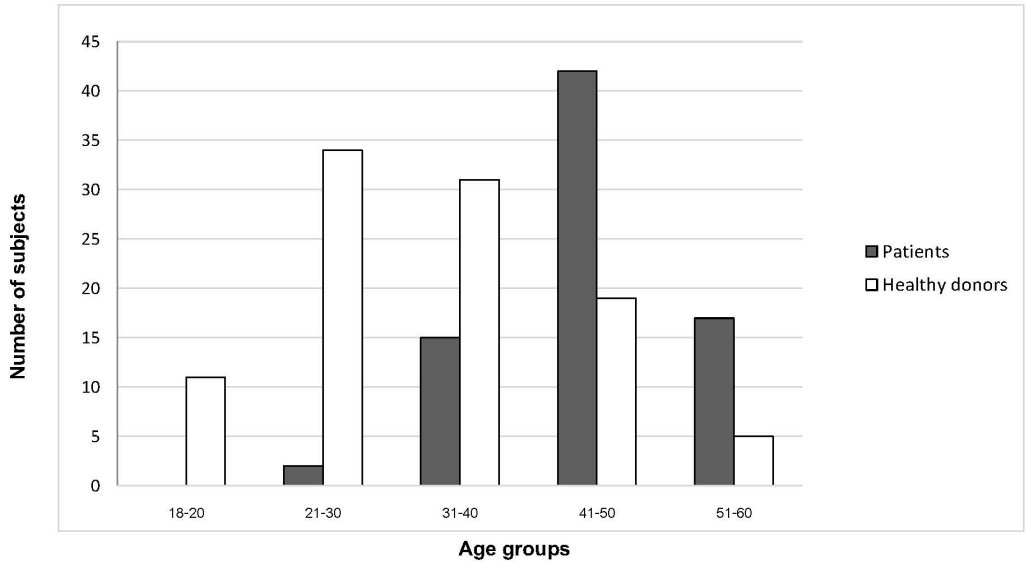


**Figure 1** Distribution of participants according to age group. The average age of healthy volunteers was significantly lower than that of HIV-infected participants ($p$-value <0.01)

For ANA test, out of 76 HIV-infected patients, 19 were positive (25%) which were accounted for 9 (31.0%) males and 10 (21.3%) females. Though the proportion of patients with ANA positive result was found to be higher in males, the difference was not reach statistical significance ($p$=0.340) (Table 2). In addition, there was no statistically significant difference in age between ANA-positive and ANA-negative groups ($p$=0.365). However, the highest proportion of ANA-positive patients was presented in the oldest group (51- to 60-years old) (Figure 2). Moreover, there was no statistically significant difference of $CD4^+$ T cell count, %CD4 and infection duration between ANA-positive and ANA-negative groups ($p$=0.792, 0.457 and 0.121, respectively). However, the highest proportion with ANA positive result was presented in the patients who had been infected with HIV for 21-25 years old (Figure 3). Among the patients with ANA positive results, there were 13 (68.4%) patients with a titer of 80 (2 homogeneous, 7 nucleoplasm granular, 3 mitotic and 1 for nucleoli patterns), 1 (5.3%) with a titer of 160 (nucleoplasm granular pattern), 2 (10.5%) with a titer of 320 (1 homogeneous and 1 nucleoli patterns) and 3 (15.8%) with a titer more than 1,280 (1 homogeneous and 1 nucleoplasm granular patterns) (Table 3, Table 4).

In addition, out of 100 healthy donors, 22 were positive for ANA (22%) of which 12 (17.6%) were males and 10 (31.2%) were females. The proportion of ANA positive donors was higher in females, though the difference did not reach statistical significance ($p$=0.126) (Table 2). There was no statistically significant difference in age between ANA-positive and ANA-negative groups of healthy volunteers ($p$=0.581). However, the highest proportion of donors who had ANA positive result was presented in the age group of 51- to 60-years old (Figure 2). Among donors with ANA positive results, there were 15 with a titer of 80 (5 homogeneous, 8 granular, 1 dotted, 1 mixed (homogeneous and granular patterns), 3 with a titer of 160 (2 granular and 1 nucleoli patterns), 1 with a titer of 640 (granular pattern) and 3 with a titer of >1280 (3 nucleoli pattern) (Table 3, Table 4).

**Table 2** ANA results, according to gender

| ANA (Count and % within Sex) | Patients (n=76) | | | Healthy donors (n=100) | | |
|---|---|---|---|---|---|---|
| | male | female | total | male | female | total |
| Negative | 20 | 37 | 57 | 56 | 22 | 78 |
| | (69.0%) | (78.7%) | (75.0%) | (82.4%) | (69.8%) | (78.0%) |
| Positive | 9 | 10 | 19 | 12 | 12 | 22 |
| | (31.0%) | (21.3%) | (25.0%) | (17.6%) | (17.6%) | (22.0%) |
| Total | 29 | 47 | 76 | 68 | 68 | 100 |
| | (100.0%) | (100.0%) | (100.0%) | (100.0%) | (100.0%) | (100.0%) |

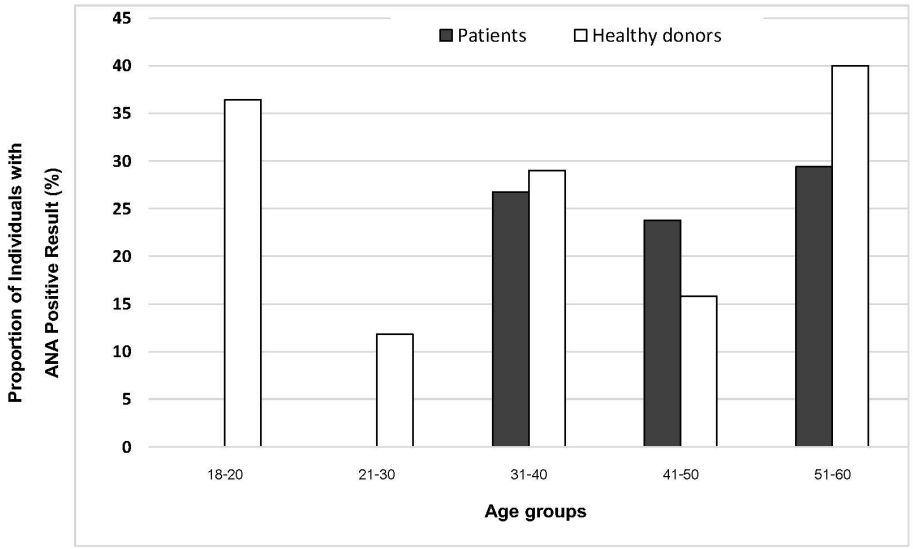


**Figure 2** ANA positive results, according to age groups. the highest proportion of ANA-positive patients was presented in the oldest group (51- to 60-years old) both in patients and healthy donors.

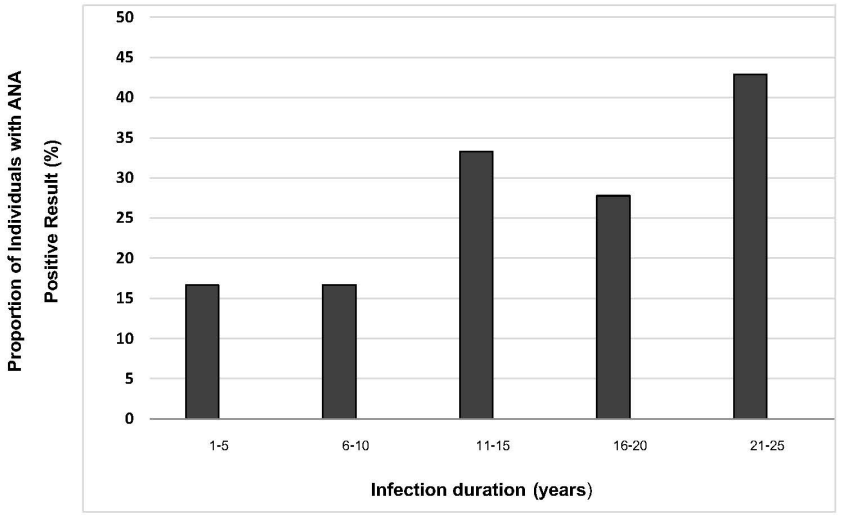


**Figure 3** ANA positive results in HIV patients, according to infection duration. The highest proportion with ANA positive result was presented in the patients who had been infected with HIV for 21-25 years old.

**Table 3** ANA positive results, according to antibody titers

| ANA (Titer) | Patients (n=19) | | | Healthy donors (n=22) | | |
|---|---|---|---|---|---|---|
| | Male | Female | Total | Male | Female | Total |
| 80 | 6 | 7 | 13 | 8 | 7 | 15 |
| 160 | 1 | 0 | 1 | 2 | 1 | 3 |
| 320 | 1 | 1 | 2 | 0 | 0 | 0 |
| 640 | 0 | 0 | 0 | 0 | 1 | 1 |
| >1280 | 1 | 2 | 3 | 2 | 1 | 3 |

**Table 4** ANA positive results, according to antibody titers and patterns

| Pattern | Patients (n=19) | | | | | | Healthy donors (n=22) | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Titers | | | | | | Titers | | | | | |
| | 80 | 160 | 320 | 640 | >1280 | Total | 80 | 160 | 320 | 640 | >1280 | Total |
| Homogeneous | 2 | 0 | 1 | 0 | 2 | 5 | 5 | 0 | 0 | 1 | 0 | 6 |
| Granular | 6 | 1 | 0 | 0 | 1 | 8 | 8 | 2 | 0 | 0 | 0 | 10 |
| Nucleoli | 1 | 0 | 1 | 0 | 0 | 2 | 0 | 1 | 0 | 0 | 3 | 4 |
| Mitotic | 3 | 0 | 0 | 0 | 0 | 3 | 1 | 0 | 0 | 0 | 0 | 1 |
| Mixed pattern | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 |
| Total | 13 | 1 | 2 | 0 | 3 | 19 | 14 | 3 | 0 | 1 | 3 | 22 |

Moreover, when compared between patient and donor groups, the proportion of individuals with ANA positive result was not statistically significant different ($p$=0.641) (Table 5).

**Table 5** Participant characteristics based on ANA result (positive/negative).

| ANA | Patients (n=76) | | | | | | Healthy donors (n=100) | | |
|---|---|---|---|---|---|---|---|---|---|
| | Male | Female | Age (years)* | CD4 (cell/μL)* | %CD4* | Infection Duration (yrs)* | Male | Female | Age (yrs)* |
| Negative | 20 | 37 | 44.8 | 524.9 | 24.4 | 12.1 | 56 | 22 | 32.4 |
| Positive | 9 | 10 | 46.4 | 542.7 | 25.8 | 14.3 | 12 | 10 | 33.9 |

## Discussion

We investigated the presence of antinuclear antibodies (ANA) in HIV-infected individuals compared to healthy donors. Upon detection of ANA in our recruited participants, we found that in HIV-infected individuals the proportion of patients with ANA positive results was 25% while proportion in healthy donors was 22%. Though the difference of the ANA positive proportion between these two groups was not statistically significant, the tendency of this proportion seems to be higher in HIV-infected group. The ANA positive proportion in HIV-positive persons presented here was higher than that were reported by Kulthanan *et al.*[11] and Krishnan and colleague[12] which

were shown at 3 and 6.8%, respectively. It is necessary to point out that the majority of positive individuals in our study were positive at significant titer, though still had low level (1:80) that might not indicate strong support between the association of ANA and ADs. However, this study was crossectional, therefore, following up the ANA level in this group of patients at later times is suggested to expand the evidence. Nevertheless, this level of ANA has also been amounted in HIV infection investigated by others as well as in other infections such as hepatitis B, hepatitis C, HTLV, and syphilis.[13-15] Autoimmune disease-mimicking symptoms like fevers, lymphadenopathy, photosensitivity, rash, renal dysfunction, neurological hematological disorders and polyarthralgia have been frequently seen in patient with HIV infection.[2,16] However, these symptoms did not attribute to ADs. ANA positive can be found in healthy individual. The prevalence found in the previous studies varies from 4% to 22.6%[14, 15, 17-20] with mostly in low titer which is in accordance with the results of our study. These findings marked that the diagnosis criteria for ADs do not solely depend on ANA. In addition, we also found participants with high titer of ANA. There were 6 (22.7%) donors and 7 (36.8%) patients who were positive with ANA titer 160 and above. It might be interesting to follow up these subjects for any sign of autoimmune disease development.

In this study, ANA positive result in female healthy donor tends to be higher than that of male group which accorded with the previous studies.[14,15,17-21] However, in HIV infected patient group, ANA positive result in male seemed to be higher than that of female. From the literature, female displayed a higher risk of ANA positive, assuming that estrogen may play an important role.[15] In addition, there was no statistic significant difference of age between ANA positive and negative groups in our study but the highest ANA positive proportion was found in the oldest group (51-60 years) which accorded the inspection reported in the literature of higher ANA positive result in elderly.[15,17,18,22] In HIV-infected patients there were no statistic significant difference of $CD4^+$ T cells and infection duration between ANA positive and negative groups. For infection duration, the highest ANA positive proportion was found in the longest infection duration group (21- 25 years), suspecting that chronic infection may play the role in ANA production.

## Conclusion

In summary, this study showed that the proportion of ANA positive individuals in HIV-infected group was not significantly different from that of healthy donors. Therefore, ANA may not commonly found in HIV-infected patients who response to ARV drugs with undetectable viral load. However, the tendency of the ANA positive proportion seems to be higher in HIV-infected group and the highest proportion was found in the longest infection duration group (21-25 years), suspecting that chronic infection may play the role in ANA production.

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