

Age associated level of estradiol (E2) and progesterone (4) influence IL-8 response to *Mycobacterium tuberculosis* (Mtb) antigens in women.

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Short report

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Abstract

Tuberculosis (TB) is an intracellular infection controlled the effective recruitment of effectors immune cells at infection site. In aged premenopausal or menopausal women, there is an increased pro-inflammatory cytokines secretion suggesting an underlying link between cytokines response and estrogen (E2) and progesterone (P4) levels.

In this study we compared women aged 40 years old and above (premenopausal) and women aged below 40 years old with and without latent TB infection to determine the link between E2, P4 and cytokines response to *Mycobacterium tuberculosis* (*M. tuberculosis*) stimuli.

E2 and P4 levels were significantly higher in women under 40 years old than in women above 40 years old irrespective their LTB status ($p < [0.0001-0.05]$). In women under 40 years old, E2 and P4 were found to correlate negatively and significantly with IL-8 response to *M. tuberculosis* antigens stimulation ($p < [0.001-0.01]$). Furthermore, *M. tuberculosis* IL-8 specific response was significantly higher in women above 40 years old than women under 40 years old. This study demonstrates that women aging and the linked hormonal changes are associated with hence IL-8 response to *M. tuberculosis* antigen, which may have implications for the age-related susceptibility or resistance to active tuberculosis.

Introduction

Tuberculosis (TB) kills more than 1.3 million lives worldwide each year according to the most recent World Health Organization (WHO) report.¹

Men have higher odds (≈ 2 times) to be infected by *M. tuberculosis* as compared to women²⁻⁵. Studies have demonstrated gender inequality in immune responses and susceptibility to infections.^{6,7} In tuberculosis, it has been shown that social the host behaviors and biological mechanisms may also contribute to gender inequality of infection risks.^{3,8} There is two-way communication between the immune and endocrine systems that facilitates and regulates the host responses during infections. Studies report different effects of estradiol (E2) and progesterone (P4) on the immune responses. It has been reported that E2 suppressed stimulated mononuclear cells cytokines production, whereas progesterone enhanced it.^{9,10} The anti-inflammatory function of E2 and the proinflammatory action of P4 are documented.^{11,12} However, it has been reported that E2 and P4 exhibit both proinflammatory and anti-inflammatory responses in a dose- and context-dependent manner.¹³⁻¹⁶ In this study we investigated the relation between cytokines response to *Mycobacterium tuberculosis* (*M. tuberculosis*) antigens, women age and the baseline levels of both E2 and P4.

Methods

Study design and participants

Succinctly, 42 HIV-negative females were included in the study. Participants with any suggestion of active TB, including fever, weight loss, prolonged fatigue or any other clinical sign of disease, were excluded from the study. Also excluded were participants with a previous history of active TB. All participants were screened with the QuantiFERON -TB (QFT) Gold in-tube test, (QIAGEN - France), following the manufacturer's guidelines. Participants with positive tests were considered to have a latent TB infection (LTB).

Sample handling, luminex cytokines analysis and specific TB antigens cytokines response were done as previous described and published.⁵

Sample handling. Whole blood was collected from all study participants into QFT test tubes including the Nil, and *M. tuberculosis*-antigen (ESAT-6/CFP-10/TB7.7) tubes. The tubes were then incubated overnight in a humidified incubator at 37 °C, with 5% CO₂. The following day, supernatants were harvested into aliquots and stored at - 40 °C.

Luminex analysis. Concentrations of GM-CSF, IFN- γ , IL-1 β , IL-10, IL-12 (p70), IL-2, IL-4, IL-5, IL-6, IL-8, and TNF- α were measured on the Bio-Plex 200 bead array system (Bio-Rad Laboratories, USA) using the Procartaplex-11-plex kits (Life Technologies -Thermo Fisher Scientific -USA).

Cytokines responses. *M. tuberculosis* antigen induced cytokine concentrations were determined by subtracting baseline cytokines concentrations (nil tube) from the concentration of cytokines measured in the TB-antigen tube.

Statistical analysis. The statistical analysis was performed using GraphPad Prism software version 6. The comparisons of the level of Estradiol (E2), progesterone (P4) and cytokines between the groups of women were analyzed using the unpaired non-parametric test (Mann Whitney test). E2 and P4 correlation to cytokines responses were assessed by computing the Pearson correlation coefficient. For all tests the threshold of significance was a p-value below 0.05.

Ethics approval and consent to participate.

The research was done in accordance with Gabonese ethical guidelines and regulations, and approval was obtained from the Gabonese National Laboratory of Public Health ethics committee. The Institute Pasteur Center for Translational Research open desk also approved the study.

Results

Age distribution

In both LTB infected and LTB non-infected women groups, the age of participants were normally distributed (D'Agostino & Pearson omnibus normality test" passed) and the Mann-Whiney test showed no significant differences in age between LTB infected and LTB non-infected women.

Levels of estradiol (E2) and progesterone (P4) in LTB-infected and non-infected women

Both E2 and P4 levels were similar in LTB infected and LTB non-infected women (Figs. 2a and 2b).

Age based levels of estradiol (E2) and progesterone (P4) in all women

Both E2 and P4 levels were significantly higher in women under 40 years old than in women above 40 years old irrespective their LTB status ($p < [0.0001-0.05]$) (Figs. 3a and 3b).

Estradiol (E2), progesterone (P4) correlation to *M. tuberculosis* antigens cytokines response – age-based analysis

In women under 40 years old, E2 and P4 were found to correlate negatively and significantly with IL-8 response to *M. tuberculosis* antigens stimulation ($p < [0.001-0.01]$) (Fig. 4a and 4b). No other correlations were observed between hormones and cytokines response to *M. tuberculosis* antigens stimulation.

Age based cytokines response to *M. tuberculosis* antigens stimuli

Age based *M. tuberculosis* antigens cytokines response in women latently infected with *M. tuberculosis* showed that women *M. tuberculosis* IL-8 specific response was significantly higher in women above 40 years old than women under 40 years old (Fig. 5). No other significant differences in *M. tuberculosis* specific cytokines responses were observed (complementary figures). Also, no significant differences were observed between ages groups in women non-infected by *M. tuberculosis*.

Discussion

The present study showed women aged above 40 years old had significant lower level of E2 and P4, an observation that maybe age related their premenopausal ages. In women under 40 years old, we observed a negative correlation between IL-8 response to *M. tuberculosis* antigens stimulation and both E2 and P4. Furthermore, the higher IL-8 responses to *M. tuberculosis* antigens stimulation in women with lower levels of E2 and P4 (women above 40 years old) suggest that the reduced secretion of ovarian sex steroid hormones at a premenopausal age increases or hence proinflammatory response.¹⁷ Menopause associated high levels of proinflammatory cytokines (including IL-8) have been reported in the literature.¹⁸ Relevant to tuberculosis, published data suggest IL-8 not only increases the ability of neutrophils and macrophages to phagocyte and kill *M. tuberculosis*, but also recruits T-lymphocytes.¹⁹⁻²¹

The high IL-8 responses to *M. tuberculosis* antigens stimulation in women above 40 years with lower levels of E2 and P4 may explained why pre- and post-menopausal women are less susceptible to tuberculosis than women in their early reproductive years.^{22,23} The negative correlation observed in women under 40 years old would be another argument tower the susceptibility of reproductive females.²³ However, the hence proinflammatory response of women aged above 40 years old may make then more prone to inflammatory disorders or chronic diseases.²⁴⁻²⁶

The absence of data on participants cycle menstrual cycles would be the principal limit of this study. However, this does not affect the intrinsic link between hormone levels and cytokines responses. Also, the study can hardly establish if a spurious relationship or independent association drives the observed correlations between hormones and cytokines secretion. The complexity of the endocrine and immune system crosstalk put us in a relatively grey area when it comes to E2 and P4 influence on the immune system and its response to infections or immune disorders.

Conclusion

This study demonstrates that aging and associated hormonal changes are associated with significantly hence IL-8 response to *M. tuberculosis* antigen, which may have implications for the age-related susceptibility or resistance to active tuberculosis.

Abbreviations

Estradiol

E2

GM-CSF

Granulocyte-macrophage colony-stimulating factor

IFN- γ

interferon gamma

IL-1 β

interleukin 1 beta

IL-10

interleukin 10

IL-12

interleukin 12

IL-2

interleukin 2

IL-4

interleukin 4

IL-5

interleukin 5

IL-6

interleukin 6
IL-8
interleukin 8
Latent tuberculosis
LTB
Progesterone
P4
QuantiFERON -TB
QFT
TNF- α
tumor necrosis factor-alpha
TB
tuberculosis

Declarations

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Author contributions. J.F.D.S is the principal investigators who conceived, designed the study, did experiments, analyzed the data and wrote the paper. BG and HT are Co-investigators involved in the materialization of the study, organized and participated in writing the manuscript. V.L.M.M, M.L and P.N.E participated in study design samples processing and experiments. M.L, A.C.M.S, AKAE, OCAB, P.H.D.T, A.M.N, D.U.E, O.M.N and G.S.P helped in the recruitment of participants, acquisition the samples, experiments and the study organization.

Competing interests. The authors declare no competing financial or non-financial interests in relation to the present work.

Consent for publication. There is no materiel needing a consent for publication

Availability of data and materials. All materials described in the manuscript, will be freely available on demand to any scientist wishing to use them.

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Figures

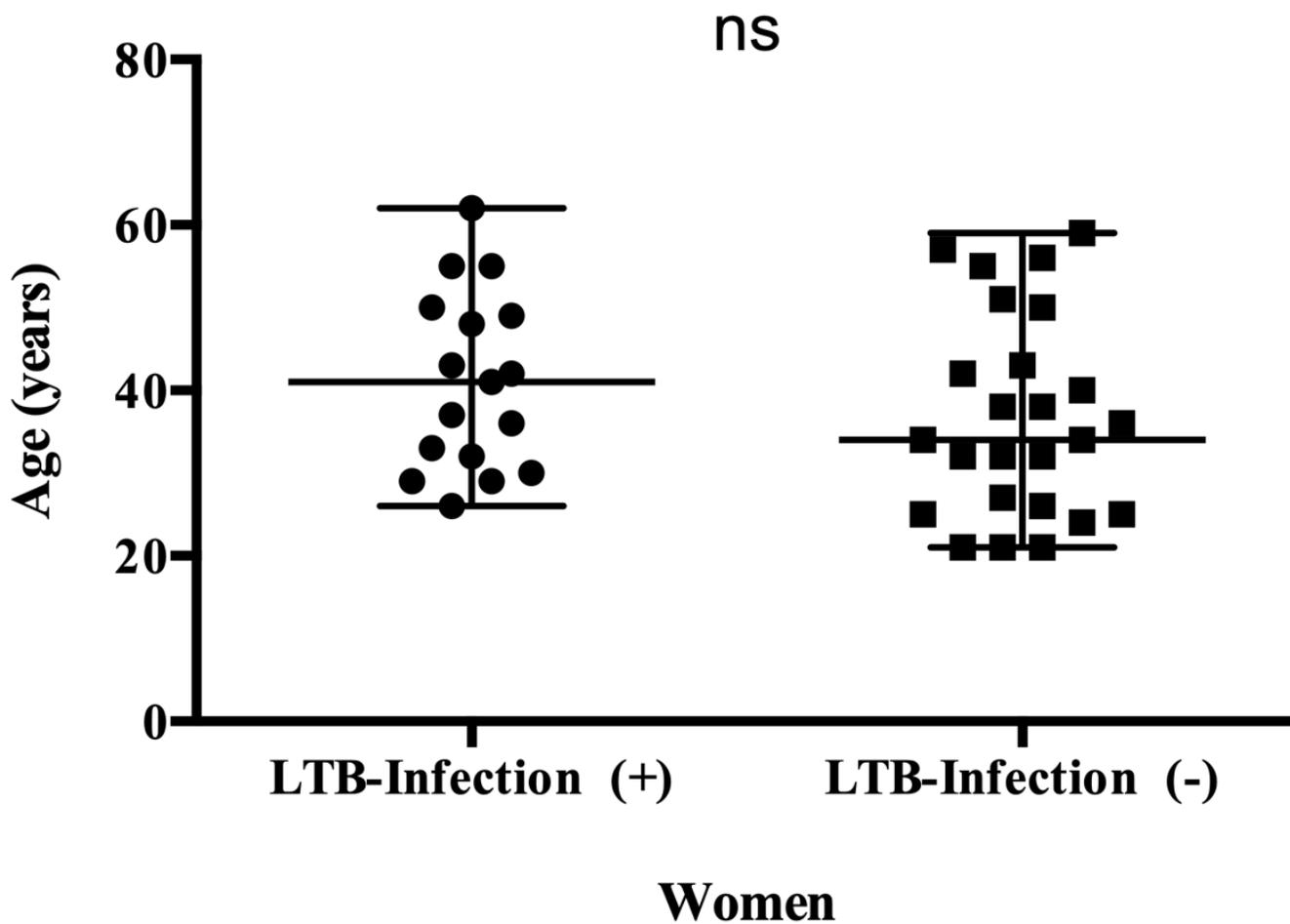
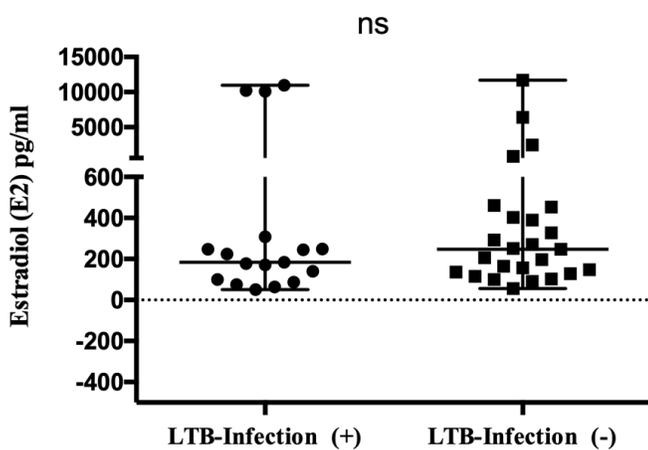


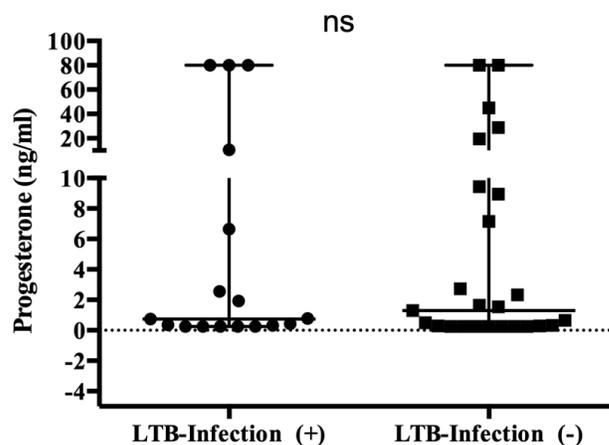
Figure 1

Age distribution of latent tuberculosis (LTB) infected and non-infected women. In the figures, bars indicate a median with ranges and ns means no significant differences between the groups.

2a



2b



Women

Figure 2

Estradiol (2a) and progesterone (2b) concentrations in latent tuberculosis (LTB) infected and non-infected women. The bars in the graphs indicate median with range and ns means no significant differences between the groups.

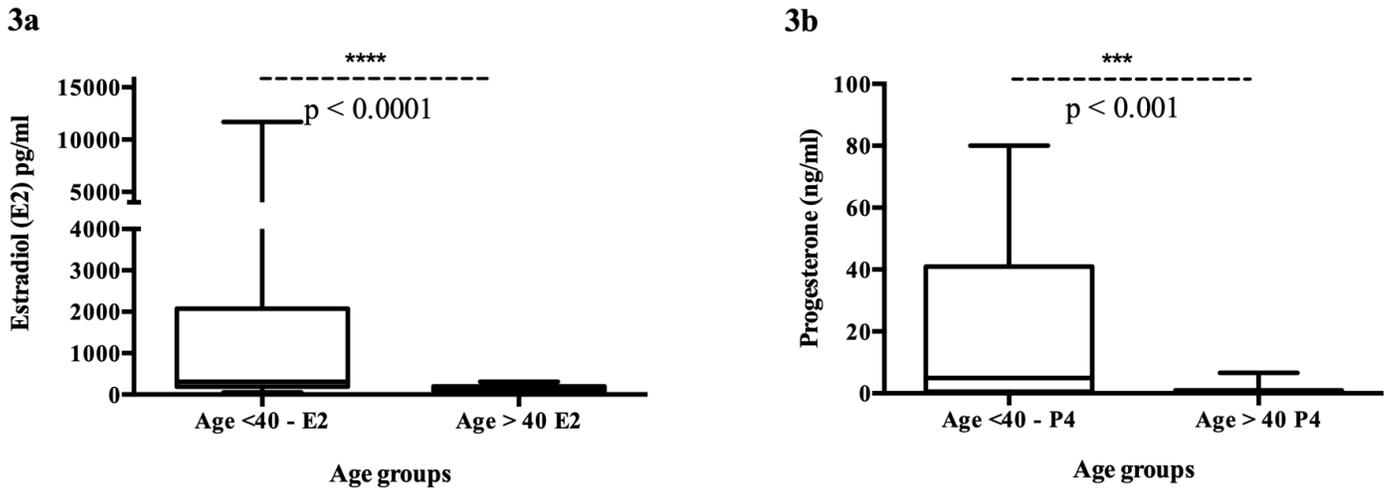


Figure 3

Age based concentration estradiol (3a) and progesterone (3b). Women aged 40 and above had lower estradiol (E2) and progesterone (P4) than younger women. The difference between women aged below 40 years old and women aged 40 and above was analyzed using the Mann–Whitney U-test. The differences were considered significant for a p-value < 0.05 . In the figures, bars indicate medians and ranges.

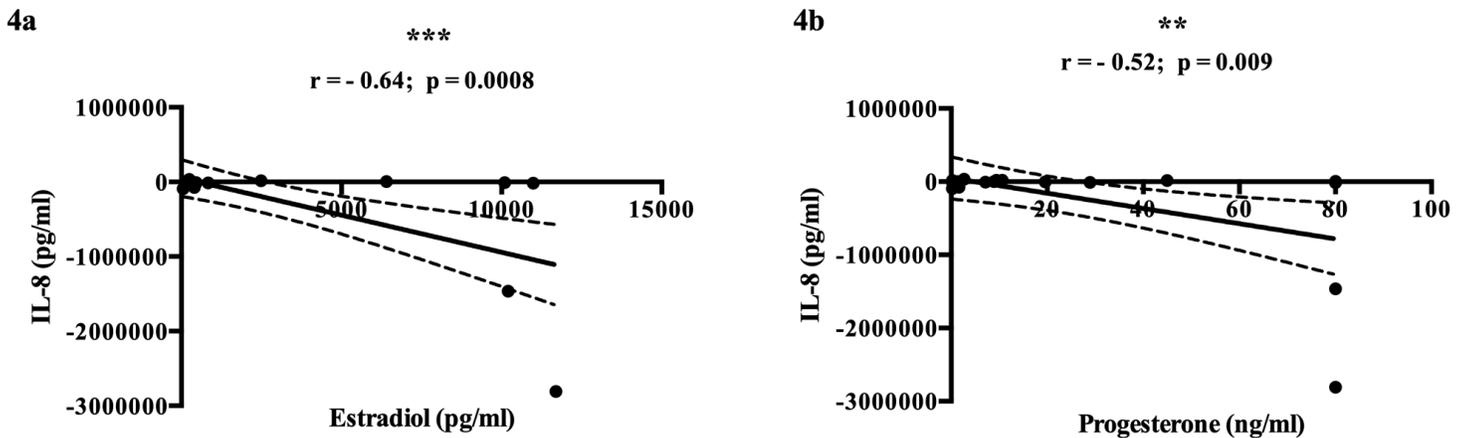


Figure 4

Estradiol (4a) and progesterone (4b) correlation to IL-8. Both estradiol (E2) and progesterone (P4) correlated negatively with IL-8 response to M. tuberculosis antigen stimuli. The correlation was considered significant for a p-value < 0.05 .

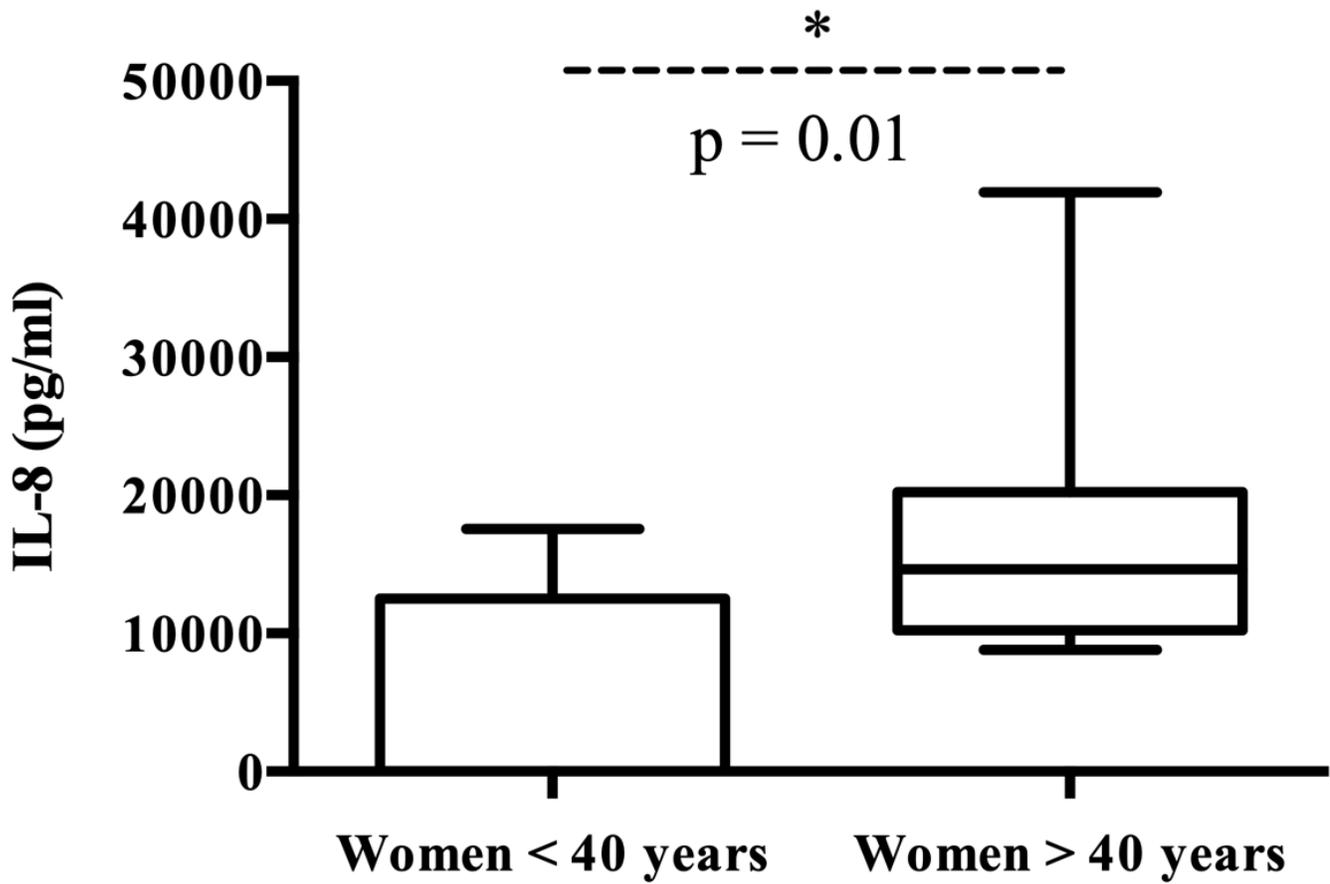


Figure 5

Age based IL-8 response to *M. tuberculosis* antigen stimuli. Women aged 40 and above had higher IL-8 response to *M. tuberculosis* antigen stimuli as compared younger women. The differences were considered significant for a p-value <0.05.

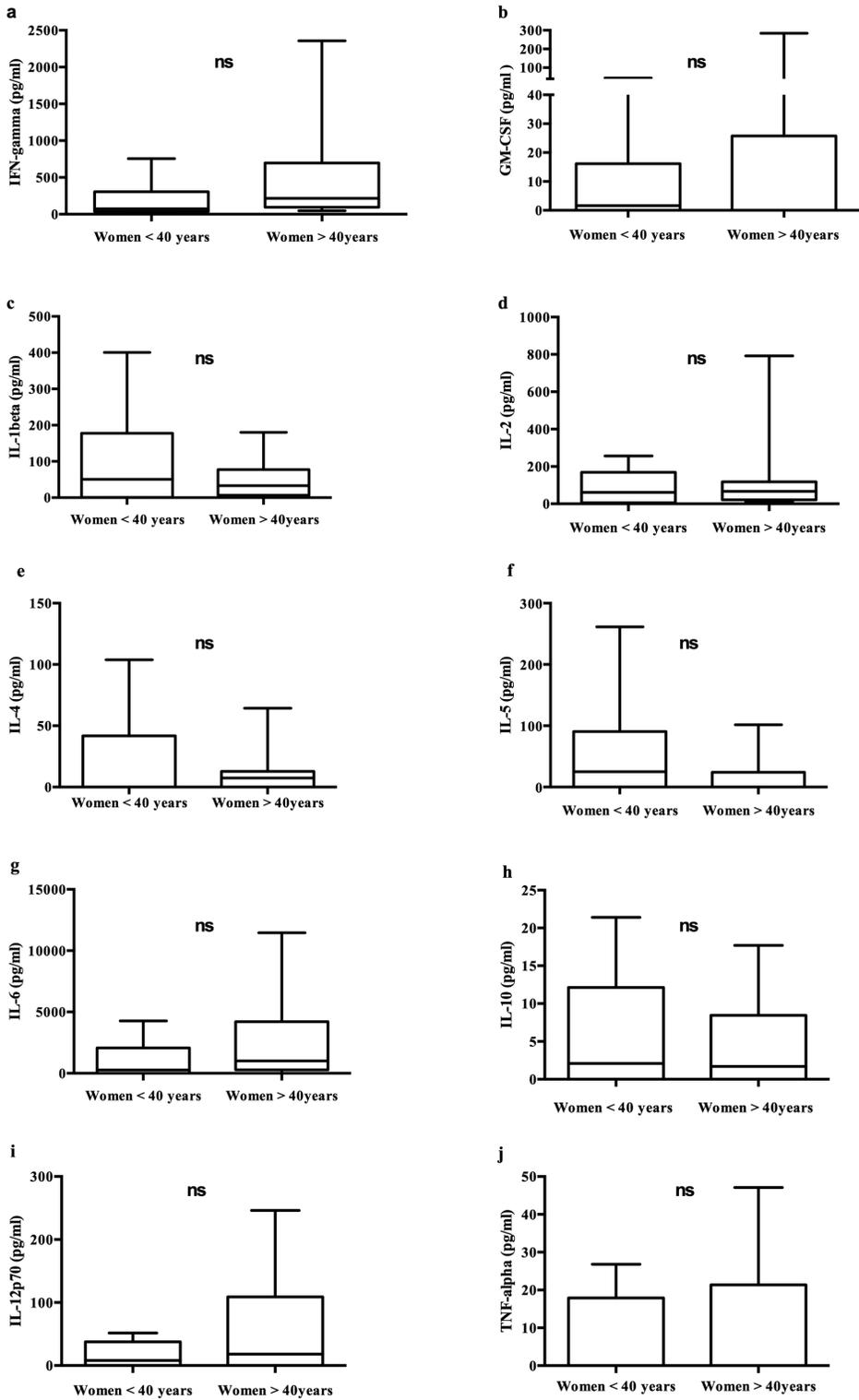


Figure 6

Age based cytokines (GM-CSF, IFN- γ , IL-1 β , IL-2, IL-4, IL-5, IL-6, IL-10, IL-12 (p70), and TNF- α) response to *M. tuberculosis* antigen stimuli. The differences were considered significant for a p-value < 0.05; ns means no significant differences between the groups.