

INF- γ and IL-2 Profile as Therapeutic Markers in Tuberculosis Patients at the Jamot Hospital of Yaounde-Cameroon

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Abstract

Background: Tuberculosis (TB) is one of the top lethal infectious diseases worldwide. In recent years, interferon- γ (INF- γ) release assays (IGRAs) have been established as routine tests for diagnosing TB infection. However, produced INF- γ assessment cannot permit to distinguish active ATB from latent TB infection (LTBI), especially in TB epidemic areas. In addition to IFN- γ , interleukin-2 (IL-2), secreted by activated T cells, is involved in immune response against *Mycobacterium tuberculosis*. This could be involved in the follow up of treatment response. The aim of our study was to determine INF- γ and IL2 cytokines profiles of patients under antituberculosis treatment. **Materials and Methods:** A six months' cross-sectional study was conducted at the Jamot Hospital of Yaoundé, from May to August 2021. Sociodemographic and clinical data as well as 5 mL of blood were collected from each participant. INF- γ and IL-2 were determined using indirect Enzyme linked Immuno-Sorbent Assay (ELISA) according to the manufacturer's recommendations and spectrum exam in combination with radiography and GeneXpert were used as standard. *P-values* < 0.05 were interpreted as statistically significant. All statistical analysis was performed using SPSS version 22.0 **Results:** The results showed that men were more infected 14/61 (31.8%) with a high presence in active and resistant TB groups. The mean age was 41.3 ± 13.1 years with a 95% CI = [38.2 - 44.7], the age group with the highest infection rate

was ranged between 31 and 40 years. The IL-2 and INF- γ means were respectively 327.6 ± 160.6 pg/mL and 26.6 ± 13.0 pg/mL in ATB patients, 251.1 ± 30.9 pg/mL and 21.4 ± 9.2 pg/mL in patients with resistant tuberculosis, while it was 149.3 ± 93.3 pg/mL and 17.9 ± 9.4 pg/mL in cured patients, 15.1 ± 8.4 pg/mL and 5.3 ± 2.6 pg/mL in participants presumed healthy ($p < 0.0001$). Significant differences in INF- γ and IL-2 rates were observed between the different groups. **Conclusion:** Monitoring the serum levels of INF- γ and IL-2 would be useful for the follow-up of anti-tuberculosis patients, particularly in the both cytokines association case.

Keywords

Tuberculosis, Antituberculosis Treatment, INF- γ , IL-2, ELISA

1. Introduction

Considered as a community diseases, tuberculosis is among the most deadly infections according to WHO [1]. It is caused by bacteria of the *Mycobacterium tuberculosis* Complex (MTBC). Tuberculosis, however, *Mycobacterium tuberculosis* (MTB) is the species mainly incriminated in humans [2]. In its 2019 report, the WHO states that approximately 10 million people were TB disease worldwide, of which 5.6 million were men, 3.2 million were women and 1.2 million children with an estimated 1.4 million deaths [3]. Africa is one of the heavily affected areas of the world, with approximately 417,000 recorded deaths per year. Cameroon is paying a heavy price for this infection, as part of the deadliest diseases behind HIV and malaria with 184 new cases per 100,000 populations of which 29% of those infected were HIV positive. In one of the WHO reports that approximately 47,000 new cases are expected in Cameroon each year up to 2017 [4]. In Cameroon, diagnosis and follow-up of tuberculosis is mainly based on radiography, which enables the localization of anatomical areas affected by the infection, microscopy (staining of Ziehl Neelsen), with a sensitivity threshold of 104 to 105 bacilli/ml of sputum allows detection of Bacilli Acido-Alcoholo-Resistant (BAARs) GeneXpert, sensitivity threshold 100 bacilli/ml of sputum is specific to MTB and based on the research of its DNA and the detection of a mono-resistance to Rifampicin, TB-Lamp which is used for negative microscopy in patients with symptoms of tuberculosis, but it is not test for resistance to TB drugs [5]. The tuberculin test (IDR) is only used when desired Check the efficacy of the vaccine and in case of suspicion of tuberculosis in children from 0 to 10 years with an infected parent [4]. But it has been found that some patients declared cured based on the routine exams results developed recurrences [6]. This could be explained by the fact that these examinations have low sensitivity level during the intensive stage of treatment which drastically reduces the burden bacillary in sputum sample used standard for microbiological examinations [7]. However, today, immunological tests are of great interest to the diagnosis and follow-up of

patients suffering from tuberculosis. The most developed are those based on quantification of interferon γ product by T lymphocytes (LT) effectors, and would be more specific and sensitive than IDR tests [8]. But on contrary to the usual tests, these tests of dosage of interferon gamma do not allow a differential diagnosis between tuberculosis latent and active tuberculosis [9]. However, other studies have shown that IL-2 is a biomarker regression of inflammatory reaction following TB therapy therefore could be used for TB patients follow-up [10]. This study aimed to determine the profiles of IFN- γ and IL2 of patients under antituberculosis treatment.

2. Materials and Methods

Study design and Setting: This cross-sectional study was conducted in the Pneumology departments of the Jamot Hospital of Yaoundé for 6 months from May 2021 to August 2021.

Participants: Our cohort included 21 years old both sex persons. Inclusion criteria permit to divide our population into four groups based on the different treatment stages and results of usual examinations (spitting examination for BARRs, radiography Lung and GeneXpert). Group 1: Patients reported cured of tuberculosis (TB cured) which had healed lungs in radiology, a negative Zielh-Neelsen stain and a negative GeneXpert. Group 2: Active TB patients who have not yet received TB treatment (active TB) that had severely damaged lungs and radiology, positive sputum staining and GeneXpert positive without resistance to the Rifampicin. Group 3: Patients with drug resistant TB in the third month of TB treatment (resistant TB) with X-ray still showing lesions pulmonary, a positive sputum exam and a GeneXpert positive with resistance to Rifampicin. Group 4: Sick guards and nursing staff with no clinical signs of tuberculosis (presumed healthy). Non-inclusion criteria were any patient with extra-pulmonary tuberculosis and/or viral co-infection.

Sociodemographic and clinical data collection: Sociodemographic and clinical data were obtained through interview with participants, supplemented by medical records. For each consenting participant, parameters required were: age, sex, marital status, educational attainment, profession or occupation, smoking, diabetes, clinical symptoms and usual examinations results.

Blood sample Collection: At Jamot Hospital, a peripheral blood was collected in a dry tube, from each participant, via venipuncture. After transfer at the Center for the Study and Control of Diseases Communicable (CSSCD) of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé 1, the serum was obtained after centrifugation of the blood sample. The sera were frozen and stored at -30°C until analysis.

Biological analysis of samples: Cytokines quantification was performed using a sandwich ELISA (Enzyme Linked-Immunosorbent Essay), with *Elabsciences* kits for human INF- γ and human IL-2, according to manufacturer instructions. The principle is based on the specific attachment of an antigen (Ag) to its Anti-

body (Ab). The antigen of interest is trapped between a capture antibody and a detection Ab. An enzymatic complex and a fluorochrome are attached to the detection of Antibodies, enabling the formation of the immune complex to be highlighted. Label the 8-well strips according to your requirements. Transfer 100 μL of each calibration standard and test sample to the corresponding wells. Apply coverslips and incubate for 2.5 hours at ambient temperature, stirring gently. Throw away the solution and wash it 4 times with 1X Lavage Solution. Wash by filling each well with wash buffer (300 μL) using a pipette or automatic washer. The complete removal of liquid at each stage is essential for good results. After the last wash, remove any residual wash buffer by aspiration or decantation. Turn the plate over and blot on clean absorbent paper. Add 100 μL of prepared 1X biotinylated antibody to each well. Incubate for 1 hour at room temperature, shaking gently. Throw away the solution and repeat the wash. Add 100 μL of prepared streptavidin solution to each well. Incubate for 45 minutes at room temperature, shaking gently. Throw away the solution and repeat washing. Add 100 μL of one-step TMB substrate reagent (element H) to each well. Incubate for 30 minutes at room temperature in the dark, shaking gently. Add 50 μL stop solution (element I) to each well. Read optical densities at 450 nm immediately. For that, we were used the Human Reader HS automated system.

Ethical consideration: To respect the ethics of medical research, the study was approved and authorized by the Ethics Committee of the Jamot Hospital of Yaoundé (No. 00752/L/MINSANTE/SG/DHTY). The CSSCD allowed the laboratories analysis. All participants read the information sheet and provided written informed consent before being included. Samples were anonymously screened, and no information made it possible to identify the patient.

Statistical Analysis: For each participant in this study, quantitative and qualitative were collected by interview and blood tests. The collected data were recorded and processed using the Excel Version 2016 (Microsoft Corp., USA). Analyses were done using the biostatistical software Statistical Package for Social Sciences (SPSS) Version 25.0. The Chi-square test allowed us to compare the proportions between the different groups and the non-parametric Kruskal Wallis test allowed us to compare the means \pm standard deviations between the different groups. A p-value of <0.05 was considered statistically significant for a 95% confidence interval.

3. Results

We obtained 61 participants from whom 16 patients were reported to be cured of tuberculosis (Group 1), 19 patients with active TB who have not yet taken treatment anti-tuberculosis (Group 2), 13 patients with Rifampicin resistance TB (Group 3) and 13 presumed healthy who gravitated around the patients (nursing staff, sick care) (Group 4).

According to usual examinations results picked in medical files, all Group 2 participants had an X-ray positive, but some had a BAARs search and a GeneX-

pert negatives (47.37%, n = 9 respectively). Group 3 participants were all positives in usual reviews and those of Group 1 were all negatives (Table 1).

Sociodemographic and clinical characteristics of participants: With a sex ratio of 2.5 (more men than women), the average age of our study population was 41.3 ± 13.1 years.

In each group, an average age was obtained: Group 2 had the most elevated age average (47.2 ± 17.6 years) and group 1 the youngest (34.5 ± 6.4 years) (Table 2).

The most observed clinical signs on our participants were chest pain (n = 34), productive cough (n = 33), night sweating (n = 28) and fever (n = 27). The more specific evocative signs (productive cough, Weight loss and chest pain) were mainly presented by the Group 2 and Group 3 participants. Presumed healthy participants (Group 4) presented chest pain and productive cough (n = 12/13) (Table 3).

Participants INF- γ and IL-2 profiles: In general, our cytokines of interest were present in all participants, but at different rates for each considered group.

With Group 2, we can have noticed that active TB showed an average of 327.6 pg/mL for IL-2 and of 26.6 pg/mL for INF- γ . The values of Group 1 were normal with a mean of 15.1 pg/mL for IL-2 and 5.38 pg/mL for INF- γ . The mean values of the presumed healthy persons of our study (Group 4) are just at the limit of the normality (Table 4).

Comparison of cytokinesis profile with usual examinations results: According to usual examinations in tuberculosis management, positive correlation was found between IL-2 values and their positive results and a negative correlation with negative usual examination results ($p < 0.0001$).

Positive correlation of INF- γ was mainly observed with positive usual examinations results ($p < 0.0001$).

4. Discussion

Our objective was to determine the profile of IFN- γ and IL-2 of patients under anti-tuberculosis treatment.

The analysis of socio-demographic data showed that more participants were young men and the age group with more infected rate was ranged between 31 to 40 years. These results joined other that showed that in endemic areas as sub-Saharan Africa, young men are most affected, in average age of 35 in Central African Republic [11], and 41.87 years \pm 12 in Cameroon [12]. This could be due to their increased exposure to risk factors such as HIV infection, tobacco abuse, drug use, alcohol consumption [2]. In all patients of our study, clinical signs have been identified but not in the same frequency. Active (Group 2) and resistant TB (Group 3) presented the high prevalence of these signs, more in chest pain, productive cough and lost weight. The author Ndishimye had repertoires cough (85%), alteration of the general condition (80%) and weight loss (75%) with an average loss of 9 kg per patient as major signs [13]. Some of our presumed healthy participants presented chest pain and productive cough (n =

Table 1. Results to usual examinations.

| | | Group 1 (cured TB) | Group 2 (active TB) | Group 3 (resistant TB) | Total (n) |
|--------------------------------|----------------|-----------------------|------------------------|---------------------------|--------------|
| Spitting examination for BARRs | Positive n (%) | // | 10 (52.63) | 13(100) | 23 |
| | Negative n (%) | 16 (100) | 9 (47.37) | // | 25 |
| Chest radiography | Positive n (%) | // | 19 (100) | 13 (100) | 32 |
| | Negative n (%) | 16 (100) | // | // | 16 |
| PCR GeneXpert | Positive n (%) | // | 10 (52.63) | 13 (100) | 23 |
| | Negative n (%) | 16 (100) | 9 (47.37) | // | 25 |

Table 2. Distribution of participants by age.

| Participant classification | Age mean \pm SD | 95% confidence interval (CI _{95%}) |
|----------------------------|-------------------|--|
| Group 1 (cured TB) | 34.5 \pm 6.5 | [31.2 - 38.1] |
| Group 2 (active TB) | 47.2 \pm 17.6 | [39.7 - 55.2] |
| Group 3 (resistant TB) | 35.2 \pm 5.7 | [32.6 - 38.5] |
| Group 4 (presumed healthy) | 44.7 \pm 11.0 | [39.5 - 50.1] |

Table 3. Evocative Signs by patient groups.

| | Group 1 (cured TB) n (%) | Group 2 (active TB) n (%) | Group 3 (resistant TB) n (%) | Group 4 (presumed healthy) n (%) | Total (n) |
|------------------------------------|--------------------------------|---------------------------------|------------------------------------|--|--------------|
| Tiredness | 2 (8.3) | 9 (37.5) | 13(54.2) | | 24 |
| Productive cough | 3 (9.1) | 13 (39.4) | 13 (39.4) | 4 (12.1) | 33 |
| Shortness of breath | 3 (13.0) | 15 (65.2) | 5 (21.7) | // | 23 |
| Weight loss | 2 (7.1) | 13 (46.4) | 13 (46.4) | // | 28 |
| Loss of appetite | 2 (10.5) | 9 (47.4) | 8 (42.1) | // | 19 |
| Chills | 2 (8.3) | 9 (37.5) | 13 (54.2) | // | 24 |
| Deterioration of general condition | // | 13 (72,2) | 5 (27.8) | // | 18 |
| Night sweating | 2 (7.1) | 13 (46.4) | 13 (46.4) | // | 28 |
| Fever | 1 (3.7) | 13 (48.1) | 13 (48.1) | // | 27 |
| Chest pains | 3 (8.8) | 10 (29.4) | 13 (38.2) | 8 (23.5) | 34 |

Table 4. Cytokines levels by group of participants.

| Cytokines | | Group 1 (cured TB) | Group 2 (active TB) | Group 3 (resistant TB) | Group 4 (presumed healthy) |
|------------------------|----------------------|-----------------------|------------------------|---------------------------|-------------------------------|
| [IL-2] pg/mL | Mean (\pm SD) | 15.1 (\pm 8.4) | 327.6 (\pm 160.6) | 251.1 (\pm 30.9) | 149.3 (\pm 93.3) |
| | [IC _{95%}] | [11.2 - 20.9] | [254.3 - 408.3] | [233.7 - 267.91] | [109.6 - 196.3] |
| [INF- γ] pg/mL | Mean (SD) | 5.38 (\pm 2.6) | 26.6 (\pm 13.0) | 21.4 (\pm 9.2) | 17.9 (\pm 9.4) |
| | [IC _{95%}] | [3.9 - 6.5] | [20.3 - 31.8] | [17.4 - 26.4] | [12.7 - 23.0] |

Normal values: IL-2 \leq 150 pg/mL; INF- γ \leq 15pg/mL.

12/13). This showed that these signs are not specific to tuberculosis, but also can illustrate the presence of tuberculosis infection in people gravitating around the patients (nursing staff, sick care).

Our results showed that the distribution of mean serum IFN- γ levels were still high in active (Group 2) and resistant TB patients (Group 3), although these values were not far from normal. With these results we can say that these cytokine levels decreased with treatment. Indeed, the goal of TB therapy is to destroy circulating mycobacteria and granulomas, that causing a regression of the immune action, the reduction of this cytokine production [14] [15]. Our IFN- γ levels results agree with those of IL-2 whose values were found high in the same groups, although some studies found that IL-2 levels can differentiate active TB from cured TB even though with inconsistent results [16]. It has been shown that the release of IL-2, after stimulation by antigens TB-specific, was significantly higher in infected patients with TB as healthy controls [17]. IL-2 is an immunoregulatory lymphokine pivot synthesized by cells T in response to antigenic or mitogenic activation. IL-2 also can have an impact on the course of infections mycobacteria, alone or in synergy with other cytokines [18]. Indeed, it is reported that the control of MTB infections is dominated by T cells of the central memory with potential co-secretion of IL-2 and possibly IFN- γ . It is suggested that IL-2 adds discriminatory power to IFN- γ , although other studies could not show the association [16].

Our study showed significant differences ($p < 0.0001$) in positive correlations between levels of cytokines of interest and gold standard examinations positive results. IL-2 was found positive with all positive results and negative with negative examinations results. This can confirm the usage of IL-2 as a potential prognosis marker for TB patient's therapeutic follow-up. Indeed, gold standards such as sputum examination and radiography cannot accurately distinguish active TB to resistant TB, we can say that the usual diagnostic methods to differentiate between active TB patients and TB patients cured are not optimal [19] [20]. Our study agrees with others in the usage of IL-2 and IFN- γ from peripheral blood as an immunodiagnostic tool to accurately distinguish between active TB, resistant TB, and cured TB [21].

Although our study will help to focus research on tuberculosis alternative diagnostic involving immunological molecules, our study had some limitations as it involved a relatively small cohort cross-sectional design. Longitudinal-type cohort studies would be required to validate the diagnostic performance of this study selected cytokines. The strength of our study is based on the fact that it can be considered as the first to propose an immunological diagnosis of the tuberculosis in Cameroon.

5. Conclusion

The radiography is a major contributor to the diagnosis of pulmonary tuberculosis, especially in developing countries, and shows invasive images with or without cavity lesions to shorten diagnostic time. Detection of BAARs is comple-

mentary and GeneXpert PCR is not always required. These examinations have low sensitivity level during the intensive stage of treatment which drastically reduces the burden bacillary in sputum sample used standard for microbiological examinations. Our results have shown that in addition to the usual tests, IFN- γ and IL-12 quantification can be used to follow up therapy response. The gradual decline of these cytokines can be used to monitor the effectiveness of treatment.

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Authors' Contributions

Njiki Bikoï Jacky, Moni Ndedi Esther Del-Florence and Riwom Essama Honorine were responsible for conception and design of the study as well as project administration. Membangbi Alexandra Emmanuelle, Mindimi Nkodo Joseph Marie were responsible for the data collection. Membangbi Alexandra Emmanuelle, Mbaga Donatien Serge, NjikiBikoï Jacky, Martha Mesembe, Ikomey Mondinde George, were involved in laboratory analysis and interpretation of results. Mbaga Donatien Serge was responsible for statistical analysis. Membangbi Alexandra Emmanuelle wrote the original draft. Mbaga Donatien Serge, Mbongue Mikangue Chris André, Makue Nguiffo Elsa, Koko-Ta Ladiiff Sharonne, Ngoutane Aicha, Elang Arnaud Franck, Touangnou-Chamda Sabine Aimée, Eric Walter Perfura-Yone, reviewed the first draft. All authors approved the final version of the paper for submission. Riwom Essama Sara Honorine oversees all steps.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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