Journal of Biomedical Research & Studies
Volume 5 | Issue 1

Research Article Open Access

Investigation of Long Intergenic Non-Coding RNA-p21 and Long Non-Coding RNA NEAT-1 and their Target Cytokines Interleukin-6 and Interleukin-8 messenger RNA respectively as Potential Biomarkers for the Diagnosis of HIV-Tuberculosis Co-infection

#Sarah Picky Sisley Ngate Nguengo[1], #Michelle Hako Toumeni[1], Fabrice Junior Tatang[1], Ulrich Stephane Fotso Simo[1], Jubi-late Afuoti Ngum[1], Cybelle Fodieu Mezajou[1], Charleine Kameni[1], Natacha Njike Ngongang[1], Alphonse God-will Tandja[1], Vanelle Fouodoung[1], Melissa Nitcheu[1], Charlotte Sabine Milong Melong[1], Mazarin Akami[1] and Annie Rosalie Ngane Ngono[1] and Ousman Tamgue[1*]

Department of Biochemistry, Faculty of Sciences, University of Douala, Douala, Cameroon
[#]Equal contribution

[*]**Corresponding Author**: Ousman Tamgue, Department of Biochemistry, Faculty of Sciences, University of Douala, Douala, Cameroon, E-mail: oustheo2014@gmail.com, Tel: +237 694791567

Citation: Sarah Picky Sisley Ngate Nguengo, Michelle Hako Toumeni, Fabrice Junior Tatang, Ulrich Stephane Fotso Simo, Jubi-late Afuoti Ngum, et al. (2025) Investigation of Long Intergenic Non-Coding RNA-p21 and Long Non-Coding RNA NEAT-1 and their Target Cytokines Interleukin-6 and Interleukin-8 messenger RNA respectively as Potential Biomarkers for the Diagnosis of HIV-Tuberculosis Co-infection. J Biomed Res Stud 5(1): 101

Received Date: November 06, 2025 **Accepted Date:** November 24, 2025 **Published Date:** December 10, 2025

Abstract

Background: HIV which targets the immune system leads to immunosuppression and thus the occurrence of several opportunistic diseases with tuberculosis being the first and deadliest one. This leads to a high rate of co-infection and deaths worldwide. Despite the different diagnostic methods recommended by the WHO for the diagnosis of this co-infection state, possible errors in the individual diagnostic and lack of unique diagnostic methods of the co-infection state remain the principal causes of the high death rate worldwide. Facing this challenge, the research of novel specific biomarkers for HIV Tuberculosis co-infection for this diagnosis remains urgent. The molecular basis for pathogen synergy that can be used to diagnose HIV-TB coinfection, however, is not clearly defined. Researchers have directed this challenge on a Host-dependent area rather than a pathogen dependent route as usually done. This was orientated on RNAs constituent of the body also called Transcriptome. This study aimed at investigating Long Intergenic Non-Coding RNA-p21 and Long Non-Coding RNA NEAT-1 and their target cytokines Interleukin-6 and Interleukin-8 mRNA respectively as potential biomarkers for the diagnosis of HIV-Tuberculosis coinfection. The study was done over a period of 08 months subdivided into sample collection and analysis respectively at LAQUINTINIE Hospital and LANAVET DOUALA respectively.

Quantitative real-time polymerase chain reaction (RT-qPCR) was performed on whole blood samples to detect the relative expression of LincRNA-p21, NEAT 1, IL-6 and IL-8 mRNA in healthy controls, TB-positive patients, HIV-positive patients and HIV-Tuberculosis coinfected patients and ROC curve analysis was used to investigate their discriminatory and biomarker potentials.

With regards to the expression level, it was found that LincRNA-p21 was significantly upregulated in TB patients ($P < 0.05$) but down regulated with HIV and co-infected patients ($P < 0.01$) meanwhile lncRNA NEAT-1 was significantly up-regulated in TB, HIV and CO compared to NC ($P < 0, 05$). On the other hand, the target cytokine IL-6 mRNA was found to be significantly upregulated in all the groups ($P < 0.05$) while IL-8 mRNA was significantly up-regulated in TB and significantly down-regulated in HIV-TB coinfected compared to healthy controls ($P < 0,05$) but no significant difference was observed between active HIV and healthy controls ($P > 0,05$). With regards to the discriminative potential, it was demonstrated that LincRNA-p21, IL-6 mRNA and NEAT-1 were able to distinguish between all the groups two by two with AUC values of 100%, 100% and 97.8% respectively meanwhile IL-8 mRNA had AUC=100% when discriminating NC from TB and CO but had poor performance in discriminating healthy control groups from active HIV with AUC = 52%.

The lncRNAs LincRNA-p21 and NEAT 1 and their target cytokines IL-6 and IL-8 mRNA respectively are potential biomarkers for the diagnosis of HIV-Tuberculosis coinfection. The study was done over a period of 08 months subdivided into sample collection and analysis respectively at LAQUINTINIE Hospital and LANAVET DOUALA respectively.

Keywords: lincRNA-p21; NEAT-1; mRNA IL-6; mRNA IL-8; HIV-Tuberculosis Co-Infection

List of Abbreviations

TB: Tuberculosis; Mtb: Mycobacterium Tuberculosis; HIV: Human Immunodeficiency Virus; AUC: Area Under the Curve; EDTA: Ethylenediaminetetraacetic acid; NEAT1: Nuclear Enriched Abundant Transcript 1; lincRNA-p21: Long intergenic noncoding RNA p21; IL: Interleukins; NF-kB: Nuclear Factor-Kappa B

Introduction

In the 20th century, it was discovered that the human immunodeficiency virus (HIV), is the causative agent of the acquired immunodeficiency syndrome (AIDS) (Korber et al., 2000), which targets specifically the immune system through its fixation on all cells having a CD4 epitope. The induced immunosuppression in the host gives way to the appearance of several opportunistic infections amongst which tuberculosis (TB) is the first. It remains a major global health problem that causes more than 36.3 million deaths (WHO, 2022).

Tuberculosis is a transmissible infectious disease caused by a strict aerobic acid -alcohol-fast bacillus, the most common of which is Mycobacterium tuberculosis (Koch's bacillus or BK) (Russell, 2007). It mainly affects the lungs, and it is spread by exhaled droplets that diffuse through the air from a sick to a healthy individual. It represents, according to estimates by the World Health Organization, one of the infectious pathologies causing the most deaths worldwide with nearly 8 million new cases each year and more than 1 million deaths per year, making it a public health problem. Despite the decrease in the incidence of this disease, the high morbidity and mortality rate can be explained in part by a high frequency of co-infection with the human immunodeficiency virus and by the emergence of resistant strains (Mjid et al., 2015). People with HIV and infected with MTB are 10 times more likely to develop tuberculosis than people without HIV (Pimpin et al., 2011). The incidence of HIV-TB coinfection in 2021 was 14 million with 4 million deaths, of which more than two thirds (79%) of coinfected people where in African regions (WHO, 2022). In 2020, approximately 6042 peoples coinfected with HIV-TB were reported in Cameroon (PLNT,

2021).

Out of this co-infection state there is thus a high level of mortality rate accompanied with distinct diagnostic methods of this co-infection state worldwide, presenting several limits and manipulation errors. The major question raised is thus how to have a unique diagnostic method and treat this infection state. The lack of a common diagnosis for HIV-TB coinfection leads researchers to seek new alternatives, such as host-specific biomarkers. However, new investigations in molecular biology offer us a new field on non -coding RNAs which represent 98% of DNA transcription products and not coding for protein synthesis. Long noncoding RNAs (lncRNAs) are defined based on their size (over 200 nucleotides) and non-protein coding capacity. Long non- coding RNAs play a role in the regulation of gene expression, but it is noted that their deregulations have effects on the occurrence and/or proliferation of pathologies and that these lncRNAs can affect cytokines functions at different regulatory levels.

Other molecular biomarkers with an interest are Cytokines. They are a small group of proteins reported to control several biological and physiological processes, including metabolism, inflammation, blood pressure etc in order to maintain homeostasis. They have also been studied to have an intertwined relationship with lncRNAs. These lncRNAs can regulate the expression of cytokines at different level. These lncRNAs and their target cytokines through their role in different pathologies could be used as potential biomarkers for a unique molecular diagnosis of TB and HIV coinfection.

Hence, this study was carried out in order to provide new reference opinions on the diagnosis of this co-infection state. This study was implemented to know whether lincRNA-p21, NEAT-1 and their target cytokines IL-6 and IL-8 mRNA respectively can be used as potential biomarkers for the diagnosis of HIV-Tuberculosis co-infection.

Materials and Methods

Study Workframe

This prospective study first had an Ethical clearance authorization (ref no 0977/Minsante/SESP/SG/DROS) to conduct this work obtained from the Institutional Ethics Committee of the University of Douala. Patient recruitment was done at Laquintinie hospital in Douala (Center of respiratory diseases (CMR) and Day hospital) and later on analyzed at the National Veterinary Laboratory Douala annex (LANAVET). A total of 146 individuals were first recruited for the study subdivided into Negative controls, Tuberculosis positive patients (TB+), HIV positive patients (HIV+) and HIV-Tuberculosis co-infected patients (CO) all aged between 18-50 years and who agreed to fill the questionnaire sheet and sign the informed consent. The final cohort of 46 patients subdivided into 11 NC, 11 TB+, 12 HIV and 12 CO used throughout the experiment resulted from a probabilistic sampling according to inclusion/exclusion criteria, RNA quality, and availability of reagents for RT-qPCR analysis.

The negative control group (NC) included 11 non-smoking subjects having no antecedent of TB and underwent a routine physical examination at LAQUINTINIE hospital, Douala, during the study period. They had no contact with tuberculosis patients within the last two months and had negative results for Human Immunodeficiency Virus (HIV), and TB LAMP tests. They had no chronic disease and were not receiving immunosuppressive therapy.

The TB positive group (TB+) comprised 11 HIV negative patients but positive at microscopy and TB LAMP test. They had no chronic diseases, were not on immunosuppressive treatment and exempt from TB treatment.

The HIV positive group (HIV+) was made up of 12 HIV patients, positive at Determine and Oraquick test, had no chronic diseases.

The co infected group comprised 12 patients positive at both HIV and Tuberculosis test, either under treatment or not but with

no chronic disease and not under immunosuppressive.

The flowchart in Figure1 summarizes the steps of the screening process.

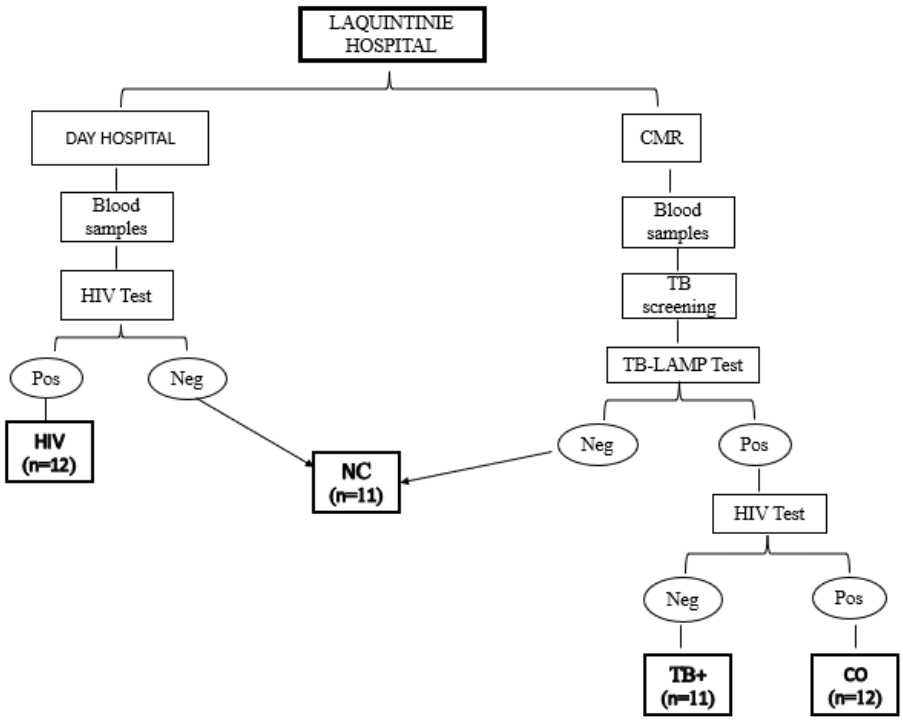

Figure 1: Study flow chart

(Pos: Positive, Neg: Negative, CMR: Respiratory disease center)

Blood Collection and Processing

A total of 4ml of venous blood was collected into a five milliliters Ethylene Diamine Tetra acetic Acid (EDTA) tube and centrifuged at 5000rpm for 5. The supernatant (plasma) was separated from the pellets. 500µl of the pellets was then transferred using a pipette to a labelled 1.5ml Eppendorf tube and 500µl of Qiazol reagent added for storage and lysis. The mixture was vortexed and then stored at -21^{0}C until total RNA extraction.

Total RNA Extraction and cDNA Synthesis

Cells were lysed in Qiazol (Qiagen, Germany) at different time points during post treatment and lysates were stored at −80 °C. Total RNA was isolated from the lysate using miRNeasy Mini Kit (Qiagen, Germany) following the manufacturer's instructions. 100ng of total RNA obtained was retro-transcribed into cDNA using the Maxima first strand cDNA synthesis with dsDnase kit according to the supplier's instructions and stored at -20°C.

Quantitative Real-Time PCR

Quantitative real-time PCR (qPCR) was performed using LightCycler® 480 SYBR Green I Master (Roche, Germany) and gene-specific primers (IDT, CA, United States). Fold change in gene expression was calculated by the ΔΔCt method and normalized to glyceraldehyde-3-phosphate dehydrogenase GAPDH, which served as an internal normalizer. The PCR amplification pro--gram was set with respect to the genes primers sequence (Table I). The qPCR primer sets used were as follows: **hs-GAPDH:** for-ward 5-GAAATCCCATCACCATCTTCCAGG -3, reverse 5- GAGCCCCAGCCTTCTCCATG -3; **hs lincRNA-p21:** forward 5'-GGG TGG CTC ACT CTT CTG GC-3', reverse 5'-TGG CCT TGC CCG GGC TTG TC-3'; **hs-NEAT-1**: forward 5'-

GCT CAC TCC ACC CCT TCT TC-3' ; reverse 5'-CAC ATT CAC TCC CCA CCC TC-3'; **hs-mRNA IL-6:** forward 5' AAA GAT GGC TGA AAA AGA TGG ATG C- 3', reverse 5' -ACA GCT CTG GCT TGT TCC TCA CTA C- 3'and **hs-IL-8:** forward 5'-GCA GCC TTC CTG ATT TCT GCA GCT C-3' ; reverse 5'-ATC TCT CCA CAA CCC TCT GCA CCC A-3'.

The 20 µL reaction system included 1X FASTstart Universal SYBR Green I Master Mix, 0.9 µL forward primer, 0.9 µL reverse primer, and 2 µL of reverse transcription product. The reaction conditions were set as follows: pre-denaturation at 95 ∘C for 15 min, 95∘C for 10 s, 60∘C for 15s, 72∘C for 30 s, and 50 cycles for lncRNA NEAT-1 and 55 cycles for IL-8 mRNA. qPCR was performed using the Thermofischer Pikoreal version 2.2 for RT-qPCR .

Table I: Amplification Program

Number of cycle (s)	Steps	Conditions
1	Pre-incubation	95 C for 15 minutes
50 and 55 cycles for lincRNA-p21, NEAT-1	Denaturation	95 C for 10 seconds
	Annealing	60 C for 15 seconds
	Elongation	72°C for 10 seconds
	Data acquisition	80°C for 1 second
1	Melting curve	Start temperature:65°C for 1 second
		End temperature:97°C for 0.2 second
		Data acquisition in a continuous mode

Graphs and Statistical Analysis

The result of RT-qPCR amplified gene is done by the described method of LIVAK according to the Ct value, and fold change in gene expression was calculated by the 2^ddCt and normalized to GAPDH, which was used as internal control (LIVAK et al., 2001).

SPSS 20.0 software (Statsoft Inc, Carey, J, USA) and easyRoc (ver. 1.3.1) were used for statistical analyses.

Data with a normal distribution was presented as Mean ± Standard deviation. After verifying with the variance test that the standard deviations were not statistically different, the ANOVA test and the t-test were used to compare the means in pairs at a significance level of 5%. These analyses were performed using SPSS 20.0 software (Statsoft Inc, Carey, J, USA). Means comparison was performed through the t-test. A p-value of <0.05 was considered to be statistically significant for a 95% confidence interval. Curves and graphs were drawn under Microsoft Excel 2013. The area under the ROC curve (AUC) is a measure of the capacity of a parameter to distinguish between two groups. In ROC curve analysis, the AUC values lie between 50% and 100% with 50% being a bad classifier and 100% a perfect classifier. Values within the range of 90 and 100% are generally considered excellent, 80 and 90% good, 70 and 80% fair, 60 and 70% poor, and 50 and 60% bad (or failed). An AUC below 50% indicates a set of random values unable to make the distinction between two groups (**Folador E, 2016**).

Results

Sociodemographic and Clinical Characteristics of the Study Population

Forty-six (46) participants were enrolled during the period of this study with 50 % being male and 50 % female. Table 2 provides details on the sociodemographic and clinical characteristics of the study population.

Table 2: sociodemographic and clinical characteristics of the study population.

Groups Effectives	Negative Control	Tb Positive	Hiv Positive	Co-Infected
Females	6	5	6	6
Males	5	6	6	6
Under TB and orHIV treatment	0	0	12	3
TOTAL	11	11	12	12

Relative Expression of lincRNA-p21, NEAT-1, mRNA IL-6 and mRNA IL-8 in the different study groups

LincRNA-p21 was Seen Upregulated in Whole Blood of Patients with Active Pulmonary TB but Downregulated with HIV and Co-Infected Patients As Compared To Negative Controls

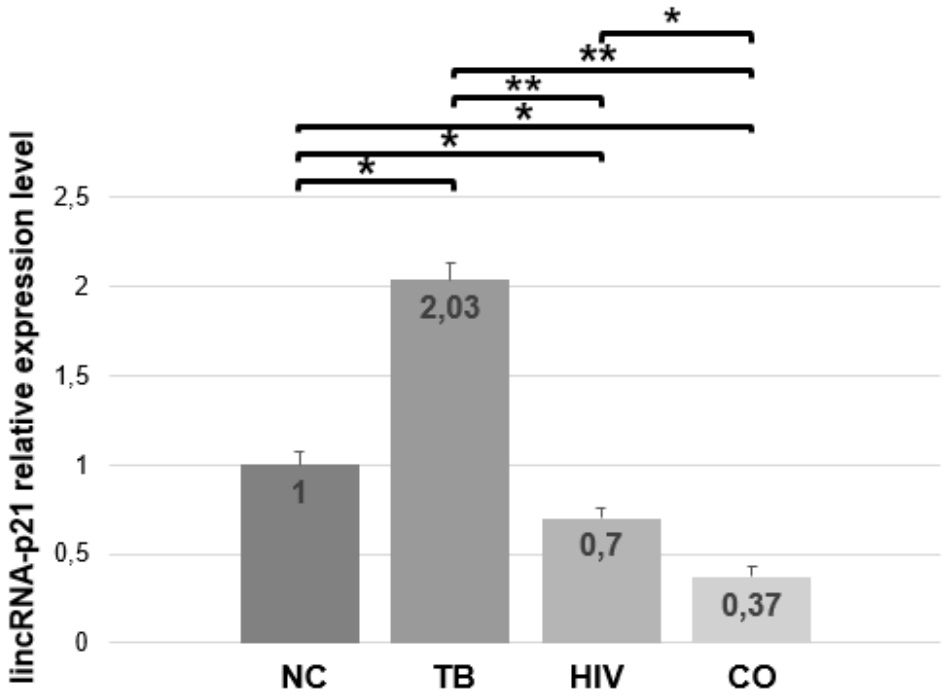

Figure 2: Expression levels of lncRNA lincRNA-P21

Relative expression of lincRNA-p21 in whole blood samples from the different subjects groups. NC: Negative Controls group; TB: Tuberculosis positive patients; HIV: HIV positive patients; CO: Co-infected patients. Total RNA was extracted, and lincRNA-p21 expression level was assessed through RT-qPCR and calculated using the $-\Delta\Delta Ct$ method with *$p < 0.05$; **$p < 0.01$: significance. The confidence interval of the null hypothesis was set at 95%, the margin of error at 5% (significant p-value if and only if $p<0.05$).

Lncrna NEAT-1 Levels Are Increased In the Whole Blood of Active Pulmonary TB, HIV Positif and HIV-Tuberculosis Coinfected Patients As Compared To Healthy Controls

The expression level of lncRNA NEAT-1 in the whole blood of active tuberculosis patients (2.25 ± 0.6) was significantly upregulated ($P < 0.05$) compared to healthy controls. Further lncRNA NEAT-1 expression level was also upregulated ($P < 0.05$) in HIV patients with (1.17 ± 0.28) and with patients co-infected (1.63 ± 0.25) (Figure 3).

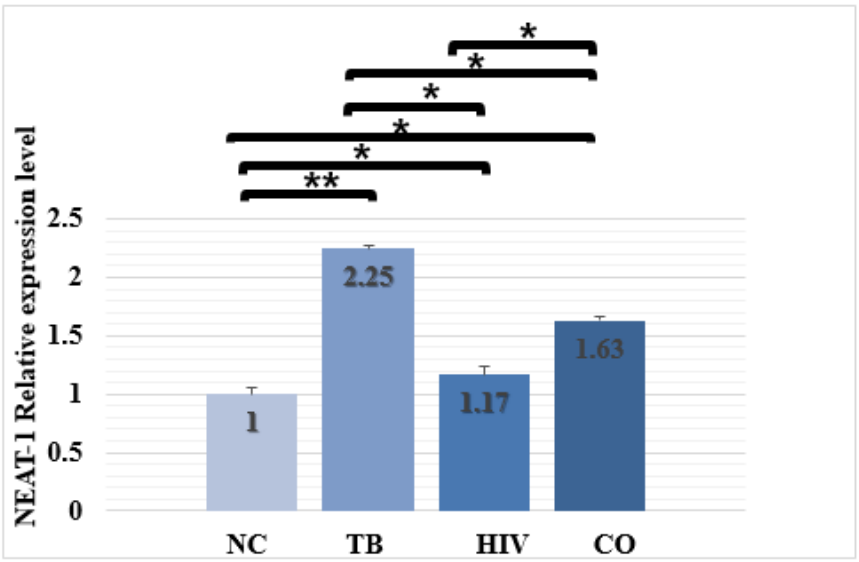

Figure 3: Expression levels of NEAT 1

Relative expression of lncRNA NEAT-1 in whole blood samples from the different groups. NC: Negative Controls; TB: active Tuberculosis; HIV: HIV positive; CO: HIV-TB Coinfected patients. After extracting Total RNA, the expression level was collect-ed through the Ct of RT-qPCR and calculated using the $-\Delta\Delta Ct$ method with *p < 0.05; **p < 0.01: significance. The confidence interval of the null hypothesis was set at 95%, the margin of error at 5% (significant p-value if and only if p<0.05).

IL-6 Mrna Was Upregulated in Whole Blood of Patients with Active Pulmonary TB, HIV and Co-Infected Patients As Compared To Negative Controls

The expression level of IL-6 mRNA in the whole blood of active tuberculosis patients (3.32 ± 0.18), HIV patients (2.93 ± 0.06) and co-infected patients (4.13 ± 0.07) was significantly upregulated (P < 0.05) compared to healthy controls (Figure 4). It was al-so observed a slight decrease in the HIV group as compared to TB and CO groups.

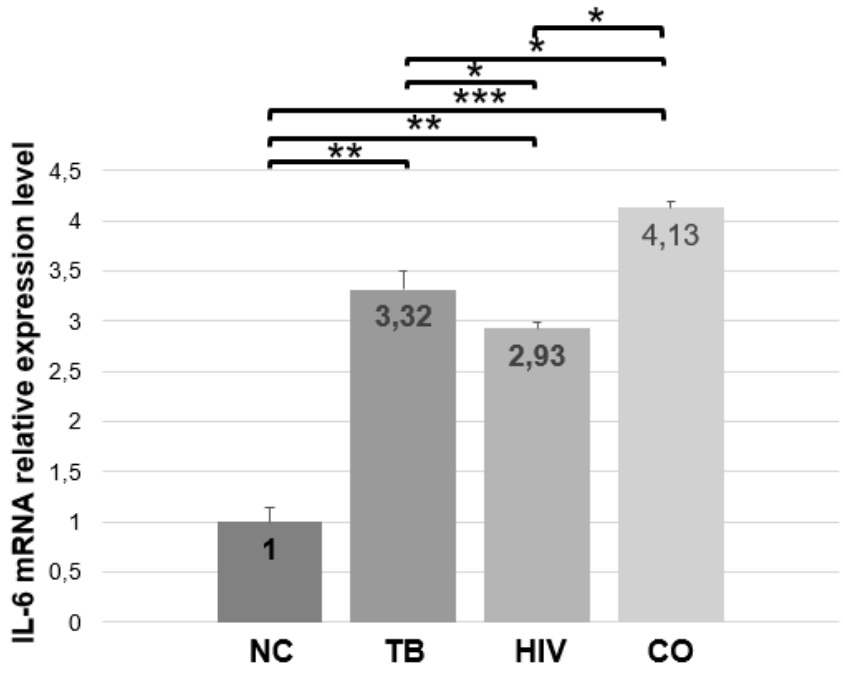

Figure 4: Expression levels of IL-6 mRNA

Relative expression of IL-6 mRNA in whole blood samples from the different subjects groups. NC: Negative Controls group; TB: Tuberculosis positive patients; HIV: HIV positive patients; CO: Co-infected patients. Total RNA was extracted, and lincR--NA-p21 expression level was assessed through RT-qPCR and calculated using the –ΔΔCt method with *p < 0.05; **p < 0.01: significance. The confidence interval of the null hypothesis was set at 95%, the margin of error at 5% (significant p-value if and only if p<0.05)

IL-8 mRNA was upregulated in whole blood of patients with active pulmonary TB but downregulated with HIV and co-infected patients as compared to Negative controls

The expression levels of IL-8 mRNA in the whole blood of active TB patients (1.6 ± 0.07) were significantly higher (P <0.05) compared to healthy controls. However, the whole blood of patients with HIV (0.98 ± 0.06) displayed similar levels of IL-8 mRNA (P ≥0.05) compared to negative controls. On the other hand, IL-8 mRNA was more lowered in CO (0.23 ± 0.02) than in the healthy groups. Thus, we observed excessive decrease in the CO group as compared to TB and HIV groups (Figure 5).

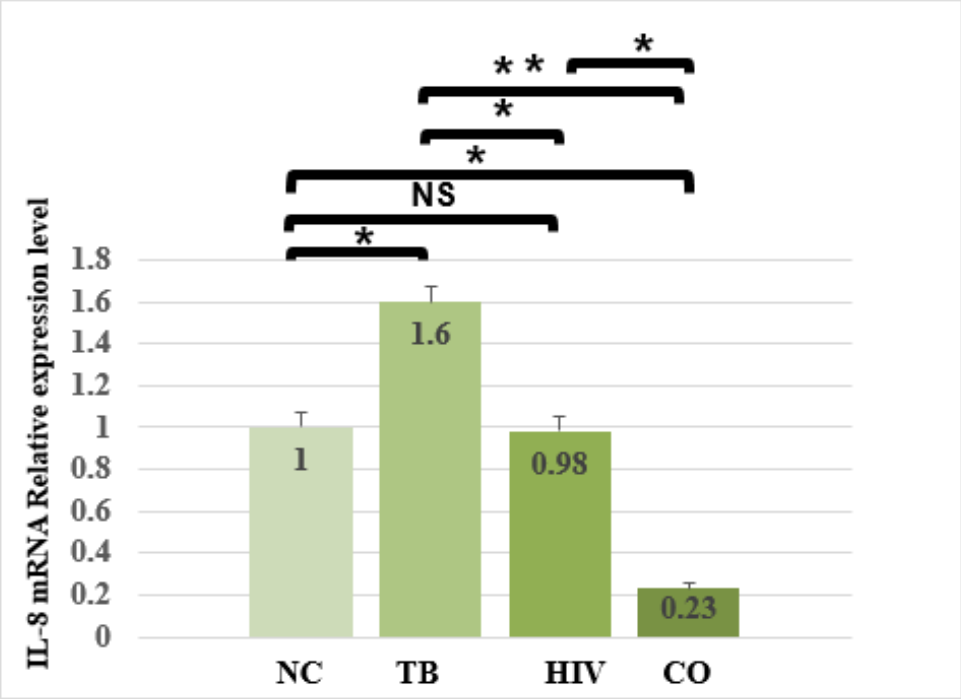

Figure 5: Expression levels of IL-8 mRNA

Relative expression of IL-8 mRNA in whole blood samples from the different groups. NC: Negative Controls; TB: active Tuberculosis; HIV: HIV positive; CO: HIV-TB Coinfected patients. After extracting Total RNA, the expression level was collected through the Ct of RT-qPCR and calculated using the –ΔΔCt method with *p < 0.05; **p < 0.01: significance. The confidence interval of the null hypothesis was set at 95%, the margin of error at 5% (significant p-value if and only if p<0.05)

LincRNA-p21 and mRNA IL-6 can discriminate between TB positive, HIV positive, co-infected and negative control groups

Using the GraphPad Prism software, the discriminative potential of lincRNA-p21 and IL-6 mRNA was evaluated between NC and TB, NC and HIV, NC and TB, TB and HIV, TB and CO then HIV and CO. This analysis verified the specificity and sensitivity of the genes and gave an AUC of 100% (Table III) between each group two by two as represented on figure 6 and 7 respectively. Thus lincRNA-p21 and IL-6 mRNA are classified as excellent discriminants.

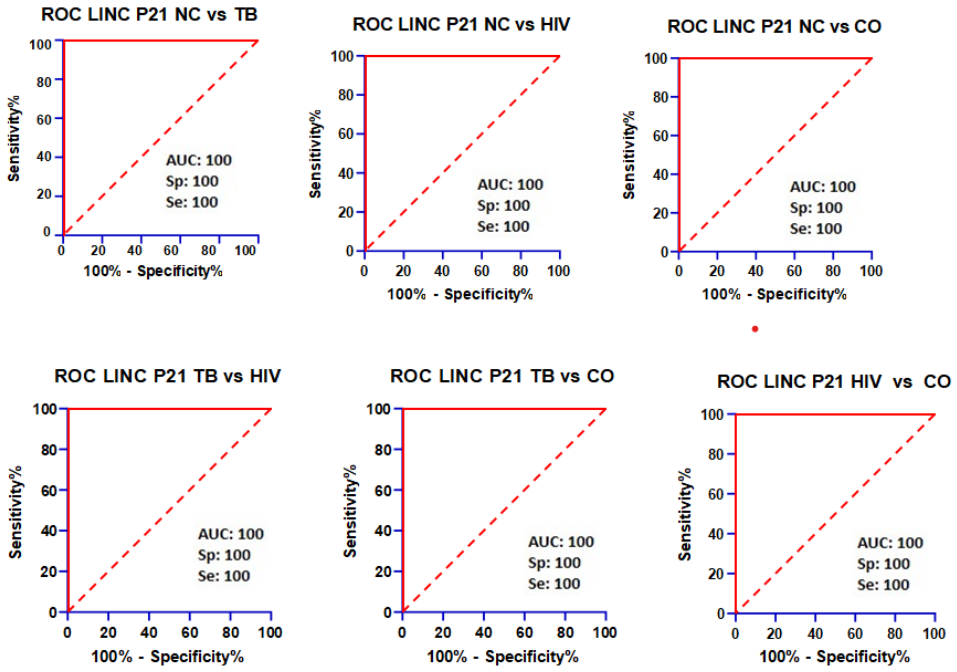

Figure 6: ROC curve for the discriminatory potential of lncRNA-P21.

LincRNA-p21 level distinguish between all the groups two by two. Total RNA was purified from the whole blood samples of active tuberculosis patients (TB) and negative controls (NC), HIV patients (HIV) and co infected patients (CO). LincRNA-p21 relative expression level was determined by RT-qPCR associated with the Livak method. The software easyROC was used to set up ROC curves and calculate the area under the curve (AUC) values.

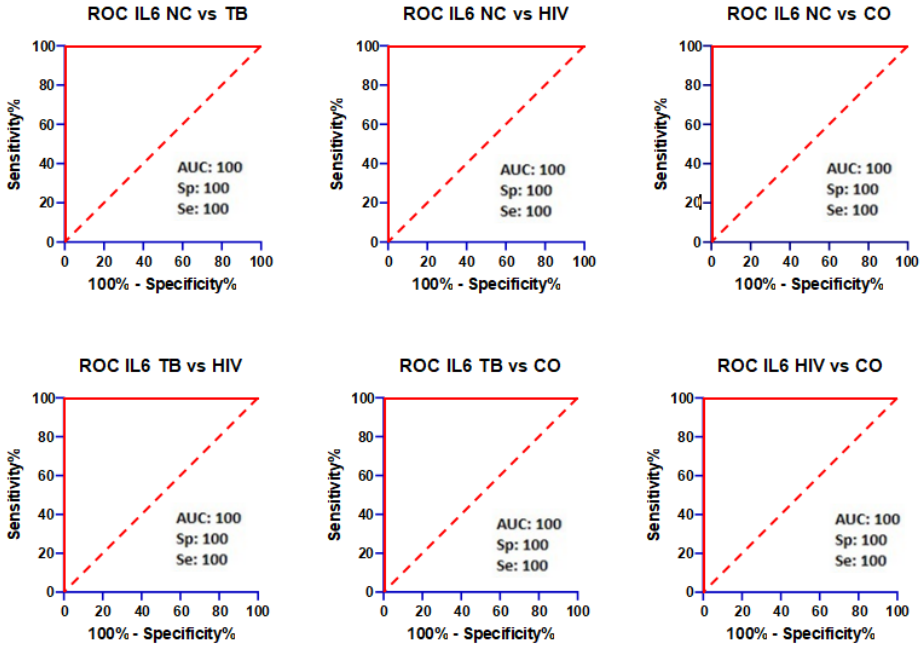

Figure 7: ROC curves for the discriminatory potential of IL-6 mRNA

IL-6 mRNA level distinguishes between all the groups two by two. Total RNA was purified from the whole blood samples of active tuberculosis patients (TB) and negative controls (NC), HIV patients (HIV) and co infected patients (CO). IL-6 mRNA relative expression level was determined by RT-qPCR associated with the Livak method. The software easyROC was used to set up ROC curves and calculate the area under the curve (AUC) values.

LncRNA NEAT-1 Levels Distinguish Active Pulmonary Tuberculosis Patients from Healthy Controls

Using the GraphPad Prism software, the discriminative potential of NEAT-1 was evaluated between NC and TB, NC and HIV, NC and TB, TB and HIV, TB and CO then HIV and CO(figure 8). The receiver operating characteristic (ROC) curve revealed that NEAT1 expression could discriminate CN from TB and CO (sensitivity and specificity of 100% and area under the curve (AUC) = 100%), but could discriminate CN from HIV whit sensitivity of 100% and specificity of 80% and area under the curve (AUC) = 97%.

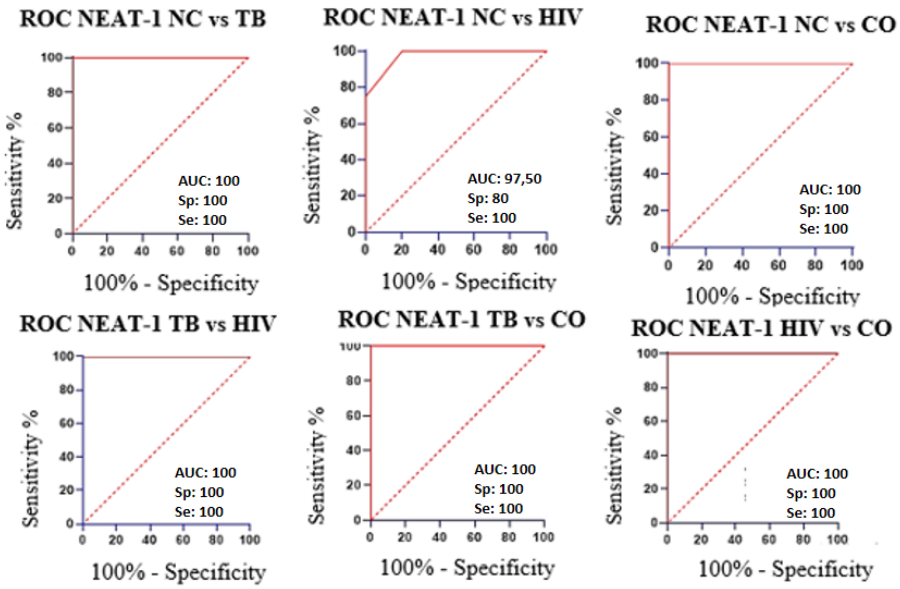

Figure 8: ROC curves for the discriminatory potential of lncRNA NEAT 1

lncRNA NEAT-1 level distinguish between all the groups two by two. Total RNA was purified from the whole blood samples of active tuberculosis patients (TB) and negative controls (NC), HIV patients (HIV) and co infected patients (CO). LincRNA-p21 relative expression level was determined by RT-qPCR associated with the Livak method. The software easyROC was used to set up ROC curves and calculate the area under the curve (AUC) values.

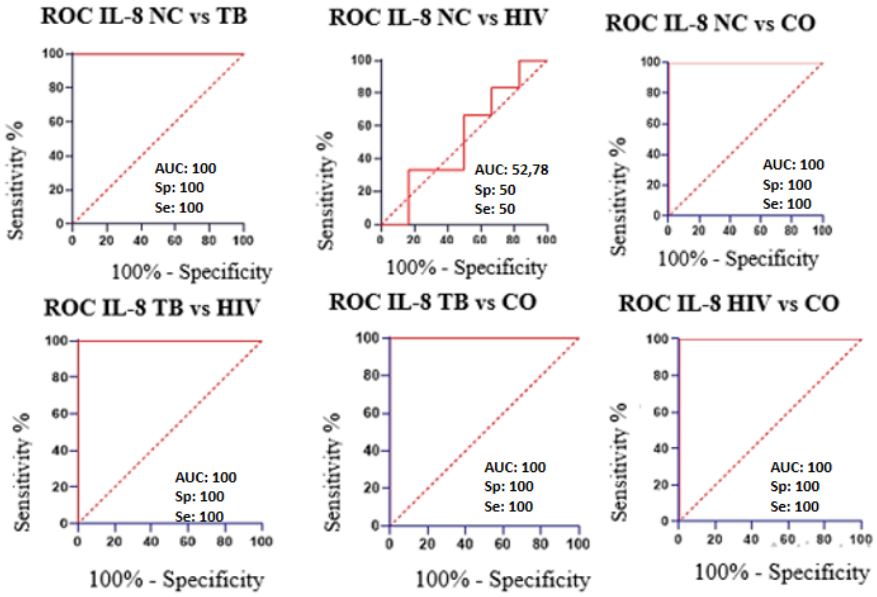

Figure 9: ROC curves for the discriminatory potential of IL-8 mRNA

IL-8 mRNA level distinguishes between all the groups two by two. Total RNA was purified from the whole blood samples of active tuberculosis patients (TB) and negative controls (NC), HIV patients (HIV) and co infected patients (CO). IL-8 mRNA relative expression level was determined by RT-qPCR associated with the Livak method. The software easyROC was used to set up ROC curves and calculate the area under the curve (AUC) values.

IL-8 mRNA Discriminates Active Pulmonary TB and HIV-Tuberculosis Co-Infected Patients with Healthy Controls but Cannot Discriminate Between HIV Patients and Healthy Controls

In our study, the potential of IL-8 mRNA in discriminating between NC from TB, HIV and CO, was evaluated by the ROC curve and Area under the Curve analysis. According to results which are illustrated in Figure 9, in the case of TB and CO compared with healthy controls, the sensitivity and specificity was 100% and area under the curve (AUC) = 100% but comparing NC and HIV we observe that could poorly discriminate NC from HIV whit sensitivity of 50% and specificity of 50% and area under the curve (AUC) = 52.78%.

Discussion

This study aimed at investigating the LincRNA-p21, NEAT-1 and their target cytokines mRNA IL-6 and mRNA IL-8 respectively as potential biomarkers for the diagnosis of Tuberculosis-HIV co-infection. The search for biomarkers is becoming a priority for the health sector in view of the many shortcomings and limitations of the individual diagnostic tests currently used and the absence of a unique diagnostic method for the co-infection state. The development of a more effective unique diagnostic test will be welcome to respond to the major concern of the WHO, which is to end TB and associated pathologies by 2030 (WHO, 2020). LncRNAs are taking the stage in recent times as biomarkers in the timely diagnosis and follow up treatment of pathologies. Several studies have demonstrated their place not only in immune response but also in host-pathogen interactions in TB. Their expression at a specific tissue and condition in an organism renders lncRNAs potential biomarkers (Statello et al., 2021; Zhang et al., 2022). Infection with Mycobacterium tuberculosis as well as HIV leads to an alteration in the expression profile of many long non-coding RNAs that could be used for the diagnosis of this co-infection. Out of all this an alternative of researching for potential biomarkers specific to the host and not those of the pathogen could guide towards a unique diagnostic method for this coinfection state.

This research was conducted on 46 individuals, with 23 males (50 %) and 23 females (50 %) including 11 healthy controls (NC), 11 active pulmonary tuberculosis (TB), 12 HIV positive (HIV) and 12 coinfected with HIV-TB (CO) at the Laquintinie hospital in Douala.

The results of this study showed a significant increase in the level of expression of lincRNA-p21 in the TB positive group but a decrease in HIV and co-infected groups as compared to the negative control group.

Concerning TB results which showed an increase in the level of expression of lincRNA-p21, these findings are concordant with those of Tamgue et al. (2021) who reported an increase in the expression level of this lncRNA in MDMs infected with MTB strains and highlighted its role in targeting IL-6 which functions in macrophages activation and immune response against MTB through the NF-kB pathway. Ngongang et al. (2022) also showed in their research that lincRNA-p21 was upregulated in TB infections. LincRNA-p21 was also investigated to have anti-inflammatory or pro inflammatory effect with respect to disease type and stimulator (Yang et al., 2018). In this study, the increased level observed with the host's lincRNA-p21 could be caused by positive regulation of p53 to inhibit cellular proliferation, migration and invasion of Mycobaterium tuberculosis and also activate the apoptosis of infected cells.

Concerning HIV results which showed a decrease in the level of expression of lincRNA-p21, these findings are concordant with

those of Barichievy et al. (2018) and Li et al. (2020) who demonstrated that HIV enables the degradation of lincRNA-p21 by sequestering HuR in the nucleus. HIV thus avoids this apoptotic mechanism by sequestrating hnRNP thereby reducing its level of expression (Xingzhu et al., 2022).

Co-infected group on the other hand showed a further decrease in the level of expression of lincRNA-p21 as compared to the negative control group. This decrease was also observed in HIV monoinfection but the level had previously increased with TB monoinfection. The level of expression of this gene in the diagnosis of HIV-Tuberculosis co-infection is reported for the first time. As the expression level of lincRNA-p21 has lowered in HIV and co-infection group but increased in TB group it can thus be hypothesized that the decrease is due to HIV and that the effect is important thus inhibiting the increase due to TB as we know that HIV generally preceeds TB. The mechanism of action remains to be elucidated.

lncRNA NEAT-1 showed an up-regulation in infected people as compared to the healthy ones. These data showing the elevated expression of lncRNA NEAT-1 in patients infected with MTB compared to healthy controls are similar to those obtained by Shuying and his group who conducted a study on The Expression of lncRNA NEAT1 in Human Tuberculosis and Its Antituberculosis Effect (Shuying et al., 2018). We also observed that in TB, NEAT-1 was highly up-regulated than in HIV patients and HIV-TB coinfected ones. The overexpression of NEAT-1 would be due to the fact that the organism should synthesize all its own molecules to reinforce the immune defense during host-MTB interactions in order to improve the bactericidal activity of the defense cells and modulate the signaling pathways of inflammatory molecules since none of these TB patients were under antituberculosis treatment.

On the other hand, we observed an upregulation of lncRNA NEAT-1 during HIV infection. This result was in line with that of Liu and collaborators during their studies on HIV-1 replication in CD4+ T cells exploits the down-regulation of antiviral NEAT1 long non-coding RNAs, which demonstrated that the HIV-1 infection increases the expression of NEAT 1 compared to that of uninfected cells because NEAT-1 is said to have broad antiviral activity (Liu et al., 2018). The potential mechanisms by which HIV infection may influence overexpression of NEAT-1 would be based on the fact that certain viral transcription factors produced by HIV may bind to the regulatory regions of NEAT-1 or that HIV may activate several cellular signaling pathways, including the NF-κB (nuclear factor kappa B) pathway which can stimulate transcription of NEAT-1, thus leading an in--crease in the expression of this lncRNA.

Regarding pulmonary HIV-TB coinfection, this study showed that the expression of lncRNA NEAT-1 is up-regulated. The lncRNA NEAT-1 was significantly up-regulated in active tuberculosis and in HIV-positive. The level of expression of this gene in the diagnosis of HIV-Tuberculosis co-infection is also reported for the first time. On a hypothetical basis, this result could be explained by the fact that the multiplication of bacteria and the replication of viruses in the body increase not only the determinants of pathogens but also the perception of the "danger" signal to the immune defense cells, thus provoking a response. This modified inflammatory response can influence the expression of NEAT-1, during HIV-TB coinfection. The exact molecular mechanisms of this interaction and its impact on the pathogenesis of HIV-TB co-infection remain to be investigated.

IL-6 mRNA was also investigated in this study, and we obtained an increase in its level of expression in all the groups.

As for the increased level in Tuberculosis infection this study is concordant with that of Correia et al. (2009) who evaluated the level of IL-6 in TB+ patients compared to healthy group and obtained a high increase in its level in this group compared to the healthy group (4.3 pg/ml vs 0.5 pg/ml). This robust increase suggests that IL-6 contributes to the inflammatory activity in the TB patients, in accordance with the proinflammatory potential of IL-6 in experimental models of acute infection. Lavanya et al. (2015) obtained the same results when evaluating the expression level of IL-6 and IL-10 in active pulmonary TB (APTB), house hold contacts (HHC) and healthy controls (HC). They found that IL-6 levels were elevated among APTB (6.044 pg/ml) com--pared to HHC (2.62 pg/ml) and HC (4.112 pg/ml). Tang et al. (2013) in their investigation of certain Cytokines response in pa-

tients with tuberculosis and chronic obstructive pulmonary disease (COPD) saw that the levels of IL-2R, IL-6, TNF-α, IFN-γ in TB patients with or without COPD were significantly higher than those in control group and COPD patients without TB (P= 0.000).

In the present study IL-6 mRNA was found upregulated with HIV infection. This result is similar to those of Breen et al. (1990) who measured the level of circulating plasma IL-6, spontaneously-produced IL-6, and IL-6 mRNA in HIV infected donors and in healthy control donors. They saw that elevated levels of plasma IL-6 and IL-6 mRNA were detected in HIV-infected donors compared to healthy donors and concluded that elevated levels of IL-6 are associated with HIV-infection, and suggest that IL-6 over-production may contribute to the polyclonal B cell activation seen in AIDS and HIV infection. Navikas et al. (1995) had the same results when evaluating the level of expression of IL-6 mRNA in HIV infected group compared to healthy group through in situ hybridization with cDNA oligonucleotide probes to enumerate blood mononuclear cells (MNCs) expressing mRNA for IL-6, IL-10, TNF-alpha, and perforin. Aurelia et al., (2013) and Alvaro et al., (2015) had the same results in the analysis of cytokines profile analysis in HIV individuals and factors associated with cytokine production in HIV patients respectively and had increased IL-6 levels in HIV patients.

The results showed a further increase in the level of expression of IL-6 mRNA in HIV-tuberculosis co-infected patients com-- pared to the negative control group. Here the mechanism of action remains to be elucidated since the level of expression of IL-6 mRNA in co-infection is reported for the first time.

The results of this study also showed a significant increase in the level of expression of mRNA IL-8 in the TB positive group but a decrease in HIV and HIV-TB coinfected groups as compared to the negative control group.

During this study, the expression level of IL-8 mRNA was also investigated. IL-8 mRNA level of expression was up regulated in active TB cases, which is identical with research of Boggaram and his team that found that IL-8 can directly bind to tubercle bacilli. Besides, it have been demonstrated that direct contact with MTB induces mononuclear cells to increase the synthesis and re-lease of IL-8 which is involved in the formation and maintenance of granuloma in tuberculosis pulmonary (Krupa A. et al., 2015). The induction and increase in IL-8 mRNA expression could be due to the stimulatory effect of determinants associated with the virulence and pathogenicity of M. tuberculosis on the transcriptional mechanisms that must activate inflammasome and granulomas to promote apoptosis and lysis of lung epithelial cells. The lung epithelium contributes to the initiation and amplification of cytokines like IL-8 which plays a key role in mediating inflammatory responses in the pathogenesis of acute and chronic lung diseases.

On the other hand, we observed a slight decrease in IL-8 mRNA in HIV-positive compared to healthy controls, which is similar to the results of Hober who found no significant differences in the level of IL-8 expression between HIV-positive people on treatment and uninfected people (Hober D et al., 1996). This could suggest that the reduction in the expression of IL-8 mRNA in infected patients is due to antiretroviral therapy, which would have beneficial effects for the host because antiretroviral molecules such as protease inhibitors, non-nucleoside reverse transcriptase inhibitors which have the ability to decrease viral re-plication and inflammation associated with HIV infection. Although there are a limited number of studies reporting that circulating IL-8 levels are significantly higher in HIV-infected individuals compared to healthy controls, depending on CD4+ T cell count < 350 cells/ mm3 due to severe immunosuppression (Ellwanger H et al., 2020) and even HIV-positive people being on ART with a suppressed viral load (Wada et al., 2015).

There is some information in the literature regarding elevated IL-8 levels in HIV-TB coinfected patients; demonstrating that the level of production of IL-8 by MDMs coinfected with HIV-MTB was 20-fold higher than uninfected control MDMs (Choi R et al., 2017). Our results disagreeing with previous findings, we observed that IL-8 mRNA expression was significantly down-regulated in HIV-TB coinfected compared to healthy controls. the decrease in IL-8 mRNA expression in our study would be

due to interactions between HIV and MTB, the influence of anti-inflammatory cytokines, the presence of other opportunistic adits, proportions of CXCR- 1/2 of IL-8, the age, physiological state of the patient and the environment. On the other hand, the mechanisms making it possible to provide explanations for the difference in the level of expression of IL-8 mRNA in coinfected compared to HIV and TB mono-infected remains to be investigated.

With regards to their discriminative potential, lincRNA-p21 showed an excellent discriminative potential with AUC of 100% when comparing the groups two by two. The comparison of NC and TB+ groups was similar to that of Ngongang et al. (2022) who showed the same results (AUC 100%) when evaluating the discriminative potential of the same between active pulmonary tuberculosis (APTB) group and negative control group.

The result of the measurement of the diagnostic potentials revealed that the lncRNA NEAT-1, presented an excellent performance to discriminate the healthy control groups from the active tuberculosis patients, seropositive and HIV-TB coinfected therefore the AUC is respectively 97% for the TB and 100% for HIV and co-infected. One study presented an ROC curve (AUC ˃97%) when discriminating between healthy controls and newly infected active pulmonary TB (Charleine K et al., 2022) which would correspond to our result. The different AUCs obtained suggest that lncRNA NEAT-1 would be an excellent biomarker for the diagnosis of HIV-TB coinfection.

IL-6 mRNA on the other hand also revealed to be an excellent discriminative potential with an AUC of 100% when comparing the groups two by two. The comparison between NC and TB+ groups appeared to be slightly similar to that of Lavanya et al. (2015) who evaluated the discriminative potential of this gene when comparing APTB group and NC group. He obtained an AUC of 70.8% classifying IL-6 as being a good discriminative potential of these groups.

This study also assessed the value of IL-8 as a biomarker that can be used for diagnosis of HIV-TB coinfection. Although the IL-8 level was statistically significantly increased in TB and reduced in CO compared to the control group, its discriminant value was excellent as confirmed by the analysis of the ROC curve with an area under the curve of 100%. On the other hand, IL-8 had a poor ability to discriminate between CS and HIV, as evidenced by the AUC of 52%. These results were different from previous reports which stated that IL-8 was a good marker in HIV (AUC=80%) (Sun J. et al., 2016) and we also noted that it is important for discriminate HIV-TB coinfected from HIV, TB and CS (AUC=100%); while it was a less valuable tool in differentiating between HIV-positive and healthy controls.

This study shows that these biomarkers could be integrated into a multiplexed molecular screening test based on RT qPCR, applicable as a first line of defense in laboratories with simple qPCR platforms. Simultaneous measurement of lincRNA-p21, NEAT-1, IL-6 and IL-8 in whole blood could enable rapid differentiation between TB, HIV and co-infection, complementing pathogen tests such as GeneXpert and Determine.

The ability of these genes to distinguish between the each group is still unknown and could constitute good avenues to explore for the enhancement of the fight against HIV-TB coinfection. Out of this results the authors intend to extend the study to a larger scale and other geographic regions compared to what was done in order to have a more detailed view of the potential of these genes. Focusing on RNA expression allowed us to explore key molecular signals, and we aim to complement this with future protein-level analyses for target cytokines of our ncRNAs. Longitudinal studies could also be done to better understand how these biomarkers evolve during treatment and disease progression.

Conclusion

The present study reported that the lincRNA-p21, NEAT-1, IL-6 and IL-8 mRNA were significantly deregulated in patients with tuberculosis, HIV, HIV-tuberculosis co-infection as compared to the negative controls. The expression level of lincR--

NA-p21 was significantly upregulated in tuberculosis patients but downregulated in HIV and co-infected patients as compared to healthy controls. lncRNA NEAT-1 was significantly up-regulated in active pulmonary TB, HIV and HIV-TB coinfected com-pared to healthy controls. The expression level of IL-6 mRNA in whole blood of patients was upregulated in TB, HIV and coinfected groups as compared to the negative control group. IL-8 mRNA was significantly up-regulated in TB and significantly down-regulated in HIV-TB coinfected compared to healthy controls but no significant difference was observed between HIV and healthy controls. LincRNA-p21, NEAT-1 and IL-6 mRNA showed excellent performance in distinguishing between each group two by two with AUC of 100%, 97% and 100% respectively meanwhile IL-8 mRNA, this has been identified as an excel--lent biomarker potential for discriminating healthy control groups from active and HIV-TB coinfected TB (AUC=100%) but poor performance in discriminating healthy control groups from HIV (AUC=52%). Despite the relatively modest sample size, the results revealed highly significant differences between groups ($p < 0.05$) and excellent diagnostic accuracy (AUC values ranging from 97% to 100%), indicating strong discriminatory power. This supports that the sample size was sufficient to detect statistically meaningful differences. LincRNA-p21, NEAT-1, IL-6 mRNA and IL-8 mRNA could constitute potential and promising biomarkers for diagnosis of HIV-tuberculosis co-infection.

Ethics Approval and Consent to Participate

Studies involving human participants were reviewed and approved by the Institutional Ethics Committee of the University of Douala for approval. Written informed consent to participate in this study was provided by the legal guardian/next of kin of the participants.

Availability of Data and Materials

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

The authors declare that the research was conducted in the absence of any external funding.

Author Contributions

SNN, MHT and OT designed the experimental design and writing plan. SNN and MHT recruited participants and performed laboratory analyses. SNN and MHT performed the statistical analysis. SNN and MHT drew all figures. SNN, MTH, JFT, USFS, JAN, CFM, CK, NNN, MFT, ESMT wrote the draft. MA, ARNN and OT reviewed the manuscript. All authors listed made a substantial, direct and intellectual contribution to the work and approved it for publication.

Acknowledgement

A big thank you to all the people who voluntarily agreed to participate in the study, to the staff from the Douala Respiratory Dis--ease Center and National Veterinary Laboratory, Cameroon. For their support throughout the conduct of this study.

References

1. Alvaro H, Jemma L, Andrew N, Frederikke F, Sarah P, et al. (2015) Factors associated with plasma IL6 levels during HIV in--fection. 212: 585-595.

2. Aurelia W, Francois S, Carolus R, Debra M (2013) The Th1/Th2/Th17 cytokine profile of HIV-infected individuals: A multi--variate cytokinomics approach, Cytokine. 61: 521-526.

3. Barichievy S, Naidoo J, Boulle M, Scholefield J, Parihar S, et al. (2018) Viral apoptosis evasion via the MAPK pathway by use of a host long noncoding RNA Front Cell Infect Microbiol. 8: 263

4. Breen E, Rezai A, Nakajima K, Beall G, Mitsuyasu R, et al. (1990) Infection with HIV is associated with elevated IL-6 levels and production. J Immunol. 144: 480-4.

5. Charleine K, Cybelle M, Natacha N, Jubilate N, Ulrich S, et al. (2022) "P50-associated Cyclooxygenase-2 Extragenic RNA (PACER) and Long Non -coding RNA 13 (LNC13) as potential biomarkers for monitoring tuberculosis treatment". Frontiers in Tropical Diseases. 84.

6. Choi R, Britigan E, Narayanasamy P (2017) "Ga (III) nanoparticles inhibit growth of both Mycobacterium tuberculosis and HIV and release of interleukin-6 (IL-6) and IL-8 in coinfected macrophages". Antimicrobial agents and chemotherapy. 61: 10-1128.

7. Correia J, Freitas M, Queiroz J, PereiraPerrin M, Cavadas B (2008) Interleukin-6 Blood Levels in Sensitive and Multiresistant Tuberculosis. Infection. 37: 138–141.

8. Ellwanger JH, Valverde-Villegas JM, de Lima Kaminski V, de Medeiros RM, de Matos Almeida E, et al. (2020). "Increased IL-8 levels in HIV- infected individuals who initiated ART with CD4+ T cell counts< 350 cells/mm3–A potential hallmark of chronic inflammation". Microbes and Infection. 22: 474-480.

9. Folador E (2016) Re: "What is the value of the area under the roc curve (AUC) to conclude that a classifier is excellent?".

10. Hober D, Hober D, Benyoucef S, Ajana F, Mouton Y, aet al. (1996) "Plasma levels of sTNFR p75 and IL-8 in patients with HIV- 1 infection - PubMed". https://pubmed.ncbi.nlm.nih.gov/8877420/.

11. Korber M, Muldoon J, Theiler F, Gao R, Gupta A, et al. (2000) "Timing the Ancestor of the HIV-1 Pandemic Strains". Science (New York, N.Y.) .288: 1789-96.

12. Krupa A, Fol M, Dziadek R, Kepka E, Wojciechowska D, et al. (2015) "Binding of CXCL8 /IL-8 to Mycobacterium tuber-cu-losis Modulates the innate immune response", Mediators of Inflammation. 11.

13. Lavanya J, Meenakshi P, Ramya S, Lakshmi K, Prathiba N, et al. (2015) Evaluation of TNF-α, IL-10 and IL-6 Cytokine Pro--duction and Their Correlation with Genotype Variants amongst Tuberculosis Patients and Their Household Contacts. https://doi.org/10.1371/journal.pone.0137727

14. Li S, Cuisong W, Jun Z, Hong X, Xiaoxia L, et al. (2020) Roles and potential applications of lncRNAs in HIV infection, In-ter-national Journal of Infectious Diseases. 92: 97-104.

15. Liu H, Pei-Wen H, Jacob C, Dorothy L, Andrew P (2018) "HIV-1 Replication in CD4+ T Cells Exploits the down Regula-

tion of Antiviral NEAT1 Long Non-Coding RNAs Following T Cell Activation". Virology. 522: 193-98.

16. Mjid M, Cherif Ben S, Toujani S, Ouahvhi Y, Zakhama H, et al. (2015) Epidemiologies de la tuberculose. Rev. Pneumol Clin. 71: 67-72.

17. Navikas V, Link J, Persson C, Olsson T, Höjeberg B, et al. (1995) Increased mRNA expression of IL-6, IL-10, TNF alpha, and perforin in blood mononuclear cells in human HIV infection. J Acquir Immune Defic Syndr Hum Retrovirol. 9: 484-9.

18. Ngongang N, Cybelle F, Kameni C, Ngum J, Ulrich S, et al. (2022) TNF and HNRNPL Related Immunoregulatory Long Non-Coding RNA (THRIL) and long intergenic noncoding RNA-p21 (lincRNA-p21) as potential useful biomarkers for the diagnosis of tuberculosis.

19. Pimpin L, Drumright L, Kruijshaar M, Abubakar I, Rice B, et al. (2011) Tuberculosis and HIV co-infection in European Union and European Economic Area countries. Eur. Respir J. 38: 1382–92.

20. PNLT (2022) "TB-HIV consultation meeting on the integration of new protocols and estimation of needs for TPT".

21. Russell David G (2007) "Who Puts the Tubercle in Tuberculosis? » NatureReviews. Microbiology 5: 39-47.

22. Statello L, Guo C, Chen L, Maite H (2021) Gene regulation by long non-coding RNAs and its biological functions.

23. Shuying H, Zikun H, Qing L, Cheng Q (2018) "The Expression of lncRNA NEAT1 in Human Tuberculosis and Its Anti-tu-berculosis Effect".

24. Tamgue O, Julius E, Frank B (2021) Triptolide Modulates the Expression of Inflammation-Associated lncRNA-PACER and lincRNA-p21 in Mycobacterium tuberculosis–Infected Monocyte-Derived Macrophages.

25. Wada I, Jacobson P, Margolick B, Breen C, Macatangay B, et al. (2015) "the effect of HAART-induced HIV suppression on circulating markers of inflammation and immune activation AIDS", vol 29, p. 463-471

26. World health organization (2020) Global tuberculosis report 2020. Geneva, World Health Organization. Global tuberculo--sis report 2020 (who.int)

27. World health organization (2022) global HIV report, July 2022

28. Xingzhu L, Mengjiao X, Ping L, Wenyuan Z, Ling-hui Z, et al. (2022) Roles of lncRNAs in the transcription regulation of HIV-1.

29. Yang Y, Yan X, Wang K, Tian R, Lu Z, et al. (2018) Triptriolide alleviates lipopolysaccharide-induced liver injury by Nrf2 and NF-kappaB signaling pathways.

30. Zhang Chan C, Yuzhong X (2022). Long Non-coding RNAs in Tuberculosis: From Immunity to Biomarkers Xianyi.