UiT The Arctic University of Norway

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Matrix metallopeptidase plasma levels among HIV-infected children and adolescents with and without HIV-associated chronic lung disease

A cross-sectional study

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Preface

The purpose of this study was to investigate plasma inflammatory biomarkers in HIV-infected children and adolescents with and without HIV-associated chronic lung disease.

The African continent as a whole has seen exponential economical and financial growth during the last decades. However, the HIV/AIDS epidemic and its associated long-term health-related complications greatly affect the younger generations in sub-Saharan Africa. Chronic lung disease (CLD) is one of the main causes of morbidity among children and adolescents with HIV. Despite its high prevalence, its pathophysiology and management is not fully understood. An effort to further understand and document the pathogenesis behind CLD and avoid unnecessary morbidity and loss of life is warranted.

This project was part of the BREATHE trial, a large randomized controlled trial under the supervision of Professor Trond Flægstad which investigated the effects of Azythromycin vs placebo treatment for HIV-associated chronic lung disease in children and adolescents. The author's previous work with pediatric microbiology and interest in a prevalent and relatively newly discovered condition lay the foundation for further cooperation. Matrix metallopeptidases (MMPs) have not, to the author's knowledge, been studied in sub-Saharan African children or adolescents.

Data was collected in Malawi and Zimbabwe and analysis was carried out at the Biomedical Research and Training Institute (BRTI) in Harare, Zimbabwe.

The author would like to thank the entire BREATHE group for introducing the author to several fields of focus, ranging from laboratory research and observational experiences in Harare, Zimbabwe to conferences in Cape Town, South Africa and Sommarøy, Tromsø where research, ideas and knowledge were exchanged freely. A special thanks to Dr. Louis-Marie Yindom (PhD), Mr. Dan Hameiri Bowen (BA Oxon, MSc) and Ms. Bethany Grace Charlton (MSci) for their exceptional support in data analysis.

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enthusiastically. The author would also like to thank Professor Trond Flægstad, Department of Clinical Medicine, UIT for his contribution in assigning the project and later proofreading the thesis, as well as including the author in the BREATHE group.
The author looks forward to working with Professor Trond Flægstad and the BREATHE group in the future. The knowledge and experiences obtained were far beyond any expectations the author may have had when starting this project.

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Abstract

Background and objectives: Among HIV-infected children and adolescents who are receiving antiretroviral therapy, chronic lung disease (CLD) is a major cause of morbidity and mortality. Matrix metallopeptidases (MMPs) are involved in a wide range of physiological processes including the breakdown and turn-over of extracellular matrix. The majority of clinical studies investigating the role of MMPs in lung pathology have been conducted among adults and none have been focused on children with HIV infection and CLD. The objectives of this study were to measure and compare the plasma levels of MMPs among HIV-infected children with and without CLD and investigate the associations between plasma MMPs levels and clinical and laboratory parameters among study participants.

Methods: Data was collected as part of the BREATHE trial, a double-blind, randomized controlled trial in Harare (Zimbabwe) and Blantyre (Malawi). In total 296 children and adolescents were included in the study. Anamnestic data, spirometry and blood samples were obtained from study participants. Statistical differences between groups were calculated using the Mann-Whitney U test and chi-square test. Associations between MMPs and other study parameters were analyzed using regression and were adjusted for age, sex, being underweight, ART regimen and prior treatment for TB.

Results: MMP-1, -7, -8, -10 and -12 were significantly higher among participants with CLD compared to participants without CLD. MMP-10 was significantly higher among those treated for TB (3.09 [IQR 2.88-3.24] vs. 2.94 [IQR 2.81-3.11], P=0.006). Logistical regression showed a significant association between presence of CLD and elevated plasma levels of MMP-1 (OR=3.169 (95% CI 1.257 – 7.988), *P=0.014)*, MMP-7 (OR= 4.981 (95% CI 1.626 – 15.262), *P=0.005)* and MMP-10 (8.487 (95% CI 2.102 – 34.265), *P=0.003)*.

Conclusions: In this population of HIV-infected sub-Saharan African children and adolescents, a significant association between CLD status and elevated plasma levels of MMP-1, -7 and -10 was found. These results suggest that those with CLD may have upregulated expression or dysfunctional regulation of MMPs which may lead to sustained lung impairment.

Abbreviations

AIDS – Acquired immunodeficiency syndrome

ALT – Alanine Aminotransferase

ART – Antiretroviral therapy

ATS – American Thoracic Society

BALF – bronchoalveolar lavage fluid

BREATHE – Bronchopulmonary function in response to azithromycin treatment for chronic lung disease in HIV-infected children

BRTI – Biomedical Research and Training Institute

CI – confidence interval

CLD – chronic lung disease

COPD – Chronic obstructive pulmonary disease

COVID – Corona virus disease 2019

CRP – C-reactive protein

CT – computed tomography

FEV1 – forced expiratory volume in 1 second

FVC – Forced vital capacity

ECM – Extracellular matrix

HIV – human immunodeficiency virus

IL-6 – interleukin 6

IPF – Idiopathic pulmonary fibrosis

IQR – Interquartile range

MMP – Matrix metallopeptidase

NSCLC – Non-small-cell lung carcinoma

OR – Odds ratio

PD-1 – Programmed death 1

RNA – Ribonucleic acid

SD – Standard deviation

TB – Tuberculosis

TB-IRIS – Tuberculosis-immune reconstitution inflammatory syndrome

TIMP – Tissue inhibitor of metalloproteinase

UNAIDS – United Nations Programme on HIV/AIDS

VL – Viral load

WHO – World Health Organization

1. Background

1.1 Historical Overview

During the last 4 decades, there has been continuous research to further our understanding of the HIV virus and the mechanisms behind its pathophysiology and the consequences of long-term infection. Although the HIV virus is believed to have existed for almost a century, with sporadic case reports on AIDS being documented up until the 1970s, it was during the early 1980s that HIV/AIDS became a household term in the western world; when the gay community in San Francisco became the epicenter for the epidemic. Following the initial outbreaks in the USA and Europe, international attention and funding was allotted to research, treatment and prevention of HIV/AIDS throughout the 1980s and 1990s. As the situation tends to be in many similar global crises, developing countries were and still are hardest affected by the epidemic. By July 2002, UNAIDS reported AIDS to be the leading cause of death sub-Saharan Africa and fourth leading cause of death globally. 2001 marked the beginning of the international effort in introducing wide-spread access to antiretroviral therapy (ART) in developing countries and has been widely regarded as a global health success story. Since the introduction of highly active ART in 1996, the number of AIDS-related deaths have declined substantially (1). WHO reports that HIV-related deaths fell by 45% between 2000-2018. ART leads to sustained virologic suppression and $CD4^+$ T-cell repletion, resulting in increased survival across multiple patient populations (2).

Despite all efforts, as of 2018, 37.9 million people were living with HIV globally. 1.7 million are children under 15 years of age, and 1.1 million of them are living in Eastern and Southern Africa (3). While the proportion of vertical transmission of HIV occurring at birth has been greatly reduced, UNAIDS has reported that 84 000 children became infected in 2018 (3). Due to widespread access to ART, these children are now surviving into adolescence and early adulthood (4).

1.2 HIV, pathogenesis and treatment

The HIV virus is able to infect the lymphocytes, replicate within the cells and subsequently migrate out of the cells to infect new immune cells. The aim of ART is to prevent HIV replication and HIV infection of vital immune cells, especially $CD4^+$ T-cells, dendritic cells

and macrophages. ART drugs utilize different mechanisms in order to deter virus propagation in the body. Some drugs aim to hinder the virus from entering the immune cells, others aim to destroy or prevent the virus's proliferation inside the immune cells. Still other therapies prevent the virus from exiting the cells and infecting new cells (5).

1.3 Lung disorders are common chronic complications associated with HIV

The scientific and medical communities have recently taken an interest in how surviving HIV-infected children and adolescents fair under long-term HIV infection and subsequently long-term ART treatment. While the increased access to ART has substantially increased the life expectancy and reduced AIDS-related mortality, the life expectancy of HIV-infected individuals is still lower than the general population. This has been attributed to a rise in the prevalence of chronic non-infectious diseases (6, 7).

Lung complications are especially common in HIV-infected individuals. Chronic lung complications encompass several chronic inflammatory conditions affecting the respiratory system. Examples of such conditions can be found in The National Cancer Institute's definition and include asthma, chronic obstructive pulmonary disease (COPD), pulmonary fibrosis, bronchiolitis obliterans and pneumonitis. Asthma, primary lung cancer, pulmonary hypertension and COPD are commonly observed in HIV infected individuals (8, 9).

While several environmental risk factors such as smoking, chronic lung infections and intravenous drug use predispose to the development of chronic lung complications, HIV infection and the use of ART may also contribute to chronic lung impairment (10). While ART treatment has generally been regarded as a facilitator to improve outcomes of infectious pulmonary complications, its influence on noninfectious lung complications has been more controversial and will be discussed further in this thesis.

Few studies have reported the prevalence of chronic respiratory conditions among HIV-infected children and adolescents. One study investigated chronic lung complications among 116 HIV-infected adolescents attending two outpatient clinics in Harare, Zimbabwe. 69% of participants were on ART at the time the study was conducted (11). 45% of the participants were reported to have a Forced Expiratory Volume at one second (FEV_1) < 80%, representing

airway obstruction. Another study performed in Malawi showed that 31,8% of the HIV infected adolescent possessed lung function abnormality (12).

1.4 Pathogenesis behind lung impairment in HIV

The pathogenesis behind chronic lung impairment in individuals with HIV is multifactorial and not completely understood (13). Some factors that may contribute to chronic lung damage are persistent HIV viremia, local and systemic inflammation and respiratory infections.

1.4.1 Persistent viremia and lung impairment

As previously stated, ART prevents HIV replication in $CD4_+$ T-cells, thus allowing the immune system to recover. This happens through the restoration of the $CD4_+$ reservoir. Loss of $CD4_+$ cells causes immune suppression and therefore increased susceptibility to infections.

The goal of ART is to suppress and sustain plasma viral load (VL) to an undetectable level. VL suppression is usually defined as plasma VL below the defined cut off (cut offs may vary depending on VL assay used, <1000, <200, <50 copies/ml etc.) in at least 2 consecutive VL tests. The majority of HIV-infected individuals achieve VL suppression after 12 months after treatment initiation (14). However, studies performed in Sub-Saharan Africa reported that up to 51.6% of perinatally HIV-infected children receiving ART treatment have detectable VL (15-17). Drummond et al reported that HIV-participants with viral loads > 200 000 copies/ml had a 3.4-fold increased odds of obstructive lung disease compared to HIV-negative participants (18). The same authors also reported that participants with HIV viral loads > 75 000 copies/ml had a faster decline in FEV1, meaning a more rapid rate of obstructivity, compared to HIV-negative participants (19). A cross sectional analysis of HIV-participants found that fibrosis-like changes found on CT images were associated with higher viral load (> 500 copies/ml) (20).

1.4.2 Persistent systemic inflammation, immune activation and lung impairment

Studies show that even in individuals with suppressed VL, levels of pro-inflammatory biomarkers in plasma remain elevated compared to uninfected individuals. For example, high-sensitivity C-reactive protein (CRP), interleukin 6 (IL-6) and cystatin C were found to be significantly increased in HIV infected virologically suppressed individuals (21). Several

inflammatory biomarkers (soluble CD14, C-X-C motif chemokine 9 and 10, and soluble interleukin-2 receptor) were found to be significantly elevated in plasma among both viremic and VL suppressed HIV-infected individuals on ART compared to healthy controls (22). Vandergeeten et al suggest that increased levels of proinflammatory cytokines may up-regulate HIV replication, leading to persistent HIV viremia even under ART (23). A study investigating lung impairment among HIV-infected adults in South-Africa found that higher CRP levels were associated with obstructive lung disease (24). Even among healthy adolescents without HIV infection, higher CRP levels were associated with lung function decline (25).

1.4.3 Local inflammation in the lungs

HIV infection can directly infect cells within the respiratory system and can persist in alveolar macrophages and $CD4_+$ T-cells (8). $CD4_+$ T-cells and macrophages in mucosal sites play a vital role in the development of inflammatory response. Among nonlymphoid tissue, animal studies have shown that mucosal sites contain high levels of simian immunodeficiency virus (26). In such sites, $CD4_+$ T cells deplete rapidly and subsequently diminish immune response regulation (27). Direct epithelial HIV infection involves the integration of viral RNA into the cellular host's genome. Such integration may disrupt or alter the cell genome's gene expression and immune response (28). Cell-cell adhesion may also be impaired in the lungs and further lead to lung tissue damage (29).

$CD8_+$ T cells play a vital role in the defense against intracellular pathogens, and aid in destroying cells infected with viruses and bacteria (30). As such, these cells target other cells infected with opportunistic pathogens, such as HIV. However, among HIV-infected individuals not receiving ART, the $CD8_+$ T cells in the respiratory system appear to be defective. One study found that both $CD4_+$ and $CD8_+$ T cells increased levels of the marker Programmed Death 1 (PD-1), indicating heightened exhaustion and subsequently dysfunction of the cells, which was associated with lymphocytic alveolitis among untreated HIV-infected individuals (31). However, under poor viral control, the defective $CD8_+$ T cells are still able to secrete proinflammatory cytokines in response to the HIV antigens expressed on infected cells within the respiratory system (31, 32). They may therefore contribute to local inflammation within the lungs in direct response to HIV infection.

1.4.4 Respiratory infections

Respiratory infections are common in HIV-infected children receiving ART treatment (33). A systematic review reported that the incidence (for 100 child/months) of respiratory manifestations in HIV-infected children initiating ART was 5.35 for upper respiratory tract infections, 9.48 for bronchitis, 2.17 for lower respiratory tract infections and 0.16 for TB (34).

While the incidence of respiratory infections decreased after the wide spread introduction of ART, respiratory events listed in the previous paragraph were still prevalent and represented 40% of all events occurring following ART initiation (34). Both viral and bacterial pneumonia remain common among HIV-infected children receiving ART. In Zimbabwe, 55% of pneumonia deaths were related to HIV (35). George et al found that ART treated HIV-infected individuals had lower FEV_1/FVC ratios, indicating airway obstructivity, if they previously had had bacterial pneumonia. The same study also found ART to be independently associated with lower FEV_1/FVC ratios. George et al found that ART treated HIV-infected individuals had lower FEV_1/FVC ratios, indicating airway obstructivity, if they previously had had bacterial pneumonia (36). Due to recurrent pulmonary infections, many children infected with HIV develop chronic lung impairment with bronchiectasis (37).

When discussing respiratory infections among individuals living with HIV, one must note tuberculosis (TB) infection. Pulmonary TB is a leading cause of mortality among HIV-infected children (37, 38). HIV-infected individuals receiving ART do not have an equivalent estimated risk of contracting TB as uninfected individuals. The incidence of TB in HIV-infected individuals receiving ART is lower than in untreated individuals, but higher than in uninfected individuals (39). In South Africa, the lifetime risk of developing TB in the general population is 10%. For the HIV-infected population, this is the estimated four-year risk of developing TB (40, 41). Furthermore, relapse and reinfection among HIV-infected children has been observed (42). Despite successful treatment, prior TB infection can cause long-term sequelae (33). One South African study found airway obstruction in 68% of participants with a history of TB several years after assessment (43). Clinical presentations following TB infection can include nodular infiltrates, fibrosis and cavitation, individually or in combination (44-46).

One can speculate whether prior TB increases susceptibility to other opportunistic pathogens for HIV-infected individuals. A recent study found that both active and latent TB increased

susceptibility for COVID-19 infection as well as disease severity. Out of the COVID-19 patients classified as severe/critical cases, 78% had TB co-infection (47).

1.5 MMPs - biomarkers that may contribute to lung impairment

1.5.1 Classification

Matrix Metalloproteinases (MMPs) are a family of extracellular proteases. They are all secreted as proenzymes and therefore require extracellular activation (48). They are also endopeptidases; therefore, their main function is to assist in proteolysis, i.e. the breakdown of proteins into peptides or amino acids caused by cleavage of peptide bonds. MMPs are responsible for the regular breakdown of the extracellular matrix (49). Indirectly, this also causes the release of signaling molecules from the matrix (50).

1.5.2 Structure

All MMPs have similar structure domain but differ in substrate specificity and function. They have three common domains; a pro-peptide, a catalytic domain and a hemopexin C-terminal (51). The pro-peptide is part of a structure known as the “cysteine switch”. This structure retains a cysteine residue that binds to a catalytic zinc ion, which inhibits the enzyme, i.e. keeping its structure in its inactive form. To activate the enzyme, the pro-peptide must be removed (52). The active sight of the domain contains a zinc ion necessary for catalyzation. The C-terminal functions as a recognition sequence for the substrates and contributes to substrate specificity (48).

1.5.3 Function

MMPs are secreted by a variety of cells and are involved in a wide range of physiological processes including inflammation, turn-over of extracellular matrix (ECM) and tissue remodeling (53). MMPs are known to facilitate wound repair by clearing out damaged ECM and preexisting damaged capillary walls, promoting angiogenesis. They also contribute to reorganization of the tissue matrix and control the timed release of signaling proteins from the ECM, many of which are stored in the ECM and can only be released and/or activated by the MMPs breaking down the matrix (54). In this way, MMPs impact wound repair in a positive way. However, excessive MMP activity is detrimental to the ECM environment by degrading both ECM and growth factors excessively. With constant degradation, the ECM will weaken and will no longer provide the structural support that the tissue requires to function properly

(55). Invading bacteria in wounds that stay open too long can release proteinases that also degrade growth factors. In defense, the body will release components such as hydrogen peroxide and inflammatory factors, which at high levels can cause tissue damage, which in turn can lead to elevated expression of MMPs (54). This cycle can keep a wound in a chronic stage and can be further facilitated by reduced levels of Tissue Inhibitors of Metalloproteinases (TIMPs)(56).

TIMPs can inhibit MMP activity in tissues and thus prohibit excessive ECM degradation and excessive inflammation. TIMPs also take part in inflammatory response within wounded tissue and the remodeling of new ECM. An overexpression of MMPs (or too little TIMPs) may contribute the chronic inflammation and delayed repair (54, 56).

1.5.4 Potential role of MMPs in HIV infection

A few studies have investigated a possible relationship between HIV progression and MMPs. Levels of MMPs were found to be significantly higher among HIV-infected individuals compared to uninfected individuals, possibly indicating an association between HIV infection and sustained immune activation and cytokine dysregulation (57). Time on ART may also influence MMP levels. Significantly higher plasma levels of MMP-1 were found among infected individuals on long term ART compared to untreated HIV-infected individuals as well as healthy uninfected individuals (58).

1.5.5 MMPs in lung impairment/ diseases

In healthy lungs, several cell types can express MMPs. These cells include structural cells within the bronchial tree and alveolae, as well as by inflammatory cells upon stimulation. The activity of these cells can be modified during pathological conditions, altering MMP (and TIMP) expression and activity and thus implicating these enzymes in various lung diseases (59). In this study, we focused on MMPs that have been regarded to be of significant interest in studies on pathogenesis of lung impairments (44, 60, 61). *Table 1.* presents the MMPs included in this study and the roles they play in the progression of lung impairment.

1.6 Justification of this study

Mapping out the functions of the individual MMPs and the role they play in various pathological processes are still at an early stage. There is however a growing interest in their possible role as either a diagnostic biomarker or therapeutic target for chronic inflammatory lung diseases (53, 95). The majority of clinical studies investigating the role of MMPs in lung pathology were conducted among adults. Very few studies were focused on MMP levels among children and adolescents, much less in those with HIV infection. There are, to our knowledge, no conducted studies investigating MMP levels among HIV-infected African children with chronic lung impairment.

Therefore, the aim of this study was to:

- Examine and compare the levels of the aforementioned plasma MMPs among HIV-infected children with and without chronic lung disease.
- Investigate the associations between plasma MMPs levels and clinical and laboratory parameters among study participants.

2. Materials and Methods

2.1 Study population

For this master project we used data collected as part of a double-blind, randomized controlled trial investigating the effect of azithromycin in children with HIV-associated chronic lung disease (BREATHE trial, clinicaltrials.gov identifier NCT02426112). The detailed study protocol has been published elsewhere (96). BREATHE trial was conducted at outpatient HIV clinics in Harare (Zimbabwe) and Blantyre (Malawi) in collaboration with Biomedical Research and Training Institute in Harare, Zimbabwe and Malawi-Liverpool-Wellcome Trust Clinical Research Programme in Blantyre, Malawi. For this study, participants from Zimbabwe were included.

2.2 Participant eligibility

In order to be included in the trial, HIV-infected participants were assumed to be infected perinatally and aged 6-19 years. Participants had to be on ART for minimum 6 months, with no evidence of active TB or other acute respiratory tract infection and with a fixed airway obstruction defined as FEV1 z-score < -1 with no reversibility. The comparison group was comprised of HIV-infected participants with normal lung function (defined as FEV_1 z-score > 0), no active TB, no acute respiratory tract infection at the time of enrolment, no history of chronic respiratory symptoms within the last 3 months and MRC dyspnea score < 2.

Consent had to be obtained from a guardian for participants < 18 years, in addition to assent from participants. Meanwhile, individual consent had to be obtained from participants ≥ 18 years. Factors leading to exclusion from the trial included having conditions that may prove fatal during the study period, active TB, acute respiratory tract infection at the time of screening, pregnancy or breastfeeding, history of cardiac arrhythmia, a prolonged QTc interval, abnormal creatinine clearance or elevated ALT, known macrolide hypersensitivity, and concomitant use of digoxin and/or fluconazole or other drugs known to prolong the QTc interval.

The HIV-infected participants were recruited during the period April 2017-January 2018.

2.3 Data collection and study procedures

All participants completed a questionnaire, underwent a clinical examination, spirometry and blood sample collection.

A questionnaire was administered to all participants in order to obtain the demographic and clinical history data. The questions were aimed at details on HIV diagnosis and treatment, respiratory symptoms within the last 3 months (chronic cough, wheezing, dyspnea). Self-reported and physician-diagnosed heart, lung (including history of prior TB and asthma) and other disorders were recorded.

The clinical examination included height, weight, heart rate, respiratory rate and oxygen saturation using a pulse oximeter (OxyWatch, Beiijing Choice Electronic Technology Co. Ltd).

All participants underwent spirometry testing in accordance with the ATS guidelines (97). The clinical implications for spirometry testing are based on its ability to reliably and non-invasively quantify lung obstruction. Up to 8 measurement attempts were made and the largest FEV_1 and FVC for each participant was recorded. Obstruction was classified using FEV_1 z-scores rather than using FEV_1 as a percentage of the predicted value (FEV_1 % pred). This was done in order to avoid certain biases that are inherent when using FEV_1 % pred, such as age, weight and sex differences pertaining to lung function distribution (98). Z-scores were presented in accordance with the GOLD (Global Lung Function Initiative) reference ranges (99) and defined as FEV_1 z-score < -1 with no reversibility. Participants with FEV_1 z-score <-1 repeated spirometry 15 minutes after administration of 200 μcg inhaled salbutamol via spacer. The largest values for post- FEV_1 and post-FVC were recorded. Reversibility was defined as < 12% improvement in FEV_1 after inhalation of a β_2 agonist, in this case 200 μg salbutamol. The spirometer used was the EasyOne™ spirometer (ndd Medical Technologies Inc., Andover, MA, USA) and was utilized by certified staff.

Blood samples were collected for full blood count tests, HIV viral load and CD4 count. HIV viral load was measured using the Gene Xpert assay (XpertTM HIV-1 Viral Load; Cepheid, Sunnyvale, CA, USA), with a lower limit of detection at 40 copies/ml and CD4 count was measured as a point of care test using a PimaTM Analyser (Alere, Orlando, FL, USA).

2.4 Plasma sampling and storage

All participant blood samples were collected in EDTA tubes and transferred to BRTI laboratory. Samples were centrifuged, aliquoted, and aliquots of plasma were stored for further analysis. Blood samples were collected from HIV-infected participants without CLD at baseline only. HIV-infected participants with CLD were sampled at 3, 6, 9, 15, 18 months of the BREATHE trial follow up period. For the present master project, only samples collected at baseline were included in the analysis.

2.5 Laboratory analysis of plasma samples

Plasma levels of biomarkers MMP-1, 3, 7, 8, 10 and 12 were analyzed using the principle of Luminex Assay. The principle of this technique is centered around the use of color-coded magnetic microspheres, also called beads (100). The surface of the beads is covered with carboxyl groups, which act as attachment sites for biological molecules, such as proteins. The bead can couple to a desired capture molecule that can then bind with a specific target. Finally, a reporter molecule with fluorescent dye is added which makes identification of the target possible. The specific dye concentrations are used by the Luminex instruments to identify each bead and the determined target it combined with, thus determining the capture molecules that are attached to the bead (101). A schematic illustration of the Luminex multiplex bead assay workflow can be found in *Figure 1*.

The aim of the multiplex assays is to quantitively measure multiple analytes using an automated 96-well plate format. The main advantage of this method is the possibility to analyze multiple analytes simultaneously without requiring large amounts of material.

The MMPs were measured on a MagPix instrument according to the manufacturer's protocol (Luminex technology, Hertogenbosch, Netherlands). All samples were run in duplicate on the same machine and any biomarker measurement falling outside of the standard curve were repeated at an appropriately adjusted dilution. MMP levels in plasma were expressed in picograms per millilitre (*pg*/*mL*).

2.6 Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics 26. A P-value of <0.05 was considered statistically significant. MMP values were presented using median and

interquartile range [IQR]. Weight-for age and height-for-age *z*-scores were calculated using British 1990 Growth Reference Curves (102) with *z*-scores less than -2 representing wasting and stunting, respectively.

Descriptive data was presented using median and interquartile range [IQR] for continuous variables, and numbers and percentages for categorical variables. Characteristics between the two study groups were compared using Fisher's exact test for categorical variables and the Mann-Whitney U-test for continuous variables. The Mann-Whitney U-test was also used to calculate pairwise differences between different groups.

The distribution of the MMP values was assessed graphically. Due to non-normal distribution of MMP levels, the values were log transformed to approximate normality. The analyses were performed with log transformed MMP data, after which the values were transformed back.

Linear and logistic regression were used in order to study the associations between MMP values and patients' characteristics. Only single MMPs were studied in analysis since each MMP has its own substrate specificity and mechanism of action. Linear regression was used to test the association between prior TB treatment and MMP levels (prior TB treatment as the explanatory variable and MMP levels as the outcome). The association between different MMP levels and CLD status was analyzed using logistic regression. Further adjustments were made for age, sex, being underweight, ART regimen and prior treatment for TB. ART regimen, particularly the use of protease inhibitor-based regimen, has been associated with CLD (103), and was therefore included in the adjusted analysis.

2.7 Ethical approval

The BREATHE trial was approved by Medical Research Council of Zimbabwe, College of Medicine and Research Ethics Committee (Malawi), London School of Hygiene and Tropical Medicine Ethics Committee (United Kingdom) and the Regional Committee for HealthResearch Ethics (Northern Norway).

3. Results

3.1 Characteristics of study population

In total, 296 HIV-infected participants were included in the study, 241 participants with CLD and 55 without CLD. Baseline characteristics of the participants and their distribution across both groups are presented in *Table 2*. There were significantly more females among those without CLD compared to participants with CLD (67.3% vs. 46.9%, *P=0.007*). HIV-infected participants with CLD were more likely to be underweight and stunted compared to those without CLD (underweight: 40.7% vs. 12.7 %, *P<0.001*; stunted: 36.5% vs. 20.0 %, *P=0.026*). The median time on ART was 6 years and comparable across two study groups. A protease inhibitor-based ART regimen was more prevalent among participants with CLD compared to participants without CLD (30.7% vs, 10.9%, *P=0.002*). Being treated for TB was more common among participants with CLD than among participants without CLD (34% vs. 14.5 %, *P=0.005*).

3.2 MMP levels among participants with CLD and participants without CLD

The median MMP levels in study groups are presented in *Table 3*. With the exception of MMP-3, all other analyzed MMPs were significantly higher among participants with CLD compared to participants without CLD when compared using the Mann–Whitney U test.

3.3 MMP levels among participants with CLD and prior TB treatment

34% of individuals with CLD were treated for TB. *Table 4.* shows the median and interquartile values for MMP among participants with CLD based on previous TB treatment. Interestingly, MMP-10 was significantly higher among those treated for TB (3.09 [IQR 2.88-3.24] vs. 2.94 [IQR 2.81-3.11], P=0.006). Borderline significant trend was also observed for MMP-7 (3.20 [IQR 3.05-3.33] vs. 3.13 [IQR 2.98-3.26], P=0.054). There was no evidence of association between CLD and CD4 count or HIV viral load.

3.4 Associations between MMPs and prior TB treatment

Using linear regression, prior TB treatment was investigated as a predictor for MMP levels (log transformed MMP values were used as the dependent variable). Prior TB treatment was significantly associated with higher MMP-10 (β= 0.118 (SD 0.099), *P=0.034)* levels adjusted

for age, sex, being underweight and ART regimen. The remaining analyzed MMPs were not found to be statistically significant, as summarized in *Table 5*.

3.5 Associations between CLD and MMP values

Logistic regression analysis was used to study the association between MMP levels and presence of CLD, adjusting for age, sex, being underweight, ART regimen and history of TB treatment. The analysis showed a significant association between CLD status and elevated plasma levels of MMP-1 (OR=3.169 (95% CI 1.257 – 7.988), *P=0.014)*, MMP-7 (OR= 4.981 (95% CI 1.626 – 15.262), *P=0.005)* and MMP-10 (8.487 (95% CI 2.102 – 34.265), *P=0.003)*. There was no significant difference in plasma levels of MMP-3 and MMP-12 between participants with and without CLD in the unadjusted or adjusted model (*Table 6*).

4. Discussion

4.1 Main findings

This study showed that HIV infected children with CLD have significantly elevated plasma levels of MMP-1, -7, -8, -10 and -12 than those without CLD. CLD status was significantly association with elevated levels of MMP-1, -7 and -10 after adjusting for age, sex, being underweight, ART regimen and prior treatment for TB.

4.2 MMPs and HIV

Few studies have investigated MMP levels among HIV-infected individuals, much less those with CLD. However, biomarkers have been investigated in association with HIV. Letizia et al compared plasma levels of inflammatory biomarkers between adults living with HIV receiving ART and healthy non-infected adults in Kenya. The study found that participants with HIV had significantly higher levels of MMP-1 and MMP-7 compared to healthy individuals. Authors suggest that the elevated levels indicated a "chronic state of immune activation and cytokine dysregulation associated with HIV disease " (57). Babu et al measured blood levels of inflammatory biomarkers in HIV-infected individuals in India. The biomarker levels were compared between healthy participants, HIV-infected participants receiving ART and untreated HIV-infected participants. MMP-1 levels among the participants receiving ART were significantly higher compared to the two other groups (58). Our study only included participants with HIV who were receiving ART and was therefore not able to demonstrate an association between HIV status and/or ART and elevated levels of MMP-1 or MMP-7. However, detectable levels of MMP-1 and MMP-7 was found in both study groups included in this study, which supports previously published findings.

MMP-1 is usually nearly undetectable in normal, healthy tissues, with elevated levels being expressed during inflammatory processes or other pathological conditions in tissues (104). MMP-1 has been associated with respiratory conditions such as COPD and idiopathic lung fibrosis (67)(66). MMP-7 is been associated with chronic respiratory conditions such as idiopathic lung fibrosis, cystic fibrosis and HIV-associated TB-IRIS (75, 76, 78). Higher serum levels of both MMP-1 and MMP-7 among participants with CLD compared to participants without CLD in our study could suggest that these biomarkers may not

necessarily be influenced by HIV status in itself or ART, but rather the subsequent pathological changes within the respiratory system.

4.3 MMPs and prior TB

TB-IRIS is an excessive immune response to *Mycobacterium tuberculosis* (105). Tadokera et al investigated involvement of several other MMPs in TB-IRIS in adult participants. Peripheral blood mononuclear cells (PBMCs) were stimulated by *Mycobacterium tuberculosis* and the cells' MMP gene expression and secreted protein were then measured. Their results showed increased expression levels of MMP-1, MMP-7 and MMP-10 among TB-IRIS participants in 24 h cultures compared to non-IRIS controls. Additionally, serum levels of MMP-7 was shown to be significantly higher among TB-IRIS participants compared to non-IRIS controls (76). While this study did not demonstrate an association between the aforementioned MMPs and prior TB treatment, an interesting finding was a significant association between elevated plasma levels of the same MMPs and CLD status. This corresponds with Tadokera et al's results among TB-IRIS participants. MMP-7 has a functional importance in wound healing and has increased expression within wounded pulmonary epithelial cells (60). Elevated serum levels of MMP-7 in both studies could suggest that MMP-7 may not be influenced by the presence of *M. tuberculosis*, but can play a role in ECM repair rather than destruction in both TB-IRIS and CLD.

Another interesting finding in our study was significantly elevated plasma levels of MMP-10 among CLD participants who were previously treated for TB compared to CLD participants without prior TB treatment. Furthermore, prior TB treatment was found to be a predictor for elevated levels of MMP-10. This is similar to the findings reported by Tadokera et al (76).

Pulmonary TB can lead to chronic lung dysfunction (44). Even after completing treatment for TB, *M. tuberculosis* can persist in alveolar macrophages. Non-replicating persistent *M. tuberculosis* can maintain inflammation in the lungs and eventually lead to lung fibrosis (33). The macrophages may also be directly infected by HIV, and lose their functions, such as their microbicidal ability. This leaves the host at increased risk of infection (106). Paradoxically, McMahan et al stated that MMP-10 functions as an essential mediator in alveolar macrophage activation and subsequent pro-inflammatory activity. Knock-out mice deficient in MMP-10 were much more susceptible to infection, indicating that MMP-10 serves a protective role in

the defense against acute infection (88). One could speculate that elevated levels of MMP-10 among CLD patients with prior TB treatment may function as an indicator of up-regulated alveolar macrophage activity in our study. Alternatively, MMP-10 may function as an indicator of macrophage dysfunction, which can occur during HIV infection as well as TB infection (33). Thus, elevated levels of MMP-10 among CLD patients with prior TB treatment may reflect upregulation and/or dysfunction of alveolar macrophages, contributing to immunological dysfunction and ultimately chronic changes within the lung.

4.4 MMPs and lung impairment

Regardless of HIV status, a fair amount of publications reports elevated MMPs in individuals with respiratory conditions such as COPD, emphysema and lung fibrosis.

One of the most common lung diseases is chronic obstructive pulmonary disease (COPD). Most commonly associated with smokers, COPD is characterized by airway obstruction caused by premature collapse of the small airways. This is partly due to composition of the ECM being different in the lungs of patients with COPD compared to the ECM in healthy lungs. One of the first changes to occur in COPD lungs is inflammation and recruitment of macrophages which secrete enzymes such as MMP-12. The enzymes lead to the degradation of elastic fibers in the parenchyme which negatively affects the recoil properties of the ECM (107). Kraen et al studied adults with co-existing COPD and carotid plaque, and found elevated blood levels of MMP-1, -3, -7 and -12 compared to participants with no carotid plaque and no COPD (108). The study also included a group who had COPD with no plaque who showed elevated levels MMP-1 compared to healthy individuals. Another study found similar results; significantly increased serum levels of MMP-1, -3 and -7 among participants with COPD compared to controls (109). While our study did not demonstrate an association between MMP-3, MMP-12 and CLD status, there is a similarity with elevated levels of MMP-1 and MMP-7 being associated with CLD status.

Emphysema is a type of COPD which is characterized by the permanent enlargement of the small airways and subsequently lead to a decline in available surface area for gas exchange (110). Emphysema leads to obstruction and reduced air flow by loss of elastic recoil in the lung parenchyme and loss of the alveoli's supporting structure. Goldklang et al presents the pathophysiology behind emphysema and points to MMP-1 being one of the main proteinases

to contribute to ECM destruction within emphysemous lungs (111). Alveolar septal cell death is additionally presented as a main facilitator of emphysema development. Through pro-apoptotic pathways, phagocytosis and pro-inflammatory processes are activated, which lead to destruction of the small airways (111). In a study discussing MMPs possible role in emphysema, MMP-10 is suggested to promote the disease through influencing macrophages, leading to inflammatory and proteolytic processes (110). It may be reasonable to assume that both MMP-1 and MMP-10 could play a similar role in CLD, as elevated levels were associated with CLD in our study.

Idiopathic pulmonary fibrosis (IPF) is a restrictive lung disease characterized as a "chronic, progressive fibrosing interstitial pneumonia" whose cause remains for the most part unknown (112). One theory is that IPF may be caused by excessive production of pro-fibrotic mediators as a result of abnormal wound healing (113). Similar to COPD, IPF is also characterized by abnormal ECM structure within the lungs. A transitional review describing evidence that links MMPs to IPF reported that several MMPs, including MMP-1, -3 and -7 are upregulated in the blood samples of patients with IPF compared to healthy controls (114). In contrast to Goldklang et al who suggested that MMP-1 contributed to ECM and lung destruction in emphysema, Herrera et al describes MMP-1 expression as an inhibitor of apoptosis in alveolar epithelial cells in IPF. MMP-1 expression was reported to be up-regulated within alveolar epithelial cells from IPF lungs(66). The difference in reported functions may point to MMP-1 having different roles when contributing to the pathophysiology behind emphysema and IPF.

Within our study, MMP-1, -7, and –10 were significantly associated with CLD status, but not MMP-3 and -12. While MMP-12 may influence elastin degradation in the lungs, it may act within a separate pathway of macrophage activation among patients who develop COPD, whose pathway converges on a common pathway that includes other MMPs, which influence ECM degradation and are active among individuals with CLD.

MMP-3 levels were not found to be significantly different between the groups in this study. While MMP-3 has been considered to be associated with a variety of diseases, such as asthma, COPD, impaired wound healing and various cancers, many recent studies have been unable to prove a positive correlation between MMP-3 and lung pathology. This may in part be due to the role of MMP-3 in activation of other MMPs, making its role in pathological pathways difficult to identify (48). Therefore, one could speculate whether MMP-3 may act

more as a facilitator rather than a direct participant in the degradation of lung tissue due to unregulated overexpression. Due to contradictory results within our and other studies, further research is needed to measure not only plasma levels of MMPs, but also gene expression and BAL levels, as MMP plasma levels may not be representative of levels in the airways.

4.5 Strengths and limitations

This is one of the first studies investigating the plasma MMPs levels in HIV-infected children with chronic lung pathology. Another advantage of the study is relatively large sample size and the use of standardized method for MMPs testing.

The limitations of this study are its cross-sectional design, thereby permitting only correlation between variables to be established, not causation. This study did not include HIV uninfected/healthy controls that would enable to establish the impact of HIV infection on MMP levels. Additionally, self-report data was utilized for collecting information regarding the demographic and clinical history data, raising concerns of recall bias. Other factors that may have an impact on MMP levels that were not included in the analysis are genetic, environmental and socioeconomic.

5. Conclusions

In order for clinicians to be able to prevent and treat CLD in young patients, it is important to understand the mechanisms that contribute to its development and sustainment. This study investigated the associations between MMP levels and CLD status in HIV-infected population. To the author's knowledge, this is the first study to investigate MMP levels among sub-Saharan African children and adolescents living with HIV and CLD. A significant association between CLD status and elevated plasma levels of MMP-1, -7 and -10 was found after adjusting for confounding factors. This shows that individuals with CLD may have upregulated expression or dysfunctional regulation of MMPs which may lead to sustained lung impairment. This study highlights the potential involvement of MMPs in lung function impairment and contributes towards further understanding of the pathogenesis behind CLD. Further studies are warranted in order to further understand their individual contribution to the pathogenesis in search of new therapeutic approaches.

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7. Tables

Table 1. *The MMPs included in this study and the roles they play in the progression of lung impairment.*

Biomarker	Classification	Function	Role in pulmonary pathology
MMP-1	Collagenase	Cleaves type I, II, III, VII and X collagens (62-64).	• Expression demonstrates emphysema-like pathology in mice (65). • Elevated levels in lungs affected by idiopathic pulmonary fibrosis (66). • Inhibits apotois of alveolar epithelial cells (66). • Increased expression in COPD lungs (67).
MMP-3	Stromelysin	• Plays a crucial role in ECM remodeling (68). • Cleaves collagens III, IV, X, IX (69) • Degrades proteoglycans, fibronectin, laminin and cartilage proteoglycans (69). • Activates other MMPs, including MMP-1 and MMP-7 (68).	• Certain alleles have been associated with an increased susceptibility to NSCLC(70). • Certain genotypes are associated with increased risk of COPD(71). • Possible biomarker for pneumoconiosis(72).
MMP-7	Matrilysin	• Degrade gelatins I, III, IV and V, fibronectin and casein (73). • Activates procollagenase (73). • Antimicrobial properties(74).	• Elevated BAL concentrations among patients with IPF(75). • Elevated levels among patients with HIV-associated TB-IRIS(76). • Increased expression in NSCLC tissue (77). • Potential overexpression in cystic fibrosis lungs (78).
MMP-8	Collagenase	• Cleaves type I, II and III collagens (79).	• Increased expression of MMP-8 in COPD lungs (67). • Associated with IPF and sarcoidosis (80). • Associated with TB-associated matrix destruction (81). • Increased levels associated with TB-IRIS (82).
MMP-10	Stromelysin	• Cleaves fibronectin, laminin and collagen IV (83).	• Elevated levels in patients with HIV-associated TB-IRIS (76) .

		• Activates procollagenase, including MMP-1(83). • Processes laminin-5, enabling cellular migration (84). • Regulates the ECM degrative potential of macrophages (85).	• Overexpression in tumors, including NSCLC(86, 87). • Increased morbidity and lethality in knock-out mice lacking MMP-10 and who were infected with *Pseudomonas aeruginosa* (88).
MMP-12	Macrophage metalloelastase	• Degrades elastin and other ECM component as well as non-matrix components (89, 90)	• Expressed by airway macrophages in emphysematous lungs, not expressed in healthy controls (91). • Linked to smoking-related lung injury and progressive lung function decline in COPD (92) • Expressed exclusively among HIV-infected individuals with early emphysema (93). • Inhibition has shown reduction in both inflammation and airspace enlargement in lung tissue (94).

Table 2. *Baseline characteristics of study participants and their distribution across groups based on CLD status.*

Variables	**CLD+ (N= 241)**	**CLD- (N= 55)**	**P-value**
Age (years), Median (IQR)	15 (13 - 18)	16 (12 - 18)	*P=0.998*
Female sex, *N* (%)	113 (46.9)	37 (67.3)	*P=0.007*
Wasting (weight for age z-score < −2), N (%)	98 (40.7)	7 (12.7)	*P<0.001*
Stunted (height-for-age z-score < −2), N (%)	88 (36.5)	11 (20.0)	*P=0.026*
Passive smoking, N (%)	49 (20.3)	12 (21.8)	*P<0.001*
Viral load > 1000 copies/ml, N (%)	100 (41.5)	17 (30.9)	*P=0.170*
CD4+ < 250 (cells/µl), N (%)	43 (17.8)	7 (12.7)	*P=0.427*
Time on ART, Median (IQR)	6.42 (4.12-8.33)	6.94 (4.08-8.74)	*P=0.404*
Protease inhibitor ART regimen, N (%)	74 (30.7)	6 (10.9)	*P=0.002*
Treated for TB, N (%)	82 (34)	8 (14.5)	*P=0.005*

Table 3. *Level of MMPs among HIV-infected participants with and without CLD.*

Biomarkers	**CLD+ (N= 241)**	**CLD- (N= 55)**	**P-value**
MMP-1, *(*pg/ml)	3.17 (2.97-3.40)	3.08 (2.85-3.28)	*P=0.023*
MMP-3, *(*pg/ml)	3.55 (3.36-3.79)	3.63 (3.35-3.75)	*P=0.750*
MMP-7, *(*pg/ml)	3.16 (3.00-3.28)	3.06 (2.85-3.15)	*P< 0.001*
MMP-8, *(*pg/ml)	3.59 (3.25-3.94)	3.43 (3.01-3.77)	*P=0.018*
MMP-10, *(*pg/ml)	2.99 (2.83-3.17)	2.83 (2.69-3.02)	*P=0.001*
MMP-12, *(*pg/ml)	1.45 (1.27-1.54)	1.36 (1.19-1.43)	*P=0.002*

Note: MMP values were log transformed and presented using median and interquartile range (IQR)

Table 4. *MMP levels among participants with CLD based on prior TB treatment.*

Biomarkers	**TB+ (N= 82)**	**TB- (N= 159)**	**P-value**
MMP-1, *(*pg/ml)	3.20 (3.00-3.40)	3.17 (2.94-3.40)	*P=0.332*
MMP-3, *(*pg/ml)	3.53 (3.32-3.76)	3.56 (3.40-3.79)	*P=0.267*
MMP-7, *(*pg/ml)	3.20 (3.05-3.33)	3.13 (2.98-3.26)	*P=0.054*
MMP-8, *(*pg/ml)	3.61 (3.29-3.92)	3.55 (3.22-3.96)	*P=0.391*
MMP-10, *(*pg/ml)	3.09 (2.88-3.24)	2.94 (2.81-3.11)	*P=0.006*
MMP-12, *(*pg/ml)	1.46 (1.26-1.55)	1.45 (1.29-1.54)	*P=0.575*

Note: MMP values were log transformed and presented using median and interquartile range (IQR)

Table 5. *Linear regression analysis of the association between MMP levels and prior TB treatment adjusted for age, sex, underweight and ART regimen.*

Biomarker	**β-coefficient (SD)**	**P-value**
MMP-1, (pg/ml)	0.049 (0.072)	*P=0.372*
MMP-3, (pg/ml)	-0.098 (0.106)	*P=0.135*
MMP-7, (pg/ml)	0.092 (0.087)	*P=0.094*
MMP-8, (pg/ml)	0.041 (0.051)	*P=0.461*
MMP-10, (pg/ml)	0.118 (0.099)	*P=0.034*
MMP-12, (pg/ml)	-0.001 (0.061)	*P=0.984*

Note: MMP values were log transformed.

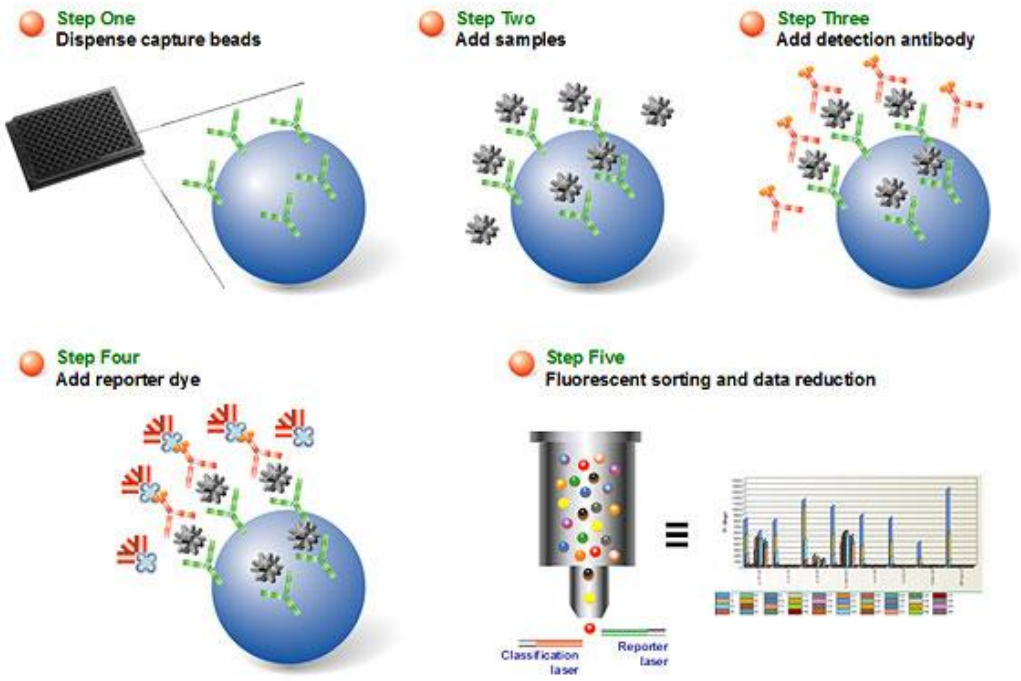
Table 6. *Logistic regression analysis of association between MMPs and presence of CLD adjusted for age, sex, underweight, ART regimen and history of TB.*

Biomarker	**Unadjusted OR (95% CI)**	**P-value**	**Adjusted OR (95% CI)**	**P-value**
MMP-1, (pg/ml)	3.323 (1.394 - 7.919)	*P=0.007*	3.169 (1.257 – 7.988)	*P=0.014*
MMP-3, (pg/ml)	1.270 (0.448 – 3.596)	*P=0.653*	1.720 (0.427 – 6.934)	*P=0.446*
MMP-7, (pg/ml)	5.791 (2.103 – 15.945)	*P=0.001*	4.981 (1.626 – 15.262)	*P=0.005*
MMP-8, (pg/ml)	1.962 (1.077 – 3.576)	*P=0.028*	1.689 (0.908 – 3.143)	*P=0.098*
MMP-10, (pg/ml)	8.630 (2.395 – 31.106)	*P=0.001*	8.487 (2.102 – 34.265)	*P=0.003*
MMP-12, (pg/ml)	1.376 (0.717 - 2.640)	*P=0.337*	1.229 (0.603 – 2-503)	*P=0.570*

Note: MMP values were log transformed

8. Figures

Figure 1. *Schematic illustration of Luminex multiplex bead assay workflow (101).*



9. GRADE

Referanse: Tadokera R, Meintjes GA, Wilkinson KA, Skolimowska KH, Walker N, Friedland JS, et al. Matrix metalloproteinases and tissue damage in HIV-tuberculosis immune reconstitution inflammatory syndrome. Eur J Immunol. 2014;44(1):127-36.			Studiedesign: Kasus-kontroll Grade - kvalitet III
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Undersøke om MMP-aktivitet påvirket immunpatologien og lungedestruksjon i TB-IRIS (TB-associated immune reconstitution inflammatory syndrome). Genuttrykket og proteinseksjon av forskjellige typer MMP ble analysert hos pasienter med TB-IRIS og i en frisk kontrollgruppe.	**Populasjon** **Kasus:** HIV-pasienter med TB-IRIS **Kontroll:** HIV-pasienter med TB uten IRIS **Inklusjonskriterier:** - Over 18 år. - Ikke gravid - Mottar ART-behandling **Eksklusjonskriter** - Behandlingsresistent Tb	**Hovedfunn** Perifere mononukleære blodceller som ble stimulert av M. tuberculosis fikk økt transkriptnivå av MMP-1, MMP-7 og MMP-10 og gener hos kasusgruppen i 24 timers kulturer (pcorr ≤ 0,05) sammenlignet med kontrollgruppen. Perifere mononukleære blodceller fra kasusgruppen hadde økt transkriptnivå av MMP-1, -3, -7 og -10 sammenlignet med kontrollgruppen. Tilsvarende var proteinsekresjon av MMP-1, -3, -7 og -10 i stimulerte kulturer høyere hos kasusgruppen sammenlignet med kontrollgruppen **Bifunn** Behandling med prednisolon førte til signifikant undertrykkelse av MMP-7-genuttrykk i løpet av behandlingsforløpet (pcorr = 0,019).	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Er kasus-kontroll design egnet for formålet?** Ja. • **Er kasus rekruttert på en «god» måte?** Ja, rekruttert som pasientgruppe på sykehus • **Diangosen validert?** Ja, basert på validert konsensusbasert definisjonskriterier. • **Er kontrollene rekrutterte på en «god» måte?** Ja, rekruttert som pasientgruppe på sykehus. • **Kan det utelukkes at kontrollgr. fri for aktuelle sykdom?** Ja. • **Var kasus-kontrollgruppene hentet fra sammenlignbare befolkningsgrupper?**Spekteret av nadir CD4-tall, dager med anti-TB-behandling, varighet av TB-behandling, sykdomsform, alder og kjønn på kasus-pasienter ble brukt til å definere lignende kontrollpasienter. **Non-responders/nekter å delta – frafalls analyser? Forskjeller kasus/kontroll-gruppe?** Ikke beskrevet • **Er gruppene sammenlignbare i forhold til viktige bakgrunnsfaktorer?***Ja, rekruttert fra samme sykehus og pasientgruppe. • **Er main exposure validert?** Ja, enten klinisk og/eller mikrobiologisk (HIV og TBjeller ved oppfyllelse av WHO sine kriterier for HIV-assosiert TB og som responderte til behandling. • **Er gruppene «behandlet» likt?** Ja, de samme prøvene ble tatt fra begge grupper. • **Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse?** Ja, andre faktorer som kan forklare resultatene er diskutert. • **Er eksponering for fare, skade, tiltak målt og gradert likt i begge gruppene?** Ikke relevant for denne studien • **Var den som målte eksponering/samlet inn data blinda mht hvem som var kasus/kontroll?** Eksponering var lik for begge grupper • **Tror du på resultatene?** Ja, et betydelig mengde arbeid var lagt ned for å gjøre gruppene mest mulig like og validerte og anerkjente metoder ble brukt for å samle og analysere data. I tillegg får studien støtte fra litteraturen. • **Kan resultatene overføres til praksis?** Uvisst, mer forskning kreves. • **Støtter litteratruen resultatene?** Ja, noe er diskutert i studien, men det finnes flere studier som støtter resultatene. **Hva diskuterer forfatterne som:** **Svakhet:** - Mekanismer relatert til regulering av MMP-ene som kunne ha forstyrt kvantifisering av dem av provene. - Antikoagulantia kan ha forstyrret MMP-aktivitet - Forskjeller i MMP-uttrykk mellom individene i kasusgruppen kan tilskrives heterogeniteten i TB-IRIS-pasientkohorten. - Små grupper Har resultatene plausible biologiske forklaringer? Ja, noe er diskutert i studien, men det finnes flere studier som støtter resultatene.
Konklusjon			
Funnene indikerer at flere MMP-er er involverte i immunopatologien til TB-IRIS, og kan utgjøre et potensielt terapeutisk mål for modulering av denne tilstanden. Siden kortikosteroider har flere skadelige immunologiske effekter og kan være skadelige i forbindelse med medikamentresistent TB, er det nødvendig med mer spesifikke immunmodulerende tilnærminger i TB-IRIS. Dysregulert MMP-aktivitet kan representere et terapeutisk mål for å redusere immunopatologi uten å forårsake steroidrelatert immunsuppresjon.	**Hovedeksponering:** HIV og tuberkulose **Viktige konfunderende faktorer** - Antikoagulantia kan ha forstyrret MMP-aktivitet -Forskjeller i MMP-uttrykk mellom individene i kasusgruppen kan tilskrives heterogeniteten i TB-IRIS-pasientkohorten **Statistiske metoder** Parvis parametriske data ble analysert ved Students paired t-test, eller via ANOVA. Parvis ikke-parametriske variabler ble analysert ved Wilcoxon signed rank test eller Friedman test. RCT-prøver ble analysert ved Kruskal – Wallis-test uten korreksjon. Uparede parametriske variabler ble analysert ved bruk av uparret t-test for parametriske data mens Mann – Whitney U-testen ble brukt for analyse av uparret ikke-parametriske data. Korreksjoner for flere sammenligninger ble gjort ved å bruke Bonferroni-korreksjonen, dvs. p-verdier multiplisert med (n-1).		
Land			
Sør-Afrika			
År data innsamling			
2005-2006			

Referanse: McHugh G, Rehman AM, Simms V, Gonzalez-Martinez C, Bandason T, Dauya E, et al. Chronic lung disease in children and adolescents with HIV: a case-control study. Trop Med Int Health. 2020;25(5):590-9.			**Studiedesign: Kasus-kontroll** **Grade - kvalitet** III
Formål	**Materiale og metode**	**Resultater**	**Diskusjon/kommentarer/sjekkliste**
Å beskrive klinikken ved HIV-assosiert kronisk lungesykdom (CLD) hos eldre barn og ungdommer som lever med HIV og for å undersøke de kliniske faktorene assosiert med CLD. **Konklusjon** Tilstedeværelsen av CLD indikerer behov for ekstra behandlingsstøtte i tillegg til antiretroviral terapi hos HIV-positive ungdom. **Land** Zimbabwe, Malawi **År data innsamling** 2016-2018	**Populasjon** HIV-positive barn og ungdom i alderen 6-19 år med og uten kronisk lungesykdom (CLD) ved to HIV-klinikker i Harare, Zimbabwe og Blantyre, Malawi. **Kasus** HIV-positive barn og ungdom i alderen 6-19 år som har mottatt ART i minst 6 måneder og som har CLD, definert som FEV1 Z-score under -1. **Kontroller** HIV-positive barn og ungdom med FEV1 z-score > 0 og ingen kronisk hoste de siste 3 måneder. **Inklusjonskriterier** Inklusjonskriterier kasus: - Positiv HIV-status - Mottatt ART i minst 6 mnd. - Alder 6-19 år. - FEV1 Z-score under -1 uten reversibilitet - Ingen tegn til aktiv tuberkulose eller akutt luftveisinfeksjon. Inklusjonskriterier kontroller: - Positiv HIV-status - Alder 6-19 år. - FEV1 z-score > 0 - Ingen kronisk hoste de siste 3 måneder. **Eksklusjonskriterier begge grupper:** - Aktiv TB - Akutte luftveissymptomer - Ikke perinatal smitte - Medisinske kontraindikasjoner **Hovedeksponering:** Positiv CLD status **Viktige konfunderende faktore** - Sosioøkonomiske faktorer - Røyking i hjemmet - Eksponering for innendørs røyk **Statistiske metoder** Til sammenligning mellom gruppene ble Mann – Whitney U-test brukt for kontinuerlige variabler og chi squared-test for kategoriske variabler. Komplett logistisk regresjon ble brukt til å undersøke risikofaktorer for CLD.	**Hovedfunn** I kasusgruppen ble aktuelle respirasjonssymptomer (hoste og kortpustethet) sjeldent rapportert (henholdsvis 9,3% og 1,8%). Imidlertid hadde 152 (43,8%) i kasusgruppen en respirasjonsrate som var over 90-percentilen for aldersgruppen. **Bifunn** Wasting og andrelinjebehandling med ART var uavhengig assosiert med CLD.	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Er kasus-kontroll design egnet for formålet?** Ja, formålet var å sammenligne klinikken hos de med CLD med de som ikke hadde CLD. • **Er kasus rekruttert på en «god» måte?** Ja, rekruttert fra HIV-klinikker. • **Diangosen validert?** Ja, mikrobiologisk validert. • **Er kontrollene rekrutterte på en «god» måte?** Ja, sykehuspopulasjon. • **Kan det utelukkes at kontrollgr. fri for aktuelle sykdom?** Ja, spirometri ble brukt for å validere/utelukke CLD • **Var kasus-kontrollgruppene hentet fra sammenlignbare befolkningsgrupper?** Ja, sykehuspopulasjon i samme aldersgruppe fra like områder. • **Non-responders/nekter å delta – frafalls analyser?** Irrelevant, kun baseline data ble brukt. • **Er gruppene sammenlignbare i forhold til viktige bakgrunnsfaktorer?** Ja, sykehuspopulasjon i samme aldersgruppe fra like områder. Assosiasjonen mellom CLD og a priori-definerte demografiske og kliniske kovariater ble undersøkt ved bruk av multivariabel logistisk regresjon. • **Er main exposure validert?** Ja, spirometri ble brukt for å validere/utelukke CLD • **Er gruppene «behandlet» likt – kan påvirke «exposure»?** Ja. • **Har forfatterne tatt hensyn til viktige konfunderende faktorer i design/analyse?** Alle mulige konfunderende faktorer kan ikke utelukkes, men begge gruppene var rekruttert fra samme type område i begge byene, slik at det er usannsynlig at konfunderende faktorer forklarer forskjellene mellom gruppene. • **Er eksponering for fare, skade, tiltak målt og gradert likt i begge gruppene?** Uklart • **Var den som målte eksponering/samlet inn data blinda mht hvem som var kasus/kontroll?** Ikke relevant • **Tror du på resultatene?** Ja, støttes av litteraturen. • **Kan resultatene overføres til praksis?** Ja • **Støtter litteratruen resultatene?** Ja, vises til i studien **Hva diskuterer forfatterne som:** • **Styrke** Alle deltakerne var screenet og en objektiv case-definisjon ble brukt under rekrutteringen. • **Svakhet** • Tverrsnittsstudie – ingen causalitet • Selvrapportering av TB, mulig CLD ble behandlet som TB. • Ingen billeddiagnostikk brukt for å bekrefte at klinisk diagnose av CLD var «constrictive obliterative bronchiolitis» Har resultatene plausible biologiske forklaringer? Ja, og støttes av litteraturen.

Referanse: Sovershaeva E, Kranzer K, McHugh G, Bandason T, Majonga ED, Usmani OS, et al. History of tuberculosis is associated with lower exhaled nitric oxide levels in HIV-infected children. Aids. 2019;33(11):1711-8.

Studiedesign: Kasus-kontroll

Grade - kvalitet II-III

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer/sjekkliste
Å sammenligne nivåene av eNO (nitrogenksid i ekspirajonsluft) hos barn med og uten HIV-infeksjon samt undersøke sammenhengen mellom eNO og tidligere tuberkulose og luftveisobstruksjon hos HIV-positive barn som mottar ART (antiviral terapi). **Konklusjon** HIV-infeksjon og tidligere tuberkulose var assosiert med lavere eNO-nivåer kan være en markør for HIV og TB-indusert endring i lungefysiologi. **Land** Zimbabwe **År data innsamling** 2007-2008	**Populasjon** HIV-positive individer som tok ART og HIV-negative barn uten aktiv tuberkulose eller akutt luftveisinfeksjon ble rekruttert fra et offentlig sykehus i Harare, Zimbabwe. **Kasus:** HIV-positive individer som mottar antiviral terapi (ART). **Kontroller:** HIV-negative friske individer. **Inklusjons-/eksklusjonskrit.** Inklusjonskriterier kasus: - Positiv HIV-status via perinatal smitte. - Mottatt ART i minst 6 mnd. - Alder 6-19 år. - Ingen tegn til aktiv tuberkulose eller akutt luftveisinfeksjon. Inklusjonskriterier kontroller: - Negativ HIV-status - Alder 6-19 år. - Ingen tidligere hjerte-/lungesykdom. - Ingen tidligere tuberkulose - Ingen rapport om brystsmerter etter aktivitet, dyspné under aktivitet eller kronisk hoste - Normal lungefunksjon. **Hovedeksponering:** HIV-status **Viktige konfunderende faktorer** - Genetiske faktorer (som påvirker aktiviteten til nitrogenoksidsyntase) - Miljøfaktorer (luftforurensning) - Sosioøkonomiske faktorer. **Statistiske metoder** Verdier for eNO var presentert som geometric mean med 95% confidence interval. Weight-for age og height-for-age z-scores var beregnet vha British 1990 Growth Reference Curves, med z-score under -2 representativt for henholdsvis wasting og stunting. Karakteristikker mellom gruppene ble sammenlignet vha Fishers exact test og Kruskal – Wallis eller Wilcoxon rank sum test. Assosiasjonen mellom eNO-nivåer og forklaringsvariabler som ble definert *a priori* ble analysert vha lineær regresjon.	**Hovedfunn** Totalt deltok 222 HIV-positive og 97 HIV-negative individer. Blant HIV-positive hadde 25,7% tidligere hatt TB; 25,2% hadde luftveisobstruksjon, men ingen tidligere TB. HIV-status var assosiert med lavere eNO-nivå [mean ratio 0.79 (95% confidence interval, 95% CI 0.65–0.97), P=0.03]. Tidligere TB hos HIV-positive deltakere var assosiert med lavere eNO-nivåer, justert for alder, kjønn og tid for eNO-testing [0,79 (95% CI 0,67–0,94), P = 0,007]. **Bifunn** Nivået av eNO økte med økende alder og høyde hos HIV-positive barn. Høyere hemoglobinnivå og antall neutrofile var assosiert med høyere eNO-nivå i justert analyse. Kvinnelig kjønn var assosiert med lavere eNO-nivå i justert analyse.	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Er kasus-kontroll design egnet for formålet?** Ja. • **Er kasus rekruttert på en «god» måte?** Ja. Rekrutteringen ble utført ved Harare Central Hospital pediatrisk HIV-klinikk. • **Diangosen validert?** Ja. Serumprøve. • **Er kontrollene rekrutterte på en «god» måte?** Ja, samme område og sammenlignbar aldersgruppe. • **Kan det utelukkes at kontrollgr. fri for aktuelle sykdom?** Ja, serumprøve. • **Var kasus-kontrollgruppene hentet fra sammenlignbare befolkningsgrupper?** Ja, fra samme område og sammenlignbar aldersgruppe. • **Non-responders/nekter å delta – frafalls analyser?** Ingen frafall ettersom kun baseline data var analysert. • **Er gruppene sammenlignbare i forhold til viktige bakgrunnsfaktorer?** Ja. • **Er main exposure validert?** Ja, via serumprøve. • **Er gruppene «behandlet» likt?** Ja. Standardisert protokoll for eNO-testing og spirometri utført av sykepleiere med godt kjennskap til prosedyrene. • **Er eksponering for fare, skade, tiltak målt og gradert likt i begge gruppene?** Ja. • **Var den som målte eksponering/samlet inn data blinda mht hvem som var kasus/kontroll?** Ikke beskrevet. • **Tror du på resultatene?** Ja. • **Kan resultatene overføres til praksis?** Mulig. Det foreslås at de lave eNO-nivåene kan være en markør for unormal lungefysiologi på grunn av perinatalt ervervet HIV-infeksjon og lungeforandringer etter TB. • **Støtter litteratruen resultatene?** Ja, det refereres til mye støttende litteratur. **Hva diskuterer forfatterne som:** • **Styrke** - Prospektiv design - Relativt mange deltakere - Standardisert protokoll for eNO-testing og spirometri utført av sykepleiere med godt kjennskap til prosedyrene. • **Svakhet** Egenrapportering av tuberkulose uten informasjon om TB-lokalisering (lunge- eller ekstrapulmonal) – mulighet for recall bias **Har resultatene plausible biologiske forklaringer?** Ja, godt diskutert med støttende litteratur.

Referanse: Mwalukomo T, Rylance SJ, Webb EL, Anderson S, O'Hare B, van Oosterhout JJ, et al. Clinical Characteristics and Lung Function in Older Children Vertically Infected With Human Immunodeficiency Virus in Malawi. J Pediatric Infect Dis Soc. 2016;5(2):161-9.

Studiedesign: Pasientserie

Grade - kvalitet II

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer/sjekkliste
Beskrive belastningen av kronisk lungesykdom og klinisk nyttige fenotyper av syksommen blant HIV-positive barn i alderen 8-16 år som får ART (antiretroviral terapi) i Blantyre, Malawi. I tillegg vurdere bronkodilatorrespons med inhalert beta-agonistbehandling.	**Populasjon** 160 HIV-positive barn i alderen 8 til 16 år som fikk HIV-behandling i Blantyre, Malawi. Pasientene var ekskludert hvis de bodde utenfor Blantyre, mottok tuberkulosebehandling, hadde Kaposis sarkom, rapporterte akutte respirasjonssymptomer eller krevde sykehusinnleggelse. **Utfall – hoved utfall** Luftveissymptomer, abnormal lungefunksjon. **Viktige konfunderende faktorer** Alder ved behandlingsoppstart. **Statistiske metoder** Univariable assosiasjoner av unormal lungefunksjon ble vurdert ved bruk av logistisk regresjon, med prediktorer ved P <.10 tatt videre til en multivariabel modell i tillegg til alder og kjønn. Utforskende analyse ble brukt for å sammenligne forskjellige prototypedefinisjoner av kronisk lungesykdom. Multivariabel logistisk regresjon ble deretter brukt for å identifisere uavhengige variabler uavhengig assosiert med disse fenotypene.	**Hovedfunn** Symptomer på kronisk lungesykdom var svært utbredt og har to viktige kliniske fenotyper: "hoste" og "hypoksi". Abnormaliteter i lungefunksjon er utbredt, med svak respons på bronkodilatorer i alle aldre inkludert i studiet. **Effektstørrelse** Av de 51 individene med unormal lungefunksjon var gjennomsnittlig økning i FEV1 etter salbutamol 3,8% (95% CI, 0,02–7,53). **Bifunn** Bronkiektasier og bronkovaskulære markeringer ble sett på røntgen thorax hos over halvparten av deltakerne.	**Sjekkliste:** • **Er formålet klart formulert?** Ja • **Var studien basert på et tilfeldig utvalg fra en egnet pasientgruppe?** Nei, kun pasienter som fikk behandling ved klinikker i sentrale Blantyre ble rekruttert. Strenge eksklusjonskriterier. • **Var inklusjonskriteriene klart definert?** Ja • **Var alle pasientene i samme stadium av sykdommen?** Uvisst • **Var responseraten høy nok?** Kun baseline data samlet inn. Dermed ingen frafall. • **Ble det brukt objektive kriterier for å vurdere/validere endepunktene?** Delvis, deltakere rapporterte subjektiv grad av hoste og funksjonsnedsettelse, men objektive mål på lungefunksjon gjennom spirometri, røntgen thorax og mikrobiologiske prøver. • **Ved sammenligninger av pasientserier, er seriene tilstrekkelig beskrevet?** Ja. • **Er prognostiske/konfunderende faktorer beskrevet?** Ja. • **Var registreringen prospektiv?** Nei • **Var oppfølgningen tilstrekkelig for å nå endepunktene?** Ikke relevant. • **Stoler du på resultatene?** Ja. • **Kan resultatene overføres til praksis?** Ja, metodene bruke for å måle lungefunksjon er utbredt. Hoste og hypoksiske fenotyper kan være en nyttig del av diagnostiske algoritmer hvis de blir validert ytterligere. • **Annen litteratur som støtter resultatene?** Ja, og vises til i artikkelen. **Hva diskuterer forfatterne som:** • **Styrke** Ikke diskutert. • **Svakhet** - Tverrsnittsstudie - Mangelen på total lungevolum og "transfer factor measurements» - Fravær av ikke-infiserte kontroller. Har resultatene plausible biologiske forklaringer? Ja, og støttes av litteraturen.
Konklusjon Symptomer på kronisk lungesykdom var svært utbredt med to viktige kliniske fenotyper: "hoste" og "hypoksi". Abnormaliteter i lungefunksjon er utbredt, med svak respons på bronkodilatorer i alle aldre inkludert i studiet. Patologiske årsaker er ikke kartlagt. Hoste og hypoksiske fenotyper kan være en nyttig del av diagnostiske algoritmer hvis de blir validert ytterligere.			
Land Malawi			
År data innsamling 2011			

Referanse: Lawn SD, Badri M, Wood R. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. Aids. 2005;19(18):2109-16.
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Studiedesign: Kohortestudie

Grade - kvalitet II

Formål	Materiale og metode	Resultater	Diskusjon/kommentarer/sjekkliste
Bestemme den langsiktige forekomsten av tuberkulose (TB) og tilhørende risikofaktorer blant individer som får HAART (Highly active antiretroviral therapy) i Sør-Afrika. **Konklusjon** Forekomsten av tuberkulose fortsetter å synke de første 5 årene av HAART-behandling, og HAART kan derfor bidra mer til TB-kontroll i lavinntektsland enn det som tidligere ble estimert. Pasienter med langkommet immunsvikt før behandlingsoppstart hadde vedvarende økt risiko for tuberkulose under HAART; Dette kan gjenspeile begrenset kapasitet for immunrestaurering blant slike pasienter. **Land** Sør-Afrika **År data innsamling** 1996-2005	**Populasjon/Kohorte:** Pasienter som mottok HAART mellom 1996 og 2005 i Cape Town. **Hovedutfall:** Positiv tuberkulosestatus **Statistiske metoder** Forskjeller i proporsjoner ble sammenlignet med $\chi 2$ test. Intraindividuelle sammenkoblede sammenligninger av median CD4-celletall og plasma-RNA-konsentrasjoner ble utført ved bruk av Wilcoxon signed rank test. Mann-Whitney U-testen ble brukt for å sammenligne disse verdiene mellom ulike grupper. Trendanalyser ble utført ved bruk av Cochrane – Armitage-test for lineær trend. Alle testene var tosidig og en P-verdi på 0,05 ble ansett som signifikant. Kaplan – Meier-plott ble brukt for TB-fri overlevelsessannsynlighet. Univariate og multivariate Cox proportional hazards regression models ble brukt for å bestemme risikoen for tuberkulose, som ble uttrykt som en rate ratio.. Variabler ble vurdert for inkludering i den multivariate modellen hvis de ble funnet assosiert med risikoen for tuberkulose i de univariate analysene ved P <0,15.	**Hovedfunn** Forekomst av TB var 3,5 / 100 personår det første året og reduserte signifikant under oppfølgingen, og nådde 1,01 / 100 personår det femte året (P = 0,002). Risiko for tuberkulose var assosiert med CD4 celletall <100 celler/mL (adjusted risk ratio [ARR], 2.38; 95% confidence interval (CI), 1.01–5.60; P = 0.04), WHO klinisk sykdomsstadie 3 eller 4 (ARR, 3.60; 95% CI, 1.32–9.80; P = 0.01) og alder < 33 år (ARR, 2.86; 95% CI, 1.29–6.34; P = 0.01). **Bifunn** Til tross for lignende virologiske respons på HAART, var økningen i CD4-celletall mye mindre blant pasienter som utviklet tuberkulose enn blant de som ikke utviklet tuberkulose.	**Sjekkliste:** • **Formålet klart formulert?** Ja. • **Er gruppene rekruttert fra samme populasjon/befolkningsgruppe?** Ja. Sykehusbasert kohorte bestående av pasienter som fikk HAART mellom 1996 og 2005 i Cape Town. • **Var gruppene sammenliknbare i forhold til viktige bakgrunnsfaktorer?** Ja, inklusjons- og eksklusjonskriterier etter eksponering (HAART-behandling). • **Var de eksponerte individene representative for en definert befolkningsgruppe/populasjon?** Ja. Inklusjonskriterier. • **Ble eksposisjon og utfall målt likt og pålitelig i de to gruppene?** Ja. • **Er den som vurderte resultatene (endepunkt- ene) blindet for gruppetilhørighet?** Ikke relevant. • **Var studien prospektiv?** Ja. • **Ble mange nok personer i kohorten fulgt opp?** Ja. • **Er det utført frafallsanalyser?** Uklart. • **Var oppfølgingstiden lang nok til å påvise positive og/eller negative utfall?** Usikkert. 5 år. • **Er det tatt hensyn til viktige konfunderende faktorer i design/ gjennomføring/analyser?** Ikke beskrevet. • **Tror du på resultatene?** Ja. • **Kan resultatene overføres til den generelle befolkningen?** Mulig, deltakerne hadde et bredt spekter av CD4-celletall og kliniske sykdomsstadier. • **Annen litteratur som styrker/svekker resultatene?** Ja, det refereres til lignende kohorter med samme resultat. • **Hva betyr resultatene for endring av praksis?** Forslag til tilleggstrategier for å redusere risikoen for tuberkulose ytterligere; tiltak som øker immunologisk gjenopprettelse under HAART, vaksinasjon under HAART for å øke TB-spesifikk immunitet eller bruk av isoniazidprofylakse samtidig med HAART. **Hva diskuterer forfatterne som:** • **Styrke** - Lengre oppfølging enn andre studier - Dokumentasjon av endringer i TB insidens over tid - Bredt spekter av inklusjonskriterier resulterte i et kohorte med forskjellige pasientkarakteristikker. - Alle diagnosene ble støttet av mikrobiologisk eller histologisk bevis. • **Svakhet** - Få TB-relaterte hendelser etter utover 5 års oppfølging. Dermed vanskelig å estimere TB-forekomst etter 5 år. - Sykehusbasert kohorte gjenspeiler ikke nødvendigvis TB-forekomst som er observert i samfunnsbaserte HAART-programmer. - Survival bias grunnet dødsfall under oppfølging som da gir et bilde av reduksjon i TB insidens.