Sex-based analysis/Differences in TB Immune Activation

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# 1. Summary/Abstract

TITLE: Sex-based analysis/Differences in TB Immune Activation.

Tuberculosis is one of the leading causes of death in the world.According to NIH reports, tuberculosis (TB), afflicts more human males than females, with a ratio of 1.7 males to female. The disease is still endemic in most of the sub-Saharan African countries. In Uganda, for every one case of tuberculosis reported, there are at least three cases of tuberculosis that are not identified and its because of these cases that the disease remains prevalent in the country. Previous studies show that there are differences in the immune activation of TB among men and women. In this study, we aim to explore possible underlying hormonal and genetic mechanisms that might contribute to differences in the immune activation among females and males.

#Methods We utilized data from a tuberculosis public repository available through Tidyverse (https://dataverse.unc.edu/dataset.xhtml?persistentId=doi:10.15139/S3/AYOFEU). The dataset comprises 60 individuals - 30 males and 30 females. All participants were HIV-negative and tuberculosis-free at the time of enrollment.

The key variables in our analysis include:

CD4 immune activation count Disease severity indicators (extent of lung disease and number of lungs involved) Fat in kilograms Sex/Gender BMI Age of participant These demographic and clinical variables form the foundation of our investigation into tuberculosis-related immune responses.

#Proposed Analysis Method.

The annalysis was conducted in R version 4.4.2. This was done to Compare immune activation levels between males and females.

We computed the male-to-female differences in disease severity using cd4 immune activation counts.We correlated immune markers with disease severity measures/Correlation between activation levels and disease severity. We did this using linear regression and machine learning models to predict which sex will show the most results. We also adjusted for potential confounders like age.

#Results # Results

Our analysis revealed a statistically significant difference in CD4 immune activation counts between males and females. On average, females showed lower CD4 activation counts than males (mean difference = 413) indicating less immune activation in females and a true difference in the body fat between females and males (mean difference = 17.76 95%CI (7.097298 29.972199).

Linear regression models adjusted for age, BMI, and fat mass demonstrated that male sex was independently associated with higher CD4 immune activation (β = 692.58 95% CI = [-786.97, 2172.13]).

Disease severity indicators were more strongly associated with immune activation in males than in females, suggesting a sex-based difference in how immune response relates to disease burden.

The LASSO and Random Forest models identified BMI, sex, CD4+, LBM as the most important predictors of immune activation. These findings support the hypothesis that sex-based physiological differences, particularly in fat composition, contribute to variability in immune activation and disease severity in tuberculosis.

#conclusions. Sex differences in TB is contributed by more than just hormonal influence. Our study shows that TB is more severe in men than it is in women. Regardless of the medical seeking behaviors of women, women tend to a have a high body fat percentage more than men.The higher the fat percentage an individual has, the lower the risk of progression of the tuberculosis infection to disease hence lowering mortality rates among females compared to men. In addition, women tend to have more lean body mass compared to men hence having a lower chance of suffering from adverse effects of TB compared to men.

# 2. Introduction

## 2.1 General Background Information

Tuberculosis (TB) remains a significant global public health challenge. According to the World Health Organization (WHO), reports show that 10 million new cases and 1.4 million deaths in 2023 (1) were as a result of Tuberculosis. The spread of M. tuberculosis, as the pathogen of TB, has long been hypothesized to occur more often in the household than in the community(2). While most of the M.Tuberculosis transmission in the community is attributed to the unknown contact networks that occurs between individuals, this accounts upto 90% of the transmission. The recent outbreak of Tuberculosis in the united states clearly explains the contact network phenomena. Overall, TB accounts for one quarter of the annual deaths that happen worldwide. An estimated 10.0 million people fell ill with TB in 2018. Geographically, Southeast Asia ranked highest (44%), followed by Africa (24%) and the Western Pacific (18%), with lesser percentages in the Eastern Mediterranean (8%), the Americas (3%), and Europe (3%). Among women of reproductive years, TB is the largest cause of death (3). Global Strategies made by WHO, aim at ending the TB epidemic, with targets to reduce the number of deaths caused by TB by 95% and to decrease new cases by 90% from 2015 to 2035(4).

Mycobacterium tuberculosis infection primarily spreads when a person shares airspace with someone who has active tuberculosis and inhales infectious droplet aerosols. Since the bacterium transmits through respiratory routes to susceptible individuals, the household of an infected person becomes a critical environment for intense M. tuberculosis transmission, where most new infections and cases can be detected and treated. Research reports indicate significant differences in tuberculosis responses between men and women. Notably, these studies suggest that women face greater barriers to early detection and treatment of tuberculosis compared to men, potentially impacting disease outcomes and progression.

The World Health Organization, among other institutions, attributes differences in tuberculosis trends between men and women to several factors: access to care and treatment, pregnancy effects, body fat mass, and gender roles that diminish women’s social capital, thereby lowering incidence among them (5). Worldwide, epidemiological data indicate that tuberculosis (TB) rates among HIV-negative men have consistently been higher than among women for many decades (6). While biological mechanisms likely influence these differences, previous studies demonstrate that men experience higher incidence and prevalence of disease, potentially leading to poorer treatment outcomes compared to women. According to an analysis conducted in 2000, TB prevalence among males exceeded that among females in 27 (93%) of 29 prevalence surveys conducted across 14 countries between 1953 and 1997 (6).

Other studies have highlighted that body fat plays an important role in supporting immune function, with women typically maintaining higher fat storage than men. This increased fat storage may contribute to women’s stronger immune responses. While these differences are partly attributed to women’s better healthcare-seeking behaviors, factors such as fat distribution, hormonal profiles, body composition, lean tissue mass, and other biological characteristics differ significantly between sexes, particularly at disease presentation. The objective of this study is to determine whether higher immune activation counts correlate with greater infection severity or illness progression and whether this relationship differs between males and females.

## 2.2 Description of data and data source

The data we used for this project was got from the TRAC dataverse and is fully accessible to everyone. This is considered a safe place where the the Tuberculosis data from the TRAc project is kept and can be used by anyone who wants contribute to TB research. More details about the data can be found [here](https://dataverse.unc.edu/dataset.xhtml?persistentId=doi:10.15139/S3/AYOFEU). The data is publicly available and can be accessed by anyone.

## 2.3 Questions/Hypotheses to be addressed

Since the balance of fat and lean tissue differ in men and women especially at the time of tuberculosis presentation, the objective of this study will be to find out whether there are differences in the Tuberculosis immune activation between males and females. We shall hypothesize on the more immune activation there is, the more infected / sicker the person and whether this relationship differs between males and females.

# 3. Methods

Study Design

This study utilized a cross sectional study design analyzing data from 2017.Sixty TB-confirmed participants (30 males, 30 females, aged 15+) were recruited from Mulago Hospital.

###inclusion and exclusion critera

Our study included patients aged 15 years and older experiencing their first episode of tuberculosis. To maintain data integrity, we excluded individuals receiving hormonal therapy or using hormonal contraceptives. Additionally, patients with comorbidities including diabetes mellitus, cancer, or HIV were excluded. Our strict exclusion criteria ensured a focused research population by omitting all individuals under 15 years of age, those with recurrent TB episodes, patients taking any form of hormonal therapy or contraception, and those diagnosed with diabetes mellitus, cancer, or HIV.

The variables of interest were extracted from the TRAC dataset and exported to an MS Excel sheet. The outcome variable was CD4+ immune activation counts. The primary independent variable was sex (cis-gender) and this was categorized as male and female. The covariates were age at diagnosis,BMI, Fat in Kg, lean body mass in kg(LBM in kg), CD4+ and CD4 immune activation counts. other variables like fat mass index (FMI) and fat-free mass index (FFMI) were also obtained.

## 3.1 Schematic of workflow

Chromosomal sex and hormones play a role in the modulation of immune responses to mTB and thereby contribute to the general outcome of disease. The first cells that encounter Mtb are known as Macrophages. These initiate a local inflammatory reaction which may differ between the sexes(7). This causes the long term containment of TB infection to differ between men and women since their adaptive immune responses are different. See (**tbfig-1?**)

|  |
| --- |
| Figure 1: Impact of sex differences on the immune response to Mtb and their influence disease outcome. |

## 3.2 Data import and cleaning

We imported the data from MS Excel to R. We explored the data and found some missing values for some variables. We decided to drop one observation since the person lacked the primary exposure and did not have most of the details we needed for our final analysis. We categorized the participants age into four categories. That is 17-<28, 28-<40, 40-<60 and >=60.

## 3.3 Statistical analysis

The final cleaned data set contains some negative fat values. Negative values occur when the deuterium dose has not had sufficient time to fully equilibrate with body water, or the dose was not completely consumed. This is seen as low deuterium enrichment, resulting in an overestimation of the size of the body water pool and hence high FFM (FFMI = LBM / height2)and low % body fat.

We applied three supervised machine learning models — linear regression, LASSO regression, and Random Forests — to predict CD4 Immune Activation Count based on Sex, Age, BMI, Lean Body Mass (LBM), Fat mass, and CD4+ cell count. Data pre processing included median imputation for missing values, dummy encoding of categorical variables, normalization, and removal of highly correlated features. Models were evaluated using RMSE and visualized through observed vs. predicted plots. Hyperparameter tuning was performed using grid search with cross-validation.

The significance of Sex as a predictor of immune activation was assessed in each model. For linear models, coefficients for Sex were examined along with their corresponding p-values. The Random Forest model’s variable importance was assessed to identify the predictive contribution of Sex.

# 4. Results

## 4.1 Exploratory/Descriptive analysis

We conducted a thorough data exploration using various visualization techniques and summary tables. Our analysis included box plots, histograms, and correlation plots to identify meaningful patterns in the dataset. We employed scatter plots to examine trends and investigate potential relationships between continuous variables. This comprehensive approach allowed us to uncover several interesting insights within the data.

(**Table1?**) shows the summary statistics of our data variables.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
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| Table 1: Summary distribution of baseline characteristics among males and females.   |  | F (N=30) | M (N=30) | Overall (N=60) | | --- | --- | --- | --- | | **CD4+** |  |  |  | | Mean (SD) | 394 (217) | 289 (181) | 341 (205) | | Median [Min, Max] | 449 [27.0, 786] | 278 [8.64, 735] | 319 [8.64, 786] | | Missing | 1 (3.3%) | 0 (0%) | 1 (1.7%) | | **CD4 Immune activation count** |  |  |  | | Mean (SD) | 1750 (1340) | 2160 (3170) | 1960 (2430) | | Median [Min, Max] | 1520 [94.0, 7350] | 1210 [84.0, 17900] | 1380 [84.0, 17900] | | Missing | 1 (3.3%) | 0 (0%) | 1 (1.7%) | | **Participant age** |  |  |  | | Mean (SD) | 29.9 (12.8) | 30.2 (6.83) | 30.0 (10.1) | | Median [Min, Max] | 26.0 [17.0, 70.0] | 28.5 [19.0, 48.0] | 28.0 [17.0, 70.0] | | **Fat in kg** |  |  |  | | Mean (SD) | 18.9 (22.4) | 0.348 (21.9) | 9.62 (23.8) | | Median [Min, Max] | 10.9 [-0.192, 81.0] | 4.37 [-96.6, 21.3] | 8.70 [-96.6, 81.0] | | **LBM in kg** |  |  |  | | Mean (SD) | 31.1 (21.0) | 54.9 (21.1) | 43.0 (24.1) | | Median [Min, Max] | 39.6 [-42.0, 45.9] | 50.0 [38.2, 151] | 43.4 [-42.0, 151] | |

Table [Table 2](#tbl-correlation) shows the correlation matrix between immune activation markers and body composition variables.

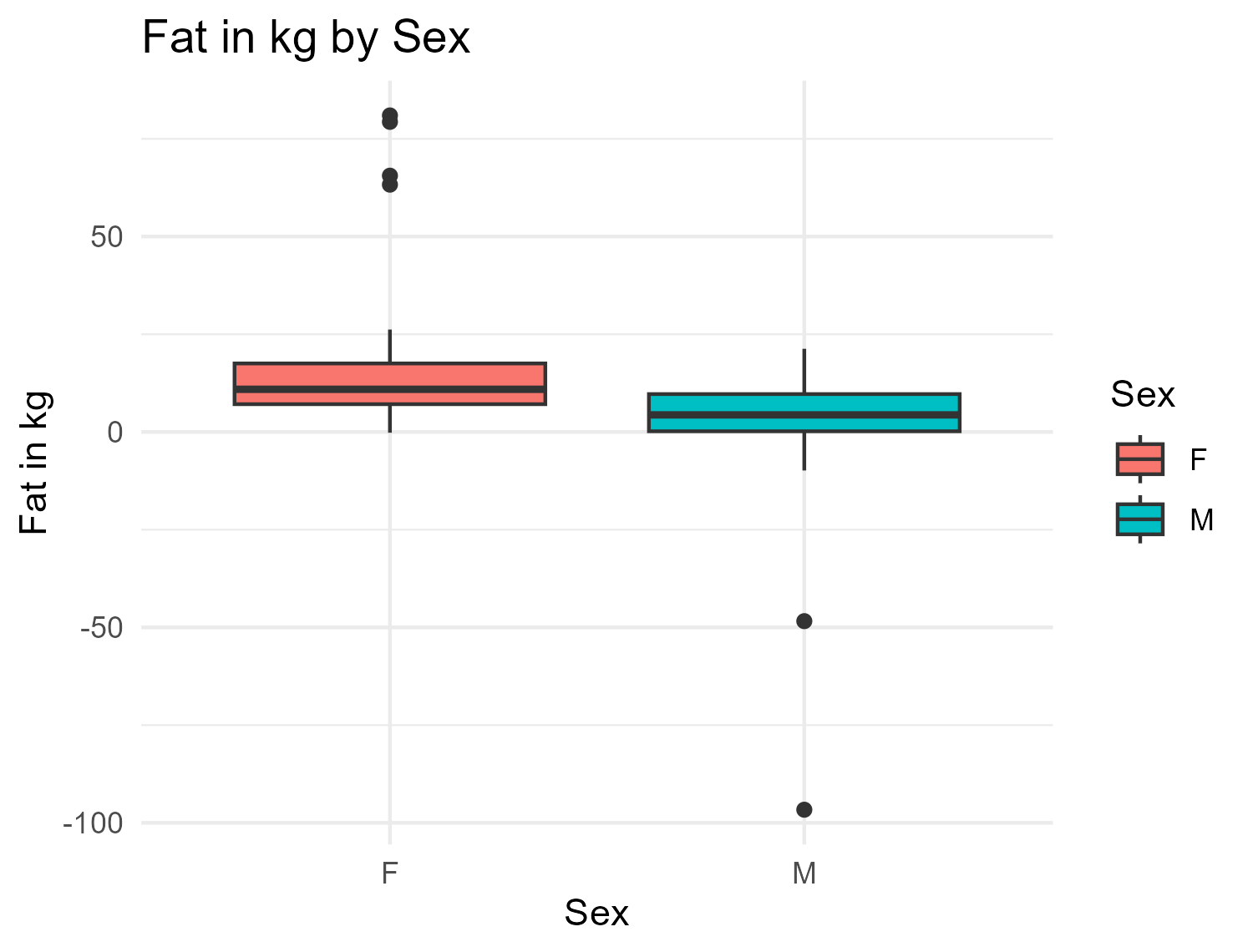
|  |
| --- |
| Table 2: Correlation matrix between immune activation markers and body composition variables.  $`\_data`  Variable CD4+ CD4 Immune activation count Fat in kg 1 CD4+ 1.00 0.22 0.17 2 CD4 Immune activation count 0.22 1.00 0.02 3 Fat in kg 0.17 0.02 1.00 4 LBM in kg -0.18 -0.04 -0.94 5 Participant age 0.08 -0.02 0.11 6 BMI 0.08 -0.15 0.19  LBM in kg Participant age BMI 1 -0.18 0.08 0.08 2 -0.04 -0.02 -0.15 3 -0.94 0.11 0.19 4 1.00 -0.10 0.06 5 -0.10 1.00 0.12 6 0.06 0.12 1.00  $`\_boxhead`  var type column\_label column\_units 1 Variable default Variable <NA> 2 CD4+ default CD4+ <NA> 3 CD4 Immune activation count default CD4 Immune activation count <NA> 4 Fat in kg default Fat in kg <NA> 5 LBM in kg default LBM in kg <NA> 6 Participant age default Participant age <NA> 7 BMI default BMI <NA>  column\_pattern column\_align column\_width hidden\_px 1 <NA> left NULL NULL 2 <NA> right NULL NULL 3 <NA> right NULL NULL 4 <NA> right NULL NULL 5 <NA> right NULL NULL 6 <NA> right NULL NULL 7 <NA> 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Table 2 presents the Pearson correlation coefficients between CD4 immune markers and body composition variables. CD4+ count showed a modest positive correlation with both CD4 immune activation count (r = 0.22) and fat mass (r = 0.17), and a negative correlation with lean body mass (r = –0.18). Notably, fat in kilograms was strongly negatively correlated with lean body mass (r = –0.94), indicating an inverse relationship between fat and lean tissue. Participant age showed very weak correlations with all variables, suggesting age may not substantially confound relationships between immune activation and body composition in this sample.

## 4.2 Basic statistical analysis

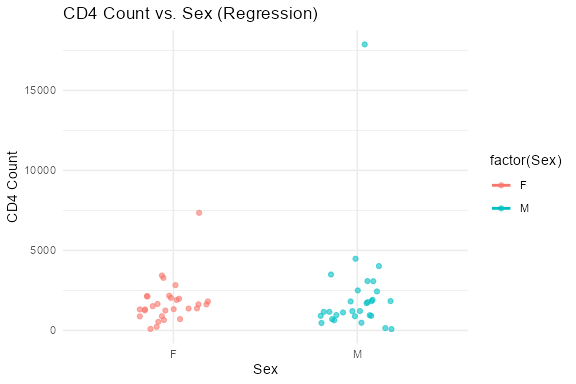
We employed a simple linear model to investigate the potential association between our primary outcome, CD4 immune activation count, and our main predictor variable, sex. To enhance the model’s explanatory power, we incorporated additional predictors that might influence immune activation counts through multivariate linear regression analysis.

Since fat content serves as a contributing factor to immune activation in disease progression, individuals with higher fat percentages typically demonstrate reduced susceptibility to tuberculosis, and vice versa. We analyzed the distribution of fat composition between men and women. This (**Fig2?**) presents a boxplot illustrating the distribution of fat in kilograms across male and female participants.



Box plot showing Fat in kg among males and females.

While this (**Fig3?**) shows the distribution of CD4 among males and females.



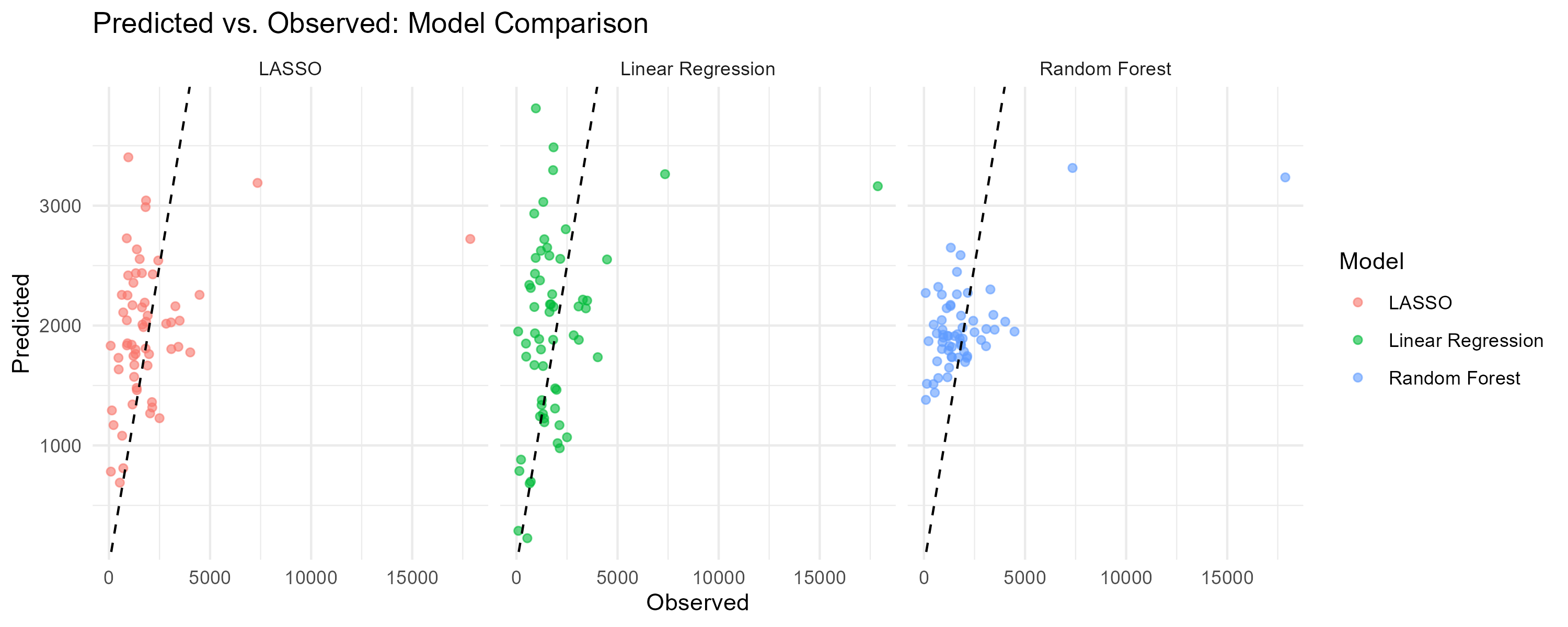
Regression plot showing CD4+ among males and females.

## 4.3 Full analysis

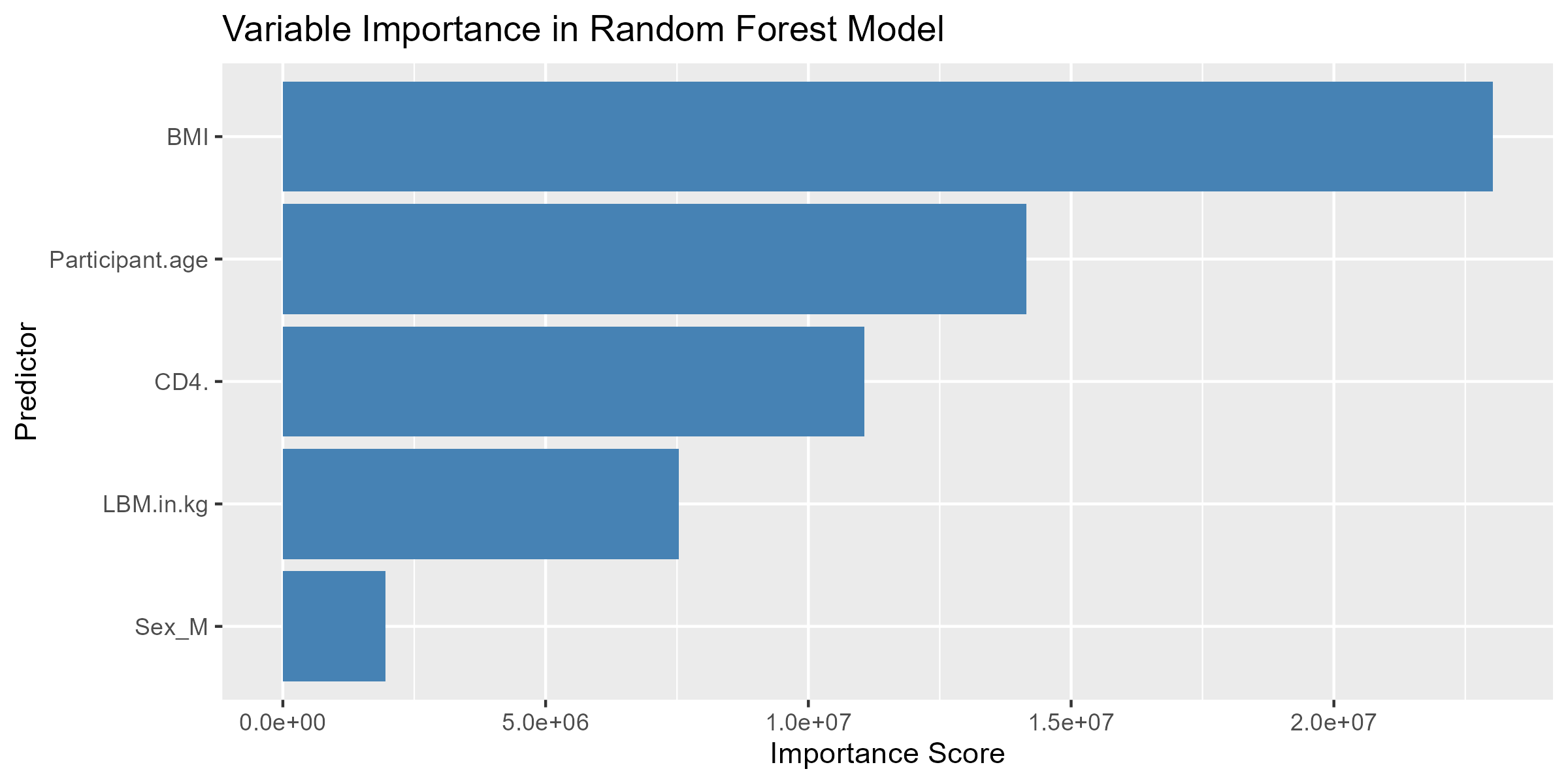
In order to take care of confounding, we performed a multi-regression analysis and the results of the analysis were not satisfactory.

We finally considered using ML algorithm to fit our data. The Random Forest model achieved the lowest RMSE (1984.8), outperforming both the linear regression and LASSO models, which had RMSEs 2123.8 and 2070.1 respectively. This figure (**Predicted\_vs\_observed?**) shows a comparison of observed vs. predicted values for each model.

This figure (**Variable\_plot?**) shows the variable importance plot from the random forest model. This highlights the most influential predictors of CD4 immune activation. BMI, participants age, CD4+, Lean body mass(LBM) and Sex emerged as the most important features, indicating they contributed most to the model’s predictive accuracy. This suggests that body composition and biological sex may play a key role in immune activation patterns.



predicted vs observed of three models



Variable plot from the random forests model.

We applied LASSO regression to identify predictors of CD4 immune activation. After removing the participant ID variable, the model retained BMI (estimate = -297), CD4+ count (estimate = 514), sex (male) (estimate = 260), and lean body mass (estimate = -22.7) as non-zero coefficients. Participant age was excluded from the final model.

Interestingly, LBM (Lean Body Mass) and Participant Age were ranked lower, suggesting they had less influence in this dataset. These findings align with the Random forest results, which also retained BMI,CD4+ lean body mass and sex as key predictors.

# 5. Discussion

In this analysis of sex-based differences in TB-related immune activation, we aimed to identify key predictors of CD4 immune activation count , which we used as a proxy for severity of disease. Our hypothesis was that higher immune activation counts indicate more severe illness, and that biological sex may influence immune responses to TB.

Both the LASSO regression and random forest models identified CD4+ count, BMI, sex, and lean body mass (LBM) as important predictors of immune activation. Our findings from the lasso model highlight that participant age was not selected, indicating it was not an important predictor after accounting for other variables.The results showed that Higher CD4 counts were associated with higher immune activation (estimate = 514), supporting our hypothesis that immune system status is closely tied to disease severity. The coefficient for lean body mass (LBM) was small (-22.7), indicating only a minor relationship.A notable difference in CD4 immune activation count rates between males and females, with males exhibiting higher risk of TB infection and disease than females. Sex was retained as a significant predictor, with a positive coefficient (estimate = 260). This indicated that males experienced a 260 increase in CD4 immune activation count compared to females, who served as the reference group.

This suggests that males may experience more severe immune activation, supporting the hypothesis that sex-based biological differences contribute to disease severity in TB. This result aligns with previous studies that have reported sex-based disparities in health outcomes and mortality risks as a result of TB(7),(8). Some studies still attribute the increased risk among males to be as a result of biological differences or differences in the accessibility of resources, especially healthcare. This is generally because women have better prognosis than men and they have better health seeking behavior as compared to their male counterparts.

Research shows that most women have higher fat repositories than men(9) and some studies show that the higher the fat percentage an individual has, the lower the risk of progression of the tuberculosis infection to disease hence lowering mortality rates among females compared to men. In addition, women tend to have more lean body mass compared to men hence having a lower chance of suffering from adverse effects of TB compared to men.

## 5.1 Strengths and Limitations

This study has several strengths. We applied simple linear regression, LASSO regression and random forest models for annalysis of our data. We were able to capture a range of relationships between predictors and immune activation, which in turn increased the robustness of our findings. The focus on sex-based differences in TB immune response addressed an important and often neglected question in infectious disease research especially among TB patients who are HIV negative. Additionally, the use of biologically meaningful predictors such as BMI, lean body mass, CD4+ count, and sex enhances the interpretability and relevance of the results. The consistency of key predictors across the LASSO model and the random forests model further supports the reliability of our conclusions. However, there are limitations to consider. The relatively small sample size may reduce the generalizability and stability of the findings.

As any observational study, we are limited to identifying associations rather than making causal claims. Important clinical variables, such as TB stage or treatment history, may not have been included, potentially introducing residual confounding. Moreover, while CD4 immune activation was used as our marker for disease severity, it may not fully capture the clinical picture.

Despite limitations of the present of our study, some studies show that Patients with wasting do not recover to the same levels of body mass as those who were not wasted. This suggests that tuberculosis may leads to permanent loss of lean tissue and fat mass.

## 5.2 Conclusions

Our results support the hypothesis that higher immune activation is associated with greater disease severity, and demonstrate that males exhibit higher CD4 immune activation levels compared to females, indicating they may experience more intense immune responses and possibly more severe disease outcomes. These findings highlight the importance of considering sex-based biological differences in TB research and clinical management.

In addition to sex, BMI and lean body mass emerged as important predictors, suggesting that body composition may influence the immune landscape in TB. These insights highlight the need for sex-specific approaches in TB monitoring and treatment, and point to CD4 immune activation as a potentially valuable biomarker for assessing disease severity. However, the need for future research is needed to validate these findings especially among larger populations to explore the underlying sex mechanism and body composition differences in regard to immune activation.

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