* Non-tuberculous mycobacteria lung disease is a chronic lung infection most often caused by the inhalation of aerosolized water droplets, soil, or biofilms harboring microbial organisms known as non-tuberculous mycobacteria
* Although NTM organisms are ubiquitous in the environment, most individuals do not develop NTM-LD through exposure. NTM-LD primarily develops in those with predisposing lung conditions or compromised immune function
* There are two main radiographic manifestations of NTM-LD: a fibrocavitary form characterized by opacification and cavitation of the upper lobes seen mostly in men with underlying emphysema and a nodular bronchiectasis form characterized by nodules and bronchiectasis that commonly affects the right middle lobe and lingula. Depending on the cohort studied, this form may be more frequently seen in women
* This disease is associated with several radiographic features that can be observed through CT scans of the lungs
  + Atelectasis: Atelectasis is the collapse or reduction in inflation of all or part of the lung and is identified in scans through a reduction in volume and increase in opacity. Depicted here is atelectasis of the right middle lobe.
  + Bronchiectasis: Bronchiectasis refers to permanent dilation of the bronchi, or airways, resulting from infection, obstruction, or congenital abnormalities. This can be identified in CT scans from the widening and lack of tapering of bronchi. Depicted here is varicose bronchiectasis, characterized by irregular dilations of the bronchi (as opposed to cylindrical [more even] and cystic [more sac/pouch like])
  + Cavities: Cavities are gas-filled spaces within areas of consolidation, masses, or nodules
  + Consolidation: Consolidation refers to the condition of the lung being rendered solid because of fluid or other disease byproduct replacing the alveolar air of the lungs.
  + Ground-glass opacities: Ground-glass opacities appear as hazy opacities in CT images of the lung and are caused by fluid in the airspaces of the lungs or thickening of lung tissue.
  + Nodules: Nodules are round opacities measuring up to 3 centimeters in diameter.
  + Tree-in-bud: Tree-in-bud opacities are small branching nodular opacities that look like a budding tree in lung CT images and are a result of mucous impaction and inflammation
* In the existing literature, the severity and frequency of the disease tends to favor some parts of the lung more than others for some studies, while this was not observed in others. For example, Reich and Johnson (1992) studied a group of 29 patients with Mycobacterium avium complex pulmonary disease and identified 6 elderly women from this group without predisposing pulmonary conditions with previously unexplained patterns of with a greater predisposition in the lingula and right middle lobe. They hypothesized that suppression of cough in these women may have led to the development of disease in these regions. Moore et al. (1993) examined 40 culture-positive NTM patients and scored ten lung zones on a 3-point scale (mild, moderate, severe) for bronchiectasis, air-space disease, and nodules. Bronchiectasis was most pronounced in the right middle lobe and lingula, whereas nodules were evenly distributed. Hazelton et al. (2000) reported CT findings in 14 patients with Mycobacterium chelonae. Two radiologists used consensus scoring; bronchiectasis and nodules were present in 13/14 cases but were diffusely distributed across lobes for the most part. Two of these patients, however, did present with more severe bronchiectasis in the right middle lobe and lingula. Lee et al. (2013) compared 369 immunocompromised versus immunocompetent patients. Three radiologists rated each lobe on a 5-point severity scale for lesions with lobar scores summed per patient, and scored bronchiectasis by determining its severity and extent and multiplying these values. Group differences were tested with paired t-tests to compare the groups and found that immunocompromised patients had significantly more nodules and cavities. For bronchiectasis, the top three most affected lobes for immunocompromised patients were the right middle lobe, left upper lobe, and lingula, while the top three most affected lobes for immunocompetent patients were the right upper lobe, right middle lobe, and lingula.
* Which brings us to our research question
* For each subject, 6 regions of the lungs were assessed for these features: the right upper lobe (RUL), right middle lobe (RML), right lower lobe (RLL), left upper segment (LUS), left lingular segment (LLS), and left lower lobe (LLL)
* The different features we will be studying are presented here. Features with ordinal outcomes are tree-in-bud, ground glass opacities, consolidation, bronchiectasis, and atelectasis, whose scores depend on the percent of the lobe affected by the disease, with 0 meaning no involvement, 1 meaning <25% of involvement, 2 meaning 25-50% involvement, and 3 meaning >50% involvement. Features with binary scores include large nodules and thin and thick wall cavities, with 0 indicating absence of this feature in the lobe and 1 indicating its presence
* Our study has 166 subjects, with 12 repeated measures per subject (6 scores for each of the lobes and 2 raters scoring each subject). The variables present in our preliminary analysis include the subject’s ID, lobe, score of the lobe, and rater. The data was provided to us in excel document in wide format. Minimal data cleaning was performed, mostly to get the data from wide to long format. I also put each feature in its own sheet for ease of extracting during modeling.
* For the features with ordinal outcomes (tree-in-bud opacities, consolidation, bronchiectasis, and atelectasis), we modeled using three approaches. The first approach, which we refer to as our full model, used an ordinal logistic regression mixed modeling approach, with ordinal score as the outcome, lobe and rater as fixed effects, a random intercept for subject, a nested random effect for rater within subject, and a cumulative logit link function.
* Our second modeling approach, which we refer to as our reduced model, again used an ordinal logistic regression mixed modeling approach, with ordinal score as the outcome, lobe and rater as fixed effects, a random intercept for subject, with a cumulative logit link function, but without a nested random effect for rater within subject
* This reduced approach was taken primarily for the features where the full model did not converge due to the complex random effects structure. We also fit this reduced model for the features where the full model did converge as a means of sensitivity analysis to check if the simpler random effects structure changes the model estimate for the effect of lobe, which is our main estimate of interest in addressing the research question.
* Our third modeling approach sought to relax the proportional odds assumption that the other two modeling approaches abide by, thereby allowing the effect of lobe to differ across the category thresholds. We again modeled this using an ordinal logistic regression mixed modeling approach, with ordinal score as the outcome, lobe and rater as fixed effects that we allowed to differ across the category thresholds, a random intercept for subject, and a cumulative logit link function.
* For the features with binary outcomes (ground-glass opacities, large nodules, thin wall cavities, and thick wall cavities), we modeled using two approaches. The first approach, which we refer to as our full model, used a logistic regression mixed modeling approach, with binary score as the outcome, lobe and rater as fixed effects, a random intercept for subject, a nested random effect for rater within subject, and a logit link function.
* Our second modeling approach, which we refer to as our reduced model, again used a logistic regression mixed modeling approach, with binary score as the outcome, lobe and rater as fixed effects, a random intercept for subject, with a logit link function, but without a nested random effect for rater within subject.
* This reduced approach, like in the ordinal features case, was taken primarily for the binary features where the full model did not converge due to the complex random effects structure. We also fit this reduced model for the features where the full model did converge as a means of sensitivity analysis to check if the simpler random effects structure changes the model estimate for the effect of lobe, which is our main estimate of interest in addressing the research question.
* Estimation of the model parameters in all our above generalized linear mixed models was performed through the adaptive Gaussian quadrature method. In the case of generalized linear mixed models, the likelihood function cannot be expressed in closed form due to the presence of random effects. These random effects must be integrated out to achieve the marginal likelihood, since the random effects are non-observable values, and this integral with respect to the random effect is what makes the likelihood estimation an intractable problem. Adaptive Gaussian quadrature is a method of numerical integration that approximates this marginal likelihood by evaluating the integrand at a set of abscissas (quadrature points) and summing weighted values of the integrand at these points.
  + L(theta) marginal likelihood, L(theta|b\_i) is conditional likelihood given random effects, phi(b\_i) is probability density of random effects, theta is placeholder for unknowns
* To address the research question of if there are differences in the frequencies and severities for each of the specific CT features among the lung lobes in NTM-LD, we constructed pairwise contrasts of the fixed-effect estimates for the lobe variable in our models using “estimate” statements for our proportional odds models fit in SAS. As our ordinal logistic regression models compare lower levels to higher levels by default (in other words, lower disease severity to higher disease severity), we first flipped the signs on these estimates before calculating the contrasts to get the comparisons in the other direction. We then exponentiated the results of these contrasts to get the odds ratios of being in a higher disease severity category between the lobes. Confidence intervals for these odds ratios were calculated by exponentiating the Wald 95% confidence limits produced by GLIMMIX.
* For the non-proportional odds model, which produces three fixed-effect estimates for the 5 non-reference lobes (one for each severity cut-point), pairwise contrasts were constructed between each of the lobes within each cut-point, again by subtracting the corresponding log-odds estimates. Standard errors were determined through the model’s variance-covariance matrix, Wald 95% confidence intervals were formed, and resulting estimates were exponentiated to yield values on the odds scale.
* As there are 6 lobes we are performing pairwise contrasts for, there are 15 unique pairwise contrasts performed in total for the models with a single fixed-effect estimate for the lobe variable and 45 unique pairwise contrasts performed in total for the non-proportional odds model with 3 fixed-effect estimates for the lobe variable. In order to correct for these multiple tests, we used the False Discovery Rate (FDR) multiple testing correction approach outlined by Benjamini and Hochberg and provided below.
* Suppose we test hypotheses H\_1,H\_2,…,H\_m based on corresponding p-values P\_1,P\_2,…,P\_m, let P\_((1) )≤P\_((2) )≤⋯≤P\_((m)) be the ordered p-values, and let H\_((i)) be the null hypothesis corresponding to P\_((i)). For independent test statistics, the following procedure controls the FDR at q^\*: let k be the largest i for which P\_((i))≤ i/m q^\*, then reject all H\_((i)),i=1,2,…,k (Benjamini & Hochberg, 1995).
* As we are evaluating these tests at a nominal significance threshold of q^\*=α=0.05, our FDR-corrected p-values are thus p\_((i))^FDR=m/i P\_((i)), and hypotheses with p\_((i))^FDR<0.05 were determined to be significant.
* For interrater reliability, we want to know how much of the total variation in scores is attributable to true differences between subjects as opposed to variation due to raters or random error.
* To determine the 95% confidence interval for our estimate of the ICC, we used a Delta Method approach proposed by Casella and Berger (2002). Let T\_1,…,T\_k be random variables with means θ\_1,…,θ\_k and let T=(T\_1,…,T\_k ) and θ=(θ\_1,…,θ\_k). For a differentiable function g(T), an approximate estimate of the variance is given by a first-order Taylor series expansion of g about θ as
* Var\_θ g(T)≈∑\_(i=1)^k▒〖[g\_i^' (θ)]^2 Var\_θ T\_i 〗+2∑\_(i>j)▒〖g\_i^' (θ) g\_j^' (θ)Cov\_θ (T\_i,T\_j)〗,
* (Casella & Berger, 2002). In our case, we have k=2 parameters, where T\_1 is our estimate for σ\_subject^2, T\_2 is our estimate for σ\_rater^2, and g(T)=T\_1/(T\_1+ T\_2+ π^2/3) is our estimate for ICC. We thus have that g\_1^' (θ)=∂g/(∂T\_1 )= (T\_2+ π^2/3)/(T\_1+T\_2+ π^2/3)^2 =A and g\_2^' (θ)=∂g/(∂T\_2 )= (-T\_1)/(T\_1+T\_2+ π^2/3)^2 =B, both by the quotient rule. Expanding the Casella and Berger formula for our case yields
* Var\_θ g(T)≈(A)^2 Var\_θ T\_1+(B)^2 Var\_θ T\_2+2 (A)(B)Cov(T\_1,T\_2),
* where the Var\_θ T\_1, Var\_θ T\_2, and Cov\_θ (T\_1,T\_2) terms are the estimated variance and covariance of the variance component estimates, obtained from the covariance matrix provided by the GLIMMIX procedure. The standard error (SE) of the ICC estimate was obtained by taking the square root of Var\_θ g(T). An approximate 95% confidence interval (CI) for the ICC was constructed as ICC\_estimate±1.96×SE
* Upper lobes have decreased blood perfusion, as the blood must work against gravity to get to the upper lobes, and follows a more tortuous path to the RUL
  + This results in decreased flow of immune cells and IV antibiotics to the upper lobes
* Upper lobes also experience greater physical stress on the lung tissue on inspiration due to the weight of the lower lobes
* This may warrant lifestyle or treatment practices, such as having patients refrain from intense exercise, and lying down more when resting or receiving antibiotics