Assessing Lung Vasculature Development and Application to Early Preterm Gestation Patients

Randi Dias, Aren Singh Saini, & Walter O'Dell *University of Florida*

Faculty Mentor: Walter O'Dell, PhD, Department of Radiation Oncology

Abstract:

Individuals who were born prematurely undergo many difficulties from the time they are born to their adult lives, with obstructive lung diseases being a common occurrence. Unfortunately, the development of lungs in premature infants has not been fully studied. This study's objective is to determine trends in lung vessel growth as a patient advances in age and discover quantitative measures that provide clinical insight regarding improving their quality of care. For each scan, the pulmonary vessel trees were extracted and characterized using in-house software to quantify the total number of vessels, branch radii, and lengths in the right hemi-lung. CT scans of 16 pediatric patients were collected retrospectively and analyzed (7 prematurely born and 9 full-term). The vessel count of full-term patients trended to increase with age, while that for premature patients appeared to be stable or decrease. A significantly larger number of vessel branches was observed between female preterm and full-term subjects. Limitations including variations in image quality, children's age, and changes in CT technology over time are being addressed to improve confidence in the results. Future works will include analysis on a larger data set and improved approaches for automatic vessel extraction from chest CTs.

Keywords: premature infant, lung vessel development, x-ray computed tomography, vessel segmentation

Background:

An infant born before their full gestation period of 37 weeks is considered to be premature. Globally, approximately 13 million babies are born prematurely each year (Simmons, 2010). Premature infants (preemies) face a number of hardships throughout their lives, one of the main ones being the underdevelopment of their lungs. The hindered development in a prematurely born infant's lungs can lead to severely inadequate gas exchange, which contributes to lung failure being the leading cause of neonatal mortality (Simmons, 2010). Moreover, the health implications of lung underdevelopment at time of birth can adversely affect these individuals throughout their life and contribute to a late onset of pulmonary diseases (Naumburg, 2019) and shorter life expectancy than their full-term gestation peers.

The prenatal lung development normally consists of five periods where vital components of the respiratory system begin to develop (Smith, 2010). In most premature infants, their lungs do not complete the saccular and alveolar periods, the final periods of lung development that occur during week 25 to week 40 of pregnancy. During the saccular period, surfactant synthesis and alveolar sac septation begin (Burri, 1997). Additionally, the primitive blood-air barrier and double-capillary network begin to form. These developments are crucial for an infant's growth

because they prevent blood from entering the alveoli and play an important role in blood/gas exchange. During the alveolar period, the surfactant system matures, and the true alveoli begin to appear (Smith, 2010). The development of the surfactant system is vital to a newborn's life because it plays a crucial role in "maintaining the functional integrity of alveoli" (Boyden, 1997). The alveoli are lined with surfactant, which allows the lungs to expand and decreases the surface tension at the air-liquid interface (Smith, 2010). Thus, when an infant is born prematurely and does not go through these crucial periods of development, they require a lot of care and attention to sustain a healthy life.

In preemies, obstructive lung diseases are commonly seen due to a variety of reasons including lung underdevelopment, oxygen therapy, and ventilator support (Jones, 2009). In the majority of cases, infants born prematurely are fitted with mechanical respirators that have been shown to maximize their likelihood of survival (Jones, 2009). Studies have also shown decreased lung function in adults who were born prematurely even though they did not display significant respiratory disease as neonates (Jones, 2009). These findings indicate a linkage between being born prematurely and developing obstructing lung diseases in later life. Although there exist some standardized methods to qualitatively measure the level of lung development in preemies, further improvements are needed to achieve for reliable, non-invasive, and quantitative assessment suitable for all age groups. The main objective of this project is to gather quantifiable data that would aid us in determining markers in vascular development in human subjects who were born prematurely, compared to subjects who were born full-term. It is a goal to collect data and establish procedures that can provide clinical guidance to improve the mortality and quality of life of prematurely born infants and provide better care as these children advance onto their adult lives.

Methodology:

For this retrospective study, an in-house software built upon the NIH ImageJ platform (Schneider, 2012) was employed to analyze chest computed tomography (CT) scans of 16 pediatric patients, ranging from ages 2 weeks to 18 years old. The scans and patient medical data were gathered retrospectively under an IRB-approved protocol: IRB201900733 *Retrospective analysis of chest CT scans in pediatric patients to monitor development of lung vasculature, particularly for those born prematurely*, abiding by all federal, state, and institutional guidelines for use of human subjects in research. The data represent a cohort of 60 patients (18 years or

younger) who received as per standard of care at least 3 chest CT scans at a single, academic, pediatric pulmonary care center between 1/1/2010 and 3/2/2019, as identified by CPT Codes 71250, 71260, 71270, 71275. The 30 patients with a premature gestational age (less than 37 weeks) with the most CT scans were chosen from all eligible subjects. Then, 30 non-premature patients, matching for age, with the most CT scans were chosen. Each patient provided 3-18 follow up scans over different stages of their lives. The first 21 patients in the list of 60 were analyzed. However, in 5 out of the 21 subjects, the software failed to achieve a complete vessel tree traversal on any of the subjects' CT scans, typically because of abnormal features in the lung tissue background. These data sets were omitted from the final analysis. Therefore, the initial analysis includes 16 subjects who were chosen without knowledge of sex, gestation age, or comorbidities to prevent selection bias. Out of these 16 subjects, all available scans were analyzed for the majority of the patients, with the exception of 5 patients where some CT scans were omitted due to the software failing to complete the vessel traversal process. The CT acquisition covered the entirety of the lung volume, and the scans were acquired at varying intervals for each subject. Among these 16 patients, an equal number of male and female subjects were included by chance. The vessel segmentation software was utilized to segment the lung volumes, extract major airways, and then identify and characterize the pulmonary tree structures (Figure 1). After completing this process, the number of branches within the lungs, along with the radius and length of each branch, were tabulated and compared across groups.

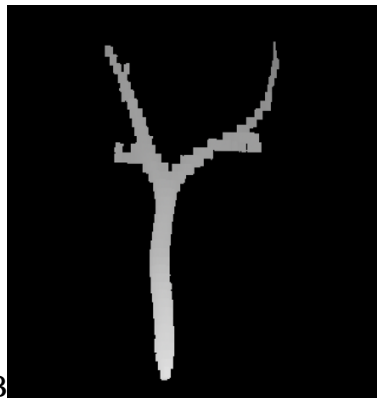
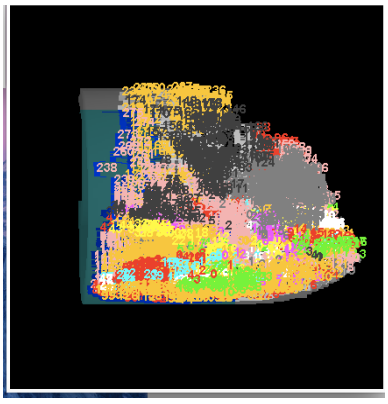
A.  B  C 

Figure 1: Process for extracting the lung vasculature structure. Shown are representative images obtained from a 4 years 10 months old full-term pediatric patient. Image A (left) shows the lung mask from a single chest CT slice, image B (middle) is a depiction of the extracted major airway, and image C (right) represented skeletonized and numbered pulmonary tree structure where each vessel tree is shown in a unique color.

In this study, the right hemi-lung was chosen both because it is larger than the left lung and because several of our initial subjects had severely diseased left hemi-lungs. The slice thickness for the patients in the sample varied, with the majority of patients having effective slice thickness of 3 mm. Some patients had a slice thickness of 1 mm while one patient had 5 mm. The in-plane pixel dimensions ranged from 0.338-0.812 mm. The analysis was initiated by running each CT scan through an automatic series of image processing and thresholding steps to define the lung outer surface in three dimensions. When needed, in regions where the border between the lung parenchyma and the chest wall were not clear, points on the lung border were manually adjusted using an active contour, aka *snake* (O'Dell, 2012). After completing the lung volume extraction step, the next major step was to segment the major airways using a seeded region-growing method. The major airways were then removed from the lung volume images to ensure that the software program does not accidentally pick up the walls of the airways as lung vessels. In a similar manner, the pulmonary vessels were also segmented from the lung volume images by applying a manually selected seed point placed in the pulmonary trunk. Afterwards, the pulmonary tree structure was automatically skeletonized to facilitate traversing and labeling of each branch in the pulmonary tree structure (O'Dell, 2014). This procedure allowed us to tabulate the total number of vessel branches in the lungs, along with their individual radius and length (O'Dell, 2017).

The gathered lung vessel metrics, along with each subject's gestational age, sex, presence of infection/disease, and health updates, were recorded in a spreadsheet where the similarities and differences between the subject factors were analyzed. Calibration curves were used to adjust for variances in imaging parameters, as well as the utilization of vascular contrast agents (Martocci, 2021). For this study, the analysis only includes vessels of radius 1 mm or larger to reduce the variability in the counts.

The comparisons were made using a non-parametric statistical analysis with the Mann-Whitney U test. A two tailed table with an alpha value of 0.05 was used to compare the critical values of U and assess significance. If the value for U critical was greater than the value of U statistic, the null hypothesis was rejected, and a significant difference was observed between the two sample groups. Furthermore, a multivariable regression test was also performed on Excel, which included both age and gestation or gender category.

Results:

CT scans of 16 patients, 8 males and 8 females, were analyzed and compared, 9 of which were full-term while the other 7 were preemies. The 9 full-term patients consisted of 5 males and 4 females, while the 7 prematurely-born patients consisted of 3 males and 4 females. Each subject provided 3-18 scans, with some as early as 2 weeks post-birth and the latest being 20 years of age. As seen on Figure 2A, for full-gestation subjects, the positive slope indicates that the number of lung vessels increased with age. However, a linear regression fit to the data with an R value of 0.2781 indicates that this is a weak association. Ignoring age, the Mann-Whitney test did not indicate a significant difference between pre-term patients and full-term patients. Furthermore, a multiple variable linear regression using age and gestation category suggested that age (p-value: 0.004) is a significant contributor but gestation category was not (p-value: 0.111). As shown in Figure 2A, in patients who were born pre-term, a trend for a slight increase in the number of lung vessels was observed between the ages 1-6. However, there was an apparent drop in the number of lung vessels from ages 7 to 11, and then the vessel count tended to stabilize between the ages 12-16. Despite not reaching the significance level of 0.05, the trend in the data from Figure 1A possibly indicates that patients who were born pre-term have fewer number of lung vessels as they grow older, relative to patients who were born full-term.

Comparisons were also made between male and female subjects, where there was a trend for greater increase in the number of vessels with age for female than male patients, which can be observed in Figure 2B. A multiple variable linear regression using age and gender category suggested that both age (p-value: 0.0002) and gender (p-value: 0.006) are significant contributors to the number of vessels. However, when the variable of age was disregarded and the vessel growth between the genders were compared, the Mann-Whitney test gave a U value of 148, being less than the critical U value 117, indicating that the difference between genders was not significant.

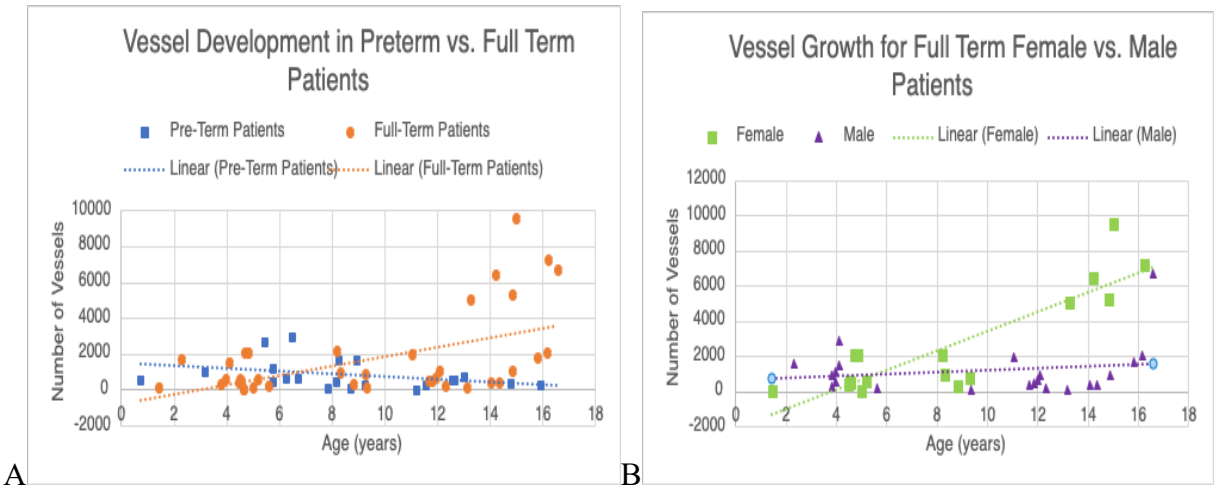
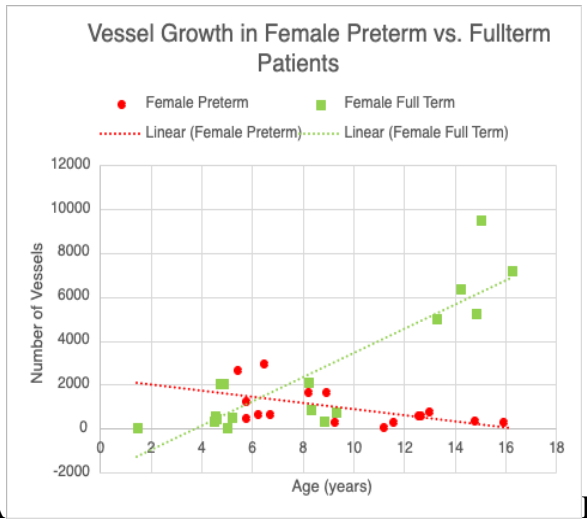


Figure 2: Plot A (left) represents a scatter plot of vessel growth in preterm vs. full-term patients as years progress. Plot B (right) represents a scatter plot of vessel growth in full-term male vs. female patients as years progress.

For female full-term subjects, there was a significantly larger increase in the number of branches over time (the slope of the graph of Figure 3A) compared to female preemies. The multivariable test indicated a p-value of 0.003 for age and a p-value of 0.03 for gestation category, indicating these variables pose as significant contributors to the vessel number. The Mann-Whitney test, disregarding age, yielded a calculated U value of 52 and U critical value of 93, indicating a statistically significant difference between female full-term and preterm patients.

A similar trend was observed when comparing the vessel development in male preterm vs full-term patients (Figure 3B). A calculated U value of 28 and a critical U value of 29 were obtained when the variable of age was ignored, and due to the calculated U value being less than the critical U value, the data indicates a slight statistical difference between the vessel growth among male pre-terms and full-terms. The multivariable linear regression using both age and gestation category suggests that age (p-value: 0.411) and gender (p-value: 0.439) are not significant contributors to the vessel growth for males.

A 

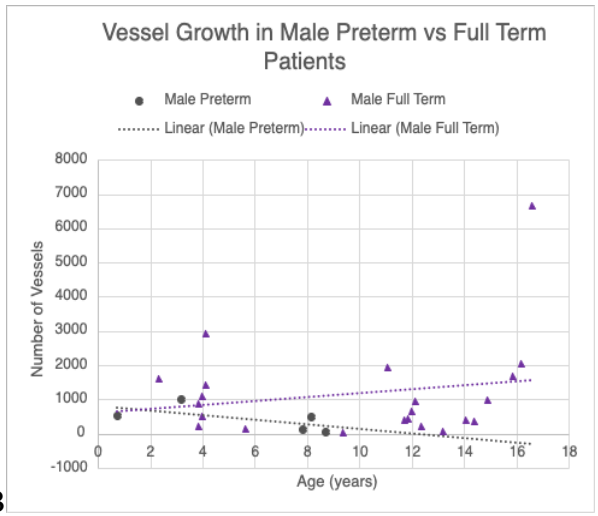
B 

Figure 3: Plot A (left) portrays lung vasculature development over time for female preterm vs full-term patients. Plot B (left) portrays lung vasculature development over time for male preterm vs full-term patients

Discussion

A quantitative analysis of pulmonary vascular development over time was conducted in pediatric patients, comparing full-term to prematurely-born children using quantitative metrics of number of vascular branches. A key finding was that the number of vascular branches increased with age in full-term subjects with a larger slope than for prematurely-born children. For female subjects, this slope difference reached statistical significance for the initial pool of subjects. The data also indicates that the differences in the number of vessel branches became more apparent in the teenage years than in younger children.

If the trends observed in this study are confirmed in larger follow up studies, physicians could use these quantitative metrics to determine the age at which prematurely born patients should begin to take preventative measures to detect any abnormalities occurring in their lungs. Since the data suggests that differences among the vessel numbers in the different sample groups begin to appear during a person's teenage years, physicians could encourage at-risk patients to prioritize quantitative assessment of lung function during this age range. Physicians could turn their focus towards patients who are at the beginning of their teenage years to detect changes in their lung health and provide treatments in a timely and efficient manner. Currently, due to the lack of data regarding lung vascular growth and angiogenesis, it has been difficult to establish clinical phenotypes that would aid with structuring treatment plans for various lung diseases

(Robbins, 2012). Therefore, the lung development tracking done in this study could potentially be used to develop treatment options for lung diseases such as pulmonary hypertension.

Limitations of this study include use of only a small number of patient samples making the current analyses mostly exploratory. Variations in image quality, imaging parameters, CT scanner technology, and the ability of patients to remain still for a long period of time (particularly difficult to achieve with younger children), resulted in large variability in the vessel counts and a few outliers, which may have affected the overall trend of the results. This variability was especially problematic at smaller branch sizes, leading us to focus here on branches > 1 mm in radius. Some of the obtained data sets date back to 2005, and during this time, the technology and machines used for CT scans were not as advanced as they are now. A calibration curve was applied to the data to account for the effects of varying scanner parameters, but a more refined calibration process would perhaps lead to more confidence in the branch counts. Also, many of the full-term subjects had underlying lung-health conditions, which prompted their receiving repeat chest CT scans. These underlying conditions may alter the appearance of the lungs and may not reflect the state of lung development in fully healthy children at the measured time points.

Future Works and Conclusion

The objective was to collect sufficient data to determine trends in lung vasculature development in prematurely born patients that, combined with future analyses with improved metrics, could serve as a tool for physicians to reference in order to improve the quality of life of prematurely born patients. The preliminary data identified a significant difference in the rate of lung vascular development between females born prematurely and those born full-term that becomes most apparent in the teenage years. This observation supports clinical findings of late-onset pulmonary disease in young adults who were born prematurely (Jones, 2009). The outcomes of this study also demonstrate the feasibility to extract quantitative metrics of lung vascular tree structure from repeat chest CT scans in pediatric patients using conventional CT technology coupled with dedicated imaging processing software tools.

The pediatric lung vessel assessment study is yet ongoing. The parent IRB protocol, IRB201900733, provided names of 60 eligible pediatric subjects with 3 or more chest CT scans. To date, 16 data sets out of those 60 have been fully analyzed. The remaining datasets will be

analyzed to increase statistical significance for discovering patterns in the data and detecting group differences. To account for the limitations mentioned above, improved calibration tools are being developed to better compare vessel metrics across patients. The lab is also constantly improving the quality and reliability of the vessel extraction algorithm and it is anticipated that upcoming versions will further improve the reliability and scope of the vessel results. With these refinements in place, the team intends to correlate patterns of lung vessel development with other patients' factors (e.g., body weight and lung volume) and disease states (e.g., adult pulmonary hypertension).

Acknowledgements

Funding for this work was provided in part from the University of Florida, College of Medicine University Scholars Program; the Florida Department of Health Bankhead-Coley Cancer Research Program (grant #6BC09), and the National Center for Advancing Translational Sciences of the National Institutes of Health under University of Florida Clinical and Translational Science Awards UL1 TR000064 and UL1TR001427.

References:

Burri P. (1997) *Structural Aspects of Prenatal and Postnatal Development and Growth of the Lung.* In: McDonald John Ah , editor. Marcel Dekker. p. 1–35

Boyden E. (1997) *Development and Growth of the Airways*. In: Hodson AW, editor. Development of the Lung. New York: Marcel Dekker Inc;. p. 3–35

Jones, M. (2009). Effect of preterm birth on airway function and lung growth. *Paediatric Respiratory Reviews*, *10*, 9-11

Martocci S, Siva Kumar S, Barreta I, O'Dell W *Effect of Pixel Intensity Threshold and Imaging Parameters on Lung Vessel Segmentation from Chest CT* Poster presentation at the BMES national conference, Orlando, FL, Oct. 6-9, 2021

Naumburg, E., & Söderström, L. (2019). Increased risk of pulmonary hypertension following premature birth. BMC pediatrics, 19(1), 1-7.

O'Dell, W. G. (2012, February). Automatic segmentation of tumor-laden lung volumes from the LIDC database. In *Medical Imaging 2012: Computer-Aided Diagnosis* (Vol. 8315, p. 831531). International Society for Optics and Photonics

O'Dell, W. G., Gormaley, A. K., & Prida, D. A. (2017). Validation of the Gatortail method for accurate sizing of pulmonary vessels from 3D medical images. *Medical Physics*, *44*(12), 6314-6328.

O'Dell, W. G., Govindarajan, S. T., Salgia, A., Hegde, S., Prabhakaran, S., Finol, E. A., & White, R. J. (2014, March). Traversing and labeling interconnected vascular tree structures from 3D medical images. In *Medical Imaging 2014: Image Processing* (Vol. 9034, pp. 847-861). SPIE

Robbins, I. M., Moore, T. M., Blaisdell, C. J., & Abman, S. H. (2012). Improving outcomes for pulmonary vascular disease. American journal of respiratory and critical care medicine, 185(9), 1015-1020.

Schneider, C. A., Rasband, W. S., & Eliceiri, K. W. (2012). NIH Image to ImageJ: 25 years of image analysis. *Nature methods*, *9*(7), 671-675.

Simmons, L. E., Rubens, C. E., Darmstadt, G. L., & Gravett, M. G. (2010, December). Preventing preterm birth and neonatal mortality: exploring the epidemiology, causes, and interventions. In *Seminars in perinatology* (Vol. 34, No. 6, pp. 408-415). WB Saunders.

Smith, L. J., McKay, K. O., van Asperen, P. P., Selvadurai, H., & Fitzgerald, D. A. (2010). Normal development of the lung and premature birth. *Paediatric respiratory reviews*, *11*(3), 135-142.