Magnetic resonance imaging of the lungs in asthma and COPD

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

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Weijuan Zhang

School of Medicine
Contents
List of tables .............................................................................................................. 6
List of figures ............................................................................................................. 8
Abstract ..................................................................................................................... 11
Declaration ............................................................................................................... 12
Copyright statement ............................................................................................... 13
Dedication .................................................................................................................. 14
Acknowledgements .................................................................................................. 15
The author .................................................................................................................. 16
The alternative format thesis .................................................................................... 17
Publications ............................................................................................................... 18
Abbreviations .......................................................................................................... 19
Symbols .................................................................................................................... 20
Chapter 1 Thesis overview ....................................................................................... 22
  1.1 Aims and objectives ......................................................................................... 22
  1.2 Thesis outline .................................................................................................. 23
  1.3 Contributions .................................................................................................. 24
Chapter 2 Background: asthma and COPD ............................................................. 26
  2.1 A snapshot of asthma and COPD: key similarities and differences .............. 26
     2.1.1 Prevalence and Burden ........................................................................... 26
     2.1.2 Clinical features ...................................................................................... 26
     2.1.3 Inflammation and structural changes ...................................................... 27
     2.1.4 Alterations in pulmonary physiology ...................................................... 28
  2.2 Assessment of asthma and COPD: non-imaging techniques ......................... 30
     2.2.1 Airway function ....................................................................................... 30
     2.2.2 Lung volumes .......................................................................................... 31
     2.2.3 Alveolar function ..................................................................................... 31
     2.2.4 Airway inflammation .............................................................................. 32
     2.2.5 Airway vasculature inflammation ............................................................ 32
     2.2.6 Pros and cons of non-imaging biomarkers .............................................. 32
  2.3 Assessment of asthma and COPD: imaging techniques ................................. 35
     2.3.1 X-ray computed tomography .................................................................. 35
     2.3.2 Radionuclide lung imaging ....................................................................... 39
     2.3.3 Bronchoscopy imaging techniques .......................................................... 41
     2.3.4 Magnetic resonance imaging ................................................................... 41
     2.3.5 Pros and cons of the imaging modalities ................................................ 41
Chapter 3 Background: proton MRI ......................................................................... 45
  3.1 Nuclear magnetic resonance .............................................................................. 45
3.2 Relaxation ................................................................. 45
3.3 Pulse sequences .......................................................... 46
  3.3.1 SE sequences ......................................................... 46
  3.3.2 GE sequences ........................................................ 47
3.4 How to generate an MR image from MR signals ...................... 49
  3.4.1 Spatial encoding ...................................................... 49
  3.4.2 Image contrast ....................................................... 50
3.5 Proton MRI of the lung: the challenges and strategies ............... 51
3.6 Relaxation time and proton density measurements ................... 52
  3.6.1 T₁ measurement ..................................................... 52
  3.6.2 S₀ measurement ..................................................... 54
  3.6.3 Application of T₁, T₂ (T₂*) and S₀ mapping ................... 54
3.7 Dynamic oxygen-enhanced MRI ....................................... 58
  3.7.1 Principles of OE-MRI .............................................. 58
  3.7.2 OE-MRI data acquisition methods ............................. 59
  3.7.3 Data analysis ....................................................... 61
  3.7.4 Application of OE-MRI in the lung ............................. 64
  3.7.5 Pros and cons of pulmonary OE-MRI ......................... 67
3.8 Dynamic contrast-enhanced MRI ...................................... 67
  3.8.1 Contrast agents and the principles of DCE-MRI ............... 67
  3.8.2 DCE-MRI data acquisition methods ........................... 68
  3.8.3 Data analysis ....................................................... 69
  3.8.4 Application of DCE-MRI in the lung ............................ 73
3.9 Other MRI techniques .................................................. 75
  3.9.1 Proton lung MRI techniques ..................................... 75
  3.9.2 Hyperpolarized gas MRI ......................................... 76
3.10 Other relevant methods employed in this PhD project .............. 78
  3.10.1 Image registration ................................................ 78
  3.10.2 Vessel segmentation ............................................. 79

Chapter 4 Paper 1: MR quantitative equilibrium signal mapping: a reliable alternative to CT for the assessment of emphysema in COPD ................................................................. 81

4.2 Introduction ................................................................... 82
4.3 Materials and methods .................................................. 83
  4.3.1 Study subjects and study design .................................. 83
  4.3.2 Spirometry ............................................................ 83
  4.3.3 MR imaging .......................................................... 84
  4.3.4 CT imaging ........................................................... 86
  4.3.5 Statistical analysis .................................................. 86
List of tables

Table 2.1 Pros and cons of non-imaging techniques in the assessment of asthma and COPD ..............................................................34
Table 2.2 Pros and cons of the imaging techniques in the assessment of asthma and COPD ........................................................................43
Table 3.1 Parameter setting for different weighting of SE and GE sequences ..............50
Table 3.2 Literature lung T1 values at normoxia and hyperoxia ........................................63
Table 4.1 Characteristics of participants and results of spirometry, MR qS0 mapping and quantitative CT ........................................................................87
Table 4.2 Sensitivity and specificity of MR qS0 readouts for the differentiation of COPD patients from healthy controls ................................................92
Table 4.3 Correlation between measurements of MR qS0 mapping and quantitative CT in patients with COPD .................................................................94
Table 4.4 Correlation between spirometric parameters and the readouts of MR qS0 mapping and quantitative CT in patients with COPD ........................................95
Table 4.5 Intraclass correlation coefficients of repeated measurements of MR qS0 readouts in healthy and COPD groups ..........................................................95
Table 5.1 Demographic information and pulmonary function tests and quantitative CT of healthy controls and COPD subjects of A or E phenotype ........................................106
Table 5.2 Dynamic OE-MRI readouts of healthy controls and COPD subjects of A or E phenotype .....................................................................................112
Table 5.3 Correlation coefficients of MRI readouts with PFT and CT measurements in the A phenotype COPD and E phenotype COPD groups ..............................................115
Table 6.1 Demographic data and clinical measurements .......................................................124
Table 6.2 Individual dynamic OE-MRI readouts ................................................................132
Table 6.3 Comparison of the dynamic OE-MRI readouts between the mild and severe asthmatic groups .................................................................133
Table 6.5 The mean bias and 95% limits of agreement of the imaging readouts between two scans .........................................................................................136
Table 7.1 Demographics, clinical measurements and pulmonary function tests .................150
Table 7.2 Comparison of baseline dynamic OE-MRI imaging parameters between subject groups .........................................................................................152
Table 7.3 MR imaging measurements in scans with and without salbutamol administration .................................................................158
Table 7.4 Pearson’s correlations between baseline pulmonary function testing parameters and the MR imaging measurements in asthmatic subjects and healthy subjects (n=38) 159
Table 8.1 Demographic information and the clinical measurements of healthy controls and patients with asthma .................................................................168
Table 8.2 Comparison of the DCE-MRI readouts between healthy subjects and patients with asthma .....................................................................................173
Table 8.3 Comparison of the DCE-MRI readouts between healthy control group and asthma subgroups of disease severity and eosinophil status ........................................177
Table 8.4 Sensitivity and specificity of different cut-off points of median SI%max, median Kwashout and median iAUC0i in the differentiation between asthma and healthy control 178
Table 8.5 Pearson’s correlation between DCE-MRI readouts and the clinical measurements in patients with asthma ................................................................181
Table 9.1 Demographic information and clinical measurements for healthy controls and patients with asthma ........................................................................................................................................191
Table 9.2 Comparison of the DCE-MRI readouts between healthy subjects and patients with asthma ........................................................................................................................................194
Table 9.3 The evaluation of independent and interactive effects of disease severity and eosinophil status of asthma on DCE-MRI readouts in patients with asthma ........................................195
Table 10.1 Reasons to investigate MR qS₀ mapping, dynamic OE-MRI and DCE-MRI 200
List of figures

Figure 3.1 SE sequence ................................................................. 47
Figure 3.2 GE sequence ................................................................. 48
Figure 3.3 A schematic of the protocol design for the acquisition of dynamic OE-MRI data in the human lung ................................................................. 59
Figure 3.4 A schematic of the extended Tofts model ................................................................. 72
Figure 3.5 An example MR image showing the manually marked outlines of the chest wall and the diaphragms ................................................................. 79
Figure 4.1 (a-e) show the examples of the raw image together with the calculated signal-to-noise ratio (SNR) for each inversion time (TI) from a healthy subject. (f) shows an example of signal intensity versus inversion time plot together with the result from fitting the inversion recovery signal equation for a region of interest drawn at the right lung (red circle in (a-e)). ................................................................................................................................. 85
Figure 4.2 Example MR qS₀ maps of the scan (a) and rescan (c), MR qS₀ thresholded map of the scan (b) and rescan (d) of a healthy subject (Male, 75 years old, FEV₁%predicted = 147%). ................................................................................................................................. 88
Figure 4.3 Example MR qS₀ maps of the scan (a) and rescan (c), MR qS₀ thresholded map of the first scan (b) and the CT image (d) of a patient with COPD (Male, 74 years old, FEV₁%predicted = 42%). ................................................................................................................................. 89
Figure 4.4 Example MR qS₀ maps of the scan (a) and rescan (c), MR qS₀ thresholded map of the first scan (b) and the CT image (d) of a patient with COPD (Male, 64 years old, FEV₁%predicted = 43%). ................................................................................................................................. 90
Figure 4.5 Histograms of lung qS₀ (green) and CT lung density (blue) of two COPD patients (patient 1, RA₀.20 =7%, RA₉₅₀ = 5%; patient 2, RA₀.20 =37%, RA₉₅₀ = 36%). ................................................................................................................................. 91
Figure 4.6 The receiver operating characteristic (ROC) curves of mean qS₀, 15th percentile of qS₀, RA₀.20 and the standard deviation of qS₀ in differentiating COPD patients from healthy controls. ................................................................................................................................. 93
Figure 4.7 Scatter plots showing the linear correlation of CT RA₉₅₀ with (a) RA₀.20, (b) mean value of qS₀, (c) 15th percentile of qS₀ and (d) the standard deviation of qS₀ in patients with COPD (n = 24). ................................................................................................................................. 96
Figure 4.8 Scatter plots showing the linear correlation of CT PD_{15} with (a) RA₀.20, (b) mean value of qS₀, (c) 15th percentile of qS₀ and (d) the standard deviation of qS₀ in patients with COPD (n = 24). ................................................................................................................................. 97
Figure 5.1 Example dynamic OE-MRI parameter maps of (a) T₁air (ms), (b) enhancing regions (white mask demonstrating effectively complete enhancement), (c) ΔPO₂_{max} (mmHg) and (d) τ_{up} (min) from a healthy subject (Male, 46 years old, FEV₁%predicted=114%). ................................................................................................................................. 107
Figure 5.2 Example maps of (a) density-mask CT image, (b) T₁air (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 50%), (d) ΔPO₂_{max} (mmHg) and (e) τ_{up} (min) from a patient with non-emphysematous COPD (Male, 72 years old, FEV₁%predicted = 82%). ................................................................................................................................. 108
Figure 5.3 Example maps of (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 54%), (d) $\Delta P_{O_{2\text{max}}}$ (mmHg) and (e) $\tau_{up}$ (min) from a patient with non-emphysematous COPD (Female, 71 years old, FEV$1\%_{\text{predicted}} = 62%$).

Figure 5.4 Example maps of a (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 50%), (d) $\Delta P_{O_{2\text{max}}}$ (mmHg) and (e) $\tau_{up}$ (min) from a patient with emphysematous COPD (Male, 64 years old, FEV$1\%_{\text{predicted}} = 43%$).

Figure 5.5 Example maps of (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 27%), (d) $\Delta P_{O_{2\text{max}}}$ (mmHg) and (e) $\tau_{up}$ (min) from a patient with emphysematous COPD (Female, 74 years old, FEV$1\%_{\text{predicted}} = 42%$).

Figure 5.6 $\Delta P_{O_{2}}$ time course curves (averaged over the lung regions) in a patient with COPD (blue curve) and a healthy subject (green curve).

Figure 6.1 Dynamic OE-MRI parameter maps from a mild asthmatic participant (female, 19 years old, FEV$1\%_{\text{predicted}} = 99%$) from scan (V1) and rescan (V2).

Figure 6.2 Dynamic OE-MRI parameter maps from a severe asthmatic participant (female, 19 years old, FEV$1\%_{\text{predicted}} = 64%$) from scan (V1) and rescan (V2).

Figure 6.3 Histograms derived from the example maps of $\Delta P_{O_{2\text{max}}}$, $\tau_{up}$ and $\tau_{down}$ shown in figure 6.1 and figure 6.2.

Figure 6.4 Group averaged histograms of $\Delta P_{O_{2\text{max}}}$ (a), $\tau_{up}$ (b) and $\tau_{down}$ (c).

Figure 6.5 The group averaged time course curves of median $\Delta P_{O_{2}}$ across the entire lung.

Figure 6.6 The scatter plots with the line of best fit to show the strongest correlation of each imaging readout with the pulmonary function test indices.

Figure 6.7 Bland-Altman plots of the agreements of two measurements of EF (a), entire-lung median $\Delta P_{O_{2\text{max}}}$ (b), median $\tau_{up}$ (c) and median $\tau_{down}$ (d) in the two groups.

Figure 7.1 Schematic of the protocol designs for the salbutamol intervention visit and the control visit.

Figure 7.2 The baseline maps of $\Delta P_{O_{2\text{max}}}$ (a, b, c), $\tau_{up}$ (d, e, f) and $\tau_{down}$ (g, h, i) from a healthy subject (F, 28 yrs, FEV$1\%_{\text{predicted}} = 107%$, a, d, g), a mild asthmatic patient (F, 23 yrs, FEV$1\%_{\text{predicted}} = 117%$, b, e, h) and a severe asthmatic patient (F, 49 yrs, FEV$1\%_{\text{predicted}} = 79%$, c, f, i).

Figure 7.3 Mean changes in median $\Delta P_{O_{2\text{max}}}$ with time after salbutamol inhalation (a, visit 1) and after scanning pause (b, visit 2) in healthy control group (closed squares), mild asthmatic group (open circles) and severe asthmatic group (closed circles).

Figure 7.4 $\Delta P_{O_{2\text{max}}}$ maps pre- and post-salbutamol (visit 1, a, b, c) and pre- and post-scanning pause (visit 2, d, e, f) from a severe asthmatic patient (M, 58yrs, FEV$1\%_{\text{predicted}} = 81%$).
Figure 7.5 Percentage changes in median $\Delta PO_{2\text{max}}$ in the severe asthmatic subgroup who attended both salbutamol intervention scan (closed circles, solid line) and control scan (open circles, dashed line).

Figure 8.1 The process of generating the vessel mask by k-means clustering method.

Figure 8.2 Group averaged relative signal enhancement curves of the lung parenchyma from healthy subjects and patients with asthma.

Figure 8.3 Example parameter maps and regional coefficient of variation (CoV-) maps of SI%$_{\text{max}}$ and $k_{\text{washout}}$ from a healthy subject (Male, 34 years old, FEV$_1$%predicted = 93%) and a patient with asthma (Male, 48 years old, FEV$_1$%predicted = 45%, severe, non-eosinophilic).

Figure 8.4 Example parameter maps of SI%$_{\text{max}}$ and $k_{\text{washout}}$ from a healthy subject (Female, 47 years old, FEV$_1$%predicted=104%) and patients with non-eosinophilic mild asthma (Female, 49 years old, FEV$_1$%predicted=90%), eosinophilic mild asthma (Male, 44 years old, FEV$_1$%predicted=106%) and eosinophilic severe asthma (Male, 48 years old, FEV$_1$%predicted=29%).

Figure 8.5 Boxplots show the comparison of median SI%$_{\text{max}}$ (a), median $k_{\text{washout}}$ (b), median iAUC$_{60}$ (c) and median local coefficient of variation of $k_{\text{washout}}$ (d) between healthy control and asthma groups.

Figure 8.6 Boxplots show the comparison of median SI%$_{\text{max}}$ (a), median $k_{\text{washout}}$ (b), median iAUC$_{60}$ (c) and median local coefficient of variation of $k_{\text{washout}}$ (d) between healthy control, mild asthma and severe asthma groups.

Figure 8.7 Boxplots show the comparison of median SI%$_{\text{max}}$ (a), median $k_{\text{washout}}$ (b), median iAUC$_{60}$ (c) and median local coefficient of variation of $k_{\text{washout}}$ (d) between healthy control, non-eosinophilic asthma and eosinophilic asthma groups.

Figure 8.8 The receiver operating characteristic curves of median SI%$_{\text{max}}$, median $k_{\text{washout}}$ and median iAUC$_{60}$ in the differentiation of asthma from healthy control.

Figure 8.9 Scatter plots showing the linear correlation of median SI%$_{\text{max}}$ and median $k_{\text{washout}}$ with FEV$_1$%predicted and FEV$_1$/FVC in patients with asthma (n=28).

Figure 9.1 ROI concentration time courses (green dots) and extended Tofts model fitting (dash line) for a healthy subject (figure 2a) and an asthmatic patient (figure 2b). The ROI was drawn in the upper lobe of the right lung. Insets highlight the individual arterial input functions.

Figure 9.2 Example parameter maps of $K_{\text{trans}}$, $v_e$, $v_p$ and iAUC$_{60}$ from a healthy subject (Male, 44 years old, FEV$_1$%predicted=106%) and a patient with asthma (Male, 48 years old, FEV$_1$%predicted=29%).

Figure 9.3 The influence of disease severity (mild vs severe) and eosinophil status (solid lines: eosinophilic; dotted lines: non-eosinophilic) on the group means of median $K_{\text{trans}}$ (a), $v_e$ (b) and $v_p$ (c) in patients with asthma. The grey dashed lines showed the group means of the health control group.
Abstract

This project focused on the pulmonary application of magnetic resonance (MR) quantitative equilibrium signal ($qS_0$) mapping, dynamic oxygen-enhanced (OE-) magnetic resonance imaging (MRI) and dynamic contrast-enhanced (DCE-) MRI in asthma and chronic obstructive pulmonary disease (COPD).

Initially, a retrospective analysis of MRI and X-ray computed tomography (CT) data from 24 COPD patients and 12 healthy controls demonstrated that MR $qS_0$ mapping had good one-week reproducibility and was comparable to CT in the localization and quantification of emphysema in patients with COPD. In the same data, a reduced oxygen ($O_2$) delivery signal was detected by dynamic OE-MRI in COPD patients regardless of the presence or absence of emphysema on CT, while a significantly reduced baseline spin-lattice relaxation time ($T_{1\text{air}}$) was only observed in emphysematous COPD. Emphysematous COPD also showed significant correlations between dynamic OE-MRI readouts, i.e. enhancing fraction (EF) and the change in the partial pressure of $O_2$ in lung parenchyma ($\Delta P_{O_2\text{max}}$), and pulmonary diffusion capacity and CT estimates of emphysema.

A prospective pilot study was conducted in 10 asthmatic patients which demonstrated that dynamic OE-MRI readouts, including EF, $\Delta P_{O_2\text{max}}$ and $O_2$ wash-in time constant ($t_{\text{washout}}$), were reproducible within one month, sensitive to asthma severity and strongly correlated with spirometric readouts of airway function and lung volume. This was followed by a second prospective intervention study in 30 asthmatic patients and 10 healthy controls which revealed a pattern of decreased $O_2$ delivery signal as a response to salbutamol inhalation in severe asthmatics but not in mild asthmatics or healthy controls using short-term repeated dynamic OE-MRI. In addition, DCE-MRI was also performed on 30 asthmatic patients and 10 healthy subjects. A semi-quantitative analysis demonstrated that contrast agent kinetics in asthmatic lungs were characterised by a reduced first-pass peak ($SI_{\text{max}}$) and a shallower downslope during the late redistribution phase ($k_{\text{washout}}$) than was observed in healthy controls, and that these were related to pulmonary function test measurements. An extended Tofts model-based quantitative analysis further revealed a significantly increased fractional extravascular extracellular space ($v_e$) in patients with asthma than in healthy controls while the contrast agent transfer coefficient ($K^{\text{trans}}$), an index related to vascular permeability, and the fractional blood plasma volume ($v_p$), did not distinguish asthmatics from controls.

In conclusion, this project demonstrated the promise of 1) MR $qS_0$ mapping for the assessment of emphysema in COPD lungs, 2) dynamic OE-MRI for the assessment of impaired pulmonary oxygenation in COPD and asthma and for the monitoring of short-term treatment effects in asthma and 3) DCE-MRI for the evaluation of pulmonary microvascular inflammation in asthma. The non-invasive non-ionizing properties and simple setup requirements make these three proton MRI techniques attractive options in the assessment of structural and functional alterations of the lungs in asthma and COPD in clinical settings.

**Thesis title:** Magnetic resonance imaging of the lungs in asthma and COPD  
**Institute:** The University of Manchester  
**Candidate:** Weijuan Zhang  
**Degree title:** Doctor of Philosophy  
**Date:** 21st Sep 2014
Declaration

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Dedication

This thesis is especially dedicated to my mother Pinghua Wu and my father Yulin Zhang, who have raised me up, loved and supported me throughout my life.

Also, this thesis is dedicated to my beloved boyfriend Jun Jiang, for his endless encouragement, understanding and company throughout my studies.
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Finally, I would not be here today without the help and support from my mother Pinghua Wu, my father Yulin Zhang and my beloved boyfriend Jun Jiang. Their understanding, patience and endless love have been my strength and courage to complete my study. My achievement is also their achievement, too. Give my greatest gratitude to them.
The author

The author completed her undergraduate medical training in 2008 and postgraduate specialized training in respiratory medicine in 2010 at Capital Medical University in China before being awarded a Dorothy Hodgkin postgraduate award in 2010 to pursue a PhD in the School of Medicine at The University of Manchester. During the past 4 years, the author participated in the lung MRI studies conducted in the Centre for Imaging Sciences, The University of Manchester. After her PhD, the author plans to start her career as a doctor in United Kingdom with a view of combining academic and clinical practice. The long-term goal of the author is to become a chest physician with all the qualities of good clinical practice, passion in research in view of improving patient care in the long term.
The alternative format thesis

This work focused on the assessment of the utility of three different lung MRI techniques, i.e. MR qS\(_0\) mapping, dynamic OE-MRI and DCE-MRI, in healthy subjects, patients with asthma and patients with COPD, resulting in many publishable findings. This project has to date yielded 1 paper accepted for publication in peer-reviewed journal, 2 papers in revision for the publication in peer-reviewed journals, 3 papers about to be submitted to peer-reviewed journals and a number of scientific abstracts accepted for oral and poster presentations in international conferences.

Since all data were intended for publication, and consisted of inter-related results forming a coherent thesis, the alternative format thesis was chosen as the most suitable way of presenting these findings.
Publications

Selected conference proceedings


<table>
<thead>
<tr>
<th>Abbreviations</th>
<th>Description</th>
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<tbody>
<tr>
<td>2D</td>
<td>Two-dimensional</td>
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<tr>
<td>3D</td>
<td>Three-dimensional</td>
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<tr>
<td>AATH</td>
<td>Adiabatic approximation to the tissue homogeneity</td>
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<tr>
<td>ACT</td>
<td>Asthma control test</td>
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<td>AIF</td>
<td>Arterial input function</td>
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<td>ANOVA</td>
<td>Analysis of variance</td>
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<td>ASL</td>
<td>Arterial spin labelling</td>
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<td>BALF</td>
<td>Bronchoalveolar lavage fluid</td>
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<td>BAT</td>
<td>Bolus arrival time</td>
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<td>BMI</td>
<td>Body mass index</td>
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<td>CO</td>
<td>Carbon monoxide</td>
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<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
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<td>CT</td>
<td>Computed tomography</td>
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<td>DCE-MRI</td>
<td>Dynamic contrast enhanced MRI</td>
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<td>DLco</td>
<td>Diffusing capacity of carbon monoxide</td>
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<td>EBUS</td>
<td>Endobronchial ultrasound</td>
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<td>EES</td>
<td>Extravascular extracellular space</td>
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<td>EOS_{b}</td>
<td>Blood eosinophil counting</td>
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<td>EOS_{s}</td>
<td>Sputum eosinophil counting</td>
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<td>EPI</td>
<td>Echo planar imaging</td>
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<td>ETL</td>
<td>Echo train length</td>
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<td>FD</td>
<td>Fourier decomposition</td>
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<td>FDG</td>
<td>Fluorodeoxyglucose</td>
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<td>FET_{25%-75%}</td>
<td>Forced expiratory flow between 25% and 75% of the forced vital capacity</td>
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<td>FEV_{1}</td>
<td>Forced expired volume in 1 second</td>
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<td>FID</td>
<td>Free induction decay</td>
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<td>FLASH</td>
<td>Fast low angle shot</td>
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<td>GRAPPA</td>
<td>Generalized autocalibrating partially parallel acquisition</td>
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<td>HASTE</td>
<td>Half fourier single shot turbo spin echo</td>
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<td>ILD</td>
<td>Interstitial lung disease</td>
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<td>IR</td>
<td>Inversion recovery</td>
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<tr>
<td>MIGET</td>
<td>Multiple inert gas elimination technique</td>
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<tr>
<td>MMEF</td>
<td>Maximum mid-expiratory flow</td>
</tr>
<tr>
<td>MR(I)</td>
<td>Magnetic resonance (imaging)</td>
</tr>
<tr>
<td>MTT</td>
<td>Mean transit time</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear magnetic resonance</td>
</tr>
<tr>
<td>O_{2}, ^{16}O_{2}</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OCT</td>
<td>Optical coherence tomography</td>
</tr>
<tr>
<td>Acronym</td>
<td>Description</td>
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<tr>
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</tr>
<tr>
<td>OE-MRI</td>
<td>Oxygen-enhanced MRI</td>
</tr>
<tr>
<td>PBF</td>
<td>Pulmonary blood flow</td>
</tr>
<tr>
<td>PBV</td>
<td>Pulmonary blood volume</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PFT</td>
<td>Pulmonary function test</td>
</tr>
<tr>
<td>RARE</td>
<td>Rapid acquisition with refocused echoes</td>
</tr>
<tr>
<td>rSIC</td>
<td>The relative signal intensity curve</td>
</tr>
<tr>
<td>RF</td>
<td>Radio frequency pulse</td>
</tr>
<tr>
<td>ROC</td>
<td>Receiver operating characteristic analysis</td>
</tr>
<tr>
<td>ROI</td>
<td>Region of Interest</td>
</tr>
<tr>
<td>RV</td>
<td>Residual volume</td>
</tr>
<tr>
<td>SABA</td>
<td>Short acting β agonist</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SE</td>
<td>Spin echo</td>
</tr>
<tr>
<td>SENSE</td>
<td>Sensitivity encoding</td>
</tr>
<tr>
<td>SNR</td>
<td>Signal to noise ratio</td>
</tr>
<tr>
<td>SPGR</td>
<td>Spoiled gradient echo</td>
</tr>
<tr>
<td>SR</td>
<td>Saturation recovery</td>
</tr>
<tr>
<td>sR_{eff}</td>
<td>Effective specific airway resistance</td>
</tr>
<tr>
<td>sR_{tot}</td>
<td>Total specific airway resistance</td>
</tr>
<tr>
<td>SSE</td>
<td>Sum of squared errors</td>
</tr>
<tr>
<td>SSFP</td>
<td>Steady-state free precession</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TLC</td>
<td>Total lung capacity</td>
</tr>
<tr>
<td>TI</td>
<td>Inversion time</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TRICKS</td>
<td>Time-resolved imaging of contrast kinetics</td>
</tr>
<tr>
<td>TSE</td>
<td>Turbo spin echo</td>
</tr>
<tr>
<td>TTP</td>
<td>Time to peak</td>
</tr>
<tr>
<td>UTE</td>
<td>Ultrashort echo-Time</td>
</tr>
<tr>
<td>VFA</td>
<td>Variable flip angle</td>
</tr>
<tr>
<td>Xe</td>
<td>Xenon</td>
</tr>
</tbody>
</table>

**Symbols**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>⊗</td>
<td>Convolution operation</td>
</tr>
<tr>
<td>B_0</td>
<td>External static magnetic field</td>
</tr>
<tr>
<td>B_1</td>
<td>External oscillating magnetic field</td>
</tr>
<tr>
<td>C_a</td>
<td>Concentration of contrast agent in arterial blood</td>
</tr>
<tr>
<td>C_t</td>
<td>Concentration of contrast agent in tissue</td>
</tr>
<tr>
<td>EF</td>
<td>Enhancing fraction</td>
</tr>
<tr>
<td>E/I_{ratio}</td>
<td>Expiratory to inspiration ratio of CT estimate lung density</td>
</tr>
<tr>
<td>H</td>
<td>Hydrogen nucleus</td>
</tr>
<tr>
<td>γ</td>
<td>Gyromagnetic ratio</td>
</tr>
<tr>
<td>iAUC_{60}</td>
<td>Initial area under the curve over the first 60 seconds</td>
</tr>
<tr>
<td>K^{trans}</td>
<td>Transfer coefficient factor</td>
</tr>
<tr>
<td>k_{up}</td>
<td>Upslope of the first-pass peak</td>
</tr>
<tr>
<td>Symbol</td>
<td>Definition</td>
</tr>
<tr>
<td>--------</td>
<td>------------</td>
</tr>
<tr>
<td>$k_{\text{washout}}$</td>
<td>Late-phase washout slope</td>
</tr>
<tr>
<td>$\lambda$</td>
<td>Pulse efficiency fraction</td>
</tr>
<tr>
<td>$M_{\text{xy}}$</td>
<td>Transverse magnetization</td>
</tr>
<tr>
<td>$M_z$</td>
<td>Longitudinal magnetization</td>
</tr>
<tr>
<td>$M_0$</td>
<td>Equilibrium magnetization</td>
</tr>
<tr>
<td>$\tau_{\text{up}}$</td>
<td>Oxygen wash-in slope</td>
</tr>
<tr>
<td>$\tau_{\text{down}}$</td>
<td>Oxygen washout slope</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Larmor frequency</td>
</tr>
<tr>
<td>PD</td>
<td>Proton density</td>
</tr>
<tr>
<td>PD$_{15}$</td>
<td>The lowest 15th percentile of the pulmonary density</td>
</tr>
<tr>
<td>$\Delta P O_2$</td>
<td>Change in partial pressure of oxygen at steady plateau after switching air to 100% oxygen</td>
</tr>
<tr>
<td>$r_{1,\text{Gd}}$</td>
<td>Longitudinal relaxivity of the contrast agent</td>
</tr>
<tr>
<td>$r_{1,\text{O}2}$</td>
<td>O$_2$ longitudinal relaxivity in water</td>
</tr>
<tr>
<td>$R_1$</td>
<td>Longitudinal, or spin-lattice, relaxation rate</td>
</tr>
<tr>
<td>RA$_{\text{-}860}$</td>
<td>Relative areas with attenuation value below -860 HU</td>
</tr>
<tr>
<td>RA$_{\text{-}950}$</td>
<td>Relative areas with attenuation value below -950 HU</td>
</tr>
<tr>
<td>S</td>
<td>Signal intensity</td>
</tr>
<tr>
<td>$S_0$</td>
<td>Equilibrium magnetization signal</td>
</tr>
<tr>
<td>q$S_0$</td>
<td>Quantitative equilibrium magnetization signal</td>
</tr>
<tr>
<td>t</td>
<td>Time</td>
</tr>
<tr>
<td>SI$_{\text{max}}$</td>
<td>Peak enhancement</td>
</tr>
<tr>
<td>$T_1$</td>
<td>Longitudinal, or spin-lattice, relaxation time</td>
</tr>
<tr>
<td>$T_2$</td>
<td>Transverse, or spin-spin, relaxation time</td>
</tr>
<tr>
<td>$T_2^*$</td>
<td>Effective transverse, or spin-spin, relaxation time</td>
</tr>
<tr>
<td>$v_e$</td>
<td>Extra-cellular extra-vascular fraction</td>
</tr>
<tr>
<td>$v_p$</td>
<td>Blood plasma fraction</td>
</tr>
</tbody>
</table>
Chapter 1 Thesis overview

Asthma and chronic obstructive pulmonary disease (COPD) are two of the most prevalent airway obstructive disorders with high morbidity and mortality, causing huge socio-economic burden across the world. It is essential to thoroughly understand the development and progress of asthma and COPD in order to better prevent, diagnose and treat these diseases.

Current diagnosis and monitoring of asthma and COPD relies on clinical symptoms and conventional pulmonary function tests that are neither useful in providing regional information nor sensitive in exploring early changes of lung function. Advanced imaging techniques, such as X-ray computed tomography (CT), scintigraphy, positron emission tomography (PET), single-photon emission computed tomography (SPECT) and hyperpolarized gas magnetic resonance imaging (HP gas MRI), enable the visualization and quantification of pulmonary structural and functional alterations at a local level, which greatly facilitates the exploration of regional pulmonary pathophysiology in asthma and COPD. However, the application of the above imaging techniques is hampered by a number of limitations including the use of ionizing radiation, the need to produce radiotracers, expense and/or practical difficulty in implementation. There is therefore a need to develop new imaging biomarkers to investigate early and regional alterations in asthma and COPD lungs with the consideration of both feasibility and usefulness.

The potential of proton magnetic resonance imaging (MRI) techniques in the evaluation of pulmonary disorders is drawing increasing interest. Quantitative MRI is based on a group of techniques that quantify lung tissue intrinsic properties, such as tissue longitudinal and transverse relaxation times (T₁, T₂), which are linked to tissue biochemical characteristics and microenvironment, and the equilibrium magnetization signal (S₀), which is linked to the tissue density. T₁-weighted dynamic oxygen-enhanced MRI (OE-MRI) allows the efficiency of regional pulmonary oxygen delivery to be assessed by using high concentration of oxygen (O₂) as an inhaled contrast agent using standard MR scanners. T₁-weighted dynamic contrast-enhanced MRI (DCE-MRI) is capable of estimating regional pulmonary perfusion and aspects of pulmonary microvascular physiology using an intravenous contrast agent. All these techniques have been proposed as exploitable and clinically accessible tools for the investigation of a variety of pulmonary diseases. However, their clinical application in asthma and COPD, especially via quantitative analysis, is still insufficiently developed, evaluated and documented.

1.1 Aims and objectives

This thesis is focused on the pulmonary application of quantitative equilibrium signal (qS₀) mapping MRI, T₁-weighted dynamic OE-MRI and T₁-weighted DCE-MRI with the aim to investigate the feasibility and usefulness of these three proton MRI techniques in asthma and COPD. The main objectives of the thesis are thus as following:
1. To evaluate the feasibility of MR quantitative equilibrium signal (qS₀) mapping in the estimation of lung density in healthy subjects and patients with COPD and to compare MR qS₀ mapping with CT in the estimation of emphysema in COPD.

2. To evaluate the feasibility of T₁-weighted dynamic OE-MRI in the estimation of pulmonary oxygenation and its response to treatment (salbutamol inhaler) in healthy subjects and patients with asthma and to explore the relationship between T₁-weighted dynamic OE-MRI and pulmonary function tests.

3. To evaluate T₁-weighted DCE-MRI in the estimation of pulmonary vascular function in asthma with the use of empirical and kinetic modelling parameters.

1.2 Thesis outline

In chapter 2, I introduce some general pathological and physiological characteristics of asthma and COPD, and outline the relevant non-imaging techniques for the diagnosis and monitoring of these two pulmonary disorders. After that, I review the current non-MR imaging techniques available for the assessment of structural and functional alterations in asthma and COPD and discuss their pros and cons. I then establish the need for novel non-invasive, non-ionizing and clinically accessible imaging methods. A special focus is given to quantitative CT techniques in this chapter, as involved in my PhD research.

In chapter 3, I introduce the theoretical basis of MRI, followed by a short description of the challenges of applying MRI in the lung and the approaches to overcome the challenges. After that, I introduce the principles of MR qS₀ mapping and T₁ mapping, T₁-weighted dynamic OE-MRI and T₁-weighted DCE-MRI and their clinical applications in the lung. A brief review of the other proton lung MRI techniques and HP gas MRI techniques and their applications in asthma and COPD are then given. The key methods of data acquisition and data post-processing used during this PhD research are also detailed in the corresponding sections.

Chapters 4-9 are written in the form of journal papers. Chapter 4 “MR quantitative equilibrium signal mapping: a reliable alternative to CT for the assessment of emphysema in COPD” is a paper accepted by “Radiology” reporting the ability and reliability of quantitative S₀ mapping in the assessment of lung density in healthy subjects and COPD plus a comparison of the methods with findings from CT. Chapter 5 reports a study which explores regional structural-functional relationships in COPD with and without emphysema using CT and T₁-weighted dynamic OE-MRI. Chapter 6 “Dynamic oxygen-enhanced magnetic resonance imaging of the lung in patients with asthma – initial experience” is a paper submitted to “European Journal of Radiology” (in revision) regarding the feasibility of T₁-weighted dynamic OE-MRI in the assessment of asthma with an assessment of correlations to pulmonary function tests in a small number of subjects. Chapter 7 reports a study regarding the feasibility of T₁-weighted dynamic OE-MRI in the assessment of pulmonary response to salbutamol inhalation in healthy subjects and asthmatic patients. Chapter 8 “T₁-weighted dynamic contrast-enhanced MRI of the lung in asthma: semi-quantitative analysis for the assessment of contrast kinetic characteristics” is a paper submitted to “Radiology” (in
revision) regarding the feasibility of empirical $T_1$-weighted DCE-MRI parameters in the assessment of contrast agent kinetic characteristics in the lungs of healthy subjects and patients with asthma. Chapter 9 reports a study with regard to tracer kinetic model parameters derived from $T_1$-weighted DCE-MRI in the assessment of pulmonary vascular permeability, extravascular extracellular space and relative capillary bed volume in the lungs of healthy subjects and patients with asthma. The 3 unsubmitted paper chapters (5, 7, 9) are in progress of the 3rd round author review, aiming for submission to peer-reviewed journals by December 2014.

Finally, conclusions are drawn from the presented research in Chapter 10.

1.3 MRI experiments and contributions

In this PhD projects, two MRI experiments were carried out in patients with asthma and healthy controls. In addition, existing datasets from a previous COPD MRI study were reanalysed as a part of the PhD work.

1) Pilot asthma study: 10 asthmatic patients underwent dynamic OE-MRI, DCE-MRI scanning and repeated dynamic OE-MRI scanning at 1 month apart. OE-MRI datasets of this study were used in chapter 6. DCE-MRI datasets contributed to chapter 8 and chapter 9.

2) Salbutamol interventional asthma study: 30 asthmatic patients and 10 healthy subjects underwent dynamic OE-MRI scanning prior to, 15 min after and 30 min after inhalation of 400 μg salbutamol. A control dynamic OE-MRI scanning and DCE-MRI scanning without salbutamol inhalation was performed in 20 asthmatics and 10 healthy subjects within 7 days. Dynamic OE-MRI datasets with and without salbutamol intervention were used in chapter 7. DCE-MRI datasets contributed to chapter 8.

3) Existing COPD datasets from a previous COPD study: Dynamic OE-MRI were performed twice at 7 days apart in 24 COPD patients and 12 healthy controls. These datasets were used on chapter 4 and chapter 5.

In chapters 6, 7, 8 and 9 (asthma related studies), the study design, application for ethical approval, participant enrolment and the clinical data collection was carried out by the candidate. Participant screening was carried out by the candidate with the assistance of Dr. Robert Niven and his team at University Hospital of South Manchester. Pulmonary function tests were performed by chest physiologists (Respiratory and Allergy Clinical Research Facility, University Hospital of Manchester). The sputum induction and processing was performed by Dr. Gael Tavernier (North West Lung Centre, University Hospital of South Manchester). All imaging data acquisitions were carried out at the Manchester NIHR/Wellcome Trust Clinical Research Facility and the Wolfson Molecular Imaging Centre by the candidate with the assistance of radiographers at University of Manchester Magnetic Resonance Imaging Facility. Subsequent data analysis was carried out by the candidate. The core MATLAB code used for data analysis was developed by Dr. Josephine Naish (The University of Manchester), including the segmentation and registration algorithms, $T_1$ mapping, quantitative dynamic OE-MRI analysis and quantitative DCE-MRI kinetic model fitting.
Chapter 4 and 5 (COPD related studies) analysed existing datasets from a previous COPD study conducted by the Centre for Imaging Sciences, The University of Manchester. Image data was acquired by Dr. Penny Cristinacce (The University of Manchester). In chapter 4, all data analysis was carried out by the candidate. In chapter 5, the dynamic OE-MRI parameter maps were generated by Dr. Penny Cristinacce. CT data analysis and the comparison between dynamic OE-MRI and CT were carried out by the candidate.
Chapter 2 Background: asthma and COPD

2.1 A snapshot of asthma and COPD: key similarities and differences

Both asthma and COPD are airway obstructive diseases characterised by widespread chronic airway inflammation. They share similarities but also have differences, some of which are discussed below.

2.1.1 Prevalence and Burden

Asthma and COPD are two of the most prevalent airway obstructive disorders with high mobility and mortality, causing huge social-economic burden across the world. Asthma affects 235 million people currently and causes 180,000 deaths per year globally [1, 2]. Although the overall mortality peaked in 1980s, the worldwide prevalence is on the rise and is set to be 400 million people in 2025 [3]. The financial costs of asthma vary between $300 to $1,300 per patient per year in western countries and 50% of the total is allocated to severe cases that only comprise 10%-20% of the total asthmatic population [4]. COPD is a more costly disease with higher morbidity and mortality than asthma worldwide. The current global prevalence of COPD is 329 million people [5]. 2.5 million people died of COPD in 2000 and the number rose to 3.1 million in 2012, accounting for 5% of total deaths worldwide [6, 7]. COPD is the fourth-leading cause of death worldwide currently and is projected to rank third in cause of death and fifth in burden of disease worldwide by 2030 [5, 6]. The urge to stem the tide of asthma and COPD and their costs provides the main impetus for the development of novel methods and biomarkers with value in improving the understanding, diagnosis, monitoring and treatment of these two diseases.

2.1.2 Clinical features

The onset of asthma is usually in early childhood but may occur at any age. Asthmatic symptoms, including wheezing, chest tightness, breathlessness and cough, initiate as intermittent and recurrent episodes with varying frequency and intensity, which can subside spontaneously or with treatment. Patients can be completely symptom free between episodes. However, in patients with severe and long-standing asthma, these symptoms become persistent. A large portion of asthmatic patients are atopic who also experience allergic conditions such as eczema, hay fever and allergic rhinitis. For these patients, an acute asthma attack is usually triggered within minutes of exposure to specific allergens, such as animal fur, mice dust, medicine, grass, pollen or food. In addition, airway hyperresponsiviness is a hallmark of asthma, i.e. bronchoconstriction provoked by a number of non-specific irritants, such as smoke, cold air and exercise at a dose which would have minimal or no effect on most of the healthy individuals. The airflow limitation is fully reversible initially but its reversibility may be diminished as fixed airway remodelling develops due to longstanding inflammation. Furthermore, the airway limitation in asthma is characteristically variable within a day, typically characterised by a morning or evening dip, due to which asthmatic patients usually experience worse symptoms in the early morning and at night [8-10].
COPD is a group of diseases characterised by persistent and progressive airway obstruction that is not fully reversible. Chronic bronchitis, emphysema or combinations of the two are the common forms of COPD. COPD symptoms usually present after several years of cumulative exposure to noxious substances, in particular tobacco smoke. Inherent deficiency of alpha-1 antitrypsin is another known cause of COPD. The diagnosing age of COPD is generally above 40 years old and the prevalence of COPD increases dramatically with aging after that. Breathlessness on exertion, long-term cough and sputum production are the respiratory symptoms of COPD, with the former prominent in patients with emphysema and the latter two prominent in patients with chronic bronchitis. These symptoms are constant and progressive at stable status and increase in frequency and intensity during COPD exacerbation. In contrast to asthma, atopy is not a feature of COPD population. The airflow limitation in COPD is usually persistent, progressive and poorly reversible without marked changes over a day or over several months. In addition, COPD is associated with a variety of extrapulmonary conditions due to its systemic inflammatory effects, e.g. weight loss, skeletal muscle dysfunction and cardiovascular diseases, etc. [11].

2.1.3 Inflammation and structural changes

The distinction in chronic inflammation profile is possibly the most important difference between asthma and COPD as it further determines the differences in the pathological changes and treatment responses between the two diseases [12, 13]. The chronic inflammation in asthma is frequently an allergic process driven by eosinophils, mast cells and CD4+ T helper type 2 cells [10, 12-14]. These inflammatory cells accumulate and infiltrate in and around the entire airways and release a number of inflammatory mediators which sustain the inflammatory process and cause airflow obstruction by contracting airway smooth muscle, causing airway wall oedema and increasing mucus secretion [10, 15, 16]. The longstanding inflammation injury then results in structural changes in the airways, termed airway remodelling, that dominate in segmental, sub-segmental, and smaller conducting airways in asthma [17]. Airway remodelling in asthma is characterised by widespread airway wall thickening and airway lumen narrowing as a result of the thickening of the sub-epithelial reticular basement membrane secondary to the deposition and reconstruction of the connective tissue components, hypertrophy and hyperplasia of airway smooth muscle, goblet cells and mucous glands and bronchial vasculature growth and remodelling [10, 18]. Shedding and damage of airway surface epithelium is also a hallmark of airway remodelling in asthma [14, 18]. On the other hand, pulmonary parenchyma in uncomplicated asthma is usually intact without the destruction of alveolar walls or the loss of capillary beds, although the characteristic inflammatory infiltration of the airways is also observed in lung parenchyma [13, 18]. Asthmatic patients with eosinophil-based inflammation usually show good responses to corticosteroid treatment (anti-inflammation effect) whereas non-eosinophilic asthmatics are more likely to be resistant to corticosteroid treatment [13].
In COPD, the chronic inflammation cascade is initiated by the inhalation of noxious irritants and is primarily driven by neutrophils, macrophages and CD8+ T cytotoxic cells [12, 13]. These inflammation cells release inflammatory mediators different from those in asthma and cause characteristic airway remodelling, parenchyma destruction and vessel damage [12, 18]. Airway remodelling also presents as thickened airway walls and narrowed airway lumen in COPD, which is particularly prominent in intermediate-sized airways (inner diameter between 2 mm-4 mm) and distal small airways (inner diameter < 2 mm) and highly characterised by squamous epithelial metaplasia and airway wall fibrosis without obvious reticular basement membrane thickening [18-20]. Shared airway structural changes between COPD and asthma include hypertrophy and hyperplasia of airway smooth muscle and mucous glands [18, 19]. Emphysematous destruction of the pulmonary parenchyma is a key feature of COPD pathology, where the airspaces are permanently enlarged and the alveolar walls are destroyed without obvious fibrotic changes. This is accompanied by damage and loss of the small airways and capillary beds [19]. Corticosteroid treatment plays a much smaller role in COPD treatment because it is not effective in controlling neutrophil-predominant inflammation [14].

In addition, the inflammatory changes in bronchial and pulmonary microvasculature are important contributors to the development and progression of asthma and COPD [21]. Characteristic alterations include increased vessel calibre due to vasodilation, increased vessel number due to angiogenesis, vascular wall swelling and peri-vascular interstitial space oedema and increased airway secretion due to increased vascular permeability, all of which further narrow the airways [21]. In COPD lungs, the destruction of pulmonary capillary beds due to emphysema causes a loss of regional pulmonary perfusion. Regulation of vessel activation is being considered as a new therapeutic avenue for asthma and COPD [21, 22].

2.1.4 Alterations in pulmonary physiology

The lung is the organ for gas exchange, where O2 is delivered from air to alveoli and across the alveolar-capillary membrane into the venous blood while carbon dioxide is moved out from venous blood and eliminated into the air. It is governed by four pulmonary physiological aspects – ventilation, perfusion, gas diffusion, and the mechanics of breathing. Impairments in pulmonary function are usually followed by pulmonary structural alterations [23, 24].

In asthma and COPD, airway obstruction impedes the airflow and leads to poor alveolar ventilation [25, 26]. Due to the varied sites and extent of airway obstruction over the lungs, ventilation distribution in asthma and COPD is substantially heterogeneous [26]. The increased resistance in peripheral airways due to airway obstruction decreases intrabronchial pressure at exhalation and causes peripheral airways to close early during expiration [23-26]. Airway obstruction in asthma is ascribed to acute bronchospasm, mucosal plugging and chronic airway remodelling, whereas in COPD, except for airway remodelling and mucosal plugging, the loss of surrounding supporting tissues and the
reduction in elastic recoil of the lung parenchyma due to emphysematous destruction also contribute to airway obstruction and make airways more prone to collapse during exhalation [23-26]. The early airway closure during expiration in asthma and COPD causes air retention and thus increases the lung volume both after the maximal expiratory effect (“air trapping”) and after normal exhalation (“hyperinflation”) and consequently changes the diaphragm geometry and increases the workload of breath. Airway obstruction is fully reversible and highly variable in asthma whilst minimally reversible without marked changes in COPD. However, the reversibility may diminish in elderly asthmatic patients or patients with longstanding asthma and increase in COPD patients with eosinophilic airway inflammation [13, 26].

Perfusion redistribution occurs in asthma and COPD as a self-compensatory response to the impaired ventilation. Pulmonary blood is shifted from the poorly ventilated regions to the well ventilated regions, attempting to retain the equality between the ventilation and perfusion [25, 26]. Hypoxic vasoconstriction in poorly ventilated regions is likely responsible for the perfusion regulation [25]. However, the compensatory changes in perfusion blood flow do not fully atone for the impaired ventilation and the balance of the distribution between ventilation and perfusion is broken down. Ventilation-perfusion (V/Q) imbalance is the major cause of hypoxemia in asthma and COPD, and exists both in severe/exacerbated and in mild/stable patients [25, 26]. It leads to inefficient use of alveolar gas and capillary blood during gas exchange and hence lowers the blood oxygenation level. In patients with chronic or acute severe asthma, the administration of short acting bronchodilators, especially via systemic routes, has a risk of worsening the V/Q imbalance [27-29]. This is likely due to the loss of the compensatory adjustment of perfusion as a result of the correction of hypoxic vasoconstriction after the reversal of airway obstruction [25, 30]. Thus, it is recommended to give short acting bronchodilators along with high flow high concentration O₂ in the management of an acute asthma attack [30]. Although pure O₂ inhalation may also aggravate V/Q imbalance by inducing vasoconstriction and predisposing the alveoli to collapsing (absorption atelectasis - O₂ absorbed without nitrogen to support alveoli), the O₂ concentration is high enough to overcome its deleterious effect on V/Q mismatch and substantially elevates the blood oxygenation level [27, 29]. True shunt (perfused but unventilated alveoli), the cause of O₂ therapy-resistant hypoxemia, is rare in COPD and asthma and the effective compensatory adjustment from collateral ventilation is likely responsible for its absence [25, 26]. In COPD lungs, except for the compensatory reduction of perfusion in poorly ventilated regions, the damage to the pulmonary capillary beds also results in genuine reduction in pulmonary perfusion that mostly matches with emphysema with regard to its location [18].

The decrease in pulmonary diffusion capacity, i.e. the capacity to diffuse O₂ and carbon dioxide across the alveolar-capillary membrane, is a characteristic feature of patients with emphysematous COPD, owing to the loss of gas exchange surface and the reduction in capillary blood volume, while it is not common in patients with asthma [25, 26].
In addition, patients with emphysematous COPD show reduced static (measured when pause breath at a given volume) elastic recoils and increased static compliance of the lung parenchyma as a result of tissue destruction whilst in patients with asthma, lung parenchyma is intact and thus the change is not prominent [23-26]. However, dynamic compliance of the lung is considerably reduced during tidal breathing in both asthma and COPD, due to the increase in airway resistance, breathing frequency and uneven ventilation [23-26].

2.2 Assessment of asthma and COPD: non-imaging techniques

2.2.1 Airway function

Spirometry provides fundamental assessment of airway function in clinical settings. The parameters derived from forced expiratory manoeuvres are the indices-of-choice for the evaluation of the presence and severity of airflow limitation in asthma and COPD, among which the forced expired volume in 1 second (FEV$_1$) and its ratio to forced vital capacity (FVC) are most widely used in routine practice [31, 32]. According to the Global Initiative for Chronic Obstructive Lung Disease guidelines, the presence of persistent airflow limitation confirmed by a post-bronchodilator FEV$_1$/FVC of less than 70% is required to make a diagnosis of COPD and the percentage of FEV$_1$ to its predicted value (FEV$_1$%predicted) is adopted to classify the severity of airflow limitation and thus to stage COPD [11]. In contrast, asthma diagnosis is based on the presence of characteristic symptoms and the severity classification is based on the level of the treatment required to achieve asthma control, where lung function tests are not mandatory. However, the assessment of airflow limitation and its reversibility and variability greatly enhances the confidence of asthma diagnosis and severity grading [8-10]. The reversibility of airflow limitation is assessed by calculating the change in FEV$_1$ measured pre- and post- the administration of acute acting bronchodilator, e.g. salbutamol. An increase of 200 ml and 12% in FEV$_1$ over the pre-bronchodilator values suggests the presence of reversible airflow limitation and thus support the diagnosis of asthma [8, 9]. Longitudinal monitoring of the change in FEV$_1$ and FVC is used to assess disease progression and/or treatment efficiency in asthma and COPD. Airway hyperresponsiveness is assessed by titrating the minimal concentration of inhaled or nebulised airway stimuli, e.g. methacholine, that is required to provoke a drop of 20% or more in FEV$_1$ [8, 10, 26]. Airway hyperresponsiveness is also observed in patients with COPD, although the prevalence and degree are lower than that in asthma population [13, 26].

Peak expiratory flow rate (PEFR), measured using either a spirometer or a peak expiratory flow meter, is an alternative to FEV$_1$ for the evaluation of airway obstruction [32]. Though PEFR is variable and less reproducible than FEV$_1$, it is more practical for the self-monitoring of the day-to-day variation of airflow limitation by patients. A diurnal variation of more than 20% in PEFR tends to confirm asthma and a sudden and significant drop in PEFR may indicate an acute asthma attack [8].
FEV₁, FVC and PEFR reflect the summed airflow limitation in the cross-sectional areas of the airways, which are insensitive to mild airway obstruction or peripheral airway obstruction. The forced expiratory flow between 25% and 75% of the FVC (FEF₂₅₋₇₅%) and maximum mid-expiratory flow (MMEF) are sensitive to airflow limitation in middle to small airways [31, 33]. However, the high variability and low reproducibility hamper their clinical application.

2.2.2 Lung volumes

Lung volume measurement is performed using plethysmographic method, the helium dilution method or the nitrogen washout method [34]. Asthma and COPD show overlapping characteristics in lung volume profile: an increased ratio of residual volume (RV, lung volume after maximal expiration) to total lung capacity (TLC, lung volume after maximal inspiration) indicates the presence of air trapping resulting from early-expiratory airway closure; an increased functional residual capacity (FRC, lung volume after tidal breath) indicates the presence of pulmonary hyperinflation secondary to air-trapping, increased inspiration muscle activity and reduced elastic recoil of the lung (mainly in COPD) [25, 26]. Significant increase in TLC is a characteristic feature of COPD patients with emphysema, ascribed to the loss of lung recoil and increase in static lung compliance due to tissue destruction [23, 26]. Patients with asthma usually show normal TLC or slightly raised TLC, which is however much smaller than that in patients with emphysema. A significant increase in TLC in asthmatic patients may indicate an acute severe asthma attack or co-existence of COPD [25, 26].

2.2.3 Alveolar function

Arterial blood O₂ tension (PaO₂) and alveolar-arterial gradient for O₂ partial pressure (P_{O2}A-a) measured by the arterial blood gas test are a useful, although rough, guides to the presence and severity of V/Q inequality, given the absence of other causes of hypoxemia including hypoventilation, true shunt and impaired pulmonary diffusion [25, 26, 35]. Pulse oximetry is a non-invasive method for monitoring the peripheral capillary O₂ saturation.

More detailed analysis of V/Q inequality is carried out by using multiple inert gas elimination technique (MIGET), which, however, mainly serves as a research tool [28, 36]. In MIGET, six inert gases with different solubility are infused into the pulmonary artery in solution and the concentrations of these gases in blood and expired air are measured. The data are used to reconstruct a profile or histogram presenting the distribution of V/Q ratio (usually on a log scale) in relation to blood flow and/or alveolar ventilation [28, 36]. The MIGET V/Q ratio histogram has been usefully utilized to reveal the V/Q inequality in chronic stable asthma and COPD and its deterioration during acute exacerbation and the administration of 100% O₂ or acute-acting bronchodilators [25-29, 37]. However, the clinical application of MIGET is hampered by the difficulty in implementation and results interpretation.

The diffusing capacity of the lung is assessed by measuring the carbon monoxide (CO) uptake from a signal maximum inspiration of gas mixture with known CO concentration.
in a standard time (usually 10 seconds) [38]. Low CO diffusion capacity (DLco) and CO transfer coefficient (Kco) are hallmarks of patients with emphysema and the degree of reduction is correlated with the extent of emphysema [26]. In contrast, asthma usually preserves normal or slightly increased DLco and Kco, unless there is presence of co-existent emphysema or longstanding impairment from smoking [25, 26].

2.2.4 Airway inflammation

The assessment of airway inflammation is performed by counting the inflammatory cells and measuring the concentrations of inflammatory mediators in spontaneous sputum, hypertonic saline-induced sputum, bronchoalveolar lavage fluid (BALF), bronchial biopsy specimens and surgical resection specimens [39]. The biopsy specimens and BALF, collected via invasive procedures, are not used as clinical routines. Regarding the sputum samples which are collected non-invasively, induced sputum samples are preferable to spontaneous sputum samples because of the higher cell quality with regard to viability and cell morphology [40]. High eosinophil level in sputum, BALF and biopsy specimens is commonly observed in patients with asthma and has been associated with the degree of airway obstruction and airway hyperresponsiveness and the response to corticosteroid treatment [39]. By contrast, COPD patients usually show an increased number of neutrophils and macrophages in sputum and BALF, which inversely correlates with the indices of airflow limitation [40]. Elevated sputum eosinophil level in COPD usually predicts a higher airway hyperresponsiveness and a better response to corticosteroids [40].

The fraction of exhaled nitric oxide (FE\textsubscript{NO}) is a non-invasive biomarker of the distal airway inflammation used in the clinical settings. The elevated FE\textsubscript{NO} in patients with asthma results from the up-regulation of nitric oxide synthase expression induced by the inflammatory mediators and has been correlated with the increase in sputum eosinophils. FE\textsubscript{NO} remains normal in stable COPD but rises in exacerbation status. Baseline FE\textsubscript{NO} value have been used to predict sputum eosinophilia, disease exacerbation and the response to corticosteroid treatment in asthma and COPD [41].

2.2.5 Airway vasculature inflammation

Non-imaging assessment of vascular morphological changes relies on direct visualization via bronchoscopy in vivo and the microscopic observation of biopsy specimens in vitro [42, 43]. The evaluation of vascular permeability is achieved by measuring plasma protein exudates, such as albumin, α-macroglobulin and fibrinogen in sputum and BALF [44-46]. These techniques are invasive, indirect and qualitative.

2.2.6 Pros and cons of non-imaging biomarkers

The pros and cons of each non-imaging technique introduced above are outlined in table 2.1. As is apparent from this table, most of these biomarkers are global measures that are poor in detecting, localizing and monitoring regional abnormalities and spatially heterogeneous abnormalities within the lungs. Regional involvement and heterogeneous distribution are two early-stage features of the functional and structural alterations in asthma and COPD that may occur even when the global measurements are still apparently normal.
Regional improvement (deterioration) in lung function may also be early signs of positive treatment response (disease exacerbation). In addition, endobronchial therapies via bronchoscopy and surgical therapies, i.e. bronchial thermoplasty in asthma, endobronchial valves insertion and lung volume reduction surgery in COPD, require detailed topographic information of pulmonary functional and structural alterations for pre-procedure planning and post-procedure estimation. There is thus strong motivation to develop reliable and non-invasive biomarkers with ability to assess regional and heterogeneous changes in asthma and COPD. Imaging has the potential to establish a new area for the direct visualization and quantitative assessment of regional lung abnormalities in asthma and COPD that has been recognised as promising and worthy of further development.
Table 2.1 Pros and cons of non-imaging techniques in the assessment of asthma and COPD

<table>
<thead>
<tr>
<th>Method</th>
<th>Parameters</th>
<th>Pros</th>
<th>Cons</th>
<th>Abnormalities observed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spirometry</td>
<td>FEV₁, FVC</td>
<td>Standardized, reproducible informative, widely accessible in clinical settings</td>
<td>Insensitive to small airways, requires good subject cooperation for forced expiratory manoeuvres</td>
<td>Airway obstruction and its variability and reversibility, airway hyperresponsiveness</td>
</tr>
<tr>
<td>Spirometry</td>
<td>FEF 25%-75%, MMEF</td>
<td>Reflect small airway function</td>
<td>Low reproducibility and high variability</td>
<td>Airway obstruction in small airways</td>
</tr>
<tr>
<td>Peak expiratory flow (peak flow meter)</td>
<td>PEFR</td>
<td>Simplest, portable, suitable for day-to-day self-monitoring</td>
<td>High variability, accuracy depends on the skills of using a flow meter</td>
<td>Daily variability of airflow limitation</td>
</tr>
<tr>
<td>Plethysmographic/helium dilution/ N₂ washout techniques</td>
<td>RV, FRC, TLC, etc.</td>
<td>Standardized, reproducible, informative, widely accessible in clinical settings</td>
<td>Plethysmographic technique tends to overestimate lung volume; claustrophobia</td>
<td>Air trapping and lung hyperinflation;</td>
</tr>
<tr>
<td>Arterial blood test</td>
<td>PaO₂, PaCO₂, PO2A-a</td>
<td>Quick, widely accessible in clinical settings</td>
<td>Low-grade invasive</td>
<td>Hypoxemia, hypercapnia, differentiation of the source of hypoxemia,</td>
</tr>
<tr>
<td>Pulse oximetry</td>
<td>SpO₂</td>
<td>Non-invasive, portable, simplest, suitable for continuous monitoring</td>
<td>Issues of accuracy, sensitivity, inability to check the source of hypoxemia</td>
<td>Hypoxemia</td>
</tr>
<tr>
<td>Multiple inert gas elimination test</td>
<td>Histogram of the V/Q ratio distribution in relation to perfusion and ventilation</td>
<td>Detailed analysis and graphic presentation of ventilation-perfusion mismatch</td>
<td>Complicated technique with regard to data collection, process and interpretation; Low grade invasive; not widely available.</td>
<td>Ventilation-perfusion mismatch</td>
</tr>
<tr>
<td>Carbon monoxide diffusion capacity</td>
<td>DLco, Kco</td>
<td>Fast, standardized, convenient, widely accessible in clinical settings</td>
<td>Affected by hemoglobin level; inhale carbon monoxide (dose in the safe range)</td>
<td>Reduced diffusion capacity of the lung in emphysematous COPD</td>
</tr>
<tr>
<td>Sputum induction and processing</td>
<td>Cell counts, concentration of inflammatory mediators and plasma proteins</td>
<td>Non-invasive, convenient, informative</td>
<td>Nebulize hypertonic saline (with a potential risk of triggering airway constriction); issues of reproducibility and standardization</td>
<td>Airway inflammation and increased airway vascular leakage –central airways</td>
</tr>
<tr>
<td>Bronchoalveolar lavage fluid</td>
<td>Cell counts, concentration of inflammatory mediators and plasma proteins</td>
<td>Informative, represent the inflammation in small airways and alveolus</td>
<td>Middle grade invasive (sample collected under bronchoscopy)</td>
<td>Airway inflammation and increased airway vascular leakage – peripheral airways</td>
</tr>
<tr>
<td>Exhaled nitric oxide</td>
<td>FeNO</td>
<td>Non-invasive, suitable for long-term monitoring, associated with inflammatory severity and sensitive to corticosteroid treatment</td>
<td>Need standardization and validation; lacking information of the inflammatory cell profile and thus not available for inflammation type classification</td>
<td>Airway inflammation</td>
</tr>
<tr>
<td>Endo-, trans-bronchial biopsy; surgical resection</td>
<td>Morphological observation</td>
<td>Enables morphological observation</td>
<td>High grade invasive; only observe the changes in biopsy sites</td>
<td>Structural changes in airways and lung parenchyma</td>
</tr>
</tbody>
</table>
2.3 Assessment of asthma and COPD: imaging techniques

2.3.1 X-ray computed tomography

Chest X-ray computed tomography (CT) is the cornerstone of the qualitative and quantitative morphological evaluation of the airways and lung parenchyma in vivo [20, 47]. The high spatial resolution (submillimetric) and the three dimensional (3D) volume data achieved by using multi-slice or multi-detector row CT scanners with a high acquisition speed guarantee the detailed presentation of structural abnormalities over the entire lungs and enable the measurement of lung volume [20, 48]. Furthermore, CT techniques with the use of tracers allow functional information relating to pulmonary ventilation and perfusion to be obtained [49, 50]. In clinical practice, CT has played an important role in diagnosis, differential diagnosis, phenotyping, emphysema staging and preoperative planning in COPD [47, 49-51], and is increasingly attractive for the diagnosis of associated diseases and the assessment of airway remodelling and air trapping in asthma [20, 52, 53]. The major drawback of CT techniques is the exposure of patients to ionizing radiation, which hampers the application of CT in exploring respiratory dynamics and longitudinal changes and in radiation-susceptible population, such as children and pregnant women [47, 51].

2.3.1.1 Techniques

Quantitative morphometric CT has been applied to assess airway remodelling in vivo. The conducting airways larger than 2 mm in diameter are visible on CT images and airway remodelling can be directly assessed by measuring the airway wall dimensions, including airway wall thickness (WT), percentage wall thickness (WT%), wall area (WA), intraluminal area (Ai), percentage wall area (WA%) and the square root of the wall area of a hypothetical airway with an internal perimeter of 10 mm. Newly introduced airway wall attenuation parameters, e.g. peak wall attenuation (PWA), are sensitive to the textural changes in the airways, e.g. calcification. Airway wall parameters are usually measured from the airways on the cross-sectional areas. With the 3D reconstruction of airway trees becoming more applicable, the measurement of a specific airway is drawing increasing interest [20, 47].

Direct measurement of airway wall dimensions for distal airways (diameter < 2 mm) is not possible on CT images as the wall thicknesses are beyond the spatial resolution threshold. Instead, measurement of air-trapping on end-expiratory CT scans is used to indirectly reflect the severity of small airway obstruction, and thus small airway remodelling. Air-trapping causes increased air retention in the lungs after exhalation, resulting in the reduction of X-ray attenuation values on end-expiratory CT images [20]. Air-trapping areas are usually quantified by using the density mask technique with a cut-off point value of -850 Hounsfield Units (HU) at expiratory CT scans (FRC level) and the extent of air-trapping is determined by the percentage of the air-trapping areas over the entire lungs [20]. Old-fashioned readouts of small airway obstruction include the expiratory to inspiration ratio of lung density (E/I ratio) and the percentage areas with attenuation value below -910 HU on full inspiration CT scans (an index of pulmonary hyperinflation), etc. [49, 52]. However, it has been
demonstrated that expiratory CT shows better correlations with pulmonary function tests than inspiratory CT, especially with the indices of air-trapping [54].

CT is the method-of-choice for the visualization and quantification of the structural changes in lung parenchyma in vivo, e.g. emphysema in COPD [47, 49-51]. Emphysema destruction causes tissue loss and lowers the lung density, which is presented as the reduction in the X-ray attenuation values on CT images. The X-ray attenuation value is related to lung density and has been used to coarsely measure lung density, i.e. lung density (g/L) = 1000+X-ray attenuation value (HU) [47]. Emphysema severity can be qualitatively assessed on CT images by using the subjective visual scoring method, which, however, shows lower reliability and reproducibility and less agreement with pathological findings than quantitative CT approaches [55]. Two common approaches for the objective quantification of emphysema on CT are the density mask method and percentile density analysis based on full inspiration CT scans. The density mask technique defines emphysema as the voxels with X-ray attenuation values below a pre-defined density threshold. The widely used threshold is -950HU and the extent of emphysema is expressed as the percentage of the low attenuation areas over the total lung area, i.e. relative areas with attenuation value below -950HU (RA_{-950}) [47, 51, 54]. Percentile density analysis adopts specific percentile density values, most commonly the lowest 15th percentile (PD\textsubscript{15}), on the frequency distribution histograms of X-ray attenuation values as emphysema indices [47, 51, 54]. Recently, clustering methods have been introduced to reconstruct contiguous emphysema on CT images, based on which the spatial extent and distribution of emphysematous regions with different volumes can be investigated separately.

The quality of quantitative CT in the estimation of airway dimensions, air-trapping and emphysema is influenced by many technical and physiological factors, such as the choice of image reconstruction algorithm, slice thickness, X-ray tube current, type of the scanner, breath-holding cooperation and the lung volume [47, 51, 52].

CT is also applicable to assess pulmonary ventilation and pulmonary perfusion, given the administration of appropriate contrast media. Nonradioactive xenon (Xe) gas is a potent X-ray attenuator (radiopaque) and has been used as an inhaled CT contrast agent to highlight the airspaces [49, 56-58]. Dual energy CT scanning has been introduced for Xe-enhanced ventilation imaging, which enables a Xe-enhancement map to be generated from simultaneously acquired images at two different X-ray energy levels [56]. However, dual energy scanners are not widely accessible and the image post-processing is technically demanding. On the other hand, the use of intravenous iodine-based contrast agents allows CT to image the blood in large pulmonary vessels (CT angiography) and capillaries (CT perfusion imaging) [49, 50]. CT angiography and CT perfusion imaging are clinically implemented on standard CT scanners but their hybrids with dual energy CT have also become available [59]. Perfusion CT imaging can be analysed either qualitatively through visual observation of perfusion defects or quantitatively through the calculation of pulmonary blood flow, pulmonary blood volume and mean transit time from dynamic acquisitions [52, 60]. However,
iodinated contrast agents are nephotoxic and have a higher risk of causing allergic reactions than gadolinium-based MRI contrast agents [61].

2.3.1.2 Application in asthma

By using quantitative CT, airway wall thickening has been demonstrated in patients with asthma in vivo and has been correlated with asthma severity and the disease duration [53, 62]. The increased airway wall thickness and airway wall area identified by CT in asthma are well correlated with the histological findings of airway remodelling and physiological measurements of airway obstruction and air trapping, including FEV$_1$%predicted, FEF$_{25%-75%}$ and RV/TLC, etc. [20, 62]. The airway wall thickening assessed by CT in asthma shows a poor response to corticosteroid treatment, which reflects the longstanding airway structural changes in asthma and is associated with the development of irreversible airflow limitation and the decline in lung function [63].

The severity of air-trapping in asthma measured by using CT has been correlated with CT-derived airway wall thickness, spirometric indices of small airway obstructions and asthma severity [33, 48, 52]. An increase in CT estimates of air-trapping is associated with an increased likelihood of severe asthma exacerbations [64]. Furthermore, some studies have successfully used CT assessed air-trapping as the outcome measure to evaluate the airway hyperresponsiveness and the therapeutic efficacy [48, 65].

Changes in inspiratory CT measurements of lung density and low attenuation area in asthma have been reported. Decreased mean lung density, PD$_{15}$ and increased RA$_{950}$ have been demonstrated in patients with asthma both at stable and at exacerbation status, correlating with FEV$_1$ and RV in all asthmatic patients and DLco in asthmatic patients with smoking history [66, 67]. It has been suggested that the changes in mean lung density, PD$_{15}$ and RA$_{950}$ in asthma are indicators of pulmonary hyperinflation while in smoking asthmatics it may also imply the presence of emphysema [66].

Xe-enhanced dual energy CT has been successfully applied to demonstrate ventilation defects in patients with stable asthma, with the Xe ventilation defect score negatively correlated with FEV$_1$, FEV$_1$/FVC, DLco and positively correlate with TLC, FRC and RV [57]. The index of Xe ventilation defects in patients with asthma was found to increase post-methacholine challenge and at least partially reverse after salbutamol inhalation, while both of the changes in the defect index were absent in healthy controls [68].

To date, there is little data about the feasibility of perfusion CT imaging in the assessment of perfusion alterations in asthma.

2.3.1.3 Application in COPD

Although the small airways (< 2 mm in diameter) are the major sites of airflow obstruction in COPD [69], the central large airways are not spared remodelling [23]. Proximal airway wall thickness and airway wall area determined by CT have been demonstrated higher in smokers with COPD than in smokers without COPD and non-smokers and increased in males, with increasing age and increasing degree of current smoking [70, 71]. Increased airway wall thickness on CT is
independently associated with increased chronic bronchitis-related symptoms, worse quality of life and increased COPD exacerbation frequency [72, 73]. WA, WT and PWA indices measured by CT in patients with COPD, which closely correlate with the histological estimation [74], are positively correlated with physiological measurements of airway obstruction and the impairment of diffusion capacity, including the percentage predicted values of FEV₁, FVC and FEF₂₅₋₇₅%, and DLco, and negatively correlated with physiological indices of air-trapping, e.g. RV/TLC [75, 76]. In addition, correlations of Ai, WA% and PWA with FEV₁%predicted in COPD were found to be strengthened as the measuring sites moved from 3rd generation airways to 5th-6th generation airways [77, 78].

Air-trapping on CT is sensitive to early airway abnormalities in current smokers and ex-smokers and correlates with the severity of neutrophilic airway inflammation [49, 79]. In addition, CT measurements of air-trapping, e.g. RA-860 and E/I_ratio, have been correlated with FVC and dyspnoea score [80]. However, the standard expiratory CT approach of quantifying air-trapping is poorly suited to distinguishing air-retention derived from airway remodelling and that from emphysema. Matsuoka et al. have proposed a method for using both inspiration and expiration CT images with different thresholds to separate the two sources of air-retention [81].

In COPD, CT has also been widely implemented to detect, characterise, quantify and grade emphysema. Increasing extent of emphysema measured using quantitative CT has been correlated with increased lung volume, worse diffusion capacity and worse health status. However, the correlations between emphysema severity assessed by CT and airflow limitation evaluated by FEV₁ are extremely variable [54, 82], which reflects the fact that emphysema-induced reduction in tissue elastic recoil is not the only cause of airflow limitation in COPD. The severity and distribution pattern of emphysema evaluated using CT is a strong predictor of COPD exacerbation, modality and outcomes of lung volume reduction surgery (LVRS) [72, 83, 84]. Homogeneous emphysema with lower lobe predominance shows substantial functional impairment and predicts high disease mortality and poor outcomes of LVRS while markedly heterogeneous emphysema with upper lobe predominance is likely to experience mortality and functional benefits from LVRS [85, 86]. Emphysema in the central regions of the lung shows stronger correlation with diffusion impairment than that in the rind of the lung [87]. The morphological subtypes of emphysema defined by CT are consistent with pathological findings and are linked to different etiologic factors and clinical characteristics. Tobacco smoking is associated with centrilobular and paraseptal emphysema that predominantly affects the upper lobes while alpha-1-antitrypsin deficiency usually leads to panlobular emphysema that either homogeneously distributes across the entire lung or shows lower lobe predominance [88]. Clinical and physiological consequences, such as dyspnoea, reduction in walk distance, pulmonary hyperinflation and pulmonary diffusion impairment, are manifest in centrilobular-predominant and panlobular-predominant emphysema but may be not in paraseptal-predominant emphysema [89].

COPD is a remarkably heterogeneous disease complex with regard to pathophysiology, clinical presentation and outcomes. Spirometric indices of persistent airway obstruction do not suffice
to depict individual differences. Therefore, different methods have been proposed to cluster COPD into different phenotypes, with the aim to understand the heterogeneous nature of the condition and to optimize its treatment [90]. Radiological phenotyping based on quantitative CT has proven a valuable COPD phenotyping method as distinct CT appearances may predict meaningful difference in clinical presentation and outcomes [90]. Kitaguchi and Fujimota et al. classified patients with COPD into three radiological phenotypes according to the CT visual scoring for emphysema and airway wall thickening: absent or little emphysema (A phenotype), emphysema without bronchial wall thickening (E phenotype) and emphysema with bronchial thickening (M phenotype) [91]. The E phenotype presents worse healthy status, worse pulmonary function, especially a low DLco, and irreversible airflow limitation. The A phenotype is more related to non-smoking related COPD and shows a normal DLco with a higher reversibility of airflow limitation. The M phenotype presents mixed characteristics of the other two phenotypes: low DLco, high reversibility of airflow limitation.

Xe-enhanced dual energy CT was adopted by Park et al. to investigate ventilation patterns in lung regions with different structural abnormalities in patients with COPD [58]. The areas with airway-predominant alterations showed low or no Xe enhancement in Xe wash-in and washout phases, indicating the presence of both inflow and outflow limitations. By contrast, emphysematous areas mostly showed normal Xe enhancement during the wash-in phase and enhancement retention during the washout phase, which suggested the presence of collateral ventilation and the predominance of outflow limitation in emphysema. Alforda et al. quantified pulmonary blood flow, pulmonary blood volume and mean transit time using dynamic perfusion CT in a group of subjects with normal spirometric readouts who were either never smokers or smokers with or without emphysema on structural CT [60]. Greater global heterogeneity in blood flow and mean transit time was demonstrated in smokers with subtle emphysema than in smokers without emphysema and never smokers, suggesting a role for quantitative perfusion CT in pinpointing emphysema susceptibility in smokers.

2.3.2 Radionuclide lung imaging

Radionuclide lung imaging techniques visualize pulmonary ventilation and pulmonary perfusion by using the gamma rays emitted directly or indirectly (via positron emission followed by annihilation) from inhaled and intravenous radioactive tracers. The three main radionuclide lung imaging techniques are scintigraphy, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [92-94]. Radionuclide lung imaging only delineates pulmonary function and provides little morphological details. Hybrid systems of SPECT and PET with X-ray CT or MRI (SPECT-CT, PET-CT and PET-MRI) facilitate the spatial matching of functional abnormalities with specific anatomic structures.

Ventilation/perfusion lung scintigraphy, also called a ventilation/perfusion or V/Q scan, uses inhaled radioactive tracers to generate ventilation phase images and uses intravenous radioactive tracers to generate pulmonary perfusion images [94]. Scintigraphy was the first established, and is the most accessible and least costly nuclear imaging technique in clinical settings. However, it is a two-dimensional (2D) planar projection imaging method and its low spatial resolution hampers the
expansion of its clinical applications. The assessment of ventilation and perfusion scans is based on the qualitative visual observation of the density and the distribution pattern of the radioactivity. However, the density of activity in 2D planar images is a projection of activity within the lung, making it impossible to determine from a single view the exact position in the lung depth from which the activity arose. Early-stage COPD may have normal V/Q scans while advanced-stage COPD may present matched sub-segmental ventilation defects and perfusion defects, i.e. regions with little or absent radioactivity, in the peripheral lungs with a focal and discrete or diffusely scattered pattern through the lungs. Some asthmatic patients only present ventilation and perfusion defects on lung scintigraphy during acute episodes. However, some patients with longstanding chronic asthma may show matched ventilation defects and perfusion defects with a similar distribution pattern to COPD even between attacks. The ventilation and perfusion defects formed during an asthma attack are spatially variable and can be resolved after treatment, which is distinct from the persistent defects seen in those with pulmonary parenchyma destruction [94].

SPECT is the 3D imaging development of lung scintigraphy. SPECT resolves the overlapping tissue issue encountered in 2D projection planar imaging and thus enables a more accurate assessment of regional radioactivity [92]. PET is a 3D nuclear medical imaging technique generating images using pairs of coincident gamma photons created during an annihilation event between positrons and electrons. Thus PET utilizes positron-emitting radioisotopes [93]. V/Q SPECT and PET have been utilized in asthma and COPD for the detection and localization of ventilation defects and perfusion defects over the entire lungs and have shown promise in measuring regional ventilation/perfusion ratio [92, 93, 95, 96]. Jögi et al. demonstrated that the qualitative assessment of ventilation and perfusion impairments by using V/Q SPECT is sensitive to mild COPD and correlated well with the severity of airway obstruction assessed by spirometry and emphysema extent measured by CT [97]. Norberg et al. proposed a quantitative analysis method to measure the inhomogeneity of ventilation on SPECT which showed potential in the discrimination between healthy subjects and patients with advanced COPD [98]. SPECT and $^{13}$nitrogen-based PET have been carried out in patients with asthma to visualize the patchiness of the regional changes in peripheral alveolar perfusion and ventilation during methacholine challenge tests [95]. $^{13}$nitrogen PET was also used to reveal the dependence of ventilation defect formation on the level of pulmonary inflation in healthy and asthmatic subjects [99]. In addition, SPECT and PET have been playing an important role in the evaluation of the lung deposition of drugs that delivered by nebulizers or different types of inhalers at different particle sizes [100]. Furthermore, SPECT and PET are promising in imaging lung inflammation [52]. PET with radiolabelled fluorodeoxyglucose ($^{18}$FDG) is sensitive to neutrophil-predominant lung inflammation. Increased $^{18}$FDG uptake has been demonstrated in animal models exposed to cigarette smoke and patients with COPD and other respiratory conditions characterised by inflammation [101, 102]. Several studies have also extended $^{18}$FDG-PET to asthma but the results are controversial [101, 103]. SPECT with a $^{99m}$technetium labelled lipophilic agent is sensitive to oxidative stress and inflammation in the lung. Increasing uptake of this radiotracer is observed in active
smokers and is correlated with increased cigarette consumption [104]. SPECT with radiolabelled leukocytes, e.g. radiolabelled eosinophils, may enable the non-invasive detection and localization of lung inflammation with specific cell profiles [105].

Compared with lung scintigraphy and SPECT, PET has higher spatial resolution and thus may be more powerful in detailing the small functional abnormalities in the lung. However, PET is a more costly and complex procedure than SPECT. In addition, all three radionuclide lung imaging techniques require the use of ionizing radiation exposures (particularly when coupled with X-ray CT in SPECT-CT and PET-CT) and thus are limited for longitudinal and paediatric studies [106].

2.3.3 Bronchoscopic imaging techniques

Optical coherence tomography (OCT) and endobronchial ultrasound (EBUS) are two emerging imaging techniques used under the fibrotic bronchoscopy for the assessment of the bronchial wall and the surrounding tissue remodelling layer-by-layer in asthma and COPD. Both OCT and EBUS offer non-ionizing radiation visualization of airway wall structure and are suitable for dynamic views, the former having much higher special resolution and the latter being more available in clinical settings. However, both have limited image coverage and airway access depth and are not applicable to image pulmonary parenchyma and distal airways [47, 52].

2.3.4 Magnetic resonance imaging

Pulmonary MRI techniques are under rapid development and have drawn accumulating attention over the past decade. Lung MRI techniques can be divided into proton MRI and hyperpolarized noble gas MRI (HP gas MRI) according to the source of the signal. Proton MRI techniques rely on the signals derived from the water hydrogen nuclei in body tissues. Hyperpolarized gas MRI allows the direct visualization of the pulmonary airspace at a high spatial and temporal resolution by using the hyperpolarized non-radioactive isotopes of noble gases as inhaled contrast agents. They permit non-invasive and non-ionizing radiation-based assessment of pulmonary physiology and morphology at a local level and provide promising biomarkers for the evaluation of asthma and COPD.

In chapter 3, a detailed introduction is given regarding the proton MRI theory, the three proton MRI techniques that applied in the current PhD work, i.e. MR qS\(_0\) mapping, dynamic OE-MRI and DCE-MRI. It is followed with a brief review of the applications of HP gas MRI and the other proton lung MRI techniques in asthma and COPD.

2.3.5 Pros and cons of the imaging modalities

The development of lung imaging biomarkers for the assessment of asthma and COPD has two main motivations: 1) the high health and societal impacts of asthma and COPD are worldwide problems that have to be addressed; 2) clear need for improved regional assessment of lung structure and function.

The pros and cons of CT, radionuclide lung imaging, proton MRI and HP gas MRI are summarized in table 2.2. A shared advantage of these imaging modalities relative to the traditional biomarkers is their ability to non-invasively visualize and assess pulmonary morphological and
function at a local level in vivo. However, the application of CT and radionuclide lung imaging techniques is hampered by a number of limitations including the use of ionizing radiation, the need to produce radiotracers, expense and/or practical difficulty in implementation. Among these imaging modalities, MRI techniques are drawing increasing attention. The major merit of non-ionizing radiation exposure makes MRI techniques ideal imaging tools for longitudinal studies and studies in radiation susceptible populations. The rapid development in MRI hardware, techniques and contrast materials have at least partially overcome the lung MRI challenges (section 3.5). HP gas MRI is one of the most powerful MRI lung techniques, providing several unique applications for lung investigations. However, HP gas MRI only has limited availability in research institutes and is relatively far from ready for clinical usage. The main limitations of HP gas MRI include the high costs of noble gases, high technical demands and high requirement of special equipment, such as laser-polarizer and special MRI coils. By contrast, the range of proton lung MRI techniques fulfils multiple needs for lung imaging, e.g. from functional imaging to morphological imaging, from imaging ventilation to perfusion to respiratory dynamics and lung elastic properties, from qualitative observation to quantitative measurement, from static imaging to dynamic acquisitions, and all of which can be implemented on the standard MR scanners with or without the administration of inexpensive and easy accessible contrast agents. Proton lung MRI shows definite clinical indications in the assessment of lung disorders with generally higher possibility than HP gas MRI to be transferred into clinic.
Table 2.2 Pros and cons of the imaging techniques in the assessment of asthma and COPD

<table>
<thead>
<tr>
<th>Imaging modality</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
</table>
| CT               | • Maximum spatial resolution  
• Method-of-choice for lung morphological imaging  
• Capable of imaging pulmonary function, given the use of contrast agents  
• Excellent general availability, cost-effective, easy to implement  
• Dedicated software for automatic quantitative analysis | • Ionizing radiation exposure  
• Limitation for dynamic acquisition, longitudinal studies, paediatric studies, and studies in pregnant women, etc. because of the radiation exposure  
• Potential risk of inducing cancer, especially in young population |
| Lung scintigraphy | • Functional imaging (V/Q)  
• Widely available  
• Cost-efficient | • Modest ionizing radiation exposure  
• 2D planar projection image low spatial resolution  
• No morphological detail |
| SPECT            | • Pulmonary functional imaging (V/Q)  
• Several other applications beyond ventilation and perfusion imaging: drug deposition and lung inflammation, etc.  
• 3D volumetric imaging  
• Widely available  
• Relatively inexpensive (compared to PET  
• Also provides morphometric information when combined with CT in SPECT-CT | • Ionizing radiation exposure  
• Long acquisition time, limited spatial resolution  
• Limitation for longitudinal studies, paediatric studies, and studies in pregnant women due to the radiation exposure and the time to clear the radioactive tracer  
• No morphological information (except for SPECT/CT hybrid but it causes more radiation exposure)  
• Moderately invasive (injection)  
• Technically demanding  
• Complicated and non-standardised image post-processing and analysis for quantification of physiology |
| PET              | • Relatively high spatial resolution  
• Others similar to SPECT | • Expensive  
• Others similar to SPECT |
<table>
<thead>
<tr>
<th>Proton MRI</th>
<th>HP gas MRI</th>
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| • Non ionizing radiation  
• High temporal and spatial resolution (but still inferior to CT); high soft-tissue contrast  
• Widely available  
• Technical requirements vary  
• Qualitative and quantitative assessment of pulmonary function  
• Improved ability to image pulmonary morphology  
• Can be safely implemented for dynamic acquisition, longitudinal studies, paediatric studies, and studies in pregnant women, etc. because of the lack of radiation exposure | • Low signal-to-noise ratio (can be improved by using contrast agent)  
• Technically demanding in data acquisition, image post-processing and analysis relative to common MRI methods  
• Relative expensive (relative to CT and lung scintigraphy; but less expensive than HP gas MRI and PET) |
| | • Limited availability  
• Limited lung anatomic information  
• High cost for noble gases  
• High requirement/cost for special equipment: laser-polarizer, special radiofrequency coils  
• Highly technically demanding in data acquisition, imaging post-processing and analysis  
• Current availability of hardware limited to specialized MR centres; $^3$helium quantities limited globally |
Chapter 3 Background: proton MRI

3.1 Nuclear magnetic resonance

MRI is based on the phenomenon of nuclear magnetic resonance (NMR). NMR can be briefly described as the process of nuclei absorbing and releasing energy with a specific resonance frequency when being placed in a static magnetic field and excited by a second oscillating magnetic field. The hydrogen nucleus ($^1$H), consisting of a single proton, is the most abundant nucleus in the body and its NMR phenomenon forms the basis of proton MRI.

In classical physics, NMR is explained as spin precession process. To start with, a population of $^1$H spin about their axes at random orientations in the equilibrium state so that the net magnetization ($M_0$) is zero. Then, a static homogeneous external magnetic field ($B_0$) is added to the z-axis of a coordinate system which aligns $^1$H with or against $B_0$ direction along z-axis. A slight excess number of $^1$H nuclei aligning with $B_0$ results in an alignment of z-axis magnetization component ($M_z$) with $B_0$ direction. $B_0$ field exerts a torque on the $^1$H magnetic moments such that the $^1$H nuclei wobble about the z-axis with an angular frequency $\omega_0$ (i.e. the Larmor frequency), termed precession. The out-of-phase magnetic moment components in the x-y plane cancel each other out so the x-y plane magnetization component ($M_{xy}$) is zero. Therefore, adding $B_0$ creates a $M_z$ aligning with $B_0$. After that, an external oscillating magnetic field ($B_1$) can be applied to the x-y plane in the form of a radiofrequency pulse (RF) with a resonance frequency $\omega_0$ to excite the $^1$H nuclei. $M_0$ then turns towards the x-y plane and the magnetic moments in x-y plane move into phase. Hence, $M_z$ is diminished and non-zero $M_{xy}$ is formed. $M_{xy}$ coherently rotates about $B_0$ at the frequency $\omega_0$ in the x-y plane and induces a voltage in the receive coil. This voltage is recorded and transformed into the MR signal.

3.2 Relaxation

When the RF pulse is switched off, the excited $^1$H nuclei release the absorbed RF energy and return to the equilibrium configuration by two independent but simultaneous relaxation processes. The dissipation of energy to the surrounding environment causes the restoration of $M_z$, which is termed spin-lattice recovery or longitudinal recovery ($T_1$-recovery). The loss of coherence between $^1$H nuclei due to local dipole-dipole interactions leads to dephasing of the transverse magnetic moments and consequently causes the reduction in $M_{xy}$, which is termed spin-spin decay or transverse decay ($T_2$-decay). The time courses of longitudinal recovery and transverse decay are both exponential, characterised by the relaxation time constants $T_1$ and $T_2$, respectively. $T_1$ is the time taken after excitation for $M_z$ to have recovered to about 63% of its initial value and $T_2$ is the time taken after excitation for $M_{xy}$ to have reduced to about 37% of its initial value. Different tissues have different $T_1$ and $T_2$ due to differences in the molecular environment within which the $^1$H nuclei reside and are also affected by temperature and static magnetic field strength $B_0$. In practice, $B_0$ inhomogeneity can further dephase the transverse magnetic moments and accelerate the decay of $M_{xy}$ by a time constant $T_2^*$, given by

$$\frac{1}{T_2^*} = \frac{1}{T_2} + \gamma^2 B_0 \Delta B / 2$$

(Eq 3.1)
where $\Delta B_0$ is the magnitude of $B_0$ inhomogeneity and $\gamma$ is the gyromagnetic ratio. This relaxation process is termed $T_2^*$-decay. $M_{xy}$ reduction leads to voltage reduction in the RF receiver coils and thus yields an oscillating MR signal with its amplitude exponentially decayed (characterised by $T_2^*$) after excitation, termed the free induction decay (FID) signal. The FID signal is a short-lived signal that does not typically directly contribute to MRI. Instead, the transverse magnetization is usually refocused to generate a MR signal in the form of echo. Spin echo and gradient echo are the two main types of echo and can be created by using appropriate pulse sequences.

3.3 Pulse sequences

Pulse sequences comprise a set of RF pulses and magnetic field gradients that are used to generate and acquire the MR signal. The two main types of pulse sequences are spin-echo (SE) sequences and gradient-echo (GE) sequences, which are the base of all other MR sequences apart from ultrashort echo-time (UTE) pulse sequences.

3.3.1 SE sequences

An RF pulse tilts the net magnetization $M_0$ away from z-axis, by a flip angle, which is governed by strength and duration of the pulse. SE sequences usually apply a 90° RF excitation pulse to flip the entire $M_0$ into the x-y plane in order to create an FID, which is followed by a phase encoding gradient to embed the spatial information (section 3.4.1). Then, a 180° RF refocusing pulse is introduced at the time $TE/2$ to invert all the transverse magnetic moments by 180° such that the out-of-phase transverse magnetic moments return to being in phase at time $TE$. The decayed MR signal is therefore “rebuilt” and a spin echo is produced at $TE$. $TE$ is the time interval between the administration of excitation pulse and echo formation, known as the echo time. A pulse sequence diagram for a simple SE pulse is shown in figure 3.1.
Figure 3.1 SE sequence.

A pulse sequence diagram for a simple spin echo sequence using 90° and 180° RF pulses with echo time TE. The slice select, phase encode and frequency encode gradients are shown on the “Slice”, “Phase” and “Read” axes, respectively.

In SE sequences, the 180° RF refocusing pulse can refocus the M_{xy} dephasing caused by the local static magnetic field inhomogeneity. Hence, SE sequences can compensate the signal loss due to T_{2}* decay and are the sequences of choice for the measurement of T_2 or in settings where T_{2}* is very short, as is the case in the lung.

In fast spin echo sequences (FSE, also known as turbo spin echo (TSE) or rapid acquisition with refocused echoes (RARE) sequences), the 90° RF pulse is followed by multiple 180° RF pulses in order to generate multiple echoes after a single excitation. By doing so, the acquisition time is substantially shortened. The number of echoes sampled after a single excitation pulse is named the echo train length (ETL), which largely determines the image acquisition time. If the echoes of an entire image are sampled after a single excitation pulse, the pulse sequences are called “single-shot” sequences. Furthermore, spin echo sequences can be further speeded up by acquiring just over half of the image information and calculating the missing half, e.g. half Fourier acquisition single shot turbo spin echo (HASTE) sequence.

3.3.2 GE sequences

The basic GE sequence applies an excitation pulse with a flip angle which is usually < 90° to tilt part of M_0 into the x-y plane, which is followed by the same phase encoding gradients as used in SE sequences. Then, a pair of magnetic field gradients with opposite polarities is used instead of an
RF pulse to first accelerate the dephasing of the transverse magnetic moments and then reverse and refocus them. The decayed MR signal is therefore regenerated and a gradient echo is produced at TE. A GE pulse sequence diagram is shown in figure 3.2.

![Figure 3.2 GE sequence.](image)

A pulse sequence diagram for a simple gradient echo sequence using an $\alpha^\circ$ RF pulse, echo time TE and repetition time TR. The slice select, phase encode and frequency encode gradients are shown on the “Slice”, “Phase” and “Read” axes, respectively.

GE sequences are generally faster than SE sequences. SE sequence uses 90$^\circ$ excitation pulse to tilt $M_0$ away from z-axis by 90 degree while GE sequence uses a low energy excitation RF pulse to tilt $M_0$ away from z-axis by a low flip angle ($< 90^\circ$). Thus it takes less time for $M_z$ to recover back to $M_0$ via $T_1$ relaxation and start next excitation. The repetition time (TR), i.e. the interval between two consecutive excitations, is usually shorter in GE sequence than in SE sequence. In addition, the gradient echo is faster to produce (shorter TE) than the spin echo due to the use of rephrasing gradients rather than 180$^\circ$ rephasing RF pulse.

Importantly, the rephasing gradient used in GE sequences is not able to cancel out the $M_{xy}$ dephasing caused by static magnetic field inhomogeneities and hence to rebuild the signal loss due to $T_2^*$-decay. In addition, the initial $M_{xy}$ induced by GE sequences is usually smaller than that induced by SE sequences, because of the use of smaller flip angle RF pulse. These two intrinsic features dictate
that GE sequences produce smaller echoes (and therefore lower MR signal) than SE sequences and are more susceptible to $B_0$ inhomogeneity and susceptibility effect.

Modified GE sequences can be broadly subdivided into two types according to how the transverse magnetization is dealt with following the acquisition of the echo. Firstly, the transverse magnetization can be effectively reduced by applying “crusher” gradients to destroy the remaining $M_{xy}$ while maintaining the $M_z$ before the next excitation. This ensures no contamination between subsequent TR periods of transverse magnetization information from previous excitations, which is important if “pure” $T_1$-weighting is desired. A similar, and in practice more effective, effect can be achieved by varying the phase of the excitation pulse between TR periods. These types of GE sequences are known as spoiled gradient-echo (SPGR) or fast low flip angle (FLASH) or fast field echo (FFE) sequences. Alternatively, it is possible to refocus the residual $M_{xy}$ after each excitation so that the signal reaches steady state after a few repetitions, which is achieved in a class of sequences known as fast imaging with steady-state precession (SSFP, FISP) sequences.

3.4 How to generate an MR image from MR signals

In order to produce an MR image, MR signals need to be encoded with spatial information. Then, the signals are stored in k-space, converted from functions of time or phase to functions of frequency, by using the Fourier transform, forming an image.

3.4.1 Spatial encoding

Spatial encoding is usually achieved by applying magnetic field gradients in 3 orthogonal directions. A slice selection gradient is superimposed on the $B_0$ at the same time as the excitation RF pulse; for simplicity we will assume that the gradient is applied along the z axis, but in practice it can be applied in any orientation. This yields a linearly changing effective $B_0$ along the z-axis and makes the $^1H$ nuclei on different x-y planes have different effective resonance frequencies. Hence, the excitation RF pulse can only excite the x-y plane, or slice, where the effective resonance frequency of $^1H$ equals the frequency of this RF pulse.

The frequency encoding gradient is a linear magnetic field gradient that we will assume is applied along the x-axis (although it could be any orientation within the selected slice) during echo formation. This makes the MR signals that are produced from the excited $^1H$ at different positions along x-axis have different precession frequencies. Therefore, by ordering the frequency spectrum of the MR signals, the x-axis location where each MR signal is from can be determined.

The phase encoding gradient is a linear magnetic field gradient applied perpendicular to the frequency gradient (along the y-axis in our example) after excitation but before echo readout. This causes the excited $^1H$ nuclei at different positions along the y-axis to precess at different speeds. When the phase encoding gradient is switched off, the phase of $M_{xy}$ of these $^1H$ nuclei are different. The phase difference is utilized to trace the y-axis location of the nuclei.

Because one phase encoding process can only identify one phase difference and fill one line in the k-space, the pulse sequence is repeated multiple times with different amplitude of phase
encoding gradient each TR. The number and size of the phase encoding steps is a major determinant of the spatial resolution and the image acquisition time.

In order to enable 3D imaging, a second phase encoding gradient is usually placed along the z-axis in order to spatially encode along the slice select direction. In this case, the excited “slice” is the slab of tissue within which the 3D encoding occurs.

### 3.4.2 Image contrast

$T_1$, $T_2$ ($T_2^*$) and proton density (PD) jointly determine the MR image contrast between different tissues. Their contributions to the image contrast, known as their weighting, can be enhanced or suppressed by altering the sequence types and adjusting the sequence parameters such as TR, TE and, in GE sequences, the flip angle. Typically the contrast of an MR image is weighted toward one out of the three intrinsic factors and the corresponding images are termed $T_1$-weighted (contrast based on differences in $T_1$), $T_2$ ($T_2^*$)-weighted (contrast based on differences in $T_2$ ($T_2^*$)) or PD-weighted (contrast based on differences in PD).

Table 3.1 shows the choices of sequence parameters for different weighting of SE sequences and GE sequences, respectively.

A short TR will result in a $T_1$-weighted image where tissues with short $T_1$, such as fat-based tissues, are bright as their $^1$H nuclei are able to recover more $M_z$ through $T_1$-relaxation during the TR and contribute more to signal in the next excitation, whereas the tissues with long $T_1$ are dark, such as fluid, as their $^1$H nuclei recover less $M_z$ before the next excitation.

A long TE will lead to a $T_2$-weighted image where tissues with long $T_2$, such as fluid, are bright as the signals they emit have not lost too much transverse magnetization during TE due to their slow $T_2$-decay, whereas the tissues with short $T_2$, such as fat-based tissues, are darker as they have lost more signal due to fast $T_2$-decay.

In GE sequences, the flip angle strongly influences the contrast weighting. A small flip angle retains a large portion of $M_0$ along the z-axis and the distance for the tilted portion of $M_0$ to relax back to longitudinal equilibrium is short. Hence, the $T_1$-recovery is usually completed before the next excitation, even for tissues with long $T_1$ and little $T_1$-weighting is possible. By contrast, the larger the flip angle the stronger the $T_1$-weighting, as tissues with longer $T_1$ are unable to fully relax before the next excitation, unless TR is very long. For further details of MRI physics the reader is referred to the following references [107-110].

### Table 3.1 Parameter setting for different weighting of SE and GE sequences

<table>
<thead>
<tr>
<th>Image contrast</th>
<th>SE sequence</th>
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<tbody>
<tr>
<td></td>
<td>TR</td>
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<tr>
<td>$T_1$-weighted</td>
<td>Short</td>
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<tr>
<td>$T_2$-weighted</td>
<td>Long</td>
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<td>PD-weighted</td>
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<table>
<thead>
<tr>
<th>GE sequence</th>
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<td>TR</td>
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### 3.5 Proton MRI of the lung: the challenges and strategies

Lung imaging with MRI is hampered by three inherent drawbacks: 1) low proton density in the lung tissue results in comparatively low MR signal intensity; 2) large air-tissue interface, which worsen the inhomogeneity of local magnetic fields, leads to extremely short $T_2^*$ and aggravates signal loss; 3) motion artefacts derived from physiological movement, including respiration and cardiac pulsation, significantly affect image quality [111].

In the past decades, the fast development of MR hardware and MR techniques has greatly progressed proton MRI of the lung. Many strategies have been proposed to overcome the challenges listed above. First, the application of paramagnetic contrast agents, such as high concentration $O_2$ and gadolinium (Gd) chelates, increases the signal intensity of the lung parenchyma and enhances the contrast between normal and abnormal lung tissues. They also give insight into functional alterations of the lung. $T_1$-weighted dynamic OE-MRI and $T_1$-weighted DCE-MRI are two contrast-enhanced proton MRI techniques for depicting regional ventilation and perfusion in the lung that are important to the work in this thesis.

SE sequences are less sensitive to magnetic field inhomogeneity than GE sequences, thus rapid SE methods are generally preferable for non-contrast enhanced or low-contrast enhanced proton MRI techniques in the lung. However, it is essential to accompany the spin-echo readout with appropriate motion compensation measures in order to counteract the increased motion effects due to the longer acquisition times than are possible using fast GE methods. In addition, short TR and short TE are recommended for lung MRI protocols as they shorten the acquisition time, reducing motion artefacts and minimizing signal loss and susceptibility artefacts, respectively.

Last but not the least, motion compensation is pivotal for proton MRI of the lung and can be achieved broadly by three approaches. 1) Reduction of the acquisition time by using high performance hardware and using fast imaging techniques. Specific examples are single-shot spin-echo imaging using half Fourier acquisition techniques (e.g., $T_1\text{-}, T_2\text{-HASTE};$ completing the acquisition in one-shot by sampling half of the k-space), ultra-short TE sequences with radial k-space readout (UTE), steady state free precession gradient echo series (e.g. SSFP-GRE, TrueFISP), time-resolved imaging of contrast kinetics (TRICKS; reducing k-space by “data sharing” technique), parallel imaging techniques (e.g. sensitivity encoding, “SENSE” and generalized autocalibrating partially parallel acquisition, “GRAPPA”; increasing the number of parallel receiver coil elements and minimizing the phase-encoding times). 2) Control motion effects during acquisition by using breath-holding techniques, respiratory/cardiac triggering, prospective gating and navigating techniques. 3) Correction of motion errors by image post-processing, such as averaging, retrospective gating and
registration techniques. The application of fast imaging sequences combined with motion controlling techniques is the most common for practical use. However, the breath-holding technique strongly depends on the compliance of subjects and may alter the physiology of the lung. Triggering, gating and navigating techniques enable free-breathing MRI with minimal requirement of subject compliance. However, they inevitably prolong the scan time, which is practically detrimental for dynamic acquisitions. Image registration is hence drawing increasing interest as a method for correcting some detrimental motion-related effects after acquisition, as it allows images to be acquired during free-breathing without increasing the scan time. It reduces the impact of motion after acquisition by spatially aligning a set of transformed images with a target image to allow image comparison between different time-points, scans, subjects and imaging modalities. It is also possible to correct some PD changes during breathing using registration, e.g. scaling the signal intensity according to the volume change detected by the registration process [112]. However, image registration does not correct phase encoding errors during image readout and changes in inflow-effects and $T_2^*$ during the breathing cycle.

In this PhD project, all the images were dynamically acquired during free-breathing with the aim to maintain the natural lung physiology. The impact of motion is minimized by using fast imaging techniques (HASTE, single short technique, etc.) along with image registration [111, 113].

3.6 Relaxation time and proton density measurements

The NMR properties $T_1$, $T_2$ and PD are dependent on different biophysical characteristics of tissue and thus can be utilized to differentiate different tissue types and identify pathological alterations [114]. The conventional practice of MRI relies on the visual inspection or simple measurement of the image signal intensities, which is however poorly comparable between different scans, subjects and institutes as the signal intensity is jointly determined by $T_1$, $T_2$ and PD at different contrast weighting and is affected by varied factors, including the scanner and sequence settings. Quantitative MRI parameters, on the other hand, probe tissue-specific intrinsic properties and are theoretically independent of the extrinsic factors. This thesis utilizes mapping of $T_1$ and the equilibrium magnetization $S_0$, an analogue of the proton density of tissue. Relevant measurement methods for these parameters are summarized below.

3.6.1 $T_1$ measurement

$T_1$ measurement is not only an important branch of quantitative MRI, but also critical for the quantitative analysis of $T_1$-weighted dynamic OE-MRI and $T_1$-DCE-MRI. The PhD project employed two common $T_1$ measurement approaches, which are introduced below.

3.6.1.1 Inversion recovery method

This $T_1$ measurement approach is accomplished by using inversion recovery (IR) sequences, in which a $180^\circ$ RF pulse (the inversion pulse) is added ahead of the excitation RF pulse by a time interval known as the inversion time (TI) [115]. The $M_z$ is first inverted by $180^\circ$ along z-axis from $M_0$ to be $-M_0$ by the inversion pulse and then starts to relax back to equilibrium. After the time period of TI,
part of the $M_z$ has recovered, which is then sampled by a $90^\circ$ excitation RF pulse to generate the MR signal. The relationship between $T_1$ and MR signal is given by

$$S = S_0'(1-2\cdot\lambda\cdot e^{-TI/T_1})$$

where $S$ is the MR signal intensity, $S_0'$ is the magnitude of the net magnetization at equilibrium state (determined by $M_0$ and other factors including the coil reception uniformity and scanner gain settings) and $\lambda$ is inversion efficiency representing the imperfection of the $180^\circ$ inversion pulse ($0\leq \lambda \leq 1$).

When $TR>>T_1$ (generally $TR \geq 5T_1$ is assumed adequate for complete $M_z$ recovery), the equation can be simplified to

$$S = S_0'(1-2\cdot\lambda\cdot e^{-TI/T_1})$$

by combining the term $e^{-TE/T_2^*}$ into term '$S_0'$, there are 3 variables: $S_0$, $\lambda$ and $T_1$. When $TE<<T_2$, we can ignore the $T_2$ dependence of the signal.

If we assume perfect $180^\circ$ inversion pulse, e.g. $\lambda = 1$, the equation can be further simplified to 2 variables ($S_0$, $T_1$) as

$$S = S_0'(1-2\cdot e^{-TI/T_1})$$

$T_1$ can be extracted along with $S_0$ and $\lambda$ by fitting Eq 3.3 (or 2-parameter fit with Eq 3.4) to signal acquired at a number of distinct TI. 3-parameter fitting is usually preferable to 2-parameter fitting as it accounts for the signal variation induced by an imperfect inversion pulse.

The IR method is the gold standard for accurate $T_1$ measurement [116]. It ensures a maximum signal intensity and hence a maximum signal-to-noise ratio (SNR) by exploiting fully inverted and full transverse $M_z$. However, the IR method is time-consuming as multiple data points are required to achieve a good fit. It is recommended that 5-8 different TI with the longest TI around $4.5 \times$ expected $T_1$ is optimal for 3-parameter fit [117]. In addition, sufficient time ($\geq 5 \times$ expected $T_1$) should be given to ensure complete recovery of $M_0$ to equilibrium before each excitation. Therefore, the IR method takes significant time, even if reducing the required TI number by using a 2-parameter fit. IR sequences used for $T_1$ measurement are usually made of an inversion pulse and a fast SE sequence, e.g. TSE and HASTE (see session 3.3.1). IR-TSE sequence provides higher SNR than IR-HASTE because of the fully sampled k-space.

### 3.6.1.2 Variable flip angle method

$T_1$ can be measured from a set of images repeatedly acquired by a spoiled gradient echo sequence with varying RF excitation flip angles. This $T_1$ mapping approach is known as the variable flip angle (VFA) method [118]. The signal intensity with a flip angle of $\alpha$ is given by

$$S = S_0'\cdot(1-E)\cdot\sin \alpha\cdot e^{-TE/T_2^*}/(1-E\cdot\cos \alpha)$$

where $E = e^{-TR/T_1}$. Perfect transverse magnetization spoiling is assumed. By combining the term $e^{-TE/T_2^*}$ in to term '$S_0'$, Eq 3.5 can be simplified to

$$S = S_0'(1-E)\cdot\sin \alpha/(1-E\cdot\cos \alpha)$$

When $TE<<T_2^*$, the $T_2^*$ effect on signal generation can be ignored.

For a given $TR$ and $T_1$, the maximum signal is achieved at Ernst angle, given by

$$\alpha = \cos^{-1}(e^{-TR/T_1})$$

(Eq 3.7)
A general recommendation from the literature is to place the two flip angles symmetrically at the each side of the Ernst angle to cover a narrow range of $T_1$ and increase to two to four flip angles to cover a larger range of $T_1$ [119-122]. Further increase the number of flip angles may reduce efficiency (precision and acquisition time) of $T_1$ mapping.

The fast image acquisition by $T_1$-weighted SPGR/FFE/FLASH sequences makes VFA a time-efficient 3D $T_1$ mapping method. It has been widely utilized to map the pre-contrast $T_1$ for 3D $T_1$-weighted DCE-MRI of many organs, including the lung. Although the intrinsic drawbacks of GE sequences may affect the accuracy and precision of $T_1$ measurement in the lung (see section 3.3.2), so far there is no better commonly available alternative to fast GE sequences that fulfils the primary requirements of 3D $T_1$-weighted DCE-MRI of the lung: volumetric coverage, reasonable spatial resolution and high temporal resolution for bolus tracking. On the other hand, the administration of strongly paramagnetic contrast agents, most commonly Gd-based contrast agents, significantly elevate the SNR, which to some extent compensate for the low SNR disadvantage of GE sequences and enables a reasonable monitoring of the post-contrast dynamic $T_1$ changes. However, the IR method in combination with a spin-echo based readout is still the choice of accurate and precise $T_1$ measurement, especially in the lung. For OE-MRI where the $O_2$ induced signal changes are much smaller (see chapter 3.7), the additional SNR from the IR-TSE method is advantageous.

### 3.6.2 $S_0$ measurement

$S_0$ is the signal associated with the equilibrium magnetization state, when the bulk magnetization of all the $^1H$ nuclei of an object is in thermal equilibrium. It is related to the equilibrium magnetization, $M_0$, by the sensitivity of the receive coil and scanner gain settings. $S_0$ is proportional to the proton density.

Acquiring images by using proton density weighted sequences provides a rapid estimation of $S_0$, and thus, the proton density of the tissue. This approach minimizes the $T_1$ and $T_2$ effects on image contrast by using very long TR and very short TE and thus yields an approximately linear relationship between the MR signal intensity and the number of protons in a given unit of imaged tissue.

More accurate and precise quantification of $S_0$ is carried out by fitting the multiple signal data points to appropriate signal equations according to the sequences that employed. This method better eliminates $T_1$ or $T_2$ ($T_2^*$) relaxation time effects on image contrast and estimates the absolute value of $S_0$ together with relaxation time, which can be carried out by using a variety of sequences.

The observed value of $S_0$ is in arbitrary units and is affected by the specific settings and performance of the scanner in individual scans. The relative $S_0$ of a tissue can be obtained by dividing by a reference $S_0$ to provide a quantitative relative $S_0$ value to account for day-to-day scanner settings. The reference $S_0$ could be the $S_0$ of a water phantom (usually gadolinium-doped) scanned simultaneously with the tissue of interest (water content being 1g/cm$^3$) [123] or, more practically, the $S_0$ of a solid tissue (a reference tissue) with higher water content such as the chest wall muscle (~75% water density) or liver (~ 70% water density) [124, 125].

### 3.6.3 Application of $T_1$, $T_2$ ($T_2^*$) and $S_0$ mapping
The measurement of lung T\textsubscript{1} on normoxia and hyperoxia has been carried out in many studies by using fast SE sequences, such as IR-TSE and IR-HASTE, and fast GE sequences, such as IR/SR-snapshot FLASH, in healthy subjects, patients with pulmonary emphysema, fibrosis and cystic fibrosis, etc.. The published T\textsubscript{1} values of normal lung tissue on normoxia at 1.5 tesla range between 900 ms -1400 ms (see table 3.2). A significant gradient of lung T\textsubscript{1} value has been found in the radial direction on sagittal images, i.e. T\textsubscript{1} value decreasing linearly from the centre to the peripheries [126]. In addition, lung T\textsubscript{1} is also affected by the changes in lung volume and O\textsubscript{2} content during respiration, with shorter values observed in end-inspiration and longer values in end-expiration in healthy subjects [127]. The alteration of the effect of respiration phase on lung T\textsubscript{1} values could be a sign of lung diseases, as suggested by a study conducted in patients with emphysema and fibrosis [128]. This study also recommended end-expiration as the favorable respiratory phase for T\textsubscript{1} mapping in pathological lungs because expiratory T\textsubscript{1} values correlated better with spirometric indices than inspiratory T\textsubscript{1} values.

In addition, the intrinsic T\textsubscript{1} value of the lung on normoxia itself delivers diagnostically-meaningful information about the health status of the lung as it is associated with tissue water content and the composition of the water i.e. the ratio of free water and molecular-bound water [129]. Lung T\textsubscript{1} shortening was observed in patients with pulmonary emphysema and has been ascribed to the reduction in the lung water content due to tissue loss and capillary bed destruction [128]. Patients with fibrosis and cystic fibrosis also presented shortened lung T\textsubscript{1} in pathological regions, which has been ascribed to the decrease in the fraction of free water to macromolecular-bound water in lung parenchyma as a consequence of the deposition of collagens [130]. Furthermore, a prenatal study revealed a linear correlation of the lung T\textsubscript{1} of human fetuses with the gestation and lung volume, suggesting the potential role of T\textsubscript{1} quantification in the non-invasive assessment of fetal lung maturation [131]. In preclinical studies, the T\textsubscript{1} value of the lung was shortened in rat models of pulmonary fibrosis [132], prolonged in dog and rat models of pulmonary oedema [133, 134] and not changed in rat models of alveolitis [132]. In addition to its potential intrinsic value, T\textsubscript{1} measurement paves the way for the quantitative analysis of T\textsubscript{1}-weighted functional pulmonary MRI techniques, including OE-MRI and DCE-MRI, which will be introduced in section 3.7 and section 3.8.

Cameron et al. state that tissues with greater surface area and lower lipid content tend to have shorter T\textsubscript{2} [114]. The very short T\textsubscript{2}* value of the lung parenchyma is ascribed to the susceptibility effect induced by enormous air-tissue interfaces which causes great local gradients [135]. Therefore, T\textsubscript{2} and T\textsubscript{2}* are related to the lung anatomy such as alveolar geometry, overall surface area, pulmonary inflation and pathology, and are considered promising biomarkers for the detection of microstructural changes in the lung. Most of the lung T\textsubscript{2} quantification studies are preclinical. Kveder et al. reported the lung T\textsubscript{2} value varying between 50 ms and 100 ms in vivo and in vitro in rodents as the magnetic field changes between 0.68 tesla and 6.34 tesla [135], which is consistent with the measurements of later in vitro studies using NMR spectroscopy [132]. Though predominantly determined by the microstructure of the lung, the T\textsubscript{2} value of the lung parenchyma has also proven
directly correlated with lung water content and inversely correlated with collagen content [132]. The rodent models of bleomycin lung injury showed prolonged lung $T_2$ in the acute stage, ascribed to the presence of pulmonary oedema, and shortened lung $T_2$ later on in the fibrotic stage as a result of the collagen deposition [132, 136]. $T_2$ was also found prolonged in rat models of pulmonary oedema secondary to increased vascular permeability, while unchanged in pulmonary oedema secondary to elevated pressure in the pulmonary circulation, implying a role of $T_2$ measurement in the identification of pulmonary oedema with different aetiologies [133, 134]. Meanwhile, no change was found in the $T_2$ value of the lung parenchyma in rodent models of alveolitis [132]. There is little literature on $T_2$ measurements of the human lung but a recent study quantified lung $T_2$ values of normal human fetuses, which positively correlated with gestation and was significantly longer than fetuses of congenital diaphragmatic hernia [131, 137]. This study indicates the potential of $T_2$ quantification in the prediction of the gestation week and the prenatal diagnosis of abnormal lung development.

In vivo $T_2^*$ mapping of the lungs by MRI has been conducted in both animals and humans at 0.2 tesla, 1.5 tesla and 3.0 tesla. Hatabu et al. successfully measured the lung $T_2^*$ in healthy adults by using a fast GE sequence with submillisecond TE at 1.5 tesla and reported a $T_2^*$ range between 0.89 ms and 2.18 ms on normoxia [124]. This is consistent with subsequent reports of 1 ms - 2.5 ms by Pracht et al. and 2.11 ± 0.27 ms by Yu et al. [138, 139]. Normoxic $T_2^*$ values of the lung in healthy adults are reported to be 10.6 ms ± 0.9 ms at 0.2 tesla and 0.74 ± 0.10 ms at 3.0 tesla [138, 140]. In addition, the inhalation of pure O$_2$ can lead to an approximately 10% reduction in $T_2^*$ values of the lung in healthy subjects at 0.2 tesla and 1.5 tesla [139, 140]. Animal experiments and a human adult study demonstrated that $T_2^*$ of the normal lung parenchyma decreased as the lung volume increased [141-143]. However, Yu et al. only noted a negligible difference between end-inspiration and end-expiration in lung $T_2^*$ of human adults measured during free breathing [138]. Moreover, lung $T_2^*$ quantification is potentially diagnostic, especially for lung disorders with architectural changes. Takahashi et al. found that $T_2^*$ of the lung parenchyma in mutant mice with pulmonary emphysema was significantly shorter than that in normal controls especially at end-expiration and the value decreased as emphysema progressed [144, 145]. Ohno et al. not only confirmed Takahashi’s finding in human adults with smoking related COPD but also revealed the underlying capability of lung $T_2^*$ quantification in the assessment of functional loss and the clinical staging of COPD in smokers, according to the significant differences of lung $T_2^*$ between COPD severity groups and the strong correlations of lung $T_2^*$ with FEV$_1$/FVC, FEV$_1$%predicted, DLco%predicted and CT readouts of emphysema and airway remodelling [146, 147]. Recently, Ohno et al. have shown that the $T_2^*$ value of the lung parenchyma in patients with connective tissue disease was prolonged than that in healthy subjects and was moderately correlated with vital capacity, diffusion capacity, a disease severity-related serum biomarker and CT estimation [148]. Although there is insufficient human data regarding the applications of lung $T_2^*$ mapping in patients with other pulmonary disorders, such as cystic fibrosis, pulmonary fibrosis and pulmonary inflammation, etc., it could be expected that $T_2^*$ may also have a role in the diagnosis of these lung diseases demonstrating microstructural alterations.
S₀ measurement has long been recognized as a non-invasive and robust method for the in vivo quantification of lung density. This relies on the fact that S₀ is proportional to the proton density of the tissue and therefore the overall tissue density, on the assumption that most lung tissue has high water content. Validation work conducted ex vivo and in vitro in animal lungs have demonstrated excellent agreement between gravimetric techniques and MRI S₀ measurement in the quantitative assessment of lung water content, the reported difference between the two techniques being less than 20% in excised rat lung (1 D line-scan technique) [149], -5% in exercised pig lungs (multi-echo SE sequence) [150], -4.1% ± 7.6% in vitro pig lungs (multi-echo SE sequence) [151] and less than 7.5% in ex vivo pig lungs (multi-GRE sequence) [143]. MRI S₀ measurement was initially performed by generating the exponential T₂-decay signal curve by using line-scan SE technique or multi-echo SE sequences and back-extrapolating the signal to zero TE [149-151]. However, the long acquisition time (> 6 min per slice) limited its application in vivo in human although early attempts were seen [152, 153]. Currently, the most popular MRI scheme for lung water quantification in vivo is extrapolating the S₀ value from the T₂*-decay curve (apparent FID signal) generated by using multi-GRE sequences [141], fast GRE sequences with a range of very short TE [123, 124] or UTE sequences [142]. After obtaining the absolute value of lung S₀, calibration is required in which lung S₀ is usually divided by a reference S₀ value from a region where the water content is known [123, 124]. In healthy subjects, the lung density measured by Hatabu et al. by using MRI fast GRE sequence was 0.29 ± 0.08 g/ cm³ [124], which is in agreement with the reports of other MRI studies [123, 141] and literature values from PET [154] and CT [155]. S₀ measurement is also capable of revealing the effects of lung volume and gravity on lung water distribution. The normalized lung S₀ decreases as lung expands. It also shows a vertical gradient in the lungs due to the gravity effect, greater at vertically higher lung zone than at lower lung zone [123]. The gravity-dependent gradient of lung density can then be utilized to calculate the pleural pressure gradient [150].

So far, S₀ measurement in the lung has been predominantly applied to understand lung water under normal conditions and its feasibility in the detection of pulmonary pathology has not been adequately explored. The limited data in rat models of bleomycin injury lung has demonstrated that S₀ measurement is sensitive to the pulmonary inflammation and fibrosis [156]. Furthermore, the good performance of a coarse surrogate of normalized S₀, i.e. the normalized signal intensity, in the assessment of lung disorders foresees the great potential of S₀ measurement in clinical settings. Animal experiments and human studies have proven that the ratio of lung signal intensity against referenced signal intensity (liver, muscle, water phantom, etc.) is sensitive to pulmonary emphysema [144], lung cancer [157], pulmonary interstitial oedema with different etiologies (e.g. heart failure, inflammation, acute respiratory distress syndrome etc.) and physiological and pathological fetal lung development at different gestation week [158-160]. Quantitative assessment of S₀ minimizes relaxation time confounds from the MR images, e.g. T₁ or T₂ (T₂*) influences, and thus more accurately and precisely quantifies the tissue proton density than the signal intensity of the raw MR
images. Therefore, $S_0$ measurement is expected to be a promising diagnostic and quantitative monitoring tool of lung diseases and deserves more investigation.

3.7 Dynamic oxygen-enhanced MRI

In 1981, Young et al. noticed the change in the signal intensity of the blood in the left ventricular cavity on MR images after subjects inhaled pure O$_2$, providing the initial evidence of using O$_2$ as an MR contrast agent [161]. T$_1$-weighted dynamic OE-MRI, which consecutively tracks the tissue signal change as a result of the T$_1$-effect of dissolved O$_2$ throughout the inhalation of high concentration of O$_2$, is capable of exploring the kinetic characteristics of O$_2$ delivery, uptake and washout of the tissue of interest. Since O$_2$ is an essential biological component of gas exchange in the lung, OE-MRI serves as a unique tool for the evaluation of lung function and investigation of pulmonary diseases.

3.7.1 Principles of OE-MRI

Molecular $^{16}$O$_2$ has two unpaired electrons in the outer shell, which induces a weak paramagnetism [161]. Most O$_2$ molecules carried in the blood are bound to haemoglobin to form oxyhemoglobin and typically less than 2% O$_2$ molecules are freely dissolved in the blood plasma when breathing air [162]. Increasing the amount of dissolved O$_2$ in the blood plasma leads to a reduction in its T$_1$ value and a signal enhancement in T$_1$-weighted MR images [163]. This T$_1$-effect of dissolved O$_2$, which forms the basis of OE-MRI technique, is distinct from the T$_2$ (T$_2^*$)-effect of the haemoglobin-bound O$_2$, which forms the basis of blood O$_2$ level dependent imaging [164].

The O$_2$ molecules in the haemoglobin-bound state and the freely dissolved state are in dynamic equilibrium. The increase in the amount of dissolved O$_2$ in blood plasma proportionally elevates the local partial pressure of O$_2$, i.e. PO$_2$, according to Henry’s law, and causes more O$_2$ to attach to haemoglobin and thus elevates haemoglobin O$_2$ saturation until the haemoglobin saturates with O$_2$, according to the O$_2$-haemoglobin dissociation curve. The concentration of dissolved O$_2$ in blood is determined by the alveolar PO$_2$ (P$_A$O$_2$), and the condition of the alveolar-capillary interface. In OE-MRI, the increase in the dissolved O$_2$ is achieved by elevating the P$_A$O$_2$ through the inhalation of high concentration, typically 100%, of O$_2$. Under normal conditions, the haemoglobin in the arterial blood is already 98% saturated when breathing air. Hence, switching the breathing gas from normal air (21% O$_2$) to pure O$_2$ only leads to a minor increase in the amount of haemoglobin-bound O$_2$ in arterial blood (O$_2$ saturation rises from 97.8% to 99.9%), governed by the sigmoid shape of the O$_2$ dissociation curve, as compared with the significant increase in the concentration of freely dissolved O$_2$ from 3 ml O$_2$ per litre to 18 ml O$_2$ per litre in the arterial blood (PO$_2$ rises from 97 mmHg to 580 mmHg), as a result of the increase of the P$_A$O$_2$ from 100 mmHg to 600 mmHg. In venous blood, however, changing breathing gas to pure O$_2$ only slightly raises the blood PO$_2$ from 38 mmHg to 49 mmHg [162].

The amplitude of the change in the concentration of dissolved O$_2$ in blood plasma and tissue water, and thus the local PO$_2$, determines the amplitude of the change in T$_1$ value of the surrounding tissue. In the lungs, the difference in the concentration of dissolved O$_2$ in local lung regions between
air inhalation and 100% O₂ inhalation is jointly determined by several factors of regional lung function: 1) ventilation, i.e. how much O₂ is delivered to the local alveoli in a given time at a given tissue volume; 2) diffusion, i.e. how efficient the O₂ crosses the air-blood membrane; 3) perfusion, i.e. how much blood flows through the local lung region in a given time at a given tissue volume for O₂ to dissolve. Therefore, the changes in the T₁ values and consequently the signal intensities of the lung parenchyma that are induced by the increase in the local dissolved O₂ in T₁-weighted OE-MRI contains mixed functional information of the lung [165].

3.7.2 OE-MRI data acquisition methods

T₁-weighted static OE-MRI consists of the acquisition of two sets of T₁-weighted images on the steady stages of air inhalation and 100% O₂ inhalation, respectively. In comparison, the core component of T₁-weighted dynamic OE-MRI is the acquisition of serial T₁-weighted images throughout the switchover of the breathing gas from room air or medical air to pure O₂ and, optionally, back to air, most commonly preceded by an acquisition of T₁-weighted images on air inhalation for generation of baseline reference MR images or baseline T₁ maps [165]. Figure 3.3 is a schematic of the protocol designs for the acquisition of dynamic OE-MRI data in the human lung. Gas is delivered at a rate of 10 L/min-25 L/min via a breathing mask. Subjects breathe freely or hold their breath during image acquisition, depending on the techniques applied for motion compensation.

<table>
<thead>
<tr>
<th>Medical air (21% O₂) breathing</th>
<th>100% O₂ breathing</th>
<th>Medical air breathing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subject set-up and structural imaging (10 min)</td>
<td>T₁₀₀ mapping (5 min)</td>
<td>T₁-weighted dynamic acquisition (140 dynamics) (15 min)</td>
</tr>
</tbody>
</table>

Figure 3.3 A schematic of the protocol design for the acquisition of dynamic OE-MRI data in the human lung

The pulse sequences applied for T₁-weighted dynamic OE-MRI of the lung aim to at least fulfil: 1) relatively high temporal resolution, permitting a sensitive trace of the signal change during gas switchover; 2) high image quality (i.e. high SNR, low artefact levels and good spatial resolution), in order to sensitise to the small signal change induced by O₂ inhalation and to ensure the quality of image analysis, especially quantitative analysis; 3) sufficient T₁-weighting, in order to increase the sensitivity to O₂ induced T₁-shortening and minimize the T₂ (T₂*) effects on signal intensities. With the additional consideration of the general challenges of lung proton MRI, fast SE sequences surpass GE sequences in their performance in pulmonary OE-MRI [165]. Single-shot SE sequences, with or without k-space undersampling, preceded by a 180° inversion pulse to achieve sufficient T₁-weighting are the most popular readout sequences for T₁-weighted dynamic OE-MRI of the lung, e.g. IR-TSE
and IR-HASTE [166-173]. Several GE sequences such as trueFISP [174] and FLASH with ultra-short TE [175-177] have also been evaluated for T₁-weighted pulmonary OE-MRI, with the aim to extend image coverage and increase the temporal resolution, but their performance is less satisfying due to their intrinsic disadvantages in lung imaging as introduced in section 3.32. The majority of T₁-weighted pulmonary OE-MRI studies have to date been limited to few 2D slices due to temporal sampling constraints while well-validated 3D sequences are still lacking. A UTE sequence has been utilized to acquire 3D static OE-MRI data in vivo in human lungs based on the T₂* shortening effect of O₂. However, the temporal resolution of this UTE sequence was not high enough to fulfill the requirement of 3D dynamic OE-MRI scanning [178]. Recently, Ulloa et al. presented a novel whole lung 3D dynamic OE-MRI protocol derived from a standard single-shot 3D magnetization prepared rapid gradient echo sequence. The preliminary results gained in 3 healthy subjects showed that this sequence can obtain 40% signal enhancement in the lung field when subjects inhaling 100% O₂ [179].

The most commonly employed IR sequence in OE-MRI uses a non-slice selective adiabatic pulse, which provides uniform inversion, but other IR types are also available. Multiple-slice selective IR pulse can be used to image more slices than single-slice selective or non-slice selective IR pulse at a given scanning time meanwhile retain reasonable T₁-weighting and temporal resolution in dynamic OE-MRI [180]. Mai et al. demonstrated that adding two IR pulses ahead of a HASTE sequence with appropriate TI, i.e. multiple IR technique, can highlight the O₂-induced signal change in the organs of interest such as lungs by improving the subtraction of signal arising from background tissues, such as subcutaneous fat and skeleton muscle via suppressing their signals [181, 182]. However, as the multiple IR technique diminishes the lung signal, reduces the absolute value of change in signal intensities and suffers more inflow effects, it is not widely used in pulmonary OE-MRI. Furthermore, by using the literature T₁ values of the lung in air and in O₂, Chen et al. have calculated the optimal TI to maximize the signal enhancement in T₁-weighted OE-MRI of the lung, which was between 900 ms and 1300 ms [167].

As with other pulmonary MRI techniques, pulmonary OE-MRI is challenged by fast cardiac and respiratory motion. The breath-holding technique has been used in some studies to overcome respiratory motion [168, 169, 182]. However, it is hampered by a high patient compliance requirement, poor reproducibility and the inability to acquire images under a normal physiological state. Cardiac triggering using a peripheral pulse oximetry or an electrocardiograph and respiratory triggering using an extensible pneumatic belt or a pneumotachograph are capable of reducing motion artefacts when performing pulmonary OE-MRI under free-breathing [170, 173, 183-186]. The synchronization of cardiac triggering and respiration triggering or navigation is available for OE-MRI of the lung [180, 185-187]. Navigator sequences can directly register diaphragm motion and better reflect respiratory motion compared with a pneumatic belt. Although triggering and navigating techniques enable images to be acquired during free-breathing, they inevitably prolong the scanning time and lower the temporal resolution and thus their application in dynamic OE-MRI is limited.
Many other settings outside of the sequence parameters affect the performance of OE-MRI. Most researchers use a concentration of inhaled O$_2$ of 100% in order to maximize the signal enhancement [166, 167, 188]. Long-timescale inhalation (> 24 hours) of 100% O$_2$ may cause acute toxic injury of the lung, but short timescale usage of pure O$_2$ in OE-MRI (about 10 min - 20 min) does not cause significant safety concerns [189]. However, severe COPD is an exception that needs closed medical cover during dynamic OE-MRI scan. Hypoxia is the key respiratory stimulus in patients with severe COPD. The rapid correction of hypoxia during dynamic OE-MRI scans via inhaling high concentration O$_2$ may suppress ventilation (due to the loss of respiratory stimulus) and subsequently cause the retention of carbon dioxide [190, 191]. The O$_2$ flow rate also influences the O$_2$-induced signal enhancement in OE-MRI and 15 L/min has been reported optimal when a non-rebreathing mask is used [192]. The type of the gas delivery system is also a confounding factor. Renne et al. has demonstrated that the OE-MRI experiments using fully closed air-cushion face masks yield more reproducible and more significant reduction in T$_1$ value of the lung than those using loose-fit face masks [177]. In addition, the static magnetic field strength matters with regard to the O$_2$-induced signal enhancement. Pulmonary OE-MRI has been successfully conducted at 0.2 tesla, 1.5 tesla and 3 tesla, with those performed at 1.5 tesla and at 3 tesla demonstrating a greater reduction in T$_1$ and increase in signal intensity and much better image quality than those performed at 0.2 tesla [168, 174, 193, 194].

### 3.7.3 Data analysis

Because O$_2$ is only weakly paramagnetic, the O$_2$-induced signal enhancement in T$_1$-weighted MR images is too small to be visually observed easily. Therefore, proper image analysis is required to evaluate the change in the signal intensity, or more quantitatively, the change in the T$_1$ value, with time-course curves, histograms and colour-coded parameter maps being used to assist the illustration of OE-MRI readouts.

The simplest way to explore O$_2$-induced signal enhancement is to generate a “static” subtraction map by subtracting a T$_1$-weighted MR image acquired on air inhalation from an image acquired on the steady state of pure O$_2$ inhalation [167, 168, 195]. In these subtraction maps, the functional regions of the lungs are bright, indicating signal enhancement, while the non-functional regions are dark, reflecting no or poor signal enhancement. This provides spatial information on the functional impairments in the lung. A closely related method is to map the relative signal enhancement ratio, which is generally given by

$$\Delta S\% = 100\% \cdot \frac{S_{O2} - S_{air}}{S_{air}}$$  \hspace{1cm} (Eq 3.8)

where $S_{O2}$ is the signal intensity on pure O$_2$ inhalation and $S_{air}$ is the signal intensity on air inhalation [166, 169, 171, 180]. Relative signal enhancement ratio maps may be more robust and comparable between subjects and scans than subtraction maps as they account for the variations of the baseline signal intensity due to variations in proton density. However, the magnitude of the relative signal intensity change is dependent on the image acquisition method and the chosen imaging parameters, as well as the baseline T$_1$ value. On the other hand, dynamic OE-MRI delivers kinetic information of
O₂ movement in the lung by serially tracing the signal change throughout air-O₂-air alteration [112, 170, 171, 183, 188, 196, 197]. The signal intensity versus time curve of a pixel or a region of interest (ROI) is usually extracted from the dynamic acquisitions for direct comparison or for the calculation of the maximum signal increase on the curve plateau (actual or relative value), signal up-slope/wash-in rate and downslope/wash-out rate. Most commonly used is the exponential function, given by

\[
\begin{align*}
O₂ \text{ wash-in:} & \quad S(t) = \Delta S_{\text{max}} (1 - e^{t/\tau_{\text{up}}}) \\
O₂ \text{ wash-out:} & \quad S(t) = \Delta S_{\text{max}} e^{t/\tau_{\text{down}}} 
\end{align*}
\]  

(Eq 3.9) (Eq 3.10)

where the fitting parameters are \(\Delta S_{\text{max}}\), the maximum change in signal intensity, \(\tau_{\text{up}}\), the O₂ wash-in time constant, and \(\tau_{\text{down}}\), the O₂ wash-out time constant. The inverse of the time constant is the corresponding signal changing rate [188]. Dietrich et al. compared the performance of six different shape functions, including exponential function, sigmoid function and box function, etc., in fitting the signal-to-time curve in the lung and a piecewise exponential function was found optimal [197].

Another useful OE-MRI readout is the enhancing fraction, i.e. the fraction of the number of pixels enhancing due to the introduction of O₂ versus the total number of pixels in the lung; this is analogous to the "ventilated fraction". Several approaches are available to define an "enhancing" pixel. The potentially easiest method would be thresholding the map of relative signal enhancement ratio. However, it is difficult to define a reasonable and standard cut-off value. A previously published approach is to estimate the similarity of the measured signal-to-time curve with a reference curve shape model by using cross-correlation analysis and the pixels with a correlation coefficient greater than 0.5 are defined as enhancing. The corresponding enhancing fraction calculated in this way was named as fraction of oxygen-activated pixels [169, 172, 197, 198]. Additionally, the enhancing pixel can be defined as one where the applied curve shape model shows a better fit to the data than a straight line of zero gradient [199].

It has been suggested that analysing the O₂-induced \(T₁\) change in a dynamic manner is more quantitative for evaluating lung function than analysing the signal intensity change [196, 200]. An increasing number of dynamic OE-MRI studies have been upgrading the analysis from the signal intensity level to the \(T₁\) level. A static \(T₁\) map on air inhalation (baseline \(T₁\) map) and on pure O₂ inhalation can be measured from the signal intensities of the image datasets acquired on the two gas inhalation states by fitting the signal equation of the employed pulse sequence. In terms of the dynamic series, the signal over time curve can be converted to a \(T₁\) over time curve by substituting the baseline \(T₁\) value and fitted \(S₀\) value into the signal equation. The multiple IR method and Look-Locker based method are most commonly used for \(T₁\) mapping in \(T₁\)-weighted pulmonary OE-MRI. Table 3.2 lists the reported \(T₁\) values of the lung parenchyma on air inhalation and on pure O₂ inhalation at 0.2 tesla, 1.5 tesla and 3.0 tesla.
<table>
<thead>
<tr>
<th>Papers</th>
<th>$T_{1\text{air}}$ (ms)</th>
<th>$T_{1\text{O}2}$ (ms)</th>
<th>Sequence</th>
<th>Field strength</th>
<th>Gas delivery</th>
<th>Sample size and breathing model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Edelman [166]</td>
<td>901 ± 55</td>
<td>826 ± 62</td>
<td>IR-HASTE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>6 healthy subjects; free breathing</td>
</tr>
<tr>
<td>Stock [168]</td>
<td>904 ± 99</td>
<td>790 ± 114</td>
<td>IR TSE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>8 healthy subjects; free-breathing</td>
</tr>
<tr>
<td>Stock [168]</td>
<td>632 ± 54</td>
<td>586 ± 41</td>
<td>IR-TSE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$; 15L/min</td>
<td>8 healthy subjects; free-breathing</td>
</tr>
<tr>
<td>Löffler [169]</td>
<td>1280 ± 85</td>
<td>1224 ± 139</td>
<td>IR-TSE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$; 20 to 25 L/min</td>
<td>4 healthy subjects; free breathing</td>
</tr>
<tr>
<td>Löffler [169]</td>
<td>1219 ± 176</td>
<td>1074 ± 92</td>
<td>IR-TSE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$; 20 to 25 L/min</td>
<td>5 healthy subjects; breath-hold</td>
</tr>
<tr>
<td>Naish [112]</td>
<td>1270</td>
<td>1080</td>
<td>IR-HASTE</td>
<td>1.5 tesla</td>
<td>Medical air (21% O$_2$) and 100% O$_2$, L/min</td>
<td>5 healthy subjects; free-breathing</td>
</tr>
<tr>
<td>Chen [167]</td>
<td>1336 ± 46</td>
<td>1162 ± 33</td>
<td>IR single-shot RARE</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>5 Healthy subject; free breathing</td>
</tr>
<tr>
<td>Arnold [176]</td>
<td>1260</td>
<td>10% shortening</td>
<td>IR-HASTE</td>
<td>1.5 tesla</td>
<td>Room air, 95% carbogen</td>
<td>10 healthy subject; free breathing</td>
</tr>
<tr>
<td>Renne [177]</td>
<td>1250 ± 52</td>
<td>1157 ± 52 (loose-fit mask); 1093 ± 38 (fully-closed mask)</td>
<td>IR-snapshot FLASH</td>
<td>1.5 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>12 healthy subjects; breath-hold</td>
</tr>
<tr>
<td>Thieme [194]</td>
<td>1281 ± 124</td>
<td>1102 ± 135</td>
<td>SR-HASTE</td>
<td>3.0 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>3 healthy subjects; breath-hold</td>
</tr>
<tr>
<td>Oechsner [140]</td>
<td>686 ± 61</td>
<td>631 ± 46</td>
<td>IR-snapshot FLASH</td>
<td>0.2 tesla</td>
<td>Room air, 100% O$_2$</td>
<td>5 healthy subjects; breath-hold</td>
</tr>
</tbody>
</table>
The spin-lattice relaxation rate (the inverse of $T_1$) of the blood plasma and tissue water depends linearly on the amount of dissolved $O_2$ [163, 166, 167, 169, 200], which is directly proportional to the local $PO_2$, according to Henry’s law [162]. Hence, the difference in $1/T_1$ between air inhalation and 100% $O_2$ inhalation is proportional to the change in $PO_2$ ($\Delta PO_2$). Therefore, the dynamic change in regional $PO_2$ ($\Delta PO_2(t)$) during gas switchover can be measured from dynamic change in $T_1$ by

$$\Delta PO_2(t) = (1/T_1(t) - 1/T_{1\text{air}})/r_{1, O_2} \quad \text{(Eq 3.11)}$$

where $r_{1, O_2}$ is the relaxivity of dissolved $O_2$. $r_{1, O_2}$ has been measured by several studies at different field strengths, in different solutions, using different pulse sequences. A literature value of $2.49 \times 10^{-4}$ /s/mmHg that was measured in distilled water was chosen for the OE-MRI work in this thesis [201].

The $\Delta PO_2$-to-time curve can be further fitted to the shape function, e.g. exponential function, to extract the maximum change in $PO_2$ ($\Delta PO_2$) and the $O_2$ wash-in and wash-out time constants [202]. Kershaw et al. compared the aorta $\Delta PO_2$-to-time curves between smokers and non-smokers by using $T_1$-weighted OE-MRI. They ascribed the gentler curve slopes and slower curve plateaus in smokers as than in non-smokers to the smoking-induced impairment of the lung function [202].

Presenting pulmonary OE-MRI data in terms of $\Delta PO_2$ is more clinically informative than doing so in terms of $\Delta T_1$ as $\Delta PO_2$ is directly related to the functional factors with great clinical interest, such as pulmonary ventilation, perfusion, ventilation-perfusion matchup and diffusion capacities, etc. $\Delta PO_2(t)$ could then be modelled using physiological models to separate the contributions of each functional component. A two compartment physiology model has been proposed to extract ventilation to perfusion ratio from pulmonary OE-MRI data, although to date this method lacks validation [203]. Instead of trying to separate these functional contributions, Jakob et al. introduced a new parameter called the $O_2$ transfer function to represent the mixed lung function information provided by OE-MRI [204]. In their study, $O_2$ with variable concentrations was administered in order to calculate $O_2$ transfer function by

$$OTF = (1/T_1(C_{O_2}) - 1/T_{1\text{air}})/C_{O_2} \quad \text{(Eq 3.12)}$$

where OTF is the $O_2$ transfer function, $C_{O_2}$ is the fractional inspired $O_2$ concentration, which was set to five different values in this study (21%, 40%, 60%, 80% and 100%), and $T_1(C_{O_2})$ is the $T_1$ value of the lung parenchyma when breathing $C_{O_2}$ concentration of gas. The $O_2$ transfer function has been reported to be significantly lower in cystic fibrosis lungs than in healthy lungs, which provides evidence for its potential for clinical use.

### 3.7.4 Application of OE-MRI in the lung

A number of preclinical studies and clinical studies of $T_1$-weighted pulmonary OE-MRI have been carried out since the first demonstration of this technique in 1996 [166]. Meanwhile, the past decade has also seen an increase in studies of OE-MRI in organs outside of the lung, which however will not be reviewed in this thesis. For simplicity, “OE-MRI” below refers to $T_1$-weighted technique unless expressly stated.

#### 3.7.4.1 Preclinical studies
Edelman et al. performed OE-MRI on a pig model fixed with an arterial line in the right femoral artery for blood pressure and gas monitoring. They demonstrated that the $R_1$ of the lung tissue has an excellent linear correlation with the $PO_2$ in the sampled arterial blood ($r^2=0.997$), which supports the use of OE-MRI as a potential non-invasive tool for the measurement of local $PO_2$ in tissue of interest [188].

Pig models of airway obstruction have been used to test the pulmonary OE-MRI technique. The distal area of balloon-blocked bronchus with relatively normal appearance in anatomical MR images was presented as an obvious ventilation defect in OE-MRI images [167, 205]. This ventilation defect was spatially matched with a perfusion defect that was detected by pulmonary perfusion MRI. These animal experiments clearly demonstrated the ability of OE-MRI to depict ventilation abnormalities caused by airway obstructions.

OE-MRI was also conducted to explore the ventilation changes in a pig model and a dog model of pulmonary embolism [205, 206]. The unmatched patterns of normal ventilation in OE-MRI images and perfusion defects in DCE-MRI images accurately reflected the pathophysiological features of this disease.

Performing OE-MRI in small animals is relatively difficult due to the small size of the lung and high frequency of respiration and cardiac pulsation. Watt et al. have successfully performed OE-MRI on mice by using an optimized cardiac-triggered, respiratory-gated fast SE sequence, with 11%-16% signal increase being obtained in mouse lungs after switching inhaled gas from air to pure $O_2$ [207].

Lee et al. repeated pulmonary OE-MRI on rabbits at normal, low and high pulmonary blood flow statuses [208]. They did not find significant difference in the signal enhancement ratio of pulmonary OE-MRI among the three statuses, although the pulmonary blood flow was significantly altered. Authors then suggested that there was little effect of the change in pulmonary blood flow on the signal enhancement in pulmonary OE-MRI under normal ventilation conditions. However, validation studies are required since the lack of significant change in signal enhancement might be ascribed to the insufficiency of the blood flow alteration.

**3.7.4.2 Clinical studies**

OE-MRI of the lung was first reported by Edelman et al. in 1996 as a ventilation imaging method [166]. The “ventilation image” was initially exhibited by the percentage signal enhancement map calculated from the static images acquired on air inhalation and on pure $O_2$ inhalation. Homogeneous signal enhancement, an analogue of normal pulmonary ventilation, was seen in healthy lungs after $O_2$ inhalation. Dynamic OE-MRI has then been implemented in healthy subjects to gain kinetic information of $O_2$ delivery, uptake and washout in the lung, with the signal enhancing upslope and downslope and maximum enhancement ratio being calculated from the signal time-course curves [188]. More recently, dynamic OE-MRI has been used to measure the specific ventilation of the lung at a local level, i.e. the ratio of fresh gas entering a lung region following an inspiration divided by its end-expiratory volume. A vertical gradient of the pulmonary specific ventilation was then demonstrated in healthy subjects using dynamic OE-MRI. [209]. Moreover, some
researchers have integrated OE-MRI with pulmonary perfusion MR techniques, including DCE-MRI and arterial spin labelling in the estimation of ventilation-perfusion matchup [195, 204, 210].

Ventilation abnormalities in COPD have been visualized by static OE-MRI and dynamic OE-MRI in many studies. Emphysema, especially bulla, generally manifests as low signal regions with less or no enhancement in OE-MRI, indicating both the poor ventilation and the loss of tissue in the emphysematous areas [166, 171, 211, 212]. A reduced and heterogeneously distributed relative signal enhancement ratio of OE-MRI has been demonstrated in patients with COPD and has been correlated with COPD severity [211, 212]. The significant correlations of OE-MRI readouts, e.g. mean relative signal enhancement ratio and signal enhancement slope, with spirometric parameters of airway obstruction and pulmonary diffusion capacity, e.g. FEV₁/FVC, FEV₁ %predicted and DLco %predicted have evidenced the feasibility of dynamic OE-MRI in the assessment of lung function [183, 211, 212]. Furthermore, OE-MRI has shown potential for more sensitive detection of early functional changes in smokers and early stage COPD than quantitative CT [211-213] and more accurate evaluation of clinical outcomes for COPD patients undergoing lung volume reduction surgery than SPECT-CT [214]. OE-MRI in combination with CT might have a place in the evaluation of regional function-structure relationship in respiratory diseases. However, there was no literature available before the current PhD work with regard to the direct comparison and correlation between these two imaging modalities in COPD (chapter 6).

Dynamic OE-MRI has also been extended to interstitial lung disease (ILD) and cystic fibrosis for the assessment of the reduction in pulmonary diffusion capacity. The signal enhancement upslope, the maximum signal enhancement ratio and the enhancing fraction of $O_2$ enhancing pixels were smaller in patients with ILD than in healthy subjects and were correlated with DLco %predicted, Kco %predicted, PaO₂ and SaO₂ in pooled data of ILD patients and healthy subjects [171, 187]. In patients with cystic fibrosis, the diseased lung regions with reduced $O_2$ transfer function in OE-MRI matched with the poor perfusion areas in DCE-MRI [204]. These findings reflect a pattern of decreased pulmonary oxygenation secondary to the impaired $O_2$ diffusion capacity in ILD and cystic fibrosis.

Dynamic OE-MRI has also been applied for lung cancer assessment [170, 176, 195]. Patients with lung cancer showed significantly lower relative signal enhancement ratios and reduced signal enhancing upslope in the lung than healthy subjects, with the signal enhancement ratio correlated with DLco %predicted, emphysema visual score from CT and the ventilation index from scintigraphy while the upslope correlated with FEV₁ %predicted. The lung cancer patients with pulmonary emphysema had reduced signal enhancing upslope than those without emphysema [170]. Pre-surgical OE-MRI has proven as useful as pre-surgical CT for the prediction of the postsurgical lung function in patients with lung cancer [184].

OE-MRI has also been successfully used to assess pulmonary vascular diseases. In patients with pulmonary embolism, the lung regions with perfusion defects presented in DCE-MR images showed normal signal enhancement in OE-MRI images [166, 195]. Pulmonary OE-MRI abnormalities
have been confirmed with moderate agreement with V/Q scintigraphy in the diagnosis of pulmonary hypertension [198].

So far, there is insufficient data regarding the utility of pulmonary OE-MRI in the estimation of lung function in asthma. Ohno et al. reported moderate correlations between the OE-MRI signal enhancement ratio and spirometric indices of airway obstruction in asthmatic lungs and demonstrated an equivalent efficacy of OE-MRI in asthma severity classification to quantitative CT [215]. However, there is no evidence available regarding the benefit and reproducibility of dynamic lung OE-MRI information in the estimation of pulmonary function and its response to treatment in patients with asthma before this PhD project.

3.7.5 Pros and cons of pulmonary OE-MRI

The major advantage of OE-MRI over other MR ventilation imaging techniques is that it induces enhancement by inhaling $^{16}$O$_2$, a natural component of gas exchange in the lung that is cheap and widely available. OE-MRI is able to acquire physiological information of the pulmonary function by using standard proton MRI systems. However, O$_2$ is only weakly paramagnetic leading to small signal change that cannot easily be directly visualized. A robust approach to separate the O$_2$-induced signal changes contributing to ventilation, diffusion and perfusion is still lacking, although relevant studies have been reported [203, 216]. The inhalation of pure O$_2$ may cause absorption atelectasis in both healthy and diseased lungs. Although it does not have significantly clinical implication in healthy adults, it may attenuate the pulmonary oxygenation in patients with lung disorders [217]. Furthermore, inhaling O$_2$ may alter the normal physiological status of the lung. One of the concerns is that breathing pure O$_2$ may cause vasodilation and potentially lead to an alteration of pulmonary perfusion, although the change in blood flow might have little effect on signal enhancement in OE-MR images [218].

3.8 Dynamic contrast-enhanced MRI

3.8.1 Contrast agents and the principles of DCE-MRI

$T_1$-weighted dynamic contrast-enhanced MRI is a contrast-enhanced proton MRI technique used for the investigation of organ perfusion and microvascular status by tracking the signal change after the intravenous administration of a contrast media bolus. It was first used to image cancer and brain perfusion and then introduced to the lungs in 1995 [219]. It can assist disease diagnosis as simply as by visually comparing the pre-contrast and post-contrast images but is also able to quantify lung perfusion and physiological characteristics of the pulmonary capillaries.

Gd chelates are the most widely used intravenous MR contrast agents. They can be further subdivided into extravascular agents, intravascular agents and specific intracellular agents [220]. The extravascular agents are the most popular in clinical use. They have small molecular weight (< 1000 daltons) and can cross capillary endothelial walls into the extravascular extracellular space (EES), typically in the first seconds to a few minutes after injection. However, they are not able to enter normal tissue cells. Contrast agent accumulated in the EES eventually re-enters the bloodstream in the late-phase (minutes and hours) and is typically excreted by the kidneys. The Gd ion is toxic and
has to be locked into chelating molecules to form a non-toxic compound before the administration in vivo. A review about the safety of MRI contrast agents could be found in reference [221].

The Gd ion used in contrast agents is strongly paramagnetic and leads to a shortening in $T_1$ and thus an increase in signal intensity on $T_1$-weighted images. In addition, Gd-based contrast agents have positive susceptibility and can increase the local magnetic field and yield field gradients, which causes a shortening in $T_2$ and $T_2^*$. Different tissues have different features in taking up the contrast medium, so the use of a contrast agent enhances the contrast between tissues on the post-contrast images. Furthermore, by using the dynamic imaging method to track the passage of contrast agent through tissue, the microvasculature characteristics can be assessed. In dynamic approaches outside the lung, the $T_1$-weighted DCE-MRI technique is preferable in the assessment of vascular endothelial permeability and EES whilst the $T_2$ ($T_2^*$) weighted bolus passage DCE-MRI technique (also called dynamic susceptibility contrast MRI) is generally used for the measurement of blood flow and blood volume [220]. As for the application in the lung, $T_1$-weighted MRI is a better option for bolus passage assessment of blood flow and volume as it is less prone to the substantial susceptibility artefacts in the lung than the $T_2$ ($T_2^*$) weighted approach. This thesis concentrates exclusively on the $T_1$-weighted DCE-MRI technique.

3.8.2 DCE-MRI data acquisition methods

In common with the dynamic OE-MRI method, a complete $T_1$-weighted DCE-MRI dataset usually consists of a set of pre-contrast images as reference images for comparison or for baseline $T_1$ mapping and a dynamic series of images over the injection and redistribution of contrast agent.

High temporal resolution, high spatial resolution and good image coverage are all important for DCE-MRI, especially for quantitative analysis. High temporal resolution is required in order to accurately delineate the time series of the signal intensities. Generally, a temporal resolution of 3 s or better (bolus transit time through the lung [222]) is needed to visualize the peak enhancement of the lung. Spatial resolution should be high enough to detect small changes as required in clinical settings. The image volume should cover the target tissue along with the feeding arteries, when the arterial input function (AIF) is to be measured.

Nowadays, almost all lung DCE-MRI studies are based on fast 3D imaging sequences. The most common are $T_1$-weighted spoiled gradient echo sequences (FFE, FLASH, SPGR) with ultra-short TE and short TR, allowing temporal resolution usually in the range of 1.0 s-2.0 s per 3D data set. The combination of fast GE sequences with parallel imaging techniques and “data sharing” techniques enables the time-resolved 3D DCE-MRI of the lung with a further improved temporal resolution (< 1 s per volume) and spatial resolution but may be more prone to image artefact [111, 223, 224]. Breath-holding 3D DCE-MRI of the first pass of contrast agent has been achieved for perfusion quantification. However, a recent study has shown that free-breathing DCE-MRI delivers quantitative parameters with higher reproducibility [225].

Several studies investigated the effects of injection-related factors on DCE-MRI and demonstrated that faster injection rates (> 3 ml/s) and lower contrast agent volume may better
separate the pulmonary circulation and systemic circulation, while higher contrast agent doses benefit SNR [226-229]. Single bolus with the dose of 0.1 mmol/kg body weight at a rate between 3 ml/L – 5 ml/L is the most widely used injection protocol in DCE-MRI of the lung [230], although half dose protocols and dual-bolus protocols are seen [231, 232].

3.8.3 Data analysis

DCE-MRI images can be analysed by visual assessment, empirical parametisations or tracer kinetic modelling [220]. Visual assessment refers to the direct identification of pathologies by visual observation of the pre-contrast and post-contrast images. Usually the pre-contrast image is subtracted from the post-contrast images to gain a better visualization of the perfusion signal. Visual assessment is the easiest to perform but not quantifiable. Empirical parametisations derive descriptive parameters that characterise the shape and structure of signal intensity-time curves or contrast agent concentration-time curves using model-free approaches. The commonly used empirical parameters include time to peak enhancement, area under the curve, maximum upslope, maximum downslope and maximum enhancement, etc. Empirical parameters are straightforward to measure and may have clinical applications. However, they are affected by scanner settings and their links to underlying physiology are not completely clear. Tracer kinetic modelling analysis refers to the quantitative measurement of physiological parameters, such as tissue blood flow, blood volume and vascular permeability, by fitting appropriate tracer kinetic models to contrast agent concentration-time curves. Quantitative parameters derived from tracer kinetic modelling are usually directly related to haemodynamics and physiology, though the post-processing is relatively complicated.

3.8.3.1 Generating tissue contrast agent concentration versus time curves and the arterial input function

It is recommended to perform the quantitative analysis in three steps: 1) measure the native tissue $T_1$ and dynamic $T_1$, using methods similar to those described for OE-MRI above; 2) quantify the tissue contrast agent concentration versus time curve ($C_t(t)$) and arterial input function (AIF); 3) extract quantitative parameters from $C_t(t)$ by model-free approaches or kinetic model fitting.

The $C_t(t)$ of the tissue of interest can be generated by using its linear relationship with the change in $1/T_1$ by

$$C_t(t) = \frac{(1/T_1(t) - 1/T_{1\text{baseline}})/r_{1\_Gd}}{1/T_{1\text{baseline}}}/r_{1\_Gd}$$  \hspace{1cm} (Eq 3.13)

where $C_t(t)$ is the tissue contrast agent concentration at time $t$, $T_1(t)$ is the $T_1$ at time $t$, $T_{1\text{baseline}}$ is the pre-contrast $T_1$, $r_{1\_Gd}$ is the spin-lattice relaxivity of the contrast agent, which is related to the type of contrast agent and $B_0$ strength.

The contrast agent concentration in the whole blood of the feeding arteries, $C_a(t)$, i.e. the AIF, is determined in the same way plus a scaling process to correct the partial volume effect due to the haematocrit. $C_a(t)$ is given by

$$C_a(t) = \frac{(1/T_1(t) - 1/T_{1\text{baseline}})/r_{1\_Gd}(1-Hct)}{1/T_{1\text{baseline}}}/r_{1\_Gd}$$  \hspace{1cm} (Eq 3.14)
where Hct is haematocrit, which can be measured individually or more commonly using an estimate value of 0.42. The AIF of lung DCE-MRI is calculated from a ROI drawn at the main trunk of the pulmonary artery.

The baseline $T_1$ and $T_2$ dynamic series are required to generate $C(t)$ and $C_a(t)$. Baseline $T_1$ is measured using one of the methods introduced in section 3.6.1 according to the type of sequence used, which is then substituted in the corresponding signal equation to convert the signal dynamic series to a $T_1$ dynamic series. This step is mostly commonly carried out by using the VFA method.

Some groups determine $C(t)$ and $C_a(t)$ directly from signal intensity versus time curves ($S(t)$), which is however only valid in certain circumstances. When the contrast agent concentration is low, the signal intensity varies approximately linearly with the $1/T_1$ and hence with $C(t)$. However, at high concentrations the signal intensity saturates and is no longer proportional to the $C(t)$. This phenomenon may affect the accuracy of $C(t)$ calculation, particularly may cause the underestimation of peak value of AIF. Although 0.1 mmol/kg body weight is the most commonly used contrast agent dose for DCE-MRI, several studies have demonstrated half dose or possibly even lower doses were needed to hold the linear relationship in the large pulmonary arteries [233, 234]. However, the reduction in contrast-to-noise ratio by using half dose contrast agent affected the accuracy of perfusion quantification in lung parenchyma [224]. The solution for this dilemma could be using a double-bolus technique, i.e. acquiring AIF by using a small pre-bolus (~ 0.01 mmol/kg body weight) followed by imaging the parenchymal perfusion by using a high dose main bolus (~ 0.04 mmol/kg body weight) [231].

Individual measurement of the AIF is optimal as it accounts for the inter-subject and intra-subject variations. When robust measurement of individual AIF is not available, a population AIF can be used as an alternative [235].

### 3.8.3.2 Modelling the concentration-time curve

$C(t)$ can be modelled by tracer kinetic models, which allow the final extraction of quantitative parameters from DCE-MRI data. There are several types of kinetic models used to quantify lung perfusion: 1) a single compartment model, which assumes contrast agent only exists, and is well-mixed, in the blood plasma space, e.g. the indicator dilution theory; 2) a two compartment model, which assumes the contrast agent is well-mixed in both the vascular plasma space and in the EES, such as general two-compartment exchange model [236], standard Tofts model and extended Tofts model [237, 238]; 3) a tissue homogeneity model, which assumes the contrast agent is well-mixed in EES but presents as a plug flow in the blood plasma space, e.g. adiabatic approximation to the tissue homogeneity (AATH) model [239]. The following paragraphs will focus on the standard Tofts model and the extended Tofts model.

#### Standard Tofts model

The MRI-visible tissue volume of an ROI equals the sum of EES, whole blood volume and intracellular space in this ROI [240]. An extracellular Gd-based contrast agent partly remains in
plasma and partly enters extracellular space but cannot enter intracellular space, so the amount of contrast agent in the tissue can be expressed as

$$C_t(t) = C_p(t) \cdot v_p + C_e(t) \cdot v_e$$

(Eq 3.15)

where $C_t(t)$, $C_p(t)$, $C_e(t)$ are contrast agent concentration at time $t$ in tissue of interest, plasma and EES; $v_p$, $v_e$ are fractional volume of plasma and EES. The amount of contrast agent in EES can be expressed as the product of the trans-membrane concentration gradient and a volume transfer constant between EES and blood plasma, $K_{trans}$, by

$$v_e \cdot dC_e(t)/dt = K_{trans} \cdot (C_p(t) - C_e(t))$$

(Eq 3.16)

The standard Tofts model ignores the contribution of the plasma compartment to the total amount of contrast agent in the tissue. Therefore, Eq 3.15 is simplified to be

$$C_t(t) = C_e(t) \cdot v_e$$

(Eq 3.17)

Combing equation Eq 3.16 and Eq 3.17, the following equation can be obtained

$$C_t(t) = K_{trans} \cdot \int_0^t C_p(t - t') \cdot \exp\left(-\frac{K_{trans} v_e}{v_p} \cdot t'\right) \cdot dt'$$

(Eq 3.18)

Assuming the time delay between the contrast agent arriving the feeding artery where AIF is measured and when it arrives in the blood plasma in the tissue of interest is $\omega$ and the concentration of contrast agent in the capillary bed is the same as the AIF, i.e. $C_p(t) = C_a(t - \omega)$, the equation can then be converted to

$$C_t(t) = K_{trans} \cdot \int_{t-\omega}^t C_a(t - t') \cdot \exp\left(-\frac{K_{trans} v_e}{v_p} \cdot t'\right) \cdot dt'$$

(Eq 3.19)

Eq 3.19 is fitted to the $C_t(t)$ measured by MRI to extract 3 free parameters $K_{trans}$, $v_e$ and $\omega$.

**Extended Tofts model**

The standard Tofts model involves a number of assumptions, the most significant of which for lung imaging is the lack of an intravascular contribution to the signal, which is very likely to be a poor assumption when studying the lung parenchyma. The extended Tofts model assumes that the blood plasma contribution to the total tissue contrast agent concentration is not negligible by converting Eq 3.21 to

$$C_t(t) = v_p \cdot C_a(t - \omega) + K_{trans} \cdot \int_{t-\omega}^t C_a(t - t') \cdot \exp\left(-\frac{K_{trans} v_e}{v_p} \cdot t'\right) \cdot dt'$$

(Eq 3.20)

This is fitted to the $C_t(t)$ measured by MRI to extract 4 free parameters $K_{trans}$, $v_e$, $v_p$ and $\omega$.

Figure 3.4 illustrates the compartments and variables in the extended Tofts model.
Figure 3.4 A schematic of the extended Tofts model.

The voxel of tissue (dashed box) consists of a plasma compartment with fractional volume \( v_p \) and an extracellular extravascular (EES) compartment with fractional volume \( v_e \). The contrast agent, delivered with the arterial input function (AIF) at the concentration \( C_a(t) \), enters the plasma compartment and leaks into the EES at the rate determined by \( K_{\text{trans}} \) and back to plasma at the rate of \( K_{\text{ep}} \) (\( K_{\text{ep}} = K_{\text{trans}}/v_e \)).

Choice of kinetic model for the lungs

Pulmonary perfusion quantification was initially performed by fitting a gamma variate function to the first-pass signal curve in DCE-MRI data [241], which was soon replaced by using the indicator dilution method [229, 242-244]. However, the assumption of no contrast agent extravasation affects the accuracy of the calculation of perfusion (to a degree dependent on the amount of leakage) and makes it impossible to estimate the vascular leakage. The standard Tofts model is inadequate to accurately describe normal pulmonary perfusion due to its lack of a vascular term. The extended Tofts model, on the other hand, accounts for the contribution of contrast agent in the blood plasma compartment and enables a more reasonable explanation of the contrast agent kinetics in the lung [245]. In addition, the extended Tofts model has less fit parameters than the more complex two compartment exchange or AATH models, although the later two models allow the independent estimation of both blood flow and microvascular permeability. Therefore, the extended Tofts model was employed in the DCE-MRI work in this thesis.

3.8.3.3 Empirical parameterisations

Apart from the kinetic modelling, DCE-MRI data can be analysed by model-free approaches to extract empirical parameters. The simplest ones are curve shape descriptors calculated from the
signal intensity-time curves, such as the maximum signal enhancement rate, time to peak and signal washout slope.

A robust model-free biomarker widely used in clinical trials outside the lung is the initial area under the concentration curve (iAUC), which is the area under $C_t(t)$ from the time of first contrast agent appearance in the feeding artery to a certain time point, $t$ [220, 246]. It is given by

$$iAUC_t = \int_0^t C_t(t') \cdot dt'$$

(Eq 3.21)

where $t$ is usually set to 60 or 90 seconds. iAUC denotes the amount of contrast agent delivered to and retained by the tissue in the first $t$ time after bolus arrival. iAUC is reproducible, easy to calculate and avoids the problems of poor model fitting, but it is difficult to link to underlying physiology.

In addition, model-free deconvolution is a simple way to quantify lung perfusion [247, 248]. $C_t(t)$ can be expressed as a convolution of an impulse response function with the product of blood flow ($F$) and AIF by

$$C_t(t) = F \cdot C_a(t) \otimes R(t)$$

(Eq 3.22)

where $F$ is the blood flow with the unit of ml plasma/ml tissue/min, $R(t)$ is the tissue residue function, which describes the amount of contrast agent remaining in the tissue at time $t$. The product of $F$ and $R(t)$, and thus $F$ (the initial value of $R(t)$ is 1), can be obtained by deconvolving $C_a(t)$ from $C_t(t)$. However, this method is sensitive to the noise and appropriate regularization methods should be employed to constrain $R(t)$ in order to guarantee the reliability.

### 3.8.4 Application of DCE-MRI in the lung

Although Gd-based contrast enhanced MRI has had been used for lung imaging in late 1980s, DCE-MRI with the bolus tracing technique was first introduced to the lung by Hittmair et al. in 1995 for the assessment of solid pulmonary nodes [219] and later adapted for imaging the perfusion of lung parenchyma by Hatabu et al. in 1996 [249]. In the last decade, DCE-MRI has been further developed and utilized. Visual observation and semi-quantitative analysis are the conventional and the most popular ways for processing DCE-MRI data in clinical routine, which are warranted in the detection and diagnosis of many pulmonary pathological alterations. In addition, a number of methods have been proposed to quantify pulmonary perfusion from DCE-MRI data. With the use of several advanced kinetic models, DCE-MRI is able to assess the characteristics of pulmonary microvasculature, such as capillary permeability and extravascular leaking space, etc.. Benefiting from the development of user-friendly analysis software, the complicated perfusion quantification process is becoming more accessible for clinicians and radiologists.

Generally, healthy subjects show homogeneous signal enhancement with few perfusion defects, which present as regions with low or no enhancement on post-contrast images. Accurate perfusion quantification is available by using DCE-MRI both in normal lungs and in diseased lungs. Ohno et al. quantified normal pulmonary perfusion by using indicator dilution theory and deconvolution analysis in 15 healthy subjects and reported a pulmonary blood flow (PBF) of 123 ml/min/100ml, a pulmonary blood volume (PBV) of 10 ml/100ml and a mean transit time (MTT) of 4.7 s [243]. These results corresponded with values from PET and other DCE-MRI studies [234, 249-251].
In addition, DCE-MRI is able to assess effects of gravity, respiration and posture, hyperoxia and hypoxia on pulmonary perfusion in healthy subjects. PBF and PBV showed gravitational gradients in both supine and prone position, the values increasing linearly from gravity-independent areas to gravity-dependent areas [234, 252, 253]. PBF and PBV derived from expiratory breath-held scans were higher than those derived from inspiratory breath-held scans, while lower than those derived from free-breathing scans [225, 244, 252, 254]. Two DCE-MRI studies revealed that pure O₂ inhalation increased PBF and PBV secondary to vasodilation while hypoxic exposure lead to uneven pulmonary perfusion secondary to vasoconstriction [218, 255].

In patients with COPD, DCE-MRI provides more consistent assessment of perfusion defects than perfusion scintigraphy at the lobar and segmental level over the entire lung [256, 257]. The perfusion defects in COPD lungs are circumscribed non-wedge-shaped or diffuse patchy, the distribution pattern matching well to that of structural destruction secondary to emphysema [218, 257, 258]. Different radiological phenotypes of COPD also show different features on MR perfusion images. Semi-quantitatively and quantitatively evaluated DCE-MRI demonstrated heterogeneously reduced peak signal intensity, PBF and PBV and MTT in COPD patients relative to that seen in healthy subjects [259]. The post-contrast signal intensity ratio between perfusion defects and normal regions, PBF, PBV and MTT are positively correlated with FEV₁ %predicted and FEV₁/FVC and negatively correlated with CT estimates of emphysema (RA₉₅₀), indicating that perfusion impairments become worse as COPD progresses [243, 258]. Pharmacokinetic parameters have been applied to explore the microvascular characteristics in smokers and patients with COPD. Significant increase in Kᵣ and vₑ has been observed in smokers relative to that seen in non-smokers and has been attributed to the smoking-related chronic inflammation in the lung [260]. On the other hand, Kᵣ and vₑ were observed to be significantly lower in COPD lungs than in healthy lungs, which reflects decreased pulmonary perfusion due to the structural destruction [261].

In patients with cystic fibrosis, lung regions of perfusion defects detected by DCE-MRI matched up with areas with low O₂ transfer function in OE-MRI [200]. The degree of pulmonary perfusion alterations in children with cystic fibrosis is correlated with the severity of tissue damage, according to visual scores derived from DCE-MRI and structural MRI [262]. More recently a morphological-functional MR scoring system has been proposed for the assessment of pulmonary alterations in cystic fibrosis and the perfusion component derived from DCE-MRI showed an acceptable intra- and inter-reader agreement [263]. In patients with idiopathic interstitial pneumonia, DCE-MRI has shown the capability of distinguishing active inflammation from fibrosis, the former being characterised by an early enhancement pattern in DCE-MRI compared to the latter [264].

DCE-MRI is also an important clinical and research tool for the assessment of pulmonary perfusion in patients with pulmonary vascular diseases or heart malformation. Several studies have demonstrated good agreement of DCE-MRI with perfusion scintigraphy in the detection and localization of the characteristically wedge-shaped perfusion defects in patients with pulmonary embolism or pulmonary arterial hypertension [256, 265]. The different patterns of perfusion defects in
idiopathic and in thromboembolic pulmonary arterial hypertension may assist in the differential diagnosis [266]. Quantitatively evaluated DCE-MRI demonstrates that PBF and PBV are reduced while MTT is prolonged in patients with pulmonary arterial hypertension [242, 243]. These quantitative parameters show moderate to fair correlations with invasive pressure measurements, including the diagnostic parameter of mean pulmonary artery pressure, which suggest the potential role of quantitative DCE-MRI in the assessment of the severity of pulmonary arterial hypertension [267, 268]. In addition, DCE-MRI has been proven equal to or more accurate than perfusion scintigraphy in the quantification of pulmonary perfusion in patients with complex pulmonary circulation, including children with congenital abnormality of pulmonary artery or tetralogy of Fallot after surgical correction, patients with Fontan circulation, etc. [269, 270].

To the best of my knowledge, there is no clinical data regarding the utility of DCE-MRI in asthma. One area in asthma that DCE-MRI may be particularly helpful is the non-invasive exploration of pulmonary microvascular remodelling and angiogenesis through the assessment of contrast agent kinetics and derived physiological characteristics.

3.9 Other MRI techniques

3.9.1 Proton lung MRI techniques

Due to its lower SNR and spatial resolution, proton MRI is generally inferior to CT in the direct visualization of morphological changes in the lung, particularly abnormalities associated with reduced density, e.g. emphysema and bulla. However, proton MRI provides excellent soft-tissue contrast and has been proposed as an alternative or adjunct to CT in the detection and evaluation of pulmonary structural changes associated with increased density, e.g. thoracic benign lesions or malignancies. [113].

Relatively high temporal resolution and no ionizing radiation allow proton MRI to be safely performed in a dynamic manner. Dynamic proton MRI has great value for the real-time visualization and regional assessment of diaphragmatic and chest wall motion, static and dynamic lung volumes and pulmonary parenchymal strain and compliance. Gierada et al. demonstrated strong correlations between static lung volumes measured by pulmonary function tests and by proton MRI in patients with emphysema both before and after the LVRS [271]. Suga et al. observed flattened diaphragms with reduced and irregular respiratory motions of both the chest wall and diaphragms in patients with emphysema by using dynamic proton MRI during free-breathing [272]. Morgan et al. proposed a method to map the dynamic compliance and strain of the lung by using dynamic proton MRI. This method has shown ability in depicting the heterogeneously altered elastic properties of the lung in patients with COPD [273].

The Fourier decomposition (FD) technique, an emerging non-contrast-enhanced non-triggered proton lung MRI technique, allows ventilation and perfusion related information to be extracted from a series of dynamic lung MR images acquired during free-breathing [274]. The core of this technique is to separate the signal components fluctuating at the respiratory motion frequency and cardiac motion frequency by using Fourier decomposition and spectral analysis. The ventilation-
and perfusion-weighted images generated by using FD-MRI have shown good agreement with those from SPECT-CT, HP gas MRI and DCE-MRI in porcine lung, healthy subjects and patients with cystic fibrosis [275-277]. Quantification approaches for the FD ventilation and perfusion maps have also been proposed recently [278, 279]. Though there have been no reports about the application of FD-MRI in asthma and COPD so far, the few studies in patients with other lung disorders already give a promising outlook for this technique in the evaluation of ventilation and perfusion alterations in these two diseases. The advantages of FD-MRI over many other MRI techniques are the lack of need for contrast agent, breath-holding or triggering. These make it an attractive tool for pulmonary functional imaging. However, FD-MRI is still restricted to 2D imaging and it is not conclusive whether the information obtained by FD technique presents the true ventilation and perfusion or predominantly reflects respiratory and cardiac motions.

Arterial spin labelling (ASL) MRI utilizes blood water as an endogenous tracer. A bolus of blood flow, with its magnetization inverted (“labelled”), has been used to image the perfusion of the organs, including the lung [106, 224]. ASL techniques do not require exogenous contrast agent to be injected and thus can be repeated several times at a short period of time. However, ASL offers low SNR ratio such that the pulmonary blood flow cannot be directly visualized and the measurement is susceptible to motion artefacts. ASL usually requires a labelled image to be subtracted from a control image for perfusion assessment, which inevitably prolongs the scanning time. ASL quantification of pulmonary perfusion by using kinetic modelling has been applied mostly in healthy subjects to explore the effects of posture, lung volume and gravity on regional pulmonary blood flow [106, 224]. Several studies have also assessed the feasibility of ASL in asthma and COPD based on the qualitative analysis of the perfusion-weighted subtraction maps. Mai et al. delineated pulmonary perfusion defects and tracked their response to salbutamol inhalation in a patient with severe asthma using ASL technique [280], Roberts et al. demonstrated good qualitative agreement between ASL and SPECT in detecting and localizing perfusion defects in patients with COPD [281]. Levin et al. then reported that ASL is able to detect the pulmonary perfusion heterogeneity not only in patients with severe COPD but also in asymptomatic smokers [282].

3.9.2 Hyperpolarized gas MRI

HP gas MRI is fundamentally different from proton MRI as its MR signals are directly derived from the inhaled hyperpolarized non-radioactive isotopes of noble gases rather than the water hydrogen nuclei in the lung. The hyperpolarized noble gases can provide MR signals 100,000 times higher than water $^1$H, enabling high quality images of the airspace to be acquired. Hyperpolarized $^3$Helium (He) is usually the HP method of choice because of the higher SNR it can provide relative to other hyperpolarized noble gases such as $^{129}$Xe and $^{83}$Krypton. $^3$He has negligible solubility and negligible chemical shift and is thus ideal for imaging the airspace. On the other hand, hyperpolarized $^{129}$Xe has its own advantages – for example it is renewable and significantly cheaper than $^3$He. $^{129}$Xe also has a large range of chemical shifts in different environments and it is soluble in tissue, lipid and blood. This unique feature enables hyperpolarized $^{129}$Xe MRI to generate images based on either the
air-phase $^{129}$Xe or the dissolved-phase $^{129}$Xe, with the former related to pulmonary ventilation and the latter associated with pulmonary diffusion and perfusion [283, 284].

HP $^3$He MRI has three major applications in the lung: HP gas ventilation MRI for static or dynamic ventilation imaging; diffusion-weighted HP gas MRI for the measurement of regional microstructure; $O_2$-weighted HP gas MRI for the measurement of regional alveolar PO$_2$.

HP gas ventilation MRI can be performed in a static manner. Normal ventilated lungs usually present uniform distribution of the HP gas in the airspaces whereas smokers, patients with COPD and asthma may show focal or diffusely scattered ventilation defects that are consistent with the findings from other image modalities. In particular, HP gas MRI has demonstrated higher sensitivity to ventilation abnormalities than spirometry and lung scintigraphy. Increasing extent, size or heterogeneity of ventilation defects has been correlated with increased symptoms, clinical severity and airway inflammation (FE$NO$) in asthma, increased CT estimates of emphysema in COPD and increased airway obstruction assessed by spirometry in both diseases [284-287]. The ventilation defects detected by HP gas MRI in asthmatic patients increase in number and spatial heterogeneity after methacholine and exercise challenge and resolve with bronchodilator therapy [287, 288]. It has also been revealed by using HP gas ventilation MRI that deep inspiration can substantially correct the methacholine-induced increase in ventilation heterogeneity in healthy subjects but not in patients with asthma [289]. Furthermore, HP gas ventilation MRI has offered the prospect of longitudinal monitoring of the regional ventilation variation in asthma and COPD without ionizing radiation. Several studies have demonstrated the striking consistency of the ventilation defects in number, size and location over the time periods from 1 hour to over 1 year in patients with asthma, which was then ascribed to the presence of persistent airway remodelling [290-292]. In a 2-year longitudinal study in ex-smokers with COPD, the significant progress of ventilation impairment was well demonstrated by HP gas ventilation MRI but not by spirometry [293]. Another attractive application of HP gas ventilation MR images is their ability to provide detailed regional information of ventilation abnormality for pre-procedure planning and post-procedure assessment of regional interventional therapies of asthma or COPD, e.g. bronchial thermoplasty, bronchoscopic airway bypass, LVRS and single lung transplantation [285, 294].

HP gas ventilation MRI can also be performed in a dynamic manner to delineate the distribution of HP gas during respiration. This requires repeated acquisitions during HP gas wash-in and wash-out. Dynamic HP gas ventilation MRI has been applied to investigate collateral ventilation in emphysematous COPD, dynamic gas trapping in asthma and the ventilation difference between the native diseased lung and lung graft with and without rejection in patients with emphysema who have undergone single lung transplantation [52, 295, 296]. Ventilation related measurements such as the HP gas arrival time, wash-out rate, and regional fractional ventilation, i.e. the specific ventilation of each breath, can also be extracted from the HP gas signal dynamics [294, 296, 297].

Diffusion-weighted HP gas MRI has been applied to assess the microstructure of the lung in vivo by measuring the diffusion distance of the noble gas molecules in the lung at a given diffusion
time, i.e. apparent diffusion coefficient. Apparent diffusion coefficient has proven increased in both COPD lungs and asthmatic lungs, the former being ascribed to the airspace enlargement due to emphysematous destruction and the latter to air-trapping [298, 299]. In addition, O₂-weighted HP gas MRI has provided the new insight in the non-invasive measurement of alveolar O₂ partial pressure, i.e. PAO₂. Scattered studies have performed HP gas PAO₂ mapping in patients with COPD that already give a promising outlook [300-302], while human date with regard to the feasibility of this technique in asthma is still lacking.

Despite the high image quality and multiple promising applications, the translation of HP gas lung MRI techniques to clinic is hampered by the high costs of noble gases, high technical demands and high requirement on special equipment, such as the laser-polarizer and specialized radiofrequency transmitter/receiver coils. In addition, noble gases have different molecular weight from O₂ and thus their behaviour due to gravity may be different from O₂.

3.10 Other relevant methods employed in this PhD project

3.10.1 Image registration

Image registration of the lung is a large topic outside of the scope of this thesis. More detailed review of this topic can be found in reference [303]. The following paragraphs only cover the specific registration method employed for all the work presented in this thesis.

The registration used in this work is a method incorporating semi-automatic lung segmentation by using active shape modelling [304] and lung registration by 1-D linear stretch in the head-foot direction (assumed to be the predominant direction of lung motion) [112]. The first step is segmentation. Chest wall outlines are marked out manually in the first image and remain unchanged during breathing. The left and right diaphragm positions in a training set of images are also marked out to build an active shape model (figure 3.5).
After that, the active shape model is implemented to automatically identify the diaphragm position and produce lung outlines in the rest of the images, according to which the lung is then segmented. The second step is registration. The diaphragm position of each image is recorded and compared in order to find the maximum lung volume (end inspiration phase) and minimum lung volume (end expiration phase), one of which is usually chosen as the target image. Each column of pixels in the rest of images is then resized by a linear 1-D stretch at the head-foot direct to fit the column in the target image. A scaling factor, defined as the pre-stretch column length to post-stretch column length ratio, is calculated and multiplied to the signal of each reshaped column of pixels with the aim to correct signal intensity variation during the breathing cycle as a consequence of the proton density variation due to lung volume changes.

This method is initially proposed by Naish et al. for dynamic OE-MRI and then extended to DCE-MRI. It has been proven capable of correcting the linear transformation at the head-foot direction on 2D coronal images and beneficial to the quantitative analysis of dynamic series of lung images. It is straightforward to perform, processing time is relatively short and the method produces satisfactory results. However, it needs to be kept in mind that this simple 1-D linear stretch is not able to register the random shift motion, deformable transformation and linear transformation in other directions (e.g. anterior-posterior, left-right) of the lung. In addition, it is not applicable for volumetric registration or multiple slice registration (proportionally increase the manual work load).

3.10.2 Vessel segmentation

In this PhD project, lung segmentation was completed semi-automatically as introduced in section 3.10.1 and the vessels were masked out afterwards by two different methods. Dynamic OE-
MRI and the MR $S_0$ mapping work in chapters 4-7 utilized the density mask method based on the quantitative $S_0$ values. A cut-off value of 0.6 for lung $S_0$ against muscle $S_0$ muscle was chosen as an empirical threshold to separate lung parenchyma and large vessels. More detail about this quantitative $S_0$-based density mask method can be found in chapter 4. This method requires a high quality $S_0$ mapping that can be fulfilled by using inversion recovery measuring method based on T$_1$-weighted IR-TSE sequence as introduced in section 3.6.1.1 However, the DCE-MRI work adopted a spoiled gradient echo sequence and the $S_0$ maps generated did not suffice to support a robust separation of lung parenchyma and vessels. Instead, a k-means clustering method is introduced to divide three different components of the lung, i.e. arteries/arterioles/arterial vessels, veins and lung parenchyma according to the different arrival time of the contrast agent bolus. More detail about this k-means clustering vessel segmentation method is provided in chapters 8 and 9.
Chapter 4 Paper 1: MR quantitative equilibrium signal mapping: a reliable alternative to CT for the assessment of emphysema in COPD

This paper has been accepted for publication in “Radiology”.

Authors: Wei-Juan Zhang, Penny L Cristinacce, Eva Bondesson, Lars H Nordenmark, Simon S Young, Yu-Zhen Liu, Dave Singh, Josephine H Naish, Geoffrey JM Parker


Contribution of authors: WJ.Z: data analysis and interpretation, statistical analysis, manuscript preparation and edition. P.L.C: study conception and design, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, manuscript reviewing. D.S.: participant enrolment, data acquisition and interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. E.B, L.H.N., S.S.Y and YZ.L: study conception and design, data interpretation, manuscript reviewing.
4.1 Abstract

Purpose: To compare magnetic resonance (MR) quantitative equilibrium signal (qS₀) mapping with quantitative computed tomography (CT) in the estimation of emphysema in chronic obstructive pulmonary disease (COPD).

Materials and methods: This study was a retrospective analysis of data from an institutional review board approved study. Written informed consent of the original study permitted future reanalysis of the data. 24 COPD patients and 12 healthy non-smokers underwent spirometry and two separate MR imaging scans at 1.5 tesla. All COPD patients underwent an extra chest CT. Lung MR qS₀ maps were generated from a set of multiple inversion time MR images by fitting the inversion recovery signal equation. Mean, 15th percentile and the standard deviation of whole-lung qS₀ and the relative lung area with qS₀ value below 0.20 (RA₀.20) were measured and compared between groups using unpaired t-test. Their reproducibilities were tested by intraclass correlation coefficients (ICC) between two scans and their associations with spirometry and CT measurements of 15th percentile density (PD₁₅) and relative lung area with attenuation value below -950 Hounsfield units (RA₋950) were assessed by Pearson’s correlation.

Results: Whole-lung mean and the 15th percentile of qS₀ were significantly lower, whereas RA₀.20 and the standard deviation of qS₀ were significantly higher, in patients with COPD than in healthy controls (P=0.014, 0.002, 0.005, <0.001, respectively). Mean qS₀, the 15th percentile of qS₀ and RA₀.20 strongly correlated with RA₋950 (r=-0.78, -0.81, 0.86, respectively; P<0.001) and PD₁₅ (r=0.78, 0.79, -0.71, respectively; P<0.001) and moderately correlated with the forced expiratory volume in 1 s (FEV₁) /forced vital capacity (FVC) ratio (r=0.63, 0.67, -0.60, respectively; P<0.001) and the percentage predicted FEV₁ (r=0.54, 0.62, -0.56, respectively; P≤0.001). Excellent reproducibilities of qS₀ readouts were found in both groups (ICC=0.89-0.98).

Conclusion: Lung MR qS₀ mapping may be a reliable, non-contrast, non-ionizing radiation alternative to CT in the assessment of emphysema in COPD.

4.2 Introduction

Chronic obstructive pulmonary disease (COPD) includes two main conditions - emphysema and chronic bronchitis. It is the fourth-leading cause of death worldwide currently and is projected to rank third in cause of death and fifth in burden of disease worldwide by 2030 [6, 7, 11]. The high health and societal impact of COPD makes research on the prevention, diagnosis and management of this disease a priority. Chest computed tomography (CT) is the standard imaging modality for the visualization and quantification of regional morphological changes in COPD lungs, particularly emphysema [51, 88, 305]. Two objective measures of CT lung density, the relative area with X-ray attenuation below -950 Hounsfield units (RA₋950, HU) and the 15th percentile density (PD₁₅), have proven reliable for emphysema assessment [305-309]. However, radiation dose considerations limit the extensive utilization of CT in longitudinal assessment [310]. Thus, imaging techniques using no ionizing radiation are preferred as alternatives for longitudinal monitoring of the emphysematous changes in COPD and treatment monitoring.
MR imaging of lung structure has been notoriously hampered by the lung's low intrinsic proton density, respiratory and cardiac motion effects and the fast signal decay caused by susceptibility differences at air/tissue interfaces [311]. Although proton MR imaging has been successful in detecting pulmonary abnormalities with increased densities, benefiting from the technical advances such as short echo time, fast imaging acquisition and motion compensation techniques, it has yet to be regarded applicable to emphysema [113]. In emphysema, the lower proton density due to tissue loss, reduction in perfusion and pulmonary hyperinflation greatly diminish the MR signal and challenge non-contrast proton MR imaging techniques [144, 312, 313].

MR quantitative equilibrium signal ($qS_0$) mapping is a method to extract robust and specific proton density information from the MR raw images, which has been used to quantify lung water content in animal models and normal human lungs [124, 141, 143, 150, 151]. Due to the elimination of relaxation effects, MR $qS_0$ is considered a more faithful probe of endogenous differences in tissue proton density than raw MR signal intensity. Therefore, we took advantage of the lung water (tissue and blood) changes in emphysema and hypothesized that MR $qS_0$ mapping may have a role in portraying emphysema distribution and quantifying lung tissue density in patients with COPD. In this study, we aimed to compare MR $qS_0$ mapping and quantitative CT in the estimation of emphysema in COPD.

4.3 Materials and methods

This study was jointly funded by AstraZeneca and the UK Engineering and Physical Sciences Research Council under the Dorothy Hodgkin Postgraduate Awards scheme. The authors who are not employees of or consultants to AstraZeneca (WJ.Z, P.L.HC, D.S, J.H.N and G.J.M.P) had control of the data and information that might present a conflict of interest for the other employee or consultant authors (E.B., L.H.N, S.S.Y, YZ.L).

4.3.1 Study subjects and study design

This study was a retrospective analysis of data from an institutional review board approved pulmonary MR imaging study conducted by the University of Manchester. 24 COPD patients and 12 age-matched healthy non-smokers were enrolled from the Medicines Evaluation Unit in University Hospital of South Manchester between 2008 and 2009. The written informed consent of the original study permitted future reanalysis of the data. The diagnosis of COPD was made according to Global Initiative for Chronic Obstructive Lung Disease criteria (forced expiratory volume in 1 s ($FEV_1$) /forced vital capacity (FVC) ratio <70 % and $FEV_1$ < 80% of the predicted value, post-bronchodilator) [111]. All COPD patients underwent spirometry, one multi-slice CT scan and two MR imaging scans, whereas the healthy participants only underwent spirometry and two MR imaging scans. The two MR imaging scans were performed 7 days apart. Spirometry and CT were performed within the 7 days prior to the first MR imaging scan.

4.3.2 Spirometry

Spirometry (V62J SensorMedics Plethysmograph, Viasys Healthcare, Carefusion, UK) was performed according to European Respiratory Society recommendations. $FEV_1$/FVC and the
percentage predicted values of pre-bronchodilator FEV₁ (FEV₁,%predicted) were collected.

4.3.3 MR imaging

Lung MR imaging was performed on a 1.5 tesla Philips Achieva MR system (Philips Healthcare, the Netherlands) at Wellcome Trust Clinical Research Facility, Manchester. A single 10-mm-thick coronal slice was placed across the descending aorta with a -4° foot-head angle to permit good lung coverage and avoid the heart. Image acquisition was performed using a two-dimensional half Fourier acquisition single short turbo spin echo (HASTE) sequence preceded by an adiabatic non-selective inversion pulse. Centric ordering was used to provide a short effective echo time (TE) in order to minimize the transverse relaxation time (T₂) effect. Five images were acquired for each inversion time (TI = 50 ms, 300 ms, 1100 ms, 2000 ms and 5000 ms) to average over the respiratory and cardiac cycles. Figure 4.1 provides examples of raw images for each TI and an example of a signal intensity versus TI plot, together with the fitted curve from a healthy subject. Signal-to-noise ratio (SNR) of each TI raw image was calculated from a round region of interest (ROI) drawn on the right upper lung zone (figure 4.1): a subtraction image is generated by subtracting 2 images acquired at the same TI; signal (S) is defined as the mean of the pixel signal intensities in the red circle marked ROI on either of the raw images; noise is defined as the standard deviation (σ) of the pixel signal intensities in the same ROI on the subtracted image; SNR is calculated as \(\sqrt{2} \cdot S/ \sigma\).
Figure 4.1 (a-e) show the examples of the raw image together with the calculated signal-to-noise ratio (SNR) for each inversion time (TI) from a healthy subject. (f) shows an example of signal intensity versus inversion time plot together with the result from fitting the inversion recovery signal equation for a region of interest drawn at the right lung (red circle in (a-e)).

In (f), the open circles are the signal intensities averaged over a stack of 5 images acquired at the same TI. The error bars are the standard deviation of the signal intensities within each stack of images at different TI. The solid line represents the least-squares curve fitting.

Other imaging parameters included: repetition time (TR) = 5500 ms; inter echo spacing (and effective TE) = 3.2 ms; field of view = 450 mm × 450 mm; 68 phase encoding steps to reconstruct a 128 × 128 matrix; pixel size = 3.52 mm × 3.52 mm. The total scan time of the original study was 1 hour but the images for $S_0$ mapping only took 4 min to acquire. All images were acquired under free-breathing and the lungs were segmented and registered to the end expiratory position (functional residual capacity) prior to processing [112]. The process of registration is essential to correct for the effects of motion, but may introduce unavoidable data smoothing, leading to a loss in effective spatial resolution. $S_0$ maps were generated by fitting images (magnitude) to the inversion recovery signal equation

$$S = |S_0 \left(1 - 2f e^{\frac{-TI}{T_1}} \right) \left(1 - 2f e^{\frac{-TR+TE}{T_1}} \right)|$$

(Eq 4.1)
where $f$ is the inversion efficiency, $T_1$ is the longitudinal relaxation time of the tissue and $n$ is the echo train length, which was 68 in this study. A circular region of interest was placed on the supraspinatus muscle and/or infraspinatus muscle of the left shoulder and the averaged muscle $S_0$ in the region of interest was calculated as a reference for each scan. Lung $S_0$ maps were then normalized, and thus quantified ($qS_0$), by dividing by the muscle $S_0$ in order to account for day-to-day scanner settings. An empirical $qS_0$ threshold of 0.60 was used to further segment out the large vessels. Pixels with $qS_0$ below 0.60 were considered lung tissue and used for further calculation. The mean value, standard deviation and the 15th percentile of $qS_0$ were measured across the entire lung in the field of view as the MR-derived parameters of the lung density. The relative lung area with $qS_0$ values below 0.20 (RA$_{0.20}$) was measured as the MR-derived emphysema index. Here, the $qS_0$ threshold of 0.20 was obtained from the linear regression model between 15th percentile $qS_0$ and CT-derived $PD_{15}$ (see results) with $PD_{15}$ substituted as 50 g·L$^{-1}$ (i.e. -950 in HU). More detailed explanation of the calculation of $qS_0$ threshold can be found in the appendix. Image analysis was performed offline on a personal computer (Dell Optiplex780) using customized components of a mathematical software package (MATLAB R2012a, The Mathworks, Natick, MA, USA).

**4.3.4 CT imaging**

Chest multi-slice CT scans were performed using a LightSpeed Plus scanner (GE Medical Systems, Amersham, UK) at Salford Royal Hospital, Manchester. Volume scans of the entire lung were acquired in the axial plane at full inspiration in the supine position, using a tube voltage of 120 kVp and exposure of 40 mAs. The matrix size was 512 × 512. The field of view was approximately 385 mm × 385 mm which was adjusted to fit each subject. The slice thickness was 1 mm - 1.25 mm. Images were reconstructed using the GE standard algorithm.

A single CT slice, which matched the MR image, was selected. Lung tissue was separated from other tissue at a threshold of -500 HU. The 15th percentile density, $PD_{15}$, was extracted from the histograms of the pixel attenuation values and converted from HU into g·L$^{-1}$ units by adding 1000. $PD_{15}$ denotes the density value in g·L$^{-1}$ at which 15% of the lung pixels had lower densities. The relative lung area with attenuation values less than -950 HU, RA$_{-950}$, was calculated via the density masks method at a threshold of -950 HU [314]. RA$_{-950}$ denotes the percentage of lung pixels with attenuation values below -950 HU. Both $PD_{15}$ and RA$_{-950}$ were extracted from the signal MR-matched CT slices. CT images were analysed using MATLAB R2012a (The Mathworks, Natick, MA, USA).

**4.3.5 Statistical analysis**

Statistical analysis was performed using IBM SPSS Statistics 20.0 software (IBM, New York, USA). The data were tested for normality using the Kolmogorov-Smirnov test. The unpaired t-test was used to compare the spirometric measurements and $qS_0$ readouts between groups. A receiver operating characteristic (ROC) analysis was conducted to assess the sensitivity and specificity of MR $qS_0$ readouts in the differentiation of COPD patients from healthy controls. The points closest to the point of (0, 1) on each ROC curve were used as the optimal threshold points to calculate the sensitivity and specificity. The distance to the point of (0, 1) was calculated as $\sqrt{((1\text{-sensitivity})^2 + (1-}$
specificity)\(^2\)). Pearson’s correlation coefficient was calculated to estimate the relationship between spirometric measurements, CT parameters and qS\(_0\) parameters. Intraclass correlation coefficients analysis (ICC) was used to evaluate the reproducibility of each qS\(_0\) readout (two-way mixed model), i.e. the consistency between the two repeated measurements of each qS\(_0\) readout at two occasions (scan and rescan) that were conducted by the same observer. Differences and correlations with a \(P\) value of 0.05 or less were considered statistically significant. Unless otherwise stated data are presented as mean ± standard deviation.

### 4.4 Results

Participant characteristics are shown in table 4.1. Age and gender were not significantly different between healthy controls and patients with COPD. Patients with COPD showed significantly lower FEV\(_1\) %\textit{predicted} (\(P < 0.001\)) and FEV\(_1\)/FVC (\(P < 0.001\)) than healthy subjects. CT measurements in COPD patients revealed mild to severe emphysematous changes and loss of lung density in the selected two dimensional CT images, with the RA\(_{-950}\) ranging from 0 % to 41% and PD\(_{15}\) ranging from 15 g·L\(^{-1}\) to 139 g·L\(^{-1}\).

The differences in qS\(_0\) readouts between the COPD and healthy groups are highlighted in table 4.1. Whole-lung mean and 15\(^{th}\) percentile of qS\(_0\) were significantly reduced, whereas the RA\(_{0.20}\) and the standard deviation of qS\(_0\) were significantly higher in patients with COPD than in healthy controls (\(P = 0.014, 0.002, 0.005, < 0.001\), respectively).

**Table 4.1** Characteristics of participants and results of spirometry, MR qS\(_0\) mapping and quantitative CT

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy controls ((n = 12))</th>
<th>COPD ((n = 24))</th>
<th>(P) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>63 ± 12</td>
<td>66 ± 8</td>
<td>NS</td>
</tr>
<tr>
<td>Sex, Male (%Total)/Female (%Total)</td>
<td>75/25</td>
<td>83/17</td>
<td>NS</td>
</tr>
<tr>
<td>FEV(_1) %\textit{predicted}</td>
<td>121 ± 14</td>
<td>53 ± 16</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV(_1)/FVC, %</td>
<td>77 ± 4</td>
<td>42 ± 11</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Mean qS(_0)</td>
<td>0.44 ± 0.06</td>
<td>0.38 ± 0.07</td>
<td>0.014</td>
</tr>
<tr>
<td>15(^{th}) percentile of qS(_0)</td>
<td>0.36 ± 0.08</td>
<td>0.26 ± 0.08</td>
<td>0.002</td>
</tr>
<tr>
<td>Standard deviation of qS(_0)</td>
<td>0.08 ± 0.02</td>
<td>0.11 ± 0.02</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RA(_{0.20}), %</td>
<td>2 ± 3</td>
<td>9 ± 11</td>
<td>0.005</td>
</tr>
<tr>
<td>RA(_{-950}), %</td>
<td>ND</td>
<td>10 ± 11</td>
<td>ND</td>
</tr>
<tr>
<td>PD(_{15}), g·L(^{-1})</td>
<td>ND</td>
<td>76 ± 36</td>
<td>ND</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. FEV\(_1\) %\textit{predicted}: percentage of predicted normal forced expiratory volume in one second; FEV\(_1\)/FVC: ratio of FEV\(_1\) to forced vital capacity; qS\(_0\): quantitative equilibrium signal; RA\(_{0.20}\): relative lung areas with qS\(_0\) values below 0.20; RA\(_{-950}\): relative lung areas with attenuation values below -950HU; PD\(_{15}\): 15\(^{th}\) percentile of lung density; NS: not significant; ND: no data.

Examples of repeat qS\(_0\) maps from a healthy subject are presented in figure 4.2. Examples of
repeat qS₀ maps, qS₀ thresholded maps and the corresponding CT images from two representative COPD patients are shown in figure 4.3 and 4.4. The repeat qS₀ maps were visually similar to each other qualitatively, indicating a good reproducibility of lung qS₀ mapping in both groups. MR qS₀ maps of COPD lungs were markedly heterogeneous with more low value regions than in healthy lungs. For COPD patients, the lung regions with values below 0.20 in qS₀ maps were highly consistent with regions with attenuation values below -950 HU in CT images according to the visual inspection, i.e. the CT detected emphysema areas.

**Figure 4.2** Example MR qS₀ maps of the scan (a) and rescan (c), MR qS₀ thresholded map of the scan (b) and rescan (d) of a healthy subject (Male, 75 years old, FEV₁%predicted = 147%).

MR qS₀ maps are highly reproducible between scan and rescan, with the scale from 0 to 0.60 (S₀ normalized to muscle). MR qS₀ thresholded maps present lung regions with qS₀ value between 0.2 and 0.6 in red, denoting normal parenchyma (marked as “P”). Vascular regions are presented in blue and marked as “V”. Few lung pixels have qS₀ values between 0 and 0.2 (RA₀.20 = 0.6%).
Figure 4.3 Example MR qS₀ maps of the scan (a) and rescan (c), MR qS₀ thresholded map of the first scan (b) and the CT image (d) of a patient with COPD (Male, 74 years old, FEV₁%predicted = 42%).

MR qS₀ maps are highly reproducible between scan and rescan, with the scale from 0 to 0.60 (S₀ normalized to muscle). In the MR qS₀ thresholded map, the lung regions with qS₀ value between 0.2 and 0.6 are shown in red, denoting normal parenchyma (marked as “P”); those with qS₀ value between 0 and 0.2 are shown in blue, denoting emphysema (marked as “E”, RA₀.20 =37%). The vascular areas are also shown in blue but marked as “V”. The CT image shows the areas with attenuation value below -950 HU in blue, i.e. CT detected emphysematous areas (RA₋950 =36%). The emphysematous regions delineated in MR qS₀ thresholded map and in CT image are highly consistent.
Figure 4.4 Example MR $qS_0$ maps of the scan (a) and rescan (c), MR $qS_0$ thresholded map of the first scan (b) and the CT image (d) of a patient with COPD (Male, 64 years old, $\text{FEV}_1\%_{\text{predicted}} = 43\%$).

$MR\ qS_0$ maps are highly reproducible between scan and rescan, with the scale from 0 to 0.60 ($S_0$ normalized to muscle). In the MR $qS_0$ thresholded map, the lung regions with $qS_0$ value between 0.2 and 0.6 are shown in red, denoting normal parenchyma (marked as “P”); those with $qS_0$ value between 0 and 0.2 are shown in blue, denoting emphysema (marked as “E”, $RA_{0.20} = 16\%$). The vascular areas are also shown in blue but marked as “V”. CT image show the areas with attenuation value below -950 HU in blue, i.e. CT detected emphysematous areas ($RA_{-950} = 25\%$). The emphysematous regions delineated in MR $qS_0$ thresholded map and in CT image are highly consistent.
The distributions of $q_{S_0}$ values and the CT HU values in COPD lungs are illustrated using histograms. As shown by the representative examples from two COPD patients in figure 4.5, the $q_{S_0}$ histograms and corresponding CT HU histograms are similar in shape and showed similar behaviour, both histograms becoming broader with the peaks shifted to the left with an increase in emphysema.

![Histograms of lung $q_{S_0}$ (green) and CT lung density (blue) of two COPD patients (patient 1, $RA_{0.20} = 7\%$, $RA_{950} = 5\%$; patient 2, $RA_{0.20} = 37\%$, $RA_{950} = 36\%$).](image)

**Figure 4.5** Histograms of lung $q_{S_0}$ (green) and CT lung density (blue) of two COPD patients (patient 1, $RA_{0.20} = 7\%$, $RA_{950} = 5\%$; patient 2, $RA_{0.20} = 37\%$, $RA_{950} = 36\%$).

The red lines are the 50th percentile values. The dashed lines are 25th and 75th percentile values. The histograms of $q_{S_0}$ and CT lung density are similar in shape. Patient 2 (c and d) has wider histograms with a broader interquartile range and a less sharp peak shifted to the left, than observed in patient 1 (a and b).
Figure 4.6 shows the ROC curves (figure 4.6a, 4.6b), sensitivity and specificity curves (figure 4.6c-4.6f) of MR qS₀ readouts for the differentiation of the COPD patients from healthy controls. Table 4.2 lists the areas under the ROC curve, optimal threshold points and the corresponding sensitivity and specificity.

### Table 4.2 Sensitivity and specificity of MR qS₀ readouts for the differentiation of COPD patients from healthy controls

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Area under the ROC curve</th>
<th>Threshold point</th>
<th>Sensitivity, % (numerator of denominator)</th>
<th>Specificity, % (numerator of denominator)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean qS₀</td>
<td>0.72</td>
<td>0.45</td>
<td>83% (20 of 24 COPD)</td>
<td>50% (6 of 12 healthy controls)</td>
</tr>
<tr>
<td>15th percentile of qS₀</td>
<td>0.80</td>
<td>0.30</td>
<td>75% (18 of 24 COPD)</td>
<td>75% (9 of 12 healthy controls)</td>
</tr>
<tr>
<td>Standard deviation of qS₀</td>
<td>0.87</td>
<td>0.10</td>
<td>71% (17 of 24 COPD)</td>
<td>92% (11 of 12 healthy controls)</td>
</tr>
<tr>
<td>RA₀.20</td>
<td>0.78</td>
<td>1.8 %</td>
<td>71% (17 of 24 COPD)</td>
<td>83% (10 of 12 healthy controls)</td>
</tr>
</tbody>
</table>

ROC curve: the receiver operating characteristic curve; qS₀: quantitative equilibrium signal; RA₀.20: relative lung areas with qS₀ values below 0.20; Threshold point: threshold point is defined as the point on the ROC curve closest to point (0,1).
Figure 4.6 The receiver operating characteristic (ROC) curves of mean $qS_0$, 15th percentile of $qS_0$, $RA_{0.20}$ and the standard deviation of $qS_0$ in differentiating COPD patients from healthy controls.

(a-b) are the ROC curves. The open and closed circles are the optimal threshold points closest to point (0, 1). AUC: area under the curve. (c-f) show the sensitivity and specificity across levels of these four imaging readouts in differentiating COPD patients from healthy controls. The solid curves are the sensitivity curves and the dashed curves are the specificity curves. The dashed vertical lines are the reference lines highlighting the sensitivity and specificity at the optimal threshold points. Please note the intervals of the x-axes are not equivalent in (c-f).
Table 4.3 provides the results of correlation analysis between the qS₀ parameters and the CT parameters in patients with COPD. There were excellent correlations between CT-derived RA₉₅₀ and MR-derived RA₀.₂₀ (r=0.86, P<0.001, figure 4.7a), mean qS₀ (r=-0.78, P<0.001, figure 4.7b) and 15⁰ percentile of qS₀ (r=-0.81, P<0.001, figure 4.7c). Similarly, strong correlations were found between CT-derived PD₁₅ and RA₀.₂₀ (r=-0.71, P<0.001, figure 4.8a), mean qS₀ (r=0.78, P<0.001, figure 4.8b) and 15⁰ percentile of qS₀ (r=0.79, P<0.001, figure 4.8c). The standard deviation of qS₀ were also significantly correlated, but with weaker strength, with RA₉₅₀ (r=0.46, P=0.024, figure 4.7d) and PD₁₅ (r=-0.46, P=0.024, figure 4.8d). The linear regression models of RA₉₅₀ and PD₁₅ with corresponding qS₀ parameters were RA₉₅₀ = 2.8 + 0.8 x RA₀.₂₀ (r² = 0.73, P<0.001) and PD₁₅ = -23 + 374 x 15⁰ percentile qS₀ (r²=0.62, P<0.001). Results of correlation analysis between spirometric parameters and the readouts of MR qS₀ mapping and quantitative CT in patients with COPD are presented in table 4.4. FEV₁ %predicted and FEV₁/FVC moderately correlated with CT parameters (RA₉₅₀ and PD₁₅) and equivalently or slightly better correlated with MR qS₀ readouts (RA₀.₂₀, mean value, 15⁰ percentile and standard deviation of qS₀).

The ICCs of repeated measurements (scan and rescan) of qS₀ readouts were between 0.89-0.97 in the COPD group and between 0.96-0.98 in the healthy group (table 4.5), suggesting an excellent short-term reproducibility in both groups.

### Table 4.3 Correlation between measurements of MR qS₀ mapping and quantitative CT in patients with COPD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RA₉₅₀, %</th>
<th>PD₁₅, g·L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean qS₀</td>
<td>-0.78 (&lt;0.001)</td>
<td>0.78 (&lt;0.001)</td>
</tr>
<tr>
<td>15⁰ percentile of qS₀</td>
<td>-0.81 (&lt;0.001)</td>
<td>0.79 (&lt;0.001)</td>
</tr>
<tr>
<td>Standard deviation of qS₀</td>
<td>0.46 (0.024)</td>
<td>-0.46 (0.024)</td>
</tr>
<tr>
<td>RA₀.₂₀, %</td>
<td>0.86 (&lt;0.001)</td>
<td>-0.71 (&lt;0.001)</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s correlation coefficient r (P value). qS₀: quantitative equilibrium signal; RA₀.₂₀: relative lung areas with qS₀ values below 0.20; RA₉₅₀: relative lung areas with attenuation values below -950HU; PD₁₅: 15⁰ percentile of lung density; NS: not significant.
Table 4.4 Correlation between spirometric parameters and the readouts of MR qS\textsubscript{0} mapping and quantitative CT in patients with COPD

<table>
<thead>
<tr>
<th>Parameters</th>
<th>FEV\textsubscript{1} %predicted</th>
<th>FEV\textsubscript{1}/FVC %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean qS\textsubscript{0}</td>
<td>0.54 (0.001)</td>
<td>0.63 (&lt;0.001)</td>
</tr>
<tr>
<td>15\textsuperscript{th} percentile of qS\textsubscript{0}</td>
<td>0.62 (&lt;0.001)</td>
<td>0.67 (&lt;0.001)</td>
</tr>
<tr>
<td>Standard deviation of qS\textsubscript{0}</td>
<td>-0.64 (&lt;0.001)</td>
<td>0.59 (&lt;0.001)</td>
</tr>
<tr>
<td>RA\textsubscript{0.20}</td>
<td>-0.56 (0.001)</td>
<td>-0.60 (&lt;0.001)</td>
</tr>
<tr>
<td>PD\textsubscript{15}</td>
<td>0.45 (0.026)</td>
<td>0.65 (0.001)</td>
</tr>
<tr>
<td>RA\textsubscript{-950}</td>
<td>-0.47 (0.021)</td>
<td>-0.56 (0.006)</td>
</tr>
</tbody>
</table>

Data are presented as Pearson's correlation coefficient $r$ (P value). FEV\textsubscript{1} %predicted: percentage of predicted normal forced expiratory volume in one second; FEV\textsubscript{1}/FVC: ratio of FEV\textsubscript{1} to forced vital capacity; qS\textsubscript{0}: quantitative equilibrium signal; RA\textsubscript{0.20}: relative lung areas with qS\textsubscript{0} values below 0.20; RA\textsubscript{-950}: relative lung areas with attenuation values below -950HU; PD\textsubscript{15}: 15\textsuperscript{th} percentile of lung density; NS: not significant.

Table 4.5 Intraclass correlation coefficients of repeated measurements of MR qS\textsubscript{0} readouts in healthy and COPD groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy (n=12)</th>
<th>COPD (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean qS\textsubscript{0}</td>
<td>0.97</td>
<td>0.94</td>
</tr>
<tr>
<td>15\textsuperscript{th} percentile of qS\textsubscript{0}</td>
<td>0.98</td>
<td>0.93</td>
</tr>
<tr>
<td>Standard deviation of qS\textsubscript{0}</td>
<td>0.97</td>
<td>0.89</td>
</tr>
<tr>
<td>RA\textsubscript{0.20} %</td>
<td>0.96</td>
<td>0.97</td>
</tr>
</tbody>
</table>

Data are presented as intraclass correlation coefficient (ICC). qS\textsubscript{0}: quantitative equilibrium signal; RA\textsubscript{0.20}: relative lung areas with qS\textsubscript{0} values below 0.20.
Figure 4.7 Scatter plots showing the linear correlation of CT RA$_{950}$ with (a) RA$_{0.20}$, (b) mean value of qS$_0$, (c) 15th percentile of qS$_0$ and (d) the standard deviation of qS$_0$ in patients with COPD (n = 24).

The correlation coefficients $r$ and the P values are presented together. The solid lines are the regression lines and the dashed lines show the 95% confidence intervals for the mean of the data.
Figure 4.8 Scatter plots showing the linear correlation of CT PD\(_{15}\) with (a) RA\(_{0.20}\), (b) mean value of qS\(_0\), (c) 15\(^{th}\) percentile of qS\(_0\) and (d) standard deviation of qS\(_0\) in patients with COPD (n = 24).

The correlation coefficients \(r\) and the \(P\) values are presented together. The solid lines are the regression lines and the dashed lines show the 95% confidence intervals for the mean of the data.

4.5 Discussion

This retrospective study demonstrates that lung qS\(_0\) mapping has good reproducibility and is comparable to CT in the qualitative description and quantitative measurement of emphysema and lung tissue density in patients with COPD, if limited to a single section through the lung parenchyma. Although MR qS\(_0\) mapping has been applied to measure lung water content in animals and normal humans [124, 141, 143, 150, 151], we are to our knowledge the first to have provided evidence of the utility and reproducibility of lung MR qS\(_0\) mapping in patients with COPD and to have compared it with CT in the assessment of emphysema.
In this study, distinct differences have been revealed in lung qS₀ measurements between healthy subjects and patients with COPD. The 14% decrease in the mean lung qS₀ and up to 27% decrease in the 15th percentile qS₀ in COPD patients when compared with healthy controls is thought to reflect the emphysema induced parenchymal loss and blood volume decrease. These drops in qS₀ values are in accord with earlier ex-vivo studies, which reported a similar reduction of lung MR signal intensity in emphysematous mice when compared with healthy controls [144, 145]. Iwasawa et al. offered a contradictory finding of significantly higher MR signals in COPD patients than in normal subjects at end inspiration, which was however suspected less reliable due to the use of an insensitive signal normalization method by the authors [315]. On the other hand, the standard deviation of qS₀ was 38% higher in the COPD group than in the healthy control group in our study, indicating increased heterogeneity in COPD. This observation presumably mirrors the non-uniform features of structural destruction and blood distribution in emphysematous lungs, as previously demonstrated [243, 316]. RA₀.₂₀ was calculated in a similar way to CT-derived RA₀.₉₅₀, using a density-masking approach [314]. The qS₀ value of 0.20 was applied as a threshold below which all pixels were assumed to be emphysema. The significantly elevated RA₀.₂₀ in COPD lungs was in agreement with the reduced qS₀ values, implying the presence of emphysema.

The four qS₀ parameters described in this work possessed good reproducibility in COPD patients and in healthy subjects. The slightly lower ICCs of the two repeated measurements (scan and rescan) of qS₀ readouts in the COPD group may indicate more physiological variation between visits for the patients. The good and reproducible performance in the distinction between normal and diseased lungs makes these qS₀ readouts potential markers for COPD staging.

The second finding was the strong correlation of lung qS₀ parameters with CT measurements of emphysema in COPD patients, suggesting the ability of MR qS₀ mapping to quantitatively evaluate the emphysema severity. Marshall et al. reported a significant correlation of CT attenuation values with signal intensities of effective transverse relaxation time (T₂ *)-weighted MR images, but not with S₀ that normalized to the S₀ from a reference vial of saline in healthy beagles’ lungs. However, the authors attributed the failure of exhibiting a significant correlation between CT and MR normalized S₀ to the inaccuracy of S₀ measurement and also speculated a better performance of normalized S₀ maps than raw MR signal intensity images in the investigation of diseased lungs [317]. Indeed, the comparative studies of lung morphological MR imaging with CT in patients with COPD have been sparsely reported, most of which revealed inferior capability of MR imaging to CT in depicting emphysema. The tested MR sequences included two dimensional balanced steady-state free precession, T₂-weighted HASTE, pre- and post-contrast T₁ weighted volumetric interpolated breath-hold examination sequence, with the sensitivity of 16%, 44%, 48% and 41% respectively in either detecting emphysema or categorizing its severity when compared with CT [318, 319]. Ultra-short TE sequences might be promising in delineating emphysema [320], but their utility has not been explored in COPD patients. As a comparison, our approach of mapping lung qS₀ values seems to have a similar efficacy to CT in identifying and, more importantly, quantifying emphysema. This is not only
supported by the excellent correlations between qS\textsubscript{0} readouts and CT parameters, but also by the similar shapes of the emphysematous regions contoured by the threshold of 0.20 in qS\textsubscript{0} maps and the threshold of -950 HU in CT images. The equally moderate or slightly better correlations of qS\textsubscript{0} readouts with FEV\textsubscript{1} and FEV\textsubscript{1}/FVC when compared with CT measurements, consistent with prior studies [54, 315], revealed the similar or slightly better sensitivities of MR qS\textsubscript{0} mapping to airway obstruction than CT and again suggested a role of qS\textsubscript{0} mapping in estimating COPD severity.

Our ROC analysis suggests a potential diagnostic value of MR qS\textsubscript{0} mapping in COPD. The sensitivity and specificity of 15\textsuperscript{th} percentile MR qS\textsubscript{0}, the standard deviation of qS\textsubscript{0} and RA\textsubscript{0.20} for the differentiation of COPD from healthy controls are comparable or slightly inferior to literature values of quantitative CT measurements from a meta-analysis study (sensitivity of 83\% (95\% confidence interval, 73\%-89\%) and specificity of 87\% (95\% confidence interval, 70\%-95\%) for COPD diagnosis. However, given the fact that the MR qS\textsubscript{0} readouts were extracted from a single MR image slice, the sensitivity and specificity of these three MR qS\textsubscript{0} readouts are encouraging. Increasing the MR image coverage may further improve the sensitivity and specificity of MR qS\textsubscript{0} mapping for COPD diagnosis. Further studies evaluating COPD diagnostic performance of MR qS\textsubscript{0} mapping are needed.

In this study, we minimized the T\textsubscript{2} effect on our qS\textsubscript{0} estimates by using very short effective TE and we eliminated T\textsubscript{1} effect using inversion recovery technique. Thus, the qS\textsubscript{0} mapping is heavily weighted towards the absolute tissue density-derived contribution. T\textsubscript{1} effects may be minimized by selecting a single image with a long TR, which is potentially more time efficient. However, our use of multiple inversion times and explicit fitting for T\textsubscript{1} and equilibrium signal value should be robust to the presence of unexpectedly long T\textsubscript{1} values, which may occur in the presence of oedema or other pathology. It is also possible that T\textsubscript{1} values themselves may prove useful in assessing lung disease, as has been suggested elsewhere [128, 130].

The normalization of lung S\textsubscript{0} values to those in muscle ensures that MR qS\textsubscript{0} mapping is independent of scanner gain settings, thus permitting the reliable comparison between different scanners and time points. In addition, the quantitative and tissue-specific nature of the measurement enables MR qS\textsubscript{0} mapping to be interpreted objectively, allowing the future possibility of automated assessment of emphysema. Furthermore, MR qS\textsubscript{0} maps can be obtained, often together with maps of relaxation times, from images acquired by a variety of sequences. However, an adequate signal-to-noise ratio is paramount for accurate qS\textsubscript{0} measurement, thus caution should be taken when choosing a sequence [321].

This present study is based on a retrospective analysis of existing MR and CT datasets, and hence subject to the following limitations. Firstly, the single slice MR imaging restricted the comparison with CT to a two dimensional assessment and the utilization of manual selection processes in the matching of MR and CT slices may introduce errors due to subjectivity. This is a potential limitation of the MR method since acquiring high-quality multi-slice or three dimensional T\textsubscript{1} maps in MR is time consuming, although further research into acquisition methods may reduce the magnitude of this as a limitation. However, given the fact that emphysema has heterogeneous
distribution within COPD lungs, improved image coverage within a clinically acceptable scanning time must be achieved before MR qS\textsubscript{0} can be adopted in routine clinical practice. Secondly, muscle S\textsubscript{0} was chosen as referencing to normalize lung S\textsubscript{0} maps. A standardized reference such as a phantom of water may be an alternative approach. However, reliable placement of phantoms in the field of view and variations in signal intensity due to field inhomogeneity and temperature may make such alternatives ineffective. Thirdly, CT images were acquired at maximal end-inspiration while MR images were acquired during free-breathing and registered to the end expiration, thus CT images reflect lung density at end inspiration whereas MR qS\textsubscript{0} maps reflect the density at functional residual capacity. Whilst this had no obvious practical impact on the correlations observed between the two measurements in our work, a meaningful change in lung qS\textsubscript{0} values is expected at different lung volumes [312], so it may be desirable that in future MR images and CT images are acquired at the same time point in a breathing cycle. However, this would require prospective or retrospective respiratory gating of the MR acquisition, which would render it less time efficient. Fourthly, our MR images had lower spatial resolution than CT images and the respiration and its related registration may lead to further reduction of spatial resolution in the MR images. Thus, early centrilobular emphysema may be missed with the MR qS\textsubscript{0} technique. Increasing MR spatial resolution causes reduction in signal-to-noise ratio, which may affect the precision of measuring lung S\textsubscript{0}. Also, image coverage or scanning time might have to be sacrificed if higher spatial resolution of MR qS\textsubscript{0} is required. Thus, a prioritization or a balance of these MR imaging factors should be considered according to the specific objective of a study when designing an MR qS\textsubscript{0} scanning protocol. Lastly, the original study was not designed to measure MR qS\textsubscript{0} so the current retrospective study was not statistically powered. Moreover, literature to assist powering a lung MR qS\textsubscript{0} study is currently lacking. The results from this retrospective study may be of utility to power future prospective studies to validate these biomarkers. Regarding these limitations, further prospective studies with alternative designs are desirable to further validate the utility of MR qS\textsubscript{0} mapping in the assessment of emphysema in COPD.

In conclusion, lung MR qS\textsubscript{0} readouts significantly differ between COPD patients and healthy controls and are strongly associated with CT density estimates, indicating the potential role of MR qS\textsubscript{0} mapping in the quantitative assessment of emphysema. The good reproducibility and the ionizing radiation-free, contrast agent-free features make MR qS\textsubscript{0} mapping a potentially attractive imaging tool for future longitudinal studies.
Chapter 5 Paper 2: The regional structural-functional relationships of COPD with and without emphysema: evaluation using quantitative CT and dynamic OE-MRI

This paper is in progress of the 3rd round author review, aiming for submission to a peer-reviewed radiology journal by December 2014.

Authors: Wei-Juan Zhang, Penny L Cristinacce, Eva Bondesson, Lars H Nordenmark, Simon S Young, Yu-Zhen Liu, Dave Singh, Josephine H Naish, Geoffrey JM Parker


Contribution of authors: WJ.Z: data analysis and interpretation, statistical analysis, manuscript preparation and edition. P.L.C: study conception and design, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, manuscript reviewing. D.S.: participant enrolment, data acquisition and interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. E.B, L.H.N., S.S.Y and YZ.L: study conception and design, data interpretation, manuscript reviewing.
5.1 Abstract

Purpose: to explore regional structural-functional relationships in patients with emphysematous and non-emphysematous chronic obstructive pulmonary diseases (COPD) by using quantitative X-ray computed tomography (CT) and dynamic oxygen-enhanced magnetic resonance imaging (OE-MRI).

Materials and methods: 15 emphysematous COPD patients, 9 non-emphysematous COPD patients and 12 healthy non-smokers underwent spirometry, dynamic OE-MRI and multislice CT (COPD patients only) within 7 days. The baseline spin-lattice relaxation time of the lung (T_{1air}), the enhancing fraction (EF), the change in the partial pressure of oxygen in the lung parenchyma (ΔPO_{2max}) and the oxygen wash-in time constant (τ_{up}) calculated from dynamic OE-MRI data were mapped and summarized using median values and interquartile ranges (IQR) and compared between groups by using one-way ANOVA. Correlations with spirometric readouts and CT measurements of 15th percentile density (PD_{15}) and relative lung area with attenuation value below -950 Hounsfield units (RA_{950}) were assessed by Pearson’s correlation analysis.

Results: EF, median ΔPO_{2max} and IQR-T_{1air} of COPD patients with and without emphysema on the selected single CT slices were similar but significantly different from those of healthy controls (P < 0.05). Median T_{1air} of patients with non-emphysematous COPD and healthy controls were similar but significantly longer than that of emphysematous COPD (P = 0.042, < 0.001, respectively). IQR-ΔPO_{2max}, median τ_{up} and IQR-τ_{up} in patients with emphysematous COPD were significantly different from those in healthy subjects (P = 0.012, 0.033, < 0.001, respectively). The correlation of median T_{1air} with RA_{950}, EF and median ΔPO_{2max} with PD_{15}, RA_{950} and the percentage predicted diffusing capacity for carbon monoxide (DLco\%predicted) were significant in emphysematous COPD (P < 0.05) but not in non-emphysematous COPD.

Conclusion: Heterogeneously reduced pulmonary oxygenation ability and prolonged pulmonary oxygenation times present in both emphysematous and non-emphysematous COPD. However, the correlations of dynamic OE-MRI readouts with DLco\%predicted and CT estimates of emphysema and lung density vary between these two COPD radiological subgroups.

5.2 Introduction

Early attempts to understand structural-functional relationships in chronic obstructive pulmonary disease (COPD) date back to the 1960s. The pioneer researchers demonstrated the independent contributions of small airway disease and emphysema to the persistent airflow limitation in COPD lungs by using macroscopic or microscopic observations and whole-lung physiological tests [23]. With the ongoing evolution of various imaging modalities such as CT and MRI, it is now possible to explore the structural-functional relationships in COPD at a local level.

X-ray computed tomography (CT) is the technique of choice for the visualization and quantification of structural alterations in COPD in vivo. CT estimates of small airway disease and emphysema are in good agreement with histopathologic findings [307] and correlate well with conventional pulmonary function tests measurements [54]. CT has also been proposed to cluster...
COPD as different radiological phenotypes are likely to have distinct clinical presentations and outcomes [86, 91, 322]. The assessment of regional pulmonary function impairment in COPD was first provided using ventilation/perfusion scintigraphy, followed by positron emission tomography (PET) and single-photon emission computed tomography (SPECT) [94, 96-98]. However, nuclear medical imaging techniques are impeded by their poor spatial resolution and the use of radioactive tracers. Xe-enhanced CT ventilation imaging and iodine-based CT perfusion imaging enable the simultaneous acquisition of functional and structural detail in COPD lungs [58, 60]. But the requirement for significant radiation exposure is a major drawback. Hyperpolarized (HP) noble gas magnetic resonance imaging (MRI) provides a non-ionizing technique enabling the evaluation of regional ventilation, alveolar microstructures and intrapulmonary oxygen (O₂) tension in COPD [293, 294, 299, 323-326]. But the high cost and currently poor accessibility restrict its clinical application. Lung perfusion MRI can be used to observe the perfusion defects in COPD lungs [257]. However, the adverse effects of the intravenous contrast agent, although very rare, cause concern. Although these functional imaging techniques, in combination with CT, have made great contributions in understanding the regional structural-functional relationships [58, 96, 325], alternatives are needed to overcome their technical and practical limitations.

Dynamic oxygen enhanced magnetic enhanced resonance imaging (dynamic OE-MRI) is a non-ionizing, non-invasive pulmonary functional imaging method using paramagnetic oxygen (¹⁶O₂) as contrast agent [166]. The inhalation of elevated levels of O₂ can cause signal enhancement in spin-lattice relaxation time (T₁) weighted images by shortening the T₁ values in lung regions where O₂ is able to reach the alveoli, cross the alveolar-capillary barrier and dissolve in the blood plasma and tissue water. This technique represents a unique tool for the assessment of regional pulmonary oxygenation, the prime function of the lung governed by the complex interplay between local ventilation, perfusion and diffusing capacity [200]. Dynamic OE-MRI has been proven feasible in COPD patients with qualitative readouts, such as relative signal enhancement ratio and signal enhancement slope, showing correlations with the spirometric indices of expiratory flow rate and diffusing capacity [183, 211, 212]. So far, however, there has been little research into the potential links between structural and functional abnormalities detected by quantitative CT and dynamic OE-MRI at a regional level. Although previous studies reported similar correlations of the signal enhancement ratio in dynamic OE-MRI and CT-based functional lung volume measurements with spirometric indices in COPD, they did not provide direct comparison of these two imaging modalities [211, 213, 214].

In this study, we compared dynamic OE-MRI of the lungs in healthy controls, patients with emphysematous COPD and patients with non-emphysematous COPD and explored regional structural-functional relationships by assessing the degree of correlation between CT measurements of emphysema and dynamic OE-MRI measurements of pulmonary function.

5.3 Materials and methods
This study formed part of a prospective exploratory study that was approved by the local ethics committee (Bolton REC: 08/H1009/39) and was conducted by the Centre for Imaging Sciences, The University of Manchester between 2008 and 2009.

5.3.1 Subjects

24 patients with COPD and 12 age-matched non-smoking healthy controls were enrolled from the Medicines Evaluation Unit in the University Hospital of South Manchester. The written informed consent was obtained. COPD diagnosis was made according to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria [11].

5.3.2 Pulmonary function testing

All subjects underwent pulmonary function tests (V62J SensorMedics Plethysmograph, Viasys Healthcare, Carefusion, UK) according to European Respiratory Society recommendations within 7 days prior to the MRI scan [32, 34, 38]. The pre- and post-bronchodilator forced expiratory volume in 1 s (FEV$_1$) and expiratory vital capacity (FVC), pre-bronchodilator carbon monoxide diffusion capacity (DLco) and the corresponding percentage of predicted values (FEV$_1$%predicted, DLco %predicted) were measured.

5.3.3 CT imaging

A chest multi-slice CT scan was carried out on all COPD subjects, using a LightSpeed Plus scanner (GE Medical Systems, Amersham, UK) within 7 days prior to the OE-MRI scan at Salford Royal Hospital, Manchester. Volume scans of the entire lung were acquired in the axial plain at full inspiration in the supine position, using a tube voltage of 120 kVp and exposure of 40 mAs. The matrix size was 512 × 512 and the slice thickness was 1 mm - 1.25 mm. The field of view was approximately 385 mm × 385 mm which was adjusted to fit each subject. Images were reconstructed using the GE standard reconstruction algorithm. For better comparison between CT and MRI, a single CT slice that visually matched the MR image plane was manually selected on Osirix workstation (http://www.osirix-viewer.com/) according to the landmarks including the shape of the chest wall, cervical spine, descending aorta and broncho-vascular markings, etc.. Density-mask CT images were generated in which the voxels with CT attenuation values less than -950 Hounsfield units were highlighted, denoting emphysematous regions. The 15$^\text{th}$ percentile of pulmonary density (PD$_{15}$) and the relative lung area with attenuation value below -950 Hounsfield units (RA$_{-950}$) were then calculated across the entire lung field in this single slice. COPD cases were classified into two radiological categories according to the absence/presence of emphysema in the selected single CT slices: 1) "non-emphysematous COPD": 0 ≤ RA$_{-950}$< 5%; 2) "emphysematous COPD": RA$_{-950}$≥ 5% [91, 327]. Image analysis was performed by using in-house codes written in MATLAB (The Mathworks, Natick, MA, USA).

5.3.4 MR imaging

MRI scans were performed on a 1.5 tesla Philips Achieva MR system (Philips Healthcare, Netherlands) at Wellcome Trust Clinical Research Facility, Manchester. Medical air and 100% O$_2$ were delivered via a non-rebreathing mask with a reservoir bag (Intersurgical Ltd, UK) at a flow rate of
15 l/min throughout the scans. Dynamic OE-MRI was carried out using a centric ordered T₁-weighted half Fourier acquisition single short turbo spin echo sequence preceded by an adiabatic non-selective inversion pulse (IR-HASTE). Single-slice coronal images were acquired with 5 inversion times (TIs = 50 ms, 300 ms, 1100 ms, 2000 ms and 5000 ms) while subjects freely breathed medical air (21% O₂) for the measurement of baseline T₁ (T₁air). This was followed by 76 dynamic acquisitions with T1=1100 ms during the gas switching over from medical air to 100% O₂ (gas switching over at 15th dynamic acquisition). Other parameters included: repetition time 5500 ms, echo time 3.2 ms, matrix size 128 × 128 matrix, 3.5 mm x 3.5 mm pixel size and 10 mm slice thickness.

Lungs were segmented and registered to the end expiration position (FVC level) [112]. Quantitative equilibrium maps (qS₀) of the lung were generated and an empirical threshold of 0.60 was used to further segment out the large vessels (see chapter 4). O₂-induced change in signal intensity was converted to the change in T₁ [115], which was further converted to the dynamic change in the partial pressure of O₂ (ΔPO₂) in the regional lung water by

\[ \Delta P_{O_2}(t) = \left( \frac{1}{T_1(t)} - \frac{1}{T_{1air}} \right) r_{1,O_2} \]  

(Eq 5.1)

using a value for the O₂ longitudinal relaxivity in water \( r_{1,O_2} \) of 2.49×10⁻⁴/s/mmHg [201]. A one-tailed independent samples t-test was performed for each pixel to compare the first 14 data points and the last 15 data points on ΔPO₂ wash-in curve. Pixels with significantly increased PO₂ (P < 0.05) were considered effectively enhancing and the percentage of enhancing lung pixels over the total lung pixels on the image slice was calculated, i.e. the enhancing fraction (EF, %), a proxy for the ventilated fraction of the lung. The ΔPO₂ (t) curves (O₂ wash-in dynamics) were then fitted using an exponential function

\[ \Delta P_{O_2}(t) = \Delta P_{O_2}^{max}(1 - e^{-t/\tau_{up}}) \]  

(Eq 5.2)

pixel-by-pixel to extract the change in ΔPO₂ at the steady plateau during O₂ inhalation (ΔPO₂^{max}, mmHg) and O₂ wash-in time constant (\( \tau_{up}, \) min). T₁air and ΔPO₂^{max} were summarized using median values and interquartile ranges (IQR, reflecting the heterogeneity of the parameter distribution) over both lungs on the image slice. \( \tau_{up} \) was summarized using median values within the enhanced lung regions. Image analysis was performed offline on a personal computer (Dell Optiplex780) using in-house code written in MATLAB (MATLAB R2012a, The Mathworks, Natick, MA, USA).

5.3.5 Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 20.0 software (IBM, New York, USA). The data were tested for normality using Kolmogorov-Smirnov test. One-way ANOVA analysis was performed to compare parameters among healthy controls, patients with emphysematous COPD and patients with non-emphysematous COPD. Bonferroni corrected post hoc tests were performed after significant ANOVA for pairwise comparison (i.e. between group comparison). An independent samples t-test was used to compare the CT parameters between COPD patients with and without emphysema. Pearson’s correlation analysis was performed to evaluate the relationships between spirometric indices, CT parameters and dynamic OE-MRI readouts in the two COPD subgroups.
Differences and correlations with a P value of 0.05 or less were considered statistically significant. Unless otherwise stated data are presented as mean ± standard deviation.

5.4 Results

Demographic information for the 24 COPD subjects and 12 healthy controls is provided in table 5.1. There were 15 COPD cases with emphysema and 9 COPD cases without emphysema in the single slice analysed. Age and gender was similar among three groups (P=0.738, 0.249, respectively). Healthy controls showed a significant higher FEV$_1$%predicted, FEV$_1$/FVC ratio and DLco%predicted than non-emphysematous COPD subjects (P < 0.001, <0.001, = 0.010, respectively) and emphysematous COPD subjects (P < 0.001). Subjects with non-emphysematous COPD had significantly higher FEV$_1$/FVC ratio (P = 0.003) and DLco%predicted (P < 0.001) than those with emphysematous COPD. The RA$_{950}$ and PD$_{15}$ in non-emphysematous COPD (1.0 ± 1.3%, 116 ± 19 g·L$^{-1}$) were significantly different from those in emphysematous COPD (16.1 ± 10.1%, 51 ± 17 g·L$^{-1}$; P < 0.001).

Table 5.1 Demographic information and pulmonary function tests and quantitative CT of healthy controls and COPD subjects of A or E phenotype

<table>
<thead>
<tr>
<th></th>
<th>Healthy controls (n = 12)</th>
<th>A phenotype COPD (n = 9)</th>
<th>E phenotype COPD (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>63 ± 12</td>
<td>65 ± 9</td>
<td>66 ± 8</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>9/3</td>
<td>6/3</td>
<td>14/1</td>
</tr>
<tr>
<td>FEV$_1$%predicted, %</td>
<td>121 ± 14</td>
<td>60 ± 17 *</td>
<td>49 ± 14 *</td>
</tr>
<tr>
<td>FEV$_1$/FVC, %</td>
<td>76 ± 4</td>
<td>50 ± 11 *</td>
<td>37 ± 8 *, †</td>
</tr>
<tr>
<td>DLco%predicted, %</td>
<td>90 ± 10</td>
<td>73 ± 10 *</td>
<td>49 ± 13 *, †</td>
</tr>
<tr>
<td>RA$_{950}$, %</td>
<td>ND</td>
<td>1.0 ± 1.3</td>
<td>16.1 ± 10.1 †</td>
</tr>
<tr>
<td>PD$_{15}$, g·L$^{-1}$</td>
<td>ND</td>
<td>116 ± 19</td>
<td>51 ± 17 †</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation; * significantly different from healthy controls (P < 0.01); † significantly different from A phenotype COPD (P < 0.01). ND: no data.

Figures 5.1-5.5 show representative example maps of T$_{1air}$, ΔPO$_{2max}$ and τ$_{up}$ and the corresponding density-mask CT images from a healthy subject (figure 5.1), two patients with non-emphysematous COPD (figure 5.2 and 5.3) and a patient with emphysematous COPD (figure 5.4 and 5.5), respectively. T$_{1air}$ maps were uniform in the healthy control subject (figure 5.1a) but heterogeneous with more regions of short T$_{1air}$ values in the patients with emphysematous COPD (figure 5.4b and figure 5.5b). Inconsistent association between areas of short T$_{1air}$ and low CT attenuation areas was observed only with a visual trend to lower T$_{1air}$ in the presence of severe focal emphysema or bullous emphysema (figure 5.5a vs 5.5b). The spatial distribution of T$_{1air}$ values in the non-emphysematous patients (figure 5.2b and figure 5.3b) is more heterogeneous than in the healthy subject, but more uniform than in the emphysematous patients.
The healthy control subject showed mostly homogeneously distributed $\Delta P_{O_{2 \max}}$ and $\tau_{up}$ values across both lungs (figure 5.1c, 5.1d). In contrast, in the patients with COPD, heterogeneously reduced $\Delta P_{O_{2 \max}}$ and prolonged $\tau_{up}$ were seen regardless of the presence or absence of emphysema (b and c of figure 5.2-5.5). Maps of the enhancing regions further illustrated that there were fewer regions being effectively enhanced by $O_2$ in COPD lungs (with and without emphysema) than seen in the lungs of healthy controls. In emphysematous COPD, apparent association between the location of non-enhanced regions and emphysematous regions was visually observed (figure 5.4a vs 5.4c, figure 5.5a vs 5.5c). However, similar non-enhancing regions were seen in the normal appearing areas on density-mask CT images in non-emphysematous COPD (figure 5.2a vs 5.2c, figure 5.3a vs 5.3c).

**Figure 5.1** Example dynamic OE-MRI parameter maps of (a) $T_{1air}$ (ms), (b) enhancing regions (white mask demonstrating effectively complete enhancement), (c) $\Delta P_{O_{2 \max}}$ (mmHg) and (d) $\tau_{up}$ (min) from a healthy subject (Male, 46 years old, FEV$_1$\%predicted=114%).

The maps of $T_{1air}$, $\Delta P_{O_{2 \max}}$ and $\tau_{up}$ are relatively homogeneous with approximately the entire lung field being enhanced by $O_2$ (EF = 98%).
Figure 5.2 Example maps of (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 50%), (d) $\Delta PO_{2\text{max}}$ (mmHg) and (e) $\tau_{up}$ (min) from a patient with non-emphysematous COPD (Male, 72 years old, $\text{FEV}_{1\%\text{predicted}} = 82\%$).

The density-mask CT image (a) highlights the areas with attenuation values below -950 HU in blue, i.e. CT detected emphysematous areas ($\text{RA}_{-950} =0.4\%$) – these areas are trivial in this patient. The maps of $\Delta PO_{2\text{max}}$ (d) and $\tau_{up}$ (e) are heterogeneous within the enhancing regions.
Figure 5.3 Example maps of (a) density-mask CT image, (b) $T_{\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 54%), (d) $\Delta P_{O_{2}}\text{max}$ (mmHg) and (e) $\tau_{\text{up}}$ (min) from a patient with non-emphysematous COPD (Female, 71 years old, FEV$_1$%$_{\text{pred}}$ = 62%).

The density-mask CT image (a) highlights the areas with attenuation value below -950 HU in blue, i.e. CT detected emphysematous areas (RA$_{-950}$ =0.4%), which are trivial in this patient. The maps of $\Delta P_{O_{2}}\text{max}$ (d) and $\tau_{\text{up}}$ (e) are heterogeneous within the enhancing regions.
Figure 5.4 Example maps of a (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; EF = 50%), (d) $\Delta P_{O_{2\text{max}}}$ (mmHg) and (e) $\tau_{\text{up}}$ (min) from a patient with emphysematous COPD (Male, 64 years old, $\text{FEV}_1/%_{\text{predicted}} = 43\%$).

The Density-mask CT image (a) highlights the areas with attenuation value below -950 HU in blue, i.e. CT detected emphysematous areas ($R_{A_{-950}} = 25\%$). The maps of $\Delta P_{O_{2\text{max}}}$ (d) and $\tau_{\text{up}}$ (e) are heterogeneous within the enhancing regions.
Figure 5.5 Example maps of (a) density-mask CT image, (b) $T_{1\text{air}}$ (ms), (c) enhancing regions (white showing areas of enhancement; grey showing areas without enhancement; $EF = 27\%$), (d) $\Delta PO_{2\text{max}}$ (mmHg) and (e) $\tau_{\text{up}}$ (min) from a patient with emphysematous COPD (Female, 74 years old, $\text{FEV}_1\%$ predicted = 42\%).

The density-mask CT image (b) highlights the areas with attenuation value below -950 HU in blue, i.e. CT detected emphysematous areas ($RA_{950}$ =36 \%). The maps of $\Delta PO_{2\text{max}}$ (d) and $\tau_{\text{up}}$ (e) are heterogeneous within the enhancing regions.
Table 5.2 allows comparison of the MRI parameters between the 3 groups. Median $T_{1\text{air}}$ was significantly shorter in emphysematous COPD ($921 \pm 66 \text{ ms}$) than in non-emphysematous COPD ($992 \pm 68 \text{ ms}$, $P = 0.042$) and healthy controls ($1044 \pm 62 \text{ ms}$, $P < 0.001$), while it was similar in non-emphysematous COPD and healthy controls ($P = 0.238$). IQR-$T_{1\text{air}}$ in emphysematous COPD ($303 \pm 71 \text{ ms}$) and non-emphysematous COPD ($303 \pm 91 \text{ ms}$) was significantly larger than that in healthy controls ($211 \pm 64 \text{ ms}$, $P = 0.009$, 0.026, respectively), indicating greater tissue heterogeneity. The EF and median $\Delta PO_{2\text{max}}$ in healthy controls ($80 \pm 12\%$, $249 \pm 73 \text{ mmHg}$) were significantly higher than in subjects with emphysematous COPD ($52 \pm 14\%$, $P < 0.001$; $153 \pm 66 \text{ mmHg}$, $P = 0.007$) and subjects with non-emphysematous COPD ($57 \pm 17\%$, $P = 0.002$; $148 \pm 90 \text{ mmHg}$, $P = 0.013$), while they were not significantly different between two COPD subgroups ($P > 0.050$). Figure 5.6 showed $\Delta PO_2$ time course curves (averaged over the lung regions) in a patient with COPD and a healthy subject. IQR-$\Delta PO_{2\text{max}}$ was significantly larger in emphysematous COPD ($355 \pm 146 \text{ mmHg}$) than in healthy controls ($196 \pm 72 \text{ mmHg}$, $P = 0.012$), demonstrating functional heterogeneity. Subjects with emphysematous COPD showed significantly longer median $\tau_{\text{up}}$ ($1.54 \pm 0.78 \text{ min}$) and broader IQR-$\tau_{\text{up}}$ ($1.99 \pm 0.81 \text{ min}$) than healthy control subjects ($0.88 \pm 0.52 \text{ min}$, $P = 0.033$; $0.77 \pm 0.48 \text{ min}$, $P < 0.001$).

Table 5.2 Dynamic OE-MRI readouts of healthy controls and COPD subjects of A or E phenotype

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy controls (n = 12)</th>
<th>A phenotype COPD (n = 9)</th>
<th>E phenotype COPD (n = 15)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF, %</td>
<td>80 ± 12</td>
<td>57 ± 17 *</td>
<td>52 ± 14 *</td>
</tr>
<tr>
<td>Median $T_{1\text{air}}$, ms</td>
<td>1044 ± 62</td>
<td>992 ± 68</td>
<td>921 ± 66 *, ‡</td>
</tr>
<tr>
<td>Median $\Delta PO_{2\text{max}}$, mmHg</td>
<td>249 ± 73</td>
<td>148 ± 90 §</td>
<td>153 ± 66 *</td>
</tr>
<tr>
<td>Median $\tau_{\text{up}}$, min</td>
<td>0.88 ± 0.52</td>
<td>0.96 ± 0.46</td>
<td>1.54 ± 0.78 §</td>
</tr>
<tr>
<td>IQR-$T_{1\text{air}}$, ms</td>
<td>211 ± 64</td>
<td>303 ± 91 §</td>
<td>303 ± 71 *</td>
</tr>
<tr>
<td>IQR-$\Delta PO_{2\text{max}}$, mmHg</td>
<td>196 ± 72</td>
<td>296 ± 169</td>
<td>355 ± 146 §</td>
</tr>
<tr>
<td>IQR-$\tau_{\text{up}}$, min</td>
<td>0.77 ± 0.48</td>
<td>1.36 ± 0.69</td>
<td>1.99 ± 0.81 *</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. § Significantly different from healthy controls ($P < 0.05$); ‡ significantly different from A phenotype COPD ($P < 0.05$); * significantly different from healthy control ($P < 0.01$).
Table 5.3 lists the correlation coefficients of MRI readouts with spirometric readouts and CT measurements in the two COPD subgroups. The EF and median \( \Delta P_{O_2} \) were positively correlated with DLco\%predicted \((r = 0.598, P = 0.024; r = 0.628, P = 0.016)\) and PD\(15\) \((r = 0.713, P = 0.003; r = 0.589, P = 0.023)\) and negatively correlated with RA\(-950\) \((r = -0.742, P = 0.002; r = -0.638, P = 0.011)\) in subjects with emphysematous COPD (figure 5.6 and 5.7) but not in subjects with non-emphysematous COPD. In emphysematous COPD, median \( T_{1air} \) was also negatively correlated with RA\(-950\) \((r = -0.523, P = 0.046)\). In contrast, the median \( \tau_{up} \) showed positive correlation with RA\(-950\) \((r = 0.697, P = 0.037)\) in non-emphysematous COPD only. There was no significant correlation of the interquartile ranges of \( T_{1air}, \Delta P_{O_2}, \) and \( \tau_{up} \) with spirometric indices or two CT measurements in either of the COPD subgroups \((P > 0.050, \text{not shown})\). There was no significant correlation of age, FEV\(_1\)\%predicted, FEV\(_1\)/FVC with MRI parameters in either of the COPD subgroups \((P > 0.050)\).

In subjects with emphysematous COPD, RA\(-950\) was negatively correlated with DLco\%predicted \((r = -0.745, P = 0.002)\), but not with FEV\(_1\)\%predicted and FEV\(_1\)/FVC. Similar but positive correlation was
found between PD_{15} and DL_{co}\%_{predicted} (r = 0.591, P = 0.026). In subjects with non-emysematous COPD, no significant correlation was found between spirometric indices and two CT measurements.

In the healthy control group, age showed a moderate and negative correlation with median T_{1air} (r = -0.594, P = 0.041) but not with other MRI readouts.
Table 5.3 Correlation coefficients of MRI readouts with PFT and CT measurements in the A phenotype COPD and E phenotype COPD groups

<table>
<thead>
<tr>
<th>MRI</th>
<th>Phenotype</th>
<th>PFT</th>
<th>CT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>FEV₁%predicted, %</td>
<td>FEV₁/FVC, %</td>
</tr>
<tr>
<td>EF, %</td>
<td>A</td>
<td>-0.631 (0.069)</td>
<td>-0.560 (0.117)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.378 (0.164)</td>
<td>0.264 (0.362)</td>
</tr>
<tr>
<td>Median T₁air, ms</td>
<td>A</td>
<td>0.191 (0.623)</td>
<td>0.132 (0.735)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.057 (0.840)</td>
<td>0.183 (0.531)</td>
</tr>
<tr>
<td>Median ΔPO₂max, mmHg</td>
<td>A</td>
<td>-0.423 (0.257)</td>
<td>-0.462 (0.210)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.256 (0.358)</td>
<td>0.211 (0.469)</td>
</tr>
<tr>
<td>Median τₜₚ, min</td>
<td>A</td>
<td>-0.071 (0.856)</td>
<td>-0.252 (0.512)</td>
</tr>
<tr>
<td></td>
<td>E</td>
<td>0.315 (0.253)</td>
<td>0.479 (0.083)</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s correlation coefficient r (P value) without Bonferroni correction; significant correlations (P < 0.05) are marked in bold. Bonferroni adjusted p-value = 0.05/20 = 0.0025.
5.5 Discussion

This study explored regional structural-functional relationships in COPD by directly comparing single coronal CT images with single slice dynamic OE-MRI parameter maps. Heterogeneously reduced pulmonary oxygenation and prolonged O\textsubscript{2} wash-in time were observed in COPD patients regardless of the presence or absence of emphysema, while significantly lower T\textsubscript{1air} was only present in emphysematous COPD. The correlations of dynamic OE-MRI readouts with diffusion capacity and CT estimates of emphysema and lung density vary in the two COPD radiological categories. To our knowledge, this is the first published investigation regarding the appearances of dynamic OE-MRI in COPD patients with different radiological features.

The first finding was the similar extent and severity of the impaired pulmonary oxygenation, as reflected by the maps of EF, \(\Delta\text{PO}_{2\text{max}}\) and \(t_{\text{up}}\), in emphysematous and non-emphysematous COPD patients. This indicated that the diverse morphological abnormalities in COPD may lead to the same functional outcome with regard to inefficient gas exchange, possibly via different mechanisms. In emphysematous COPD patients, the significant correlations of RA\textsubscript{950}, PD\textsubscript{15} with EF, \(\Delta\text{PO}_{2\text{max}}\) suggest that the greater levels of emphysema may cause a decrease in \(O_2\) enhancement in dynamic OE-MRI. This finding accords with a previous OE-MRI study conducted in lung cancer patients with and without emphysema which reported a significant and negative correlation between the maximal signal enhancement ratio and CT emphysema visual score [170]. Spatial associations were shown between the non-\(O_2\)-enhanced regions in EF maps and areas of emphysematous destruction in CT images in this dataset (figure 5.4a vs 5.4c and figure 5.5a vs 5.5c), although they did not exactly match. The poorly enhanced or unenhanced regions appeared to be centred at CT-detected emphysema areas but in many cases to additionally extend into surrounding tissue. In Muller et al.’s paper, the example figures from a chest X-ray and an OE-MRI scan on a patient with a large emphysematous bulla show a clear overlap between the bullous region and the areas without \(O_2\) enhancement [171]. Ohno et al. presented OE-MRI signal enhancement ratio maps of 4 COPD patients with different GOLD stage along with the corresponding density-mask CT images [211]. It is difficult to comment on the spatial relationships between the functional and structural abnormalities according to these 4 examples as the emphysema of these COPD patients was either mild in severity or homogeneous in distribution. Nevertheless, a trend indicating that greater levels of emphysema led to lower the signal enhancement could be visually observed from these images [211]. In emphysematous COPD, the longer \(O_2\) wash-in time is likely due to the airway obstruction and the low \(\Delta\text{PO}_{2\text{max}}\) may be attributed to a local increase in perfusion for \(O_2\) washout or, perhaps more likely, the diminishing and collapse of small airways for \(O_2\) delivery (ventilation could be reduced or maintained while the specific ventilation is often reduced due to air trapping during exhalation [58]) [23]. The ventilation/perfusion (V/Q) mismatch (high V/Q ratio predominant) could further compound pulmonary oxygenation inefficiency [96]. Although the relative contribution of each factor to the reduced pulmonary oxygenation cannot be determined from our results, the significant correlations of EF and \(\Delta\text{PO}_{2\text{max}}\) with DL\textsubscript{co}%predicted, consistent with earlier findings [170, 171, 183], implies that the diffusion limitation secondary to the
alveolar-capillary membrane destruction would override others to be the predominant contributor in emphysematous COPD. In contrast to the earlier reports in patients with smoking-related COPD [211-213], however, no evidence of a correlation of O₂ enhancement-related parameters with FEV₁ and FEV₁/FVC was detected in this study. This is possibly attributable to the use of a single-slice based imaging protocol, which may be inferior to multi-slice based method in reflecting global functional status. On the other hand, although no CT estimates of airway remodelling were available in this study, small airway disease was expected to exist as it seems unlikely that the trivial emphysema observed in these patients would cause such drop in FEV₁%predicted and pulmonary oxygenation (ΔPO₂max) relative to the healthy controls. Airway narrowing as a result of chronic airway inflammation may cause reduction in regional ventilation by limiting gas inflow and outflow [58] and alter V/Q balance (low V/Q ratio predominant) [96], and thus lower pulmonary oxygenation efficiency. Further studies with precise estimation of the airway disease are needed to clarify this inference.

The second finding was that significantly shorter median lung T₁air was found in the patients with emphysematous COPD than in controls, but this relationship was not observed in those with the non-emphysematous COPD. However, a broader interquartile range of T₁air, as a reflection of more heterogeneous distribution of these values, was present in both COPD radiological subgroups. T₁air is an inherent property of a specific tissue and is related to the biophysical compositions of the tissue [129]. It is suspected that the destruction of capillary beds and the reduction of regional perfusion due to emphysema may lead to a decrease in the fraction of free water in the pulmonary parenchyma and thus result in the significantly shorter T₁air [128]. It is not surprising that the median T₁air of non-emphysematous COPD was within the normal range, as the pulmonary parenchyma of these COPD cases was relatively intact. In comparison to the median value, the interquartile range of T₁air might be more sensitive to pinpoint deranged microstructure in non-emphysematous COPD lungs and distinguish them from the healthy lungs. Stadler et al. measured lung T₁air in healthy individuals and in a group of emphysema patients in two separate studies and the emphysema patients showed shorter T₁air values in the lung [127, 128]. However, no information on the emphysema severity was given in their reports. As far as we are aware, we are the first to demonstrate T₁air alterations in COPD lungs with and without emphysema using a control group in the same study. Also, this is the first report of a linear correlation between median T₁air and RA₉₅₀ in emphysematous lungs, suggesting that the more severe the emphysema, the shorter the lung T₁air. In addition, the negative correlation of median T₁air with age in healthy controls indicates aging-induced lung T₁ shortening, possibly due to the degeneration of lung tissue.

This study has several limitations. Firstly, the spatial comparison between CT images and dynamic OE-MRI maps was performed according to visual observation, which may introduce subjective errors. More objective analysis could be achieved by co-registering the images from these two modalities. Secondly, the two dimensional parameter maps were compressed to one dimensional data points, i.e. the median value and the interquartile range, for the purpose of statistical analysis. Important spatial information was inevitably lost during this process. Thirdly, the 2D image coverage
of our method hampers the application of dynamic OE-MRI for characterising whole lung effects and may partly explain the lack of correlation with spirometry. New MRI sequences or relevant techniques are needed to expand the coverage from two dimensional acquisitions to three dimensional acquisitions. Finally, the number of non-emphysematous COPD subjects was relatively small and there was no CT index of airway remodelling available.

In conclusion, this study demonstrates that emphysematous COPD and non-emphysematous COPD both present a pattern of reduced pulmonary oxygenation and slow gas delivery in dynamic OE-MRI despite their distinct CT characteristics. However, the reduction in lung $T_{1\text{air}}$ and the correlations of dynamic OE-MRI readouts of pulmonary oxygenation with diffusion capacity and CT estimates of emphysema and lung density were only observed in emphysematous COPD. These findings suggested that pulmonary oxygenation impairment is a shared feature of these two COPD radiological subgroups. Dynamic OE-MRI, when used alone, might not be capable of distinguishing the COPD radiological phenotypes. However, the use of a non-ionizing, easily accessible and biological gas as the source of contrast makes this technique an attractive option in the assessment of regional pulmonary oxygenation in COPD, particularly when used with other quantitative MRI indices, such as $T_1$ measurements, to provide additional specificity.
Chapter 6 Paper 3: Dynamic oxygen-enhanced magnetic resonance imaging of the lung in patients with asthma – initial experience

This paper has been submitted to “European Journal of Radiology” and now it is in the progress of revision.

Authors: Wei-Juan Zhang, Robert M Niven, Simon S Young, Yu-Zhen Liu, Geoffrey JM Parker and Josephine H Naish

From the Centre for Imaging Sciences, Institute of Population Health (WJ.Z., G.J.M.P., J.H.N.), Biomedical Imaging Institute (WJ.Z., G.J.M.P., J.H.N.), The University of Manchester, Oxford Road, Manchester, U.K., M13 9PT; North West Lung Research Centre, University Hospital of South Manchester (R.M.N.), Southmoor Road, Manchester, U.K., M23 9LT; Personalised Healthcare and Biomarkers, AstraZeneca R&D (S.S.Y., YZ.L.), Alderley Park, Macclesfield, U.K., SK10 4TF; Bioxydyn Limited (G.J.M.P.), Pencroft Way, Manchester, U.K., M15 6SZ

Contribution of authors: WJ.Z: study conception and design, approval of ethics, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript preparation and edition. R.M.N: study conception and design, participant enrolment, data interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. S.S.Y and YZ.L: study conception and design, data interpretation, manuscript reviewing.
6.1 Abstract

Purpose: To prospectively estimate the feasibility and reproducibility of dynamic oxygen-enhanced magnetic resonance imaging (OE-MRI) in the assessment of regional oxygen delivery, uptake and washout in asthmatic lungs.

Materials and methods: The study was approved by the National Research Ethics Committee and written informed consent was obtained. Dynamic OE-MRI was performed twice at one month apart on four mild asthmatic patients and six severe asthmatic patients. The enhancing fraction (EF), the change in the partial pressure of oxygen in lung tissue (ΔPO_{2\text{max}}_l) and arterial blood of the aorta (ΔPO_{2\text{max}}_a), and the oxygen wash-in (τ_{\text{up}, l}, τ_{\text{up}, a}) and wash-out (τ_{\text{down}, l}, τ_{\text{down}, a}) time constants were extracted and compared between groups using the independent samples t-test. Correlations between imaging readouts and clinical measurements were assessed by Pearson’s correlation analysis. Bland-Altman analysis was used to estimate the levels of agreement between the repeat scans.

Results: The severe asthmatic group had significantly smaller EF and median ΔPO_{2\text{max}}_l and significantly larger interquartile range of τ_{\text{up}, l} than the mild asthmatic group (P = 0.014, 0.004 and 0.005, respectively). EF, median ΔPO_{2\text{max}}_l and τ_{\text{down}, l} and the interquartile range of τ_{\text{up}, l} and τ_{\text{down}, l} were significantly correlated with age and pulmonary function test parameters. Median ΔPO_{2\text{max}}_l was significantly correlated with ΔPO_{2\text{max}}_a (r = 0.745, P = 0.013). Imaging readouts showed good reproducibility.

Conclusion: The study results demonstrate the feasibility, sensitivity and reproducibility of dynamic OE-MRI in the estimation of regional oxygen delivery, uptake and washout in asthmatic lungs.

6.2 Introduction

The rapid advance of imaging techniques has enabled exploration of functional impairment in asthmatic lungs from the global level to the regional level [52]. Imaging is an appealing monitoring tool in this condition, as asthma is characterised by heterogeneous lung function with variable regional involvement. In addition, imaging techniques may play an important role in phenotyping patients and guiding targeted therapies in severe asthma [53, 328].

Functional lung imaging modalities utilized in asthma studies to date include scintigraphy [94], positron emission tomography (PET) [95], single-photon emission computed tomography (SPECT) [92], xenon-enhanced dual-energy computer tomography (CT) [57] and hyperpolarized noble gas (HP-) magnetic resonance imaging (MRI) [286]. Most previous work has focused on a component of lung function, such as ventilation, perfusion, or ventilation-perfusion mismatch. Whilst the investigation of individual contributors to functional impairment is of interest and potentially important, the estimation of the integrated functionality of a regional lung unit is equally important, for example how oxygen (O_{2}) is delivered and taken up locally. In addition, the application of previously-described imaging techniques is hampered by a range of limitations including the use of ionizing radiation, the need to produce radiotracers, expense and/or practical difficulty in implementation. A non-invasive, non-ionizing and widely available imaging technique with the capability to assess regional lung
function may allow more widespread use of imaging in asthma, potentially leading to better characterization of severe asthma patients and/or treatment stratification, i.e. guiding tropical therapy and monitoring treatment effect.

Dynamic oxygen-enhanced (OE-) MRI can provide spatial and temporal information on regional delivery and uptake of O\textsubscript{2} in the lung by using \textsuperscript{16}O\textsubscript{2} as a contrast agent [166]. The paramagnetic O\textsubscript{2} molecules dissolve in the tissue water and blood plasma within the lung and increase the lung spin-lattice relaxation rate (R\textsubscript{1}) in proportion to dissolved O\textsubscript{2} concentration and thus the local O\textsubscript{2} partial pressure (PO\textsubscript{2}) [201]. By measuring the change in lung R\textsubscript{1} between breathing air and breathing elevated levels of O\textsubscript{2}, the change in lung tissue PO\textsubscript{2} (ΔPO\textsubscript{2}) can be quantified. The steady-state ΔPO\textsubscript{2} in lung water in response to a step change in inspired oxygen fraction (FiO\textsubscript{2}) reflects the efficiency of alveolar oxygenation and ventilation-perfusion matching. Additionally, the dynamic change of ΔPO\textsubscript{2} yields information on regional alveolar ventilation [188, 196, 203, 212].

Although OE-MRI has been used to investigate lung function changes in many pulmonary disorders [171, 198, 200, 212], there is little data regarding its utility in asthma. Ohno et al. demonstrated an equivalent efficacy of OE-MRI to quantitative CT in the classification of asthma severity [215]. However, this work was based on a static observation of the O\textsubscript{2}-induced signal enhancement in spin-lattice relaxation time (T\textsubscript{1}=1/R\textsubscript{1}) weighted images, which is potentially less informative than dynamic and quantitative measurement of R\textsubscript{1} changes in reflecting lung function [196, 200].

The aim of this study was to explore the feasibility and reproducibility of dynamic OE-MRI in the assessment of the local efficiency of pulmonary oxygenation in patients with asthma.

6.3 Materials and methods

6.3.1 Study subjects

10 non-smoking asthmatic patients were recruited from University Hospital of South Manchester, Manchester, between February 2012 and June 2012. 4 were mild asthmatic patients who matched the criteria of: 1) the percentage predicted forced expiratory volume in 1 second (FEV\textsubscript{1} (%pred\textsubscript{predicted}) ≥ 85%; 2) required treatment of low dose inhaled corticosteroid (≤ 400 μg/day beclomethasone dipropionate or equivalent) or short-acting inhaled β\textsubscript{2}-adrenergic receptor agonists only; 3) no requirement for oral steroid in last 12 months. The other 6 were severe asthmatic patients who matched the criteria of: 1) FEV\textsubscript{1} (%pred\textsubscript{predicted} < 85%; 2) treatment required consistent with step 4 or step 5 of British Thoracic Society guideline on the management of asthma [9]; 3) a minimum of two courses of oral corticosteroid in the last 12 months. All patients withheld short-acting bronchodilators for 6 hours and long-acting bronchodilators for 12 hours prior to each visit. The study was approved by the National Research Ethical Committee (Ref: 11/NW/0086) and written informed consent was obtained from each subject. The study was registered in UK Clinical Research Network study portfolio database (Ref: 10270).

6.3.2 Clinical visit
All subjects underwent spirometry, body plethysmography and gas transfer measurements at University Hospital of South Manchester, Manchester. The pulmonary function tests (PFT) were performed using a plethysmograph (CareFusion Ltd., Germany) according to European Respiratory Society recommendations [32, 34, 38]. The PFT indices included the actual values and the percentage predicted values (%predicted) of forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), functional residual capacity (FRC), FEV₁ to FVC ratio, maximum mid-expiratory flow (MMEF), total lung capacity (TLC), residual volume (RV), RV to TLC ratio, total specific airway resistance (sRtot), effective specific airway resistance (sReq) and diffusing capacity of carbon monoxide (Dlco).

Asthma Control Test (ACT) questionnaires were completed. Sputum and blood samples were collected for eosinophil counting (EOSs, EOSb). Subjects with either increased EOSs (EOSs > 3% of all sputum cells) or increased EOSb (EOSb > 0.4×10⁹/L) were defined as “current eosinophil positive”. The others were “current eosinophil negative”. The blood and sputum eosinophil testing results within the last 3 years were also checked, according to which subjects were divided into “3-year eosinophil positive/negative” subgroups. The highest blood eosinophil results in the last 3 years were recorded (EOSbmax).

6.3.3 Image acquisition

All subjects underwent MRI scanning at Wellcome Trust Clinical Research Facility, Manchester, within 7 days of the clinical visit (median 4 days, interquartile range 3-5 days). A rescan was carried out on 9 subjects approximately 4 weeks later (median 28 days, interquartile range 26-30 days). 1 severe asthmatic subject reported claustrophobia and withdrew from the second scan.

Scans were performed using a 1.5 tesla whole-body scanner (Philips Achieva, Philips Healthcare, The Netherlands). Subjects breathed medical air (21% O₂) and 100% O₂ throughout the scans via non-rebreathing masks (Intersurgical Ltd., Wokingham, UK) at a flow rate of 15 L/min. A single 10 mm-thick coronal oblique slice was positioned posteriorly in the chest, angled to intersect the descending aorta. Baseline lung T₁ (T₁air_l) measurements were made using a set of T₁-weighted centric ordered inversion-recovery turbo spin echo sequence (IR-TSE), using a non-selective inversion pulse, with 5 inversion times (TI = 60/300/1100/2000/5000 ms). There were 5 repetitions for each TI to average over the cardiac and respiratory cycles. This was followed by 70 dynamic acquisitions with a TI of 1100 ms, during which the gas supply was switched to 100% O₂ at the 15th acquisition. After that, an additional 70 images were acquired dynamically with the gas supply switched back to medical air at the 15th acquisition. The total scanning time was approximately 22 min. Other scanning parameters included: repetition time (TR) 6000 ms, effective echo time (TE) 3.2 ms, matrix 128 x 128 and pixel size 3.52 mm x 3.52 mm. Images were acquired during free breathing. No breath-holding, respiratory or cardiac triggering was used.

6.3.4 Image analysis

Image analysis was performed by using code written in MATLAB R2012a (MathWorks, Natick, Mass, USA). Lungs were segmented and registered semi-automatically to the end inspiration position
Central major blood vessels were manually segmented based on the images with TI of 5000 ms. $T_{\text{air, l}}$ maps were generated by using the multiple inversion recovery images acquired on air inhalation. The signal time course curves throughout the gas switchover were converted to dynamic $T_1$ curves [115], which were further converted to dynamic changes in the partial pressure of $O_2$ dissolved in the tissue water and plasma of the lung by

$$\Delta P_{O_2}(t) = \left( \frac{1}{T_1(t)} - \frac{1}{T_{\text{air}, l}} \right) r_{\text{l, O}_2}$$

(Eq 6.1)

using a value for the $O_2$ longitudinal relaxivity in water ($r_{\text{l, O}_2}$) of $2.49 \times 10^{-4} / \text{s/mmHg}$ [201]. $T_1$ maps on $O_2$ plateau ($T_{\text{oxy, l}}$) were calculated as the average of the last 10 points in the dynamic $T_1$ curves. For each pixel within the lung, a one-tailed independent samples t-test was performed to compare the first 14 data points and the last 15 data points on the $O_2$ wash-in $\Delta P_{O_2}(t)$ curve. Pixels with significantly increased $P_{O_2}$ were considered as demonstrating enhancement due to oxygen breathing. The fraction of the enhancing pixels over the lung was denoted as the enhancing fraction (EF). The lung $\Delta P_{O_2}$ (t) curves of the enhancing pixels were then fitted using an exponential function for the $O_2$ wash-in portion of the dynamic curve and for the $O_2$ wash-out portion of the dynamic curve. $O_2$ wash-in ($t_{\text{up}, l, \text{min}}$) and wash-out ($t_{\text{down}, l, \text{min}}$) time constants and the lung $\Delta P_{O_2}$ at the steady plateau after switching air to 100% $O_2$ ($\Delta P_{\text{max, l, mmHg}}$) were calculated using the following:

Wash-in: $\Delta P_{O_2}(t) = \Delta P_{\text{max}} \left( 1 - e^{\frac{-t}{t_{\text{up}}}} \right)$

(Eq 6.2)

Wash-out: $\Delta P_{O_2}(t) = \Delta P_{\text{max}} e^{\frac{-t}{t_{\text{down}}}}$

(Eq 6.3)

$\Delta P_{\text{max, l}}$ was summarized using the median and the interquartile range 1) across the entire lungs in the field of view with the non-enhancing pixels assigned zero $\Delta P_{\text{max, l}}$, and 2) over the enhancing lung regions only. $t_{\text{up}, l}$ and $t_{\text{down}, l}$ were summarized over the enhancing lung regions only. Median $T_{\text{air, l}}$ and median $T_{\text{oxy, l}}$ across the entire lungs in the field of view were measured. Similar imaging parameters of the arterial blood in aorta ($T_{\text{air, a}}, \Delta P_{\text{max, a}}, t_{\text{up, a}}$ and $t_{\text{down, a}}$) were calculated in the same way using a region of interest defined in the descending aorta [202].

6.3.5 Statistical analysis

Data were tested for normality using the Kolmogorov-Smirnov test. The independent samples t-test and $\chi^2$ test were applied for between-group comparisons. Pearson's correlation analysis was used to explore the association between clinical measurements and imaging readouts. All inter-group analysis was performed using the first scan data. Bland-Altman analysis was used to estimate the scan-rescan agreements. A P value of less than 0.05 was taken to indicate statistical significance. Analysis was performed using IBM SPSS Statistics 20.0 software (IBM, New York, USA).

6.4 Results

6.4.1 Demographic and clinical information

Demographic information and clinical measurements are summarized in table 6.1. The severe asthmatic group was on average older ($P = 0.030$) with a larger body mass index (BMI, $P = 0.020$) and worse PFT results than the mild asthmatic group. 2 out of 4 mild asthmatics and 4 out of 6 severe
asthmatics were genuine eosinophilic asthma patients (3-year eosinophil positive). There was no statistically significant difference in the ACT score, EOB\textsubscript{S}, EOB\textsubscript{B}, EOB\textsubscript{Smax} between groups.

### Table 6.1 Demographic data and clinical measurements

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild Asthma(^\dagger) (<em>n = 4</em>)</th>
<th>Severe Asthma(^\dagger) (<em>n = 6</em>)</th>
<th>P value(^\dagger)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>23 ± 5</td>
<td>41 ± 12</td>
<td>0.033</td>
</tr>
<tr>
<td>Sex, Male/Female</td>
<td>2/2</td>
<td>3/3</td>
<td>1.000</td>
</tr>
<tr>
<td>BMI, kg/m(^2)</td>
<td>25 ± 2</td>
<td>34 ± 6</td>
<td>0.021</td>
</tr>
<tr>
<td>ACT score</td>
<td>20 ± 3</td>
<td>14 ± 6</td>
<td>0.125</td>
</tr>
<tr>
<td>EOS\textsubscript{S}, %</td>
<td>0.5 ± 0.7</td>
<td>11.1 ± 19.1</td>
<td>0.384</td>
</tr>
<tr>
<td>EOS\textsubscript{B}, 10(^9)/l</td>
<td>0.4 ± 0.2</td>
<td>0.5 ± 0.8</td>
<td>0.766</td>
</tr>
<tr>
<td>EOS\textsubscript{Bmax}, 10(^9)/L</td>
<td>0.4 ± 0.2</td>
<td>1.2 ± 0.8</td>
<td>0.060</td>
</tr>
<tr>
<td>Current EOS status, +/-</td>
<td>2/2</td>
<td>2/4</td>
<td>0.598</td>
</tr>
<tr>
<td>3-year EOS status, +/-</td>
<td>2/2</td>
<td>4/2</td>
<td>0.598</td>
</tr>
<tr>
<td>FEV(_1), % predicted</td>
<td>96 ± 3</td>
<td>60 ± 14</td>
<td>0.001</td>
</tr>
<tr>
<td>FVC, % predicted</td>
<td>110 ± 1</td>
<td>94 ± 9</td>
<td>0.011</td>
</tr>
<tr>
<td>FEV(_1)/FVC, %</td>
<td>75 ± 3</td>
<td>54 ± 12</td>
<td>0.005</td>
</tr>
<tr>
<td>MMEF, % predicted</td>
<td>62 ± 3</td>
<td>18 ± 11</td>
<td>0.001</td>
</tr>
<tr>
<td>FRC, % predicted</td>
<td>120 ± 16</td>
<td>136 ± 35</td>
<td>0.423</td>
</tr>
<tr>
<td>TLC, % predicted</td>
<td>111 ± 5</td>
<td>118 ± 17</td>
<td>0.453</td>
</tr>
<tr>
<td>RV, % predicted</td>
<td>142 ± 39</td>
<td>181 ± 54</td>
<td>0.263</td>
</tr>
<tr>
<td>RV/TLC, % predicted</td>
<td>32 ± 5</td>
<td>49 ± 6</td>
<td>0.003</td>
</tr>
<tr>
<td>sR(_{tot}), kPa·s</td>
<td>1.3 ± 0.4</td>
<td>3.6 ± 1.1</td>
<td>0.007</td>
</tr>
<tr>
<td>sR(_{tot}), % predicted</td>
<td>116 ± 25</td>
<td>321 ± 74</td>
<td>0.002</td>
</tr>
<tr>
<td>sR(_{eff}), kPa·s</td>
<td>1.1 ± 0.4</td>
<td>3.4 ± 1.1</td>
<td>0.008</td>
</tr>
<tr>
<td>sR(_{eff}), % predicted</td>
<td>102 ± 25</td>
<td>303 ± 76</td>
<td>0.003</td>
</tr>
<tr>
<td>DL(_{co}), % predicted</td>
<td>82 ± 13</td>
<td>78 ± 8</td>
<td>0.509</td>
</tr>
</tbody>
</table>

BMI: body mass index; ACT: Asthma Control Test questionnaire; EOS\textsubscript{S}: sputum eosinophil counting; EOS\textsubscript{B}: blood eosinophil counting; EOS\textsubscript{Bmax}: the highest blood eosinophil counting in the last 3 years; Current EOS status: current eosinophil status; 3-year EOS status: long term eosinophil status according to the sputum and blood eosinophil testing results in the last 3 years; FEV\(_1\): forced expiratory volume in 1s; %predicted: percentage of predicted normal value; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; FRC: functional residual capacity; TLC: total lung capacity; RV: residual volume; RV/TLC: ratio of RV to TLC; sR\(_{tot}\): total specific airway resistance; sR\(_{eff}\): effective specific airway resistance; DL\(_{co}\): diffusing capacity for carbon monoxide.

\(^{\dagger}\) Data are means ± standard deviations.

\(^{\dagger}\) P value is from the independent samples t-test or the \(\chi^2\) test. P values < 0.05 are presented in bold.
6.4.2 Dynamic OE-MRI of the lung

Example imaging parameter maps (scan and rescan) from a mild asthmatic patient (19 years old, female, FEV$_1$%predicted = 99%) are shown in figure 6.1. Example maps from a severe asthmatic patient (19 years old, female, FEV$_1$%predicted = 69%) are presented in figure 6.2. The corresponding parameter histograms for these two subjects are shown in figure 6.3. The mild asthmatic patient shows relatively homogeneous maps of ΔPO$_{2\text{max}_l}$, $\tau_{\text{up}_l}$ and $\tau_{\text{down}_l}$ with approximately all the lung regions enhancing (EF is 100% and 97% in the two scans, respectively). The repeat parameter maps were visibly similar to each other. By contrast, the parameter maps from the severe asthmatic patient are relatively heterogeneous, with some areas of low ΔPO$_{2\text{max}_l}$ values and some areas of high $\tau_{\text{up}_l}$ and $\tau_{\text{down}_l}$ values. Moreover, a large portion of the lungs (lower part, both sides) did not enhance after the O$_2$ administration (EF is 82% and 67% in the two scans, respectively) in both scans. There is visibly less agreement between the maps obtained from the two visits for the severe asthmatic (figure 6.2) patient than for the mild asthmatic patient (figure 6.1). The histograms of ΔPO$_{2\text{max}_l}$, $\tau_{\text{up}_l}$ and $\tau_{\text{down}_l}$ from the mild asthmatic patient (figure 6.3a, 6.3b, 6.3c) are narrower with a higher and sharper peak than the histograms from the severe asthmatic patient (figure 6.3d, 6.3e, 6.3f).
Figure 6.1 Dynamic OE-MRI parameter maps from a mild asthmatic participant (female, 19 years old, FEV$_1$%predicted = 99%) from scan (V1) and rescan (V2).

(a-c) are the maps of ΔPO$_{2\text{max,}l}$, $\tau_{\text{up,}l}$ and $\tau_{\text{down,}l}$ from the first scan, respectively. (d-f) are the maps of ΔPO$_{2\text{max,}l}$, $\tau_{\text{up,}l}$ and $\tau_{\text{down,}l}$ from the second scan, respectively. The enhancing fraction is 100% for the first scan and 97% in the second scan.
Figure 6.2 Dynamic OE-MRI parameter maps from a severe asthmatic participant (female, 19 years old, FEV₁ %predicted = 64%) from the scan (V1) and rescan (V2).

(a-c) are the maps of ΔPO₂max_l, τup_l and τdown_l from the first scan, respectively. (d-f) are the maps of ΔPO₂max_l, τup_l and τdown_l from the second scan, respectively. The enhancing fraction is 82% in the first scan and 67% in the second scan.
Figure 6.3 Histograms derived from the example maps of $\Delta P_{O_{2\text{max}}_l}$, $\tau_{\text{up}_l}$ and $\tau_{\text{down}_l}$ shown in figure 6.1 and figure 6.2.

Figures 6.3(a-c) are the histograms derived from figures 6.1 (a-c), which are from the first scan of the mild asthmatic participant. Figures 6.3 (d-f) are the histograms derived from figures 6.2 (a-c), which are from the first scan of the severe asthmatic participant. The solid lines represent the median values. The intervals between two dashed lines represent the interquartile ranges (the dashed lines are at the 25th centile and the 75th centile, respectively). The black bars represent the percentage of non-enhancing voxels in the lung. The histograms of $\Delta P_{O_{2\text{max}}_l}$ are the distribution across the entire lung while the histograms of $\tau_{\text{up}_l}$ and $\tau_{\text{down}_l}$ are distribution across the enhancing lung regions.
The group averaged histograms of the dynamic OE-MRI parameter maps are provided in figure 6.4. The histogram of \( \Delta P_{O_2_{\text{max} \cdot l}} \) has markedly lower values in the severe asthmatic group than in the mild asthmatic group. The histograms of \( \tau_{\text{up} \cdot l} \) and \( \tau_{\text{down} \cdot l} \) in the severe asthmatic group were wider, as demonstrated by the larger interquartile range, with a lower peak than those in the mild asthmatic group.

The time course curves of the entire-lung median \( \Delta P_{O_2} \) were averaged across the groups and then plotted in figure 6.5. The curve from the severe asthmatic group showed a lower \( \Delta P_{O_2} \) plateau than that of the mild asthmatic group, consistent with the findings shown in figure 6.4a.
Figure 6.4 Group averaged histograms of $\Delta P_{O_{2}}^{\text{max},l}$ (a), $\tau_{\text{up},l}$ (b) and $\tau_{\text{down},l}$ (c).

The solid lines representing the median values and the dashed lines representing the 25th percentile and the 75th percentile. The red colour denotes the mild asthma group and the blue colour denotes the severe asthma group. The histograms are derived from the parameter maps obtained from the first scans. The histograms of $\Delta P_{O_{2}}^{\text{max},l}$ are the distribution across the entire lung while the histograms of $\tau_{\text{up},l}$ and $\tau_{\text{down},l}$ are the distribution across the enhanced lung regions.
Figure 6.5 The group averaged time course curves of median ΔPO$_2$ across the entire lung.

The red solid line is the median ΔPO$_2$(t) curve averaged over the mild asthma group. The blue solid line is the median ΔPO$_2$(t) curve averaged over the severe asthma group. The red stars (mild asthma) and blue dots (severe asthma) are the individual values.

The imaging readouts of each individual are listed in table 6.2 and the comparison of these parameters between the two asthmatic groups is shown in table 6.3. There was no significant difference in median T$_{1\text{air},l}$ and median T$_{1\text{oxy},l}$ between mild and severe asthmatic groups. There were significantly lower EF (P = 0.014) and entire-lung median ΔPO$_{2\text{max},l}$ (P = 0.004) as well as significantly wider interquartile range of τ$_{\text{up},l}$ (P = 0.001) in the severe asthmatic group than in the mild asthmatic group, while no significant difference was observed in the median τ$_{\text{up},l}$, median τ$_{\text{down},l}$, entire-lung interquartile range of ΔPO$_{2\text{max},l}$ and interquartile range of τ$_{\text{down},l}$. The median ΔPO$_{2\text{max},l}$ remained significantly different between the groups when the calculation was performed over the enhancing lung regions only (P = 0.005). No significant between-group difference was found in the imaging readouts when asthmatic subjects were divided according to the current or the 3-year eosinophil status (not shown).
<table>
<thead>
<tr>
<th>ID/Age (y)/Sex</th>
<th>Visit</th>
<th>EF (%)</th>
<th>( \Delta P_{O_{2 max}} ) (mmHg)</th>
<th>( t_{up} ) (min)</th>
<th>( t_{down} ) (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Median, entire lung( ^{†} )</td>
<td>IQR, entire lung( ^{†} )</td>
<td>Median, EF( ^{‡} )</td>
</tr>
<tr>
<td><strong>Mild asthma</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1/19/F</td>
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<td>100</td>
<td>243</td>
<td>69</td>
<td>243</td>
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<td></td>
<td>2</td>
<td>97</td>
<td>262</td>
<td>112</td>
<td>265</td>
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<tr>
<td>2/20/M</td>
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<td>93</td>
<td>252</td>
<td>132</td>
<td>258</td>
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<tr>
<td></td>
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<td>277</td>
<td>108</td>
<td>284</td>
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<tr>
<td>3/29/F</td>
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<td>151</td>
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<td></td>
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<td>252</td>
</tr>
<tr>
<td>4/25/M</td>
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<td></td>
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<td>289</td>
<td>90</td>
<td>292</td>
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<tr>
<td><strong>Severe asthma</strong></td>
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<td></td>
<td></td>
</tr>
<tr>
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<td>158</td>
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<td>166</td>
<td>208</td>
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<tr>
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<td>176</td>
<td>350</td>
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<td>226</td>
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<tr>
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</tr>
<tr>
<td>8/19/F</td>
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<td>82</td>
<td>122</td>
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<td>2</td>
<td>67</td>
<td>112</td>
<td>211</td>
<td>178</td>
</tr>
<tr>
<td>9/44/F</td>
<td>1</td>
<td>88</td>
<td>127</td>
<td>101</td>
<td>134</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>94</td>
<td>151</td>
<td>107</td>
<td>158</td>
</tr>
<tr>
<td>10/35/F</td>
<td>1</td>
<td>73</td>
<td>187</td>
<td>167</td>
<td>210</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>53</td>
<td>129</td>
<td>304</td>
<td>211</td>
</tr>
</tbody>
</table>

*EF: enhancing fraction; \( \Delta P_{O_{2 max}} \): the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water of the lung; \( t_{up} \): oxygen wash-in time constant of the lung; \( t_{down} \): oxygen wash-out time constant of the lung.

\( ^{†} \) Median, entire lung: median value over the entire lungs in the field of view; IQR, entire lungs: interquartile range over the entire lungs in the field of view.

\( ^{‡} \) Median, EF: median value over the enhancing lung regions in the field of view; IQR, EF: interquartile range over the enhancing lung regions in the field of view.
There was no statistically significant linear correlation of BMI, ACT score, or EOS with PFT parameters.

There was no significant linear correlation of the interquartile range of RV% and the clinical measurements in all asthmatic subjects. Example scatter plots are provided in figure 6.

Table 6.3 Comparison of the dynamic OE-MRI readouts between the mild and severe asthmatic groups

<table>
<thead>
<tr>
<th>Parameters*</th>
<th>Mild Asthma † (n = 4)</th>
<th>Severe Asthma † (n = 6)</th>
<th>P value ‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>EF, %</td>
<td>95 ± 3</td>
<td>70 ± 16</td>
<td>0.014</td>
</tr>
<tr>
<td>Calculation performed over the entire lung in the field of view</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median T_{1air, l}, ms</td>
<td>1204 ± 43</td>
<td>1158 ± 70</td>
<td>0.273</td>
</tr>
<tr>
<td>Median T_{1oxy, l}, ms</td>
<td>1009 ± 47</td>
<td>1113 ± 59</td>
<td>0.894</td>
</tr>
<tr>
<td>Median ΔPO_{2max, l}, mmHg</td>
<td>281 ± 40</td>
<td>156 ± 52</td>
<td>0.004</td>
</tr>
<tr>
<td>Interquartile range of ΔPO_{2max, l}, mmHg</td>
<td>137 ± 53</td>
<td>173 ± 91</td>
<td>0.508</td>
</tr>
<tr>
<td>Calculation performed over the enhancing lung regions in the field of view</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median ΔPO_{2max, l}, mmHg</td>
<td>286 ± 42</td>
<td>183 ± 42</td>
<td>0.005</td>
</tr>
<tr>
<td>Interquartile range of ΔPO_{2max, l}, mmHg</td>
<td>127 ± 49</td>
<td>127 ± 76</td>
<td>0.998</td>
</tr>
<tr>
<td>Median t_{up, l}, min</td>
<td>0.56 ± 0.10</td>
<td>0.76 ± 0.20</td>
<td>0.099</td>
</tr>
<tr>
<td>Interquartile range of t_{up, l}, min</td>
<td>0.20 ± 0.07</td>
<td>0.84 ± 0.26</td>
<td>0.001</td>
</tr>
<tr>
<td>Median t_{down, l}, min</td>
<td>0.68 ± 0.52</td>
<td>0.86 ± 0.71</td>
<td>0.676</td>
</tr>
<tr>
<td>Interquartile range of t_{down, l}, min</td>
<td>0.97 ± 1.49</td>
<td>1.41 ± 1.47</td>
<td>0.651</td>
</tr>
<tr>
<td>Aorta</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T_{1air, a}, ms</td>
<td>1338 ± 82</td>
<td>1333 ± 122</td>
<td>0.945</td>
</tr>
<tr>
<td>ΔPO_{2max, a}, mmHg</td>
<td>306 ± 99</td>
<td>199 ± 90</td>
<td>0.112</td>
</tr>
<tr>
<td>t_{up, a}, min</td>
<td>0.68 ± 0.60</td>
<td>1.58 ± 0.76</td>
<td>0.035</td>
</tr>
<tr>
<td>t_{down, a}, min</td>
<td>0.53 ± 0.18</td>
<td>1.29 ± 0.81</td>
<td>0.071</td>
</tr>
</tbody>
</table>

* EF: enhancing fraction; T_{1air, l}: longitudinal relaxation time of the lung parenchyma when subject breathing medical air; T_{1oxy, l}: longitudinal relaxation time of the lung parenchyma when subject breathing 100% oxygen; ΔPO_{2max, l}: the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water of the lung; t_{up, l}: oxygen wash-in time constant of the lung; t_{down, l}: oxygen wash-out time constant of the lung; T_{1air, a}: longitudinal relaxation time of the arterial blood in aorta; t_{up, a}: oxygen wash-in time constant of the arterial blood in aorta; t_{down, a}: oxygen wash-out time constant of the arterial blood in aorta.
† Data are means ± standard deviations.
‡ P values are from Independent samples t-test. P values < 0.05 are presented in bold.

Table 6.4 lists the Pearson’s correlation coefficients between the imaging readouts of the lung and the clinical measurements in all asthmatic subjects. Example scatter plots are provided in figure 6.6 to show the strongest correlation of imaging readouts with the PFT indices. EF and median ΔPO_{2max, l} showed strong and positive correlations with FEV$_1$%predicted, FVC%predicted, FEV$_1$/FVC and MMEF%predicted (r = 0.750 - 0.879, P = 0.001 - 0.012) while strong and negative correlations with age, sR$_{tot}$%predicted, sR$_{eff}$%predicted, RV%predicted and RV/TLC (r = -0.926 - -0.817, P = 0.001 - 0.004). The interquartile range of t_{up, l} was significantly and negatively correlated with FEV$_1$%predicted, FVC%predicted, FEV$_1$/FVC and MMEF%predicted (r = -0.927 - -0.734, P = 0.001 - 0.016) while significantly and positively correlated with sR$_{tot}$%predicted, sR$_{eff}$%predicted and RV/TLC (r = 0.678 - 0.748, P = 0.020 - 0.045). The median and interquartile range of t_{down, l} were positively correlated with FRC%predicted, TLC%predicted and RV%predicted (r = 0.722 - 0.905, P = 0.001 - 0.028), but not with age or the other PFT indices. There was no significant linear correlation of the interquartile range of ΔPO_{2max, l} and median t_{up, l} with the PFT parameters. There was no statistically significant linear correlation of BMI, ACT score, EOS, EOS, EOS or DLco%predicted with any imaging readouts. There was no statistically significant linear correlation of ACT score, EOS, EOS or EOS with PFT parameters.
**Table 6.4 Pearson’s correlation coefficients between the dynamic OE-MR imaging readouts of the lung and the clinical measurements in all asthmatic subjects**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>EF (%)</th>
<th>Median $\Delta$PO$_{2\max_l}$ (mmHg), entire lung</th>
<th>Median $\Delta$PO$_{2\max_l}$ (mmHg), EF</th>
<th>Interquartile range of $\tau_{up_l}$ (min), EF</th>
<th>Median $\tau_{down_l}$ (min), EF</th>
<th>Interquartile range of $\tau_{down_l}$ (min), EF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years</td>
<td>-0.835 (0.003)</td>
<td>-0.817 (0.004)</td>
<td>-0.742 (0.014)</td>
<td>0.678 (0.031)</td>
<td>0.465 (0.176)</td>
<td>0.444 (0.198)</td>
</tr>
<tr>
<td>FEV$_1$, %predicted</td>
<td>0.858 (0.002)</td>
<td>0.750 (0.012)</td>
<td>0.676 (0.032)</td>
<td>-0.847 (0.002)</td>
<td>-0.297 (0.405)</td>
<td>-0.167 (0.644)</td>
</tr>
<tr>
<td>FVC, %predicted</td>
<td>0.787 (0.007)</td>
<td>0.539 (0.108)</td>
<td>0.451 (0.191)</td>
<td>-0.927 (&lt;0.001)</td>
<td>-0.034 (0.925)</td>
<td>0.024 (0.947)</td>
</tr>
<tr>
<td>FEV$_1$/FVC, %</td>
<td>0.836 (0.003)</td>
<td>0.773 (0.009)</td>
<td>0.700 (0.024)</td>
<td>-0.734 (0.016)</td>
<td>-0.432 (0.212)</td>
<td>-0.280 (0.433)</td>
</tr>
<tr>
<td>MMEF, %predicted</td>
<td>0.879 (0.001)</td>
<td>0.831 (0.003)</td>
<td>0.771 (0.009)</td>
<td>-0.889 (0.001)</td>
<td>-0.329 (0.353)</td>
<td>-0.257 (0.473)</td>
</tr>
<tr>
<td>sR$_{tot}$, kPa·s</td>
<td>-0.873 (0.002)</td>
<td>-0.868 (0.002)</td>
<td>-0.835 (0.005)</td>
<td>0.684 (0.042)</td>
<td>0.467 (0.205)</td>
<td>0.327 (0.391)</td>
</tr>
<tr>
<td>sR$_{tot}$, %predicted</td>
<td>-0.863 (0.003)</td>
<td>-0.872 (0.002)</td>
<td>-0.850 (0.004)</td>
<td>0.700 (0.036)</td>
<td>0.403 (0.282)</td>
<td>0.264 (0.492)</td>
</tr>
<tr>
<td>sR$_{eff}$, kPa·s</td>
<td>-0.860 (0.003)</td>
<td>-0.860 (0.003)</td>
<td>-0.829 (0.006)</td>
<td>0.677 (0.045)</td>
<td>0.458 (0.215)</td>
<td>0.314 (0.411)</td>
</tr>
<tr>
<td>sR$_{eff}$, %predicted</td>
<td>-0.851 (0.004)</td>
<td>-0.864 (0.003)</td>
<td>-0.844 (0.004)</td>
<td>0.694 (0.038)</td>
<td>0.399 (0.287)</td>
<td>0.257 (0.505)</td>
</tr>
<tr>
<td>FRC, %predicted</td>
<td>-0.634 (0.066)</td>
<td>-0.624 (0.072)</td>
<td>-0.577 (0.104)</td>
<td>0.196 (0.613)</td>
<td>0.905 (0.001)</td>
<td>0.770 (0.015)</td>
</tr>
<tr>
<td>TLC, %predicted</td>
<td>-0.562 (0.120)</td>
<td>-0.620 (0.075)</td>
<td>-0.596 (0.090)</td>
<td>0.067 (0.864)</td>
<td>0.886 (0.001)</td>
<td>0.724 (0.027)</td>
</tr>
<tr>
<td>RV, %predicted</td>
<td>-0.594 (0.090)</td>
<td>-0.628 (0.070)</td>
<td>-0.612 (0.080)</td>
<td>0.361 (0.340)</td>
<td>0.794 (0.011)</td>
<td>0.722 (0.028)</td>
</tr>
<tr>
<td>RV/TLC, %</td>
<td>-0.904 (0.001)</td>
<td>-0.926 (&lt;0.001)</td>
<td>-0.911 (0.001)</td>
<td>0.748 (0.020)</td>
<td>0.613 (0.080)</td>
<td>0.552 (0.123)</td>
</tr>
</tbody>
</table>

EF: enhancing fraction; Median $\Delta$PO$_{2\max\_l}$, entire lung: the median value of the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water over the entire lungs in the field of view; Median $\Delta$PO$_{2\max\_l}$, EF: the median value of the $\Delta$PO$_{2\max\_l}$ over the enhancing lung regions in the field of view; Interquartile range of $\tau_{up\_l}$, EF: the interquartile range of oxygen wash-in time constant over the enhancing lung regions in the field of view; Median $\tau_{down\_l}$, EF: oxygen wash-out time constant over the enhancing lung regions in the field of view; Interquartile range of $\tau_{down\_l}$, EF: the interquartile range of $\tau_{down\_l}$ over the enhancing lung regions in the field of view; FEV$_1$: forced expiratory volume in 1 s; %predicted: percentage of predicted normal value; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; sR$_{tot}$: total specific airway resistance; sR$_{eff}$: effective specific airway resistance; FRC: functional residual capacity; TLC: total lung capacity; RV: residual volume; RV/TLC: ratio of RV to TLC. Data are presented as correlation coefficient (P value). P values < 0.05 are presented in bold.
Figure 6.6 The scatter plots with the line of best fit to show the strongest correlation of each imaging readout with the pulmonary function test indices.

(a) EF vs RV/TLC; (b) median ΔPO$_{2\text{max}}$ vs RV/TLC; (c) interquartile range of τ$_{\text{up}}$ vs MMEF%$_{\text{predicted}}$; (d) median τ$_{\text{down}}$ vs FRC%$_{\text{predicted}}$; (e) interquartile range of τ$_{\text{down}}$ vs FRC%$_{\text{predicted}}$. $r$ is the Pearson's correlation coefficient. The solid dots are the individual data points (red for mild asthma; blue for severe asthma).
6.4.3 Dynamic OE-MRI in the aorta

A statistically significant difference was found in $\tau_{\text{up}_a}$ between the two groups ($P = 0.030$), but not in $T_{\text{air}_a}$, $\Delta\text{PO}_{2\text{max}}_a$ and $\tau_{\text{down}_a}$ (table 6.3). $\Delta\text{PO}_{2\text{max}}_a$ was positively correlated with median $\Delta\text{PO}_{2\text{max}}_l$ (entire-lung median and enhanced-region median, both $r = 0.745$, both $P = 0.013$, Spearman's rank correlation).

6.4.4 One-month reproducibility

The imaging parameter maps from the scan and rescan (1 month apart) were similar in mild asthmatic subjects while relatively more variable in severe asthmatic subjects, as shown in the examples in figure 6.1 and figure 6.2. The degree of agreement between the two measurements of the imaging readouts is illustrated by the Bland-Altman plots in figure 6.7. The mean bias, 95% confidence interval of the bias and the limits of agreement between the two measurements for each dynamic OE-MRI parameter are listed in table 6.5. The Bland-Altman plots demonstrate that the mean bias between the imaging measurements did not significantly deviate from zero ($P > 0.05$, one-sample t-test). The mild asthmatic group presents less variation between repeat measurements with narrower limits of agreements and bias closer to zero than the severe asthmatic group.

### Table 6.5 The mean bias and 95% limits of agreement of the imaging readouts between two scans

<table>
<thead>
<tr>
<th>Parameter *</th>
<th>Mean bias [95% limits of agreement]†</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF (%)</td>
<td></td>
</tr>
<tr>
<td>Calculation performed over the entire lung in the field of view</td>
<td></td>
</tr>
<tr>
<td>Median $\Delta\text{PO}_{2\text{max}}_l$, mmHg</td>
<td>12 [-66, 90] -4 [-191, 181]</td>
</tr>
<tr>
<td>Interquartile range of $\Delta\text{PO}_{2\text{max}}_l$, mmHg</td>
<td>28 [-98, 147] -57 [-162, 49]</td>
</tr>
<tr>
<td>Calculation performed over the enhanced lung regions in the field of view</td>
<td></td>
</tr>
<tr>
<td>Median $\Delta\text{PO}_{2\text{max}}_l$, mmHg</td>
<td>13 [-72, 98] -18 [-122, 86]</td>
</tr>
<tr>
<td>Interquartile range of $\Delta\text{PO}_{2\text{max}}_l$, mmHg</td>
<td>27 [-92, 146] -44 [-109, 21]</td>
</tr>
<tr>
<td>Median $\tau_{\text{up}_l}$, min</td>
<td>-0.12 [-0.33, 0.10] 0.04 [-0.65, 0.73]</td>
</tr>
<tr>
<td>Interquartile range of $\tau_{\text{up}_l}$, min</td>
<td>-0.06 [-0.21, 0.09] -0.43 [-2.35, 1.49]</td>
</tr>
<tr>
<td>Median $\tau_{\text{down}_l}$, min</td>
<td>0.23 [-0.44, 0.89] 0.28 [-1.03, 1.59]</td>
</tr>
<tr>
<td>Interquartile range of $\tau_{\text{down}_l}$, min</td>
<td>0.68 [-1.83, 3.19] 1.07 [-2.16, 4.30]</td>
</tr>
</tbody>
</table>

* EF: enhancing fraction; $\Delta\text{PO}_{2\text{max}}_l$: the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water of the lung; $T_{\text{up}_l}$: oxygen wash-in time constant of the lung; $T_{\text{down}_l}$: oxygen wash-out time constant of the lung.

† 95% limits of agreement are calculated as mean bias $\pm 1.96 \times$ standard deviation of the difference between two measurements.
Figure 6.7 Bland-Altman plots of the agreements of two measurements of EF (a), entire-lung median $\Delta \text{PO}_{2\text{max}_l}$ (b), median $\tau_{\text{up}_l}$ (c) and median $\tau_{\text{down}_l}$ (d) in the two groups

The solid dots are the individual data points (red for mild asthma; blue for severe asthma). The solid lines are the mean of the difference between the two measurements (red for mild asthma; blue for severe asthma). The black dashed line is at zero difference. The dotted lines are the mean of the difference between the two measurements $\pm 1.96 \times$ the standard deviation of the difference between two measurements (red for mild asthma; blue for severe asthma).

6.5 Discussion

This prospective pilot study provides initial evidence of the feasibility and the one-month reproducibility of dynamic OE-MRI in the quantitative estimation of $O_2$ delivery, uptake and washout in asthmatic lungs.

We have demonstrated that the dynamic OE-MRI technique is able to visualize regional functional abnormalities in patients with asthma. The patchier appearance of the imaging parameter maps and the broader parameter histograms in severe asthmatic lungs than in mild asthmatic lungs clearly reflects the increased heterogeneity of lung functional impairment in more severe asthma. These inhomogeneous patterns are consistent with those seen in other asthma imaging studies [95,
The non-enhancing regions observed in the lungs may indicate the presence of severe airway occlusion, probably due to mucus plugging, airway remodelling or airway spasm, resulting in low alveolar ventilation. ΔPO$_{2\text{max}_l}$ maps provide valuable spatial information about pulmonary oxygenation, i.e. the maximum increase in lung water PO$_2$ observed after a step change in inspired oxygen fraction. The low value regions in ΔPO$_{2\text{max}_l}$, which are more prominent in severe asthmatic lungs, imply reduced ability to deliver O$_2$, as we would expect a low ventilation-perfusion ratio, which can occur even in asymptomatic asthma [329], to lead to low local ΔPO$_2$. Averaged across the whole lung the ventilation-perfusion impairment in severe asthmatics was not sufficiently large to significantly reduce ΔPO$_{2\text{max}_a}$, although the wash-in time, $\tau_{\text{up}_a}$, was significantly longer in the severe asthmatic group, probably indicating reduced total ventilation across the lung, consistent with the worse lung function demonstrated in the PFTs. Although diffusion impairment may also lead to decreased pulmonary oxygenation efficiency, this is not the case in this study as approximately all subjects had normal diffusion capacity according to DLco%predicted (78%-82%). The $\tau_{\text{up}}$ and $\tau_{\text{down}}$ maps represent the time constants for regional pulmonary oxygen delivery and the prolonged wash-in and wash-out time constants in severe asthmatics reflect regional airflow limitation.

We have also shown that OE-MRI readouts are sensitive to asthma severity. EF and median ΔPO$_{2\text{max}_l}$ are significantly lower in severe asthmatic patients than in mild asthmatic patients. These differences are expected as studies have shown that poor ventilation, ventilation-perfusion inequality and shunt are exacerbated with increased asthma severity [330], all of which could contribute to inefficient pulmonary oxygenation. Furthermore, not only the entire-lung median but also the enhancing-region median of ΔPO$_{2\text{max}_l}$ differed significantly between groups, which indicates that ΔPO$_{2\text{max}_l}$ was lower even in the absence of gross obstruction or constriction in the severe asthmatics’ lungs. The significant correlation of median ΔPO$_{2\text{max}_l}$ with ΔPO$_{2\text{max}_a}$, an index of the overall oxygenation efficiency within the lungs, accords with an early OE-MRI experiment on a pig where an excellent linear correlation was demonstrated between the lung tissue R1 and the PO$_2$ of the arterial blood sampled from right femoral artery ($r^2 = 0.997$) [188]. This correlation suggests that single slice ΔPO$_{2\text{max}_l}$ measurement could to some extent reflect global lung functional status in addition to providing unique regional information.

The interquartile range of $\tau_{\text{up}_l}$ is also sensitive to asthma severity. The significantly wider interquartile range of $\tau_{\text{up}_l}$ and the correspondingly broader $\tau_{\text{up}_l}$ histogram in the severe asthmatic lungs implies a more heterogeneously distributed airflow limitation than in mild asthmatic lungs. The median $\tau_{\text{up}_l}$ and median $\tau_{\text{down}_l}$ in our two asthmatic groups are comparable to previously published values in patients with chronic obstructive pulmonary disease while longer than that in healthy subjects [188, 211, 212]. The between-group difference in median $\tau_{\text{up}_l}$ and median $\tau_{\text{down}_l}$ did not reach statistical significance, most likely owing to the small sample size and the relatively large variation. Baseline T1 of the lung was comparable in the two asthmatic groups and was similar to normal lung T1 [112]. However under hyperoxia, lung T1 was shortened due to oxygen inhalation by about 7.5 % in mild asthmatic patients, which was a larger change than the 3.6 % T1 shortening in severe asthmatic patients in this study while comparable to the literature values in healthy lungs (6% -
17% $T_1$ shortening; note that $T_1$ shortening contains essentially the same information as the $\Delta PO_2$ data we have presented due to the nature of the $\Delta PO_2$ calculation) [112, 188, 331].

The third finding is the substantial correlations between the imaging readouts and the PFT measurements in asthma. EF, median $\Delta PO_{2\text{max}}$ and the interquartile range of $\tau_{\text{up}}$ were strongly correlated with PFT indices of airway function. The entire-lung median $\Delta PO_{2\text{max}}$ showed slightly better correlations with PFT parameters than the enhancing-region median $\Delta PO_{2\text{max}}$. Similar correlations of OE-MRI readouts with FEV$_1$$\%_{\text{predicted}}$, FVC$\%_{\text{predicted}}$, FEV$_1$/FVC ratio and MMEF$\%_{\text{predicted}}$ have been demonstrated in asthmatics and patients with other lung diseases [127, 128, 171, 200, 211, 212, 215], while the correlations with the airway resistance indices of $sR_{\text{tot}}$ and $sR_{\text{eff}}$ are to our knowledge the first reported. In a previous asthma OE-MRI study, Ohno et al. reported significant but much weaker correlations between the mean signal enhancement ratio and FEV$_1$ ($r = 0.55$, $P < 0.05$) and the average forced expiratory flow between 25% point and 75% point of FVC (FEF$_{25\%-75\%}$, $r = 0.55$, $P < 0.05$) [215]. The stronger correlations presented in our study probably benefit from our use of quantitative $T_1$ measurements in a dynamic manner, which is likely to be more precise in evaluating lung function than the simple observation of static signal intensity change, as changes in relaxation rate relate linearly to changes in oxygen partial pressure [196, 200]. Unlike other imaging parameters, median and interquartile range of $\tau_{\text{down}}$ showed strong correlations with TLC$\%_{\text{predicted}}$ and RV$\%_{\text{predicted}}$ but not with other PFT indices. This observation might imply the underlying differences between the $O_2$ wash-in and wash-out processes, with $O_2$ wash-in more prominently associated with the forced expiratory flow rate [196, 211, 212] while $O_2$ wash-out is more related to lung volume measurements. Significant correlations between DL$\text{CO}$$\%_{\text{predicted}}$ and the OE-MRI imaging readouts have been observed in patients with COPD and interstitial lung disease [171, 211, 212] but not in healthy subjects [196] or in the asthmatic patients in the current study. DL$\text{CO}$$\%_{\text{predicted}}$ is more affected in emphysematous COPD and interstitial lung disease than in asthma and thus is an essential determinant of OE-MRI readouts in the former two diseases. In contrast to the good sensitivity to lung function, dynamic OE-MRI readouts failed to differentiate the airway inflammatory phenotypes (eosinophilic/non-eosinophilic) and were not associated with the level of airway inflammation in this study. However, considering most patients were undergoing anti-inflammatory/anti-allergic treatment that alter the eosinophil levels, this observation needs to be further explored by future studies with larger sample size and controlled therapies.

Dynamic OE-MRI showed good one-month reproducibility in mild asthmatic patients, as evidenced by the similar parameter maps between scans and the narrow limits of agreement in the Bland-Altman plots. The higher visit-to-visit variability in the severe asthmatic group is likely to be derived from the true disease-related variation of airway changes over the one month interval. It seems less likely that potential technical issues, for example the difference in image location between repeat scans, are the cause of differences, as the differences were much smaller in the mild asthmatic group. The stability of the geographic location of ventilation defects in severe asthmatic patients at the lobe level (as the example in figure 6.2), also seen with serial hyperpolarized $^3$helium MRI ventilation imaging, implies the existence of fixed airway obstruction due to airway remodelling in
severe asthma [291]. To our knowledge, this is the first report of the reproducibility of the OE-MRI technique in asthma.

There are several limitations to this study, including the small patient cohort, the different age distribution between groups, limited image volume coverage and the lack of normal controls. These issues will be addressed in future studies. In addition, we assumed that the $O_2$ relaxivity in water is approximately the same as that in the lung tissue. However, they may be different and thus cause errors in $\Delta PO_{2\text{max}}$ estimation. Experiments are needed to measure the $O_2$ relaxivity in lung tissue but this is a non-trivial measurement to perform.

In conclusion, our work supports a potential role of dynamic OE-MRI in visualizing and quantifying regional lung functional abnormalities in asthma. Quantitative dynamic OE-MRI readouts, with good one-month reproducibility, are sensitive to disease severity and the localised nature of lung function deficits in asthma. The spatial and temporal information of $O_2$ delivery, uptake and washout in the lungs captured by dynamic OE-MRI using a cheap and non-ionizing source of contrast makes it an attractive option in the assessment of asthma. The simple setup requirement makes this technique practicable for clinical usage. Further work is required to confirm and extend findings in large age-matched cohorts and to assess sensitivity of dynamic OE-MRI to lung function changes due to intervention.
Chapter 7 Paper 4: Short-term repeated dynamic OE-MRI measures response to salbutamol inhalation in asthma and distinguishes severity of the disease

This paper is in progress of the 3rd round author review, aiming for submission to a peer-reviewed clinical journal by December 2014.

Authors: Wei-Juan Zhang, Robert M Niven, Simon S Young, Yu-Zhen Liu, James PB O’Connor, Geoffrey JM Parker and Josephine H Naish

From the Centre for Imaging Sciences, Institute of Population Health (WJ.Z., J.P.B.O, G.J.M.P., J.H.N.), Biomedical Imaging Institute (WJ.Z., J.P.B.O, G.J.M.P., J.H.N.), The University of Manchester, Oxford Road, Manchester, U.K., M13 9PT; North West Lung Research Centre, University Hospital of South Manchester (R.M.N.), Southmoor Road, Manchester, U.K., M23 9LT; Personalised Healthcare and Biomarkers, AstraZeneca R&D (S.S.Y., YZ.L.), Alderley Park, Macclesfield, U.K., SK10 4TF; Department of Radiology, Christie Hospital (J.P.B.O), 550 Wilmslow Road, Manchester, U.K., M20 4BX; Bioxydyn Limited (G.J.M.P.), Pencroft Way, Manchester, U.K., M15 6SZ

Contribution of authors: WJ.Z: study conception and design, approval of ethics, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript preparation and edition. R.M.N: study conception and design, participant enrolment, data interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. J.P.B.O, S.S.Y and YZ.L: study conception and design, data interpretation, manuscript reviewing.
7.1 Abstract

Purpose: To evaluate the feasibility and sensitivity of dynamic oxygen-enhanced magnetic resonance imaging (OE-MRI) to detect pulmonary functional changes due to salbutamol inhalation in patients with asthma and healthy subjects.

Materials and methods: 20 severe asthmatics, 10 mild asthmatics and 10 healthy subjects underwent dynamic OE-MRI scanning prior to, 15 min after and 30 min after inhalation of 400 μg salbutamol. A control scan without salbutamol inhalation was performed in 10 severe asthmatics, 9 mild asthmatics and 10 healthy subjects within 7 days. A two dimensional inversion-recovery turbo spin echo sequence was used to measure $T_1$ during the inhalation of medical air and 100% oxygen ($^{16}$O$_2$). The enhancing fraction (EF) and the median values of $O_2$ wash in and wash out time constants ($\tau_{up}$, $\tau_{down}$), the change in the partial pressure of $O_2$ ($\Delta P_{O_2\text{max}}$) after gas switchover in the blood plasma and tissue water of the lung parenchyma were measured and compared between pre- and post-salbutamol time points in each subject group.

Results: Before salbutamol, the severe asthmatic group showed significantly lower EF ($P = 0.002$) and median $\Delta P_{O_2\text{max}}$ ($P = 0.029$) than the healthy control group. In severe asthmatics, but not mild asthmatics or healthy volunteers, whole-lung median $\Delta P_{O_2\text{max}}$ was significantly decreased at 30 min after salbutamol inhalation relative to baseline ($P = 0.011$) and 15 min post-salbutamol ($P=0.017$). These differences were not found in the non-salbutamol scan.

Conclusions: Short-term repeated OE-MRI revealed a heterogeneous pattern of decreased $O_2$ delivery in the lungs of severe asthmatics in response to salbutamol inhalation, which supports the unique potential role of this imaging technique in the assessment of treatment effect in asthma.

7.2 Introduction

Short acting $\beta$ agonists (SABA) are the mainstream drugs used for the quick relief of acute asthma symptoms and asthma attack. Their acute effects on reversing bronchoconstriction and correcting ventilation abnormalities in patients with asthma are well established. Physiological tests including spirometry, plethysmography, forced impulse oscillometry have provided evidence of increased expiratory flow rates and volumes, reduced airway resistance and improved ventilation inhomogeneity after SABA administration in asthma [332-336].

Despite the mostly favourable changes in airway function, SABA may cause a detrimental effect on gas exchange - the core of lung function. A fall in arterial oxygen saturation ($SaO_2$) and arterial $O_2$ tension ($PaO_2$), the outcome indices of the overall oxygenation adequacy within the lung, following SABA administration have been observed in patients with asthma [30, 337, 338]. The possible hypoxemia caused or aggravated by SABA has been attributed to the worsened ventilation-perfusion imbalance, as suggested by early experiments using the multiple inert gas elimination technique [27-29, 329].

In addition to the global physiological measurements, the development of imaging techniques has shed further light on the response of regional pulmonary function to SABA in asthma [52]. By using quantitative computed tomography (CT), researchers have demonstrated that SABA can significantly increase airway internal luminal diameter but cannot change airway wall thickness, gas trapping and CT estimates of lung density in asthmatics [68, 339-341]. Scintigraphy, positron emission...
tomography (PET), hyperpolarized noble gas magnetic resonance imaging (MRI) and xenon-inhaled dual CT have shown bronchoconstrictor-induced deterioration and SABA-induced improvement in regional ventilation defects and ventilation inhomogeneity in asthmatic lungs [68, 95, 287, 342]. However, few imaging techniques have explored the SABA-induced change in regional gas exchange in asthmatic lungs. One of the significant obstacles to using such imaging methods in the evaluation of the short-term effects of SABA is the fact that most imaging approaches are either challenging or impossible to repeat over the short timescales required to characterise the evolution of the functional impact of the intervention. X-ray CT and nuclear medicine techniques require the use of ionizing radiation, which discourages their use for multiple examinations over short timescales. The requirement for radioactive or non-radioactive tracer administration makes repeat functional imaging within a few tens of minutes impossible, or at best extremely challenging, for nuclear medicine and methods such as dynamic contrast-enhanced (DCE) MRI and perfusion CT. Hyperpolarized gas MRI methods can be applied for the required repeat measurements but require hyperpolarized gas preparation and delivery and non-standard imaging equipment, which is expensive and available at only a small number of leading lung imaging sites worldwide. There is therefore a need to develop practical, safe, cost-effective and reproducible functional lung imaging to characterise lung function changes in response to SABA.

Dynamic oxygen enhanced (OE-) MRI is a promising technique for quantifying regional pulmonary oxygen delivery efficiency [166, 183, 200]. In this method, high levels of isobaric oxygen ($^{16}$O$_2$) are inhaled by subjects, usually 100% O$_2$ at a rate of 15 litres per minute. As the excess O$_2$ reaches exchange tissues via ventilation, it is dissolved in the plasma and tissue fluid, which leads to a shortening in the lung longitudinal relaxation time ($T_1$) [161]. The change in $T_1$ is determined to the change in the local O$_2$ tension (PO$_2$) in the lung water, which is in turn determined by the interplay of regional ventilation, perfusion and diffusion [201, 202]. Dynamic OE-MRI has been applied to respiratory studies of healthy subjects and patients with different lung disorders, including asthma and also to studies of non-respiratory tissues and tumour biology [343-345]. Significant correlations have been reported between the dynamic OE-MRI derived readouts, e.g. relative signal enhancement ratio and O$_2$ wash-in slope, and the pulmonary function tests (PFT) indices of airway obstruction and diffusion capacity [170, 183, 184, 198, 211, 212, 214, 215, 346].

In this study, we aimed to assess the regional pulmonary response to the inhalation of a typical SABA, i.e. salbutamol, in healthy subjects and patients with asthma using dynamic OE-MRI. We scanned patients three times within approximately 45 minutes in order to assess the short-term regional effects of salbutamol.

7.3 Materials and Methods

7.3.1 Study subjects

The study was approved by the National Research Ethical Committee (Ref: 11/NW/0387) and written informed consent was obtained from each subject. The study was registered in UK Clinical Research Network study portfolio database (Ref: 11431). 30 asthmatic patients and 10 healthy subjects were recruited from University Hospital of South Manchester, Manchester and the public respectively between July 2012 and August 2013.
10 out of 30 patients were mild asthmatics who matched the following criteria: 1) the percentage predicted forced expiratory volume in 1 second (FEV₁ %predicted) ≥ 85 %; 2) requirement for treatment with low dose inhaled corticosteroid (≤ 400 μg/day beclomethasone dipropionate or equivalent) or short-acting inhaled β₂-adrenergic receptor agonists only; 3) no requirement for oral steroids in the last 12 months. The remaining 10 patients were severe asthmatics who matched the following criteria: 1) FEV₁ %predicted < 85%; 2) treatment required consistent with step 4 or step 5 of British Thoracic Society guidelines on the management of asthma [9]; 3) a minimum of two courses of oral corticosteroids in the last 12 months. Healthy subjects were within the same age range as the patient groups and had no history of asthma or other significant respiratory conditions.

Main exclusion criteria for all subjects included being a current smoker or ex-smoker with pack-years > 10 or smoking cessation < 1 year; recent respiratory tract infection; past or current lung or other significant disease (other than asthma for the patient groups); evidence of bronchiectasis or emphysema according to previous chest CT (patients group only); being unable to perform spirometry or plethysmography manoeuvres; daytime O₂ saturation < 90% on room air; interfering medicines or MRI contraindications.

All patients withheld short-acting bronchodilators for 6 hours and long-acting bronchodilators for 12 hours prior to each visit.

7.3.2 Clinical visit

All subjects underwent spirometry, body plethysmography and gas transfer measurements at University Hospital of South Manchester, Manchester. The PFT was performed using a plethysmograph (CareFusion Ltd., Germany) according to European Respiratory Society recommendations [32, 34, 38]. Spirometry was carried out at baseline to measure the forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC) and the maximum mid-expiratory flow (MMEF). Three maximal forced expirations were recorded to ensure reproducibility and were followed by the measurement of static lung volumes including total lung capacity (TLC) and residual volume (RV). Total specific airway resistance (sRtot) and effective specific airway resistance (sReff) were also measured. Carbon monoxide diffusion capacity (DLco) was then obtained by the single-breath technique. Predicted values (%predicted) for PFT parameters were calculated.

Subjects were then given 400 μg inhaled salbutamol sulphate (Salamol®, IVAX Pharmaceuticals, Waterford, Ireland) via a pressurized metered-dose inhaler and a large-volume spacer device (Volumatic®, GlaxoSmithKline Ltd, Middlesex, UK). Spirometry was repeated at 15 min and 30 min post salbutamol administration. The PFT and the administration of salbutamol took place in a seated position.

Asthma Control Test (ACT) questionnaires were completed by all asthmatic subjects. Blood samples from all subjects and spontaneous or induced sputum samples from 29 asthmatic subjects were obtained for eosinophil cell counting (EOS₆, EOS₅). Healthy control subjects did not undergo a sputum test.

7.3.3 MR imaging

All subjects underwent MRI scanning at Wolfson Molecular Imaging Centre, Manchester, within 7 days of the clinical visit (median days 4, interquartile range 3 – 5 days). A repeat scan was
carried out on the first 10 severe asthmatic subjects, 9 mild asthmatic subjects and 10 healthy subjects within 7-10 days of the first scan. 1 mild asthmatic patient did not attend the rescan.

The scans were performed on a 1.5 tesla whole body scanner (Philips Achieva, Philips Healthcare, The Netherlands) in the coronal plane. In the first scan (with salbutamol intervention visit), dynamic OE-MRI was performed prior to, 15 min after and 30 min after the administration of 400 μg inhaled salbutamol sulphate. Salbutamol was delivered in the same way as in PFT but in a supine position. Subjects were instructed to keep lying in the supine position without moving to minimize the shift of the imaging slice location between scans. In the second visit (control scan), there was no salbutamol administration but scanning was paused for the same time length. Dynamic OE-MRI was repeated 3 times using the same scanning protocol as that in the first scan i.e. prior to, 15 min after and 30 min after the scan pause. Figure 7.1 illustrates the design of the scanning protocol.
Figure 7.1 Schematic of the protocol designs for the salbutamol intervention visit and the control visit.
Each OE-MRI scan consisted of a series of 25 image acquisitions, with the subject breathing medical air (21% O\textsubscript{2}), in order to provide a measurement of native lung T\textsubscript{1}. This was followed by a series of 140 dynamic images acquired throughout the gas switchover between medical air and 100% O\textsubscript{2}. Images were acquired using a 10 mm thick single coronal oblique slice positioned posteriorly in the chest and angled to intersect the descending aorta by using a two dimensional (2D) T\textsubscript{1} weighted centric ordered single-shot turbo spin echo sequence preceded by a non-selective inversion recovery pulse (IR-TSE). The 25 T\textsubscript{1} measurement images were grouped into series of 5 images acquired with different inversion times (TI = 60 ms, 300 ms, 1100 ms, 2000 ms and 5000 ms), permitting the measurement of baseline lung T\textsubscript{1}; 5 images were collected in each series to provide an average signal intensity over the cardiac cycle. The 140 dynamic images were acquired with a TI of 1100 ms, which permitted the dynamic observation of the change in T\textsubscript{1} throughout the O\textsubscript{2} wash-in and wash-out with optimum sensitivity. The gas supply was switched to 100% O\textsubscript{2} at the 15\textsuperscript{th} acquisition and switched back to air at the 85\textsuperscript{th} acquisition. Other imaging parameters for all images included: repetition time (TR) = 6000 ms, echo time (TE) = 3.2 ms, field of view 450 mm × 450 mm, full k-space sampling and reconstructed to a 128 × 128 matrix, pixel size 3.52 mm × 3.52 mm. Images were acquired during free breathing. No respiratory or cardiac triggering was used. Medical air and 100% O\textsubscript{2} was delivered at a flow rate of 15 L/min via a non-rebreathing mask (Intersurgical Ltd., Wokingham, UK). The inspired O\textsubscript{2} concentration was continuously monitored by a gas analyzer (ML206, ADInstruments, Oxford, UK), which achieved the real-time gas sampling through a small tube placed directly into the masks.

### 7.3.4 Image analysis

To correct for motion effects, the lungs on the images were segmented and registered to the end expiration position (FVC level) using a semi-automatic registration method [112]. Baseline T\textsubscript{1} (T\textsubscript{1air}) and the equilibrium signal (S\textsubscript{0}) maps were generated by a three parameter fit to the data (magnitude) using

\[
S = |S_0 \left(1 - 2f e^{\frac{TI}{T_1}} \cdot e^{\frac{TR + TE}{T_1}}\right)| \quad \text{(Eq 7.1)}
\]

where f is the inversion efficiency (also a free fitting variable); n is the echo train length, which was 128 in this study. The dynamic T\textsubscript{1} of the lung (T\textsubscript{1}(t)) throughout gas switchover was obtained by rearranging the same signal equation, using a single TI = 1100 ms and with S(t), S\textsubscript{0} and f as known inputs. T\textsubscript{1}(t) was then converted to dynamic changes in the partial pressure of O\textsubscript{2} dissolved in the tissue water and blood plasma of the lung (ΔP\textsubscript{O2}(t)) due to the administration of O\textsubscript{2} by

\[
\Delta P_{O2}(t) = \left(\frac{1}{T_1(t)} - \frac{1}{T_{1air}}\right) r_{1_O2} \quad \text{(Eq 7.2)}
\]

using a value for the O\textsubscript{2} longitudinal relaxivity in water (r\textsubscript{1,O2}) of 2.49 × 10\textsuperscript{-4} /s/mmHg [201].
Each dynamic OE-MRI scan (three for each visit) involved the acquisition of an individual $T_1\text{air}$ map for the $T_1(t)$ and $\Delta PO_2(t)$ calculation. The $\Delta PO_2(t)$ time course curve was then fitted using the exponential equations 7.3 ($O_2$ wash-in) and 7.4 ($O_2$ wash out) for the calculation of $O_2$ wash-in and wash-out time constants ($t_{up}$, $t_{down}$, min) and $\Delta PO_2$ at the steady plateau after switching air to 100% $O_2$ ($\Delta PO_{2\text{max}}$, mmHg).

\[
\text{Wash-in: } \Delta PO_2(t) = \Delta PO_{2\text{max}} \cdot (1 - e^{-t/t_{up}}) \quad (\text{Eq 7.3})
\]

\[
\text{Wash-out: } \Delta PO_2(t) = \Delta PO_{2\text{max}} \cdot e^{-t/t_{down}} \quad (\text{Eq 7.4})
\]

For each lung pixel, the first 14 data points at baseline and the last 15 data points of the $O_2$ plateau of the $\Delta PO_2(t)$ time course (i.e. immediately before the switch from 100% $O_2$ back to medical air) were compared using a one-tailed independent samples $t$-test (significance was assumed at the 5% significance level). Pixels with significantly increased $\Delta PO_2$ were considered as demonstrating enhancement and the fraction of these pixels over the entire lung was measured and denoted as the enhancing fraction (EF). Parameter maps of $\Delta PO_{2\text{max}}$, $t_{up}$ and $t_{down}$ were generated for visual observation and the median values of these parameters were calculated across the entire imaged lung ($\Delta PO_{2\text{max}}$) or the enhancing lung ($t_{up}$ and $t_{down}$) for subsequent statistical analysis. All image analysis was completed using MATLAB R2012a (Mathworks, Natick, USA).

7.3.5 Statistical analysis
Statistical analysis was performed using IBM SPSS Statistics 20.0 software (IBM, New York, USA). Data were tested for normality by Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) with Bonferroni’s post hoc testing, $\chi^2$ testing and independent samples $t$ testing were performed to compare the demographics, baseline clinical measurements and baseline MR imaging readouts between subject groups. One-way repeated measures ANOVA with multiple comparisons by Bonferroni’s correction was employed to compare the pre- and post- salbutamol (pre- and post- scanning pause in the control scan) MR imaging measurements for each subject group. A 3 by 3 two-way mixed-design ANOVA with multiple comparisons by Bonferroni’s correction was employed to compare the changes in MR imaging readouts over time between subject groups and to determine the interaction between subject type and salbutamol time course (scanning pause time course in the control scan). The between-subject independent variable was subject type (3 levels: healthy control, mild asthma, severe asthma) and the within-subject independent variable was the time course (3 levels: baseline, 15 min and 30 min post salbutamol/scanning pause). A paired sample $t$ test was then carried out in the subjects who attended both scans to compare the change in MR imaging measurements over time between the salbutamol intervention scan and the control scan. Pearson’s correlation analysis was carried out on the pooled data of all subjects to evaluate the association between the clinical measurements and MR imaging measurements. Two-tailed $P < 0.05$ was considered to indicate statistical significance.

7.4 Results
7.4.1 Clinical characteristics and pulmonary function tests
Subject demographics, clinical measurements and PFT readouts are provided in table 7.1. Age, gender, body mass index and eosinophil cells in sputum and blood were not statistically different between subject groups. As expected the ACT score was significantly higher in mild asthmatic subjects (21 ± 4) than in the severe asthmatic group (13 ± 6, P <0.001) and PFT parameters were significantly worse in the severe asthmatic group than in the mild asthmatic group and the healthy control group, except for TLC%predicted and DLco%predicted. No significant difference was found between the mild asthmatic subjects and the healthy subjects in PFT parameters.
Table 7.1 Demographics, clinical measurements and pulmonary function tests

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy Controls (H) (n=10)</th>
<th>Mild Asthma (M) (n = 10)</th>
<th>Severe Asthma (S) (n = 20)</th>
<th>P value</th>
<th>Significant difference between groups (P value)</th>
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<td>Age, year</td>
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<td>BMI, kg/m²</td>
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<td>29 ± 4</td>
<td>31 ± 8</td>
<td>0.512</td>
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</tr>
<tr>
<td>ACT score</td>
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<td>21 ± 4</td>
<td>13 ± 6</td>
<td>&lt;0.001</td>
<td>--</td>
</tr>
<tr>
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<td>H-S (0.046)</td>
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<td>FEV&lt;sub&gt;1&lt;/sub&gt;%predicted, %</td>
<td>105 ± 10</td>
<td>101 ± 12</td>
<td>60 ± 17</td>
<td>&lt;0.001</td>
<td>H-S (&lt;0.001), M-S (&lt;0.001)</td>
</tr>
<tr>
<td>FVC%predicted, %</td>
<td>114 ± 12</td>
<td>110 ± 11</td>
<td>88 ± 18</td>
<td>&lt;0.001</td>
<td>H-S (0.001), M-S (0.002)</td>
</tr>
<tr>
<td>FEV&lt;sub&gt;1&lt;/sub&gt;/FVC, %</td>
<td>79 ± 6</td>
<td>76 ± 8</td>
<td>56 ± 10</td>
<td>&lt;0.001</td>
<td>H-S (&lt;0.001), M-S (&lt;0.001)</td>
</tr>
<tr>
<td>MMEF%predicted, %</td>
<td>73 ± 16</td>
<td>67 ± 24</td>
<td>30 ± 17</td>
<td>&lt;0.001</td>
<td>H-S (&lt;0.001), M-S (&lt;0.001)</td>
</tr>
<tr>
<td>TLC%predicted, %</td>
<td>114 ± 14</td>
<td>113 ± 8</td>
<td>115 ± 16</td>
<td>0.868</td>
<td>--</td>
</tr>
<tr>
<td>RV%predicted, %</td>
<td>118 ± 33</td>
<td>122 ± 24</td>
<td>181 ± 42</td>
<td>&lt;0.001</td>
<td>H-S (0.001), M-S (0.001)</td>
</tr>
<tr>
<td>RV/TLC, %</td>
<td>33 ± 8</td>
<td>36 ± 8</td>
<td>50 ± 10</td>
<td>&lt;0.001</td>
<td>H-S (&lt;0.001), M-S (0.001)</td>
</tr>
<tr>
<td>FRC%predicted, %</td>
<td>120 ± 19</td>
<td>110 ± 12</td>
<td>134 ± 28</td>
<td>0.036</td>
<td>M-S (0.032)</td>
</tr>
<tr>
<td>sR&lt;sub&gt;eff&lt;/sub&gt;%predicted, %</td>
<td>85 ± 34</td>
<td>112 ± 38</td>
<td>273 ± 149</td>
<td>&lt;0.001</td>
<td>H-S (&lt;0.001), M-S (&lt;0.001)</td>
</tr>
<tr>
<td>DLco%predicted, %</td>
<td>86 ± 13</td>
<td>89 ± 11</td>
<td>82 ± 14</td>
<td>0.414</td>
<td>--</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation. BMI: body mass index; ACT: Asthma Control Test questionnaire; EOSS<sub>S</sub>: sputum eosinophil counting; EOSB<sub>B</sub>: blood eosinophil counting; FEV<sub>1</sub>: forced expiratory volume in 1s; %predicted: percentage of predicted normal value; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; FRC: functional residual capacity; TLC: total lung capacity; RV: residual volume; RV/TLC: ratio of RV to TLC; sR<sub>eff</sub>: effective specific airway resistance; DLco: diffusing capacity for carbon monoxide; ND: no data; H-S: healthy control vs severe asthma; M-S: mild asthma vs severe asthma. * P value from one way ANOVA except for P value of sex from χ² test, P values of ACT and EOSS<sub>S</sub> from independent samples t-test, P values of EOSB<sub>B</sub> and sR<sub>eff</sub>%predicted from welch’s ANOVA. + P values are from Turkey-Kramer test except for the P values of EOSB<sub>B</sub> and sR<sub>eff</sub>%predicted from Games-Howell test. P values < 0.05 are presented in bold.
7.4.2 MR imaging measurements

Example parameter maps of baseline $\Delta P_{O_{2_{\text{max}}}}$, $\tau_{\text{up}}$ and $\tau_{\text{down}}$ from a healthy control subject, a mild asthmatic subject and a severe asthmatic subject are provided in figure 7.2. At the first visit baseline scan, the severe asthmatic group showed significantly lower EF ($P = 0.002$) and median $\Delta P_{O_{2_{\text{max}}}}$ ($P = 0.029$) than the healthy control group (table 7.2). EF in the severe asthmatic group was also significantly lower than that in the mild asthmatic group ($P = 0.003$). There was no significant difference in baseline MR imaging measurements between the healthy control group and the mild asthmatic group. There was no significant difference in median $\tau_{\text{up}}$ and median $\tau_{\text{down}}$ between groups.

**Figure 7.2** The baseline maps of $\Delta P_{O_{2_{\text{max}}}}$ (a, b, c), $\tau_{\text{up}}$ (d, e, f) and $\tau_{\text{down}}$ (g, h, i) from a healthy subject (F, 28 yrs, FEV$_1$%predicted = 107%, a, d, g), a mild asthmatic patient (F, 23 yrs, FEV$_1$%predicted = 117%, b, e, h) and a severe asthmatic patient (F, 49 yrs, FEV$_1$%predicted = 79%, c, f, i).
Table 7.2 Comparison of baseline dynamic OE-MRI imaging parameters between subject groups

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Healthy controls (H) (n=8)</th>
<th>Mild asthma (M) (n=10)</th>
<th>Severe asthma (S) (n=20)</th>
<th>P value</th>
<th>Significant difference between groups (P value) *</th>
</tr>
</thead>
<tbody>
<tr>
<td>EF, %</td>
<td>93 ± 5</td>
<td>92 ± 4</td>
<td>81 ± 13</td>
<td>0.004</td>
<td>H-S (0.002), M-S (0.003)</td>
</tr>
<tr>
<td>Median ΔPO_{2max}, mmHg</td>
<td>253 ± 28</td>
<td>243 ± 43</td>
<td>198 ± 56</td>
<td>0.013</td>
<td>H-S (0.029)</td>
</tr>
<tr>
<td>Median τ_{up}, min</td>
<td>0.63 ± 0.18</td>
<td>0.72 ± 0.42</td>
<td>0.91 ± 0.42</td>
<td>0.191</td>
<td>-</td>
</tr>
<tr>
<td>Median τ_{down}, min</td>
<td>0.54 ± 0.29</td>
<td>0.87 ± 0.89</td>
<td>1.27 ± 0.98</td>
<td>0.128</td>
<td>-</td>
</tr>
</tbody>
</table>

Data are means ± standard deviations. EF: enhancing fraction; ΔPO_{2max}: the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water of the lung; τ_{up}: oxygen wash-in time constant of the lung; τ_{down}: oxygen wash-out time constant of the lung; H-S: healthy control vs severe asthma. *P values are from one way ANOVA except for the P value of EF from Welch’s ANOVA. *P values are from Turkey-Kramer test except for the P value of EF from Games-Howell test. P values < 0.05 are presented in bold.
According to the 3 × 3 mixed-design ANOVA in the scans with salbutamol intervention, the “subject type” but not the “salbutamol time course” showed a significant main effect on EF (F(2, 32)=5.09, P=0.012, \( \eta^2_p = 0.241 \)) and median \( \Delta PO_{2\max} \) (F(2, 32)=5.176, P= 0.011, \( \eta^2_p = 0.244 \)). The averaged EF and median \( \Delta PO_{2\max} \) over the pre- and post-salbutamol measures were significantly lower in the severe asthmatic group than in the healthy control group (P = 0.024 and 0.026). There was no significant interaction between the “subject type” and the “salbutamol time course”. One-way repeated measures ANOVA with Bonferroni’s corrected pairwise comparison further revealed a significant effect of salbutamol time course on whole-lung median \( \Delta PO_{2\max} \) for the severe asthmatic group (F(2, 32)=6.565, P=0.004, \( \eta^2_p =0.291 \)). There was a significant reduction in whole-lung median \( \Delta PO_{2\max} \) at 30 min after inhaling salbutamol from the baseline (P= 0.011) and the 15 min post-salbutamol (P= 0.017) in subjects with severe asthma, but not in the healthy control subjects and the subjects with mild asthma.

The same statistical analysis was performed on the control scans. There was a significant main effect of “subject type” on median \( \Delta PO_{2\max} \) (F(2,25)=3.626, 0.041, 0.225) but no significant main effect of “scanning pause time course” or significant interaction between the “subject type” and “scanning pause time course”, according to 3 × 3 mixed-design ANOVA. There was no significant change in MR imaging readouts pre- and post-scanning pause in any subject group according to one-way repeated measures ANOVA. Figure 7.3 shows the three repeated measures of median \( \Delta PO_{2\max} \) in three subject groups in two visits. Figure 7.4 shows the examples maps of pre- and post- salbutamol inhalation/scanning pause \( \Delta PO_{2\max} \) from a severe asthmatic subject.
Figure 7.3 Mean changes in median $\Delta P_{O_{2max}}$ with time after salbutamol inhalation (a, visit 1) and after scanning pause (b, visit 2) in healthy control group (closed squares), mild asthmatic group (open circles) and severe asthmatic group (closed circles).

Error bars are the standard error of the mean. * $P = 0.011$, 30 min post salbutamol vs baseline; $\xi P = 0.017$, 30 min post salbutamol vs 15 min post salbutamol. Differences between time points were not statistically significant if not shown.
Figure 7.4 $\Delta P\text{O}_{2\text{max}}$ maps pre- and post-salbutamol (visit 1, a, b, c) and pre- and post-scanning pause (visit 2, d, e, f) from a severe asthmatic patient (M, 58yrs, FEV$_1$%predicted=81%).
To ensure that the significant post-salbutamol changes in median ΔPO_{2max} observed in the severe asthmatic group was due to the salbutamol administration rather than the effect of pure O_2 inhalation or posture [27, 347], paired-samples t tests were carried out to compare the salbutamol intervention scans and the controls in the subgroup (n=7) who attended both visits (figure 7.5). In the severe asthmatic subjects, the change in median ΔPO_{2max} from baseline was significantly different from zero at 30 min-post salbutamol inhalation (-44 ± 22 mmHg, P = 0.002) but not at 30 min post-scanner pause (-11 ± 25 mmHg, P = 0.306) and it was significantly different between the time points of 30 min post-salbutamol inhalation and 30 min post-scanner pause (P = 0.002).

**Figure 7.5** Percentage changes in median ΔPO_{2max} in the severe asthmatic subgroup who attended both salbutamol intervention scan (closed circles, solid line) and control scan (open circles, dashed line).

*P = 0.002, 30 min post salbutamol vs baseline; †P = 0.002, 30 min post salbutamol vs 30 min post scanning pause. Differences between time points and between scan and rescan were not statistically significant if not shown.
In addition, the inhaled O$_2$ concentration and the whole-lung median value of the baseline lung T$_1$ was not significantly different across the 3 time points in any of the subject groups in both scans, which further suggested than observed post-salbutamol change in imaging parameters were genuine (table 7.3).

### 7.4.3 Correlations between pulmonary function tests and MR imaging readouts

There are moderate correlations of EF, median ΔPO$_{2\text{max}}$, and median τ$_{up}$ with PFT readouts when the asthmatic patient data and healthy control data are pooled (table 7.4).
Table 7.3 MR imaging measurements in scans with and without salbutamol administration

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Visit 1: with salbutamol administration</th>
<th>Visit 2: without salbutamol administration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Healthy control (n=8)</td>
<td>Mild asthma (n=10)</td>
</tr>
<tr>
<td>Maximum $O_2$ concentration, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>97 ± 3</td>
<td>94 ± 3</td>
</tr>
<tr>
<td>T15</td>
<td>96 ± 3</td>
<td>95 ± 4</td>
</tr>
<tr>
<td>T30</td>
<td>95 ± 4</td>
<td>95 ± 3</td>
</tr>
<tr>
<td>$T_1 \text{air, ms}$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1330 ± 84</td>
<td>1321 ± 82</td>
</tr>
<tr>
<td>T15</td>
<td>1326 ± 68</td>
<td>1303 ± 70</td>
</tr>
<tr>
<td>T30</td>
<td>1318 ± 78</td>
<td>1310 ± 76</td>
</tr>
<tr>
<td>EF, %</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>93 ± 5</td>
<td>92 ± 4</td>
</tr>
<tr>
<td>T15</td>
<td>94 ± 4</td>
<td>93 ± 5</td>
</tr>
<tr>
<td>T30</td>
<td>94 ± 5</td>
<td>89 ± 13</td>
</tr>
<tr>
<td>Median $\Delta P_{O_{2_{\max}}}$, mmHg</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>253 ± 28</td>
<td>243 ± 43</td>
</tr>
<tr>
<td>T15</td>
<td>225 ± 56</td>
<td>229 ± 35</td>
</tr>
<tr>
<td>T30</td>
<td>245 ± 53</td>
<td>223 ± 63</td>
</tr>
<tr>
<td>Median $\tau_{\uparrow}$, min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.63 ± 0.18</td>
<td>0.72 ± 0.42</td>
</tr>
<tr>
<td>T15</td>
<td>0.70 ± 0.29</td>
<td>0.88 ± 0.47</td>
</tr>
<tr>
<td>T30</td>
<td>0.73 ± 0.27</td>
<td>1.16 ± 1.75</td>
</tr>
<tr>
<td>Median $\tau_{\downarrow}$, min</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>0.54 ± 0.29</td>
<td>0.87 ± 0.89</td>
</tr>
<tr>
<td>T15</td>
<td>1.11 ± 1.25</td>
<td>1.57 ± 2.55</td>
</tr>
<tr>
<td>T30</td>
<td>0.55 ± 0.16</td>
<td>0.81 ± 0.86</td>
</tr>
</tbody>
</table>

Data are means ± standard deviations. T15: 15 min post salbutamol inhalation or scanning pause; T30: 30 min post salbutamol inhalation or scanning pause; $C_{O_{2_{\max}}}$: maximum inspired $O_2$ concentration; $T_1 \text{air}$: longitudinal relaxation time of the lung parenchyma when subject breathing medical air; EF: enhancing fraction; $\Delta P_{O_{2_{\max}}}$: the maximal change in the partial pressure of dissolved oxygen in the blood plasma and tissue water of the lung; $\tau_{\uparrow}$: oxygen wash-in time constant of the lung; $\tau_{\downarrow}$: oxygen wash-out time constant of the lung; * Value is significantly different from the baseline value (P =0.011). ξ Value is significantly different from the 15min post salbutamol (P =0.017).
<table>
<thead>
<tr>
<th>Parameters</th>
<th>EF</th>
<th>Median ΔPO_{2\text{max}}</th>
<th>Median $\tau_{\text{up}}$</th>
<th>Median $\tau_{\text{down}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI, kg/m$^2$</td>
<td>-0.554 (&lt; 0.001)</td>
<td>-0.518 (0.001)</td>
<td>-0.361 (0.026)</td>
<td>-0.053 (0.752)</td>
</tr>
<tr>
<td>FEV$_1$%predicted, %</td>
<td>0.514 (0.001)</td>
<td>0.410 (0.011)</td>
<td>-0.305 (0.063)</td>
<td>-0.250 (0.130)</td>
</tr>
<tr>
<td>FVC%predicted, %</td>
<td>0.293 (0.074)</td>
<td>0.252 (0.127)</td>
<td>-0.161 (0.333)</td>
<td>-0.182 (0.274)</td>
</tr>
<tr>
<td>FEV$_1$/FVC, %</td>
<td>0.572 (&lt; 0.001)</td>
<td>0.421 (0.008)</td>
<td>-0.360 (0.026)</td>
<td>-0.226 (0.173)</td>
</tr>
<tr>
<td>MMEF%predicted, %</td>
<td>0.337 (0.038)</td>
<td>0.208 (0.210)</td>
<td>-0.349 (0.032)</td>
<td>-0.198 (0.233)</td>
</tr>
<tr>
<td>FRC%predicted, %</td>
<td>-0.314 (0.055)</td>
<td>-0.259 (0.117)</td>
<td>0.369 (0.023)</td>
<td>0.235 (0.156)</td>
</tr>
<tr>
<td>TLC%predicted, %</td>
<td>-0.203 (0.221)</td>
<td>-0.206 (0.215)</td>
<td>0.250 (0.131)</td>
<td>0.196 (0.239)</td>
</tr>
<tr>
<td>RV%predicted, %</td>
<td>-0.492 (0.002)</td>
<td>-0.427 (0.008)</td>
<td>0.458 (0.004)</td>
<td>0.299 (0.069)</td>
</tr>
<tr>
<td>RV/TLC%predicted, %</td>
<td>-0.449 (0.005)</td>
<td>-0.412 (0.010)</td>
<td>0.373 (0.021)</td>
<td>0.273 (0.097)</td>
</tr>
<tr>
<td>sR$\text{eff}$%predicted, %</td>
<td>-0.486 (0.002)</td>
<td>-0.346 (0.034)</td>
<td>0.452 (0.004)</td>
<td>0.387 (0.016)</td>
</tr>
</tbody>
</table>

Data are presented as Pearson’s correlation coefficient (P value). Significant correlations with P value < 0.05 are presented in bold. BMI: body mass index; FEV$_1$: forced expiratory volume in 1s; %predicted: percentage of predicted normal value; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; FRC: functional residual capacity; TLC: total lung capacity; RV: residual volume; RV/TLC: ratio of RV to TLC; sR$\text{eff}$: effective specific airway resistance.

7.5 Discussion

In the current study, dynamic OE-MRI has revealed: 1) significantly lower baseline pulmonary oxygen delivery in patients with severe asthma than in healthy controls; 2) decreased pulmonary oxygen delivery as a response to inhaled salbutamol in patients with severe asthma. These findings suggest that dynamic OE-MRI is sensitive to the baseline pulmonary oxygenation impairment in patients with severe asthma and the change in pulmonary oxygenation caused by salbutamol intervention. These data support further evaluation of dynamic OE-MRI as a non-invasive non-ionizing technique for monitoring short-term treatment effects in asthma.

We have shown that dynamic OE-MRI can depict the heterogeneous pattern of impaired baseline pulmonary oxygen delivery in patients with severe asthma. The imaging parameter maps in patients with severe asthma were characterised by increased patchiness and decreased enhancing fraction with lower ΔPO$_{2\text{max}}$ throughout the lung relative to those in healthy controls and patients with mild asthma. The non-enhancing regions may indicate the location of severe airway obstruction due to mucus plugging, airway constriction and airway...
remodelling, which expand as asthma becomes severe. The generally lower \( \Delta P_{O_2} \text{max} \) across the lung, i.e. the maximum increase in lung water \( P_{O_2} \) observed after a step change in inspired oxygen fraction, in patients with severe asthma indicates a reduced capacity of pulmonary oxygen delivery in severe asthmatic lungs that is most probably to be attributed to poor alveolar ventilation and ventilation-perfusion mismatch [28]. On the other hand, the patients with mild asthma presented similar \( \Delta P_{O_2} \text{max} \) maps to the healthy controls, high in value and homogeneous in distribution, which mirrors the preservation of a normal pulmonary oxygen delivery capacity in mild asthmatics with normal PFT [348]. The significantly lower EF and median \( \Delta P_{O_2} \text{max} \) in the severe asthmatics than in the mild asthmatics and the healthy controls suggests a good sensitivity of dynamic OE-MRI to asthma severity.

The significant, moderate correlations of a subset of the MR imaging indices with PFT agree with previous reports [215, 349] and further support the feasibility of dynamic OE-MRI in the assessment of lung function loss in asthma. However, the current study has provided evidence for the first time that dynamic OE-MRI readouts may lack the power to differentiate mild asthmatics who have normal PFT from the healthy controls. This imaging technique may thus provide limited assistance to the diagnosis and assessment of early stage asthma.

This study presents quantitative regional measurements of pulmonary oxygenation (\( \Delta P_{O_2} \text{max} \)) using dynamic OE-MRI in response to salbutamol in patients with asthma and healthy controls. To our knowledge, this is the first report in which dynamic OE-MRI was used to track short term treatment effects on lung function in asthma. The post-salbutamol reduction in \( \Delta P_{O_2} \text{max} \) in patients with severe asthma indicates a decreased pulmonary oxygen delivery as a response to salbutamol inhalation, which is in accordance with existing evidence that the administration of salbutamol may lead to a transient reduction in arterial blood \( O_2 \) tension and saturation in asthmatic patients, including those with chronic stable asthma [337, 338]. The salbutamol-induced hypoxia and the subsequently enhanced cardiovascular side effects are suspected to be responsible for the raised risk of asthma death associated with salbutamol [30, 350, 351]. An aggravated ventilation-perfusion mismatch has proven the reason of decreased pulmonary oxygenation after salbutamol administration [30]. Salbutamol abrogates compensatory pulmonary vasoconstriction and increases pulmonary perfusion in poorly ventilated regions due to its vasodilation effect, leading to a worsening of the ventilation-perfusion imbalance in asthmatic lungs [28, 30].

Unlike arterial blood gas tests, which provide global measurements of the pulmonary oxygenation adequacy, dynamic OE-MRI derived \( \Delta P_{O_2} \text{max} \) maps offer valuable spatial information and may act as a unique tool to visualize and quantify the treatment effect on pulmonary oxygenation at a regional level, which may facilitate the detection of subtle but meaningful post-therapeutic changes [28]. In addition, the lack of improvement in \( \Delta P_{O_2} \text{max} \) and EF despite the rise in forced airflow rates and volumes after salbutamol administration in patients with asthma strengthens the assertion of other researchers that the
ventilation/perfusion mismatch may predominantly be associated with mucus plugging, swelling and remodelling of the small peripheral airways that is hardly mitigated by salbutamol inhalation, rather than reversible bronchoconstriction in large, central airways. In addition, the significant moderate correlation between the percentage change in $\tau_{\text{up}}$ and percentage change in FVC suggests a concomitant improving but not significant trend in $O_2$ delivery rate after salbutamol inhalation partially in relation to the relief of large airway obstruction. Salbutamol resulted in a statistically significant, but small and transient, increase in the heterogeneity of $O_2$ wash-in time above baseline values in healthy controls. This may be due to uneven airway reaction due to salbutamol aerosol intolerance.

The absence of any significant change in dynamic OE-MRI readouts in the control scans excludes the effects of pure $O_2$ and posture on gas exchange as plausible causes of the significant post-salbutamol changes observed in median $\Delta P_{O_2\text{max}}$ and provides evidence of the good immediate (minutes) reproducibility of the dynamic OE-MRI in the assessment of lung function in both asthmatics and healthy controls. Furthermore, the imaging readouts were not significantly different between scans and rescans at baseline, reflecting a good short-term (days) reproducibility of this technique.

There were several limitations to this exploratory study. Firstly, we did not adopt an inhaler placebo in the control scans to account for potential placebo effect of salbutamol nor did we counterbalance the order of scans with and without salbutamol intervention to minimize carryover effects between visits. Secondly, the sample size was relatively small and unequal between groups. However, with the given sample size, the post-salbutamol reduction of median $\Delta P_{O_2\text{max}}$ in the severe asthmatic group survived our conservative statistical analysis and proved significant. It gave us the confidence that the observed changes were genuine. The results from this current study could be used to power future studies. Thirdly, no referencing methods were employed to validate the post-salbutamol changes in pulmonary oxygenation that were detected by dynamic OE-MRI. Arterial blood tension and saturation measured by an arterial blood gas test could be potentially used as coarse references of the adequacy of the overall pulmonary oxygenation. However, the validation of pulmonary oxygenation change at the regional level is difficult because of the lack of gold standard techniques. Fourthly, the single slice MR acquisition restricted the image coverage and may introduce errors in the regional comparisons between repeated measures in a scan and between scan and rescan due to the variation of the slice position. Technical developments in volumetric dynamic OE-MRI are urgently required in order to overcome this limitation.

In conclusion, our work has provided initial evidence of the sensitivity of dynamic OE-MRI to the effect of salbutamol inhalation on pulmonary oxygenation in patients with severe asthma. It supports the potential role of this non-invasive and non-ionizing radiation imaging technique in the evaluation of short-term treatment effects on pulmonary oxygenation in asthma at both regional and global levels.
Chapter 8 Paper 5: T₁-weighted dynamic contrast-enhanced MRI of the lung in asthma: semi-quantitative analysis for the assessment of contrast kinetic characteristics

The asthma DCE-MRI data in this PhD work were collected at two sites: Manchester Welcome Trust Clinical Research Facility for 10 asthmatic patients; Wolfson Molecular Imaging Centre for 20 asthmatic patients and 10 healthy controls. The quality of all datasets was sufficient for a semi-quantitative analysis and thus is included in study reported in this chapter. However, only the datasets collected using the scanner at Wolfson Molecular Imaging Centre enabled robust T₁ mapping necessary for quantitative DCE-MRI analysis which is reported in Chapter 9.

This paper has been submitted to “Radiology” and now it is in the progress of revision.

Authors: Wei-Juan Zhang, Robert M Niven, Simon S Young, Yu-Zhen Liu, Geoffrey JM Parker and Josephine H Naish

From the Centre for Imaging Sciences, Institute of Population Health (W.J.Z., G.J.M.P., J.H.N.), Biomedical Imaging Institute (W.J.Z., G.J.M.P., J.H.N.), The University of Manchester, Oxford Road, Manchester, U.K., M13 9PT; North West Lung Research Centre, University Hospital of South Manchester (R.M.N.), Southmoor Road, Manchester, U.K., M23 9LT; Personalised Healthcare and Biomarkers, AstraZeneca R&D (S.S.Y., YZ.L.), Alderley Park, Macclesfield, U.K., SK10 4TF; Bioxydyn Limited (G.J.M.P.), Pencroft Way, Manchester, U.K., M15 6SZ

Contribution of authors: WJ.Z: study conception and design, approval of ethics, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript preparation and edition. R.M.N: study conception and design, participant enrolment, data interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. S.S.Y and YZ.L: study conception and design, data interpretation, manuscript preparation and edition.
8.1 Abstract

Purpose: to evaluate the contrast agent kinetics of dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) in healthy lungs and asthmatic lungs by using non-model-based semi-quantitative parameters and to explore their relationships with pulmonary function testing and eosinophil level.

Materials and methods: 10 healthy subjects and 30 patients with asthma underwent pulmonary function tests, blood and sputum eosinophil counts and 1.5 tesla DCE-MRI scanning within 7 days. Semi-quantitative parameters of contrast agent kinetics were calculated from the relative signal enhancement time course curves on a pixel-by-pixel basis and summarized using whole-lung median values. Their distribution heterogeneity was assessed using the regional coefficient of variation. DCE-MRI readouts were compared between groups using one-way ANOVA and their relationships with pulmonary function testing and eosinophil count were assessed using Pearson’s correlation analysis.

Results: Asthmatic patients showed significantly lower peak enhancement (SI%max) and initial area under the curve in the first 60 seconds (iAUC60) and significantly lower late-phase washout slope (kwashout) than healthy controls (P < 0.01). The distribution heterogeneity of bolus arrival time (BAT), time-to-peak (TTP), upslope of the first-pass peak (kup) and kwashout, estimated by the median regional coefficient of variation, were significantly higher in asthmatic patients than in healthy controls (P < 0.05). These imaging readouts also showed significant linear correlations with measurements of pulmonary function testing (P < 0.05) but not with eosinophil level in patients with asthma.

Conclusion: The contrast agent kinetic characteristics of T1-weighted DCE-MRI of asthmatic lungs are different from those in healthy lungs and are related to measurements of pulmonary function testing.

8.2 Introduction

Circumstantial evidence has suggested vascular remodelling and angiogenesis, characteristics of chronic airway inflammation, as important contributors to the development and progression of asthma [24, 42, 352]. Because of this, regulation of vessel activation is being considered as a new therapeutic avenue [22]. Characteristic alterations in asthma bronchial vasculature include increased vessel calibre due to vasodilation, increased vessel number due to angiogenesis, vascular wall swelling and perivascular interstitial space oedema due to increased plasma extravasation secondary to increased vascular permeability [352]. However, little human data are available regarding the involvement of the pulmonary circulation in asthma, partly due to the lack of appropriate non-invasive investigation approaches. Nevertheless, limited data from animal models of allergic airway inflammation have provided initial evidence of the existence of pulmonary vascular remodelling characterised by endothelial cell proliferation and vascular muscularization [353, 354]. Such observations motivate our development of a non-invasive method for the in vivo estimation of pulmonary vasculature in patients with asthma.
Dynamic contrast enhanced magnetic resonance imaging (DCE-MRI) is a well-established imaging technique for the estimation of tissue microvascular function in clinical settings. It works by acquiring a number of images to track the passage of a bolus of an intravenously administered diffusible contrast agent through the vasculature, and its distribution within the organ of interest. The contrast agent kinetics can be monitored using either T1-weighted or T2*-weighted methods. The T1-weighted DCE-MRI approach is more sensitive to contrast agent extravasation and is less sensitive to the high magnetic susceptibility differences between lung tissue and air, which can lead to substantial signal loss and artefact [220]. T1-weighted DCE-MRI thus has been employed to explore pulmonary microvascular properties, such as capillary perfusion, microvascular permeability and extracellular leakage space, in healthy non-smokers, smokers, patients with pulmonary embolism, pneumonia, chronic obstructive pulmonary disease and benign or malignant pulmonary nodules [243, 245, 258, 260, 355-357]. However, there is insufficient data demonstrating the feasibility and utility of DCE-MRI in the evaluation of asthma. We hypothesized that the underlying structural and functional changes in pulmonary microvasculature in patients with asthma may alter contrast agent kinetics and that this will be reflected in the shape of signal intensity time course obtained using DCE-MRI.

8.3 Materials and methods
8.3.1 Subjects
30 asthmatic subjects and 10 healthy subjects were recruited between February 2011 and August 2013. All asthmatic subjects withheld short-acting bronchodilators for 6 hours and long-acting bronchodilators for 12 hours prior to each examination. None of the subjects were current smokers and none had smoked within the previous 1 year or had total pack-years > 10. None of the subjects had had any respiratory tract infection or asthma exacerbation (for asthmatics) during the 4 weeks preceding the examinations. Approval for this prospective study was given by the local ethics committee and written informed consent was obtained from all subjects.

8.3.2 Pulmonary function testing
Pulmonary function testing was performed within 7 days prior to the DCE-MRI scan using a plethysmograph (CareFusion, German) according to European Respiratory Society recommendations [32, 34, 38]. Parameters including forced expiratory volume in 1 second (FEV1), forced vital capacity (FVC), maximum mid-expiratory flow (MMEF), total lung capacity (TLC), residual volume (RV) and diffusing capacity of carbon monoxide (DLco) were measured and expressed as a percentage of the measured value to the predicted normal value. FEV1 to FVC ratio and RV to TLC ratio were then calculated as absolute values.

8.3.3 Blood and sputum eosinophil count
Measurements were performed on the same day of pulmonary function testing. A blood sample was obtained from all subjects for full blood cell count. Spontaneous or induced sputum samples were successfully collected from 26 asthmatic patients for the cell
count. The blood eosinophil count was expressed as the actual cell number per volume of blood while sputum eosinophil count was expressed as the percentage to the total cell count. Healthy control subjects did not undergo sputum test. The blood and sputum eosinophil count results within the last 3 years were also checked to determine historic eosinophilic inflammatory pattern.

8.3.4 DCE-MRI scan

DCE-MRI scanning was performed on one of two 1.5 tesla whole-body MR scanners (Philips Achieva, Philips Healthcare, The Netherlands) at two sites: Wellcome Trust Clinical Research Facility, Manchester and Wolfson Molecular Imaging Centre, Manchester. All scans were acquired using the body resonator for radiofrequency transmission and reception.

A three dimensional (3D) T₁- fast field echo (T₁-FFE) with flip angle of 20° was employed to acquire rapid serial volumetric pulmonary images in the coronal plane during free-breathing. 180 dynamic acquisitions were obtained over a total period of 6 min. A bolus of 0.1 mmol/kg gadoterate meglumine (Dotarem®, Guerbet, Paris, France) was injected into the antecubital vein at the 10th acquisition using an automatic power injector at a flow rate of 3 ml/s. Other imaging parameters included: repetition time (TR) = 2.5 ms; echo time (TE) = 0.8 ms; field of view: 375 mm × 375 mm; matrix: 128 × 128 in-plane (88 phase encoding steps); 20 slices with a thickness of 8 mm (16 mm over-contiguous). Over-contiguous slicing (i.e., interpolation in the slice direction within the 3D slab) was utilized to achieve good volume coverage with a temporal resolution of 1.98 s per volume.

8.3.5 Image analysis

For each subject, image analysis was limited to a single coronal slice posterior to the heart and across the descending aorta, in order to minimize through-plane motion and image signal contamination from cardiac motion. Both lungs were segmented and registered to the end expiration position (FVC level) using a semi-automatic registration method [112]. The heart, large hilar vessels and surrounding structures were excluded by k-means clustering analysis. Figure 8.1 illustrates the process of generating the vessel mask by k-means clustering. In brief, the post-registered dynamic acquisitions with the earliest contrast agent passage peak (pulmonary arterial phase) and latest contrast agent passage peak (pulmonary venous phase) were selected and the signal intensities on the two images were classified into 3 clusters by using the k-means clustering method. The cluster with the largest number of pixels was considered as the lung parenchyma and the rest was masked out as large pulmonary arteries and pulmonary veins.
Figure 8.1 The process of generating the vessel mask by k-means clustering method

The post-registered contrast enhanced images (a, pulmonary artery phase; d, pulmonary venous phase) were each classified into 3 clusters by using the k-means clustering method, (b and e). The cluster with the largest number of pixels was considered as the lung parenchyma and the rest was masked out as pulmonary arteries (c) and pulmonary veins (f). The final vessel mask (g) was the combination of the artery mask and vein mask.

Next, the relative signal intensity curve (rSIC) of each pixel was determined by plotting the relative signal intensity within this pixel against imaging time:

\[ \text{SI}\% (t) = \left( \frac{\text{SI}(t) - \text{SI}_0}{\text{SI}_0} \right) \times 100\% \]  

(Eq 8.1)

where SI\% (t) is the percent signal intensity over baseline at time t, SI(t) is the signal intensity at time t, and SI_0 is the baseline (pre-contrast) signal intensity.

Baseline signal intensity (SI_0) was calculated as the average of the first 10 dynamic acquisitions in the pixel. Semi-quantitative parameters were extracted pixel-by-pixel from the rSICs, including the bolus arrival time (BAT), time from BAT to peak (TTP), the maximum relative enhancement during the first passage (SI\%_{max}), the upslope (mean gradient) of the first-pass peak (k_{up}), the late-phase washout slope (k_{washout}) calculated by fitting rSIC segment between the 40th acquisition and the last acquisition with a straight line, the relative signal enhancement retention at the end of the acquisitions (SI\%_{retention}) and the initial area under the rSIC over the first 60 seconds (iAUC_{60}) after bolus arrival, calculated using the trapezoidal rule. BAT was defined as the time interval between the contrast agent administration to the time point of bolus arrival in the pixel, which was automatically detected by fitting the piecewise linear regression from the start to the first pass peak of rSIC [358]. Parameter maps were generated for visual observation and the median values of the parameters were calculated across the lung in the selected slice for statistical analysis. In addition, the regional distribution heterogeneity of each parameter was quantified by generating a local coefficient of variation map [289, 359]. The value of each pixel on the map.
was the coefficient of variation calculated from the $5 \times 5$ neighbourhood pixels. All image analysis was completed using MATLAB R2012a (Mathworks, Natick, USA).

### 8.3.6 Statistical analysis

Statistical analysis was carried out by using IBM SPSS Statistics 20.0 software (IBM, New York, USA). The Kolmogorov-Smirnov test was performed to test for the normality of data. The comparison between healthy subjects and the whole group of patients with asthma was performed using the independent samples t-test for the continuous variables and the $\chi^2$ test for the categorical variables. One-way analysis of variance followed by the Bonferroni’s corrected pairwise comparison was performed to compare imaging parameters between healthy subjects, patients with mild asthma and patients with severe asthma as well as between healthy subjects, patients with eosinophilic asthma and those with non-eosinophilic asthma. Receiver operating characteristic (ROC) analyses were performed to retrospectively evaluate the utility of the imaging readouts as indices for differentiation of asthmatics versus healthy controls. Sensitivity, specificity and accuracy were calculated for each parameter by varying the cut-off values. The correlations between the imaging readouts and the clinical measurements were assessed by using Pearson’s correlation analysis (two-tailed).

### 8.4 Results

DCE-MRI scans were successfully performed in 28 patients with asthma and 10 healthy subjects without adverse event. The injection of contrast agent was unsuccessful in 2 asthmatic patients, due to the failure of intravenous cannulation and breakdown of the power injector. Table 8.1 summarizes the demographic information of the 28 asthmatic patients and 10 healthy subjects. Age, gender, BMI and DLco%predicted were similar between healthy subjects and asthmatic patients. As expected patients with asthma showed significantly lower FEV$_1$%predicted, FEV$_1$/FVC ratio and higher RV/TLC ratio than healthy subjects, indicating the presence of airflow limitation and air trapping. 19 out of 28 asthmatic patients were on inhaled or/and oral corticosteroid treatment. The group averaged Asthma Control Test (ACT) score was $17 \pm 5$. 16 out of 28 asthmatic patients fulfilled the criterion of FEV$_1$%predicted < 85% and treatment requirement = British Thoracic Society asthma guideline step 4-step 5 and were clinically labelled as “severe asthma”. The other 12 asthmatic patients who fulfilled the criteria of FEV$_1$%predicted $\geq$ 85% and treatment requirement = British Thoracic Society asthma guideline step 1-step 2 and were clinically labelled as “mild asthma” [9]. 16 out of 28 asthmatic patients were diagnosed as having an “eosinophilic phenotype” according to the testing results in the past 3 years and 12 of them present raised eosinophil in either blood or sputum during the study period. The other 12 asthmatic patients did not have abnormal blood and sputum eosinophil count in the past 3 years and during the study period and were categorized as demonstrating a “non-eosinophilic phenotype”. The eosinophilic asthma and non-eosinophilic asthma groups had similar age, gender, ACT score and proportion of severe disease cases (50 % vs 62.5%, $P = 0.508$), while the mild asthma group and severe asthma group had similar age, gender, proportion of eosinophilic
cases (50 % vs 62.5%, P = 0.508) but significantly different ACT score (20 ± 3 vs 15 ± 5, P = 0.003).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy controls (n = 10)</th>
<th>Asthma (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>45 ± 9</td>
<td>43 ± 11</td>
<td>0.679</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>4/6</td>
<td>13/15</td>
<td>0.726</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>28 ± 6</td>
<td>30 ± 6</td>
<td>0.337</td>
</tr>
<tr>
<td>ACT score</td>
<td>-</td>
<td>17 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>EOS blood, 10⁹/L</td>
<td>0.09 ± 0.03</td>
<td>0.31 ± 0.27</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>EOS sputum, %</td>
<td>-</td>
<td>4 ± 7</td>
<td>-</td>
</tr>
<tr>
<td>EOS current status, +/-</td>
<td>-</td>
<td>12/16</td>
<td>-</td>
</tr>
<tr>
<td>EOS 3 year status, +/-</td>
<td>-</td>
<td>16/12</td>
<td>-</td>
</tr>
<tr>
<td>Regular ICS, +/-</td>
<td>-</td>
<td>19/9</td>
<td>-</td>
</tr>
<tr>
<td>FEV₁%predicted, %</td>
<td>105 ± 9</td>
<td>75 ± 25</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>FEV₁/FVC ratio, %</td>
<td>78 ± 5</td>
<td>63 ± 14</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>RV/TLC ratio, %</td>
<td>34 ± 7</td>
<td>44 ± 11</td>
<td>0.013</td>
</tr>
<tr>
<td>MMEF%predicted, %</td>
<td>74 ± 15</td>
<td>39 ± 23</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>DLco%predicted, %</td>
<td>86 ± 14</td>
<td>85 ± 12</td>
<td>0.736</td>
</tr>
</tbody>
</table>

Data was presented as mean ± standard deviation; P value < 0.05 is presented in bold; BMI: body mass index; ACT: Asthma Control Test questionnaire; EOS: eosinophil; ICS: inhaled corticosteroids; FEV₁: forced expiratory volume in 1s; FEV₁%predicted: percentage of predicted FEV₁; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; TLC: total lung capacity; RV: residual volume; DLco: diffusing capacity of carbon monoxide; %predicted: percentage of predicted value. Comparison is performed by using independent samples t-test or χ² test.

Figure 8.2 illustrates the group averaged mean rSICs of the lung parenchyma from healthy subjects and patients with asthma. Both rSICs comprised a sharp first-pass peak, a smaller second-recirculation peak and a steady contrast agent washout during the late redistribution phase. Though the first-pass peaks were reached approximately at the same time in the two groups, the rSICs of asthmatic group presented a generally lower signal enhancement level, a smaller first-pass peak, a more gradual late-phase downslope and thus a smaller area under the curve than seen in the rSICs of the healthy control group.
Figure 8.2 Group averaged relative signal enhancement curves of the lung parenchyma from healthy subjects and patients with asthma.

(a) shows the group averaged curves together (healthy control in blue; asthma in red, y axis scaling from -200% to 1400%). (b) shows the group averaged curve from healthy subjects along with the error bars (± standard deviation), y axis scaling from -200% to 1800%. (c) shows the group averaged curve from asthmatic patients along with the error bars (± standard deviation), y axis scaling from -200% to 1800%.
Figure 8.3 provides example parameter maps of SI%$_{\text{max}}$ and $k_{\text{washout}}$ from a healthy subject and a patient with severe non-eosinophilic asthma. The parameter value maps for the asthmatic patient are heterogeneous and show lower SI%$_{\text{max}}$ and slower $k_{\text{washout}}$ whilst they are homogeneous with relatively higher SI%$_{\text{max}}$ and faster $k_{\text{washout}}$ distributed across the lung field for the healthy subject. The regional heterogeneity of the distribution of SI%$_{\text{max}}$ and $k_{\text{washout}}$ in these two subjects were quantified and displayed using the parameter coefficient of variation maps in figure 8.3. The coefficient of variation maps of SI%$_{\text{max}}$ appeared similar between the two subjects while regionally elevated coefficient of variation of $k_{\text{washout}}$, the indicator of increased contrast agent extravasation heterogeneity, is present in the asthmatic subject. The example maps of SI%$_{\text{max}}$ and $k_{\text{washout}}$ for the other patient subgroups are presented in figure 8.4 along with the reference maps from another healthy subject. As shown in figure 8.3 and 8.4, the widespread reduction in SI%$_{\text{max}}$ occurs in the examples of all four asthmatic subgroups relative to the healthy control examples, while the slow $k_{\text{washout}}$ seems more apparent in the severe asthmatic examples than in the mild asthmatic examples.
Figure 8.3 Example parameter maps and regional coefficient of variation (CoV-) maps of $SI_{\text{max}}$ and $k_{\text{washout}}$ from a healthy subject (Male, 34 years old, $FEV_1\%_{\text{predicted}} = 93\%$) and a patient with asthma (Male, 48 years old, $FEV_1\%_{\text{predicted}} = 45\%, \text{severe, non-eosinophilic}$).
Figure 8.4 Example parameter maps of $SI_{\text{max}}$ and $k_{\text{washout}}$ from a healthy subject (Female, 47 years old, FEV$_1$%predicted=104%) and patients with non-eosinophilic mild asthma (Female, 49 years old, FEV$_1$%predicted=90%), eosinophilic mild asthma (Male, 44 years old, FEV$_1$%predicted=106%) and eosinophilic severe asthma (Male, 48 years old, FEV$_1$%predicted=29%).
Table 8.2 and figure 8.5 compare the imaging readouts between healthy subjects and patients with asthma. Patients with asthma showed significantly lower medians of $\text{SI}_{\text{max}}^\text{a}$ and $\text{iAUC}_{60}$ and significantly slower median $k_{\text{washout}}$ than healthy controls. However, medians of $\text{BAT}$, $\text{TTP}$, $\text{SI}_{\text{retention}}^\text{a}$ and $k_{\text{up}}$ were similar between groups. In addition, patients with asthma also presented significantly higher median coefficient of variations of $\text{BAT}$, $\text{TTP}$, $k_{\text{up}}$ and $k_{\text{washout}}$ than seen in healthy controls, whilst median coefficient of variations of $\text{SI}_{\text{max}}^\text{a}$, $\text{SI}_{\text{retention}}^\text{a}$ and $\text{iAUC}_{60}$ were similar between groups.

**Table 8.2** Comparison of the DCE-MRI readouts between healthy subjects and patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Health controls (n=10)</th>
<th>Asthma (n=28)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{BAT}$, min</td>
<td>0.42 ± 0.02</td>
<td>0.41 ± 0.02</td>
<td>0.600</td>
</tr>
<tr>
<td>$\text{TTP}$, min</td>
<td>0.12 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.779</td>
</tr>
<tr>
<td>$\text{SI}_{\text{max}}^\text{a}$, %</td>
<td>1575 ± 337</td>
<td>1195 ± 191</td>
<td>$&lt; 0.001$</td>
</tr>
<tr>
<td>$\text{SI}_{\text{retention}}^\text{a}$, %</td>
<td>201 ± 32</td>
<td>175 ± 41</td>
<td>0.078</td>
</tr>
<tr>
<td>$k_{\text{up}}$, %·min$^{-1}$</td>
<td>14059 ± 5208</td>
<td>11370 ± 3643</td>
<td>0.083</td>
</tr>
<tr>
<td>$k_{\text{washout}}$, %·min$^{-1}$</td>
<td>-32 ± 11</td>
<td>-22 ± 7</td>
<td>$0.002$</td>
</tr>
<tr>
<td>$\text{iAUC}_{60}$, au</td>
<td>520 ± 97</td>
<td>406 ± 87</td>
<td>$0.002$</td>
</tr>
<tr>
<td><strong>Median coefficient of variation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\text{BAT}$, %</td>
<td>2.5 ± 0.7</td>
<td>3.3 ± 1.0</td>
<td>0.029</td>
</tr>
<tr>
<td>$\text{TTP}$, %</td>
<td>16 ± 5</td>
<td>24 ± 8</td>
<td>0.008</td>
</tr>
<tr>
<td>$\text{SI}_{\text{max}}^\text{a}$, %</td>
<td>26 ± 4</td>
<td>27 ± 3</td>
<td>0.257</td>
</tr>
<tr>
<td>$\text{SI}_{\text{retention}}^\text{a}$, %</td>
<td>26 ± 6</td>
<td>29 ± 5</td>
<td>0.268</td>
</tr>
<tr>
<td>$k_{\text{up}}$, %</td>
<td>28 ± 6</td>
<td>36 ± 9</td>
<td>0.011</td>
</tr>
<tr>
<td>$k_{\text{washout}}$, % §</td>
<td>30 ± 6</td>
<td>36 ± 7</td>
<td>$0.032$</td>
</tr>
<tr>
<td>$\text{iAUC}_{60}$, %</td>
<td>26 ± 5</td>
<td>26 ± 4</td>
<td>0.945</td>
</tr>
</tbody>
</table>

Data is presented as mean ± standard deviation; P value < 0.05 is presented in bold; $\text{BAT}$: bolus arrival time; $\text{TTP}$: time to peak; $\text{SI}_{\text{max}}^\text{a}$: maximum relative signal enhancement; $\text{SI}_{\text{retention}}^\text{a}$: relative signal enhancement retention; $k_{\text{up}}$: upslope of the first-pass peak; $k_{\text{washout}}$: downslope in the late-redistribution phase; $\text{iAUC}_{60}$: initial area under the curve in the first 60 seconds; § calculated as the coefficient of variation of the magnitude of $k_{\text{washout}}$. Comparison is performed by using independent samples t-test.
Figure 8.5 Boxplots show the comparison of median SI%\textsubscript{max} (a), median k\textsubscript{washout} (b), median iAUC\textsubscript{60} (c) and median local coefficient of variation of k\textsubscript{washout} (d) between healthy control and asthma groups.

* indicates P value < 0.05 and ** indicates P value < 0.01

Table 8.3, figure 8.6 and figure 8.7 compare the imaging readouts between healthy subjects and asthma subgroups of disease severity and eosinophil status. Significantly lower medians of SI%\textsubscript{max} and iAUC\textsubscript{60} and significantly slower median k\textsubscript{washout} were found in both the eosinophilic asthma group and the non-eosinophilic asthma group than seen in the healthy group and they were comparable between the two asthmatic subgroups. Median SI%\textsubscript{max} was also significantly lower in both the mild asthma group and the severe asthma group than in healthy group. However, only the severe asthma group but not the mild asthma group showed significantly lower medians of iAUC\textsubscript{60} and significantly slower median k\textsubscript{washout} than the healthy group. These three parameters were also significantly different between the mild asthma group and the severe asthma group. In addition, the eosinophilic asthma group but not the non-eosinophilic asthma group showed significantly higher median
coefficients of variation for BAT, TTP, \( k_{up} \) and \( k_{washout} \) than the healthy group, indicating greater local functional vascular heterogeneity. The severe asthma group but not the mild asthma group also showed significantly higher median coefficient of variation of \( k_{washout} \) than the normal group.

\[\text{Figure 8.6} \text{ Boxplots show the comparison of median SI\%\text{max} (a), median } k_{washout} \text{ (b), median } \text{iAUC}_{60} \text{ (c) and median local coefficient of variation of } k_{washout} \text{ (d) between healthy control, mild asthma and severe asthma groups.} \]

* indicates P value < 0.05 and ** indicates P value < 0.01.
Figure 8.7 Boxplots show the comparison of median SI%\textsubscript{max} (a), median k\textsubscript{washout} (b), median iAUC\textsubscript{60} (c) and median local coefficient of variation of k\textsubscript{washout} (d) between healthy control, non-eosinophilic asthma and eosinophilic asthma groups.

* indicates P value < 0.05 and ** indicates P value < 0.01.
Table 8.3 Comparison of the DCE-MRI readouts between healthy control group and asthma subgroups of disease severity and eosinophil status

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Health controls (n=10)</th>
<th>Mild asthma (n=12)</th>
<th>Severe asthma (n=16)</th>
<th>Health controls (n=10)</th>
<th>Non-eosinophilic asthma (n=12)</th>
<th>Eosinophilic asthma (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT, min</td>
<td>0.42 ± 0.02</td>
<td>0.42 ± 0.03</td>
<td>0.41 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.42 ± 0.02</td>
<td>0.41 ± 0.02</td>
</tr>
<tr>
<td>TTP, min</td>
<td>0.12 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.12 ± 0.03</td>
<td>0.11 ± 0.03</td>
<td>0.11 ± 0.03</td>
</tr>
<tr>
<td>SI%&lt;sub&gt;max&lt;/sub&gt;, %</td>
<td>1575 ± 337</td>
<td>1274 ± 220 *</td>
<td>1136 ± 147 **</td>
<td>1575 ± 337</td>
<td>1219 ± 192 **</td>
<td>1176 ± 195 **</td>
</tr>
<tr>
<td>SI%&lt;sub&gt;retention&lt;/sub&gt;, %</td>
<td>201 ± 32</td>
<td>192 ± 44</td>
<td>162 ± 35 *</td>
<td>201 ± 32</td>
<td>182 ± 45</td>
<td>170 ± 39</td>
</tr>
<tr>
<td>k&lt;sub&gt;up&lt;/sub&gt;, %·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>14059 ± 5208</td>
<td>11627 ± 4471</td>
<td>11178 ± 3023</td>
<td>14059 ± 5208</td>
<td>11788 ± 4358</td>
<td>11057 ± 3118</td>
</tr>
<tr>
<td>k&lt;sub&gt;washout&lt;/sub&gt;, %·min&lt;sup&gt;-1&lt;/sup&gt;</td>
<td>-32 ± 11</td>
<td>-27 ± 6</td>
<td>-19 ± 6 **, †</td>
<td>-32 ± 11</td>
<td>-23 ± 8 *</td>
<td>-21 ± 7 **</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;60&lt;/sub&gt;, au</td>
<td>520 ± 97</td>
<td>456 ± 78</td>
<td>369 ± 76 **, †</td>
<td>520 ± 97</td>
<td>417 ± 82 *</td>
<td>398 ± 93 **</td>
</tr>
</tbody>
</table>

Median coefficient of variation

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Health controls (n=10)</th>
<th>Mild asthma (n=12)</th>
<th>Severe asthma (n=16)</th>
<th>Health controls (n=10)</th>
<th>Non-eosinophilic asthma (n=12)</th>
<th>Eosinophilic asthma (n=16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT, %</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
<td>3 ± 1</td>
<td>3 ± 1</td>
<td>4 ± 1 **, †</td>
</tr>
<tr>
<td>TTP, %</td>
<td>16 ± 5</td>
<td>22 ± 9</td>
<td>25 ± 7</td>
<td>16 ± 5</td>
<td>20 ± 6</td>
<td>26 ± 8 **</td>
</tr>
<tr>
<td>SI%&lt;sub&gt;max&lt;/sub&gt;, %</td>
<td>26 ± 4</td>
<td>26 ± 3</td>
<td>28 ± 4</td>
<td>26 ± 4</td>
<td>27 ± 3</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>SI%&lt;sub&gt;min&lt;/sub&gt;, %</td>
<td>26 ± 6</td>
<td>26 ± 3</td>
<td>31 ± 6</td>
<td>26 ± 6</td>
<td>27 ± 7</td>
<td>30 ± 4</td>
</tr>
<tr>
<td>k&lt;sub&gt;up&lt;/sub&gt;, %</td>
<td>28 ± 6</td>
<td>33 ± 8</td>
<td>38 ± 9</td>
<td>28 ± 6</td>
<td>33 ± 8</td>
<td>38 ± 9 **</td>
</tr>
<tr>
<td>k&lt;sub&gt;washout&lt;/sub&gt;, % 6</td>
<td>30 ± 6</td>
<td>32 ± 5</td>
<td>39 ± 7 **, ††</td>
<td>30 ± 6</td>
<td>34 ± 9</td>
<td>37 ± 6 *</td>
</tr>
<tr>
<td>iAUC&lt;sub&gt;60&lt;/sub&gt;, %</td>
<td>26 ± 5</td>
<td>25 ± 3</td>
<td>27 ± 5</td>
<td>26 ± 5</td>
<td>26 ± 5</td>
<td>27 ± 4</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation; * significantly different from healthy control (P < 0.05); ** significantly different from healthy control (P < 0.01); † significantly different from mild asthma/non-eosinophilic asthma (P < 0.05); †† significantly different from mild asthma/non-eosinophilic asthma (P < 0.01); § calculated as the coefficient of variation of the magnitude of k<sub>washout</sub>. Comparison is performed by using one-way analysis of variance followed by the Bonferroni post hoc test.
Figure 8.8 shows the ROC curves of median SI%\textsubscript{max}, median \( k_{\text{washout}} \) and median iAUC\textsubscript{60} in the differentiation of asthma from healthy controls and table 8.4 lists the sensitivity, specificity and accuracy of the differentiation according to different cut-off points. Areas under the ROC curves were 0.90 (95% confidence interval (CI): 0.81, 1.0) for median SI%\textsubscript{max}, 0.79 (95% CI: 0.64, 0.94) for median \( k_{\text{washout}} \) and 0.82 (95% CI: 0.69, 0.96) for median iAUC\textsubscript{60}. The sensitivity and specificity were 82% and 90% for a threshold value of 1323% for median SI%\textsubscript{max}, 68% and 70% for a threshold value of -25%/min for median \( k_{\text{washout}} \) and 68% and 70% for a threshold value of 445 for median iAUC\textsubscript{60}.

Table 8.4 Sensitivity and specificity of different cut-off points of median SI%\textsubscript{max}, median \( k_{\text{washout}} \) and median iAUC\textsubscript{60} in the differentiation between asthma and healthy control

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cut-off point</td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>Median SI%\textsubscript{max} (%) = 1323</td>
<td>82 (23/28)</td>
<td>90 (9/10)</td>
<td>84 (32/38)</td>
</tr>
<tr>
<td>Median ( k_{\text{washout}} ) (%/min) = -25</td>
<td>68 (19/28)</td>
<td>70 (7/10)</td>
<td>68 (26/38)</td>
</tr>
<tr>
<td>Median iAUC\textsubscript{60} (au) = 445</td>
<td>68 (19/28)</td>
<td>70 (7/10)</td>
<td>68 (26/38)</td>
</tr>
</tbody>
</table>
Figure 8.8 The receiver operating characteristic curves of median SI%\textsubscript{max}, median $k_{\text{washout}}$ and median iAUC60 in the differentiation of asthma from healthy control.

(a) the receiver operating characteristic (ROC) curves of median SI%\textsubscript{max} (red curve), median $k_{\text{washout}}$ (blue curve) and median iAUC60 (green curve) in differentiating asthma from healthy control. (b-c) show the sensitivity and specificity across levels of these three imaging readouts in differentiating asthma from healthy control.

Figure 8.9 and table 8.5 show the Pearson’s correlation coefficients between imaging readouts and the clinical measurements in patients with asthma. Medians of SI%\textsubscript{max}, SI%\textsubscript{retention} and iAUC60 were positively correlated with FEV\textsubscript{1}\%predicted, FEV\textsubscript{1}/FVC and MMEF\%predicted. Median $k_{\text{washout}}$ was negatively correlated with ACT score, FEV\textsubscript{1}\%predicted, FEV\textsubscript{1}/FVC and MMEF\%predicted and positively correlated with RV/TLC. There were also negative correlations of median SI%\textsubscript{max} with age and RV/TLC, median $k_{\text{up}}$ with age and positive correlation of median BAT with ACT score. In addition, the median coefficient of variations of BAT, TTP, SI%\textsubscript{retention}, $k_{\text{up}}$ and $k_{\text{washout}}$ were negatively correlated with...
FEV₁%predicted, FEV₁/FVC and MMEF%predicted. The median coefficient of variations of SI%retention and k\textsubscript{washout} were also positively correlated with RV/TLC. However, no correlation was found between imaging readouts and blood and sputum eosinophil count.

Figure 8.9 Scatter plots showing the linear correlation of median SI\textsubscript{max} and median k\textsubscript{washout} with FEV₁%predicted and FEV₁/FVC in patients with asthma (n=28).

The correlation coefficients r and the P values are presented together. The dash lines show the 95% confidence intervals.
Table 8.5 Pearson’s correlation between DCE-MRI readouts and the clinical measurements in patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Age</th>
<th>ACT score</th>
<th>FEV$_1$% predicted</th>
<th>MMEF% predicted</th>
<th>FEV$_1$/FVC ratio</th>
<th>RV/TLC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Median</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAT, min</td>
<td>0.240 (0.220)</td>
<td>0.392 (0.039)</td>
<td>0.281 (0.148)</td>
<td>0.280 (0.149)</td>
<td>0.148 (0.453)</td>
<td>-0.289 (0.144)</td>
</tr>
<tr>
<td>TTP, min</td>
<td>0.252 (0.195)</td>
<td>0.373 (0.051)</td>
<td>0.188 (0.338)</td>
<td>0.261 (0.180)</td>
<td>0.177 (0.368)</td>
<td>-0.076 (0.705)</td>
</tr>
<tr>
<td>SI$_{\text{max}}$, %</td>
<td><strong>-0.492 (0.008)</strong></td>
<td>0.163 (0.406)</td>
<td><strong>0.428 (0.023)</strong></td>
<td>0.421 (0.026)</td>
<td>0.551 (0.002)</td>
<td><strong>-0.387 (0.046)</strong></td>
</tr>
<tr>
<td>SI$_{\text{retention}}$, %</td>
<td>-0.089 (0.652)</td>
<td>0.078 (0.692)</td>
<td><strong>0.411 (0.030)</strong></td>
<td>0.380 (0.046)</td>
<td>0.544 (0.003)</td>
<td>-0.190 (0.342)</td>
</tr>
<tr>
<td>$k_{\text{up}}$, %</td>
<td><strong>-0.418 (0.027)</strong></td>
<td>-0.199 (0.310)</td>
<td>-0.018 (0.926)</td>
<td>-0.069 (0.728)</td>
<td>0.048 (0.808)</td>
<td>-0.068 (0.735)</td>
</tr>
<tr>
<td>$k_{\text{washout}}$, %</td>
<td>0.058 (0.771)</td>
<td><strong>-0.418 (0.027)</strong></td>
<td>-0.597 (0.001)</td>
<td>-0.607 (0.001)</td>
<td>-0.696 (&lt; 0.001)</td>
<td><strong>0.422 (0.028)</strong></td>
</tr>
<tr>
<td>iAUC$_{60}$, au</td>
<td>-0.307 (0.112)</td>
<td>0.308 (0.111)</td>
<td>0.576 (0.001)</td>
<td>0.566 (0.002)</td>
<td>0.696 (&lt; 0.001)</td>
<td><strong>-0.417 (0.031)</strong></td>
</tr>
</tbody>
</table>

**Median coefficient of variation**

<table>
<thead>
<tr>
<th></th>
<th>BAT, %</th>
<th>TTP, %</th>
<th>SI$_{\text{max}}$, %</th>
<th>SI$_{\text{retention}}$, %</th>
<th>$k_{\text{up}}$, %</th>
<th>$k_{\text{washout}}$, %</th>
<th>iAUC$_{60}$, au</th>
</tr>
</thead>
<tbody>
<tr>
<td>BAT, %</td>
<td>-0.023 (0.908)</td>
<td>-0.065 (0.741)</td>
<td>-0.434 (0.021)</td>
<td>-0.409 (0.030)</td>
<td>-0.514 (0.005)</td>
<td>0.200 (0.318)</td>
<td></td>
</tr>
<tr>
<td>TTP, %</td>
<td>-0.025 (0.898)</td>
<td>-0.258 (0.185)</td>
<td>-0.419 (0.026)</td>
<td>-0.477 (0.010)</td>
<td>-0.512 (0.005)</td>
<td>0.332 (0.090)</td>
<td></td>
</tr>
<tr>
<td>SI$_{\text{max}}$, %</td>
<td>0.080 (0.686)</td>
<td>-0.012 (0.950)</td>
<td>-0.333 (0.083)</td>
<td>-0.369 (0.054)</td>
<td>-0.355 (0.064)</td>
<td>0.192 (0.339)</td>
<td></td>
</tr>
<tr>
<td>SI$_{\text{retention}}$, %</td>
<td>0.093 (0.639)</td>
<td>0.032 (0.871)</td>
<td><strong>-0.588 (0.001)</strong></td>
<td><strong>-0.568 (0.002)</strong></td>
<td><strong>-0.604 (0.001)</strong></td>
<td><strong>0.435 (0.023)</strong></td>
<td></td>
</tr>
<tr>
<td>$k_{\text{up}}$, %</td>
<td>0.032 (0.872)</td>
<td>-0.160 (0.416)</td>
<td><strong>-0.455 (0.015)</strong></td>
<td><strong>-0.502 (0.006)</strong></td>
<td><strong>-0.546 (0.003)</strong></td>
<td>0.232 (0.371)</td>
<td></td>
</tr>
<tr>
<td>$k_{\text{washout}}$, %</td>
<td><strong>-0.088 (0.656)</strong></td>
<td>0.308 (0.311)</td>
<td><strong>0.643 (&lt; 0.001)</strong></td>
<td><strong>0.659 (&lt; 0.001)</strong></td>
<td><strong>0.653 (&lt; 0.001)</strong></td>
<td><strong>-0.516 (0.006)</strong></td>
<td></td>
</tr>
<tr>
<td>iAUC$_{60}$, %</td>
<td>0.122 (0.536)</td>
<td>-0.061 (0.759)</td>
<td><strong>-0.377 (0.048)</strong></td>
<td><strong>-0.421 (0.026)</strong></td>
<td><strong>-0.404 (0.033)</strong></td>
<td>0.327 (0.095)</td>
<td></td>
</tr>
</tbody>
</table>

Data was presented as correlation coefficient (P value), P values < 0.05 is presented in bold; BAT: bolus arrival time; TTP: time to peak; SI$_{\text{max}}$: maximum relative signal enhancement; SI$_{\text{retention}}$: relative signal enhancement retention; $k_{\text{up}}$: upslope of the first-pass peak; $k_{\text{washout}}$: downslope in the late-redistribution phase; iAUC$_{60}$: initial area under the curve in the first 60 seconds; ACT: Asthma Control Test questionnaire; FEV$_1$: forced expiratory volume in 1s; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; TLC: total lung capacity; RV: residual volume; %predicted: percentage of predicted value; § calculated as the coefficient of variation of the magnitude of $k_{\text{washout}}$. 

181
8.5 Discussion

The present study demonstrates that the characteristics of contrast agent kinetics monitored using DCE-MRI differ between asthmatic lungs and healthy lungs and are related to measurements of pulmonary function. To the best of our knowledge, this is the first published study regarding the feasibility of DCE-MRI parameters in reflecting pulmonary microvasculature alterations in patients with asthma.

We have shown that the rSIC of asthmatic lungs is characterised by a smaller first-pass peak and a slower washout during the late redistribution phase than observed in healthy controls. Previous simulation and in vivo studies have demonstrated that the first-pass peak may be predominantly affected by fractional blood flow and vascular permeability while the late-redistribution segment seems mainly determined by the volume of extravascular extracellular space [360, 361]. In addition, the rise in the volume of blood plasma present in the tissue may amplify the signal enhancement in the first passage, but have a much smaller effect at later time-points [361]. Normal lung is highly vascular with rich blood supply and small vascular permeability and small extravascular interstitial space. These properties determine that normal lung tissue has a similar rSIC shape to the blood in its feeding arteries, with a sharp and narrow first-pass peak as the hallmark. Lack of a first-pass peak has been observed in the cases of pulmonary embolism, lung malignancies, benign tumors and acute pneumonia and has been attributed to the reduction of regional perfusion and the high extravasation of contrast agent due to the substantially increased vascular permeability, respectively [245, 355-357]. Early reports also described a reduced but still well-defined signal peak during the first passage of contrast agent in 11 of 13 COPD patients, which is similar to the rSIC we observed in asthma [356]. In emphysema, capillary beds are damaged with reduced capillary density and this leads to a reduction in regional pulmonary blood flow and pulmonary blood volume. This has been proposed as a plausible reason for the low first-pass peak seen in COPD [356]. However, there is no known loss of capillary beds in asthmatic lungs. We suggest however, that the presence of oedema and mucus may be responsible for the low peak in the lungs of patients with severe asthma. In lung MRI, the measurements of DCE-MRI reflect the kinetics of the concentration of the contrast agent in the MRI-visible tissue of the lung. The presence of oedema and mucus in severe asthmatic lungs can increase the amount of MRI-visible tissue of the lung and may lead to a lower concentration of contrast agent during the first pass peak even without any changes in the blood volume or blood flow to the lung as a whole.

iAUC$_{60}$ reflects the amount of contrast agent delivered to and retained by the lung tissue in the first 60 seconds after bolus arrival. It covers the duration of the first and second passage of the contrast agent and hence predominantly associated with the blood flow and vascular permeability [362]. The information provided by iAUC$_{60}$ is therefore similar to SI%$_{max}$.

In addition, the slower contrast agent elimination rate seen during the late-redistribution phase in asthmatic lungs may reflect an increased extravascular extracellular
space. The larger the extravascular extracellular space the greater the potential for accumulation of contrast agent and the longer it takes to wash out the contrast agent from the lung tissue. The enlarged extravascular extracellular space may be a marker of interstitial oedema or accumulation of fluid within the alveoli and small airways and has been previously confirmed in asthmatic airways by histological observation [352]. The current study seems to imply that the enlarged extravascular extracellular space may also occur in asthmatic lung parenchyma. On the other hand, the vascular permeability may be altered in asthma and add further complexity to the interpretation of the curve shape. In fact, the relationship between the rSIC shape and variable vascular properties are complex and it is difficult to elucidate the pure contribution of each vascular factor on the shape of each curve segment. Therefore, caution must be employed when using the curve-shape related semi-quantitative parameters to interpret the underlying pathophysiology. Further specificity may be possible with the application of pharmacokinetic modeling methods to the DCE-MRI data.

Nevertheless, the semi-quantitative DCE-MRI parameters appear to be promising biomarkers for asthma assessment in clinical settings. SI%\max, k\washout and iAUC\_60 are sensitive descriptors of the altered kinetic characteristics in asthma and correlate with the degree of airflow limitation and air trapping, with SI%\max being especially sensitive to the early changes in mild asthmatic patients whose FEV\_1%\_predicted and FEV\_1/FVC are still normal. A threshold of 1323% for the median SI%\max differentiated asthma subjects from healthy controls with a sensitivity of 82% and a specificity of 90% in this dataset. Furthermore, most of the parameters showed uneven distribution in asthmatic lungs, as determined by measurement of the local coefficient of variation of each parameter, and the magnitude of uneven distribution increases as airway function worsens. This mirrors the heterogeneous distribution of the underlying functional and structural impairments, a recognized feature of asthma [24]. Mapping the regional coefficient of variation of certain parameter is a simple way to quantify functional heterogeneity and meanwhile retain the spatial information [289, 359].

The inflammation phenotype seems not to affect the shape of rSIC in the group of asthmatics studied in this work and there is no linear correlation between the semi-quantitative parameters and eosinophil level. This suggests that contrast agent kinetic characteristics in asthma are insensitive to the type of inflammation. It is possible that the mechanisms leading to altered capillary permeability and oedema build up in asthma are independent of the inflammatory process and mask any additional effects on the vasculature due to inflammation per se. In addition, the sample size is relatively small and all patients were under different treatments which may affect the eosinophil levels. It is possible that if larger groups of eosinophilic and non-eosinophilic phenotypes with well-controlled treatment were available then a subtle effect could be apparent that is beyond the sensitivity of our study to detect.

This current study has several limitations. Firstly, the semi-quantitative DCE-MRI parameters are based on relative signal enhancement, which can be reasonably assumed
linearly proportional to the contrast agent concentration in the tissue under some circumstances. However, this linearity is lost when the contrast agent concentration is high, which can be the case in studies of the lung, due to its high blood volume. Under these circumstances, the parameters derived from the relative signal intensity curve, especially the peak height, might be underestimated relative to what may be expected from direct measurements of contrast agent concentration. This problem could be solved by calculating these parameters from the contrast agent concentration curves, which could be determined by measuring the baseline $T_1$ and the dynamic $T_1$ change [363]. Secondly, inter-subject variations in the contrast agent concentration in the feeding arteries may affect rSIC, especially the first-pass behavior [246, 361]. This effect could be minimized by normalizing the lung rSIC to the rSIC of the feeding arteries. However, additional care must be taken to minimize the inflow effect and partial volume effect on arterial rSIC. Similarly, a normalizing method such as this could also account for the day-to-day scanner settings. However, such variations are unlikely to lead to systematic differences between the subject groups, although they could serve to introduce unwanted random variation and therefore reduce the sensitivity of the method to detect differences. Third, the pathophysiological meanings of the reduced signal enhancement peak and the slower contrast agent elimination rate in asthma are still not fully understood. This may be elucidated in future studies by exploring the relationships between the semi-quantitative DCE-MRI parameters and the tracer kinetic modelling-derived quantitative DCE-MRI parameters in asthmatic lungs.

In conclusion, this study confirms the feasibility of $T_1$-weighted DCE-MRI in the assessment of asthma. The contrast kinetics differ between asthmatic lungs and normal lungs and can be evaluated using semi-quantitative DCE-MRI parameters, which we have shown are closely related to airway function. This non-invasive and non-ionizing imaging technique may have a role to play in the evaluation and monitoring of pulmonary microvasculature in asthma in vivo and thus may be of value in studies of disease characteristics and in clinical trials of novel interventions.
Chapter 9 Paper 6: T$_1$-weighted dynamic contrast-enhanced MRI of the lung in asthma: quantitative analysis for the assessment of microvascular function alterations in disease

This paper is in progress of 3$^{rd}$ round author review, aiming for submission to a peer-reviewed radiology journal by December 2014.

Authors: Wei-Juan Zhang, Robert M Niven, Simon S Young, Yu-Zhen Liu, Geoffrey JM Parker and Josephine H Naish

From the Centre for Imaging Sciences, Institute of Population Health (WJ.Z., G.J.M.P., J.H.N.), Biomedical Imaging Institute (WJ.Z., G.J.M.P., J.H.N.), The University of Manchester, Oxford Road, Manchester, U.K., M13 9PT; North West Lung Research Centre, University Hospital of South Manchester (R.M.N.), Southmoor Road, Manchester, U.K., M23 9LT; Personalised Healthcare and Biomarkers, AstraZeneca R&D (S.S.Y., YZ.L.), Alderley Park, Macclesfield, U.K., SK10 4TF; Bioxydyn Limited (G.J.M.P.), Pencroft Way, Manchester, U.K., M15 6SZ

Contribution of authors: WJ.Z: study conception and design, approval of ethics, participant enrolment, data acquisition, analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript preparation and edition. R.M.N: study conception and design, participant enrolment, data interpretation, quality control of data and algorithms, manuscript reviewing. G.J.M.P and J.N: study conception and design, data analysis and interpretation, quality control of data and algorithms, statistical analysis, manuscript reviewing. S.S.Y and YZ.L: study conception and design, data interpretation, manuscript preparation and edition.
9.1 Abstract

Purpose: To assess the feasibility of modelling dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) contrast kinetics in the lung in order to extract quantitative parameters associated with capillary permeability, extravascular extracellular space and blood volume in asthmatic patients and to assess differences in these microvascular parameters between patients and healthy subjects.

Materials and methods: 10 healthy subjects and 20 asthmatic patients underwent pulmonary function tests, eosinophil count and T₁-weighted DCE-MRI at 1.5 tesla within 7 days. The extended Tofts model was fitted to the contrast agent concentration time course curves on a pixel-by-pixel basis within the lung to extract measurements of the contrast agent transfer coefficient ($K^{\text{trans}}$), fractional leakage volume ($v_e$) and fractional blood plasma volume ($v_p$). Initial area under the contrast agent concentration-time curve over the first 60 seconds was also calculated. Parameters were summarized using median values over the lung field of view and evidence for differences between groups was assessed.

Results: Median $v_e$ was significantly higher in asthmatic patients ($0.30 \pm 0.06 \text{ ml/ml tissue}$) than in the healthy subjects ($0.23 \pm 0.02 \text{ ml/ml tissue}$, $P = 0.001$). No difference was observed between mild and severe asthmatics. Median $K^{\text{trans}}$ showed borderline significant difference between patients with severe asthma ($0.28 \pm 0.07 \text{, ml/ml tissue/min}$) and those with mild asthma ($0.19 \pm 0.07 \text{, ml/ml tissue/min}$, $P = 0.040$), but was not significantly different between the combined asthmatic group and healthy controls. $v_p$ and $\text{iAUC}_{60}$ were not significantly different between healthy subjects and asthmatic patients.

Conclusion: T₁-weighted DCE-MRI quantitative parameters are promising biomarkers of pulmonary inflammation in asthma, with the fractional extravascular extracellular space $v_e$ in particular sensitive to the inflammation-associated increase in extracellular space in asthma. The contrast agent transfer coefficient $K^{\text{trans}}$, which is related to vascular permeability and blood flow, and $v_p$, a measurement of blood volume, do not distinguish asthmatics from controls, although there is some evidence that $K^{\text{trans}}$ may be sensitive to the severity of asthma.

9.2 Introduction

Vascular remodelling has been recognized as one of the hallmarks of asthma, affecting tracheobronchial circulation and pulmonary circulation, both in patients during acute asthma attack and those with chronic stable asthma [24, 42, 352-354, 364]. These vascular changes occur in response to chronic airway inflammation in asthma and may aggravate airflow limitation in many ways. For instance, enhanced microvascular permeability increases airway secretions and causes mucosal oedema which, accompanied with vasodilation and congestion due to the increased blood infusion, further narrows the airways [364]. Slowing down or reversing the altered microvasculature may indicate a new therapeutic strategy in asthma [22].

Current assessment of vascular morphological changes relies on direct visualization via bronchoscopy or X-ray computed tomography (CT) in vivo and the microscopic
observation of biopsy specimens in vitro [42]. The evaluation of vascular permeability is achieved by measuring plasma protein exudates in sputum and bronchoalveolar lavage fluid [44-46]. These techniques are invasive, indirect and qualitative. Imaging techniques including CT, scintigraphy, positron emission tomography (PET) and single-photon emission computed tomography (SPECT) have been used to evaluate regional pulmonary perfusion in asthma with the help of intravenous tracers [53, 94, 95]. However, they have the drawback of ionizing radiation exposure in common and none of them has been used successfully to measure vascular permeability. There is therefore a strong motivation to develop a non-invasive, non-ionizing tool to provide reliable and quantitative estimation of pulmonary perfusion and vascular leakage in asthma.

Quantitative dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) has a substantial history of application to measure microvascular functional status. Its use has been well-documented for the evaluation of tumour vascularity and it has recently been introduced to imaging of the lung [220, 230]. It works by acquiring a series of MR images, usually longitudinal relaxation time ($T_1$)-weighted, during the passage and redistribution of an intravenous bolus of an MR contrast agent. The time series of the tissue concentration of the contrast agent determines the observed change in signal intensity. This signal can be converted to measurements of contrast agent concentration variation over time, which can be interpreted using an appropriate tracer kinetic model to extract quantitative parameters related to the tissue vascular properties. The extended Tofts model is one of the most commonly used kinetic models for the description of the extravasation of contrast agent, and has been applied in a small number of studies in the lung [245]. It yields parameters closely linked to vascular physiology: the contrast agent transfer coefficient ($K_{trans}$), a compound factor associated with blood flow, capillary surface area and transendothelial permeability; the extravascular extracellular fraction ($v_e$), the space per unit volume of tissue for contrast agent to leak; the blood plasma fraction ($v_p$), the plasma space per unit volume of tissue.

Quantitative $T_1$-weighted DCE-MRI has been reported to be feasible in healthy smokers, non-smokers, patients with chronic obstructive pulmonary disease (COPD), pulmonary hypertension, pulmonary embolism, and lung cancer, with the imaging readouts being related to the conventional pulmonary function tests and global perfusion measurements [242, 243, 258, 260, 261, 357]. However, there is no clinical data regarding the utility of this imaging technique in asthmatic lungs. In addition, most of the previous studies quantified pulmonary perfusion from the first-passage of the contrast agent based on the indicator dilution theory. Unlike the application of the extended Tofts model, however, the limited data acquisition period and assumption of no extravasation of contrast agent in indicatory dilution theory makes this approach unsuitable to probe vascular leakage.

We hypothesize that $T_1$-weighted DCE-MRI with the use of extended Tofts model applied to a data acquired over an extended time-series is feasible in asthmatic patients and that it is sensitive to the potentially altered pulmonary perfusion and microvascular permeability in patients with asthma.
9.3 Material and methods

9.3.1 Subjects

A total of 20 non-smoking asthmatic subjects and 10 non-smoking healthy subjects were enrolled between July 2012 and August 2013. All asthmatic subjects withheld short-acting bronchodilators for 6 hours and long-acting bronchodilators for 12 hours prior to each examination. None of the subjects were current smokers and none had smoked within the previous 1 year or had pack-years > 10. None of the subjects had had any respiratory tract infection or asthma exacerbation (for asthmatics) during the 4 weeks preceding the examinations. Approval for this prospective study was given by the local research ethics committee and written informed consent was obtained from all subjects.

9.3.2 Pulmonary function testing

Pulmonary function testing was performed within 7 days prior to the DCE-MRI scan by using a plethysmograph (CareFusion, Germany) according to European Respiratory Society recommendations [32, 34, 38]. Parameters including forced expiratory volume in 1 second (FEV₁), forced vital capacity (FVC), specific total airway conductance (sGtot), specific total airway resistance (sRtot), functional residual volume (FRV), total lung capacity (TLC), residual volume (RV), diffusing capacity of carbon monoxide (DLco) were measured and expressed as a percentage of the measured value to the predicted normal value. FEV₁ to FVC ratio and RV to TLC ratio were then calculated.

9.3.3 Enumeration of blood and sputum eosinophils

Measurements were performed on the same day as pulmonary function testing. A blood sample was obtained from all subjects for the full blood cell count. Spontaneous or induced sputum samples were successfully collected from 17 asthmatic patients for the cell count. The blood eosinophil count was expressed as the actual cell number per volume of blood while sputum eosinophil count was expressed as the percentage of the total cell count. Healthy control subjects did not undergo sputum test. The blood and sputum eosinophil count results within the last 3 years were also checked.

9.3.4 DCE-MRI scan

DCE-MRI scanning was performed on all subjects using a 1.5 tesla whole-body MR system (Philips Achieva, Philips Healthcare, The Netherlands) using the body resonator for radiofrequency transmission and reception.

A three dimensional (3D) T₁-weighted radiofrequency spoiled fast field echo (FFE) sequence with four flip angles (FA = 2°, 10°, 20°, 30°) was employed to acquire volumetric pulmonary images in the coronal plane during free-breathing in order to measure the baseline T₁ of the lung. This was followed by 180 dynamic acquisitions throughout the passage and redistribution of the contrast agent using the same sequence with a flip angle of 20° over 6 min. A bolus of 0.1 mmol/kg gadoterate meglumine (Dotarem®, Guerbet, Paris, France) was injected into the antecubital vein at the 10th acquisition using an automatic power injector at a flow rate of 3 ml/s. Other imaging parameters included: repetition time (TR) = 2.5 ms; echo time = 0.8 ms; field of view: 375 mm × 375 mm; matrix: 128 × 128 in-
plane (88 phase encoding steps); 20 slices with a thickness of 8 mm (16 mm over-contiguous). Over-contiguous slicing (i.e., interpolation in the slice direction within the 3D slab) was utilized to achieve a temporal resolution of 1.98 s per volume.

9.3.5 Image analysis

For each subject, image analysis was limited to a selected slice posterior to the heart and across the descending aorta. Both lungs were segmented and registered to the end expiration position (functional residual volume, FRC, level) by using a semi-automatic registration method [112]. Large vessels were removed by using the masks generated by a k-mean clustering method (see figure 8.1 in chapter 8). In brief, the post-registered dynamic acquisitions with the earliest (pulmonary arterial phase) and latest (pulmonary venous phase) arrived first-peak were selected and the signal intensities on the two images were classified into 3 clusters using k-means clustering. The cluster with the largest number of pixels was considered lung parenchyma and the rest was masked out as pulmonary arteries and pulmonary veins. The quantitative analysis, including the kinetic model fitting, was then performed on a pixel-by-pixel basis as detailed previously [245]. The baseline $T_1$ was measured using variable flip angle approach and the signal intensity-versus-time curve was converted into a $T_1$-versus-time curve. The tissue contrast agent concentration at each time point in the dynamic series was then determined by the change in $T_1$, which was further fitted by the extended Tofts model to extract the parameters including $K^{\text{trans}}$ (ml/ml tissue/min), $v_e$ (ml/ml tissue) and $v_p$ (ml/ml tissue). The contrast agent concentration of the feeding artery (i.e. the arterial input function) used in the model fitting was calculated in the same way as tissue concentration from a manually defined region of interest defined in a cross-section of the main pulmonary artery. In addition, the initial area under the tissue contrast agent concentration curve from the time of first contrast agent appearance in the main pulmonary artery to the 60 seconds after that ($\text{iAUC}_{60}$, min·mmol/l) was calculated as a model-free parameter (the calculation of $\text{iAUC}_{60}$ in this chapter was based on concentration-time curve that is different to the $\text{iAUC}_{60}$ in the chapter 8 which was calculated based on the relative signal intensity-time curve). Parameter maps were generated for visual observation and the parameters were also summarized for the statistical analysis using the median values across the lung field in the selected slice. Any pixels with $v_e + v_p > 1$ were excluded from calculating the median on the basis that they represent model fitting errors. All image analysis was completed using MATLAB R2012a (Mathworks, Natick, USA).

9.3.6 Statistical analysis

Statistical analysis was carried out by using IBM SPSS Statistics 20.0 software (IBM, New York, USA). The Kolmogorov-Smirnov test was performed to test for the normality of data. The comparison between healthy subjects and the whole group of patients with asthma was performed using the independent samples t-test for the continuous variables and the $\chi^2$ test for the categorical variables. In patients with asthma, the two-way factorial ANOVA analysis was performed to estimate the independent and interactive effects of eosinophilic status and disease severity on each imaging parameter. The correlations
between the imaging readouts and clinical measurements were assessed by Pearson’s correlation analysis.

9.4 Results

DCE-MRI scans were carried out safely and there were no adverse events reported by study participants. The injection of contrast agent was unsuccessful in 2 asthmatic patients, due to failure of intravenous cannulation and breakdown of the power injector. 2 patient datasets and 1 healthy control dataset were extremely noisy and did not allow good arterial input function extraction due to motion artefact, and gave unrealistically high values in $v_e$ and $v_p$ maps. These datasets were removed from statistical calculation. In total, the datasets from 8 healthy subjects and 17 asthmatic patients were finally included in the statistical analysis.

Table 9.1 lists the demographic information for the 8 healthy subjects and 17 asthmatic patients. There was no significant difference in age, gender, BMI or DLco%predicted between groups. Patients with asthma showed significantly lower FEV$_1$%predicted and FEV$_1$/FVC ratio and higher RV/TLC ratio and blood eosinophil level than healthy subjects, reflecting the presence of airflow limitation, air trapping and airway inflammation. 9 out of 17 asthmatic patients were categorized as eosinophilic asthma phenotype because they presented abnormally raised eosinophil count either in blood (> 0.4x10$^9$/L) or in sputum (>3% of cell counts) during the study period and/or frequently within the last 3-years were categorized as eosinophilic phenotype. The other 8 patients were of the non-eosinophilic asthma phenotype as there was no evidence of elevated eosinophils during the study period and within the last 3 years. In addition, 10 out of 17 asthmatic patients fulfilled the criteria of FEV$_1$%predicted < 85% and treatment requirement = British Thoracic Society asthma guideline step 4-step 5 and were clinically labelled as “severe asthma”. 7 asthmatic patients fulfilled the criteria of FEV$_1$%predicted ≥ 85% and treatment requirement = British Thoracic Society asthma guideline step 1-step 2 and were clinically labelled as “mild asthma”. All 10 severe asthmatic patients and 2 out of 7 mild asthmatic patients were receiving treatment with inhaled corticosteroids. The eosinophilic asthma group and non-eosinophilic asthma group had similar asthma control test (ACT) score (18 ± 5 vs 18 ± 5, P = 0.925) and proportion of severe disease cases (66% vs 50%, P = 0.486), while the mild asthma group and the severe asthma group had similar proportions of eosinophilic cases (43% vs 60%, P = 0.486) but significantly different ACT scores (22 ± 2 vs 16 ± 4, P = 0.001).
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Healthy controls (n=8)</th>
<th>Asthma (n=17)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, year</td>
<td>42 ± 11</td>
<td>47 ± 10</td>
<td>0.241</td>
</tr>
<tr>
<td>Gender, male/female</td>
<td>4/3</td>
<td>8/9</td>
<td>0.653</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>25 ± 5</td>
<td>29 ± 5</td>
<td>0.091</td>
</tr>
<tr>
<td>ACT score</td>
<td>-</td>
<td>18 ± 5</td>
<td>-</td>
</tr>
<tr>
<td>EOS status, -/+</td>
<td>-</td>
<td>8/9</td>
<td>-</td>
</tr>
<tr>
<td>Blood EOS count, 10⁹/L</td>
<td>0.08 ± 0.03</td>
<td>0.36 ± 0.32</td>
<td>0.002</td>
</tr>
<tr>
<td>Sputum EOS count, %</td>
<td>-</td>
<td>5.5 ± 8.0</td>
<td>-</td>
</tr>
<tr>
<td>Asthma severity, mild/severe</td>
<td>-</td>
<td>7/10</td>
<td>-</td>
</tr>
<tr>
<td>ICS treatment, -/+</td>
<td>-</td>
<td>5/12</td>
<td>-</td>
</tr>
<tr>
<td>FEV₁,%predicted, %</td>
<td>104 ± 10</td>
<td>75 ± 28</td>
<td>0.001</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>77 ± 5</td>
<td>63 ± 15</td>
<td>0.002</td>
</tr>
<tr>
<td>MMEF,%predicted, %</td>
<td>73 ± 16</td>
<td>41 ± 23</td>
<td>0.001</td>
</tr>
<tr>
<td>sRₜot,%predicted, %</td>
<td>198 ± 106</td>
<td>84 ± 18</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>sGₜot,%predicted, %</td>
<td>109 ± 29</td>
<td>60 ± 33</td>
<td>0.002</td>
</tr>
<tr>
<td>RV/TLC, %</td>
<td>34 ± 7</td>
<td>44 ± 12</td>
<td>0.030</td>
</tr>
<tr>
<td>DLco,%predicted, %</td>
<td>86 ± 16</td>
<td>87 ± 12</td>
<td>0.848</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation; BMI: body mass index; ACT: Asthma Control Test questionnaire; EOS: eosinophil; ICS: inhaled corticosteroids; FEV₁: forced expiratory volume in 1 s; FVC: forced vital capacity; MMEF: maximum mid-expiratory flow; sRₜot: specific total airway resistance; sGₜot: specific total airway conductance; TLC: total lung capacity; RV: residual volume; DLco: diffusing capacity of carbon monoxide; %predicted: percentage of predicted value.

The extended Tofts model was found to provide a good fit to the tissue contrast agent concentration time course curves both for healthy subjects and asthmatic patients (figure 9.1). The asthmatic lung shows a broadly similar concentration curve shape to healthy normal lung tissue in the region shown in figure 9.1, including a sharp and fast reached first-pass peak followed with a second-recirculation peak and a smooth washout during the late redistribution. However, the asthmatic curve shows a slower washout, indicating a greater quantity of contrast agent leaking into the lung tissue from the vasculature. Figure 9.2 shows example parameter maps of $K^{\text{trans}}$, $V_e$, $V_p$, and $\text{iAUC}_{60}$ for a healthy subject and an asthmatic patient. The parameter maps for this asthmatic patient are heterogeneous and show regionally elevated $K^{\text{trans}}$ and $V_e$ and reduced $V_p$. In the healthy subject all parameters are relatively homogeneous across the lungs.
Figure 9.1 ROI concentration time courses (green dots) and extended Tofts model fitting (dash line) for a healthy subject (figure 2a) and an asthmatic patient (figure 2b). The ROI was drawn in the upper lobe of the right lung. Insets highlight the individual arterial input functions.
Figure 9.2 Example parameter maps of $K_{\text{trans}}$, $v_e$, $v_p$ and $i\text{AUC}_{60}$ from a healthy subject (Male, 44 years old, FEV$_1$ %predicted=106%) and a patient with asthma (Male, 48 years old, FEV$_1$ %predicted=29%).
Table 9.2 compares the DCE-MRI readouts between the asthmatic group and the healthy control group. Patients with asthma presented with significantly higher median $v_e$ (0.30 ± 0.06 ml/ml tissue) than healthy subjects (0.23 ± 0.02 ml/ml tissue, $P = 0.001$) whilst median $K^{\text{trans}}$, median $v_p$ and median $i\text{AUC}_{60}$ were not significantly different between healthy subjects and asthmatic patients.

Table 9.2 Comparison of the DCE-MRI readouts between healthy subjects and patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Median $K^{\text{trans}}$ (ml/ml tissue/min)</th>
<th>Median $v_e$ (ml/ml tissue)</th>
<th>Median $v_p$ (ml/ml tissue)</th>
<th>$i\text{AUC}_{60}$ (min-mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy (n=8)</td>
<td>0.23 ± 0.11</td>
<td>0.23 ± 0.02</td>
<td>0.29 ± 0.09</td>
<td>1.73 ± 0.33</td>
</tr>
<tr>
<td>Asthma (n=17)</td>
<td>0.25 ± 0.08</td>
<td>0.30 ± 0.06 **</td>
<td>0.29 ± 0.10</td>
<td>1.89 ± 0.55</td>
</tr>
<tr>
<td>P value</td>
<td>0.597</td>
<td>0.001</td>
<td>0.982</td>
<td>0.463</td>
</tr>
</tbody>
</table>

Data are presented as mean ± standard deviation; ** significantly different from the healthy control group ($P = 0.001$); $K^{\text{trans}}$: transfer coefficient; $v_e$: fractional extravascular extracellular space volume; $v_p$: fractional blood plasma volume; $i\text{AUC}_{60}$: initial area under the contrast agent concentration curve in the first 60 seconds.

In patients with asthma, a two-way factorial ANOVA was carried out to evaluate the independent and interactive effects of disease severity (mild and severe) and eosinophil status (eosinophilic and non-eosinophilic) of asthma on each of the DCE-MRI readouts (table 9.3; figure 9.3). Disease severity ($F = 5.326, P = 0.040$) was an independent influence factor for median $K^{\text{trans}}$ in asthma, with significantly increased median $K^{\text{trans}}$ seen in severe asthmatic patients (0.28 ± 0.07 ml/ml tissue/min) relative to the mild asthmatic patients (0.19 ± 0.07 ml/ml tissue/min). Eosinophil status ($F = 0.606, P = 0.451$) did not affect median $K^{\text{trans}}$ in asthmatic patients and there was no significant interaction between disease severity and eosinophil status ($F = 0.265, P = 0.616$) in their effect on median $K^{\text{trans}}$. In addition, neither disease severity nor eosinophil status was an independent influence factor for median $v_e$, median $v_p$ and median $i\text{AUC}_{60}$ in asthma, and they did not have significant interaction in their effects on the three imaging readouts ($P > 0.05$).

When the asthmatic patient data and healthy control data are pooled, median $v_e$ was found to be correlated with blood eosinophil count ($r = 0.488, P = 0.013$), $R_{102}\%_{\text{predicted}}$ ($r = 0.490, P = 0.013$), $sG_{100}\%_{\text{predicted}}$ ($r = -0.492, P = 0.017$), $RV\%_{\text{predicted}}$ ($r = 0.418, P = 0.038$) and $RV/TLC$ ($r = 0.408, P = 0.043$) and median $K^{\text{trans}}$ was correlated with $sG_{100}\%_{\text{predicted}}$ ($r = -0.486, P = 0.022$) and $FRC\%_{\text{predicted}}$ ($r = 0.448, P = 0.028$). However, these linear correlations disappear when calculated within the asthmatic patient group or the healthy control group, respectively. Instead, asthmatic patients showed a significant correlation between the ACT scores and median $v_e$ ($r = -0.650, P = 0.005$). No correlation was found between median $i\text{AUC}_{60}$ and pulmonary function tests. No correlation was found between imaging readouts and age or BMI for pooled data or for asthmatic patients.
Table 9.3 The evaluation of independent and interactive effects of disease severity and eosinophil status of asthma on DCE-MRI readouts in patients with asthma

<table>
<thead>
<tr>
<th></th>
<th>Disease severity (mild vs severe)</th>
<th>Eosinophil status (eos vs non-eos)</th>
<th>Disease severity X eosinophil status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median $K_{\text{trans}}$ (ml/ml tissue/min)</td>
<td>F = 5.326, P = 0.040*</td>
<td>F = 0.606, P = 0.451</td>
<td>F = 0.265, P = 0.616</td>
</tr>
<tr>
<td>Median $v_e$ (ml/ml tissue)</td>
<td>F = 0.071, P = 0.794</td>
<td>F = 0.826, P = 0.380</td>
<td>F = 0.647, P = 0.436</td>
</tr>
<tr>
<td>Median $v_p$ (ml/ml tissue)</td>
<td>F = 3.056, P = 0.104</td>
<td>F = 0.831, P = 0.379</td>
<td>F = 0.014, P = 0.901</td>
</tr>
<tr>
<td>iAUC$_{60}$ (min mmol/l)</td>
<td>F = 0.026, P = 0.874</td>
<td>F = 1.321, P = 0.271</td>
<td>F = 2.923, P = 0.111</td>
</tr>
</tbody>
</table>

Data are presented as "F value, p value" derived from two-way factorial ANOVA; two independent variables are asthma severity (mild, severe) and eosinophil status (eosinophilic, non-eosinophilic); X: interactive effect between disease severity and eosinophil status on DCE-MRI readouts. $K_{\text{trans}}$: transfer coefficient; $v_e$: fractional extravascular extracellular space volume; $v_p$: fractional blood plasma volume; iAUC$_{60}$: initial area under the contrast agent concentration curve in the first 60 seconds. * P value < 0.05.

Figure 9.3 The influence of disease severity (mild vs severe) and eosinophil status (solid lines: eosinophilic; dotted lines: non-eosinophilic) on the group means of median $K_{\text{trans}}$ (a), $v_e$ (b) and $v_p$ (c) in patients with asthma. The grey dashed lines showed the group means of the health control group.

9.5 Discussion

In this dataset, the extended Tofts model provided a good description of the contrast agent kinetics both in normal healthy lungs, as reported previously [245, 260], and in asthmatic lungs. The median $v_e$ significantly increased from 0.23 ml/ml lung tissue in healthy subjects to 0.30 ml/ml lung tissue in patients with asthma. This indicates either an enlargement of the extravascular extracellular space in asthmatic lungs or the build-up of excess fluid into which the contrast agent may leak, probably resulting from the inflammation induced bronchial wall and parenchymal oedema. This finding fits with an early study which found widened gaps between the collagen fibrils in perivascular structures of airway.
specimens of patients dying of acute asthma attack and biopsy specimens obtained from the middle lobe bronchus of asthmatic patients relative to those of healthy controls [352, 365]. Although no published histological data are available regarding the interstitial space of the lung parenchyma in patients with asthma, partly ascribed to the difficulty in obtaining the specimens, limited investigations have proven the occurrence of inflammatory infiltration in alveolar walls which is similar but more severe than the airway inflammation [366, 367]. Thus, it is reasonable to deduce that a similar enlargement in the extravascular extracellular space may be present in the lung parenchyma of patients with asthma, as suggested by DCE-MRI. In addition, interstitial oedema within the airway wall has been widely demonstrated in fatal asthma, acute severe asthma and asthma under methacholine challenge [368, 369]. Contrast agent that has leaked from the vasculature may be able to pass from the interstitial space to neighbouring oedema, which then effectively becomes an extension of the space described by the raised $v_e$. Furthermore, the current study demonstrates that the increase in the apparent leakage space (either due to the enlargement of extravascular extracellular space or oedematous fluid build-up) generally exists in asthmatic lungs regardless of the disease severity and eosinophilic status and there is no clear correlation between the extent of $v_e$ increase and the level of airway dysfunction and peripheral eosinophilia in the asthmatic patient group. These seem to indicate that the occurrence of asthma per se, rather than the disease severity or inflammatory type, may be the critical determinant of $v_e$.

Normal lung tissue is rich in blood supply and low in vascular permeability. Thus, $K_{\text{trans}}$ extracted from the extended Tofts model is expected to be predominantly limited by the vascular permeability rather than by the blood flow [245]. This assumption should also be valid in the asthmatic lung unless a substantial reduction or absence of regional perfusion is present (which might occur in acute severe asthma). Hence, we speculate that the significantly higher $K_{\text{trans}}$ across the lung field in severe asthmatic patients than in mild asthmatic patients observed in our study reflects elevated vascular leakage in the bronchial and pulmonary circulation as asthma progresses. This finding is in agreement with previous research that found that the amount of plasma proteins that extravasates into the sputum and bronchoalveolar lavage fluid is higher in asthmatic patients and is associated with asthma severity [44-46]. Whilst the $K_{\text{trans}}$ difference between healthy subjects and asthmatic patients was not statistically significant in this dataset, possibly owing to the small sample size and the higher interindividual variation of $K_{\text{trans}}$ than $v_e$, this study did give the insight into the relationship between vascular leakage and asthma severity by using the non-invasive DCE-MRI technique. The negative correlation between $K_{\text{trans}}$ and airway conductance and the positive correlation between $K_{\text{trans}}$ and functional residual capacity found in this study also added support to this relationship. However, the level of vascular leakage is not significantly different between the eosinophilic and non-eosinophilic asthma subgroups, implying a potentially less effect of the type of the inflammation on vascular permeability. This is not surprising as neutrophil infiltration is also seen and is even more
common than eosinophil infiltration in the bronchial mucosal capillaries and venules and may also contribute to vascular leakage [370]. The raised vascular leakage in asthma could be pathologically attributed to enhanced permeability due to the formation of interendothelial gaps on venular walls and the neovascularization of permeable vessels, according to histological findings in the bronchial vasculature in patients with asthma [370]. There is insufficient human data to clarify the cause of increased leakage in pulmonary vasculature in asthma, but the limited data from animal models of allergic airway inflammation has indicated the existence of vascular remodelling in the pulmonary circulation [353, 354].

The DCE-MRI measurements of fractional blood plasma volume, \( v_p \), were found to be similar between healthy subjects and patients with asthma, indicating a comparable blood volume per MRI-visible volume of lung tissue between two groups. In asthma, inflammatory vasoactive mediators dilate the tracheobronchial vessels and angiogenesis expands the bronchial capillary beds, while poor ventilation induces hypoxic vasoconstriction in the pulmonary circulation. These two opposite alterations might counteract each other and maintain the overall fractional blood volume in the normal range, at least when measured at the whole lung level, as in this study. However, the significant negative correlation between ACT score and \( v_p \) in the asthma group tends to imply a link of decreased \( v_p \) linked to asthma symptoms. We suspect that the worsened hypoxia in more severe asthma may induce further pulmonary vasoconstriction such that the fractional blood volume is accordingly reduced. The picture of increased \( v_e \) with comparable \( v_p \) in asthmatic lungs could also be attributed to the presence of interstitial oedema and mucus in the lungs. \( v_e \) and \( v_p \) are the fractions of the MRI-visible tissue rather than the fractions of the whole lung. It is likely that there is an overall increase in MRI-visible tissue/oedema in asthma due to an increased oedema and blood volume with the absolute amount of cellular material staying roughly the same. \( v_e \) and \( v_p \) could then behave as observed.

In terms of application in the lung, the quantitative DCE-MRI technique using the extended Tofts model has previously only been exploited for healthy non-smokers, smokers, patients with COPD and patients with lung cancer [245, 260, 261]. This current study extends this method to patients with asthma for the first time. Significant increases in \( K_{\text{trans}} \) and \( v_p \) have been observed in smokers relative to non-smokers and these have been attributed to smoking-related chronic inflammation in the lung [260]. The combination of this early finding and the results of the present study indicate that \( K_{\text{trans}} \) and \( v_p \) may be general biomarkers of lung inflammation, independent to the type of inflammation. However, care must be taken when interpreting \( K_{\text{trans}} \) as it is a composite parameter reflecting both blood flow and capillary leakage. In situations with blood flow limitation, such as in COPD, the change in \( K_{\text{trans}} \) may be a reflection of the alteration in regional perfusion rather than inflammation-related vascular permeability [261]. In addition, \( v_p \) was found to be similar between patients with asthma and healthy non-smokers in the current study, but has been reported to be reduced significantly in smokers and patients with COPD [260, 261]. This suggests that \( v_p \) may be a biomarker of the underlying destruction of pulmonary capillary
beds and may thus provide additional information in the differentiation between asthma and COPD.

This study has several limitations. First, the sample size is relatively small and the effect of current asthma treatment is not possible to ascertain. In addition, recruited patients had to be stable and not in an acute asthma attack, limiting the data to “stable” mild and severe asthma, with no ability to assess the impact of asthma “instability”. Second, no gold-standard tests were performed to validate the findings of DCE-MRI, although well-documented evidence from the literature has been presented to support them. Therefore, further studies with a larger cohort and well-controlled treatment factors are required to validate the observed DCE-MRI differences and correlations by comparing DCE-MRI with other established techniques, such as PET and SPECT for pulmonary perfusion estimation, bronchoscopy examination and histological observation for vascular morphometric estimation, nitric oxide tests for airway inflammation estimation and bronchoalveolar lavage fluid analysis for the estimation of vascular leakage. Third, the extended Tofts model was employed to ensure a reliable model fitting at a temporal resolution of 1.98 seconds per volume. However, this model is not able to independently quantify the blood flow component and capillary permeability component from the compound parameter $K^{\text{trans}}$. This may not be an important issue if the observed alterations are dominated by one of the two components. However, there is inevitably some residual uncertainty in using $K^{\text{trans}}$ to interpret the underlying pathophysiology, particularly where vascular permeability and perfusion alterations are expected to occur together. Alternative kinetic modelling methods, such as the adiabatic approximation to the tissue homogeneity model (AATH), which has been proven available in human lungs, could address this issue but a high temporal resolution is needed, particularly in the lung, where the first passage of contrast agent is very rapid [245].

In conclusion, the present study demonstrates two DCE-MRI quantitative parameters as potential biomarkers of pulmonary inflammation in asthma, with the fractional extravascular extracellular space $v_e$ sensitive to the presence of asthma and the transfer factor $K^{\text{trans}}$, an analogue of vascular permeability, sensitive to the severity of asthma. To our best of knowledge, this is the first published study using DCE-MRI to quantitatively assess the lung in human adults with asthma. The added spatial information of extravascular extracellular space and vascular leakage of the lung obtained non-invasively by DCE-MRI with the use of a non-radioactive tracer makes it an attractive tool for the investigation of the pulmonary microstructural response to inflammation in patients with asthma in vivo.
Chapter 10 Summary and conclusion

This thesis aimed to investigate the feasibility and usefulness of three proton MRI techniques, MR qS\textsubscript{0} mapping, dynamic OE-MRI and DCE-MRI, in the estimation of pulmonary structural and functional changes in patients with asthma and COPD.

The main impetuses of the body of work were 1) the urge to address the high health and societal burdens of asthma and COPD and 2) the clear need for improved regional assessments of lung structure and function to become available for clinical usage and longitudinal monitoring. The rapid development of imaging techniques has allowed the visualization, localization and quantification of structural and functional changes in the lung. Within the available imaging methods proton MRI techniques surpass CT, radionuclide lung imaging and HP gas MRI techniques in the key feature that they do not require ionizing radiation exposure or complicated and expensive setup requirements. Hence, proton MRI techniques have great potential to be adopted in clinical settings for lung assessment in patients susceptible to ionizing radiation and for longitudinal monitoring of local changes and treatment effects in diseased lungs. Although proton MRI techniques have been previously demonstrated as promising candidates to fulfil clinical needs in the characterisation of the lung, there are still relatively few clinical studies, particularly in the investigation of asthma. This strongly motivated the current PhD project. Several studies were then carried out within the past 4 years by the author with the aim to fill the gap and add to the evidence on the utility of proton MRI techniques in patients with asthma and COPD.

There is a variety of proton MRI techniques that are available for lung imaging, of which three specific techniques considered most promising were explored in the current work due to the limits of time and funds: 1) MR qS\textsubscript{0} mapping for the assessment of emphysematous changes in lung structure in patients with COPD; 2) dynamic OE-MRI for the assessment of pulmonary oxygenation impairment in patients with COPD and asthma and for the assessment of treatment effects of salbutamol inhalation on the lung function in patients with asthma; 3) DCE-MRI for the assessment of pulmonary capillary perfusion and pulmonary microvasculature in patients with asthma. The choice to investigate these three proton MRI techniques rather than the others in this PhD project was driven by three reasons. Firstly, these three techniques were assessed and developed to attempt to solve important clinical issues and fill clinical gaps in the asthma and COPD fields (see table 10.1). Secondly, these three techniques are not novel but are either well-established or have been under development for use in the clinical practice (but as yet poorly evaluated in the chosen areas of COPD and asthma). Thirdly, these methods are straightforward to implement, analyse and interpret by radiologists and clinicians. Further motivation for evaluating these techniques is provided in table 10.1.
Table 10.1 Reasons to investigate MR $q_S_0$ mapping, dynamic OE-MRI and DCE-MRI

<table>
<thead>
<tr>
<th>The clinical issue attempting to solve/ the clinical gap attempting to fill</th>
<th>What proton MRI technique was chosen for investigation and why</th>
</tr>
</thead>
</table>
| A non-ionizing radiation imaging alternative to chest CT for emphysema assessment in patients with COPD in clinical settings has long been desired. | Technique: MR $q_S_0$ mapping  
Reason to choose this technique:  
- Recognized method for the estimation of lung density and lung water (see section 3.6.3)  
- Given the fact that emphysematous change causes the reduction in the lung water and thus lung density, MR $q_S_0$ mapping was hypothesized sensitive to emphysema.  
- Straightforward to implement on standard clinical MR scanners without additional need for any contrast agents  
- MR $q_S_0$ calculation is relatively simple. |
| The lack of methods for the in vivo estimation of the integrated functionality of a regional lung unit in terms of the pulmonary oxygen delivery. | Technique: dynamic OE-MRI  
Reason to choose this technique:  
- Straightforward to implement on standard clinical MR scanners with only one additional but cheap requirement: inhaling high concentration $O_2$ as contrast agent.  
- Due to the use of $O_2$ as contrast agent, OE-MRI represents a unique tool for the assessment of regional pulmonary oxygen delivery, the prime function of the lung governed by the complex interplay between local ventilation, perfusion and diffusing capacity.  
- Cumulative literature and studies have proven the feasibility of OE-MRI techniques in the assessment of lung function in a variety of lung disorders other than asthma. |
| Given the importance of pulmonary microvascular remodelling in the presence and progress of asthma, there is a clear need to develop a non-invasive method for the in vivo estimation of pulmonary microvascular characteristics. | Technique: DCE-MRI  
Reason to choose this technique:  
- DCE-MRI has a substantial history of application to measure microvascular functional status outside the lung. Its use has been well-documented for the evaluation of tumour vascularity. This experience gained in tumours and other organs can be adapted to facilitate the application of DCE-MRI in the lung.  
- DCE-MRI is a relatively well-established technique for clinical usage; straightforward to implement on standard clinical MR scanners with an additional requirement of intravenous contrast agent. |
The following paragraphs will briefly summarize the findings of each study, discuss their clinical implications and the potential pulmonary application of these techniques in patients with asthma, COPD and other lung disorders.

Chapter 4 and chapter 5 present two studies conducted in patients with COPD. The retrospective study reported in chapter 4 explored the ability of MR qS₀ mapping in portraying emphysema distribution and quantifying lung tissue density in patients with COPD by comparing it with quantitative CT. In patients with COPD, the lung regions with values below 0.20 in qS₀ maps showed high spatial consistency with CT defined emphysematous regions, indicating that MR qS₀ mapping is capable of localizing and delineating emphysema in COPD lungs. The quantitative measurements of lung MR qS₀ mapping, including RA₀.2₀, the mean, standard deviation and the 15th percentile of qS₀, were significantly different between healthy subjects and patients with COPD and were strongly correlated with CT estimates of emphysema and lung density, i.e. PD₁₅ and RA₁₅. In patients with COPD, suggesting MR qS₀ is sensitive to emphysema and is capable of emphysema quantification. The ROC analysis further revealed the high sensitivity and specificity of MR qS₀ mapping in distinguishing COPD patients from healthy controls. In addition, the measurements of lung MR qS₀ mapping showed good one-week reproducibility between two scans in both healthy control and COPD groups, reflecting the robustness of this technique for clinical use. In terms of the implication of MR qS₀ mapping for patient care, lung MR qS₀ mapping may be potentially used as an alternative to CT for the longitudinal monitoring of the emphysematous changes or treatment effects in COPD patients to reduce radiation exposure in future, although further validation is needed to allow widespread application to clinical practice. Additional studies are required in order to standardize this technique, achieve 3D coverage and test the sensitivity of MR qS₀ mapping to the changes in emphysema caused by disease progress or treatment. Moreover, lung MR qS₀ mapping may have a role to play in patients with lung diseases but susceptible to ionizing radiation such as children with cystic fibrosis or pregnant COPD patients. Another prospective application of MR qS₀ mapping in COPD is radiological phenotyping. Given that the distribution pattern and the severity of emphysema evaluated using CT have proven strong predictors of COPD exacerbation, modality and outcomes of LVRS, MR qS₀ mapping may also have input in these areas, particularly when it is performed as part of an MRI scanning session that provides significant additional physiological information – for example via OER-MRI or DCE-MRI. It would be worthwhile to investigate the feasibility of predicting COPD exacerbation, modality and outcomes of LVRS by using MR qS₀ derived emphysema assessment in the near future. Furthermore, MR qS₀ mapping has been demonstrated feasible in depicting the location and the extent of emphysema in this project, which raises the possibility for its applications in COPD in future for pre-operation/procedure planning for LVRS and pre-procedure planning for the insertion of endobronchial valves (a minimally invasive non-surgical procedure that involves the insertion of endobronchial valve under bronchoscope to reduce the lung volume in COPD patients). Lung structural information is
necessary for both procedures that can be collected by CT. However, the radiation exposure of CT is an inevitable concern. It is worthy exploring the feasibility of MR qS₀ mapping as a potential alternative to CT for pre-LVRS and pre-bronchial valve insertion assessment in future studies. Last but not the least, simply adding the MR qS₀ mapping into functional lung MRI scans, such as DCE-MRI, OE-MRI and even HP gas MRI, or hybridizing MR qS₀ mapping with radionuclide lung imaging, such as PET and SPECT, can facilitate the correspondence of functional abnormalities with emphysematous structures in COPD.

The study reported in chapter 5 explored regional structural-functional relationships in COPD by directly comparing single coronal CT images with single slice dynamic OE-MRI parameter maps. Reduced pulmonary oxygenation and prolonged O₂ wash-in time were observed in COPD patients regardless of the presence or absence of emphysema on CT images, while the reduction in lung T₁air and the correlations of dynamic OE-MRI readouts of pulmonary oxygenation with diffusion capacity and CT estimates of emphysema were only observed in emphysematous COPD. To our knowledge, this is the first published investigation regarding the characteristics of dynamic OE-MRI in COPD patients with different radiological features. These findings suggested that pulmonary oxygenation impairment is a shared feature of these two COPD radiological subtypes (emphysematous-, non-emphysematous-). Dynamic OE-MRI might not be capable of distinguishing the COPD radiological phenotypes. However, the use of a non-ionizing, readily available clinical gas as the source of contrast makes this technique an attractive option in the assessment of regional pulmonary oxygenation in COPD. On the other hand, the significantly lower T₁air of the lung in patients with emphysematous COPD compared to patients with non-emphysematous COPD and healthy controls, and the significant correlations between T₁air and CT estimates of emphysema in patients with emphysematous COPD but not in patients with non-emphysematous COPD, suggested the underlying biophysical link between the decreased lung T₁air and the emphysematous damage of the lung structure in COPD. The decrease in the fraction of free water in the pulmonary parenchyma secondary to emphysema-induced destruction of capillary beds and the reduction of regional perfusion may be responsible for the T₁air shortening in emphysematous lungs. However, inconsistent spatial association between areas of short T₁air and low CT attenuation areas was observed only with a visual trend to lower T₁air in the presence of severe focal emphysema or bullous emphysema, which implied that the reduction of T₁air in emphysematous COPD lungs were only partially explained by the emphysema-induced structural damage and there could be other unknown factors affecting T₁air values in the lung. T₁air mapping was unreliable in depicting the location and the shape of emphysema and thus is not as a good candidate as MR qS₀ mapping for the pulmonary structural assessment in COPD lungs.

Chapter 6 and chapter 7 presented dynamic OE-MRI studies conducted in patients with asthma. The pilot study reported in chapter 6 provided initial supportive evidence of the feasibility and usefulness of dynamic OE-MRI in the assessment of pulmonary oxygen delivery in asthmatic patients. Quantitative dynamic OE-MRI readouts, including EF,
\( \Delta P_{\text{O}_2}^{\text{max}} \) and \( \tau_{\text{sp}} \), were found to be sensitive to disease severity and strongly correlated with pulmonary function tests of airway function and lung volume in the 10 asthmatic patients. Dynamic OE-MRI parameter maps also reflected the heterogeneous nature of functional impairment in asthmatic lungs. Moreover, dynamic OE-MRI showed the ability to pinpoint the severe and focal functional defects (see figure 6.2), which supported a potential role of dynamic OE-MRI in localizing the focal functional abnormalities for pre-procedure planning and post-procedure assessment of focal interventional therapies of asthma, e.g. bronchial thermoplasty (a bronchoscopy procedure that employs controlled radiofrequency energy to the airway wall under bronchoscope to physically ablate the hypertrophic and highly contractible airway muscles in order to relieve airflow limitation in patients with asthma). The one-month reproducibility of dynamic OE-MRI readouts was only moderate in patients with asthma, which however may be at least partly attributed to the genuine variation of the lung function during the time span (since high variability in lung function is a hallmark of asthma).

Chapter 7 further explored the ability of dynamic OE-MRI in detecting short-term treatment effects of inhaled salbutamol in patients with asthma. Salbutamol inhalation has been known to attenuate airflow limitation and improve pulmonary ventilation in patients with asthma but with a possibility to aggravate V/Q imbalance and thus reduce pulmonary oxygenation. Thus salbutamol was used as a known intervention in the current study to test the imaging technique. Dynamic OE-MRI successfully revealed a decreased pulmonary oxygen delivery as a response to salbutamol inhalation in patients with severe asthma but not in patients with mild asthma and healthy controls. These findings not only confirmed the high sensitivity of dynamic OE-MRI to the effect of salbutamol inhalation on lung function in patients with asthma but also helped answer a question that has yet not been clearly elucidated, i.e. what dynamic OE-MRI actually measures. Because the post-salbutamol reduction in \( \Delta P_{\text{O}_2}^{\text{max}} \) in severe asthmatics was 1) demonstrated genuinely to derive from salbutamol intervention and 2) can be explained by a deteriorated pulmonary oxygenation likely due to a V/Q imbalance secondary to salbutamol inhalation, it is reasonable to conclude that dynamic OE-MRI measures the integrated function of a regional lung unit that determines the efficiency of pulmonary oxygen delivery and uptake, rather than any single contributor to lung function. Before now, the adequacy of pulmonary oxygenation, the primary function of the lung, could only be indirectly estimated by measuring the arterial blood oxygenation level using arterial blood gas tests, while the direct, non-invasive and regional assessment method was still lacking. Dynamic OE-MRI may be able to fulfill the clinical need and become a unique tool for the direct and regional assessment of pulmonary oxygen delivery at baseline and after treatment in patients with asthma and other lung disorders. Moreover, dynamic OE-MRI showed good short-term (\( \leq 7 \) days) reproducibility and, more importantly, good immediate (minutes) reproducibility in the assessment of lung function in both asthmatics and healthy controls. The ability to repeat measurements within a few tens of minutes allows dynamic OE-MRI to detect the evolution of the functional impact of the intervention, which is however very challenging using those functional imaging
techniques which are impossible or discouraged to repeat within a short timescale, e.g. PET, SPECT, Xe-enhanced CT, etc. In addition, the good one-week reproducibility of dynamic OE-MRI in both asthmatics and healthy controls that demonstrated in chapter 7 lends weight to the idea that the moderate reproducibility of dynamic OE-MRI over a month in asthmatic patients that demonstrated in chapter 6 is likely caused by the genuine disease variability.

Chapter 8 and chapter 9 presented DCE-MRI studies in patients with asthma. To the best of my knowledge, these were the first investigations and evidence of the ability of DCE-MRI parameters to reflect pulmonary microvasculature alterations in patients with asthma. The non-model-based semi-quantitative analysis of the DCE-MRI data in chapter 8 demonstrated that the relative signal intensity curve in asthmatic lungs was characterised by a smaller first-pass peak and a reduced downslope during the late redistribution phase than observed in healthy controls, the peak enhancement (SI%max) and late phase washout slope (kwasout) being related to the spirometric indices of airflow limitation. The presence of oedema and mucus in severe asthmatic lungs can increase the amount of MRI-visible tissue of the lung and may lead to a lower concentration of contrast agent during the first pass peak even without any changes in the blood volume or blood flow to the lung as a whole. In addition, the increased leakage space or interstitial fluid build-up was likely responsible for the slower enhancement elimination rate seen during the late-redistribution phase in asthmatic lungs. These hypotheses were then substantiated by the subsequent model-based quantitative analysis that is reported in chapter 9, where the fractional extravascular extracellular space, ve, was found to be significantly higher in asthmatic lungs than in healthy lungs regardless the type of airway inflammation (eosinophilic and non-eosinophilic). The increased leakage space could be either due to the enlargement of extravascular extracellular space or to oedematous fluid build-up within the alveoli and small airways secondary to the microvascular inflammation in asthmatic lungs. ve derived from DCE-MRI was sensitive to the presence of asthma and is a promising inflammatory biomarker for the assessment of microvascular characteristics of the lung in patients with asthma. On the other hand, the contrast agent transfer coefficient (Ktrans), an index related to vascular permeability, and the fractional blood plasma volume (vp), a measurement of blood volume, did not distinguish asthmatics from healthy controls. The picture of increased ve but not increased Ktrans and vp in asthmatic lungs was different from that in COPD lungs (decreased vp) [264] and in smokers’ lungs (increased ve and increased Ktrans) [263], which reflected the different underlying pathophysiology and indicated the potential of quantitative DCE-MRI in assisting the differentiation between asthmatic patients, COPD patients and asymptomatic smokers.

In conclusion, I hope to have shown the promise of 1) MR qS0 mapping for the assessment of emphysema in COPD lungs, 2) dynamic OE-MRI for the assessment of impaired pulmonary oxygenation in COPD and asthma and for the monitoring of the short-term treatment effects in asthma and 3) DCE-MRI for the evaluation of pulmonary microvascular inflammation in asthma. The non-invasive non-ionizing properties, simple setup requirements, good safety profile, and cost-efficient and reproducible features make
these three proton MRI techniques attractive options in the assessment of structural and functional alterations of the lung in asthma and COPD in clinical settings.
Appendix: Why was the threshold of 0.20 used for the MR qS₀ map in chapter 4?

The threshold of 0.20 for MR qS₀ corresponded to the threshold of -950 HU for CT. The threshold of 0.20 was calculated from -950 HU according to the linear regression model between CT lung attenuation parameter and MR qS₀ parameter. Because there was only one CT parameter related to lung attenuation (i.e., PD₁₅) in chapter 4, the linear regression model of PD₁₅ with its corresponding MR qS₀ readout (i.e., 15th percentile qS₀) was used to predict the qS₀ threshold corresponding to -950 HU. The linear regression model we obtained was PD₁₅ = -23 + 374 × 15th percentile qS₀ (r² = 0.62, P < 0.001). The attenuation of -950 HU was corresponding to the CT lung attenuation of 50 g·L⁻¹ (CT lung attenuation = 1000 + attenuation value). Thus, PD₁₅ in the regression model was set to be 50 g·L⁻¹ to calculate corresponding qS₀ value, which resulted in 0.20. CT-estimated mean lung attenuation (MLA) was measured as well (but not reported), and its relevant regression model with mean qS₀ was mean qS₀ = 0.151 + 0.001 × MLA (r² = 0.517, P < 0.001). Setting mean lung attenuation to 50 g·L⁻¹ (i.e., -950 HU) also gave 0.20 as the corresponding qS₀ threshold. To simply validate the calculated threshold of 0.20, we empirically compared the performance of 0.20 with other thresholds, including 0.15, 0.25, etc. The MR qS₀ threshold maps generated by using a threshold of 0.20 showed visually better spatial consistency with -950 HU thresholded CT images than those obtained by using other qS₀ thresholds (not shown). Also, the correlation of RA₉₅₀ with RA₉₂₀ was stronger than the correlation with relative lung area with qS₀ value below thresholds other than 0.20 (not shown).
References


4. Braman SS. The global burden of asthma. Chest. 2006;130(1 Suppl):4S-12S.


131. Duncan KR, Gowland PA, Freeman A, Moore R, Baker PN, Johnson IR. The changes in magnetic resonance properties of the fetal lungs: a first result and a potential tool for the


