Lung heterogeneity as a predictor for disease severity and response to therapy

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Word count = 2,472 words

ABSTRACT (111 words)

Heterogeneity is an intrinsic property of the lungs. Structurally, it is evident in the complex branching of the airways and the spatial distribution of tissue throughout the lung. Functionally, this translates to variation in the distribution of airway resistance and lung compliance, resulting in disparity in the filling and emptying rates between individual compartments, and consequently heterogeneous distribution of ventilation. Disease causes pathological alterations to structure and function, causing corresponding changes to heterogeneity, which can be measured via functional imaging, oscillometry or gas washout methods. This review presents established and recent evidence for the significance of heterogeneity as a marker of disease severity and potential predictor of treatment or intervention response.

1. Heterogeneity characterises lung structure and function

Heterogeneity is an intrinsic property of the lung. Structurally, the branching of the airways is complex and asymmetric, having evolved following a fractal pattern to maximise space filling within a finite space[1]. There is wide variation in the number of branching generations before an airway path terminates in an alveolar sac, as well as in length and diameter between airways of the same generation[1]. There is also variation in the regional distribution of tissue throughout the lung[2], and heterogeneity in airway smooth muscle pathology across lung lobes, in terms of smooth muscle mass and reticular basement membrane thickness[3]. Consequently, regional variations in mechanical properties would be expected.

Functionally, these factors translate to disparities between individual lung regions or compartments in the rate at which they fill and empty with air during breathing. The rate of emptying can be quantified by the time constant, i.e. the slope of exponential decay in flow during emptying. A short time constant indicates a lung compartment with fast/good ventilation, conversely a long time constant indicates one with slow/poor ventilation. The time constant links structural mechanics with function, as it is also the product of airway resistance and lung compliance associated with the compartment.

Of the two factors contributing to the time constant, compliance is thought to be dominant in determining heterogeneity in ventilation distribution between regions[2, 4], along with overall determinants such as gravity, shape of the lung, and interaction with chest wall and diaphragm[2, 4]. Regional lung compliance is dependent on tissue mechanical properties as well as the amount of communicating lung units[5]. An additional physiological complexity is the role of collateral ventilation between lung units, but this effect is difficult to measure[6]. As a result, a diverse range of pathophysiological processes which cause loss of communicating lung units, e.g. gas trapping,

small airway closure, expiratory flow limitation etc, can result in mild to dramatic changes in ventilation heterogeneity.

Studying heterogeneity may help us link single airway mechanics and the behaviour of the airway tree, as heterogeneity may manifest as variations in airway calibre or as changes to airway-interdependence and parenchymal tethering exerting different mechanical stresses to airways located at different depths of the airway tree. Multiscale modelling would allow these relationships to be explored in an integrative manner, and reconcile in vitro investigations of airway smooth muscle properties with in vivo mechanics studies[7].

2. Measuring of heterogeneity *in vivo*

Much of the current empirical evidence relating to heterogeneity in the lung stems from functional measures. **Functional imaging methods** allow us to visualise heterogeneity in terms of topographical distribution of ventilation. Single-photon emission computed tomography (SPECT) measures the distribution of inhaled Technegas, an ultrafine, carbon particle aerosol labelled with ^{99m}Tc that emits radiation, to reflect ventilation within the lung. Regions with low Technegas activity indicate non- or poorly-ventilated lung units (i.e. ventilation defects), the size, number and location of which can be quantified. Oxygen and hyperpolarised xenon (^{129}Xe) or hyperpolarised helium (^{3}He) magnetic resonance imaging (MRI) can also be used to determine ventilation distribution in three-dimensional space, without requiring ionising radiation. Similar to SPECT, regions with low signal are reflective of ventilation defects - often quantified by the Ventilation Defect Percentage (VDP), i.e. the ventilation defect volume as a percentage of thoracic cavity volume. Parametric Response Mapping (PRM) is a voxel-based image-analysis technique that uses co-registered inspiratory and expiratory computed tomography (CT) scans. Although PRM does not

measure ventilation heterogeneity per se, it can identify topological areas of normal parenchyma, small airways disease and emphysema[8].

The **multiple breath washout (MBW)** measures heterogeneity in terms of temporal distribution of flow, resulting from distribution of time constants within the lung. By tracking flow and concentration at the airway opening of a tracer gas (nitrogen, helium or sulfur hexafluoride) as it is progressively washed out of the lungs, we can derive a measure of global ventilation heterogeneity (lung clearance index, LCI). Modelling of the gas flow contributions from fast versus slow compartments enables us to partition heterogeneity into convective (predominant in the conductive airways, Scond) and convective-diffusive interactions (predominant in the acinar airways, Sacin)[4]

Oscillometry (also known as the forced oscillation technique, FOT) measures respiratory mechanics, i.e. resistance Rrs and reactance Xrs (inversely related to elastance), as a function of frequency. Theoretically, this enables us to assess heterogeneity because lung regions of different time constants characteristically respond to forcing pressure at different oscillation frequencies - the peripheral airways and lung parenchyma, which have slower time constants, have the greatest contributions at lower frequencies, whereas the more proximal airways, which have fast time constants, contribute mostly to higher frequencies. Different parallel pathways along the airway tree also have disparities in time constants and consequently dependence on frequency. The frequency dependence of resistance, in particular, is often quantified by the difference in resistance at 5 and 20 (or 19) Hz (Rrs_{5-20} or Rrs_{5-19}). Multiple other factors contribute to frequency dependence, such as tissue viscoelasticity, central airway wall shunting[9] even intrinsic variations in mechanics over time[10]. Elastance, derived from Xrs, has also been related to small airway heterogeneity[9], while the area under the reactance-frequency curve (AX) could be considered an integrated measure of frequency dependence of reactance, that is often sensitive to disease.

3. Heterogeneity worsens with bronchoconstriction and determines airway hyperresponsiveness

Earlier modelling studies revealed a critical role for heterogeneity in explaining the effects of bronchoconstriction[11, 12]. Mild constriction applied heterogeneously in the peripheral airways results in massive changes in airway resistance and its dependence on frequency[12], provided constriction was sufficient to close or nearly close a small number of airways[11, 12]. The effect of heterogeneous constriction was more pronounced than homogeneous or more central constriction.

The seminal work of Venegas, Winkler *et al* allowed us to visualise the patchiness of ventilation using PET, with clusters of poorly ventilated airways evident during bronchoconstriction in mild-to-moderate asthmatics[13]. Importantly, using modelling they showed that these clusters can arise in a catastrophic manner even from the presence of mild structural heterogeneity, e.g. a small variation in the distribution of wall thickness across the airway tree, in response to smooth muscle activation. The emergence and persistence of these ventilation defect clusters can be attributed to the underlying structural asymmetry of the airway tree[14].

The mechanistic role of heterogeneity in bronchoconstriction and airway hyperresponsiveness has been validated in multiple physiological studies in health and asthma. Functional measures of ventilation heterogeneity at baseline determines extent of airway narrowing[15] and closure[16] with bronchoconstriction, and the degree of airway hyperresponsiveness in both health[17] and asthma[18, 19], independently of the effect of airway inflammation[19]. Furthermore asthmatic airways narrow more heterogeneously than healthy airways during bronchoconstriction, contributing to even greater airway closure[20].

The worsening of heterogeneity with bronchoconstriction is apparent whether assessed via imaging[15, 21], MBW[22] or oscillometry[15, 22]. The relationship between changes in these measures can be variable but may provide further insight, e.g. worsening Xrs_5 but not Rrs_{5-19} correlated with Sacin changes, which may suggest Xrs_5 is more sensitive to pathological chances in the acinar airways or distal lung[22]. It has been posited that MBW may be more sensitive to severity of constriction, while FOT may be more useful for determining depth (i.e. proximal vs distal) of constriction[23].

4. Heterogeneity is a predictor of disease severity

Correspondingly, functional measures of heterogeneity have been related to clinical measures of disease severity. In doing so they provide us with a better understanding of the disease process. In this review, we will limit our discussion to asthma and chronic obstructive pulmonary disease (COPD), where most of the evidence has been gathered.

In mild-to-moderate asthma, Scond (and to a lesser extent, Sacin) was greater both in patients with poor or partly-controlled asthma control compared to those who had well-controlled asthma[24], while elevated Sacin has been observed in higher proportions in severe asthma[25]. Both MRI-derived VDP[26] and CT-based markers of heterogeneity, as well as Rrs_{5-19} from oscillometry[27], have been strongly related to Asthma Quality of Life Questionnaire (AQLQ) and Asthma Control Questionnaire (ACQ) scores. Reversibility of X_5 and AX is better than spirometry in identifying patients with poor ACQ[28]. VDP and Rrs_{5-19} have also been significantly related to St. George Respiratory Questionnaire (SGRQ) in COPD[29].

Correlations between the different functional measures of heterogeneity can reveal further insights into disease pathophysiology. For example, VDP is more strongly related to Rrs_{5-19} in COPD

without emphysema, and to Xrs_5 in COPD with emphysema, suggesting different mechanisms in the emergence of ventilation defects and heterogeneity in COPD[30].

Recently, there has been renewed interest in characterising small airways dysfunction (SAD) as a distinct disease phenotype, partly facilitated by the increasing use of Rrs_{5-20} in cohort studies, where cut-offs have been derived to define SAD[31, 32]. Rrs_{5-20} was found to be a strong contributor to SAD[31], and related to asthma severity[31] and control[33]. Another emerging marker for SAD comes from the use of CT-based PRM to distinguish between air trapping due to SAD (PRM^{fSAD}) versus emphysema (PRM^{Emph}) in COPD[34], where SAD has been identified as the stronger determinant of FEV1 decline in COPD. Both PRM^{fSAD} and PRM^{Emph} affect MBW-derived ventilation heterogeneity in smokers[35].

There has also been renewed interest in the loss of terminal bronchioles as a disease mechanism[36]: with the availability of high-resolution micro-CT scans, we now know that loss of terminal bronchioles occurs early in the disease process in COPD, preceding the development of emphysema[37] and becoming worse with severity of GOLD stage[38]. Loss of terminal bronchioles has also been related to PRM^{fSAD} in COPD[39]. Increased ventilation heterogeneity has been previously observed in established COPD[40]; recent simulations suggest that gradual loss of terminal bronchioles is associated with an increase in Sacin, and matches observations in smokers with COPD exhibiting impaired diffusion capacity[41]. More recently, loss of small airways determined by CT imaging has also been documented with increasing severity of asthma, and related to increased wall thickness as well as MRI-derived VDP[42].

5. Heterogeneity predicts response to therapy

In asthma, both Sacin and Scond improve following the administration of a short-acting β2-agonist (SABA). However, these parameters may remain abnormal even in patients with mild disease[43, 44] perhaps suggestive of early structural changes that are difficult to reverse. MBW and oscillometry indices are also responsive to combination inhaled corticosteroids (ICS) and long-acting β2-agonist (LABA) treatment, both in mild-to-moderate asthma and severe uncontrolled asthma[19, 24, 45, 46]. Improvements in Scond and Sacin have been associated with parallel improvements in asthma symptoms[24].

Heterogeneity is also an important predictor of treatment response. Baseline Scond, Rrs_5 and Rrs_{5-19} predicted improvement in asthma control following ICS uptitration in uncontrolled asthma[45, 46], while baseline Sacin correlated with loss of asthma control with ICS downtitration, suggesting that patients who are well-controlled but have abnormal Sacin may not tolerate stepping down of their asthma treatment[45]. More recently, Sacin and LCI in patients with severe eosinophilic asthma improved rapidly following treatment with the anti-IL5 biologic, mepolizumab[47]; the early improvement in Sacin was associated with the improvement in symptoms and may indicate a more sustained symptomatic response to these treatments. Oscillometry indices including Xrs have also been shown to improve early with mepolizumab along with symptoms, and continued to do so despite no further improvements in spirometry or eosinophil counts[48]. Meanwhile, improvements in LCI but not Scond or Sacin have been shown following bronchial thermoplasty (BT)[49], suggesting that while overall ventilation heterogeneity is improved via alterations to the large airways, the effects on the smaller airways are minimal.

MRI measures of heterogeneity also improve following BD treatment. Like oscillometry, MRI imaging can identify the presence of a significant BD response in asthma despite a negative

spirometric response[50]. Furthermore, ventilation defects at baseline in mild-to-moderate asthma may predict risk of developing BD irreversibility over 6 years[51]. In severe asthma, VDP abnormalities persisted post BD in patients in whom eosinophilic inflammation was not adequately controlled, suggesting BD-nonresponsive ventilation defects may help distinguish airway inflammation from non-inflammatory smooth muscle response, thus guiding treatment decisions[52]. Furthermore, patients with prednisone-dependent asthma showed improved VDP following biologic treatments; greater improvement was found among patients with worse airway eosinophilia at baseline[53]. VDP has also been shown to improve following BT. Importantly, when BT treatment was guided by MRI (to prioritize the most involved airways), there was a greater reduction in the percentage of poorly and non-ventilated lung when compared with unguided BT, as well as number of patients experiencing exacerbations [54]. The utility of SPECT imaging is less established; a recent case-series demonstrated that improvements in ventilation distribution with mepolizumab can be detected, though these were heterogeneous and not always concordant with symptoms[55].

In COPD, oscillometry and MRI indices are responsive to SABA, LABA or LABA/LAMA treatment, and similar to asthma, may be more sensitive than spirometry in detecting a bronchodilator response[50, 56]. Baseline oscillometry indices (Rrs, Xrs and R_{5-19}) predicted improvements in gas trapping following LABA treatment[57]. In moderate-to-severe COPD, BD treatment improved respiratory conductance as measured by oscillometry, which was associated with an improvement in exertional dyspnoea[58]. Most recently, a study of patients with stable COPD showed significant improvements in oscillometry indices at 6 weeks following ultrafine ICS/LABA treatment that were maintained at 12 weeks; the improvement in Rrs_{5-20} was related to the improvements in gas trapping and health status[59]. Thus, there is good evidence for the ability of oscillometry to predict which COPD patients might benefit from BD treatment.

In contrast, Scond and Sacin do not improve following SABA or LABA in COPD, despite reported improvements in FEV_1[60, 61]. We speculate that the lack of sensitivity of MBW to treatment in COPD may reflect the irreversibility by BD of loss of terminal bronchioles or emphysema, which are likely dominant contributors to Scond and Sacin.

In smokers with normal spirometry (i.e. without clinical COPD), however, MBW may have utility in identifying treatment responsiveness. While most smokers with abnormal Scond are able to reverse their abnormal Scond values with BD treatment, most with abnormal Sacin failed to normalise their Sacin values, implicating irreversible structural damage to the acinar airways which were correlated with smoking history[62]. This is corroborated by observations following smoking cessation, where Sacin and Scond were shown to improve rapidly, but only Scond showed persistent improvement after 12 months[63].

6. Conclusions and future directions

In summary, heterogeneity in the lung has a profound impact on its function and plays a key role in determining airway hyperresponsiveness. In addition to providing insight into disease mechanisms, functional measures of heterogeneity derived from imaging, washout and oscillometry are associated with clinical markers of disease severity, often with greater sensitivity than spirometry. These measures also help predict response to therapy and may guide treatment decisions in specific patient groups. Aided by new tools and emerging data from large cohorts allowing us to establish thresholds for clinical applicability, physiologic characterisation of heterogeneity will increasingly form an important and novel dimension to disease phenotyping, potentially becoming another treatable trait in airways disease.

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