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Commentary on “The influence of lung airways branching structure and diffusion time on measurements and models of short-range ^3He gas MR diffusion”

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In a recently published paper in this journal by Parra-Robles and Wild (1), the authors present results of computer simulations of hyperpolarized ^3He gas diffusion MR experiments in lungs, focusing on the effects of airway branching structure. In the abstract of their paper (1) Parra-Robles and Wild conclude: “The results demonstrate that the cylinder model can produce inaccurate estimates of the airway dimensions as a consequence of incompletely accounting for the diffusion-time dependence in the model equations and confirmed the predicted limitations of the cylinder model for reliable lung morphometry measurements”. In the article, the authors identified “the cylinder model” as the theoretical model of gas diffusion in lung acinar airways developed and used in publications by our Washington University (WU) research group (e.g., (2-6)) that forms the basis for our MRI-based lung morphometry technique employing hyperpolarized gases (4). We feel some commentary and clarification are in order and we offer such herein.

The main focus of Parra-Robles and Wild paper is on studying effects of lung airways branching in a rather broad range of diffusion times Δ (up to 6 ms) on diffusion MRI signal and criticizing us for not taking these effects into account. Apparently they missed one of the major ideas of our approach – selecting diffusion time that allows minimizing the effects of acinar airways branching. The diffusion time Δ used for lung morphometry technique is a crucial parameter and only the specified diffusion time interval should be used in experiments. This diffusion time is selected such that diffusing gas atoms are expected to diffuse away from single alveoli but remain mostly in the same acinar airway (alveolar duct or alveolar sac) throughout the duration of the bipolar diffusion-sensitizing gradient pulse – i.e., a short diffusion time but not too short. For ^3He gas this restricts the diffusion time Δ in human lungs to about 2 ms (4,5) and for ^{129}Xe , with its smaller free diffusivity, to about 15 ms (7). In small animal lungs, where the alveolar ducts and sacs are shorter, the diffusion time Δ should be much shorter, a fraction of a millisecond (7-10). This constraint recognizes acinar airways as the elementary geometrical units contributing to the gas diffusion MR signal. It also allows us to dramatically decrease the number of parameters required for quantifying the diffusion attenuated MR signal from hyperpolarized gas delivered to lung airspaces and to obtain valuable information on lung microstructure at the alveolar level

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from a quick (less than 10 seconds) *in vivo* experiment, hence the term “*in vivo* lung morphometry”.

Interestingly, the Parra-Robles and Wild's own experimental results obtained for diffusion times within the interval prescribed (see their data for $\Delta = 1.6\text{--}1.8$ ms in Fig. 11 in (1)) are in good agreement with those obtained by our group in excised (4) and *in vivo* (6) human lungs. Given this agreement, we are puzzled by the contention of (1) that the “cylinder model” of lung morphometry (4) does not work, i.e., “produces inaccurate estimates of the airway dimensions”. To the contrary, we argue that considerable theoretical and experimental evidence suggests that this technique (if used correctly with specified diffusion times) provides robust and quite accurate information on lung microstructure at the alveolar level. We offer the following evidence in support of this contention.

- In (4) the MRI-based measurements of lung morphometric parameters were validated in explanted human lungs against direct invasive morphometric measurements – the current gold standard. The results shown in Figure 1 demonstrate excellent agreement between direct histological and ^3He MRI-based measurements of L_m (mean linear intercept) in normal lungs and lungs with different levels of emphysema. Surprisingly, despite of our approach was developed for lungs with preserved airway structure (normal lungs and lungs with mild alterations) our measurements of L_m are in a good agreement with direct histology even for lungs with severe emphysematous destruction (two points on the right in Fig. 1).
- Our results for the surface-to-volume ratio from diffusion MRI obtained in (4) ($S/V \sim 200\text{--}240$ cm²/ml for healthy lungs, $\sim 100\text{--}140$ cm²/ml for subjects with mild emphysema, and $\sim 50\text{--}55$ cm²/ml for the case of severe emphysema) are also in good agreement with the results of morphometric measurements of Coxson et al (11) obtained from excised lung specimens (256 ± 24 cm²/ml for control human subjects, 165 ± 23 cm²/ml for subjects with mild emphysema, and 43 ± 6 for severe emphysema).
- Our measurements of alveolar number density N_v (4) are in agreement with available direct morphometric results. In two normal lung specimens, our data (120 and 146 per mm³) compare well with direct measurements by Ochs and colleagues (12) (the average N_v varies between 132 and 177 per mm³).
- Similar results as above were obtained *in vivo* in humans by Quirk *et al.* (6).
- Osmanagic *et al.* (8) applied our lung morphometry technique to study explanted lungs in mice. The MR protocol was adjusted to acquire data with much shorter diffusion times to accommodate the substantially smaller acinar airway length as compared to human lungs. These measurements yielded mean values of lung surface-to-volume ratio of 670 cm⁻¹, alveolar density of 3200 per mm³, alveolar depth of 55 μm and L_m of 62 μm , all consistent with published data obtained histologically in mice by independent methods.
- The lung morphometry technique was further implemented by Wang *et al.* (9) for *in vivo* lung imaging in mice. Again, the results indicated good agreement between *in vivo* morphometry via ^3He MRI and microscopic morphometry after sacrifice. It should be noted here that even shorter diffusion times would likely be optimal for measurements in mice due to their short airways. However, hardware restrictions did not allow that in experiments reported in (8) and (9).

As is clear from the above, the MRI-based lung morphometry technique has strong experimental validation when implemented appropriately within the prescribed diffusion

time regime. To add perspective, it is useful to briefly recast the major ideas and results behind the lung morphometry technique with hyperpolarized gases.

In 2002, we proposed a theory (2) explaining experimental results on hyperpolarized ^3He gas diffusion MRI obtained in human lungs. This theory (2) captures the salient feature of this diffusion *in the millisecond diffusion-time range* - its microscopically anisotropic but macroscopically nearly isotropic nature. Our theory was based on the well-established lung model developed by Weibel and colleagues (13,14), in which the lung geometrical structure is treated in terms of acinar airways – cylindrical passages covered with alveolar sleeves. We proposed that the diffusion in such airways should be anisotropic and described it by two distinct diffusion coefficients – parallel (D_L) and perpendicular (D_T) to the airway axis. While diffusion in each airway is anisotropic, it is almost *isotropic on a macroscopic scale* since each imaging voxel contains a multitude of acinar airways with a nearly isotropic distribution of directions. This fundamental property of gas diffusion in lung acinar airways was confirmed by experimental MRI measurements in humans (2,4,6), canines (15), mice (8,9) and rats (10,16) using hyperpolarized ^3He gas. It was also recently confirmed in humans (17) and rats (18) using hyperpolarized ^{129}Xe gas.

The next step was reported in (3), where gas MR diffusion measurements were related to lung microstructure parameters (main radius of acinar airways R and depth of alveolar sleeve h). All calculations and considerations in this paper were conducted for specific diffusion time of 1.8 ms. This paper also has numerous discussions on the assumptions and restrictions of our approach but only a very brief mentioning on the role of branching effects: “In our simulations, we considered the acinar airways as infinitely long, ignoring their branching. This simplification is valid when a mean displacement of ^3He atoms along the airway's principal axis during diffusion sensitizing gradients, $(2D_L\Delta)^{1/2}$, is smaller than the mean length of alveolar ducts and sacs. In human lungs, this mean length is about 1 mm (13). For a typical longitudinal diffusivity $D_L \sim 0.4 \text{ cm}^2/\text{s}$, this condition imposes a restriction on diffusion time: $\Delta < 10 \text{ ms}$. For longer diffusion times, a more general theory accounting for branching of acinar airways is required.” This estimate has never meant to be precise and only provided a qualitative estimate; it was not placed in the Abstract or Introduction or Conclusion sections of our manuscript (3). It is difficult to understand why this brief comment became the central point of Parra-Robles and Wild critique of our work while they practically ignored discussions and clarifications of diffusion timing effects in several follow-up publications from our group (4,5,7). These are briefly discussed below.

The timing requirement was formulated in our key paper (4) where lung morphometry technique for human lung was finalized in its current form: “Our method of measuring airway geometrical parameters is based on MRI measurements of hyperpolarized ^3He gas diffusion. We use a rather short diffusion time, $\Delta=1.6 \text{ ms}$. For this diffusion time, and the free (unrestricted) diffusion coefficient D_0 of ^3He in air of $0.88 \text{ cm}^2/\text{s}$, the characteristic free diffusion length $L_1 = \sqrt{2D_0\Delta}$ is about $500 \mu\text{m}$. Hence, each ^3He atom will have a chance on its diffusion path to diffuse out of its initial alveolus and across the airway (the average alveolar radius is about $100 \mu\text{m}$). *Thus, in this time regime, we consider acinar airways rather than alveoli as independent elementary geometrical units.* On the other hand, L_1 is smaller than the average acinar airway length ($730 \mu\text{m}$ for ducts and $1012 \mu\text{m}$ for sacs (13)). Hence for these diffusion times the effects of the airways' finite length are not important and we can neglect both collisions with the terminal ends of sacs and branching effects when ^3He atoms migrate from one airway to another. *In this case, the total MR diffusion signal can be considered as a sum of the signals from individual, non-communicating airways.*”

The above consideration is, of course, qualitative. A detail theoretical analysis of the accuracy of the approach proposed in (4) was provided by Sukstanskii *et al.* (5). For this purpose, computer simulations were used to calculate the MR signal for the diffusion time of 1.6-1.8 ms in a realistic model of human acinus (per (13)) that accounted for the branching structure of acini and the finite length of alveolar ducts and sacs, as well as the distribution of airway geometrical parameters. As a result, the relative errors in estimation of the main acinus parameters using lung morphometry were found. The main conclusion was that all these effects, including branching structure, produce rather small changes in the evaluation of lung geometrical parameters. In particular, for the surface-to-volume ratio, the average difference is 6.5%. For a non-invasive technique which provides 3D tomographic information on lung microstructural parameters in less than 10-second MRI scan, this degree of potential error is easily tolerable.

Is the approach developed by the WU group ideal? Certainly it is not. From the beginning, it was recognized and emphasized that the lung acinus is an extremely complex system and cannot be described without assumptions and simplifications. The goal of our series of publications was not to develop an all-encompassing detailed theory of ^3He gas diffusion in lungs for all time scales but to develop a practical method – MRI-based lung morphometry – that would capture lung salient structural features and allow the study of human and animal lungs *in vivo* and *ex vivo*. Such a method was developed and validated both in humans and small animals. At the same time, the method relies on theoretical modeling of the gas MR diffusion signal from lungs, which can be further improved as more experimental data are accumulated.

In summary, the conclusion of (1) that our MRI-based lung morphometry technique “produces inaccurate estimates of the airway dimensions” does not have scientific basis and is not in agreement with a considerable body of peer-reviewed scientific reports and Parra-Robles and Wild's own data. Our technique has strong theoretical background, it is validated, and it has already produced a number of results related to lung morphology and function that were not previously accessible (see our recent review paper (19) for details).

Appendix

In addition to the issues discussed above, the paper (1) also has a number of other incorrect statements and assumptions. Due to space restrictions, we herein address only some of them.

A substantial part of (1) is devoted to studying how branching effects influence the parameters D_{L0} , D_{T0} , β_L , β_T of our model (4). Apparently, the authors miss our definition of these parameters introduced in (4), which, obviously, have their longitudinal or transverse meaning only for infinitely long airways. Therefore the numerous figures and discussions of these dependences in (1), including the summary in Table 1, are misleading.

In their paper (1), Parra-Robles and Wild criticize our approach (4) to validate lung morphometry technique. However their critique has no basis. They mistakenly assumed that the gas mixture in the lungs in our experiments contained 40% of ^3He gas which is incorrect; the concentration of ^3He gas in the lungs in our experiments never exceeded 15-20% (as a result, the free diffusion coefficient D_0 never exceeded $0.9 \text{ cm}^2/\text{s}$ rather than $1.03 \text{ cm}^2/\text{s}$ used in (1)). They also suggested that the presence of oxygen in the air can create magnetic field gradients that would substantially affect diffusion measurements. Simple calculations show this assumption is also incorrect – contribution of oxygen to the magnetic susceptibility difference between fresh lung tissue (that we used) and air does not exceed a few percent.

When discussing our branching model (5), Parra-Robles and Wild criticize our approach used in computer simulations as not reflecting “actual physical process of MR signal formation”. Our approach is absolutely correct and it does reflect a real MR-based diffusion measurement process when the diffusing spins are “labeled” by diffusion-sensitizing gradients and the signal is measured at the end of applied diffusion wave-form no matter where their trajectories end (analog of the Lagrangian specification of the flow field). Though another approach (similar to the Eulerian specification of the flow field), that focuses on specific locations in the space, is also correct. This is a trivial detailed balance principle.

We also note that the branching model proposed in (1) does not include a number of important lung features accounted for in our model (5). Specifically, their model (1) consists of a central duct and a branching node at each end, to which two closed ducts are connected. This is unrealistic situation which imposes additional restrictions for diffusing ^3He atoms, especially for those which start their diffusion paths in the closed airways. Thus, the model is “over-restricted” and biased toward the motion narrowing regime. The “bridges” between airways (bottlenecks for the diffusing particles) also make the model biased toward the motion narrowing regime. The model in (1) also completely ignores the well-known difference between ducts and sacs: these two types of airways have substantially different lengths: on average, 0.75mm for ducts vs. 1 mm for sacs (13). Moreover, the model used in (1) doesn't take into account the distribution of airways' radii and alveolar sleeve depths (variation of about 17% according to (13)). Note that all the above-mentioned features are properly accounted for in our branching model (5).

Another shortcoming of the model (1) is the fixed (90°) relative orientation of the attached ducts' planes. This choice (even though used before in several simulation studies) is not validated by any histological data. In our paper (5) these orientations were chosen randomly.

Unfortunately, no discussion of errors produced by any of the above-mentioned choices, made by Parra-Robles and Wild in their model, is present in their paper (1).

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- In vivo lung morphometry technique is based on hyperpolarized gases diffusion MRI.
- It provides accurate 3D tomographic information on lung microstructural parameters.
- This information was previously available only from invasive biopsy specimens.

Figure 1.

(Adopted from (4)). Plot of mean linear intercept (Lm) obtained by means of lung morphometry with hyperpolarized ^3He diffusion MRI vs. direct measurement under the microscope. Each point represents an average across one explanted lung (two points on the left correspond to normal lungs, two points in the middle – lungs with moderate emphysema, and two points on the right – lungs with severe emphysema). The dashed line represents equality of the two measures of Lm .