

RESEARCH ARTICLE

Quantitative Gd-DOTA-based aerosol deposition mapping in the lungs of asthmatic rats using 3D UTE-MRI

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Asthma is a chronic respiratory disease, commonly treated with inhaled therapy. Better understanding of the mechanisms of aerosol deposition is required to improve inhaled drug delivery. Three-dimensional ultrashort echo time (UTE) MRI acquisitions at 1.5 T were combined with spontaneous nose-only inhalation of aerosolized gadolinium (Gd) to map the aerosol deposition and to characterize signal enhancement in asthmatic rat lungs. The rats were sensitized to ovalbumin (OVA) to develop asthmatic models and challenged before imaging by nebulization of OVA to trigger asthmatic symptoms. The negative controls were not sensitized or challenged by nebulization of saline. The animal lungs were imaged before and after administration of Gd-based aerosol in freely breathing rats, by using a T_1 -weighted 3D UTE sequence. A contrast-enhanced quantitative analysis was performed to assess regional concentration. OVA-sensitized rats had lower signal enhancement and lower deposited aerosol concentration. Their enhancement dynamics showed large inter-subject variability. The signal intensity was homogeneously enhanced for controls while OVA-sensitized rats showed heterogeneous enhancement. Contrast-enhanced 3D UTE was applied with aerosolized Gd to efficiently measure spatially resolved deposition in asthmatic lungs. The small administered dose (around 1 μ mol/kg body weight) and the use of standard clinical MRI suggest a potential application for the exploration of asthma.

KEYWORDS

aerosol deposition, asthma, contrast enhancement, Gd-DOTA, lung MRI, ultrashort echo

1 | INTRODUCTION

Asthma is the most common chronic respiratory disease, especially in children. According to the World Health Organization, around 300 million patients suffer from this disease and 80% of chronic respiratory disease deaths occur in low and middle income countries.¹ Asthma affects the lower respiratory tract and especially bronchioles. The symptoms include periodic shortness of breath, chest tightness and cough. This is explained by three characteristic mechanisms: (i) inflammation with bronchial epithelium edema, corresponding to the thickening of the bronchial wall as a

Abbreviations: CT, computed tomography; ET, expiration time; Gd, gadolinium; Gd-DOTA, gadoterate meglumine; HI, heterogeneity index; IT, inspiration time; MMAD, mass median aerodynamic diameter; OVA, ovalbumin; PEF, peak expiratory flow; PIF, peak inspiratory flow; RD, relative dispersion; ROI, region of interest; RR, respiratory rate; RSE, relative signal enhancement; SEM, standard error of the mean; SI, signal intensity; SNR, signal-to-noise ratio; TTP, time to peak; TV, tidal volume; UTE, ultrashort echo time

result of liquid accumulation; (ii) bronchoconstriction by bronchospasms, which are sudden and involuntary contractions of airway smooth muscles of distal bronchi; and (iii) airway remodeling with bronchial hyper-responsiveness, developed by an increased secretion of mucus. The constricted smooth muscles and the inflammatory thick wall narrow the bronchial airways, thus limiting air flow therein.

Asthma is commonly treated with inhaled therapy.² Delivering aerosolized drugs directly into the lungs showed a number of advantages over systemic (oral or intravenous) administration, such as a higher drug concentration delivered more effectively to the airways, a faster onset of action, and reduced systemic adverse effects.^{3,4} However, aerosol deposition patterns are difficult to predict, as they depend on airway geometry, breathing parameters, and aerosol and inhaled gas properties. Besides computational fluid dynamics modeling, imaging methods are thus necessary to better understand deposition mechanisms and improve inhaled drug therapy.

X-ray-based lung imaging techniques, such as gamma scintigraphy⁵ or dual-energy computed tomography (CT) combined with xenon ventilation,⁶ have been used to characterize the efficiency of aerosol delivery or the ventilation defects in asthmatic patients. Positron emission tomography or single photon emission computed tomography, together with co-registered high resolution CT anatomical data, provide accurate three-dimensional information to evaluate regional distribution of inhaled pharmacological agents^{7,8} and lung function^{9,10} in asthma. However, ionizing radiation exposure from inhaled radiotracers and X-ray sources limits their extensive use. Many studies have been made to visualize local ventilation using hyperpolarized gases in MRI such as helium-3 ³He,¹¹ and xenon-129 ¹²⁹Xe.¹² Aerosol deposition could be further mapped with phase-contrast ³He MRI.¹³ Despite relatively high temporal and spatial resolutions, the sophisticated polarization procedure and the limited availability of ³He restrict clinical dissemination. Several alternative techniques were proposed to map lung ventilation/perfusion, by using self-gated 3D ultra-short echo time (UTE),¹⁴ Fourier decomposition¹⁵ and self-gated non-contrast-enhanced functional lung imaging.¹⁶ Another promising alternative to hyperpolarized gas lung MRI relies on contrast enhancement with the use of gadolinium (Gd)-based aerosol administration.¹⁷⁻¹⁹ More recently nebulized Gd-based nanoparticles, developed as multimodal imaging probes and radiosensitizing agents, were combined with UTE MRI to detect lung tumor nodules and improve radiotherapy.²⁰ To favor translational developments, 3D UTE MRI was implemented at 1.5 T with spontaneous nose-only inhalation of aerosolized Gd-DOTA.²¹ The distributions of deposited aerosol were found homogeneous throughout the lungs and aerosol clearance followed similar trends in healthy subjects.²¹

Here, 3D mapping of aerosol deposition was evaluated in asthmatic rats compared with controls. Aerosol distribution and clearance exhibited different spatial and temporal patterns. Hence, aerosolized Gd-based UTE MRI may provide additional insights into lung diseases.

2 | METHODS

2.1 | Animal model

Animal model development and validation were performed at the Centre de Recherches Biologiques (CERB), Baugy, France. The standard study protocol related to this study was approved by the CERB Internal Ethics Committee. Animal use and care were in accordance with the Directive 86/609/EEC European Convention for the Protection of Vertebrate Animals used for Experimental and Other Scientific Purposes.²² Imaging experiments were carried out following the ethical committee guidelines of the local institution (Service Hospitalier Frédéric Joliot, CEA/SHFJ) regarding fair treatment of animals.

2.1.1 | Sensitization to OVA

Rats ($n = 6$, female Wistar, 180 ± 20 g) (Janvier Labs, Saint Berthevin, France) were sensitized to OVA (1 mg/rat) by an intraperitoneal injection emulsified in aluminum hydroxide (50 mg/animal).²³ Undosed rats ($n = 5$, female Wistar, 180 ± 20 g) were used as a negative control. Hereafter, the animals are called 1 AC–5 AC for negative control rats, and 6A–11A for OVA-sensitized ones.

2.1.2 | Asthma challenge

OVA challenges were performed to induce the asthmatic symptoms. OVA-sensitized rats spontaneously breathed an aerosol of 5% OVA generated by a nebulizer (ATOMISOR® NL9 minidose, Diffusion Technique Française, Saint-Etienne, France) in a closed induction chamber for 20 min. The nebulizer produced the aerosol with a mass median aerodynamic diameter (MMAD) of 4.4 μ m. In contrast, the negative control rats were exposed to aerosolized saline for 20 min.

Two challenges were undertaken 7 and 11 weeks after sensitization to verify the asthma symptoms stability. The reference challenge was realized 7 weeks after sensitization with the previously described protocol. The reproducibility of asthma symptoms was verified by a new challenge one month later, 11 weeks after sensitization. MRI was performed at 19 weeks after sensitization.

2.2 | Respiration measurement

Thirty minutes after each challenge, respiratory parameters were recorded for 4 h by unrestrained whole-body plethysmography (PLY/UN2P, EMKA Technologies, Paris, France). This simple, precise, and non-invasive technique allows for the measurement of the relevant parameters, respiratory rate (RR), peak inspiratory and peak expiratory flows (PIF, PEF), inspiration and expiration times (IT, ET), tidal volume (TV), and minute volume, which provide a comprehensive understanding of the respiratory physiology of the rats.

2.3 | Imaging and nebulization protocols

The entire protocol, which lasted about 2 h, has been detailed in a previous study²¹ and was implemented on a clinical whole-body 1.5 T system (Achieva; Philips Healthcare, Best, The Netherlands) at the Service Hospitalier Frédéric Joliot (SHFJ, I2BM; CEA, France).

Two hours before imaging, OVA-sensitized rats (294 ± 8 g) and control rats (332 ± 12 g) were challenged for 20 min by the nebulization of OVA and saline, respectively. Rats, immobilized in the prone position, were anaesthetized with 2% isoflurane in 0.8 L/min pure O₂. While inserted into a 47 mm diameter receiver surface coil (microscopy coil; Philips Healthcare, Best, The Netherlands), they spontaneously breathed through a modified nose cone (Rothacher Medical, Heitenried, Switzerland). 3D isotropic T_1 -weighted UTE radial acquisitions were performed once before, then continuously repeated during nebulization and up to 1 h after nebulization, with the following parameters: TR/TE = 14/0.4 ms, $\alpha = 30^\circ$, 64 mm isotropic field of view, acquisition of 144 points for each of the 32 770 radial spokes (with ramp-sampling) reconstructed into an acquisition matrix of $256 \times 256 \times 256$, nominal acquisition voxel size $(0.5 \times 0.5 \times 0.5)$ mm³, and reconstructed isotropic voxel size of 250 μ m obtained after zero-padding the k -space by a factor of 2. The 3D UTE sequence had a hard RF pulse with duration of 89.6 μ s, corresponding $B_1 = 21.8$ μ T achievable by the system body coil. Echo time was defined as the time between the end of the RF pulse and the start of acquisition. Imaging gradients included a trapezoid (ramp of 479 ms and plateau of 2.2 ms). With an acquisition bandwidth of 255 Hz per pixel, 144 acquisition points were sampled. The readout gradient was fully refocused during the application of a spoiling gradient. T_1 weighting was enhanced using RF spoiling with a quadratic phase transition of 150° increment. Radial encoding was carried out with a 3D radial sampling “Koosh-ball”,²⁴ containing 32 770 radial spokes sampled uniformly in a sphere. With one single excitation, the acquisition duration was 7 min 29 s. R_2^* mapping was performed separately before and after the series of UTE imaging, at the time point of pre-contrast and 1 h after Gd-DOTA nebulization, with double use of the radial UTE sequence: TE₁/TE₂ = 0.4/1.4 ms, TR = 14.7 ms, $\alpha = 30^\circ$. Each scan lasted 8 min with matrix size and resolution the same as the previously described sequence. All the reconstructions were performed with 0.25 mm isotropic voxels using Philips software without phase correction and with an absolute MR scale for storage. The gradient delay for radial reconstruction was calibrated iteratively in a pilot study using several retro-reconstructions and choosing the value (7 μ s) that visually provided the smallest reconstruction artifacts. This gradient-delay parameter was then set for all subsequent scans providing consistent image quality indicating stability of the gradient hardware response.

A fraction of 7 mL of gadoterate meglumine (Gd-DOTA) (Dotarem®; Guerbet, Villepinte, France) was aerosolized on line by a vibrating-mesh nebulizer (Aeroneb® Solo; Aerogen, Galway, Ireland) and was administered to the free-breathing rats during approximately 14 min. The aerodynamic size of the aerosols was measured by laser diffraction (Helos/BF; Sympatec, Germany) with an MMAD of 2.5 μ m.²¹ The TV was estimated using a cine sequence synchronized with the animal's respiration, with the following parameters: TR/TE = 5.2/2.6 ms, $T_{acq} = 5.6$ min, $\alpha = 60^\circ$, BW = 854 Hz, phases = 50, voxel = 1 mm and a gating adapted to respiratory cycles per minute.

2.4 | Lung segmentation

Data were processed offline using MATLAB (Version 2012a; MathWorks, Natick, MA, USA). A semi-automatic segmentation approach was used to regionally analyze the acquired data as described in our previous study.²¹ The segmentation relied on baseline signal intensity (SI) to distinguish voxels corresponding to the lung parenchyma (lower SI) from those corresponding to the vessels and the surrounding tissues (higher SI). Because the SI varied by about 50% on the slice direction perpendicular to the surface coil over the lung volume, the intensity bias along the slice direction of the baseline long TE dataset (TE = 1.4 ms) was corrected by an estimation based on the SI profile of the surrounding tissues. The signal histogram was therefore corrected to show better distinguished components in a Gaussian mixture model. The corrected histograms of the baseline long TE and the 3D UTE datasets were fitted to multi-component Gaussian distributions, providing parenchyma SI mean, μ , and standard deviation, σ . Thresholding at $\mu + 2\sigma$ discriminated the lung parenchyma as lower SI and provided an estimate of the lung volume V_{ROI} . Lung parenchyma density was assessed from sensitivity-corrected UTE, by considering surrounding tissue density equal to 1 g/mL.

2.5 | Image analysis

The relative signal enhancement (RSE) in the segmented lung regions of interest (ROIs) was analyzed for each time point of UTE acquisitions by $RSE = SI(C)/SI(0) - 1$, with pre- and post-administration signal intensities $SI(0)$ and $SI(C)$, where C denotes the contrast agent concentration. RSE at the end of nebulization was converted into a concentration map,²⁵ with longitudinal molar relaxivity $r_1 = 3.7$ /mM/s and baseline $T_{1,0} = 1.1$ s for rat lung of both models at 1.5 T:

$$R_1 = -\frac{1}{TR} \ln \left[\frac{SI(C)/SI(0)(E_{1,0} - 1) + E_{2,C}(1 - E_{1,0} \cos \alpha)}{SI(C)/SI(0)(E_{1,0} - 1) \cos \alpha + E_{2,C}(1 - E_{1,0} \cos \alpha)} \right], \quad (1)$$

$$C = \frac{1}{r_1}(R_1 - R_{1,0}) \quad (2)$$

with $E_{1,0} = \exp(-TRR_{1,0})$ and $E_{2,C} = \exp(-TECr_2^*)$, where $R_{1,0}$ and R_1 are the pre- and post-contrast longitudinal relaxation rates, respectively, and r_2^* is the transverse relaxivity. We have neglected the R_2^* -related variation term $E_{2,C}$ on the quantification of deposition because of the low concentration range and the short TE,²¹ and assumed that lung collapses in the asthmatic case do not affect $T_{1,0}$.²⁶

The relative dispersion (RD) was used to evaluate the heterogeneity of concentration and pre- and post-administration SI as well as R_2^* maps to assess the global aerosol distribution. The RD was calculated as the ratio between the standard deviation, σ , and the mean value, μ :

$$RD = \frac{\sigma}{\mu} \quad (3)$$

Besides the global evaluation of aerosol distribution, local heterogeneity was further assessed to investigate local airway collapse.²⁷ For each pixel within the lung ROI, a heterogeneity index ($HI_{i,j,k}$) was calculated by computing the RD of the voxel aerosol fractional concentration ($FC_{i,j,k}$) in its neighborhood, which was defined as a cubic kernel of local length scale L centered around the voxel of interest. The voxels outside the ROI were excluded from the neighborhood. The voxel aerosol fractional concentration $FC_{i,j,k}$ was calculated as

$$FC_{i,j,k} = \frac{C_{i,j,k}}{\sum_v (C_{i,j,k} \cdot V_{\text{voxel}})} \quad (4)$$

where $C_{i,j,k}$ is the aerosol concentration in the voxel located at (i,j,k) within the lung volume V , and V_{voxel} is the voxel volume. The local length scale L was set to 10% of the global length scale, which was defined as the average of the left and right lungs' maximum width.

To assess regional distribution, the lung was divided into two equal volumes along four anatomical directions: left/right (L/R), anterior/posterior (A/P), head/feet (H/F), and central/peripheral (C/P) (see Figure 4A later). Relative deposited aerosol concentrations were calculated in each region. Group analysis was expressed as mean $\pm$ standard error of the mean (SEM). Because of small sample sizes and the absence of distribution information, a Wilcoxon rank-sum test (two tailed, or one tailed in some cases) for two groups and Wilcoxon signed rank test for the same group before and after contrast were performed, with a significance level of 0.05.

2.6 | Histological analysis

The rats were euthanized at the end of the imaging protocol by an overdose of isoflurane (5%) followed by intracardiac pentobarbital injection. Lungs were removed and fixed by intratracheal instillation of 10% neutral buffered formalin. Three transverse sections of the right lung (cranial, middle, and caudal lobes) and one longitudinal section of the left lobe were performed. Sections were then processed routinely and embedded in paraffin. Four micrometer thick sections were stained with hematoxylin and eosin and mounted on glass slides (Novaxia, St Laurent-Nouan, France). The lung sections were examined by light microscopy on a Leica Diaplan microscope (Le Net Pathology Consulting, Amboise, France). The histopathological findings were graded in severity using a five-point system: minimal (Grade 1), slight (Grade 2), moderate (Grade 3), marked (Grade 4), or severe (Grade 5), and we assigned 0 for histopathological absence.

3 | RESULTS

All rats survived the challenge procedure and the administration protocol so they could be followed up until the end of the imaging protocol. The respiratory parameters, MRI outcomes, and histopathological findings grades are summarized in Table 1.

3.1 | Respiratory parameters

For OVA-sensitized and OVA-challenged rats, a significant increase in RR ($p < 0.05$, left tailed), PIF ($p < 0.05$, left tailed), and PEF ($p < 0.05$, left tailed) and a shortening in IT ($p < 0.05$, right tailed) and ET ($p < 0.05$, right tailed) were observed progressively over 2 h after challenge and remained quasi-stable for more than 2 h, in comparison with the negative control animals (Figure 1). These OVA-challenge reactions lasted more than 4 h and had disappeared 24 h later. Respiratory measurements measured during another challenge 1 month later showed no significant difference and were thus reproducible (Figure 1). The respiratory parameters (RR, PIF, PEF, IT, ET) on the quasi-stable plateau of the challenge were averaged over the 2 h sensitization, from 2 h after challenge to 4 h after challenge, to assess the sensitization state.

3.2 | Global measurements

At baseline, lung mean signal-to-noise ratios (SNRs) between OVA-sensitized rats and control rats are comparable ($p = 0.052$, two tailed). After aerosol administration, the lung SI is significantly enhanced in control ($p < 0.05$, one tailed) and OVA-sensitized groups ($p < 0.05$, one tailed), and a significant difference was observed between the two groups ($p < 0.05$, two tailed). Compared with the control group after administration, OVA-sensitized rats have a significantly lower SNR within the lung (30.61 ± 3.14 versus 49.11 ± 5.13 , with $p < 0.05$, two tailed). OVA-sensitized rats have a larger lung volume, (5.81 ± 0.43) mL versus (3.60 ± 0.42) mL ($p < 0.05$, two tailed), and a slightly smaller TV, (1.33 ± 0.18) mL versus (1.43 ± 0.17) mL ($p = 0.42$, two tailed).

The mean R_2^* calculated from R_2^* maps showed a higher value in the OVA-sensitized group compared with controls before contrast, (760.60 ± 16.95)/s versus (662.14 ± 35.25)/s ($p < 0.05$, two tailed) respectively, and a comparable level 1 h after nebulization,

Parameter	Rat no.	Control					OVA-sensitized					Mean	SEM	P	
		1 AC	2 AC	3 AC	4 AC	5 AC	6A	7A	8A	9A	10A				11A
Breathing profile	RR (cycles/min)	84	80				177	119	128	146	156	195	153	12	0.036 (l)
	PIF (mL/s)	3.3	2.9				4.4	10.1	4.8	7.2	6.2	6.0	6.5	0.8	0.036 (l)
	PEF (mL/s)	1.9	1.8				4.2	10.8	4.1	5.7	6.1	5.4	6.1	1.0	0.036 (l)
	IT (ms)	286	308				146	186	175	187	173	174	173	6	0.036 (r)
	ET (ms)	428	460				146	311	290	363	259	259	271	30	0.036 (r)
	V _{ROI} (mL)	3.74	4.82	2.77	2.58	4.08	4.64	7.34	6.18	5.42	6.48	4.78	5.81	0.43	0.017
	TV (mL)	1.85	1.62	0.83	1.35	1.50	1.29	1.58	1.41	1.08		1.30	1.33	0.07	0.42
MRI	S _I _{pre}	5.71	5.79	7.68	8.36	6.57	5.83	4.30	4.91	6.10	4.51	5.62	5.21	0.30	0.052
	S _I _{post}	9.55	9.38	12.99	13.19	11.89	7.50	4.83	5.94	7.55	5.58	9.65	6.84	0.71	0.017
	SNR _{pre}	23.96	25.75	32.66	37.92	21.74	25.14	20.53	23.84	27.67	20.01	25.62	23.80	1.23	0.25
	SNR _{post}	40.83	41.52	63.34	59.84	40.03	32.18	22.38	28.01	34.09	23.76	43.23	30.61	3.14	0.03
	R ₂ [*] _{pre} (1/s)		727.70	664.55	550.61	705.72	723.04	813.19	813.20	748.08	732.00	734.07	760.60	16.95	0.019
	R ₂ _{post} (1/s)	578.11	660.92	631.55	627.47	744.09	695.15	610.26	633.77	656.53	804.81	544.32	657.47	35.93	0.93
	post-RSE (%)	93.41	59.71	87.34	62.43	87.11	28.54	12.50	21.05	23.84	23.88	71.81	30.27	8.59	0.017
	max RSE (%)	100.64	59.71	103.29	65.21	109.13	38.24	33.22	22.61	25.98	29.19	95.16	40.73	11.11	0.017
	TTP (min)	27.08	23.22	65.33	28.00	59.15	53.73	88.30	88.10	11.28	41.72	41.75	54.15	12.20	0.54
	C (mM)	0.24	0.19	0.22	0.20	0.27	0.09	0.07	0.07	0.08	0.09	0.24	0.11	0.03	0.026 (r)
	ρ (g/mL)	0.41	0.45	0.42	0.46	0.36	0.41	0.37	0.38	0.44	0.34	0.43	0.40	0.02	0.43
	σ _ρ (g/mL)	0.15	0.14	0.13	0.16	0.13	0.12	0.14	0.12	0.12	0.10	0.13	0.122		
	Q (μmol/kg BW)	1.17	1.33	0.74	0.59	1.20	0.65	0.59	0.54	0.63	0.61	1.82	0.81	0.20	0.25
	RD _C	2.06	0.81	0.57	0.63	1.21	0.79	6.32	0.85	1.65	2.13	1.87	2.27	0.84	0.18
	RD _{Spre}	0.35	0.24	0.21	0.22	0.23	0.22	0.43	0.31	0.25	0.31	0.23	0.29	0.03	0.43
	RD _{Spost}	0.32	0.28	0.23	0.19	0.27	0.24	0.45	0.35	0.27	0.41	0.51	0.37	0.04	0.13
	RD _{h₂pre}		0.53	0.44	0.61	0.61	0.56	0.51	0.59	0.53	0.46	0.57	0.50	0.53	0.02
RD _{h₂post}		0.53	0.48	0.45	0.49	0.44	0.46	0.74	0.64	0.52	0.71	0.79	0.64	0.05	0.052
HL _C	0.76	0.53	0.47	0.52	0.57	0.57	0.63	1.04	0.72	0.70	0.83	0.68	0.77	0.06	0.026 (l)
Histology	AI	0	0				3	3	3	3	4	4	3.33	0.21	0.036 (l)
	PVI	0	0				2	2	2	2	2	2	2	0	0.036 (l)
	PVE	0	0				3	3	3	3	3	3	3	0	0.036 (l)
	PBI	0	0				2	2	2	2	2	2	2	0	0.036 (l)
	HS	0	0				1	1	1	1	1	1	1	0	0.036 (l)
	VA	0	0				2	2	2	1	2	2	1.83	0.17	0.036 (l)
	IAE	0	0				0	1	1	1	0	1	0.67	0.21	0.036 (l)
	HE	0	0				0	1	0	1	1	0	0.50	0.22	0.036 (l)
	GR	0	0				0	0	0	0	0	1	0.17	0.17	1
	FC	0	1				0	0	0	0	0	0	0.17	0.17	0.46
SUM	0	1				13	15	14	14	16	16	14.67	0.49	0.036 (l)	

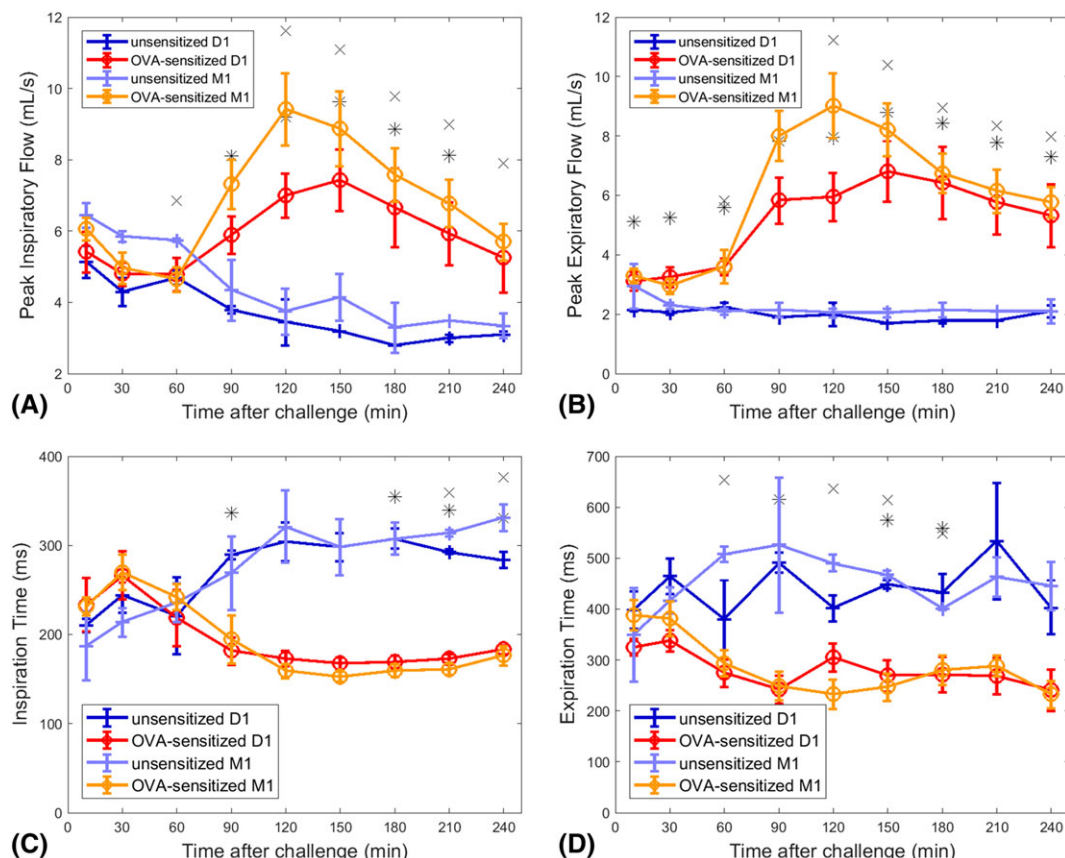


FIGURE 1 Temporal evolution of breathing profile parameters, PIF (A), PEF (B), IT (C), and ET (D), after asthma challenge for OVA-sensitized rats ($n = 6$) and control rats ($n = 2$), 7 (D1) and 11 (M1) weeks after sensitization. Statistical analysis of repeated measures was performed by unpaired two-tailed t -tests and compared with the control group. A significant difference with $p < 0.05$ is marked by an asterisk for 7 (D1) and a cross for 11 (M1) weeks after sensitization

(657.47 ± 35.93)/s versus (648.43 ± 27.37)/s ($p = 0.93$, two tailed). We noticed a mean R_2^* reduction after aerosol administration in most rats of both groups.

In the control rats, the RSE dynamics followed a rapid increase during aerosol administration and a slow decrease once the aerosol administration had stopped (Figure 2). Post-administration average RSEs corresponded to the ones obtained in the healthy rats in the previous study (50% on average),²¹ at the end of administration ($(78 \pm 7.02)\%$) and maximal peak ($(87.60 \pm 10.39)\%$). In the OVA-sensitized rats, the RSE dynamics reveal different trends for contrast agent deposition and clearance, both inter-individually and in comparison with control rats (Figure 2). Post-administration mean RSE and maximal RSE are three times lower in the OVA-sensitized rats than in the control ones ($p < 0.05$, two tailed). They are respectively ($30.27 \pm 8.59\%$) and ($40.73 \pm 11.11\%$).

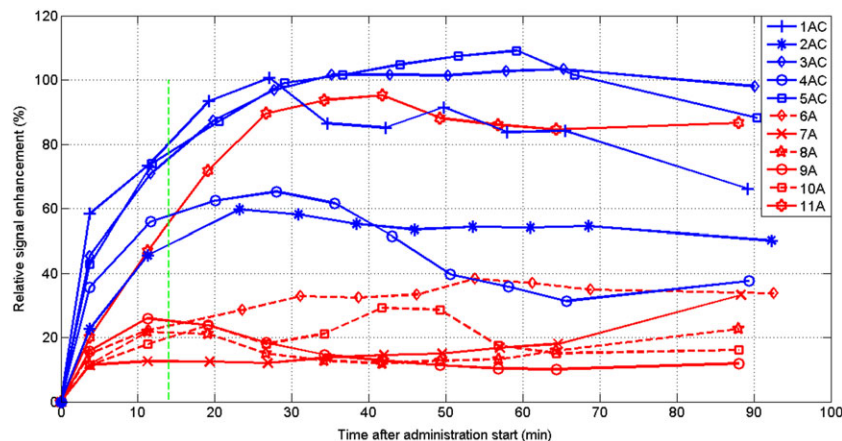


FIGURE 2 Whole-lung RSE as a function of time. Pre-administration acquisition is used for reference ($t = 0$ min) and the end of aerosol administration is indicated by the vertical dotted line ($t = 14$ min). For control rats, RSE curves show similar trends: an increase during administration to a maximum enhancement followed by a slow decay during the observed time up to 90 min. For OVA-sensitized rats, RSE curves show individual trends with lower peaks, which underlines different clearance behaviors in diseased lungs

3.3 | Aerosol concentration maps

The contrast in the lungs of control rats is significantly and homogeneously enhanced with aerosol administration (Figure 3A-C). The resulting mean concentration of deposited aerosol is estimated to be (0.22 ± 0.02) mM. For the OVA-sensitized rats the mean concentration of deposited aerosol is half the value ($p < 0.05$, right tailed) for the control rats, (0.11 ± 0.03) mM, and the distribution of aerosol deposited in the lungs is relatively heterogeneous (Figure 3D-F), as characterized by the HI. The average parenchyma density was estimated as 0.42 ± 0.14 g/mL in control lungs and 0.4 ± 0.12 g/mL ($p = 0.43$, two tailed) in OVA-sensitized lungs. While depending on the effective volume fraction of the lung parenchyma by considering each measured parenchyma density, the total aerosol deposition in the lungs was estimated to be (1.01 ± 0.14) $\mu\text{mol/kg}$ BW (dosage based on body weight) for controls and (0.81 ± 0.20) $\mu\text{mol/kg}$ BW for the OVA-sensitized rats ($p = 0.25$, two tailed).

Regarding the specific trend of the RSE curve observed in Rat 11A, the mean concentration of deposited aerosol is three times higher than in the other OVA-sensitized rats (0.24mM versus 0.08mM), which is comparable to the mean aerosol concentration for control rats (0.22mM). Some hot spot patterns of aerosol concentration were observed in some of the OVA-sensitized rats (rats 10A and 11A).

3.4 | Aerosol regional distribution

For the control rats, as shown by the bar graph in Figure 4B, where blue bars are centered around 50%, the aerosol distribution is relatively homogeneous in the four directions (relative difference $< 10\%$): left versus right ($(50.05 \pm 2.24)\%$ versus $(49.95 \pm 2.24)\%$), anterior versus posterior ($(47.66 \pm 1.28)\%$ versus $(52.34 \pm 1.28)\%$), foot versus head ($(48.68 \pm 1.57)\%$ versus $(51.32 \pm 1.57)\%$), and central versus peripheral ($(52.32 \pm 1.43)\%$ versus $(47.68 \pm 1.43)\%$).

For the OVA-sensitized rats (red bars in Figure 4B), the aerosol distribution is relatively heterogeneous in the three directions (relative difference $\geq 10\%$): anterior versus posterior ($(54.13 \pm 3.24)\%$ versus $(45.87 \pm 3.24)\%$), feet versus head ($(43.10 \pm 4.65)\%$ versus $(56.90 \pm 4.65)\%$), and central versus peripheral ($(43.33 \pm 2.15)\%$ versus $(56.67 \pm 2.15)\%$) with significant difference $p < 0.01$. However, it is rather homogeneous in the left versus right direction with $(51.84 \pm 1.93)\%$ versus $(48.16 \pm 1.93)\%$.

3.5 | Heterogeneous distribution and HI maps

The RD is similar in the pre-contrast SI/R_2^* for OVA-sensitized and control rats, and it is 45% higher ($p = 0.13$, two tailed) in the post-contrast SI and 34% higher ($p = 0.052$, two tailed) in the post-contrast R_2^* for OVA-sensitized rats, but the difference is not significant. Remarkably, the RD on the aerosol concentration maps (RD_c) is close to 115% higher for the OVA-sensitized rats (2.27 ± 0.84) than for the control rats (1.06 ± 0.28) ($p = 0.18$, two tailed). Moreover, RD_c is positively correlated to the PIF ($R^2 = 0.88$, $p < 10^{-5}$) and to the PEF ($R^2 = 0.99$, $p < 10^{-10}$) for OVA-sensitized rats.

HI is mapped in Figure 5 with L as 2.25 mm (nine voxels in each direction). In the control rats, homogeneous aerosol distributions are observed in most of the lung parenchyma, with mean HI 0.57 ± 0.05 . A few heterogeneous depositions around the trachea and at the base of the lungs are revealed (Figure 5A and 5B). In the OVA-sensitized rats, significantly highly heterogeneous aerosol distributions ($p < 0.05$, left tailed) are observed

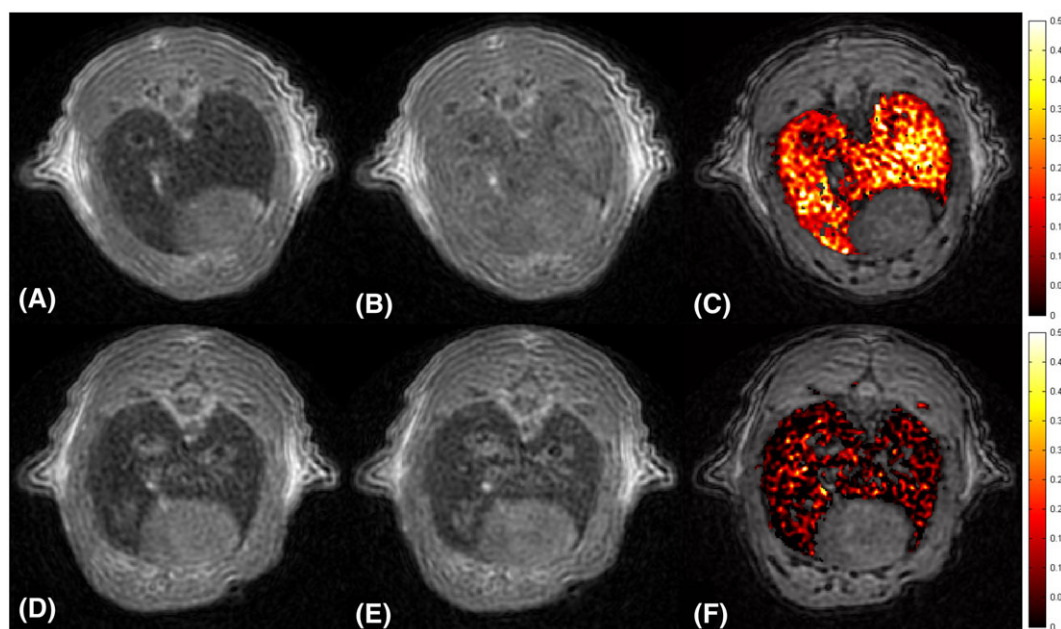


FIGURE 3 Pre- (A, D) and post- (B, E; 20 min after nebulization) aerosolization UTE-MRI ($TE = 0.4$ ms) and resulting aerosol concentration maps (C, F; color scale in mM) superposed on the anatomic image (grey scale). A homogeneous distribution with higher concentration in control rats (A-C; 2 AC) and heterogeneous distribution with lower concentration in OVA-sensitized rats (D-F; 7A) are observable in the concentration maps

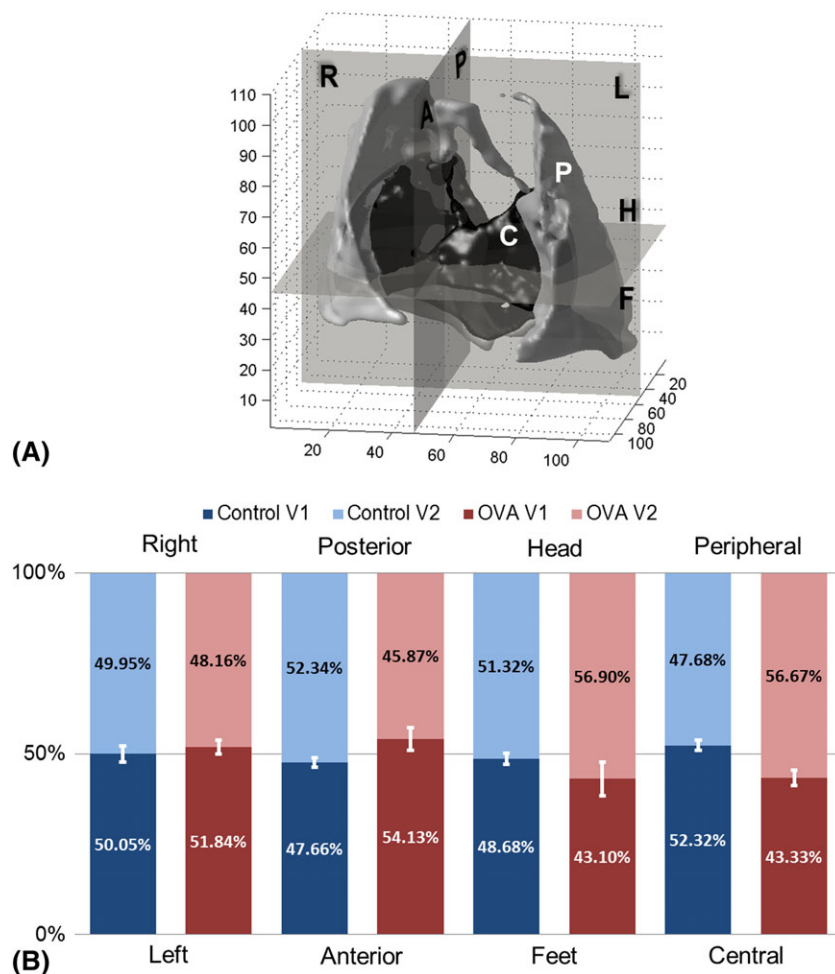


FIGURE 4 A, Four anatomical directions (left/right (L/R), anterior/posterior (A/P), head/feet (H/F), and central/peripheral (C/P)) are defined in the overall lung volume. B, Regional heterogeneity along the defined directions. For control rats the aerosol distribution is relatively homogeneous in the four directions, whereas for OVA-sensitized rats it is observed in most directions (corresponding regional relative difference $\geq 10\%$), except the left versus right direction

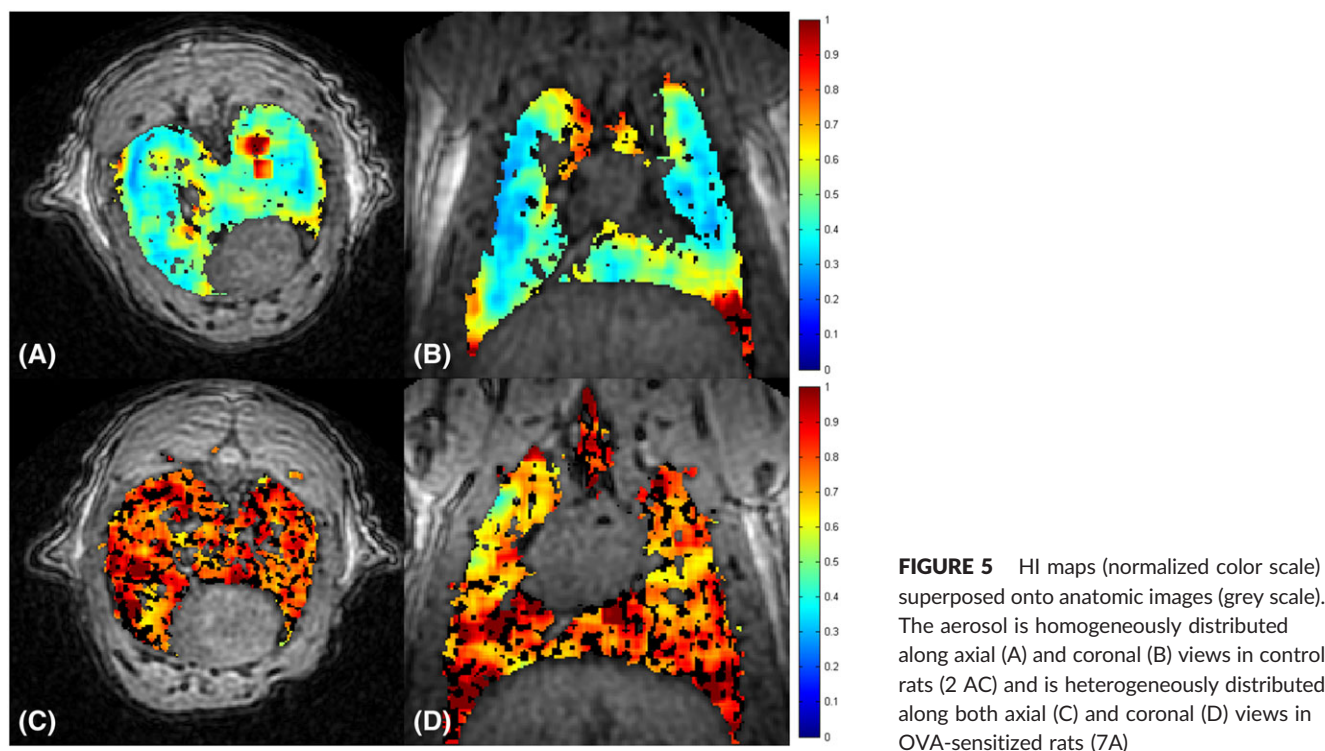


FIGURE 5 HI maps (normalized color scale) superposed onto anatomic images (grey scale). The aerosol is homogeneously distributed along axial (A) and coronal (B) views in control rats (2 AC) and is heterogeneously distributed along both axial (C) and coronal (D) views in OVA-sensitized rats (7A)

throughout the lung, with mean HI of 0.77 ± 0.06 . Higher heterogeneity occurs in the tracheal, intermediary, and basal regions (Figure 5C and 5D). Furthermore, the mean HI of aerosol concentration is also positively correlated to the PIF ($R^2 = 0.75$, $p < 10^{-3}$) and to the PEF ($R^2 = 0.89$, $p < 10^{-5}$) for OVA-sensitized rats.

3.6 | Histopathological analysis

For the control rats histology reveals no sign of inflammation, whereas inflammatory changes are obvious on histological slices in all OVA-sensitized rats (Figure 6), with a significant level ($p < 0.05$, one tailed) for the majority of histological observations. These acute changes are generally characterized here by moderate to marked multifocal alveolar inflammatory cell infiltration (mainly neutrophils), moderate perivascular edema and slight perivascular and peribronchiolar infiltration of inflammatory cells (mainly polynuclear cells), as well as minimal hemosiderosis and minimal to slight vasculitis. Moreover, minimal intra-alveolar edema (7A, 8A, 9A, 11A), minimal granuloma including one with cholesterol clefts (11A), foci of minimal hemorrhage (7A, 9A, 10A) and foam cell (10A) are also occasionally present in some rats. Moderate negative correlations are observed between the sum of histopathological grades and TV ($R^2 = 0.56$, $p < 0.01$) as well as after ($R^2 = 0.48$, $p < 0.01$) (Table 1).

4 | DISCUSSION

First, as demonstrated by the negative histopathological outcomes for inflammation in control rats, the overall experimental protocol, which includes sensitization by intraperitoneal injection, aerosol challenges, anesthesia with isoflurane, aerosolized Gd-based contrast agent administration, and MRI acquisition, has no visible histopathological effects. Here, T_1 -enhanced 3D UTE radial sequence was applied to quantify aerosol deposition, as it is robust to respiratory motion, allowing free breathing during acquisition, and it is less sensitive to short T_2^* in the lung.

4.1 | Respiratory profile, SI, and R_2^* effect

The significant differences of respiratory parameters between control and OVA-sensitized models indicated that the OVA-induced sensitization, like the airway narrowing,^{28,29} was effective. In the present protocol, MRI was not performed in control and OVA-sensitized groups in the pre-challenge state, as the changes in respiratory profiles were deemed sufficient to validate the models.

The R_2^* -related variation term $E_{2,C}$ in Equation 1 on the quantification of deposition was neglected. In our pilot study, we have compared two models to show limited variations on concentration from the R_2^* effect, first by assuming the R_2^* effect performing an exponential decay on the steady-state signal and linearity between R_2^* and concentration, and second by neglecting R_2^* changes. The concentration was overestimated by 5% if R_2^* was assumed not to vary as compared with assuming an exponential decay and linearity with concentration.²¹ With the same

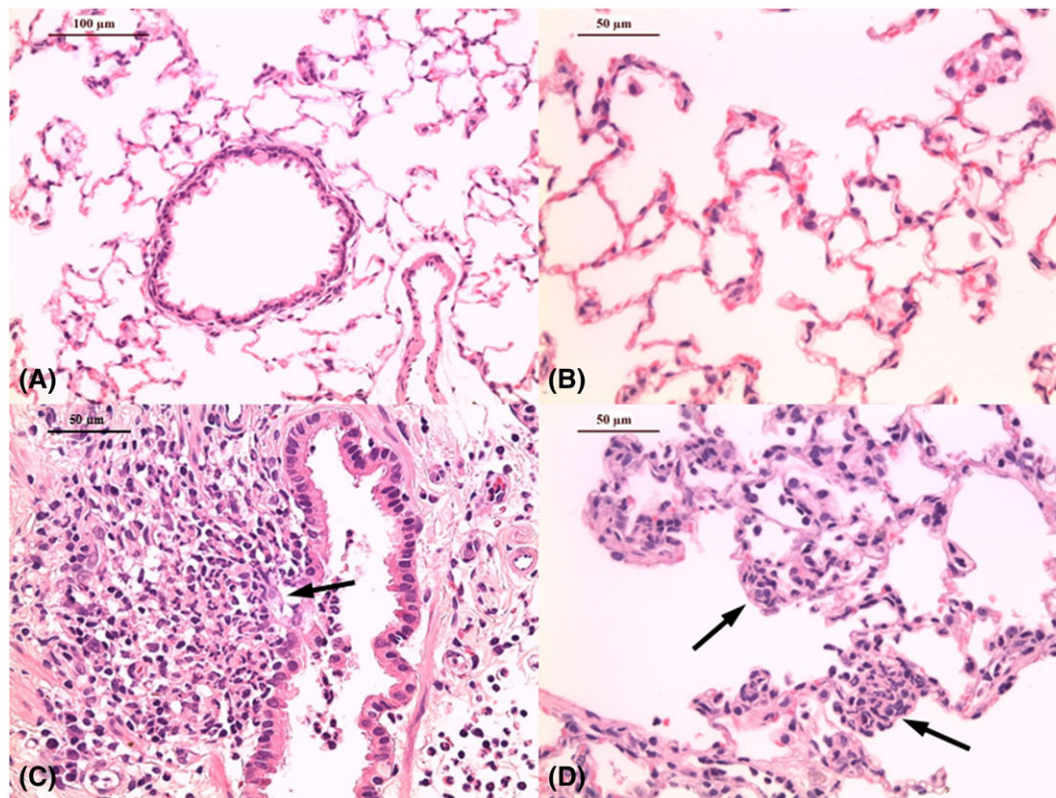


FIGURE 6 Representative photomicrographs of lung tissue from control rat 1 AC (A, HE×25; B, HE×40) and OVA-sensitized rats 8A and 10A (C, HE×40 from rat 8A; D, HE×40 from rat 10A). Examples of local pathological cases are shown. C, focal peribronchiolar infiltration of inflammatory cell, mainly polynuclear neutrophils with lesser number of macrophages and lymphocytes. The focal erosion of the bronchiolar epithelium was marked (arrow), with the presence of a few neutrophils within the lumen. D, Multifocal infiltration of inflammatory cells, mainly polynuclear neutrophils, within the alveolar septa (arrows)

consideration in the present study, the pre- and 1 h post-administration average R_2^* measurements lead to an estimate for $E_{2,C} = 1.0055$ and an average overestimation of 0.08% on concentration in control animals, and an estimate for $E_{2,C} = 1.0421$ and an average overestimation of 1.76% on concentration in OVA-sensitized animals. Therefore, the R_2^* effect was neglected in the concentration quantification.

A quasi-systematic reduction of R_2^* between the pre- and post-administration scans was recorded for both OVA-sensitized and control rats (Table 1), as formerly observed in healthy animals.²¹ Such a reduction is expected as long as the adjunction of Gd-DOTA paramagnetic agent in the airways compensates, at least partially, the magnetic susceptibility difference between the gas and the tissue in the lung.³⁰ Asthmatic symptoms did not substantially alter this compensation effect.

4.2 | Dynamic contrast enhancement

The evolution of the RSE for OVA-sensitized rats showed important inter-subject variability, with different maximal RSEs and times to peak (TTPs). This variability could be explained by the variability of both the disease severity level and the heterogeneous distribution of asthmatic symptoms. It is supported by the differing inter-subject pathophysiological observations made in histology. The higher RSE recorded in rat 11A may be related to the marked alveolar inflammatory cell infiltration, which is known to change the mechanical properties of the airway wall,³¹ and to be present with pulmonary granuloma (compact collection of phagocytes³²). Despite the observed variability, the RSE, which is on average twice as high in the control rats as in the OVA-sensitized ones, could be considered as a relevant indicator of the airway narrowing during asthma challenge.

4.3 | Aerosol concentration and distribution in the lung

Asthmatic lung volume enlargement was observed, presumably due to gas trapping in the airways. Although the lung parenchyma densities of the two groups were comparable, density-related T_2^* was slightly reduced in the asthmatic lungs. However, considering the concentration quantification from Equation 1, the density-compensated effect had little influence on the enhancement ratio $SI(C)/SI(0)$, and the variation of the transverse term $E_{2,C}$ before and after contrast is expected to be small and the ratio was considered equal to 1. Here, the same baseline $T_{1,0}$ between control and asthmatic lungs was considered. However, T_1 reduction in the asthmatic lungs could be observed and oxygen-breathing in this study could further reduce T_1 relaxation, because of oxygen's paramagnetic properties.³³ Nevertheless, as the assessment of concentration based on $E_{1,0}$ from Equation 1, the variation ratio of $E_{1,0}$ between healthy and asthmatic lungs was close to 1, which has negligible effect on the quantification. Taken together, the difference measured in the concentration between healthy and OVA-sensitized rats is considered to be related to asthmatic symptoms. Here the longitudinal relaxivity value in Equation 2 was the calibrated value measured for the contrast agent homogeneously dissolved in the blood/water solution provided by the manufacturer, as the relaxivity in the lung is not known.

The low aerosol concentration, deduced from the weak RSE, in OVA-sensitized rats suggests that OVA-induced airway narrowing restricts aerosol deposition accordingly. A heterogeneous distribution of deposited aerosol is systematically observed in asthmatic rats. Therefore, relative deposition homogeneity could be a sign of limited effect. As observed in rat 6A, no specific pathological symptoms other than the principal findings in OVA-sensitized cases (inflammatory cell infiltration) was observed in histology and a relatively homogeneous aerosol distribution was recorded. The hot spots observed in some OVA-sensitized rats (10A and 11A) could be the result of droplet accumulation when impacting at asthma-induced constrictions. Rats 10A and 11A show marked (highest level) alveolar inflammatory cell infiltration, focusing within the alveolar septa, which could narrow the peripheral airway.³⁴ Furthermore, rat 10A had minimal foam cell focus, which is associated with inflammation,³⁵ leading to airway remodeling.^{34,36} Rat 11A presents minimal granuloma, which could be an additional indicator of respiratory tract narrowing.³⁷

Regionally, the distribution of deposited aerosol is relatively homogeneous for the control rats, whereas for OVA-sensitized rats the heterogeneity of aerosol distribution in most directions is a clear signature of airway narrowing and even airway obstruction, which usually occur at varying locations in the lungs during asthma.

4.4 | Dispersion of NMR parameters

The higher RD of post-administration SI and R_2^* in OVA-sensitized rats results from both the remodeled pulmonary structure and the heterogeneous distribution of aerosol deposition. The higher RD and HI of aerosol concentration maps in OVA-sensitized rats corroborate the heterogeneity of the aerosol deposition. These findings are related to the breathing profile change, along with increased PIF and PEF. Positive correlations between the aerosol heterogeneity and peak flow rate observed in the OVA-sensitized group lead to the deduction that a higher sensitization grade leads to a greater dispersion of aerosol distribution in the lung. Therefore the deposition heterogeneity could be a pathophysiological marker for asthmatic symptoms. Such HI mapping based on local RD could be used to explore voxel-wise local heterogeneity and potential airway pathophysiological modification; it may be further compared with local histological analyses to facilitate diagnosis of asthmatic grades.

4.5 | Lung morpho-pathological modification and aerosol deposition

Asthmatic symptoms such as inflammation, mucous hypersecretion and airway narrowing act together to yield higher gas velocities and can generate turbulence in the affected airways.³⁸ The heterogeneity of peripheral bronchoconstriction, accompanied by an increase in peripheral

resistance, is a major determinant of airway obstruction.³⁹ This effect favors more important aerosol deposition in the proximal airways. However as observed in this study, it globally yields a lower deposition concentration and more heterogeneous deposited aerosol distribution in the lungs.

Post-administration contrast-enhanced MRI, providing the temporal evolution of aerosol deposition as well as the voxel-wise, regional, and global aerosol concentration distributions in the lung, carries rich information on breathing patterns and the underlying pathology. These highlighted parameters, obtained with the aerosolized Gd-DOTA contrast-enhanced UTE MRI technique, provide spatial and temporal characterization of both the pathophysiological state of the probed airways and the aerosol deposition distribution in the airways. Thus, it could guide the development of novel inhalation administration methods.

4.6 | Clinical transfer perspective

The imaging protocol was performed *in vivo* on rats with a clinical RF coil and a conventional MRI contrast agent using a clinically relevant administration route on a standard 1.5 T MRI system. It thus provides insight into the expected contrast and feasibility in humans for free-breathing aerosolized contrast agent protocols. Notably, administration was performed using an MR-compatible nebulizer, without having to move the animal, thus enabling robust pre- versus post-administration comparison and quantitative voxel-wise analysis.

Here, 3D radial UTE allowed fairly long (7.5 min) acquisitions with negligible motion artifacts. These limited artifacts benefitted from the stable respiratory cycles of the anesthetized rats. UTE images reflect the temporal average of positions and lung parenchyma that could be segmented properly from surrounding vessels with limited blurring. In humans, to have reasonable acquisition durations and to take into account the effects of respiratory fluctuations, 3D UTE with respiratory compensation or navigation by prospective/retrospective gating could be employed, or alternatively 2D UTE breath-hold acquisitions could be implemented.

In the present study, the integrated human-size body coil (birdcage geometry with a diameter larger than 50 cm) was used for transmission, so that the flip angle could reasonably be considered homogeneous within the imaging field of view, smaller than 50 mm.⁴⁰ The body-coil flip angle calibration relied on the default optimization protocol of the manufacturer, which can also lead to a bias smaller than 3%.⁴¹ These biases may add on to a maximum estimated bias of 6% that propagates to the estimation of concentration, leading to a relative bias in the concentration measurement of less than 2%.²⁵

The reception coil sensitivity profile varied by approximately 50% over the lung volume. To help with the histogram-based segmentation procedure, the estimation of the coil's sensitivity profile was self-calibrated, by using the surrounding tissues, which did not require a separate calibration or additional body-coil scan. It is similar to the bias correction performed routinely in brain imaging, and was successful in refining the histograms here. The coil sensitivity profile could have been calibrated with the body coil; however, the size of the rat and of the small reception coil, as well as the target resolution, were not adapted to a standard procedure as done for humans. In any case, the quantitative analysis is based on the signal enhancement ratio, and it is thus intrinsically normalized by the reception coil sensitivity. In humans, the reception coil sensitivity profiles could also accommodate spatial selectivity in 3D UTE when using a non-selective excitation. A cardiac array or a specific lung array would provide such spatial selection. Additionally, the approaches to optimize selective excitations could be combined for UTE imaging, such as using the variable rate selective excitation method.⁴²

5 | CONCLUSION

Contrast-enhanced 3D UTE MRI combined with aerosolized Gd-DOTA was applied to characterize asthmatic rats. The heterogeneity of aerosol distribution measured in the lungs of asthmatic rats was correlated to the breathing patterns characterized by peak flow rate, so they could be advantageously used as regional pathophysiological markers for asthma. The fairly low-dose and highly heterogeneous aerosol deposition recorded in asthmatic lungs was related to the OVA-induced asthmatic reactions. With 3D UTE, MRI is an efficient tool to measure spatially resolved aerosol deposition in the lungs. The use of small Gd doses in a 1.5 T whole-body clinical MRI system suggests a potential clinical transfer for the evaluation of aerosol deposition in healthy and diseased human lungs.

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