Prospectively and retrospectively gated lung MR imaging sequences compared for visualizing pneumonial cryptococcosis

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TARGET AUDIENCE: Researchers interested in the non-invasive and longitudinal assessment of lung pathology in small animal models of lung transplantation, infection, fibrosis and/or emphysema.

PURPOSE: *Cryptococcus neoformans* and *C. gattii* are encapsulated yeast that cause life-threatening disease in both immune competent and immune suppressed individuals. Cryptococcosis mostly affects the lung of a host and may spread to the brain, manifesting itself by cryptococcal meningitis and/or pseudocystic lesions in the brain. As in animal models of this disease, the mice show no phenotypical signs indicating disease onset, it is essential to dynamically monitor cryptococcosis non-invasively to establish the kinetics of cryptococcal lung infection and the time profile of spreading to the CNS for each animal individually. Because currently available imaging tools to evaluate lung infection with good temporal and spatial resolution *in vivo* are lacking (μCT is limited because of radiotoxicity concerns), advances made in lung MRI techniques to follow-up disease progression will greatly enhance research in lung infections. We have aimed at optimizing, evaluating and fine-tuning novel MRI protocols to visualize lung infection in a mouse model for pneumonial cryptococcosis. We compared prospectively gated spin echo and retrospectively gated gradient echo MRI sequences and validated our results with CT imaging of the lung, histology and quantification of fungal load.

METHODS: Animal model: Balb/C mice were infected by inhalation of a *C. gattii* R265 cell suspension (50 000 cfu's) in PBS (n = 4) or sham (n = 2). Mice were scanned with CT and MRI at 2 to 3 weeks post infection and afterwards sacrificed followed by lung isolation for histology and quantification of fungal load.
MRI methods: MRI images were acquired on a horizontal Bruker Biospec (9.4T, 20 cm Bruker Biospin, Ettlingen, GE) equipped with a gradient insert, with a maximal gradient strength of 1200mT/m, and in combination with a 3.5 cm quadrature resonator. The following sequences were used: (1) an ecg - and respiration triggered RARE sequence (TR~ 6000ms, TEeff=15.9ms, 11slices of 0.5mm, in plane resolution of 100μm and 2 averages) (2) a retrospectively gated FLASH sequence (IG-FLASH; IntraGate®, Bruker) with specific parameters: TR/TE =11.9/1.26ms, 10 deg flip angle, bandwidth of 150kHz, 1 slices of 0.5 mm thick, in plane resolution of 100 μm, 80 repetitions resulting in a 4 min acquisition (3) retrospectively gated ultra short echo time imaging using IntraGate® (IG-UTE) in fid mode with TR/TE=10/0.3ms, slice position and thickness as for IG FLASH, in plane resolution of 133μm, 100 movie cycles. CT methods: retrospectively gated CT images were acquired on a small animal μCT scanner (SkyScan 1076, Bruker microCT) software as described before [1].

RESULTS: While the mice showed no phenotypical signs of cryptococcosis, the progression of the lung pathology can be non-invasively visualized using prospective T_2 weighted imaging or IG FLASH imaging. The contrast in the current IG UTE sequence is however more apparent and extensive than for the IG FLASH. IG UTE images of control animals confirm the absence of contrast without infection. The extent of infection was validated using CT and confirmed the inter-subject infection variability seen on the IG-UTE (figure1). Cryptococcal invasion of the lung was also confirmed by histology and lung fungal load quantification, confirming the in vivo results.

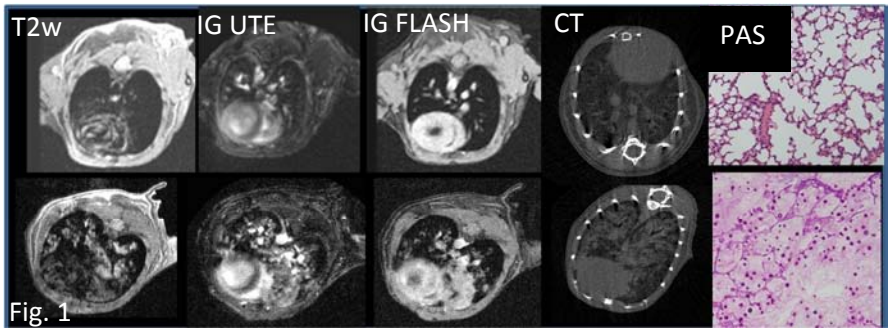

Fig. 1

DISCUSSION: In contrast to prospective methods which are tedious and difficult to setup due to the requirement of combined respiration and cardiac triggering for lung imaging, the IG-UTE is an efficient alternative that seems to provide better contrast than conventional gradient echo imaging. In contrast to CT, with its low soft tissue contrast, the IG UTE methodology could potentially also provide contrast difference between fibrotic area's and inflammation.

CONCLUSION: To the best of our knowledge, this is the first study showing that non-invasive monitoring of pneumonial cryptococcosis is feasible with retrospectively gated UTE MRI (Intragate, Bruker) resulting in images with high resolution and contrast. This MRI approach will allow longitudinal screening of animals, without radiotoxicity concerns as for CT, thereby visualizing infection onset and progression on an individual basis and far before the appearance of any phenotypical signs of disease. MR imaging of cryptococcosis will greatly help unraveling the still enigmatic pathogenesis of this life-threatening disease.

REFERENCES:
(1) De Langhe E, Vande Velde G, *et al.* PLoS ONE 7, 2012: (8):e43123