below ±17.8%, comparable to volume using Bland-Altman and also had high repeatability {CCC (0.84≤CCC≤0.99)}.
Conclusion This study has highlighted the potential clinical utility of CTTA in the risk stratification of PNs. It has also shown that CTTA is a highly repeatable imaging biomarker of malignancy, akin to volume measurements but with the advantage of not requiring imaging follow-up.

Understanding and treating those irritating infections

S16 CIRCADIAN CONTROL OF PRIMARY LUNG ALLOGRAFT DYSFUNCTION, MEDIATED BY THE CLOCK PROTEIN, REVERBα

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Introduction The circadian clock regulates murine immune responses by time of day, partly through the clock protein REVERBα, resulting in altered mortality after infection. The mechanisms regulating time of day differences are poorly understood in humans, where performing circadian studies presents a number of challenges. Lung transplantation, which is performed at any time of day to minimise organ ischaemic time, is an ideal model to study circadian effects on human immune responses.
Methods Primary graft dysfunction (PGD) incidence after lung transplantation was examined for an eight year retrospective (2004–2012) cohort (n=563) in one centre. Patients were excluded, *a priori*, if they had significant intra-operative complications, had a previous lung transplant, or if the donor lung had undergone *ex-vivo* perfusion. Circadian factors were also studied using PER2::Luc and REVERBα$^{-/-}$ mice and by pharmacological targeting of the circadian clock in primary alveolar macrophages from lung transplant recipients.
Results The incidence of PGD grades 2/3 at 24 hours was temporarily elevated when organs were reperfused between 4 and 8 am ($p<0.02$) compared to other time points. Similar observations were made when the cohort was examined by operation start time ($p<0.01$). Sub-cohort analysis, defined using ISHLT relative contraindications, revealed that PGD incidence oscillated in a circadian manner ($r^2=0.87$, $p=0.046$). Investigations in PER2::Luc mice, which allows real time tracking of circadian oscillations, revealed that temperature and serum fluctuations, mimicking organ preservation, shifts the donor organ clock by 4–12 hours depending on time of retrieval. This could create circadian desynchrony between the transplanted organ and recipient. In macrophages, genome-wide gene expression analysis of the role of REVERBα identified gene ontology terms linked to the regulation of lymphocyte function and activation, suggesting a functional link from the macrophage to the adaptive immune response. Furthermore, key PGD biomarkers are elevated ($p<0.05$) in macrophages from REVERBα$^{-/-}$ mice and are repressed ($p<0.05$) by REVERB ligands (GSK2945 and GSK2667) in macrophages from lung transplant recipients.
Conclusion This study suggests that the circadian clock could temporarily affect outcomes after lung transplantation due to recipient-donor circadian desynchrony. Ligands targeting the clock protein REVERBα repress key PGD biomarkers showing that this is a tractable therapeutic pathway.

S17 LATENT CLASS MODELLING FOR PULMONARY ASPERGILLOSIS DIAGNOSIS IN LUNG TRANSPLANT RECIPIENTS

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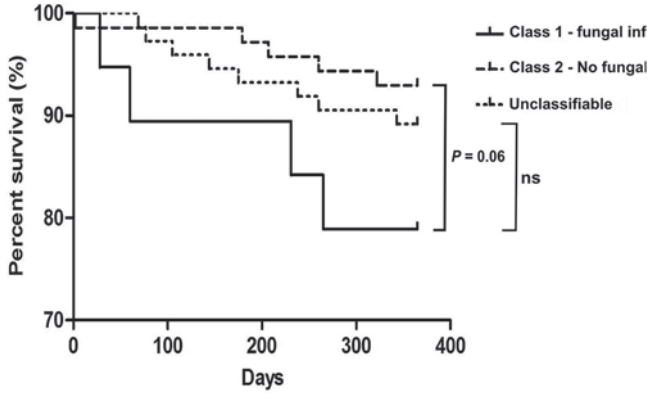
Rationale Timely, accurate diagnosis of invasive aspergillosis (IA) is key to enable initiation of antifungal therapy in lung transplantation. Despite promising novel fungal biomarkers, the lack of a diagnostic gold-standard creates difficulty in determining utility.
Objectives This study aimed to use latent class modelling of fungal diagnostics to classify lung transplant recipients (LTR) with IA in a large single centre.
Methods Regression models were used to compare composite biomarker testing of bronchoalveolar lavage to clinical and EORTC-MSG guideline-based diagnosis of IA with mortality used as a surrogate primary outcome measure. Bootstrap analysis identified radiological features associated with IA. Bayesian latent class modelling was used to define IA.
Measurements and Main Results A clinical diagnosis of fungal infection ($P=<0.001$) and composite biomarker positive Results ($P=<0.001$) had significantly increased 12 month mortality. There was poor correlation between clinical diagnosis, EORTC-based IA diagnosis and composite biomarker positivity. Tracheobronchitis was positively predictive of a clinical and composite biomarker positive diagnosis of IA ($p=0.004$;95% CI–1.79–21.28 and $p=0.03$;95% CI–0.85–15.62 respectively). Latent class modelling resulted in the formation of 3 groups: Class 1: likely fungal infection; Class 2: unlikely fungal infection; Class 3: unclassifiable. *A. fumigatus* PCR was positive in ~90% of class 1 LTRs compared to only 1% in class 2. Analysis of mortality showed a trend towards significance comparing class 1 with class 2 ($p=0.06$;HR–4.7;95% CI(0.91–24)) (figure 1).



Abstract S17 Figure 1

Conclusions This study demonstrates a latent class modelling approach for IA diagnosis in LTR with a combination of culture, composite biomarker testing, and radiology required for optimal IA diagnosis.

S18 MECHANISMS REGULATING COLLAGENOLYTIC AND ELASTOLYTIC ACTIVITY IN M. AVIUM INFECTION

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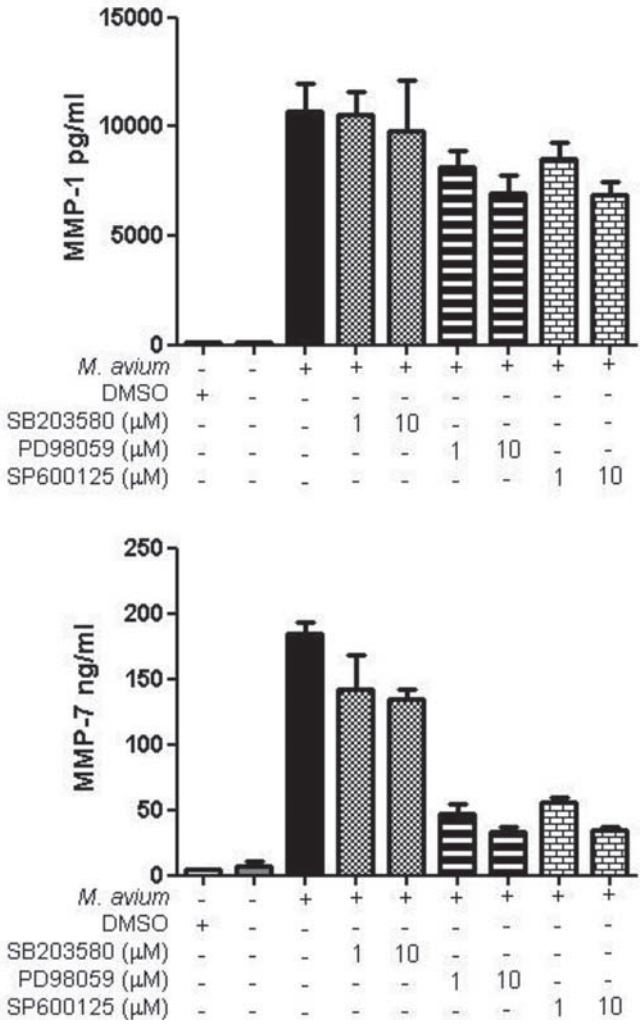
Background The incidence of pulmonary non-tuberculous mycobacterial (NTM) infection is increasing. In the UK mycobacterium avium complex (MAC), is the commonest NTM infection outside of CF lung disease. Patients with pulmonary MAC infection develop cavitating lung disease or nodular bronchiectasis, but the mechanisms of tissue destruction are not well-characterised, unlike *M. tuberculosis* infection. We have previously shown that clinical isolates of *M. avium* surprisingly do not drive secretion of MMP-9 by infected macrophages. Instead, *M. avium* drives functionally unopposed MMP-1, previously thought to be an *M. tuberculosis*-specific response, and MMP-7. We investigated the mechanisms regulating MAC-induced MMP-1 and −7 secretion.

Methods Monocytes were isolated from healthy human volunteer blood by density centrifugation and adherence, before incubation in GM-CSF for 7 days to generate monocyte-derived macrophages (MDMs). MDMs were stimulated with four different clinical isolates of *M. avium* at MOI 100 for up to 72 hours. Whole cell lysates, and cytoplasmic and nuclear extracts were collected 15 mins −4 hours after infection, and analysed by western blot for protein phosphorylation or TransAm assay for NF-κB activation. Supernatants collected at 72 hours were analysed by ELISA for MMP-1 and 7.

Results Infection with *M. avium* caused activation of all 3 MAPK (p38, JNK, ERK) pathways as early as 15 min post exposure with maximal phosphorylation at 30 min. *M. avium* infection drove maximal nuclear translocation of NF-κB and degradation of cytosolic IκBα at 30 min, returning to baseline by 4 hours. *M. avium*-induced MMP-1 secretion from MDMs is ERK and JNK, but not p38- dependant (figure 1). Treatment with caffeic acid phenethyl ester (CAPE), an NF-κB inhibitor, reduced *M. avium*-induced MMP-1 secretion by 30%. Both MMP-1 and −7 upregulation were suppressed by PI3 kinase inhibitor LY294002. *M. avium*-induced MMP-7 upregulation was not inhibited by indomethacin.

Conclusions MMP-1 and −7 may drive the destructive pulmonary pathophysiology that characterises MAC infection. However, regulation of the host macrophage response to *M. avium* is divergent to that *M. tuberculosis*, with p38- independent MMP-1 secretion. This divergence in intracellular signalling may necessitate deviation in potential adjunctive patient therapies for *M. tuberculosis* and *M. avium*.



Abstract S18 Figure 1 MMP-1 and −7 secretion from *M. avium* stimulated MDMs, pre-incubated with p38, ERK or JNK inhibitor prior to infection. Supernatants were harvested at 72 hours and MMP concentration analysed by ELISA.

S19 THE ROLE OF LYMPH NODE-RESIDENT NEUTROPHILS IN ADAPTIVE IMMUNITY

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Introduction Neutrophils play a key role in the early response to a diverse range of infectious and inflammatory stimuli. However, persistent neutrophilic inflammation can result in collateral tissue damage, as evident in a number of chronic respiratory diseases. In addition to their role in innate immunity, neutrophils can also shape the adaptive immune response, in part through antigen presentation. Whilst there is accumulating evidence that neutrophils can migrate to draining lymph