Pulmonary Aspergillosis in Patients with Suspected Ventilator-associated Pneumonia in UK Intensive Care Units

Loughlin, L., Hellyer, T. P., White, P. L., McAuley, D. F., Conway Morris, A., Posso, R. B., Richardson, M. D., Denning, D. W., Simpson, A. J., & McMullan, R. (2020). Pulmonary Aspergillosis in Patients with Suspected Ventilator-associated Pneumonia in UK Intensive Care Units. *American Journal of Respiratory and Critical Care Medicine*, *202*(8). https://doi.org/10.1164/rccm.202002-0355OC

Published in:
American Journal of Respiratory and Critical Care Medicine

Document Version:
Peer reviewed version

Pulmonary Aspergillosis in Patients with Suspected Ventilator-associated Pneumonia in UK Intensive Care Units

Laura Loughlin MB BCh[1], Thomas P Hellyer PhD[2], P Lewis White PhD[3], Danny F McAuley MD[1], Andrew Conway Morris PhD[4], Raquel B Posso MSc[3], Malcolm D Richardson PhD[5], David W Denning FRCP[6], A John Simpson PhD*[2], Ronan McMullan MD*[1].

1. Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom
2. Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne NE2 4HH, United Kingdom
3. Public Health Wales, Microbiology Cardiff, University Hospital of Wales, Heath Park, Cardiff CF14 4XW, United Kingdom
4. Division of Anaesthesia, Department of Medicine, University of Cambridge, Addenbrooke's Hospital, Hills Road, Cambridge CB2 0QQ, United Kingdom
5. UK NHS Mycology Reference Centre, Manchester University NHS Foundation Trust, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, United Kingdom.
6. The University of Manchester and Manchester Academic Health Science Centre, National Aspergillosis Centre, Wythenshawe Hospital, Southmoor Road, Manchester M23 9LT, United Kingdom

* These authors contributed equally to overseeing the work presented

Correspondence to: Dr Ronan McMullan

Address: Wellcome-Wolfson Institute for Experimental Medicine, Queen's University Belfast, 97 Lisburn Road, Belfast BT9 7BL, United Kingdom

Email: ronan.mcmullan@belfasttrust.hscni.net

Phone: 0044 28 9063 5304

Author Contributions

LL was involved in data acquisition, analysis & drafting manuscript. TPH was involved in data acquisition, analysis and revising the manuscript. PLW was involved in data analysis, interpretation and revising the manuscript. ACM and RP were involved in data acquisition, analysis and revising the manuscript. MR, DWD and DFMcA were involved in conception and design of study and revising the manuscript. AJS and RMcM were involved in conception and design of study, drafting and revising the manuscript.

Funding

This publication presents independent research supported by the Health Innovation Challenge Fund (HICF-0510-078 and WT094949/Z/10/Z), a parallel funding partnership between the UK Department of Health and Wellcome Trust. The views expressed in this publication are those of the author(s) and not necessarily those of the Department of Health or Wellcome Trust.
The research data presented was also supported by an investigator-led funding award from Pfizer UK.

Role of the funding sources

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had the final responsibility for the decision to submit for publication**.**

Running head

Aspergillus infection in suspected VAP in UK ICUs

Subject category

10.13 Pneumonia: Fungal Infections

Word count 3500

At a Glance Commentary

Scientific Knowledge on the Subject

Aspergillus infection is the commonest missed infectious cause of death at autopsy in ICU. There have been several studies estimating incidence and prevalence in critically ill patients but they have used definitions of disease which are not tailored to this population and rely on the culture of Aspergillus species as a mycological criterion. Recent evidence has suggested that Aspergillus infection is likely to be underdiagnosed in this population but, to our knowledge, no study has assessed patients with suspected ventilator associated pneumonia to estimate the burden of Aspergillus infection.

What This Study Adds to the Field

Clinically suspected ventilator associated pneumonia is a commonly encountered clinical scenario and arises in a large patient population, globally. This multicentre study is the first robust estimate of the prevalence of *Aspergillus* infection in that patient group. We estimated its prevalence at 12·4%, which is higher than expected and builds on the growing body of evidence that *Aspergillus* infection may be underdiagnosed. This study points towards an opportunity to improve diagnostic pathways using non-culture tests for *Aspergillus*, such as galactomannan, to identify patients who may benefit from further investigation towards a diagnosis of aspergillosis.

Abstract

Rationale

Aspergillus infection in patients with suspected ventilator-associated pneumonia remains uncharacterised due to absence of a disease definition and limited access to sensitive diagnostic tests.

Objectives

To estimate the prevalence and outcomes of *Aspergillus* infection in adults with suspected ventilator associated pneumonia.

Methods

Two prospective UK studies recruited 360 critically ill adults with new or worsening alveolar shadowing on chest X-ray and clinical/haematological parameters supporting suspected ventilator-associated pneumonia. Stored serum and bronchoalveolar lavage fluid were available from 194 non-neutropenic patients and underwent mycological testing.

Patients were categorized as having probable *Aspergillus* infection using a definition comprising clinical, radiological and mycological criteria. Mycological criteria included: positive histology or microscopy; positive bronchoalveolar lavage fluid culture; galactomannan optical index ≥1 in bronchoalveolar lavage fluid or ≥0·5 in serum.

Measurements and main results

Of 194 patients evaluated, 24 met the definition of probable *Aspergillus* infection, giving an estimated prevalence of 12·4% (95% CI 8·1-17·8). All 24 patients had positive galactomannan in serum (n=4), bronchoalveolar lavage fluid (n=16), or both

(n=4); three patients cultured *Aspergillus* sp. in bronchoalveolar lavage fluid. Patients with probable *Aspergillus* infection had significantly longer median duration of critical care stay (25·5*vs*15·5 days, p=0·02). ICU mortality was numerically higher in this group although was not statistically significant (33·3%*vs*22·8%, p=0·23).

Conclusions

The estimated prevalence for probable *Aspergillus* infection in this geographically dispersed multicentre UK cohort indicates that this condition should be considered when investigating patients with suspected ventilator-associated pneumonia, including patient groups not previously recognised to be at high risk of aspergillosis.

Word count: 249

Key words: Aspergillus, critical care, diagnostic tests, prevalence

Introduction

Invasive pulmonary aspergillosis is a life-threatening disease classically affecting severely immunocompromised individuals, particularly those with prolonged neutropenia. Recently, non-neutropenic, critically ill patients have been recognised as an at-risk population, although the nature of this risk remains uncertain (1). Reported estimates of incidence and prevalence vary substantially between studies of patients in intensive care units (ICUs). One single centre study estimated the rate of proven or probable invasive aspergillosis (IA) to be as high as 3·7% of all ICU patients without malignancy (2). A more recent large retrospective study reported a prevalence of 0·017% in all ICU admissions during a 3-year period excluding those with severe immunosuppression, a well-established host factor (3). It has previously been estimated that between 821 and 9665 cases of aspergillosis develop per year in UK ICU patients (4,5). In a multicentre study of patients with influenza who were admitted to critical care, the prevalence of IA was 19% with a 5% prevalence in patients with community acquired pneumonia (CAP) but not influenza (6).

Aspergillosis is consistently found to be among commonly missed diagnoses in critically ill patients, demonstrated by the results of multiple autopsy studies (7,8,9). Multiple factors contribute to delayed and missed diagnosis in this patient group which creates difficulties with estimating the burden of disease. Firstly, the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) definitions of IA are intended for use in the research setting and in severely immunocompromised patients (10). Consequently, when patients do not present with severe immunosuppression described in this definition, physicians are less likely to consider the diagnosis. Secondly, the

definition of probable disease relies on a combination of host factors, generally not applicable to the ICU population (11,12).

A definition of aspergillosis based on non-culture tests, without the requirement for host factors, has the potential to identify more patients with this disease and allow disease prevalence to be assessed more reliably. Recently published American Thoracic Society clinical practice guidelines for the diagnosis of fungal infection in critical care highlight the importance of non-invasive diagnostic tests in this population with strong recommendation given to both serum and bronchoalveolar lavage fluid galactomannan (GM) testing and detection of *Aspergillus* DNA by polymerase chain reaction (PCR). However, the guidance is aimed at severely immunocompromised patients, advising caution in their application to other potentially susceptible groups (13).

The *Asp*ICU algorithm was developed to guide investigation of patients with *Aspergillus* cultures from respiratory tract specimens (14). Although useful, it relies on positive culture as a starting point. Furthermore, international clinical guidelines do not address the investigation of patients with ventilator-associated pneumonia (VAP) towards a diagnosis of aspergillosis in the absence of substantial immunosuppression (15,16). As a result, this condition may be under-diagnosed.

A recent critical care-based study by Schauwvlieghe et al on IA post-influenza (6) used a modified definition of disease; this did not rely on host factors and included GM as a mycological criterion for defining the presence of aspergillosis. The aim of the present study was to apply this principle to estimate the prevalence of *Aspergillus* infection in non-neutropenic ventilated patients with suspected pneumonia in the UK; this represents a common scenario in which the diagnosis of

aspergillosis may be considered. Some of the results of this study have been previously reported in the form of abstracts (17,18).

Methods

Study design and patient recruitment

Participants with suspected ventilator associated pneumonia (VAP) who had been recruited to two prospective multicentre UK studies (The VAP-Rapid studies) between February 2012 and September 2016 were re-evaluated for the diagnosis of aspergillosis (19,20). All participants had undergone standardised bronchoalveolar lavage (BAL) and serum sampling and were eligible for inclusion in the present study if sufficient volumes of stored serum and bronchoalveolar lavage fluid (BALF) were available for mycological testing.

Eligibility criteria

Criteria for clinically suspected VAP required for recruitment to the two studies (19,20) were fulfilled when a patient:

1. Had been intubated and mechanically ventilated for at least 48 hours and
2. Had new or worsening alveolar infiltrates on chest x-ray (CXR) or CT scan and
3. Had two or more from
    - Temperature >38°C or <35ºC,
    - White cell count >11x10^9 or <4x10^9 /L
    - Purulent tracheal secretions.

Patients with the following characteristics were excluded from these studies:

1. Recent history of neutropenia (<0·5 x 10^9 neutrophils/L for >10 days).

2. Features considered to predict poor tolerance of BAL.

BALF underwent conventional culture for bacteria and fungi as part of the original study protocols. A panel of mycological tests was subsequently performed on paired BALF and serum samples for the present study. These were:

1. GM enzyme immunoassay (EIA) on BALF and serum samples using the Platelia *Aspergillus* EIA (Bio-Rad Laboratories, France), according to the manufacturer's instructions.
2. PCR amplification for detection of *Aspergillus* DNA on BALF and serum samples according to a method outlined previously and in line with the recommendations of the European *Aspergillus* PCR initiative (21). Each sample was tested in duplicate. Given the likely higher fungal burden in BALF, a positive result was defined by both replicates generating a positive signal at any cycle during the PCR. In serum, where fungal burden is limited, at least one replicate generating a positive signal at any cycle was considered a positive result.
3. (1→3) β-D-glucan (BDG) detection was carried out on serum samples as per the manufacturer's instructions (Fungitell assay, Associates of Cape Cod, USA). A threshold for positivity of 80pg/ml was applied, consistent with previous literature (22).

Definition of *Aspergillus* infection

To overcome previously encountered problems arising from inaccessibility of tissue samples, low sensitivity of culture, poor applicability of traditional host factors and absence of typical clinical and radiological findings, a definition of disease that is not reliant on these was adopted. Patients were categorised as having probable *Aspergillus* infection based on the principles used in a recent ICU study (6),

comprising clinical, radiological and mycological criteria. All three criteria were required for the definition of probable *Aspergillus* infection to be met.

Clinical criteria

At least two of the following signs or symptoms had to be present:

- Temperature <35 °C or >38 °C
- White cell count of $<4\times10^9$/L or $>11\times10^9$/L
- Purulent tracheal secretions

Radiological criteria

Any new or progressive infiltrate on pulmonary imaging by chest X-ray or CT scan of the lungs.

Mycological criteria

One or more of the following had to be present:

- Histopathology or direct microscopic evidence of dichotomous septate hyphae with positive culture for Aspergillus from tissue.
- Positive Aspergillus culture from BALF.
- Galactomannan optical density (OD) index in BALF of ≥1.
- Galactomannan OD index in serum of ≥0·5.

Risk Factors and Clinical outcomes

Where available from the databases of the two studies that patients had been originally recruited to, possible risk factors for aspergillosis were noted including: age; acute physiology and chronic evaluation (APACHE) II score; need for renal replacement therapy; corticosteroid exposure; duration of ICU stay prior to enrolment; and concurrent diagnosis of influenza. BALF specimens from patients categorized as probable aspergillosis also underwent PCR for detection of Influenza

A & B RNA. Presence of microbiologically-confirmed bacterial VAP, length of ICU stay after enrolment and ICU mortality were the outcome measures used.

Secondary analyses

The prevalence of *Aspergillus* infection was determined using the same disease definition but applying a range of BALF GM OD index threshold values from 0·7 to 3·0. A further secondary analysis was undertaken in which more than one positive *Aspergillus* biomarker was required to meet the disease definition.

Statistical analysis

Categorical variables were compared by $\chi 2$ test, and continuous variables with the student *t* test or Mann-Whitney *U* test, as appropriate.
Statistical tests were performed using SPSS version 25 (IBM, Armonk, NY, USA) with the significance level set at 5%.

Results

One-hundred and ninety-four patients with paired samples, from 17 UK centres, were eligible for inclusion (Figure 1). Characteristics of the cohort are summarised in table 1. The median age of participants was 57 years (IQR 44-69) and mean APACHE II score on admission was 17·93 with males comprising 70·6% of the cohort.

Prevalence estimate for probable *Aspergillus* infection

All 194 patients met the clinical and radiological criteria due to the eligibility criteria for the original two studies. The mycological criteria were met by 24 patients, giving an estimated prevalence of *Aspergillus* infection of 12·4% (95% CI 8·1-17·8). GM

was positive in BALF alone in 16 patients, serum alone was positive in four, and a further four patients had a positive GM in both serum and BALF samples (table 2). Three of the 24 patients also cultured *Aspergillus sp.* in BALF.

The median BALF GM OD index in patients with aspergillosis was 1·84 (IQR 1·43-5·51) and 0·12 (IQR 0·07-0·25), in those without aspergillosis (p<0.0001).

The median serum GM OD index in those with aspergillosis was 0·15 (IQR 0·08 to 0·51) and 0·07 (IQR 0·05 to 0·09), in those without aspergillosis (p=0.001).

Prevalence estimates using different BALF GM OD index threshold values

A range of BALF GM OD index threshold values were used to assess prevalence of aspergillosis, (table 3), in addition to the value of 1.0 used in the main analysis. Using a more stringent BALF GM OD index cut-off value of 1·5, six fewer patients would be classified as having probable *Aspergillus* infection giving a prevalence of 9·3% (95% CI 5·6-14·3%).

Other Biomarkers

An additional biomarker (BDG in serum or PCR in either BALF or serum) was positive in 15 of the 24 patients with a positive GM in either serum or BALF (Table 2). If the disease definition required more than one positive biomarker, the estimated prevalence would be 7·8% (95%CI 4·74-12·4%). Ten of the 24 with a positive GM test in either specimen type had a positive BDG; 10 had a positive *Aspergillus* PCR in BALF; and 5 had a positive *Aspergillus* PCR in serum. Of the three patients whose BALF cultured *Aspergillus* species, one was positive for all biomarkers; the other two patients had four of the five biomarkers positive. All patients with a positive serum GM had at least one other *Aspergillus* biomarker positive.

Possible risk factors for aspergillosis

There were no statistically significant differences in age or APACHE II score between the groups with and without probable *Aspergillus* infection, although the age of patients in the probable aspergillosis group was numerically higher (Table 1). The median duration of ICU stay prior to enrolment was numerically higher (7·5*vs*6.0 days) in the probable aspergillosis group but was also not statistically significant (p=0·19). No patients had documented influenza infection at the time of enrolment to the original studies. Of the 24 patients categorized as having probable aspergillosis in the main analysis, none had detectable Influenza A or B RNA in BALF.

Use of steroids prior to bronchoscopy appeared higher in patients who had probable *Aspergillus* infection (25%*vs*15·8%) and renal replacement therapy also appeared to be used more frequently in this group (12·5%*vs*7·5%); neither of these differences were statistically significant. White cell count and use of vasopressors was similar between groups.

Clinical outcomes

The proportion of patients with bacteria cultured in significant numbers (>10^4 CFU/mL) from BALF was similar in those with and without *Aspergillus* infection (Table 1).

Median length of critical care stay was significantly longer in those with probable *Aspergillus* infection compared with those who did not have *Aspergillus* infection (25·5*vs*15 days, p=0·02). ICU mortality was numerically higher in the group with probable *Aspergillus* infection (33·3%*vs*22·8%, p=0·27), however, this did not reach significance with statistical testing. Only one patient classified as having probable

aspergillosis received mould-active antifungal treatment during their ICU episode. Data regarding treatment subsequent to ICU discharge were not available.

Discussion

This study represents the first robust estimate of the prevalence of *Aspergillus* infection in a population of non-neutropenic, ventilated, critically ill patients with suspected pneumonia. The 12·4% prevalence estimate is higher than expected based on previous literature.

We acknowledge uncertainty in this estimate arising from the definition of probable aspergillosis; however, we address this uncertainty by presenting a range of prevalence data using more stringent disease definitions. We considered: (i) the lower bound of the confidence interval for the main analysis; (ii) the effect of using a more stringent cut-off value of 1·5 for BALF GM OD; and (iii) requiring more than one biomarker to be positive for the disease definition to be met. In these scenarios, the range of prevalence estimates obtained is 7·8-9·3%. While lower than the prevalence estimated in our main analysis, this more conservative range of estimates may indicate that a genuine burden of illness exists in these patients. This builds on the work by Schauwvlieghe et al, where 5% of non-immunocompromised critically ill patients with CAP had IA (6). We provide further evidence that, in the ICU population, there is potential for under diagnosis in clinical practice.

One of the reasons for potential under diagnosis is the lack of a validated definition of aspergillosis in this population. Such a definition is difficult to produce because of insufficient clinical evidence. The *Asp*ICU algorithm, primarily intended to guide management of patients with a positive lower respiratory tract culture for *Aspergillus*

(14), does not enhance detection of disease because of the low sensitivity of culture (23). Galactomannan detection has been suggested as an alternative entry route to better capture patients with aspergillosis (24).

It is acknowledged that the disease definition we used is not perfect; for example, there is debate about the most appropriate threshold value to use for GM positivity. D'Haese et al (25) demonstrated that a threshold value of 1.0 OD index in BALF offers a 93·8% specificity for IA. Zhou et al (26) demonstrated 0·7 as an optimal threshold OD index in non-neutropenic patients. As there is uncertainty regarding the optimal threshold value for BALF GM in this heterogeneous population of non-neutropenic critically ill patients, a secondary analysis used a range of BALF GM OD index threshold values. This demonstrated that even when more stringent BALF GM threshold values, offering higher specificity, are applied (eg 1·5 OD, with 95% specificity) the estimated prevalence remained higher than would be expected, at 9.3%. Using an extremely stringent OD threshold of 3.0 for BALF GM, and excluding serum GM from the disease definition, would give a prevalence estimate of 4.1%. This might be considered the ‘minimum’ estimate since a threshold of 3.0 for the BALF GM index has been reported to have specificity of 100%; however, with sensitivity that may be as low as 56% this approach is likely to yield an underestimate (25). It is clear that the true prevalence of aspergillosis in this population remains subject to uncertainty given the variation seen across a range of plausible BALF GM threshold values.

Compounding this uncertainty is the potential effect of disease prevalence on the false positive rate, even for a test with such high specificity as GM. Consequently, our disease definition may overestimate prevalence and, similarly, use of GM alone may lead to overdiagnosis and overtreatment in clinical practice. Collectively, these

issues underscore the importance of not relying on a single diagnostic test as a trigger for starting antifungal therapy in the context of suspected VAP. However, we propose that a high level of GM in BALF has potential to identify patients who should undergo more extensive evaluation towards the diagnosis of aspergillosis, even in patient groups that are not classically regarded as being at high risk. Such further evaluation is likely to be multimodal, since no single alternative test (direct microscopy, culture, serum GM or β-D-glucan, Aspergillus PCR, or CT scan) is more sensitive or specific than BALF GM.

Other biomarkers (serum β-D-glucan, serum and BALF PCR for *Aspergillus* DNA) did not form part of the disease definition in this study, however, they provided useful corroboration of our results since 15 of the 24 probable cases had other biomarkers positive. These could be useful in a panel of rapid, non-culture based tests, but require further assessment of diagnostic accuracy in the ICU population to establish their utility.

This study suggests that detection of galactomannan in BALF appeared to be more sensitive than in serum for detection of probable *Aspergillus* infection in this population. Twenty (88.3%) of the 24 patients with probable infection had a positive BALF result whereas only eight (33·3%) had a positive serum result. This is in keeping with previous studies, where BALF GM sensitivity has been demonstrated to be significantly higher than culture or serum GM in this patient population (24,26). In non-neutropenic patients, several studies have found that serum galactomannan lacks sufficient sensitivity when used alone (26, 27, 28). Our data support that view since, not all patients with the highest values for BALF GM had a positive serum result. Although serum GM lacks sensitivity, all eight patients in our dataset who had a positive serum GM had at least one other biomarker positive. This suggests that,

although not a sensitive test, it may still have an important role in a diagnostic panel as it seems likely to represent established disease.

We note with interest that patients categorized as having probable *Aspergillus* infection had significantly greater length of stay in critical care after enrolment. This group also had mortality that was numerically higher (though not statistically significant). Whether these findings represent an association with increased risk of aspergillosis or are a consequence of other factors cannot be ascertained within the present study design.

The same is true of risk factors that were noted, more generally. The risk factor data are incomplete, hence subject to bias, because the patients had been originally recruited to studies that focused on bacterial VAP; as a result, data collected were not ideally tailored towards risk factors for aspergillosis. Use of steroids and renal replacement therapy, which are previously identified risk factors for aspergillosis (29), were not significantly higher in the probable aspergillosis group; however, insufficient details of the intensity, timing or duration of exposure limit inference from these data. Moreover, since the epidemiology of aspergillosis in this patient group has not been well characterised, our understanding of risk factors is incomplete; this, along with the missing details referred to, may lead to futile risk factor analysis in the present study.

Because of recent attention that influenza has received as a possible risk factor, it is interesting that none of our probable aspergillosis cases had detectable Influenza RNA in BALF. Nonetheless, this finding does not necessarily exclude the possibility of antecedent influenza infection.

Only one of the patients had treatment active against aspergillosis during their ICU admission. An incomplete picture of whether other patients received such treatment

subsequently during their hospital stay makes this finding difficult to interpret; for that reason, we cannot infer that it materially undermines the definition we used.

Likewise, the co-existence of a significant bacterial pathogen in BALF from several patients classified as having probable *Aspergillus* infection is difficult to contextualise. There is no evidence to indicate whether co-infection is likely in such patients and it is not possible to determine whether either organism had a dominant role in the pathogenesis of such patients' pneumonia.

Limitations of our study design included the sample size, which was limited by the size of the VAP-Rapid studies, and the retrospective analysis of prospectively collected data which may introduce bias from incomplete information. More complete data on host factors such as COPD, liver cirrhosis and immunosuppressant drugs, as well as details of clinical response to antibiotic treatment may have strengthened the analysis. Incomplete risk factor, antifungal treatment and long-term outcome data were such that we could not triangulate these findings to provide additional support for the disease definition used. Because this placed additional reliance on GM to classify patients, we considered the effect of applying different GM index thresholds and requiring more than one positive Aspergillus biomarker in the disease definition. Sample storage and retrospective testing may have influenced the performance of non-culture tests. Furthermore, as discussed earlier, the risk of error from diagnostic misclassification arises from the lack of a robust definition of aspergillosis in this patient group.

Strengths of the study included the number of sites participants were recruited from and their geographic spread. Additionally, the study addresses the critically ill cohort, which is a patient group of interest with broad applicability in healthcare and has been understudied to date in the context of aspergillosis. Standardised testing was

performed on all patient samples leading to a complete laboratory data set, strengthening the analysis. Another strength is that the definition used overcomes the weaknesses of previous definitions with its lack of reliance on host factors or non-applicable clinical and radiological findings.

In conclusion, we present a range of estimates for the burden of aspergillosis in this multicentre UK study of non-neutropenic critically ill patients which highlights the potential for under-diagnosis in clinical practice. We suggest that these data demonstrate the need for increased awareness among clinicians. More widespread use of galactomannan when patients with suspected VAP undergo bronchoalveolar lavage may provide a means to identify patients who might benefit from extensive clinical investigation to seek a diagnosis of aspergillosis.

Declaration of interests

PLW reports payment to cover testing costs from Queen's University Belfast for the conduct of the study. Outside the submitted work, he reports personal fees from Gilead, Pfizer and F2G. DFMcA reports a grant from the Wellcome Trust, NIHR and Pfizer for the conduct of the study. Outside the submitted work, he reports personal fees from consultancy for GlaxoSmithKline, Boehringer Ingelheim and Bayer. In addition, his institution has received funds from grants from the UK NIHR, Wellcome Trust, Innovate UK and others. He is a Director of Research for the Intensive Care Society and NIHR EME Programme Director. ACM is supported by a Clinical Research Career Development Fellowship from the Wellcome Trust (WT 2055214/Z/16/Z).MR reports a grant from Pfizer for the conduct of the study. Outside the submitted work, he reports personal fees from Gilead Sciences and Pfizer, grants from Matoke Holdings and NW Lung Charity**.** DWD reports a grant from Pfizer for the conduct of the study. DWD and family hold founder shares in F2G Ltd, a

University of Manchester spinout antifungal discovery company. He acts or has recently acted as a consultant to Scynexis, Pulmatrix, Zambon, iCo Therapeutics, Roivant, Biosergen and Fujifilm. In the last 3 years, he has been paid for talks on behalf of Dynamiker, Hikma, Gilead, Merck, Mylan and Pfizer. He is a longstanding member of the Infectious Disease Society of America Aspergillosis Guidelines group, the European Society for Clinical Microbiology and Infectious Diseases Aspergillosis Guidelines group and the British Society for Medical Mycology Standards of Care committee. AJS reports a grant from the Wellcome Trust, Department of Health and Pfizer for the conduct of the study. His institution has received funds from grants around sepsis/pneumonia from the UK MRC, UK NIHR and others; and he is Director of the NIHR Newcastle In Vitro Diagnostics Co-operative. AJS is a National Institute for Health Research (NIHR) Senior Investigator. The views expressed in this article are those of the authors and not necessarily those of the NIHR, or the Department of Health and Social Care. RMcM reports grants from Wellcome Trust, Department of Health (HICF) and Pfizer UK Ltd for the conduct of the study. Outside the submitted work, he reports grants from the UK NIHR HTA Programme, Innovate UK, Invest Northern Ireland and Randox Laboratories Ltd and personal fees from Gilead Sciences Ltd. All of the other authors confirm that they have no declarations of interest in relation to this work.

References

1. Bassetti M, Peghin M, Vena A. Challenges and Solution of Invasive Aspergillosis in Non-neutropenic Patients: A Review. *Infect Dis Ther* 2018;**7**:17-27. doi: 10.1007/s40121-017-0183-9.

2. Meersseman W, Vandecasteele SJ, Wilmer A, Verbeken E, Peetermans WE, Van Vijngaerden E. Invasive aspergillosis in critically ill patients without malignancy. *Am J Respir Crit Care Med* 2004; **170**(6): 621-25.

3. Baddley JW, Stephens JM, Ji X, Gao X, Schlamm HT, Tarallo M. Aspergillosis in Intensive Care Unit (ICU) patients: epidemiology and economic outcomes. *BMC Infect Dis* 2013; **13**:29.

4. Pegorie M, Denning DW, Welfare W. Estimating the burden of invasive and serious fungal disease in the United Kingdom. *J Infect* 2017; **74**: 60-71.

5. Dorgan E, Denning DW, McMullan R. Burden of fungal disease in Ireland. *J Med Microbiol* 2015; **64**: 423-6.

6. Schauwvlieghe AFAD, Rijnders BJA, Philips N, Verwijs R, Vanderbeke L, Van Tienen C, Lagrou K, Verweij PE, Van de Veerdonk FL, Gommers D, Spronk P, Bergmans DCJJ, Hoedemaekers A, Andrinopoulou E, van den Berg,Charlotte H S B., Juffermans NP, Hodiamont CJ, Vonk AG, Depuydt P, Boelens J, Wauters J. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. *Lancet Respir Med* 2018; **6**: 782-792. DOI:https://doi.org/10.1016/S2213-2600(18)30274-1.

7. Winters B, Custer J, Galvagno Jr S,M., Colantuoni E, Kapoor SG, Lee H, Goode V, Robinson K, Nakhasi A, Pronovost P, Newman-Toker D. Diagnostic errors in the intensive care unit: a systematic review of autopsy studies. *BMJ QUAL SAF* 2012; **21**: 894-902.

8. Danion F, Rouzaud C, Duréault A, Poirée S, Bougnoux M, Alanio A, Lanternier F, Lortholary O. Why are so many cases of invasive aspergillosis missed? *Med Mycol* 2019; **57**:S94-S103.

9. Tejerina EE, Padilla R, Abril E, Frutos-Vivar F, Ballen A, Rodríguez-Barbero JM, Lorente JÁ, Esteban A. Original contribution: Autopsy-detected diagnostic errors over time in the intensive care unit. *Hum Pathol* 2018; **76**: 85-90.

10. Donnelly JP, Chen SC, Kauffman CA, Steinbach WJ, Baddley JW, Verweij PE, Clancy CJ, Wingard JR, Lockhart SR, Groll AH, Sorrell TC, Bassetti M, Akan H, Alexander BD, Andes D, Azoulay E, Bialek R, Bradsher RW,Jr, Bretagne S, Calandra T, Caliendo AM, Castagnola E, Cruciani M, Cuenca-Estrella M, Decker CF, Desai SR, Fisher B, Harrison T, Heussel CP, Jensen HE, Kibbler CC, Kontoyiannis DP, Kullberg B, Lagrou K, Lamoth F, Lehrnbecher T, Loeffler J, Lortholary O, Maertens J, Marchetti O, Marr KA, Masur H, Meis JF, Morrisey CO, Nucci M, Ostrosky-Zeichner L, Pagano L, Patterson TF, Perfect JR, Racil Z, Roilides E, Ruhnke M, Prokop CS, Shoham S, Slavin MA, Stevens DA, Thompson GR,III, Vazquez JA, Viscoli C, Walsh TJ, Warris A, Wheat LJ, White PL, Zaoutis TE, Pappas PG. Revision and Update of the Consensus Definitions of Invasive Fungal Disease From the European Organization for Research and Treatment of Cancer and the Mycoses Study Group Education and Research Consortium. *Clin Infect Dis* 2019:ciz1008.

11. Roosen J, Frans E, Wilmer A, Knockaert DC, Bobbaers H. Comparison of Premortem Clinical Diagnoses in Critically Ill Patients and Subsequent Autopsy Findings. *Mayo Clin Proc* 2000; **75**: 562-7

12. Meersseman W, Lagrou K, Maertens J, Van Wijngaerden E. Invasive Aspergillosis in the Intensive Care Unit. *Clin Infect Dis* 2007; **45**: 205-16.

13. Hage CA, Carmona EM, Epelbaum O, Evans SE, Gabe LM, Haydour Q, Knox KS, Kolls JK, Murad MH, Wengenack NL, Limper AH. Microbiological Laboratory Testing in the Diagnosis of Fungal Infections in Pulmonary and Critical Care Practice. An Official American Thoracic Society Clinical Practice Guideline. *Am J Respir Crit Care Med* 2019; **200**(5): 535-50.

14. Blot SI, Taccone FS, Van dA, Bulpa P, Meersseman W, Brusselaers N, Dimopoulos G, Paiva JA, Misset B, Rello J, Vandewoude K, Vogelaers D. A Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients. *Am J Respir Crit Care Med* 2012; **186**: 56-64.

15. Guidelines for the Management of Adults with Hospital-acquired, Ventilator-associated, and Healthcare-associated Pneumonia. *Am J Respir Crit Care Med* 2005; **171**: 388–416.

16. Torres A, Niederman MS, Chastre J, Ewig S, Fernandez-Vandellos P, Hanberger H, Kollef M, Li Bassi G, Luna CM, Martin-Loeches I, Paiva JA, Read RC, Rigau D, Timsit JF, Welte T, Wunderink R. International ERS/ESICM/ESCMID/ALAT guidelines for the management of hospital-acquired pneumonia and ventilator-associated pneumonia. *Eur Respir J* 2017; **50**: 1700582

17. Loughlin L, Simpson AJ, Hellyer TP, White PL, Posso R, Palmer M, McAuley DF, Richardson M, Denning DW, McMullan R. Probable Aspergillus infection in UK patients with suspected ventilator-associated pneumonia (abstract P1242). ECCMID,2018. Available online ESCMID E-library https://www.escmid.org/escmid_publications/escmid_elibrary/

18. Loughlin L, Simpson AJ, Hellyer TP, White PL, Posso R, McAuley DF, McMullan R. *Aspergillus* Infection in UK Patients with Suspected Ventilator Associated Pneumonia (abstract). ASM Microbe Conference, 2019. Oral presentation.

19. Hellyer TP, Morris AC, McAuley DF, Walsh TS, Anderson NH, Singh S, Dark P, Roy AI, Baudouin SV, Wright SE, Perkins GD, Kefala K, Jeffels M, McMullan R, O'Kane CM, Spencer C, Laha S, Robin N, Gossain S, Gould K. Diagnostic accuracy of pulmonary host inflammatory mediators in the exclusion of ventilator-acquired pneumonia. *Thorax* 2015; **70**:41.

20. Hellyer TP, McAuley DF, Walsh TS, Anderson N, Conway Morris A, Singh S, Dark P, Roy AI, Perkins GD, McMullan R, Emerson LM, Blackwood B, Wright SE, Kefala K, O'Kane C,M., Baudouin SV, Paterson RL, Rostron AJ, Agus A, Bannard-Smith J, Robin NM, Welters ID, Bassford C, Yates B, Spencer C, Laha SK, Hulme J, Bonner S, Linnett V, Sonksen J, Van Den Broeck T, Boschman G, Keenan DJ, Scott J, Allen AJ, Phair G, Parker J, Bowett SA, Simpson AJ. Biomarker-guided antibiotic stewardship in suspected ventilator-associated pneumonia (VAPrapid2): a randomised controlled trial and process evaluation. *Lancet Respir Med* 2020;8:182-191.

21. White PL, Barnes RA, Springer J, Klingspor L, Cuenca-Estrella M, Morton CO, Lagrou K, Bretagne S, Melchers WJG, Mengoli C, Donnelly JP, Heinz WJ, Loeffler J. Clinical Performance of Aspergillus PCR for Testing Serum and Plasma: a Study by the European Aspergillus PCR Initiative. *J Clin Microbiol* 2015; **53**: 2832-7.

22. Hoenigl M, Prattes J, Spiess B, Wagner J, Prueller F, Raggam RB, Posch V, Duettmann W, Hoenigl K, Wölfler A, Koidl C, Buzina W, Reinwald M, Thornton CR, Krause R, Buchheidt D. Performance of galactomannan, beta-d-glucan, Aspergillus lateral-flow device, conventional culture, and PCR tests with bronchoalveolar lavage fluid for diagnosis of invasive pulmonary aspergillosis. *J Clin Microbiol* 2014; **52**(6):2039-45.

23. Meersseman W, Lagrou K, Maertens J, Wilmer A, Hermans G, Vanderschueren S, Spriet I, Verbeken E, Van Wijngaerden E. Galactomannan in bronchoalveolar lavage fluid: a tool for diagnosing aspergillosis in intensive care unit patients. *Am J Respir Crit Care Med* 2008; **177**: 27-34.

24. Schroeder M, Simon M, Katchanov J, Wijaya C, Rohde H, Christner M, Laqmani A, Wichmann D, Fuhrmann V, Kluge S. Does galactomannan testing increase diagnostic accuracy for IPA in the ICU? A prospective observational study. *Crit Care* 2016; **20**:1-10.

25. D D'Haese J, Theunissen K, Vermeulen E, Schoemans H, De Vlieger G, Lammertijn L, Meersseman P, Meersseman W, Lagrou K, Maertens J. Detection of galactomannan in bronchoalveolar lavage fluid samples of patients at risk for invasive pulmonary aspergillosis: analytical and clinical validity. *J Clin Microbiol* 2012; **50**:1258-63.

26. Zhou W, Li H, Zhang Y, Huang M, He Q, Li P, Zhang F, Shi Y, Su X. Diagnostic Value of Galactomannan Antigen Test in Serum and Bronchoalveolar Lavage Fluid Samples from Patients with Nonneutropenic Invasive Pulmonary Aspergillosis. *J Clin Microbiol* 2017; **55**: 2153-61

27. Pfeiffer CD, Fine JP, Safdar N. Diagnosis of Invasive Aspergillosis Using a Galactomannan Assay: A Meta-Analysis. *Clin Infect Dis* 2006;**42**:1417-27.

28. van de Groep K, Verboom DM, van de Veerdonk FL, Haas PJA, van der Poll T, Schultz MJ, Bonten MJM, Cremer OL. Detection of Invasive Aspergillosis in Critically Ill Patients with Influenza: The Role of Plasma Galactomannan. *Am J Respir Crit Care Med* 2019; **200**: 636-8.

29. Taccone FS, Van den Abeele A, Bulpa P, Misset B, Meersseman W, Cardoso T, Paiva J, Blasco-Navalpotro M, De Laere E, Dimopoulos G, Rello J, Vogelaers D, Blot SI, AspICU Study Investigators. Epidemiology of invasive aspergillosis in critically ill patients: clinical presentation, underlying conditions, and outcomes. *Crit Care* 2015; **19**;7.

Table 1 Characteristics and outcomes of suspected VAP patient cohort

	Suspected VAP [§] cohort n=194	With probable *Aspergillus* infection n=24	Without probable *Aspergillus* infection n= 170	p value
Median age (years) (IQR*)	57 (44-69)	66·5 (49·8-72·5)	56 (43-69)	0·07
Male	137 (70·6%)	15 (62·5%)	122(71·8%)	0·35
Mean APACHE II on admission[†] (SD[‡])	17·93 (7·5)	19·25 (7·5)	17·74 (7·7)	0·36
Reason for admission-medical	113 (58·2%)	17 (70·9%)	96 (56·5%)	0·18
Reason for admission - surgical	81 (41·8%)	7 (29·1%)	74 (43·5%)	0·18
Median pre-enrolment length of stay (IQR)	7 (4-11)	7.5(7-12)	6(4-10.75)	0.19
Steroids	33 (16·9%)	6 (25%)	27 (15·8%)	0·27
WCC[ǁ] on day of BAL**	15·3	14·95	15·3	0·83
Renal replacement therapy	16 (8·3%)	3 (12·5%)	13 (7·7%)	0·42
Vasopressors	61 (31·4%)	8 (33·3%)	53 (31·2%)	0·83
Microbiologically confirmed VAP	78 (40·2%)	9 (37·5%)	69 (40·6%)	0·77
Median Length of stay critical care (IQR)	17 (11-31·5)	25·5 (17·25-32·8)	15 (10-30·5)	0·02
Median Length of stay in hospital (IQR)	34 (17-62)	34 (24·5-61)	34 (14·25-62)	0·39
ICU mortality	47 (24·1%)	8 (33·3%)	39 (22·8%)	0·27

*IQR= Interquartile range, [†]APACHE II = acute physiology and chronic evaluation, [‡] SD= standard deviation, [§]VAP=ventilator associated pneumonia, [ǁ]WCC=White cell count, **BAL= Bronchoalveolar lavage

Table 2 Biomarker and culture results of serum and BALF for patients who met the definition of probable Aspergillus infection

Patient	BALF* GM†	Serum GM	Serum BDG‡	Serum PCR	BALF PCR	BALF Culture >10^4 CFU§/ml
1	14·67	1·22	+	+	+	*Aspergillus sp.*‖
2	13·95	0·21	+		+	*Acinetobacter sp.*
3	11·59	0·50	+		+	*Aspergillus fumigatus* ‖
4	8·46	0·04			+	*Moraxella catarrhalis*
5	8·04	0·11	+	+	+	*Aspergillus fumigatus* ‖
6	4·66	0·08				
7	3·32	0·12				*E.coli*
8	3·16	0·07				*Klebsiella pneumoniae*
9	2·40	0·08	+			*Proteus mirabilis*
10	2·02	0·03				
11	1·65	0·05				*Pseudomonas aeruginosa*
12	1·52	0·05				*S. aureus*
13	1·47	0·17	+			
14	1·47	0·08				
15	1·44	0·08				
16	1·41	0·05		+		
17	1·36	0·18				
18	1·24	0·52	+		+	
19	1·05	0·21		+	+	
20	1·03	0·51	+	+		
21	0·14	2·65	+			*Enterobacter cloacae*
22	0·32	1·54			+	
23	0·43	0·79			+	*S. aureus, Serratia marcescens*
24	0·92	0·50	+			

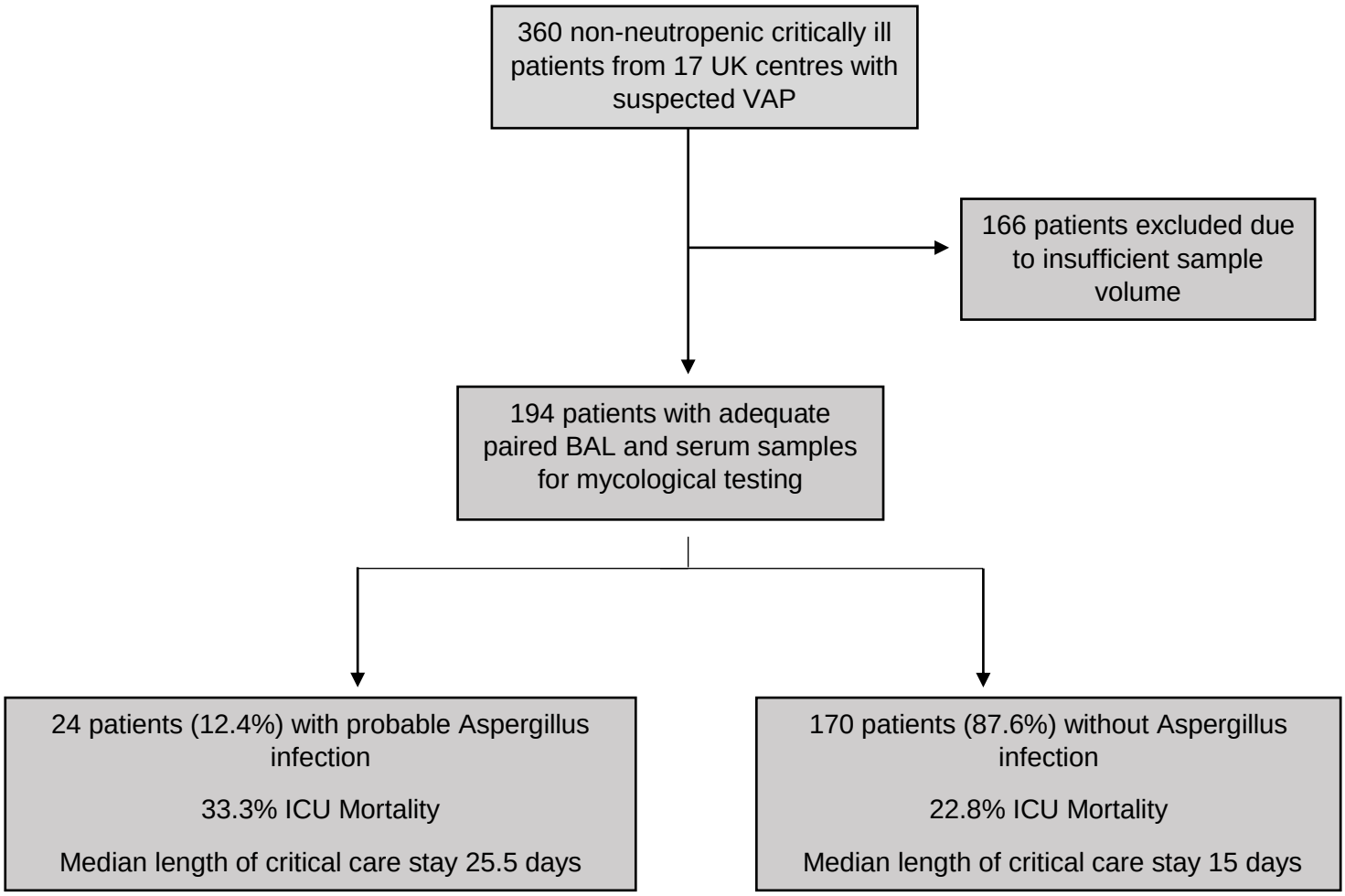
*BALF= Bronchoalveolar lavage fluid; †GM=galactomannan; ‡BDG= β-D-glucan; §CFU=colony-forming units; ‖ Any growth of Aspergillus sp. reported)no minimum CFU/ml applied)

Table 3 Prevalence of *Aspergillus* infection at a range of GM OD index threshold values

BALF* GM† Threshold OD‡	**Number of patients with BALF positive**	**Total Number of aspergillosis cases (BALF or serum positive)**	**Prevalence % (95% CI)**
0·7	27	30	15·5 (10·7-21·3)
0·8	25	28	14·4 (9.8-20·2)
1·0	20	24	12·4 (8·1-17·8)
1·5	12	18	9·3 (5·6-14·3)
3·0	8	14	7·2 (4·0-11·8)

*BALF= Bronchoalveolar lavage fluid; †GM=galactomannan; ‡OD=Optical Density

Figure 1


360 non-neutropenic critically ill patients from 17 UK centres with suspected VAP
166 patients excluded due to insufficient sample volume
194 patients with adequate paired BAL and serum samples for mycological testing
24 patients (12.4%) with probable Aspergillus infection
33.3% ICU Mortality
Median length of critical care stay 25.5 days
170 patients (87.6%) without Aspergillus infection
22.8% ICU Mortality
Median length of critical care stay 15 days