A national strategy to diagnose COVID-19 associated invasive fungal disease in the ICU

P. Lewis White, PhD[1], Rishi Dhillon, MD[1], Alan Cordey, MD[1], Harriet Hughes, MD[1], Federica Faggian, MD[1], Shuchita Soni, MD[1], Manish Pandey, MD[2], Harriet Whitaker, BSc[3], Alex May, MD[1], Matt Morgan, MD[2], Matthew P. Wise, MD[2], Brendan Healy, MD[4], Ian Blyth, MD[4], Jessica S. Price, BSc[1], Lorna Vale, MSc[1], Raquel Posso, MSc[1], Joanna Kronda, BSc[1], Adam Blackwood, BSc[1], Hannah Rafferty, BSc[1], Amy Moffitt, BA[1], Alexandra Tsitsopoulou, MD[5], Soma Gaur, MD[6], Tom Holmes, MD[2], Matthijs Backx, MD[1]

[1]Public Health Wales Microbiology Cardiff, UHW, Cardiff, UK CF14 4XW. [2]Intensive Care Medicine, UHW, Heath Park, Cardiff, UK, CF14 4XW. [3]Department of Pharmacy, UHW, Cardiff, UK CF14 4XW. [4]Public Health Wales Microbiology Swansea, Singleton Hospital, Swansea, UK. [5]Cwm Taf Microbiology Department, Royal Glamorgan, Ynysmaerdy, RCT, UK. [6]Aneurin Bevan Microbiology Department, Royal Gwent Hospital, Newport, Gwent, UK.

Corresponding Author: Dr P. Lewis White,

Mycology Reference Laboratory, Public Health Wales, Microbiology Cardiff, UHW, Heath Park, Cardiff, UK. CF14 4XW.

Telephone Number: +44 (0)29 2074 6581; **Fax Number:** +44 (0)29 2074 2161

Email: lewis.white@wales.nhs.uk

Summary: Invasive fungal disease represents a significant complication associated with severe COVID-19 infection, resulting in increased mortality. The use of early antifungal therapy, directed by strategic mycological testing infers a survival benefit. Antifungal prophylaxis may be warranted in certain patients.

© The Author(s) 2020. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved. For permissions, e-mail: journals.permissions@oup.com.

Accepted Manuscript

ABSTRACT

Background: Fungal co-infection is a recognised complication of respiratory virus infections, increasing morbidity and mortality, but can be readily treated if diagnosed early. An increasing number of small studies describing aspergillosis in COVID-19 patients with severe respiratory distress are being reported, but comprehensive data is lacking. The aim of this study was to determine the incidence, risk factors and impact of invasive fungal disease in adult COVID-19 patients with severe respiratory distress.

Methods: An evaluation of a national, multi-centre, prospective cohort evaluation of an enhanced testing strategy to diagnose invasive fungal disease in COVID-19 intensive care patients. Results were used to generate a mechanism to define aspergillosis in future COVID-19 patients.

Results: One-hundred and thirty-five adults (median age: 57, M/F: 2·2/1) were screened. The incidence was 26.7% (14.1% aspergillosis, 12·6% yeast infections). The overall mortality rate was 38%; 53% and 31% in patients with and without fungal disease, respectively (P: 0.0387). The mortality rate was reduced by the use of antifungal therapy (Mortality: 38·5% in patients receiving therapy versus 90% in patients not receiving therapy (P: 0.008). The use of corticosteroids (P: 0.007) and history of chronic respiratory disease (P: 0.05) increased the likelihood of aspergillosis.

Conclusions: Fungal disease occurs frequently in critically ill, mechanically ventilated COVID-19 patients. The survival benefit observed in patients receiving antifungal therapy implies that the proposed diagnostic and defining criteria are appropriate. Screening using a strategic diagnostic approach and antifungal prophylaxis of patients with risk factors will likely enhance the management of COVID-19 patients.

Key words: *Aspergillus*, COVID-19, critical care, Incidence, Risk factors and diagnosis

Accepted Manuscript

INTRODUCTION

The emergence of the novel coronavirus COVID-19 has placed a major strain on healthcare services globally, and efforts are focussed on the management of this disease. Secondary infections, including invasive pulmonary aspergillosis (IPA), are a recognised complication of respiratory virus infections. [1] A strong association has been observed in patients presenting with acute respiratory failure due to influenza (IAPA incidence: 19%), possibly a result of damage to epithelial cells and/or immune dysregulation. [2-3]

An increased incidence of IPA in those suffering with severe respiratory virus infection has led to concerns that this may also occur in patients with acute respiratory failure due to COVID-19 infection, particularly as this infection causes pulmonary damage and an inflammatory environment permissive for fungal infection. [4-8] However, data on COVID-19 associated IPA (CAPA) are currently limited to anecdotal reports or small case studies. Larger studies are needed to determine an accurate incidence, optimize diagnostics and improve patient management. [9-15]

The Public Health Wales Mycology Reference Centre has 20 years' experience of using non-culture fungal diagnostics to assist in the management of patients at risk of invasive fungal disease (IFD). [16] Given the urgent need for evidence to guide diagnostic and antimicrobial prescribing policy, a testing strategy to diagnose IFD in critically-ill COVID-19 patients across Wales was recommended with aim of determining the incidence, impact and risk factors (Figure 1). This manuscript describes the findings and is, to our knowledge, the first national, prospective screening of PCR confirmed COVID-19 patients for IFD, incorporating novel diagnostics.

Accepted Manuscript

MATERIALS AND METHODS

Testing Strategy and patient population

Enhanced mycological testing of intensive care unit (ICU) patients with refractory severe respiratory illness or deterioration of respiratory function one week post-COVID diagnosis was recommended. The optimum strategy, in line with recent international opinion, involved obtaining both blood and deep respiratory samples for mycological investigation of both yeast and mold infections (Figure 1). [6] To enhance the detection of yeast infection, blood culture was combined with (1-3)-β-D-Glucan (BDG) testing, the latter also of benefit for the diagnosis of IPA, when combined with molecular, antigen and culture investigation of respiratory samples. The service was available to all ICUs across Wales with samples sent as part of the routine investigations for COVID-19-associated secondary infections. Antifungal therapy (AFT) was administered at the clinicians' discretion. The appropriateness of AFT was determined by considering the type of IFD diagnosed (yeast or mold infection), the degree of identification when the first AFT wad administered and how this related in international therapy guidelines. All data generated and interpreted was part of routine patient management, forming a prospective, consecutive cohort study covering the first seven weeks of service, with one-month follow-up, not requiring ethical approval.

Novel definitions, their justification for classifying CAPA and comparison with previous definitions used to classify IPA in the ICU are described in Table 1. Novel definitions are in line with recent opinion, stratifying the confidence of IPA diagnosis according to the degree of clinical/mycological evidence. [6]

Routine investigations for Invasive Fungal disease

Samples were tested by the BioRad *Aspergillus* Ag assay (GM-EIA) (BioRad, Hemel Hempstead, UK) following manufacturer's instructions, using a positivity threshold of ≥0·5 in serum and ≥1·0 in deep respiratory samples (non-directed bronchial lavage (NBL) or bronchoalveolar lavage (BAL)). *Aspergillus* PCR testing was performed on 0·5ml serum/plasma and NBL/BAL, following international

Accepted Manuscript

recommendations, using the BioMerieux Emag extractor, with a well validated “in-house” Q-PCR assay performed on the Qiagen Rotorgene Q-HRM . [17,18] Serum BDG was detected using the Fungitell assay (Associates of Cape Cod, Liverpool, UK) following manufacturer’s instructions, with a positivity threshold of 80pg/ml. Samples were tested in duplicate and the mean value used for interpretation.

Blood and central venous catheter culture was performed following national guidance for investigating sepsis, with 5-10ml of blood incubated up to 10 days on the BD Bactec FX Automated Blood Culture Analyser. [19] Yeast were identified using the Bruker MALDI-TOF system.

Radiological investigations were performed at the clinicians’ discretion. The investigations included computed tomography of the thorax (CT-Thorax), with or without high-resolution enhancement, and CT-pulmonary angiogram (CTPA). Data was retrieved from prospective reports generated by the consultant radiologist, no independent analysis was performed. Radiological evidence such as nodules, halos, cavities, wedge shaped, lobar or segmental consolidation and tree in bud presentation were recorded as evidence typical of IPA, given these findings are not usually associated with COVID-19 infection and following well-established international definitions for IFD. [20, 21] All other evidence of chest infection was considered non-specific. Alternative reasons for the chest radiology considered typical of IPA was documented. Given sinusitis is a frequent presentation of aspergillosis, evidence of sinusitis on CT head/sinus was recorded but not deemed typical of CAPA, due its presence in ventilated and/or COVID-19 patients. Due to the lack of bronchoscopic investigation it was not possible identify mucosal plaques suggestive of *Aspergillus* tracheobronchitis, evident in IAPA. [2]

Accepted Manuscript

Statistical Analysis

The positivity rate for each test was determined for both specimens and patients. For proportionate values ninety-five percent confidence intervals and, where required, *P* values (Fishers exact test; *P*: 0·05) were generated to determine significance. Median values were compared using a Mann-Whitney T-test for pairwise analysis when comparing multiple median values. Associations between clinical factors were determined for combined IFD, and IPA and candidosis individually.

RESULTS

Over the period, 257 patients were admitted to Welsh ICUs with COVID-19 infection. Fifty-three percent (135 patients) were screened for IFD, 123 patients had blood cultures and BDG testing performed, 60 patients had a NBL tested, and 48 of these patients had all tests (Figure 1). Patient demographics, clinical information and associated mycology are shown in Table 2.

Positivity rates of mycological testing

Fifty-one of the 135 patients (37·8%. 95% CI: 30·0-46·2) had ≥1 positive mycological test (culture, BDG, GM-EIA or PCR) (Table 2). Seventeen patients (12·6%, 95% CI: 8·0-19·2) had evidence of invasive yeast infection, mainly (93·8%) *Candida* (Table 3). There was one case of *Rhodotorula* fungaemia. Thirty patients (22·2%, 95% CI: 16·0-30·0) had ≥1 *Aspergillus* positive results, 14 having just a single positive result and 16 having ≥2 *Aspergillus* positive results (Table 4). In addition, four patients, potentially with unspecified IFD, were BDG positive on multiple occasions. There were no documented cases of *Pneumocystis* pneumonia.

Sample and patient positivity rates for the primary diagnostic investigations are shown in table 2 and Figure 1. Testing more samples and the optimal approach of combining NBL with BDG testing were associated with an increased likelihood of mycological positivity.

Accepted Manuscript

Associations between clinical/pharmaceutical factors and IFD

There was a significant association between patients with positive mycology and patients diagnosed with or treated for a solid malignancy (Table 1). Among the 57 patients where corticosteroids data was available, there was a strong association between patients with multiple *Aspergillus*/BDG (≥2) positive results and the use of high-dose systemic corticosteroids (13/15 patients, Odds ratio 7.9, 95% CI: 1.6-39.3, *P*: 0·007), compared to 19 of 42 with ≤1 positive result. There was a significant association for patients with an underlying chronic respiratory condition to have multiple positive *Aspergillus*/BDG tests (7/16) compared to 23/116 patients without multiple positive results (OR: 3·15, 95% CI: 1·06-9.34, *P*: 0·05). There were no significant associations between underlying conditions/co-morbidities and yeast infections (results not shown).

Procalcitonin, C-reactive protein, total leucocytes, neutrophils and lymphocytes were similar across cohorts (Table 1).

Timing of Mycology positivity

In the 16 patients with multiple *Aspergillus* positive results, the median time to positivity post admission to the ICU was eight days, although this ranged from 0-35 days (90^{th} percentile: 23.8 days). Post PCR diagnosis of COVID-19 infection the median time to positivity was 6.5 days, with a range of -20 to 22 (90^{th} percentile: 19.9 days). In the 17 patients with yeast infection the median time to culture positivity post ICU admission was 9 days (range 0-38 days, 90^{th} percentile: 26 days) and time elapsed post PCR diagnosis of COVID-19 infection was 10 days (range 1-38 days. 90^{th} percentile 29 days). Positive mycology results extended the ICU admission duration (Table 2).

Radiological evidence of IA

In 7/16 of patients with multiple *Aspergillus* positive results CT-Thorax/CTPA was non-specific, indicative of progressing respiratory infection and indistinguishable from COVID-19 pneumonia (e.g. bilateral airspace opacification). However, in 56% chest CT was typical of IPA (cavities (n=5), nodules (n=5) and “tree in bud” (n=1)) (Table 4). Seven patients had one typical chest sign and two patients

Accepted Manuscript

had two signs. Three patients (6, 14 and 16 in table 4) had potential bacterial respiratory infection possibly explaining the CT evidence, although each patient had 3-5 mycological positive tests supportive of IPA. Four patients with typical chest radiology also had CT evidence of sinusitis. One additional patient with non-specific chest radiology had evidence of sinusitis. In total, 62.5% of patients with multiple *Aspergillus* positive tests had radiology that could be attributed to IFD.

Of the 14 patients with a single *Aspergillus* positive test two had nodules and one patient had evidence of sinusitis. One patient had *Aspergillus* cultured from a NBL, with a GMI of 0·5, another was *Aspergillus* PCR positive on NBL and the third had a single NBL with a GMI of 1·0, but also had a potential bacterial pneumonia and likely lung metastases. None of the patients received antifungal therapy and two died. Two of the four patients that were positive by BDG alone had radiological evidence (one potential fungal ball in the sinuses, one lung nodule). Two of the 84 patients who were negative for mycology had evidence of chest cavitation.

Comparing the chest radiology typical of IPA from patients with multiple *Aspergillus* positive results (n=16) to those with yeast infection (radiology typical of IPA: 0/17) combined with patients with negative mycology (radiology typical of IPA: 2/84) generates sensitivity and specificity of 56.3% (95% CI: 33.2-76.9) and 98·0% (95% CI: 93·1-99·5), respectively. The subsequent positive likelihood ratio (28.2) was highly predictive of IPA (probability: 82·2% at a 14.1% incidence).

Defining IPA in ICU COVID-19 patients

The incidence of IPA varies, dependent on the definitions used to classify disease (Tables 1 and 4). Using the AspICU, IAPA and novel CAPA definitions the incidence of IPA was 5·9% (8/135), 14·8% (20/135) and 14.1% (19/135), respectively. [2,22] Between the three methods 25 patients were classified with IPA (Table 4). The eight patients classified by the AspICU definitions were supported by the IAPA definitions, but one patient was not classified using the CAPA definitions. This patient had *Aspergillus fumigatus* cultured from a single NBL sample but radiology was non-specific. Seven of the 12 additional IPA cases classified by IAPA were supported by CAPA, five had radiology

Accepted Manuscript

attributable to IPA and two had non-specific radiology, all were supported with multiple *Aspergillus* positive results (Table 4). Three patients classified by IAPA alone had non-specific radiology with a single *Aspergillus* positive result. Two patients had radiology that could be attributed to IFD (one sinusitis and one nodules), both had a single supporting mycology result. Given the broad aetiological diversity associated with sinusitis, including COVID-19, the lack of multiple positive *Aspergillus* markers prevented classification using the CAPA definitions (Table 1). The patient with nodules had secondary lung metastases and a bacterial pathogen, possibly explaining the radiology; subsequently multiple positive *Aspergillus* results would be required to classify CAPA. Of the five IPA patients classified by CAPA alone, two had nodules detected on chest CT with supporting mycological evidence and two had non-specific radiology but multiple *Aspergillus* positive results. The final patient had radiological evidence of a fungal ball in the sinuses and was supported by multiple strongly positive BDG results.

Patient prognosis

The overall mortality rate for COVID-19 patients on ICU was 38% (Table 1). Mortality rates in patients with negative mycology were similar irrespective of AFT (*P*: 1·000). The mortality rate in patients defined with CAPA was 57.9% (95% CI: 36.3-76.9), ranging from 46.7% (95% CI: 24.8-69.9) in patients receiving appropriate AFT to 100% (95% CI: 51.1-100) in patients not receiving appropriate AFT. In patients with invasive yeast infection mortality was 47·1% (95% CI: 26·2-69·0), ranging from 27·3% (95% CI: 9.8-56.6) in patients on appropriate AFT to 83·3% (95% CI: 43.7-97.0) in those not receiving appropriate AFT (*P*: 0.0498). For combined IFD (CAPA and yeast infections) the mortality rate was 52.8% (95% CI: 37.0-68.0), being 38.5% (95% CI: 22.4-57.5) in patients receiving appropriate AFT and 90.0% (95% CI: 59.6-98.2) in those not receiving appropriate AFT (*P*: 0.008). All four patients with unspecified IFD died, two received appropriate AFT.

Accepted Manuscript

DISCUSSION

There is urgent need for structured IFD testing in COVID-19 patients given the likely poor prognosis in untreated patients. [5,10,12] BDG was incorporated as a primary test as it provides broad-fungal detection in easily obtainable samples and has been associated with improved sensitivity over serum GM-EIA testing for the diagnosis of ICU associated IPA. [23] Unfortunately, BDG testing is not universally available and while GM-EIA screening of blood is highly specific it cannot be used to exclude CAPA, leaving the testing of respiratory samples as the preferred option. While testing BAL would be preferable, obtaining this invasive sample from a large number of COVID-19 patients represents a significant infection control risk. Obtaining NBL is less intrusive and is a routine microbiological investigation in many Welsh ICU units. Mycological testing of NBL is less validated and could be associated with the detection of upper airway fungal colonization/contamination, but a recent evaluation in COVID-19 supports NBL fungal testing and there is an argument for a low threshold for initiating AFT, given early AFT significantly improves prognosis of IA. [24,25] In this study the mortality rate of IFD patients on appropriate AFT (38.5%) was comparable to patients suffering from COVID-19 alone (31.0%).

The incidence of CAPA varied according to the definitions applied (Table 1). Using the definitions proposed in this manuscript, the CAPA incidence was 14.1% of patients screened (7.4% of all COVID-19 ICU admissions), lower than two previous studies in France (30%) and Germany (26%). Patient numbers in this current study were 5-7 fold higher and its prospective, consecutive multicentre nature should provide more robust data, although geographical differences need to be considered. [10,11] A limitation of our study is that not all ICU patients were screened, and of those that were <50% were tested by the optimal combined respiratory/circulatory approach. Consequently, the incidence of CAPA is likely underestimated, nevertheless considerable. While 257 COVID-19 patients were admitted to the ICU during the testing period, not all would have met the inclusion criteria

Accepted Manuscript

(Figure 1), so calculating incidence based on all patients, or extrapolating the incidence to entire population to determine a total disease burden would not be accurate. Given 68.4% and 84.2% of CAPA defined cases were positive by serum BDG and NBL testing, respectively, it is possible that CAPA cases were missed when combination testing was not performed, accounting for this increases the incidence to 31%, in line with other studies. [10,11,26]

The AspICU definitions significantly underestimate the rate of CAPA (5·9%), with classification based on respiratory culture that lacks sensitivity, is slow and of limited utility in the ICU, with mortality rates similar in patients with positive *Aspergillus* respiratory culture, irrespective of AFT. [27] Applying the IAPA definitions, which incorporate GM-EIA, increases the incidence to 14·8%, similar to the proposed CAPA definitions (14.1%), but considerable discordance was evident (Table 4). Given the IAPA definitions allow non-specific radiology with a single GM-EIA positive result it is hoped that the CAPA definitions would provide enhanced specificity. Overall mortality rates from cases classified according to the CAPA and IAPA definitions were 58% and 45%, respectively, 46·7% of CAPA patients died despite AFT, while 100% not receiving AFT died. In IAPA defined patients, 42·9% died on AFT and 50% died while not receiving AFT. As a high mortality would be expected in untreated IPA patients, it appears that the IAPA definitions are misclassifying patients. While this could be a result of testing NBL over BAL, the utility of NBL testing has been demonstrated. [25] Receiver operator characteristic analysis of NBL GM-EIA testing, with CAPA defined using the proposed definitions showed that using GM threshold of 1.2 generated a specificity of 97.4%, with values >4.5 associated with 99% specificity, implying a high likelihood of IPA (Positive likelihood ratio >16) and performance comparable to BAL testing. [28] In patients with no mycological evidence of IFD, the use of AFT did not improve patient outcome indicating that the CAPA definitions were not missing cases. The prognosis of untreated CAPA is unclear, sub-acute or chronic disease could occur in this heterogeneous group. The overuse of AFT is obviously of concern, but the incidence of CAPA was not excessive, and the administration of AFT on the basis of radiology typical of IPA or positive

Accepted Manuscript

mycology represents an improvement over empirical AFT use (29% of mycology negative patients in this study received empirical AFT, without improving prognosis).

CT of the chest and head provided signs that could be attributed to IFD in 15 patients. However, many patients presented with non-specific radiology, which makes diagnosing an additional respiratory infection in a patient with underlying respiratory disease challenging. In this scenario, progression of non-specific radiology can be suggestive of IFD. Chest signs more typical of IA are highly specific (98%) and should increase concern of IA, unless they can be attributed to alternative clinical reason (e.g. lung metastases). [29] Given the variability in reporting of chest radiology, independent review of images by a radiologist with experience of discriminating IFD is recommended.

Clinical risk factors associated with CAPA included an underlying chronic respiratory condition and the use of corticosteroids. The latter has implications in the UK COVID-19 treatment randomized control trial where one arm recommends the use of dexamethasone, which been associated with reduced COVID-19 mortality (RECOVERY trial, www.recoverytrial.net). Adverse outcomes in this arm could be potentially attributable to CAPA rather than COVID-19, and the benefits of this approach could be enhanced if CAPA was systematically screened for and treated. In 31 COVID-19 patients requiring ventilator support on ICU in the Netherlands the incidence of putative CAPA was (10%). [30] In this current study, 79% of CAPA patients were ventilated. As noted previously, there was a significant incidence of invasive yeast infections (13%). [31] The reasons for this are unclear, but may be a consequence of difficult working conditions, rather than COVID-19. Given cases of IFD present 1-5 weeks post ICU admission frequent and prolonged testing of easily obtained specimens is recommended.

To conclude, there is substantial IFD in ICU-COVID-19 patients, potentially associated with poorer prognosis. The proposed systematic screening programme using a combination of markers from easily obtainable samples provides a sufficiently sensitive and specific way of identifying IFD in patients with COVID-19 and has the potential to reduce mortality from this relatively frequent

Accepted Manuscript

complication. Radiology when typical of IA, is highly specific for CAPA, AFT should be administered and further investigation considered. Multiple positive mycology results are also indicative of IFD. The CAPA definition provided enables clinicians to use a strategic approach for identifying and classifying IPA in critically unwell COVID-19 patients. It provides a framework for introduction of AFT in this cohort, which is likely to confer a survival benefit, if initiated early, but prospective validation is required. The use of steroids and an underlying chronic respiratory condition increase the likelihood of developing CAPA, and prophylactic AFT may benefit this group.

Accepted Manuscript

DISCLOSURES

PLW: Performed diagnostic evaluations and received meeting sponsorship from Bruker, Dynamiker, and Launch Diagnostics; Speakers fees, expert advice fees and meeting sponsorship from Gilead; and speaker and expert advice fees from F2G and speaker fees MSD and Pfizer. Is a founding member of the European *Aspergillus* PCR Initiative.

MB: Speakers fees, expert advice fees and meeting sponsorship from Gilead. Meeting sponsorship form Abbvie.

RD: Educational grants from Gilead and MSD, and expert opinion fees from MSD.

AC, HH, FF SS, MP, HW AM, MM, MPW, BH, IB, JSP, LV, RP, JK, AB, HR, AMo, AT, SG and TH: Disclose no COI

Accepted Manuscript

REFERENCES

1) White PL, Parr C, Barnes RA. Predicting Invasive Aspergillosis in Hematology Patients by Combining Clinical and Genetic Risk Factors with Early Diagnostic Biomarkers. J Clin Microbiol. **2017**;56:e01122-17
2) Schauwvlieghe AFAD, Rijnders BJA, Philips N, et al. Invasive aspergillosis in patients admitted to the intensive care unit with severe influenza: a retrospective cohort study. Lancet Respir Med. **2018**;6:782-792.
3) Dewi IMW, Cunha C, Vanderbeke L, et al. Oseltamivir affects host defense against invasive pulmonary aspergillosis. European Conference on Clinical Microbiology and Infectious Diseases; Madrid; April 21–24, **2018**.Abstract O1115.
4) Chen X, Zhao B, Qu Y, et al.Detectable serum SARS-CoV-2 viral load (RNAaemia) is closely correlated with drastically elevated interleukin 6 (IL-6) level in critically ill COVID-19 patients. Clin Infect Dis. **2020**;Epub ahead of print.
5) Verweij PE, Gangneux J-P, Bassetti M et al. Diagnosing COVID-19-associated pulmonary aspergillosis. Lancet Microbe. **2020**;Epub ahead of print.
6) Armstrong-James D, Abdolrasouli A, Bicanic T. Pre-empting and mitigating the risk that invasive pulmonary fungal infections present to patients with COVID-19. Euro Resp J. **2020**;In press.
7) Ackermann M, Verleden SE, Kuehnel M, et al. Pulmonary Vascular Endothelialitis, Thrombosis, and Angiogenesis in Covid-19. N Eng J Med. **2020**;Epub Ahead of print.
8) Arastehfar A, Carvalho A, van de Veerdonk FL, Jenks JD, Koehler P, Krause R, Cornely OA, S Perlin D, Lass-Flörl C, Hoenigl M_COVID-19 Associated Pulmonary Aspergillosis (CAPA)-From Immunology to Treatment. Fungi (Basel). 2020;24;6(2):91.
9) Lescure F-X, Bouadma L, Nguyen D, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis. **2020**;Epub ahead of print.

Accepted Manuscript

10) Alanio A, Delliere S, Fodil S, Bretagne S, Megarbane B. High prevalence of putative invasive pulmonary aspergillosis in critically ill COVID-19 patients. Lancet Resp Medicine. **2020**;Epub ahead of print.

11) Koehler P, Cornely OA, Böttiger BW, et al. COVID-19 associated pulmonary aspergillosis. Mycoses. **2020**;63:528-534

12) Zhang G, Hu C, Luo L, et al. Clinical features and short-term outcomes of patients with COVID-19 in Wuhan, China. J Clin Virol. **2020**;127:104364.

13) Rawson TM, Moore LSP, Zhu N, et al. Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis. **2020**;Epub ahead of print.

14) Blaize M, Mayaux J, Nabet C, et al. Fatal invasive aspergillosis and coronavirus disease in an immunocompetent patient. Emerg Infect Dis. **2020**;26:Epub ahead of print.

15) Gangneux J, Bougnoux M-E, Dannaoui E, Cornet M and Zahar JR. Invasive fungal diseases during COVID-19: We should be prepared. J Mycol Med. **2020**;Epub Ahead of print.

16) Barnes RA, Stocking K, Bowden S, Poynton MH, White PL. Prevention and diagnosis of invasive fungal disease in high-risk patients within an integrative care pathway. J Infect. **2013**;67:206-14.

17) White PL, Linton CJ, Perry MD, Johnson EM, Barnes RA. The evolution and evaluation of a whole blood polymerase chain reaction assay for the detection of invasive aspergillosis in hematology patients in a routine clinical setting. Clin Infect Dis. **2006**;42:479-86.

18) White PL, Bretagne S, Klingspor L, Melchers WJ, McCulloch E, Schulz B, Finnstrom N, Mengoli C, Barnes RA, Donnelly JP, Loeffler J; European Aspergillus PCR Initiative. Aspergillus PCR: one step closer to standardization. J Clin Microbiol. **2010**;48:1231-40.

19) NICE Guideline 51. Sepsis: Recognition, diagnosis and Early management. National Institute for Health and Care Excellence, Published 2016, updated 2019 www.nice.org.uk/guidance/ng51.

Manuscript

20) Donnelly JP, Chen SC, Kauffman CA, et al. 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**; Online ahead of print.

21) Prokop M, van Everdingen W, van Rees Vellinga T, Quarles van Ufford H, Stöger L, Beenen L, Geurts B, Gietema H, Krdzalic J, Schaefer-Prokop C, van Ginneken B, Brink M; COVID-19 Standardized Reporting Working Group of the Dutch Radiological Society. Radiology. CO-RADS: A Categorical CT Assessment Scheme for Patients Suspected of Having COVID-19-Definition and Evaluation. 2020;296(2):E97-E104

22) Blot SI, Taccone FS, Abeele A-MV den, et al. A Clinical Algorithm to Diagnose Invasive Pulmonary Aspergillosis in Critically Ill Patients. Am J Resp Crit Care. **2012**;186:56–64.

23) Lahmer T, Neuenhahn M, Held J, et al. Comparison of 1,3-β-d-glucan with galactomannan in serum and bronchoalveolar fluid for the detection of Aspergillus species in immunosuppressed mechanical ventilated critically ill patients. J Crit Care. **2016**;36:259-264.

24) Maertens J. Selleslag D, Heinz WJ, et al. Treatment outcomes in patients with proven/probable vs possible invasive mold disease in a Phase III trial comparing isavuconazole vs voriconazole. Mycoses. **2018**;61:868-876.

25) Van Biesen S. Kaw D, Osman RJ and Juffermans NP. Detection of Invasive Pulmonary Aspergillosis in COVID-19 with Non-directed Bronchoalveolar Lavage. Am J Resp Crit Care Med. 2020; Epub ahead of print.

26) Bartoletti M, Pascale R, Cricca M, Rinaldi M, Maccaro A, Bussini L, Fornaro G, Tonetti T, Pizzilli G, Francalanci E, Giuntoli L, Rubin A, Moroni A, Ambretti S, Trapani F, Vatamanu O, Ranieri VM, Castelli A, Baiocchi M, Lewis R, Giannella M, Viale P; PREDICO study group. Epidemiology of invasive pulmonary aspergillosis among COVID-19 intubated patients: a prospective study Clin Infect Dis. 2020; Epub ahead of print

27) Paiva JA, Mergulhao P, Gomes A, et al. Drivers and Impact of Antifungal Therapy in Critically Ill Patients With Aspergillus-positive Respiratory Tract Cultures. Int J Antimicrob Agents. **2017**;50:529-535.

28) 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.

29) Blot S, Rello J and Koulenti D. Diagnosing invasive pulmonary aspergillosis in ICU patients: putting the puzzle together. Curr Opin Crit Care. **2019**;25:430-437.

30) Van Arkel ALE, Rijpstra TA, Belderbos HNA van Wijngaarden P, Verweij PE and Bentvelsen RG. COVID-19 associated pulmonary aspergillosis. Am J Resp Crit Care Med. **2020**;Epub ahead of print.

31) Heard KL, Hughes S, Mughal N, Moor LSP.COVID-19 and fungal superinfection. *Lancet Microbe* 2020; **1:** e107.

Accepted Manuscript

Table 1. Strategies for defining invasive pulmonary aspergillosis (IPA) in intensive care patients with COVID-19 infection. Given the limitations of previous definitions for classifying fungal disease in ICU patients, it was decided to develop novel definitions to enhance both sensitivity and specificity. The format of the definitions, using clinical, radiological and mycological criteria was maintained. The EORTC/MSGERC definitions are, generally, not applicable to the ICU setting, due to the lack of host factors in ICU patients, and have not been included. [20] The ASPICU definitions are based on the recovery of *Aspergillus* from a respiratory tract specimen, an investigation that lacks sensitivity and is slow. [22,27] The recently proposed definitions for classifying influenza associated pulmonary aspergillosis (IAPA) enhanced the mycology criterion by incorporating GM-EIA of BAL and serum, but the radiological criterion, based on non-specific chest radiology is difficult to interpret when evaluating a secondary chest infection in a patient with an underlying chest infection. [2] In the novel COVID-19 associated pulmonary aspergillosis (CAPA) the radiological criterion was enhanced to reflect a progression of respiratory illness due to a secondary chest infection, but also the possible presence of chest radiology typical of IPA. The mycological criterion were extended to reflect the availability of further diagnostic investigations, including the testing of blood samples where sensitivity may be compromised but specificity is high. [24] *Aspergillus* PCR testing was included to reflect the recent acceptance of this testing format due to methodological standardization. [18,20] 1-3-β-D-Glucan testing of serum was included, despite not being specific for *Aspergillus*, due to the documented improved sensitivity over GM-EIA when testing serum for the diagnosis of aspergillosis in ICU patients. [23] The reliance on mycology for completing a classification was dependent on the presence of radiology typical of IPA. Outside of the neutropenic population typical IPA radiology is usually absent, and signs of IPA are non-specific. It was predicted that the presence of typical IPA radiology would be highly specific, and if present would only require the support of a single positive mycological result. [29] This still represents a likely increased specificity over the IAPA definitions that combine non-specific radiology with a single positive GM-EIA result. In the presence of non-specific chest radiology, the novel CAPA definitions are designed to maintain increased specificity by combined the radiology with ≥2 positive mycology results. The

Accepted Manuscript

definitions were retrospectively applied to the current patient cohort to determine respective incidences.

Strategy (Abbreviation/reference)	Requirement		
	Clinical	Radiological	Mycological
Aspergillosis in the ICU (AspICU) (22)	One of: Refractory fever despite at least 3 days antibiotics Recrudescent fever of at least 48 hours despite antibiotics Dyspnoea Haemoptysis Pleural rub or chest pain Worsening respiratory function despite antibiotics and ventilatory support	Abnormal imaging on chest X-ray or chest CT	**Proven**: Histology/Microscopy demonstrating dichotomous septate hyphae in tissue Positive culture from tissue **Putative**: Positive culture from lower respiratory tract specimen in a patient with host risk factors (neutropenia, underling haematological/oncological malignancy, corticosteroids (20mg/day), congenital/acquired immunodeficiency, COPD, decompensated cirrhosis). Semi-quantitative positive culture from BAL with a positive cytological smear in the

Accepted Manuscript

			absence of bacterial growth in patient without host factors
Dutch/Belgian Influenza Associated pulmonary (IAPA) aspergillosis (2)	One of: Refractory fever despite at least 3 days antibiotics Recrudescent fever of at least 48 hours despite antibiotics Dyspnoea Haemoptysis Pleural rub or chest pain Worsening respiratory function despite antibiotics and ventilatory support	Any infiltrate on chest x-ray or chest CT	At least one of: **Proven**: Histology/Microscopy demonstrating dichotomous septate hyphae in tissue Positive culture from tissue **Putative**: Positive culture from BAL Positive GM-EIA in BAL (I≥1·0) Positive GM-EIA in serum (I≥0·5)
COVID-19 Associated pulmonary aspergillosis (CAPA)	PCR confirmed COVID-19 infection and one of: Refractory fever despite	New infiltrates on chest x-ray or chest CT when compared to admission,	**Proven**: Histology/Microscopy demonstrating dichotomous septate hyphae in tissue

Accepted Manuscript

	at least 3 days antibiotics Recrudescent fever of at least 48 hours despite antibiotics Dyspnoea Haemoptysis Pleural rub or chest pain Worsening respiratory function despite antibiotics and ventilatory support	including progression of signs attributed to viral infection. Radiological signs typical of invasive pulmonary aspergillosis (nodules, halos, cavities, wedge-shaped and segmental or lobar consolidation) or evidence of sinusitis should be associated with heightened suspicion of fungal disease (20, 29).	Positive culture from tissue **Putative**: Non-specific radiology: Two or more positives across different test types, or multiple positives within one test type, from the following: Positive culture from NBL/BAL Positive GM-EIA in NBL/BAL (I≥1·0) Positive GM-EIA in serum (I≥0·5) Positive *Aspergillus* PCR in BAL or blood Positive 1-3-β-D-Glucan in serum/plasma Radiology typical of IA: One positive mycological tests as listed above, unless the typical radiological signs can be attributed to a different underlying infection (e.g. lung cancer or alternative infection). In this scenario multiple positive results would be required to attain a diagnosis of putative IPA. **Please note:** Given the aetiological diversity

Accepted Manuscript

			associated with sinusitis, multiple positive tests from the list above are required to attain a diagnosis of putative IPA.

Accepted Manuscript

Table 2. Basic demographics, comorbidities, risk factors and test performance according to population. P-Values compared data from the mycology positive and negative populations. Significant differences highlighted in bold text. * Data is only available for patients admitted to the intensive care unit of the University Hospital of Wales, Cardiff, UK.

	Population			
	All ICU patients (n=135)	Mycology positive (n=51)	Mycology negative (n=84)	P-value
Median Age (25^{th}/75^{th} percentile)	57 (48/64)	58 (50/69)	57 (47/63)	0·2305
Male/female	2.2/1	2/1	2.36/1	0·7038
Comorbidities (n/N)	112/131 (4 not available)	41/51	71/80 (4 not available)	0·2097
Comorbidities-listed	Diabetes mellitus: 38 Hypertension: 35 Chronic respiratory illness: 30 Obesity/Hyperlipidaemia: 27 Cardiac/Vascular disease: 18 Autoimmune/Inflammatory conditions: 18	Diabetes mellitus: 13 Hypertension: 16 Chronic respiratory illness: 14 Obesity/Hyperlipidaemia: 10 Cardiac/Vascular disease: 6 Autoimmune/Inflammatory conditions: 8	Diabetes mellitus: 25 Hypertension: 19 Chronic respiratory illness: 16 Obesity/Hyperlipidaemia: 17 Cardiac/Vascular disease: 12 Autoimmune/Inflammatory conditions: 10	0·5559 0·4185 0·3947 1·0000 0·7954 0·6083

Accepted Manuscript

	Solid Cancer: 10 Kidney disease: 8 Haematology malignancy: 4 Other infection: 4 Other: 8	Solid Cancer: 7 Kidney disease: 4 Haematology malignancy: 2 Other infection: 0 Other: 4	Solid Cancer: 3 Kidney disease: 4 Haematology malignancy: 2 Other infection: 4 Other: 4	**0·0466** 0·7106 0·6424 0·1563 0·7106
Antibacterials administered (n/N, %,)	115/122 (94·3%) (13 unavailable)	50/51, (98·0%)	65/71 (91·5%) (13 unavailable)	0·2369
Antifungals administered (n/N, %)	54/121, 44·6% (14 unavailable)	35/50, 70·0% (1 unavailable)	19/71, 36·5% (13 unavailable)	**<0.0001**
Invasive Ventilatory Support (n/N, %)	122/134, 91·0% (1 unavailable)	44/51, 86·3%	78/83. 94·0% (1 unavailable)	0·2108
Corticosteroids* (n/N, %)	32/57, 56·1% (2 unavailable)	20/35 (57·1%)	12/22 (54·5%)	1·0
ICU LOS (days median, $25^{th}/75^{th}$ percentile))	17·5 (5.3/27.8)	19·5 (12.3/33.3)	12·0 (2.8/22.3)	0·0504
Total Leucocytes (median, ($25^{th}/75^{th}$ percentile))	12·95, (8.95/19.30)	13·20, (9.00/20.80)	12·70, (8.80/18.70)	0·4475
Neutrophils (median,	9·90, (6.60/16.10)	9·95, (6.40/16.15)	9·50, (6.70/16.10)	0·8376

Accepted Manuscript

(25th/75th percentile))				
Lymphocytes (median, (25th/75th percentile))	1·20, (0.70/1.60)	1·20, (0.80/1.825)	1·10, (0.70/1.60)	0·2545
PCT (median, (25th/75th percentile))	0·85, (0.29/2.20)	0·77, (0.18/2.22)	1·02, (0.35-2.20)	0·4509
CRP (median, (25th/75th percentile))	139, (82.5/249)	136, (79/243)	141, (95/260)	0·5300
Mortality rate (%, 95% CI)	38·3 (30·3-46·9)	47·1 (34·1-60·5)	31·3 (22·2-42·1)	0·0952
Mycology				
Significant Yeast culture (n/N, (%, 95% CI))	17/135 (12·6, 8.0-19.2)	17/51 (33·3, 22.0-47.0)	-	-
Aspergillus respiratory culture (n/N (%, 95% CI))	11/135 (8.2, 4.6-14.0)	11/51 (21·6, 12.5-34.6)	-	-
Combined NBL/BDG testing strategy (n/N	48/135 (35·6, 28·0-43·9)	30/51 (58·8, 45·2-71·3)	18/84 (21·4, 14·0-31·4)	**<0·0001**

Accepted Manuscript

(%, 95% CI))				
(1-3)-β-D-Glucan Mean Concentration (pg/ml, (95% CI))	85·6 (67·7-103·4)	151·1 (114·6-187·7)	33·5 (31·9-35·1)	**<0·0001**
(1-3)-β-D-Glucan (Median tests per patient, (25^{th}/75^{th} percentile))	2·0, (1.0/2.0)	2·0, (1.0/3.0)	1·0, (1.0/2.0)	**0·0006**
(1-3)-β-D-Glucan sample positivity (n/N, (%, 95% CI))	38/217 (17·5, 13·0-23·1)	38/96 (39·6, 30·4-49·6)	-	-
(1-3)-β-D-Glucan patient positivity (n/N (%, 95% CI))	19/122 (15·6, 10·2-23·1)	19/45 (42·2, 29·0-56·7)	-	-
GM-EIA-NBL Mean GMI (95% CI)	1·2 (0.7-1.7)	1·7 (1.0-2.4)	0·1 (0.09-0.14)	**0·0024**
GM-EIA-NBL (Median tests per patient, (25^{th}/75^{th} percentile))	2·0 (1.0/3.0)	2·0 (1.0/4.0)	1·0 (1.0/2.0)	**0·0205**
GM-EIA-NBL sample	27/135 (20·0, 14·1-27·5)	27/93 (29·0, 20·8-38·9)	-	-

Accepted Manuscript

positivity rate (n/N (%, 95% CI))				
GM-EIA-NBL patient positivity rate (n/N (%, 95% CI))	17/60 (28·3, 18·5-40·8)	17/35 (48·6, 33·0-64·4)	-	-
Aspergillus PCR-NBL (Median tests per patient, (25^{th}/75^{th} percentile))	2·0 (1.0/3.0)	2·0 (1.0/4.0)	1·0 (1.0/2.0)	**0·0205**
Aspergillus PCR-NBL sample positivity rate (n/N (%, 95% CI))	31/131 (23·7, 17·2-31·6)	31/91 (34·1, 25·2-44·3)	-	-
Aspergillus PCR-NBL patient positivity rate (n/N (%, 95% CI))	20/60 (33·3, 22·7-45·9)	20/35 (57·1, 40·9-72·0)	-	-

Key:

ICU: Intensive care unit

PCT: Procalcitonin

CRP: C-reactive protein

Accepted Manuscript

LOS: Length of stay

NBL: Non-directed bronchial lavage

BDG: 1-3-β-D-Glucan

GM-EIA: Galactomannan Enzyme Immunoassay

GMI: Galactomannan index value

95% CI: 95% Confidence interval

Accepted Manuscript

Table 3. Cases of culture confirmed invasive yeast disease. * Antifungal therapy was deemed appropriate if it were in-line with international guidelines. For instance, if a yeast was recovered from blood or a central venous catheter, but not identified to species level and the patient was commenced on fluconazole, then this would considered inappropriate in the absence of an antifungal with a broader spectrum of activity.

Case No	Comorbidities	Corticosteroids	Ventilatory support	Radiological Evidence	Mycological Evidence	Antifungal therapy*	Type of infection		Died (Day 30)
							Line	Deep	
1	HTN, obesity	N/A	Yes	Non-specific	Yeast (No ID) (CVC) tip	Fluconazole	Yes		Yes
2	HTN	None	Yes	Non-specific	*Rhodotorula* (BC)	Caspofungin, L-Amb		Yes	Yes
3	Oesophagectomy, cancer	Hydrocortisone	Yes	Non-specific	Yeast (No ID) sterile fluid (Chest drain)	Fluconazole		Yes	Yes
4	Ulcerative colitis	None	Yes	Non-specific	*C. albicans* (CVC)	None	Yes		No
5	DM, HTN, obesity, asthma	None	Yes	Non-specific	*C. albicans* (CVC)	Fluconazole	Yes		No
6	HTN, asthma	None	Yes	Non-specific	*C. albicans* (BC),	Caspofungin,		Yes	No

Accepted Manuscript

					Candida PCR positive, BDG 156, 95,86	fluconazole			
7	Haem, cardiac	None	Yes	Non-specific	*C. albicans* (BC),	None		Yes	Yes
8	None	N/A	Yes	Non-specific	*C. albicans* (CVC)	Fluconazole	Yes		No
9	Cardiac, CKD, cancer (bowel)	N/A	Yes	Non-specific	*C. albicans* (CVC)	Caspofungin,	Yes		Yes
10	Inflammatory, asthma, IBS	N/A	Yes	Non-specific	*Candida* sp (CVC) BDG: (60)	Voriconazole	Yes		Yes
11	None	N/A	Yes	Non-specific	*C. parapsilosis* (CVC)	Caspofungin	Yes		No
12	None	N/A	Yes	Non-specific	*C. albicans* (BC, CVC)	Fluconazole	Yes	Yes	Yes
13	None	N/A	Yes	Non-specific	*C. albicans* (BC), BDG: >500, Candida PCR positive	Fluconazole, caspofungin		Yes	No

Accepted Manuscript

14	DM, HTN, obesity	N/A	Yes	Non-specific	*C. albicans* (CVC)	Fluconazole	Yes		No
15	Hepatitis, IVDU, neutropenia, cellulitis	N/A	Yes	Non-specific	*C. albicans* and *C. parapsilosis* (BC), BDG: 386	Fluconazole, L-Amb		Yes	No
16	DM, inflammatory, Alcoholic	Yes, not specified	Yes	Non-specific	*C. albicans* (ascites)	Caspofungin, voriconazole		Yes	No
17	DM, HTN	N/A	Yes	Non-specific	*C. albicans* (CVC), BDG: >500	Fluconazole, voriconazole	Yes		Yes

Key: **HTN:** Hypertension **CVC:** Central venous catheter **BC:** Blood culture **L-amb:** Liposomal amphotericin B

DM: Diabetes Mellitus **BDG:** 1-3-β-D-Glucan **Haem:** Haematological malignancy **CKD:** Chronic kidney disease

IBS: Irritable bowel syndrome **IVDU:** Intravenous drug user **No ID:** No species identification available

Accepted Manuscript

Table 4. Cases of COVID-19 associated invasive aspergillosis (CAPA) classified according to the various definitions (described in Table 1). Seven patients with positive *Aspergillus* mycology insufficient for classification by any of the definitions have been excluded. Shaded cells reflect agreement between the definitions.

Case No	Comorbidities	Corticosteroids	Ventilatory support	Radiological Evidence	Mycological Evidence	Antifungal therapy [a]	Case definition			Died (day 30)
							AspICU	IAPA [b]	CAPA	
1	Vasculitis, essential thrombocytohaemia	Hydrocortisone	Yes	Cavities, sinusitis	BDG: >500 (x3) Asp PCR NBL Positive (x2) Asp PCR plasma: Positive GM-EIA NBL: 8·3, 7·6 GM-EIA plasma: 4·9 *A. fumigatus*	Voriconazole	Yes	Yes	Yes	Yes

Accepted Manuscript

					from NBL					
2	None specified	Dexamethasone	Yes	Non-specific	BDG: 251, 237, 164 Asp PCR NBL Positive (x2) Asp PCR plasma: Positive GM-EIA NBL: 8·2, 8·4 GM-EIA plasma: (0·4) *A. fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	No
3	Solid Cancer, CR	None	Yes	Non-specific	*A. fumigatus* from NBL	None	Yes	Yes	No	Yes
4	DM, CR	Prednisolone	Yes	Nodule	Asp PCR NBL Positive GM-EIA NBL:	L-Amb	Yes	Yes	Yes	Yes

Accepted Manuscript

					12·8, (0·7) *A. fumigatus* from NBL					
5	Solid cancer	Hydrocortisone, Prednisolone, dexamethasone	Yes	Tree-in-bud, nodule	BDG: 85, 105, 154 Asp PCR NBL Positive (x7) Asp PCR serum Positive (x2) GM-EIA NBL: 16·6, 3·8, 3·6, 3·2, (0·9) *A. fumigatus* from NBL	Voriconazole, L-Amb	Yes	Yes	Yes	No
6	DM, CR	IV Hydrocortisone	Yes	Nodule, sinusitis	Asp PCR NBL Positive (x2) GM-EIA NBL: 5·6, 3·7 *A. fumigatus*	Voriconazole	Yes	Yes	Yes	No

Accepted Manuscript

					from NBL					
7	CR, autoimmune	Prednisolone (methotrexate prior to COVID-19)	No	Non-specific, but not typical of COVID-19	Asp PCR NBL Positive GM-EIA NBL: 16·4, 5·2 *A. fumigatus* from NBL	Voriconazole	Yes	Yes	Yes	Yes
8	HM, liver dysfunction	Methylprednisolone, IV hydrocortisone	Yes	Nodules, cavities, sinusitis	BDG: 292, 445 Asp PCR NBL Positive (x2) Asp PCR serum Positive (x2) GM-EIA NBL: 16·4, 5·2 GM-EIA serum: 0·9 *A. fumigatus* from NBL	Anidulafungin, L-Amb	Yes	Yes	Yes	Yes

Accepted Manuscript

9	CR (Asthma), obesity	No systemic, but did received inhaled beclometasone dipropionate and formoterol	Yes	Non-specific, sinusitis	*A. fumigatus* from NBL GM-EIA NBL: (0·5)	None	No [c]	Yes	No	No
10	DM	N/A	Yes	Cavitation, sinusitis	BDG: >500 (x2), 485 Asp PCR NBL Positive *A. fumigatus* from NBL	Caspofungin, voriconazole	No [c]	Yes	Yes	No
11	CR, obesity	N/A	Yes	Non-specific	BDG: >500 GM-EIA NBL: 1·8	None	No	Yes	Yes	Yes
12	CR	Low dose hydrocortisone and Inhaled beclometasone dipropionate and	Yes	Non-specific	GM-EIA NBL: 6·8	Caspofungin, voriconazole	No	Yes	No	No

Accepted Manuscript

		formoterol								
13	CR, HTN	Dexamethasone	No	Nodule	BDG: >500, 489, 367 Asp PCR NBL Positive (x2) GM-EIA NBL: 6·8, 1·2	Voriconazole	No	Yes	Yes	No
14	CR, DM, HTN	Prednisolone	No	Cavitation	BDG: 142 Asp PCR NBL Positive GM-EIA NBL: 1·5	Voriconazole	No	Yes	Yes	Yes
15	None Specified	N/A	Yes	Non-specific	GM-EIA NBL: 1·1 BDG: 109	None	No	Yes	Yes	Yes
16	HTN, obesity	Methyl-prednisolone	Yes	Cavitation	Asp PCR NBL Positive GM-EIA NBL:	Voriconazole, Ambisome	No	Yes	Yes	No

Accepted Manuscript

					1·6 (x2)					
17	Alzheimers, HTN	None	No	Non-specific	GM-EIA NBL: 4·2	Voriconazole	No	Yes	No	Yes
18	HTN, solid cancer	Dexamethasone	Yes	Non-specific	GM-EIA NBL: 1·1, (0.7)	None	No	Yes	No	No
19	DM, HTN, Obesity	None	Yes	Non-specific, sinusitis	Asp PCR NBL Positive (x2) GM-EIA NBL: 2·2 *A fumigatus* and *A. versicolor* cultured from NBL, BDG: 137 (>1 month later)	Voriconazole	No [c]	Yes	Yes	No
20	CR, Solid Cancer	None	No	Nodules (secondary lung	GM-EIA NBL: 1·0	None	No	Yes	No	No

Accepted Manuscript

				metasteses)						
21	CKD, solid cancer	Hydrocortisone, fludrocortisone	Yes	Nodules	BDG: >500, 467	Voriconazole, fluconazole	No	No	Yes	No
22	CR, obesity	Prednisolone	No	Nodule	Asp PCR NBL Positive	None	No	No	Yes	Yes
23	DM, Obesity, Cardiac	Methylprednisolone	Yes	Non-specific	BDG: (70) Asp PCR NBL Positive (x2) GM-EIA NBL: (0·7)	Voriconazole	No	No	Yes	Yes
24	CR, CKD, HTN	No systemic, Fluticasone nasal spray and Symbicort inhaler	Yes	Non-specific	BDG: 103 Asp PCR plasma: Positive	Fluconazole	No	No	Yes	Yes
25	Auto-immune, HTN	Dexamethasone, hydrocortisone, prednisolone	No	Fungal ball in sinus	BDG: >500 (x2) GM-EIA	L-Amb	No	No	Yes	Yes

Accepted Manuscript

serum: (0·3)

[a] Antifungal therapy was deemed appropriate if it were in-line with international guidelines. For instance, if a patient was diagnosed with CAPA but had only received caspofungin, then this would considered inappropriate, as it is not recommended as frontline therapy for invasive aspergillosis.

[b] IAPA guidelines have been modified to accepted NBL GM-EIA positivity in place of testing bronchoalveolar lavage fluid

[c] Cases did not meet AspICU definitions as the patient lacked a host factor and the *Aspergillus* culture was not performed in quantifiable manner.

Key: **HTN:** Hypertension **BDG:** 1-3-β-D-Glucan **Asp PCR:** *Aspergillus* PCR **NBL:** Non-directed bronchial lavage

GM-EIA: Galactomannan Enzyme immunossay **L-amb:** Liposomal amphotericin B **DM:** Diabetes Mellitus

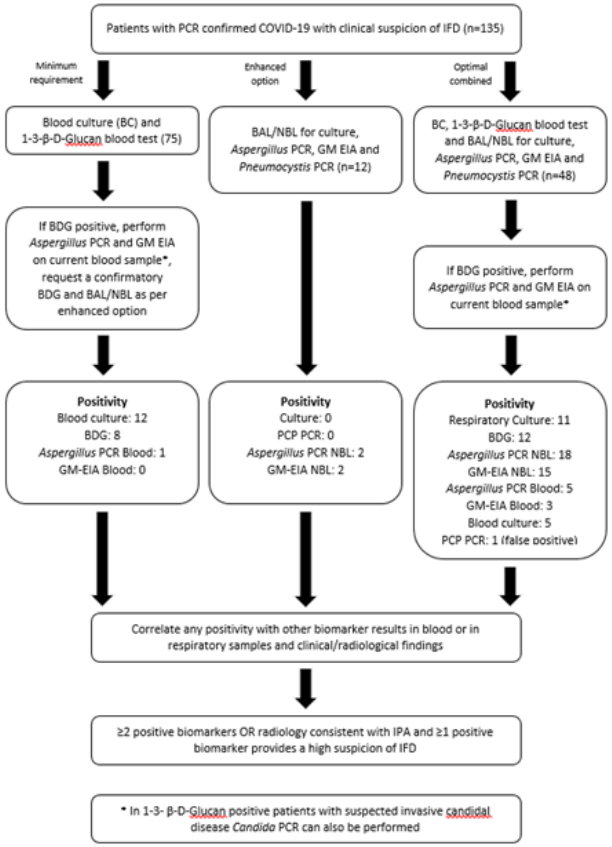
HM: Haematological malignancy **CKD:** Chronic kidney disease **CR:** Chronic respiratory illness

Accepted Manuscript

- **Figure 1**. Diagnostic screening algorithm when managing COVID-19 patients at risk of invasive fungal disease (n = patient numbers). Samples were sent to the Public Health Wales Mycology reference laboratory at the discretion of clinicians from PCR confirmed COVID-19 adult (≥18 years) patients requiring critical care management for prolonged (>7 days) or worsening severe respiratory dysfunction despite clinical intervention. As part of the diagnostic work-up, *Aspergillus* PCR/GM-EIA and *Pneumocystis* PCR testing on NBL/BAL fluid was recommended. In addition, (1-3)-β-D-Glucan (BDG) testing of serum was advised, and if positive lead to further fungal investigations (e.g. *Aspergillus* PCR/GM-EIA). For optimal diagnosis, both respiratory and blood testing was recommended, but in the absence of a respiratory sample BDG testing of serum was a minimum requirement. Blood culture was performed according to national guidelines on the investigation of sepsis. [19] Once weekly testing was recommended while the patient was in a critical state.
- In 16 patients with ≥2 *Aspergillus* positive results, 15 had a NBL tested, 86.7% (95% CI: 62·1-96·3) were GM-EIA positive, 80.0% (95% CI: 54.8-93.0) were *Aspergillus* PCR positive, 10 patients positive by both tests. All 16 had BDG testing of serum and 68.8% (95% CI: 44.4-85·8) were positive, being positive in one patient where NBL testing was not available, but *Aspergillus* PCR was also positive in blood (Table 4). In the five patients where BDG was negative, both *Aspergillus* PCR and GM-EIA were positive in NBL from four patients, with GM-EIA on NBL being positive on multiple occasions in one patient. *Aspergillus fumigatus* was cultured from NBL from a total of 11 patients (8.2%, 95% CI: 4.6-14.0), and in 56.3% (95% CI: 33.2-76.9) of patients with multiple *Aspergillus* positive results. In two of the four patients, potentially with unspecified IFD, the BDG assay was serially positive in two patients where NBL was negative by both GM-EIA and *Aspergillus* PCR, despite radiological evidence of IFD, in the remaining patients NBL was not available for testing but BDG was serially positive.

Key: **IFD:** Invasive fungal disease **BDG:** 1-3-β-D-Glucan **BAL:** Broncholvleolar lavage fluid **NBL:** Non-directed bronchial lavage fluid **GM-EIA:** Galactomannan Enzyme immunoassay

Figure-1


Patients with PCR confirmed COVID-19 with clinical suspicion of IFD (n=135)
Minimum requirement
Enhanced option
Optimal combined
Blood culture (BC) and 1-3-β-D-Glucan blood test (75)
BAL/NBL for culture, Aspergillus PCR, GM EIA and Pneumocystis PCR (n=12)
BC, 1-3-β-D-Glucan blood test and BAL/NBL for culture, Aspergillus PCR, GM EIA and Pneumocystis PCR (n=48)
If BDG positive, perform Aspergillus PCR and GM EIA on current blood sample*, request a confirmatory BDG and BAL/NBL as per enhanced option
If BDG positive, perform Aspergillus PCR and GM EIA on current blood sample*
Positivity
Blood culture: 12
BDG: 8
Aspergillus PCR Blood: 1
GM-EIA Blood: 0
Positivity
Culture: 0
PCP PCR: 0
Aspergillus PCR NBL: 2
GM-EIA NBL: 2
Positivity
Respiratory Culture: 11
BDG: 12
Aspergillus PCR NBL: 18
GM-EIA NBL: 15
Aspergillus PCR Blood: 5
GM-EIA Blood: 3
Blood culture: 5
PCP PCR: 1 (false positive)
Correlate any positivity with other biomarker results in blood or in respiratory samples and clinical/radiological findings
≥2 positive biomarkers OR radiology consistent with IPA and ≥1 positive biomarker provides a high suspicion of IFD
* In 1-3- β-D-Glucan positive patients with suspected invasive candidal disease Candida PCR can also be performed