DIAGNOSTIC CHALLENGES OF FUNGAL DISEASE IN WALES

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Summary

Invasive and serious fungal diseases carry a high degree of morbidity and mortality. Despite the seriousness of these conditions, no national fungal disease registry exists. In this thesis, the incidence and prevalence of fungal disease in Wales was estimated through a literature review exploring the expected burden of the most common or most serious pathogens in at-risk populations. Local laboratory reporting was also utilised to retrospectively analyse Wales's local incidence data. The discrepancy between the expected incidence of pneumocystosis and the high number of laboratory-confirmed cases prompted a more comprehensive review. Aside from HIV, in which mortality was low, there was no significant difference in mortality between the various aetiologies of immunosuppression responsible for contracting pneumocystosis.

Microbiological investigation of fungal pathogens is difficult. Biomarker and culture techniques are applied to samples such as bronchoalveolar lavage which can be problematic to obtain. The SPutum Induction Trial For Improved Respiratory Evaluation (SPITFIRE) was designed and implemented to investigate the opportunity to use sputum induction as a novel method of obtaining deep respiratory samples in an unwell, immunocompromised haematology cohort. The data suggests this to be an acceptable procedure for both operator and patient and produces microbiologically similar results to deep respiratory samples obtained at bronchoscopy. There is evidence of concordance with bronchoscopy in diagnosing fungal disease.

It is a challenge to correctly interpret the significance of a positive result. *Exophiala dermatitidis* is frequently isolated from patients with cystic fibrosis but its impact on lung function was unknown. Following a retrospective case-controlled review, it was demonstrated that *E.dermatitidis* isolation is associated with a more rapid lung function decline than in the same individuals pre-isolation and compared to control. A healthcare environment-focussed patient-patient transmission model was also postulated.

This thesis highlights multiple diagnostic challenges in fungal disease and proposes Wales-wide approaches to address them.

Publications and Presentations

- **September 2020**: European Cystic Fibrosis Society Annual Conference – poster presentation - *Exophiala dermatitidis* infection in cystic fibrosis patients accelerates lung function decline: a retrospective single-centre review of historical lung function
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- **June 2022**: Welsh Fungal Interest Meeting – oral presentation – prevalence of fungal respiratory disease in Wales
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Abbreviations

ABPA	Allergic bronchopulmonary aspergillosis
AIDS	Acquired immune deficiency syndrome
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ART	Antiretroviral therapy
AU/ml	Arbitrary units per millilitre
BAL	Bronchoalveolar lavage
BMI	Body mass index
BMT	Bone Marrow Transplant
Bp	Base pairs
BR	Bronchoscopy
BROW	Bronchial washing
BTS	British Thoracic Society
CF	Cystic fibrosis
CFS	Clinical Frailty Score
CFTR	Cystic fibrosis transmembrane conductance regulator
CLED	Cystine lactose electrolyte deficient
CLL	Chronic lymphocytic leukaemia
CML	Chronic myeloid leukaemia
CMV	Cytomegalovirus
CNS	Central nervous system
COPD	Chronic obstructive pulmonary disease
CPA	Chronic pulmonary aspergillosis
Cq	Quantification cycle
CrAg	Cryptococcal antigen
CRF	Case report form
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed tomography

CTAE	Common Terminology Criteria for Adverse Events
DGH	District general hospital
DLBCL	Diffuse large B cell lymphoma
DMARD	Disease-modifying anti-rheumatic drug
DNA	Deoxyribonucleic acid
ECFS CTN	European Cystic Fibrosis Society Clinical Trial Network
ECG	Electrocardiogram
ED	Emergency department
FBC	Full blood count
FEV1	Forced expiratory volume in 1 second
FEV1%	Percent predicted forced expiratory volume in one second
GCS	Glasgow Coma Score
GM	Galactomannan
HIV	Human immunodeficiency virus
HL	Hodgkin's lymphoma
HRA	Health Research Authority
HTS	Hypertonic saline
IA	Invasive Aspergillosis
ICNARC	Intensive Care National Audit & Research Centre
ICU	Intensive Care Unit
IgE	Immunoglobulin E
IgG	Immunoglobulin G
ILD	Interstitial lung disease
IS	Induced sputum
IS1	1st induced sputum procedure
IS2	2nd induced sputum procedure
ITP	Immune thrombocytopenia
L	Litre
Mcg	Micrograms
MDS	Myelodysplastic syndrome
MDT	Multidisciplinary team

Mg/L	Milligrams per litre
MM	Multiple myeloma
N	Number
NBL	Non-directed bronchoalveolar lavage
NEWS	National Early Warning Score
NHL	Non-Hodgkin lymphoma
NPA	Naso-pharyngeal aspirate
ONS	Office of National Statistics
PcP	*Pneumocystis jirovecii* pneumonia
PCR	Polymerase chain reaction
PET	Positron Emission Tomography
PHW	Public Health Wales
PPI	Patient and public involvement
rDNA	Ribosomal deoxyribonucleic acid
REC	Research ethics committee
RSV	Respiratory syncytial virus
SAFS	Severe asthma with fungal sensitisation
SAIL	Secure Anonymised Information Linkage
SCT	Stem cell transplant
SNS	Sino-nasal secretions
SOP	Standard Operating Procedure
SPITFIRE	Sputum induction trial for improved respiratory evaluation
STR	Single tandem repeat
TB	Tuberculosis
TBI	Total body irradiation
UPGMA	Unweighted pair group method with arithmetic mean
VAP	Ventilator associated pneumonia
WIMD	Welsh Index of Multiple Deprivation

Chapter 1 – Outline of serious or invasive fungal disease

1.1. Introduction and definitions

Invasive infection is defined as pathogens breaching the basement membrane of tissue and spreading or gaining access to usually sterile deeper tissue, organs or distant sites (1,2). The pathogenicity of some organisms, such as *Aspergillus* species, are well documented and others are emerging (3). However, there are a number of disease processes related to fungal pathogens which carry a high degree of mortality or morbidity but are not truly microbiologically invasive. There is no clear accepted definition for grouping these diseases in the literature. For the purposes of this thesis, conditions caused by fungal organisms that require medical intervention in a specialist medical (secondary) care setting or are responsible for a level of physiological compromise or decompensation have been termed "serious".

Invasive and serious fungal disease encapsulates a wide variety of pathological processes. Typically, these organisms are ubiquitous in the environment and so, in order to cause opportunistic infection, require a level of host immunological dysfunction. The nature of this dysfunction often dictates the impact of disease; from dysregulated hypersensitivity reactions to immunological failure leading to invasive infections. Overall, fungal disease carries a high degree of morbidity and mortality and their severity mandates a high healthcare utilisation cost burden (4). However, individual patient outcomes can be highly variable depending on the organism and the underlying immune deficit.

There are a number of reasons for Wales to have a particular problem with fungal disease. As a country, Wales has a maritime climate. It features weather that is mild in temperature but cloudy, wet and windy, which serves as suitable growing conditions for many environmental pathogens, including moulds (5). This is unlikely to improve with global climate change. There is a growing concern that global warming may create environmental change that results in new or re-emergence of fungal pathogens rather than their extinction (6).

Wales has a poor socioeconomic status with more people of all ages living in poverty than the United Kingdom (UK) average. It has the highest rate of child poverty of any nation in

the UK with one in three children living in poverty and 14% in severe poverty (7,8). This has a direct relationship to the overall health of the nation as there is a strong negative effect of poverty on health (9). This relationship between socioeconomic deprivation and health is analysed in the Welsh Index of Multiple Deprivation (WIMD) and the Office of National Statistics (ONS) regularly concludes that areas of Wales with significantly more poverty suffer with decreased life expectancy and a greater burden of chronic illness (10,11). Of particular relevance to fungal disease, Wales has significantly older housing stock than other nations in the UK and a higher percentage of those houses (6% versus 4%) are affected by damp or visible mould (12). This has recently been highlighted as a major public health concern following the death of a two-year-old boy in England, ruled to be directly attributable to mould exposure in the home (13).

Wales has a relatively small population of just over three million and a population density of 150 per square kilometre (compared to 434 per sq. km in England) with a concentration around the coast and English border (14). This means, that in order to provide effective healthcare to all in an accessible manner, secondary care medical services are spread out over a wider area with minimal centralised tertiary referral services. This has the potential to lead to local variation in the availability or timeliness of investigations including their reporting. An example of improvements being made in this area is the provision of Positron Emission Tomography (PET) Computed Tomography (CT) scanners. Previously a single PET CT scanner was available in Cardiff, serving all of South Wales. As Cardiff is located in the South East of Wales, this had the potential to create significant healthcare inequality through restriction of access and so this is attempting to be mitigated through the investment in further scanners in Swansea, Cardiff and North Wales (15). However, even the development of Cardiff and Swansea as diagnostic hubs for their respective wider areas still leaves a large number of patients in the catchment of District General Hospitals (DGH) outside these cities with suboptimal provision. An example of where this phenomenon has been addressed is in the diagnostic pathway of patients with Interstitial Lung Disease (ILD). This is a diverse group of severe respiratory conditions with a high degree of morbidity and mortality which are usually progressive. They require expert opinion on the interpretation of imaging, clinical and histopathological investigations in order to determine a diagnosis through consensus. These expertise are not available in each DGH and so a virtual

multidisciplinary team (MDT) has been created in order that all patients can access the same level of expertise regardless of location (16).

To address the challenges of investigating fungal disease in Wales, some key organisms and disease process associated with them have been identified and these are introduced below. Whilst these are not a complete list of all pathology-causing organisms, they represent the most common or the most likely to be encountered in Wales as pathogenic.

Organism	**Disease process**
***Aspergillus* species**	Invasive aspergillosis
	Chronic pulmonary aspergillosis
	Allergic bronchopulmonary aspergillosis
	Severe asthma with fungal sensitisation
***Candida* species**	Invasive candidiasis
***Cryptococcal* species**	Cryptococcosis
Order of fungi Mucorales	Mucormycosis

Table 1: Key pathogenic fungal organisms in Wales and their associated disease states

1.2. Aspergillosis

1.2.1. Invasive aspergillosis

Invasive aspergillosis (IA) is the most severe presentation of infection by *Aspergillus* species. The commonest organism associated with IA is *Aspergillus fumigatus*. Far less common, but also of significance are *Aspergillus niger*, *Aspergillus flavus* and *Aspergillus terreus* (17). The lung is the most common portal of entry of infection into the body, where inadequate immunological defences allow fulminant infection to breech the basement membrane of epithelial cells and allow variable amounts of invasion (17). IA requires a profound level of immunosuppression to occur. This level of immunosuppression usually takes place rapidly as more gradual reductions in immune function are more likely to result in infection by other opportunistic organisms before the level by which IA becomes a risk. More likely aetiologies include induction chemotherapy and total body irradiation (TBI) prior to bone marrow transplant (BMT), breakdown of mucosal barriers and acute immunocompromise due to other chemotherapy agents, and human immunodeficiency virus (HIV)(18). Pulmonary

symptoms are common clinical manifestations and include a cough which is sometimes complicated by haemoptysis, pain, fever and breathlessness. Invasion into other organ systems correlates with additional symptoms and signs. For example, dissemination involving the central nervous system (CNS) can produce additional neurological symptoms but is associated with more impaired immune function (18).

The mortality rate of IA is variable depending on the extent of invasion or dissemination of the infection. For example, it is approximately 40% in pulmonary disease and over 90% in disseminated disease with CNS involvement (19). However, untreated IA has a mortality approaching 100% (17). As such, an appropriate level of clinical suspicion in at-risk groups and prompt investigation followed by intensive antifungal treatment is crucial.

1.2.2. Chronic pulmonary aspergillosis

Chronic pulmonary aspergillosis (CPA) is a focal, progressive pulmonary infection caused by *Aspergillus* species. It carries a high degree of morbidity with the potential for a progressive decline in lung function and death. As inhalation of this species is not associated with any ill effects in healthy people, the spectrum of pathology is highly reliant upon the nature of the underlying immunosuppression and degree of underlying structural lung disease. For example, a simple aspergilloma has been shown to occur when an *Aspergillus* mycetoma forms within a pre-existing cavity, usually as a result of prior sarcoidosis or *Mycobacterium tuberculosis* (TB) infection. This can progress to CPA with immunological activation and inflammation in the surrounding lung parenchyma. A more rapid onset and progressive version of CPA can also occur with multifocal consolidation, pleural involvement, cavity formation and fibrosis but this requires greater immune dysfunction (20).

Diagnosis of this variable condition requires evidence of radiological progressive change of the aforementioned cavities, fibrosis and consolidation. There must also be evidence of infection by *Aspergillus* species as demonstrated microbiologically or through host-interaction with serology testing (20).

1.2.3. Allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitisation (ABPA/SAFS)

ABPA and SAFS are separate disease entities which occupy a similar clinical niche in that they are likely to present in a similar manner or through the same pathway in primary or secondary care. Patients suffering from these conditions do not have features of invasive disease but rather a sensitisation to *Aspergillus* leading to worsening breathlessness, cough and mucous plugging.

ABPA is most commonly a complication of a patient's asthma diagnosis and can lead to bronchiectasis if not detected in a timely manner (17). ABPA is also a recognised complication of cystic fibrosis (CF) and exacerbates a patient's difficulty in the clearance of airways and breathlessness. Multiple diagnostic criteria for ABPA exist. However, all have the consistent core requirements of pre-existing lung disease (asthma or cystic fibrosis) in the presence of an immunoglobulin E (IgE) driven allergy response which is specific to *Aspergillus* through either serum or skin testing (21,22). Other supportive diagnostic features exist which are variations of the prior criteria. These include radiological evidence of the pre-existing lung disease, blood eosinophilia as a biomarker of the aforementioned allergy response or include microbiological isolation of *Aspergillus* from the respiratory tract.

Asthma is a prerequisite of SAFS but the true pathogenesis of this condition is not fully understood. Fungal components are important aeroallergens for asthma development and progression in patients with SAFS but, unlike in ABPA, this is not restricted to *Aspergillus* species and there is an absence of structural lung damage (23). Diagnostic criteria have not been fully established but require the presence of severe asthma, fungal sensitisation and the exclusion of ABPA (24). Its categorisation as a separate clinical entity from asthma alone is characterised by the clinical responsiveness of antifungal therapy and remains a pragmatic definition away from ABPA (25). SAFS is generally considered to sit within an overlapping spectrum of allergic fungal airways diseases characterised by immunologically dysfunctional reactions to the presence of fungi within airways (26). This concept, in particular the potential for diagnostic criteria overlap with these conditions is illustrated below in figure 1, adapted from Dupont et al (26):

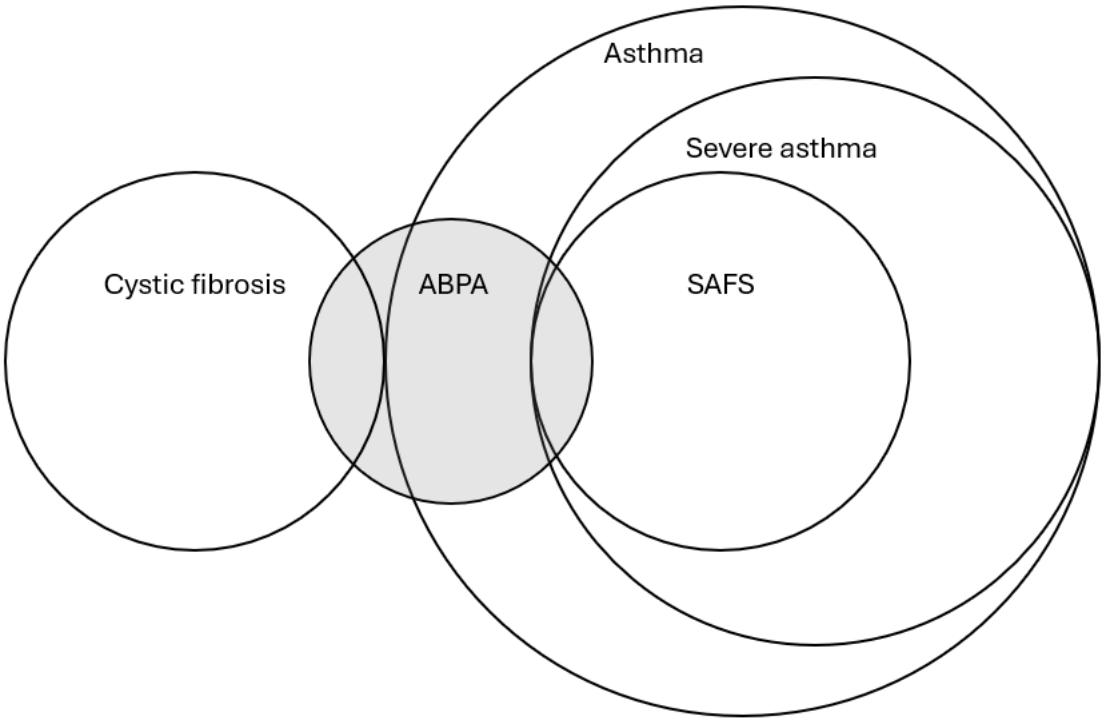


Figure 1: An illustration of the suggested crossover and interaction between fungal organisms and airways disease

ABPA and SAFS do not carry a significant mortality rate alone but rather are examples of conditions that carry a high degree of morbidity as these patients have often a high symptom and, potentially, treatment burden. The mucous plugs can dramatically reduce lung function and the potential for developing bronchiectasis risks longer term clinical sequelae. There is evidence of patients with ABPA changing their phenotype to one of CPA which carries a higher mortality risk (20).

1.3. Invasive candidiasis

Candida organisms are yeasts which are found as commensals throughout the skin, oral cavity, gastrointestinal and genitourinary tracts (27). Candidiasis is an opportunistic infection that can be caused by a number of *Candida* species in circumstances arising from impaired immunity. However, the immunological insults required for infection to become established are extremely broad including, but not limited to; malignancy, surgery, burns, chemotherapy and the use of long term invasive medical devices such as central venous

catheters (28). *C.albicans, C.glabrata, C.krusei, C.tropicalis*, and *C.parapsilosis* are found in more than 90% of cases of invasive candidiasis (29). *Candida* detected in the bloodstream (candidaemia) is the commonest type of invasive candidiasis, with haematological spread then leading to deep-seated infection at end-organ sites (29). The non-specific nature of this end-organ damage and the normal commensal nature of *Candida* species makes diagnosis more difficult (30). Diagnosis is usually reliant upon microbiological cultures or histology from normally sterile sites which often takes a significant amount of time but polymerase chain reaction (PCR) detection in blood is being increasingly utilised alongside other serological markers (29).

The mortality of invasive candidiasis is usually high (30-60%) but is dependent upon the underlying issues that led to candidiasis (31,32).

1.4. Pneumocystosis

Pneumocystis pneumonia (PcP), caused by the organism *Pneumocystis jirovecii,* is an opportunistic infection requiring a significant degree of immunocompromise. It remains an infection that is particularly prevalent in individuals that present late with HIV and transplant recipients (33). It features almost exclusively respiratory symptoms such as a dry cough, breathlessness and hypoxia and has classical radiological features of bilateral perihilar ground glass opacifications (34).

The estimated global mortality is around 10-30% but this is regardless of underlying cause and is likely to be skewed by a large proportion of patients with HIV who have a disproportionately low mortality risk if given appropriate anti-retroviral therapy (ART) (35).

1.5. Cryptococcosis

This infection occurs through the inhalation of *Cryptococcus* species, most commonly *Cryptococcus neoformans*, and is mostly associated with a high degree of immunosuppression such as organ transplantation, malignancy or as an acquired immune deficiency syndrome (AIDS)-related opportunistic infection. The advent of antiretroviral

therapy (ART) has significantly decreased the incidence of this rare infection in developed countries, although the incidence in other at-risk populations has not changed (36).

The *Cryptococcus* yeast is capable of direct invasion through the blood-brain barrier and, therefore, the predominant clinical features are neurological in nature such as headache, cranial neuropathies, memory loss and signs of meningeal irritation (37,38). As the organism is most likely to enter the body through the lungs, there can be a number of respiratory findings on investigation such as nodules and cavities. However, these can be entirely asymptomatic, even in those with AIDS-associated cryptococcal meningoencephalitis (36,39).

The mortality in countries with a higher HIV burden and reduced access to ART is extremely high, with estimates of mortality directly related to cryptococcosis of around 70% (40). In developed nations the mortality is significantly lower (13-19%) but there is increasing recognition that mortality related to cryptococcosis is commonly measured within the first three months of diagnosis and longer term outcome data indicates that overall mortality may be as high as 50%; particularly in those that are not adequately virally suppressed (41).

1.6. Mucormycosis

Mucormycosis is caused by infection by pathogens of the taxonomic order Mucorales and requires a high level of immunocompromise. The commonest underlying condition leading to mucormycosis is haematological malignancy. Stem cell transplantation remains the most significant risk factor in developed nations. The incidence of mucormycosis is increasing (42). It is possible that this is an artefact of better diagnostic techniques but is more likely due to an increase in the immunosuppressive treatment burden of more modern therapies. In developing countries, uncontrolled diabetes has emerged as a significant risk factor (43). Corticosteroid use is also an identified risk factor for mucormycosis. However, it is unclear if this is a confounder of worsening diabetes control through raising blood glucose and increasing gluconeogenesis and reducing glycogen storage or through its immunosuppressive qualities (44).

Most infection is through the inhalation of fungal spores although cutaneous infection can also occur (43). As such, the commonest manifestations of Mucormycosis are sinus and

pulmonary disease and feature respiratory and facial or neurological symptoms such as cranial nerve palsies, sinus pain, breathlessness and cough (43). The treatment often requires a multi-modal approach including systemic antifungals as well as surgery to gain microbiological control of this invasive disease. The expected mortality of mucormycosis is high as it is often compounded by multiple other co-morbidities. However, an absolute figure ranges from 20-100% depending on the underlying immunosuppression and the nature and quality of treatment (45,46).

1.7. Investigation of invasive fungal disease – diagnostic tools

1.7.1. Radiology

Chest radiographic findings are usually seen in patients with serious and invasive fungal disease, as the lung is the most common portal of entry of the infection (47). However, their differentiation from other infective agents such as bacteria is not always straightforward.

CT scanning of the thorax is a vital tool for the diagnosis of most serious mould infections and less useful in yeast infections such as by *Candida* or *Cryptococcus*. Here, pulmonary features are present in the form of nodules but this is in the clinical presence of overwhelming critical illness with neurological features and other diagnostic tools are more likely to achieve the diagnosis (48).

CT scanning is commonplace and widely available in secondary care. However, access to the expertise required to interpret these scans effectively is more variable. Many of the diagnostic features seen in invasive disease have a good sensitivity but low specificity such as consolidation, cavities and ground-glass opacifications and so proficiency in collating these signs in the at-risk patient is key (43,47,49). Whilst radiology interpretation is a key component of making the diagnosis, making the diagnoses in a multi-disciplinary team (MDT) manner allows for clinical correlation that would otherwise be unavailable to the reporting radiologist.

1.7.2. Microbiological culture and histology

Histological demonstration of the invasion of *Aspergillus* hyphae is an effective method of diagnosing IA(50). Hyphal growth is a characteristic of all mould infections in tissues; hyphae

are not produced as much as they are part of the fungal lifecycle and are demonstration of fungal growth and replication. Hyphae are found in other fungi organisms beside *Aspergillus* species, so histology is used alongside culture to definitively identify *Aspergillus* as the causative organism rather than other hyphae producing fungi such as *Scedosporium* and *Fusarium* species (51). Similarly, microbiological culture of *Candida* species from otherwise sterile sites in an at-risk individual remains the gold-standard criteria for the diagnosis of invasive candidiasis (52). However, the sensitivity of this test is low and the ability to obtain samples in this unwell cohort may be prohibitive (29). As such, it is often more appropriate to rely upon a combination of serological, microbiological, biochemical, radiological and clinical evidence.

In contrast, *Cryptococcus* species are able to be cultured from a variety of biological specimens including blood and cerebral spinal fluid (CSF). However, the time required for culture, especially in those already on antifungal treatment and the lower sensitivity for *Cryptococcus* in blood culture makes this technique unsuitably slow and has largely been replaced by serology techniques (53).

1.7.3. Serology and Molecular testing

A major disadvantage of fungal culture is the inability to separate colonisation from infection, particularly in the case of samples obtained from the respiratory tract which has a high burden of commensal microorganisms (54,55). Therefore, the culture may only be positive when there is a high degree of burden i.e. a later stage of disease, which is unhelpful in attempting to target these patients early to reduce mortality. Despite this, the source of respiratory sampling is important as the presence of *Aspergillus* in a deep respiratory sample obtained at bronchoscopy is more likely to be pathologically significant than a sputum sample (20). The use of serological tests for markers of fungal cell walls, fungal deoxyribonucleic acid (DNA) and measures of specific immunology have therefore been developed.

Molecular detection techniques such as polymerase chain reaction (PCR) are more sensitive than culture. PCR has the added benefit of demonstrating a signal strength through the interpretation of the PCR cycle threshold required to reach positivity. A lower cycle number indicates a likely higher burden of the organism and greater chance of infection rather than

colonisation (20). In the case of PcP, PCR of bronchoaleveolar lavage (BAL) fluid is considered the gold standard and maintains a high degree of sensitivity and specificity (56,57). However, the difficulty obtaining this fluid means that testing other sites such as sputum, oropharynx or serum is often attempted (34).

Galactomannan (GM) is a polysaccharide present in the cell walls of *Aspergillus* species. It is released during infection and is detectable in blood and respiratory samples in patients suffering from IA (58). However, it is less sensitive in the serum of non-neutropenic patients as it is quickly cleared systemically but its value in specificity is high (58). It remains an important tool in the screening of severely neutropenic patients such as transplant recipients (59). Interpretation should be used with caution as there is evidence of false-positivity in concurrent use of antibiotics such as beta-lactams when used intravenously due to the presence of GM in the manufacturing process of these antibiotics and therefore confirmatory PCR testing is often required in order to increase operator confidence in interpreting these tests (59). In CPA, the presence of cell wall markers such as GM demonstrates low sensitivity and invasive procedures are intolerable or risky in this vulnerable cohort. Antibody response, namely *Aspergillus*-specific IgG, is a key diagnostic tool with superior sensitivity and specificity than GM (60).

(1–3)-β-D-Glucan is another cell wall polysaccharide that is detectable in patients suffering from disseminated fungal disease such as IA. It is also found in the cellulose matrix of dialysis filters as well as the filters used in the preparation of blood products and in the manufacturing process of a number of antibiotics (61). This can lead to false-positive results as this constellation of clinical possibilities is also reflective of the sort of patient that is at higher risk of serious fungal disease. (1–3)-β-D-Glucan is found in the cell wall of *Candida* yeasts and so is a useful tool in the diagnosis of candidaemia (62,63). Its presence as a compound in other organisms such as *Aspergillus* and *Pneumocystis*, which would also be within the frame of clinical suspicion in these patients, makes it sensitive but less specific. However, its high negative predictive value for all of these organisms described still makes it helpful, particularly when used in combination with the less sensitive but more specific GM (64). Crucially, it is not seen in the cell wall of *Rhizopus* organisms causing mucormycosis and so a negative (1–3)-β-D-Glucan test does not confidently rule out all invasive fungal diseases.

Serum IgE to *Aspergillus* is a readily available tool for the diagnosis of ABPA (65). Whilst this is a key investigation, the cross-reactivity between crude fungal extracts other than *Aspergillus* makes the diagnosis of allergic syndromes to other fungi challenging (66).

The cryptococcal polysaccharide capsular antigen (CrAg) is shed during cryptococcal infection and can be used for serological testing. It demonstrates high sensitivity and specificity of >98% for serum and CSF and can be performed in under an hour with minimal equipment (67,68).

1.8. Fundamental concepts explored by this thesis

Understanding the burden of fungal disease in Wales is currently a significant challenge. The incidence of fungal disease is unclear and so there is likely to be an undiagnosed cohort. This makes the morbidity and mortality impact of fungal disease in Wales largely undocumented. There is also an unmet need for the provision of a coordinated and funded service for workforce, diagnostics and therapeutics in Wales. The true scope of this practice is yet to be established. The mortality risk associated with many of the conditions described in this thesis is high and this is strongly associated with delay to diagnosis and treatment. As such, it is essential that, in the presence of clinical suspicion, diagnostic tests can be undertaken swiftly and reliably. However, as described above, obtaining relevant samples in a safe and acceptable manner in these unwell patients is a considerable contributor to the delay to diagnosis.

This thesis will address some of these issues through a number of chapters separated into three parts:

Chapters 2 and 3: Estimating the incidence and prevalence of serious or invasive fungal disease in Wales

Using literature-derived incidences of various fungal pathogens and laboratory reporting mechanisms the following questions will be explored:

- Can the numerical burden of cases of serious fungal disease by various important pathogens be estimated?

- If this estimation does not correlate with laboratory reporting schemes, can the aetiology of this difference be identified?
- Can at risk groups be identified in order to better develop diagnostic strategies in Wales?

Chapters 4 and 5: New diagnostic approaches to fungal disease

Given the variability of diagnostics, one area to explore was the availability of new options for obtaining useable samples. An interventional feasibility study, SPITFIRE (SPutum Induction Trial For Improved Respiratory Evaluation) was undertaken in order to address the following questions:

- Is an induced sputum procedure a safe and acceptable (to both patients and medical professionals) method of obtaining deep respiratory samples?
- What is the microbiological correlation between induced sputum and the current standard of care?

Chapters 6 and 7: Discovering new impacts of fungal disease

Once organisms are identified through diagnostic processes, their role as pathogen or commensal is not always clear. In the complex microorganism milieu of the cystic fibrosis lung, fungi are often disregarded as either contaminants or harmless colonisers of the respiratory tract, with the possible exception of *Aspergillus* species (69). This leads to a poor understanding of the mycobiota of the respiratory tract and the opportunistic organisms that are potentially pathogenic. An example of this is the fungus *Exophiala dermatitidis* which is being identified with microbiological techniques with increasing frequency. This thesis will also explore the following:

- In cystic fibrosis, are ubiquitous organisms such as *Exophiala dermatitidis* pathogenic?
- Can the potential for person-person transmission be identified in *Exophiala dermatitidis* by combining results reporting records and hospital attendance records?

Chapter 2 – Estimating the annual burden of invasive and serious fungal disease in Wales

2.1. Introduction

While the significance and impact of fungal infections are increasingly recognised by health organisations, cases of infection are usually not considered as reportable (notifiable) diseases. This limits understanding of the burden and consequences of fungal disease and subsequent interventions required to manage these conditions. Currently, no unifying registry or database of significant fungal disease exists in the UK. This complicates our understanding of the need for service provision, workforce planning and the assessment of success of measures such as antifungal stewardship and the requirement for prophylaxis in existing and novel "at-risk" clinical cohorts.

The following work estimates the expected numbers of patients being diagnosed annually in Wales with invasive or chronic fungal infections requiring substantial clinical involvement and that cannot be managed by purchase of "over the counter" antifungal medication (i.e. for superficial fungal infections). In fungal disease complicating asthma, it was more appropriate to estimate the prevalence of this chronic condition as rates of new diagnosis could not be found. However, there was information available as to the expected percentages of asthma populations living with these fungal disease.

Estimates of the burden of fungal disease in the UK have been made previously in research by Pegorie et al.(70). This group identified the significant occurring infections and utilised up to date literature to report the incidence of these conditions either per risk group or to a whole national population. They then used national databases and other reporting measures to apply these incidences to estimate the burden of fungal disease in the UK. They estimated 382-1,049 cases per 100,000 population. As a first national look at fungal disease of its kind, it highlighted the need for more national monitoring or reporting. However, a criticism of the structure of the research was that the heterogeneity of the populations of the UK, the variable availability of the underlying population databases and the often outdated or contradicting incidence figures from the literature led to large ranges being introduced into the final estimates to cope with the expected error.

While it would be straightforward and convenient to derive data directly from the UK estimates according to Welsh population figures, it was anticipated that rates in Wales could be unduly impacted by various social, clinical, demographic, environmental and meteorological factors specific to Wales. For example, post-transplant immunosuppression is seen as a key risk factor for the development of fungal disease. Whilst transplants are undertaken in Wales, they are more limited in number than in England and so are likely to reflect a difference in local risk for those inpatients.

2.2. Methods

Patient populations at risk of invasive, chronic and allergic fungal disease were identified as previously described in the prior literature (70). Figures for at-risk populations were taken from databases available prior to the COVID-19 pandemic i.e. beginning of 2017 to the end of 2019. Fungal disease occurs frequently in critically ill, mechanically ventilated COVID-19 patients (71). However, with the reduction in requirement of ventilation for COVID-19 following the uptake of the vaccination program and the advent of antiviral strategies, the true current and projectable burden of this phenomenon is unclear. Given this uncertainty, it was discounted from the methodology of this thesis. The databases used included, but were not limited to, the HIV Public Health Report, Annual Transplant Report, local stem cell transplant reports, regional cancer incidence data, National chronic obstructive pulmonary disease (COPD) audit, National Intensive Care audit, British Lung Foundation reports, Secure Anonymised Information Linkage (SAIL), Asthma UK, All Wales Cystic Fibrosis Centre database, Wales tuberculosis (TB) report and the Wales dialysis report. Where populations are steadily increasing e.g. in the case of year on year transplantation numbers, the most recent relevant pre-COVID figure was found. Where populations are largely static e.g. stem cell transplantation, a three year average was calculated.

Where possible, more up to date incidence data was included per infection using local laboratory database queries and published articles.

2.2.1. Invasive Aspergillosis

Some of the populations identified as high-risk for invasive aspergillosis have reliable monitoring systems in place in Wales; Critical Care departments and transplant units have

routine screening for fungal biomarkers. This provides accurate baseline numbers and comprehensive databases exist for certain populations (e.g. Stem cell transplantation). Given the highest-risk population to be those within the first 12 months of transplant, a mean of those transplanted between 2017-2019 was taken. For the other key malignancies of Non-Hodgkin lymphoma (NHL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), multiple myeloma (MM) and lung cancer, annual data held by the Welsh Cancer Intelligence and Surveillance Unit was used to determine a mean number of cases per malignancy over the three year period 2016-2018.

Incidence rates of IA in these patients were taken from the prior estimations (72,73). This included an incidence rate of 8.1% in allogeneic stem cell transplants and between 0.2% and 3.8% for other haematological malignancies. However, Wales-specific data exists, demonstrating a rate of proven or probable aspergillosis of 9.7% in Welsh haematology patients undergoing chemotherapy or stem cell transplant treatment (74,75). This rate of 9.7% was applied to all allogeneic transplant patients and acute leukaemias as a reflection of those that are most likely to be neutropenic or receive neutropenia-inducing chemotherapy. This was considered the upper limit of likely diagnosis and the contemporaneous rate, as documented in the literature, considered a lower limit as this is not treatment dependent. Given the treatment options, there is likely to be a small overlap with the numbers of stem cell transplants and acute leukaemias but given that the annual rate of these remains small, they have been included.

As with PcP, the number of solid organ transplants were derived from annual reports on solid organ transplantation in Wales (76). With the exception of renal transplants, the number of transplants in Wales is not large. For example, in the 2018-2019 transplantation report there were 109 renal transplants and 50 other transplants as a combination of all heart, lung, liver and intestinal transplants. The incidence of IA in this cohort was derived from literature (72). The rates of IA post heart and lung transplant are between 4-5% and 0-1% in liver and kidney transplant.

Prior estimations of fungal burden in the UK identified a study by Yan X et al which suggested an IA incidence of 2.63% in patients with lung cancer (70,77). No significant articles were found for a more up to date incidence. This higher rate suggests a role of underlying respiratory tract disease, lung irradiation and use of corticosteroids (78).

Aspergillosis is an uncommon AIDS-defining illness, occurring with an incidence of 4.4% (79). As with the PcP calculations, a mean of late presenting HIV diagnoses was used.

Understanding how many chronic obstructive pulmonary disease (COPD) admissions there are annually is difficult due to inconsistencies regarding the definition of admission. The National COPD Audit is contributed to by all hospitals in Wales so this was used for the 2018-2019 financial year. The COPD Audit does not take into account Emergency Department (ED) attendances that did not lead to an admission so biases towards the more unwell patient. However, it was the work of Guinea et al that strongly influenced the prior estimates of fungal burden in the UK and they suggested that there is an incidence of 1.3% of those requiring hospital admission but that their underlying characteristics strongly favoured the more severe ends of the COPD phenotypic spectrum; a group which are unlikely to be able to return home immediately after presenting to ED (70,80).

There is a high degree of diagnostic uncertainty in critical care patients and it will likely contain cross-over from pathology groups such as transplant recipients, but also represents a unique cohort. Observational studies often report this group of patients as a mixed patient cohort including those intubated, not intubated and medical high dependency unit patients. In Wales, separating data from medical critical care and non-medical units is not feasible, so all data on all adult critical care admissions as reported to the Intensive Care National Audit & Research Centre (ICNARC) in 2018 was collected. This excluded paediatric cases. Where the same patient was admitted more than once to critical care, they were only counted once. Prior estimations of fungal burden in the UK undertook a sensitivity analysis for estimation of burden of invasive aspergillosis amongst patients in critical care and, in particular, the works of Garnacho-Montero et al and Vandewoude et al were highlighted (54,70,81). These studies into a pooled critical care cohort suggested incidence rates of IA between 0.33-1.1%. Since the publication of UK-wide estimations of fungal disease, articles have been published looking specifically at IA in the intubated patient showing signs of ventilator-associated pneumonia (VAP); demonstrating that the incidence is far higher than previously suggested, with IA complicating VAP in 12.4% of those with evidence of probable VAP (82). This was diagnosed using a definition that comprised of clinical, radiological and mycological criteria that included positive histology or microscopy, positive BAL fluid culture, positive galactomannan in BAL fluid or in serum. The data from ICNARC was

interrogated to understand the number of patient-days in which level 3 care (requiring mechanical ventilation) was required. The diagnosis of ventilator associated pneumonia is somewhat contentious, with a variety of clinical and chemical scoring mechanisms available (83,84). A pooled European rate of ventilator-associated pneumonia in 18.3 episodes per 1,000 patient-days was used from the CAP/VAP study (84).

2.2.2. Chronic pulmonary aspergillosis (CPA)

Patients with CPA are poorly identified and underdiagnosed. CPA complicates a number of respiratory conditions such as bronchiectasis or sarcoidosis which share respiratory symptoms as well as the potential for multisystem symptomatology such as fatigue ,thereby potentially leading to diagnostic uncertainty. As such two approaches were used to determine the burden of disease. Initially, two at-risk populations were identified; sarcoidosis and pulmonary tuberculosis (TB). Sarcoidosis is a systemic granulomatous disease with a heterogenous and variable clinical course. Around 20% of patients are chronically progressive and develop pulmonary fibrosis although many are likely to suffer multisystem disorders as the sarcoidosis commonly affects organ systems outside of the respiratory tract (85). Rates of sarcoidosis in Wales were identified from British Lung Foundation documentation (86). Identifying an incidence of chronic pulmonary aspergillosis in the entirety of the group is unclear when the group is pooled by severity as the underlying sarcoidosis can be largely innocuous at an early stage and it is only at later fibro-cavitatory disease does the patient become more at risk. An estimation aspergillosis complicating 6% of sarcoidosis was available (87).

In TB, it is evident that the risk of pulmonary aspergillosis is different depending on the extent of the underlying structural lung damage following TB infection. Estimates from the literature indicate CPA complicates TB in 22% of those with cavities of 2.5 cm or greater and 2% in those without a residual cavity (88). The 2019 Wales TB report was used to identify the at risk population and a rate of residual cavitation after pulmonary TB of 12% was assumed using a synthesis of the literature available (70,89,90).

Following this, a laboratory derived approach was employed, based on *Aspergillus*-specific immunoglobulin G (IgG) testing, which has a high specificity (>95%) for the diagnosis of chronic aspergillosis, irrespective of the method used (91). The results of all tests performed

between 2015-2018 were scrutinised. All negative tests were removed. Patients with more than one result in the sample set were only counted once, utilizing their highest antibody concentration. As CPA is a chronic condition with mortality rates at 32% and 45%, one and five years post-diagnosis, patients are likely to demonstrate positive results over a long period of time leading to a cumulative annual increase in burden (92). By eliminating any patient replication each result reflects a unique positive patient but, being a chronic condition with an uncertain cure rate, it cannot be assumed that all of these patients are new to the health service with this diagnosis. For the *Aspergillus*-specific IgG antibody assay used across Wales (BioRad *Aspergillus* IgG) local data indicated an antibody concentration of ≥35 arbitrary units (AU)/ml is highly specific (>95%) and should be considered to be a true positive (L. White, Personal Communication, May 2022). Patients with an antibody concentration of 20-35 arbitrary units per millilitre (AU/ml) were considered to be possibly positive for CPA, serving as an upper range of possible annual new cases.

2.2.3. Allergic Bronchopulmonary Aspergillosis (ABPA)

Data from health economy databases in Wales such as the primary care-oriented Secure Anonymised Information Linkage (SAIL) and Asthma UK provided an estimate for the total asthma population in Wales. No defined prevalence data exists for ABPA in asthma but a previous estimation of 2.5% of those with a diagnosis of asthma was used in prior UK-wide estimations as a synthesis of multiple studies looking at secondary care referral rates with ABPA complicating asthma (70).

A review of the patients at the All-Wales Adult CF centre was performed to identify cases of ABPA as diagnosed by their treating clinical team.

Cases of ABPA in the paediatric CF population were identified in the annual CF report (2019) that also provided a mean ABPA rate in this cohort (93). For the purposes of this work, it was deemed unnecessary to estimate paediatric ABPA rates in the non-CF cohort, as this appears to be a rare clinical entity.

2.2.4. Severe asthma with fungal sensitisation (SAFS)

Estimating the rates of severe asthma with fungal sensitisation is more difficult than ABPA, as there is likely to be some overlap in the clinical presentation of those diagnosed with

severe asthma and ABPA. Regardless, these two airways diseases likely exist in the same space as those with severe asthma. Prior estimations as to UK wide burden of fungal disease utilised a sensitivity analysis and a synthesis of the literature to assume a 5% frequency of asthma as severe (70). This 5% was used for the Wales-specific estimations and included treatment-refractory, steroid dependent and poorly compliant patients. There appears to be an increased frequency of fungal sensitisation in those with poor asthma control. There is a prevalence range in the literature of 25% of those with severe asthma requiring secondary care referral with up to 75% of those requiring repeated hospital admissions (94–96). The synthesis of Pegorie et al was to assume 60% of these severe adult patients were assumed to have an element of fungal sensitisation (70). This estimation was not extended to children due to its rarity.

2.2.5. Invasive candidiasis

Candidiasis is subject to a voluntary national laboratory reporting scheme. This report was used to identify the number of culture-proven cases of candidiasis in Wales per 100,000 population (97). The reporting scheme uses blood culture positivity. However, there is recognition that the culture positivity rate in the laboratory of proven invasive candidiasis is low at 38% (CI: 29-46%) and that more contemporary techniques such as PCR have greater diagnostic accuracy but were not available for this analysis as they were not included in the report or compiled in Wales national databases (98).

Candida peritonitis as a complication of surgery will likely be incorporated in the candidaemia cohort so are not counted here.. A small number of patients that experience localised infection and not develop candidaemia will be missed by this methodology but the number is assumed to be small.

The total number of patients undergoing continuous abdominal peritoneal dialysis (CAPD) was derived from a national database (99). The estimated number of episodes per patient year attributable to *Candida* in this patient group was previously 0.05 (70). However, newer estimations puts this number of episodes at 0.02 (100) which act as the lower and higher ends of the prediction respectively.

2.2.6. Pneumocystis pneumonia (PcP)

Prior estimations for incidence of pneumocystosis utilised solid organ transplantation rates and patients with AIDS at first presentation of HIV. This was coupled with death certificate data to derive an estimation. This did not include stem cell transplantation or other modality of immunosuppression.

The 2017, 2018 and 2019 HIV Public Health report for Wales was used to identify an approximate population of HIV patients with AIDS at diagnosis (101). The HIV transmission report for the same years was used and suggested that *Pneumocystis* was the diagnosed organism in 37% of the AIDS diagnoses in 2018 (102).

The risk of PcP in the solid organ transplant population is variable and uncertain. It is likely to be related to degree of graft versus host disease and compliance with PcP prophylaxis. Annual reports for solid organ transplantation in Wales were used (76). The most recent (pre COVID) transplantation activity report was chosen as the rates of transplant in Wales are steadily increasing, so an average utilising previous years was not felt to be representative of current rates. The transplantation report uses the financial year model so the 2019-2020 report would have been impacted by COVID therefore the 2018-2019 report was used. Rates of kidney, heart, lung and liver transplantation was used. Pancreatic transplants could not be included as it is not clear in the annual reports how many are occurring as they are often combined with renal transplants.

The rate of stem cell transplantation in Wales is well documented by the tertiary haematology centre. The mean number of transplants between 2017-2019 was identified and separated by allogeneic and autologous. Studies identified in the literature suggested incidence rates of PcP of up to 2.5% in allogeneic and 1.4% in autologous stem cell transplants; predominantly occurring within the first year post-transplant (103,104).

To confirm the accuracy of the estimated rates of PcP and to identify cases of PcP related to other, less well defined, risk factors for PcP such as steroid use in pulmonary fibrosis or biologics therapy in rheumatoid disease; a second dataset based on laboratory derived data was compiled. Public Health Wales Mycology laboratory data was interrogated for PcP PCR positive results between 1st January 2015 and 31st December 2018. Positive results were

analysed and true positive results in unique patients were identified. The methodology behind this technique is further explored in Chapter 3.

2.2.7. Cryptococcosis

Two techniques were used to estimate case numbers of cryptococcal disease. Two significant at-risk populations were identified; HIV and solid organ transplantation. The number of new HIV patients with AIDS at diagnosis were identified and documented incidence was used to calculate an expected incidence in Wales. Prior UK estimates makes reference to a paper by Patel et al which identified an incidence of cryptococcal meningitis of 5% (105). Evaluating a cohort of HIV patients in the UK, this paper had a small sample size. More recently in Spain, a larger cohort was evaluated suggesting the incidence may be as high as 7.5% in those with low CD4 counts (106). The figure used here was 7.5% as the maximal likely incidence as cryptococcal disease does not appear in the top four most commonly diagnosed AIDS defining illness according to the 2019 Public Health England HIV report (102). This same report states that pneumocystis pneumonia was the more commonly diagnosed AIDS-defining illness, accounting for 37% of AIDS diagnoses. This was followed by candidiasis (22%), tuberculosis (8%) and Kaposi's sarcoma as the 4th most common diagnosis at 8% of this population(73). Therefore, cryptococcal disease will be less than 8%. A US paper has defined the risk of cryptococcosis in solid organ transplant as a rare but important cause of death in the first year after transplantation with a pooled risk in solid organ transplants of 0.37% (107).

The second approach used Public Health Wales mycology reference laboratory-derived data for cryptococcal antigen testing between 2015-2018. Given the excellent performance of the cryptococcal antigen lateral flow assay (sensitivity and specificity (97.7-100%) it was reasonable to presume that positive results were indicative of cryptococcal infection (68).

2.2.8. Mucormycosis

The difficulty in establishing a diagnosis of mucormycosis hinders our understanding of the burden of this infection. Increasing numbers of patients are at risk of this infection (e.g. those with risk factors such as uncontrolled diabetes, chronic renal failure, haematological malignancy and transplant recipients) but there is only limited understanding of incidence.

To estimate burdens in Wales the population incidence generated in the French population of 0.09 per 100,000 per year, averaged over 10 years, was applied (108).

2.2.9. Other Rare Fungal infections

There are a small number of rare fungal infections that are unlikely to be encapsulated by this data review. The Public Health Wales (PHW) Mycology Reference Laboratory was asked to comment on annual cases of endemic fungi such as histoplasmosis. Rarer fungal pathogens such as *Scedosporium* species can complicate the health of patients with cystic fibrosis and the All Wales Adult Cystic Fibrosis unit's database was reviewed to establish an annual incidence. An estimate of 0.04 cases of rare fungal infections per 100,000 was used in the literature and this was applied to the Welsh population (70).

2.3. Results

2.3.1. Invasive aspergillosis (IA)

For 2017-2019, a mean of 52 patients underwent allogeneic stem cell transplantation (SCT) and in 2016-2018 there was a mean of 146 acute myeloid leukaemia (AML) patients and 31 acute lymphoblastic leukaemia (ALL) patients. When using documented incidence rates of IA there is a projected incidence of 15 cases annually (72–75). When the Wales-specific rate for neutropenic patients with haematological malignancy is used there is an incidence of 22 annually which forms a higher end of disease burden. This is illustrated in table 2.

Invasive aspergillosis – neutropenic patients with haematological malignancy				
Risk Group	Risk population in Wales, N[i]	Incidence from literature, %	Wales-specific incidence, %	Yearly number of cases (literature incidence to Wales-specific incidence), N
Allogeneic stem cell transplant	52	8.1	9.7	4-5
Acute myeloid leukaemia	146	7.1	9.7	10-14
Acute lymphoblastic leukaemia	31	3.8	9.7	1-3
Total	229			15-22

Table 2: Estimated annual burden of invasive aspergillosis in patients with haematological malignancy causing neutropenia or undergoing treatment causing neutropenia

[i] Number

The incidence of autologous stem cell transplants and other haematological malignancies including Non-Hodgkin lymphoma (NHL), Hodgkin's lymphoma (HL), CML, CLL and multiple myeloma were assessed from prior literature (72,73). Their populations are variable but have an annual incidence of invasive aspergillosis of <1% each. It was not appropriate to apply the Wales-specific incidence rate as there was no information as to how many received chemotherapy. The expected numbers of IA in this group of other haematological malignancies is 5-10.

The expected number annually for the pooled solid organ transplant group is 0-4.

The expected number of IA in new HIV annually is 0-1.

There was a mean of 2,512 lung cancer diagnoses per annum in Wales in 2016-2018 inclusive. With an expected incidence of 2.63%, 66 cases per year are predicted.

These are demonstrated in table 3 below.

Invasive aspergillosis – transplant and other at-risk groups			
Risk Group	Risk population in Wales, N[i]	Incidence, %	Yearly number of cases, N
Autologous stem cell transplant	51	0.9	0-1
Heart transplant	7	4.8	0-1
Lung transplant	8	4.1	0-1
Liver transplant	35	0.8	0-1
Kidney transplant	109	0.3	0-1
AIDS[ii]	10	4.4	0-1
CML[iii]	33	2.3	0-1
CLL[iv]	153	0.5	0-1
NHL[v]	642	0.8	5
HL[vi]	105	0.4	0-1
Myeloma	264	0.2	0-1
Lung cancer	2,512	2.63	66
Total			71-81

Table 3: Estimated annual burden of invasive aspergillosis in the transplant and haematology groups

[i] Number
[ii] Acquired immune deficiency syndrome
[iii] Chronic myeloid leukaemia
[iv] Chronic lymphocytic leukaemia
[v] Non-Hodgkin- lymphoma
[vi] Hodgkin's lymphoma

The National COPD audit suggests there are 7,805 admissions in Wales. As with prior UK estimates of fungal disease prevalence, the assumption is made that that up to 25% of admissions will be double-counted patients with recurrent admissions (70). Given that the IA incidence rate is 1.3% it is assumed that these patients generate an incidence of 76-101 cases of IA annually.

In 2018, 9,479 admissions of 8,646 patients were reported from critical care units across Wales. There is a distinction between levels of treatment offered in Intensive Care Units (ICUs). Level 2 care suggests intensive monitoring and support of single organ failure, whereas level 3 care is used to describe patients that require multiple organ support or advanced respiratory support including intubation and ventilation (109). Utilising the pooled Intensive Care Unit (ICU) statistics (as opposed to the medical ICU only in which there are no level 3 patients), this gives an approximate expected incidence of 28-95 cases. There were 30,069 level 3 bed-days in 2018. As per the conclusions of the EU VAP/CAP study, there is an expectation of 550 episodes of ventilator associated pneumonia (VAP) (18.3 episodes per 1,000 ventilator days). If 12.4% of these are associated with aspergillosis, 62 cases would be expected annually. This sits within the range estimated of 28-95, therefore 28-95 is considered to be a fair upper and lower figure. However, it is assumed that 50% of these cases are likely to be double counted as COPD patients or transplant recipients so the adjusted rate is 14-48 cases annually, dependent on level of care.

Invasive aspergillosis (IA) – Respiratory and Critical Care				
Risk Group	Risk population in Wales, N[i]	Incidence, %	Yearly potential population, N	Yearly burden of disease minus double counting, N
COPD[ii] admissions	7,805	1.3	101	76-101
Critical Care (all patients)	8,646	0.33-1.1	28-95	14-48
Critical Care (level 3 only[iii])	550	12.4	62	31

Table 4: Estimated annual burden of IA in Wales in respiratory and critical care groups

[i] Number
[ii] Chronic obstructive pulmonary disease
[iii] Intubated and ventilated patients

2.3.2. Chronic pulmonary aspergillosis

Based on British Lung Foundation estimates of 7 cases per 100,000, (irrespective of locality) 222 pts in Wales will be diagnosed with sarcoidosis annually (86). Assuming rates of chronic pulmonary aspergillosis (CPA) in this condition to be 6%, this generates an estimate of 13 cases of CPA per annum.

The 2019 Wales TB report describes 97 new diagnoses of tuberculosis. In the absence of UK data, a rate of 12% residual cavitation was assumed as per prior estimations (70). CPA complicates TB in 22% of those with cavities and 2% in those without a residual cavity; between 3-5 cases of CPA could therefore be expected annually in Wales (88).

Prior UK estimates for fungal disease approximates that these two conditions account for 30% of total annual CPA incidence so the estimated 16-18 becomes 53-60 cases per year.

In the second methodology of determining annual incidence, the laboratory data request yielded 1,203 positive *Aspergillus* IgG results over four years. Once duplicate patients were removed, this equated to 277 unique patients with an *Aspergillus* concentration of >35 and 386 with a concentration of >20 in total. This is a mean of 69-97 CPA cases being detected per year. A French prevalence study found the one year mortality of CPA to be 32% (92).This is applied to this estimate to update this estimate to 47-66 which is similar to, and encompasses, the range found using the first method. It is not possible to identify how many of these patients are new patients as the cure rate is not established alongside the mortality. As many patients are asymptomatic at the early stages of this disease, 47-66 is likely to represent an overestimate as to the severe end of this disease spectrum. This is wider than the extrapolations based on incidence of underlying conditions and so is utilised for the pooled estimations.

2.3.3. Allergic bronchopulmonary aspergillosis (ABPA)

There is an approximate population of 255,000 patients with asthma in Wales (110). Using published literature, an assumed rate of 2.5% of patients with asthma have ABPA generating an ABPA population of 6,375.

Sixty-nine of the 340 patients (19%) in the All Wales Adult CF centre had a diagnosis of ABPA. In addition, for patients under the age of 16, the annual CF report (2019) suggests an average ABPA rate of 3.5%, with 175 registered paediatric CF patients six cases of ABPA would be expected.

2.3.4. Severe asthma with fungal sensitisation (SAFS)

An estimated 255,000 people in Wales have asthma (110). If 3.6-10% have severe asthma and 60% of these severe patients have a degree of fungal sensitivity then there is an estimated prevalence of SAFS in Wales of between 5,508 and 15,300 patients. This is illustrated below in figure 2.

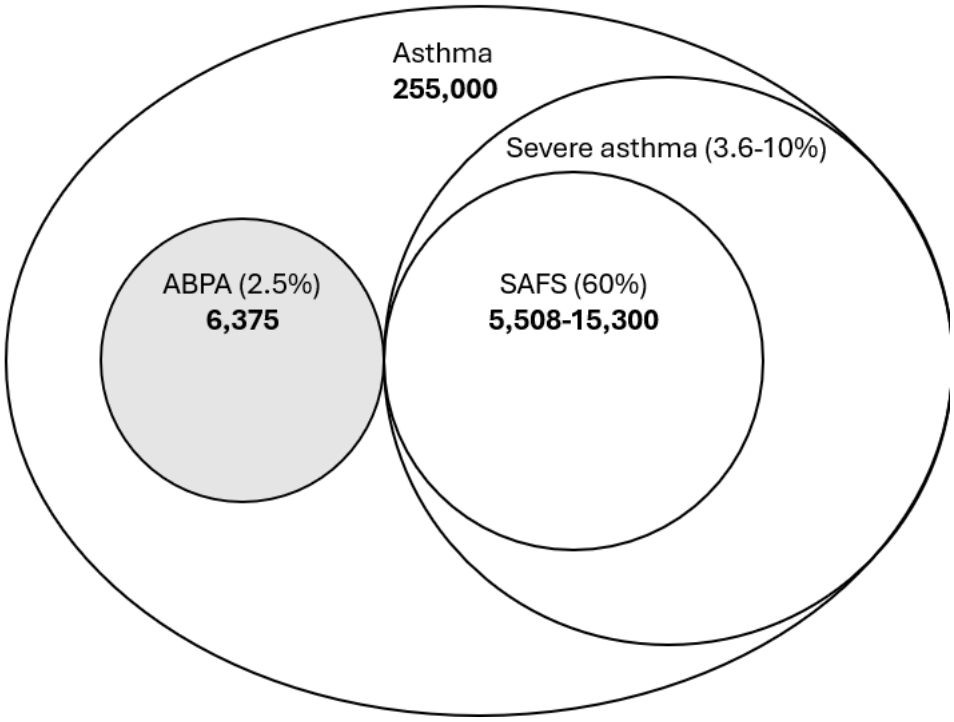


Figure 2: An illustration of the relationship between allergic bronchopulmonary aspergillosis, asthma and severe asthma with fungal sensitisation. Circles are not to scale.

However, there is likely to be a double-counting effect here as some patients who are sensitised to *Aspergillus* species may meet the criteria for both ABPA and SAFS. The degree of crossover is uncertain but a similar range as demonstrated in the UK-wide fungal burden estimation was used in which the overlap was estimated to vary from 20-50%. For this estimate to be made, a severe asthma prevalence of 5% was assumed. Applying this criteria

and assumption generates a prevalence estimate of those with SAFS of 7,013-11,220. Figure 3 below demonstrates the effect of this crossover and it is totalled in table 5.

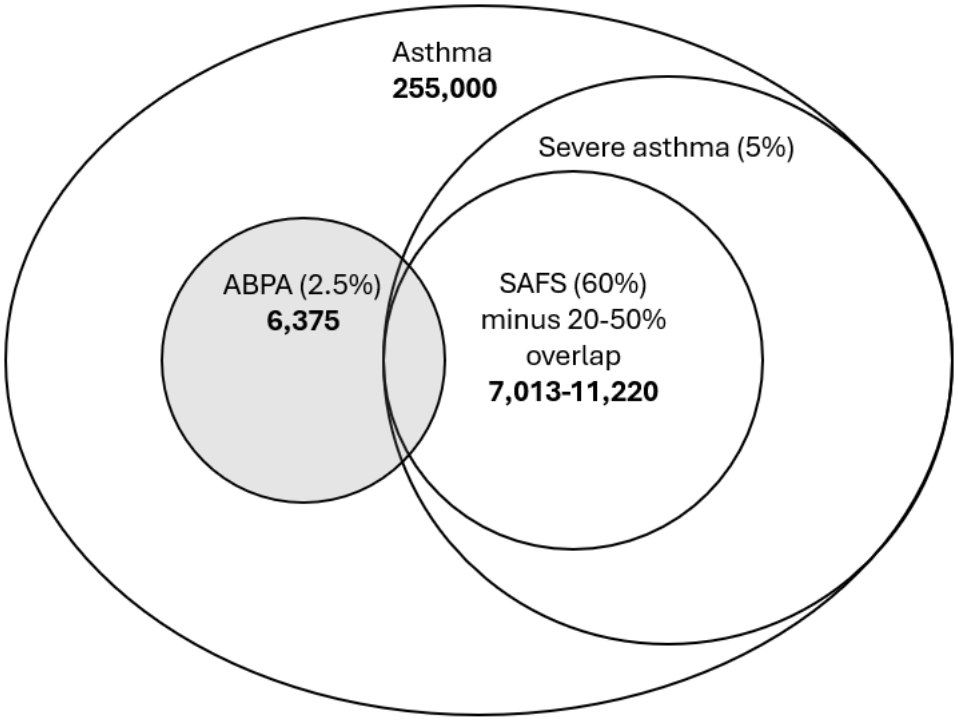


Figure 3: An illustration of the likely burden of severe asthma with fungal sensitisation in Wales if there is a variable overlap between this and allergic bronchopulmonary aspergillosis. Circles are not to scale.

Allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS)		
Condition	Assumptions	**Cases in Wales, N**
Asthma	Wales database	255,000
ABPA prevalence	2.5% of all asthma	6,375
Severe asthma prevalence	3.6% of all asthma	5,508
	5% of all asthma	7,650
	10% of all asthma	15,300
SAFS prevalence	50% overlap between severe asthma and ABPA	7,013
	33% overlap between severe asthma and ABPA	9,397
	20% overlap between severe asthma and ABPA	11,220

Table 5: Number of cases of allergic bronchopulmonary aspergillosis and severe asthma with fungal sensitisation in Wales

2.3.5. Invasive candidiasis

Utilising the 2017 candidaemia reporting scheme, the current rate of cases in Wales is 5.2 per 100,000, generating an estimated 164 candidaemia cases per annum (97). With the diagnosis based only on blood culture positivity and given the sensitivity limitations of such an approach, there could be up to be 357-566 cases of candidaemia or invasive candidiasis expected annually.

In 2021, there were 189 patients in Wales on continuous abdominal peritoneal dialysis (CAPD). If the same rate of 0.05 events per patient year is maintained, there is an expectation of nine cases per year. More up to date literature is used to extrapolate 0.02 events per patient year for an estimated incidence of four. Therefore, 4-9 cases is an expected range.

2.3.6. Pneumocystis pneumonia (PcP)

There were 109 kidney transplants, 7 heart transplants, 8 lung transplants and 35 liver transplants in Wales in the 2018-2019 transplant report. Each would contribute 0-1 expected cases per year. Between 2017-2019, the mean number of stem cell transplants in Wales per annum was 51 for autologous transplants and 52 for allogeneic transplants. This would give an annual incidence of up to 1 case annually for the autologous transplant and 1-2 from the allogeneic.

Over three years a mean of 10 patients per annum presented with AIDS at HIV diagnosis. The annual number of PcP cases is estimated to be 0-4 from solid organ transplants, up to 3 from bone marrow transplants and 3-4 from HIV for a total of 4-11 cases.

Pneumocystosis			
Risk Group	**Annual at-risk population in Wales, N[i]**	**Incidence, %**	**Yearly burden of disease, N**
Heart transplant	7	5.5	0-1
Kidney transplant	109	0.3	0-1
Liver transplant	35	1.15	0-1
Lung transplant or Heart and Lung transplant	8	5.78	0-1
AIDS[ii]	10	37	3-4
Allogeneic stem cell transplant	52	2.5	1-2
Autologous stem cell transplant	51	1.4	0-1
Total			4-11

Table 6: Estimated annual burden of pneumocystosis in Wales, broken down by risk group

[i] Number
[ii] Acquired immune deficiency syndrome

In addition to estimated rates, laboratory testing data in Wales returned 197 PcP PCR positive results between 2015 and 2018 inclusive, excluding duplicates, where the result associated with the highest fungal burden was retained for analysis. During this time, 159 patients were diagnosed, representing 40 cases annually. This number was derived from a combination of cases with a higher fungal burden or those with a lower fungal burden but with additional mycological or clinical evidence supporting a diagnosis of PcP. The methodology and results are further discussed in chapter 3 of this thesis. This is significantly higher than the extrapolated incidence of pneumocystosis identified by risk groups but is also likely to be more accurate as it is based on laboratory testing. Therefore, this will be the number used later in the chapter when identifying incidence for pooled fungal disease in Wales.

2.3.7. Cryptococcosis

The same HIV/AIDS population used in the *Pneumocystis* pneumonia cohort was utilised here. The incidence of cryptococcosis in this cohort is 5-7.5%, resulting in 1-2 cases per annum in Wales. Given a pooled risk in solid organ transplant of 0.37%, the projected incidence in Wales is 0-1 cases per year.

Over four years there was a laboratory yield of 48 CrAg positive results generated from 15 patients, equating to 3-4 cases per year, although this included one year where there were no positive cases. The backgrounds of these patients are not available. As such, the annual rate of cryptococcal meningitis cases is expected to be 0-4.

2.3.8. Mucormycosis

Based on a population incidence of 0.09 per 100,000, the annual expected burden of mucormycosis in Wales will be 2-3 cases.

2.3.9. Rare infections

It has been previously postulated that there are fewer than 25 cases of rare infections not encapsulated by the rest of this list annually in the UK (70). Using a population incidence of 0.04 cases per 100,000, the annual expected burden of rare infections in Wales will be 1-2 cases (70).

From a laboratory perspective, up to one case of rare fungal infection is recorded in Wales by the regional reference laboratory each year and 0-2 rare fungal organisms are isolated each year from the adult cystic fibrosis cohort, supporting the annual estimate.

2.3.10. Final estimations

Prior estimates of the burden of fungal disease in the UK could be extrapolated to gross Welsh population numbers. This would give an expected disease population of 12,109-33,253, or 475-729 (15.0-23.0 per 100,000) if the ABPA and SAFS patients are removed as they are chronic conditions, therefore requiring counting as prevalence rather than incidence.

Wales-specific estimates are detailed in the tables below. Laboratory-driven results are used preferentially over derived incidences as they are more accurate and reflective of real world data. Where two analytical options are available, they are used in conjunction to create an estimated range.

Pooled rates of invasive fungal disease in Wales				
Invasive fungal infection	Data source	At risk population	Number of cases expected Wales, N[i]	Cases per 100,000 Wales, N
Invasive aspergillosis	Extrapolating at-risk populations	Neutropenic haematology patients	15-22	0.47-0.69
		COPD	76-101	2.4-3.19
		Critical care	14-48	0.44-1.51
		All other risk groups	71-81	2.24-2.56
Chronic pulmonary aspergillosis	Retrospective laboratory analysis	All patients	47-66	1.48-2.08
Invasive candidiasis	Extrapolating candidaemia reporting		357-566	11.3-17.9
	CAPD patients		4-9	0.13-0.28
***Pneumocystis* pneumonia**	Retrospective laboratory analysis	All patients	40	1.26
Cryptococcal meningitis	Retrospective laboratory analysis	All patients	0-4	0-0.13
Mucormycosis	Extrapolating population literature	Whole population	2-3	0.06-0.09
Other rare infections	Extrapolating population literature	Whole population	0-2	0-0.06
Total			626-942	19.7-29.7

Table 7: Pooled annual incidence of invasive fungal disease in Wales, separated by data source and risk group where appropriate

[i] Number

Prevalence of allergic bronchopulmonary aspergillosis (ABPA) and severe asthma with fungal sensitisation (SAFS) in Wales			
	Risk group	**Total, N[i]**	**Total per 100,000, N**
ABPA	Asthma	6,375	201
	CF	75	2.37
SAFS	-	7,013-11,220	221-354
Total		13,463-17,670	425-557

Table 8: Estimation of total prevalence of ABPA and SAFS in Wales

[i] Number

2.4. Discussion

There is a degree of variability in the estimates that are able to be produced for Wales. However these estimates are significantly less variable than previously reported for the rest of the UK (70). This is due to improved disease monitoring and reporting and a smaller population with less heterogeneity of healthcare provision. That said, fungal disease remains a difficult entity to diagnose at times and there remains uncertainty as to the true burden of disease. There is a reliance here on use of published incidences derived from literature where there is the assumption that the populations quoted in literature are an exact match for those in Wales. An example where this assumption is particularly visible is using the French population estimate of mucormycosis. It is likely that this is not reflective of Wales's population but the actual impact on the total estimates is likely to be low as it is a rare disease.

This work has used more focussed disease populations and updated incidence data than prior UK estimations to give a more narrow estimate to the burden of disease in Wales. This new estimate has an expected population of 626-937 of patients with serious or invasive fungal disease (the non-asthma related conditions). This is on the higher range of the original extrapolated UK estimate, the cause of which is likely to be multifactorial. One such

driver of this higher estimation is a markedly variable estimation in candidaemia. This is mathematically driven by diagnostic uncertainty around the accuracy of blood culture versus PCR techniques and the extrapolation of poor detection in blood culture. This could be the subject of further work locally to evaluate the laboratory reporting scheme and understand the processes by which samples are collected Wales-wide for testing.

The estimates around pneumocystosis in particular is of interest. Original estimates of fungal burden in the UK suggested that there was an expectation of 207-587 cases per year in the UK (70). Given that Wales's population is maintained at 4.7% of the UK population, this would imply that there is an expected case number of 10-28. Wales has a transplant population that is lower than would be proportionately expected due to the reduced provision of these services and so it is not surprising that the estimated number of cases per year was found to be lower at 4-11. However, looking at retrospective Wales mycology reference laboratory data, there are approximately 40 cases per year, highlighting not only that PcP is under-recognised or suspected but also that less well documented forms of immunosuppression causing PcP infection are more impactful than recognised. It is also likely to be rising as forms of immunosuppression such as biologics are in more widespread use across a variety of conditions (111–113). This implies an inappropriately low index of suspicion for this infection when it is outside the more well documented cohort of HIV and transplant therefore the true incidence of this important infection may be higher still but remain undetected. This interest was developed further through the work done in Chapter 3; where a deeper exploration into the underlying cause and associated mortality was reviewed.

The estimated rates of aspergillosis remain very wide. There is increased appreciation that chronic pulmonary aspergillosis can be seen in a number of chronic conditions and may be responsible for patient increasing morbidity, if not mortality, but the true numbers remain elusive. The rates of *Aspergillus*-specific IgG positivity being significantly higher than the projected figures suggest that there is a large undiagnosed population, but this is entirely reliant on the clinician having the clinical suspicion to request the test and that IgG positivity correlates completely with active disease. Further work identifying the underlying pathologies in the cases of those that are testing positive may go some way to identifying at risk groups.

In invasive aspergillosis, the greatest threat to patients remains treatment or pathology induced neutropenia and it is uncertain whether this rate is maintained with more up to date treatment or strategies which may not render a patient neutropenic Examples include the rise of biologic therapy or altered treatment strategies such as reduced induction chemotherapy pre-transplantation. It is also uncertain as to which of those that are diagnosed with these at-risk conditions went onto receive treatment. In the case of solid organ transplantation in particular, the incidence of IA is likely related to both level of immunosuppression required and any underlying condition preceding transplantation that caused immunological dysfunction. This explains the high incidence in the literature of IA in heart transplantation versus kidney for example and also impacts on the relative reduction in IA in Wales in this cohort as fewer heart transplants are taking place than kidney transplants in this region (76).

There is an argument that, with the invention and significant use of biologics in asthma patients, the outcomes of patients with SAFS and ABPA remains similar regardless of overall diagnosis. As such, there is even less need to record or track the outcomes of these patients. However, accounting for the influence of the complex asthma patients, there remains a large number of patients throughout Wales annually that are not provided for with a unified diagnostic regimen, access to expertise and bespoke recommended treatment pathways.

2.5. Conclusion

An estimate for the number of cases of invasive or serious fungal diseases in Wales can be made. In several cases, when broken down per disease, it is higher than the previous estimates made per 100,000 for all of the UK. It is not completely apparent why this is the case but possible explanations include Wales-specific socio-economic dynamics, variability in at-risk populations, more recent literature describing the incidence and the possibility of novel at-risk populations. Novel at-risk populations and their associated mortality will be further assessed in the PcP cohort in the subsequent chapter through the utilisation of centralised Welsh laboratory data.

Chapter 3 – Pneumocystosis in Wales; a retrospective review of burden, mortality and associated immunocompromised populations

Adapted from publication in Journal of Fungi, June 2023:

Ayling-Smith J., Backx M., Grant E., Dhillon R., Duckers J., Hood K., White P.L. Gaining an Understanding of Pneumocystosis in Wales. J. Fungi. 2023;9:660. doi: 10.3390/jof9060660

3.1. Introduction

Pneumocystis pneumonia (PcP) is a serious infection with a significant mortality rate of 10-30% (114,115). Its ongoing importance as a global public health concern warranted its inclusion in the 2022 World Health Organisation fungal priority pathogen list (116). Within the UK, the incidence of this condition is poorly understood due to a lack of active surveillance, the fact that fungal diseases, in general, are not notifiable and the diagnosis of PcP is inconsistent. Estimated rates of PcP in the UK are 0.33-0.93 per 100,000 people (70). The incidence of PcP in the established HIV positive population has declined, a result of effective antiretroviral therapy and prophylaxis. However, it remains one of the leading causes of opportunistic infections among people living with advanced HIV. Given that immunosuppressed patients are at higher risk of developing PcP and that this population is expanding annually through the use of novel immunomodulatory therapies (117), the incidence of PcP will vary according to local demographics and healthcare utility. However, immunosuppression has been shown to drive rates of PcP in national studies (118). Patients with solid organ or bone marrow transplants and haematological cancers are well documented as being at particular risk (119,120).. However, cases of PcP are increasingly documented in patients who are immunosuppressed secondary to treatment for a variety of rheumatological, renal, respiratory and haematological disorders which is likely to contribute a significant burden of pathology (114,121). At present, for those undergoing treatment for these conditions, the true mortality risk due to opportunistic infections such as PcP is unknown.

While the burden of underlying diseases in Wales are broadly similar to other developed countries, unique clinical distinctions are evident. One in twelve adults report a

longstanding respiratory condition and Wales had the highest avoidable mortality rate in Great Britain for respiratory diseases in 2019 (122). Wales has a lower proportion of solid organ transplants compared to other UK nations with the majority being renal transplants (76). The overall rates of HIV in Wales are low with 2,358 (2.4%) of the population of Wales receiving HIV care in 2019 (101). However, in 2021, 42% of new cases of HIV in Wales were late diagnoses; generally presenting with AIDS defining diseases such as PcP (123,124). The Welsh tertiary haematology service manage 140-160 acute myeloid patients, 30 acute lymphoblastic leukaemia patients and undertake approximately 100 stem cell transplants annually.

The PcP diagnostic options across Wales are enhanced, with rapid access to both PcP PCR and serum (1–3)-β-D-glucan testing available, which potentially provides information to accurately define an incidence of PcP in a large population. Microscopic detection of *P. jirovecii* is not routine practice in Wales; this approach has not been used for almost a decade due to its limited sensitivity and interpretative subjectivity.

To identify and understand existing and emerging service demands, assess patient risk, and improve diagnostic pathways, accurate knowledge of the incidence of PcP and associated mortality rates in different clinical cohorts is critical. This study describes attempts to both estimate the rates of PcP in Wales based on pre-established clinical risk and correlates this data to laboratory-confirmed diagnoses of PcP based on PCR testing by the Public Health Wales Mycology Reference laboratory. It also investigates diagnostic accuracy and the impact of PcP on the patient outcome, depending on the underlying condition.

3.2. Methods

A UK study stated that the rate of PcP cases was 0.33–0.93 cases per 100,000 of the population. This estimate was applied to the population of Wales to predict the national number of PcP cases (70). The objective was to corroborate the accuracy of this estimate through correlation with laboratory-diagnosed PcP cases, with the accuracy of diagnosis of each case determined by retrospective evaluation of clinical evidence.

PcP PCR was performed at the Wales mycology reference laboratory using an in-house real-time PCR amplifying 77 base pairs (bp) of the mitochondrial 26S rDNA multi-copy gene with

nucleic acid extracted using the BioMerieux EasyMag generic 2.0 protocol and PCR performed on the ABI 7500 real-time PCR platform, with oligonucleotides as previously described in the literature, with technical procedures and clinical performance validated through ISO15189 accreditation, and analytical performance confirmed in a recent multicentre evaluation of PcP PCR methods and participation in external quality control schemes (125–127). All PcP PCR-positive results from the four year period 2015 to 2018 inclusive were extracted from the Public Health Wales Mycology reference laboratory database. This laboratory processes samples from all hospital sites in Wales. This time period was chosen to avoid confounding from the COVID-19 pandemic and to ensure an adequate follow-up period when calculating rates of mortality within the population. All testing was performed as part of routine clinical diagnostics at the request of a consulting clinician, and this current study formed an audit of the clinical accuracy of this clinical service, not requiring ethical approval. All patients with more than one positive result were included only once. For patients with multiple positive results, the lowest PCR cycle number (quantification cycle, Cq) representing the highest fungal burden was included. One individual had multiple positive tests with greater than 12 months in between tests with negative tests in between, potentially indicating separate episodes of infection. This was included as two separate episodes of infection.

Based on meta-analyses and systemic reviews of the performance of PcP PCR testing of respiratory samples, PCR was assumed to be 95% sensitive and 90% specific for the diagnosis of PcP (128). Subsequently, the total defined by laboratory testing was initially adjusted to reflect the 5% of cases missed and the 10% false-positivity rate to create a representative value independent of assay variability, which could be compared with estimates derived using the local assay.

Based on local clinical validation of the specific PcP PCR assay prior to the implementation into routine clinical use, all patients with a throat-swab PcP PCR-positive testing at <38 cycles were assumed to represent a true positive result, as locally, these results were highly specific (>98%). PcP PCR testing of upper respiratory tract samples has been associated with high specificities (≥96%) in other studies (129,130). When testing deeper respiratory samples (bronchoalveolar lavage (BAL), non-directed bronchoalveolar lavage (NBL), or

bronchial washings (BROW)), a Cq of <36 cycles was applied, which was again associated with high specificity (>95%).

All patient results with a positive PcP PCR result above the designated thresholds were individually reviewed, incorporating other laboratory results useful for the diagnosis of PcP (e.g., serum (1–3)-β-D-glucan, using the associate of Cape Cod Fungitell assay), radiology reports, as well as exploring clinical details gained from letters and discharge summaries. Examples of clinical details reviewed are past medical histories, comments regarding other clinical findings in keeping with pneumocystosis such as profound hypoxia and the improvement (or not) on antimicrobial therapies that should be effective in pneumocystosis patients. A decision was made on whether it represented a true clinical diagnosis based on the combination of results. Patients were considered not to have PcP if the combination of results did not represent a true clinical diagnosis (Figure 4). In line with international consensus definitions, all cases were classified as probable PcP (120).

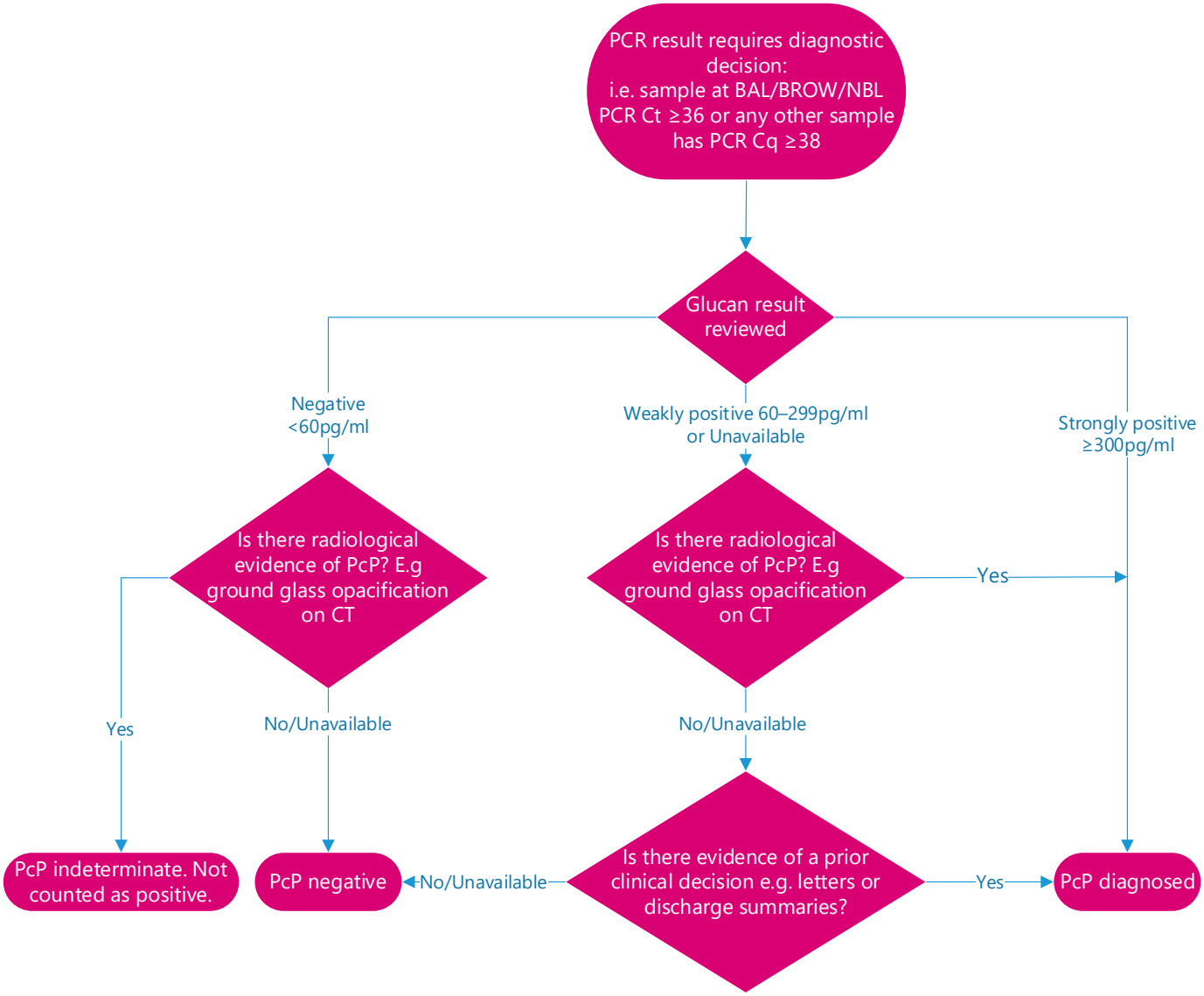


Figure 4: Diagnostic pathway of reviewing patients with positive PcP PCR

When testing respiratory throat swabs, 126 out of 132 samples from patients with PcP generated a positive PcP PCR result (Specificity: 95.5%, 95% CI: 88.0–98.5. Positive likelihood ratio: 18.5). However, only two of these false-positive results generated a cycle threshold (Ct value) < 38 cycles generating a specificity and positivity likelihood ratio of 98.5% (95% CI: 92.3–99.8) and 52.1, respectively, indicating that a PcP PCR-positive results with a Ct < 38 cycles on a throat swab was highly predictive of PcP. Similarly, when testing bronchoalveolar lavage fluid (*n* = 41), the specificity of PcP PCR when applying a positivity threshold of <36 cycles was 95.1% (39/41), and the positive likelihood ratio was 18.0. In relation to combination testing, involving reviewing patients who were deemed weakly positive by PcP

PCR, the Fungitell (1–3)-β-D-Glucan threshold to exclude the need for clinical/radiological review was selected based on data from a recent study, where patients, who were PcP PCR-positive and had serum (1–3)-β-D-Glucan >200 pg/mL, and the specificity of a PcP diagnosis was 100% (131). However, to address any concern that replying solely on this test on those that were approaching a borderline result may introduce inaccuracies, it was decided to increase the (1–3)-β-D-Glucan positivity threshold to ≥300 pg/mL for the purposes of this study (L. White, Personal Communication, April 2023). It was also assumed that this would also minimise the impact of using different PcP PCR assays that may have been introduced between studies.

All remaining patients were reviewed via their health records with their underlying diagnoses and the reason for immunosuppression and their survival data recorded in an Excel database on a password-protected NHS network. The immunosuppression was clinically grouped for analytical purposes. Statistical analysis, including Chi-squared tests and 95% confidence intervals, were generated when comparing proportions (e.g., the mortality between different groups) and *t*-test used for continuous variables that were approximately normally distributed. p values < 0.05 were considered to be statistically significant.

3.3. Results

When applying the UK-wide estimate of 0.33–0.93 cases of PcP per 100,000 to the population of Wales, an estimated 3,170,000 people, between 10 and 29 cases of PcP would be expected annually in Wales (14). Across the 4-year period (2015–2018), this would equate to 40–116 cases. However, a total of 289 PcP PCR-positive results were derived from the laboratory data, although this included 94 duplicate results, leaving 195 unique records. Of these, 103 (52.6%, 95% CI: 45.6–59.4) patients had PCR results associated with high assay specificity (>95%) and were considered true positives.

The remaining 92 records were individually clinically reviewed, and 56 patients were considered to be clinically positive. This process is outlined in figure 5. A more detailed, anonymised view of these results is tabulated as appendix A. In total, 159 cases were identified (mean age 57, 66% male), indicating that 81.5% (95% CI: 75.5–86.6) of positive PcP PCR results were associated with actual disease; 73% (95% CI: 64–80%) of PcP PCR-

positive BAL/NBL/BROW compared to 94% (95% CI: 86–97) of upper respiratory tract samples.

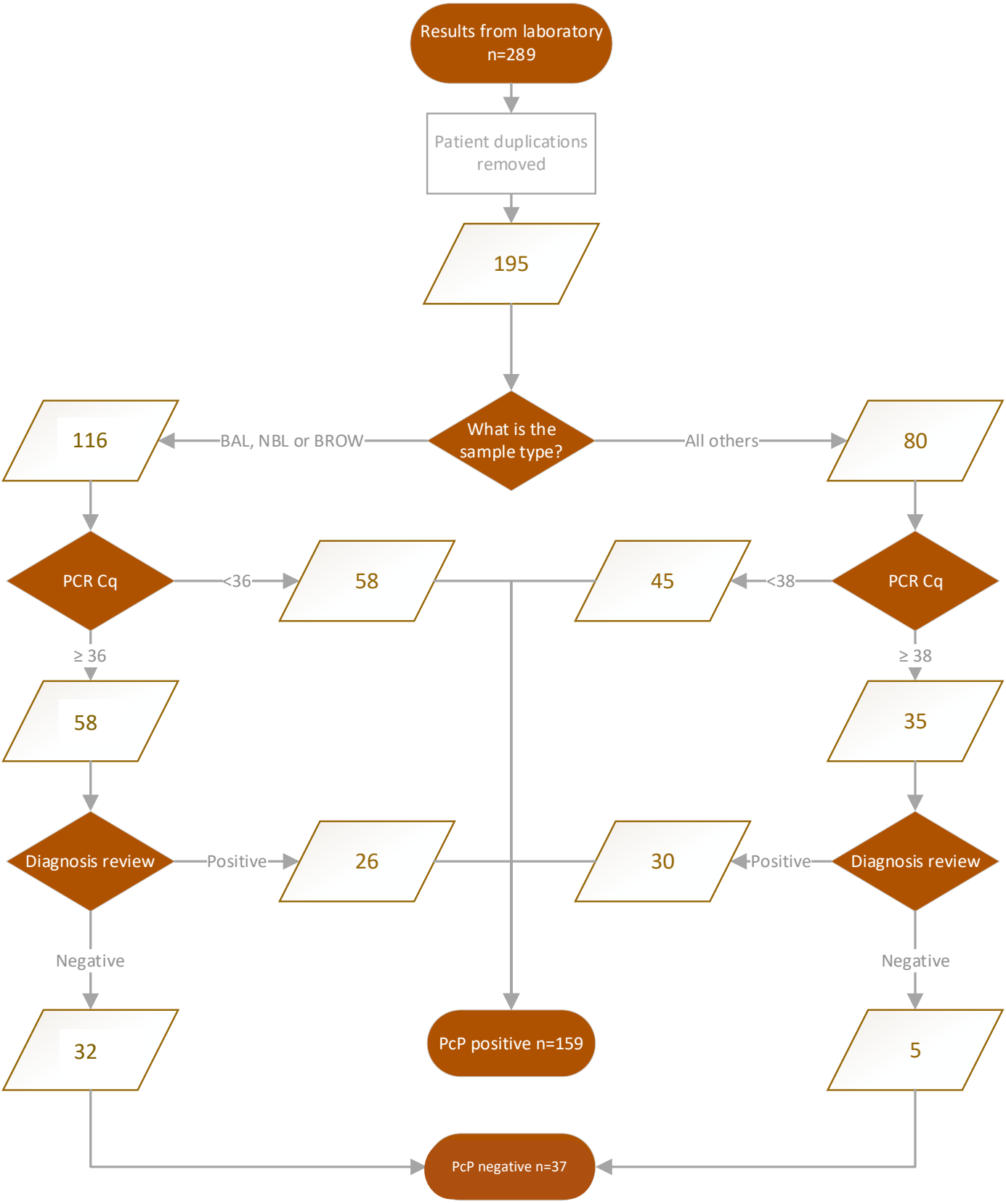


Figure 5: Flowchart of PcP PCR positivity and association with clinical pneumocystosis

Fifty-three percent of samples involved bronchoscopy (58 bronchoalveolar lavage fluid, 25 bronchial washings, and 1 non-directed bronchial lavage fluid). Forty-two percent of the samples were throat swabs. The median cycle threshold for positivity in the throat swab samples was 37 (range: 26–41). The median cycle threshold for PCR positivity in BAL was 32 cycles (range: 20–41), which is significantly different to the throat swab ($p < 0.0001$) (Table 9). Sample sites include bronchoalveolar lavage (BAL), bronchial washing (BROW), non-directed bronchial lavage (NBL), throat swab, nasopharyngeal aspirate (NPA), sino-nasal secretions (SNS), and serum. A full tabulated view of the sample positivity is seen in table 9.

***Pneumocystis jirovecii* PCR[i] sample analysis**						
Site of Sample	Samples with PCR Cq[ii] < 36 in deep respiratory samples and <38 in all others, N	Samples with PCR Cq ≥ 36 in deep respiratory samples and ≥38 in others, N	Samples with PCR Cq ≥ 36 in deep respiratory samples and ≥38 in others and in keeping with PcP, N	Total samples in which PcP diagnosed, N	Overall median PCR Cq in PcP-positive Patients	PCR Cq range in PcP-positive patients
BAL[iii]	44	38	14	58	32	20–41
BROW[iv]	14	19	11	25	34	20–41
NBL[v]	0	1	1	1	37	37
Throat swab	40	31	26	66	37	26–41
NPA[vi]	2	0	0	2	33.5	31–36
SNS[vii]	3	3	3	6	37	26–40
Serum	0	1	1	1	38	38
Total	103	93	56	159		

Table 9: Pneumocystis jirovecii PCR Cq by site of sample

[i] Polymerase chain reaction
[ii] Quantification cycle
[iii] Bronchoalveolar lavage
[iv] Bronchial washing
[v] Non-directed bronchial lavage
[vi] Nasopharyngeal aspirate
[vii] Sinonasal secretion

The difference in median Cq does not translate to a difference in mortality either at 1 month or 1 year and likely reflects the greater fungal burden in the deeper respiratory samples compared to throat swabs. The mortality at 1 month of patients diagnosed by throat swab and BAL was 29% and 36%, respectively (p = 0.378). Mortality at one year was also not significantly different between sample types (42% vs. 48%; p = 0.514).

If sensitivities and specificities from meta-analyses of PcP PCR are applied, there would be a minimal 5% reduction in cases resulting in 185 patients with possible PcP, supporting our initial process for defining PcP based on Cq value and combination with other fungal tests and clinical presentation. The 159 laboratory-positive cases equate to a mean number of 39.75 cases annually, but the study period is not a long enough period to determine a trend in incidence but provides an annual incidence of PcP in Wales of 1.23–1.26 cases per 100,000 and 32–35% greater than the upper limit estimated in the previous UK study (70).

Of the 159 patients considered to be cases of PcP, mortality at one month was 35.2% (56/159, 95% CI: 28.2–42.9). One-year mortality was 49.1% (78/159, 95% CI: 41.4–56.8). Mortality between those that were considered definitively PCR-positive (Cq below the designated threshold) and those with a higher Cq value but with a clinical diagnosis of PcP was similar at one month (36% and 34%, respectively, where p = 0.802) or one year (49% and 50%, respectively, where p = 0.861). This is separated by year in figure 6.

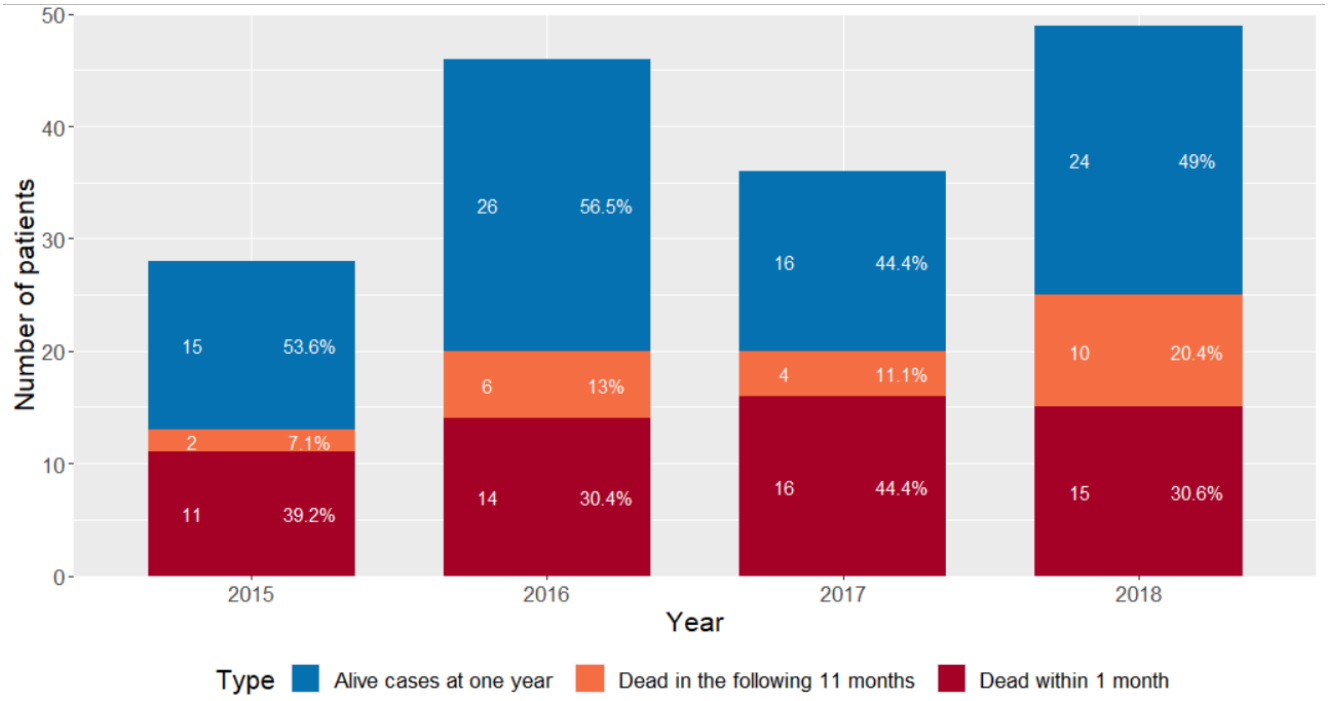


Figure 6: Pneumocystosis mortality in Wales by year, separated into 1 month and 1 year

Regression analysis was undertaken to determine if there is a relationship between Cq value and mortality. In those that were HIV-positive, mortality was low regardless of Cq value and sample type. In those that were HIV-negative, when testing BAL/NBL or BROW (i.e, respiratory samples), the mortality rate for Cq < 37 was 45.7% (21/46, 95% CI: 32.1–59.8); for Cq ≥ 37, it was 35.0% (7/20, 95% CI: 18.1–56.7), which was not significantly different (p = 0.589). In the same HIV-negative cohort that had been tested using throat swabs, the mortality rate for Cq < 38 was 41.9% (13/31, 95% CI: 26.4–59.2); for Cq ≥ 38, it was 28.6% (6/21, 95% CI: 13.8–50.0), which was also not significantly different (p = 0.3887).

Underlying immunosuppressive conditions were grouped, as summarised in table 10, and mortalities at one month and one year were calculated for groups with at least five participants to avoid bias associated with smaller cohorts and reduce identifiability. Rheumatology diseases included rheumatoid arthritis, psoriatic disease, lupus, and other autoimmune diseases. Patients were included in the rheumatology, vasculitis, respiratory, autoimmune haematology, inflammatory bowel disease, dermatology, or autoimmune neurological disease only if they were on at least one of steroids, disease-modifying anti-rheumatic drug (DMARD) or biological therapy and not by the presence of condition alone.

Patients were included in the solid organ cancer or haematological cancer groups if they were on treatment for that condition. Some patients had more than one cause for their immunosuppression and so belonged to more than one group; hence, a total is not applicable.

PcP mortality				
Aetiology of Immunosuppression	Total Number	Mortality at 1 Month (95% CI)	Mortality at 1 Year (95% CI)	Acute Life Threatening?
HIV	33	9% (3–24%)	12% (5–27%)	No
Lymphoma	21	33% (17–55%)	62% (41–79%)	Yes
Renal +/− pancreatic transplant	17	35% (17–59%)	35% (17–59%)	No
Allogeneic stem-cell transplant	16	38% (18–61%)	44% (23–67%)	No
Rheumatology disease	15	60% (36–80%)	67% (42–85%)	No
Solid organ cancer	15	47% (25–70%)	80% (56–93%)	Yes
Haematology malignancy	12	8% (1–35%)	50% (25–75%)	Yes
Vasculitis	11	45% (21–72%)	64% (35–85%)	No
Respiratory disease	9	56% (26–81%)	67% (35–88%)	No
Inherited immunodeficiency	3	N/A	N/A	No
Autologous stem-cell transplant	3	N/A	N/A	No
Liver failure	3	N/A	N/A	Yes
Non-malignant renal disease	2	N/A	N/A	No
Autoimmune haematology disease	2	N/A	N/A	No
Inflammatory bowel disease	1	N/A	N/A	No
Dermatology disorder	1	N/A	N/A	No
Autoimmune neurological disease	1	N/A	N/A	No

Table 10: Mortality of PcP broken down by aetiology of immunosuppression. Mortality rates are only given in populations greater than five, otherwise they are listed as N/A. "Acute life threatening" conditions are those with a mean life expectancy of less than 12 months.

The most common single underlying condition associated with PcP remains HIV, with a total of 33 (21%, 95% CI: 15–28) patients diagnosed, but nowadays, almost four-fold more cases of PcP occur in HIV-negative patients (126 cases, 79%, 95% CI: 72–85). Mortality rates associated with HIV-positive PcP were low at one month (9%, 95% CI: 3–24) and one year (12%, 95% CI: 5–27). Thirty-one percent of the cases occurred in the heterogenous

haematology/allogeneic stem-cell population, while the overall mortality at one month was 29% (95% CI: 18–42); mortality varied considerably depending on the underlying condition and clinical intervention. A significant burden of PcP (approximately 10% of cases) was associated with renal transplantation, rheumatological conditions, and solid cancer (Table 2). Compared to the HIV cohort, mortality was significantly increased in PcP patients with underlying rheumatological conditions, respiratory disease, and solid organ cancer. Patients with PcP receiving treatment for solid organ cancer represented the group with the third highest mortality rate within one month of PcP infection, 47% (7/15, 95% CI: 25–70), and the highest mortality at one year, 80% (12/15, 95% CI: 55–93), both being significantly greater than mortality associated with PcP in HIV (one-month *P*: 0.0059; one-year $p < 0.0001$). The solid organ cancers group ($n = 15$) represented a heterogenous group of cell types/original organ disease, with lung cancer being the most common ($n = 4$). Clinical information regarding the modality of chemotherapy in these patients was limited, preventing further analysis. The 2016 Public Health Wales and Cancer Research UK reports of one-year survival statistics by cancer type for Wales was used to identify the average percentage survival, weighted by the number of this population, with each solid organ cancer (132,133). This is demonstrated in table 11 below:

Solid organ cancers	Patients, N[i]	1 year survival, %
Lung	4	36.9
Colorectal	3	74.7
Brain	2	54.0
Breast	2	96.0
Ovarian	1	72.3
Oesophageal	1	46.5
Renal	1	77.7
Skin	1	97.5
Total	15	

Table 11: List of solid organ cancers affecting patients with pneumocystosis. Survival statistics taken from the 2016 Public Health Wales and Cancer Research UK reports

[i] Number

The weighted mean survival at one year was 64.4%The average mortality at one year was, therefore, expected to be 35.6%, significantly different ($p < 0.01$) compared to the measured cumulative mortality of 80% (12/15, 95% CI: 55–93) associated with PcP in this cohort.

Subgroup analysis comparing "acute life-threatening" conditions with a mean life expectancy of 12 months or less with "non-acute or non-life-threatening" conditions demonstrated statistically significantly higher mortality for patients with "life-threatening conditions" and a PcP diagnosis compared to other conditions (66%, 33/50, 95% CI: 52–78 vs. 41%, 45/109, 95% CI: 32–51, $p = 0.004$). The breakdown of the conditions described as "acute life-threatening" or not is described in table 10.

It was felt that HIV infection represents a unique cohort. Patients with HIV are often younger (mean age of HIV patients 49 (SD: 16.9) vs. 59 (SD: 16.5) in the non-HIV group, ($p < 0.001$) and clinically do well post-swift treatment with antimicrobials and antiretrovirals, as represented by the significant difference between PcP mortality at one year in the HIV group (12%, 4/33, 95% CI: 5–27) and non-HIV group (59%, 75/128, 95% CI: 50–67) ($p <$

0.00001). As such, if the HIV group is removed from the non-acute/non-life-threatening arm of the subgroup analysis, the difference in mortality between the two groups becomes non-significant (66%, 33/50, 95% CI: 52–78 vs. 54%, 41/76, 95% CI: 43–65, $p = 0.149$), highlighting the negative impact of PcP in non-acute/non-life-threatening/non-HIV conditions.

HIV, solid organ transplantation and stem-cell transplants are groups that have a large evidence base behind their established PcP risk. Further subgroup analysis was undertaken to compare the one-month and one-year mortality in patients with these three well-established risks of PcP compared with all other patients, which defined conditions less associated with PcP. The new risk factor patients consistently had both a one-month (43%, 39/91, 95% CI: 33–53) and one-year (65%, 59/91, 95% CI: 55–74) mortality that was significantly higher than the well-documented group (25%, 17/68, 95% CI: 16–36 at one month, $p \leq 0.02$ and 28%, 19/68, 95% CI: 19–40 at one year, $p < 0.0001$). This significant difference in mortality at one month, however, does not remain the case when patients with HIV are removed (40%, 14/35, 95% CI: 26–56 vs. 43%, 39/91, 95% CI: 33–53 at one month, $p = 0.771$), but this difference is present at one year (43%, 15/35, 95% CI: 28–59 vs. 65%, 59/91, 95% CI: 55–74, $p = 0.02$).

3.4. Discussion

Rates of PcP in Wales, assuming the level of diagnostic accuracy in this study (1.23–1.26 per 100,000) are significantly higher than in previous UK-wide estimations (0.33–0.93 per 100,000), likely because the cases of PcP in immunosuppressed patients outside those cohorts with well-documented risk of PcP (e.g., solid-organ transplant and haematological malignancy) were not accounted for in previous studies (70). In 2013, a review by Maini et al identified a significant rise in the incidence of PcP outside of HIV and focussed on the transplant and malignancy groups. However, there was also the appreciation at this point that other novel groups such as those with chronic lung disease were emerging as underrecognized cohorts (134). The overall mortality of PcP in this study is 49.1% at one year and was57.6% in non-HIV patients. Mortality remains high (54%) in non-HIV cohorts with conditions that do not pose an acute, significant threat to life at one year. This

represents a group that is at a considerable risk of PcP but is possibly being under-investigated and likely subsequently undertreated, therefore, contributing to increased morbidity and mortality. While the literature describes the risks and strategies associated with more usual conditions, such as transplantation, there is a paucity of information regarding these other patients (114,119–121,135). However, their mortality post-PcP infection is consistently in excess of those cohorts where the risk of PcP is well-established. There are currently calls for clinical guidance for the diagnosis and management of PcP in rheumatology patients, with rheumatologists recognising the increased risk of PcP in their patients associated with prolonged use of high-dose corticosteroids but also biologic therapies (135). The increased use of immuno-suppressive but also immunomodulatory therapies (e.g., infliximab) is leading to an expanding patient population potentially at risk of PcP, but currently not necessarily recognised as such (113,135). This is more problematic as there are likely to be a number of other patients in these cohorts who have died without the relevant investigations, as the index of suspicion was low. Indeed, 80% of PcP cases with underlying solid organ cancer died within one year, of which 47% died within a month of PcP diagnosis. The Wales national database for these specific cancers suggested that there was an expectation of a 68% mortality at 12 months, but the numbers of patients in this study to compare are small. The mortality of rheumatology conditions that were implicated in this work is expected to be low (<5%), but their mortality post-PcP is high (60% at 1 month and 67% at 1 year) (136). It is not possible to compare to a rheumatology non-PcP cohort due to the multifactorial nature of these conditions and PcP's relative rarity.

In the subgroup analysis, the risk of death is not significantly different whether there is a life-threatening condition vs. a non-life-threatening condition underpinning the immunosuppression and PcP infection. Therefore, there is the implied possibility that PcP may be responsible for a number of deaths in patients who have a condition with a mean life expectancy of greater than 12 months, such as rheumatoid arthritis and psoriasis. As an opportunistic infection with a well described treatment pathway, at least a proportion of these deaths may be described as preventable. However, the risk of PcP in this cohort overall remains low, and there is limited information as to the level of immunosuppression required in this cohort to put them at increased risk (137,138). Pragmatic solutions have been proposed regarding the amount of corticosteroid required to render a patient at risk,

but this has not reached a consensus agreement . It is, therefore, difficult to initiate and champion any prophylactic strategies in these cohorts, but it should lead to heightened awareness of the risk of PcP and the need for enhanced diagnostic surveillance when patients present with acute respiratory illness.

It is not possible to derive disease-specific prevalence or incidence rates from this data as this work lacks a denominator, but the nature of these previously under-recognized cohorts suggests that diagnostic pathways for these novel at-risk patients can be improved. This is evident in the renal transplantation group, where PcP outbreaks have led to heightened awareness for diagnostics and infection control measures in this now-recognised high-risk group. The subsequent low mortality rate is likely a product of heightened awareness and rapid interventions in these patients (139).

A limitation of this study is the necessity of removing replicated patients. In each patient, the lowest Cq value was used to represent them during that acute illness. In circumstances where a throat swab was positive with a high Cq value and the patient went on to have a confirmatory bronchoscopy, in which the BAL yielded a low Cq value PCR result, the throat swab would be removed in preference to the BAL. This inherently introduces bias to those patients that had both but has no impact on the conclusions drawn regarding the underlying condition. While the lack of a reference method, such as immunofluorescence microscopy, may have resulted in the over-diagnosis of PcP based on PCR, an independent meta-analysis of PcP PCR on respiratory specimens has confirmed its accuracy as a diagnostic test (128). Indeed, the use of microscopic diagnosis would likely lead to an under-representation of the PcP burden. Furthermore, in our study, weak PcP PCR-positive results were investigated to confirm the diagnosis. It was then shown that the diagnosis and mortality rates were similar when compared to cases where the PcP PCR result was low i.e. indicative of a high burden. This study also lacks a denominator for most underlying conditions, and it is, therefore, not possible to derive cohort-specific incidence rates.

3.5. Conclusions

The PcP case rate in Wales is higher than previously estimated, and although the true rates of these conditions in their subgroups are not known, it highlights non-transplant and non-

cancer as significant contributors to both the caseload and the mortality of PcP in Wales. However, developing diagnostic-driven pathways in this large group of patients remains problematic due to its size and complexity. Nevertheless, through the dual use of serum (1–3)-β-D-glucan and PcP PCR on respiratory samples, PcP can be confidently excluded or diagnosed. PcP can be diagnosed using a variety of samples from throat swab to bronchoalveolar lavage, particularly when used in conjunction with other modalities, such as radiology and serology, and every effort should be made to attain a diagnosis using these relatively accessible tests. Further work needs to build on this audit with a focus on quantifying the rates of PcP and outcomes for patients with non-life-threatening conditions and exploring the mechanisms underpinning these observational findings. A heightened awareness of PcP in these groups will reduce delays in diagnosis and potentially improve mortality.

Chapter 4 – New diagnostic approaches to fungal disease, designing the SPITFIRE study

4.1. Study Design

4.1.1. Scientific justification and background

Guidelines for the management of febrile immunocompromised haematology patients advocate early initiation of antibiotics, often followed by the addition of antifungals, due to the recognised high mortality risk from infection in this patient group (140). Regardless of whether an empirical antifungal model or targeted diagnostic-driven approach is used, microbial culture is vital in streamlining the antimicrobial choice and this is often achieved through culture of blood and respiratory samples (141,142). A bronchial washing or lavage obtained through bronchoscopy is considered gold standard for deep respiratory sampling of the lung microbiota and access to this is essential for haematology cancer services (143,144). Obtaining suitable respiratory samples for microbiological assessment can, however, be difficult in unwell immunocompromised patients. Bronchoscopy can be inappropriate in patients with a high oxygen demand or cardiovascular instability. As an in-demand procedure with finite operator availability, this aerosol generating procedure must be undertaken in a particular area in the hospital with appropriate facilities and support, making it often logistically difficult to arrange in a timely manner. The potential for any combination of low levels of red blood cells (anaemia), low levels of white cells (leukopenia) and low levels of platelets (thrombocytopenia) in this cohort offer additional risks not found in other patients. Many of these patients are thrombocytopenic, giving the patient a risk of bleeding during the procedure which can be life threatening. As such, all current guidance is that this should be corrected before or during the procedure using blood products which is costly both monetarily and with scarce resources (145).

Induced sputum (IS) is the method of inhaling nebulised (converted to a fine mist) hypertonic saline and then using physiotherapy techniques to cough the saline out. By inhaling the saline it mixes with the microbiological flora of the respiratory tract and its hypertonic nature allows for the breakdown of thick mucus secretions (146). It is a

technique that has been used extensively in paediatric cystic fibrosis (CF) clinics and is increasingly being used in adults with CF who are no longer spontaneously producing sputum (146,147). Previous research has demonstrated an induced sputum sample is comparable to bronchial lavage and superior to cough swabs for bacterial yield when sampling the respiratory tract of cystic fibrosis patients, although this research was performed in children (148). There is data looking at tolerability and patient satisfaction regarding bronchoscopy but no research exists directly comparing the patient experience of induced sputum with bronchoscopy (149). Other evidence has shown benefit of induced sputum over bronchoscopy in obtaining samples for *Mycobacterium* culture in patients who are not producing sputum (6). The inference here being that induced sputum also carries a more favourable risk profile to the staff than a bronchoscopy. In both of these studies the patients were not unwell. Whilst it is logical to assume that this good level of tolerance and non-inferior yield is also achievable in patients with haematological conditions who have respiratory infections, there is no evidence to confirm this.

Nasosorption devices provide a non-invasive method licensed for sampling the nasal mucosal lining fluid. They have been shown to be effective in obtaining samples in order to measure cytokine levels following a nasal antigen challenge and measuring viral loads in respiratory syncytial virus (RSV) infection (151,152). They have documented good tolerance in patients but their use in unwell patients for bacterial and fungal culture has not been assessed.

Respiratory infections often occur in haematology patients who are immunosuppressed due to their pathology or as a consequence of their haematological treatment. However, it is often unclear as to why patients with seemingly similar immunosuppression can have very different incidence of respiratory infection. The immunological basis for differing respiratory infections is poorly understood.

The SPITFIRE study's objectives centred on these gaps in research by assessing the feasibility and acceptability of induced sputum versus bronchoscopy in unwell haematology patients. This was assessed indirectly through patient questionnaires but with the added opportunity to ask the operators of the procedure their perception of tolerance and comfort. There was an anticipation that induced sputum carried a lower risk of adverse events and a much-reduced requirement for sedating medications. The SPITFIRE trial aimed to assess and

compare the diagnostic yield of these procedures to allow for comment as to how effective induced sputum is in the acute setting. As a feasibility study, SPITFIRE was not powered to allow for strong statistical conclusions based on diagnostic yield, however a positive correlation allows for the planning of further research in this area. SPITFIRE allowed for the feasibility testing of nasosorption sticks as bedside/point of care tools in the unwell patient in a way that had not been done before. This study also allowed for the coordinated collection of samples from unwell patients which will provide an interesting insight into the immunological variability between these patients. As such, an additional (and optional for the participant) part of the study was planned where excess samples were stored in biobank for later immunological testing. This would be supported in later developments of the study.

4.1.2. Ethics/Research and Development approval

The SPITFIRE study was reviewed by Queen Square Research Ethics Committee (REC) and was granted Health Research Authority (HRA) and Health and Care Research Wales approval on 13th July 2021 (project ID: 301001, REC reference: 21/PR/0803).

Cardiff and Vale University Health Board agreed to be the sponsor of the study and the study was adopted into the Research portfolio.

4.1.3. Study recruitment

All admissions to the Haematology and Bone Marrow Transplant and Teenage Cancer Trust wards at University Hospital of Wales with signs and symptoms of infection were considered for the study by the clinical haematology medical team. The Teenage Cancer Trust ward admits patients from 13 to 25 years of age with a variety of malignant medical conditions, usually haematology in nature. Patients are looked after by a combination of paediatricians up to the age of 16 and adult haematologists. The haematology team had a copy of the inclusion and exclusion criteria and if they met the criteria and felt that the patient was a candidate for the study, they asked permission from the patient to contact the research team to inform them of the new admission. If permission was not given then the research team was not contacted. A log of patients approached was updated, regardless of outcome. This allowed basic analysis of how many patients were not interested in the study in order to inform the approach of any future studies.

The research team counselled the patient in detail regarding the study and answered any questions they had. They were given a copy of the patient information sheet (PIS). This can be found as appendix B. If the research team also felt the patient met eligibility criteria the patient was invited to take part in the study and consented. A copy of the consent form is included as Appendix C. If at any time the patient felt that they needed more time to consider participation then more time was given. If consent is not granted, the patient was given an opportunity to explain their decision not to participate in order to better guide future research in this area.

A summary of the recruitment process is shown below in figure 7:

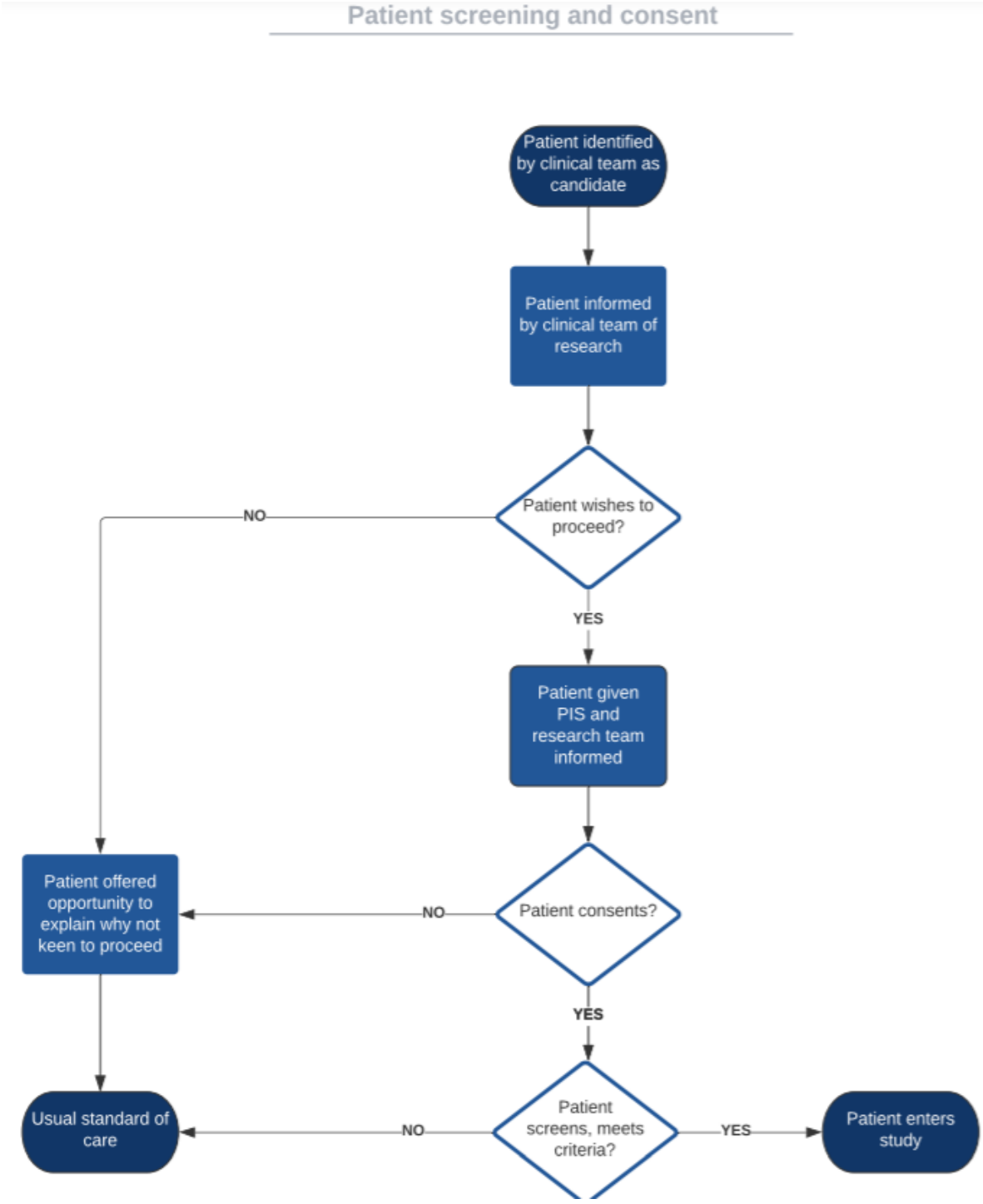


Figure 7: Summary of the SPITFIRE screening and consent process. In this flowchart, patient information sheet uses the acronym PIS. A copy of the PIS is available as appendix B

4.1.4. Inclusion criteria

- Age 18 or over
- Able to consent to the trial in its full extent including the procedures
- Haematological condition present which predisposes to opportunistic infection

E.G: prior allogenic or autologous haematopoietic stem cell transplantation, acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML), chronic lymphocytic leukaemia (CLL), Non-Hodgkin lymphoma (NHL), Hodgkin lymphoma (HL), and multiple myeloma (MM)

- Reasonable clinical evidence of pulmonary infection
- Current inpatient in University Hospital of Wales
- Usual haematology team or microbiology or respiratory team feel that airway sampling through bronchoscopy is indicated

4.1.5. Exclusion criteria

- Age under 18
- Deemed without capacity to consent to the trial and practical procedures included
- COVID-19 respiratory swab positive during this admission or COVID-19 respiratory swab positive in the past without a subsequent negative test
- Deemed by haematology clinical care team to be unsuitable for induced sputum procedure.

There was target recruitment rate of approximately 1-2 every two weeks. An estimation was made that up to 25% of patients would not consent for the bronchoscopy in addition or be too unwell for this procedure. The study was scheduled to be open to recruitment for approximately 9 months. As such, there was a recruitment goal of 30-40 patients who had a haematological condition leading to immunosuppression with clinical suspicion of respiratory infection, aiming for 30 who have both a bronchoscopy and induced sputum. This number was not used based upon power calculations as this was a feasibility study to explore recruitment and acceptability prior to a larger study. It also allowed for the recruitment goal to be adjusted as necessary due to fluctuation in bronchoscopy demand due to COVID or winter pressures.

4.2. Methods

4.2.1. Study procedures

A summary chart of the schedule of study procedures is below in table 12.

SPITFIRE study procedures					
Procedures	**Visits (all study visits performed as an inpatient)**				
	Baseline	Visit 1	Visit 2	Visit 3	Follow up
Timeline	N/A	As soon as possible, up to four days	Within 24 hours prior of scheduled bronchoscopy	Next available bronchoscopy slot	Up to three months later
Informed consent	X				
Demographics	X				
Medical history	X				
Targeted physical examination	X				
Vital signs (heart rate/blood pressure/O_2 saturations/requirement)	X	X	X	X	
Glasgow Coma Score (GCS)	X				
Electrocardiogram (ECG)*	X				
Eligibility assessment	X	X	X	X	
Allocation	X				
Concomitant medications	X	X	X	X	
Blood tests* - full blood count, Clotting, Renal, Bone profile, C-reactive protein (CRP)	X				
Blood cultures*	X				
Serum fungal biomarkers*	X				
CT thorax +/- venous phase contrast*	X				
Nasosorption sampling		X			
Induced sputum procedure		X	X		
Patient questionnaire		X	X	X	
Bronchoscopy and washings*				X	
Adverse event assessments		X	X	X	X
Diagnosis and prognosis data					X

Table 12: A summary of SPITFIRE study procedures at each study visit

Note * denotes procedures which will have taken place as part of routine care, the results/reports of which will be included in analysis but will not be performed by the study/required by the study protocol

An oxygen requirement of 5 litres/minute or more at any time before bronchoscopy precludes the bronchoscopy but does not exclude the patient from the trial. In this instance the patient does not undergo Visit 3 but remains included for analysis of IS acceptability.

4.2.2. Baseline data collection

Inclusion in the trial was as soon as possible after screening for eligibility and consent, because there are concerns about reduction in yield over time, particularly if already on antimicrobial therapy. Once the clinical care team had conducted the eligibility screening, the rest of the procedures were conducted following consent obtained by the research team. The maximum accepted length of time following consent to start the trial at visit 1 was four days. However, this was extended in circumstances where the procedures were unable to be organised due to logistical issues which may be directly or indirectly COVID related including unavailability of side room, physiotherapist or experienced bronchoscopist.

It was important to establish a number of safety parameters in order that the success or failure of the IS or bronchoscopy could be viewed in an appropriate clinical context. As such a number of assessments were required to better characterise the overall health of the participant. It was also important to not rely upon deep respiratory sampling only and to treat each participant equally so a number of baseline microbiology investigations were performed.

Baseline assessments included:

- Demographic data collection
- Clinical review of medical history
- Targeted physical examination
- Vital signs (including heart rate, oxygen saturations, blood pressure)
- National Early Warning Score (NEWS)

- Glasgow Coma Score
- Electrocardiogram (ECG)
- COVID swab for PCR if not already done
- Concomitant medications review including antimicrobial medication
- Blood tests: full blood count (FBC), Clotting profile, renal profile, bone profile, C-reactive protein (CRP), blood cultures, serum fungal biomarkers including beta-d glucan, *Aspergillus* (galactomannan) antigen and *Aspergillus* PCR
- CT scan of thorax with venous phase contrast (unless contrast contraindicated)
- CT scan of sinuses as per local Invasive Fungal Infection protocol if has localising signs in keeping with sinus infection
- Swab for common respiratory viruses (not including COVID)

Results were termed valid and included in analysis if the baseline investigations were performed up to 7 days before enrolment in the trial. Invalid results were only repeated if clinically indicated and never solely for the purposes of being included in the trial. Any repeat tests were known to the original clinical team and were acted upon during the trial as needed. The collection of these results or reports as part of the trial were consented for.

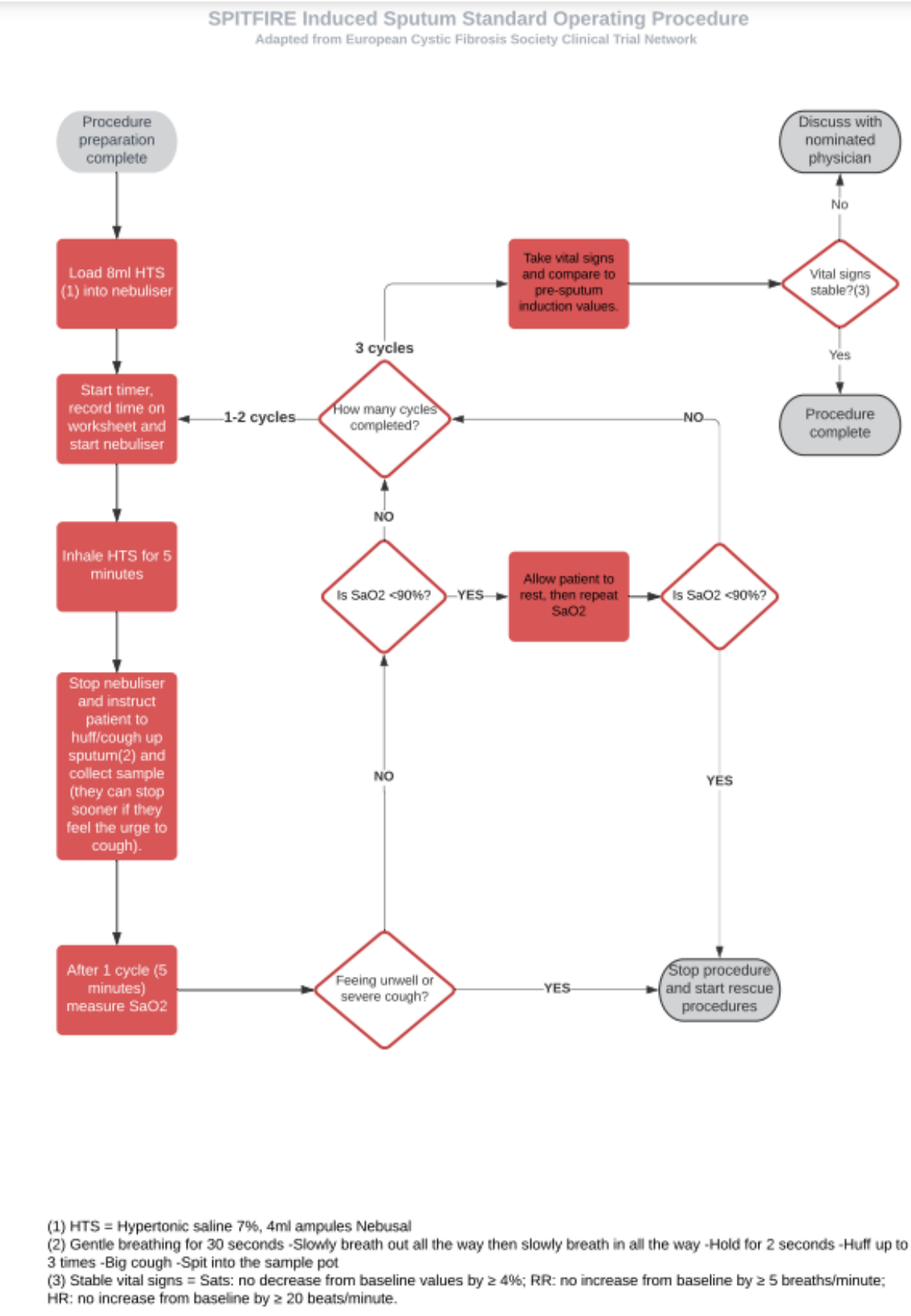
4.2.3. Induced sputum method

There was a need to develop an induced sputum protocol including a Standard Operating Procedure (SOP) as this did not exist for unwell inpatients. It was important that this stipulated the presence of an appropriately trained professional, the administration of the hypertonic saline and the number of times this could be attempted before it was declared an unsuccessful procedure. It was sensible to include safety parameters and the ability to monitor patients throughout.

This procedure is utilised on the CF ward and in outpatients with great regularity with experienced operators and expert patients. The CF-SPiT clinical trial was undertaken locally and led to changes across paediatric care as to the delivery of this investigation (148). There was an SOP for a standardised and safe method of performing sputum induction in clinical trials developed by the European Cystic Fibrosis Society Clinical Trial Network (ECFS CTN)

with focusses on identification and prevention of bronchospasm secondary to hypertonic saline use through the use of spirometry which has then been largely adopted by other studies and societies (153–155). This requires good patient understanding and collaboration, specialist training and is not readily available on the ward. It was felt that this was unnecessary in patients who are being closely monitored on the ward with respiratory intervention available in short notice.

It was felt that this SOP could be adapted because it is not fit for use in unwell patients who are being regularly monitored and needed to be more appropriately user-friendly for physiotherapists who have not necessarily received respiratory subspecialty training. As such the SOP was adapted to create the following SOP for ward-based IS and was reviewed by respiratory physiotherapists, general physiotherapists, respiratory physicians and haematologists. Included in the revised addition was the removal of spirometry at the bedside and the addition of ensuring that salbutamol rescue therapy was prescribed and readily available. The SOP is outlined below with the full sputum induction protocol included as Appendix D.



(1) HTS = Hypertonic saline 7%, 4ml ampules Nebusal
(2) Gentle breathing for 30 seconds -Slowly breath out all the way then slowly breath in all the way -Hold for 2 seconds -Huff up to 3 times -Big cough -Spit into the sample pot
(3) Stable vital signs = Sats: no decrease from baseline values by ≥ 4%; RR: no increase from baseline by ≥ 5 breaths/minute; HR: no increase from baseline by ≥ 20 beats/minute.

Figure 8: Induced sputum standard operating procedure for SPITFIRE. Acronyms and procedure explained in more detail in appendix. In this diagram RR is respiratory rate and SaO2 is arterial saturation of oxygen in the blood

A case report form (CRF) was then designed to collect information regarding these procedures, to be filled out by the physiotherapist undertaking the IS on each patient. This included baseline information data as to the clinical frailty score for the patient, time taken to perform the procedure from the point of saline nebulisation starting to final sample being produced, adverse events and the outcome. Frailty was assessed visually by the operator using a copy of the Clinical Frailty Score provided to them. A copy of this scoring system is in figure 9 below:

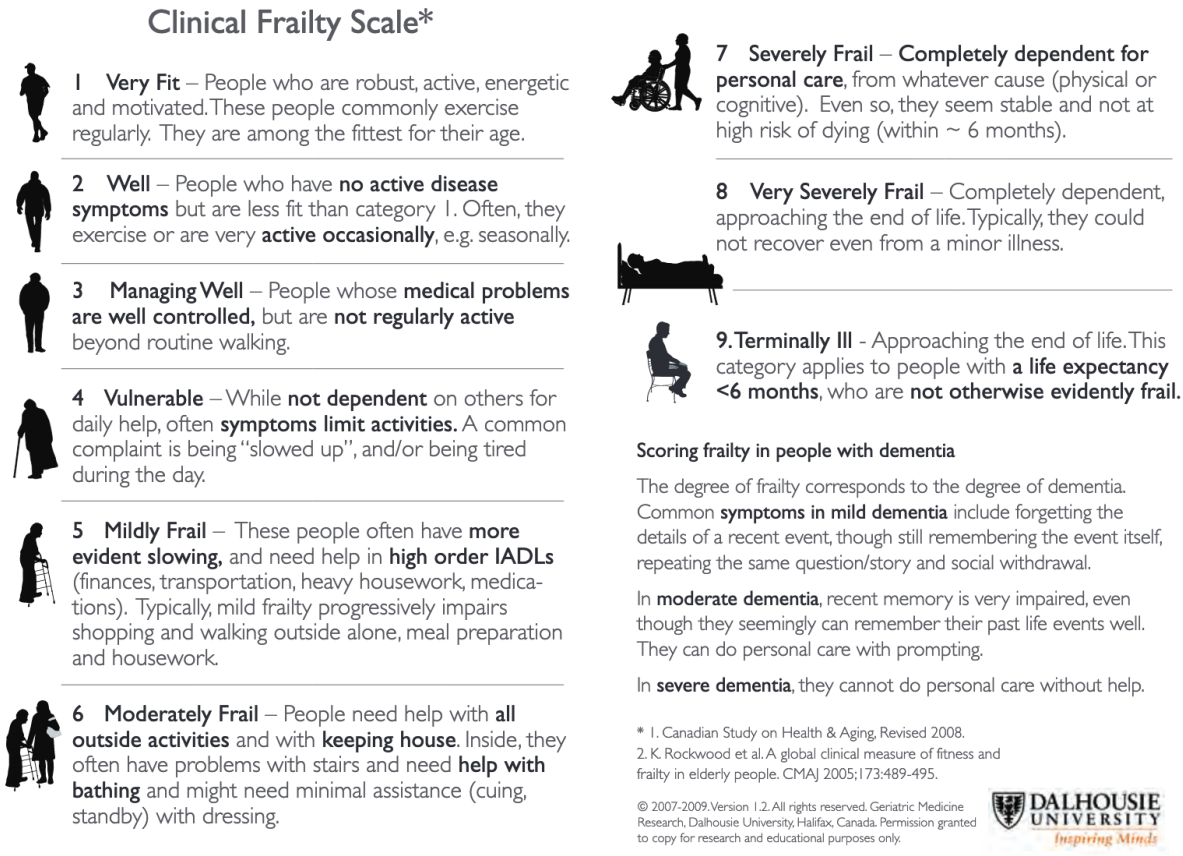


Figure 9 - Clinical frailty scale used in the SPITFIRE study. Taken from Rockwood et al. (156)

This is a validated reliable predictor of acute care outcomes and was an appropriate scoring system to assess patients in a swift and standardised way (157–159). A copy of the IS CRF is found as appendix E.

It was important to perform the induced sputum as soon as feasible following the consent process and baseline investigations as it was appropriate to prove clinical acceptability through speed of delivery of test. It also is in the context of patients receiving empirical antimicrobials so It was important to get accurate results as early as possible. However, in order to better evaluate the results concordance with bronchoscopy washings it was also appropriate to perform the test as close to the bronchoscopy as possible. As such, the protocol was written to accommodate two procedures.

4.2.4. Bronchoscopy washings

Although listed as a procedure as part of the study, the bronchoscopy is part of standard of care. Bronchoalveolar lavage (BAL) is a minimally invasive procedure that involves the instillation of sterile saline into a subsegment of the lung, followed by the suction and collection of the fluid for analysis. Bronchial washings are obtained through bronchoscopy by the aspiration of sterile saline solution applied to a targeted area of the lung through the tip of the bronchoscopy. These two techniques are used variably in clinical practice. Although BAL has a better diagnostic yield for difficult to investigate organisms, the co-investigator team felt that there was more heterogeneity of training and delivery of a BAL (160). This therefore could introduce operator bias. A BAL requires the instillation of a greater volume of fluid and so can take longer than a bronchial washing. A bronchial washing was felt to be acceptable given that the patients would be potentially unwell and needing supplemental oxygen so a swift approach was preferable and there was less chance of inter-operator variability. The collection of the BAL sample also is prone to variability as the equipment to collect it is not standardised. This would be more of an issue in a multicentre study but will still felt to be noteworthy as there were two hospitals in which the procedure could take place. As such the instruction to the limited number of trained bronchoscopy operators was to perform a bronchial washing at a diseased lung lobe targetable at CT scan and, if able, at least one other lobe. This was felt to be well within the realms of normal practice, even allowing for some personal variation and would be considered usual clinical practice for this indication. A case report form for bronchoscopy was written for completion by the operator and includes the same baseline information data as the IS CRF, time taken to perform the procedure from initial use of sedation to

extubation, sedation given, lobes targeted at bronchoscopy, adverse events and outcome. The bronchoscopy CRF is indicated in Appendix F.4.2.5. Nasosorption sampling

The nasosorption sampling involves the wiping of the nasosorption sample applicator on the inside of the nasal mucosa surface. This was done as soon as possible after consent and baseline investigations are taken.

The procedure results in an applicator secured in a sampling tube. This will be eluted in 300uL to yield a sample. This sample was frozen at -80°C +/- 10°C as soon as practically possible and registered in the existing biobank for future research planned in Immunological assays in fungal disease.

4.2.6. Acceptability data collection

The IS and bronchoscopy acceptability was divided into operator acceptability and participant acceptability. The operator acceptability domains were determined through discussion with those undertaking the procedure to define a series of safety parameters as well as the perception of patient comfort. These parameters, such as lowest oxygen saturation, were included in the procedure case report form for each test. Participant acceptability was harder to define and so a patient who had undertaken both of these tests before for different conditions was consulted as Patient and Public Involvement (PPI). Their feedback regarding the study in general helped formulate questions in a questionnaire including patient-reported pre-procedure anxiety, willingness to repeat the test, comfort and adequate analgesia/sedation. Each question was phrased as an affirmatory statement, for example, “I would be prepared to have the procedure again”. Participants would then be invited to score their agreement with this statement on a Likert scale of 1-5 where 1 is strongly disagree and 5 is strongly agree. These questions were compiled into a questionnaire and were distributed to the participant for completion following each investigation. A copy of the questionnaire is attached as Appendix E.

4.2.7. Statistical analysis

The study was not powered to demonstrate similarity in microbiological outcomes from the samples generated by the two procedures. As such, each patient who had paired tests were described as having an induced sputum that, in addition to radiology and serological

markers, generated a concordant diagnosis with the standard of care (bronchoscopy). A kappa statistic was used to identify the level of agreement between bronchoscopy and IS results where possible. Microbiological outcomes will be described where proportions or measures of relatedness are not appropriate.

When analysing for acceptability, basic statistical comparisons were made between those who underwent IS alone and those who underwent bronchoscopy in addition to IS. This included representing the demographics through percentages and proportions. This was to identify if any patients would tolerate IS when they might not be able to tolerate bronchoscopy. There would also be an opportunity to identify if some microbiologically isolates or haematological conditions were associated with profound enough reductions in clinical state that they are more appropriate for IS alone because they would not tolerate bronchoscopy. Failures to complete the protocol and withdrawals were described. Test-related outcomes such as duration, ability to produce a sample and lowest oxygen saturations were recorded and compared. Continuous variables were compared by way of a T-test.

The CRF for bronchoscopy and IS were reviewed and operator-perceived patient comfort and frailty were compared between the two tests by way of Wilcoxon unpaired rank. Questionnaire outcomes regarding the tests were compared using Mann-Whitney or Wilcoxon tests.

4.2.8. Utilisation and cost analysis

Without being powered for microbiological outcomes it is unclear how induced sputum will best be utilised in clinical practice. However, in order to suggest effective policy alternative to current practice, an appreciation for relative cost is needed.

The price of a single induced sputum procedure was calculated using the stock price of consumables used in the procedure along with the time taken for a physiotherapist to complete the procedure. During the SPITFIRE study the consumables were donated by Trudell Medical. Cost of laboratory time was not included as it was assumed in many instances that IS would be a like for like replacement for bronchoscopy and the tests would be identical. The cost of a single bronchoscopy procedure was identified through prior financial reports obtained directly from the health board finance department.

As well as cost, likely frequency of utilisation is needed and so the referral rate to the respiratory department from haematology was audited.

4.3. Discussion

The SPITFIRE study has the potential to address a problem with inpatient investigations in the unwell patient as access to bronchoscopy is often a rate determining step. There is the potential for this to be applicable to the multiple other unwell patients such as those immunosuppressed for solid organ transplants. In those cases, percussive physiotherapy techniques could be more appropriately applied as they are far less likely to be thrombocytopenic.

A strength of the SPITFIRE study is the use of an established procedure in a highly investigated group of patients. The novelty lies in the unfamiliarity of this procedure which is usually reserved for the outpatient setting. There are appropriate procedures in place to mitigate for any possible adverse events related to the procedure however there is a risk of observation bias in that bronchoscopy can be requested by a separate team who will take the patient off the ward and perform the test. They may not appreciate the unwellness of a patient themselves and so the test may be performed differently than if the same team is responsible for the tests; such as in the SPITFIRE study.

The SPITFIRE study is highly reliant upon patients remaining in a similar clinical status throughout which is often unrealistic. Should they deteriorate, they are likely to receive an induced sputum procedure and not the bronchoscopy. Clinically, this is effective as it provides some microbiological data to empirically treat but is less effective given the primary and secondary outcomes require paired questionnaire answers. Also, by waiting several days until the bronchoscopy is available, there is the potential that the induced sputum is not suitably compared to the bronchoscopy. In order for this to be a better example of a diagnostic accuracy study then they should ideally take place on the same day as close together as possible. Whilst this is clearly not suitable in this small feasibility study it is worth consideration in the possible application of this protocol in a wider study. In order to address this within the confines of a feasibility study, two IS procedures were undertaken. The first procedure was as close as possible to the consent process in order to

provide as accurate as possible information for the clinical team before the patient is commences on empirical antimicrobial agents, and the second as close as possible to the bronchoscopy in order to assess if the IS is an accurate reflection of the results obtained at bronchoscopy.

There was concern that the rate of referral to respiratory may be influenced by the presence of the SPITFIRE study taking place in the hospital and therefore generate an element of performance bias. As such, the referral audit evaluated a year prior to SPITFIRE initiation, the SPITFIRE recruitment numbers as an annual rate and the year following SPITFIRE recruitment. Whilst this helps to give a more accurate estimate as to the annual number of haematology patients requiring deep respiratory sampling, how IS is best utilised in this setting remains unclear.

4.4. Conclusion

The SPITFIRE study was set up to evaluate the acceptability of the induced sputum procedure to unwell haematology patients, including in their ability to produce a useable sample. The population size is small and therefore unlikely to generate significance in the analysis of microbiological outcomes but as a feasibility study gained approval as a worthwhile and safe study.

Chapter 5 – SPITFIRE Results: acceptability of induced sputum versus bronchoscopy

5.1. Introduction and objectives

The inpatient haematology cohort represent an unwell and often clinically unstable group for whom it is important to ensure that investigation of additional clinical problems, including infection, takes place correctly, swiftly and accurately in order to ensure good application of results to their ongoing management strategy. At present, bronchoalveolar lavage (BAL) at bronchoscopy remains the gold standard for deep respiratory sampling for patients with suspected pulmonary infection with an atypical organism such as fungi, viruses or bacteria that do not usually cause disease in healthy individuals. However, bronchoscopy access can be limited by logistical constraints such as availability of equipment, trained staff and space or by clinical risks which are heightened in unwell haematology patients with thrombocytopenia. Induced sputum (IS) is a procedure used in cystic fibrosis care whereby hypertonic saline is inhaled and respiratory physiotherapy techniques are applied in order to expectorate a sample. However, it is important in this cohort to establish if increased frailty, reduced platelet counts due to sickness or drugs and the exertion of respiratory physiotherapy are barriers to the administration of this procedure. Regardless of patient acceptability, if the sample is diagnostically inaccurate it will not serve as an effective adjunct or replacement to bronchoscopy.

When trying to establish if induced sputum is an appropriately acceptable test, it was decided to look at predominantly two questions:

1) Is it acceptable to the patient? i.e., are they willing to have the procedure and are they willing to have a repeat procedure.
    a. This was measured through patient feedback questionnaires using Likert scales and analysis of adverse events
2) Does the sample generate clinically valid results? i.e., the administration of the test is likely to yield a result concordant with the diagnosis made using the bronchoscopy and in a cost-effective and timely manner.

If patients are unable to participate in the test or are unable to provide a sample, a further objective was agreed to assess if these patients are able to be identified before administration of the test to avoid unnecessary procedures.

5.2. Study population and methods

Haematology inpatients at the University Hospital of Wales who were unwell with a reasonable clinical suspicion of respiratory infection that would benefit for deep respiratory sampling were enrolled in the SPITFIRE study. Patients were consented to undergo a two induced sputum procedures before a bronchoscopy, where the bronchoscopy was the standard of care for which they would have been referred regardless of trial participation. A full description of the recruitment procedure is described in the prior chapter.

Patients were also consented for nasosorption samples to be taken at time of consent. It was made clear to them that these samples would be frozen and used in retrospective analysis as part of later biobank projects.

Enrolment in the SPITFIRE study involved baseline data collection, CRF completion by the operator and patient questionnaire completion following each of the three planned procedures. Data was collected on paper and recorded on a dedicated SPITFIRE spreadsheet on Microsoft Excel 2016. All patients enrolled in the SPITFIRE study were analysed.

Participation in the trial and completeness of follow-up was illustrated by a flow diagram (figure 10). The numbers of patients screened, eligible and recruited are described. The reasons for recruitment failures withdrawals are also described. Participants have the right to withdraw consent for participation in any aspect of the study at any time. If a participant initially consented but subsequently withdraws from the study, clear distinction was made as to what aspect of the study the participant is withdrawing from, including:

- Withdrawal from individual interventions (such as bronchoscopy or induced sputum)
- Partial withdrawal from further data collection (questionnaires etc)
- Complete withdrawal from further data collection

A participant may have withdrawn or been withdrawn from study intervention for the following reasons:

- Intolerance to intervention
- Loss of capacity to consent to an intervention or in person data collection
- No longer wishes to take part
- Discharge from secondary care

IS and bronchoscopy procedures were defined as a success if a sample was produced that was suitable for analysis. Adverse events and serious adverse events were recorded in real time by the operator in the relevant case report form (CRF) and whether they were related to the study/intervention/procedure by each time point and treatment option. The operators recorded the time taken for each of the procedures to be complete. The IS procedure was defined as started when the nebulised saline was started and finished when the last sample or cycle had completed. Bronchoscopy procedures were defined as started at the point of sedation being administered and finished when the patient was extubated. Time taken to attend endoscopy or return to the ward after the bronchoscopy was not included. Statistical analysis was performed in SPSS v26.0 and R. Paired comparisons of continuous data was analysed by T test, comparisons of medians was done by Mann Whitney U tests and comparisons of paired discrete data was by Wilcoxon. A *p* value of <0.05 was deemed significant.

The radiology, serology and microbiological evidence of bacterial or fungal disease in each patient was reviewed alongside the bronchoscopy results to diagnose infection. IS and BAL samples underwent processing according the local standard of practice followed by identical microbiological testing as dictated by the relevant clinical information provided per patient. Where opportunistic pulmonary infection was suspected, each sample was processed for microbiology culture on blood agar, chocolate agar and cystine lactose electrolyte deficient (CLED) agar plates. Fastidious anaerobic agar plates were also used when indicated through clinical information given at the time of the test. Each sample was also tested on fungal agar plates for culture as well as *Pneumocystis* PCR, *Aspergillus* PCR, *Aspergillus* antigen (galactomannan) and *Mycobacterium* microscopy and culture. Any remaining samples were saved in accordance with repeat testing policy.

The results of these microbiological assessments were performed as part of routine diagnostic service and made available to the clinical team responsible for the patient's

inpatient admission. This was a deliberate decision as part of the ethical informed consent process; a test was being performed on an unwell patient in a timely manner and they should benefit directly if possible, with the understanding that IS is an unvalidated sample.

The questionnaire responses were collated and compared. All responses were used when analysing procedure-specific factors such as perception of tolerance. Responses to the first post-IS questionnaire were paired with the post-bronchoscopy questionnaire to analyse factors such as patient-reported anxiety pre-procedure, comfort and willingness to have again.

If IS is suitable for a role in the diagnostic pathway of unwell haematology patients, then consideration needs to be made for the cost effectiveness and frequency of utilisation of this test in various contexts. As such, further work was undertaken to establish if there is a cost saving and how frequently IS could be applied in order for this to be realised.

The cost of the equipment required to undertake IS (including staff time) was identified and an approximate cost per procedure was compiled. Prior financial reports were used to establish the cost per bronchoscopy for an inpatient. The two costs were compared and opportunities to model this against the referral frequency to respiratory medicine for the unwell haematology patient was explored through analysis of referral patterns before, during and after the SPITFIRE study. Pathways were described that could assimilate IS with current practice.

5.3. Results

5.3.1. Patient demographics

Nineteen participants were enrolled in the SPITFIRE study. There was one screen failure due to worsening confusion. The demographics of the study population are in table 13. A more comprehensive, anonymised table of patient demographics and medical background is attached as appendix H. All 19 patients had abnormalities reported on their CT thorax that would be consistent with infection. The results of their baseline investigations is attached as appendix I.

	Total patients	Paired IS[i] and bronchoscopy	IS only (no bronchoscopy)
Number enrolled, N[ii]	19	12	7
Median age (range)	52 (24-80)	51.5 (24-80)	56 (24-78)
% Male	42.1	58.3	14.2
Median NEWS[iii] score at consent	3 (0-7)	2.5 (0-7)	3 (1-4)

Table 13: SPITFIRE participant demographics

[i] Induced sputum
[ii] Number
[iii] National early warning score

The most frequent underlying condition was acute myeloid leukaemia (AML, 13). Other conditions included myelodysplastic syndrome (MDS, 2), myeloma (1), chronic immune thrombocytopenia (ITP, 1), diffuse large B cell lymphoma (DLBCL, 1), chronic myeloid leukaemia (CML, 1) and chronic lymphocytic leukaemia (CLL, 1). Some patients had more than one underlying haematology disease. This is demonstrated in table 14 below.

Underlying haematological disease	Number of patients, N[i]
Acute myeloid leukaemia (AML)	13
Myelodysplastic syndrome (MDS)	2
Myeloma	1
Chronic immune thrombocytopenia (ITP)	1
Diffuse large B cell lymphoma (DLBCL)	1
Chronic myeloid leukaemia (CML)	1
Chronic lymphocytic leukaemia (CLL)	1

Table 14: List of underlying haematological diseases of the patients enrolled in SPITFIRE. Some patients had more than one condition and so a total is not appropriate.

[i] Number

Six patients (32%) had previously had an allogeneic stem cell transplant. The mean number of days since transplant was 119 (S.D 102.3).

Of the participants, 19 (100%) underwent induced sputum testing. Fourteen (74%) underwent a second induced sputum test as per protocol. The reasons for not undertaking the second IS was patient declined (2), patient too unwell to continue (1), patient improved (1) and patient had self-discharged from hospital (1). A bronchoscopy was undertaken in 12 patients. One patient who had declined the second IS consented to bronchoscopy. Otherwise, the withdrawals described above remains the same. In addition, 2 further patients declined bronchoscopy and a further 1 patient was too confused for it to be safely performed. This is illustrated in a diagram below (figure 10).

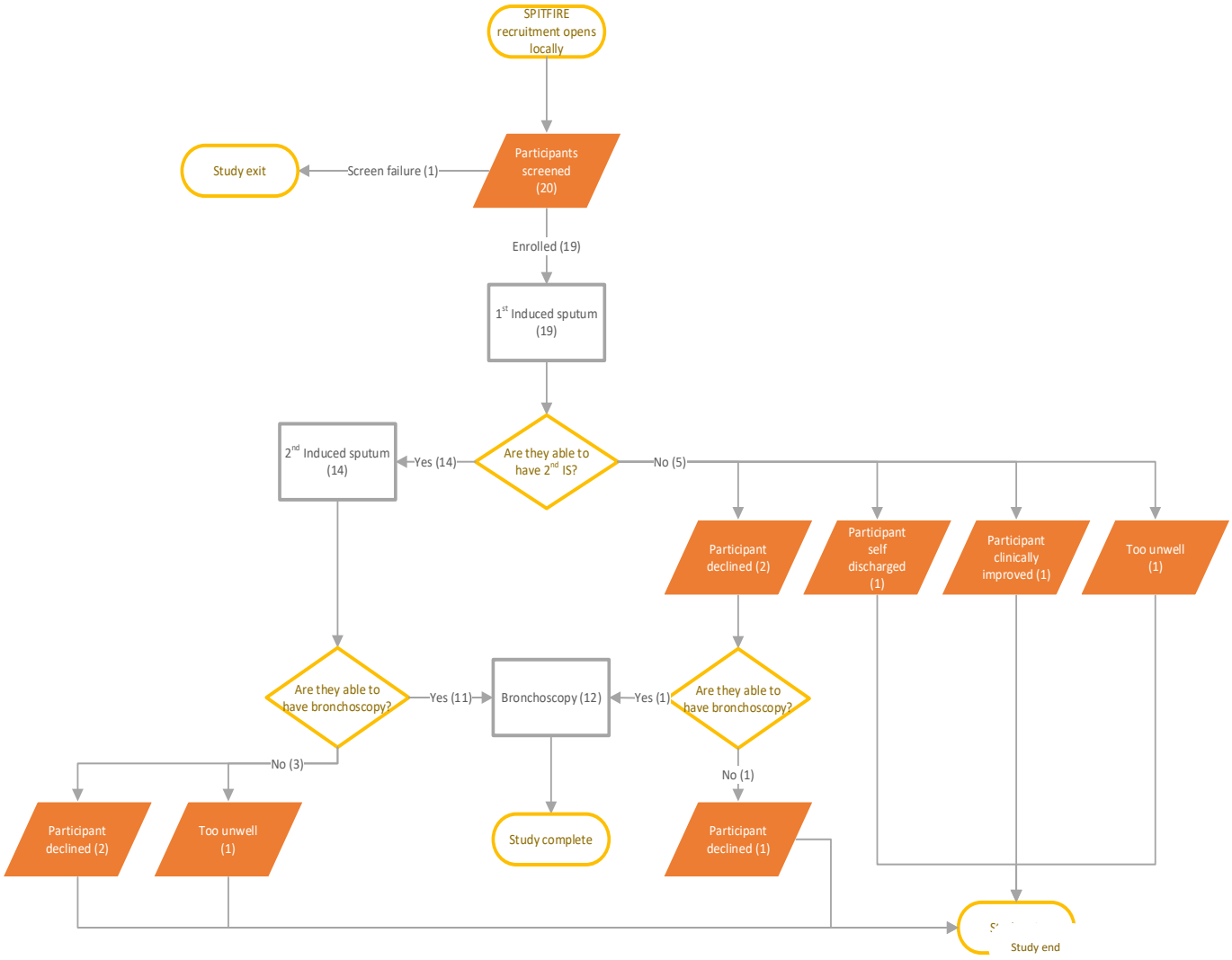


Figure 10: Outcome of all SPITFIRE participants

5.3.2. Procedure results

Seventeen out of the nineteen (89.5%) enrolled participants in SPITFIRE consented for nasosorption samples to be taken. These were successfully eluted and stored frozen as intended for future analysis.

The median time taken from consent to the first induced sputum procedure was 1 day (range 0-10). The median time taken from consent to bronchoscopy was 5 days (range 1-11). This difference in time was statistically significant (p<0.001).

There was a total of 33 IS procedures performed. They were successful (a useable sputum sample being produced) in 27 cases (81.8%). Fifteen out of the 19 patients (78.9%) were

able to produce a sample on induction. On no occasion did a patient who had produced a sample at the first induced sputum procedure (IS1) go on to later fail to produce a sample at the second induced sputum procedure (IS2), excepting those who exited the study for reasons listed above. All 12 (100%) of the bronchoscopy procedures were successful in providing BAL fluid.

All 33 IS procedures had a corresponding completed CRF. One bronchoscopy CRF was not completed. The lowest oxygen saturations during the test were recorded. During induced sputum the mean lowest saturation was 95.6%. In bronchoscopy this was 88.9% despite patients being started on supplemental oxygen at a minimum of two litres per minute via nasal cannulae as a matter of local policy at the start of the procedure. Oxygen saturation levels were significantly lower during bronchoscopy (p<0.001).

There was a significant difference (p<0.0001) in duration of procedure. The mean duration of an IS procedure was 19 minutes 38 seconds and 9 minutes 38 seconds for bronchoscopy.

Patient frailty was assessed during the IS procedures using the Clinical Frailty Score (CFS). The median frailty score during all IS procedures was 3. It was also 3 in the those that were successful (range 1-5). It was 6.5 (range 3-7) in the IS procedures that were not successful i.e. sample obtained versus not obtained (p=0.002).

For the first IS procedure, the position of the patients was recorded as either lying prone (in bed) or sat (on edge of bed or in the chair). Patients who were sat to do the procedure were more likely to have a successful procedure (11/12) than those lying (4/7) but this was not a significant difference (p=0.117). When this analysis was expanded to all IS procedures the proportion of those sat that were successful remains high (18/19) compared to lying (9/14) which is suggestive of an emergent trend despite the small numbers (p=0.062).

The median baseline heart rate difference between those successful was lower than those that were unsuccessful. The upper limit of the range was similar but the lower limit of the range was higher in the unsuccessful group, implying that there were more patients in the unsuccessful group that were more systemically unwell i.e. had a higher baseline heart rate.

Further analysis of the differences between those patients that are successful and those not is shown below (table15):

Induced sputum procedures, understanding the difference between successful and unsuccessful			
	Successful IS procedures	Unsuccessful IS procedures	*P* value
Clinical Frailty Score, median (range)	3 (1-5)	6.5 (3-7)	0.002
Baseline heart rate in beats per minute, median (range)	95.5 (66-145)	107.5 (99-147)	0.046
Fraction of inspired oxygen, median (range)	21% (21-35%)	21% (21-32%)	0.575
Baseline respiratory rate, median (range)	18 (16-22)	20 (17-22)	0.213

Table 15: Comparing clinical characteristics of patients who were either successful or unsuccessful at producing a sputum sample at induced sputum

The fraction of inspired oxygen and baseline respiratory rate were not significantly different between those that were successful at induced sputum and those not.

5.3.3. Patient acceptability

The post-procedure questionnaire responses were compared. Responses to the first post-IS questionnaire were paired with the post-bronchoscopy questionnaire to analyse factors such as patient-reported anxiety pre-procedure, comfort and willingness to have again. One questionnaire was not performed post-bronchoscopy due to patient confusion and two post-IS questionnaires were not performed by patients who declined to answer them. As such the number of paired questionnaires was eleven.

Eleven patients had a questionnaire response for both IS1 and bronchoscopy which could be paired. Patient reported anxiety was higher before bronchoscopy (median 5 = strongly agree) than IS (median 2 = disagree) and this reached statistical significance (p=0.02). There was no statistical difference in how patients felt they had tolerated both procedures, how

the operator felt they had tolerated them (*n*=10 due to missing data) or the patient reported comfort levels. However, patients were less prepared to have a bronchoscopy again (*p*=0.025). This is summarised in table 16 below:

Results of the SPITFIRE questionnaires compared between 1st induced sputum (IS1) and bronchoscopy			
	IS1	**Bronchoscopy**	***P* value**
Patient reported anxiety, median (range)	2 (1-5)	5 (1-5)	0.020
Patient felt able to ask questions, median (range)	5 (5)	5 (1-5)	0.083
Patient reported quality of care, median (range)	5 (5)	5 (1-5)	0.317
Patient reported comfort, median (range)	5 (3-5)	5 (1-5)	0.655
Patient reported tolerability, median (range)	5 (4-5)	4 (1-5)	0.655
Patient happy to have procedure again, median (range)	5 (4-5)	4 (1-5)	0.025
Operator perception of tolerance, median (range)	5 (1-5)	5 (1-5)	0.059

Table 16: SPITFIRE questionnaire responses

When analysing all IS procedures, the operator-reported patient comfort at all IS procedures where a matching patient questionnaire was available (*n*=31) was a median of 5 (range 1-5) and the patients reported their tolerability at median 5 (range 3-5).

The distribution of scores associated with all first IS procedures and all bronchoscopies i.e. including those that are not paired, are summarised in the graph below (figure 11).

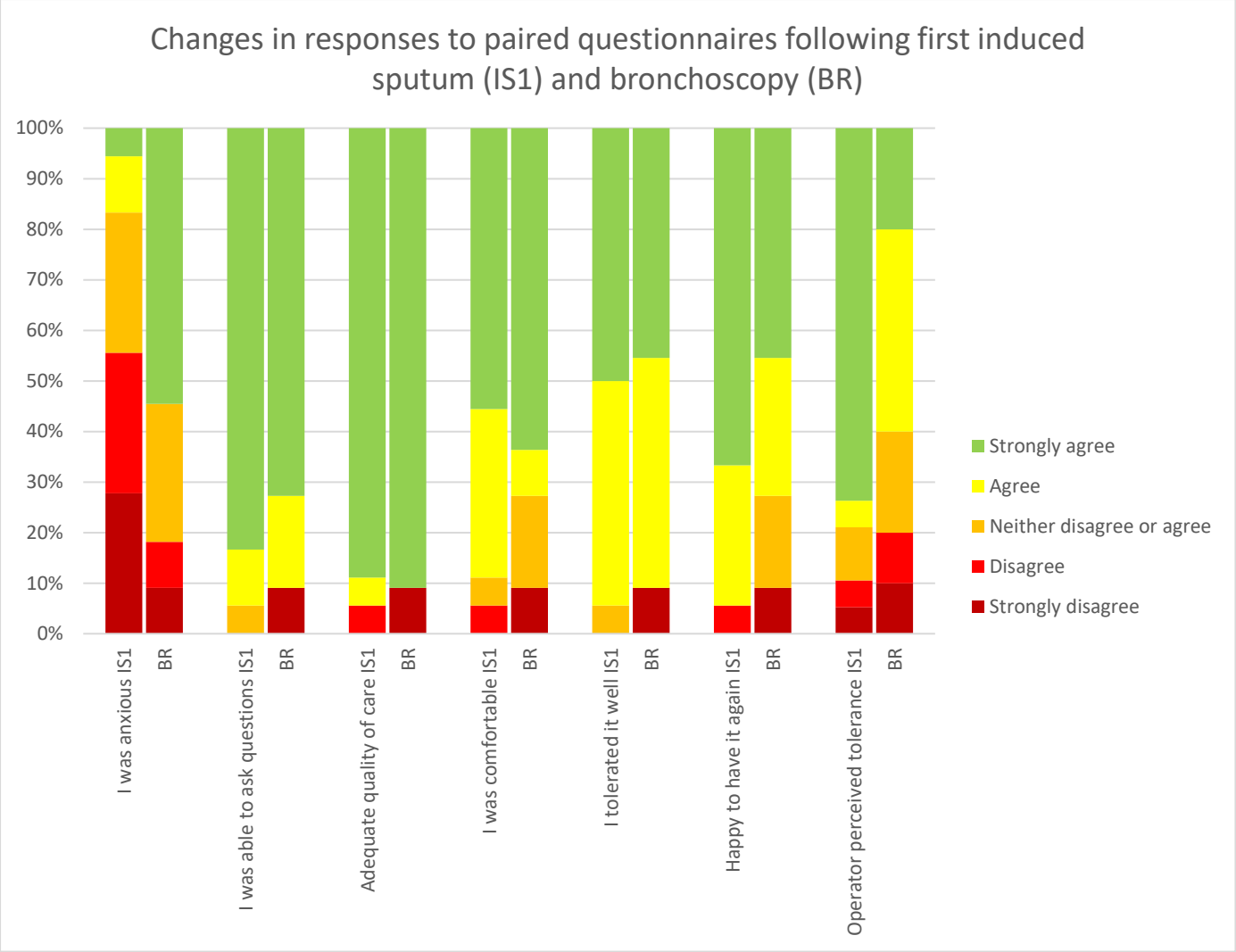


Figure 11: Comparing responses to paired SPITFIRE questionnaires

Nine patients had a recorded platelet count of less than $20x10^9$/L (range, 7-19) at time of enrolment which is the lower limit under which patients require transfusion for bronchoscopy according to British Thoracic Society (BTS) guidance (145). In cases where patients had a low platelet count, they did not receive platelets for the IS procedure. There were two adverse event reports of blood-stained sputum post procedure which was Common Terminology Criteria for Adverse Events (CTAE) grade 1 and did not require further intervention. There was one report of epistaxis which was CTAE grade 1 and did not require intervention and a CTAE grade 1 report of cough. On one occasion a patient in known atrial fibrillation had a faster ventricular response during the IS procedure; likely due to the administration of salbutamol. This was CTAE grade 1. Adverse events that occurred during IS

procedure were not only experienced by patients that were too unwell for bronchoscopy. In keeping with BTS guidelines, four patients with low platelet counts received appropriate platelet transfusion prior to bronchoscopy. There were no bleeding events during bronchoscopy. There was a cough adverse event that was grade 1 reported following bronchoscopy, a sore throat (grade 1) and three reported events of low oxygen saturations, all of which were CTAE grade 3 and limited the procedure.

5.3.4. Microbiology outcomes

All patients recruited into SPITFIRE had their baseline investigations recorded. An anonymised version of these results can be found in appendix I. From the 19 patients recruited into SPIRFIRE, 33 induced sputum procedures were undertaken with 27 (81.8%) yielding a sample. Two IS samples from the same patient suffered a transport processing error and so were not available to be analysed. Of those that were available to the laboratory, all (25, 100%) of the IS samples were suitable for microbiological and mycology analysis and produced an interpretable result. All 12 (100%) bronchoscopy procedures undertaken yielded a useable sample.

Nine patients had paired samples (i.e., at least one successful IS and one bronchoscopy). The radiology, serology and culture evidence of bacterial or fungal disease in each patient was reviewed alongside the bronchoscopy results to diagnose disease or infection. Four patients met the diagnostic criteria for probable invasive pulmonary aspergillosis. Two patients had *Pneumocystis jirovecii* pneumonia (PcP). One patient had radiological and biochemical features of pneumonia but no causative organism was identified and no atypical or fungal pathogens were suggested by serology or molecular investigations. In two patients, the bronchoscopy was negative for serological markers or culture. However, other culture and radiological features indicated different diagnoses (central venous catheter infection and tonsillar abscess) making the negative bronchoscopy results helpful in achieving this diagnosis by supporting the idea of radiological findings being inflammatory infiltrates rather than separate pathologies. A tabulated, anonymised list of these participant diagnoses and details of positivity at both induced sputum and bronchoscopy is found as appendix J.

There were no false positive PcP results from IS. The PCR cycle number at which they became positive (Cq values) of these patients are described in table 17, the multiple values represent the number of samples taken. The Cq threshold cut offs used here were the same as in chapter 3. In participant 9, the prior PcP throat swab taken was negative but the induced sputum results were both positive. The patient was considered at risk of PcP and presented with radiology typical of PcP (bilateral ground glass opacification) and the patient was treated with PcP-appropriate antibiotics for seven days prior to bronchoscopy and repeat IS sampling. Testing of bronchial washing samples was negative in bilateral upper lobes but positive on testing bilateral lower lobes, generating Cq values that were comparable to those for the repeat IS sample (Cq: 39 cycles). The Cq values generated indicate a likely reduction in *Pneumocystis* burden within the respiratory tract post initiation of therapy, but also indicate that the IS procedure samples across the lung parenchyma, including the lower lobes.

***Pneumocystis* PCR[i] result review**			
Participant	**IS1[ii] PCR Cq[iii] values**	**IS2[iv] PCR Cq values**	**Bronchoscopy PCR Cq values**
9	32	39	39/40
20	39	Negative	34/35/38/38

Table 17: Review of the two patients with positive PcP tests

[i] Polymerase chain reaction
[ii] Induced sputum 1
[iii] Quantification cycle
[iv] Induced sputum 2

As described in chapter 1, diagnosis of fungal disease often requires more than one test to indicate the presence of disease. Appropriate radiological findings in an at-risk patient in the context of positive microbiological tests is threshold needed, with diagnostic certainty labels ranging from proven to possible depending on the nature of the microbiological proof (161).

Aspergillus PCR positivity was seen with IS in 100% (4/4) of those diagnosed with probable invasive pulmonary aspergillosis using bronchoscopy, serology and radiology as seen in appendix J. However, perfect agreement between the two tests was not reached as three patients had false positive *Aspergillus* PCR results at IS. Here, IS positivity is declared in the presence of at least one positive in a patient's one or both IS results (where appropriate). This is further illustrated in table 18

***Aspergillus* PCR**[i]				
		Induced sputum		
		Aspergillus PCR positive, N[ii]	*Aspergillus* PCR negative, N	Total, N
Bronchoscopy	*Aspergillus* PCR positive, N	4	0	4
	Aspergillus PCR negative, N	3	9	12
	Total, N	7	9	16
81.25% agreement, Kappa 0.6; indicating moderate agreement				

Table 18: Comparing Aspergillus *PCR results between induced sputum and bronchoscopy*

[i]Polymerase chain reaction
[ii] Number

Of note, on three occasions an IS sample demonstrated a high galactomannan result (5, 3.5, >8) which was deemed to be spurious as it was not in keeping with the patient's clinical condition, not repeatable on further IS testing or did not correlate with bronchoscopy. The level of agreement with GM was lower. There was only one false negative IS result and is mostly due to a number of false positive results at IS. This is demonstrated in table 19.

Galactomannan				
		Induced sputum		
		Galactomannan positive, N[i]	Galactomannan negative, N	Total, N
Bronchoscopy	Galactomannan positive, N	6	1	7
	Galactomannan negative, N	4	5	9
	Total	10	6	16
68.75% agreement, Kappa 0.394; indicating fair agreement				

Table 19: Comparing Galactomannan results between induced sputum and bronchoscopy

[i] Number

There was strong agreement of fungal culture between the two tests. On four out of the sixteen occasions patients have fungal culture positivity from an IS procedure. This is compared to five out of sixteen occasions at bronchoscopy. This is further illustrated in table 20.

Fungal Culture				
		Induced sputum		
		Fungal culture positive, N[i]	Fungal culture negative, N	Total, N
Bronchoscopy	Fungal culture positive, N	4	1	5
	Fungal culture negative, N	0	11	11
	Total, N	4	12	16
93.75% agreement, Kappa 0.846; indicating almost perfect agreement				

Table 20: Comparing fungal culture results between induced sputum and bronchoscopy

[i] Number

Bacteriological culture was positive in 7/9 (77.8%) bronchial washing samples and 8/16 (50%) IS, supporting the clinical diagnosis on one occasion (table 21). The strong growth of *Pseudomonas aeruginosa* at bronchoscopy altered management in one patient but was not felt to be the causative organism in their deterioration. It was not detected in the same patient at IS but only one IS procedure was undertaken. The reverse was true in participant 5; an induced sputum procedure yielded a sample that grew *Pseudomonas aeruginosa* which was not replicated at bronchoscopy.

Microbiological culture		
Participant number	**Bronchoscopy microbiological culture result**	**Induced sputum microbiological culture result**
3	*Pseudomonas aeruginosa*	IS1[i]: Respiratory microflora
4	*Enterococcus faecium*	IS1: Negative IS2[ii]: Negative
5	Coagulase-negative staphylococci	IS1: *Pseudomonas aeruginosa* IS2: Respiratory microflora
6	Coagulase-negative staphylococci	IS1: Respiratory microflora IS2: Respiratory microflora
9	Coagulase-negative staphylococci	IS1: Negative IS2: Negative
13	Alpha-haemolytic streptococci	IS1: Respiratory microflora IS2: Respiratory microflora
14	Negative	IS1: Respiratory microflora IS2: Negative
20	*Escherichia coli*	IS1: Yeast IS2: Yeast

Table 21: Comparing bacterial culture results between induced sputum and bronchoscopy

[i] Induced sputum 1
[ii] Induced sputum 2

5.3.5. Cost analysis and future impact of IS on diagnostic pathways

As a feasibility study, SPITFIRE was not powered for microbiological outcomes. As such it is not appropriate to include detection rates in any assessment of the opportunity that IS presents to replace bronchoscopy in certain circumstances. The microbiological results above indicate that pathogen detection is achievable with this test and so for purposes of

calculations it was assumed that they are comparable whilst accepting bronchoscopy as the gold standard.

The aerobika and breath-actuated nebuliser used to perform the IS in the study was donated by Trudell Medical. Prices for this equipment was requested by their representatives. Costs for physiotherapy time was taken from the local clinical trial costing matrix (R. Norman, Personal Communication, July 2021). Costs for an inpatient bronchoscopy was obtained from the most recent local health board financial audit (2019,2020, pre-COVID). Recruitment numbers for the study period were used to estimate potential savings if IS procedures were implemented as early screening tests. Laboratory cost calculations were not included as they were the same for both IS and bronchoscopy but would be impactful if multiple IS procedures were needed per patient.

Some of the equipment is single use or is purchasable in bulk. Nebuliser machines are utilised on the ward and are not single use and so were not included in the cost analysis. The table below illustrates the cost of a single induced sputum procedure (table 22).

Induced sputum cost		
Component item	**Cost**	**Cost per procedure**
Physiotherapist time	£29.53 per hour	£14.77
Aerobika	£45.50	£45.50
Hypertonic saline	£27 per box of 30	£0.90
Breath actuated nebuliser chamber	£184.80 per box of 10	£18.48
Total		£79.65

Table 22: Calculating the cost per induced sputum procedure

This enabled a calculation of £79.65 per IS procedure. The bronchoscopy is costed in the 2019/2020 financial year as £584 each.

It was important to identify the annual burden of bronchoscopy or induced sputum requests that are likely to occur. SPITFIRE recruited between 16/8/21 and 10/6/22 (298 days/43

weeks). In addition to the 20 patients identified suitable for SPITFIRE, of which 19 were enrolled (and consent took place within 24 hours in all cases), a further five patients were referred to the respiratory team (time to see from referral was median 1 day, range 0-6) and one of these needed a bronchoscopy (time to complete 4 days). This is a total respiratory referral pathway cohort of 25 patients in 43 weeks (1 every 12 days or 31 annually), of whom bronchoscopy was indicated or consented for in 13 (52%). At present, inpatient bronchoscopy is undertaken in the site of the SPITFIRE study weekly so this reflects one haematology patient every three bronchoscopy lists.

From end of recruitment to end of December 2022 (203 days) there were 17 referrals (1 every 12 days, time to see was median 1 day, range 0-3). Of these 17 referrals, 6 (35.3%) had bronchoscopy (time from assessment/consent 4.5 days, range 0-11). The length of time from being seen to having a bronchoscopy is not significantly different from those in SPITFIRE and although there is a trend to taking longer than having an induced sputum, this did not reach significance (p=0.06).

In order to evaluate if this frequency of referrals outside of SPITFIRE and the rate of bronchoscopy requests is an accurate reflection of practice or a bias of SPITFIRE having been run recently locally, a one year period of respiratory referrals from haematology was evaluated (30/9/16-30/9/17). In this time there were 39 referrals (1 every 9 days). Seven referrals resulted in a bronchoscopy (17.9%) which was organised within a median of four days (range 1-11). This is significantly slower than SPITFIRE was able to obtain an IS(p=0.024) but not significantly different to the time taken to obtain a bronchoscopy during SPITFIRE or afterwards. There was no significant difference in the rate of bronchoscopy in this 2016-2017 window compared to local bronchoscopy rates after SPITFIRE but this was significant compared the bronchoscopy rates during SPITFIRE (p=0.004). This suggests that bronchoscopy was able to be arranged in a consistent time frame but that the number of patients that warranted a bronchoscopic evaluation varied over time.

Considering the above data and cost implications there are a number of ways in which IS utility could be modelled

1) All patients that met the criteria for bronchoscopy have a preceding IS. The annual referral rate from haematology was 39 in 2016-2017 and extrapolated to be 31

during SPITFIRE recruitment. The patients that were referred to the respiratory department that went on to have bronchoscopy is a wide range (17.9%-52%) and this is applied to provide upper and lower limits of a range. The reason for doing IS would be to provide an opportunity to obtain a sample and microbiological diagnosis in the four to five days that most patients are waiting for their bronchoscopy. This can also include a number of people who may well have been appropriate for bronchoscopy but refused or were too unwell. The acceptability work in SPITFIRE showed that seven patients (36.8%) had IS only. This can be extrapolated to estimate the total number of patients who have been referred who needed investigation.

2) Some patients were not appropriate for bronchoscopy for medical reasons or did not consent to take part in that part of SPITFIRE. Another way that IS could be used is by applying this test only to individuals that are unable to have a bronchoscopy. This would allow an opportunity for otherwise unavailable microbiological testing.
3) There was good correlation between the microbiological outcomes of IS and bronchoscopy. As such, if microbiological diagnosis is possible through a successful IS procedure, confirmatory testing through a second IS procedure would be financially preferable than to continue to bronchoscopy. As such, bronchoscopy could be reserved only for those that are unable to produce a sputum sample. During SPITFIRE, patients were consistent in their ability or inability to produce a sample. Therefore, the result that 78.9% of participants were successful in sputum production was used to identify how many bronchoscopies would be required. It was assumed that these individuals do not cross over with the group that would be too unwell to have a bronchoscopy but in reality there is likely to be some cross over which would need to be explored.
4) The above point using IS as a complete replacement of bronchoscopy is unrealistic as there is not enough evidence that the pathogen-detection rate of IS is comparable enough with bronchoscopy. However, the assumption that IS can achieve a microbiological diagnosis faster than bronchoscopy, negating the need for this test, is a dramatic improvement in the time lost to diagnosis.
5) There is a potential to use IS in an even more targeted way; as a screening tool for aspergillosis through routinely testing at-risk individuals in the same way that a diagnostics-driven approach investigates the potential for fungal infection in the

immunocompromised (142). It is assumed that there will be a significant increase in IS procedures done. For the purposes of these calculations, it is assumed that each allograft bone marrow transplant patient in the local transplant centre performed weekly IS procedures for the first month after their transplant which is the usual time that they take to engraft and thus at highest risk of infection. In 2017-2019, a mean of 52 patients underwent allogeneic stem cell transplantation locally. This would equate to 208 IS procedures for screening alone. This could be further targeted if only those patients with febrile neutropenia are screened but the proportion of this group that meet that criteria is unknown. In SPITFIRE, the detection of aspergillosis showed good correlation between IS and bronchoscopy and the local calculated incidence of pulmonary or invasive aspergillosis in this cohort (9.7%, 5 patients) was taken to be the number of bronchoscopies which could be replaced by IS procedures. These patients are likely to be unwell and so two IS procedures were assumed to have taken place at the point that infection was detected. Invasive fungal disease is life threatening and patients are more likely to be more frail on account of their illness so the 18% chance that patients are unable to produce a sputum sample and so needed a bronchoscopy was also considered. This number was between 0-1 so the provision for one further bronchoscopy was included.

These opportunities are described in table 23 below

Cost analysis of variations of induced sputum (IS) utilised alongside bronchoscopy (BR)							
Utilisation of tests	**IS, N**	**BR, N**	**Total procedures, N**	**IS cost, £**	**BR cost, £**	**Total cost, £**	**Cost difference, £**
Lower control (100% BR)	0	5	5	0	2,920	2,920	-
Upper control (100% BR)	0	21	21	0	12,264	12,264	-
100% IS +36% unsuitable for BR + 100% BR (lower)	6	5	11	477.90	2,920	3,397.9	+477.90
100% IS +36% unsuitable for BR + 100% BR (upper)	29	21	50	2,309.85	12,264	14,573.85	+2,309.85
36% IS + 100% BR (lower)	1	5	6	79.65	2,920	2,999.65	+79.65
36% IS + 100% BR (upper)	8	21	29	637.20	12,264	12,901.20	+637.20
100%+36% IS x2 + 21% BR (unable to IS) (lower)	12	1	13	955.80	584	1,539.80	-1,380.20
100%+36% IS x2 + 21% BR (unable to IS) (upper)	58	5	63	4,619.70	2,920	7,539.70	-4,724.30
100%+36% IS x2 + 50% BR (lower)	12	2	14	955.80	1,168	2,130.80	-789.20
100%+36% IS x2 + 50% BR (upper)	58	11	69	4,619.70	6,424	11,043.70	-1,220.30
Targeted transplant patients with screening IS and remainder BR (lower)	213	1	214	16,965.45	584	17,549.45	+14,629.45
Targeted transplant patients with screening IS and remainder BR (upper)	213	17	230	16,965.45	9,928	26,893.45	+14,629.45

Table 23: Costs of variations of number of induced sputum procedures (IS) and bronchoscopies (BR) performed. The number of procedures is determined by analysis of patterns of referrals for bronchoscopy by haematology locally where the upper and lower limits are defined by the minimum and maximum number likely to be referred.

5.4 Discussion

As part of the study, participants were able to have their bronchoscopy organised in a timely manner. However, induced sputum is a procedure that was regularly performed locally and in a more timely manner than could have been achieved with bronchoscopy. This shows promise for its application as a screening tool for the at-risk unwell patient. The high chance of successful sampling (78.9%) per patient, highlighting the potential for re-sampling at a level of ease superior to bronchoscopy. The duration of the IS procedure was longer than bronchoscopy but this calculation was made from the start of the procedure i.e. moment of bronchoscopy intubation or saline nebulisation, so would not take into account the transport time from the ward to endoscopy unit, preparation and sedation delivery and recovery. It is likely that being able to perform the IS procedure at the patient's bedside drastically reduced the comparative time but this was not fully recorded.

Patients were consistent in their ability to produce a sputum sample during the IS procedure i.e. On no occasion did a patient who had produced a sample at the first induced sputum procedure (IS1) go on to later fail to produce a sample at the second induced sputum procedure (IS2), excepting those who exited the study for reasons listed previously. . This suggests that the success of the test in producing a sample is not operator dependent but rather a reflection either of the patient's disease or their current clinical state. Each procedure can be measured in isolation as being successful and unsuccessful as a measure of procedural success but this risks pseudoreplication of patients as patients are likely to succeed repeatedly or fail and be too unwell to repeat the test.

There is a difference which did not reach significance in the median National Early Warning Score (NEWS) per patient suggesting that the more unwell patients are less likely to be able to produce a sample. There may be complex relationship between these factors as features of the NEWS score (respiratory rate, oxygen requirement and heart rate) could not individually predict success or failure of the IS procedure. There was a signal that heart rate was more likely to be elevated in the group that was unable to produce a sample. However, there is a confounding relationship with heart rate; the propensity of the more unwell patient to go into atrial fibrillation with a rapid ventricular response as a result of their

infection and therefore have both a high heart rate and be too unwell to produce a sample. Frailty could be a predictor of ability to perform the test as the difference between the successful group (median 3) and unsuccessful group (median 6.5) was significant but the number of unsuccessful patients is limited. In addition, the range of frailty in these two groups overlaps, which suggests that a future model predicting procedure success will need to rely upon multiple measurable features. The measurement of frailty may need to be reviewed as clinical frailty scores are often estimations based on first impression by a clinician and will likely vary according to the clinician's own inherent biases, despite visual guides accompanying the clinical frailty score indicator.

Another predictor of procedural success could also be patient position. It is likely that this is related to frailty or NEWS score, as a more frail or more unwell patient is less likely to be able to sit in a chair to have the procedure or even sit on the edge of the bed. When the successful and unsuccessful groups are divided by the position the test was performed there is a suggestion that being able to sit up to do the test is more likely to lead to success but this also was not statistically significant. Future work could look at these possible predictors of success in more detail and gather more information at time of procedure. A principle component analysis could be undertaken to understand the relationship between various suggested factors that may lead to an unsuccessful procedure. A model can then be suggested for future work to expand upon to generate a pre-test probability score of success.

A concern prior to the initiation of the study was that the hypertonic saline could cause bronchospasm which could be problematic for these patients who are already unwell, some of whom are also on oxygen. However, regardless of inspirated oxygen percentage, patients desaturated more during bronchoscopy than during IS and this was to the extent that three patients had their procedure limited during bronchoscopy due to safety concerns. None of the IS procedures required additional oxygen usage.

Platelets are used during bronchoscopy in those that are thrombocytopenic. Platelets have a financial cost which could be included in any future more comprehensive cost analysis but their value comes also from their status as a limited human tissue product and so any procedure that offers an opportunity to reduce reliance upon them should be explored. Despite severe thrombocytopenia ($<20x10^9/L$) in nine individuals, no significant bleeding

events took place in the induced sputum group and no platelet transfusions were required. Usual respiratory physiotherapy could also use percussive techniques to enhance the possibility of sputum production and this was not undertaken in this study. Later work could review this possibility as an optional add on but only in patients with an appropriate platelet count and adequate coagulation.

There is a signal of good concordance between IS and bronchial washing, particularly with PcP and *Aspergillus* testing. Any loss of agreement is mostly through false positivity with IS; likely as a result of either mouth commensal contamination or artefact from a handling error at time of collection. Given that the polysaccharide galactomannan is present in many organisms, it is understandable that this is detected more in a potentially contaminated IS sample than the more specific *Aspergillus* PCR. The patient who tested negative on throat swab for PcP but positive at IS demonstrates that IS is a good representation of the yield of the whole lung. This is reinforced by variable PcP PCR results when testing BAL fluids, with PcP PCR negative upper lobes but PcP PCR positive lower lobes.

Bacterial analysis shows success of bacterial culture in the samples with a suggestion of parity between IS and BAL. However, there are a number of examples in which there is evidence of artefactual contamination at IS which Is likely related to handling or because the sample must pass through the mouth and may be contaminated with oral commensals. *Pseudomonas aeruginosa* is a pathogen with a high impact on morbidity and mortality in those with airways disease in particular (162,163). A positive *P.aeruginosa* result at bronchoscopy that was not replicated in IS in one patient and vice versa in a different patient is a cause for concern for the reliability of the test.

The presence of the SPITFIRE investigator team did not significantly alter the rate of referrals to respiratory but likely influenced the number of bronchoscopy requests. This higher rate of bronchoscopy has persisted following SPITFIRE's closure to recruitment but was not significantly from the prior baseline. This rate of requirement for microbiological investigations in the unwell immunocompromised patient is likely to rise. There is a great appreciation for fungal organisms as pathogens in this unwell cohort and with more complex chemotherapy and transplant regimes available.

IS is much cheaper to perform than bronchoscopy. Cost effectiveness analysis is not appropriate in a small study but there is an opportunity to reduce the number of expensive bronchoscopies in an inpatient setting in favour of IS in selected patients although the best placed role of IS is currently unclear. In reality, it would not be appropriate to replace bronchoscopy with induced sputum, however the microbiological outcomes from SPITFIRE support the idea of confirmatory testing with a second IS procedure which still have the potential to streamline the diagnostic pathway for these patients as they may not require a bronchoscopy. A diagnostic pathway that is suggested by these results is demonstrated in the below figure:

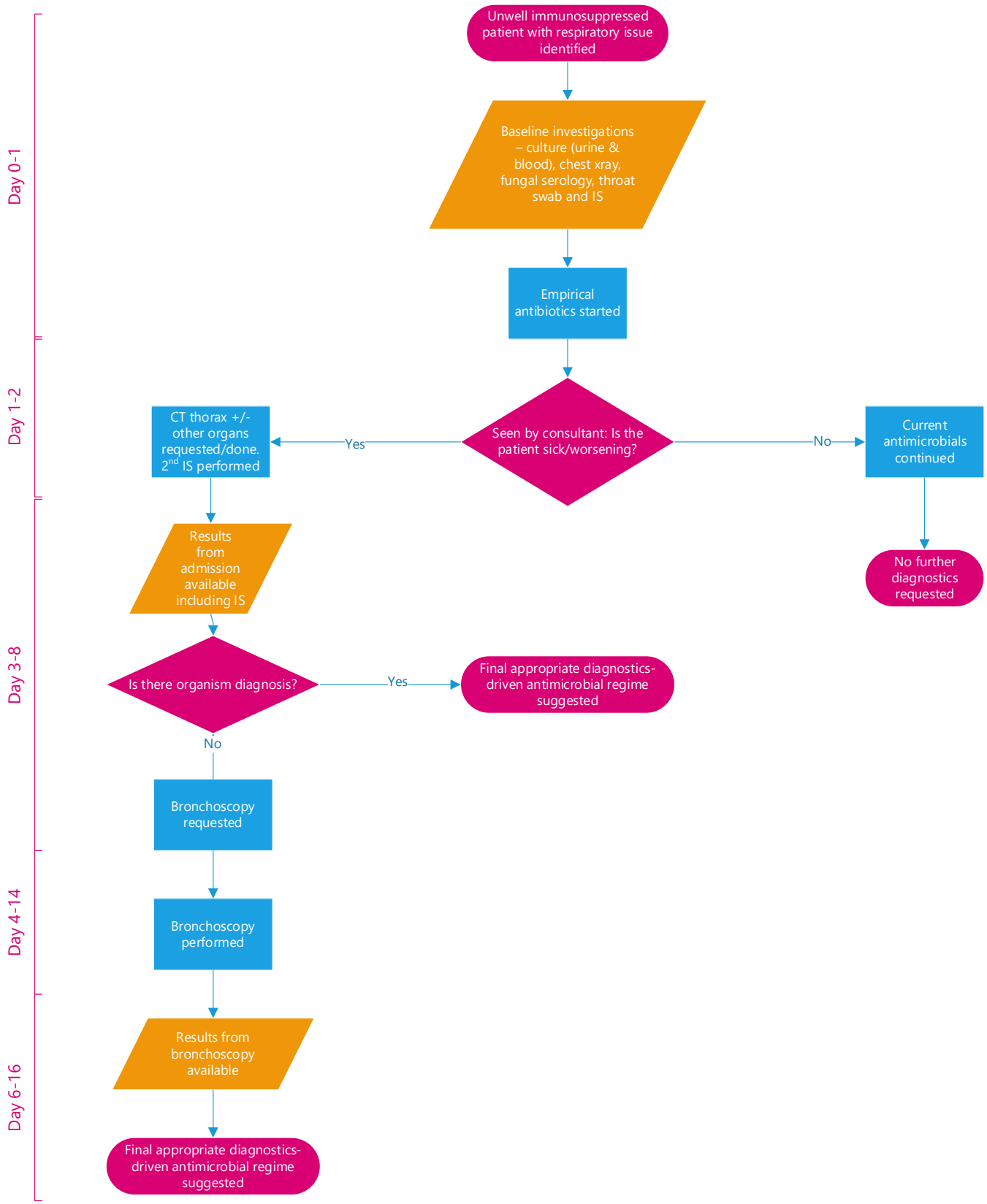


Figure 12: Suggested adaptation to the current diagnostic pathway

In this particular example, IS is utilised early in the identified at-risk patient and increases the likelihood at Day 6-8 that diagnostics-driven antimicrobial therapy can be used rather

than waiting for the results of a bronchoscopy at Day 6-16. That said, the pathway synergistically uses other tests as needed (including bronchoscopy) in order to reduce a potential delay in diagnosis. The acceptability data shows that not all patients will be able to produce a sputum sample and not all patients will consent to bronchoscopy which provides a much more variable utility model which is able to be tested in further work.

5.5. Summary and conclusion

IS has potential as a procedure that can be used in the context of the unwell, immunocompromised haematology patient for the swift investigation of potential respiratory infection. It is not as effective as bronchoscopy at producing a viable sample in the more frail or unwell patient but has several advantages over bronchoscopy such as time to organise procedure, lack of additional blood products, patient reported comfort, a lack of patient reported anxiety and the feasibility and willingness to replicate the test if needed. There is evidence of microbiological parity but the variability of some markers and the variation in bacterial yield suggests that a larger study could be performed to investigate its use as an early investigation tool in the identified at risk individual rather than as a replacement test for bronchoscopy.

Chapter 6 – Discovering new impacts of fungal disease: *Exophiala dermatitidis* in the respiratory tract of cystic fibrosis patients accelerates lung function decline

Adapted from publication in Journal of Fungi, April 2022:

Ayling-Smith J., Speight L., Dhillon R., Backx M., White P.L., Hood K., Duckers J. The Presence of *Exophiala dermatitidis* in the Respiratory Tract of Cystic Fibrosis Patients Accelerates Lung Function Decline: A Retrospective Review of Lung Function. J. Fungi. 2022 Apr 7;8(4):376. doi: 10.3390/jof8040376

6.1. Introduction

Diagnostic challenges are not unique to the task of obtaining appropriate samples as described in the SPITFIRE study and how best to interpret the result obtained can also be difficult. Patients with cystic fibrosis (CF) are at an increased risk of pulmonary colonisation by opportunistic microorganisms. These microorganisms include the thermophilic black yeast *Exophiala dermatitidis*, which is isolated in 1-16% of the bronchial secretions of patients with CF (164,165). *E.dermatitidis* rarely causes infection in the immunocompetent patient but is more prevalent in the immunocompromised and has been identified as both a commensal bystander and important cause of invasive disease (164). Aside from superficial skin infection, the respiratory system is the commonest site of *E.dermatitidis* infection (166). Despite this, the pathological mechanism of respiratory infection remains unclear. In the CF lung in particular, it is likely that the nutrient-rich, heavy mucous burden within the lung coupled with inadequate mucociliary clearance promotes fungal growth whilst the ongoing use of broad-spectrum antimicrobials may positively select fungi through the impairment of the growth of their competitors (167). However, the variability of morbidity associated with this organism in both the immunocompromised and immunocompetent patient suggests that there is a more complex interaction of virulence factors, risk factors and host responses at play than wholly the pulmonary-specific CF defects (166). It is likely that the mode of infection is through respiratory inhalation of this ubiquitous organism. Man-made humid and hot habitats have a far higher isolation rate than the natural environment and therefore

it has been further postulated that aerosolization of the organism through hot equipment such as dishwashers or saunas may be a route of transmission (164,168).

Despite the growing body of evidence of the importance of fungi, there is no current consensus on the clinical management of the CF patient where *E.dermatitidis* has been isolated from the respiratory tract (167). When deciding to treat *E.dermatitidis*, it is important to balance the potential risk/benefits of treatment initiation. The azole family of antifungals is often selected as first line therapy but have considerable side effects and drug-drug interactions, including interactions with cystic fibrosis transmembrane conductance regulator (CFTR) modulators, leading to a requirement for therapeutic drug monitoring. However, balancing the risk against the potential benefits of treatment is difficult when there is limited evidence on the clinical consequences of *E.dermatitidis* presence in the CF airways.

Despite treatment advances, lung disease remains the major cause of mortality and morbidity in CF. As such, strategies to minimize pulmonary exacerbations and maintain lung health remain of paramount importance. Percent predicted forced expiratory volume in one second (FEV1%) is routinely used by clinical teams and registries to monitor clinical stability, stratify disease severity and assess treatment outcomes (169). The rate of lung function decline, determined by comparison of FEV1% values over time, is perhaps an even more relevant and better predictor of clinical deterioration (170). Prior studies have suggested that *E.dermatitidis* does not contribute to lung function decline but patient numbers were limited and the length of follow up was short at usually one or two years maximum (171,172). The primary objective of this current study was to assess if the presence of *E.dermatitidis* in respiratory cultures impacted on the trajectory of lung function decline of CF patients, which would help better inform the decision whether to initiate treatment targeting *E.dermatitidis* in future patients.

6.2. Study population and methods

A service evaluation in the form of a single-centre retrospective case-control study was performed using data gained as part of routine clinical practice with no impact on patient management. Adult patients with CF receiving care at the All Wales Adult CF Centre from

whom *E.dermatitidis* had been cultured from the respiratory tract was compiled from retrospective review of routinely collected clinical data to generate the case population. They were compared with a randomly selected control group of *E.dermatitidis*-negative CF patients managed over the same period at the same CF centre. The control group was chosen to be twice the size of the proven case group and potential confounding factors such as age, CF-genetics, body mass index (BMI) and pancreatic sufficiency status were recorded to ensure the two groups were comparable.

The lung function data in the form of forced expiratory volume in 1 second (FEV1) was documented using clinic letters and local spirometry databases. With data collected over several years, calculations to derive the percentage-predicted FEV1 will likely have varied as population-based algorithms are updated so the actual FEV1 was documented and all percentage-predicted measurements were individually recalculated using a single algorithm.

Patient data was included up to lung transplantation, starting CFTR modulator therapy (including clinical trials), moving out of area or death. A historical limit of 2007 or one year of paediatric data before coming under the care of the adult CF team (whichever was later) was used as, prior to 2007, the availability of local lung function data was variable and would likely introduce significant numbers of paediatric measurements making predicted FEV1 data unreliable due to significant changes in height over a short period of time and ability to perform repeatable testing in much younger patients (173). Patients with less than 48 months of continuous data available were also excluded, to allow for two calculations of annual lung function decline per patient; one prior to *E.dermatitidis* infection and one following.

The rate of decline in lung function in each patient prior to infection with *E.dermatitidis* was calculated as FEV1%/year by finding the difference between the mean of all FEV1% from the earliest 12 month period on record and the mean FEV1% from the most recent 12 month period, ending at the date *E.dermatitidis* was first isolated, divided by the time studied. A second “post-infection” rate of decline was calculated in the same patient in a similar manner using the means of the FEV1% measurements for the immediate 12 months after infection and the most recent 12 months, subject to the limitations described above (transplant, CFTR use etc). The control group rate of decline was calculated as FEV1%/year, documenting the difference between the mean of all FEV1% from the earliest 12-month

period on record and the mean of the FEV1% from the most recent 12-month period, subject to the same limitations as before. This difference in each patient's paired means was divided by the time studied (in months) multiplied by 12 to achieve a FEV1% change/year. The rates of change of all the patients were pooled by finding a mean decline to give a control group rate of decline.

All respiratory cultures conducted over the period of interest from both groups were reviewed in order to establish if growth of non-tuberculous *Mycobacterium*, *Aspergillus fumigatus* or *Pseudomonas aeruginosa* was documented and potentially associated with FEV1% decline. Patients were recorded as being positive or negative for these organisms at the start and end date of data collection and "point of infection" in the *E.dermatitidis* group. This positive status was determined by at least one isolation culture. The control group was subject to the same patient and data selection limitations as the *E.dermatitidis* group

All of the data was collated in a password protected database stored on a hospital networked PC and analysed using Microsoft Excel 2016 and IBM SPSS statistics 26.

Patients with a positive sputum sample for *E.dermatitidis* were compared with the control group. Clinical, demographic and microbiological characteristics were compared. The *E.dermatitidis* group (pre infection) and the control group were compared to see if patients were similar in demographics and their lung function declined at the rate that was similar to the control group. The pre and post infection time points in the *E.dermatitidis* group were then compared to assess if they underwent a significant change after infection including their rates of decline. Demographic, microbiological and baseline clinical characteristic data were compared by way of T-tests for continuous variables, Chi-2 for binary variables and Fisher's exact for binary variables with less than 10 cases. A Poisson regression model was utilised to provide a relative risk ratio for a participant belonging to the E.dermatitidis group. Similar tests were used when comparing within the *E.dermatitidis* group pre and post infection except a paired T-test was used for continuous variables and McNemar for binary variables. A p value of <0.05 was considered significant.

6.3. Results

From the 306 adult CF patients managed by the CF unit over the given period, a total of 61 patients had *Exophiala dermatitidis* cultured from the respiratory tract. This is an incidence of 19.9% over the study period. After exclusion criteria were applied, 31 *E.dermatitidis* positive patients remained and subsequently the control comprised 62 patients. The control group and *E.dermatitidis* group were comparable in regards to gender, CF genotype, proportion that were pancreatic sufficient and amount of time studied. The demographics of this are shown in table 24 and the relative risk of belonging to the *E.dermatitidis* group is illustrated in the forest plot (figure 13). There was a slight, but statistically significant, age difference between the two groups at the start of data collection.

Clinical characteristics of the *E.dermatitidis* and control groups			
Parameter	Population		
	E.dermatitidis group (*n*=31)	Control group (*n*=62)	*P* value
Sex, male *N*[i](%)	16 (52)	36 (58)	0.555
Mean age at start of data collection (SD[ii])	20 (7.92)	25 (10.5)	0.033
Mean age at date of positivity (SD)	24 (9.24)		
Mean BMI[iii] at start of data collection kg/m^2 (SD)	20.8 (2.85)	22.0 (3.30)	0.113
Mean BMI at end of data collection kg/m^2 (SD)	21.9 (2.92)	23.0 (4.12)	0.167
Genotype: delta-F508 homozygous *N(*%)	14 (45.1)	28 (45.1)	1.000
Pancreatic insufficient *N(*%)	28 (90.3)	57 (91.9)	0.79
Mean study period, months (SD)	97.8 (33.5)	102.6 (41.2)	0.580
Mean FEV1%[iv] at start of data collection (SD)	71.4 (17.5)	69.2 (21.3)	0.629
Mean FEV1% change/year (SD)	-0.34 (2.38)	-0.82 (1.36)	0.212
Microorganisms at start of data			
Nontuberculous mycobacteria *N*(%)	4 (12.9)	4 (6.5)	0.43
***Aspergillus fumigatus N*(%)**	4 (12.9)	9 (14.5)	1.00
***Pseudomonas aeruginosa N*(%)**	26 (83.9)	47 (75.8)	0.43
Microorganisms at end of data			
Non-tuberculous mycobacteria *N*(%)	10 (32.2)	14 (22.6)	0.33
***Aspergillus fumigatus N*(%)**	23 (74.2)	26 (41.9)	0.004
***Pseudomonas aeruginosa* N(%)**	25 (80.6)	44 (71.0)	0.45

Table 24: Clinical characteristics of E.dermatitidis group and control group

[i] Number
[ii] Standard deviation
[iii] Body mass index
[iv] Forced expiratory volume in 1 second

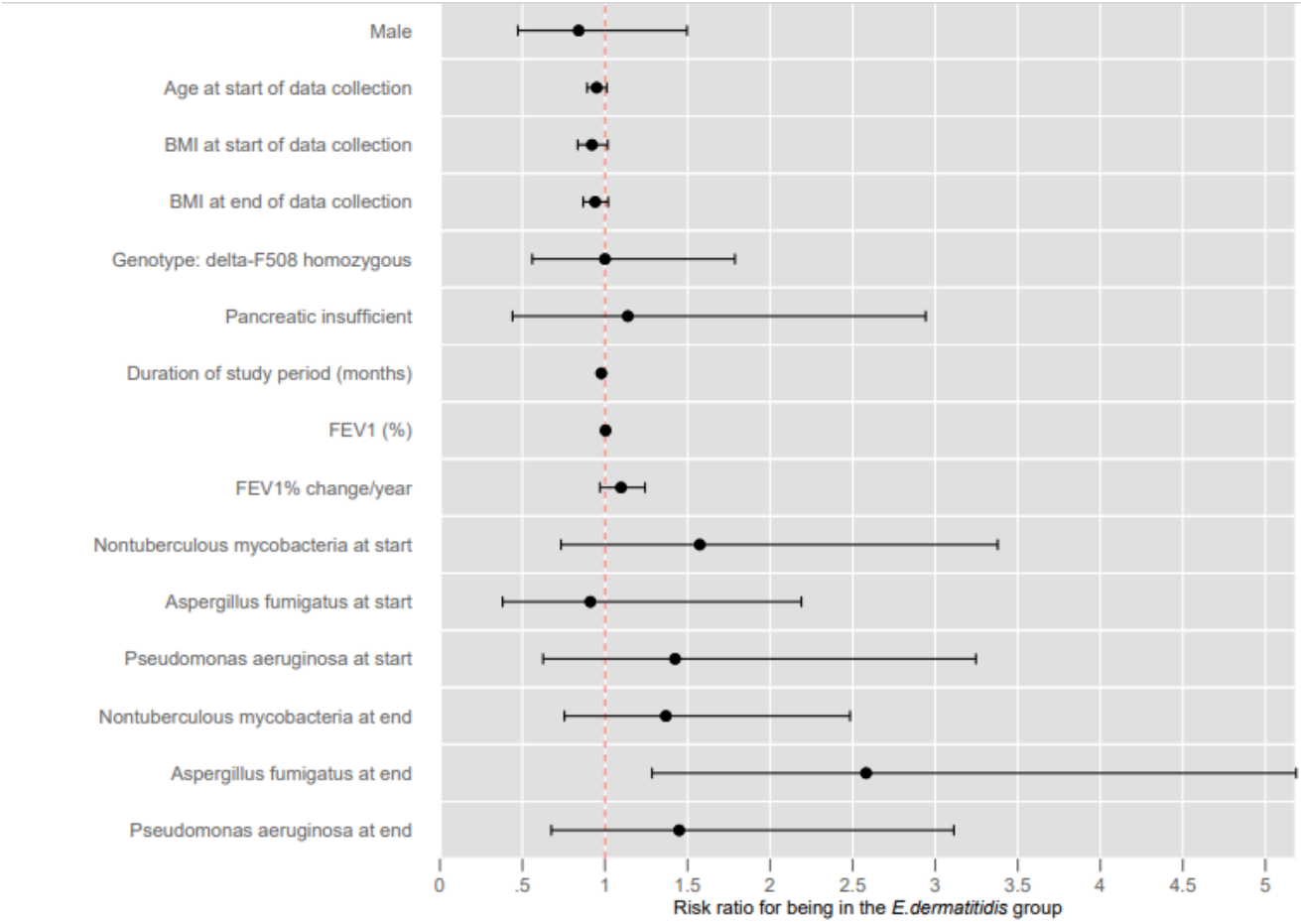


Figure 13: Relative risks of each clinic factor for a participant belonging to the E.dermatitidis group compared to control

A total of 1840 lung function measurements were reviewed and predicted FEV1% recalculated, from the 62 control patients at a mean of 15 measurements per patient. The average time between the first measurement recorded and most recent (subject to exclusion criteria) was 102.6 months. A total of 884 lung function measurements were recorded for the 32 patients in the *E.dermatitidis* group, which is 28.5 per patient, over an average time period of 97.8 months. Pre-infection with *E.dermatitidis* there were 365 measurements, at a mean of 11.7 per patient, over 51.9 months and post-infection there were 519 measurements, at a mean of 16.7 per patient, over 45.9 months. Their clinical characteristics before and after infection are tabulated below in table 25.

Clinical characteristics before and after infection			
Parameter	*E.dermatitidis* group (pre-infection) (*n*=31)	*E.dermatitidis* group (post-infection)(*n*=31)	*P* value
Mean study period per patient in Months (SD[i])	51.9 (29.5)	45.9 (18.7)	0.36
Mean BMI[ii] change per year kg/m2/year (SD)	0.34 (1.75)	-0.02 (0.44)	0.011
Mean FEV1%[iii] change per year (SD)	-0.34 (2.38)	-1.82 (2.35)	0.005
Microorganisms			
Non-tuberculous mycobacteria N[iv](%)	4 (6.5)	10 (32.2)	0.250
***Aspergillus fumigatus* N(%)**	9 (14.5)	23 (74.2)	0.022
***Pseudomonas aeruginosa* N(%)**	47 (75.8)	25 (80.1)	1.00

Table 25: Clinical characteristics of the E.dermatitidis group before and after isolation

[i] Standard deviation
[ii] Body mass index
[iii] Forced expiratory volume in 1 second
[iv] Number

The pooled rate of decline of the control group was -0.82%/year (S.D 1.36) was not statistically different (*p*=0.212) from the rate of decline in the *E.dermatitidis* group, prior to positive culture (-0.34%/year, S.D 2.38). However, there was a significant difference (p=0.003) in rate of decline between the *E.dermatitidis* group pre and post first isolation (-0.34%/year compared to -1.82%/year, S.D 2.35), seen in figure 14. The rate of decline between the control group was also significantly different to the *E.dermatitidis* group post-infection (*p*=0.011), seen in Table 20. This is also worse than the UK national average CF lung function decline.

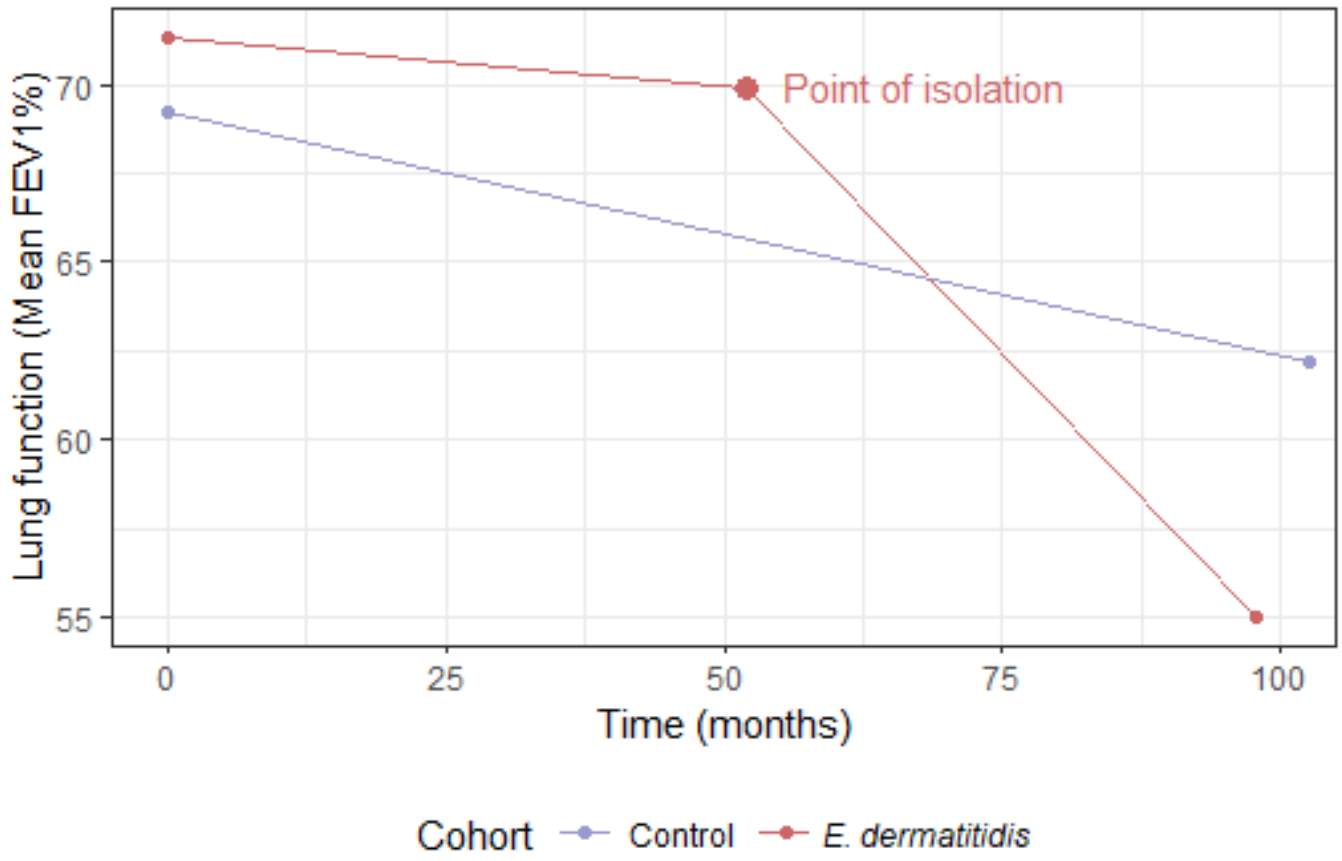


Figure 14: Lung function decline before and after infection in E.dermatitis group compared with lung function decline in control group

The BMI measurements of the control group and the *E.dermatitidis* group were not significantly different at the start of the data collection but there was a significant increase in rate of decline of BMI post *E.dermatitidis* infection (*p*=0.011). Further analysis in comparing the endpoint BMI of the two groups did not show a significant difference (23.0, S.D. 4.12 in control group compared to 21.9, S.D. 2.92 in the *E.dermatitidis* group).

The *E.dermatitidis* group and the control group were comparable in rates of positive respiratory culture for non-tuberculous *Mycobacterium* at the start (*p*=0.434) and at the end (p=0.326) of the data collection. This was also the case for *Pseudomonas aeruginosa* culture (p=0.433 and p=0.451). Rates of *Aspergillus fumigatus* positive culture were comparable at the start of the data (*p*=1.00). However, when comparing study end data, whilst rates of *A.fumigatus* increased in the control population, they increased significantly more in the *E.dermatitidis* group (*p*=0.004).

Eighteen control patients (29%) changed from *Aspergillus* culture negative to positive during the data review. In the *E.dermatitidis* group 20 patients (64.5%) changed *Aspergillus* culture status, representing significant increase in the recovery of *Aspergillus* (*p*=0.001). Further analysis showed that in the *E.dermatitidis* group, 12 (38.7%) changed *Aspergillus* status before *E.dermatitidis* growth and 11 (35.5%) after they had grown *E.dermatitidis*, suggesting that the presence of *E.dermatitidis* in the respiratory tract does not promote the presence of *Aspergillus* (*p*=0.157).

Further subgroup analysis was undertaken to understand if *Aspergillus* positivity was driving the lung function decline. The rates of decline after *E.dermatitidis* infection in those with negative *A.fumigatus* culture (n=6) were compared to patients with positive Aspergillus culture (*n*=11). Patients with persistent recovery of *Aspergillus* were excluded. The amount of time measured in each group was not statistically different. The rates of decline were not significantly different with a rate of -1.64 (SD 2.31) in the *A.fumigatus* positive subgroup and a rate of -1.71 (SD 2.33) in the negative subgroup (*p*=0.92).

Further subgroup analysis was done to establish if the presence of antifungals affected the rate of decline. The *E.dermatitidis* group were evaluated for all antifungal prescriptions issued to them and divided into an antifungal negative group if they had never received a prescription for antifungals or an antifungal group if they were issued with an antifungal at any time after testing positive for *E.dermatitidis* up to the end point of their data collection subject to the prior limits of data.. In the *E.dermatitidis* group 14 individuals were given antifungals and their rate of decline was -1.47% per year (SD 3.26) over an average of 45.0 months (SD 19.1), 17 individuals did not receive antifungals and they declined at a rate of -1.77% per year (SD 3.19) over an average of 45.9 months (SD 18.7). Even though the rate of decline if given antifungals appears to be slower, there was no significant difference between the two groups (*p*=0.73). This is also illustrated in figure 15 below:

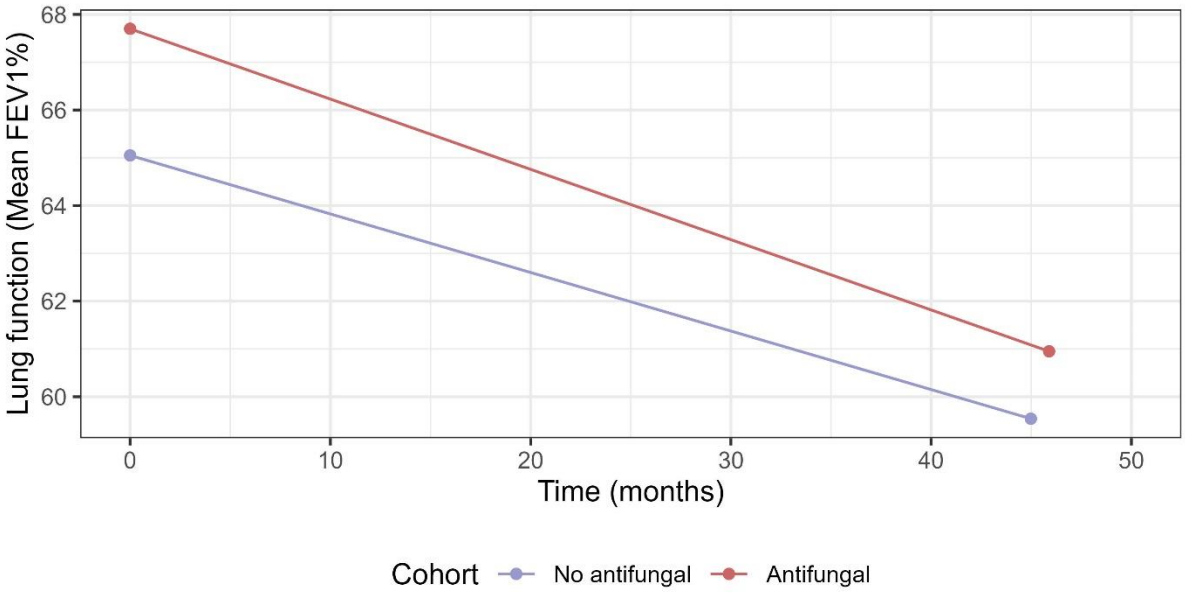


Figure 15: Lung function decline in the E.dermatitidis group separated into those that received antifungals and those that did not

6.4. Discussion

The results indicate an increased rate of lung function decline after isolation of *E.dermatitidis* in the CF lung. At present it is not clear if this enhanced rate of lung function decline is cause or effect of isolating *E.dermatitidis*. In this current study, the relationship with *A.fumigatus* is unclear, but the presence of *E.dermatitidis* within the respiratory tract appears to increase the recovery rates of *A.fumigatus*, but the presence of *A. fumigatus* was not necessarily associated with decline in lung function. Previous work suggesting that *E.dermatitidis* could be a colonising bystander of a dysregulated airway could be supported by this study but the temporal nature of the isolation and rate of decline makes this less likely (164). Previous research described an association between the recovery of *E.dermatitidis* and that of *Aspergillus fumigatus* (171). This may be related to the hypothesis that increased use of broad-spectrum antibiotics in the unwell CF patient positively selects all fungi in the respiratory tract and the presence of both *A.fumigatus* and *E.dermatitidis* could be a marker of CF disease progression (55,165).

However, the presence of *A.fumigatus* in the CF respiratory tract is unlikely to be solely responsible for the decline observed in this study. Individuals from whom *A.fumigatus* was recovered prior to the presence of *E.dermatitidis* did not show significant decline in lung function. The limited difference between rates of *Aspergillus* growth before and after *E.dermatitidis* isolation implies that *E.dermatitidis* itself does not make the individual more susceptible to *Aspergillus*. The significant difference in the recovery of *Aspergillus* in control and *E.dermatitidis* groups at the end of the study period implies that both *E.dermatitidis* and *A.fumigatus* are both markers of CF disease progression or have a synergistic affect. This is also supported by the antifungal subgroup analysis; that the *E.dermatitidis* group did not decline in lung function slower on antifungals suggests that *E.dermatitidis* presence is a signal of severe disease rather than driving the deterioration. However, the numbers of individuals included in the subgroup analysis was small.

There is a significant signal that *E.dermatitidis* isolation may be associated with BMI decline, which is not translated into a significant difference in the end measurement of BMI so is unlikely to be a further feature of CF disease progression but rather may be a confounder of the anorexia experienced by the unwell patient during exacerbation; either due to the infection itself of the antimicrobials they will be receiving. The prescription of antifungals was only measured in the *E.dermatitidis* group before and after isolation and, whilst the difference in decline was not significant, the numbers in this subgroup analysis was small. In addition, an increase in these drugs commonly associated with anorexia and nausea could explain the apparent temporary BMI decline that did not translate to significant weight loss.

This study reviews patients with an uncommon condition from which a rare pathogen was isolated from their respiratory tract and as such the numbers in each group are lower in this single centre compared with other published works (171). A total of 7 patients (22.6%) had paediatric readings in the *E.dermatitidis* group. Removing them would have made the mean age 21.8 which would have been comparable to the control group (p=0.242). However, the decision was made to keep these patients in the analysis as reducing the sample size by 7 would have been statistically costly and the paediatric bias was mitigated as much as possible through the recalculation of the predicted FEV1% data.

The overall incidence of *E.dermatitidis* isolation in this centre is greater than described previously (164,165). This may be an artifact of increased interest around this organism but

the possibility of localised patient-patient infections or transmission from an environmental source could also be considered. This study reviews historic lung function over a far longer timeframe than previously studied which may account for the elucidation of a modest decline difference (171,172). Despite the smaller numbers, the patients were well matched in each group; beyond the relationship with *A.fumigatus*, the only significant difference between the groups was age. This is likely to be a confounding result brought about by the necessity of gathering more retrospective data in the *E.dermatitidis* group in order calculate a decline in lung function before and after infection. as the minimum amount of data needed was greater in this group. As such, patients were more susceptible to exclusion in the *E.dermatitidis* group than the control. This is a potential source of bias. Whilst the proportion of delta-F508 homozygous patients is similar in each group, there is still a limitation in the comparability to national data.

When considering sample size, the rate at which *E.dermatitidis* is being detected locally is increasing, potentially due to increased awareness of the organism. While many patients were excluded from the study as insufficient time had elapsed following positive culture to effectively measure a rate of decline, within two years the number of patients that could be included in this study could be much higher. This could allow for future work to be powered for multivariate logistic regression modelling and better matching of the controls, including matching by other microorganisms present within the respiratory tract of the CF patient.

6.5. Conclusions

This study suggests that *E.dermatitidis* isolation from the respiratory tract of adults with CF has a temporal relationship with an increased rate of lung function decline (1.82% compared to 0.33% p.a.) that exceeds the control group of adult CF patients at the same centre (0.82%) and the previously reported UK average (1.5%) (174). Evidence indicates that *E.dermatitidis* is an organism of significance in the CF lung, whether it plays an active or passive role in the lung microbiota it represents a priority signal to the CF clinician, particularly considering the importance of preserving lung function in CF (175). Given the limited population studied, the evidence generated is not sufficient to support clinical decisions, such as antifungal use, following isolation of *E.dermatitidis* and performing larger,

confirmatory studies would be a significant contributory step to resolving this clinical dilemma.

Chapter 7 – Exploring *Exophiala dermatitidis* as a transmissible pathogen

7.1. Introduction

The adult CF cohort described in Chapter 6 has a much greater incidence of *E.dermatitidis* than the literature suggests to expect. This may partly be an artifact of the way that patients were labelled as positive or colonised. However, it must also be considered that there is a higher than usual incidence of this organism locally. One such reason for this was postulated that patient-patient transmission could be occurring and a model to describe this was envisioned.

In the cystic fibrosis (CF) world, patient-patient interaction has transformed drastically over the last 30 years. CF patient groups and summer camps disappeared overnight with the discovery of highly infectious and deleterious organisms such as *Burkholderia cepacia*. Instead, exclusive side rooms, organism-specific outpatient clinics and other quarantine measures were implemented. The advent of genotyping organisms has led to the identification of specific strains of organisms such as *Pseudomonas aeruginosa* which explains its variable virulence and infectivity. The risk of patients being colonised by one of these organisms has led to CF outpatients and wards having some of the strictest infection control policies in the hospital. There is evidence emerging of aerosol transmission of fungi such as *Aspergillus fumigatus* through analysis of cough aerosols, suggesting that current infection control policies which centre around skin contact may be inadequate (176).

Anecdotally, it was noticed that the black yeast *Exophiala dermatitidis* was being grown more frequently in the local CF population. Initially this was felt to be a chance finding given that prior reviews have postulated that common, hot, moist environments such as dishwashers, steam baths and saunas may be an aerosolising transmission route (177). Certainly, there is no evidence at present of human-human transmission. However, a recent increase in the number of positive cases identified in a single CF unit, led to the uncovering of a number of genetically identical isolates. Given that CF patients share designated facilities such as wards and outpatient departments it was conceivable that they would cross paths in corridors, car parks or cafes nearby. Prior work by Brynant and Grogono et al

has shown that whole genome sequencing in conjunction with patient epidemiological data can be helpful in identifying potential reservoirs or transmission events (178). Retrospective review of patient data was used to evaluate trends of patient interaction and to investigate the hypothesis that CF patients were either transmitting *E.dermatitidis* to each other in hospital-facilitated settings or were coming into contact with a reservoir within secondary care.

7.2. Methods

In order to investigate this hypothesis, short tandem repeat (STR) genotyping was undertaken. A random sample of stored *Exophiala dermatitidis* cultures from the same laboratory grown on agar plates were used. Automated DNA extraction using the EMAG (Biomerieux) platform took place following ceramic bead beating. Multiplex PCR was performed on a thermocycler and PCR products were analysed on a 3500 XL genetic analyzer (Applied Biosystems) (L. White, Personal Communication, January 2024). Copy numbers of all nine short tandem repeat (STR) markers were determined using GeneMapper 5 software (Applied Biosystems). Relatedness between isolates was analyzed using BioNumerics software version 7.6.1 (Applied Maths) via the unweighted pair group method with arithmetic means (UPGMA), using the multistate categorical similarity coefficient (T. de Groot, Personal Communication, January 2024).

This identified a clone and a more heterogeneousmix of isolates. The laboratory details of the clone was cross referenced with local health board databases to identify the patients who had produced these samples.

A retrospective review was planned in which the contact each of these patients had with healthcare services could be compared to find matching dates. The dates of first *E.dermatitidis* growth was known so each contact could be evaluated for significance. Local health board databases were used to identify all contact with secondary care inpatient and outpatient services in Wales, as well as attendances to the tertiary transplant referral centre. This data was used to generate a complete list of contact dates for each patient, from the year 2012 (the year of the first growth of *E.dermatitidis* in the cohort) to the present day. The list of dates per patient were cross-referenced with other patients to find

potential transmission events which were termed clashes. Clashes were filtered by macrolocation (hospital site) and also only included if clashing patients were of opposing statuses i.e. positive and negative. The remaining dates were then focussed on by individually reviewing each patient's remaining dates in Excel and health board databases to help identify dates of significant clashes where a positive and negative patient clashed which led to both patients testing positive less than six months later.

Microbiological swab samples were taken from higher risk inpatient and outpatient facilities that would be shared patient locations and specific fungal cultures undertaken with these specimens. Patient postcodes were taken from hospital databases and plotted against a local map to identify clusters of similarity or shared areas.

7.3. Results

Thirty four stored isolates of *Exophiala dermatitidis* were available for analysis. Following genome sequencing of all 34 and construction of the UPGMA dendrogram it was identified that, of these 34 organisms, 21 were a clone. The dendrogram is illustrated below with patient identifiable locations anonymised (figure 16).

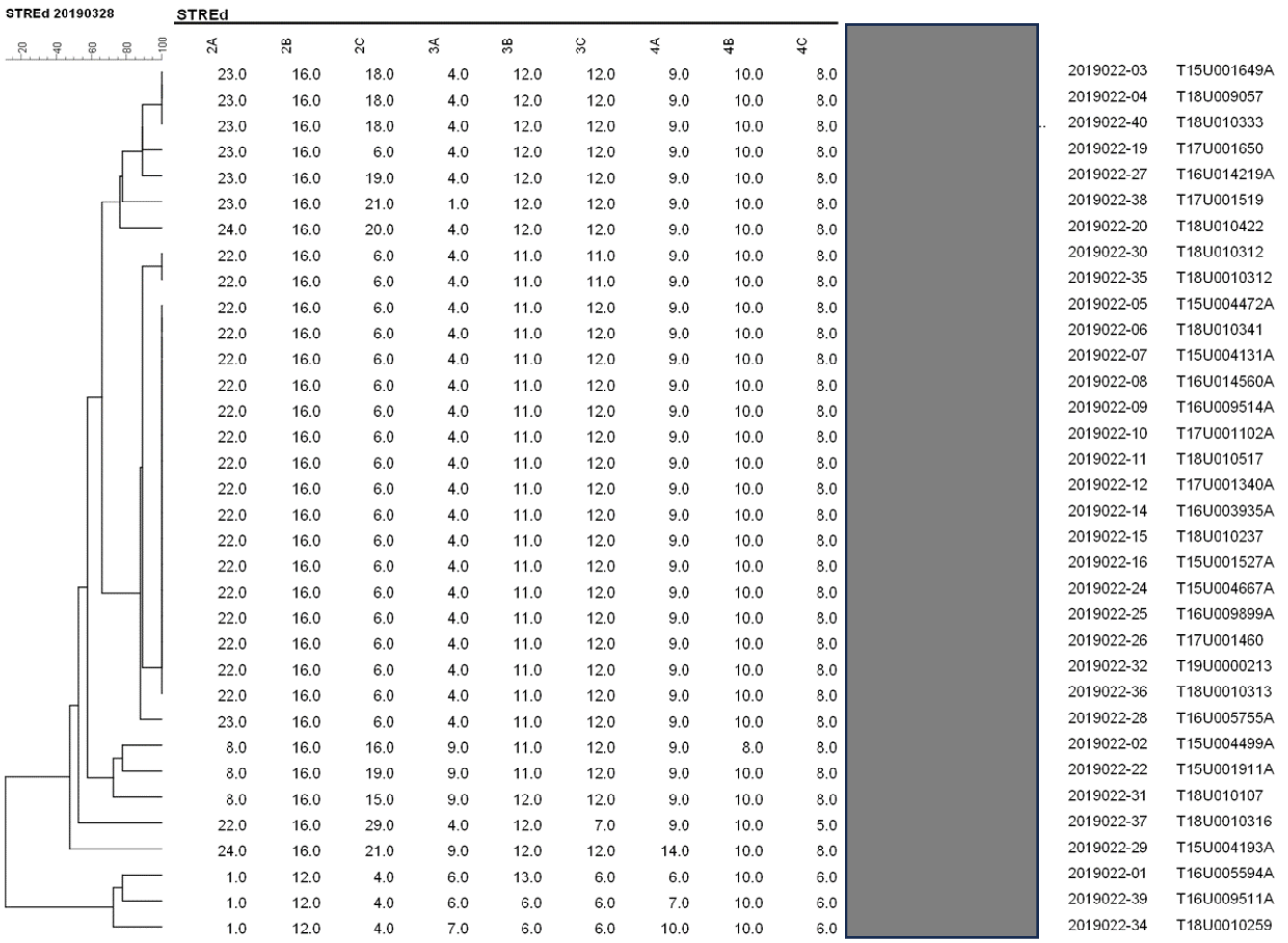
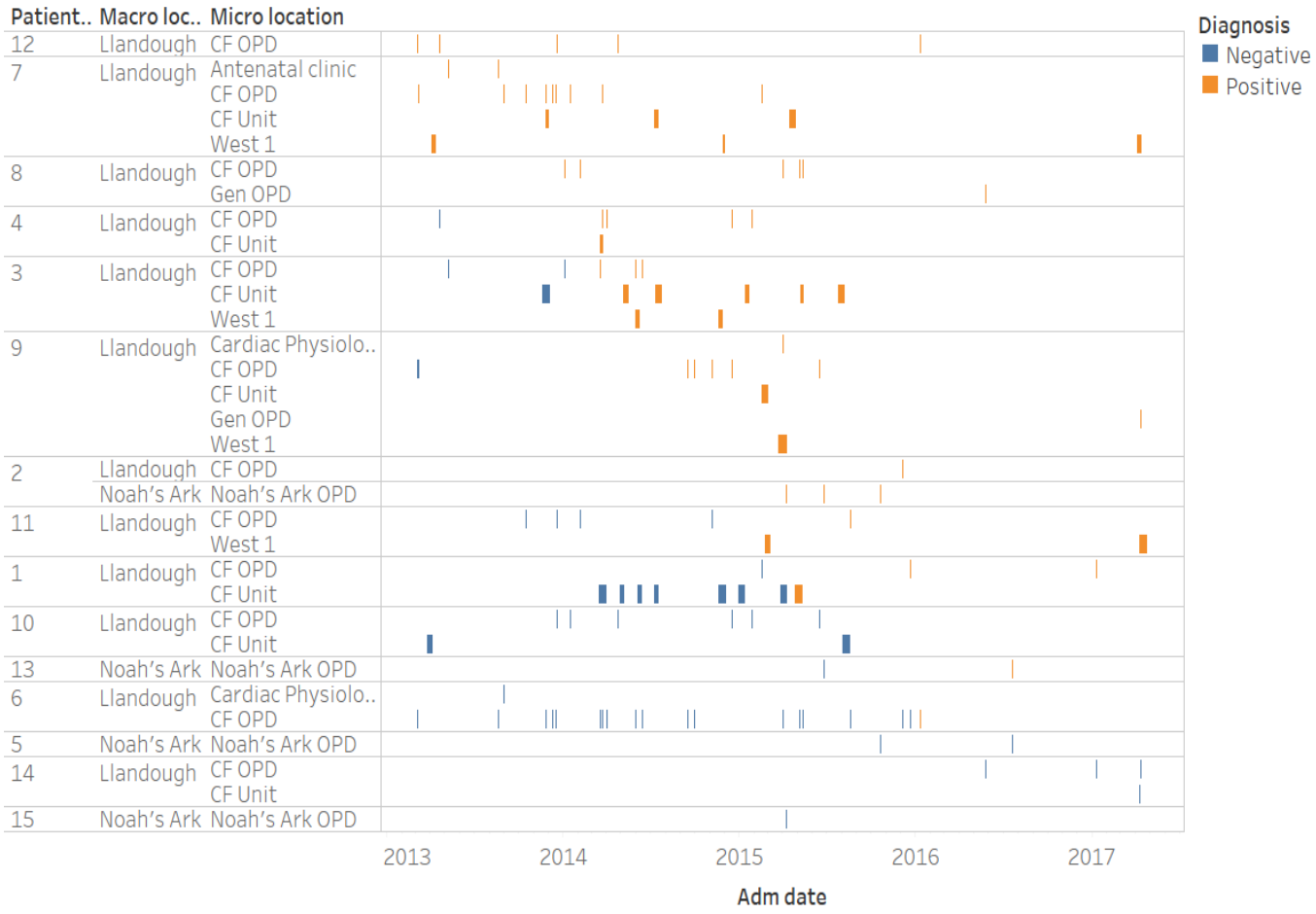


Figure 16: Short tandem repeat typing of E.dermatitidis (STREd) isolates demonstrated as an UPGMA dendrogram. The scale in the upper left corner represents similarity (%). Sample-related information is listed on the right. Patient identifiable information is blocked out.

The laboratory details of these 21 clonal organisms were traced and had been cultured from 15 CF patients and 2 non-CF patients. The other 13 organisms were a heterogenous genetic mix of the same species which implies that these 13 represent a good background mix of *E.dermatitidis* and the 21 organisms likely stem from the same original organism.

For the 15 CF patients, 57% were male. Their mean age at time of testing positive for *E.dermatititis* was 22 (range 15-41). The total number of outpatient appointments reviewed (after Did Not Attend codes and replications were removed) was 1,506. 557 potential clashes events in an outpatient setting between patients was identified. The total number of inpatient patient-days was 2,196. 542 clashing inpatient patient-days were identified. Clash

events where a negative patient met a positive patient occurred 17 times on the ward. 11 of these clash events (65%) led to a negative patient becoming positive less than 6 months later. These interactions are seen in Figure 12. All the inpatient clashes involved 5 of the negative patients (36%). Clash events where a negative patient met a positive patient occurred 49 times in outpatients. 12 of these clash events (24%) led to a negative patient becoming positive less than 6 months later. 11 negative patients (79%) experienced positive/negative clash events and 8 of them (57% of total) became positive less than 6 months after a clash. Four patients had no identifiable positive/negative clash event before turning positive. This is illustrated by way of a variation of a cascading Gantt chart (figure 17). Here, a patient's interaction in the healthcare service is recorded when they are likely to be on site with another patient. The size of marking indicates length of stay. The colour change occurs when a patient becomes positive.



Admission date for each Patient ID broken down by Macro location and Micro location. Colour shows details about Diagnosis. Size shows sum of Duration of admission. Patient 5, 14 and 15 become positive after the limit of the X axis. Admissions in which a patient becomes positive during admission is labelled as positive for the purposes of this chart

Figure 17: All clashes between patients testing positive/negative for E.dermatitidis cascaded by positivity date, filtered by Macrolocation and Microlocation

There was no correlation between patient home postcode and positive result with the clonal organism. Environmental swabs taken on the ward including from known possible reservoirs such as the washing machine and dishwasher were negative for any significant growth. Environmental swabs taken from windowsills, doorframes, handles, sinks and radiators of the CF outpatients department were also negative for fungal growth.

7.4. Discussion

There is a possibility that *E.dermatitidis* can be transmitted between patients and, if this was to be proven, this could represent a model of that transmission event.

The relatively small size of group analysed emphasises the importance of any found clash event and suggests that the four patients with no identifiable clash event may be explainable if all positive isolates are genotyped. A further explanation that should be considered is that as CF patients progress in their lung function decline, visits to hospital settings may become more frequent. If *E.dermatitidis* is an organism which becomes more prevalent in the more diseased lung then the footfall in hospital becomes a confounder and not a potential transmission event.

Further research could evaluate a larger group or, in the future, work could be done to more regularly genotype organisms in order to look at potential trends of transmission. Environmental flora could be genotyped to establish how likely these patients are to pick up strains from each other versus the environment.

7.5. Conclusion

This data suggests that *E.dermatitidis* could be transmitted from patient-patient and the most likely location for this within the hospital setting is CF outpatients. There is therefore the possibility of inadequacy in scope or implementation of current local infection control policies regarding outpatient clinics. This chapter highlights the possibility that fungal disease is identified fungi may have pathogenic potential which at present is not clearly identified and that there are complex patient-patient interactions which need to be considered carefully.

Chapter 8 – Summary and Future Work

8.1. Summary of preceding chapters

A multi-stranded approach has been taken in the investigation of the diagnostic challenges of fungal disease in Wales. The individual pathogens of particular interest were identified from the literature and estimations as to the predicted burden of disease in Wales were made. These estimations were consistently higher than those made previously and provably occurring in hitherto clinically overlooked patient groups. This was further explored in a closer analysis into pneumocystosis as one example. The SPITFIRE study was designed to investigate if induced sputum was acceptable to patients and had potential as a viable test to be used in conjunction with bronchoscopy to investigate unwell patients with respiratory infection. This was a success in that patients reported less pre-test anxiety and a willingness to have the test again over bronchoscopy with fewer adverse events reported than the standard of care procedure. Detection of pathogenic organisms was achieved in many patients, but further work is needed to better identify those that are likely to benefit from this alternative. Understanding the significance of a positive result is a challenge, therefore a project to identify the impact of *Exophiala dermatitidis* infection in a CF cohort was undertaken. There was a discovery of probable contribution to long term lung function decline in the CF patient. The possibility of patient-patient transmission was explored and a model for this possibility described.

A more detailed summary of these findings and how they impact the future direction of research in this area is further explored in the subsequent sections.

8.2. Understanding the burden of fungal disease in Wales

There is no dedicated provision for the healthcare service of patients with invasive or serious fungal disease in Wales. At present, the responsibility for the identification, diagnosis and management of these conditions lies with the clinical team responsible for the cause of severe immunosuppression; whether that is disease or treatment induced, with support from other specialties with expertise such as respiratory or infectious diseases. Whether this is an appropriate model of care is difficult to support or refute, as a working

understanding of the number of cases of fungal disease in Wales is difficult to establish. In chapter 2, this thesis has established that the current published estimates for fungal disease in Wales are inaccurate. In the instances in which laboratory results are available, it is consistently demonstrated that a higher than expected positivity rate is found, often in non-traditional risk populations. Whilst it would then be reasonable to assume that therefore these new insights are a better reflection of the rates of disease in Wales, there is highly likely to be a significant population that remains hidden. This is because the clinical suspicion for fungal disease in these patients is very low. An example of this is in the pneumocystosis chapter (chapter 3). This chapter highlights cohorts such as patients with rheumatology disease as populations of great interest; with a similar PcP mortality rate to those with severe disease such as cancer but do not share such a poor prognosis outside of PcP diagnosis (136). PcP is not tested for in the same timely manner in these cohorts than in the post-renal transplant patient and this is likely to due to a lower index of clinical suspicion. As such, whilst the conclusions are valuable, the whole project suffers from that level of bias. Future work could include prospective testing in some of these patients that become unwell and recording those that are established on medications that are identified as higher risk. This could help better establish both the numerator and denominator for these large populations. There is also a cohort of patients that could be said to be "hiding in plain sight". An example of this are the chronic pulmonary aspergillosis patients. As demonstrated in the earlier chapter 2, there are several patients identified from laboratory records that have demonstrably high serological markers for aspergillosis. Many of these patients have COPD or bronchiectasis and have a high degree of healthcare utilisation due to the high severity classification of their airways disease (92). A patient having a positive serology test suggests that their care is managed by a clinician who recognises that fungal disease may be present but does not give any insight as to the difficulty in interpreting the result. However, despite the numerous patients with severe airways disease, these tests are not routine in the secondary care outpatient setting. In contrast to the patients who have ABPA, at present, there is no regional for patients with CPA in Wales. There is an expectation that the local clinician recognises the possibility of CPA, diagnoses it correctly and manages this long term condition. Regardless of whether this is considered a reasonable burden for the respiratory generalist, the true burden of this condition on this large cohort of patients with severe COPD is unknown due to the variability of testing and

the definition of disease (20,49). It would be an interesting development of this work to prospectively test for aspergillosis in a cohort of patients with severe COPD, where severe is defined either by exacerbation frequency criteria or by hospitalisation frequency. Patients, if meeting criteria of severity, would have radiological and serological assessment in addition to usual care with the possible inclusion of microbiology testing through sputum or even bronchoscopy. This is likely, as per the hypotheses generated by this thesis, to reveal a hitherto hidden group of patients whose potential fungal disease is being inadequately managed. These patients are likely to benefit from an expert multidisciplinary clinical group to assist the long term management of their condition (179,180).

8.3. Investigating fungal disease with induced sputum

Fungal disease is not easily investigated at present. This thesis reviewed (in chapter 1) a number of the current commonly used techniques including the manner by which the samples for testing are obtained. The SPITFIRE study was undertaken to establish if induced sputum is a possible option to obtain deep respiratory samples in unwell haematology patients; a complex cohort that are high risk for opportunistic infections. This feasibility study demonstrated, in chapter 5 of this thesis, that induced sputum is a procedure that can be undertaken swiftly by physiotherapists and is tolerated well with a good likelihood of generating a useable sample. The population was limited to a single cohort of immunocompromised patients in a single centre. This was also undertaken whilst restrictions were in place, particularly for aerosol generating procedures, for the COVID pandemic. Ultimately, these issues led to fewer patients than targeted being recruited. There were a number of diseases diagnosed in these patients, mostly from the respiratory tract and the microbiological information obtained from the bronchoscopy was key in understanding the relevance of the induced sputum results. However, the rare nature of invasive fungal infections makes statistical analysis of the microbiological results inappropriate and a more narrative description of the outcomes was undertaken. Whilst the results of SPITFIRE demonstrate the possibility of induced sputum as a test, a more detailed diagnostic accuracy study is difficult to power straight away as the numbers of each infection were small. Ergo, understanding the role induced sputum plays in the landscape of investigating fungal disease is also unclear.

Further work in this area would require improvements in three key areas in order to develop this technique further.

8.3.1. Developing the target population for evaluation of induced sputum

As reviewed in the SPITFIRE set up, paediatric CF patients and patients with suspected *Mycobacterium tuberculosis* infection are capable of producing a useable sample. The haematology cohort were a natural choice for SPITFIRE as they represented a large burden of inpatient respiratory reviews and bronchoscopy time with a motivated clinical team, keen to improve the pathway for these patients. However, there are a number of other key patient cohorts that are at risk for invasive fungal disease in which this would be a valuable test and may be under investigated as the risk of bronchoscopy may outweigh the benefits of obtaining a deep respiratory sample. This includes the HIV population or those that are post-solid organ transplantation but, as demonstrated in the prior chapters, there are a several key novel populations that are also at risk and could be investigated further. Secondary care settings are usually organised in an organ system manner with minimal interaction in between, even with allied healthcare professionals. Therefore, in order to best engage with these variable populations with different healthcare teams, an interventional study would initially be needed before the advent of a multi-disciplinary team to act as a longitudinal constant and standard of care for these patients.

8.3.2. Understanding patient suitability characteristics

SPITFIRE demonstrated the benefits of using IS to produce a deep respiratory sample. However, a number of patients were unable to expectorate. This was likely to be due to factors related to the patient, rather than the operator. Sub-analysis of this suggested that the patient's Clinical Frailty Score held correlation with their ability to be successful, and there was also a signal that their position in the bed or chair may also be related. However, it is likely that these two things are linked as a more frail patient is unlikely to be able to sit out in the chair and so may well be measuring the same thing. There is not enough evidence to preclude frail patients from the opportunity to participate in further study relating to induced sputum. It would therefore be helpful to gather more objective parameters to produce a more predictive model in order to determine ahead of time who is likely to benefit from this test and consequently better utilise healthcare resources. Options for

these objective parameters would include vital signs as these were collected at the time of consent but, beyond oxygen saturations, were not recorded again. Frailty could be assessed more rigorously and more care could be taken to ensure that the procedure was being undertaken in a more consistent way with regards to positioning i.e. patients were encouraged to sit in the chair but future protocols could mandate that the patients be stood if able, sat in a chair if not, sat on the edge of the bed or lying in bed as a more hierarchical way of demonstrating mobility and frailty.

8.3.3. Understanding the impact of a positive test

As demonstrated in prior chapters, there is no single diagnostic test of fungal disease and the level of immunosuppression required in order that patients be considered at risk of disease by these organisms puts them similarly at risk of other unusual pathogens. In addition, fungal disease as an umbrella concept takes into account multiple organisms which each may need to be identified by different microbiological strategies outside of fungal culture. As such, it is reasonable to assume that induced sputum is a technique that is most suited to certain fungal diseases or tests over others. This may only be apparent when adequately comparing the recovery or detection of organisms at IS with bronchoscopy. In the small cohort of SPITFIRE patients, induced sputum samples that underwent fungal culture had good correlation with the culture taken from the bronchoscopy samples. However, in order to establish if this is a persistent trend, a much greater sample size would be needed in order to investigate the multiple organisms and relevant testing techniques. This sample should also have a similar or standardised aetiology of immunosuppression as there is likely to be a relationship between level of immunocompromise, microorganism burden and chance of detection by differing sampling methods.

8.4. Developing an understanding of myco-diversity in the lung

This thesis has made mention previously of the difference between colonisation and infection. This is particularly of relevance in fungal disease as a number of fungal organisms can exist, largely inert, within the microbiota of the lung (181). The complex interplay between microbiota and mycobiota has not been explored in this thesis but remains a consideration of the SPITFIRE study and the work looking at *Exophiala dermatitidis* as

described in chapter 6. This research has demonstrated that *E.dermatitidis* is associated with accelerating lung function decline in CF patients but the relationship between cause and effect in these circumstances is still unclear. It would be unsurprising to find that cause and effect are not so easily delineated and an element of synergism exists between the two. Further work could explore this through the analysis of genomics of the respiratory samples obtained in these patients; to identify if *E.dermatitidis* growth is associated with a reduction in bacterial and fungal species heterogeneity in sputum samples.

8.5. Appreciation for the uncertainty of fungal transmission

Fungi are largely ubiquitous in the environment with some exceptions. *E.dermatitidis* is found naturally in the environment but its optimum growth conditions of hot, wet areas makes specific environmental circumstances a much more likely transmission environment to human hosts. In the thesis it was postulated that transmission could be occurring patient to patient in the healthcare setting and a patient movements were tracked and clash events were suggested in chapter 7 to account for a number of these potential transmission events. Whilst this is far from definitive, there is an appreciation that more could be done in at risk individuals to attempt to modify their risk of getting serious or invasive fungal disease. At present, advice is given to transplant recipients to avoid compost heaps, but minimal other lifestyle advice is given. The evidence base for this is not robust enough to warrant further clinical advice but further work could investigate risk factors of organisms with specific growth environments such as *Exophiala dermatitidis* and the possibility of patient-patient transmission to help identify outbreaks early and reduce healthcare transmission.

8.6. Final remarks

In short, the diagnostic challenges of fungal diseases in Wales are varied and multifocal. It is difficult to address these issues without an appreciation for how common the conditions are. This thesis provides a new bespoke estimate as to the fungal disease burden in Wales. In particular, it has highlighted new populations revealed by new therapies and cohorts of patients that may have been overlooked for some time. The current model of centralising the testing of samples within Wales has enabled a structured response to positive results.

The methods of obtaining these samples, however, remains unchanged and not evolving to meet the increased volume and complexity of patients today. Whilst induced sputum may not be a fully-fledged replacement for bronchoscopy, it provides opportunities to explore other models of testing including targeted screening before empirical treatment which is a novel undertaking. Lastly, positive results are rarely easily interpretable. Questions over the true clinical impact on some organisms remain, whilst others have clear pathogenic potential. At present, interpretation of these results, taken in a non-standardised way, remains solely the responsibility of the patient's clinician who may have had variable experience in treating fungal disease. This thesis demonstrates a need for the conditions explored in these chapters to be continued by a dedicated team that would be best placed to respond to the threats posed by this emerging clinical issue.

Appendices

Appendix A: Anonymised patient database of positive *Pneumocystis* results, ordered by PCR value.

Sex	Specimen type	Age at positive	Year collected	PCR[i] Cq[ii] value	Disease theme	Glucan result	Radiology comment
M	BAL[iii]	38	2016	20	HIV[iv]	N/R[v]	N/R
M	BAL	50	2017	20	HIV	N/R	N/R
M	BROW[vi]	28	2018	20	HIV	N/R	N/R
M	BAL	43	2015	21	HIV	N/R	N/R
M	BAL	34	2015	21	HIV	N/R	N/R
F	BAL	61	2016	21	Renal+/-pancreatic transplant	N/R	N/R
M	BAL	66	2016	22	HIV	N/R	N/R
M	BAL	63	2015	22	HIV	N/R	N/R
M	BAL	38	2018	22	HIV	N/R	N/R
M	BAL	30	2017	23	HIV	N/R	N/R
M	BAL	63	2015	23	HIV	N/R	N/R
M	BAL	55	2015	24	HIV	N/R	N/R
M	BAL	56	2015	25	HIV	N/R	N/R
F	BAL	66	2015	25	HIV	N/R	N/R
F	BAL	22	2015	25	Inherited immunodeficiency	N/R	N/R
M	BAL	69	2017	26	Haem cancer on treatment, Autoimmune haem disease	N/R	N/R
M	BAL	38	2015	26	HIV	N/R	N/R
M	BAL	41	2015	26	Renal+/-pancreatic transplant	N/R	N/R
M	BAL	70	2015	26	Respiratory disease on steroids/immunosuppression	N/R	N/R

F	SNS[vii]	71	2017	26	Rheumatology disease on DMARDs[viii]/steroids/biologics, Respiratory disease on steroids/immunosuppression	N/R	N/R
M	TS[ix]	42	2017	26	Renal+/-pancreatic transplant	N/R	N/R
M	BAL	47	2018	27	HIV	N/R	N/R
F	BAL	22	2016	27	Inherited immunodeficiency	N/R	N/R
M	BAL	67	2015	27	Vasculitis on steroids/DMARD/biologic	N/R	N/R
M	BROW	58	2015	27	Lymphoma	N/R	N/R
F	BAL	62	2015	28	Renal+/-pancreatic transplant	N/R	N/R
F	BROW	23	2017	28	Allogeneic SCT[x]	N/R	N/R
M	BAL	79	2015	29	Immunosuppressed for renal disease	N/R	N/R
F	BAL	67	2016	29	Lymphoma	N/R	N/R
M	BAL	65	2015	29	Rheumatology disease on DMARDs/steroids/biologics	N/R	N/R
M	SNS	56	2016	29	HIV	N/R	N/R
M	TS	35	2018	29	Allogeneic SCT	N/R	N/R
M	TS	59	2015	29	HIV	N/R	N/R
F	TS	52	2017	29	Renal+/-pancreatic transplant	N/R	N/R
M	BAL	78	2018	30	Haem cancer on treatment	N/R	N/R
F	BAL	70	2015	30	Lymphoma	N/R	N/R
F	BROW	70	2018	30	Respiratory disease on steroids/immunosuppression	N/R	N/R
M	BROW	71	2015	30	Respiratory disease on steroids/immunosuppression	N/R	N/R
F	BROW	74	2018	30	Solid organ cancer on chemotherapy	N/R	N/R
F	TS	40	2017	30	Renal+/-pancreatic transplant	N/R	N/R

M	TS	74	2017	30	Rheumatology disease on DMARDs/steroids/biologics		N/R
F	BAL	70	2016	31	Solid organ cancer on chemotherapy	N/R	N/R
M	BROW	67	2017	31	Haem cancer on treatment	N/R	N/R
M	BROW	50	2016	31	Vasculitis on steroids/DMARD/biologic	N/R	N/R
F	NPA[xi]	51	2017	31	Vasculitis on steroids/DMARD/biologic	N/R	N/R
M	BAL	46	2018	32	HIV	N/R	N/R
M	BAL	74	2017	32	Renal+/-pancreatic transplant	N/R	N/R
M	BAL	36	2016	32	Respiratory disease on steroids/immunosuppression	N/R	N/R
F	TS	76	2018	32	Haem cancer on treatment	N/R	N/R
M	TS	27	2016	32	HIV	N/R	N/R
F	TS	68	2016	32	HIV	N/R	N/R
F	BAL	24	2016	33	Allogeneic SCT	N/R	N/R
M	BAL	58	2016	33	Lymphoma	N/R	N/R
M	BAL	52	2016	33	Lymphoma	N/R	N/R
F	BAL	74	2016	33	Rheumatology disease on DMARDs/steroids/biologics	N/R	N/R
M	BROW	65	2016	33	Allogeneic SCT	N/R	N/R
M	BROW	55	2018	33	HIV	N/R	N/R
M	BROW	55	2017	33	Lymphoma	N/R	N/R
F	TS	7	2018	33	Allogeneic SCT	N/R	N/R
M	TS	52	2016	33	HIV	N/R	N/R
M	TS	29	2017	33	HIV	N/R	N/R
M	TS	60	2018	33	HIV	N/R	N/R
M	TS	53	2018	33	HIV	N/R	N/R
M	BAL	70	2015	34	Autoimmune haem disease	N/R	N/R

M	BAL	75	2015	34	Lymphoma	N/R	N/R
M	BAL	58	2018	34	Rheumatology disease on DMARDs/steroids/biologics	N/R	N/R
M	BAL	77	2015	34	Rheumatology disease on DMARDs/steroids/biologics	N/R	N/R
M	BAL	65	2016	34	Vasculitis on steroids/DMARD/biologic	N/R	N/R
F	BAL	67	2018	34	Vasculitis on steroids/DMARD/biologic	N/R	N/R
M	BROW	66	2016	34	Liver failure	N/R	N/R
F	BROW	70	2017	34	Lymphoma	N/R	N/R
F	TS	56	2016	34	Allogeneic SCT	N/R	N/R
M	TS	1	2016	34	Allogeneic SCT	N/R	N/R
M	TS	64	2017	34	Lymphoma	N/R	N/R
M	TS	41	2018	34	Renal+/-pancreatic transplant	N/R	N/R
M	TS	76	2016	34	Solid organ cancer on chemotherapy	N/R	N/R
M	BAL	70	2016	35	Autologous SCT	N/R	N/R
F	BAL	49	2016	35	Haem cancer on treatment	N/R	N/R
M	BAL	74	2015	35	Immunosuppressed for renal disease, Respiratory disease on steroids/immunosuppression	N/R	N/R
M	BROW	71	2018	35	Haem cancer on treatment	N/R	N/R
M	TS	58	2018	35	Allogeneic SCT	N/R	N/R
F	TS	13	2016	35	Lymphoma	N/R	N/R
F	TS	75	2018	35	Renal+/-pancreatic transplant	N/R	N/R
M	TS	68	2017	35	Solid organ cancer on chemotherapy	N/R	N/R
M	BAL	59	2018	36	Allogeneic SCT	>500	N/R
M	BAL	79	2016	36	Lymphoma	Unavailable	Patchy ill-defined opacification bilaterally, deteriorating

M	BROW	67	2017	36	Rheumatology disease on DMARDs/steroids/biologics	Unavailable	Widespread interstitial opacity, progressive
F	BROW	63	2017	36	Solid organ cancer on chemotherapy	Unavailable	Thickening of intra and interlobular septae with superimposed ground glass opacity
M	BROW	64	2018	36	Solid organ cancer on chemotherapy	Unavailable	Bilateral nodular and interstitial shadowing
M	NPA	81	2017	36	Rheumatology disease on DMARDs/steroids/biologics	N/R	N/R
F	SNS	81	2016	36	Solid organ cancer on chemotherapy	N/R	N/R
M	TS	24	2016	36	Allogeneic SCT	N/R	N/R
M	TS	55	2016	36	HIV	N/R	N/R
M	TS	70	2016	36	HIV	N/R	N/R
F	TS	66	2016	36	Lymphoma	N/R	N/R
M	TS	70	2017	36	Renal+/-pancreatic transplant	N/R	N/R
F	TS	72	2016	36	Solid organ cancer on chemotherapy	N/R	N/R
F	TS	71	2017	36	Vasculitis on steroids/DMARD/biologic	N/R	N/R
M	BAL	66	2015	37	Autologous SCT	Unavailable	Multifocal areas of ground glass opacification throughout lung parenchyma
M	BAL	55	2017	37	Lymphoma	65	Widespread interstitial opacity, progressive
F	BAL	68	2017	37	Solid organ cancer on chemotherapy	57	Patchy inflammatory changes in both upper lobes with ground glass opacification
M	BAL	69	2017	37	Vasculitis on steroids/DMARD/biologic	Unavailable	Confluent right upper lobe consolidation and patchy ground glass attenuation throughout both lungs
M	BROW	78	2018	37	Solid organ cancer on chemotherapy	Unavailable	Widespread ground glass change and small areas of consolidation within both lungs
M	BROW	79	2018	37	Vasculitis on steroids/DMARD/biologic	167	Patchy ground glass change throughout lung fields

M	NBL[xii]	67	2018	37	Vasculitis on steroids/DMARD/biologic	382	N/R
M	TS	45	2015	37	Allogeneic SCT	N/R	N/R
M	TS	32	2017	37	Allogeneic SCT	N/R	N/R
M	TS	58	2018	37	Autoimmune neurological disease	N/R	N/R
M	TS	79	2018	37	Haem cancer on treatment	N/R	N/R
M	TS	71	2016	37	Haem cancer on treatment, Respiratory disease	N/R	N/R
F	TS	56	2016	37	Inflammatory bowel disease on steroids/DMARD/biologics	N/R	N/R
F	TS	66	2017	37	Lymphoma	N/R	N/R
M	TS	60	2018	37	Lymphoma	N/R	N/R
F	TS	65	2018	37	Renal+/-pancreatic transplant	N/R	N/R
M	TS	54	2017	37	Solid organ cancer on chemotherapy	N/R	N/R
F	Serum	66	2015	38	Haem cancer on treatment	>500	N/R
F	BAL	28	2016	38	HIV	Unavailable	Widespread ground glass change with confluent area of consolidation
F	BAL	41	2018	38	Inherited immunodeficiency	195	Widespread ground glass change throughout both lungs with interlobular septal thickening at the lung bases
F	BAL	37	2018	38	Rheumatology disease on DMARDs/steroids/biologics	>500	N/R
F	BROW	31	2016	38	Vasculitis on steroids/DMARD/biologic	Unavailable	Scattered widespread patchy air space shadowing
M	SNS	67	2017	38	Vasculitis on steroids/DMARD/biologic	>500	N/R
M	TS	66	2016	38	Allogeneic SCT	Unavailable	Widespread airspace shadowing on CXR
M	TS	58	2016	38	Allogeneic SCT	Unavailable	Reticular shadowing with airspace opacification noted in both lower lobes and right middle lobe

M	TS	47	2018	38	HIV	Unavailable	Bilateral airspace shadowing, worsening appearances
F	TS	37	2018	38	HIV	301	
M	TS	56	2018	38	HIV	Unavailable	Diffuse bilateral ground glass opacities predominantly perihilar
M	TS	50	2017	38	HIV	181	Perihilar confluent ground glass opacifications
M	TS	30	2018	38	HIV, Renal+/-pancreatic transplant	241	Widespread ground glass opacifications
M	TS	58	2018	38	Renal+/-pancreatic transplant	>500	N/R
M	TS	67	2016	38	Renal+/-pancreatic transplant	>500	N/R
F	TS	50	2018	38	Rheumatology disease on DMARDs/steroids/biologics	214	Diffuse non-segmental airspace shadowing throughout the both lungs including perihilar region
F	TS	46	2018	38	Rheumatology disease on DMARDs/steroids/biologics	Unavailable	Diffuse ground glass opacifications, worsening extent
F	TS	52	2017	38	Solid organ cancer on chemotherapy	238	Extensive lung parenchymal abnormality is present with multiple patchy areas of air space shadowing and consolidation
F	TS	62	2018	38	Solid organ cancer on chemotherapy	396	Significant ground glass shadowing, interstitial infiltrates and ill-defined consolidation
F	BAL	43	2015	39	Liver failure	Unavailable	Widespread fluffy opacifications throughout lung fields
M	BAL	30	2018	39	Respiratory disease on steroids/immunosuppression	392	Bilateral interstitial changes, extensive air space opacification and small areas of consolidation
F	BAL	62	2016	39	Rheumatology disease on DMARDs/steroids/biologics	80	Bilateral severe airspace opacifications
M	BROW	79	2018	39	Rheumatology disease on DMARDs/steroids/biologics	Unavailable	Patchy ground glass opacity
F	BROW	61	2016	39	Solid organ cancer on chemotherapy	Unavailable	Widespread pneumonitis changes.
M	SNS	59	2016	39	Dermatology disorder needing immunosuppression	Unavailable	Widespread ground glass opacifications

F	TS	49	2016	39	Liver failure, Lymphoma	Unavailable	Unavailable - documentation of likely PCP according to radiology and biochemistry and cause of death but actual results not seen
M	TS	84	2017	39	Lymphoma	Unavailable	Extensive bilateral lung opacification
M	TS	51	2018	39	Renal+/-pancreatic transplant	>500	N/R
M	TS	72	2016	39	Rheumatology disease on DMARDs/steroids/biologics	>500	N/R
M	BAL	55	2018	40	Renal+/-pancreatic transplant	32	Widespread patchy ground glass opacification.
F	BROW	35	2018	40	Allogeneic SCT	Unavailable	Widespread ground glass opacifications
M	BROW	63	2017	40	Haem cancer on treatment	110	Bilateral lower and midzone airspace opacifications
M	SNS	57	2018	40	Renal+/-pancreatic transplant	>500	N/R
F	TS	62	2018	40	Autologous SCT	>500	N/R
M	TS	80	2018	40	Haem cancer on treatment	166	Severe consolidation Left base
M	TS	54	2016	40	Lymphoma	81	Bibasal interstitial shadowing and airspace opacifications
M	TS	61	2017	40	Lymphoma	Unavailable	Patchy ground glass opacification worsening
M	TS	64	2015	40	Lymphoma	Unavailable	Airspace opacifications bibasal in distribution
M	TS	67	2017	40	Rheumatology disease on DMARDs/steroids/biologics	Unavailable	Patchy alveolar air space opacification in both lower lobes
M	BAL	50	2018	41	Respiratory disease on steroids/immunosuppression	151	Bilateral infiltrates
M	BROW	66	2018	41	Haem cancer on treatment	Unavailable	Multiple areas of lung consolidation with predominantly symmetrical distribution
F	TS	62	2018	41	Allogeneic SCT	441	N/R
F	TS	77	2017	41	Lymphoma	Unavailable	Extensive bilateral air space opacification
F	TS	57	2016	41	Solid organ cancer on chemotherapy	>500	N/R

[i] Polymerase chain reaction
[ii] Quantification cycle
[iii] Bronchoalveolar lavage
[iv] Human immunodeficiency virus
[v] Not required as per the project decision making flowchart
[vi] Bronchial washing
[vii] Sino-nasal secretions
[viii] Disease-modifying anti-rheumatic drug
[ix] Throat swab
[x] Stem cell transplant
[xi] Naso-pharyngeal aspirate
[xii] Non-directed bronchoalveolar lavage

Appendix B: SPITFIRE patient information sheet

PARTICIPANT INFORMATION SHEET

Study Title: SPITFIRE: Sputum Induction Trial For Improved Respiratory Evaluation

Chief Investigator (CI): Dr Jamie Duckers

You are being invited to take part in a medical study called SPITFIRE conducted at University Hospital of Wales and Llandough Hospital, Cardiff and Vale University Health Board. In conjunction with Cardiff University. You have been invited because you are a patient at University Hospital of Wales with a haematology condition and your clinical team are concerned that you have an infection. They feel you may benefit from a bronchoscopy procedure. The study involves comparing the planned bronchoscopy with alternative techniques of getting respiratory samples from the airways of the lung.

It is important that you take the time to read this information sheet carefully before considering whether to take part. If you are unclear about any of the information provided or would like further information, please let us know when we next speak to you or by contacting us using the details at the end of this letter. We will be happy to discuss any questions with you. If you decide not to take part, please be reassured that this will not affect your ongoing clinical care.

What is the purpose of the study?

Many haematology conditions affect the body's ability to fight infection. The haematology team also often give drugs (including chemotherapy) in order to try and treat haematological conditions which also suppress the immune system, making infections more likely.

Infections are treated with antimicrobials but treatments are most effective when the antimicrobial is targeted to the specific bacteria or fungus causing the infection. In order to find out the organism responsible, a sample of the organism needs to be found somewhere in the body and tested in the hospital laboratory. One of the commonest sites of infection in the body is the lungs. It is not always straightforward to get samples of organisms from the lungs; sometimes when people have an infection they produce lots of sputum (phlegm, spit) which can be brought up and analysed. But this is not always the case.

The best way to get a good sample from the lungs is to do a bronchoscopy (also called a camera test or magic eye test) and "wash out" a patch of the lung which looks like it might be infected. These "washings" are then hoovered up by the camera and collected and sent to the lab. A bronchoscope (the camera itself) is very small; thinner than a Biro and the test is done hundreds of times a year by very experienced operators in this hospital. Local anaesthetic spray is used around the mouth and throat and sedation is often used which makes the patient feel more relaxed or even fall asleep. It is not done under general anaesthetic in your circumstances. Common side effects last less than a day and include a cough, occasionally coughing up a small (less than a teaspoon) amount of blood, a sore throat, hoarse voice and drowsiness if sedation is used. Serious risks such as damage to the lung itself is very uncommon but this will always be explained fully by the operator before the procedure. Despite its usefulness in helping to guide effective treatment by identifying the bugs causing infection not all patients undergo a bronchoscopy. This may be because patients feel anxious about

the procedure and choose not to have it or are too unwell to undergo the procedure. It may also be because a trained operator is not available or suitable clinical space available.

A different technique exists called 'induced sputum' which we think may be helpful. Induced sputum is where a patient spends up to 30 minutes breathing in a highly salty mist through a loose-fitting mask. The salty solution helps break down any thick phlegm in the deep parts of the lung (where there might be infection). A physiotherapist then helps the patient do some breathing and coughing techniques to enable them to cough up samples from deep in the airways which can then go to the lab.

Induced sputum has been used safely for many years in the field of Cystic Fibrosis which is a condition where patients often struggle to bring up thick phlegm. Comparing induced sputum to bronchoscopy has been successfully tried before in patients with possible TB (Mycobacterium tuberculosis) infection and in children. No-one has specifically looked at this procedure in haematology patients before and nobody has ever asked the patients who have had both techniques which one they prefer.

In addition, we plan to use an absorbent pad to absorb some of the mucous just on the inside of your nostril. This technique is uses a very small pad called a Nasosorption stick. There is good evidence that it is a good technique to detect viruses but we are interested in taking this further. Because the nose is an important "entry point" of a lot of respiratory infections we want to look at the immune cell that we pick up here and compare it to what we find in the lungs to see if we can explain why some patients become more unwell than others. . We would be looking at some of the same samples in more detail at a later date. We want to test all the samples that we have in one go and so we will freeze and store the nasosorption samples and the bronchoscopy samples for the future and need to ask you for your specific permission for storage of your samples.

Why have I been asked to take part?
We are inviting patients aged 18 and upwards who have a haematological condition which predisposes them to infection, or who already have an pulmonary infection and are an inpatient in the University Hospital of Wales.

How can I take part?
A member of your care team will check if you are eligible to take part and if you are interested, a member of the research team will explain the study to you and answer any questions you might have. You will be asked whether or not you want to take part. If you would like to, you will be asked to sign a consent form and then we can begin the study.

Do I have to take part?
It is up to you to whether or not to take part. If you do decide to take part you will be given this information sheet to keep and a copy of your signed consent form. If you decide to take part, you are still free to withdraw at any time without giving a reason. Should you decide not to take part, you do not have to provide a reason for this choice and it will not change the care you receive.

What does the study involve?

The study would involve:

- Signing an informed consent form
- Having a physical examination performed by a member of the research team, specifically looking at your heart, lungs and abdomen. This can be performed on the ward in whatever environment is comfortable for you and will take no more than 5 minutes

- Vital signs measurements (observations) – these are the same as the observations done by the nursing staff on the ward (pulse, temperature, oxygen levels and blood pressure) but we will want to repeat these when you agree to take part in the study and we will do them during any procedure you have done
- Some blood tests – you may have already had these done when you first arrived in hospital. In which case they don't need to be done again
- An ECG (electrical tracing of your heart) – you may have already had this done on the ward, in which case it does not need to be done again
- We would like to look at your CT scan – your haematology team would usually want you to have a CT scan of your chest and sometimes sinuses if they are worried about serious bacterial or fungal infection. We would like to look at the scan and the report but will not ask you to have another one
- An induced sputum procedure done here on the ward. This involves breathing a highly salty mist through a loose-fitting mask for up to 30 minutes. You will do this in a side room with a physiotherapist helping you. This will be interrupted every few minutes by the physiotherapist helping you cough up a sample of phlegm from your chest. The water can leave a salty taste in the mouth temporarily. The physiotherapist is there to help you cough effectively but you may find that the salty water also makes you feel like you want to cough. The only other risk of this procedure is that, rarely, some patients temporarily feel slightly breathless using this technique. This is very short lived and the physiotherapist will be present throughout, monitoring your oxygen levels for your safety and ready to give you any medication you may need. We would like you to have this procedure twice. All the samples will be sent to Public Health Wales for analysis.
- A nasosorption stick procedure. This involves wiping a small (approximately 2cm) absorbent pad on the inside of your nostril. This will then be frozen and stored here at University Hospital of Wales, Cardiff ready for a future piece of research into why some Haematology patients become more unwell than others. We are freezing them so that we can test these small samples altogether but need your permission to do so. This is an entirely optional part of the SPITFIRE study.
- One bronchoscopy procedure (your clinical team would be planning this anyway)
- A questionnaire after each procedure about how you feel the procedures went and how you felt before, during and afterwards

The study would not involve any additional hospital visits, it would occur whilst you are an inpatient on the ward.

For clarity we have summarised the above in this table:

Procedures that you can expect to have normally (your "standard of care")	Extra procedures we would like you to have as part of SPITFIRE
Vital signs	Vital signs during procedures
ECG	Physical examination
Blood tests	Nasosorption stick procedure – optional
CT scan	Induced sputum procedure (done on two separate occasions)
Bronchoscopy (camera test/magic eye test)	Questionnaires after each procedure

What are the possible benefits?

You are being invited to take part in this study because your haematology team is concerned that you may have an infection and they are considering you having a bronchoscopy in the future to help plan your treatment. By taking part in the study and also having the induced sputum procedures, more samples than “normal treatment” will be taken to the lab. This may allow your team to make a diagnosis earlier and treat you more accurately. This study may change how we investigate unwell haematology patients in the future and you will be contributing to us improving this.

The tests into how your immune system is working is purely for our research and has no benefit for you directly. Indirectly you will be helping to improve our understanding of the body’s fight against infection which will benefit the wider haematology community.

There is no financial benefit to you taking part.

What are the possible risks?

The risks of the bronchoscopy are no different whether you take part in the study or not.

The induced sputum procedure has a low chance of making you feel more breathless temporarily. As part of the procedure, we regularly test your oxygen levels and a trained member of staff monitors you throughout the test and can give you inhalers if the breathlessness becomes uncomfortable. The salty mist may affect your taste briefly and may make you cough.

We appreciate you are in hospital because you are unwell and you may feel tired or unwell when completing the questionnaires with us. If you do not want to continue at any time we can pause the questionnaire and return another time or not at all if you prefer.

The saving of samples for future use

As part of the optional extra part of the study, with your permission, your sample will be passed to the Cardiff University Biobank (CUB) when it is no longer required for this study. You can withdraw your sample from CUB at any point without giving a reason, if you do withdraw your consent any sample that has not already been used for research will be destroyed according to local practices.

In order to maximise the potential of your sample, information collected through this study might also be passed to CUB, such as your NHS number and information from your medical records (both now and in the future). Neither your name nor any other identifying information from any of these sources will be shared with researchers and your identity will always be protected.

Researchers in the UK may request permission to use your sample. Samples will only ever be used in research that has the ultimate goal of helping patients or the general public.

CUB will seek to recover costs for the work it does but your samples will never be sold for profit. By signing the biobanking statement on the consent form you are agreeing for your sample to be used for these purposes.

Will my taking part in this study be kept confidential?

All information which is collected about you during the course of the research will be kept strictly confidential. Your data will be anonymised and will be stored on a secure server at C&VUHB on a password protected computer and only the research team will be able to access it. Identifiable information (e.g. contact details) will not be shared outside the research team and will be kept separate to the study data. Your personal information and results from the study will be stored on secure computers within C&VUHB and only accessed by the research team involved in the study. Should you wish to withdraw from the study, you are free to do so but data collected will be

retained. At the end of the study your data will be archived for 5 years in accordance with good research practice and C&VUHB data protection regulations and archiving procedure.

What will happen to the data collected in the study?
Data will be kept secure for 5 years in line with good research practice and data protection regulations imposed by C&VUHB in line with the General Data Protection Regulations 2018. All data obtained during the study will be kept confidential. Access to data will only be available to the investigators attached to the project and C&VUHB.

C&VUHB is the sponsor for this study based in the United Kingdom. We will be using information from you in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. C&VUHB will keep identifiable information about you for up to 5 years after the study has finished. Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally identifiable information possible. You can find out more about how we use your information by contacting cav.ig.dept@wales.nhs.uk.

How will we use information about you?

We will need to use information from you and your medical records for this research project.

This information will include your initials and NHS number. People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

We will keep all information about you safe and secure.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

What are your choices about how your information is used?

You can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have.

If you choose to stop taking part in the study, we would like to continue collecting information about your health from your hospital records. If you do not want this to happen, tell us and we will stop.

We need to manage your records in specific ways for the research to be reliable. This means that we won't be able to let you see or change the data we hold about you.

If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study. Further information about the Cardiff University Biobank can be found in this Information Sheet or by speaking to one of the research team.

Where can you find out more about how your information is used?

You can find out more about how we use your information

- at www.hra.nhs.uk/information-about-patients/
- our leaflet available from www.hra.nhs.uk/patientdataandresearch

- by asking one of the research team
- by sending an email to cav.ig.dept@nhs.uk

Some questions you may have

Am I able to have the induced sputum procedure without having the bronchoscopy?

Yes, this is possible. This is also an option that we will be offering to patients who are unable to have a bronchoscopy for whatever reason (e.g. they currently need a lot of oxygen) but are still keen to be part of the study. However, we are looking for as many people as possible to have both the bronchoscopy and induced sputum procedures to allow us to compare them as much as possible

Will my haematology consultant and their team know I am taking part in this study?

Yes, they will need to be informed that you are taking part or have declined. This is because we will need their help with the logistics of organising the procedures and to make sure you remain safe afterwards. The team will also have access to the results of any procedures done.

Will my GP know I am taking part in this study?

It is not necessary for us to contact your GP to inform them that you are taking part in this study.

Will I be paid for taking part in the study?

You will not be paid for taking part in the study. The whole study will take place whilst you remain an inpatient and so no travel expenses will be needed. The bronchoscopy procedure will be booked as soon as possible after you consent (if it has not been booked already) and sometimes this takes place in Llandough Hospital. If this is the case, you would be transported there and back via ambulance for the procedure. We have no control over where the bronchoscopy takes place.

What if I don't want to take part?

If you decide to not take part you will continue to be looked after by your regular haematology team and the standard care you receive will not be affected. . All the treatment that you will need will still be available. If the team feel that you need a bronchoscopy (outside of the trial) this can still be arranged.

What if I decide halfway through the study that I don't want to take part?

You can withdraw your consent at any time and withdraw from the study without giving reason. If you remain in hospital we will approach you to ask questions regarding what helped you make this decision to withdraw from the study but this is entirely optional and is purely to help us adapt the study for the future. If you have already had one or more of the procedures, we will include the results of these in our analysis.

You are free to withdraw from the study at any time and do not have to give a reason. If you withdraw from the study, we will retain your data and samples collected up until this point.

Who is organising and funding the study?

This study is being organised by a group of researchers based in Cardiff and Vale University Health Board. It is chiefly run by the Respiratory department. Dr Jonathan Ayling-Smith is a postgraduate research student and is undertaking SPITFIRE as part of an MD qualification with supervision from Respiratory and Microbiology departments and Cardiff University.

The study has been funded by Cardiff and Vale University Health Board through the provision of researcher time and testing. Trudell have provided the study with equipment needed to perform the procedure.

Who has reviewed the study?
Before any research goes ahead it has to be checked by a research ethics committee (REC) to make sure that the research is safe to conduct. This study has been reviewed and approved by REC.

What if there is a problem?
This study is being led by Dr Jamie Duckers at C&VUHB. If you have any problems about this study, our contact details are at the end of the information sheet. If you are harmed by taking part in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for a legal action but you may have to pay for it. Regardless of this, if you wish to complain, or have any concerns about any aspect of the way you have been approached or treated during the course of this study, the normal C&VUHB complaints mechanisms should be available to you. Please discuss any complaints about the way our study has been carried out with us first. If you are still unhappy and want to formally complain, please contact concerns@wales.nhs.uk.

Thank you for reading this information sheet.

If you have any further questions please contact Dr Jonathan Ayling-Smith or Dr Jamie Duckers using the contact details below, or ask a member of your care team to get in touch and we will attend you to answer any questions you may have;

Jonathan.ayling-smith@wales.nhs.uk

Jamie.duckers@wales.nhs.uk

Appendix C: SPITFIRE consent form

INFORMED CONSENT FORM

Study Title: SPITFIRE: Sputum Induction Trial For Improved Respiratory Evaluation

Chief Investigator: Dr Jamie Duckers

Participant ID: …….. Initials: …… Date of Birth: ……..

1. I confirm that I have read the information sheet dated **(version.....)** for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily	☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. Data and samples collected up until my withdrawal may still be used in the study.	☐
3. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by healthcare professionals from Cardiff and Vale University Health Board, Cardiff University, regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	☐
4. I understand that as part of this study I will undergo up to 2 induced sputum procedures in addition to what my usual medical team have planned. The samples generated from these procedures will be analysed in Public Health Wales in accordance with local Microbiology standards of practice.	☐
5. I understand that I will be asked to complete questionnaires about all of the procedures I undertake whilst part of this study	☐
6. I understand that the information provided by me will be held confidentially. Data from all parts of this study will be stored using a code so that only the researchers can trace this information back to me individually (anonymised-linked). I understand that any information that could identify me will be kept strictly confidential and that no personal information will be included in the study report or other publication. The information will be retained for 5 years.	☐

7. I understand that the information collected about me will be used to support other research in the future, and may be shared anonymously with other researchers	☐
8. I agree to take part in the above study	☐
9. (OPTIONAL) I agree to a nasosorption procedure and the processing and subsequent frozen storage of this sample in preparation for future planned research	☐ ***(OPTIONAL)***
10. (OPTIONAL) I agree for my sample to be passed to the Cardiff University Biobank (CUB) when it is no longer needed in this study. I understand information, including my NHS number and medical records, may also be passed to CUB and my identity will never be released to researchers. I understand my samples may be accessed by researchers from the UK. I agree for my sample and associated information to be used for these purposes.	☐ ***(OPTIONAL***

Person agreeing to take part	
Signature:	Date:
Name:	
Person conducting consent (STAFF CONFIRMATION)	
By signing below, I show that: • I have explained the study to the potential study participant and what will happen to his/her blood and sputum samples collected during the study. • I have given the potential study participant the chance to ask questions and I have answered them to his/her satisfaction. • I have given the potential study participant enough time to think and decide whether or not he/she wants to take part in the study. • I explained that he/she may talk with others before making a decision.	
Signature:	Date:
Name:	
Witness (only required if the potential study participant is unable to read or write due to literacy or illness or if required per local legislation)	
I confirm that: • I am not linked to the study	

• I attended the consent process • I have read the information for the study.	
Signature:	Date:
Name:	

Appendix D: SPITFIRE Sputum Induction Protocol

SPUTUM INDUCTION PROTOCOL

Introduction

The aim of sputum induction is to collect an adequate sample of secretions from the lower airways in order to obtain a respiratory sample for microbiological analysis

Objective

This SOP ensures that sputum induction is performed in a safe, reproducible and standardised manner. To ensure quality and reliability of the laboratory tests carried out on sputum.

In Preparation

- Discuss with the PI / medical personnel prior to the procedure whether the patient requires a clinical evaluation prior to sputum induction and whether medical personnel are required to be present in the room during the procedure (e.g. patients with a perceived high risk of bronchospasm), or whether it is sufficient for the medical personnel to be immediately available (i.e. physically available in the proximity of the procedure room, so as to respond immediately in the case of an emergency). Respiratory medical personnel should be trained in advanced life support.
- Induced sputum is an aerosol-generating procedure and so the following additional precautions should be taken in preparation
    - Do not proceed with sputum induction if there is any clinical suspicion of COVID-19 regardless of test result.
    - Do not proceed with sputum induction if the patient has a documented positive COVID-19 test without a subsequent documented negative COVID-19 test
    - Procedure should only be performed in single occupancy rooms.
    - Anyone assisting this procedure with the patient should be wearing universal protective equipment as well as a respiratory mask for which they have been fit tested
- Set up equipment in designated room.
- Not necessary to brush teeth/wash mouth unless patient has been drinking/eating immediately prior to procedure
- Give detailed instructions to patient prior to the procedure
- Obtain consent from patient prior to procedure being carried out.
- Have a blank copy of the study-specific Sputum Induction Worksheet ready

Personnel and Equipment

- Should be conducted by an experienced health care professional and trained resuscitation personnel should be available
- Spirometer and pulse oximeter
- Supplemental oxygen
    - Oxygen can be passed through the Aerobika if necessary, to maintain saturations
- Full resuscitation equipment should be available at close proximity to the procedure room.
- Hypertonic Saline solution (7% as directed by study-specific protocol)
- Patient's own rescue bronchodilator medication (inhaled or nebulised salbutamol or other similar) or prescribed rescue medication e.g. Salbutamol 100mcg/dose, with anti-static valved holding chamber
- Ombra table top compressor
- Aerobika
- Calculator
- Stop watch
- Single patient / disposal equipment: nebuliser mouthpiece/mask, spirometry bacteria filter, collection container for sputum
- Tissues
- Cup of water
- Alcohol wipes
- Gloves
- Gown, if deemed necessary
- Mask as per local policy for aerosol generating procedures (for health professional)

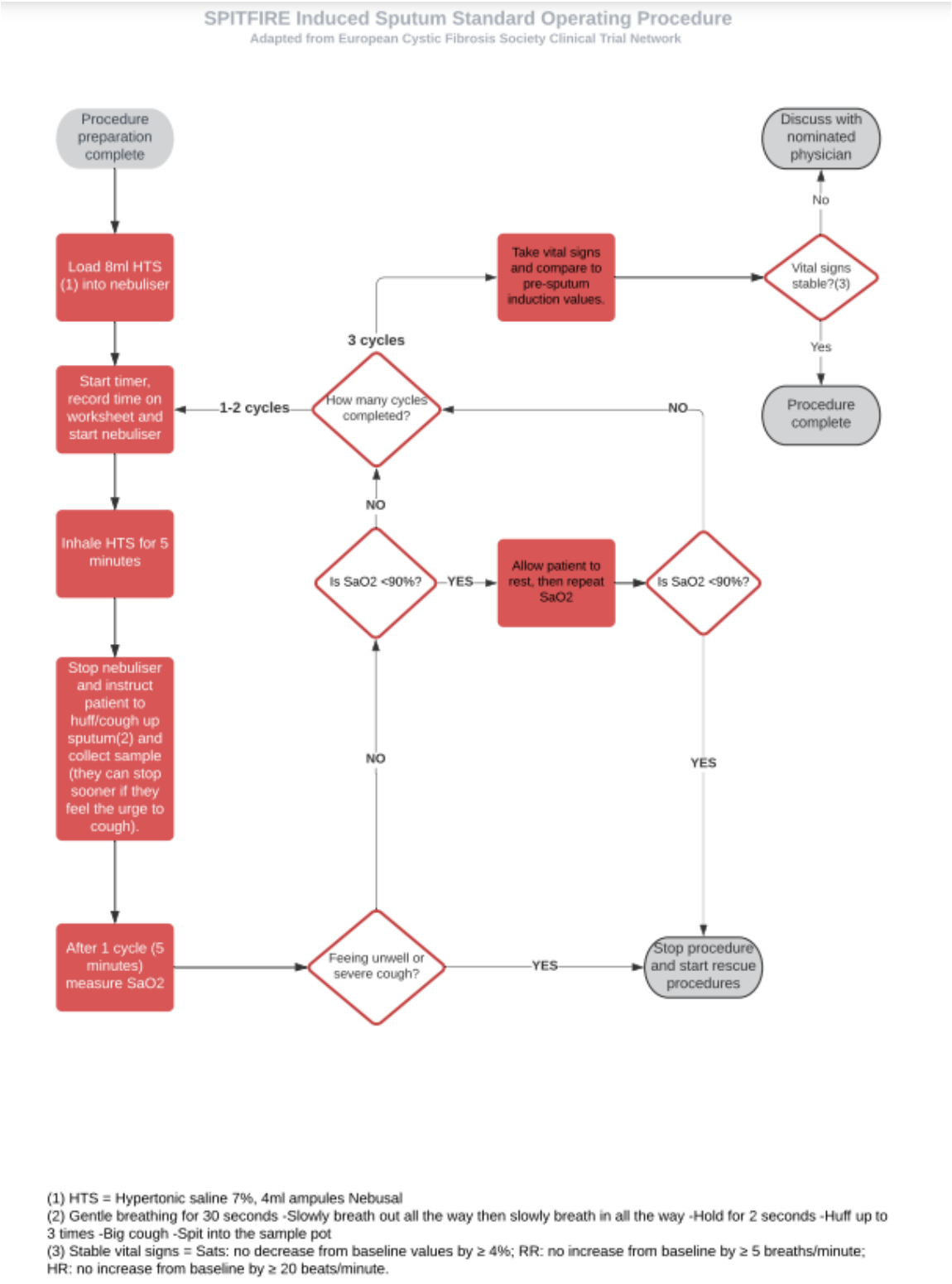
Care/Contraindications

- Oxygen therapy
- Nausea/vomiting
- Severe bronchospasm
- Pneumothorax
- Pleural effusion
- Haemoptysis
- COVID-19 positive

Procedure instructions/flowcharts

1. Assemble and check the equipment as per manufacturer's guidelines. Label the sputum containers.
2. Encourage the participant to blow his/her nose as often as needed during the procedure to prevent nasal secretions from becoming mixed with the induced sputum sample.

3. Record vital signs (Pulse oximetry, respiratory rate (RR) and heart rate(HR)), record on the worksheet
4. Patient should take an inhaled bronchodilator (patient's own or prescribed by medic) regardless of whether the patient has an obstructive disorder. (200-400micrograms-usually 2-4 puffs from a standard metered dose inhaler +/- spacer device).
5. Instruct patient on how the procedure will be performed, what is expected of them and potential adverse symptoms (salty taste, wheeze, chest tightness, nausea, light headedness, cough, need to swallow, sore throat, shortness of breath, headache). Explain that these are transient. Instruct the patient to breathe in HTS solution by taking 2-3 slow deep breaths (± breath hold) interspersed with tidal breathing.
6. Begin sputum induction (see Flow chart)



(1) HTS = Hypertonic saline 7%, 4ml ampules Nebusal
(2) Gentle breathing for 30 seconds -Slowly breath out all the way then slowly breath in all the way -Hold for 2 seconds -Huff up to 3 times -Big cough -Spit into the sample pot
(3) Stable vital signs = Sats: no decrease from baseline values by ≥ 4%; RR: no increase from baseline by ≥ 5 breaths/minute; HR: no increase from baseline by ≥ 20 beats/minute.

Stopping the procedure

- The procedure should be stopped when:
  - 3 cycles have been completed
  - The patient complains of dyspnoea, chest tightness or wheeze

    - The patient shows signs of respiratory distress or is light-headed or feels nauseated
- Mouthwash or drink should be offered to remove any unpleasant after taste
- An interval of 2 days is recommended between subsequent inductions

Rescue Procedures

Rescue procedures will be used when the oxygen saturations drop below 90% or if oxygen saturation decreases by 4% or more, or if participant feels unwell or has severe coughing
For the patient's safety, sputum induction should be discontinued once rescue procedures are initiated.

1. Begin the rescue procedure by administering the bronchodilator - 2 puffs of salbutamol MDI, 100mcg/dose
2. 10 minutes after the last puff of bronchodilator, perform the forced expiratory manoeuvre.
3. If oxygen saturation has returned to baseline, the rescue procedure is complete. Proceed to discharge patient. DO NOT resume the sputum induction.
4. If oxygen saturation has not returned to baseline, try having the participant perform 3 huffs and a cough in order to try to clear secretions from the central airways. Repeat O_2 saturation measurement and then repeat from step 1.
5. If the patient has not recovered after 15 minutes consider discussing with the nominated physician.

Infection control

- Do not proceed with sputum induction if there is any clinical suspicion of COVID-19 regardless of test result.
- Do not proceed with sputum induction if the patient has a documented positive COVID-19 test without a subsequent documented negative COVID-19 test
- Procedure should only be performed in single occupancy rooms.
- Anyone assisting this procedure with the patient should be wearing universal protective equipment as well as a respiratory mask for which they have been fit tested
- Wash and thoroughly dry hands before and after contact with the patient regardless of use of gloves
- Any reusable equipment should be disinfected according to Trust policies (for example use alcohol wipes and allow equipment to air dry completely before storage or re-use)
- Nebuliser should be cleaned with tap water after each use to avoid clogging of valves with salt.
- The nebuliser should be sterilised as per manufacturer's instructions

- The participant's own equipment should also be cleaned and disinfected thoroughly
- If saliva and sputum are collected in separate containers: after sputum induction is completed, cover the saliva collection container and discard following institutional guidelines for hazardous materials

Regulations, Guidelines, References

Paggiaro PL, Chanez P, Holz O et al. Sputum Induction, *Eur Resp J* 2002, 20, Suppl 37, 3s-8s

Pizzichini E, Pizzichini MM, Leigh R et al. Safety of sputum induction, *Eur Resp J* 2002, 20, Suppl 37, 9s-18s

Appendix E: SPITFIRE Induced Sputum CRF

Induced Sputum Worksheet

Patient Initials/#:		Date:	
Operator role/grade:		Operator name:	
Contact physician in emergency:		Clinical frailty score[1]:	
Volume+%HTS used:	ml %		

Contraindications checked: ☐ Verbal consent ☐

Pre-sputum induction vital signs

Heart rate	**Respiratory rate**	**Pulse oximetry**
beats/min	breaths/min	%
Bronchodilator administration:	Dose taken	
	Time taken (HH:MM)	:

Sputum induction

Induction cycles	**Time (HH:MM)**	**Pulse oximetry**	**HR**	**Comments**
Start time	:			
End of cycle 1	:	%	beats/min	
End of cycle 2	:	%	beats/min	

End of cycle 3	:	%	beats/min	
Stop time	:			

Successful procedure (sample obtained): ☐

Please indicate your perception of patient tolerance and comfort:

Poorly tolerated, uncomfortable throughout	1	2	3	4	5	Well tolerated, comfortable throughout

Please complete as appropriate:			
Adverse event	Descriptor	(please tick)	Comment
Hypoxaemia	Transient hypoxemia (saturations <94%) responding to an increase in FiO2		
Lowest saturation	Please record here the lowest recorded O2 saturation		
Minor bleeding/haemoptysis	Coughing up <20ml blood or blood stained sputum up to 1 hour post procedure		
Major bleeding/haemoptysis	Coughing up ≥20ml blood or blood stained sputum up to 1 hour post procedure		
Hypotension	A ≥20 mmHg change in systolic or diastolic blood pressure, mean arterial pressure <60		

	mmHg, or need for pressors, crystalloid or colloids.		
Wheezing	Audible wheezing or need treatment with bronchodilators		
Cough	Only include if uncontrollable coughing requiring cessation of procedure		
Arrythmia	New or changed rhythm not present at the start of procedure, including acceleration or deceleration (>20 beats/min change from baseline) of the existing rhythm (excluding sinus tachycardia) or any rhythm requiring pharmacological therapy or cardioversion/defibrillation.		
Respiratory failure	Oxygen saturation <88% for ≥2 minutes despite increasing fraction of inspired oxygen or any length of time of SaO2 ≤88% accompanied by bag mask ventilation for apnea/hypoventilation		
Please document here any other adverse events and any additional comments			

1. For reference, the Clinical Frailty Score is outlined below

Clinical Frailty Scale*

1 **Very Fit** – People who are robust, active, energetic and motivated. These people commonly exercise regularly. They are among the fittest for their age.

2 **Well** – People who have **no active disease symptoms** but are less fit than category 1. Often, they exercise or are very **active occasionally**, e.g. seasonally.

3 **Managing Well** – People whose **medical problems are well controlled,** but are **not regularly active** beyond routine walking.

4 **Vulnerable** – While **not dependent** on others for daily help, often **symptoms limit activities.** A common complaint is being "slowed up", and/or being tired during the day.

5 **Mildly Frail** – These people often have **more evident slowing,** and need help in **high order IADLs** (finances, transportation, heavy housework, medications). Typically, mild frailty progressively impairs shopping and walking outside alone, meal preparation and housework.

6 **Moderately Frail** – People need help with **all outside activities** and with **keeping house**. Inside, they often have problems with stairs and need **help with bathing** and might need minimal assistance (cuing, standby) with dressing.

7 **Severely Frail** – **Completely dependent for personal care**, from whatever cause (physical or cognitive). Even so, they seem stable and not at high risk of dying (within ~ 6 months).

8 **Very Severely Frail** – Completely dependent, approaching the end of life. Typically, they could not recover even from a minor illness.

9. **Terminally Ill** - Approaching the end of life. This category applies to people with **a life expectancy <6 months**, who are **not otherwise evidently frail.**

Scoring frailty in people with dementia

The degree of frailty corresponds to the degree of dementia. Common **symptoms in mild dementia** include forgetting the details of a recent event, though still remembering the event itself, repeating the same question/story and social withdrawal.

In **moderate dementia**, recent memory is very impaired, even though they seemingly can remember their past life events well. They can do personal care with prompting.

In **severe dementia**, they cannot do personal care without help.

* 1. Canadian Study on Health & Aging, Revised 2008.
2. K. Rockwood et al. A global clinical measure of fitness and frailty in elderly people. CMAJ 2005;173:489-495.

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Appendix F: SPITFIRE Bronchoscopy CRF

Bronchoscopy Case Report Form

Patient Initials/#:		Date:	
Operator name:		Operator grade:	
Hospital Site:		Duration of bronchoscopy: (from intubation to extubation)	
Route of intubation:			

Pre-medication/sedation/analgesia used: (include Lidocaine flushes)		
Drug *(+ strength if known e.g. 1%):*	Route:	Dose:
e.g. Midazolam	*e.g. IV*	*e.g. 2mg*

Indicate lobe(s) with a tick:	Showing disease/abnormality on CT	Washed during bronchoscopy:	Approximate volume of sample aspirated (mls):
Left Upper Lobe			
Lingula			

Left Lower Lobe			
Right Upper Lobe			
Right Middle Lobe			
Right Lower Lobe			

Please indicate your perception of patient tolerance and comfort:

Poorly tolerated, uncomfortable throughout	1	2	3	4	5	Well tolerated, comfortable throughout

Please complete as appropriate:			
Adverse event	Descriptor	(please tick)	Comment
Hypoxaemia	Transient hypoxemia (saturations <94%) responding to an increase in FiO2		
Lowest saturation	Please record here the lowest recorded O2 saturation		

Minor bleeding/haemoptysis	Coughing up <20ml blood or blood stained sputum up to 1 hour post procedure		
Major bleeding/haemoptysis	Coughing up ≥20ml blood or blood stained sputum up to 1 hour post procedure		
Hypotension	A ≥20 mmHg change in systolic or diastolic blood pressure, mean arterial pressure <60 mmHg, or need for pressors, crystalloid or colloids.		
Wheezing	Audible wheezing or need treatment with bronchodilators		
Cough	Only include if uncontrollable coughing requiring cessation of procedure		
Arrythmia	New or changed rhythm not present at the start of procedure, including acceleration or deceleration (>20 beats/min change from baseline) of the existing rhythm (excluding sinus tachycardia) or any rhythm requiring pharmacological therapy or cardioversion/defibrillation.		
Respiratory failure	Oxygen saturation <88% for ≥2 minutes despite increasing fraction of inspired oxygen or any length of time of SaO2 ≤88% accompanied by bag mask		

	ventilation for apnea/hypoventilation		
Please document here any other adverse events and any additional comments			

Appendix G: SPITFIRE Participant Questionnaire

Post-Procedure Questionnaire

Patient Initials/Study number (if known):		Date:	

Which procedure are you answering this questionnaire about? (please circle)

1st induced sputum 2nd induced sputum Bronchoscopy

Other than during this study have you had this procedure before? (please circle) Yes / No

For each of these statements please indicate to what extent you agree by circling a number, where 5 is strongly agree and 1 is strongly disagree. If you feel the question does not apply to you, please leave blank and "not applicable" will be assumed.

I was anxious prior to the procedure				
1	2	3	4	5
Strongly disagree				Strongly agree
The procedure was explained to me (either verbally or written) to an adequate extent prior to the procedure				
1	2	3	4	5
Strongly disagree				Strongly agree
I was given ample opportunity to ask questions about the procedure				

1	2	3	4	5
Strongly disagree				Strongly agree
I was satisfied by the quality of care during the procedure by the staff				
1	2	3	4	5
Strongly disagree				Strongly agree
The amount of pain relief given was adequate				
1	2	3	4	5
Strongly disagree				Strongly agree
The amount of sedation given was adequate				
1	2	3	4	5
Strongly disagree				Strongly agree
I was comfortable during the procedure				
1	2	3	4	5
Strongly disagree				Strongly agree
I tolerated the procedure well				
1	2	3	4	5

Strongly disagree				Strongly agree

If my medical team felt it was necessary, I would be happy to have the procedure again

1	2	3	4	5
Strongly disagree				Strongly agree

We are keen to improve our pre-procedure explanations and after-care. Can you describe the symptoms or side effects (if any) that you experienced

Any other comments that you would like to make about this procedure

Thank you for taking the time to complete this questionnaire

Appendix H: SPITFIRE participants demographics, past medical history, drug history and clinical observations at consent

Participant number	Age	Sex	Primary haematology diagnosis	BMT[i] type	Immunosuppression	Past medical history	Antibiotics	Observations at consent		
								FiO2[ii]	Temperature	NEWS[iii]
1	56	F	Multiple myeloma	Autologous	None	Primary biliary cirrhosis, systemic sclerosis	Meropenem, Levofloxacin, Aciclovir	28%	37.2	3
2	52	F	AML[iv]	Allogeneic	Azacitidine	Previous supraventricular tachycardia	Tazocin, Aciclovir, Voriconazole, Levofloxacin	21%	37.4	1
3	65	F	AML	Allogeneic	Low dose cytarabine	None	Aciclovir, Tazocin, Voriconazole	28%	37.1	3
4	62	F	Myelodysplasia	Allogeneic	Ciclosporin,Mycophenolate	CLL[v], MGUS[vi]	Meropenem, Levofloxacin, Voriconazole, Aciclovir	21%	37.3	1
5	80	M	AML	N/A	Azacitadine	Atrial fibrillation	Meropenem	21%	37.5	0
6	51	F	AML	N/A	Cytarabine, daunorubicin	Crohn's disease, non-epileptic seizures, polycystic ovarian syndrome, anxiety, psoriasis	Co-trimoxazole, Aciclovir, Fluconazole	21%	36.6	2

7	78	F	AML	N/A	Low dose cytarabine	Hypertension, diverticulosis	Meropenem, Aciclovir, Fluconazole	21%	38.8	4
8	26	M	AML	N/A	Cytarabine, daunorubicin, Gemtuzumab-ozogamicin	None	Tazocin, Amikacin	21%	37.3	0
9	63	M	AML	Allogeneic	None	Hypothyroidism, hypoadrenalism	Meropenem, Levofloxacin	28%	38.1	6
11	67	F	AML	N/A	None	Type 2 diabetes mellitus	Aciclovir, Fluconazole, Meropenem, Levofloxacin	21%	38.9	3
12	70	M	AML	N/A	None	Benign prostate hypertrophy, hyperlipidaemia	Meropenem	21%	37.2	1
13	51	F	Chronic immune thrombocytopenia	N/A	Azathioprine, prednisolone	Recurrent venous thromboembolism, myocardial infarction, subdural haemorrhage, CMV[vii] viraemia, colitis	Voriconazole, valganciclovir	21%	37.1	0
14	24	M	AML	N/A	Cytarabine, daunorubicin, gentuzumab	None	Meropenem, Clarithromycin, Vancomycin, Aciclovir, Fluconazole	21	37.1	5
15	50	F	Myelodysplastic syndrome	N/A	Cytarabine, daunorubicin	Sjogrens syndrome, microscopic colitis	Voriconazole, Aciclovir, Ciprofloxacin	21%	37.1	1

16	24	F	AML	N/A	Fludarabine, high dose cytarabine, idarubicin	Fibromyalgia, recent covid	Co-trimoxazole, Meropenem, Levofloxacin, Amphotericin B	21%	36.6	4
17	54	M	CML[viii]	Allogeneic	Imatinib	None	Meropenem, Aciclovir, Cotrimoxazole	28%	37.3	3
18	52	F	AML	N/A	Irinotecan and floxuridine	*E.faecium* in blood cultures with hydronephrosis and nephrostomy	Fluconazole, Meropenem, Linezolid, Voriconazole	32%	37	7
19	51	M	AML	N/A	None	Prior alcohol excess. Prior pneumothorax	Tazocin, Clarithromycin, Fluconazole	21%	37.1	0
20	50	M	Diffuse large B cell lymphoma	N/A	Rituximab	Porcine aortic valve replacement 2020	Tazocin, Clarithromycin	32%	36.8	5

[i] Bone marrow transplant
[ii] Fraction of inspired oxygen
[iii] National early warning score
[iv] Acute myeloid leukaemia
[v] Chronic lymphocytic leukaemia
[vi] Monoclonal gammopathy of undetermined significance
[vii] Cytomegalovirus
[viii] Chronic myeloid leukaemia

Appendix I: Results of SPITFIRE participant baseline investigations

Participant	**Neutrophils ($x10^9/L$[i])**	**Platelets ($x10^9/L$)**	**CRP[ii] (mg/L[iii])**	**Blood culture**	**Device culture**	**Viral result**	**Sputum culture**	**Fungal biomarkers**	**CT thorax**
1	1.7	45	38	Negative	Negative	Parainfluenza	N/A	Negative	Extensive bilateral airspace opacification and centrilobular nodules. Bibasal consolidation
2	0.7	14	265	Negative	N/A	Negative	N/A	Negative	Consolidation within medial right lung demonstrates peripheral groundglass opacification. Nodular opacities also demonstrating some surrounding groundglass opacification
3	0.1	19	163	Negative	N/A	Negative	Scanty *Pseudomonas* growth	Negative	Cavitating lesion, multiple foci of consolidation and groundglass opacification in the right lung with mucus plugging of right sided bronchi
4	1.8	13	58	Negative	N/A	Negative	Negative	Negative	Dense area of mass like consolidation with poorly defined margins, peripheral satellite nodularity and possible

									a degree of adjacent groundglass. Small bilateral pleural effusions
5	0	78	217	Negative	N/A	Negative	N/A	Glucan mild positive	Progressive extensive dense cavitating consolidation in right lung. Includes substantial fluid components reflecting likely liquefied necrotic lung and some central cavitation
6	0	35	103	Negative	N/A	Negative	N/A	Negative	Peribronchovascular nodularity and atelectasis. Groundglass opacification in perilymphatic distribution. Upper zone groundglass opacifications
7	0	58	240	Negative	N/A	Negative	Negative	*Aspergillus* PCR[iv] positive. Glucan and galactomannan negative	Patchy groundglass change and interstitial thickening within left lower lobe. Some ground glass change in right middle lobe persists.
8	0	113	9	Negative	N/A	Negative	N/A	Negative	Wedge shaped predominantly ground glass change at right lung base.

9	2.9	104	1	Negative	N/A	Negative	Negative	Glucan positive	Patchy airspace shadowing throughout both lungs, progressive
11	0.5	7	360	Negative	Negative	Negative	N/A	Negative	Patchy atelectasis throughout both lungs with some septal thickening. Small bilateral pleural effusions
12	0.1	15	141	Negative	N/A	Rhinovirus	N/A	Negative	Small area of consolidation at the right lung base with background emphysema. Nodular change throughout the right lung
13	8.9	24	14	Negative	*Candida metapsilosis* from central line	CMV[v] in serum	N/A	Glucan positive	Minor groundglass change in bilateral lower lobes unchanged compared to previous images.
14	0	16	353	Coag. Negative Staph	Coag. Negative Staph	Negative	N/A	Negative	Several subpleural nodules, background scattered groundglass nodules in lower lobes with tree in bud changes.
15	0.1	16	40	Negative	N/A	Negative	N/A	Negative	Groundglass nodules in left lung.

16	0	9	388	*Candida dubliniensis*	Negative	Covid positive low level 3 weeks prior	N/A	*Aspergillus* PCR positive. Glucan positive	Diffuse peribronchovascular nodularity throughout all lobes with more patchy groundglass changes in bilateral upper lobes. New left pleural effusion
17	5.1	108	165	Negative	N/A	Epstein-barr virus low level positive	N/A	Negative	Groundglass opacification in both lungs. Background emphysematous change
18	0	15	468	*Enterococcus faecium*	N/A	Negative	N/A	Negative	Patchy consolidation throughout both lungs with background emphysema. Reactive lymph nodes
19	0.1	210	93	Negative	N/A	Negative	N/A	Negative	Numerous inflammatory nodules in middle and lower lobes with groundglass halos and coalescing into larger areas of consolidation.
20	8.8	96	187	Negative	N/A	Seasonal coronavirus and adenovirus	N/A	Negative	Extensive bilateral groundglass opacification in periphilar distribution. Septal thickening in associated regions.

[i] $x10^9$ per litre
[ii] C-reactive protein
[iii] Milligrams per litre
[iv] Polymerase chain reaction
[v] Cytomegalovirus

Appendix J: SPITFIRE microbiology results and diagnoses for participants with paired induced sputum and bronchoscopy results

Participant number	**Bronchoscopy result**	**Diagnosis utilising bronchoscopy (standard of care)**	**1st induced sputum result**	**2nd induced sputum result**
3	*Pseudomonas* growth, *Aspergillus* growth, galactomannan positive	Probable aspergillosis, *Pseudomonas* pneumonia	Not processed (transport error)	Low positive *Aspergillus* PCR[i], no growth. Galactomannan positive
4	*Enterococcus faecium* growth, *Aspergillus* PCR positive, galactomannan positive, *Candida* growth	Probable aspergillosis	*Aspergillus* PCR positive, galactomannan negative, yeast growth	*Aspergillus* PCR positive, galactomannan positive, yeast growth
5	*Aspergillus* PCR positive, Coagulase negative *Staphylococcus* growth	Probable aspergillosis	*Aspergillus* PCR positive, *Pseudomonas* growth	*Aspergillus* PCR positive, respiratory flora growth
6	Coagulase negative *Staphylococcus*	Pneumonia	Respiratory flora, high galactomannan	Respiratory flora, negative galactomannan
8	Negative	Tonsillar abscess	Negative	Stored and not processed
9	*Pneumocystis* PCR positive, Coag negative *Staphylococcus*	Pneumocystosis	*Pneumocystis* PCR positive, galactomannan positive	*Pneumocystis* PCR positive (lower Cq[ii])

13	Positive galactomannan (low), alpha haemolytic *Streptococcus*	Probable aspergillosis	*Aspergillus* PCR positive, galactomannan positive, respiratory flora	Positive galactomannan (reducing from IS1[iii])
14	Negative	Central line infection	Galactomannan positive (low), respiratory flora	Galactomannan positive
20	*Pneumocystis* PCR, galactomannan positive, *candida* positive, *Escherichia coli* growth	Pneumocystosis	*Pneumocystis* PCR positive, galactomannan positive, *Penicillium* growth	Galactomannan positive, *Candida* growth

[i] Polymerase chain reaction
[ii] Quantification cycle
[iii] 1st induced sputum

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