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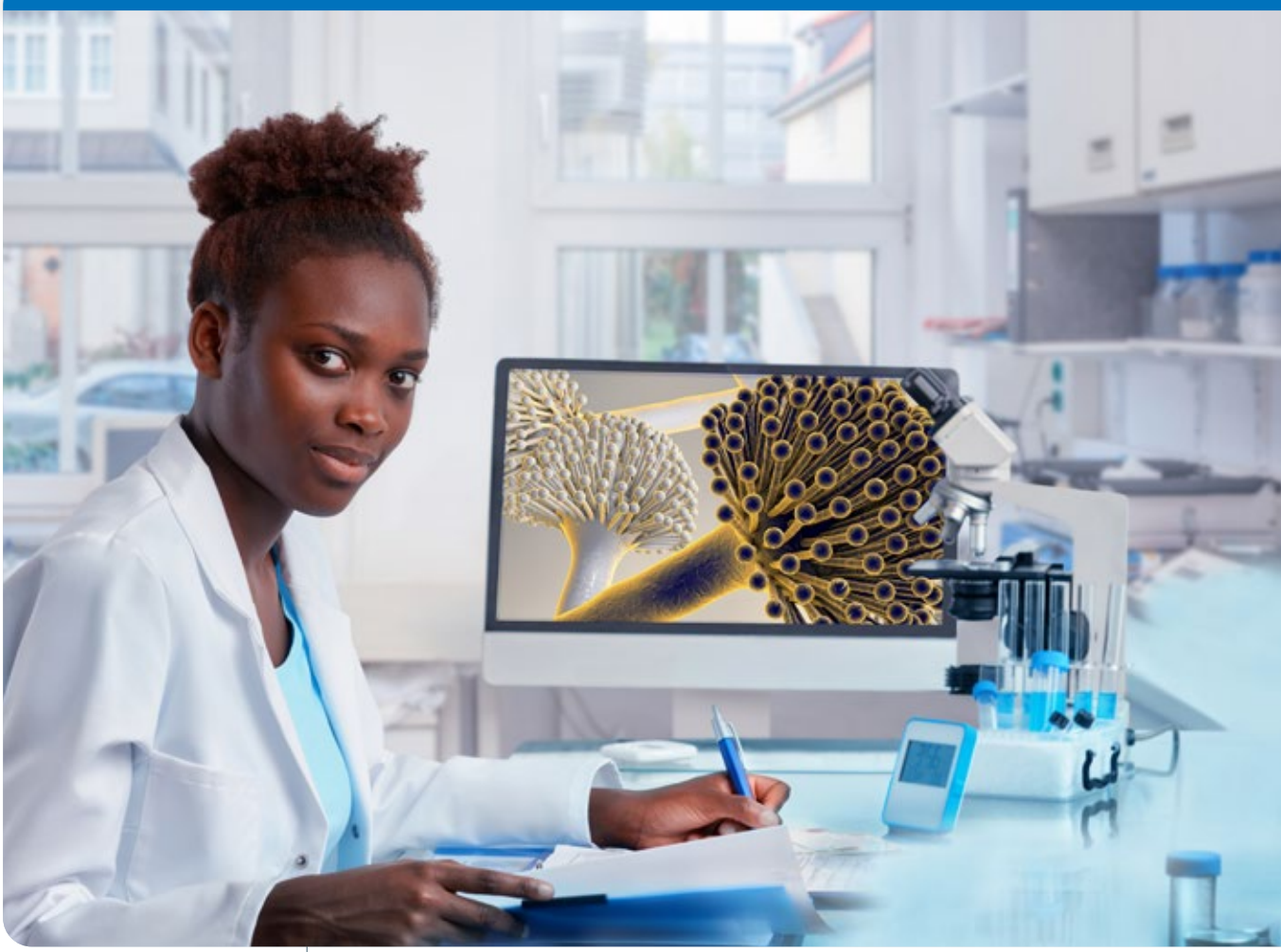


Expert Insights

- Improving Patient Outcomes of Aspergillosis: Rapid Diagnosis by PCR

PCR

Innovation with Integrity

Improving Patient Outcomes of Aspergillosis: Rapid Diagnosis by PCR

Clinical scientists at Public Health Wales are pioneering rapid *Aspergillus* PCR tests, with the potential to reduce morbidity and mortality in high risk patients

Bruker Fungiplex® Aspergillus PCR

Rapid, reliable tests are needed to diagnose invasive aspergillosis, caused by the fungus *Aspergillus*. PCR is used as part of biomarker testing for this disease and Dr Lewis White, Clinical Scientist at the Regional Mycology Reference Laboratory, Cardiff, comments on its use:

"Bruker's Fungiplex Aspergillus PCR assay is a highly sensitive test for patients at risk of invasive aspergillosis. Clinicians, by using this test, have the potential to provide an earlier diagnosis, therefore minimising mortality and morbidity."

Synopsis

The use of polymerase chain reaction (PCR) in clinical practice has undergone a standardization process in recent years, to ensure accurate analytical performance in *Aspergillus* diagnosis. Aspergillosis, until recently, has often been treated empirically with antifungal medication – an expensive approach – which potentially leads to unnecessary side-effects. PCR technologies, such as Fungiplex Aspergillus, can now be used to reduce the broad application of these drugs. Their high predictive value can rule out aspergillosis, and high specificity can rule in a diagnosis of aspergillosis earlier. In combination with other biomarkers, PCR allows pre-emptive treatment and the potential to reduce morbidity and mortality. This case study highlights the work of a specialized laboratory in Cardiff, Wales, which use a novel PCR test for the diagnosis of aspergillosis in a clinical setting. There are also commercial tests available such as Bruker's Fungiplex Aspergillus PCR which is a CE-IVD validated kit for this purpose.

Regional Mycology Reference Laboratory, Public Health Wales

Public Health Wales – the national public health organization for Wales – is composed of a number of specialised services conducting research and providing health advice. One such service, delivered by the Mycology Regional Reference Unit, undertakes both routine and specialist services for the diagnosis of fungal diseases. Conducting the work is the Regional Mycology Reference Laboratory, headed by Dr Lewis White, Clinical Scientist, who oversees the daily mycology services. Dr White's role at the laboratory drives the service forward, by introducing new tests in order to meet new clinical demand, as well as providing technical liaison between the laboratory and clinicians.

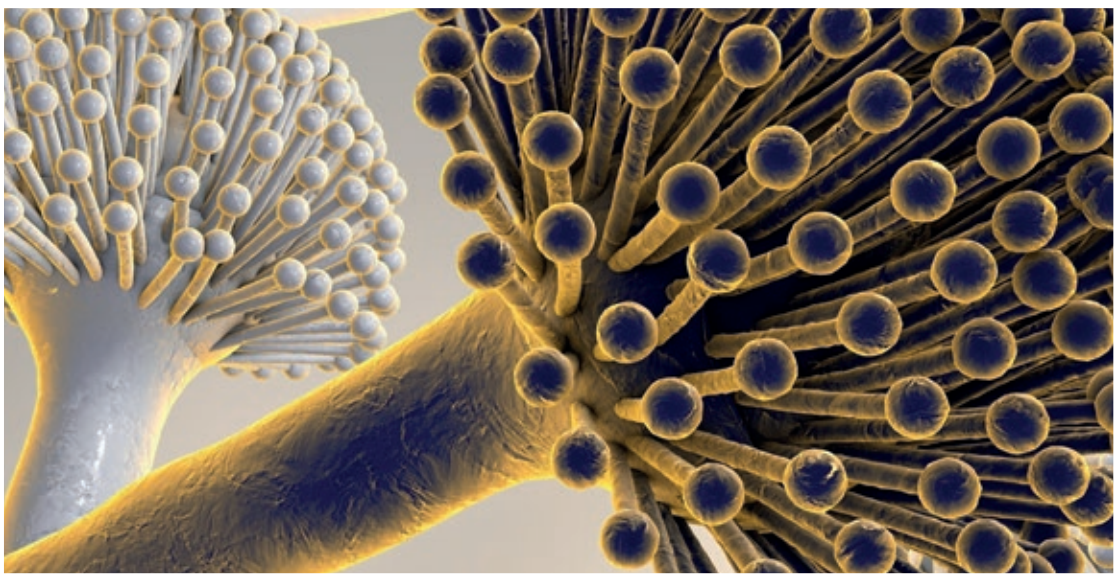
Professor Rosemary Barnes, emeritus Professor of Medical Microbiology at Cardiff University and honorary Consultant Microbiologist at Public Health Wales, recruited Dr White in 2000 as a clinical scientist, to introduce novel diagnostics for fungal disease. Professor Barnes and Dr White have ongoing research projects and work closely with the Fungal Polymerase Chain Reaction Initiative (FPCRI).

Aspergillus, a widespread fungus

Aspergillus spores are ubiquitous throughout the environment in most countries and are readily airborne. We come into contact with the fungus on a regular basis but only immuno-compromised individuals – for example, those on immunosuppressant drugs following a transplant or on chemotherapy – and patients with pre-existing lung conditions are susceptible to disease. In these individuals, the fungus can cause aspergillosis, a broad term which encompasses a group of diseases including invasive aspergillosis, allergic bronchopulmonary aspergillosis (ABPA), chronic pulmonary aspergillosis (CPA) and aspergilloma (a fungal ball). Acute invasive aspergillosis requires urgent treatment and causes a high degree of morbidity and mortality in susceptible patients.

Although they do not normally cause illness, high numbers of *Aspergillus* spores are commonly found in air conditioning units, soil, compost heaps, soft furnishings, and particularly in damp or flooded housing where circulating levels of spores can be high. When a patient is known to be immunocompromised in hospital, every precaution is taken to prevent the individual coming into contact with infectious agents. However, building projects close to, or within a hospital have been associated with increased levels of infection, where large quantities of spores from the environment are released into the surrounding atmosphere exposing patients in the hospital during building work. Furthermore, many immunocompromised patients are now treated through day units and will inevitably be exposed to *Aspergillus* spores during their daily life.

Symptoms of aspergillosis vary depending on the degree of disease state, and also depending on the patient. General symptoms include a cough, fever, fatigue, coughing blood, breathlessness and weight loss, but these symptoms are not definitive of the disease. As *Aspergillus* is airborne, the primary organ affected is the lungs, but with invasive disease there is the potential for secondary infection in other parts of the body.



Combatting aspergillosis

Depending on the disease presentation, an array of antifungal drugs are used to treat aspergillosis. 'Azoles' such as itraconazole, voriconazole, posaconazole, and now isavuconazole and the polyene liposomal Amphotericin B are common antifungal drugs, with the specific medication depending on the type of aspergillosis. The challenge facing clinicians is that the symptoms of invasive aspergillosis are non-specific, so they commonly resort to broad spectrum empirical antifungal treatment which often exposes the patient to unnecessary medication with potential side effects and at high cost. UK antifungal expenditure is estimated to be over £100 million per annum, and rising. The drugs are in the top ten most expensive items for most haematology and critical care units. Diagnostic tools traditionally include radiological investigations, blood tests, bronchoscopy or biopsies to provide samples for histopathology and microscopic and microbiological investigations. Radiological investigations, particularly CT scans can visualize abnormalities such as dense lesions, lung cavities, or the presence of an aspergilloma (fungal ball). With invasive aspergillosis the pulmonary abnormalities can be transient and only detectable by CT scan in the early stages of invasive aspergillosis, after which radiological signs become non-specific or appear too late to be therapeutically useful.

Research undertaken over the past 20 years has revealed polymerase chain reaction (PCR) as an invaluable tool for diagnosing invasive aspergillosis. Initially the use of *Aspergillus* PCR was limited, due to a lack of standardised commercial testing solutions [1]. However, Fungiplex Aspergillus is a CE-IVD test that is now commercially available.

Collaborating with Bruker

The laboratory provided Bruker with the analytical and clinical specimens needed for the retrospective evaluation of its Fungiplex® Aspergillus PCR test. By working with Bruker in the validation of its test, the laboratory is able to collaborate on the development of innovative diagnostics. Fungiplex Aspergillus is a real-time PCR designed to detect the main species of *Aspergillus* associated with invasive aspergillosis, and differentiates species resistant to first-line treatment. It provides results in under two hours from extracted DNA, enabling laboratories to support time-critical clinical decision-making.

PCR as a diagnostic tool

The ability of PCR to detect slow-growing, difficult to culture microorganisms has propelled its popularity as a microbiological detection technique. By not requiring growth of the pathogenic organism, PCR offers detection in a fraction of the time, also contributing highly sensitive and specific detection of nucleic acids from organisms such as fungi. For patient management to be directly influenced, PCR results need to be available within 24 hours, to enable pre-emptive treatment and targeted therapy. Early diagnosis and treatment of invasive aspergillosis is essential for effective patient management, and therefore a rapid technique such as PCR can contribute to the timely treatment of high-risk patients.

In 2008, the European Organisation for the Research and Treatment of Cancer/Mycoses Study Group (EORTC/MSG) definitions of invasive fungal disease were revised, but molecular methods such as PCR were still not used due to limited standardization and a lack of clinical validation [2]. The European *Aspergillus* PCR Initiative (EAPCRI) was formed in 2006 – now known as the Fungal PCR Initiative (FPCRI) – with the goal of standardising *Aspergillus* PCR methodology to overcome these barriers. Significant progress has been made towards this goal, with the ultimate outcome of improving diagnosis times and the resultant patient management. The Regional Mycology Reference Laboratory works closely with the FPCRI for collaborative research and contribution to the *Aspergillus* standardisation process and there is a proposal to include *Aspergillus* PCR in the second revision of the EORTC/MSG definitions. The laboratory also collaborates with many academic institutions globally, to progress research and evaluate PCR as a standardised tool for *Aspergillus* diagnosis.

Currently, PCR can be used for two objectives: ruling out aspergillosis during a screening strategy in high risk immunocompromised patients, or ruling in a diagnosis of invasive aspergillosis in patients with suspected disease. The former is possible due to the high negative predictive value of the test, which is conferred by its high sensitivity and means a negative test result can exclude a diagnosis of disease when used in combination with other biomarker tests. The predictive value of a positive PCR test can be enhanced by combining PCR with serological tests (galactomannan antigen) for invasive aspergillosis.

The laboratory work

At the forefront of mycobiological research, Dr White and Professor Barnes use a PCR assay for *Aspergillus* detection and diagnosis. As a result of this, the laboratory conducts *Aspergillus* testing for the whole of Wales, splitting time between assay research and development, and commercial testing. The laboratory's goals are two-fold: to reduce the number of patients receiving unnecessary empirical and long-term antifungal treatment, and to pre-emptively treat high-risk patients for aspergillosis before the disease clinically manifests itself. For the past decade, the laboratory has been optimising a standardised *Aspergillus* process, to increase the number of aspergillosis cases diagnosed in the clinical setting, and to reduce the use of empirical antifungal drugs and minimise the duration of targeted therapy. Professor Barnes describes the challenges in the field which spurred the laboratory's interest in this work:

"Historically, when a patient was prescribed antifungal medication, that person would stay on treatment for much longer than necessary. Not only does this drive up costs, but has an impact on patient wellbeing. Follow up tests can determine when an appropriate time is to stop treatment, so we now implement biomarker screening so patients can be on treatment for less time."

In terms of diagnostic assays, and in addition to the *Aspergillus* PCR, the laboratory performs routine culture and microscopic investigations, including testing for dermatophyte infections, the galactomannan ELISA (enzyme-linked immunosorbent assay), an Aspergillus resistance PCR, a

Pneumocystis PCR, cryptococcal antigen testing, *Aspergillus* IgG testing and ß-D-glucan testing. The laboratory also provides a range of reference facilities, such as the identification of fungi referred from other hospitals and antifungal susceptibility testing. The laboratory is accredited to ISO 15189 standards.

Dr White describes the frequency of these tests in the laboratory:

"We're currently doing two Aspergillus *PCR runs per week, the galactomannan ELISA three times per week, when required. The* Aspergillus *IgG – for chronic aspergillosis – we perform once a week as the turnaround time is less critical for most patients. We conduct the ß-D-glucan test twice per week, and the PCP (*Pneumocystis Pneumonia*) PCR is done four times per week. On top of this we have our reference service: currently if a specimen comes in for culture, the requesting clinician may be interested in more than just fungi. There could be a range of bacterial or viral infections causing symptoms in the patient. So rather than splitting the specimen for processing in multiple different labs, the microbiology laboratory will perform the prerequisite culture. If a fungus is cultured on the plate, it is then referred to the mycology lab for identification.*

In addition to the 15,000 tests per year that the laboratory conducts, it is also heavily involved in research, and published 14 papers in 2017. The majority of tests that the laboratory receive are ordered by clinicians:

"What we're doing is quite different from other labs" explains Professor Barnes. She continues: *"Our work is translational, and we have to complete the journey from lab bench to bedside effectively."*

Developing PCR

Dr White, under a research grant, has developed an analytically validated PCR which was offered to haematologists for evaluation as a service improvement for their patients. The purpose of the work was to evaluate the impact of the *Aspergillus* PCR test on patient management.

Following the first audit and publication in 2008, *Aspergillus* PCR was introduced rigidly and the laboratory has been auditing and publishing ever since. Professor Barnes explains:

"We were able to show within six months to a year that we had reduced antifungal expenditure within the Trust very markedly, and certainly more than the cost of the PCR testing itself."

Due to this success, the Public Health Wales Trust accepted *Aspergillus* PCR as a workable test, partly due to the evidence that the test had a good negative predictive value. Initially, a trial period of two years was agreed on a pay-per-test basis. After a further three years, the PCR was fully engrained into the Public Health Wales contracts with the hospitals. Frequent testing is required, either to rule out or to diagnose invasive aspergillosis: the laboratory screens high-risk patients two times per week with *Aspergillus* PCR for early detection of *Aspergillus* DNA in patient blood samples. Constant audits are conducted to show an improvement in service and over the years, the laboratory has shown improved PCR standardisation, particularly in combination with galactomannan. Professor Barnes describes these improvements:

"We have also gone one stage further, in that we no longer have to wait for a patient who is positive by multiple biomarkers to develop clinical signs of aspergillosis.

We treat patients pre-emptively on the basis of targeting infection rather than overt disease. In doing this we have reduced our rates of invasive fungal disease within the patient population without increasing the costs."

This process is multidisciplinary across haematology, pharmacology and microbiology. The laboratory, using PCR, can ensure that all patients receiving antifungals in the haematology setting are only doing so on the basis of a clinical need, driven primarily by biomarker testing. Once a patient has begun antifungal treatment, the utility of the biomarkers change and PCR screening is stopped. Dr White explains the follow-up process after testing:

"Individual patients are discussed in stewardship meetings, and advice is given to clinicians from microbiologists whether treatment should be given and whether it is appropriate. We provide advice downstream of testing."

Evaluating the use of PCR

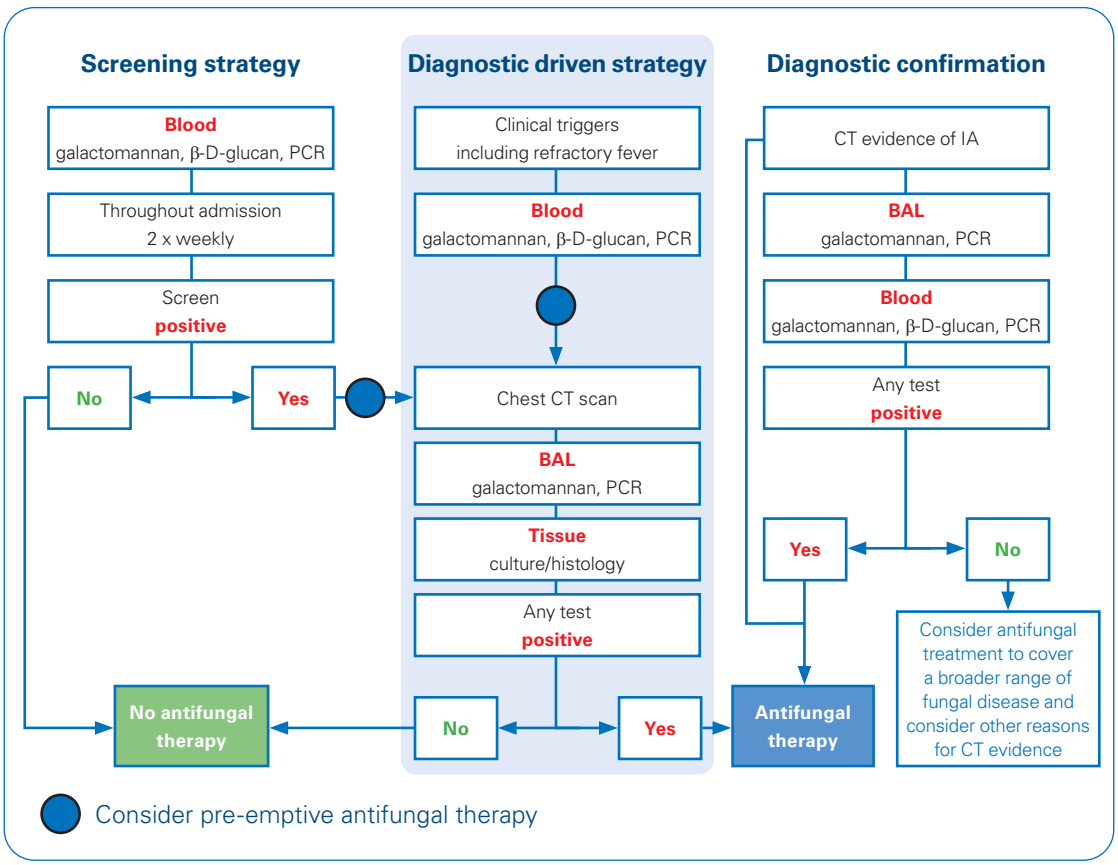
The key strategy of the laboratory has been to convince the clinician that a positive biomarker is often a driver for a CT scan. This can be a challenge, because a scan can make it seem as if there is no infection, when the galactomannan and PCR results are positive. Clinicians must be convinced that a negative CT scan may not necessarily mean the patient is not at risk of developing aspergillosis. Pre-emptive treatment – providing antifungal treatment on the basis of positive biomarkers – is administered before the development of disease in those patients, and most will never go on to develop aspergillosis. Since the implementation of regular weekly testing of high risk patients with PCR and biomarker tests, the laboratory has reduced its incidence of invasive fungal disease by 75% as a result of using pre-emptive treatment to ensure patients do not go on to develop disease as defined by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group/Mycoses Study Group (EORTC/MSG).

"We were looking at the incidence of invasive fungal disease and mortality and the performance of the test – its positive predictive value and negative predictive value – as well as its use in combination with other biomarkers" explains Professor Barnes, continuing: *"This is where we showed a real benefit of combining* Aspergillus *PCR with galactomannan. We often found that PCR was positive prior to the galactomannan test, and certainly prior to radiology."*

This supports PCR as an early indication of disease.

Challenges and solutions

Currently, Public Health Wales Trust is one of a handful of centres in the United Kingdom (UK) to adopt PCR for *Aspergillus* testing. Scepticism arises from a lack of understanding of the difference between screening a high-risk group and introducing a diagnostic test in patients with suspected disease. Funding structures are another barrier to the acceptance of PCR as an acceptable technique. For Wales, the cost savings from the introduction of an *Aspergillus* PCR are directly seen by the Trust. Institutions within countries such as Spain



and Australia are implementing similar protocols to Public Health Wales and are achieving impressive results [3, 4].

"Whilst our research has directly proven cost savings through reduced empirical antifungal treatment, a study in Spain has achieved results showing less invasive fungal death in patients where clinicians have been convinced of the benefits of proving a patient doesn't have a disease, rather than the other way round [3]. In microbiology, we are familiar and comfortable with diagnosing a patient with a disease based on a positive test result. It takes time and a change of mind-set to show the benefits of believing a negative test result" explains Dr White.

Animal models have shown that PCR becomes positive within an hour of exposure to *Aspergillus*, which can be perceived as problematic in the clinical setting because of the high numbers of the population exposed to the fungus on a regular basis. Dr White explains why this is challenging:

"If you have an patient who has been exposed to an infectious agent and is at high risk of developing disease caused by that agent, that is something we should react to pre-emptively and prevent the disease manifestation, rather than looking at the results as potential false positives. The difficulty we have in the clinical setting is that we're never going to know that – even though it's classified as acute invasive aspergillosis – the overt manifestation is acute. We will never know how long it has been persisting as a sub-clinical presentation, post-exposure. We have had patients with a strong positive Aspergillus *result, which can be several weeks if not months before they present with overt disease. This is why it's so important to treat pre-emptively."*

Treating early exposure would mean administering therapy before any clinical signs are observed on a CT scan, increasing the patient's chances of survival and reducing overall incidence of fungal disease. Currently, the laboratory is creating its own evidence base for its approach to treating aspergillosis, both through its pragmatic methods and through work with other institutions in Europe.

Antifungal drug resistance

Similarly to the widely-reported mounting problem of antibiotic resistance, resistance to azoles for the treatment of fungal disease is a rising concern in the field. PCR can be used to detect markers of resistance in a clinical specimen without the need for isolation of the organism. Being able to detect molecular markers associated with resistance to frontline antifungal drugs for aspergillosis is crucial, but is impeded by the classically low sensitivity of culture of *Aspergillus* from bronchoalveolar lavage (BAL) in the laboratory. Such resistance is usually environmentally driven; it is not necessarily the clinical use of azoles that make organisms resistant, but environmental strains of *Aspergillus* that acquire azole resistance through agricultural use of the antifungals. These strains can subsequently infect susceptible patients. Current guidelines recommend voriconizole as a frontline therapy for aspergillosis but many strains are resistant to this drug. Dr White explains this problem:

"The difficulty is, if you have a patient with invasive aspergillosis the mortality rate is high, full stop. If you haven't cultured an organism and conducted susceptibility tests, you wouldn't know whether the fungus was resistant or not. It may be that we're underestimating the number of cases of aspergillosis that are attributed to azole resistant Aspergillus strains. Most of our cases are defined as probable aspergillosis on the basis of radiology and the galactomannan test, not the culturing of an organism. This is really important to consider for the future of aspergillosis diagnosis and treatment."

The future of *Aspergillus* PCR

It is currently unknown why some patients go on to develop aspergillosis, when others who have received almost identical treatments will not, despite both inevitably being exposed to *Aspergillus*.

"Even when you have a high risk population, not all those patients are at the exact same risk of developing aspergillosis" explains Professor Barnes.

"This is the area in which we are moving forward" adds Dr White, continuing: *"Incorporating genomics with other clinical tests will help us see which patients have higher probabilities of developing disease, therefore enabling clinicians to manage them appropriately. We're probably a few years off implementing this in the real world, but it will really transform the way patients are managed [5]."*

For more information on Bruker's Fungiplex Aspergillus IVD PCR, please visit https://www.bruker.com/products/molecular-diagnostics/fungiplex-Aspergillus-pcr-kits.html.

For more information about the Regional Mycology Reference Laboratory, please visit http://www.wales-snhs.uk/sites3/page.cfm?orgid=457&pid=25381.

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