Candida species in cystic fibrosis: A road less travelled

SANJAY H. CHOTIRMALL*,#, CATHARINE M. GREENE* & NOEL G. McELVANEY
Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland

Candida species are isolated with high frequency from cystic fibrosis patients, yet their definitive role in the disease remains unclear. Previously considered to have minimal inherent virulence owing to their commensal ability, the last decade has heralded an increasing recognition of Candida infection among patients with cystic fibrosis. What has been more recently hypothesized is that the organism possesses virulence factors that play diverse roles at different body sites during varied stages of an infection. Currently, limited data is accessible in the area of cystic fibrosis. This review aims to provide an overview of the role of Candida species in cystic fibrosis as it is currently understood including the common local and systemic infections observed in clinical practice. The uncertain role of airway colonization and insight into emerging fields such as Candida–bacterial interactions are also addressed. Finally, we outline the current understanding of the innate, cellular and humoral immune responses associated with this genus which has been the major focus of work performed to date.

Keywords Candida, infection, colonization, immune response, cystic fibrosis

Introduction

Major advances in the care of cystic fibrosis (CF) patients have positively influenced prognosis over the last decade. Significant inroads into understanding the basic defect have accelerated the development of targeted therapies. However, the disease continues to present new challenges to clinicians and researchers, for example fungal airway colonization. The consequences of bacterial infection, colonization and need for segregation in outpatient clinics have all been confronted and curbed to the point that fungal colonizers with an undetermined role on the course of disease and progression are becoming increasingly recognized. Both yeasts and filamentous fungi have been identified as microbial pathogens in CF, particularly in the context of invasive disease in the transplanted population and allergic responses, for instance allergic bronchopulmonary aspergillosis (ABPA). One particular fungal genus isolated at high frequencies from sputum cultures is Candida but limited literature is available addressing the issues of Candida colonization and infection in CF. This is possibly because its manifestations are still considered relatively minor in comparison to other infectious agents. As a consequence, the role of Candida has received little attention in terms of clinical and scientific research. This review aims to provide an overview of our current understanding of Candida species in CF, its associated local and systemic infections and an insight into emerging data on its role in airway colonization and associated immune response.

The Candida genus

Oral thrush was the first infection of the Candida species described in humans and following identification of its reproductive potential by budding, the fungus was originally named Oidium albicans. Candida albicans became the adopted name used and since then many other species have been identified as playing roles in human infection.
The most common of these are *C. albicans*, *Candida glabrata*, *Candida krusei*, *Candida parapsilosis* and *Candida tropicalis* [1,2].

The genus has exponentially grown with hundreds of newer species being identified and described, but unlike dimorphic fungi, the morphology of a given *Candida* species remains fundamentally comparable *in vitro* or *in vivo*. *Candida* species are capable of causing chronic, localized or systemic infection collectively termed ‘candidiasis’ although most commonly act as commensals within the oropharynx, skin folds, gastrointestinal tract and vagina, but on occasion they can cause opportunistic infections [3]. Once infection ensues, significant morbidity and mortality results and consequently, systemic candidiasis has high mortality rates (>75%) [4–6].

Identification in the microbiology laboratory is achieved on Sabouraud dextrose media and *C. albicans* can be identified through germ tube testing or colorimetric detection of L-proline aminopeptidase and beta-galactosaminidase. Since recent isolation of *Candida dubliniensis* which produces false positive results in the above tests, a chromogenic agar culture method that allows isolation and identification of *C. albicans*, *C. tropicalis* and *C. krusei* has been widely adopted. A modified version of this media is available to more clearly distinguish *C. dubliniensis* [7]. Alternative older methods to distinguish between *C. albicans* and *C. dubliniensis* include assessment of their growth ability at higher temperatures (45°C for *C. albicans*) or specific DNA sequencing.

Patients with CF are at an increased risk of *Candida* acquisition and colonization due to use of inhaled steroids, diabetes mellitus and lifelong antibiotic treatment. However despite its frequent isolation from sputum, oral and vaginal swabs, it remains unclear what such positive cultures actually mean in practical terms for CF clinicians. We believe that a spectrum of ‘commensal–colonizer–pathogen’ most likely exists for the organism and where specifically the organism is on this spectrum at a particular time point may be dictated by the clinical state of the CF patient and whether bacterial co-colonizers are present in the airway.

Although frequently identified in CF, the clinical role of *Candida* species has yet to be definitively determined [8–10]. Bakare *et al.* [11] identified *Candida* as the second most frequent fungus to *Aspergillus* in the CF airway and as such the growth of the yeasts has been associated with more severe CF in patients who have received prolonged treatment with antibiotics, glucocorticoids and probiotics [12–14]. In terms of infection, Cimon *et al.* [15] performed a 5-year epidemiological study assessing the frequency of bronchopulmonary mycoses in a CF population and examined the aetiological role of individual fungal species in disease. The filamentous fungi *Aspergillus* and *Scedosporium apispermum*, together with *Candida* contributed the largest burden. Despite high isolation of *Candida* from CF patients, only a single case of candidiasis was observed and this low rate was attributed to the antifungal ability of various bacterial colonizers in CF. However, while invasive airway infections are rare events, the extent of airway damage from hypersensitivity phenomena remain unknown. We will now address the localized and systemic infections associated with *Candida* species in CF and subsequently tackle the complex issues of airway colonization, cross kingdom interaction and the immune response.

**Localized Candida infection in CF**

**Oral candidiasis**

*Candida* species are isolated from the oral mucosa in up to 40% of healthy adults and therefore considered to be commensals [16]. A cut-off point to distinguish between commensalism and colonization remains undetermined. Common risk factors associated with oral recovery include poor dentition, older age, diabetes mellitus, use of inhaled or systemic steroids, smoking, malignancy and frequent antibiotic use. Oral thrush usually presents as discomfort associated with a dry mouth and associated dysphagia. In some cases, altered taste is experienced. The diagnosis is usually straightforward and by direct observation of white membranous plaques on the buccal mucosa or soft palate. This may be confirmed microbiologically by staining a swab or culturing a rinse from the associated area. Atypically, foci of oral erythematous inflammation or angular cheilitis may present. There are clearly significant risk factors in the CF state that predispose to oral colonization and subsequent infection including impaired salivary secretion, steroid use, CF-related diabetes and recurrent courses of antibiotics. Antibiotics alter the homeostasis of oral flora and as such have a permissive action on *Candida* growth. In a study from Manchester, patients on direct questioning of those with major symptoms of CF, 40% (*n* = 17) complained of a sore mouth, 24% (*n* = 10) of thrush five times annually and 38% (*n* = 16) of a hoarse voice every three months [17]. In our own institution’s experience, we encounter regular instances of oral candidiasis annually following courses of antibiotics, but they resolve after a short burst of antifungal treatment (Fluconazole). We recommend microbiological confirmation in all cases by scrapings unless white plaques are directly observed on oral examination. This is because some of the symptoms described are not specific to oral thrush but can be found in associated vitamin deficiencies (Bző, B₁₂) or by simple blistering. We recommend that CF patients attending routine clinic be screened for risk factors and questioned at 3-month intervals with regard to the symptoms of oral...
Genital candidiasis

Genital candidiasis is a common occurrence in the general population with rates of a single occurrence of up to 75% and 50% recurrent episodes [2]. It may be asymptomatic or present with balanitis in males and pruritis with vaginal discharge in females. Most infections are caused by *C. albicans* (95%), but *C. glabrata* (5%) infections have been described [2]. There is limited but important literature available addressing these infections in CF patients with the majority focused on female manifestations. It has been more than a decade since Sawyer *et al.* [18] first reviewed the subject with a self-administered questionnaire in young women with CF (*n* = 55). Vulvovaginal candidiasis was more common in CF (35%) versus controls (13%) and additionally more persistent and difficult to treat. Antifungal use was a significant association and the work concluded that ‘health professionals generally trivialize illnesses and diseases that are common, easily treated and not life-threatening’. More recent work has included male patients and addressed symptomatic partners. Lyon *et al.* [19] evaluated 40 adults with CF (19 male, 21 female) and similarly found large proportions (62.5%, *n* = 25) experiencing symptoms of infection, but few (15%, *n* = 6) of them had been directly questioned about it at CF clinics. Patients refused to discuss if their partners were symptomatic. This highlighted to CF clinicians a major deficiency of clinical practice and questions about candidiasis should be featured during annual review clinic consultations. It is also important to consider that vaginal discharge in young CF women can be caused by other pathogens such as *Chlamydia, Gonococcus* or *Trichomonas* species and that there is an observed unexplained high incidence of genital *Chlamydia* in CF.

A third study addressing the same subject was performed by interviews of 101 CF patients and addressed symptom frequency and medical risks associated with *Candida* [20]. Patients were asked to report on personal risk factors for *Candida* infection and their desire to be questioned about ‘thrush’ in CF clinic. Many had two or more risk factors (92.1%, *n* = 93), however the only significant factor associated with genital *Candida* was long-term antibiotics (87.1%, *n* = 88, *P* = 0.001). Over 70% of patients, both males and females had symptoms of either oral or genital *Candida* or both simultaneously. Forty percent (18/45) of those with oral and two-thirds (33/50) of those reporting genital candidiasis described ‘distress’, but it did not affect desire for treatment. Most cases of oral infection were diagnosed by a CF physician while genital infections were mainly self-diagnosed. It is noteworthy that general practitioners diagnosed more cases of genital infection as compared to CF physicians. This is potentially explained by differing doctor-patient relationships in different settings or alternatively, because the focus of the CF unit remains on respiratory or gastrointestinal symptoms leading patients to believe that this forum is inappropriate for discussing other complaints. Most patients in the study did, however, want to discuss such issues and were unconcerned who their discussant was although some females predictably preferred to discuss the issues with female staff. This Manchester-based study is the largest to date and detected similar patterns as found in previous work. The most concerning new discovery was the high incidence of symptoms among CF patients, but only on direct questioning which places a future onus on CF clinics. A major criticism of all these studies was that symptom recording was not supported by microbiological confirmation of infections, an important point for future work. Additionally, although several publications have assessed the benefits and efficacy of antifungal treatment in vulvovaginal candidiasis in the ‘normal’ population, none have been performed in CF [21]. Despite this clear lack of available literature, we strongly recommend screening questions for infections at all CF clinic visits and depending on clinical findings, antifungal treatment be prescribed either empirically or following microbiological confirmation.

Systemic Candida infection in CF

Post-transplant Candidiasis

CF is the 3rd most common indication for lung transplantation and the opportunistic nature of *Candida* species suggest that the post-transplant period is a major source for such infections [22]. Despite this, candidiasis post-transplant remains rare and *Aspergillus* species are in fact more commonly encountered in this setting [23]. The main *Candida* infection following transplantation is surprisingly tracheobronchitis which includes anastomotic site infections. Bloodstream and other invasive infections secondary to *Candida* are rare and will not be addressed in any detail within this review except to state that, when present, they occur within the first month following transplantation.
[24,25]. This is primarily a consequence of the major surgical intervention and intensive care unit stay experienced by patients.

**Totally implantable venous access device (TIVAD) infection**

A more commonly encountered systemic infection associated with Candida involves the presence of a TIVAD commonly referred to as a ‘port’. Candida species in this setting are recognized as the most common source of infections resulting in septicemia [26–28]. Important risk factors for infection remain the same as that for other Candida infections. These are diagnosed by the presence of swinging pyrexia, systemic septicemia and positive blood cultures for Candida species taken from both the port site and peripherally. The first intervention remains to remove the offending device whose tip should also be sent for microbiological evaluation. Device removal results in significant clinical improvement, but aggressive antifungal therapy is concurrently administered during which time patients on the active transplant list have to be temporarily removed. Recently, we encountered a case series at our centre which presented a different setting to the traditional Candida port infection. We experienced three cases of TIVAD thrombosis and superior vena cava obstruction that required use of thrombolytic therapy [29]. In two of the three patients, their post-thrombolysis course was complicated by systemic candidiasis secondary to TIVAD infection. In these cases, we achieved a successful outcome following removal of the device coupled with aggressive antifungal treatment. Another more traditional case series conducted over a 6-year period in Manchester earlier this decade described that 15 adults with CF were diagnosed via positive blood cultures with a Candida port infection [17]. Here, a variety of Candida isolates were identified including C. albicans, C. parapsilosis and C. glabrata and excellent clinical outcomes again were achieved via device removal and systemic antifungal treatment dictated through in vitro susceptibility testing. Our own practice continues to evolve with regard to optimal treatment and we routinely look for at least two negative blood cultures following completion of the prescribed treatment course. Replacing ports depends on need, but we try not to replace them before 8 weeks following the last negative blood culture. To date, there remain no clinical trials or evidence-based guidelines to support these treatment practices. Another important point is that many Candida infections involve biofilm formation particularly with indwelling vascular catheters in the context of CF. These biofilms are microbiologically complex containing matrix enclosed microcolonies containing yeasts and hyphae in a bilayer structure [30]. Such Candida biofilms can be resistant to conventional antifungals through a multitude of mechanisms and as such future research needs to be conducted to determine the best and optimally standardized treatment of Candida port infections.

**Airway Candida colonization in CF**

It remains controversial as to whether Candida species are transient or persistent colonizers of the respiratory tract in CF patients. A study by Muthig et al. [31] showed that the mean persistence of Candida species was at least 9 months and that the species identified were genetically related and transmissible but susceptible to all antifungals tested. Although concerns of transmissibility persist, it is unsure whether they conclusively contribute to chronic infection and the inflammatory milieu in CF. We have assessed colonization rates at our own centre over prolonged time periods and found persistence rates in excess of that previously described. We have established that the main factors predicting colonization by C. albicans in CF patients are pancreatic insufficiency, osteopenia and co-colonization with Pseudomonas [32]. At first glance, this suggests that the more advanced a patient’s disease, the likelier their sputum contained C. albicans, a view of many clinicians and it may be that the organism acts as nothing more than a microbiological marker of disease severity in CF. To challenge this paradigm, we performed a complex multivariate regression analysis that allowed for the adjustment of confounders commonly found in CF to determine the strongest predictors of major clinical effects. The clinical outcomes we assessed included FEV1, BMI, hospitalizations for infective exacerbations and sputum colonization with Pseudomonas or Aspergillus species. Through such analyses, we found that C. albicans colonization significantly predicted hospital-treated exacerbations (P = 0.004) after adjustment for confounders. Exacerbation rate was also significantly increased following first acquisition of C. albicans. This latter finding is vitally important as it refutes the alternate view that frequent antibiotic treatment for large numbers of exacerbations gave rise to the C. albicans in sputa that we observed. We additionally detected that although C. albicans colonization was not a significant predictor of FEV1 or BMI, it longitudinally accelerated rates of decline for both parameters (P < 0.001 and <0.0001 respectively). The FEV1 dataset followed the trend suggested by a previous cross sectional analysis of a European CF registry which stated that C. albicans colonization was associated with 5–10% predicted decrease in pulmonary function [33]. Our study concluded that sputum colonization with C. albicans presaged a greater rate of FEV1 decline and increased hospital-treated exacerbations in CF and was the first longitudinal study in this area to suggest a potential pathogenic role for the organism in the CF airway [32]. What our data could not establish was
whether the fungus itself, a virulent co-colonizer or cross-kingdom interaction, was promoting the increased hospital admissions recorded. This is an avenue for future clinical investigation.

Newer airway Candida species have also emerged over the last decade and one pertinent example is the high recovery rates (10–25%) of C. dubliniensis from the oral cavity of HIV patients [34]. This new organism was subsequently described in the non-HIV population particularly in individuals receiving high antibiotic burdens [35]. Therefore, its detection in CF came as no surprise, but did involve a complex isolation procedure involving Staib agar [36]. What was surprising was that its prevalence rate in CF was higher than that found in HIV. However virtually nothing about its potential for virulence is known. There is no clear clinical or experimental evidence of differences in terms of pathogenic potential but cell surface hydrophobicity is known to play a role as compared to C. albicans [37]. C. dubliniensis is reported to exhibit cell surface hydrophobicity not observed in the adhesion of C. albicans [38] and thus can take advantage of the dehydrated respiratory secretions in CF and consequently proliferates. These observations may also explain why patients who are older (>30 years) and have more advanced disease are colonized by this yeast. Peltroch et al. [36] followed six CF patients with C. dubliniensis and while all patients remained stable with no invasive infection detected, its effect on lung function could not be conclusively established because of the small numbers. A larger epidemiological study of this Candida species in CF is warranted.

Candida-bacterial interactions

According to Costerton et al. [39], biofilms are ‘a structured community of bacterial cells enclosed in a self-produced polymeric matrix and adherent to an inert or living surface’. Traditionally biofilms have been thought to comprise a single bacterial species, however it is now increasingly recognized that mixed biofilms exist involving interactions between both prokaryotes and eukaryotes. Bacteria and fungi are found together in a variety of environments, but particularly in biofilms, where adherent species interact through diverse signaling mechanisms. In the host, C. albicans can often be found growing with bacteria in polymicrobial biofilms and interspecies interactions occur that can impact on the transition of C. albicans between virulent and nonvirulent states [30]. Under conditions of immune dysfunction, such as in the CF lung, colonizing C. albicans can become opportunistic pathogens causing mucosal and disseminated infections potentially impacting on patient mortality. In the biofilm environment, microbial species use ‘quorum-sensing’ (QS) molecules for cell-to-cell communication to promote collective behaviour within the population, enhance access to nutrients and niches, and provide a combined defense against competitor organisms [40,41]. The process of QS can cross the prokaryote–eukaryote boundary [40–43].

Interactions with Pseudomonas aeruginosa

Pseudomonas aeruginosa is the most prevalent opportunistic pathogen in individuals with CF and is the principal organism associated with biofilm formation in the CF lung, but Staphylococcus aureus and Burkholderia spp. are also considered important pulmonary pathogens in CF. The dimorphic yeast C. albicans is the most common eukaryotic microbe isolated from CF patient sputum [11,12,44]. Candida albicans can exist in a mixed biofilm where the prokaryotic and eukaryotic communities exhibit either synergistic or antagonistic interactions. Several studies suggest that P. aeruginosa and C. albicans interact with each other in vivo, and they are commonly found together in mixed infections [45]. In their seminal paper, Hogan and Kolter [42] first reported a pathogenic relationship between P. aeruginosa and C. albicans. They demonstrated how P. aeruginosa can form a dense biofilm on C. albicans filaments and kill the fungus. Interestingly this only occurred when C. albicans was growing in its filamentous (or hyphal) form – an essential feature associated with its virulence [46]. Pseudomonas aeruginosa neither bound to nor killed the yeast form of C. albicans and the ability of P. aeruginosa to kill filamentous C. albicans was dependent on a number of physiological factors including growth phase, nutrient availability, surface structures including flagellae and type IV pili, secreted QS factors and regulatory molecules such as rpoN [47]. By forming a biofilm on fungal filaments, P. aeruginosa may be able to obtain nutrients from C. albicans in a nutritionally scarce environment.

Prior to the killing of C. albicans by P. aeruginosa, signaling can occur between both organisms. The QS molecules of both species are responsible for this communication. For example the bacterial molecule 3-oxo-C12 homoserine lactone can affect Candida morphology, whilst the fungal 12-carbon sesquiterpene metabolite, farnesol, can interfere with Pseudomonas quinolone and pyocyanin production and swarming motility [41,43,45,48–53]. Thus eukaryotes and prokaryotes possess diverse signaling mechanisms to detect and respond to each other through QS signal molecules.

Interactions with Staphylococcus aureus

In oral biofilms a mutually beneficial interaction called coaggregation can occur where the adhesion of C. albicans to oral bacteria facilitates its colonization of the oral cavity [54–56]. In contrast, the interaction between C. albicans and...
and *P. aeruginosa* as described above is competitive and antagonistic in nature. A third mechanism of interaction that can occur is that evident between *Staphylococci* and *C. albicans*, which appears to be initially synergistic [57–59]. Carlson et al. [60,61] described a synergistic effect between *C. albicans* and *S. aureus* in a mouse infection model leading to enhanced animal mortality following dual infection suggesting that *C. albicans* can either enhance the virulence of *S. aureus* or impair the host’s immune defences. Extensive physical interactions are known to occur between *S. aureus* and both the yeast and hyphal forms of *C. albicans* in a mixed biofilm [62] and it has been suggested that farnesol has a role in orchestrating these interactions. After the initial synergy during *C. albicans–S. aureus* biofilm formation, farnesol then negatively affects staphylococcal biofilm formation, compromises cell membrane integrity, viability and susceptibility of *S. aureus* to a variety of clinically important antibiotics [62]. Thus farnesol may represent a therapeutic target for inhibiting the development of a mixed biofilm in the CF lung. However once the biofilm has been established, farnesol may actually behave as an antibacterial factor. It remains to be seen which has the more detrimental effect in the CF lung, *C. albicans* growing alone or in combination with *S. aureus* in a mixed biofilm.

**Interactions with other microbes**

Notwithstanding the ability of *C. albicans* to modulate bacterial growth, reciprocal evidence indicates that other bacteria may also play an important role in the pathogenesis of *C. albicans* infections. For example in the urinary tract, *Escherichia coli* can enhance adhesion of *C. albicans* to bladder mucosa [63], whereas in the gut indigenous microbes can inhibit mucosal adhesion of *C. albicans* [64]. Consequently alterations in the normal bacterial flora following treatment with broad-spectrum antibiotics may allow *C. albicans* to proliferate and invade tissues, greatly affecting its pathogenicity [64]. This is an important consideration for individuals with CF who are frequently prescribed antibiotics.

This may be most clearly studied in the oral cavity where adhesion of *C. albicans* to saliva-coated surfaces and proline-rich proteins is an important early step in colonization [54,65–67]. Many species of oral bacteria may compete with *C. albicans* for primary adhesion receptor sites [54,65,68,69], however once resident in the mouth, *C. albicans* can adhere to the major microbial constituents of early dental plaque.

**Candida and the immune response**

The outer strata of *Candida* species contain elements with antigenic potential, which include mannans and mannosproteins which upon human exposure induce an immunogenic response [70–73]. Where mannans-deficient, *Candida* strains are clinically less virulent and during the course of a *Candida* infection, cellular, humoral and innate immune responses all play a role [71,72,74–76].

**Innate immunity**

Recognition of microbes by the innate immune system depends on activation of specific pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs). For fungi the first PAMPs encountered by the immune system are those present in the fungal cell wall. The cell wall of *C. albicans* is composed of a core structure of β-(1,3)-glucan polysaccharide fibrils covalently linked to chitin (αβ-(1,4)-linked polymer of N-acetyl glucosamine) and β-(1,6)-glucan. The outer layer consists of N-linked [77] or O-linked mannosylated proteins called mannans [78]. Two classes of PRRs in particular play an important role in antifungal immunity – the C-type lectin receptors (CLRs) and Toll-like receptors (TLRs). Neutrophils, monocytes, macrophages and airway epithelial cells are all involved in defense against fungal pathogens. Dendritic cells (DCs) also respond to fungal PAMPs leading to activation of T-cell-mediated specific immunity. These various cell populations differ in their expression of CLRs and TLRs on the cell membrane, and are therefore capable of initiating different responses. CLRs and TLRs recognize the major polysaccharide cell wall components, N- and O-linked mannans, β-mannosides, β-(1,6)-glucan and phospholipomannan. CLRs comprise a large family of receptors, including the mannose receptor (MR), the dendritic cell-specific ICAM3-grabbing nonintegrin (DCSIGN), dectin-2, galectin-3 that share at least one carbohydrate recognition domain originally identified in mannose binding lectin (MBL) and recognize the mannann structures like TLR4. In contrast, complement receptor 3 (CR3) and dectin-1, two other CLRs, as well as TLR2 detect the β-glucans.

Much is known regarding the expression and function of TLRs in the CF lung [79,80]. The most important TLRs involved in recognition of *Candida* species in the lung are TLRs 1, 2, 4, 6, while TLR9 has a more minor role. In macrophages and dendritic cells TLR9 resides in the endoplasmic reticulum (ER) and redistributes to uCpG-containing lysosomal compartments for ligand binding and signal transduction [81]. Cell surface expression of TLR9 has been detected by fluorescence microscopy on a CF tracheal epithelial cell line and by flow cytometry on both immortalized and differentiated primary airway epithelial cells [82,83].

Claeys et al. [84] found higher mRNA expression of TLR2, but not TLR4, in nasal polyps in patients with cystic fibrosis versus healthy controls and it appears that this
distribution of TLRs also extends to the lower respiratory tract. Two groups independently examined the distribution and function of TLRs in cystic fibrosis airway epithelial cells. Greene et al. [82] showed that CF tracheal epithelial cell lines express functional TLRs 1-6 and 9, whilst Muir et al. [85] reported that TLRs 1-10 were expressed in CF cells. Interestingly TLR2 transcription was modestly increased in CF compared with normal epithelial cells following bacterial stimulation but not all investigators have confirmed this observation [86]. TLR4 was present in a more basolateral distribution and appeared to have a limited role in epithelial responses. John et al. [87] found a similar distribution of TLR4 in the CF bronchial epithelial cell line CFBE410- and in histologic lung sections of patients with CF. Overall it appears that TLR2 is the predominant TLR expressed on the surface of airway epithelial cells in vivo with TLR4 residing mainly intracellularly or displaying only low level surface expression. It can however be mobilized to the membrane following stimulation with microbial factors, e.g., RSV infection [88].

The airway epithelium provides a vast surface area and contributes significantly to pulmonary innate immunity [79]. However as CF is a neutrophil-dominated disease the expression of TLRs by neutrophils is also likely to be important in the host’s immune response to infection with Candida. Hauber et al. [86] reported that the number of TLR4-positive neutrophils in the submucosa of patients with CF was higher than in control subjects. From other reports it appears that expression of TLR2, TLR4, and TLR9 are all increased on airway neutrophils compared with circulating neutrophils in CF patients [89,90].

Little is known regarding the differential expression of TLRs by monocytes from CF versus healthy controls. However, accurate TLR2 and TLR4 responses in these cells are believed to depend on TLRs localizing to lipid rafts [91]. Although it has been suggested that CF monocytes are locked in an LPS-tolerant state, the mechanism responsible does not involve altered regulation of TLR expression but rather is due to down-regulation of TREM-1 [92]. Nonetheless there may be altered regulation of TLR4 in monocytes from CF patients [93]. It will be interesting to determine the effect of Candida infection on TLR expression in various cell types in the CF lung.

**Humoral immunity**

The role of the humoral immune response during Candida exposure and infection remains controversial. Despite this, the majority of literature with regards to Candida in CF exists in this area. In the late 1980s, Przyklenk et al. [101] first assessed serum IgG antibodies to both Aspergillus fumigatus and C. albicans in CF versus control patients and found that antibody levels were higher in CF patients irrespective of sputum isolation. In contrast to A. fumigatus, antibodies to C. albicans were observed to increase significantly with its increased isolation from sputum culture. Minimal work followed until Maiz et al. [102] assessed the prevalence of Aspergillus and Candida species in CF sputa and the serologic IgE responses to these fungi. For the first time, they additionally investigated whether the immune response had direct effects on clinical status in CF. Candida species were isolated in nearly half of all sputum samples analyzed (47.5%), but 87.9% of patients had at least one growth of C. albicans during the study course. One-quarter (26.7%) were sensitized to C. albicans and only patients who grew C. albicans at least once during the study developed an IgE response to the fungi. The clinical parameters assessed (FEV1 and CT scores) were not worse in those sensitized versus the non-sensitized. Interestingly, half of the sensitized group had confirmed ABPA whilst the remaining patients had some immunologic characteristics of ABPA. In conclusion, the group found a high prevalence of both colonization and sensitization to C. albicans in CF, but could not relate this to disease severity or clinical status. Although serum IgE to C. albicans appeared to represent an immunological marker of ABPA in CF, it is important to note that the
studied group was small \((n = 20)\) and only FEV1 and CT scores assessed as clinical measures. The same group extended this work recently to assess serum IgG, IgA and IgM against \(A. fumigatus\) and \(C. albicans\) and found that although no correlation was detected between the presence of \(A. fumigatus\) in sputum and an immune response, the converse was true of \(C. albicans\). Increasing sputum isolation heralded an elevated serum response however again this could not be related to respiratory impairment [103].

**Conclusion and future directions**

In this review, we have highlighted the current knowledge base and infections caused by the *Candida* species in CF. The dearth of CF-specific literature available illustrates that it evidently is a ‘road less travelled’. Despite this lack of literature and audit, what remains clear is that the species is isolated frequently and has importance in contributing to morbidity and in some cases mortality in CF. There is a high rate of undetected symptomatic oral and genital infections in the adult CF population and the problem should not be ignored with newer antibacterial agents on the horizon that will likely select out these fungal pathogens. Long-term use of antibiotics has recurrently emerged as a contributing factor to *Candida* infection and an alteration of flora post therapy lends survival advantages to the pathogenicity of this species. In doing so, *Candida* probably contributes to the inflammatory milieu observed in CF. Although post-transplant candidiasis is a rare occurrence, port infections do occur frequently. When a port is infected, it should be removed promptly and combined with antifungal therapy results in excellent clinical outcomes. The *Candida* species interestingly elicits its innate, cellular and humoral immune responses that we have yet to fully understand in the context of CF. Clearly an increasing amount of work remains left to be done to address the many unanswered questions. Future avenues for focus in this field lie within clinical care, isolation techniques and biomedical research. Healthcare professionals should maintain a positive approach in looking for manifestations of *Candida* infection during annual reviews at CF clinics and subsequently pursue microbiology in symptomatic cases. In terms of isolation techniques, selective media needs to be developed to suppress the growth of gram negative pathogens such as *Pseudomonas* and *Burkholderia* species and enhance fungal identification and isolation. Standardization of detection protocols needs to be pursued for fungi in CF as currently lab and international variation persists. Finally, basic science and clinical research avenues with regard to *Candida* species in CF need to be actively pursued so as to enable an improved understanding of its role in the CF airway, *Candida*-bacterial interaction and its potential use as a microbiological marker of CF disease severity and progression.

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