Biomarker tests for invasive *Candida* infection in critically ill patients in the UK (A-Stop): a multicentre diagnostic test accuracy study

*Ronan McMullan, Laura Loughlin, Sorcha Toase, Christina Campbell, Mike Clarke, Tanya Curran, Paul Dark, Mary Guiney, David A Harrison, Jaime Lindsay, Clare Mills, Kevin P Morris, Gavin D Perkins, A John Simpson, Matt Stevenson, Timothy Walsh, P Lewis White, Danny F McAuley, on behalf of The A-Stop Study Investigators**

Summary

Background The current practice of empirical antifungal treatment for suspected invasive *Candida* infection in the intensive care unit (ICU) leads to overtreatment. Biomarkers might better target treatment; however, their diagnostic accuracy in this patient group is uncertain. This study aimed to evaluate whether a biomarker or a combination of biomarkers has sufficiently high negative predictive value (NPV) to enable early cessation of empirical antifungal treatment in patients in ICUs.

Methods In this prospective, multicentre, diagnostic test accuracy study, we recruited patients who had been started on empirical antifungal treatment for suspected candidiasis and had blood cultures drawn within 24 h of starting treatment from 43 UK ICUs. We tested a research blood sample, collected within 24 h of the standard-of-care care blood culture (ie, within 48 h of starting empirical antifungal treatment), with three DNA detection tests (Fungiplex, T2Candida, and CandID) and one (1,3)-β-D-glucan test (Fungitell). We assessed the accuracy of these tests against an international consensus definition of proven invasive *Candida* infection and a constructed reference standard for proven or probable infection. The primary outcome was NPV for exclusion of proven *Candida* infection; we also reported sensitivity, specificity, and likelihood ratios.

Findings Among 4005 patients screened, 1251 were enrolled. A total of 1225 participants, recruited between April, 2018 and Feb, 2023, were evaluable, among whom 54 (4·4%) had proven invasive *Candida* infection. The median duration of antifungal treatment was 9 days (IQR 5–15), and survival at 28 days was 76·8%. The index tests had similar NPVs: 96·6% (95·3–97·6) for Fungiplex, 97·1% (94·7–98·6) for T2Candida, 96·7% (95·4–97·8) for CandID, and 97·2% (95·5–98·4) for Fungitell; however, sensitivity varied widely among the tests, from 27·5% to 68·6%. The optimal test combination was Fungitell and CandID, which had an NPV of 97·5% (95·7–98·7) and a sensitivity of 75·0%. Combining these biomarkers with standard-of-care blood culture testing further increased the NPV to 98·6% (97·0–99·4), with a sensitivity of 85·1%.

Interpretation No single biomarker had sufficiently high sensitivity to justify stopping empirical antifungal treatment. Combining Fungitell and CandID with standard-of-care blood culture testing could provide a sufficiently high NPV to allow early cessation of empirical antifungal treatment in this patient group, thereby reducing overtreatment, although this gain in sensitivity is accompanied by a reduction in specificity. Randomised trials are needed to confirm the clinical and cost-effectiveness of this diagnostic strategy before it is adopted in practice.

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Introduction

Patients in intensive care units (ICUs) frequently receive empirical antifungal treatment; however, fungal disease is confirmed in only a small proportion (approximately 5%) of patients who receive treatment.[1] Diagnostic uncertainty among patients in ICUs at risk of candidiasis drives precautionary empirical prescribing because observational datasets show that delayed treatment initiation has been associated with increased mortality in a small proportion of patients later confirmed to have infection. This precautionary approach results in substantial overtreatment as most patients receiving empirical treatment do not have invasive *Candida* infection.[2,3]

Candida and related species account for two of the four crucial fungal pathogens on the WHO fungal priority pathogens list.[4] Antifungal treatment is associated with adverse effects, drug interactions, resistance pressure, and drug acquisition cost. *Candidozyma auris* shows the health consequences of drug resistance in *Candida* and related species.[5–7]

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*Members of The A-Stop Study Investigators are listed in appendix (pp 49–54)

Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast, UK (Prof R McMullan MD, J Lindsay PhD, C Mills PhD, Prof D F McAuley MD); **Belfast Health and Social Care Trust, Belfast, UK** (Prof R McMullan MD, L Loughlin MD, T Curran PhD, Prof D F McAuley MD); **Northern Ireland Clinical Trials Unit, Belfast Health and Social Care Trust, Belfast, UK** (S Toase MSc, C Campbell BSc, Prof M Clarke DPhil, M Guiney); **Centre for Public Health, Queen's University Belfast, Belfast, UK** (Prof M Clarke); **Division of Immunology, Immunity to Infection and Respiratory Medicine, Faculty of Biology, Medicine and Health, The University of Manchester, Manchester, UK** (Prof P Dark MD PhD); **Intensive Care National Audit & Research Centre, London, UK** (D A Harrison PhD); **Birmingham Women's and Children's Hospital, Birmingham, UK** (K P Morris FRCPCH); **Warwick Medical School, University of Warwick, Coventry, UK** (Prof G D Perkins DSc); **Translational and Clinical Research Institute, Newcastle University, Newcastle upon Tyne, UK** (Prof A J Simpson PhD); **Department of Respiratory Medicine, Newcastle upon Tyne Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK** (Prof A J Simpson); **Sheffield Centre for Health and Related Research, University of Sheffield, Sheffield, UK**

(Prof M Stevenson PhD); **Usher Institute, University of Edinburgh, Edinburgh, UK** (Prof T Walsh MD); **Public Health Wales Mycology Reference Laboratory and Cardiff University Centre for Trials Research, Cardiff, UK** (P L White PhD)

Correspondence to: Prof Ronan McMullan MD, Wellcome-Wolfson Institute for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University Belfast, Belfast BT9 7BL, UK
r.mcmullan@qub.ac.uk

Research in context

Evidence before this study

Across different patient populations, several studies, often assessing performance of only one or two biomarkers, have reported good, although variable, sensitivity of both β-D-glucan and PCR-based tests, mainly when evaluated for detecting candidaemia. Whether these tests perform sufficiently well to guide treatment discontinuation in the large population of patients in the intensive care unit (ICU) who receive empirical antifungal treatment remains uncertain. We searched PubMed from inception until Dec 31, 2025 for manuscripts that included the MeSH terms (Candida OR fungal) AND diagnostic accuracy AND (biomarker OR PCR OR DNA OR T2 OR beta-D-glucan OR BDG) AND (intensive care OR critical care), without language restriction. We identified 377 studies. Most studies were not relevant to our research question, were of single-centre design, or were restricted to *Candida* infection syndromes that did not involve the bloodstream. No large multicentre studies prospectively evaluated the diagnostic accuracy of several biomarkers in patients in ICUs who were receiving empirical antifungal treatment for suspected invasive *Candida* infection.

Added value of this study

This study presents new evidence evaluating the diagnostic accuracy of four biomarkers for *Candida* among critically ill patients without neutropenia who were receiving empirical antifungal treatment for suspected *Candida* infection. The study provides new evidence to guide targeted antifungal treatment in intensive care. We propose an optimal combination of biomarkers that, when used with blood cultures, has a negative predictive value of approaching 99·0% (ie, 98·6%) in patients considered to be at sufficient risk of *Candida* infection to have been started on treatment empirically.

Implications of all the available evidence

No single test performs sufficiently well to allow treatment to be stopped. The incremental gain in diagnostic accuracy from combining biomarkers with blood culture might be sufficient to exclude infection in at-risk patients in ICUs; however, randomised trials should confirm the clinical and cost-effectiveness of such a diagnostic strategy.

Several commercially available biomarkers could help to reduce overtreatment. These biomarkers are based on the detection of either pathogen DNA or antigen but are not widely used in practice for antifungal treatment decision making in the UK, likely because of uncertainty about their clinical effectiveness and cost-effectiveness. Therefore, in this study, we aimed to assess the diagnostic accuracy of four biomarkers to evaluate their potential utility for excluding *Candida* infection and enabling early cessation of empirical antifungal treatment.

Methods

Study design and participants

A-Stop was a prospective, multicentre, diagnostic test accuracy study for evaluating biomarkers for *Candida* infection in patients in ICUs receiving empirical antifungal treatment. We published the study protocol online, registered it with ISRCTN (ISRCTN43895480), and detail it in the appendix (pp 2–26).[8] The study was conducted according to the principles of the Declaration of Helsinki.[9] A Research Ethics Committee (South Central Hampshire A, ref 17/SC/0613) and the UK Health Research Authority reviewed the protocol, participant information, and consent forms.

See Online for appendix

We recruited participants between April, 2018, and Feb, 2023. To be eligible, participants had to have received empirical systemic antifungal treatment for suspected invasive *Candida* infection, initiated by their clinical care team within the preceding 24 h, and had to have blood culture samples obtained as part of usual clinical care within 24 h (before or after) of starting treatment. Patients at significant risk of, or suspected to have, non-Candida fungal infection (eg, Aspergillosis) were excluded. The complete eligibility criteria are provided in the appendix (pp 9–10). We obtained informed consent from the participants directly or, when appropriate, based recruitment decisions on the opinion of a parent, guardian, personal consultee, or a nominated consultee.

Study procedures

We obtained a research blood sample for biomarker testing as soon as possible within 24 h of the blood culture (ie, within 48 h of starting empirical antifungal treatment). We separated samples into aliquots. Some aliquots were centrifuged to obtain serum; these serum aliquots were then frozen at participating sites and shipped in batches to the central laboratories in Belfast and Cardiff, UK, where they were stored at –80°C. We evaluated four index tests: one (Fungitell; Associates of Cape Cod, East Falmouth, Massachusetts, USA) detects (1,3)-β-D-glucan (BDG), a fungal cell wall antigen; the remaining three detect *Candida* DNA (Fungiplex *Candida*, Bruker Diagnostics, Nehren, Germany; T2Candida, T2 Biosystems, Lexington, Massachusetts, USA; CandID, IMMY, Braintree, England). Scientists trained by the manufacturers performed all tests according to manufacturers' instructions.

For the main analysis, we classified all positive results from DNA detection tests (irrespective of cycle threshold value or signal strength) as positive, including weak positives, and set the threshold for BDG positivity at 80 pg/mL. For the Fungitell BDG test, we considered an intermediate result (60–79 pg/mL) inconclusive and excluded it from the main analysis.

We performed an additional test for combined *Candida* mannan or anti-mannan (Platelia *Candida* Ag plus and

Platelia *Candida* Ab plus, BioRad Laboratories, Feldkirchen, Germany) to support diagnostic classification of study participants as part of a constructed reference standard for probable *Candida* infection. Scientists performing the index tests were masked to standard-of-care culture results, mannan or anti-mannan results, and clinical characteristics of participants. We did not make research test results available to clinical care teams.

We assessed diagnostic accuracy by comparing each index test to disease definitions for proven invasive *Candida* infection and separately for proven or probable invasive *Candida* infection. These definitions are provided in the appendix (pp 27–31), which are based on consensus disease definitions developed for patients in ICUs.[10] Briefly, the reference standard for the main analysis was proven infection, defined as meeting at least one of the following criteria: (1) definitive histological evidence; (2) identification of *Candida* species in blood culture; or (3) isolation of *Candida* species from a normally sterile anatomical site in a specimen obtained either intraoperatively or via a freshly placed drain (<24 h). For analysis of secondary outcome measures, we developed a constructed reference standard for probable infection, in which one test used for the mycological component of the definition was a combined mannan or anti-mannan test. To account for uncertainty in the accuracy of the mannan or anti-mannan test, we repeated the secondary analysis using an alternative mycological test (DNA or BDG).

Outcome measures

The primary outcome measure was the negative predictive value (NPV) for each index test, individually, with reference to the definition for proven *Candida* infection. We also reported sensitivity, specificity, positive predictive value, and negative or positive likelihood ratios.

The secondary outcome measures were diagnostic accuracy metrics for combinations of index tests, with reference to proven invasive *Candida* infection; diagnostic accuracy metrics for each index test, individually and in combination, with reference to the constructed reference standard of proven or probable invasive *Candida* infection; estimated proportion of patients receiving empirical antifungal treatment who might not require treatment based on the disease definitions used; and cost-effectiveness of testing strategies and risk preferences of clinicians and patients for biomarker testing as an antifungal stewardship intervention. These findings will be reported separately.

We planned subgroup analysis using the outcome of NPV for each index test or, where more appropriate for some subgroups, using test sensitivity instead. Subgroups comprised children, patients with candidaemia, and patients grouped by severity of organ dysfunction using the Sequential Organ Failure Assessment or Paediatric Logistic Organ Dysfunction 2 score. We also planned subgroup analysis for patients with infection caused by *Candida albicans* and *Nakaseomyces glabratus* (formerly *Candida glabrata*) versus other species.

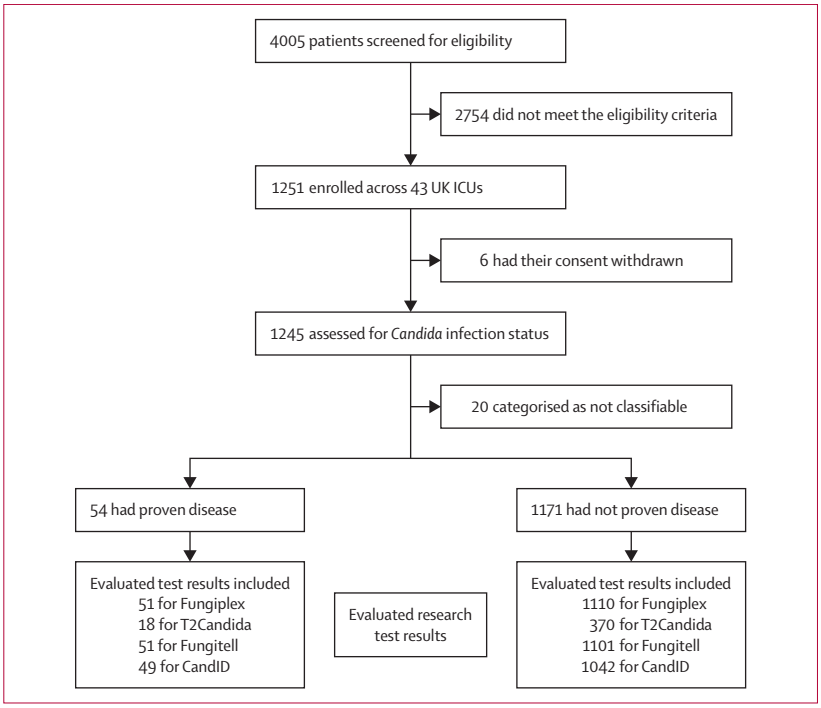


Figure 1: **Study profile**
ICU=intensive care unit.

Statistical analysis

The planned sample size of 1250 provided a power of 90% to detect an NPV of more than 99%, with a likelihood of 93% that the point estimate for sensitivity would exceed 90%. The calculation assumed a disease prevalence of 4% for proven disease, dropout rate of 5%, true test sensitivity of 95%, and specificity of 92%.

We analysed data using the statistical software Stata/IC version 15.1 for Windows in accordance with the statistical analysis plan, which was finalised before database lock. We presented baseline characteristics and follow-up data using descriptive statistics. We used the diagt command to estimate sensitivity, specificity, positive predictive value, NPV, and likelihood ratios for each index test and test combination, expressing precision with their 95% CIs. From the main analysis, we excluded missing samples and those for which the index test could not be completed or interpreted for technical reasons (eg, assay failure or failure of internal controls to run as expected, mostly applicable to T2Candida).

Role of the funding source

The funders of this study had no role in study design (except for providing peer review before the decision to fund), data collection, analysis, interpretation, or manuscript writing.

Results

We screened 4005 potentially eligible patients and enrolled 1251 patients with informed consent from 43 UK ICUs. Among the 2754 patients who were not enrolled, the most

	Proven infection (n=54)	No proven infection (n=1171)	All evaluable patients (n=1225)
Age, years (adults and children older than 1 year)	60·3 ± 13·6 (33–86)	57·9 ± 16·8 (1–93)	58·0 ± 16·7 (1–93)
Age, months (children younger than 1 year [n=14])	..	3·4 ± 2·8 (1–10)	3·4 ± 2·8 (1–10)
Sex			
Male	28 (51·9%)	716 (61·1%)	744 (60·7%)
Female	26 (48·1%)	455 (38·9%)	481 (39·3%)
Main diagnosis on ICU admission			
CNS	2 (3·7%)	36 (3·1%)	38 (3·1%)
Cardiovascular system	4 (7·4%)	88 (7·5%)	92 (7·5%)
Respiratory system	10 (16·7%)	219 (18·5%)	229 (18·7%)
GIT or hepatology	21 (38·9%)	518 (44·2%)	539 (44·0%)
Renal	0	27 (2·3%)	27 (2·2%)
Toxicology	0	13 (1·1%)	13 (1·1%)
Haematology	0	4 (0·3%)	4 (0·3%)
Orthopaedic	1 (1·9%)	18 (1·5%)	19 (1·6%)
Sepsis	11 (20·4%)	195 (16·6%)	206 (16·8%)
Other	5 (9·3%)	54 (4·6%)	59 (4·8%)
Relevant medical history			
Diabetes	19 (35·2%)	204 (17·4%)	223 (18·2%)
Neutrophil abnormality	1 (1·9%)	12 (1·0%)	13 (1·1%)
Solid organ transplant	1 (1·9%)	31 (2·7%)	32 (2·6%)
Scores			
SOFA score	9·1 ± 4·1	9·0 ± 4·3	9·0 ± 4·3
APACHE 2 score (adults)	17·2 ± 6·7	17·2 ± 6·1	17·2 ± 6·2
PELOD 2 score (children)	0	7·6 ± 4·1	7·6 ± 4·1
Candidiasis risk factors			
Pancreatitis	3 (5·6%)	118 (10·1%)	121 (9·9%)
Total parenteral nutrition	12 (22·2%)	251 (21·4%)	263 (21·5%)
Abdominal surgery	25 (46·3%)	604 (51·5%)	629 (51·3%)
Other major surgery	12 (22·2%)	169 (14·4%)	181 (14·8%)
Renal replacement therapy	10 (18·5%)	300 (25·6%)	310 (25·3%)
Number of central venous catheters			
0	3 (5·6%)	90 (7·7%)	93 (7·6%)
1	40 (74·1%)	673 (57·5%)	713 (58·2%)
2 or more	11 (20·4%)	408 (34·8%)	419 (34·2%)
Number of drains in situ			
0	18 (33·3%)	577 (49·2%)	595 (48·5%)
1, 2, or 3	34 (63·0%)	567 (48·4%)	601 (49·0%)
4 or more	2 (3·7%)	28 (2·4%)	30 (2·5%)
Glucocorticoid treatment within 30 days of enrolment	13 (24·1%)	295 (25·2%)	308 (25·1%)
Systemic immunosuppressant drugs within 30 days	2 (3·7%)	67 (5·7%)	69 (5·6%)
Impaired gut wall integrity in last 14 days	28 (51·9%)	630 (53·8%)	658 (53·7%)
Impaired cutaneous barriers within 14 days before enrolment	41 (75·9%)	875 (74·7%)	916 (74·7%)

(Table 1 continues on next page)

common reason for exclusion was antifungal treatment duration exceeding 24 h. 140 patients declined consent for participation. Ultimately, 1245 participants had evaluable research blood samples and 1222 had results for standard-of-care blood cultures (figure 1; appendix pp 32–33). In total, the invasive *Candida* infection status of 1225 participants was evaluable, among whom three had positive cultures from a normally sterile body site despite the absence of standard-of-care blood culture results. We categorised 54 participants as having proven invasive *Candida* infection, 1171 as not having proven invasive *Candida* infection, and 20 as having insufficient data for categorisation. None of the 39 children who participated had proven infection.

Not all participants had interpretable results for every index test (appendix p 38). We obtained fewer results for the T2Candida test because the assay could not be completed on several occasions despite substantial remedial effort.

The prevalence of proven invasive *Candida* infection among 1225 evaluable participants was 4·4% (n=54). The prevalence of documented candidaemia was 2·9% (n=36), with *C albicans* being the most common species (35·9%), followed by *N glabratus* (15·4%). The prevalence of proven or probable infection was 10·2% (n=125) when the main definition of probable infection was applied; this prevalence ranged from 9·9% to 11·7% when alternative definitions were used.

Participant characteristics at trial entry are summarised in table 1. The median duration of the primary course of antifungal treatment was 7 days (IQR 4–11). Subsequently, 25·2% (308 of 1224) of participants received further antifungal treatment. Overall, the median treatment duration was 9 days (IQR 5–15). At 28 days after enrolment, 942 (76·8%) of 1225 participants with evaluable *Candida* infection status had survived (63·0% [34 of 54] in those with proven *Candida* infection compared with 77·5% [908 of 1172] in those without); at 90 days, 863 (70·4%) of 1225 participants had survived.

Diagnostic accuracy metrics for each test, using proven *Candida* infection as the reference standard, are summarised in table 2. All the index tests had similar NPVs, ranging between 96·6% and 97·2%. In contrast, sensitivity estimates varied between 27·5% and 68·6% and specificity ranged from 52·8% to 94·5%. For participants with candidaemia, NPV and sensitivity estimates were higher. Fungitell BDG had the highest NPV of 98·8% and sensitivity of 78·8%; it also had the lowest specificity (appendix pp 36, 41–43). Of note, 11 (30·6%) of 36 participants with documented candidaemia had either no recorded species or a species not detectable by DNA detection tests.

We evaluated performance of combinations of two index tests to identify a pair that maximised both NPV and sensitivity for proven *Candida* infection. For this analysis, we combined pairs of tests and considered the combination positive if either test was positive. The optimal pair of tests

was Fungitell BDG and CandID DNA, with an NPV of 97·5% and a sensitivity of 75·0%. This combination was also optimal for detecting candidaemia, with an NPV of 99·0% (97·6–99·7) and a sensitivity of 84·4% (67·2–94·7). In a post-hoc analysis, we evaluated the performance of Fungitell BDG and CandID DNA in combination with standard-of-care blood cultures for detection of proven *Candida* infection, to reflect clinical use. Collectively, these three tests had an NPV of 98·6% and a sensitivity of 85·1% (appendix pp 46–48).

The decision model estimated the effect of treating study participants according to the combined biomarker and blood culture diagnostic algorithm (figure 2). Among 1023 participants with results available for all three tests, 483 (47·2%) could have had treatment stopped early, which we estimate would have occurred after 3–4 days of treatment. The algorithm would have falsely categorised seven participants as negative, who would subsequently have been found to have proven invasive *Candida* infection (based on other routinely collected clinical samples) and would have required restarting treatment. This finding represents 15·0% of participants with proven *Candida* infection. A further 540 (52·8%) participants would have continued treatment, among whom 500 would not have had proven invasive *Candida* infection.

Using the main constructed reference standard for probable invasive *Candida* infection, each individual index test had an NPV between 90·8% (Fungiplex DNA) and 93·4% (T2Candida) for detecting proven or probable infection. Sensitivity estimates ranged between 17·7% (Fungiplex DNA) and 57·7% (Fungitell BDG), and specificity estimates ranged from 53·2% (Fungitell BDG) to 95·0% (Fungiplex DNA). For comparison, blood cultures had a sensitivity of 28·8% for detecting proven or probable infection, with an NPV of 92·5%.

Using an alternative constructed reference standard for probable invasive *Candida* infection in which we adopted Fungitell BDG testing as part of the reference standard, each DNA detection test had a similar NPV for detecting proven or probable infection; sensitivity estimates were 14·1% (Fungiplex), 18·4% (T2Candida), and 24·6% (CandID), although 95% CIs for these estimates overlapped. We used an additional alternative constructed reference standard, in which we adopted CandID DNA detection as part of the constructed reference standard for probable infection, to further estimate the performance of the Fungitell BDG test. In that analysis, we estimated sensitivity of Fungitell to be 59·7% and specificity to be 53·6% for detecting proven or probable infection. Details are provided in the appendix (p 39).

The prevalence of proven or probable *Candida* infection ranged between 10·2% and 18·1%, depending on the disease definition used. Therefore, we estimate that 81·9–89·9% of participants might be receiving unnecessary antifungal treatment in the course of current usual-care practice in the UK.

	Proven infection (n=54)	No proven infection (n=1171)	All evaluable patients (n=1225)
(Continued from previous page)			
Otherwise unexplained clinical finding consistent with an infectious disease process	19 (35·2%)	341 (29·1%)	360 (29·4%)
Clinical findings specified			
Fever	3/19 (15·8%)	72/341 (21·1%)	75/360 (20·8%)
Leucocytosis	0	20/341 (5·9%)	20/360 (5·6%)
Increasing inotrope requirement	2/19 (10·5%)	16/341 (4·7%)	18/360 (5·0%)
New or worsening organ failure	3/19 (15·8%)	49/341 (14·4%)	52/360 (14·4%)
Signs of intra-abdominal sepsis	5/19 (26·3%)	94/341 (27·6%)	99/360 (27·5%)
Failure to show improvement with broad-spectrum antibacterial treatment	4/19 (21·1%)	65/341 (19·1%)	69/360 (19·2%)
Other	2/19 (10·5%)	25/341 (7·3%)	27/360 (7·5%)

Data are n (%) or mean (SD), unless otherwise specified. Race or ethnicity data were not collected. APACHE 2=Acute Physiology and Chronic Health Evaluation 2. GIT=gastrointestinal tract. ICU=intensive care unit. PELOD 2=Paediatric Logistic Organ Dysfunction 2. SOFA=Sequential Organ Failure Assessment.

Table 1: **Baseline characteristics of participants at enrolment**

Compared with the main analysis, subgroup analyses revealed no important differences in any subgroup when assessed using either NPV or sensitivity for proven infection as appropriate for each subgroup (appendix pp 44–45). We could not evaluate the subgroup of children because none had proven infection.

Discussion

This multicentre clinical diagnostic test accuracy study provides evidence of the performance of four biomarker tests for invasive *Candida* infection. The focus of this study was to identify a diagnostic strategy appropriate for early discontinuation of empirical antifungal treatment in patients in ICUs by confidently excluding this serious, although uncommon, infection. No single test had sufficiently high sensitivity to justify its use for this purpose. Although all the tests had high NPV, this result was influenced more by low disease prevalence than by test performance, although none reached the pre-specified level of more than 99%.

Test combinations showed improved sensitivity but lower specificity. The optimal pairing, which prioritised both NPV and sensitivity, was Fungitell BDG and CandID DNA. This pairing is rational because it targets different *Candida* cell constituents. Maximising sensitivity inevitably compromises specificity; all combinations that include BDG show low specificity since approximately half of the evaluable participants had a positive BDG result.

Biomarker testing would presumably be an adjunct to conventional culture in clinical practice; it is therefore

	Evaluable participants (proven infection prevalence in each group)	NPV	Sensitivity	Specificity	PPV	NLR	PLR
Fungiplex alone	1161 (4·4%)	96·6% (95·3–97·6)	27·5% (15·9–41·7)	94·5% (93·0–95·8)	18·7% (10·6–29·3)	0·8 (0·7–0·9)	5·0 (3·0–8·3)
Fungitell alone	1114 (4·6%)	97·2% (95·5–98·4)	68·6% (54·1–80·9)	52·8% (49·7–55·8)	6·5% (4·6–8·9)	0·6 (0·4–0·9)	1·5 (1·2–1·8)
T2Candida alone	388 (4·6%)	97·1% (94·7–98·6)	44·4% (21·5–69·2)	90·5% (87·1–93·3)	18·6% (8·4–33·4)	0·6 (0·4–0·9)	4·7 (2·6–8·6)
CandID alone	1091 (4·5%)	96·7% (95·4–97·8)	38·8% (25·2–53·8)	85·6% (83·3–87·7)	11·2% (6·9–17·0)	0·7 (0·6–0·9)	2·7 (1·8–4·0)
Fungiplex + Fungitell	1085 (4·6%)	97·3% (95·5–98·5)	70·0% (55·4–82·1)	51·3% (48·2–54·4)	6·5% (4·6–8·9)	0·6 (0·4–0·9)	1·4 (1·2–1·7)
Fungiplex + T2Candida	364 (4·4%)	97·5% (95·1–98·9)	50·0% (24·7–75·3)	88·5% (84·7–91·7)	16·7% (7·5–30·2)	0·6 (0·4–0·9)	4·4 (2·5–7·7)
Fungiplex + CandID	1063 (4·5%)	96·8% (95·4–97·9)	41·7% (27·6–56·8)	83·2% (80·7–85·4)	10·5% (6·5–15·7)	0·7 (0·6–0·9)	2·5 (1·7–3·6)
Fungitell + T2Candida	361 (4·4%)	97·4% (93·9–99·1)	68·8% (41·3–89·0)	53·3% (47·9–58·7)	6·4% (3·2–11·2)	0·6 (0·5–0·7)	1·5 (1·0–2·1)
Fungitell + CandID	1026 (4·7%)	97·5% (95·7–98·7)	75·0% (60·4–86·4)	48·7% (45·5–51·9)	6·7% (4·7–9·1)	0·5 (0·3–0·8)	1·5 (1·2–1·7)
T2Candida + CandID	337 (4·5%)	97·8% (95·4–99·2)	60·0% (32·2–83·7)	84·8% (80·4–88·5)	15·5% (7·3–27·4)	0·5 (0·3–0·9)	3·9 (2·4–6·4)

Data are % (95% CI) for NPV, sensitivity, specificity, and PPV and estimates (95% CI) for NLR and PLR. CI=confidence interval. NLR=negative likelihood ratio. NPV=negative predictive value. PLR=positive likelihood ratio. PPV=positive predictive value.

Table 2: **Diagnostic accuracy of each index test and pairs of tests for proven infection**

notable that adding blood cultures to the optimal biomarker pair enhanced diagnostic performance. The pretest probability of proven invasive *Candida* infection in this study was 4·4%, which was reduced to a post-test probability of 1·4% when the optimal pair of biomarkers and blood cultures were both negative. If this diagnostic combination had been used to stop empirical treatment, 15·0% of patients with proven infection would have had antifungal treatment discontinued. However, since non-protocolised culture testing detected proven infection, these patients would likely have had treatment restarted soon afterwards based on standard-of-care culture results.

We obtained T2Candida DNA results for relatively few study participants owing to recurrent technical difficulties with the assay. Despite repeated attempts to resolve these difficulties, made in liaison with the manufacturer's technical support team, the assay continued to produce unsatisfactory results with the manufacturer's control specimens. In addition, a high proportion of research samples produced invalid results. These issues developed only after the assay had been established and running successfully for an initial period. We observed no apparent correlation between the observed technical problems and any variable such as operator or duration of specimen storage. We excluded T2Candida when selecting a preferred test or test combination. Notably, T2Candida is no longer commercially available.

Several strategies for improving the stewardship of antifungal treatment in the ICU have been tested, although none of them have replaced the convention of empirical treatment for patients with fever and risk factors for *Candida* infection—a practice endorsed by the Infectious Diseases Society of America.[11] The EMPIRICUS trial showed that patients with sepsis in ICUs who had risk factors for *Candida* infection did not have greater infection-free survival after 28 days despite 2 weeks of empirical antifungal treatment.[12] Two additional trials of antifungal treatment strategies based on clinical risk assessment of infection similarly did not show a patient benefit.[13,14] A recent Cochrane review concluded that moderate-quality evidence shows that untargeted antifungal treatment in the ICU is not associated with a substantial reduction in all-cause mortality.[15]

Previous attempts to improve the targeting of antifungal drug prescribing in the ICU using clinical risk prediction scores did not resolve the problem of overtreatment. These scores are either too complex to apply or have insufficient accuracy. With moderate specificity but low sensitivity, the scores do not provide a safe basis for stopping empirical treatment.[1,16–18]

A biomarker-based algorithm offers the best opportunity for improving antifungal stewardship in ICUs. Previous smaller studies of BDG test accuracy indicate that BDG generally has higher sensitivity than clinical risk scores and provides high NPV in low-prevalence populations such as patients with fever in ICUs.[19–22] Although we have reported good performance for the Fungitell BDG test, it would be

inappropriate to infer that all manufacturers' BDG tests on different platforms produce similar results.

Individual biomarkers show different diagnostic performance across various clinical scenarios; this finding supports the principle of using a combination of tests.[23] Risk-stratified strategies incorporating molecular and antigen-based tests for diagnosing invasive *Candida* infection offer the greatest potential for excluding disease when multiple tests are negative.[24] However, no such strategy has been rigorously evaluated in a sufficiently robust manner to re-define the standard of care. Two small, single-centre, randomised trials (approximately 100 participants) showed biomarkers enabled early cessation of empirical antifungal treatment—in 54·0% of patients in one trial and 70·0% in the other—although neither trial had sufficient power to assess safety and other clinical outcomes required to change practice.[25,26]

Previously reported diagnostic accuracy of biomarkers shows variable performance between different tests and different studies for the same tests. A meta-analysis of BDG accuracy across diverse patient groups, fungal diseases, and reference standards reported a sensitivity of 75–80%.[27] A subsequent evaluation estimated this test would yield an NPV of more than 90% in patients in ICUs, principally for excluding invasive candidiasis.[28] A 2011 meta-analysis of *Candida* PCR tests for detecting candidaemia, which formed the basis for our sample size calculation, reported a sensitivity of 95% and specificity of 92%; however, substantial heterogeneity existed among included studies.[29] The 2018 DIRECT2 study reported a sensitivity of 89% for the T2Candida test for candidaemia.[30] A reference standard based solely on candidaemia does not reflect non-candidaemic invasive candidiasis encountered in the ICU.

Together, the evidence indicates that although the diagnostic performance of biomarkers is generally good, it might vary across fungal disease definitions, patient groups, and different testing platforms for a given analyte. A recent systematic review documented this heterogeneity between reported studies.[31] Our study provides new evidence of biomarker performance in the ICU population. The strengths of the study include design features intended to minimise sources of bias: prospective design, blinding of index test assessors, large sample size (both in terms of number of sites and participants), and evaluation of multiple biomarkers. The eligibility criteria for enrolment strengthen the applicability of the study to the clinical decision problem we address.

Despite these strengths, the study has some limitations. First, blood and serum specimens frozen for several months before central laboratory testing might compromise the accuracy of biomarker test results. Serum samples stored at –80°C are considered stable for BDG detection for up to 4 years;[32] however, the stability of DNA quality and quantity in blood and serum over prolonged periods remains uncertain.

Second, uncertainty exists regarding the definition of invasive *Candida* infection in non-neutropenic ICU populations. Because no perfect reference standard for *Candida* infection exists, definitions with varying levels of stringency have been developed (ie, proven *vs* probable). Probable infection definitions have lower specificity and consequently might include patients without candidiasis. This finding likely underestimates sensitivity, and hence NPV, of tests evaluated against these definitions.

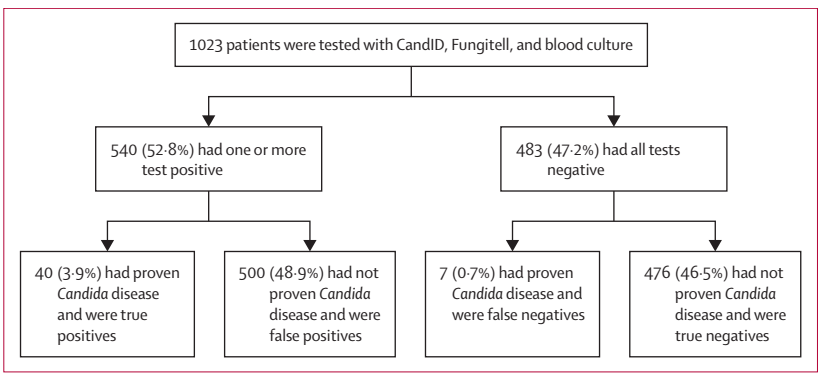


Figure 2: **Estimated effect of the optimal biomarker and blood culture testing algorithm for early cessation of antifungal treatment**

Third, two distinct international consensus definitions were published during our recruitment period.[10,33,34] Consequently, the disease definitions used in this study did not completely align with either of these consensus definitions, although they closely resembled both. Notably, in the most recently published consensus, the expert panel specifically recommended that PCR and fungal antigen-based tests represent important areas for future research to inform the development of future disease definitions in patients without neutropenia in ICUs. The present study addresses this research need.

Finally, our reference standard relied on blood cultures collected during routine clinical care. Although all evaluable patients underwent blood culture testing, timing was not standardised. Clinicians sent non-protocolised samples for culture as they deemed appropriate, and proven infection was identified in several instances based on these non-protocolised culture results.

In conclusion, the optimal test combination identified in this study, when used as an adjunct to standard-of-care blood culture testing, could allow early cessation of empirical antifungal treatment. Decision modelling based on an algorithm comprising the three tests estimated that approximately half of the patients receiving empirical antifungal treatment could have treatment stopped early. We acknowledge that this finding is an estimate since the present study was not an interventional study. A diagnostic algorithm enabling treatment cessation in a larger proportion of patients would require sacrificing sensitivity for greater specificity, which clinicians might be less willing to accept. Randomised trials should address remaining uncertainties regarding the clinical effectiveness and cost-effectiveness of implementing this diagnostic

test-based strategy for antifungal stewardship in clinical practice.

Contributors

RM was the chief investigator and was responsible for the overall conduct of the study, including study design, funding acquisition, project management, and initial manuscript drafting. ST and CC were study statisticians who contributed to data curation and were responsible for data analysis. MC, TC, PD, DAH, KPM, GDP, AJS, MS, TW, PLW, and DFM contributed to study design, funding acquisition, and project supervision. TC, PLW, JL, and CM completed laboratory aspects of data curation and analysis. LL and MG contributed to project management and data curation. All authors contributed to drafting this manuscript, approved the final version of the manuscript, and agree to be accountable for all aspects of the study. All authors had full access to all the data in the study and accept responsibility for the decision to submit for publication. ST and CC had direct access to the underlying data reported at the level of the individual study participant and verified these data.

Declaration of interests

RM has received funding for this study to his institution from the National Institute for Health and Care Research (NIHR) and Research Charitable Trust Fund of the Belfast Health & Social Care Trust. Beyond this study, RM reports institutional research funding from the NIHR Health Technology Assessment Programme, NIHR Efficacy and Mechanisms Evaluation programme, Wellcome Trust, Medical Research Council, NI Chest, Heart & Stroke Association, and Randox Laboratories. RM has also been a member of an NIHR Health Technology Assessment Programme Prioritisation Committee and the NIHR Efficacy and Mechanisms Evaluation Funding Committee and is an NIHR Senior Investigator. PD has received funding for this study to his institution from the NIHR. Beyond this study, PD reports institutional research funding from various NIHR funding programmes and the Manchester NIHR Biomedical Research Centre and is a NIHR Senior Investigator. During the study, PD served as the NIHR Clinical Research Network National Deputy Medical Director and a member of the NIHR Efficacy and Mechanisms Evaluation Strategy Advisory Committee. DAH has received funding for this study to his institution from the NIHR. Beyond this study, DAH reports grant funding to his institution from the NIHR Health Technology Assessment Programme. DAH is also a member of the NIHR Health Technology Assessment Programme Funding Committee (General). AJS reports institutional research funding from the NIHR, Medical Research Council, Wellcome Trust, and UK Department of Health. AJS holds grants on which Clinigen, Aerogen, and Partner Therapeutics are collaborators and has previously held grants on which Becton Dickinson was an applicant. AJS reports membership of an independent data monitoring committee for Synairgen and independent adjudication panel for Pulmovant. AJS is Programme Director for the NIHR/MRC Efficacy and Mechanism Evaluation Panel and was previously a member of the same panel. AJS is the Director of the NIHR Healthtech Research Centre in Diagnostic and Technology Evaluation and was previously Deputy Chair and a member of the MRC Clinical Training and Career Development panel. AJS is an NIHR Senior Investigator. GDP has received funding for this study to his institution from the NIHR. Beyond this study, GDP reports institutional research funding from the NIHR Health Technology Assessment Programme and NIHR Efficacy and Mechanisms Evaluation programme. MS reports institutional research funding from the NIHR Health Technology Assessment Programme. PLW has received funding for this study to his institution from the NIHR. Beyond this study, PLW has performed diagnostic evaluations and received meeting sponsorship from Associates of Cape Cod, Bruker, Dynamiker, and Launch Diagnostics; received speaker fees, expert advice fees, and meeting sponsorship from Gilead and Mundipharma; received speaker and expert advice fees from Pfizer; and received expert advice fees from F2G. DFM has received funding for this study to his institution from the NIHR and Research Charitable Trust Fund of the Belfast Health & Social Care Trust. Beyond this study, DFM has received funding to his institution for studies from the NIHR, Medical Research Council, Wellcome Trust, Northern Ireland Health and Social Care Research and Development Office, Novavax, and Randox. DFM holds a patent application issued to his institution and has also received consultancy fees from Bayer, Aptarion, Direct Biologics, Healios, Novartis, and MSD. DFM was previously Director of Research for the UK Intensive Care Society and MRC/NIHR Director for the EME Programme and is currently NIHR Scientific Director for Programmes. MC and KPM have received funding for this study to their institutions from the NIHR and Research Charitable Trust Fund of the Belfast Health & Social Care Trust. All other authors declare no competing interests.

Data sharing

Requests for data sharing from bona fide researchers should be directed to the corresponding author. Fully anonymised data will be shared subject to approval by the chief investigator and Sponsor.

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