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Usefulness of β -D-glucan for diagnosis and follow-up of invasive candidiasis in onco-haematological patients

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A B S T R A C T

Objectives: Definitive diagnosis of invasive candidiasis (IC) may be difficult to achieve in patients with haematological malignancy (PHM). We aimed to evaluate the performance of BDG for the diagnosis and the follow-up of IC in PHM.

Patients and methods: We retrospectively reviewed the serological data of BDG assay in adult and paediatric PHM, who developed candidemia or chronic disseminated candidiasis (CDC) through a 4-year period. Sensitivity and kinetics of BDG were determined for both clinical forms.

Results: In a panel of 3027 PHM, incidence rates of candidemia and CDC ranged between 0.74 and 0.77 and 0.30 and 0.44 according to the group of patients. At the time of diagnosis, 43.5% and 73% of cases of candidemia and CDC had a positive BDG assay, respectively. We found a significant correlation between the level of BDG at diagnosis and the outcome of candidemia ($p = 0.022$). In all cases of CDC, BDG negative results were obtained 2 to 6 months before recovery of the CT-scan lesions.

Conclusions: BDG exhibits a low sensitivity to detect IC in PHM, but its kinetics correlates the clinical outcome. Additional studies are warranted in patients with CDC to evaluate the interest of monitoring BDG levels to anticipate the discontinuation of antifungal maintenance therapy.

Keywords:

β -D-glucan

Candidemia

Chronic disseminated candidiasis

Diagnosis

Outcome

Introduction

Candida spp. remains a major cause of invasive fungal infection (IFI) in patients with haematological malignancy.¹ In those patients, candidemia is the most common form, but specifically in the onco-haematological setting, some patients develop a chronic disseminated infection.^{2,3} Chronic disseminated candidiasis (CDC) is characterised by multiple organ involvements, mainly the liver and the spleen. The pathogenesis of CDC is thought to rely on a first candidemia episode associated with organ infectious metastases, which are further revealed when the neutropaenia resolves.

In recent studies, an incidence of candidemia between 0.5% and 1.4% and between 1.4% and 9.4% has been reported in adult and paediatric population of patients with haematological malignancy,

respectively.^{2,4-6} Many case reports and some series of CDC have been published but, to the best of our knowledge, incidence rates are lacking for this clinical entity.⁷⁻⁹

The diagnosis of candidemia and CDC may be difficult.¹⁰ Candidemic patients usually present without specific clinical sign, and even fever may be inconstant. The sensitivity of blood culture is considered about 50% and may be even lower in the case of an ongoing antifungal therapy.¹¹ The diagnosis of CDC is most often based on the demonstration of small targeted lesions in the liver and/or the spleen on a CT-scan or ultrasonography in a patient who had recently recovered from neutropaenia. A previous history of candidemia during the neutropaenia phase may have been documented or not.¹² Culture of biopsy specimens of infected organs is most often unsuccessful, making the diagnosis most often probable.¹⁰

The development of new diagnostic biomarkers is thus highly desirable to improve the diagnosis and guide the therapeutic management. For adults, *Candida* antigen (mannan) and anti-mannan antibodies assays are moderately recommended by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) and the European Conference on Infections in Leukaemia (ECIL) for the diagnosis of candidemia and CDC.^{11,13} In paediatric population,

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those tests have not been validated, and no recommendation could be proposed by the ECIL group.¹⁴

In 2008, the revised definitions of IFI from the EORTC-MSG consensus group introduced the detection of β -D-glucan (BDG) among the biological diagnostic criteria.¹² BDG is a polysaccharide component of the cell wall of the majority of pathogenic fungi. Since BDG is a panfungal marker looking specifically at the performances of the test for the diagnosis of invasive candidiasis could be difficult. Nevertheless, sensitivity between 60% and 100% for candidemia emerging in adult patients has been reported.^{15–18} Data in paediatric populations are scarcer. Furthermore, it has been suggested that concentration levels may be higher in healthy children leading to a higher rate of false positive.¹⁹ Nevertheless, in a previous study focused on the diagnosis of invasive fungal infections in a paediatric population with haematological malignancy, we found that the use of the threshold recommended by the manufacturer, similar for both adult and children, offers the best performance both in terms of sensitivity and specificity.¹⁷

To the best of our knowledge, none of these studies focused on the diagnosis and follow-up of CDC. In this study, we reviewed the data on BDG assay collected in patients with haematological malignancy who developed candidemia or probable CDC. We evaluated the performances of the test for the diagnosis of both clinical forms and looked at the potential interest of BDG as a surrogate marker of clinical outcome.

Patients and methods

Patients

We retrospectively reviewed all the adult and paediatric patients admitted to the haematology–oncology wards of our institution between 01/01/2013 and 31/12/2016 (4 years), diagnosed with either candidemia or CDC (see definitions below). For each patient, demographic data, type of haematological disorder and other underlying conditions were collected. Antifungal treatments received before and after the diagnosis of candidiasis were also recorded.

Non-targeted antifungal management

The standard procedures of care are detailed in [Supplementary Table S1](#). In the paediatric department, patients presenting with a prolonged neutropaenia (>10 days) associated with *Candida* colonisation, received antifungal prophylaxis with micafungin. In the adult ward, posaconazole was the preferred prophylactic therapy during induction phase of chemotherapy for Acute Myeloid Leukaemia and in case of graft-versus-host disease (GVHD) treated with corticosteroids. An empirical antifungal treatment consisting of liposomal amphotericin B or caspofungin was started in the case of persistence of fever 48 to 96 hours after the initiation of antibacterial therapy.

Diagnosis of IFIs

Routine procedures were applied for the diagnosis of invasive candidiasis. Multisite sampling (at least 3 among nose, rectum, armpit, and groin) for bacterial and fungal cultures was performed weekly during the neutropaenic phase. Blood cultures were performed in case of fever. Mannan antigenemia assay (Platelia *Candida* Antigen Plus, Biorad, France) was performed on demand, according to the manufacturer's recommendations.

During periods of neutropaenia, BDG serial assays were routinely performed once a week, according to the manufacturer's recommendations (Fungitell, Capecode, USA), on a thermostat-controlled spectrophotometer (ELX 808, Biotek Instruments, France).

Sera were tested in duplicate, and the mean was assigned as the final result for the specimen.

Candidiasis definition

Candidemia was defined by the positivity of at least one blood culture for *Candida sp.* CDC was defined by the demonstration on a CT-scan or ultrasonography of small, target-like abscesses in the liver and/or spleen areas at the time of neutrophil recovery after a prolonged phase of neutropaenia. CDC was considered as probable if it occurs after an episode of candidemia within the previous month (we extend the EORTC proposed delay considering the fact that the date of imaging following the first clinical signs appeared very variable). Taking into account the ESCMID guidelines for the diagnosis and management of candidiasis, we also considered probable CDC in patients with radiological lesions associated with multisite *Candida* colonisation and positive biomarker(s) (mannan/anti-mannan assay, or BDG assay).¹¹ Finally, CDC was considered as possible if typical CT-scan or ultrasonographic lesions occur in the absence of positive biomarkers but with multisite *Candida* colonisation. Day 0 of the diagnosis was defined as the day of sampling of the first positive blood culture and the date of demonstration of the imaging lesions for candidemia and CDC, respectively.

Ethical considerations

All samples and data were collected as a part of routine diagnostic procedures. The study was retrospective, and indeed the results of the analysis did not influence the management of the patients. The study has been registered to the National Agency of Drug and Health Product Safety (ANSM) under the reference **2017-A01358-45**.

Results

Description of the population

Between January 2013 and December 2016, 28 episodes of candidemia, 12 probable CDC and one possible CDC were diagnosed in patients suffering from haematological malignancies. Five episodes of candidemia were excluded due to insufficient BDG data or mixed infection with another IFI ([Fig. 1](#)). Among the remaining 23 episodes, five evolved towards a probable CDC. Furthermore, seven episodes of probable CDC and one episode of possible CDC, without any previously detected candidemia, were diagnosed. Two episodes were excluded due to concomitant polyvalent immunoglobulin administration or insufficient BDG data. Thus, 10 cases of probable CDC and one possible CDC were retained for analysis ([Fig. 1](#)).

There were 12 children and 16 adult patients. The main characteristics of those patients are described in [Table 1](#) (detailed description in [Supplementary Tables S2 and S3](#)).

The 23 episodes of fungemia were due to *Candida albicans* (n = 7), *Candida tropicalis* (n = 6), *Candida parapsilosis* (n = 4), *Candida kefyr* (n = 4), *Candida lusitanae* (n = 1), and *Candida dubliniensis* (n = 1). Median duration of the candidemia episodes was 3.0 ± 2.85 days. One case was considered as persistent candidemia as positive blood cultures returned positive over a 14-day period despite catheter removal. Four episodes of candidemia were breakthrough infections. In one of these cases, a *C. tropicalis* isolate was found resistant to the antifungal used in prophylaxis, caspofungin in this case. All the 5 patients who developed CDC after candidemia received antifungals at the time of CDC diagnosis. Two among 6 patients who developed CDC without candidemia previously detected were treated with liposomal amphotericin B prophylaxis.

Incidence rates of candidemia and CDC varied between 0.74% and 0.77% and 0.30% and 0.44 % according to the population of

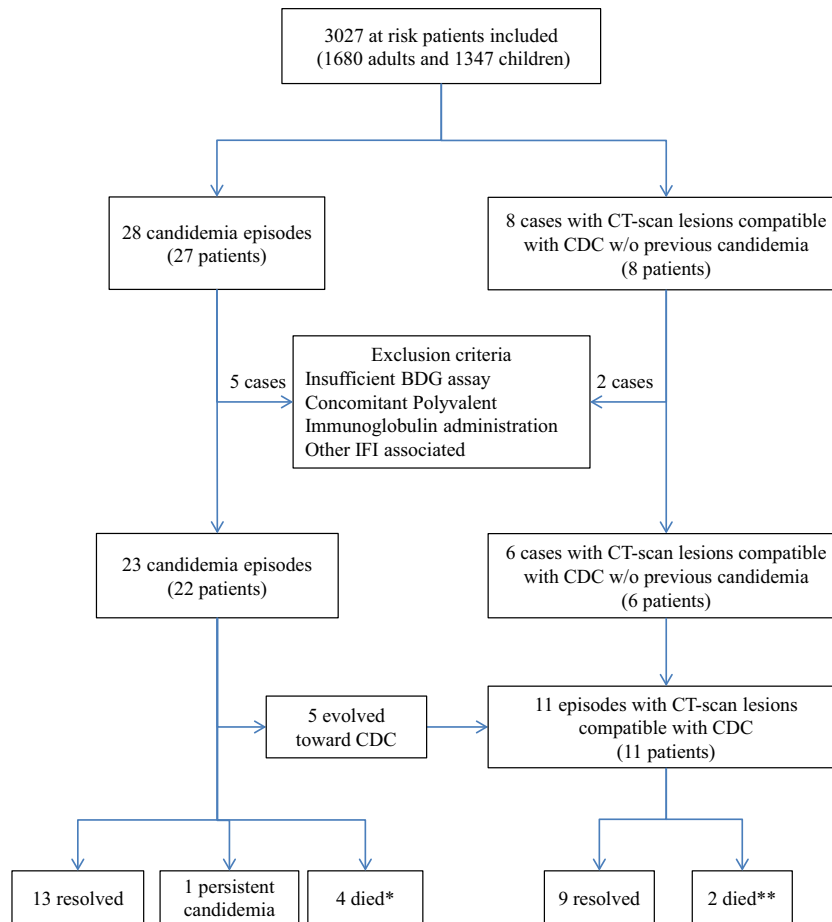


Fig. 1. Flow chart of patient's inclusion and classification of candidiasis. BDG: β -D-glucan, CDC: chronic disseminated candidiasis, IFI: invasive fungal infection, w/o: without. *Among which one had persistent *Candida* infection (positive blood cultures over a 10-day period). **Within 15 days. ***Within 3 months.

patients (Supplementary Table S4). In 5 cases, the diagnosis of CDC occurred after a median of 16 ± 12 days following a candidemia episode. The global mortality rate was at 17% on day 15 post-diagnosis for candidemia and 18.2% on day 90 for CDC.

β -D-glucan assay performance for the diagnosis of IC and CDC

The first BDG assay was performed with a mean delay of 1.4 days (-17 to $+3$) before the first positive blood culture sampling. BDG was found positive, at the time of diagnosis or before, in 10 candidemia episodes, leading to a positivity rate of 43.5%. In comparison, 8 cases out of the 14 patients (57.1%) who benefited of a mannan assay, had a positive test at the same time. When the BDG assay was positive, the diagnosis could have been suspected before the first positive blood culture in 5 out of 9 cases with available data (55%), anticipating the diagnosis from 2 to 15 days. Among the 13 patients with a negative or equivocal BDG at the time of diagnosis, 5 (38%) turned positive between D4 and D13 after the candidemia diagnosis.

All the 5 patients who developed CDC after candidemia had a positive BDG assay with a high level of BDG (median: 509 pg/ml, range: 500–523 pg/ml) at the time of CDC diagnosis (Supplementary Table S3). In contrast, the test was positive for 3 out of the 6 patients (50%) presenting CDC without previous candidemia. One CDC patient with a negative BDG at the time of diagnosis turned positive 7 days later. Among the three BDG-positive patients who developed CDC without previous episode of candidemia, BDG anticipated the diagnosis by one, six, and 22 days.

Potential factors leading to false negative result in β -D-glucan assay

Because the sensitivity of BDG was quite low, we looked for possible factors that may have influenced the result of the test (Supplementary Table S5 and S6). Neither *Candida* species (*albicans* vs non-*albicans*; $p = 0.67$), nor an ongoing antifungal treatment ($p = 0.64$) impacted the result of the test. In five patients who had only positive blood cultures when drawn from the catheter, the BDG assays were negative, whereas the test was positive in 8 out of the other 18 patients (44.4%) ($p = 0.046$).

Timeline and kinetics of β -D-glucan in IC and CDC patients

Serological follow-up of candidemia and CDC patients are described in Fig. 2. Among the 13 candidemia episodes which resolved clinically, all but 1 presented with a sharp decline in their BDG level (Fig. 2A). In contrast, among the 10 other episodes which had persistently high levels of BDG (Fig. 2B), 4 patients died, 5 evolved towards a CDC and 1 had persistent positive blood culture. We also found a correlation between the level of BDG at the time of candidemia diagnosis and the outcome at D15. Indeed, the patients who recovered had a mean level at 94 ± 119 pg/ml whereas those who died, evolved towards CDC and those who had persistent infection had a mean level at 297 ± 236 pg/ml (Supplementary Table S5, Student test, $p = 0.022$).

Looking at the kinetics of BDG after CDC (Fig. 2C and D), 2 patients with persistently high levels of BDG died. Regarding the 9

Table 1
Demographics, underlying diseases and biological results of patients presenting candidiasis infection.

	Adult patients	Paediatric patients
Sex ratio (F/M)	9/7	7/5
Mean age (years \pm SD)	55.8 \pm 11.7	7.9 \pm 4.2
Underlying disease		
ALL	2	7
AML	3	5
NHML	3	0
Other*	8	0
Candidemia	13	10
CDC post-candidemia	2	3
CDC w/o previous candidemia	3	3
Causative species (blood cultures/colonisation)		
<i>C. albicans</i>	5/9	2/6
<i>Candida non-albicans</i>	8/8	8/6
Biomarkers % of positivity at time of diagnosis (Candidemia/CDC)		
BDG [†]	46.1%/100%	40%/50%
Mannan	66.7%/60%	66.7%/50%
Mannan or BDG	61.5%/100%	40%/83.3%
Outcome		
Candidemia	7	6
Favourable outcome	4 (7.5 \pm 4.7)	0
Death (day post-diagnosis)	0	1
Persistent infection	2	3
Evolution towards CDC	3	6
CDC	2 (44.5 \pm 21.9)	0
Favourable outcome		
Death (mean day \pm SD)		

* Other underlying diseases: refractory anaemia with excess blasts, myelofibrosis, multiple myeloma, Hodgkin lymphoma.

[†] Values lower than 8 pg/ml and higher than 523 pg/ml were considered as being 8 and 523 pg/ml. Manufacturer's recommendations are to consider values lower than 60 pg/ml as negative, higher than 80 pg/ml as positive and equivocal between 60 and 80 pg/ml.

F: female, M: male, SD: standard deviation, AML: acute myeloid leukaemia, ALL: acute lymphoid leukaemia, NHML: non Hodgkin myeloid lymphoma, BDG: β -D-glucan, CDC: chronic disseminated candidiasis, w/o: without.

patients who survived, 7 presented positive BDG during the follow-up, in which levels decreased within 2 to 6 months. Resolution of imaging lesions occurred 5 months to 2 years after the CDC diagnosis with remaining imaging sequel in 3 cases. In those 7 patients, BDG turned negative 2 to 14 months before a partial CT-scan recovery.

Discussion

To the best of our knowledge, our data are among the very rare to document the incidence of both candidemia and CDC in adult and paediatric patients with haematological cancer. We also show that the overall mortality rate, below 20%, is lower than expected according to previous studies.^{4,7,9}

Current mycological tools for the diagnosis of these infections are unsatisfactory^{10,11} and evaluation of a new promising biomarker such as BDG is always desirable. Nevertheless, our study has some limitations. First, because it was retrospective, BDG follow-up was missing for some patients. As candidemia became quite uncommon in those patients, we were able to analyse only 23 episodes of candidemia and 11 cases of CDC, limiting the statistical analysis. Finally, as we focused on *Candida*-infected patients, we could not calculate the specificity of the test in this cohort of patients. Other studies performed in onco-haematology setting had estimated this specificity between 82% and 92% in adults,²⁰ and only 46% and 56% in children.^{17,21}

In our study, the sensitivity of the BDG test, performed 3 days before to 3 days after the diagnosis of candidemia, was low at 43.5%, in agreement with previous reports.^{15,16} In a prospective study

focused on adult haematological patients admitted in ICU, including 8 cases of candidemia, sensitivity was found at 87.5% (for specimen drawn within 5 days before the candidemia) but with a specificity of only 35%.¹⁶ Another prospective study reported a BDG sensitivity at 63.5% when looking at specimen sampled 1 day before to 7 days after the positive blood culture.¹⁵ Using such an interval of time in our cohort would have led to a similar sensitivity of 65%. However, using such a post-diagnosis interval (7 days) clearly reduces the interest of the test for which an anticipated diagnosis is expected. In our case, the anticipation of the diagnosis was only seen for a small number of patients, 22% of candidemia and 50% of CDC without previous candidemia.

The reasons for this overall low sensitivity remain uncertain. In their study, Angebault et al and Liu et al found the sensitivity lower for non-*Candida albicans* species as compared to *C. albicans* ($p < 0.01$), a factor not retrieved in our study.^{15,22} However, in our study, in 5 episodes of candidemia diagnosed with negative BDG, one can hypothesise that candidemia was only due to catheter colonisation and that this particular clinical form is not associated with the release of a significant amount of BDG in the bloodstream. Previously, we had suggested that a previous antifungal treatment, especially with echinocandin or amphotericin B, administered for more than 6 days, may be associated with negative BDG assay in a paediatric cohort.¹⁷ Again, in this larger cohort of candidemia this factor did not appear significant. Both *in vitro* and clinical studies are needed to decipher this issue and possibly adopt new diagnostic strategy with the BDG test.

We also looked at the potential value of the BDG in the follow-up of those patients. We found a correlation between the level of BDG at the time of candidemia diagnosis and the outcome at D15. This was concordant with a previous study where patients with a negative BDG at candidemia diagnosis had more chance to recover than those with a positive test at this time.¹⁵ Seric BDG levels may reflect higher persistent fungal load indicative of a poor outcome which may be either fatal or turn into CDC.

CDC diagnosis is always difficult, notably in the absence of previous candidemia, as the EORTC criteria do not include biomarkers for the classification as probable or possible CDC.¹² As positive candidemia is rarely obtained in the CDC context, the ECIL and ESCMID added mannan and anti-mannan to the definition of CDC in adult patients. Furthermore, Rammaert et al also suggested adding BDG in the CDC diagnosis criteria.²³ In our study, the sensitivity of blood positive culture, mannan detection, BDG detection, and the combination of these 3 criteria to detect CDC would be at 45.4%, 66.9%, 72.7% and 90.9%, respectively. Similar to candidemia, the kinetics of BDG correlated the clinical outcome of the CDC. Negativation of test occurred 2 to 6 months after the diagnosis and was associated with recovery or stabilisation of the imaging lesions. This is of significant importance since the duration of antifungal therapy is not defined for those infections and prolonged treatments expose to the risk of emergence of resistant pathogens.

In conclusion, the BDG test exhibits a low sensitivity to detect and especially anticipate the diagnosis of candidiasis. However, looking at the kinetics of the marker, the level of BDG at diagnosis can predict the outcome of those infections and thus guide the therapeutic management and future studies should determine if it can be used to discontinue the antifungal maintenance therapy.

Conflict of interest

No conflict of interest.

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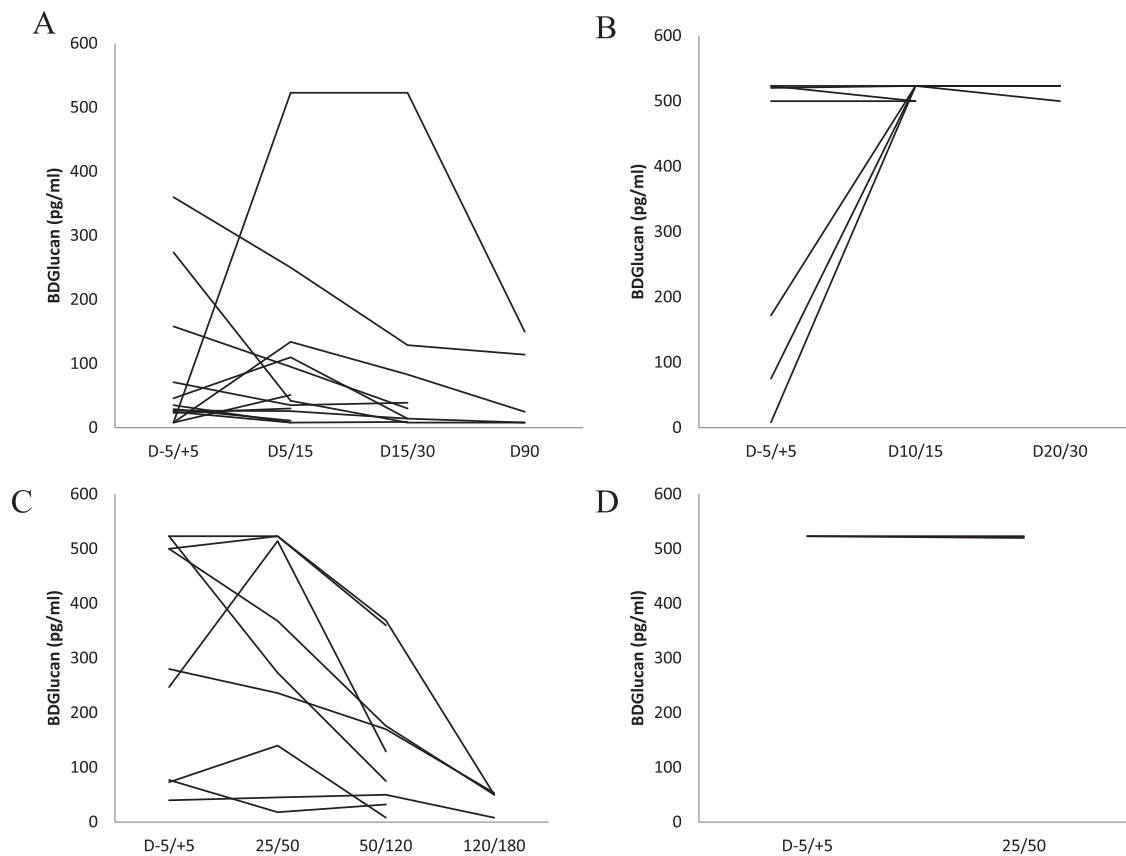


Fig. 2. Kinetics of BDG levels in patients with candidemia (A–B) and CDC (C–D) according to the outcome. BDG: β -D-glucan, CDC: chronic disseminated candidiasis. A: Rapid recovery of candidemia (n = 13). B: Persistent candidemia, evolution towards CDC or fatal outcome (n = 10). C: CDC with favourable outcome (n = 9). D: CDC with fatal outcome (n = 2).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jinf.2018.01.011>.

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