Determination of urinary D-/L-arabinitol ratios as a biomarker for invasive candidiasis in children with cardiac diseases

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A non-invasive, non-culture-based method of determining urinary D-/L-arabinitol (D-/L-ARA) ratios was investigated as a tool for the diagnosis of invasive candidiasis in nosocomial paediatric infection cases. The study encompassed 138 children aged 4 days to 16 years (mean $\pm$ SD = 1.6 ± 4.2 years) with congenital heart defects (91.4 %) or with rhythm disorders or circulatory failure (8.6 %). ARA enantiomers were detected by GC using an electron capture detector. Positive D-/L-ARA ratios were found for 11/11 patients with proven candidiasis and 17/19 patients with clinically suspected invasive candidiasis. Thirty children were undergoing antifungal chemotherapy. D-/L-ARA ratios (mean $\pm$ SD) were 2.601 ± 0.544 in hospitalized cardiac patients without fungal infection and 5.120 ± 1.253 in those receiving antifungal therapy ($P < 0.001$). The sensitivity of the method was 100 %, the specificity 97.2 %, the positive predictive value was 78.6 % and the negative predictive value was 100 %.

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INTRODUCTION

Progress in modern methods of cardiac diagnostics and cardiac surgery has significantly improved the detection and effectiveness of treating these conditions, even early in life. Unfortunately, the development of highly specialized treatment modalities has been accompanied by an increased incidence of nosocomial infections. Although *Staphylococcus* spp., *Enterococcus* spp. and *Pseudomonas* spp. are considered to be the main pathogens responsible for nosocomial cardiac infection (Giamarellou, 2002; Levy *et al.*, 2003), in recent decades a significant rise in infections caused by *Candida* spp., particularly *Candida albicans* and, increasingly, *Candida parapsilosis*, has been noted (Weems, 1992; Ellis *et al.*, 2001; Benjamin *et al.*, 2004; Tissières *et al.*, 2005). Children with congenital heart defects or circulatory disorders are at high risk of fungal infections as treatment is often invasive (including surgery) and they are frequently hospitalized in intensive care units, have immune disorders, undergo long-term antibiotic

treatment and are hospitalized for long periods (MacDonald *et al.*, 1998; Zaoutis *et al.*, 2004).

Cultures are the standard method of diagnosing infections. Although they are effective in bacterial infections, the sensitivity of various blood culture systems is unsatisfactory with respect to fungal infections (24–53 %), with many patients with disseminated infections having negative culture results (McDonald *et al.*, 2001; Benjamin *et al.*, 2003). The absence of typical clinical features, and a lack of fast and satisfactory microbiological methods make the final diagnosis in patients with a suspicion of fungal infection very difficult.

D-Arabinitol (D-ARA) is a characteristic major biomarker of several *Candida* species (Bernard *et al.*, 1981). GC, a rapid alternative to culture, has been used to determine the D-/L-ARA ratio in urine samples (Roboz, 1994; Larsson *et al.*, 1994; Reiss *et al.*, 2000; Stradomska & Mielniczuk, 2002). In an earlier study addressing the urinary D-/L-ARA ratio as a function of age, we determined the normal values for age in children (Stradomska & Mielniczuk, 2002). The objective of the current study was to determine the urinary D-/L-ARA ratios in a group of children undergoing

Abbreviations: ARA, arabinitol; NPV, negative predictive value; PPV, positive predictive value.

cardiological treatment and to evaluate the usefulness of this diagnostic method in identifying *Candida* infections in this risk group.

METHODS

Study population and case definition. Among 4143 patients hospitalized in the Cardiology Department of the Children's Memorial Health Institute over a 3 year period, a total of 138 children aged from 4 days to 16 years (mean $\pm$ SD = 1.6 ± 4.2 years) was studied. The age profile of this group was: ≤ 1 year (range 4 days–1 year), 46.4%; >1 to ≤ 3 years (range 1.1–3 years), 26.8%; >3 to ≤ 7 years (range 3.1–7 years), 15.9%; >10 years (range 11–16 years), 10.9%. The primary disease was a congenital heart defect in 91.4% of the patients and rhythm disorders or circulatory failure in 8.6%. All of the children were considered to be at high risk of invasive candidiasis: 91.4% were treated in the intensive care unit, 93.5% had central venous catheters and 90.6% underwent cardiac surgery.

The patients were grouped as follows: group A comprised hospitalized children with a heart defect or cardiac disease without fungal infection, whilst group B comprised 30 children who presented clinical signs of fungal infection, had a fever $>38.8^\circ\text{C}$, did not respond to broad-spectrum antibiotic treatment (3–5 days) and were treated with antifungal chemotherapy. Group B patients were subdivided into subgroups: B₁, those with microbiologically proven candidiasis with positive blood cultures; B₂, those with *Candida* antigenaemia; and B₃, those with suspected but unproven candidiasis.

Invasive candidiasis was clinically suspected in patients presenting clinical signs of infection, a fever $>38.8^\circ\text{C}$ and not responding to broad-spectrum antibiotic treatment. Empirical antifungal treatment was instituted despite blood cultures for *Candida* and the remaining mycological tests being negative. None of the studied patients had *Candida* funguria during the study.

Urine samples were collected from each patient 4–7 days after hospitalization; subsequent samples were obtained, depending on clinical condition, every 2–4 days after the onset of fever and the start of antibiotic treatment, and 2–5 days after the start of antifungal therapy. Altogether, 389 urine samples were collected, with a mean $\pm$ SD of 2.8 ± 4.2 samples per patient (range 1–11 per patient). The mean numbers of specimens per patient for each group were: group C (controls), 1.0; group A, 1.1; group B₁, 5.8; group B₂, 6.1; and group B₃, 6.3. The control group (C) comprised 80 healthy children ranging in age from 1 month to 13 years without clinical symptoms of either superficial or invasive candidiasis. The highest peak values of D-/L-ARA were used for analysis state for each patient.

GC and microbiological analysis. Samples (~2 ml urine) were collected from patients and stored at -20°C before analysis. The D-/L-ARA ratio was determined as trifluoroacetic derivatives according to a previously described method (Stradomska & Mielniczuk, 2002).

The BacT/Alert method was used for blood cultures. *Candida* was cultured on Sabouraud agar from samples of blood, urine and mucous membranes. The serum of each child was tested for the presence of *Candida* antigen using the Pastorex *Candida* test and for anti-*Candida* mannan antibodies with an immunofluorescence test (Gutiérrez *et al.*, 1993).

Statistical analysis. The results are presented as means $\pm$ SD. The significance of the differences was determined by the Levene test for determining the homogeneity of variances, and an analysis of variance Kruskal–Wallis rank test was applied to compare the three samples. All *P* values <0.05 were considered significant.

RESULTS AND DISCUSSION

The urinary D-/L-ARA ratios (means $\pm$ SD) and results of microbiological analysis in the studied groups are presented in Table 1. The urinary D-/L-ARA ratios in children according to age subgroup are given in Table 2. The mean urinary D-/L-ARA ratios in group A were higher than in the control group C in the respective age groups and declined with age. The distribution of D-/L-ARA levels in groups A and B was normal. The differences in D-/L-ARA ratios among the three groups (A, B and C) were statistically significant ($P < 0.001$). The normal range of urinary D-/L-ARA levels was defined (mean ± 2 SD) on the basis of findings from a study of healthy children as was the cut-off limit (mean ± 3 SD) for diagnosis of candidiasis (Table 2).

In 108 hospitalized children with cardiac disease but without fungal infection (group A), the mean D-/L-ARA level was higher than in the control group of healthy children (2.601 ± 0.544 vs 2.21 ± 0.626), but was still within the upper normal range. The results of microbiological tests remained within normal limits, and blood cultures for *Candida* species were negative, as was the test for *Candida* antigen, with mannan antibodies at normal levels (Table 1). Three patients without evidence of invasive candidiasis had above-normal D-/L-ARA ratios. In one of these patients, this value later normalized, whilst in two it remained elevated.

Elevated D-/L-ARA ratios were found in the 30 children in group B: 5.120 ± 1.253 (range 3.8–8.2) (Table 1). All of them presented clinical signs of infection. Their temperature remained constant or rose to 40.5°C , and they did not respond to broad-spectrum antibiotics.

The 11 patients in group B₁ were diagnosed with invasive candidiasis on the basis of positive blood cultures for *Candida*. All of these patients had D-/L-ARA ratios elevated to above cut-off levels (5.209 ± 0.735). In group B₂, the five patients were *Candida* antigen positive and had antibody levels $>1:160$. All these patients also had positive D-/L-ARA ratios (mean 4.730 ± 0.659 ; Table 1, Fig. 1). Both groups of patients received antifungal chemotherapy (fluconazole or amphotericin B). In all of these patients, the value of the D-/L-ARA biomarker suggested infection. The D-/L-ARA ratio for groups B₁ and B₂ was 5.034 ± 0.792 .

Fever also persisted in each of the remaining 14 patients (group B₃), despite antibiotic treatment, and it was not possible to establish a cause. Despite the results of mycological blood cultures for *Candida* being negative, and *Candida* antigens and serological tests for antibodies being within normal limits, these patients were classified as having clinically suspected but unproven invasive candidiasis and received empirical antifungal therapy. Three of these patients had *Candida* colonization of mucosal membranes. Two children had D-/L-ARA levels of 3.8–3.9 (subgroup 1–3 years), which was below the pathological cut-off value (mean ± 3 SD = 4.0), but above the normal range. The D-/L-ARA ratio in this group was 5.322 ± 1.590 .

Table 1. Urinary D-/L-ARA levels in children with heart defects or cardiac disease

Group	D-/L-ARA ratio (mean $\pm$ SD)	D-/L-ARA ratio (range)	Blood culture	Pastorex <i>Candida</i> antigen test	Anti- <i>Candida</i> mannan antibodies*
A ($n=108$)	2.601 $\pm$ 0.544	1.7–4.3	–	–	1:10–1:40
B ($n=30$)	5.120 $\pm$ 1.253	3.9–8.2	+ ($n=11$); – ($n=19$)	+ ($n=5$)	1:40–1:640
B ₁ ($n=11$)	5.209 $\pm$ 0.735	4.5–6.9	+	–	1:20–1:80
B ₂ ($n=5$)	4.730 $\pm$ 0.659	4.1–5.85	–	+	1:160–1:640
B ₃ ($n=14$)	5.322 $\pm$ 1.590	3.9–8.2	–	–	1:10–1:80
C (control) ($n=80$)	2.210 $\pm$ 0.626	1.3–3.3	ND	ND	ND

ND, Not determined.

*Normal range 1:10–1:80.

(Fig. 1). No statistically significant differences were found in the levels of D-/L-ARA ($H=2.257$, $P>0.324$) among groups B₁ (proven invasive *Candida* infection), B₂ (patients with *Candida* antigenaemia) and B₃ (clinically suspected invasive candidiasis).

During antifungal chemotherapy, D-/L-ARA levels were normalized in 11/11 patients with proven candidiasis (group B₁), and in 5/5 patients with antigenaemia (group B₂), but only in 11/14 patients in group B₃ with suspected but unproved candidiasis. In summary, antifungal therapy led to clinical improvement and normalization of D-/L-ARA levels in 27/30 patients. The remaining three patients died without a lowering of this biochemical marker. It is

very difficult to evaluate the response to antifungal treatment, particularly in dead patients. Simultaneous clinical improvement and reduced D-/L-ARA levels during antifungal treatment are indicative of probable infection. It seems that persisting clinical symptoms, with increased D-/L-ARA ratios, can attest to possible infection. Treatment was monitored in ten patients. It appeared that D-/L-ARA ratios responded rapidly to clinical changes during treatment. Monitoring D-/L-ARA levels facilitates decision making about changing medications if the D-/L-ARA level remains high (Figs 2 and 3). However, the example of one patient (Fig. 4) showed that negative results of traditional mycological diagnostics cannot exclude fungal infection if D-/L-ARA levels are elevated.

Table 2. D-/L-ARA levels in the different age subgroups

Group A, hospitalized children with a heart defect or cardiac disease without fungal infection; group B, hospitalized children with a heart defect or cardiac disease who received antifungal treatment; group C, control group (healthy children aged 1 month–13 years). The mean age $\pm$ SD (in years) in each particularly study subgroup is given in square brackets.

Age subgroup (years)	D-/L-ARA ratio (no. of patients) [mean $\pm$ SD]						Normal value (mean $\pm$ 2 SD)	Cut-off (mean $\pm$ 3 SD)
	Group A ($n=108$)	Group B ₁ ($n=11$)	Group B ₂ ($n=5$)	Group B ₃ ($n=14$)	Group B ($n=30$)	Control C ($n=80$)		
0–1	2.65 $\pm$ 0.46 ($n=48$) [0.37 $\pm$ 0.36]	5.13 $\pm$ 0.68 ($n=4$) [0.22 $\pm$ 0.27]	5.22 $\pm$ 0.88 ($n=2$) [0.39 $\pm$ 0.50]	5.32 $\pm$ 1.63 ($n=8$) [0.55 $\pm$ 0.36]	5.16 $\pm$ 1.32 ($n=16$) [0.47 $\pm$ 0.38]	2.48 $\pm$ 0.58 ($n=23$) [0.53 $\pm$ 0.24]	3.6	4.2
1–3	2.61 $\pm$ 0.50 ($n=28$) [1.89 $\pm$ 0.58]	5.60 $\pm$ 0.92 ($n=4$) [2.12 $\pm$ 0.25]	4.30 $\pm$ 0.28 ($n=2$) [1.34 $\pm$ 0.23]	5.38 $\pm$ 1.91 ($n=6$) [1.48 $\pm$ 0.43]	5.14 $\pm$ 1.37 ($n=9$) [1.78 $\pm$ 0.53]	2.30 $\pm$ 0.58 ($n=18$) [2.19 $\pm$ 0.62]	3.5	4.0
3–7	2.51 $\pm$ 0.57 ($n=17$) [4.63 $\pm$ 1.47]	4.80 $\pm$ 0.40 ($n=3$) [4.51 $\pm$ 2.17]	4.60 $\pm$ 0.68 ($n=1$) [3.50]	– –	4.90 $\pm$ 1.16 ($n=5$) [4.20 $\pm$ 1.25]	2.09 $\pm$ 0.59 ($n=22$) [5.19 $\pm$ 1.15]	3.3	3.9
>10	2.34 $\pm$ 0.42 ($n=15$) [14.37 $\pm$ 2.17]	–	–	–	–	1.64 $\pm$ 0.39 ($n=17$) [14.87 $\pm$ 1.97]	2.4	2.8

Fig. 1. Graph showing the urinary D-/L-ARA ratios in groups A, B1, B2 and B3. White bars, 25–75 %; □, median value; error bars, minimum–maximum values.

In group A (hospitalized children without fungal infection), a negative correlation was found between D-/L-ARA ratios and age (Table 2), which confirmed our earlier

Fig. 2. Urinary D-/L-ARA ratios in a 1½-year-old boy with a congenital heart defect (complete atrio-ventricular septal defect; sepsis 6 months earlier). The pre-surgery D-/L-ARA ratio was 3.7. On day 6 post-surgery, the D-/L-ARA ratio rose to 5.5 and was accompanied by a deterioration in clinical condition. Fluconazole (↑D) was started and on the sixth day the D-/L-ARA ratio normalized to 2.3 with improvement of the patient's condition. Solid line, treatment time; dashed line, upper limit of normal values; ↑P, positive blood culture for *C. albicans*; ↑N, negative blood culture for *C. albicans*.

Fig. 3. Urinary D-/L-ARA ratios in a 3-year-old girl with a congenital heart defect, pneumonia, mycotic and purulent lesions of a forearm wound (*C. albicans*), in a serious condition with a high fever not responding to broad-spectrum antibiotic therapy. The initial D-/L-ARA ratio was 5.6. Fluconazole (↑D) lowered the level of this biomarker over a period of 19 days, but improvement of the clinical condition and normalization of the D-/L-ARA ratio was achieved only after the introduction of amphotericin treatment (↑A).

reports on the normal values of D-/L-ARA ratios in the paediatric population (Stradomska & Mielniczuk, 2002). Similarly to a group of at-risk hospitalized infants undergoing long-term antibiotic therapy (Stradomska *et al.*, 2005), elevated mean D-/L-ARA ratios and medians were found in group A children in comparison with healthy children. Other authors have also observed higher urinary D-/L-ARA ratios in hospitalized children and adults (Larsson *et al.*, 1994; Christensson *et al.*, 1997). This rise probably reflects the high sensitivity of this biomarker to risk factors (such as antibiotic therapy and hospitalization), and its level seems to point towards an increased fungal load in hospitalized at-risk patients.

As the intervals delineated by the means and SD were nearly identical, the numbers of patients in the age subgroups low and the sets disjunctive (Table 1), it seemed justified to determine the mean results and to conduct a sensitivity and specificity analysis of this method on the entire group studied. On the basis of the results for patients in groups B₁ and A, the sensitivity and specificity of urine ARA as a biomarker in the diagnosis of invasive candidiasis were 100 and 97.2 %, respectively, and the positive predictive value (PPV) and negative predictive value (NPV) were 78.6 and 100 %, respectively. If it is assumed that the empirically treated patients (groups B₂ and B₃) indeed also had invasive candidiasis, the calculated validation parameters for the ARA test were: sensitivity, 93.3 %; specificity,

Fig. 4. Urinary D-/L-ARA ratios in a child after cardiac surgery. Despite antibiotic therapy, fever rose to 40.5 °C. Although the results of mycological diagnostic tests were negative, antifungal therapy, fluconazole (↑ D), was started. The D-/L-ARA ratio rose to 6.1. The patient died on day 14 of treatment.

97.2 %; false-negative results, 6.7 %; false-positive results, 2.8 %; NPV, 98.1 %; PPV, 90.9 %. The estimated diagnostic values of this method in this study are similar to those presented previously: 94 % sensitivity and 88 % specificity in adults (Roboz & Katz, 1992), and 100 % sensitivity and 94 % specificity, with a PPV of 71 % and an NPV of 100 %, in children with neutropenia (Christensson *et al.*, 1997). It should be noted that assessment of the clinical utility of the reported method is based on a prevalence of 30/138. If the ARA test were to be used for screening for invasive candidiasis in the hospital, the prevalence would be 0.7 % and the NPV could change significantly.

Infants (range 16 days–1 year) were the largest age group, accounting for 53.3 % of group B, whilst children ≤3 years (range 1.1–2.5) made up 30.0 % and children aged >3 to ≤7 years (range 3.5–7) comprised 16.7 %. Similar data (63 % children <1 year) were presented in a review by Millar *et al.* (2005). Overall, *Candida* infection developed in 0.7 % of the patients hospitalized in this 3 year period. Although similar data were presented by Millar *et al.* (2005), higher figures have been reported by others [6.3 % in infants following cardiac surgery, 10 % in children with an underlying diagnosis of cardiac diseases (Chakrabarti *et al.*, 2003; Zaoutis *et al.*, 2004)]. The percentage of children who were given antifungal treatment (group B) in

relation to the number of patients in particular age subgroups was similar (<1 year, 25.0 %; <3 years 24.3 %; >3 to <7 years, 22.7 %), but in the age group >10 years it was 0 %.

Three children in our study (<3 years) died, despite antifungal treatment, without normalization of the biomarker; thus, mortality was 10 %. Mortality in children with invasive candidiasis has been reported to range from 26 to 54 % in infants (McDonald *et al.*, 2001; Zaoutis *et al.*, 2004), but as high as 83 % following cardiac surgery in children (Chakrabarti *et al.*, 2003). *C. albicans* is considered to be the most virulent pathogen and also causes the highest mortality [59 % (Rabalais *et al.*, 1996) and 26 % (Kossoff *et al.*, 1998) have been reported], although other authors report that mortality from *C. parapsilosis* can be as high as 39 % (Saxen *et al.*, 1995).

Successful treatment of fungal infections depends mainly on early and accurate diagnosis, and implementation of the appropriate antifungal therapy (Debusk *et al.*, 1994; Denning *et al.*, 2003). Diagnostic difficulties and high mortality are why prophylactic antimycotic therapy is a standard procedure in some risk groups (Rex *et al.*, 2000; Eggimann *et al.*, 2003). As discussed by other authors, more aggressive drugs (such as amphotericin B) are usually preferred (Rex *et al.*, 2000; Chandrasekar, 2001). However, this approach can lead to highly resistant pathogens, which has already been observed (such as a 50 % increase in *Candida glabrata* infections known to be resistant to fluconazole in patients after empirical treatment; Sendid *et al.*, 2006).

In patients with fungal endocarditis, especially children, mortality reaches 70–80 % due to a recurrence rate of 30 %. For this reason, they should be followed for up to 4 years while on prophylactic antifungal therapy (Johnston *et al.*, 1991; Rex *et al.*, 2000; Ellis *et al.*, 2001). In our opinion, determining D-/L-ARA levels during treatment creates optimal conditions for monitoring the condition of a patient with deep fungal infection (aiding selection of the appropriate drug and duration of therapy; Figs 2 and 3). It may be especially helpful in managing children at risk due to cardiac disorders.

The known diagnostic difficulties (delayed or mistaken diagnosis in 82 % of patients; Ellis *et al.*, 2001) suggest that better diagnostic monitoring by heightened diagnostic acumen, including detection of other biomarkers, is required to allow earlier identification of patients at high risk of fungal infection (Ellis *et al.*, 2001; Millar *et al.*, 2005). Recently, a very interesting study on the clinical utility of fungal cell wall components detected as biomarkers has been presented (Ellis *et al.*, 2008).

D-ARA (pentane-1,2,3,4,5 pentitol) belongs to the group of polyols. Polyols are involved in the pentose phosphate pathway. Studies have shown that serum D-ARA/creatinine ratios are elevated in animals and humans with invasive *Candida* infection compared with uninfected or colonized

controls (Wong *et al.*, 1982). Comparison of serum D-ARA secretion with creatinine has made it possible to eliminate false-positive results for renal dysfunction patients. The experiments by Roboz (1994) and our unpublished results show that serum D-/L-ARA 'translates into' urine D-/L-ARA. In addition, this marker is not influenced by renal dysfunction. The availability of material and the non-invasiveness of the test, and thus the possibility of taking multiple samples, are why we determine urinary ARA levels, especially because the study dealt with the paediatric population. It must be noted that the measured D-/L-ARA ratio is a product of human D-/L-ARA and, possibly, a metabolite of *Candida* sp. existing in the host. The D-/L-ARA ratio rises if the D-ARA concentration increases or if the L-ARA concentration decreases. The pentose pathway in humans is not well understood. Metabolic dysfunctions or disorders in the pathways of polyols may affect the diagnostic value of the D-/L-ARA ratio. In recent years, new diseases involving disorders of polyol metabolism resulting in accumulation of D-ARA in body fluid have been found, which may generate false-positive results. Also, one child with L-arabinosuria has been reported, which may, in turn, cause false-negative results. Nevertheless, these are very rare cases and are accompanied by other clinical symptoms (Tyłki-Szymańska *et al.*, 2009). However, relatively less is known about ARA enantiomer secretion, metabolism and kinetics, which may cause some potential problems with interpreting results.

Earlier reports on increases in D-/L-ARA ratios preceding positive blood culture results by 1–3 weeks (Christensson *et al.*, 1997; Walsh *et al.*, 1995), and clinical experience from other medical centres (Yeo *et al.*, 2006; Sigmundsdottir *et al.*, 2007), as well as the results presented above, support the suggestion that the use of the described method in combination with traditional methods can improve the reliability of fungal infection diagnosis and facilitate the early initiation of antifungal treatment. The reported method does not permit the identification of the pathogen in terms of species, but because the assayed metabolite is produced mainly by the major aetiological factor (Millar *et al.*, 2005), *Candida* spp., and given the rapidity, non-invasiveness and micro-scale of the assay, it should be a useful tool in the diagnosis of fungal infections in the panel of diagnostic methods used for children at risk.

REFERENCES

- Benjamin, D. K., Jr, DeLong, E. R., Steinbach, W. J., Cotton, C. M., Walsh, T. J. & Clark, R. H. (2003). Empirical therapy for neonatal candidemia in very low birth weight infants. *Pediatrics* **112**, 543–547.
- Benjamin, D. K., Jr, Miro, J. M., Hoen, B., Steinbach, W. J., Fowler, W. G., Jr, Olaison, L., Habib, G., Abrutyn, E., Perfect, J. & other authors (2004). *Candida* endocarditis: contemporary casus from the International Collaboration of Infectious Endocarditis Merged Database (ICE-mD). *Scand J Infect Dis* **36**, 453–455.
- Bernard, E. M., Christiansen, K. J., Tsang, S., Kiehn, T. E. & Armstrong, D. (1981). Rate of arabinitol production by pathogenic yeast species. *J Clin Microbiol* **14**, 189–194.
- Chakrabarti, C., Sood, S. K., Parnell, V. & Rubin, L. G. (2003). Prolonged candidemia in infants following surgery for congenital heart disease. *Infect Control Hosp Epidemiol* **24**, 753–757.
- Chandrasekar, P. H. (2001). Empirical antifungal therapy for persistent fever in patients with neutropenia. *Clin Infect Dis* **32**, 320.
- Christensson, B., Wiebe, T., Pehrson, K. & Larsson, L. (1997). Diagnosis of invasive candidiasis in neutropenic children with cancer by determination of D-arabinitol/L-arabinitol ratios in urine. *J Clin Microbiol* **35**, 636–640.
- Debusk, C. H., Daoud, R., Thirumoorthi, M. C., Wilson, F. M. & Khatib, R. (1994). Candidemia: current epidemiologic characteristics and a long-term follow-up of the survivors. *Scand J Infect Dis* **26**, 697–703.
- Denning, D. W., Kibbler, C. C. & Barnes, R. A. on behalf of the British Society for Medical Mycology (2003). British Society for Medical Mycology proposed standards of care for patients with invasive fungal infections. *Lancet Infect Dis* **3**, 230–240.
- Eggimann, P., Garbino, J. & Pittet, D. (2003). Management of *Candida* species infections in critically ill patients. *Lancet Infect Dis* **3**, 772–785.
- Ellis, M. E., Al-Abdely, H., Sandridge, A., Greer, W. & Ventura, W. (2001). Fungal endocarditis: evidence in the world literature, 1965–1995. *Clin Infect Dis* **32**, 50–62.
- Ellis, M., al-Ramadi, B., Finkelman, M., Hedstrom, U., Kristensen, J., Ali-Zadeh, H. & Klingspor, L. (2008). Assessment of the clinical utility of serial β -D-glucan concentrations in patients with persistent neutropenic fever. *J Med Microbiol* **57**, 287–295.
- Giamarellou, H. (2002). Nosocomial cardiac infections. *J Hosp Infect* **50**, 91–105.
- Gutiérrez, J., Maroto, C., Piédrola, G., Martin, E. & Perez, J. A. (1993). Circulating *Candida* antigens and antibodies: useful markers of candidemia. *J Clin Microbiol* **31**, 2550–2552.
- Johnston, P. G., Lee, J., Domanski, M., Dressler, F., Tucker, E., Rothenberg, M., Cunnion, R. E., Pizzo, P. A. & Walsh, T. J. (1991). Late recurrent *Candida* endocarditis. *Chest* **99**, 1531–1533.
- Kossoff, E. H., Buescher, E. S. & Karlowicz, M. G. (1998). Candidemia in a neonatal intensive care unit: trends during fifteen years and clinical features of 111 cases. *Pediatr Infect Dis J* **17**, 504–508.
- Larsson, L., Pehrson, C., Wiebe, T. & Christensson, B. (1994). Gas chromatographic determination of D-arabinitol/L-arabinitol ratios in urine: a potential method for diagnosis of disseminated candidiasis. *J Clin Microbiol* **32**, 1855–1859.
- Levy, I., Ovadia, B., Erez, E., Rinat, S., Ashkenazi, S., Birk, E., Konisberger, H., Vidne, D. & Dagan, O. (2003). Nosocomial infections after cardiac surgery in infants and children: incidence and risk factors. *J Hosp Infect* **53**, 111–116.
- MacDonald, L., Baker, C. & Chenoweth, C. (1998). Risk factors for candidemia in a children's hospital. *Clin Infect Dis* **26**, 642–645.
- McDonald, L. C., Weinstein, M. P., Fune, J., Mirrett, S., Reimer, R. G. & Reller, L. B. (2001). Controlled comparison of BacT/ALERT FAN aerobic medium and BACTEC fungal blood culture medium for detection of fungemia. *J Clin Microbiol* **39**, 622–624.
- Millar, B. C., Jugo, J. & Moore, J. E. (2005). Fungal endocarditis in neonates and children. *Pediatr Cardiol* **26**, 517–536.
- Rabalais, G. P., Samiec, T. D., Bryant, K. K. & Lewis, J. J. (1996). Invasive candidiasis in infants weighing more than 2500 grams at birth admitted to a neonatal intensive care unit. *Pediatr Infect Dis J* **15**, 348–352.

- Reiss, E., Obayashi, T., Orle, K., Yoshida, M. & Zancopé-Oliveira, R. M. (2000). Non-culture based diagnostic tests for mycotic infections. *Med Mycol* **38**, 147–159.
- Rex, J. H., Walsh, T. J., Sobel, J. D., Filler, S. G., Pappas, P. G., Dismukes, W. E. & Edwards, J. E. (2000). Practice guidelines for the treatment of candidiasis. *Clin Infect Dis* **30**, 662–678.
- Roboz, J. (1994). Diagnosis and monitoring of disseminated candidiasis based on serum/urine D/L-arabinitol ratios. *Chirality* **6**, 51–57.
- Roboz, J. & Katz, R. N. (1992). Diagnosis of disseminated candidiasis based on serum D-/L-arabinitol ratios using negative chemical ionization mass spectrometry. *J Chromatogr* **575**, 281–286.
- Saxen, H. S., Virtanen, M., Carlson, P., Hopppu, K., Pohjavuori, M., Vaara, M., Vuopio-Varkila, J. & Peltola, H. (1995). Neonatal *Candida parapsilosis* outbreak with a high case fatality rate. *Pediatr Infect Dis J* **14**, 776–781.
- Sendid, B., Cotteau, A., François, N., D'Haveloose, A., Standaert, A., Camus, D. & Poulain, D. (2006). Candidemia and antifungal therapy in a French University Hospital: rough trends over a decade and possible links. *BMC Infect Dis* **6**, 80.
- Sigmundsdottir, G., Larsson, L., Wiebe, T., Björklund, L. J. & Christensson, B. (2007). Clinical experience of urine D-arabinitol/L-arabinitol ratio in the early diagnosis of invasive candidiasis in paediatric high risk populations. *Scand J Infect Dis* **39**, 146–151.
- Stradomska, T. J. & Mielniczuk, Z. (2002). Gas chromatographic determination of D-/L-arabinitol ratio in healthy Polish children. *J Chromatogr B Analyt Technol Biomed Life Sci* **773**, 175–181.
- Stradomska, T. J., Bobula-Milewska, B., Bauer, A., Mielniczuk, Z., Dąbkowska, M., Syczewska, M. & Dzierżanowska, D. (2005). Urinary D-/L-arabinitol levels in infants undergoing long-term antibiotic therapy. *J Clin Microbiol* **43**, 5351–5354.
- Tissières, P., Jaeggi, E. T., Beghetti, M. & Gervaix, A. (2005). Increase of fungal endocarditis in children. *Infection* **33**, 267–272.
- Tylki-Szymańska, A., Stradomska, T. J., Wamelink, M. M. C., Salomons, G. S., Taybert, J., Pawłowska, J. & Jakobs, C. (2009). Transaldolase deficiency in two new patients with a relative mild phenotype. *Mol Genet Metab* **97**, 15–17.
- Walsh, T. J., Merz, W. G., Lee, J. W., Schaufele, R., Sein, T., Whitcomb, P. O., Ruddel, M., Burns, W., Wingard, J. R. & other authors (1995). Diagnosis and therapeutic monitoring of invasive candidiasis by rapid enzymatic detection of serum D-arabinitol. *Am J Med* **99**, 164–172.
- Weems, J. J., Jr (1992). *Candida parapsilosis*: epidemiology, pathogenicity, clinical manifestations, and antimicrobial susceptibility. *Clin Infect Dis* **14**, 756–766.
- Wong, B., Bernard, E. M., Gold, J. W. M., Fong, D. & Armstrong, D. (1982). The arabinitol appearance rate in laboratory animals and humans: estimation from the arabinitol/creatinine ratio and relevance to the diagnosis of candidiasis. *J Infect Dis* **146**, 353–359.
- Yeo, S. F., Huie, S., Sofair, A. N., Campbell, S., Durante, A. & Wong, B. (2006). Measurement of serum D-arabinitol/creatinine ratios for initial diagnosis and for predicting outcome in an unselected, population-based sample of patients with *Candida* fungemia. *J Clin Microbiol* **44**, 3894–3899.
- Zaoutis, T. E., Greves, H. M., Lautenbach, E., Bilker, W. B. & Coffin, S. E. (2004). Risk factors for disseminated candidiasis in children with candidemia. *Pediatr Infect Dis J* **23**, 635–641.