Chronic obstructive cholestasis with gallbladder masses associated with invasive fungal infection in a preterm neonate: a case report

Anne Geurden [a], Anneleen Engelen [a], Luisa Vonghia [b], Nicolette Moes [c], Veerle Matheeussen [d], Bart Bracke [e], Koen Vanden Driessche [c], Ludo Mahieu [a]

[a] Department of neonatology, University Hospital of Antwerp and University of Antwerp, Antwerp, Belgium
[b] Department of gastroenterology and hepatology, University Hospital of Antwerp and University of Antwerp, Antwerp, Belgium
[c] Department of paediatrics, University Hospital of Antwerp and University of Antwerp, Antwerp, Belgium
[d] Department of clinical microbiology, University Hospital of Antwerp and University of Antwerp, Antwerp, Belgium
[e] Department of hepatobiliary, transplant and endocrine surgery, University Hospital of Antwerp and University of Antwerp, Antwerp, Belgium

a_geurden@hotmail.com

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Abstract

A preterm boy, born at the gestational age of 26 weeks, developed a sepsis followed by obstructive cholestasis at the age of 4 weeks despite broad spectrum antibiotic and fluconazole treatment. Abdominal ultrasound showed masses of unknown origin in the gallbladder. An invasive fungal infection was diagnosed via serum (1,3)-beta-D-glucan. After initiation of the echinocandin caspofungin, inflammation and cholestasis parameters subsided quickly and the patient recovered.

Accumulation of fungal balls in the gallbladder has been described as a cause of chronic obstructive cholestasis in immunocompromised adults, this is the first reported case in a preterm neonate.

Introduction

Neonatal cholestasis and invasive fungal infections are both associated with important morbidity and mortality in preterm neonates. Neonatal cholestasis is defined by a serum conjugated bilirubin of more than 1 mg/dL or more than 20% of the total serum bilirubin. Up to 10 to 20% of premature babies will develop cholestasis due to various causes, including but not excluded to those mentioned in figure 1. Some of these require urgent interventions so a prompt and full diagnostic work up of neonatal cholestasis is key (1).

Invasive fungal infections are mostly caused by *Candida* species. Risk factors include presence of central venous catheters, use of broad-spectrum antibiotics, intravenous lipid emulsion, endotracheal tubes and antenatal antibiotics (2). The gold standard for microbiological diagnosis is a fungal culture, but this lacks sensitivity (3). Therefore, additional culture-independent tests such as galactomannan and (1,3)-beta-D-glucan (BDG) can be of added value for diagnosing invasive fungal infections. BDG is a major cell wall constituent of most pathogenic fungi (including *Candida* and *Aspergillus* species) which is released in serum in case of an invasive fungal infection. The most important diagnostic value of a BDG assay is its high negative predictive value (4). In case of invasive *Candida* infections in neonates, the first choice of empirical antifungal therapy is still under debate. Amphotericin B deoxycholate, fluconazole and echinocandins are appropriate choices. If a non-*albicans Candida* species is being isolated such as *C. glabrata*, or *C. krusei*, which are intermediate or resistant to fluconazole, amphotericin or an echinocandin should be administered. This is also recommended when the patient has received fluconazole prophylaxis (3). Although it is under discussion as studies have shown that there is no increase in fluconazole resistance after prophylaxis (5).

In adult immunocompromised patients the accumulation of fungal balls in the gallbladder during a systemic fungal infection has been described to cause chronic obstructive cholestasis (6). To this day, this had not yet been reported in preterm neonates.

Patient information

A male baby was born at the gestational age of 26 weeks, weighing 800 grams, through spontaneous vaginal delivery. APGAR scores were 8-10-10. He received surfactant and respiratory support through nasal continuous positive airway pressure. Regarding premature labour of unknown origin antibiotics were administered for 5 days. Blood culture remained negative. Fluid intake consisted solely of breast milk by the age of 10 days and there were no more central lines. No major particularities were reported during the first month.

At the age of 4 weeks, he developed a clinical late onset sepsis of unknown origin with hypothermia, a distended abdomen and a septic appearance for which broad-spectrum antibiotics were initiated. C-reactive protein (CRP) rose to 23 mg/L (normal < 4 mg/L) and blood culture became positive for *S. epidermidis*. A central venous catheter was placed and empirical therapy with amikacin and piperacillin-tazobactam initiated for which the antibiogram confirmed sensitivity. Therapy was subsequently narrowed to vancomycin monotherapy for a total of 10 days. The patient improved clinically, nevertheless CRP remained elevated. Therapy was switched again to various (broad-spectrum) antibiotics: vancomycin, cefotaxime, piperacillin-tazobactam and the antifungal azole drug fluconazole. Despite this, CRP increased to a value of 109 mg/L at the age of 7 weeks. Echocardiography showed no signs of endocarditis. Serial blood and urine cultures remained sterile. The central catheter was removed and replaced, no pathogens were cultured from the catheter tip. A viral

Figure 1: Flowchart for management of neonatal cholestasis and when to suspect an invasive fungal infection.
HSV = herpes simplex virus, CMV = cytomegalovirus, HAV = hepatitis A virus, HBV = hepatitis B virus, HCV hepatitis C virus, BDG = beta-D-glucan

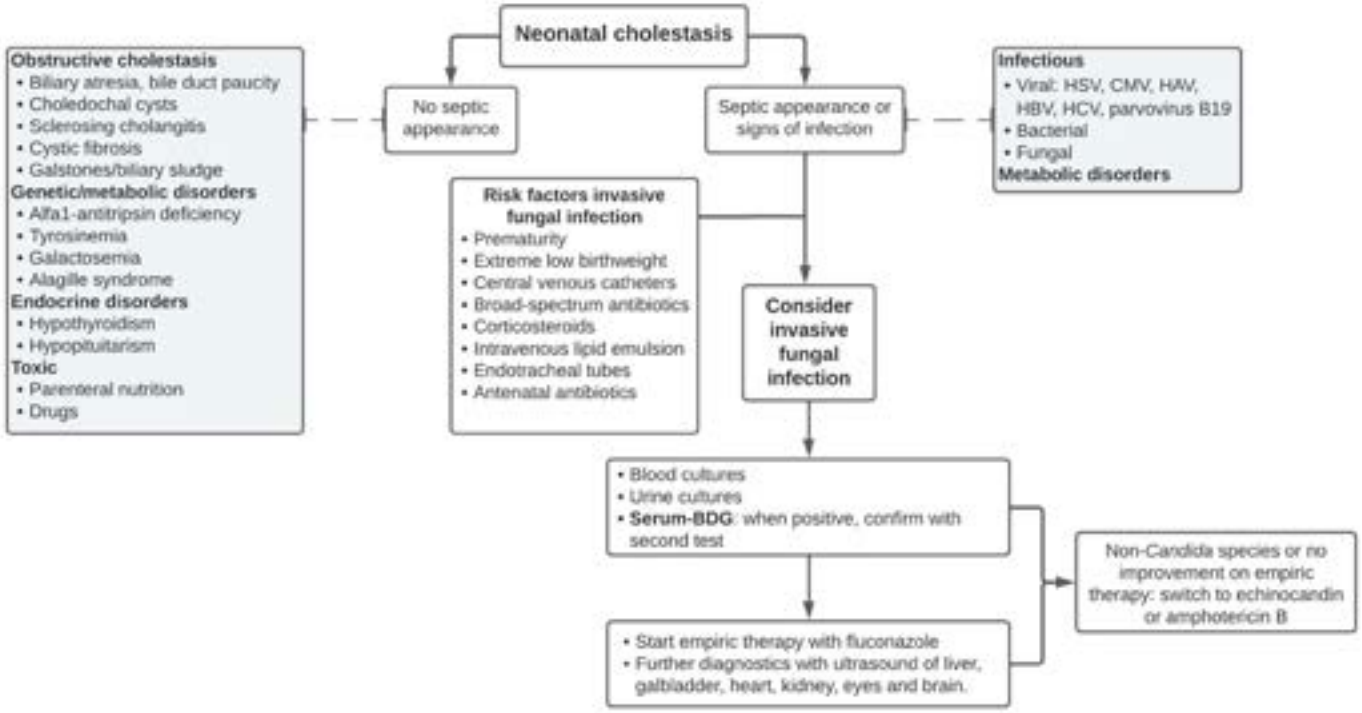
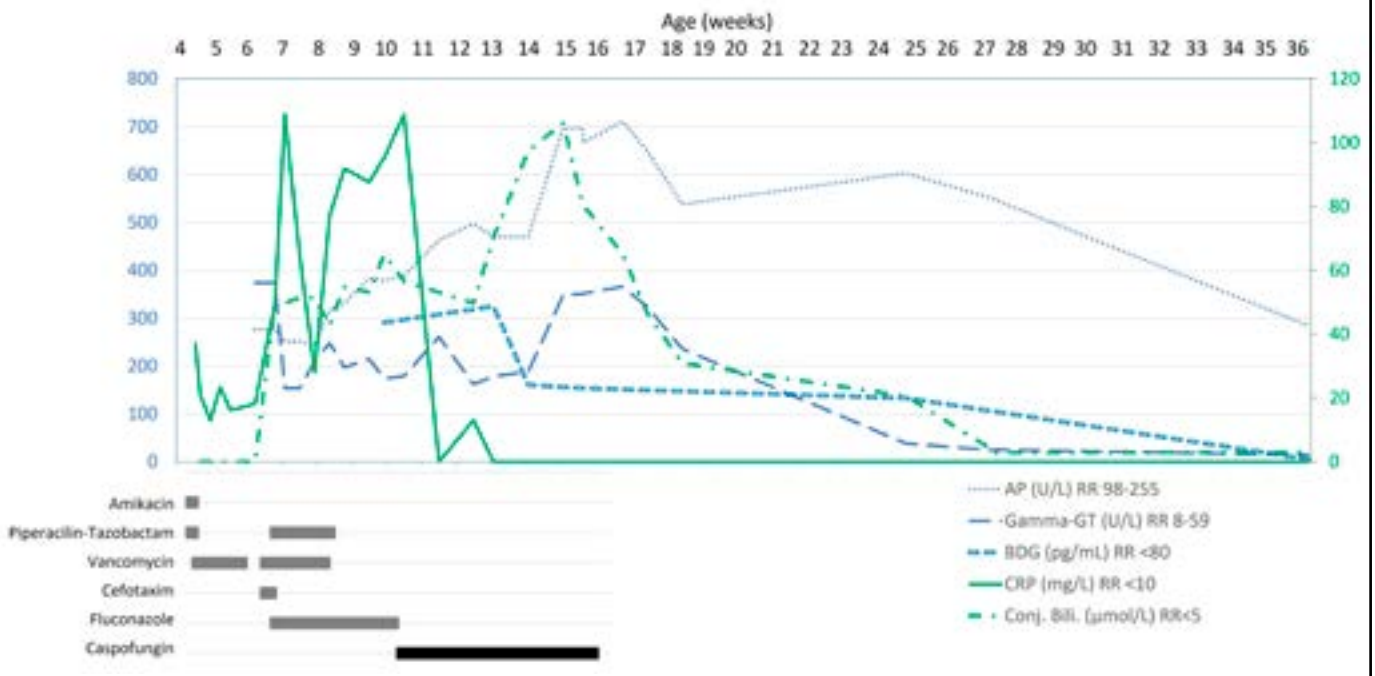


Figure 2: Evolution of laboratory results and reaction to antibiotic and antifungal treatment. AP, gamma-GT and BDG are displayed on the left axis, CRP and Conj. Bili. in the right axis.
AP = alkaline phosphatase, BDG = beta-D-glucan, CRP = C-reactive protein, Conj. Bili. = conjugated bilirubin, Gamma-GT = gamma-glutamyl transferase, RR = reference range.



respiratory multiplex molecular panel performed on a nasopharyngeal aspirate could not identify any pathogens.

During this period, his stool became acholic and he developed jaundice and failure to thrive. Further investigations revealed cholestasis with a conjugated hyperbilirubinemia, elevated gamma-glutamyl transpeptidase and liver alkaline phosphatase. The evolution of the biochemical parameters is visualised in figure 2.

Furter diagnostic tests could not indicate any viral cause for the cholestasis and screening for alfa1-antitripsin deficiency, cystic fibrosis, tyrosinemia and galactosemia came back negative. An abdominal ultrasound showed hyperechogenic material in the gallbladder and magnetic resonance imaging confirmed cholecystolithiasis without distention of the biliary tract.

Weight gain was already insufficient from the age of 4 weeks despite enriching breast milk with human milk fortifier and medium chain triglycerides. At 7 weeks of age breast milk was substituted for total parenteral feeding. After a small catch up in weight the evolution ceased again by 8 weeks.

As no bacterial or other cause could be found for the cholestasis, inflammation and failure to thrive, at the age of 8 weeks all antibiotics were stopped and fluconazole continued in therapeutic dosages. There was no effect on the clinical evolution or growth. Blood cultures taken within this antibiotic free window remained negative. At the age of 10 weeks, a serum BDG test (performed at the Belgian mycosis reference centre at the laboratory of University Hospital Leuven) suggested an invasive fungal infection with a value of 294 pg/mL (cut-off value for positivity = 80 pg/mL) while receiving fluconazole in therapeutic dosages for over two weeks (7). Subsequently, therapy was switched to intravenous caspofungin (an echinocandin) at a dosage of 2 mg/kg/day, once daily. Within one week CRP normalized and a remarkable catch up growth could be noted (figure 3). The serum BDG rose further to a maximum of 325 pg/mL, followed by a gradual decrease (figure 2). Transaminases declined slowly under the ongoing therapy, though the cholelithiasis remained visible on ultrasound exams up to 7 months after therapy. We believe this history is compatible with the presence of fungal collections in the gallbladder. This could however not be confirmed. Firstly, because endoscopic retrograde cholangiopancreatography (ERCP) was not feasible at his young age and low body weight, and secondly, because it was decided to hold off on cholecystectomy given the clinical improvement following initiation of caspofungin.

Caspofungin was discontinued after 6 weeks. Afterwards, our patient was observed for another 2 weeks at the neonatology ward, without signs of relapse. The boy could be discharged from the hospital in good clinical condition at the age of 18 weeks (postconceptional age of 44 weeks).

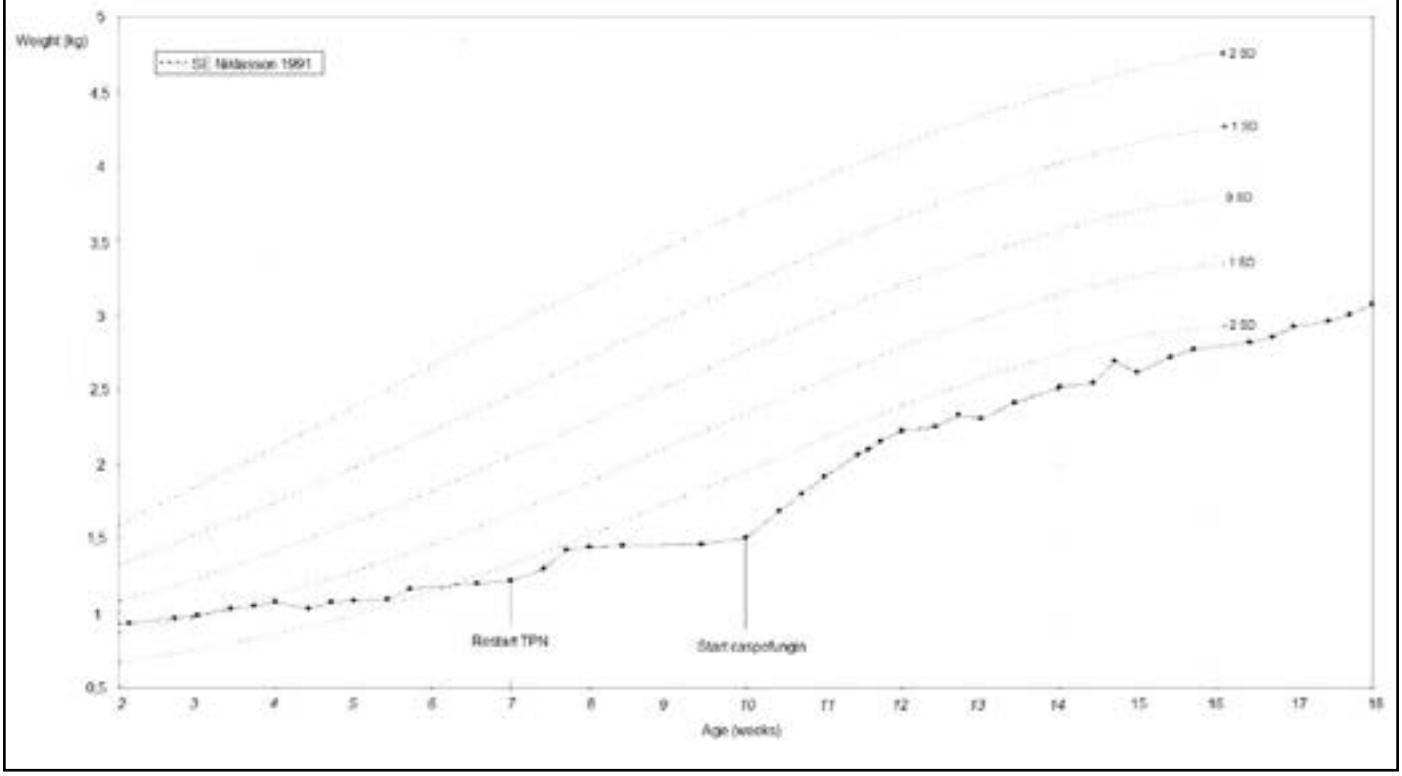
Over the following weeks cholestasis parameters declined to normal values. His growth curve returned within the normal range. BDG became negative at the age of 36 weeks (20 weeks after termination of caspofungin therapy).

Discussion

The presence of fungal balls in the gallbladder, obstructing bile flow, as a result of a systemic fungal infection have been described in adult immunodeficient patients but is very rare (6). One case report describing a *Candida tropicalis* infection with gallbladder masses in a term neonate has been published but to our knowledge systemic fungal infections with gallbladder concrements have not been described in preterm neonates (8).

Diagnosis of a systemic fungal infection can be challenging. *Candida* sensitivity of blood cultures has been reported to be only 50%, and most only become positive after 48 hours of incubation. BDG is a component of the fungal wall of the *Aspergillus*, *Candida*, *Pneumocystis*, *Coccidioides* and *Histoplasma species* and its presence in serum can be an indication for an invasive fungal infection. BDG can be positive up to 10 days before growth of *Candida* on culture and seem to be more sensitive in neonates

Figure 3: Fenton Preterm weight-for-age 22-50 weeks, boys.
Born at 26 weeks, a failure to thrive is setting in at the age of 4 weeks. At the age of 10 weeks Caspofungin was initiated, resulting in catch-up growth.



compared to adults, possibly caused by a higher fungal load during infection due to a poorly developed immune system (7). False positive BDG tests are possible in case of antibacterial treatment (mostly with amoxicillin-clavulanate and piperacillin-tazobactam), hemodialysis, intravenous treatment with immunoglobulins, albumin or coagulation factors. Two consecutive positive serum BDG tests have been associated with a high likelihood of invasive fungal infection (10). In our case broad-spectrum antibiotics (piperacillin-tazobactam) were given until 10 days before the first BDG test, a second positive test was performed 3 weeks later, both of which make a false positive result unlikely.

Risk factors for biliary tract candidiasis described in adults are immunosuppressive drugs or antibiotics and admission to an intensive care unit. The diagnosis is best achieved by direct culture of the bile post-ERCP (6). Both ERCP and cholecystectomy were considered in our case but judged not feasible. Low weight or age have been reported as a risk for ERCP failure (10). Moreover, the patient was clinically improving so an invasive procedure seemed redundant.

First line drug therapy is fluconazole, second line is caspofungin or amphotericin B. In our case we observed no improvement after two weeks of fluconazole treatment, assuming infection due to a fluconazole resistant *Candida* strain and therefore switched to caspofungin treatment with success.

Conclusion

A disseminated fungal infection should be considered in a preterm neonate with signs of sepsis with obstructive cholestasis and/or gallbladder stones, especially in the presence of other risk factors for invasive fungal disease, such as a central venous catheter or recent exposure to broad-spectrum antibiotics or steroids. A serum BDG test can help establish a presumptive diagnosis when microbiological cultures remain negative, even when patient is under fluconazole therapy. Empirical antifungal therapy is warranted in these cases, as early recognition and treatment are associated with decreased morbidity and mortality. In case of treatment failure with azoles, a switch to caspofungin to target fluconazole resistant *Candida* species should be considered (Figure 1).

Conflicts of interest

We know of no conflicts of interest associated with this publication, and there has been no financial support for this work that could have influenced its outcome.

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