ARTICLE

Prophylactic fluconazole protocol in very low birth weight infants: invasive candidiasis prevention in a Latin American neonatal intensive care unit

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OBJECTIVE: To evaluate the effect of prophylactic fluconazole for very low birth weight infants (VLBWI) in a neonatal intensive care unit (NICU) with a 7.8% incidence of invasive candidiasis (IC).
STUDY DESIGN: Interventional pre-post cohort study comparing 2 years with and without fluconazole prophylaxis protocol (2016–2018 = 228 infants and 2019–2021 = 125 infants). Fluconazole was administered to all extremely low birth weight infants (ELBWI) and infants with BW 1001–1500 g with risk factors or positive carrier cultures. Liver function tests were performed weekly.
RESULTS: The incidence of IC decreased from 7.8% to 2.4% (OR:0.3, $p = 0.05$) with the use of prophylactic fluconazole for VLBWI and in ELBWI decreased from 16,7% to 3,7% (OR:0.1, $p = 0.04$). No significant differences were seen in mortality.
CONCLUSIONS: Fluconazole is a safe, effective, and feasible strategy to prevent IC in a Latin American country.

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INTRODUCTION

Very low birth weight infants (VLBWI, BW ≤ 1500 g) and extremely low birth weight (ELBWI, BW ≤ 1000 g) survival has improved because of the medical advances in the last decades, but this population is still at high risk of morbidities and mortality related to their immature systems and their dependence on invasive care [1, 2]. In this context, invasive candidiasis (IC) defined by the presence and isolation of *Candida spp* in a sterile site (blood, urine, or cerebrospinal fluid), is the third cause of late-onset sepsis (LOS) in preterm neonates. IC incidence is inversely proportional to birth weight (BW) and gestational age (GA) (1.6–9% in VLBWI, 10–16% in ELBWI, and up to 20% in premature infants with BW ≤ 750 g) [3]. Also, its mortality is between 20 to 75% and neurological sequelae of 60% despite appropriate treatment [1, 2]. Risk factors described include an extreme premature condition, immature barriers (skin and mucosa), *Candida spp* colonization, central lines use, parenteral nutrition, broad-spectrum antibiotics, postnatal systemic corticosteroids, histamine-2-receptor antagonists and proton-pump inhibitors, invasive mechanical ventilation, necrotizing enterocolitis (NEC) and abdominal surgeries [1, 2, 4–6]. Certainly, the most important risk factor is the skin and gastrointestinal colonization with *Candida spp*.

Epidemiological data on invasive fungal infections in VLBWI from Chile is limited. However, a prospective surveillance study from 23 hospitals throughout 8 countries of Latin America (including Chile) between 2008 and 2010, described an incidence of 0.98/1000 admissions of <18 years old, from which 29% of candidemia episodes were neonatal. The median age of presentation was 16 days and the main species isolated was *Candida albicans* [2]. Between January 2013 and October 2017, a prospective, multicenter, laboratory-based candidemia study in 26 Chilean tertiary centers was conducted. In this study, 780 episodes of candidemia were included, with 35% occurring in the pediatric population. Of the 25 episodes in neonates, the median age of presentation was 10 days and *Candida albicans* was the microorganism responsible for 60% of the infections [5].

The use of prophylactic fluconazole in neonatal intensive care units (NICUs) has showed to decrease the incidence of IC in premature neonates with birth weight ≤1500 g, with no significant effect on mortality or long-term neurological outcome [3, 4, 6, 7]. Therefore, international guidelines now recommend the use of prophylactic fluconazole in NICUs with an incidence of IC above 5–10% [8, 9]. The "Latin American Invasive Mycosis Network " recommends the use of fluconazole 3 mg/kg twice weekly for 6 weeks, for ELBWI in NICUs with ≥5% incidence of IC [9]. An increase in *Candida spp* resistance to fluconazole has not been evidenced, and there have been no difference in transaminases between treatment and placebo groups [1, 3, 4, 6, 10].

Considering an incidence of 7.8% in VLBWI (16.7% in ELBWI) in our hospital, a protocol for prophylactic fluconazole prescription was initiated in July 2019. This study reviews demographic and clinical data comparing the pre-prophylaxis control group (2016-2018) with the prophylaxis intervention group (2019–2021). The aim was to evaluate the effects of fluconazole prophylaxis in IC incidence, mortality and hepatotoxicity safety.

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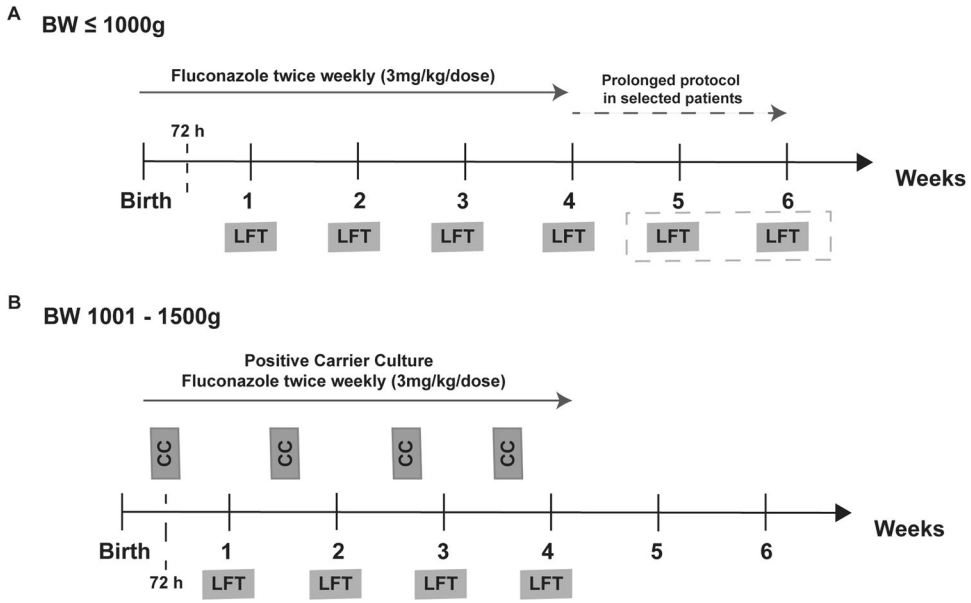

Fig. 1 Schematic representation of fluconazole prophylaxis protocol in the intervention cohort. A All infants with a birth weight under 1000 g (ELBWI) received fluconazole prophylaxis. The protocol had a duration of 4 weeks, and it was extended in patients who had central lines, endotracheal tubes or urinary catheters (Segmented arrow in 5–6 weeks). **B** Infants with birth weight between 1001–1500 g were screened with weekly carrier cultures (CC; Vertical grey boxes) for 4 weeks. In case of a positive culture, they received fluconazole prophylaxis. All patient has liver function test weekly (LFT; Horizontal grey boxes).

MATERIALS/SUBJECTS AND METHODS

Patient population

A prospective longitudinal cohort study was performed with all VLBWI born at, or transferred to, Complejo Asistencial Dr. Sótero del Río Hospital's tertiary NICU between January 2016 and August 2021. Patients with a diagnosis of congenital candidiasis, previously using systemic antifungal therapy or with acute liver failure diagnosis were excluded. All patients were followed until NICU discharge. A non-concurrent historical cohort (pre-prophylaxis control group, 2016–2018) was used to compare with the prophylaxis intervention cohort (2019–2021). There were no major differences in antibiotic guidelines, respiratory strategies, nutritional management, and breastfeeding interventions between the 2 epochs. All demographic and clinical information of infants were collected from physical and electronic medical records. A database was created and maintained by the research team for the development of this study. A cross-check with the hospital's list of newborn babies and transferred patients was made to ensure all patients were included.

Fluconazole administration protocol and safety monitoring

The prophylactic fluconazole protocol was started in July 2019. The protocol (Fig. 1**)** consisted of the administration of fluconazole 3 mg/kg intravenously (IV), or through a nasogastric tube if the IV route was not available, as soon as they were born or transferred to the hospital. The administration of fluconazole was done twice a week to all ELBWI for a minimum of 4 weeks, extended up to 6 weeks if the patient still had central lines, endotracheal tube or urinary catheter. Patients with BW1001–1500 g with risk factors were screened with skin cultures (colonization culture) after the first 72 hours of life, and then once a week for 4 weeks or until the culture become positive. Cultures were obtained from three sites: perianal, axillary and inguinal. If one culture turned positive, prophylaxis was started with the same dose as ELBWI. The risk factors for IC in patients with BW 1001–1500 g were defined as: rupture of membranes >18 hours, cerclage, intrauterine device in pregnancy, chorioamnionitis with intact membranes, suspicious amniotic fluid, ≥2 postnatal antibiotic courses, ≥2 invasive devices, parenteral nutrition, NEC stage II or greater by Bell's criteria, or abdominal surgery. The latter two were indications of prophylaxis despite culture status. All patients receiving fluconazole required weekly assessment of gamma-glutamyl transferase (GGT), alanine aminotransferase (ALT) and aspartate transferase (AST). The primary outcome was the invasive candidiasis incidence in VLBWI. The incidence in subgroups (BW 1001–1500 g and ELBWI) and overall mortality were studied as secondary outcomes. To evaluate the safety of fluconazole, trends of liver enzymes were analyzed.

Definition of *Candida* and bacterial infections, microbiological identification, and fluconazole-susceptibility testing

Invasive candida infection was defined by positive blood, urine or cerebrospinal fluid culture for *Candida spp*. Urinary tract infection was defined as the presence of any colony-forming unit of *Candida spp* in urine culture obtained by sterile urinary catheterization or bladder puncture. Regarding bacterial infections, bloodstream infection was defined as a positive blood culture for a causative bacterium (except for coagulase-negative *Staphylococcus* which required two positive blood cultures to be considered an infection [11]), and for intravascular catheter-related infection definitions from the IDSA guidelines were used [12]. Urinary tract infections were defined as a positive urine culture obtained by sterile urinary catheterization with a growth of bacteria greater than or equal to 10 000 CFU/ml, or a suprapubic puncture with any growth (1 CFU/ml) [13]. Lower respiratory tract infection was defined as a positive tracheal aspirate with bacterial growth greater than or equal to 100 000 CFU/ml associated with abnormal chest X-ray and worsening respiratory function [14]. Lastly, IDSA definitions for central nervous system infection were used [15].

Statistical analysis

Demographic, clinical and laboratory data are described as frequency (%) for categorical variables and median with an interquartile range for quantitative variable data. Odds ratios (ORs) are reported with a 95% confidence interval (CI). The clinical variables exhibited by patients were analyzed using a two-tailed Fisher exact test for each variable, or U-Mann Whitney test, or Kruskal-Wallis test performed in the Statistical Package for the Social Science Software (Version 25, SPSS Inc). Differences were

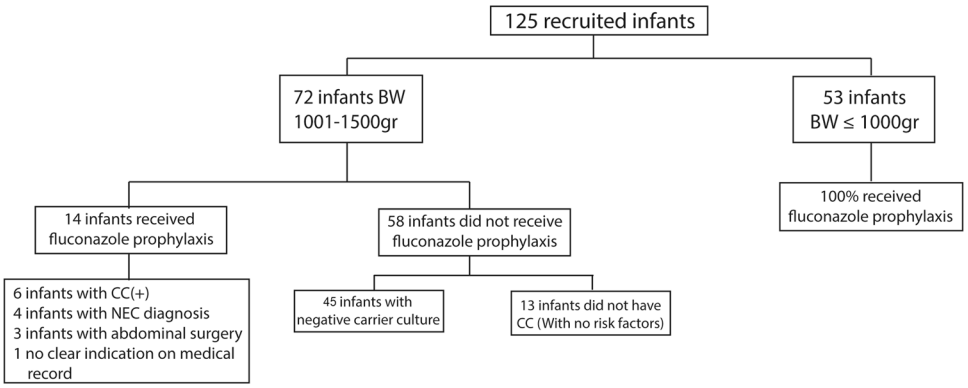

Fig. 2 Flowchart of enrolled patients during intervention cohort. They recruited 53 patients in the group of ELBWI (BW ≤ 1000 g) and 72 infants with birth weights between 1001–1500 g. (BW Birth weight, CC Carrier culture).

Table 1. Demographical features of pre and post-fluconazole protocol cohorts.

	2016–2018 ($n = 228$)	2019–2021 ($n = 125$)	OR (CI 95%)	*p*-value
Maternal age, median (IQR)	28 (23–33)	30 (25–36)		**0.012***
Maternal morbidity, *n* (%)[a]	172 (76.1)	110 (88.7)	2.5 (1.3–4.7)	**0.005***
Gestational age, weeks, median (IQR)	28 (27–30)	28 (26–30)		0.68
Birth weight, g, median (IQR)	1102 (858–1331)	1080.5 (837–1322)		0.88
ELBWI, *n* (%)	96 (41.7)	54 (42.9)	1.04 (0.68–1.6)	0.91
Female, *n* (%)	101 (44.3)	54 (43.2)	0.97 (0.63–1.5)	0.9
Complete prenatal corticosteroid, *n* (%)	137 (60.9)	92 (73.6)	1.8 (1.1–2.9)	**0.048***
Premature membrane rupture > 18 h, *n* (%)	39 (13.8)	18 (14.4)	0.53 (0.21-1-35)	0.23
Emergency C-section, *n* (%)	176 (78.9)	92 (74.2)	0.74 (0.45–1.24)	0.29
APGAR 5 minutes, median (IQR)	8 (6–9)	8 (7–9)		0.44
Clinic chorioamnionitis, *n* (%)	29 (12.8)	8 (6.5)	0.46 (0.2–1)	0.07

n number, *OR* odds ratio, *CI* confidence interval, *IQR* Interquartile range, *C-section* Cesarean section, *ELBWI* extremely low-birth-weight infants.
Bold values were given for the factors that were statiscally significant as described by the *.
[a]Maternal morbidity includes pregestational and gestational diabetes, hypertension, obesity, hypothyroidism, smoking.

considered significant at $p < 0.05$. The study was approved by the ethics institutional review board of the Medicine School of the Pontificia Universidad Católica de Chile (Institutional Review Code: 200416008) and Servicio de Salud Metropolitano Sur Oriente and was conducted according to the Declaration of Helsinki principles.

RESULTS

Demographic and clinical characterization

One hundred twenty-six VLBWI were born after the protocol was implemented, while the pre-protocol group consisted of a cohort of 228 infants. No patients were lost in the study. One patient was excluded because of congenital candidiasis diagnosis. The 53 ELBWI (43%) born in the interventional period received fluconazole prophylaxis per protocol. From the 72 neonates with BW 1001–1500 g, 14 received fluconazole (19%) (6 for positive colonization cultures, 4 for NEC, 3 for abdominal surgery and 1 had no register of the indication), 45 had negative colonization cultures and 13 did not receive fluconazole without having cultures taken because they had no risk factors (Fig. 2). The baseline characteristics of both groups are described in Table 1. Statistically significant differences were present in maternal age average (28 vs 30 years, $p = 0.012$), maternal morbidity (76.1% vs 88.7%, $p = 0.005$) and prenatal corticosteroid prescription (60.9% vs 73.6%, $p = 0.048$). Regarding their clinical course, the intervention group had higher antibiotic use, confirmed bacterial infections, necrotizing enterocolitis and postnatal corticosteroid use. Details of the clinical course differences are presented in Table 2. Transferred patients ($n = 10$) were not excluded from our protocol, their demographic characteristics and clinical course were compared with inborn patients in Supplementary Tables 1 and 2.

Protocol outcomes and fluconazole safety

The incidence of invasive candidiasis in VLBWI decreased significantly from 7.8% to 2.4% ($p = 0.05$) with prophylactic fluconazole protocol, keeping significance in the ELBWI sub analysis (Fig. 3, 16.7% vs 3.7%, $p = 0.04$). There was no difference in mortality between the two cohorts. Two cases of IC presented in the post-intervention cohort, caused by *Candida lusitaniae* and *Candida glabrata*, all without antifungal resistance (Supplementary Table 3). The first case was a 28 weeks preterm, BW 1213 g, transferred at day 10 of life. Skin carrier culture were taken upon admission. Fluconazole prophylaxis was started at day 15 postmenstrual age when skin carrier culture was reported positive for *Candida lusitaniae*. Two days later, while on non-invasive mechanical ventilation, with parenteral nutrition and a peripherally inserted central catheter, the patient presented a urinary tract infection for *Candida lusitaniae* and was successfully treated with amphotericin B deoxycholate for 4 days and then fluconazole for 10 days. The second infant had completed 5 weeks of prophylaxis and three days after discontinuing fluconazole presented a urinary tract infection for *Candida glabrata* at day 38 of postmenstrual age. The infection was successfully treated with fluconazole for 7 days. When comparing risk factors for IC between the infected populations in both cohorts, there were no statistically significant differences (Table 3).

Table 2. Clinical features of pre and post-fluconazole protocol cohorts.

	2016–2018 ($n = 228$)	2019–2021 ($n = 125$)	OR (IC 95%)	*p*-value
Postnatal antibiotics use, *n* (%)	161 (71.2)	112 (90.4)		**<0.001***
Antibiotics courses, number, median (IQR)	1 (1–3)	2 (1–3)		**0.001***
Bacterial infection, *n* (%)	77 (34.4)	58 (46.8)	1.6 (1.1–2.6)	**0.029***
NEC, *n* (%)	33 (14.5)	35 (28)	2.3 (1.4–4)	**0.003***
Invasive mechanical ventilation, *n* (%)	159 (69.7)	84 (67.7)	0.9 (0.6–1.5)	0.71
Duration of invasive mechanical ventilation, days, median (IQR)	10 (3–20)	8 (3–28)		0.8
Duration of umbilical catheters, days, median (IQR)	5 (3–6)	5 (4–6)		0.07
Duration of a peripherally inserted central catheter, days, median (IQR)	17 (12–19)	19 (11–27)		0.99
Duration of parenteral nutrition, days, median (IQR)	17 (10–29)	19 (12–28)		0.24
Postnatal corticosteroid use, *n* (%)	26 (11.5)	34 (27)	2.8 (1.6–5)	**<0.001***
Length of hospitalization, days, median (IQR)	62 (34–89)	68 (43–95)		0.1

n number, *OR* odds ratio, *CI* confidence interval, *IQR* Interquartile range, *C-section* Cesarean section, *NEC* Necrotizing enterocolitis.
Bold values were given to the factors that were statistically significant as described by the *.

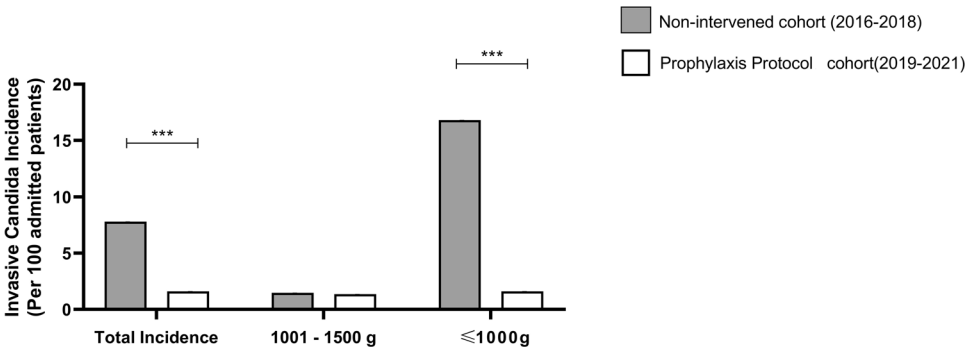

Fig. 3 Incidence of invasive candidiasis comparing historical and intervention cohort. The incidence was analyzed using global data, infants under 1000 g and 1000–1500 g with a two-tailed Fisher exact test.

Of the 67 patients receiving fluconazole 78% completed liver enzyme follow-up (34 for 4 weeks and 19 for six weeks). Trends of liver enzymes (ALT, AST, GGT) showed no significant increase up to 4 weeks, with values below standard cutoffs for neonates. ALT had a significant increase at weeks 5 and 6, while AST and GGT had a significant increase at week 6 (Fig. 4), reaching abnormal levels [16].

DISCUSSION

This study supports fluconazole prophylaxis as an effective and safe method to prevent invasive candidiasis in VLBWI, especially in ELBWI, in agreement with the previous studies [4, 6, 10, 17] and systematic reviews [3, 8, 17]. Our study provides data on the health situation of *Candida* infections in a Chilean population of neonates, thus providing valuable information for anti-fungal interventions. Different regimes of fluconazole have been used from 3 mg to 6 mg/kg every 72 hours for 4–6 weeks in these populations. In a study by Manzoni et al. [1], no difference was found between both doses (3 mg/kg vs 6 mg/kg), probably secondary to a low infection incidence in their multicenter randomized trial. On the other hand, Lee et al. [18] published an interventional pre-post cohort study analyzing the efficacy and safety of fluconazole prophylaxis in ELBWI (3 mg/kg twice a week for 4 weeks) without observing a reduction in invasive fungal infection, although it lowered the fungal colonization. However, their pre-intervention incidence was 4.4%, which is lower than the cutoff for international guideline recommendations for the use of prophylaxis with fluconazole (5-10%). As previously reported, [6, 7, 18] fluconazole use did not reduce mortality in the intervened cohort despite the reduction in IC. In a recent systematic review [8], mortality associated with IC was decreased in only one of the four studies which reported that outcome. This has raised doubt about the clear benefit of fluconazole prophylaxis [19]. It is worth noting that none of the studies have been powered to examine mortality, therefore no strong evidence can be obtained from them. The international recommendation continues to be the use of prophylaxis with fluconazole in high-risk patients in units with a high incidence of IC.

In our study, demographic and clinical data showed statistically significant differences in both cohorts, which were likely to give the intervened cohort a higher risk for IC. But, when comparing risk factors for IC in the populations that develop the infection, there were no differences between both groups, only a trend towards less use of carbapenems an third-generation cephalosporin antibiotics, highlighting antifungal prophylaxis as the main cause of IC decrease.

Antifungal drugs are potentially hepatotoxic, a concern raised regarding the use of fluconazole prophylaxis. In our study, there was no significant increase in liver enzymes during the first 4 weeks, as described by Benjamin et al. [6], confirming the safety of the drug. AST, ALT, and GGT were raised towards weeks 5 and 6, but only high-risk patients continued the protocol for six weeks. These patients have several factors that could be influencing the increase in transaminases, not only the use of fluconazole for example a chronic use of parenteral nutrition and infections. Considering it was not possible to obtain liver enzyme data of the pre-intervention cohort, no comparison could be done, so the authors cannot make any conclusion about this topic. The augmentation of resistant species is another important factor in the risk-benefit evaluation of fluconazole prophylaxis. In our study,

Table 3. Risk factors in the population with IC in pre and post-cohort prophylaxis.

	2016–2018 ($n = 14$)	2019–2021 ($n = 2$)	OR (IC 95%)	*p*-value
Number of postnatal antibiotics courses, median (IQR)	4 (3–6)	4 (3–6)		0.9
Cerclage, *n* (%)	3 (22.2)	1 (33.3)	1.56 (0.11–20.6)	1
NEC, *n* (%)	3 (22.2)	0 (0)		
Focal bowel perforation, *n* (%)	3 (16.6)	0 (0)		
Abdominal surgery, *n* (%)	5 (27.8)	0 (0)		
Proton-pump inhibitor use, *n* (%)	3 (16.7)	0 (0)		
Carbapenems use, *n* (%)	11 (61.1)	1 (33.3)	0.2 (0.01–2.4)	0.29
3rd generation cephalosporin use, *n* (%)	3 (16.7)	0 (0)		
Postnatal corticosteroid use, *n* (%)	6 (33.3)	1 (50)	1.8 (0.1–34.8)	1.0

IC invasive candidiasis, *n* number, *OR* odds ratio, *CI* confidence interval, *IQR* Interquartile range, *NEC* Necrotizing enterocolitis.

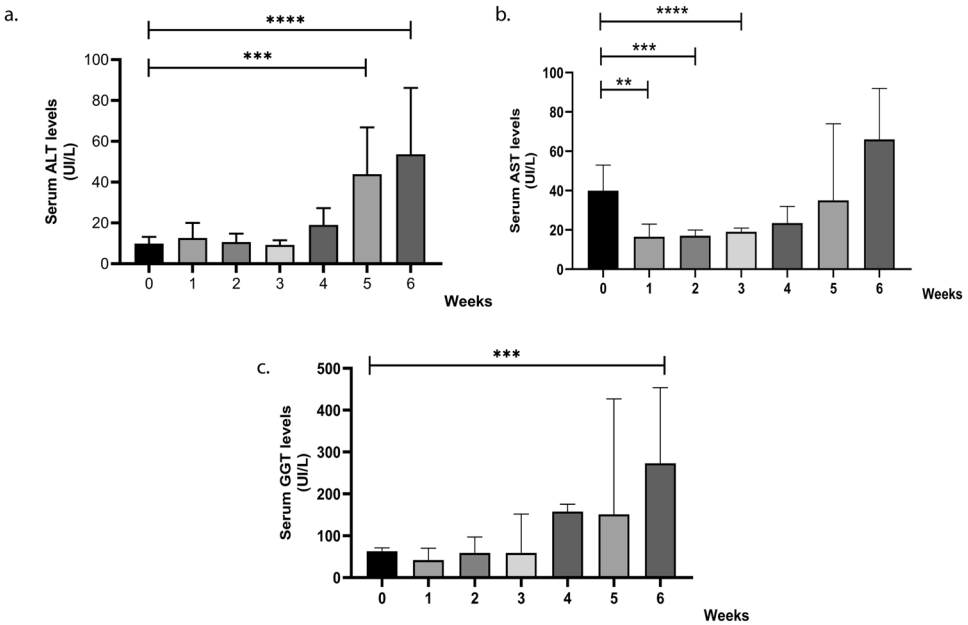

Fig. 4 Trends in LFTs during first 6 weeks after birth. Serum levels of ALT, AST and GGT transaminase in the intervention cohort were evaluated during fluconazole prophylaxis until 6 weeks of administration. The values shown are the median (IQR). Statistical analysis was performed by a Kruskal-Wallis test and Dunn´s multiple comparisons test (***$p < 0.01$).

no resistant species developed with a 2-year prophylaxis protocol. Luparia et al. [19] analyzed fungal ecology in a NICU for 16 years and concluded that fluconazole prophylaxis is effective in reducing IC without causing resistant *Candida spp* emergence.

We did not exclude patients that were transferred from another hospital, despite starting fluconazole prophylaxis later. Of the 2 patients in the protocol cohort that had IC, 1 of them was transferred from another hospital, hence, he could have already been colonized on admission and a late start of prophylaxis was not effective. He lost the window of opportunity to start the prophylaxis during the first 72 hours, increasing his risk of colonization with *Candida spp*. Previous studies excluded patients recruited after 72 hours [4, 7], missing the chance to analyze this group.

Invasive candidiasis prevention with fluconazole was effective, in 2 years our unit's incidence became lower than the cutoff set by the "Latin American Mycosis Network" to start prophylaxis [11]. There is no algorithm defined to stop universal prophylaxis in this scenario and to determine which high-risk patients should continue receiving fluconazole. Beyond the scientific contribution, this study is an approach to improving the prevention of IC diseases. Future research will allow us to evaluate mortality associated with invasive candidiasis. Being also a non-randomized single-centered study with a small number of participants could affect the results. Despite the low number of patients in our study, the effect of prophylaxis could be observed, probably due to the 7.8% incidence of IC in the unit. The lack of liver enzyme data in the pre-intervention cohort, is another limitation of our study.

We showed that fluconazole is an effective and safe prophylaxis for invasive candidiasis in VLBWI, in a NICU with a high incidence. This study has also indicated that when working in a network it is essential to follow up on the same guidelines and policies, to be able to eventually eradicate IC.

DATA AVAILABILITY

Raw data used for Tables 1–3, Supplementary Tables 1, 2 and Figs. 1–4 is not available to protect patient's privacy but it may be requested for further research to the corresponding author or the unit's email (neosotero@ssmso.cl).

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AUTHOR CONTRIBUTIONS

DVS collected data and wrote the manuscript. AMR developed the protocol of fluconazole prophylaxis in the unit, collected data and reviewed the manuscript. PA conducted the statistical analysis and reviewed the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

ETHICS APPROVAL

Institutional review board of the Medicine School of the Pontificia Universidad Católica de Chile (Institutional Review Code: 200416008) and Servicio de Salud Metropolitano Sur Oriente. The study was conducted according to the Declaration of Helsinki principles.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41372-023-01699-0.

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