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Research article

The underestimated role of major skin commensal *Malassezia furfur* in the development of neonatal invasive fungal infections

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ABSTRACT

In recent years, some new evidence on the role of *Malassezia* in late-onset sepsis in immunocompromised patients have been published, but there are still very few studies with special focus on newborns.

The prevalence of *Malassezia*-associated conditions in 3519 newborn patients of general and surgical neonatal intensive care units (NICU) was assessed. All patients underwent pharyngeal and rectal swab screening for *Malassezia* spp. Identification of *Malassezia* spp. was carried out with the use of an adapted nutrient media, microscopic assessment of yeast cell morphology, and real-time PCR analysis.

Malassezia furfur-induced invasive mycoses (IM) were developed 2.5 times more often in very low birth weight (VLBW) *M. furfur*-positive newborns, than in neonates with birth weight ≥1500 g, and affecting 15.8 % of VLBW infants. Funguria occurred 16 times more often in VLBW babies, but fungemia incidence was similar for both weight categories. Gastrointestinal (GI) colonization was found in 94.6 % of *Malassezia*-positive population, and in 8 % of all studied neonates. Among IM patients, death rate was 6.5 %. The specific pathogen was highly detectable by a combination of real-time PCR and an adapted nutrient media. Colonization with *M. furfur* in newborns was associated with low gestational age, VLBW, and long stay in NICU.

The findings emphasize the need to monitor colonization and infection with *M. furfur* in neonates, staying in ICU for more than two weeks and to improve current diagnostic approaches by using real-time PCR and an adapted nutrient media for *M. furfur* isolation.

1. Introduction

Fungi, including rare yeasts such as *Malassezia*, are increasingly being identified as emerging pathogens, especially in individuals with compromised immune systems [1,2]. *Malassezia* is a basidiomycetous yeast genus, partaking in the human skin microbiome as the most dominant fungal component where it generally involves in a commensal interaction with human tegument [3]. In recent years, application of novel DNA sequencing and genomics approaches has increasingly implicated a role for *Malassezia* in deep-seated conditions such as late-onset sepsis (LOS), gut inflammation and pancreatic cancer. Multiple direct sequencing studies have

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identified *Malassezia* presence in the human gut where under certain circumstances the pathogen may be involved in afore mentioned diseases [2,4–6]. In contrast to the gut, the human bloodstream normally is a sterile environment and potential pathogens require a porte d'entrée. The abundant presence of *Malassezia* yeasts on human skin provides ample opportunity for intrusion into the bloodstream, for example in cases when immunocompromised individuals receive intravenous nutrition. Limited information about *Malassezia* LOS incidence is available but there is increasing evidence suggesting that this condition is underdiagnosed. *Malassezia* fungemia is generally described for immunocompromised individuals with most cases pertaining to neonates, and only three species have been linked to LOS: *Malassezia furfur* ((Robin) Baillon H., 1899), *Malassezia pachydermatis* ((Weidman) Dodge, 1935) and in few cases *Malassezia sympodialis* (Simmons & Gueho, 1990*)* [2]. Interestingly, *M. pachydermatis* is not a common human skin colonizer, but is associated with pet animals such as cats and dogs, and a previous study traced an outbreak of this species in a neonatal intensive care unit to transmission from a health care worker's pet dogs to a patient [7]. *M. furfur* is the most frequently observed etiology of *Malassezia* fungemia [2]. A striking feature of *Malassezia* is their lipid dependance, requiring specialized culture media for their *in vitro* growth. As most clinics do not yet include such processing in their diagnostic setup, *Malassezia* may be missed as an underdiagnosed agent causing fungemia [2,3]. Additionally, there is a worldwide emergence of resistance to antifungal drugs, which may pose a further risk for increased *Malassezia* infections; moreover, a reduced susceptibility to frequently used azoles has already been observed [8,9]. Additionally, the frequent use of fluconazole for prophylaxis in combination with reduced susceptibility to this drug may lead to an additional rise of *Malassezia* LOS, and has been linked to a replacement from *Candida* to *Malassezia* fungemia in neonates [2,10]. Besides culturing and identification challenges, initial symptoms of the disseminated disease are often minimal, and correct identification is important for effective treatment and better outcomes [1,11]. This all illustrates the need for more surveillance studies to better understand the role and impact of *Malassezia* in LOS. Therefore, we carried out a three-year survey of neonatal intensive care unit (NICU) patients, evaluating a total of 3519 infants for the prevalence of *Malassezia* invasive infections.

2. Material and methods

2.1. Study design and procedures

This retrospective study included 3519 babies, admitted between January 2016 and December 2018 to the General NICU (G-NICU) and Surgical NICU (S-NICU) of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov of Ministry of Healthcare of Russian Federation, within the first 24 h after birth. All patients, including those who had no signs of infection, underwent pharyngeal and rectal swab screening for *Malassezia* spp. At the start of late-onset sepsis (LOS) clinical signs, blood and urine cultures were additionally studied; central venous catheter (CVC) cultures were investigated in cases of suspected central line-associated bloodstream infection (CLABSI). If meningitis was suspected, cerebrospinal fluid (CSF) culture was analyzed. In case of death, postmortem microbiology of heart blood and tests for other tissues (spleen, liver, GI tract, lungs) were carried out. In the absence of specific diagnostic criteria for *M. furfur*-associated invasive mycosis (IM), we used, with some modifications, the basic categories of invasive candidiasis (IC): fungemia without deep-seated infection, fungemia with disseminated infection, deep-seated infection without fungemia. The findings were analyzed in reference to cumulative and specific populations of the two NICUs, and to the *M. furfur*-positive population (i.e. patients for whom *M. furfur* was found in any of the biomaterials).

Limited information is available about the neonatal and child skin mycobiome but data suggests that skin colonization begins directly after birth [2]. Previous internal observations for some preterm infants with sepsis–like syndrome (SLS) signs and negative blood culture (BC) showed positive PCR results with universal fungal primers, and occasionally, yeast colonies were isolated from fecal samples incubated on Lactobacagar (State Collection of Pathogenic Microorganisms and Cell Cultures, Obolensk) while no growth was observed on Sabouraud agar. The initial yeast isolate was identified as *M. furfur* with Vitek 2 Compact (bioMérieux, France). Due to major limitations of this approach, optimizations were incorporated by overlaying the Lactobacagar with olive oil to accommodate potential growth of *Malassezia*. Identification was additionally supported by microscopic assessment of yeast cell morphology. Further improvement resulted in a final pipeline including the use of a *Malassezia* selective culture medium modified Dixon agar (mDA), and specific detection of *Malassezia* spp. and *M. furfur* with the Mycozoscreen real-time PCR detection kit (DNA-Technology LLC, Russia). Pharyngeal and rectal swabs, and urine samples, were cultured on mDA. Blood and CSF samples were inoculated into pediatric bottles, then loaded into the Bact/Alert system (bioMérieux). In addition, 0·5 ml of blood and other biologic fluids were cultured in liquid modified Dixon medium. Postmortem blood was cultured on liquid modified Dixon medium, and tissue samples were cultured on solid modified Dixon medium. Culture media were incubated at 32 °C ± 2 °C for 72 h with longer incubation (up to five days) for blood culture (BC). Fungal growth was microscopically examined for budding yeast cells, and further identified using the PREP-NA DNA extraction kit and the Mycozoscreen real-time PCR detection kit (DNA-Technology LLC, Russia), according to the manufacturer's recommendations.

2.2. Outcomes and definitions

The primary aim of our study was to assess *Malassezia* prevalence in neonatal invasive fungal infections, and to obtain a general understanding of *Malassezia* colonization of the neonatal GI tract and *Malassezia* presence in urine and blood. Additionally, we were able to assess death rates in *Malassezia*-positive neonates and to identify possible risk factors for invasive fungal infections, caused by *Malassezia*.

2.3. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics Version 21; the data normality was tested using Kolmogorov-Smirnov test and reported as median (Me) and interquartile range [LQ; UQ]. The Mann-Whitney rank-sum two-tailed test was used to compare non-normally distributed continuous variables; the Pearson's exact chi-square test - to compare categorical variables, while for dichotomous data we used logistic regression and calculated odds ratio (OR) and 95 % confidence interval (CI). Two-tailed P values < 0.05 were considered statistically significant.

3. Results

The average NICU stay for the total population of 3519 infants was 12 days, the average death rate was 4 %. Of all NICU patients, 40.7 % were preterm, and 9.3 % were VLBW infants (<1500 g). Of the G-NICU patients, 15.4 % were VLBW, in contrast to only 0.8 % in the S-NICU; but average length of stay and death rate in S-NICU were higher (16 vs 8 days, and 7.5 % vs 28 %, respectively) (Table 1).

Surgical procedures were performed in 639 neonates: for congenital malformations of the lungs in 16 % (n = 105); urinary system in 29 % (n = 187); GI tract or necrotizing enterocolitis in 55 % of patients (n = 355).

In 1077 isolates from 299 (8.5 %) NICU patients *M. furfur* was identified morphologically from cultures derived from one or multiple sources (i.e. GI, blood, urine, CVC), determining the diagnosis for each patient. The identity of 380 isolates from patients with clinical signs of SLS were confirmed with Mycozoscreen real-time PCR. S-NICU Infants that were positive for *M. furfur* had a higher birth weight (BW) and gestational age (GA), when compared to G-NICU (Table 2).

For 285 (8.1 %) infants of the total NICU population, *M. furfur* colonization of the GI tract (rectal and pharyngeal swabs) was observed, but with a much higher frequency in infants with VLBW compared to infants with birth weight ≥1500 g: 34 % (112/329) vs 5.4 % (173/3190), respectively (Fig. 1). When considering the *M. furfur*-positive population as a whole, GI tract colonization did not differ significantly between infants with VLBW and infants with birth weight ≥1500 g: 97.3 % (112/115) vs. 92.9 % (173/184).

Among infants with clinical signs of infection (SLS), 19 % (58/299) of patients had a *M. furfur*-positive blood culture, 14.7 % (44/299) a positive urine culture, 4.3 % (13/299) demonstrated CVC colonization (Fig. 1). Fungemia and funguria rates in the total NICU population were higher in VLBW infants than in newborns ≥1500 g: 6.7 % (22/329) vs 1.1 % (36/3190) and 12.1 % (40/329) vs 0.1 % (4/3190), respectively. In the total *M. furfur*-positive population, the rates of fungemia did not differ significantly: 19.1 % (22/115) in the VLBW vs 19.7 % (36/184) in infants with birth weight above 1500 g, while funguria was much more frequently observed in VLBW neonates (34.8 % = 40/115 vs 2.2 % = 4/184) in infants ≥1500 g (Fig. 1). Positive CVC cultures were detected in 13 infants (103 were tested). The majority of CVC-colonized infants had BW ≥ 1500 g (n = 12), and for two infants (both ≥1500 g) only CVC-colonization was observed.

3.1. Colonization and invasive mycosis categories in M. furfur-positive populations

Of the 299 *M. furfur*-positive patients, in 205 cases (68.5 %) only GI-tract colonization was observed, in two cases (0.7 %) only CVC-colonization occurred, and 92 (30.8 %) patients suffered from invasive mycosis (Table 3). *M. furfur*-associated IM affected 2.6 % of the total NICU population. Fungemia was observed in 63 % (58/92) of children with MI caused by *M. furfur*. Six (6.5 %) *M. furfur*-associated IM patients died and their postmortem cultures of heart blood, liver and lungs appeared yeast-positive in two, three and one case, respectively; four infants with these positive tissue cultures were considered to have disseminated mycosis (DM). The IM rate in *M. furfur*-positive VLBW infants was much higher than in that for BW ≥ 1500 g: 45.2 % (52/115) vs 21.7 % (40/184), respectively. 47.8 % (44/92) of infants with *M. furfur*-associated IM had funguria, and VLBW infants suffered significantly more often from funguria than ≥1500 g neonates: 76.9 % (40/52) vs 10 % (4/40) (p = 0.0001), OR 30.0 (95%CI: 8.8–101.4). IM was often associated with CVC-related infection in newborns with BW ≥ 1500 g (in 25 % (10/40)) and very rarely – in VLBW (1.9 % (1/52)).

3.2. Detection of fungemia and funguria in M. furfur-positive neonates: key points

The median birth weight and gestation age [LQ; UQ] in neonates with *Malassezia* fungemia (n = 58) was 2257 g [1100; 3036] and 36 weeks [29; 38], respectively. BC appeared positive on average (the median of detection fungemia) on the 23rd [17.0; 34.5] day of life/stay in the NICU. Only in two cases (3.5 %) fungemia was detected during the first week of life in neonates with BW ≥ 1500 g without surgical interventions. During the second week of life fungemia was detected in seven neonates (12.0 %) with median BW 2655 g [850; 3160], and GA 38 weeks [25; 39]. After two weeks of life, fungemia was detected in 49 neonates (84.5 %) with median

Table 1
NICU definitions.

	G-NICU	S-NICU	Total (%)
Number of infants	2073	1446 (639 infants underwent surgery)	3519
Preterm infants	1316 (63.5 %)	116 (10 %)	1432 (40.7 %)
VLBW infants	320 (15.4 %)	9 (0.8 %)	329 (9.3 %)
Death rate	59 (2.8 %)	86 (7.5 %)	145 (4 %)
Length of stay in days, mean [min, max]	8 [1, 125]	40.3 [1, 154]	

Table 2
Gestational Age and Birth Weight comparison between infants that were positive for *M. furfur*.

	Infants with *M.furfur* (n = 299)			
	G-NICU (n = 126)		S-NICU (n = 173)	
GA (wks)	28.9 [27; 31]		38 [36; 39]	
BW (g)	993.5 [823.7; 1350]		2820 [2200; 3370]	
	VLBW (n = 107)	**BW ≥ 1500 (n = 19)**	**VLBW (n = 8)**	**BW ≥ 1500 (n = 165)**
GA (wks)	28.0 [27; 30]	32.0 [31.0; 34.0]	30.5 [28.5; 32.5]	38.0 [36.0; 39.0]
BW (g)	970 [820; 1184]	1946 [1657; 2380]	1210 [1112.5; 1370]	2905 [2290; 3412]

GA = gestational age, BW = birth weight, VLBW = very low birth weight. Data presented as median [interquartile range].

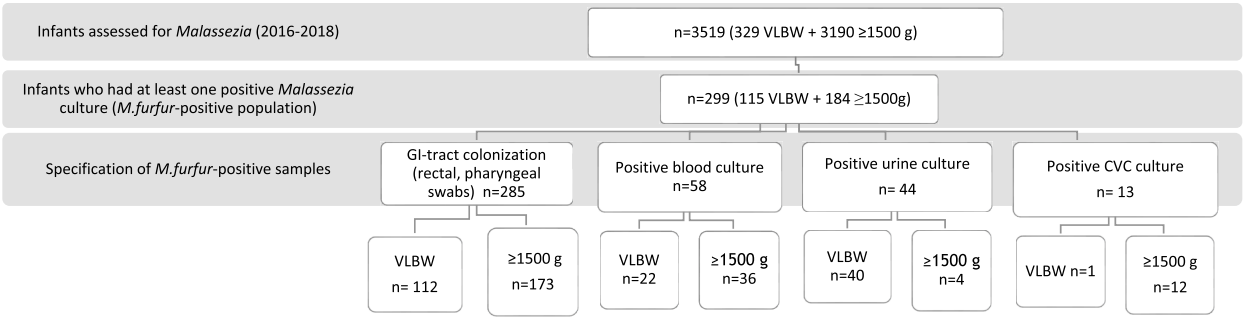

Fig. 1. *Malassezia* detection with new microbiologic testing technique.

Table 3
M. furfur-associated conditions.

M.furfur-positive sites	VLBW (n = 115)	≥1500 g (n = 184)	Total, number of infants (n = 299)	Categories
GI tract colonization only	63	142	205	*Colonization*
CVC colonization only	0	2	2	*Colonization*
	63	**144**	**207 (69.2 %)**	***Colonization (Total)***
Colonization of GI-tract and funguria	29	3	32	*IM without fungemia*
Funguria only	1	1	2	*IM without fungemia*
Colonization of GI-tract and fungemia	10	19	29	*IM with fungemia*
Colonization of GI-tract, CVC and fungemia	0	9	9	*IM with fungemia*
Colonization of GI-tract, fungemia and funguria	10	0	10	*IM with fungemia*
Fungemia only	1	7	8	*IM with fungemia*
Fungemia and CVC colonization	1	1	2	*IM with fungemia*
	52	**40**	**92 (30.8 %)**	***IM (Total)***

BW 2198 g [1102.5; 3054.0] and GA 36 weeks [29–38], and 35 (71.4 %) of these infants underwent abdominal surgery. Thus, in most cases the primary detection of fungemia occurred after the 14-th day of life/stay in the NICU (2 cases occurred in the first week (1–7 days), seven cases – in the second week (8–14 day), 49 cases – after second week (after 14th day) (Fig. 2). Fungemia was detected significantly earlier in babies with BW ≥ 1500 g than in VLBW neonates: after 21.5 vs 27.5 days of life, respectively (p = 0.04).

The median BW and GA in neonates with *M. furfur*-positive urine culture (n = 44) were 930 g [820.0; 1147.5] and 28 weeks [26; 29]. The first detection of funguria on average took place on the 24th [14; 33] day of life/stay in the NICU. Only one case of funguria (2.3 %) was originally reported during the first week of life in a baby with a BW of 850 g. During the second week of life, funguria was detected in 12 (273 %) neonates; median BW and GA were 935 g [825; 1393] and 28·0 weeks [26.25; 29.0]. After two weeks of life funguria was primarily detected in 31(70.4 %) neonates; median BW and GA were 940 g [740; 1130] and 28 weeks [26; 29]. Of these only one infant underwent surgery earlier (Fig. 2).

4. Discussion

Historically, *Malassezia* yeasts have primarily been linked to various skin diseases but there is increasing evidence that they are emerging pathogens with a role in various deep seated body sites and pathologic conditions such as Crohn's disease and pancreatic cancer [4–6]. Its emergence in bloodstream infections of immunocompromised patients - and specifically in neonates – has been suggested, but only limited information is thus far available about *Malassezia* prevalence in neonatal invasive fungal infections. The limited knowledge is a result of similarity in clinical features, patient management and outcomes between *Candida* and *Malassezia*; in combination with the fact that *Malassezia* requires lipid supplementation for growth, and most clinics currently do not use culture

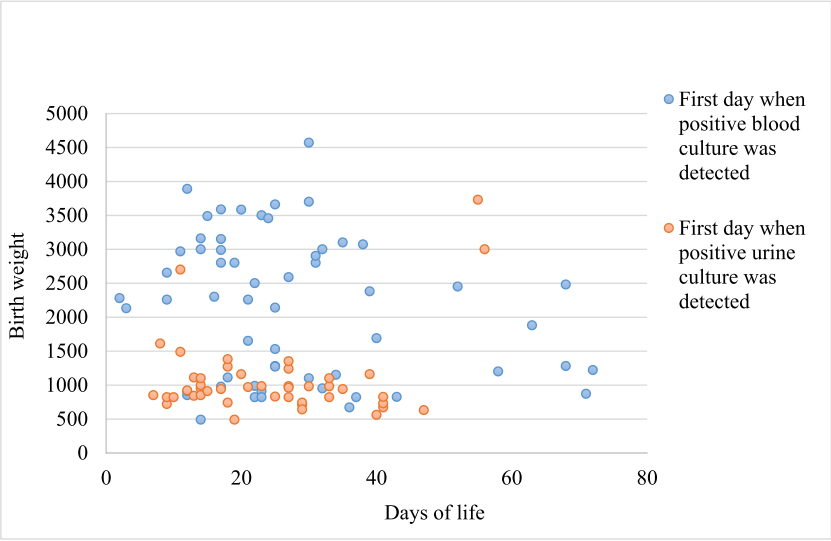

Fig. 2. Correlation between BW and the age at which blood cultures vs urine cultures were first detected positive for *M. furfur*.

media that meet this requirement [2,10]. Only two surveillance studies are known that specifically evaluated *Malassezia* LOS prevalence in neonatal and pediatric patients, both performed in the same Italian hospital, in different years. The first one year study (2011) found a higher prevalence for *Malassezia* (2.1 %) than for *Candida* (1.4 %) in a population of 290 neonatal and 17 pediatric patients [12]; the second study (2016) even reported 4.4 % *Malassezia* prevalence in a population of 202 neonates [13].

In our current retrospective study, we set out to evaluate *Malassezia* prevalence in neonatal invasive fungal infections in the general and surgical NICU's in Moscow; for this we assessed a total of 3519 neonates during a period of three years. We found a *Malassezia* related IM prevalence of 2.5 %, comparable to the previous findings in the Italian surveys. Among IM patients, the mortality rate was 6.5 %.

IM developed 2.5 times more often in VLBW *M. furfur*-positive infants, compared to nenonates with BW $\geq$ 1500 g; for funguria this was 16 times more often but fungemia frequency was similar for both weight categories. In reference to total population of the two NICUs, IM affected 15.8 % of VLBW infants, and only 0.12 % of those with BW above 1500 g, mostly the patients after surgery.

The first detection of *M. furfur* in blood and/or urine culture occurred most often (in 70 % of cases) after the second week (usually on the 23rd or 24th day) of life/admission to the NICU. Since 30 % of VLBW neonates of the *M. furfur*-positive population had a positive urine culture already after the first week of life, we recommend urine culture of infants with clinical signs of late onset sepsis on mDA from the eighth day of life onward. We also believe that monitoring of *M. furfur* colonization and infection in infants staying in the NICUs longer than two weeks can improve infection control practices. Our results confirm that the routine use of a medium with lipid supplementation, such as mDA, supports successful identification of clinically important *Malassezia* in preterm VLBW infants and term infants receiving long-term parenteral nutrition or undergoing abdominal surgery. A limitation of this study was a large diversity of the studied population and too many factors contributing to morbidity; however it allowed to obtain a general picture of *M. furfur* colonization and associated infections in NICU patients. Further studies in carefully selected groups will be necessary. *Malassezia* yeasts colonize human skin after birth and become the most abundant fungal component of the skin microbiome in healthy adults, but they are also associated with various skin diseases [2,14]. Their abundant presence on the skin can provide a port d'entrée to deeper body sites, either naturally via the GI-tract or when the skin is damaged in a hospital setting, e.g. during parenteral nutrition or surgical activities. As some recent studies have implicated *Malassezia* in recurrent aphthous stomatitis (RAS) [15] and various pathologies of the central nervous system (CNS) [16,17], we verified the absence of *Malassezia* in mouth ulcers and did not observe any *Malassezia* related meningitis or meningoencephalitis.

Inflammatory bowel disease (IBD) is associated with fungi, particularly *Malassezia*. Caspase recruitment domain-containing protein (CARD)-9 polymorphism in Crohn's disease patients may promote *Malassezia* colonization contributing to gut inflammation [18]. Moreover, recent studies suggest that a dysregulated immune response to *Malassezia* in the gut plays a role in pathogenesis of Crohn's disease in genetically predisposed asymptomatic individuals [4]. Since fungi-mediated gut changes may be associated with the pathogenesis of IBD, neonatal gut colonization by *Malassezia* might provide a trigger for later intestinal inflammation in infants. We have found GI colonization in 94.6 % of *Malassezia*-positive population, and in 8 % of all studied neonates. Further clinical studies are needed to clarify the possible impact of neonatal GI colonization on the health of children at a later age.

Azoles, including fluconazole (FLZ), are frequently used antifungal drugs for the treatment fungal systemic infections of preterm infants. Though generally successful for prophylaxis and treatment of invasive candidiasis, these drugs frequently fail to treat *Malassezia*-related IM, due to increasingly observed reduced susceptibility or resistance for these drugs [2,9]. Use of prophylactic FLZ may in part possibly contribute to a shift in observed *Malassezia* IM cases, compared to *Candida* [10]. Lack of standardized antifungal susceptibility testing methods, treatment guidelines, and a high level of inter- and intraspecies variation in antifungal susceptibility

profiles, are complicating factors in combatting *Malassezia* invasive infections [2]. In addition, the strains of *Malassezia* can produce mycelial forms which, through interactions with saprophyte bacteria, may build biofilms on skin and on medical devices surfaces [19].

Malassezia has all prerequisites to act as a hospital pathogen, is able to overcome selective antimycotic barriers, and the prevention of patient colonization is quite challenging. Alike *Staphylococcus epidermidis*, the yeast is a saprophytic organism living on human skin and for this reason, colonization of medical personnel and patients is hardly preventable with standard anti-epidemic measures, especially in the absence of generally accepted recommendations for antifungal prophylaxis against skin-associated commensal fungi.

Typing of each *Malassezia* species isolates into "pathogenic" and "nonvirulent" subtypes, might be useful for infection control and prevention; however, virulence may be not sole intrinsic feature of the yeast, but may arise as a combination of host and environmental factors, possibly in combination with the complexity of interactions including other members of the microbiome. Until now, only limited information is available to connect specific subtypes with increased virulence and disease onset [2,11,20,21].

The nosocomial nature of *Malassezia*-associated invasive mycosis in NICUs is supported by recent reports on hospital outbreaks caused by *M. furfur* and *M. pachydermatis* [2].

The first reports of Malassezia-associated generalized infections date back to the 1980s, including eight cases of *M. pachydermatis* infection and colonization in NICUs over a six-month period, reported by Chryssanthou et al. [2]. The available literature provide evidence that infants, children, and adults with profound immunosuppression, serious concurrent health problems, and the infusion of TPN with LS through central CVCs are at most vulnerable to *M. furfur* infection and colonization [2,11]. However, we suggest that IM in neonates should not be linked to CVC only, since in our study this association occurred less frequently than other IM, and mostly in term neonates undergoing surgical interventions; while preterm VLBW infants were reported to have fungal invasion associated with primary gut colonization and funguria. Since there is some evidence that an innate immune response to changes in the intestinal mycobiome may contribute to the development of Crohn's disease associated with *M. restricta* [4], it can be speculated that the immature and weak immune system of preterm neonates may allow the inflammatory response and fungal invasion. It is quite challenging to prevent *Malassezia* colonization of GI-tract in neonates because it may be derived from the mother by skin contact or with breast milk, shortly after birth. According to metagenomic mycobiota analysis of transient and mature human milk, the most abundant species in both milk types in women after normal spontaneous term delivery was *M. globosa* [22]. The infants may also become colonized from healthcare workers, who may further transmit the yeast from an infected or colonized infant to other patients [2].

In our study, *M. furfur* IM rarely manifested before two weeks of life/stay in NICU; on average, fungemia and/or funguria were revealed on days 23–24. This correlates with observations that colonization in neonates and infants is associated with low gestational age, admission to a NICU and length of hospital stay [23].

A comprehensive understanding of *Malassezia* infection, including diagnostic and therapeutic modalities, is necessary to ensure better patient care and outcomes, especially in NICU. The importance of appropriate culture methods for identification of the new opportunistic pathogens can hardly be overstated [2,3]. Implementation of mDA made it possible to confirm a relatively high *Malassezia* infection morbidity and death rate in NICUs, though this method is currently not widely used. Continuation of our study [26] showed that the incidence of *M. furfur* IM in newborns was 2.6 %. Though this corresponds to the lower limit of the reported average invasive candidiasis rate (from 2 % to 4 % with fluconazole prophylaxis and from 13.2 % to 16.7 % without preventive treatment) [24,25], the prevalence of *M. furfur* IM is much higher (15.8 %) in VLBW infants. Since NICU patients are vulnerable to *Malassezia*-associated infections, it is essential to improve current diagnostic approaches with real-time PCR and suitable culture media, in an effort to obtain more rapid and accurate identification, crucial for further patient management.

Ethics approval and consent to participate

The study was approved by the Ethics Committee of the National Medical Research Center for Obstetrics, Gynecology and Perinatology named after Academician V.I.Kulakov of the Ministry of Healthcare of Russian Federation (Protocol No. 13, December 10th, 2015).

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CRediT authorship contribution statement

Tatiana V. Priputnevich: Supervision, Conceptualization. **Alexey B. Gordeev:** Formal analysis. **Natalia E. Shabanova:** Writing – original draft, Validation. **Pavel Denisov:** Writing – review & editing. **Dmitry Yu Trofimov:** Validation, Data curation. **Ekaterina N. Balashova:** Writing – original draft. **Andrey E. Donnikov:** Data curation. **Ekaterina L. Yarotskaya:** Writing – original draft. **Viktor V. Zubkov:** Writing – original draft, Validation. **Gennady T. Sukhikh:** Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The data were presented at international conferences, including Priputnevich T.V. "Congress of the International Society of

Human and Animal Mycology", Amsterdam, 2018 with the report "Morbidity of fungal infections caused by Malassezia furfur in neonatal intensive care units". I would like to comment on the data and its storage. Our center's servers store all patient information. With the help of special software developed by us, we can analyze almost all patients and provide this information upon request. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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