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ORIGINAL ARTICLE

Fungal and bacterial sepsis and threshold ROP in preterm very low birth weight neonates

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Objective: To determine whether an association exists between either fungal or bacterial sepsis and retinopathy of prematurity (ROP).

Study Design: Retrospective cohort study on all neonates with birth weight <1500 g admitted to a large Italian third Level Neonatal Intensive Care Unit in the years 1997–2001 and screened for ROP. Univariate analysis and multiple logistic regression were used to detect significant associations with ROP (all grades and threshold) in neonates with birth weight <1000 g (extremely low birth weight (ELBW)) and 1000–1500 g.

Results: Among 301 enrolled neonates, ROP (all grades), threshold ROP, fungal and bacterial sepsis occurred in 31.9, 12.9, 11.6 and 40.5% of the infants, respectively. At multivariate analysis, only gestational age ($P = 0.03$), colonization by *Candida non-albicans* spp ($P = 0.03$) and fungal sepsis ($P = 0.03$) were independent predictors of threshold ROP, and only in ELBW neonates.

Conclusions: Fungal (but not bacterial) sepsis is significantly and independently associated with ROP, but only in ELBW neonates and only with threshold ROP.
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Keywords: retinopathy of prematurity; Candida; sepsis; preterm infant; extremely low birth weight; NICU

Introduction

The incidence of both candidemia and threshold retinopathy of prematurity (ROP) in extremely premature infants is quite high, and has indeed increased worldwide over the last 10 years as a consequence of higher preterm survival rates.[1–4] *Candida spp*. are the third most common cause of late-onset sepsis in neonatal intensive care units (NICUs),[1] and ROP occurs in 40–80% of preterm neonates.[4] Severe ROP, known as threshold ROP,[5–7] may result in retinal detachment and poor visual outcome if ablative surgery is not performed.[7,8]

An association between candidemia and ROP in extremely low birth weight (ELBW) neonates has been suggested, but it is uncertain if this association depends on the fungus or on the sepsis itself. Ophthalmologic involvement with Candida sepsis, as part of disseminated end-organ damage, has been described:[8] *Candida spp*. infiltrate the eyes via the bloodstream and may cause endophthalmitis and chorioretinitis in premature infants,[9] as well as choroidal neovascularization 2 weeks to 2 years after *Candida albicans* chorioretinitis in adults.[10]

In 1998, Mittal *et al*.[11] reported that Candida sepsis in ELBW neonates was significantly associated with severe ROP and a more than five-fold increase in the need for laser surgery; later on, this association was extended to all VLBW infants by Noyola *et al*.[12] Other authors have also described a significant association between ROP and the concomitance of fungal sepsis plus either Caucasian race[13] or treatment with dexamethasone.[14] However, a larger series investigated by Karlowicz *et al*.[8] indicated that candidemia may not be an independent risk factor for threshold ROP in ELBW infants, and that gestational age determines the association.

The aim of this study, in a large population of preterm neonates, was to determine whether Candida sepsis is associated with ROP and, if so, which ROP grades of severity and which preterm subset(s) are involved. Additionally, we evaluated the relationships between bacterial sepsis and ROP in the same population.

Methods

Study design, setting and population

This retrospective, cohort study was conducted at the Sant'Anna Hospital, Turin, Italy. The NICU is a third-level unit located in the greater Turin area (1 500 000 inhabitants and 15 000 births per year) with a mean (M) delivery rate of 4000 per year and 400 admissions.

All VLBW neonates born from January, 1st 1997, when all medical records of NICU patients began to be stored in a computerized database, to December, 31st 2001, and who had not

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died or been transferred to other institutions before ophthalmologic screening for ROP, were considered for eligibility, and their clinical and microbiological records were reviewed. Criteria for exclusion from the study were as follows:

- Not inborn;
- Incomplete data or charts, or unavailable computerized medical record, or unavailability of results from at least one surveillance culture per week and from at least three different sites during the stay in NICU for each infant;
- Having undergone prophylaxis with antifungal systemic drugs;
- Informed written parental consent not released prior to any investigation or treatment.

Methods

Demographic, gestational and perinatal data of the neonates included in the study were reviewed and the following groups were identified:

- NE-VLBW (1000–1500 g of birth weight) and ELBW (<1000 g) neonates;
- Neonates affected by ROP of all grades, both NE-VLBW and ELBW;
- Neonates affected by threshold ROP, i.e. infants requiring urgent (within 72 h) ablative ROP surgery, both NE-VLBW and ELBW;
- Neonates affected by SFI, both NE-VLBW and ELBW.

Data were obtained from computerized medical records, and were personally entered into the NICU database by three of the authors. The database was regularly maintained and routinely cross-referenced with two other databases: one was maintained and regularly checked by the Consultant Ophtalmologists, and the other by the Hospital Service of Microbiology. This assured that there could be no missed cases of either ROP, SFI or bacterial sepsis.

Incidence of ROP of all grades, threshold ROP and SFI was calculated for all infants and separately for both birth-weight groups. Mortality prior to discharge was recorded for all infants. The following antenatal and postnatal risk factors for both ROP and SFI were evaluated: chorioamnionitis, pre-eclampsia, maternal and fetal diabetes, Apgar score at 5 min, presence of bacterial sepsis (both by Gram-positive and -negative species), duration of supplemental oxygen, duration of parenteral nutrition, use of steroids, need for major surgery, overall fungal colonization, colonization by non-albicans fungal spp, duration of stay in NICU, hyperglycaemia, neutropenia, thrombocytopenia, treatments with antibiotics, incidence of necrotizing enterocholitis (NEC) (surgical), bronchopulmonary dysplasia (BPD), intraventricular hemorrhage (IVH) (grade 2 or more), and a check was also made to ensure that all neonates with a diagnosis of SFI displayed the microbiological laboratory and clinical criteria required. The presence of candidal endophthalmitis was also sought in these neonates within 1 week of their SFI diagnosis.

Neutropenia was defined according to the reference ranges established by Manroe *et al*.,[15] revised by Mouzinho *et al*.,[16] and adapted by Funke *et al*.:[17] that is, <500 neutrophils/ml to <1100/ml in neonates 0–6 h of life and more than 72 h respectively. A neonate was defined as neutropenic if having at least one hemogram with values below the above mentioned reference levels. Treatment with Filgrastim (rhG-CSF) was performed in 12 of the 18 neutropenic patients, following the implementation of this policy in the NICU since the third year of study.

BPD was defined according to the definition of Jobe *et al*.[18] The oxygen saturation range during hospitalization was kept above 90%, and infants were weaned off oxygen supplementation when they could maintain HbO_2 saturations above 90% for more than 12 h.

Bacterial sepsis was defined by the presence of a positive blood culture from a sample drawn from a peripheral site.

Fungal isolation and identification from cultures

Fungal isolates were obtained from cultures of surveillance (ear canal swab at birth, and then weekly at least two from stool, gastric aspirate and nasopharynx secretions (endotracheal if intubated), from surgical and mechanical devices when removed (endotracheal tubes, intravascular catheter, drains and similar devices), and cultures from any site clinically indicated (e.g. skin, respiratory secretions, etc.). Stool, gastric aspirates, surgical and intravascular devices were collected in sterile containers; respiratory secretions were obtained with an infant mucus sterile extractor kit supplied with two 3.3 mm suction catheters (Vygon, Ecouen, France); skin, ear, nasopharynx specimens were obtained on swabs (Labobasi, Switzerland); blood draws for culture were submitted in dedicated specimens (BacT/Alert PF; BioMerieux Inc., Durham, NC, USA). Urine samples were obtained by sterile urethral catheterization or suprapubic aspiration of the bladder; samples collected from indwelling catheters or from urine bags were not considered.

Fungi were identified following inoculation on chromogen culture plates (Albicans ID, Biomerieux Inc., Durham, NC, USA) that allow rapid *C. albicans* identification by blue staining of the colonies after 48 h incubation at 37°C. Differently stained colonies were speciated through a miniaturized system of biochemical tests (Vitec Yeast, Biomerieux Inc., Durham, NC, USA).

The surveillance and culture collected and analysis procedures and methods were not changed at any time.

Definition of SFI

SFI was defined as a positive culture from (1) blood withdrawn from peripheral sites; or (2) from urine collected by suprapubic sterile puncture or sterile bladder catheterization, with growth of more than 10 000 fungal organisms/ml; or (3) from cerebrospinal fluid; or (4) from intravascular catheter tip (but only limited to patients with prior peripheral colonization by the same species; otherwise, positivity was considered as colonization). These

diagnostic criteria derive from from international consensus documents,[19,20] as well as the Italian Neonatology Society's Fungal Infections Task Force.[21]

All SFI episodes were treated with Liposomal Amphotericin B given intravenously (i.v.) at 2.5 (start) to 5.0 (steady state) mg/kg/day for 12–28-day courses, as decided by the physician in charge. When diagnosing an episode, removal of central intravascular catheter(s) was standard policy. When SFI was only clinically presumed, liposomal amphotericin B was started empirically until the culture results were known. 5-Fluorocytosine was added in five cases with end-organ damage and one neonate received liposomal amphotericin B plus itraconazole. No drug-related adverse effects or reactions were recorded and there were no discontinuances in antifungal treatment once started.

Definition of ROP

Ophthalmologic screening for ROP was performed by one of two board-certified consultant ophthalmologists. Infants were first screened at 3–4 weeks of age and then at 1–2 week intervals, depending on the clinical picture and the severity of the retinopathy. Gestational age was determined by the attending neonatologist when the infant was admitted to the NICU.

Severity of ROP was determined according to the International Classification.[5] Threshold ROP was defined as 'stage 3 ROP, zone I or II in 5 or more continuous clock hours or 8 cumulative clock hours with the presence of plus disease', according to the criteria of the American Academy of Pediatrics Section on Ophthalmology ROP subcommittee.[6] Neonates were all classified by their most severe ROP examination. In case of discharge prior to the 36th week of gestational age, infants with ongoing not threshold ROP lesions were considered still at risk of progression to most severe stages, and therefore the screening was not discontinued. The infants were revisited also after discharge by the same Ophtalmologists in the Hospital Unit, at scheduled intervals, until either the development of ROP or the disappearance of the lesions. Infants with threshold ROP were transferred to a referral Ophtalmology Unit in another Hospital of Turin, where other ophthalmology specialists confirmed the diagnosis and performed retinal ablative therapy if indicated.

The screening ophthalmologists were unaware of histories of SFI, bacterial sepsis or any other potential risk factors for ROP other than very low birth weight (VLBW) or gestational age $\leqslant 32$ weeks. The decision whether or not to treat the ROP was always taken according to the stage of the disease, and in no cases was influenced by the clinical status of the neonate, or by the knowledge of whether the infant had severe ongoing systemic illnesses, including SFI.

Statistics

Data are expressed as means, medians (Md) and standard deviation (s.d.). Statistical analysis was performed using SPSS 8.0 version for Windows statistical software by means of *T*-test for independent data (ANOVA for continuous variables, and the χ^2 test, or Fisher's exact test when appropriate, for categorical variables). A univariate analysis was performed to look for significant associations between ROP (all grades and threshold) and each one of the risk factors listed in the Methods section. When an association was indicated by $P<0.05$, multiple logistic regression was used to check the factors significantly associated with ROP in the univariate analysis. All tests were two-tailed and a $P<0.05$ was chosen as the significance cut-off. Yates' corrected χ^2, relative risk (RR), Wald test, odds ratio were also calculated with SPSS 8.0. Analysis of dichotomous outcomes and interpretation of results were performed as suggested in Cochrane Reviewers' Handbook 4.2.2.[22]

Results

In total, 351 VLBW inborn neonates were admitted to our NICU during the study period and underwent ophthalmologic screening for ROP. Of the neonates, 40 were excluded because of incomplete or unavailable data, and 10 were excluded neonates because they received antifungal prophylaxis. The final number of enrolled neonates was thus 301 (118 ELBW and 183 NE-VLBW).

Table 1 (published electronically, Supplementary material), shows the demographics of the neonates and the main ROP, SFI and bacterial sepsis data. SFI occurred at a Md age of 22 days. The episodes were caused by *C. albicans* (30 cases), *C. parapsilosis* (3), *C. glabrata* (3), *C. krusei* (2), *C. tropicalis* (1) and *C. guillermondii* (1). Five neonates were infected by two species, and they were assigned to one of the two different Candidal subspecies infections groups (albicans/non-albicans) according to the type of subspecies involved (in two cases *C. albicans* + *C. parapsilosis*, in three cases an association of non-albicans different species). The final analysis was not affected, and same was true for the inclusion of the episodes diagnosed by catheter tip (predominantly *C. albicans* infections). Three neonates eventually died with SFI due to *C. albicans* as the primary cause (2 ELBW and 1 NE-VLBW). All had been screened for ROP (one had threshold ROP, one had not-threshold ROP and the third had nothing): inclusion of their data in the study did not affect the final analysis. None of the neonates with SFI had signs of endophthalmitis.

Risk factors significantly associated with ROP (of all grades and threshold, respectively) in ELBW and NE-VLBW neonates at univariate analysis are reported in Tables 2 and 3. ROP of all grades correlated significantly in ELBW neonates with birth weight, gestational age, days on supplemental oxygen. In NE-VLBW neonates, ROP correlated significantly only with gestational age. Threshold ROP correlated significantly in ELBW neonates with birth weight, gestational age, IVH grade 2 or more, days on supplemental oxygen, colonization by *Non-albicans Candida spp*, and inversely with early-onset neutropenia. In NE-VLBW neonates,

Table 1 Demographics, incidence of ROP and incidence of SFI in the study neonates

	All population	*ELBW neonates*	*NE-VLBW neonates*
Demographics			
No. of patients	301	118	183
Sex (male/female)	150/161	62/66	88/95
Caucasian race (%)	83	89	81
Birth weight in grams, M (±s.d.) (Md)	1108 (±266) (Md 1100)	835 (±235) (Md 825)	1208 (±245) (Md 1230)
Gestational age (weeks), M (±s.d.) (Md)	28.6 (±4) (Md 29)	26.9 (±3) (Md 27)	29.8 (±3) (Md 30)
Mortality rate (prior to hospital discharge)	34/301 (11.3%)	19/118 (16.1%)	15/183 (8.2%)
Duration of stay in NICU in days, M (±s.d.) (Md)	37 (±29) (Md 36)	55 (±29) (Md 58)	30 (±16) (Md 29)
ROP			
ROP of all grades (not-threshold + threshold)	96/301 (31.9%)	55/118 (46.6%)	41/183 (22.4%)
Threshold ROP	39/301 (12.9%)	30/118 (25.4%)	9/183 (4.9%)
Age (dol) at the diagnosis of threshold ROP (all infants), Md and range	69 (35–160)	75 (44–160)	59 (35–134)
SFI			
SFI (total)	35/301 (11.6%)	21/118 (17.7%)	14/183 (7.6%)
SFI caused by *Non-albicans Candida spp*	9/301 (2.9%)	6/118 (5.0%)	3/183 (1.6%)
Age (dol) at the onset of SFI, Md and range	22 (9–78)	25 (9–78)	21 (10–52)
Duration of stay in NICU in days, M (±s.d.) (Md), in SFI patients	39 (±26) (Md 39)	61 (±32) (Md 60)	34 (±13) (Md 35)
ROP in SFI			
ROP of all grades (not-threshold + threshold) in patients with SFI	17/35 (48.1%)	14/21 (66.6%)	3/14 (21.4%)
Threshold ROP in patients with SFI	11/35 (31.1%)	10/21 (47.6%)	1/14 (7.1%)
Age (dol) at the diagnosis of threshold ROP in infants with SFI, Md and range	64 (39–134)	67 (44–134)	51 (39–110)
Bacterial sepsis			
Bacterial sepsis (all episodes)	122/301 (40.5%)	62/118 (52.5%)	60/183 (32.7%)
Bacterial sepsis (Gram negative)	80/301 (26.5%)	41/118 (34.7%)	39/183 (21.3%)
Bacterial sepsis (Gram positive)	42/301 (14.0%)	21/118 (17.8%)	21/183 (11.4%)

threshold ROP correlated significantly only with birth weight and gestational age.

Both ROP of all grades and threshold ROP correlated significantly with SFI in ELBW, but not in NE-VLBW neonates. The association of ROP with SFI remained significant when removing the catheter tip culture positive infections ($n = 5$) from the SFI analysis. Similarly, significance remained also when clustering the SFI analysis for causal fungal subspecies (*albicans* and *non-albicans Candida spp*).

Table 4 shows results and statistical analysis about threshold ROP and ROP of all grades in neonates with SFI. The incidence of threshold ROP and ROP of all grades were significantly higher in neonates with SFI than without SFI, but the difference were significant only in ELBW ($P = 0.003$ and $= 0.02$, respectively).

Table 5 shows results of multiple logistic regression for threshold ROP, ROP of all grades and SFI in ELBW neonates. After controlling for all significant factors found at univariate analysis, only gestational age, colonization by *Candida non-albicans spp*, presence of SFI and presence of SFI caused by *non-albicans Candida spp* remained significant and independent predictors of threshold ROP, while none remained independently associated with ROP of all grades.

No significant association with ROP of all grades and threshold ROP was found for the other risk factors analyzed: Apgar score at 5 min, overall presence of fungal colonization, presence of high grade fungal colonization (>3 sites involved), mean duration of stay in NICU in days, incidence of NEC (surgical), BPD, use of steroids, hyperglycaemia, need for major surgery, maternal diabetes, thrombocytopaenia, chorioamnionitis, maternal preeclampsia, number of days on TPN, maternal vaginal colonization by *Candida spp*. Importantly, no association with ROP of all grades and threshold ROP was found for the presence of bacterial sepsis, both by Gram-positive and -negative microrganisms.

Table 2 Risk factors for ROP (all grades) and threshold ROP in ELBW infants at univariate analysis

Risk factors	*ELBW with threshold ROP (n = 30)*	*ELBW without threshold ROP (n = 88)*	P-*value*	*ELBW with ROP all grades (n = 55)*	*ELBW without ROP all grades (n = 63)*	P-*value*
SFI	10/30 (33.3%)	11/88 (12.5%)	0.004	14/55 (25.4%)	7/63 (10.1%)	0.01
Gestational age	26.4(±3)	27.5(±4)	0.007	26.8 (±3)	27.8 (±3)	0.02
Days on supplemental oxygen	32 (±7)	24(±9)	0.009	30 (±8)	22 (±7)	0.03
Birth weight	742 (±212)	866(±195)	0.02	768 (±205)	894 (±188)	0.01
IVH grade 2 or more	8/30	11/88	0.008	10/55	9/63	0.21
Overall fungal colonization	9/30	37/88	0.25	22/55	33/63	0.20
Colonization by non-albicans Candida spp	7/30	2/88	0.01	7/55	3/63	0.12
Colonization by *Candida albicans*	12/30	35/88	0.40	17/55	30/63	0.19
SFI caused by *C. albicans*	6/30	9/88	0.05	9/55	6/63	0.05
SFI caused by non-albicans Candida spp	4/30	2/88	0.03	5/55	1/63	0.01
Absence of neutropenia	28/30	72/88	0.04	48/55	52/63	0.10
Bacterial sepsis (all episodes)	18/30	44/88	0.20	30/55	32/63	0.25
Bacterial sepsis (Gram negative)	14/30	27/88	0.12	21/55	20/63	0.20
Bacterial sepsis (Gram positive)	4/30	17/88	0.30	9/55	12/63	0.35

Table 3 Risk factors for ROP (all grades) and threshold ROP in NE-VLBW infants at univariate analysis

Risk factors	*NE-VLBW with threshold ROP (n = 9)*	*NE-VLBW without threshold ROP (n = 174)*	P-*value*	*NE-VLBW with ROP all grades (n = 41)*	*NE-VLBW without ROP all grades (n = 142)*	P-*value*
SFI	1/9 (11.1%)	13/174 (7.5%)	0.22	3/41 (7.3%)	11/142 (7.6%)	0.45
Gestational age	28.5 (±3)	30.1 (±4)	0.01	29.2 (±4)	30.9 (±5)	0.03
Birth weight	1045 (±58)	1234 (±230)	0.008	1088 (±73)	1285 (±145)	0.08
Bacterial sepsis (total episodes)	4/9	56/174	0.13	24/41	36/142	0.18
Bacterial sepsis (Gram negative)	3/9	36/174	0.11	16/41	22/142	0.14
Bacterial sepsis (Gram positive)	1/9	20/174	0.18	8/41	14/142	0.20

Table 4 ROP (all-grades and threshold) in SFI infants: results

	Threshold ROP in SFI infants, and (rate of incidence)	*Threshold ROP in not-SFI infants, and (rate of incidence)*	*R.R.*	*95% C.I.*	P-*value*
All neonates	11/35 (31.1%)	28/266 (10.5%)	3.012	1.904–9.202	0.008
ELBW neonates	10/21 (47.6%)	20/97 (20.6%)	3.353	1.268–9.107	0.003
NE-VLBW neonates	1/14 (7.1%)	8/169 (4.7%)			0.25
	ROP of all grades in SFI infants, and (rate of incidence)	*ROP of all grades in not-SFI infants, and (rate of incidence)*	*R.R.*	*95% C.I.*	*P-value*
All neonates	17/35 (48.1%)	79/266 (34.9%)	2.082	1.125–5.242	0.04
ELBW neonates	14/21 (66.6%)	41/97 (42.2%)	2.664	1.194–6.065	0.02
NE-VLBW neonates	3/14 (21.4%)	38/169 (22.4%)			0.42

Discussion

The etiology of ROP has been the subject of extensive research and the discovery of several associated factors has shown that supplemental oxygen is not its only cause. Our study sheds a clearer light on a controversial issue, namely the association between SFI and ROP. This is the first study to systematically

Table 5 Multiple logistic regression for association between threshold ROP and SFI, and ROP all grades and SFI, in ELBW neonates (controlling for all factors found significantly $-P<0.05$ – associated at univariate analysis)

	Beta coefficient	*Odds Ratio*	*Wald test*	*95% C.I.*	P-*value*
Threshold ROP					
Gestational age	−0.2297	0.79	4.649	0.645–0.979	0.03
SFI	1.2566	3.44	4.579	1.109–13.679	0.03
Colonization by non-albicans candidal spp.	1.7836	4.92	4.634	1.098–12.255	0.03
SFI caused by non-albicans Candida spp	1.9886	3.65	4.244	1.008–14.890	0.05
Absence of neutropenia	1.1017	1.55	3.062	0.799–3.025	0.08
IVH	0.1870	1.40	0.288	0.834–6.556	0.12
Day on supplemental oxygen	0.2112	1.25	0.453	0.990–1.112	0.35
Birth weight	0.0013	1.00	0.376	0.997–1.005	0.53
ROP all grades					
SFI	1.1095	0.51	3.2692	0.9190–6.6755	0.07
Gestational age	0.1359	1.15	2.5182	0.9686–1.3549	0.11
Birth weight	−0.0007	0.99	0.1493	0.9958–1.0028	0.69
Day on supplemental oxygen	0.9850	1.05	1.6581	0.9050–1.2152	0.40

evaluate the association of Candida sepsis and both all-grades and threshold ROP in ELBW and NE-VLBW neonates.

Our data show that an association exists, and that SFI is a risk factor independently associated with ROP, although confined to ELBW neonates and threshold ROP. Additionally, our findings show that an association with bacterial sepsis may be excluded.

We deliberately focused on both ELBW and NE-VLBW neonates since in the present literature it was uncertain which subset might be at risk of the association with fungal sepsis, and whether there was a birth-weight cutoff to identify preterm neonates at high risk of increased severity of ROP in the event of SFI.[8,11,12] Our data reflect those of Mittal *et al*.,[11] limited, as already mentioned, to ELBW neonates, but are in conflict with those of Karlowicz *et al*.[8] and Noyola *et al*.,[12] since the former ruled out an independent association between Candida sepsis and threshold ROP, while the latter extended the association to NE-VLBW neonates. The overall incidence of threshold ROP and of SFI in our study were 25.4 and 17.7%, respectively, in ELBW neonates, and 4.9 and 7.6% in NE-VLBW neonates. While the rates of SFI are comparable, those of threshold ROP (as for ELBW neonates) are slightly higher than described by Mittal (14.5% for SFI and 16.5% for threshold ROP) and Karlowicz (13% for both features): possible explanations include the accuracy of our postdischarge controls, who ensured that none of the infants discharged with ongoing retinal lesions could be dismissed by the scheduled controls until our consultants Ophtalmologists had certified the disappearance of his/her lesions.

Threshold ROP is related specifically to fungal sepsis, and – based on our data – not to sepsis itself of any etiology. This is in contrast with the data of Hussain *et al*.,[23] who found that culture-positive sepsis of any etiology was significantly associated with ROP at univariate analysis, whereas multiple logistic regression analysis failed to show its independent or additional contribution to the risk of ROP.

Which factors differentiating a fungal from a bacterial sepsis could account for this increased ability of the former to worsen the natural history of a ROP? None of our neonates displayed signs of endophtalmitis, although we know that end-organ involvement (eyes included) is very frequent and often misidentified in SFI. Nonspecific lesions that could be due to candidal endophthalmitis (cotton wool spots, superficial retinal hemorrhages, and Roth spots) are frequent in adult candidemic patients, with an incidence of 11–20%.[24]

In our series, fungal infection appears to be temporally associated and specifically related to the severity of ROP as opposed to its mere occurrence: SFI and threshold ROP are not two overlapping events, as they are separated in time by a widely ranging interval. *Candida spp*. may thus accelerate progression or otherwise constitute an additional hazard in ways that are still unclear.

The risk of severe ROP is increased, even if the patient recovers from SFI. In our series, 32/35 neonates were successfully treated, but progression to threshold ROP was not impeded even though the SFI episodes were resolved. A role of *Candida spp*. in promoting angiogenesis has been proposed. In rats, *Candida spp*. induces neovascularization in the kidney and the brain,[25] and *C. albicans* has been shown to interact with vascular endothelial cells to induce phagocytosis, endothelial cell damage, and release of cytokines and prostanoids.[26,27] According to Mittal, SFI might injure developing blood vessels in the retina and promote release of proinflammatory cytokines (such as vascular endothelial growth

factor, a cytokine specifically implicated in the pathogenesis of ROP),[28,29] that may be responsible for the development of severe ROP. This would be consistent with our finding that neutropenia is somewhat protective towards the risk of progression to severe ROP. This scenario, however, does not explain why this should only happen for inflammation caused by systemic fungal infections (SFI), and not for that caused by bacterial infection. Different proinflammatory cytokines may be released by different infectious agents, and *Candida spp*. may be able to release those that are specifically angiogenic, while bacterial agents may not. Induction of retinal angiogenesis by fungi may thus be envisaged.

Finally, and limitedly to the small sample size in our study (six infections and nine colonized infants), our data arise the question whether *non-albicans Candida spp*. may be particularly involved in the development of threshold ROP in our ELBW infants. If *C. parapsilosis*, *C. krusei* or *C. glabrata* have a particular tropism for the eye, this would be consistent with the view that different strains may be associated to different pathological features or to different severity of the same feature, and might allow early identification of subjects at higher risk of progression to threshold ROP. Prospective investigation of larger series is obviously needed to secure a clearer picture.

In conclusion, our data show that SFI is independently associated with threshold ROP and need for surgical intervention in ELBW neonates, with a magnitude wider than the simple mediation of gestational age, whereas there is no such association in neonates weighing 1000–1500 g at birth. *Non-albicans Candida spp* could play a role in determining threshold ROP in ELBW neonates. Neonates with SFI, especially if caused by these species, require close monitoring to detect the advanced stages of ROP.

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Supplementary Information accompanies the paper on the Journal of Perinatology website (http://www.nature.com/ jp).