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Leucocytosis is associated with retinopathy of prematurity in extremely preterm infants

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Leucocytosis, namely a markedly elevated white blood cell (WBC) count, occurs in 1.3–17.0% of infants admitted to neonatal intensive care units and can be induced by infection, inflammation, stress or medication. Postnatal leucocytosis can also reflect immature granulocytopoiesis in the bone marrow or systemic foetal inflammatory response syndrome, which particularly affects preterm infants (1). Chorioamnionitis and neonatal morbidities, such as sepsis, necrotising enterocolitis, intraventricular hemorrhage, prolonged oxygen support and bronchopulmonary dysplasia, have been associated with leucocytosis in preterm infants (2).

Retinopathy of prematurity (ROP) may involve prenatal and neonatal inflammation (3), but leucocytosis has not been associated with its development (2,4).

Our retrospective Swedish study of 164 extremely preterm infants, born at <27 weeks in 2008–2011, evaluated any associations between leucocytosis and neonatal morbidities, including ROP. We found that 18.3% developed severe ROP requiring treatment (Table 1).

Variables were presented using means, standard deviations, numbers and percentages and analysed using chi-square, Fisher's exact test and the Mann-Whitney *U* test, as appropriate. Univariate logistic regression analysis evaluated the impact of different crude and adjusted

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CONFLICT OF INTEREST

The authors declare no conflicts of interest.

risk factors for ROP requiring treatment as odds ratios (ORs) and 95% confidence intervals (CIs) were presented. A two-sided p value of <0.05 was significant.

Blood was drawn according to local guidelines and clinical indications and WBC and C-reactive protein (CRP) values from birth until 32 weeks of post-menstrual age were retrospectively retrieved. The highest WBC value each day indicated if leucocytosis was present. Definitions of leucocytosis have been debated and studies have used values of 30 , 40 and $50 \times 10^9/L$ (2,4). We found that leucocytosis was a significant risk factor for ROP that required treatment regardless of the definition. A receiver operating characteristic curve showed that 30 had the highest sensitivity and specificity and we used that definition. The first day of leucocytosis occurred within four weeks of life in 97.0% of the 67 affected infants and that period was used for some variables.

Leucocytosis was associated with a lower gestational age (GA) (25.1 vs 25.6 weeks) and higher rates of maternal chorioamnionitis (17.9% vs 7.2%). It was positively associated with days of mechanical ventilator support (16.0 vs 12.2 days), CRP (13.1 vs 7.6 g/L), sepsis (57.9% vs 41.2%), bronchopulmonary dysplasia (62.2% vs 40.4%) and ROP treatment (31.3% vs 9.3%), but not antenatal steroids (Table 1).

Leucocytosis was evaluated as a risk factor for ROP that required treatment, by using logistic regression analysis, adjusted for weeks of GA at birth and CRP during the first four weeks of life. Leucocytosis (OR 3.43, 95% CI: 1.37-8.59, $p < 0.01$) and GA (OR 0.61, 95% CI: 0.40-0.93, $p < 0.05$) persisted as independent risk factors, but CRP did not.

We believe that this was the first documented association between leucocytosis – defined as above – and severe ROP requiring treatment. Morag et al. assessed leucocytosis using $40 \times 10^9/L$ with moderate-to-severe ROP (stages >2) and Duran et al. used $50 \times 10^9/L$ in relation to any ROP (2,4). However, no associations were found, possibly because of the different definitions and outcomes.

Limited data are available on the aetiology, consequences and mechanism of leucocytosis. Numerous factors may alter WBC levels, including infection, inflammation, stress and medication (1). Healthy neonates and preterm infants display postnatal peaks in WBC, possibly caused by stress-related catecholamine-mediated marginalisation of WBC after labour or immature granulocytopoiesis in the bone marrow. Further research is needed into whether associations between leucocytosis and ROP development are due to infection, inflammation, immaturity or stress. Notably, leucocytosis persisted as a risk factor that had a major impact when the logistic regression analysis was adjusted for GA, indicating immaturity, and adjusted for CRP, which could indicate infection and inflammation.

Prenatal and neonatal inflammation have been suggested as possible key contributors to ROP initiation and development. Inflammation could suppress retinal neuro-vascular development during the first phase of ROP, causing more vaso-obliteration and thus more hypoxia. This increases the risks of more severe neovascularisation and severe ROP in the second phase (3). Biomarkers of inflammatory responses in preterm infants in the first few weeks of life, including increased blood levels of cytokines and CRP, have been associated

with later ROP (5). Further research is needed to understand the timing and pathophysiology of inflammation and leucocytosis and their correlation to ROP development.

Our study's strength was its longitudinal design, with WBC data collected for several months. A receiver operating characteristic curve confirmed our cut-off point was relevant. The limitations were the retrospective design, with sampling driven by clinical routines, and no data on the WBC characteristics.

When leucocytosis was defined as $WBC \geq 30 \times 10^9/L$, it was robustly associated with an increased risk of severe ROP requiring treatment in extremely preterm infants and could be used as a potential predictor. Large prospective studies are required to assess its applicability.

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Table 1

Associations between factors and leucocytosis

	Total (n = 164)	No leucocytosis (n = 97)	Leucocytosis (n = 67)	p-value
Birth characteristics				
Gestational age (weeks)	25.4 (1.0)	25.6 (0.9)	25.1 (1.0)	<0.01
Birth weight (grams)	799 (160)	819 (163)	771 (153)	0.067
Gender, boy	56.1% (92/164)	60.8% (59/97)	49.3% (33/67)	0.14
Maternal and prenatal variables				
Chorioamnionitis	11.6% (19/164)	7.2% (7/97)	17.9% (12/67)	<0.05
Pre-eclampsia	10.4% (17/164)	12.4% (12/97)	7.5% (5/67)	0.31
Premature rupture of membranes	14.0% (23/164)	14.4% (14/97)	13.4% (9/67)	0.86
Caesarean section	53.0% (87/164)	58.8% (57/97)	44.8% (30/67)	0.078
Antenatal steroids	2.66 (1.14)	2.68 (1.1)	2.63 (1.2)	0.83
Neonatal variables				
Sepsis, 0–28 days	48.8% (80/164)	41.2% (40/97)	57.9% (40/67)	<0.05
Mechanical ventilator treatment (days) 0–28 days	13.7 (10.0)	12.2 (9.6)	16.0 (10.2)	<0.05
Bronchopulmonary dysplasia	49.4% (79/164)	40.4% (38/94)	61.2% (41/66)	<0.01
Intraventricular haemorrhage (any)	33.5% (55/164)	27.8% (27/97)	41.8% (28/67)	0.063
Necrotising enterocolitis	11.0% (18/164)	8.2% (8/97)	14.9% (10/67)	0.18
CRP (g/L) 0–28 days	9.8 (13.3)	7.6 (11.1)	13.1 (15.5)	<0.001
ROP treatment, % (n)	18.3% (30/164)	9.3% (9/97)	31.3% (21/67)	<0.001

Leucocytosis was defined as a white blood count of $30 \times 10^9/L$.

For categorical variables, % (n) is presented.

For continuous variables, mean (standard deviation) is presented.

For continuous variables, the Mann–Whitney *U* test were used to analyse the difference between two group means and the chi-square test and Fisher's exact test were used to compare the proportions between two groups.