The association of the carrier state of the tumor necrosis factor-α (TNFα) $^{-308}$A allele with the duration of oxygen supplementation in preterm neonates

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ABSTRACT. ***Background.*** **High levels of inflammatory cytokines lead to lung damage in premature newborns. We investigated whether single nucleotide polymorphisms (SNP) of innate immunity cytokine genes influence the length of oxygen supplementation.** ***Methods*****. We genotyped 123 very low birth weight (VLBW) infants for the tumour necrosis factor (TNF)- α $G^{-308}A$, interleukin (IL)-1β $C^{3954}T$, IL-6 $G^{-174}C$ and IL-10 $G^{-1082}A$ SNPs. Genomic DNA was isolated from remnant dried blood samples from the neonates. We tested the association between SNPs and ventilation characteristics using a stepwise multiple regression analysis model.** ***Results.*** **The carrier state of the TNF-α $G^{-308}A$ allele was associated with a 40-hour longer period of mechanical ventilation (p=0.004) and, on average, an additional 36 hours of oxygen supplementation (p=0.0008). The association was significant after its adjustment for perinatal risk factors for lung damage.** ***Conclusions.*** **The TNF-α $G^{308}A$ genotype – which is associated with increased TNF-α levels – might influence the supplemental oxygen requirement of VLBW infants.**

Keywords: cytokine, genetic polymorphism, preterm neonate, oxygen supplementation, tumour necrosis factor alpha

Underdeveloped lung (incomplete alveolarization and surfactant deficiency), systemic infection and even medical intervention (postnatal oxygen toxicity, mechanical injury, volutrauma, barotrauma and overextension) are contributing factors to respiratory dysfunction in the preterm neonate. Each leads to the inflammation and destruction of lung tissue [1-3]. Inflammatory cytokines play a key part in this process [1, 4-6]. Cytokines trigger leukocyte invasion, release of proteolytic enzymes and production of reactive oxygen intermediates. In addition, they interfere with the signaling pathways of lung development in perinatal life [2, 3, 7].

The clinical sign of lung damage is respiratory dysfunction, which is characterized by an increased need for supplemental oxygen therapy and mechanical ventilation. Oxygen supplementation lasts for a couple of days in the majority of preterm neonates, but in some cases it might be extended for several weeks or even months [2, 6]. Chronic lung disease (CLD) (formerly referred to as bronchopulmonary dysplasia or BPD) is defined as oxygen dependency at 28 days' postnatal age or 36 weeks' postconceptional age with clinical and radiological signs of the disease. CLD is the cause of morbidity and mortality in a large number of preterm neonates affected with the disease [1, 3, 7].

Cytokine production is affected by the individual's genetic background, as well as by the developmental state and perinatal conditions [1-7]. The single nucleotide polymorphism (SNP) of the TNF-α $G^{-308}A$ gene is associated with high TNF-α cytokine levels, the SNP of the IL-1β $C^{3954}T$ gene with high IL-1β cytokine levels, the SNP of the IL-6 $G^{-174}C$ gene with low IL-6 cytokine levels, and the SNP of the IL-10 $G^{-1082}A$ gene with low IL-10 cytokine levels [8]. Our aims in this study were to test, in very low birth weight preterm babies, whether these SNPs are associated : a) with the need for supplemental oxygen requirement and mechanical ventilation; b) with the duration of oxygen supplementation in ventilated newborns; and c) with the risk of CLD.

PATIENTS AND METHODS

We retrospectively analysed the medical records of 189 neonatal intensive care unit (NICU) singleton patients born consecutively with very low birth weight (VLBW) (≤ 1500 grams). Of these, 25 died before the end of the first postnatal week (and they were not included in phenylketonuria screening). Twelve neonates died after the first postnatal week, but before the end of oxygen supplementation; they were also excluded. We requested the dried blood samples of the remaining 152 neonates who survived until the end of oxygen supplementation, from the central office of the Metabolic Screening Program. Of these, 123 samples were available for our analysis.

The patient group (59 boys and 64 girls) was heterogeneous in terms of birth weight (median [range]: 1200 [640-1500], gestational age at birth (30 [24-36]), presence

of perinatal complications (patent ductus arteriosus: 46; respiratory distress syndrome: 59; sepsis: 30; pneumonia: 38; necrotizing enterocolitis: 41), duration of mechanical ventilation (2 [0-80] days) and duration of oxygen supplementation (10 [0-83] days). Ninety-nine patients required oxygen supplementation; of those, 82 also required mechanical ventilation. Twenty-six subjects suffered CLD.

After isolating DNA from dried blood samples, genotyping was done by PCR and RFLP methods as previously described [8]. At the beginning of therapy at the NICU, we obtained the informed consent of the babies' parents to collect the dried blood samples for diagnostic and scientific purposes. The University Ethical Committee approved our retrospective analysis.

The association between genotypes and the need for oxygen supplementation, mechanical ventilation and risk of CLD was tested using the chi-square test; logistic regression analysis was used for the adjustment for gestational age and perinatal complications. Then, the number of days of oxygen supplementation and mechanical ventilation in neonates was log-transformed to achieve normal distribution. We applied stepwise multiple regression analysis using gestational age, genotypes and perinatal complications as independent variables, and duration of oxygen supplementation and mechanical ventilation as dependent variables. We also examined whether the common carrier state of the combination of two cytokine SNP variants influenced ventilation characteristics.

RESULTS

The distribution of tested genotypes fulfilled Hardy-Weinberg criteria. The TNF-α $^{-308}$A allele prevalence was 0.12, IL-1β ^{3954}T prevalence was 0.23, IL-6 $^{-174}$C prevalence was 0.27 and IL-10 $^{-1082}$A prevalence was 0.46. These values correspond to Hungarian reference values in this population [8].

None of these genotypes were associated with the need for mechanical ventilation, oxygen supplementation and CLD risk. (However, due to the low number of subjects in the study, further investigation is needed to exclude the impact of these genotypes on CLD.) The results of stepwise multiple regression analysis indicate that the carrier state of the TNF-α $^{-308}$A allele was associated with a 40-hour longer period of mechanical ventilation (p=0.004) and with, on average, an additional 36 hours of oxygen supplementation (p=0.0008) *(table 1)*. The carrier state of the combination of cytokine alleles was not associated with prolonged respiratory support.

Table 1
Parameters significantly associated with duration of oxygen supplementation and mechanical ventilation. Results of stepwise multiple regression analysis

Factors	Beta value	P value	Additional length of ventilation in hours (e^{β}*24)
Duration of oxygen supplementation			
Respiratory distress syndrome	0.60	0.014	44
Pneumonia	0.41	0.020	36
Presence of TNF-α $^{-308}$A allele	0.48	<0.001	39
Duration of mechanical ventilation			
Respiratory distress syndrome	0.97	<0.001	63
Birth weight (under 1000 g)	-0.28	0.007	4.6/100g
Presence of TNF-α $^{-308}$A allele	0.74	0.004	50

DISCUSSION

The central role of cytokines in the pathogenesis of perinatal lung damage is undeniable, although the exact interactions have yet to be revealed [2, 3, 6]. In other studies, the levels of proinflammatory cytokines (IL-1β, TNFα) increased, while the levels of anti-inflammatory cytokines (IL-6, IL-10) decreased in tracheal aspirates and blood samples of neonates on long-term oxygen therapy [2, 4, 5]. We aimed to determine whether the functional SNPs of these cytokines are associated with the risk of CLD and length of supplemental oxygen therapy [8].

We could not demonstrate an association between any of the examined SNPs and CLD. This result is in line with that of Adcock *et al.*, who also found that the risk of CLD is independent of the TNF-α G^{-308}A genotype [9]. However, given the complex pathomechanism of CLD and the heterogeneity of the affected population, further studies with larger number of VLBW neonates are needed to exclude the hypothesized impact of these cytokine genotypes on CLD risk.

In patients with a milder form of lung damage however, we demonstrated that the TNF-α G^{-308}A genotype – which is associated with increased TNF-α levels – might still influence the supplemental oxygen requirement of VLBW infants. This finding corresponds with the central role of high TNF-α levels in pulmonary pathogenesis [1, 5, 8, 9].

Interestingly, our finding is not in line with those obtained in adult patients. Yende *et al.* found that the length of mechanical ventilation following coronary artery bypass surgery increases in carriers of TNF-α haplotypes with low levels of TNF-α secretion (i.e. lymphotoxin-α $^{+250}$G and TNF-α $^{-308}$G allele) [10]. Different pathology and patient characteristics could account for this inconsistency. Whereas Yende *et al.* enrolled postoperative adult subjects without apparent lung disease, we examined VLBW neonates who are susceptible to lung damage.

In summary, our results support the hypothesis that the TNF-α G^{-308}A genotype contributes to the diseased, preterm neonates' need for mechanical ventilation and oxygen supplementation.

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