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CLINICAL RESEARCH ARTICLE OPEN

Can serum periostin predict bronchopulmonary dysplasia in premature infants?

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BACKGROUND: Bronchopulmonary dysplasia (BPD) is the most common morbidity complicating preterm birth and affects long-term respiratory outcomes. The objectives of this study were to establish whether serum periostin at birth, day of life (DOL) 28, and corrected 36 weeks' gestational age could be potential biomarkers for BPD.

METHODS: A total of 98 preterm Japanese infants born at <32 weeks and comparing 41 healthy controls born at term, were divided into BPD ($n = 44$) and non-BPD ($n = 54$) cohorts. Serum periostin levels were measured using an enzyme-linked immunosorbent assay.

RESULTS: Among 98 preterm infants, the median serum periostin levels at birth were higher with BPD (338.0 ng/mL) than without (275.0 ng/mL, $P < 0.001$). Multivariate analysis revealed that serum periostin levels at birth were significantly associated with BPD ($P = 0.013$). Serum periostin levels at birth with moderate/severe BPD (345.0 ng/mL) were significantly higher than those with non-BPD/mild BPD (283.0 ng/mL, $P = 0.006$).

CONCLUSIONS: Serum periostin levels were significantly correlated with birth weight and gestational age, and serum periostin levels at birth in BPD infants were significantly higher than that in non-BPD infants.

Pediatric Research (2022) 92:1108–1114; https://doi.org/10.1038/s41390-021-01912-w

IMPACT:

- This study found higher serum periostin levels at birth in preterm infants subsequently diagnosed with bronchopulmonary dysplasia.
- It also emerged that serum periostin levels at birth significantly correlated with gestational age and birth weight.
- The mechanism by which serum periostin is upregulated in BPD infants needs further investigation.

BACKGROUND

Bronchopulmonary dysplasia (BPD) is the most common morbidity complicating preterm birth and is associated with neurodevelopmental impairment and long-term respiratory outcomes such as childhood wheezing and asthma.[1,2] BPD results from various perinatal factors including maternal inflammation, surfactant deficiency, ventilation, and oxygen toxicity.[3,4] Premature infants are often exposed to positive pressure ventilation and supplemental oxygen, contributing to the development of BPD. An important pathophysiological feature of infants affected with BPD is the developmental arrest of alveolarization.[4] Such structural alterations are accompanied by characteristic inflammatory changes and extensive remodeling of the extracellular matrix (ECM), together with increased smooth muscle mass in small pulmonary arteries and airways.[5] Periostin is characterized as both a matricellular protein and extracellular matrix (ECM) protein of the fasciclin family.[6,7]

Periostin plays an important role in the development of allergic, pulmonary, and other diseases.[7,8] Lung periostin is expressed in human lung fibroblasts and human bronchial epithelial cells.[9] Since periostin is regulated by interleukins IL-4 and IL-13 and is involved in the pathogenesis of fibrosis and allergy in various diseases, many studies reported that serum and plasma periostin levels were potential biomarkers for various diseases such as idiopathic lung fibrosis in adults and asthma.[7,10–12] Furthermore, various factors such as transforming growth factor-beta (TGF-β), IL-4, IL-13, mechanical stress, and connective tissue growth factor upregulate periostin.[13] In the pathogenesis of BPD, TGF-β is involved in lung vascular development. Periostin is associated with TGF-β-mediated fibrosis and lung development in response to hyperoxia.[8,14] TGF-β, in turn, is associated with the pathogenesis of BPD during lung vascular development.

Although periostin expression is increased in autopsy lungs of preterm neonates with BPD,[14] few reports have suggested a

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Received: 18 December 2020 Revised: 23 November 2021 Accepted: 24 November 2021
Published online: 27 December 2021

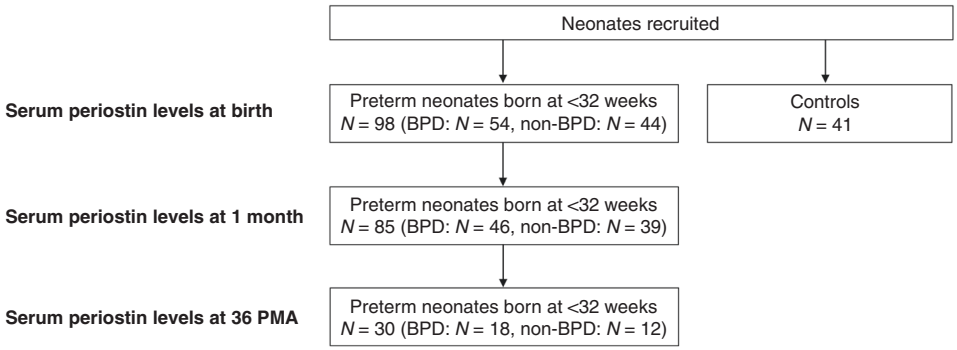

Fig. 1 Flow diagram in neonates included in the analysis of serum periostin levels at each time point. BPD bronchopulmonary dysplasia, PMA postmenstrual age.

Table 1. Characteristics of subjects.

	Healthy controls (*N* = 41)		Preterm infants <32 weeks (*N* = 98)	
Gestational age, median (IQR), weeks	38.7	(37.7–39.9)	26.0	(24.2–28.2)
Birth weight, median (IQR), g	2948	(2588–3122)	743	(621–1068)
Male gender, *n* (%)	18	(43.9)	48	(49.0)
CAM, *n* (%)	0	(0)	43	(43.9)
Antenatal steroid, *n* (%)	0	(0)	90	(91.8)
PROM, *n* (%)	0	(0)	26	(26.5)
HDP, *n* (%)	0	(0)	8	(8.2)
RDS, *n* (%)	0	(0)	71	(72.4)
SGA, *n* (%)	0	(0)	15	(15.3)
PDA, *n* (%)	0	(0)	55	(56.1)
NEC, *n* (%)	0	(0)	9	(9.2)
ROP, *n* (%)	0	(0)	49	(50.0)
BPD, *n* (%)	0	(0)	44	(44.9)
Oxygen supplementation at DOL14, *n* (%)	0	(0)	51	(52.0)
Invasive mechanical ventilation at DOL28, *n* (%)	0	(0)	61	(62.2)
Apgar score at 1 min <3, *n* (%)	0	(0)	26	(26.5)
Apgar score at 5 min <3, *n* (%)	0	(0)	10	(10.2)

IQR interquartile range, *CAM* chorioamnionitis, *PROM* premature rupture of membranes, *HDP* hypertensive disorders of pregnancy, *ROP* retinopathy of prematurity, *SGA* small for gestational age, *RDS* respiratory distress syndrome, *PDA* patent ductus arteriosus, *NEC* necrotizing enterocolitis, *ROP* retinopathy of prematurity, *BPD* bronchopulmonary dysplasia, *DOL* days of life.

relationship between serum periostin levels at birth and BPD. Ahlfeld et al. reported elevated plasma periostin levels in BPD patients on day 28 of life (DOL28) compared with non-BPD patients in a study somewhat limited by their low sample number and their two sampling times (DOL7 and DOL28).[15]

While some studies propose reference intervals for serum periostin in children,[16,17] reports correlating serum periostin levels in term and preterm births with other perinatal factors are lacking. Periostin plays an important role in the pathogenesis of inflammation and pulmonary fibrosis. BPD is known to be associated with intrauterine inflammation.

Therefore, we hypothesized that serum periostin at birth might increase in BPD patients, and could serve as a biomarker of BPD. The objectives of the present study are to evaluate the perinatal factors affecting serum periostin levels at birth in preterm and healthy infants and to validate whether serum periostin at birth, DOL28, and corrected 36 weeks' gestational age can be potential biomarkers for BPD.

MATERIALS AND METHODS

Ethics approval and compliance

This research (protocol 2020-020) was approved by the Institutional Review Board of Fukushima Medical University, which is guided by local policy, national law, and the World Medical Association Declaration of Helsinki. As our human subjects were neonates, informed consent was solicited from parents or other legal guardians, and documented in writing.

NICU patients

Serum samples were obtained from mechanically ventilated or oxygenated Japanese patients in the neonatal intensive care unit (NICU) of Fukushima Medical University from November 2014 to July 2020. We examined cord serum at birth and venous serum at 36 weeks postmenstrual age and DOL28. Newborns with congenital anomalies or those who died prior to postnatal day 28 were excluded. Data for analysis included gestational age, phenotypic sex, body weight at birth, invasive mechanical ventilation at DOL28, supplemental oxygen at DOL14, respiratory distress syndrome (RDS), retinopathy of prematurity (ROP), being small for gestational age (SGA), patent ductus arteriosus (PDA), necrotizing enterocolitis (NEC), Apgar scores, and maternal complications: chorioamnionitis (CAM), premature rupture of membranes (PROM), hypertensive disorders of pregnancy (HDP).

BPD was defined in accordance with the National Institutes of Health consensus definition for infants.[18] At a postmenstrual age of 36 weeks, the premature infants were classified into the following groups: non-BPD if outside the above definition, mild BPD if needing supplemental oxygen at ≥28 days but not at 36 weeks postmenstrual age; moderate BPD if needing supplemental oxygen at 28 days, in addition to supplemental oxygen at FiO_2 (fraction of inspired oxygen) ≤ 0.30 at 36 weeks postmenstrual age; and severe BPD if needing supplemental oxygen at 28 days and, at 36 weeks postmenstrual age, needing mechanical ventilation and/or having $FiO_2 > 0.30$.[18] SGA was defined as a birth weight of below 1.5 standard deviations, corrected for the gestational age and sex in accordance with previously published criteria.[19]

Healthy neonatal subjects

Healthy Japanese neonates who were born from 36.6 weeks to term in our hospital were included if informed consent was obtained from parents and/or legal guardians and documented in writing.

Serum periostin measurements

Serum samples (cord blood or venous blood) were obtained from neonates at birth, DOL28, and corrected 36 weeks' postmenstrual age. Using serum samples stored at −80 °C until assay, serum periostin levels were measured using an enzyme-linked immunosorbent assay (Periostin

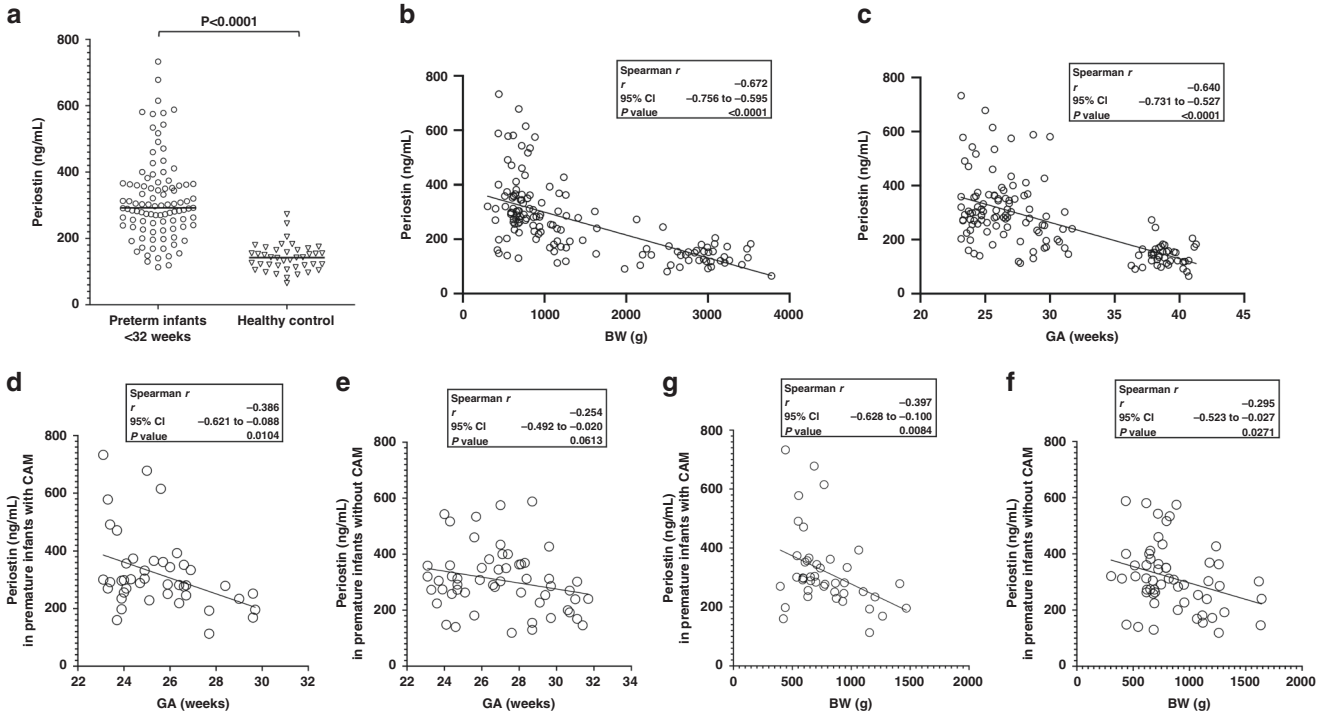

Fig. 2 Serum periostin levels. a Comparing healthy neonates and preterm neonates born at <32 weeks' gestational age, serum periostin levels at birth were higher in the preterm neonates. **b**, **c** Serum periostin levels at birth were significantly correlated with BW (birth weight) and GA (gestational age). **d**–**f** Correlation between GA, BW, and serum periostin levels at birth in the preterm neonates born at <32 weeks' gestational age with and without CAM (chorioamnionitis). Horizontal bars denote the median values in each group of infants.

ELISA Kit (Human)) performed at Shino-Test (Kanagawa, Japan), as previously described.[20–22]

Statistical analysis

All numerical data are presented as the medians. The Mann–Whitney *U*-test and χ^2 test were used to compare continuous variables and nominal variables, respectively. To evaluate the correlation between two parameters, Spearman's correlation coefficient was calculated. We performed multivariate analyses to determine factors significantly associated with serum periostin levels at birth as BW, GA, RDS, ROP, BPD, and Apgar score at 1 min < 3 in premature infants born at <32 weeks. Next, we also performed multivariate analyses to determine factors significantly associated with BPD as potential confounding factors such as BW, GA, invasive mechanical ventilation at DOL28, Apgar score at 1 min < 3, oxygen supplementation at DOL14, and serum periostin levels at birth in premature infants born at <32 weeks. BPD diagnostic accuracy was evaluated by receiver operating characteristic (ROC) curves with areas under the curve (AUC) use to quantify the sensitivity of independent risks for BPD. The levels of significance were set at $P < 0.05$. Data analysis was performed with IBM SPSS (version 21.0, IBM Corp., Armonk, NY) and GraphPad Prism (version 8 GraphPad Software, San Diego, CA). A post hoc power analysis was performed using G*Power 3 with an effect size = 0.5, $\alpha = 0.05$, and sample sizes.

RESULTS

Clinical characteristics and serum periostin levels at birth in preterm and term infants

A total of 139 infants, preterm ($n = 98$) and healthy controls ($n = 41$) were included in this study (Fig. 1). The clinical characteristics of preterm infants born at <32 weeks and healthy controls are summarized in Table 1. Figure 2 shows the serum periostin levels at birth in preterm and term infants. The median serum periostin levels at birth among preterm infants born at <32 weeks was significantly higher than those among healthy infants (292.0 ng/mL vs 142.0 ng/mL, $P < 0.0001$) (Fig. 2a). Furthermore, there were significant inverse correlations between BW ($r = -0.672$, $P < 0.0001$), GA ($r = -0.640$, $P < 0.0001$), and serum periostin levels at birth in 139 preterm and term infants (Fig. 2b, c). Furthermore, we investigated whether CAM affects periostin levels at birth in premature infants born at <32 weeks (Fig. 2d–g). As shown in Fig. 2d, there was a significant correlation between GA and periostin levels at birth in preterm infants with CAM ($r = -0.386$, $P = 0.0104$). However, there was no significant correlation between GA and periostin levels at birth without CAM ($r = -0.254$, $P = 0.0613$) (Fig. 2e). As shown in Fig. 2g, f, there were significant correlations between birth weight and periostin levels at birth in premature infants born at <32 weeks with ($r = -0.397$, $P = 0.0084$) and without CAM ($r = -0.295$, $P = 0.0271$).

Perinatal factors and serum periostin levels at birth in preterm infants born at <32 weeks

Next, among 98 preterm infants born at <32 weeks, we analyzed the correlation between serum periostin levels at birth and perinatal factors (Table 2). GA and BW were negatively correlated with serum periostin levels at birth. Additionally, serum periostin levels at birth were significantly higher in RDS, ROP, BPD, and Apgar score at 1 min < 3. In particular, the median serum periostin levels at birth were higher with BPD than without (338.0 ng/mL vs 275.0 ng/mL, $P < 0.001$). A post hoc power analysis with an effect size = 0.5, $\alpha = 0.05$, and sample size (BPD: $n = 44$, non-BPD: $n = 54$) showed a power of 0.85. Multivariate analysis revealed that serum periostin levels at birth were significantly associated with BPD ($P = 0.026$).

Serum periostin levels in BPD infants

To investigate whether serum periostin levels were associated with BPD, preterm infants born at <32 weeks were divided into BPD neonates ($n = 44$) and non-BPD neonates ($n = 54$) (Table 3). The median GA in BPD infants was significantly lower than those in non-BPD neonates (24.4 weeks vs 27.2 weeks, $P < 0.001$). The median BW in BPD neonates was also significantly lower than those in non-BPD infants (638 g vs 952 g, $P < 0.001$).

Table 2. Associations between serum periostin levels at birth and perinatal factors in preterm infants born at <32 weeks.

	Serum periostin levels at birth (ng/mL) (median)	Coefficient	Univariate analysis (*P*-value)	Multivariate analysis (*P*-value)
Gestational age	–	−0.265	**0.008**	0.610
Birth weight	–	−0.313	**0.002**	0.345
Male vs female	306.5 vs 287.5	–	0.234	–
CAM vs non-CAM	302.0 vs 289.0	–	0.994	–
Antenatal steroid vs no antenatal steroid	300.0 vs 266.5	–	0.078	–
PROM vs non-PROM	295.5 vs 291.5	–	0.554	–
HDP vs non-HDP	300.0 vs 266.6	–	0.315	–
RDS vs non-RDS	303.0 vs 256.0	–	**0.008**	0.080
SGA vs non-SGA	321.0 vs 290.0	–	0.598	–
PDA vs non-PDA	299.0 vs 291.0	–	0.155	–
NEC vs non-NEC	292.0 vs 291.0	–	0.629	–
ROP vs non-ROP	312.0 vs 270.0	–	**0.003**	0.596
BPD vs non-BPD	338.0 vs 275.0	–	**<0.001**	**0.026**
Apgar score at 1 min < 3 vs Apgar score at 1 min ≥ 3	326.5 vs 283.0	–	**0.022**	0.370
Apgar Score at 5 min < 3 vs Apgar score at 5 min ≥ 3	300.0 vs 291.5	–	0.469	–

NA not applicable, *CAM* chorioamnionitis, *PROM* premature rupture of membranes, *HDP* hypertensive disorders of pregnancy, *SGA* small for gestational age, *RDS* respiratory distress syndrome, *PDA* patent ductus arteriosus, *NEC* necrotizing enterocolitis, *ROP* retinopathy of prematurity, *BPD* bronchopulmonary dysplasia. *P*-values in bold are those that show statistical significance.

The occurrence of Apgar scores at 1 min < 3 was significantly higher in BPD infants compared with those in non-BPD infants (40.9% vs 14.8%, $P < 0.005$). Furthermore, the incidence of invasive mechanical ventilation at DOL28 and oxygen supplementation at DOL14 in BPD infants were significantly higher than in non-BPD infants. There were no significant differences between BPD and non-BPD infants in terms of phenotypic sex, CAM, antenatal steroid usage, PROM, HDP, RDS, SGA, and PDA (Table 3). In multivariate analysis of the correlation between serum periostin at birth and clinical parameters, serum periostin levels at birth significantly correlated with BPD ($P = 0.013$), BW ($P = 0.027$), and oxygen supplementation at DOL14 ($P = 0.041$) (Table 3). Receiver operating characteristic analysis for serum periostin levels at birth in infants with and without BPD yielded an area under the curve of 0.725 (95% CI 0.627–0.822, $P = 0.0001$) (Fig. 3). A threshold of serum perisostin > 305 ng/mL at birth identified BPD with 71.7% sensitivity and 63.4% specificity (Fig. 3).

Figure 4 shows the serum periostin levels at birth, DOL28, and corrected 36-weeks' postmenstrual age in BPD and non-BPD infants. The median periostin levels on DOL28 in BPD infants (281.0 ng/mL) were significantly lower compared with those at birth (338.0 ng/mL, $P = 0.003$). However, the median serum periostin levels on DOL28 and corrected age of 36-weeks' gestation were not significantly different in BPD infants compared with non-BPD infants (DOL28: 281.0 ng/mL vs 254.5 ng/mL; corrected 36-weeks' postmenstrual age: 309.0 ng/mL vs 293.0 ng/mL) (Fig. 4). Next, we evaluated the relationships between serum periostin at birth and the severity of BPD (Fig. 5). Serum periostin levels at birth with moderate/severe BPD were significantly higher than those with non-BPD/mild BPD (345.0 ng/mL vs 283.0 ng/mL, $P = 0.006$). A post hoc power analysis with an effect size = 0.5, $\alpha = 0.05$, and sample size (moderate/severe BPD: $n = 71$, non-BPD/mild BPD: $n = 27$) showed power was 0.83.

DISCUSSION

This study describes an association between serum periostin levels at birth and perinatal factors in preterm and term infants and the correlation between serum periostin levels at birth and BPD. The present study revealed that higher serum periostin levels at birth in preterm infants born at <32 weeks' gestational age are independent risk factors for BPD and reflect the severity of BPD. Although there are many studies trying to demonstrate an association between serum biomarkers and the risk of BPD, few suggest a correlation between serum periostin levels and BPD.[15,16] In this study, we also demonstrated that serum periostin levels on DOL28 and corrected 36 weeks' postmenstrual age could not serve as potential biomarkers for BPD. Ahlfeld et al. suggested that 7-day-old infants who went on to have the most severe lung disease, requiring tracheostomy and ventilator dependence, had measurably higher periostin levels, and early elevation of plasma periostin levels that persisted to DOL28 were significantly associated with chronic ventilator-dependent bronchopulmonary dysplasia.[15] Differences with our results may be due to differences in the study cohorts and methods of analysis. Ahlfeld's cohort was small (no BPD = 11, BPD/died = 20), and they measured periostin only on DOL7 and DOL28, to minimize phlebotomy loss. We measured serum periostin levels at birth, DOL28, and corrected 36 weeks' postmenstrual age in a larger sample size that allowed multivariate analyses. Moreover, they used a different immunoassay.

In terms of the relationship between periostin and lung disease, previous studies demonstrated that elevated serum periostin levels were associated with various lung diseases such as asthma, idiopathic pulmonary fibrosis, and COPD in children and adults.[5,8,23,24] Furthermore, the expression of lung periostin was upregulated in patients with idiopathic lung fibrosis.[8,11] Bozyk et al. also reported that periostin expression increased in autopsy lungs of preterm neonates with BPD.[14] In a murine model of BPD exposed to hyperoxia, hyperoxia upregulated periostin expression in neonatal mice lung.[14] Furthermore, lung periostin levels were also increased during the saccular stage, as previously shown.[25] Although the mechanism by which periostin is associated with the pathogenesis of BPD remains poorly understood, we speculate that the linkage of periostin and TGF- β might be associated with

Table 3. Characteristics of BPD and non-BPD infants.

	Non-BPD ($N = 54$)	BPD ($N = 44$)	Univariate analysis *P*-value	Multivariate analysis *P*-value
Gestational age, median (IQR), weeks	27.2 (25.6–29.6)	24.4 (23.7–26.3)	<0.001	0.242
Birth weight, median (IQR), g	952 (622–1210)	638 (438–734)	<0.001	**0.027**
Male gender, *n* (%)	26 (48.1)	22 (50.0)	0.508	–
CAM, *n* (%)	23 (42.6)	20 (45.4)	0.311	–
Antenatal steroid, *n* (%)	50 (92.6)	40 (90.9)	0.522	–
PROM, *n* (%)	13 (24.1)	13 (29.5)	0.647	–
HDP, *n* (%)	7 (13.0)	1 (2.3)	0.070	–
RDS, *n* (%)	35 (64.8)	36 (81.8)	0.072	–
SGA, *n* (%)	6 (11.1)	9 (20.5)	0.262	–
PDA, *n* (%)	34 (63.0)	21 (47.7)	0.155	–
Oxygen supplementation at DOL14, *n* (%)	20 (37.0)	31 (70.5)	0.001	**0.041**
Invasive mechanical ventilation at DOL28, *n* (%)	21 (38.9)	40 (90.9)	<0.001	0.391
Apgar score at 1 min <3, *n* (%)	8 (14.8)	18 (40.9)	0.005	0.571
Apgar score at 5 min <3, *n* (%)	3 (5.6)	7 (15.9)	0.107	–
Serum periostin levels at birth, median (ng/mL)	275.0	338.0	<0.001	**0.013**

IQR interquartile range, *CAM* chorioamnionitis, *PROM* premature rupture of membranes, *HDP* hypertensive disorders of pregnancy, *SGA* small for gestational age, *RDS* respiratory distress syndrome, *PDA* patent ductus arteriosus, *DOL* days of life.
P-values in bold are those that show statistical significance in multivariate analysis.
P-values are for BPD vs. non-BPD neonates.

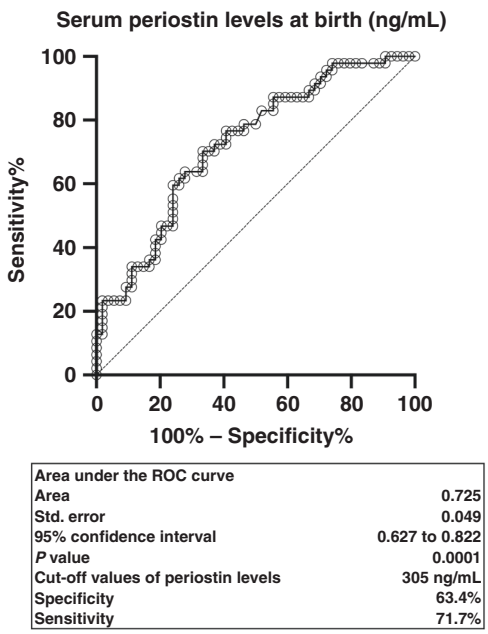

Fig. 3 Comparison of receiver operating characteristic (ROC) curve analyses of serum periostin levels at birth that distinguish infants with and without BPD. ROC receiver operating characteristic.

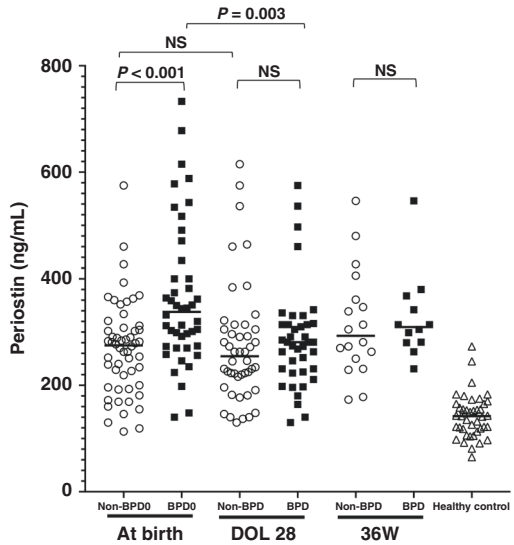

Fig. 4 Serum periostin levels at birth were significantly higher in BPD neonates compared with non-BPD neonates. Serum periostin levels on DOL28 and corrected 36 week's postmenstrual age did not differ in infants with or without BPD. Horizontal bars denote the median values in each group of infants. NS not significant.

the pathogenesis of BPD. Periostin and TGF-β are known to play a critical role in the proliferation of lung fibroblasts.[9] Furthermore, many studies in different animal models of BPD confirm elevated TGF-β expression levels and activation of its associated pathways as an important part of lung disease pathophysiology.[22,26,27] Also, we previously reported that serum TGF-β levels were upregulated in BPD patients.[28]

Another new finding in this study was the significant correlation of serum periostin levels at birth with BW and GA. Fujitani et al. reported that periostin levels in non-allergic children from 0 to 15 years were almost 91.9–124.8 ng/mL.[17] They also suggested that serum periostin levels gradually increased after age 10 years. On the other hand, a previous study proposed a periostin threshold of 95 ng/mL based on values from healthy adult controls.[29]

Anderson et al. also reported that serum periostin levels at ages 2–6 years ranged from 120 to 150 ng/mL.[16] In this study, serum periostin levels of healthy neonates were around 140 ng/mL. Furthermore, serum periostin levels at birth in neonates born at <32 weeks' gestational age were almost 340 ng/mL. Although our periostin levels are not directly comparable to those of Anderson's study, due to differences in the method of analysis, serum periostin levels in infants were the highest when comparing infants, children, and adults. These developmental changes of

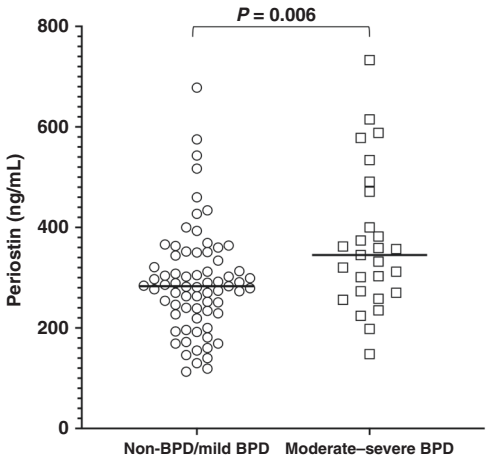

Fig. 5 Serum periostin levels at birth were higher in moderate/severe BPD compared with no BPD/mild BPD. Values are the median (no BPD/mild BPD: $n = 71$, moderate/severe BPD: $n = 27$). Horizontal bars denote the median in each group of infants. The other *P*-values were calculated using the Mann–Whitney *U*-test.

serum periostin levels may be related to metabolic turnover and growth, as periostin is a component of the extracellular matrix and regulates serum type I collagen formation, which is an essential component of skin, tendon, and bone development.[16,30] Compared with term infants, the cord blood serum procollagen type I C-terminal propeptide (PICP) as bone information in preterm infants was significantly higher and influenced by fetal age.[31] Furthermore, our study suggests that serum periostin levels at birth in premature infants born at <32 weeks with CAM were strongly correlated with gestational age and birth weight compared with those without CAM. Although periostin is well known to be involved in inflammation, few have reported any relationship between CAM and periostin. However, some studies suggest that cord blood IL-4 and IL-13, which regulate periostin, were significantly higher in neonates with CAM compared to those without CAM.[32,33] Further study is needed to investigate the relationship between IL-4, IL-13, and periostin levels at birth.

Our study has several limitations. First, it was performed at a single center and the sample size of BPD patients was small. Furthermore, we did not have validation cohorts. To validate our observations, a larger sample size with multiple centers and different ethnic cohorts would be invaluable. Second, we could not evaluate lung periostin. A previous study demonstrated that lung periostin in BPD infants was higher than in healthy lungs at term.[9] Third, we could not detect the cellular sources of periostin. Thus, our next goal is to determine the cell types secreting periostin as well as the mechanism(s) of upregulation of periostin in BPD neonates; this will advance understanding of the pathogenesis of BPD. Lastly, in this study, we did not investigate the correlation between periostin levels and Th2 cytokines such as IL-4 and IL-13. It is noteworthy that upon stimulation by IL-4 and IL-13, periostin could be detected in lung fibroblasts.[34] One of the main consequences of BPD is lung fibrosis. Although a previous study suggested that IL-4 and IL-13 levels of tracheal aspirates from premature infants were very low and did not correlate with BPD,[35] premature infants born at <32 weeks' gestational age have increased nasal airway IL-4 and IL-13 secretion during rhinovirus infections.[36] Although various commercial ELISA kits and multiplex assays are available, there are no reliable, commercially available assays for periostin levels. In this study, an enzyme-linked immunosorbent assay (ELISA) was performed at Shino-Test (Kanagawa, Japan). It was previously reported that this assay, using two monoclonal antibodies (SS18A and SS17B) recognizing the R1 and R4 domains of periostin, had good intra-assay and inter-assay performance, with CV ≤ 3%.[29,37] This ELISA has been used in a wide range of research but without being commercially available. Furthermore, periostin levels have still not been standardized, and caution is needed in interpreting serum periostin levels using different assays.[38] Lastly, we demonstrated that periostin levels at birth among moderate/severe BPD patients are significantly higher than those of non-BPD/ mild BPD patients. However, there are significant overlaps between these two populations. Furthermore, ROC analysis revealed that serum periostin levels at birth would not reliably predict BPD in clinical practice, because periostin levels in BPD and non-BPD infants overlapped and were affected by birth weight and gestational age.

CONCLUSION

We conclude that serum periostin levels were significantly correlated with birth weight and gestational age. Furthermore, serum periostin levels at birth in BPD infants were significantly higher than those in non-BPD infants. The mechanism by which serum periostin is upregulated in BPD infants and inversely correlated with gestational age and birth weight remains to be further elucidated.

REFERENCES

1. Gough, A., Spence, D., Linden, M., Halliday, H. L. & McGarvey, L. P. A. General and respiratory health outcomes in adult survivors of bronchopulmonary dysplasia: a systematic review. *Chest* **141**, 1554–1567 (2012).
2. Gough, A. et al. Impaired lung function and health status in adult survivors of bronchopulmonary dysplasia. *Eur. Respir. J.* **43**, 808–816 (2014).
3. Kinsella, J. P., Greenough, A. & Abman, S. H. Bronchopulmonary dysplasia. *Lancet* **367**, 1421–1431 (2006).
4. Baraldi, E. & Filippone, M. Chronic lung disease after premature birth. *N. Engl. J. Med.* **357**, 1946–1955 (2007).
5. Anderson, P. J. & Doyle, L. W. Neurodevelopmental outcome of bronchopulmonary dysplasia. *Semin. Perinatol.* **30**, 227–232 (2006).
6. Izuhara, K. et al. Periostin in allergic inflammation. *Allergol. Int.* **63**, 143–151 (2014).
7. Izuhara, K. et al. Periostin: an emerging biomarker for allergic diseases. *Allergy* **74**, 2116–2128 (2019).
8. Nanri, Y. et al. Cross-talk between transforming growth factor-β and periostin can be targeted for pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **62**, 204–216 (2020).
9. Yoshihara, T. et al. Periostin plays a critical role in the cell cycle in lung fibroblasts. *Respir. Res.* **21**, 38 (2020).
10. Okamoto, M. et al. Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur. Respir. J.* **37**, 1119–1127 (2011).
11. Murata, K. et al. Accumulation of periostin in acute exacerbation of familial idiopathic pulmonary fibrosis. *J. Thorac. Dis.* **10**, E587–E591 (2018).
12. Naik, P. K. et al. Periostin promotes fibrosis and predicts progression in patients with idiopathic pulmonary fibrosis. *Am. J. Physiol. Lung Cell Mol. Physiol.* **303**, L1046–L1056 (2018).
13. Nakazawa, T. et al. Gene expression of periostin in the early stage of fracture healing detected by cDNA microarray analysis. *J. Orthop. Res.* **22**, 520–525 (2004).
14. Bozyk, P. D. et al. Neonatal periostin knockout mice are protected from hyperoxia-induced alveolar simplication. *PLoS ONE* **7**, e31336 (2012).
15. Ahlfeld, S. K., Davis, S. D., Kelley, K. J. & Poindexter, B. B. Early elevation of plasma periostin is associated with chronic ventilator-dependent bronchopulmonary dysplasia. *Am. J. Respir. Crit. Care Med.* **194**, 1430–1433 (2016).
16. Anderson, H. M. et al. Relationships among aeroallergen sensitization, peripheral blood eosinophils, and periostin in pediatric asthma development. *J. Allergy Clin. Immunol.* **139**, 790–796 (2017).
17. Fujitani, H. et al. Age-related changes in serum periostin level in allergic and non-allergic children. *Allergol. Int.* **68**, 285–286 (2019).
18. Jobe, A. H. & Bancalari, E. Bronchopulmonary dysplasia. *Am. J. Respir. Crit. Care Med.* **163**, 1723–1729 (2001).
19. Itabashi, K., Miura, F., Uehara, R. & Nakamura New Japanese neonatal anthropometric charts for gestational age at birth. *Pediatr. Int.* **56**, 702–708 (2014).
20. Matsusaka, M. et al. Phenotype of asthma related with high serum periostin levels. *Allergol. Int.* **64**, 175e80 (2015).
21. Kimura, H. et al. Sinus computed tomographic findings in adult smokers and nonsmokers with asthma. Analysis of clinical indices and biomarkers. *Ann. Am. Thorac. Soc.* **14**, 332e41 (2017).

22. Oak, P. & Hilgendorff, A. The BPD trio? Interaction of dysregulated PDGF, VEGF, and TGF signaling in neonatal chronic lung disease. *Mol. Cell Pediatr.* **4**, 11 (2017).
23. Golpe, R. et al. Differences in systemic inflammation between cigarette and biomass smoke-induced COPD. *Int. J. Chron. Obstruct. Pulmon. Dis.* **12**, 2639–2646 (2017).
24. Pavlidis, S. et al. "T2-high" in severe asthma related to blood eosinophil, exhaled nitric oxide and serum periostin. *Eur. Respir. J.* **53**, 1800938 (2019).
25. Ahlfeld, S. K. et al. Periostin downregulation is an early marker of inhibited neonatal murine lung alveolar septation. *Birth Defects Res. A Clin. Mol. Teratol.* **97**, 373–385 (2013).
26. Mokres, L. M. et al. Prolonged mechanical ventilation with air induces apoptosis and causes failure of alveolar septation and angiogenesis in lungs of newborn mice. *Am. J. Physiol. Lung Cell Mol. Physiol.* **298**, L23–L35 (2010).
27. Hilgendorff, A. et al. Neonatal mice genetically modified to express the elastase inhibitor elafin are protected against the adverse effects of mechanical ventilation on lung growth. *Am. J. Physiol. Lung Cell Mol. Physiol.* **303**, L215–L227 (2012).
28. Go, H. et al. Extracellular vesicle miRNA-21 is a potential biomarker for predicting chronic lung disease in premature infants. *Am. J. Physiol. Lung Cell Mol. Physiol.* **318**, L845–L851 (2020).
29. Kanemitsu, Y. et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J. Allergy Clin. Immunol.* **132**, 305e12 (2013).
30. Kudo, A. Periostin in fibrillogenesis for tissue regeneration: periostin actions inside and outside the cell. *Cell Mol. Life Sci.* **68**, 3201–3207 (2011).
31. Aly, H. et al. Gestational age, sex and maternal parity correlate with bone turnover in premature infants. *Pediatr. Res.* **57**, 708–711 (2005).
32. Zein, H. et al. Cord blood cytokine levels correlate with types of placental pathology in extremely preterm infants. *Front. Pediatr.* **9**, 607684 (2021).
33. Romero, R. et al. Clinical chorioamnionitis at term V: umbilical cord plasma cytokine profile in the context of a systemic maternal inflammatory response. *J. Perinat. Med.* **44**, 53–76 (2016).
34. Takayama, G. et al. Periostin: a novel component of subepithelial fibrosis of bronchial asthma downstream of IL-4 and IL-13 signals. *J. Allergy Clin. Immunol.* **118**, 98–104 (2006).
35. Baier, R. J., Loggins, J. & Kruger, T. E. Interleukin-4 and 13 concentrations in infants at risk to develop bronchopulmonary dysplasia. *BMC Pediatr.* **3**, 8 (2003).
36. Perez, G. F. et al. Rhinovirus-induced airway cytokines and respiratory morbidity in severely premature children. *Pediatr. Allergy Immunol.* **26**, 145–152 (2015).
37. Kou, K. et al. Periostin levels correlate with disease severity and chronicity in patients with atopic dermatitis. *Br. J. Dermatol.* **171**, 283–291 (2014).
38. Ono, J., Takai, M., Kamei, A., Azuma, Y. & Izuhara, K. Pathological roles and clinical usefulness of periostin in type 2 inflammation and pulmonary fibrosis. *Biomolecules* **11**, 1084 (2021).

ACKNOWLEDGEMENTS

Junya Ono is a salaried employee of Shino-Test Co., Ltd., which provided ELISA for periostin without stipulations or influence over the interpretation of test results. The authors have no other potential or actual conflicts of interest pertaining to the contents of this article.

AUTHOR CONTRIBUTIONS

H.G. designed the study, carried out the analyses, and drafted multiple iterations of the manuscript. H.O., K.N., S.N., K.I., and M.H. supervised the study and edited the manuscript. J.O. carried out the analyses and reviewed the manuscript. H.M., K.O., M.S., K.S., M.C., Y.K., H.I., Y.K., N.K., and K.H. collected the samples and reviewed the manuscript. All authors concur with the final manuscript are accountable for all aspects of the work.

FUNDING

This work was supported by Grants-in-Aid Scientific Research from the Japan Society for the Promotion of Science, KAKENHI (Grant number: 20K08233).

COMPETING INTERESTS

Junya Ono is a salaried employee of Shino-Test Co., Ltd. The remaining authors declare no competing interests.

PATIENT CONSENT STATEMENT

Informed consent was solicited from parents or other legal guardians, and documented in writing.

ADDITIONAL INFORMATION

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