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Juliana, Amadu; Plötz, Frans B; Achten, Niek; Bultman, Anita; Jongman, Rianne M; van Meurs, Matijs; Wilschut, Jan C; Zonneveld, Rens

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Requirement of respiratory support in acute bronchiolitis in infants is linked to endothelial and neutrophil activation

Amadu Juliana MD[1] | Frans B. Plötz MD, PhD[2,3] | Niek Achten MD, PhD[1] | Anita Bultman MD[1] | Rianne M. Jongman PhD[4,5] | Matijs van Meurs MD, PhD[5,6] | Jan C Wilschut PhD[7] | Rens Zonneveld MD, PhD[1]

[1]Academic Pediatric Center Suriname, Academic Hospital Paramaribo, Paramaribo, Suriname

[2]Department of Pediatrics, Tergooi Hospitals, Hilversum, The Netherlands

[3]Emma Children's Hospital, Amsterdam Medical Center, University of Amsterdam, Amsterdam, The Netherlands

[4]Department of Pathology and Medical Biology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[5]Department of Anesthesiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[6]Department of Critical Care, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

[7]Department of Medical Microbiology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands

Correspondence
Amadu Juliana, MD, Academic Pediatric Center Suriname, Academic Hospital Paramaribo, Flustraat 1 Paramaribo, Suriname.
Email: ajuliana@azp.sr

Abstract

Background: Evidence shows that activation of pulmonary vascular endothelium and neutrophils are involved in the pathophysiology of acute bronchiolitis. We hypothesized that levels of markers of endothelial activation and leukocyte counts are associated with requirement and duration of respiratory support.

Methods: Thirty-four infants with bronchiolitis and eight controls were included. Nasopharyngeal swabs and blood samples were taken at admission. Serum levels of Angiopoietin (Ang)-1, Ang-2, sP-selectin, sE-selectin, vascular cell adhesion molecule-1 (sVCAM-1), intercellular adhesion molecule-1 (sICAM-1), and leukocyte counts were measured. For univariate analysis, bronchiolitis cases were grouped into two groups, namely those not requiring and those requiring any form of respiratory support. To control for known risk factors for poor outcome (i.e., age, prematurity, and congenital heart disease), and for days post symptom onset, linear regression analysis was performed with duration of any type of respiratory support in days.

Results: Ang-2 levels, Ang-2/Ang-1 ratios, sE-selectin levels, immature neutrophil count, and neutrophil/lymphocyte ratio (NLR) were higher in acute bronchiolitis versus controls. Ang-2, and NLR levels were significantly higher, and lymphocyte counts significantly lower, in infants that required respiratory support versus those that did not. Ang-2 levels (β: .32, 95% confidence interval [CI]: 0.19–1.19) and NLR (β: .68, 95% CI: 0.17–1.19) were positive predictors for the duration of respiratory support.

Conclusions: Markers of endothelial and neutrophil activation are associated with respiratory support for acute bronchiolitis. Admission Ang-2 levels and NLR may be promising markers to determine requirement of respiratory support and deserve further study.

KEYWORDS
acute bronchiolitis, endothelium, infants, neutrophils, severity

Abbreviations: Ang, angiopoietin; CAM, cell adhesion molecule; LOS, length of stay; nCPAP, nasal continuous positive airway pressure; NLR, neutrophil/lymphocyte ratio; RSV, respiratory syncytial virus; sICAM-1, soluble intercellular adhesion molecule-1; SpO_2, transcutaneous hemoglobin oxygen saturation; sVCAM, soluble vascular cellular adhesion molecule-1.

1 | INTRODUCTION

Acute bronchiolitis is the leading lower respiratory tract infection among infants.[1] Viruses, especially respiratory syncytial virus (RSV), are the predominant cause of bronchiolitis.[2,3] The mechanisms that drive severe disease and the requirement of respiratory support remain to be elucidated.

It is currently widely accepted that neutrophil influx into the airways is a hallmark of the pathophysiology of acute bronchiolitis.[4–6] However, recent evidence indicates that the severity of bronchiolitis may be related to the involvement of the pulmonary vascular endothelium and interaction of neutrophils with and migration across endothelial cells.[7] Both indirect activation through alveolar epithelial cell signaling and direct viral infection of endothelial cells drives upregulation and release of cell adhesion molecules (CAMs), particularly intercellular adhesion molecule-1 (ICAM-1).[8–10] These molecular changes indicate a "pro-adhesive state" of the vascular endothelium during acute bronchiolitis as a prerequisite for leukocyte adhesion. Higher neutrophil counts in lung aspirates are associated with the severity of acute bronchiolitis.[11–13] While circulating neutrophil counts increase, lymphocyte counts decrease in acute bronchiolitis, driving a higher neutrophil/lymphocyte ratio (NLR).[14] Angiopoietin (Ang)-1 and Ang-2 are both ligands of the endothelial Tie-2 receptor. Circulating levels of Ang-1 decrease and Ang-2 increase during inflammation and infection, representing decreases in endothelial barrier integrity.[15] Clinically, increased levels of Ang-2 are associated with the development and outcome of acute lung injury and ARDS.[16,17] It remains to be elucidated if changes in the levels of these biomarkers are also associated with the requirement of respiratory support in infants with acute bronchiolitis.

For this study, we hypothesized that Ang-1 levels are lower, and that Ang-2 levels, Ang-2/Ang-1 ratio, sCAM levels (immature) neutrophil counts, and NLR, are higher among infants with acute bronchiolitis versus controls at admission, and that these levels are associated with requirement, and duration of respiratory support during admission.

2 | MATERIALS AND METHODS

2.1 | Study design and subjects

A prospective observational cohort study was performed at the Academic Pediatric Center Suriname at the Academic Hospital Paramaribo, Suriname. Patients were included in a period of four months between December 5th, 2016 and April 10th, 2017. Infants aged 0–12 months admitted with a clinical diagnosis of acute bronchiolitis were considered for inclusion. The clinical diagnosis consisted of presence of rhinorrhea, coughing, rales, crackles, and/or wheezing on physical examination. Informed consent was obtained of at least one parent or guardian before inclusion. Infants with (known) congenital lung disease, immunodeficiency, or malignancy were excluded. Also excluded were infants of whom no serum was available for biomarker analysis. Controls were infants that visited the outpatient clinic at our hospital for other reasons than infectious illnesses, and for whom blood sampling was indicated for other reasons, and without presence of any of the exclusion criteria. Supportive therapy consisted of frequent nasal aspiration and, according to local protocol, respiratory support and/or supplemental oxygen therapy was started when oxygen saturations were constantly <90%. At the time of this study, our pediatric high and intensive care unit facility was not yet operational, and patients were only transferred to the neonatal intensive care unit when mechanical ventilation was indicated. The study protocol was approved by the Surinamese Medical-Ethical Board (VG 02-14A-2015_16).

2.2 | Data collection

For all infants a nasopharyngeal swab and blood sample were taken at admission. Medical history, risk factors for severe disease (i.e., age, prematurity, and congenital heart disease [CHD]), days post symptom onset, and findings on initial physical examination (i.e., bodyweight, temperature, respiratory rate, pulse rate, transcutaneous hemoglobin oxygen saturation (SpO2), and presence of tachypnea, nasal flaring, and chest retractions) were recorded on a case report form. Weight was corrected for age using weight-for-age *z*-scores according to the WHO Growth Standard.[18] Tachypnea was defined as a respiratory rate above 60 or 50 breaths per minute for infants below 2 months or those between 2 and 12 months of age, respectively. Type and duration of respiratory support, laboratory results, including total leukocyte and neutrophil counts, viral PCR results, length of stay (LOS), and mortality were extracted from the medical charts and laboratory information systems after the inclusion period. Infants were divided into two groups, namely those without or with the need for any form (i.e., supplemental oxygen via nasal cannula, nasal continuous positive airway pressure [nCPAP], mechanical ventilation) of respiratory support.

2.3 | Sample collection and analysis

Nasopharyngeal swabs sample handling and analysis were done as described before.[19] In short, extraction of genetic material was performed with the QIAamp MinElute Virus Spin Kit (Qiagen) and detection of viruses was performed with the Respifinder SMART 22 multiplex PCR kit (Pathofinder BV) on a Lightcycler 480 (Hoffmann La Roche), according to the manufacturers' instructions. Blood samples were collected in serum microtainers using venipuncture, capillary collection, or blood collection during the insertion of a venous cannula. Total cell counts were measured routinely with a Sysmex XT 2000i analyzer (Sysmex). To obtain serum, blood was allowed to clot at room temperature and serum was separated by centrifugation at 2300*g* for 8 min and stored at −80°. Analysis was performed after only one freeze-thaw cycle to prevent stability issues of the biomarkers. Levels of Ang-1 and Ang-2 were measured using ELISA

(R&D Systems DANG10 and DANG20 Quantikine ELISAs). Levels of sE-selectin, sP-selectin, soluble vascular cell adhesion molecule (VCAM-1), and soluble intercellular adhesion molecule (ICAM-1) were measured using Luminex (Human Adhesion 6-plex; Thermo Fisher Scientific). All assays were performed according to the manufacturers' instructions. Ang-2/Ang-1 ratios and NLR were calculated.

2.4 | Statistical analysis

Categorical variables were presented as numbers and percentages, and analyzed with χ^2 or Fisher exact test. Continuous variables were presented as median and interquartile range (IQR). Mann–Whitney or in case of more than two groups, Kruskal–Wallis test, were used for analysis of continuous variables, controlling the false discovery rate at 0.1 for omnibus testing with a Benjamini-Hochberg[20] adjusted *p* value. Post-hoc testing was done according to Dunn's method with Holm's correction for multiple comparisons. Robust linear regression analysis was performed to control for age, prematurity, CHD, and days post symptom onset with total days of respiratory support of any type as the dependent variable to maximize the use of available data points. We entered the independent admission variables age, gender, weight-for-age *z*-score, prematurity, congenital heart disease, days post symptom onset, levels of the Angiopoietins and sCAMs, and leukocyte counts, and subsequently removed the variables with the highest *p*-values, except for age, prematurity, CHD, and days post symptom onset. Results of linear regression analysis were expressed as the coefficient beta (β) with 95% confidence intervals (CI). All calculations were made using computer software JASP version 0.14.1 (University of Amsterdam), R version 3.5.2 (R Foundation), and Graphpad Prism, Version 9.0.2 (Graphpad Software, Inc.).

3 | RESULTS

3.1 | Descriptive statistics

A total of 34 infants with acute bronchiolitis were included, of whom 12 did not, and 22 did require respiratory support (Table 1). A total of eight controls were included, which were three infants evaluated for maternal HIV (and whom tested negative), three evaluated before anorectal malformation surgery, and two recruited at follow-up visits for ABO blood group antagonism and convulsions, respectively. Median age of infants with acute bronchiolitis was 126 days (IQR: 126) with no differences between groups. No differences in weight-for-age *z*-score, gender and presence of prematurity and congenital heart disease were observed. Median days post symptom onset was higher in infants not requiring versus those requiring respiratory support ($p = .008$). A positive viral polymerase chain reaction (PCR) result was found in 66% of the patients, of which 76% was RSV, with no differences between the groups.

3.2 | Levels of the angiopoietins

Median Ang-1 levels were not different between groups (Table 2 and Figure 1). Median Ang-2 levels were higher in infants requiring respiratory support (7.4 ng/ml, IQR: 4.1) versus controls (4.9 ng/ml, IQR: 2.4), and versus infants not requiring respiratory support (5.4 ng/ml, IQR: 1.4), $p = .042$ and $p = .042$, respectively. Ang-2/Ang-1 ratios were higher in infants requiring respiratory support (0.14, IQR: 0.09) versus controls (0.09, IQR: 0.01), $p = .019$. Linear regression analysis revealed that admission Ang-2 level was a predictor of duration of respiratory support (Table 3).

3.2.1 | Levels of soluble CAM

Median sE-selectin levels were higher in cases not requiring and those requiring respiratory support (1134 ng/ml, IQR: 599 and 1415 ng/ml, IQR: 843) versus controls (709 ng/ml, IQR: 194), $p = .034$ and $p = .001$, respectively (Table 2 and Figure 1). The levels of other sCAMs were not different between the groups, and none of the sCAMs predicted the duration of respiratory support.

3.3 | Leukocyte counts

Immature neutrophil counts were higher in cases not requiring and those requiring respiratory support (0.02 $\times 10^9$/L, IQR: 0.01 and 0.02 $\times 10^9$/L, IQR: 0.01) versus controls (0.005 $\times 10^9$/L, IQR: 0.01), $p = .025$ and $p = .001$, respectively (Table 2 and Figure 1). Neutrophil counts were higher in infants requiring respiratory support (6.1 $\times 10^9$/L, IQR: 5.5) versus controls (2 $\times 10^9$/L IQR: 2.7), $p = .016$. Lymphocyte counts were lower in infants requiring respiratory support (4.7 $\times 10^9$/L, IQR: 3.5) versus controls (7.2 $\times 10^9$/L, IQR: 2.5) and versus infants not requiring respiratory support (5.9 $\times 10^9$/L, IQR: 3.8), $p = .041$ and $p = .048$, respectively. NLR was higher in infants requiring respiratory support (1.07, IQR: 2.45) versus controls (0.36, IQR: 0.21), and versus infants not requiring respiratory support (0.58, IQR: 0.94), $p = .01$ and $p = .027$, respectively. Admission NLR was a predictor of duration of respiratory support (Table 3).

4 | DISCUSSION

In this translational observational study, we found substantial evidence for endothelial and neutrophil activation in infants with acute bronchiolitis. Levels of the Angiopoietins, and neutrophil and lymphocyte counts were associated with respiratory support for acute bronchiolitis as a proxy for severity of disease. Although sE-selectin levels were higher in acute bronchiolitis compared to controls, sCAM levels were not associated with the severity of disease. After controlling for age, prematurity, CHD, and days post symptom onset, Ang-2 levels and NLR at admission predicted the total number of days of respiratory support of any

TABLE 1 Descriptive statistics of the study group at hospital admission

				Acute bronchiolitis		
			Controls $n = 8$	Not requiring respiratory support $n = 12$	Requiring respiratory support $n = 22$	p values
Demographic variables	Age in days, median (IQR)		116 (256)	157 (89)	99 (102)	.135
	Weight-for-age z-score, median (IQR)		1.9 (2.7)	-0.5 (1.4)	−0.8 (2.3)	.934
	Female, n (%)		2 (25)	3 (25)	7 (32)	.888
Comorbidity	Prematurity, n (%)			1 (8)	5 (23)	.389
	Congenital heart disease, n (%)			0	2 (9)	.529
Clinical variables	Days post symptom onset, median (IQR)			6.5 (2.3)	3 (3.5)	**.008**
	Tachypnea, n (%)			4 (33)	11 (50)	.476
	Nasal flaring, n (%)			4 (33)	10 (46)	.717
	Chest retractions, n (%)			6 (50)	21 (95)	**.004**
	SpO_2, median (IQR)			98 (4)	92 (7)	**.002**
	Maximum respiratory support received (%)	Low flow nasal canula			15 (68)	
		nCPAP			5 (23)	
		Mechanical ventilation			2 (9)	
	Total days of respiratory support, median (IQR)			0	4 (5.5)	
	Length of stay in days, median (IQR)			2 (2)	5 (2)	**<.01**
	Death, n (%)			0	1 (5)	1
Viral cause	Respiratory Syncytial Virus, n (%)			5 (42)	11 (50)	.617
	Rhino/Enterovirus, n (%)			1 (8)	1 (5)	
	Parainfluenzavirus, n (%)			1 (8)	1 (5)	
	Adenovirus, n (%)			1 (8)	0	
	Negative, n (%)			3 (25)	8 (36)	
	Missing, n (%)			1 (8.3)	1 (5)	

Note: Bold values are statistically significant.

Abbreviations: IQR, interquartile range; nCPAP, nasal continuous positive airway pressure; SpO_2, transcutaneous hemoglobin oxygen saturation.

type. These findings have both pathophysiological and clinical implications.

This is the first study to report an association between the serum levels of Angiopoietins and severity of acute bronchiolitis. Admission Ang-2 levels were associated with acute bronchiolitis and predicted the requirement of respiratory support. Ang-2 is released from endothelial Weibel-Palade bodies upon stimulation by bacterial or viral infection, hypoxia, or altered shear stress.[15] Ang-2/Tie-2 signaling leads to loss of endothelial integrity and upregulation of CAMs.[15] Our findings are similar to observations in earlier studies among children, in which increased Ang-2 levels predicted worse outcome in acute respiratory distress syndrome and sepsis.[21,22] Admission Ang-1 levels were not associated with the requirement of respiratory support. Ang-1 is constitutively released from vascular pericytes and Ang-1/Tie-2 signaling leads to endothelial stability and downregulation of CAMs.[15] The association of Ang-1 with severity of disease is less pronounced compared to Ang-2, and therefore less likely to reach statistical significance in a small study group.[15,23–25] In summary, when the requirement of respiratory support is regarded as a proxy of severity of acute bronchiolitis, loss of endothelial integrity appears to play a role in severe bronchiolitis.

The first sCAM to appear in the circulation after a shift in Ang-2/Ang-1 balance is sE-selectin.[26] We found higher median sE-selectin levels in infants with acute bronchiolitis versus controls. This finding is similar to results from animal models that show the importance of sE-selectin in neutrophil rolling on endothelial cells.[27] No associations between levels of sCAMs and the requirement of respiratory support were found. However, levels of sCAMs are a result of a complex interplay between their production, upregulation, and shedding from endothelial cells.[28] Shedding of CAMs results in a decrease of their

TABLE 2 Serum levels of the angiopoietins and soluble cell adhesion molecules, and neutrophil and lymphocyte counts

		Acute bronchiolitis				Pair-wise post-hoc comparisons (z, p^{holm})		
	Controls $n = 8$	Not requiring respiratory support $n = 12$	Requiring respiratory support $n = 22$	η^2	p	Controls vs. bronchiolitis not requiring respiratory support	Controls vs. bronchiolitis requiring respiratory support	Bronchiolitis not requiring vs. requiring respiratory support
Angiopoietins	$n = 7$	$n = 11$	$n = 21$					
Ang-1 (ng/ml)	58.1 (21.2)	53.6 (29.9)	49.6 (16.6)	0.04	.762	0.401, 0.701	0.727, 0.701	0.333, 0.701
Ang-2 (ng/ml)	4.9 (2.4)	5.4 (1.4)	7.4 (4.1)	**0.14**	**.030**	−0.396, 0.346	**−2.197, 0.042**	**−2.061, 0.042**
Ang-2/Ang-1 ratio	0.09 (0.01)	0.10 (0.04)	0.14 (0.05)	**0.233**	**.006**	−1.456, 0.081	**−3.101, 0.003**	−1.744, 0.081
Cellular adhesion molecules	$n = 7$	$n = 11$	$n = 21$					
P-Selectin (ng/ml)	730 (157)	904 (526)	839 (429)	0.045	.823	−0.507, 0.810	−0.612, 0.810	−0.060, 0.810
E-Selectin (ng/ml)	709 (194)	1134 (599)	1415 (843)	**0.248**	**.004**	**−2.120, 0.034**	**−3.302, 0.001**	−1.117, 0.132
VCAM-1 (ng/ml)	7016 (2473)	12325 (6063)	11841 (4549)	0.078	.090	−1.919, 0.055	−2.086, 0.055	0.045, 0.482
ICAM-1 (ng/ml)	1537 (979)	2037 (786)	1942 (1390)	0.091	.071	−2.266, 0.035	−1.244, 0.137	1.485, 0.137
Leukocytes	$n = 8$	$n = 12$	$n = 20$*					
Total WBC count ($\times 10^9$/L)	11 (2.73)	13.4 (8.9)	13.4 (6.2)	0.033	.678	−0.734, 0.594	−0.849, 0.594	−0.055, 0.594
Neutrophil count ($\times 10^9$/L)	2 (2.7)	5 (4.7)	6.1 (5.5)	**0.123**	**.038**	−1.640, 0.101	**−2.556, 0.016**	−0.879, 0.190
Immature Neutrophils ($\times 10^9$/L)	0.005 (0.01)	0.02 (0.01)	0.02 (0.01)	**0.258**	**.003**	**−2.234, 0.038**	**−3.397, 0.001**	−1.020, 0.154
Lymphocyte count ($\times 10^9$/L)	7.2 (2.5)	5.9 (3.8)	4.7 (3.5)	**0.127**	**.037**	0.440, 0.330	**2.208, 0041**	**1.979, 0.048**
NLR	0.36 (0.21)	0.58 (0.94)	1.07 (2.45)	**0.347**	**<.001**	−1.593, 0.056	**−3.662, <0.001**	**−2.214, 0.027**

Note: Results per group as median and interquartile range. Eta squared (η^2) effect sizes (0.01–0.039: small effect, 0.04–0.110: intermediate effect, 0.140–0.200: large effect) and p-values (Benjamini-Hochberg20 adjusted $p = .058$.) of the Kruskal-Wallis test for differences between groups including controls. *$n = 19$ for lymphocytes and NLR. The infants with missing Ang and CAM serum levels were not the same as those with missing leukocyte counts. Bold values are statistically significant.

Abbreviations: Ang-1, angiopoietin-1; Ang-2, angiopoietin-2; ICAM-1, intercellular adhesion molecule-1; NLR, neutrophil/lymphocyte ratio; VCAM-1, vascular cellular adhesion molecule-1; WBC, white blood cell.

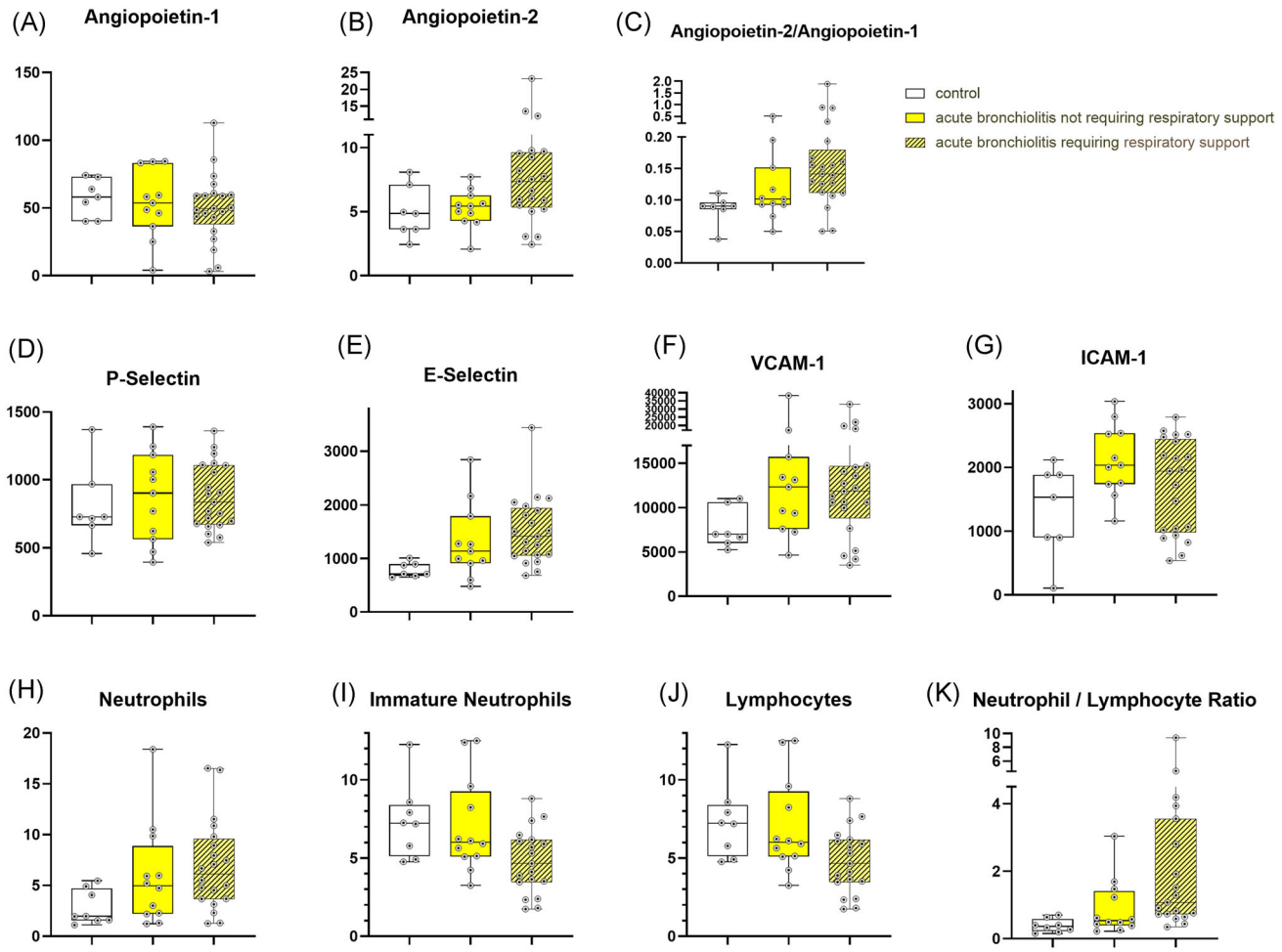

FIGURE 1 Levels of the angiopoietins (ng/ml), cellular adhesion molecules (ng/ml), and leukocyte counts ($\times 10^{9}$/L) in infants with acute bronchiolitis ($n = 34$) and controls ($n = 8$). (A) Angiopoietin-1. (B) Angiopoietin-2. (C) Ang-2/Ang-1, ratio. (D) sP-Selectin. (E) sE-Selectin. (F) sVCAM-1. (G) sICAM-1. (H) Neutrophils. (I) Immature neutrophils. (J) Lymphocytes. (K) Neutrophil/Lymphocyte ratio. Bars represent the interquartile range with median line. Dotted circles represent individual serum levels [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Robust linear regression analysis with total days of respiratory support as the dependent variable

Independent variables	Coefficient β	95% CI	t value
Age in days	−.0076	−0.01594, 0.00079	−1.7738
Prematurity	.0429	−1.92971, 2.01558	0.0427
DPSO	−.0967	−0.24798, 0.05459	−1.2527
CHD	4.5925	2.03236, 7.15266	3.5159
Ang-2	.2830	0.10243, 0.46354	3.0719
NLR	.6299	0.311692, 0.94806	3.8799

Note: Residual standard error: 1.275 on 22 degrees of freedom (5 missing observations deleted).

Abbreviation: CI, confidence interval; DPSO, days post symptom onset; NLR, neutrophil/lymphocyte ratio.

concentration on endothelial cell and leukocyte surfaces, thereby reducing interactions between these two cell types. Shedding also produces sCAMs as scavengers that bind circulating CAM ligands.[28] Last, evidence shows CAM-independent sequestration of neutrophils in the particularly narrow pulmonary capillaries.[29–32] It is not known whether and to what extent this plays a role in neutrophil efflux in acute bronchiolitis. Thus, in summary and in our opinion, sCAMs have a limited role as markers of acute bronchiolitis, consistent with findings in bacterial infection.[33,34]

Neutrophils are the predominant cells found in the lungs in acute bronchiolitis, and higher neutrophil counts in lung aspirates are associated with severe bronchiolitis.[6,11–13] Infants also appear more prone to severe disease due to the deleterious innate immune response, as opposed to a more targeted adaptive response in older children.[35–37] We found that higher neutrophil counts and lower

lymphocyte counts in peripheral blood are associated with the requirement of respiratory support and that the NLR is a significant predictor of total days of respiratory support. This agrees with an earlier retrospective study, in which lower lymphocyte counts were associated with RSV bronchiolitis and intensive-care admission.[14] The higher immature neutrophil counts in the bronchiolitis group indicate bone marrow activation after neutrophil efflux into the bronchiolitic lungs.[6,7] Last, the mechanistic and temporal relationship between increased serum Ang-2 levels and neutrophil efflux into the lungs appears complex. An ex vivo study showed that interaction of neutrophils and endothelial cells actually induced increased Ang-2 production in a mice model of acute lung injury.[38] This observation indicates that neutrophil efflux on itself causes loss of endothelial integrity, thereby augmenting more severe disease. Regardless of the pathophysiological underpinnings, based on our findings, we propose Ang-2 and NLR as promising markers of the requirement of respiratory support in acute bronchiolitis that deserve further study.

The results of this study show that Ang-2 and NLR may be used to determine the need for respiratory support at first evaluation. Acute bronchiolitis is generally thought to peak 3–5 days post symptom onset.[39] With regard to this, it is important to notice that infants that did not require respiratory support presented at Day 6 after symptom onset versus Day 3 in those that required respiratory support ($p = .008$). Thus, infants that did not require respiratory support may have passed peak severity of disease and resolution of symptoms could explain differences between groups in the levels of the investigated biomarkers. However, the infants not requiring respiratory support did not present sooner, which indicates mild disease before presentation. Moreover, days post symptom onset was not associated with duration of respiratory support in robust linear regression analysis. Nonetheless, further studies are necessary that address biomarker dynamics from onset to resolution of disease for complete understanding of the relationship of neutrophil and endothelial activation with the need for respiratory support and severity of acute bronchiolitis.

Strengths of this study are the prospective character, presence of a control group, and the fact that the study group is representative of daily clinical practice. Limitations include the absence of nasal aspirates to test for neutrophil presence and activity in the airways. Also, the relatively small number of infants with only two cases of mechanical ventilation, precluded further stratification of disease severity, yet *post-hoc* analysis after exclusion of these two cases did not significantly change results.

In conclusion, this translational study shows that the vascular endothelium and neutrophils are activated in acute bronchiolitis. Admission Ang-2 level and NLR are promising markers of the requirement of respiratory support and deserve further study.

ETHICS STATEMENT

The study protocol was approved by the Surinamese Medical-Ethical Board (VG 02-14A-2015_16).

AUTHOR CONTRIBUTIONS

Frans B Plötz: formal analysis (supporting); methodology (supporting); supervision (supporting); validation (supporting); writing original draft (supporting); writing review & editing (supporting). Anita Bultman: data curation (equal); formal analysis (supporting); investigation (equal); project administration (equal); validation (supporting); writing review & editing (supporting). Rianne M. Jongman: investigation (equal); writing review & editing (supporting). Matijs van Meurs: investigation (supporting); supervision (supporting); writing review & editing (supporting). Jan C. Wilschut: conceptualization (supporting); methodology (supporting); supervision (supporting); writing original draft (supporting); writing review & editing (supporting).

ORCID

Amadu Juliana http://orcid.org/0000-0002-9844-7235

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