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Correlation between urinary and serum NT-proBNP in acute bronchiolitis: A pilot study

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Abstract

Background and Aims: We aimed to analyze the correlation of urinary with serum N-terminal pro-brain natriuretic peptide (NT-proBNP) concentrations and its association with severity in acute bronchiolitis.

Material and Methods: A pilot observational study was conducted between October 1, 2021 and March 31, 2022 including acute bronchiolitis cases who attended our institution. Serum and urinary NT-proBNP concentrations were determined using the Alere i NT-proBNP assay in time-matched urine and blood samples. The Mann–Whitney *U* test, Spearman's correlations, and simple linear regression were utilized to analyze the association of urine NT-proBNP levels with serum NT-proBNP and with variables indicative of severe bronchiolitis.

Results: Seventeen infants (median age 68 [IQR: 36–91] days) with 36 time-matched samples were included. The urine NT-proBNP was positively and strongly correlated with the serum NT-proBNP concentrations (Spearman's $\rho = 0.81$ & R^2 coefficient = 0.751; $p < 0.001$), and increased with higher C-reactive protein, ($p = 0.004$), procalcitonin ($p = 0.001$), and pCO_2 ($p = 0.029$) levels. The initial urinary NT-proBNP concentrations were higher in those infants that required ventilatory support compared with those without this outcome (1.85 [IQR: 1.16–2.44] vs. 0.63 [IQR: 0.45–0.84] pg/mg); $p < 0.001$); and resulted positively and strongly correlated with the duration of the ventilatory support (Spearman's $\rho = 0.76$; $p < 0.001$) and the length of stay hospitalization (Spearman's $\rho = 0.84$; $p < 0.001$).

Conclusion: The urinary NT-proBNP concentrations could be a reliable surrogate for serum NT-proBNP levels and resulted elevated in cases of acute bronchiolitis with complicated evolution, suggesting a potential as a noninvasive tool to assess severity in this setting.

KEYWORDS

acute bronchiolitis; biomarkers; infections: pneumonia, TB, viral; myocardial strain; natriuretic peptides; pulmonary hypertension; urinary NT-proBNP

1 | INTRODUCTION

N-terminal pro-brain natriuretic peptide (NT-proBNP) is a low molecular weight peptide (8.5 kDa) expressed in the ventricular myocardium secondary to pressure and volume increases that has diuretic and natriuretic effects and is the most used biomarker for diagnosis and prognosis in heart failure.[1–3] There has been recent research interest in NT-proBNP as a reliable biomarker in several pediatric scenarios.[4] Increasing evidence supports the use of serum NT-proBNP levels as a potential biomarker of myocardial strain and disease severity for respiratory conditions, including acute bronchiolitis.[5–8] Serial monitoring of serum NT-proBNP concentrations in these infants would require multiple blood sampling through venipuncture in an otherwise vulnerable population.

NT-proBNP is a nonbiologically active molecule with no active clearance mechanisms that is removed from plasma via passive excretion mainly by the kidney.[1,3,9,10] The investigations in premature newborns and infants with congenital heart diseases (CHD) suggest that urine NT-proBNP determination could be easily performed with current kit assays for serum NT-proBNP determination.[11–14] Therefore, urinary NT-proBNP may have the potential as a noninvasive and reliable biomarker of severity in acute bronchiolitis that has not yet been investigated.

This pilot study aims to determine urinary NT-proBNP in a cohort of infants with acute bronchiolitis, analyze its correlation with serum NT-proBNP concentrations, and explore its association with the severity of the disease. The hypothesis was that concentrations of both serum and urine NT-proBNP are correlated, and therefore, NT-proBNP levels would indicate severe acute bronchiolitis by using urine instead of blood analysis.

2 | MATERIALS AND METHODS

2.1 | Design, setting, and patients

This prospective observational study was conducted between October 1, 2021 and March 31, 2022 in the Pediatric Department of a Tertiary University Hospital in Spain after the approval by the ethics committee of our institution (approval number: 1338-N-20; October 2021). We included infants less than 1-year-old hospitalized with acute bronchiolitis of any severity. The diagnosis and management of infants with bronchiolitis were made following current international recommendations.[15] Infants with significant congenital anomalies, including cardiac diseases, chronic renal diseases, acute kidney injury, incomplete data, and refusal of parental consent, were excluded from the final analysis.

2.2 | Specimen collection

Time-matched urine and blood samples were collected at the time of inclusion, always before initiation of any inotropic or ventilatory support. If follow-up blood or urine laboratory analysis were solicited during the episode of hospitalization at the discretion of the attending pediatrician, new time-matched samples of blood and urine were collected in those patients. Blood samples were obtained by venipuncture, and urine samples were obtained by urethral catheterization and urine bag. The attending pediatrician chose the method of urine collection according to the clinical characteristics of the patients. All blood samples were always obtained after collecting urine samples, with a variable time interval never exceeding 10 min. The attending physician evaluated the respiratory involvement of the included cases by the clinical severity score of San Joan de Deu Hospital (BROSJOD)[16] at the time of specimen collection. The pH, pCO_2, HCO_3, lactate, C-reactive protein (CRP), procalcitonin, creatinine, and sodium were also determined in these blood samples.

2.3 | NT-proBNP analysis

Fresh samples (at least 2 ml) were immediately sent for analysis, without being frozen, to our institution's certified clinical chemistry laboratory. Serum and urinary concentrations of NT-proBNP were determined using a chemiluminescent micro-particle immunoassay, Alere NT-proBNP, for Alinity i assay (Abbott). The intra-assay and inter-assay coefficients of variation were 1.9% to 2.9% and 2.6% to 5.4%, respectively, with an analytical range of 8.3–35,000 pg/ml. No subjects had serum and urine NT-proBNP concentrations lower or higher than the assay linearity limit. These assays and quality controls were performed according to the manufacturer's recommendations. Because the samples were not obtained in a predefined time in all patients, we corrected the urine NT-proBNP by urine creatinine levels (urine NT-proBNP/creatinine ratio in pg/mg) to address a potential bias caused by different urine concentrations and to reduce intersubject variability. To address the limitation of the substantial age-dependency of the levels of NT-proBNP in neonates and infants, we calculated Z-log values (Z-score for skewed variables) adjusted for age in days as previously reported.[17]

2.4 | Research endpoints

The primary outcome of this study was the relationship between serum and urine NT-proBNP levels. The secondary outcome was the development of severe acute bronchiolitis. We selected the longer length of stay (LOS) hospitalization, the need of PICU admission for ventilatory support (invasive or noninvasive), and the longer duration of ventilatory support as clinical outcomes indicative of severe bronchiolitis.

2.5 | Statistics

Mean ± standard deviation (SD) (median and 25th–75th percentiles [IQR] where appropriate) and proportions were reported for continuous and categorical variables, respectively. Since the serum NT-proBNP and urinary NT-proBNP/creatinine ratio concentrations exhibited skewed distributions, the log-10 transformed (log10) values were used in the statistical analysis to stabilize variances. The Z-log-NT-proBNP presented a normal distribution and was directly used in the statistical analysis. The

TABLE 1 Baseline characteristics at the time of admission and outcomes of the study population

Variable	Admission samples ($n = 17$)
Demographic and clinical data	
Age (days)[a]	68 (36–91)
Weight (kg)[a]	4.9 (4.4–5.5)
Gender (male)[b]	11 (65)
GA (weeks)[a]	38 (33^{+4} to 39^{+5})
pCA (weeks)[a]	46^{+1} (45^{+5} to 50)
Comorbidities[b]	4 (23) prematurity (all GA < 36 weeks)
	1 of them (5) BDP & hs-PDA
BROSJOD score[a]	7 (6–10)
BROSJOD >10 points[b]	5 (29)
RSV positive[b]	7 (41)
Laboratory data	
pH[a]	7.34 (7-28–7.39)
pCO_2 (mmHg)[a]	50 (45–56)
HCO_3 (mEq/L)[a]	25.5 (24.3–28.1)
Lactate (mmol/L)[a]	2 (1.6–3.5)
CRP (mg/dl)[a]	13.2 (2–64)
Procalcitonin (mg/dl)[a]	0.14 (0.09–0.38)
Serum creatinine (mg/dl)[a]	0.39 (0.38–0.41)
Plasmatic sodium (mEq/L)[a]	138 (136–139)
Urine creatinine (mg/dl)[a]	22 (13–34)
NT-proBNP values	
Serum NT-proBNP (pg/ml)[a]	899 (307–2279)
Log-10-serum-NT-proBNP[a]	2.95 (2.48–3.35)
Serum Z-log for age of NT-proBNP[a]	1.25 (0.31–1.57)
Z-log-NT-proBNP >1.96[a]	4 (23)
Urine NT-proBNP (pg/ml)[a]	218 (74–625)
NT-proBNP/creatinine (pg/mg)[a]	7 (4.1–14.7)
Log-10-urine NT-proBNP/creatinine (pg/mg)[a]	0.84 (0.61–1.16)
Treatment	
Oxygen (nasal canulae)[b]	13 (76)
Noninvasive ventilation[b]	6 (35)
Mechanical ventilation[b]	3 (17)
Inotropic support[b]	1 (7)
Antibiotics[b]	3 (17)
Diuretics[b]	1 (6)
Clinical outcomes	
PICU admission[b]	6 (35)
Duration of respiratory support (nasal canulae + MV + NIV) (days)[a]	2 (1–5)
LOS hospitalization (days)[a]	4 (2–11)
Death or sequel[b]	0 (0)

Abbreviations: BPD, bronchopulmonary dysplasia; BROSJOD score, bronchiolitis score of Sant Joan de Deu; CRP, C-reactive protein; GA, gestational age; hs-PDA, hemodynamically significant patent ductus arteriosus; LOS, length of stay; NT-proBNP, N-terminal pro-brain natriuretic peptide; pCA, post conceptual age; PICU, pediatric intensive care unit; *p* value, statical significance; RSV, respiratory syncytial virus.

[a]Data presented in median y interquartile range.

[b]Data presented in frequency and percentage.

relationship between urine and serum NT-proBNP concentrations was assessed using Spearman's correlation coefficient and linear regression analysis, where the strength of correlation was evaluated by the squared correlation coefficient (R^2). All time matched samples were used ($n = 36$ samples from 17 patients) for this purpose. The Mann–Whitney *U* test, Spearman's correlations, and simple linear regression were utilized to describe and analyze the association of urine NT-proBNP levels with different clinical and laboratory variables indicative of severity. For this analysis we used only the first sample obtained from each patient before starting any inotropic or ventilatory support ($n = 17$) to avoid possible influences of these treatments on the value of urine NT-proBNP. Because of the potential for type I error due to multiple comparisons, findings for analyses should be interpreted as exploratory. A sample size estimation was not performed due to the exploratory nature of our study. All tests were two-sided, and a $p < 0.05$ was considered statistically significant. We used Stata v.16 software (StataCorp).

3 | RESULTS

3.1 | Descriptive analysis

Twenty patients were initially assessed for enrollment, and 3 cases were excluded due to incomplete laboratory data. Therefore, 17 infants (median age 68 [IQR: 36–91] days; 11 [65%] male sex) with 36 time-matched samples were included in the final analysis. Four (23%) patients were premature infants born at $28,^{+3}$ $33,^{+4}$ $34,^{+5}$ and 36^{+1} weeks of gestational age (GA), who presented with a post conceptual age (pCA) of $59,^{+3}$ $37,^{+2}$ 47, and 45^{+6} weeks respectively. The infant born at 28^{+3} weeks had a hemodynamically significant patent ductus arteriosus (hs-PDA) closed percutaneously with a pCA of 33^{+2} weeks, and moderate bronchopulmonary dysplasia (BPD), with normal echocardiogram and under any treatment at neonatology discharge with 38^{+3} weeks of pCA. Two samples were determined at the emergency room in 2 infants that did not require hospitalization, 18 samples were determined in 9 cases at

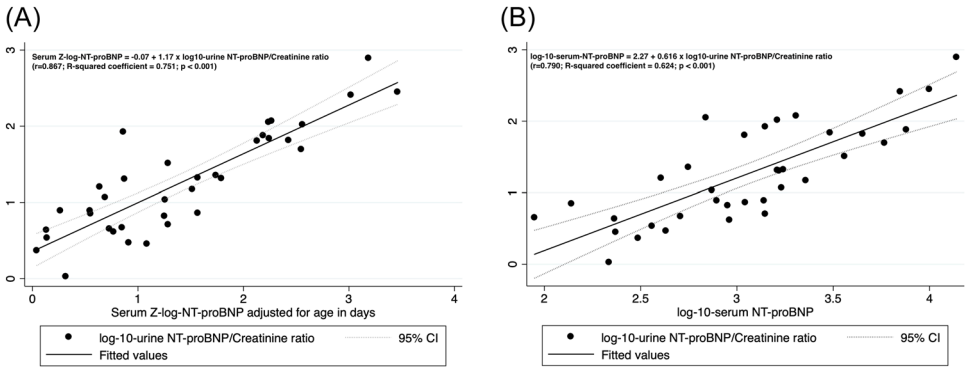

FIGURE 1 Correlation between urinary and serum concentrations of NT-proBNP. The vertical axis (urinary NT-proBNP/creatinine ratio) is log-10-transformed because of its skewed distribution; and the horizontal axis is expressed as Z-log values adjusted for age (A) and log-10-transformed serum NT-proBNP (B). The linear regression curve is shown in a continuous line and the 95% confidence interval (95% CI) in discontinued lines. NT-proBNP, N-terminal pro-brain natriuretic peptide.

the pediatric ward, and 16 samples were determined in 6 patients that required PICU admission and ventilatory support. Echocardiography was performed only in the patient that required inotropic support, showing signs of severe biventricular dysfunction secondary to the respiratory involvement that recovered progressively until complete spontaneous normalization, with no final diagnosis of any primary cardiac disease. Table 1 shows the baseline characteristics and clinical outcomes of the 17 cases included.

3.2 | Correlation between urinary and serum NT-proBNP

In the 36 samples obtained, the median concentrations of serum NT-proBNP resulted significantly higher than those of urine NT-proBNP (1246 [IQR: 470–2158] vs. 300 [QR: 114–1192] pg/ml; $p < 0.001$). The mean log-10-serum NT-proBNP values were 3 (SD: 0.52) pg/ml. The mean serum Z-log-NT-proBNP was 1.41 (SD: 0.91), with 11 (30%) samples showing raised serum NT-proBNP (Z-log >1.96). The median urine NT-proBNP/creatinine ratio was 15.3 (IQR: 4.8–66.6) pg/mg, and the mean log-(10)-urine NT-proBNP/creatinine ratio was 1.26 (SD: 0.67) pg/mg. The log-(10)-urine NT-proBNP/creatinine ratio was positively and strongly correlated with the log-10-serum-NT-proBNP concentrations (Spearman's $\rho = 0.78$ & R^2 coefficient = 0.624; $p < 0.001$). This correlation resulted improved when using the serum Z-log-NT-proBNP values (Spearman's $\rho = 0.81$ & R^2 coefficient = 0.751; $p < 0.001$). The scatter plots and linear equations for these relationships are shown in Figure 1.

TABLE 2 Correlations and linear regression analysis between urinary concentrations of NT-proBNP and several clinical and serum parameters at the time of admission in this cohort ($n = 17$)

Log-10-urine NT-proBNP/ creatinine ratio	Correlation analysis		Simple linear regression analysis	
	Spearman's coefficient	*p* Value	Beta coefficient (SE)	*p* Value
Age	−0.27	0.111	−0.002 (0.001)	0.889
Weight	−0.56	0.018	−0.18 (0.09)	0.066
GA	−0.20	0.428	−0.08 (0.06)	0.221
pCA	−0.44	0.072	−0.04 (0.02)	0.136
BROSJOD score	0.11	0.680	0.02 (0.06)	0.709
Serum creatinine	−0.03	0.853	1.52 (2.33)	0.519
Serum sodium	0.15	0.381	0.02 (0.03)	0.602
CRP	0.54	0.025	0.006 (0.002)	0.004
Procalcitonin	0.65	<0.001	0.19 (0.05)	0.001
pH	−0.15	0.391	−1.22 (1.56)	0.441
pCO_2	0.38	0.034	0.02 (0.01)	0.029
HCO_3	0.42	0.018	0.05 (0.03)	0.084
Lactate	−0.20	0.270	−1.03 (0.12)	0.401

Abbreviations: BROSJOD score, bronchiolitis score of Sant Joan de Deu; CRP, C-reactive protein; GA, gestational age; NT-proBNP, N-terminal pro-brain natriuretic peptide; pCA, post conceptual age; *p* value, statical significance.

3.3 | Clinical factors associated with urinary NT-proBNP levels at admission

The linear regression analysis showed that urinary NT-proBNP concentration increased with higher CRP, PCT, and pCO_2 levels. We did not observe significant correlation with neither the chronological age, GA, nor the pCA (Table 2). The patient with BPD and closed hs-PDA presented non-statistically significant higher levels of NT-proBNP at admission compared with the other 16 cases (1.83 [IQR: 1.83–1.83] vs. 0.83 [IQR: 0.57–1.10] pg/mg; $p = 0.307$). The 4 premature infants also presented a tendence to have higher levels of urinary NT-proBNP than the 13 full-term infants (1.35 [IQR: 0.85–2.13] vs. 0.70 [IQR: 0.52–1.03] pg/mg; $p = 0.141$).

3.4 | Association of urinary NT-proBNP levels with outcomes

The log-10-NT-proBNP/creatinine ratio was higher at the time of hospital admission in those infants that required PICU admission with ventilatory support compared with those without this management (1.85 [IQR: 1.16–2.44] vs. 0.63 [IQR: 0.45–0.84] pg/mg); $p < 0.001$) (Figure 2), and resulted positively and strongly correlated with the duration of the ventilatory support (Spearman's $\rho = 0.76$; $p < 0.001$) and the LOS hospitalization (Spearman's $\rho = 0.84$; $p < 0.001$).

4 | DISCUSSION

4.1 | Main findings

This pilot study showed the feasibility of analyzing urinary NT-proBNP in young infants with acute bronchiolitis with the Alere NT-proBNP, for Alinity i assay. We observed a strong positive linear relationship between serum and urinary NT-proBNP concentration, with higher levels in patients who presented with a higher degree of inflammatory response (CRP and procalcitonin), a worse respiratory state (pCO_2), and who required longer ventilatory support and hospitalizations.

4.2 | Feasibility of urinary NT-proBNP in acute bronchiolitis

Several studies have demonstrated that NT-proBNP levels are detectable in the urine of preterm newborns with significant associations between elevated urinary NT-proBNP concentrations and neonatal morbidities such as hs-PDA, retinopathy of prematurity, and BPD with pulmonary hypertension.[12–14,18] Urinary NT-proBNP also seems promising as a screening tool for CHD in term newborns and has shown potential to differentiate simple and complex CHD.[11,19] Furthermore, urinary-NT-proBNP would be helpful in the pediatric ambulatory setting to assess heart failure when combined with clinical scores.[20] Like these populations, obtaining blood samples for testing in acute bronchiolitis is technically challenging for the healthcare provider and stressful and painful for the patient, especially in cases where repeated tests are needed for monitoring evolution. Evidence shows that it is possible to collect urine non-invasively, efficiently, and quickly, especially in children under 3 months of age, who constitute most of the hospitalized population with bronchiolitis.[21,22] With this study, we point out that urinary NT-proBNP could be adequately analyzed with the same laboratory kit as serum NT-proBNP (Alere i) in acute bronchiolitis. Therefore, using urine samples could be beneficial in these patients as it would replace the need for stressful blood sampling to measure NT-proBNP, even more, if repeated samples for monitoring evolution are required. We did not find previous studies using the Alere i assay to measure urinary NT-proBNP. Most relevant studies about urinary NT-proBNP in infants have used the Roche immunoassay, which was validated by Palmer et al.[9] in 2009 for this determination. Recently, Lau et al.[23] have demonstrated an almost perfect correlation ($r = 0.999$, 95% CI: 0.999–0.999, $p < 0.0001$) between plasmatic NT-proBNP values measured by Alere and by Roche assay. Awaiting a similar validation study, we assumed that urinary values determined by the Alere Assay would reflect plasmatic concentrations adequately. The results of our correlation analysis are in line with this affirmation.

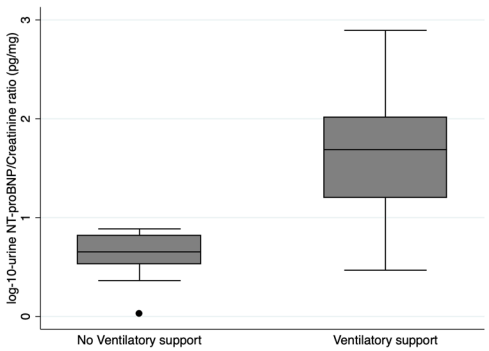

FIGURE 2 Comparison of log-10-urine NT-proBNP/creatinine ratio values of the first samples obtained from each patient after inclusion, between cases requiring or not PICU admission and ventilatory support. Urinary NT-proBNP/creatinine is log-10-scaled because of its skewed distribution. Patients that required PICU admission presented significantly higher median levels of log-10-urinary NT-proBNP/creatinine ratio before initiating ventilatory support (1.85 [1.16-2.44] vs. 0.63 (0.45–0.84) pg/mg); $p < 0.001$). NT-proBNP, N-terminal pro-brain natriuretic peptide.

4.3 | Urinary NT-proBNP as a surrogate of serum NT-proBNP

Several previous studies in adults demonstrated that NT-proBNP levels are detectable in the urine of patients with heart failure with a good correlation with plasma NT-proBNP levels in matched measurements.[24,25] The evidence on whether urinary NT-proBNP can replace serum NT-proBNP as a biomarker in pediatrics is scarce. In 2011, Kurihara et al.[26] reported a significant correlation with an R^2 coefficient of 0.548 in 36 urinary and plasmatic samples from 9 neonates aged 0–25 days. Recently, Müller et al.[11] studied the correlation between plasma and urine NT-proBNP in 83 children undergoing cardiac surgery using age-adjusted values for age and creatinine correction as we did. Notably, they also observed a significant strong positive correlation between the two parameters ($r = 0.78$ preoperatively and 0.87 postoperatively; $p < 0.001$). Another small sized study ($n = 33$) by these authors also showed an excellent correlation between plasma and urine NT-proBNP levels in 33 children with CHD ($r = 0.902$).[27] Our results are consistent with the scarce previous evidence, suggesting that urinary NT-proBNP concentrations could be used to surrogate serum levels in the acute bronchiolitis setting.

4.4 | Influence of clinical variables on urinary NT-proBNP levels

The plasmatic concentration of NT-proBNP in infants is clearly influenced by the chronological age, prematurity, and its related complications (BPD, hs-PDA, …).[17,28–32] As all these variables are well-known risk factors for worse outcomes in acute bronchiolitis, their influence on NT-proBNP concentrations should be considered before stablishing the use of this biomarker in this setting. In our exploratory analysis the urinary NT-proBNP levels resulted not associated with any of these parameters. The dynamics and values of NT-proBNP levels are similar in premature infants compared with healthy term infants, reaching stable plateau values after the first month of life.[30,31] The pCA and prematurity related comorbidities (BPD and hs-PDA) seem to affect NT-proBNP concentrations more than the GA, but their influence is rarely present after a pCA of >36 weeks.[28–32] Therefore, our small sample size including infants with a median pCA of 46^{+1} weeks, 4 premature infants all with pCA >36 weeks, and only 1 patient with BPD could explain our unexpected results. The observed tendence of higher levels of urinary NT-proBNP in premature infants and comorbidities suggests that including more patient with these characteristics would lead to find the influence previously described on NT-proBNP concentrations. Therefore, all these variables should be considered when interpreting NT-proBNP values in this setting.

4.5 | Association of urinary NT-proBNP with severity

There is increasing evidence supporting the role of NT-proBNP as a biomarker for myocardial strain in infants with severe bronchiolitis, and that it could help screen patients with a worse clinical evolution when used in conjunction with clinical scores.[5,33,34] In acute bronchiolitis, the airway obstruction and inflammation would affect pulmonary vascular tone increasing the right ventricular afterload with subsequent biventricular dysfunction,[8,35,36] leading to an increased release of NT-proBNP. This could be an explanation for the significant association observed between elevated urinary NT-proBNP concentrations and increased pCO_2, CRP, and procalcitonin levels in our patients. The enhancement of NT-proBNP synthesis and secretion would be more significant in those infants with a more severe respiratory impairment. Accordingly, we observed higher values of urinary NT-proBNP at early stages of hospitalization in those infants requiring PICU admission and with longer respiratory support and LOS hospitalization. As the urine and serum concentration of NT-proBNP resulted strongly and positively correlated in our patients, it is not surprising that urine NT-proBNP presented similar results for clinical outcomes of severity in acute bronchiolitis than those previously reported for serum NT-proBNP. We found only one previous work evaluating the utility of urinary NT-proBNP in acute bronchiolitis. Çullas-ilarslan et al.[37] designed a prospective nonrandomized study that included 160 patients diagnosed with lower respiratory tract infection. They also demonstrated the feasibility of analyzing urine NT-proBNP in this setting, but contrary to our observations, they did not find differences between severity groups. The inclusion of a very heterogeneous population with mixed bronchiolitis and pneumonia cases with a wide age range from 0 to 6 years, and the use of a different laboratory kit assays to measure NT-proBNP, could explain their different results.

A growing body of evidence shows the applicability and benefits of noninvasive complementary exams such as cardiopulmonary ultrasound to evaluate the severity of acute bronchiolitis.[38–40] Our findings suggest that urinary NT-proBNP measurements could be another useful noninvasive tool, overall, in settings where ultrasound expertise is not available. There are no standard values for urine NT-proBNP concentrations, and we were not powered for cut-off estimations. Therefore, our exploratory study could not provide enough evidence to stablish urinary NT-proBNP as a prognostic biomarker in bronchiolitis. As one of the major handicaps of performing studies about biomarkers of severity in bronchiolitis is the need for venipuncture in a clinical scenario in where laboratory exams are not currently recommended, our most relevant contribution would be that we would have a noninvasive candidate biomarker that could facilitate future investigations. If our results are confirmed and urinary NT-proBNP reflects the severity of bronchiolitis, it would be interesting to assess whether it provides any extra benefit to the current recommendations to sit and clinically monitor the evolution, in addition to studying its cost-effectiveness as a prognostic biomarker.

4.6 | Limitations

The small sample size and exploratory nature of this study that included mostly moderate to severe cases (35% required ventilatory support) of acute bronchiolitis, with few cases of premature infants with associated comorbidities, preclude the generalization of our results regarding our secondary objective. The diagnostic performance of NT-proBNP assays in urine may be assay-specific, necessitating validation of biomarker performance on an assay-by-assay basis.[41,42] Therefore, our results would not be fully comparable with studies using a different assay for NT-proBNP measurement. We also acknowledge that the Alinity i assay is still awaiting validation for urinary NT-proBNP measurement before its application to the clinical practice. Several clinical factors such as the age, GA, and prematurity related comorbidities can influence on urinary NT-proBNP levels, but we could not demonstrate this influence in our study probably due to the lack of patients with these characteristics. Urinary NT-proBNP concentration can be influenced by various clinical criteria such as the age, the GA, and prematurity associated comorbidities such as BPD or PDA, that could not be demonstrated in our study probably due to the lack of patients with these comorbidities in this study. The influence of the inflammatory response and respiratory state would be interpreted as a reflect of clinical severity more than a limitation. Finally, routine echocardiographic evaluation was not performed unless suspicion of heart disease or failure was raised. Therefore, data about the cardiac status were not systematically recorded, and we could miss the diagnosis of simple CHD. We believe that this limitation does not alter our results as all patients were discharged without any specific cardiac treatment. Despite these limitations, our results are strengthened by the

methodology used, overcoming the major application limitation in pediatrics, the strong age dependence. We also controlled the variations of urinary concentration of NT-proBNP across the day with correction by urinary creatinine, as samples were taken at different times for each patient. Therefore, our results are promising and encourage us to continue the study by recruiting more patients.

5 | CONCLUSIONS

The present study showed that the measurement of urinary NT-proBNP concentrations could be a good and reliable surrogate for serum NT-proBNP levels in acute bronchiolitis. We further observed a significant association of higher urinary NT-proBNP concentrations with clinical characteristics and outcomes indicative of severe bronchiolitis. These findings suggest the potential value of urinary NT-proBNP as a noninvasive tool to assess severity in this setting. Due to the limitations mentioned, further research is warranted to clarify its usefulness before its application in clinical practice.

AUTHOR CONTRIBUTIONS

Moises Rodríguez-González: conceptualization; investigation; writing – original draft; methodology; validation; visualization; writing – review & editing; formal analysis; supervision; data curation; software; project administration. **Ana Castellano-Martínez**: writing – original draft; software; writing – review & editing; visualization; investigation. **Ana Estalella-Mendoza**: writing – review & editing; resources; visualization; investigation. **Patricia Rodríguez-Campoy**: writing – review & editing; visualization; resources; investigation. **Lorena Estepa-Pedregosa**: writing – review & editing; visualization; formal analysis; investigation. **María Mercedes Calero-Ruiz**: writing – review & editing; validation; resources; formal analysis; methodology. **Ana Sáez-Benito Godino**: validation; writing – review & editing; formal analysis; resources; methodology. **Jose Carlos Flores-González**: investigation; funding acquisition; writing – review & editing; methodology; formal analysis; resources; project administration; supervision; data curation; conceptualization; validation.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ETHICS STATEMENT

The Institutional Review Board approved the study (Ethics Committee approval number 1338-N-20; October 2021).

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