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

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July 5, 2022

Abstract

Background and aims: We aimed to analyze the correlation of urinary with serum NT-proBNP concentrations in acute bronchiolitis and its association with the severity of the disease. **Material and Methods**: A pilot observational study conducted between 1st October and 31st March 2022, including acute bronchiolitis cases who attended our institution. Serum and urinary NT-proBNP concentrations were determined using the Alere NT-proBNP assay in time-matched urine and blood samples. We explored the linear relationship between both concentrations and compared clinical outcomes indicative of severe acute bronchiolitis between groups of raised and normal urinary NT-proBNP. **Results:** 17 infants (median age 68 (36-91) days) with 36 time-matched samples were included. The urinary and serum concentrations of NT-proBNP were significantly correlated with (r=0.867 & R-squared coefficient=0.751; p<0.001). The log-10-transformed urinary NT-proBNP concentrations were higher at the time of hospital admission in those infants that required PICU admission with ventilatory support compared with those without this outcome (1.85 (1.16-2.44) pg/mg vs 0.63 (0.45-0.84) pg/mg); p<0.001); and resulted positively and strongly correlated with the duration of the ventilatory support (rho=0.76; p<0.001) and the LOS hospitalization (rho=0.84; p<0.001) **Conclusion:** The measurement of urinary NT-proBNP concentrations could be a reliable surrogate for serum NT-proBNP levels highlighting the potential value of the urinary NT-proBNP as a non-invasive tool to assess severity in acute bronchiolitis.

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

Short title: Urinary NT-proBNP in acute bronchiolitis.

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Funding: This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Ethical approval: The Institutional Review Board approved the study (ethics committee approval number 1338-N-20; October 2021).

Declarations of interest: None.

Type of article: Research article

Word count: 2718

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 non-biologically 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 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 non-invasive 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. Material and methods

2.1 Design, setting and patients: This prospective observational study was conducted between 1st October 2021 and 31st March 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 one-year-old hospitalized with acute bronchiolitis of any severity. The diagnosis and management of infants with bronchiolitis were made per the attending physician's discretion, 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 (UC) and urine bag (UB). The attending pediatrician chose the method of urine collection according to the clinical characteristics of the patients. 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, pCO2, HCO3, lactate, C-reactive protein (CRP), procalcitonin, creatinine and sodium were also determined in these blood samples.

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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 (CMIA), Alere NT-proBNP, for Alinity i assay (Abbott, Spain). 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 to 35 000 pg/mL. No subjects had serum and urine NT-proBNP concentrations lower or higher than the assay linearity limit. This equipment has not previously been found suitable for measuring urinary NT-proBNP levels. 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 inter-subject 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 non-invasive) 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 and used this value in the statistical analysis. As the Z-log-NT-proBNP presented a normal distribution, the serum Z-log-NT-proBNP value was directly used in the statistical analysis. The relationship between urine NT-proBNP concentrations and all blood parameters (including serum NT-proBNP) were assessed using Pearson's correlation coefficient and linear regression analysis, where the strength of correlation was evaluated by the squared correlation coefficient (R-squared). All time matched samples were used (n=36 samples from 17 patients) for this correlation analysis. To explore the association of urine NT-proBNP levels with clinical outcomes indicative of severity, 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. The Mann-Whitney U test and Spearman correlations were utilized to assess associations of log-10-urine NT-proBNP/creatinine ratio levels with categorical and continuous variables respectively. 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-value of < 0.05 was considered statistically significant. We used Stata v.16 software (StataCorp, College Station, Texas).

3. Results

Twenty patients were initially assessed for enrollment, and 3 cases were excluded due to incomplete laboratory data. Therefore, 17 infants (median age 68 (36-91) days; 11 (65%) male sex) with 36 time-matched samples were included in the final analysis. Two samples were determined at the emergency room in 2 infants that did not require hospitalization, 18 samples were determined in 9 cases at 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.

In the 36 samples obtained, the median concentrations of serum NT-proBNP resulted significantly higher than those of urine NT-proBNP (1246 (470-2158) pg/ml vs 300 (114-1192) pg/ml; p<0.001). The mean log-10-serum NT-proBNP values were 3 (0.52) pg/ml. The mean serum Z-log-NT-proBNP was 1.41 (0.91), with 11

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(30%) samples showing raised serum NT-proBNP (Z-log > 1.96). The median urine NT-proBNP/creatinine ratio was 15.3 (4.8-66.6) pg/mg, and the mean log-(10)-urine NT-proBNP/Creatinine ratio was 1.26 (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 (r = 0.790; p<0.001). This correlation resulted improved when using the serum Z-log-NT-proBNP values (r = 0.867; p<0.001). The scatter plots for these relationships are shown in Figure 1 and fitted by the following linear equations: 1) log-10-serum-NT-proBNP = 2.27 + 0.616 x log10-urine NT-proBNP/Creatinine ratio (R-squared coefficient = 0.624; p < 0.001); and 2) Serum Z-log-NT-proBNP = -0.07 + 1.17 x log10-urine NT-proBNP/Creatinine ratio (R-squared coefficient = 0.751; p < 0.001). The log-10-urine NT-proBNP/creatinine ratio showed also a moderate positive correlation with CRP, procalcitonin and pCO2 levels, and a moderate negative correlation with the weight (Table 2).

Regarding the association of initial urinary NT-proBNP values with severity in these cohort (n=17), we found that 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 (1.16-2.44) pg/mg vs 0.63 (0.45-0.84) pg/mg); p<0.001) (Figure 2), and resulted positively and strongly correlated with the duration of the ventilatory support (rho=0.76; p<0.001) and the LOS hospitalization (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 association between serum and urinary NT-proBNP concentrations in this setting, with both parameters being higher in patients who developed more severe disease requiring longer 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 and infants with congenital heart diseases (CHD). These investigations have reported significant associations between elevated urinary NT-proBNP concentrations and neonatal morbidities such as hemodynamically significant persistent ductus arteriosus, retinopathy of prematurity, and bronchopulmonary dysplasia with pulmonary hypertension [12,14,18,19]. Urinary NT-proBNP also seems promising as a screening tool for congenital heart diseases in newborns and has shown potential to differentiate simple and complex CHD [11,20]. Recently, urinary-NT-proBNP has been helpful in the pediatric ambulatory setting to assess heart failure in children with congenital heart diseases when combined with clinical scores [21]. 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 three months of age, who constitute most of the hospitalized population with bronchiolitis [22,23]. With this study, we point out that urinary NT-proBNP can 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.

4.3. Urinary NT-proBNP as a surrogate of serum NT-proBNP: Several previous studies in adults demonstrate 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. measured the serum and urinary NT-proBNP levels in 36 samples from 9 neonates aged 0–25 days and reported a significant correlation with an R-squared coefficient of 0.548 between those variables [26]. Recently, Muller et al. 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) [11]. 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) [26].

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Our results are consistent with previous evidence, suggesting that urinary NT-proBNP concentrations could be used to surrogate serum levels in the acute bronchiolitis setting.

4.4 . Association of urine 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. Recent work shows that serum NT-proBNP levels are associated with echocardiographic signs of pulmonary hypertension and subclinical myocardial dysfunction. It could help screen patients with a worse clinical evolution when used in conjunction with clinical scores [5,27,28]. In acute bronchiolitis, the airway obstruction and inflammation would affect pulmonary vascular tone increasing the right ventricular afterload [8,29,30], 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 pCO2, CRP, and procalcitonin levels at any time of hospitalization in our patients. As the significant stimuli that upregulate the NT-proBNP synthesis from the ventricular myocardium are conditions of sustained ventricular blood volume and pressure overload, the enhancement of NT-proBNP synthesis and secretion would be more significant in those infants with acute bronchiolitis with a more severe respiratory impairment that will need PICU admission and mechanical ventilation. Noteworthy, increased values of urinary NT-proBNP at early stages of hospitalization and before initiation of inotropic or ventilatory support were higher in those infants requiring PICU admission and resulted strongly associated with longer respiratory support and LOS hospitalization, our clinical outcomes indicative of severe bronchiolitis in this study. We found only one previous work evaluating the utility of urinary NT-proBNP in acute bronchiolitis. Çullas-İlarslan et al. designed a prospective non-randomized study that included 160 patients diagnosed with lower respiratory tract infection [31]. 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. However, these authors included a very heterogeneous population with mixed bronchiolitis and pneumonia cases and a wide age range from 0 to 6 years. This fact, joined with the different laboratory kit assays used to measure NT-proBNP, could explain the differences between studies. In our study, urine and serum concentration of NT-proBNP resulted strongly and positively correlated. Therefore, 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, pointing out the potential value of the measurement of urinary NT-proBNP as a non-invasive tool to assess severity in acute bronchiolitis. A growing body of evidence shows the applicability and benefits of non-invasive complementary exams such as cardiopulmonary ultrasound to evaluate the severity of acute bronchiolitis in previously healthy infants [32–34]. Our findings suggest that urinary NT-proBNP measurements may be another useful non-invasive tool for this, overall, in settings where ultrasound expertise is not available. As there are no standard values for urine NT-proBNP concentrations and we were not powered for cut-off estimations, we could not establish a reliably cut-off point for outcomes in this study.

4.5. 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 preclude the generalization of our results. The diagnostic performance of NT-proBNP assays in urine may be assay-specific, necessitating validation of biomarker performance on an assay-by-assay basis [35,36]. Therefore, our results would not be fully comparable with studies using a different assay for NT-proBNP measurement. Although we acknowledge that the Alere NT-proBNP for Alinity i assay has not been previously validated to determine urinary NT-proBNP, our correlation analysis suggests that the urinary levels provided by this assay adequately reflect NT-proBNP serum levels. 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 the need for 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.

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5. Conclusions

The present study demonstrated a strong positive correlation between the serum and urinary concentrations of NT-proBNP in a small cohort of infants with acute bronchiolitis. We further confirmed a significant association of elevated urinary NT-proBNP concentrations with clinical outcomes indicative of severity in this setting. These findings suggest that the measurement of urinary NT-proBNP concentrations could be a good and reliable surrogate for serum NT-proBNP levels and highlight the potential value of the measurement of urinary NT-proBNP as a non-invasive tool to assess severity in acute bronchiolitis. Due to the limitations of our study, further research on urine NT-proBNP measurement in the setting of acute bronchiolitis is warranted to clarify its potential role as a non-invasive biomarker.

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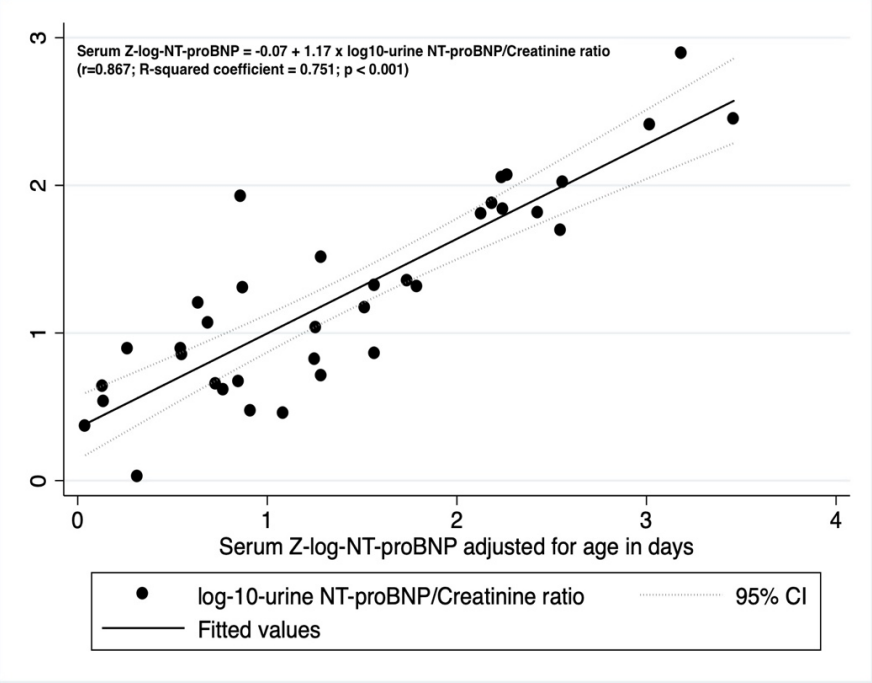
Figure 1. Correlation between urinary and serum concentrations of NT-proBNP. Abbreviations: NT-proBNP, N-terminal pro–B-type natriuretic peptide; The vertical axis (urinary NT-proBNP/creatinine ratio) is log-10-transformed because of its skewed distribution; and the horizontal axis is expressed as Z-log values adjusted for age**(panel A)** and log-10-transformed serum NT-proBNP **(panel B)** . The linear regression curve is shown in a continuous line and the 95% confidence interval (CI95%) in discontinued lines.



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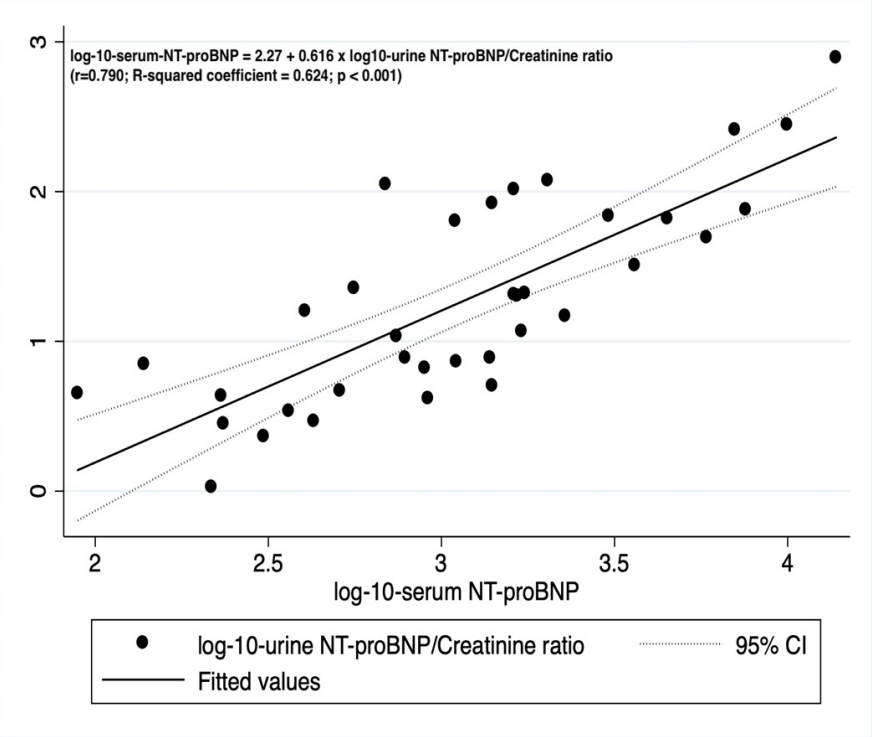

Figure 2. Comparison of log-10-urine NT-proBNP/creatinine ratio values of the first samples obtained from each patient after inclusion, between cases requiring or not PICU admission and ventilatory support. Urinary NT-proBNP/creatinine is log-10-scaled because of its skewed distribution. Patients with 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) pg/mg vs 0.63 (0.45-0.84) pg/mg); p<0.001).

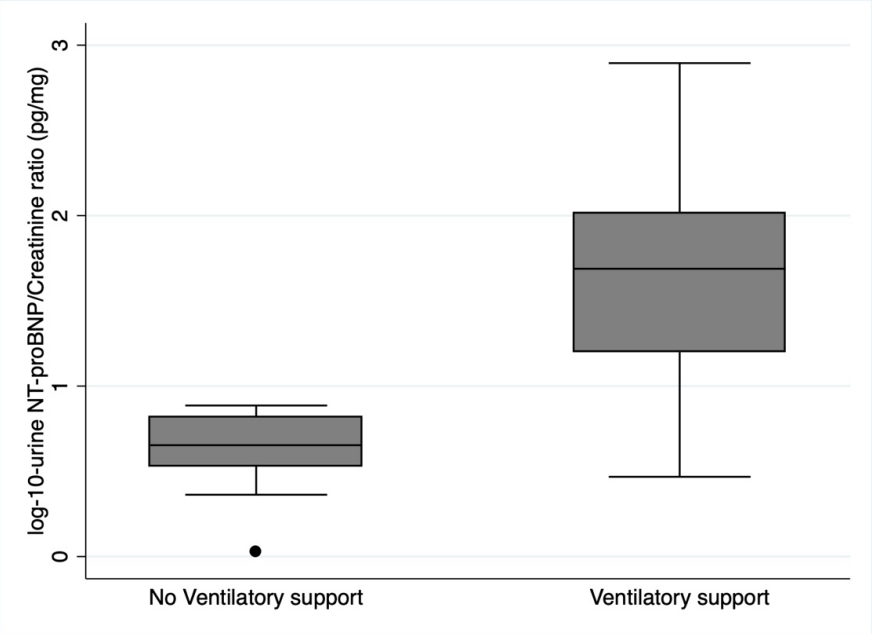

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Table 1. Baseline characteristics and outcomes of the study population

Variable	**Admission samples (n=17)**
Demographic and clinical data	Demographic and clinical data
Age (days)*	68 (36-91)
Weight (kg)*	4.9 (4.4-5.5)
Gender (male)ˆ	11 (65)
Comorbidityˆ	4 (23) (all prematurity)
BROSJOD score*	7 (6-10)
BROSJOD $>$ 10 pointsˆ	5 (29)
RSV positiveˆ	7 (41)
Laboratory data	Laboratory data
pH*	7.34 (7-28-7.39)
pCO2 (mmHg) *	50 (45-56)
HCO3 (mEq/L)*	25.5 (24.3-28.1)
Lactate (mmol/L)*	2 (1.6-3.5)
CRP (mg/dl) *	13.2 (2-64)
Procalcitonin (mg/dl)*	0.14 (0.09-0.38)
Serum Creatinine (mg/dl) *	0.39 (0.38-0.41)
Plasmatic Sodium (mEq/L) *	138 (136-139)
Urine Creatinine (mg/dl) *	22 (13-34)
NT-proBNP values	NT-proBNP values
Serum NT-proBNP (pg/ml)*	899 (307-2279)
Log-10-serum-NT-proBNP*	2.95 (2.48-3.35)
Serum Z-log for age of NT-proBNP*	1.25 (0.31-1.57)
Z-log-NT-proBNP $>$ 1.96*	4 (23)
Urine NT-proBNP (pg/ml)*	218 (74-625)
NT-proBNP/creatinine (pg/mg) *	7 (4.1-14.7)
Log-10-urine NT-proBNP/Creatinine (pg/mg) *	0.84 (0.61-1.16)
Treatment	Treatment
Oxygen (nasal canulae) ˆ	13 (76)
Non-invasive ventilationˆ	6 (35)
Mechanical ventilationˆ	3 (17)
Inotropic supportˆ	1 (7)
Antibioticsˆ	3 (17)
Diureticsˆ	1 (6)
Clinical outcomes	Clinical outcomes
PICU admissionˆ	6 (35)
Duration of respiratory support (nasal canulae + MV + NIV) (days)*	2 (1-5)
LOS hospitalization (days)*	4 (2-11)
Death or sequelˆ	0 (0)
ˆ Data presented in frequency and percentage. * Data presented in median y interquartile range. BROSJOD score: Bronchiolitis Score of Sant Joan de Deu. RSV: respiratory syncytial virus. CRP: C-Reactive Protein. NT-proBNP: N-terminal pro-brain natriuretic peptide. PICU: pediatric intensive care unit. LOS: Length of stay. p-Value: statical significance.	ˆ Data presented in frequency and percentage. * Data presented in median y interquartile range. BROSJOD score: Bronchiolitis Score of Sant Joan de Deu. RSV: respiratory syncytial virus. CRP: C-Reactive Protein. NT-proBNP: N-terminal pro-brain natriuretic peptide. PICU: pediatric intensive care unit. LOS: Length of stay. p-Value: statical significance.

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Table 2. Spearman correlations between urinary concentrations of NT-proBNP and several clinical and serum parameters in all samples obtained (n=36).

Log-10-urine NT-proBNP/Creatinine ratio
Age
Weight
BROSJOD score
Serum creatinine
Serum sodium
CRP
Procalcitonin
pH
pCO2
HCO3
Lactate
Log-10-serum NT-proBNP
Serum Z-log for age of NT-proBNP

ˆ Data presented in frequency and percentage. * Data presented in median y interquartile range. BROSJOD score: Bronchi