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Plasma concentration of N-terminal pro-atrial and N-terminal pro-brain natriuretic peptides and fluid balance in children with bronchiolitis

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Abstract. The aim of this study was to evaluate the plasma levels of N-Terminal pro-brain natriuretic peptide (N-BNP), N-Terminal pro-atrial natriuretic peptide (N-ANP) and antidiuretic hormone (ADH) over time and their relationship to clinical indicators in hospitalized children with bronchiolitis. Prospective crossover clinical investigation. Hospitalized children in a university-affiliated hospital. Twenty-seven children (birth to 24 mo) with first episode of bronchiolitis and 34 age-matched healthy controls. Daily blood samples up to five consecutive days were obtained for N-BNP, N-ANP and ADH in the bronchiolitis group and on the initial blood draw in the control group. Daily total fluid intake, net fluid balance and clinical bronchiolitis severity levels were recorded. N-BNP and N-ANP levels were measured by enzyme-linked immunosorbent assay. ADH levels were measured by a double antibody technique. The mean age (months ± SD) in the bronchiolitis group was 4.2 ± 5.9 mo and 12.0 ± 6.1 mo in the control group; 51.9% of bronchiolitis patients were positive for respiratory syncytial virus (RSV). In patients with bronchiolitis on admission, plasma N-BNP measurements (mean ± SD) were elevated (996.0 ± 570.2 fmol/mL) compared to controls (552.7 ± 264.7 fmol/mL $P < 0.005$). Serum N-ANP levels were also initially elevated (3,889 ± 1,769.7 fmol/mL) compared to controls (2,173 ± 912 fmol/mL $P < 0.005$). The serum levels of N-BNP and N-ANP remained significantly elevated from day 2 through day 5. Similarly, ADH levels were significantly higher on admission in the bronchiolitis group (10 ± 7.49 pg/mL) vs. the control group (5.8 ± 5.5 pg/mL $P < 0.05$), but quickly decreased from day 2 through day 5. N-BNP, N-ANP and ADH concentrations were elevated in hospitalized children with bronchiolitis at admission. Based on our observation, judicious fluid management is indicated in children hospitalized with bronchiolitis.

Keywords: Brain natriuretic peptide, atrial natriuretic peptide, arginine vasopressin, antidiuretic hormone, bronchiolitis, respiratory distress, fluid management, intensive care unit, pediatric

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1. Introduction

Bronchiolitis is the most common lower respiratory tract infection in infants and children less than 2 yr of age [1]. This respiratory disease is characterized by inflammation and edema formation in the bronchiolar tree along with small airway obstruction, which can result in worsening of oxygenation. Supportive care with supplemental oxygen, suctioning, on occasion inhaled β-agonist therapy and administration of intravenous fluid is often part of the medical care of infants and children with bronchiolitis.

Intravascular volume and serum osmolality are tightly regulated by a balance of various neurohumoral mediators [2]. During critical illness, these regulatory mediators may be modified by disease or treatment, either benefiting or harming the patient. Such regulatory mediators of intravascular volume and fluid balance include atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and vasopressin (also called antidiuretic hormone [ADH]). Although well studied in adult cardiac patients, these neurohormones are infrequently studied in pediatric respiratory diseases such as bronchiolitis. Understanding the effects of pediatric respiratory diseases and treatment interventions on the concentrations of these neurohormonal regulators is a crucial first step in formulating more effective and safer fluid therapy in the treatment of bronchiolitis.

ANP is synthesized in cardiac atria initially as pre-pro-ANP containing 151 amino acids [3,4]. It then undergoes proteolytic cleavage and is stored in granules within atrial myocytes as a prohormone (Pro-$ANP_{1–126}$) that contains 126 amino acids. When needed, it undergoes another proteolytic cleavage step before release, giving rise to the inactive molecule, Pro-$ANP_{1–98}$ (N-ANP) and the biologically active 27 amino acid carboxy-terminal $ANP_{99–126}$ (ANP) [4]. ANP acts through membrane associated guanylyl cyclase receptors that are found throughout the body especially on vascular smooth muscle [4,5]. ANP was shown previously to be elevated in pediatric patients with pneumonia [6]. In addition, ANP has direct pulmonary vasorelaxant activity [7]. Hypoxemia stimulates ANP secretion in normal humans and in animals [8,9].

In contrast to ANP, BNP is found mainly in the cardiac ventricles [10]. Human BNP is synthesized as a 132 amino acid prepropeptide that is processed by endoprotease cleaving to a 108 amino acid precursor protein (pro$BNP_{1–108}$). Subsequently, this propeptide is cleaved into the biologically active 32 amino acid carboxy-terminal fragment and an inactive 76 amino acid N-terminal fragment [4,11,12]. BNP is produced and secreted in response to increased ventricular end-diastolic volume and pressure [13,14]. BNP's pulmonary vascular effects seem similar to ANP. BNP levels are used as an established marker of congestive heart failure in adults [15]. In addition, BNP levels are increased in obstructive lung disease in the presence of cor pulmonale and acute hypoxemia [16,17] and correlate positively with the severity of hypoxemia in both human and animal models [8,18]. However, BNP levels were never studied in children with bronchiolitis who may have severe lung hyperinflation and on-going hypoxia. If severe lung hyperinflation is present it may decrease both right and left atrial preload by impairing systemic venous return resulting in lower ventricular volumes and thus lower BNP levels, despite the presence of hypoxemia.

Elevated plasma ADH concentrations in bronchiolitis was observed in several studies; the stimulus for this increase is unclear, [19–21] but may be related to reduced venous return as noted above. A recent case report of a critically ill infant implicated RSV infection as the cause of the syndrome of inappropriate ADH (SIADH) though confounding factors make this relationship unclear [22]. Increased ADH concentration in bronchiolitis represents one proposed mechanism for the increased potential risk of water retention and risk of hyponatremia in children with severe bronchiolitis [23]. The concurrent administration of hypotonic intravenous fluid and elevated ADH concentrations can decrease serum sodium concentration and osmolality while resulting in a positive fluid balance that may worsen lung compliance. Since ANP and BNP antagonize the action of ADH, simultaneous measurement of all three hormones in acutely ill children may help elucidate the neurohumoral mechanisms of fluid balance in bronchiolitis.

Therefore, the aim of this study was to determine the serum concentration of ANP, BNP and ADH over time in children with bronchiolitis compared with a cohort of normal children and compare the relationship of these neurohumoral agents with each other and with fluid balance.

2. Materials and methods

This was a prospective observational clinical study performed on consecutive bronchiolitis patients admitted to Shands Teaching Hospital between October 2005 and April 2007. The study was approved by the institutional

review board of the University of Florida, and informed consent was obtained from the parents of all participants. The study followed all standards set by the Health Insurance Portability and Accountability Act.

2.1. Patients

Children with bronchiolitis were enrolled based on: 1) age less than 2 yr (corrected for gestational age); 2) two of the following: nasal secretions with upper airway congestion, inter-and/or subcostal retractions, or expiratory wheezing; and 3) a chest x-ray that demonstrated peribronchial cuffing and/or air trapping. Each chest x-ray was individually analyzed by two radiologists blinded to the patient's clinical status. In addition, all enrolled patients had rapid RSV antigen testing; however, patient inclusion was not based upon a positive RSV antigen test since bronchiolitis is also caused by other viruses such as adenovirus and parainfluenza.

In the control group, children between 37 wk of gestation and 2 yr of age (corrected for gestational age) were approached for enrollment when they were in the outpatient surgery center for minor surgical operations similar to previous studies [24–29]. This was done to establish control values in a similar cohort of healthy children without respiratory disease who were instructed not eat or drink several hours before arrival. The minor surgical operations included, but were not limited to hernia repair, cleft lip and/or palate repair, circumcision and any cosmetic surgery. We excluded children with chronic renal disease, known congenital cardiac disease, bronchopulmonary dysplasia, pituitary abnormalities, a history of reactive airway disease, asthma, cystic fibrosis, recent history of receiving steroids, thyroid disease or patients on chronic diuretic therapy.

2.2. Collections of samples and other data

Following the admission of patients with bronchiolitis, an initial and then daily blood samples were drawn from an existing indwelling catheter (central venous line, arterial line, or peripheral venous line) or in conjunction with routine laboratory draws for up to five consecutive days for analysis of N-BNP, N-ANP, ADH, and serum osmolality. Samples were drawn for neurohumoral testing at the time of admission. During the study period, we recorded daily weight, urine output, all sources and amounts of fluid intake, net fluid balance, and vital signs along with serum sodium and potassium levels.

For the control group, a study blood sample was obtained with placement of an indwelling catheter at the time of surgical center admission. After the initial blood draw, no further blood collections were done in this group of patients.

Bronchiolitis severity of illness scoring (BSI) was adopted and modified from previous scores [30,31]. BSI was assessed daily and independently by a bedside nurse (RN) and the principal investigator (PI). Each BSI category was then averaged from the RN and PI daily assessments and a total composite score was created and described in Table 2. All children with bronchiolitis were managed using a clinical pathway established prior to initiating this study.

2.3. Samples processing and neuropeptide measurement

To process neuropeptides, blood samples were immediately transferred to chilled plastic tubes containing potassium ethylene diamine tetra-acetic acid (EDTA) and promptly centrifuged. The plasma fraction of the samples were then separated and an aliquot stored at −80°C and thawed only once at the time of assay. NT-ProBNP (N-BNP) and NT-ProANP (N-ANP) were analyzed by a competitive Enzyme Immuno Assay (ALPCO diagnostics, NH). This kit incorporated an immunoaffinity purified sheep antibody specific for N-BNP (8–29) and for N-ANP (1–98). The intra- and inter-assay variation coefficients for N-BNP were 4.5% and 6.5% respectively and for N-ANP were 6% and 7% respectively.

ADH was analyzed by a double antibody radioimmunoassay labeled with 125-Iodine arginine vasopressin obtained from (ALPCO diagnostics, NH). This kit followed a modified method as previously explained and perfected by Glick et al. [32] Intra- and inter-assay precisions were 6% and 9.9% respectively with analytical sensitivity of 0.75 pg/mL and functional sensitivity of 1.3 pg/mL. Serum and urine osmolality were analyzed by Advanced 3D3 Osmometer (Shands laboratory, Florida).

2.4. Statistical analysis

Data were analyzed using the statistical analysis system (SAS) program (version 9.1; SAS Institute,

Cary, NC.). Demographic data were presented as mean ± SD. Mean daily neuropeptide levels (N-ANP, N-BNP and ADH) for bronchiolitis subjects were compared to controls via two-sample t-tests. Pearson's correlation coefficients were used to evaluate the relationship between neuropeptide plasma levels, BSI and fluid intake. We considered $P < 0.05$ (2-sided) to be statistically significant for these analyses. Daily percent change in these parameters was also evaluated and compared to the null hypothesis of no change via one-sample t-tests. Finally, we employed Cohen's Kappa statistic in order to evaluate inter-rater reliability between the two BSI raters.

3. Results

We evaluated 61 infants and children with respiratory distress hospitalized consecutively between October 2005 and April 2007. Of these, 27 children were diagnosed with bronchiolitis and 34 cohorts were enrolled in the control group. The demographic characteristics for the study participants are shown in Table 1. The mean age in the control subjects was 12 ± 6.1 months versus 4.2 ± 5.9 months in patients with bronchiolitis. There were no differences among the major ethnic groups or mean gestational weeks at study entry. Patients with bronchiolitis were admitted to the hospital with an average of a 4 day history of rhinorrhea and cough and a 2 day history of fever, chest wall retractions and wheezing. Respiratory syncytial virus was identified in 51.9% of children with bronchiolitis enrolled in this study. Most of the control patients were male because one of the common outpatient surgeries performed at our institution on younger infants was circumcision. Each patient had a BSI assessment independently by the bedside RN and the PI at the time of admission (Table 2) and daily thereafter. The overall BSI agreement between RN and PI was $\kappa = 0.71$ (95% CI, 0.55–0.86). Over the course of the study, 8 patients in the bronchiolitis group did not complete the full five day study period. In the control group, 2 patients did not have their plasma samples tested for N-ANP or N-BNP and 3 patients did not have their ADH levels measured.

Table 1
Baseline Characteristic of Study Population, N (%)

Characteristic	Control	Bronchiolitis	*P* value
Sample Size (n)	34	27	
Age at study entry, months [mean (SD)]	12 (6.1)	4.2 (5.9)	<0.0001*
Age Groups			<0.0001**
0–3 months	3 (8.8)	19 (70.4)	
3.1–6 months	5 (14.7)	2 (7.4)	
6.1–12 months	8 (23.5)	4 (14.8)	
12.1–24 months	18 (52.9)	2 (7.4)	
Male, %	30 (88.2)	14 (51.9)	0.003**
Ethnicity			0.36**
White	20 (58.8)	14 (51.9)	
Black	10 (29.4)	12 (44.4)	
Hispanic	3 (8.8)	0 (0.0)	
Other	1 (2.9)	1 (3.7)	
Premature Birth <36 weeks	5 (14.7)	7 (25.9)	0.34**
Resided with tobacco smokers	10 (29.4)	14 (51.9)	0.11**
Gestational Age, weeks [Mean (SD)]	38.6 (2.4)	36.8 (3.4)	0.02*

*tested via one-way ANOVA; **tested via Fisher's Exact test.

In children with bronchiolitis, admission serum N-ANP levels were elevated (3,889 ± 1,770 fmol/ml) compared with controls (2,173 ± 912 fmol/mL; Fig. 1). Admission serum N-BNP concentrations were also elevated (996.0 ± 570.2 fmol/mL) in children with bronchiolitis compared with controls (552.7 ± 264.7 fmol/mL; Fig. 2). The serum concentrations of both neuropeptides remained significantly elevated from days 2 through 5 as compared to control patients. Similarly, ADH levels were significantly higher than controls on admission in the bronchiolitis group (10 ± 7.49 pg/mL vs. 5.8 ± 5.5 pg/mL), but quickly decreased towards control concentrations from days 2 through 5 (Fig. 3).

Five out of 27 children were intubated during this study. The mean N-ANP concentration in intubated patients with bronchiolitis rapidly decreased from a mean of 4,800 to 2,600 fmol/mL between days 1 to 5. However in nonintubated patients with bronchiolitis, N-ANP levels did not vary significantly over the 5 day period (mean range of 3,750 to 4,250 fmol/mL) though they were markedly elevated compared with controls (mean of 2,000 fmol/mL) (Fig. 4). There were no significant variations in N-BNP levels in patients with bronchiolitis over the 5 day period regardless of their intubation status (Fig. 5). Measured ADH levels in nonintubated patients decreased after day 3 to approximately the same levels of intubated patients with bronchiolitis (Fig. 6).

On day one, the nonintubated patients with bronchiolitis had severe clinical disease (BSI of 4.8 ± 2.0). However, their BSI score decreased to moderate-severe intensity (range 3.0 to 3.9) from days 2 to 4 and further declined to mild-moderate intensity (2.9 ± 0.7) on

Table 2
Bronchiolitis Severity of Illness (BSI)

Principal investigator and bedside nurse examine the patient for the following three components of the scoring system

Respiratory-effort score

Examine intercostal recession, subcostal recession, substernal recession, tracheal tug and nasal flaring. Assign score as follows: 0 (not present), 1 (mild to moderate), or 2 (severe) for each factor.

Multiply each score by a weighting factor, as follows: intercostal recession (x1), subcostal recession (x1), substernal recession (x1), tracheal tug (x1.5), and nasal flaring (x1.5). Total the weighted scores for respiratory effort as follows: score between 0 to 4.9 assigned as mild (1), between 5 to 8.9 assigned as moderate (2), between 9 to 12 assigned as severe (3).

Oxygen saturation in ambient air score

Infants and children receive scores for oxygenation saturation levels as follows: (0) for 95 to 100 percent, (1) for 90 to 94 percent, (2) for less than 90 percent.

Respiratory rate score

	Clinical Score		
Age	1	2	3
Infant less than 2 months	RR ≤ 60	RR between 61–69	RR ≥ 70
2 months to 12 months	RR ≤ 50	RR between 51–59	RR ≥ 60
1 year to 2 years	RR ≤ 40	RR between 41–45	RR ≥ 45

RR = Respiratory Rate.

Feeding, vocalization and activity scores

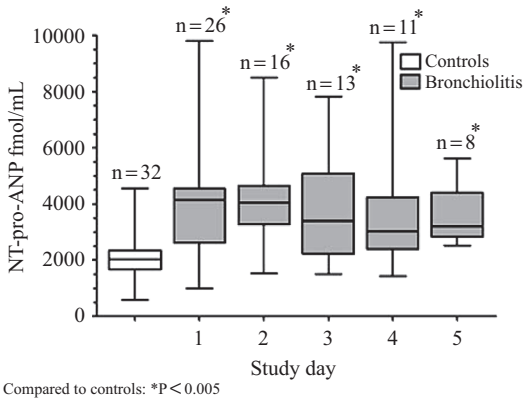
Assign a score of (0) for normal feeding, vocalization and activity; (1) for one of the following: difficulty with feeding, decreased vocalization, or agitated; (2) for two of the following: difficulty with feeding, decreased vocalization, or agitated; (3) for stopped feeding, no vocalization, or drowsy.

Overall severity score

Total the above four scores. Infants and children with bronchiolitis are classified for the severity of their illness according to the following composite score:

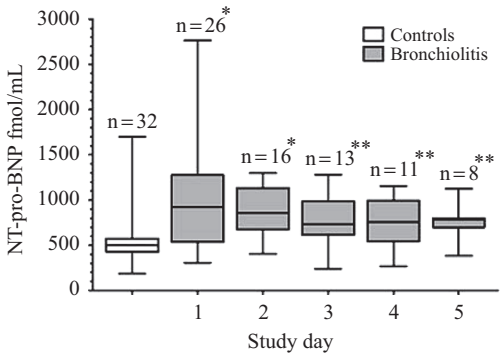
Less than 2 correspond to mild, 2–4 as moderate, greater than 4 as severe bronchiolitis.

Modified scoring system described by Wainwright et al. [30] and Liu et al. [31].



Compared to controls: *P < 0.005

Fig. 1. Serum N-ANP levels by day (all patients).



Compared to controls: *P = 0.0002; **P < 0.05

Fig. 2. Serum N-BNP levels by day (all patients).

day 5. The total fluid input in children with bronchiolitis was 6.9 (±3.6) mL/kg/hour on day one and this gradually decreased to 4.2 (±1.6) mL/kg/hour by day 5 of admission. However, the net fluid balance was nearly even after the first 24 hr. Serum sodium, potassium, chloride, and mean serum and urine osmolalities were unchanged during the study period. ADH plasma levels were (11.1 ± 7.8 pg/mL) on the day of admission, (12.7 ± 13.6 pg/mL) by day two, and then trended lower on day 3 (6.5 ± 4.7 pg/mL), but remained unchanged by day 5 (6.5 ± 4.4 pg/mL).

4. Discussion

The aim of this study was to begin to understand the potential role of N-ANP, N-BNP and ADH in children with bronchiolitis by characterizing their concentrations

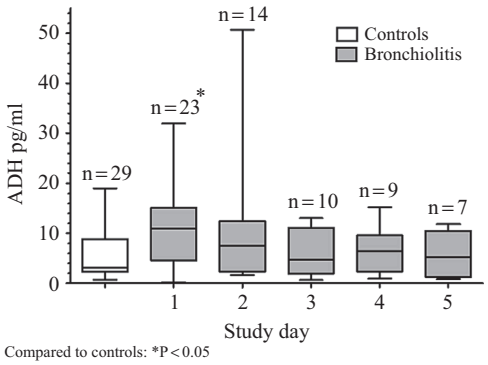


Fig. 3. Serum ADH levels by day (all patients).

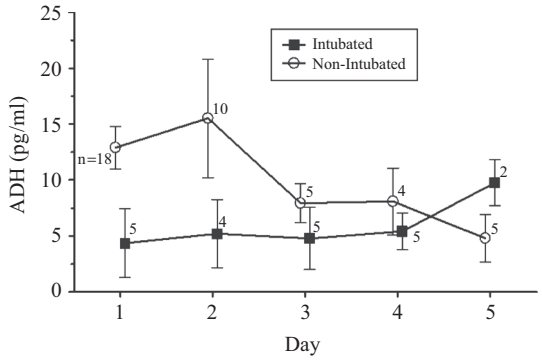


Fig. 6. Antidiuretic hormone (ADH) differences between intubated and non-intubated patients with bronchiolitis.

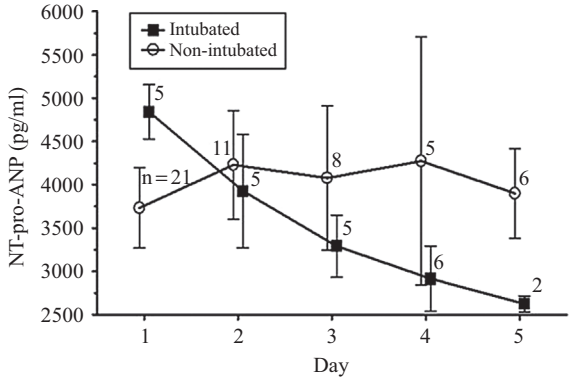


Fig. 4. N-Terminal Pro-Atrial Natriuretic Peptide (N-ANP) differences between intubated and non-intubated patients with bronchiolitis.

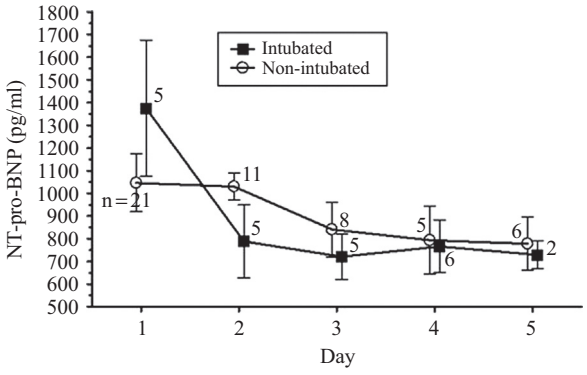


Fig. 5. N-Terminal Pro-Brain natriuretic peptide (N-BNP) differences between intubated and non-intubated patients with bronchiolitis.

over time. By recognizing the patterns of N-ANP, N-BNP and ADH concentrations in patients with bronchiolitis, one may make a more informed decision prescribing effective fluid therapy in the management of this common pediatric respiratory illness. In this study of 27 children with bronchiolitis, both N-ANP and N-BNP levels were increased upon admission as compared to controls. The N-ANP and N-BNP levels then decreased over the 5 day observation period along with the severity of bronchiolitis. This decrease was especially evident in the N-ANP concentrations in intubated patients with bronchiolitis. Moreover, the ADH levels followed a similar pattern in these same children.

Since both N-ANP and N-BNP serum concentrations have not been examined in the context of bronchiolitis severity, we reviewed previous studies regarding the possible causes of these neuropeptide elevations. Acute hypoxemia stimulates ANP secretion in experimental animals [9] and in humans [33–35]. We expected to find low levels of N-ANP and N-BNP serum concentrations due to the inhibitory effects of ADH as previously described in animal models [36,37]; in fact, this was not found. One explanation could be that the effect of hyperinflation from air trapping does not impact the levels of N-ANP and N-BNP as much as hypoxemia if this occurred before clinical presentation. In this study, the N-ANP levels in intubated patients were lower than non-intubated patients after the second day of admission (Fig. 4) despite the possibility that air trapping effects and increased intrathoracic pressure caused by positive pressure ventilation can be more pronounced than the hyperinflation caused by severe bronchiolitis [38]. It is also noteworthy that the ADH levels were relatively lower in nonintubated patients compared to intubated

patients with bronchiolitis (Fig. 6), which is opposite from what would be expected if hyperinflation played a major role. Therefore, hyperinflation may have less of an effect clinically on these neuropeptides than other factors in children with bronchiolitis.

Serum ADH levels were not elevated throughout the acute phase of the bronchiolitis as compared with controls although ADH was initially elevated at the time of admission. Respiratory infection, specifically RSV infection, elevates ADH levels during the acute phase of the illness presumably due to the effects of lung hyperinflation (by causing air trapping) reducing venous return and thus atrial volume [19,39,40]. A case series by Rivers et al looking at 4 patients with RSV bronchiolitis demonstrated increased levels of ADH along with documented hyponatremia [20]. In contrast to our observations, a larger study of 48 patients (mean age 104 days) with RSV bronchiolitis demonstrated elevated ADH levels averaging 9.3 pg/mL ± 1.4 (units converted from original manuscript) while the intubated cohorts had a mean serum ADH level of 18 pg/mL ± 6.7 [19]. We did not observe the level of hyponatremia seen by Rivers et al, but rather serum sodium levels averaging 136 to 139 mmol/L as in van Steensel-Moll et al.

Patients with severe bronchiolitis are expected to have increased insensible fluid loss secondary to their tachypnea and increased work of breathing. When combined with reduced oral intake, these infants are often thought to be relatively hypovolemic on admission, which should increase ADH concentrations and predispose them to low sodium and low osmolality. The serum osmolalities and sodium levels were normal on admission and throughout the hospitalization in our study population. This was similar to a study by Gozal et al [23]. Initially, the ADH concentration in our 23 patients with bronchiolitis were elevated and then decreased to the same concentration as observed in healthy subjects. Since no previous research followed ADH levels in patients with bronchiolitis for several days, comparable studies were not available. Previous studies measured ADH levels only at the time of admission, but not on subsequent days as the disease process improved or worsened [19,20,23]. It was suggested by Dreyfuss et al. that respiratory disease may affect the osmoregulation of serum ADH level by changing its set-point [41,42]. This is consistent with the normal levels of serum sodium and plasma osmolalities seen in our patients with bronchiolitis. However, another plausible cause for normal serum osmolality throughout the study period could be that hospitalized children with bronchiolitis may not be as dehydrated as previously thought.

In addition, the elevation of ADH may not follow the pathophysiology that one would expect when comparing ANP and BNP levels [43]. Rather, it is possible that ADH levels are inversely related to ANP and BNP serum concentrations except in the case of stress and hypoxemia [44]. Therefore, the initial elevated levels of ADH with normal serum osmolality and sodium concentration may be appropriate as patients with severe bronchiolitis are relatively more stressed and hypoxemic on admission. As patients improve and are less distressed, ADH levels decreases rapidly in subsequent days.

Our study had several limitations. First, we had a small number of patients with bronchiolitis enrolled. We prospectively enrolled 34 healthy cohorts as controls so comparisons of neuropeptide concentrations could be performed with the same technique and standards. We believe that this design enabled more accurate comparisons to verifiable serum concentrations of N-ANP, N-BNP and ADH in children. Nevertheless, the small size of this group precluded determination of clinically relevant effects of bronchiolitis on these neuropeptide concentrations. We stratified our patients based on disease severity in which oxygenation was only one of four factors that determined this measure. Thus, precise correlation of oxygenation to measureable fluid volume metrics in patients with bronchiolitis is lacking. In addition, the term "hypoxia" is rather nebulous, and we did not measure lactate concentrations in our patients. Nevertheless, a larger number of patients would have allowed us to perhaps make more valid assertions on whether any correlations existed between the levels of neuropeptides and BSI in addition to more precise measures of both oxygen delivery and evidence of low oxygenation. Second, drawing daily blood samples imposed a limitation on our study due to the small circulatory blood volume in children compared to adults. We did not measure aldosterone and renin levels. Renin measurement may have shed further light on the causation of elevated ADH on admission. Third, we used input and output data collected by the bedside nurses even though these data may be unreliable as explained by Wise et al. [45]. However, the focus of our study was on obtaining admission values for neuropeptides in children with bronchiolitis stratified according to disease severity. More stringent data on fluid intake/output as well as perhaps the degree of insensible fluid changes will be important in future studies with a larger numbers of patients where the effect of

each neuropeptide on fluid balance may be assessed with greater accuracy.

In conclusion, this is the first study in children evaluating the relationship of natriuretic peptides and ADH in both intubated and nonintubated children with RSV bronchiolitis. Our study shows that N-ANP and N-BNP plasma levels are elevated in bronchiolitis and remain elevated for at least 5 days. However, ADH concentrations are elevated initially and decrease to levels similar to control subjects. Furthermore, patients with bronchiolitis may not be as volume depleted as previously thought and therefore may be at risk of receiving excessive fluids on admission to the hospital. Nevertheless, our work suggests that a larger study is justified in evaluating the neurohormonal interactions with fluid administration, severity of disease and other measures of intravascular volume in children with RSV bronchiolitis.

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