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Local production of inflammatory mediators during childhood parainfluenza virus infection

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Abstract

Objective—To describe the clinical manifestations of PIV infection and to characterize biochemical markers of PIV disease severity.

Patients and Methods—We reviewed the medical records of 165 children who had a nasal wash culture positive for PIV at our institution between 1998 and 2008. Nasal wash samples were assayed for 26 inflammatory mediators using Luminex bead proteomics.

Results—153 patients, ages 2 weeks to 12 years, with single virus infection were included in our final analysis. 52 patients were infected with PIV1, 19 with PIV2, 74 with PIV3, and 8 with PIV4. LRTI was diagnosed in 67 (44%) patients, 21 (14%) had LTB, and 49 (32%) had a URI other than LTB. LRTI was diagnosed in 54% of patients infected with PIV3, 35% of those infected with PIV1, 26% of those with PIV2 and 50% of those with PIV4. Compared to uninfected control patients, PIV-infected patients had higher nasal wash concentrations of interleukin (IL)-6, CXCL8 (IL-8), CCL3 (macrophage inflammatory protein (MIP)-1α), CCL4 (MIP-1β), CXCL9 (monokine induced by interferon gamma (MIG) and CCL5 (regulated upon activation, normal T cell expressed and secreted (RANTES). Patients with LRTI, moderate or severe illness, and respirovirus infection (PIV 1 or 3) had higher nasal wash concentrations of CXCL8 when compared to patients with URI, mild illness, or rubulavirus infection (PIV 2 and 4) ($p<0.05$).

Conclusions—PIV infection causes a spectrum of illnesses associated with the expression and release of several proinflammatory mediators. Of note, elevated levels of CXCL8 in nasal wash samples are associated with more severe forms of PIV disease.

Keywords

Parainfluenza virus; innate immunity; respiratory viral infection

Introduction

Parainfluenza viruses (PIVs) are responsible for more than a third of all acute respiratory tract infections in infants and children [1, 2]. Most primary PIV infections are symptomatic.

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Re-infections are milder and continue to occur at all ages. The clinical spectrum of illness caused by PIVs include upper (URIs) and lower respiratory tract infections (LRTIs) encompassing rhinosinusitis, otitis media, pharyngitis, laryngotracheobronchitis (LTB), bronchiolitis and pneumonia. As a group, PIVs are among the most common causes of lower respiratory tract infections in young children[3,4] accounting for up to 40% of acute lower respiratory tract illnesses from which a virus is recoverable[5]. PIVs account for the second largest number of pediatric hospitalizations for community-acquired respiratory illnesses, second only to respiratory syncytial virus[6–8] and account for almost 7% of hospitalizations for fever and/or acute respiratory illnesses in young children[9].

Four human parainfluenza viruses are recognized (types 1, 2, 3 and 4) which are divided into two genera based upon complement fixation and hemagglutinating antigens. The genus respirovirus includes PIV types 1 and 3 and the genus rubulavirus includes PIV types 2 and 4[10]. PIV 1 and 2 are leading causes of LTB in children, while PIV 3 is more frequently associated with LRTIs including bronchiolitis and pneumonia[10,11]. The seasonal patterns of human PIV infections differ according to the virus type with PIV 3 most prevalent in the late spring and summer, and PIVs 1 and 2 occurring most commonly in the late fall and early winter. Outbreaks of PIV 2 infections usually follow PIV 1 outbreaks. PIV 3 infections continue throughout the year, primarily in the spring and summer, while PIV 4 is isolated infrequently [1,2, 10–16]. Seroepidemiologic studies indicate that approximately half of US children older than one year, and nearly all of those over five years of age have been infected with PIV 3. Antibodies against PIV 1 and 2 develop somewhat later, with 50–74% of children demonstrating seropositivity by age five years. Although infections are less commonly identified by culture, between 70 and 90% of young adults are seropositive for PIV 4 [2, 10, 12].

The pathophysiology of PIV infection is generally accepted to be a result of direct virus cytotoxicity accompanied by a robust virus-induced inflammatory response in the infected airway. The initial host response to infection is dictated by events occurring within the infected respiratory epithelial cells. Proinflammatory mediators released from infected cells direct the recruitment of inflammatory cells to the site of infection and contribute to the clinical manifestations of disease. We have previously demonstrated that the epithelial cell line HEp-2 and explanted human bronchial epithelial cells respond to PIV infection with the production and release of the pro-inflammatory chemokines CCL3 (macrophage inflammatory protein 1-α) and CXCL8 (interleukin-8) [17]. Interestingly, while local production of proinflammatory mediators in response to RSV infection have been documented extensively[18–28], there are no analogous published studies evaluating inflammation in response to PIV.

In this study, we describe the clinical manifestations of PIV in children, identify differences in presentation and severity according to the PIV type and document the proinflammatory mediator ‘fingerprint’ detected in the nasal wash samples obtained from these patients.

Patients and Methods

Patient cohort and controls

Nasal wash samples obtained from children evaluated in our emergency room for suspected respiratory viral infection have been archived in our laboratory since 1998. The protocol was approved by the SUNY Upstate Medical University IRBPHS, #4460. Children were included in the present study if they were evaluated for a suspected acute respiratory viral infection, had a nasal wash sample collected between 1998–2008, were culture positive for PIV from that nasal wash sample, and had residual nasal wash sample available for further characterization. We identified 165 such patients. Chart review was performed to collect

patient information including demographics, details regarding clinical presentation, information as to whether or not the patient was hospitalized, and if so, details regarding the hospital course. Three categories of illness severity were used for the purposes of this study; mild, moderate and severe. Mild disease was defined as an illness not requiring hospitalization. Moderate disease was any illness requiring hospitalization, but not requiring administration of supplemental oxygen, and severe disease was defined as any illness requiring hospitalization and treatment with supplemental oxygen and/or admission to the pediatric intensive care unit. In addition, after obtaining informed consent, nasal wash samples from 79 asymptomatic children between the ages of two weeks and 4 years were obtained for use as controls. The age and gender distribution for the control group was similar to the research subjects with 55 children under the age of one year, and 15 children between the age of one and four years. Forty-four of the control subjects were males.

Co-detection of other viral pathogens

Nucleic acid was extracted from 100 μl of the archived nasal wash sample from all PIV culture positive patients and from 79 asymptomatic control patients. Nucleic acid was subjected to amplification (Luminex ID-TAG™ Respiratory Virus Panel [RVP]) to determine whether patients had been infected or co-infected with enterovirus, rhinovirus, human metapneumovirus, RSV A or B, coronavirus OC43, 229E, HKU1, or NL93, adenovirus, parainfluenza 1, 2, 3, or 4, influenza A or B. Twelve samples (7%) were documented to be culture positive or RVP positive for viruses other than PIV. Those patients were excluded from further analysis as it was then not possible to determine which of the infecting viruses contributed to the clinical presentation or pattern of induced pro-inflammatory mediators. None of the patients examined were simultaneously infected with more than one PIV type.

Measurement of pro-inflammatory mediators present in nasal wash samples

Cytokine profiles were determined in nasal wash fluid using a human cytokine twenty six-plex bead immunoassay kit designed to detect interleukin (IL)-1α, β, IL-1RA, IL-2, 3, 4, 5, 6, 7, 8, 10, 12, 13, 15, 17, interferon (inf) α and γ, granulocyte macrophage colony stimulating factor, TNF-α, CCL2 (macrophage chemotactic protein (MCP)-1), CCL3 and 4 (macrophage inflammatory protein (MIP)-1α, β), CCL11 (eotaxin), CCL5 (regulated upon expression normal T-cell expressed and secreted (RANTES)), CXCL10 (inducible protein (IP)-10) and CXCL9 (monokine induced by gamma interferon (MIG)) (Biosource International, Camarillo, CA). Nasal wash samples were stored at −80°C until use, then thawed at room temperature and diluted 1:1 prior to analysis according to the manufacturer's instructions. Following a two hour incubation with spectrally encoded beads coated with analyte-specific biotinylated primary antibodies, samples were incubated with streptavidin R-phycoerythrin and analyzed with a Luminex 100 IS xMap™ multiplex system (Luminex Corporation, Austin, TX). Data were analyzed with Star Station V. 2.0 software (Applied Cytometry Systems, Sacramento, CA). Protein concentrations were determined by the Bradford microassay (Biorad, Richmond, CA) against bovine serum albumin standards (Sigma Chemical Corporation, St. Louis, MO). Concentrations of inflammatory mediators are reported herein as per mg of total protein to correct for differences in nasal wash collection techniques [25, 29].

Statistical analyses

Chi square tests were used for associations between categorical variables. Student t-tests were used to compare inflammatory mediator concentrations between different groups. Analyses were performed using SPSS, version 17.

Results

We identified 165 children with a positive nasal wash culture for PIV. Twelve patients were excluded because of co-infection with another infectious agent known to cause respiratory illnesses. Respiratory virus panel analysis on nasal wash samples of 79 asymptomatic patients were negative for respiratory virus pathogens.

The seasonal distribution of the PIV infections by virus type is shown in Figure 1. PIV 3 infections were most common in the spring and early summer, while PIV 1 infections showed peaks in both spring and fall. The median age of the PIV infected patients was 6 months (range 2 wks to 12 yrs). Forty-one percent were females. Eighty (52%) of the 153 patients had one or more underlying chronic medical conditions. Thirty one patients were born prematurely (<36 weeks gestational age), 30 had asthma or reactive airway disease , 7 had chronic lung disease, 5 had trisomy 21, 22 had gastro-esophageal reflux disease, 9 had congenital heart disease, 2 had cystic fibrosis, 1 had CHARGE syndrome, and 1 had primary hypogammaglobulinemia.

The most common presenting symptom was cough (77%) but other signs and symptoms of acute respiratory tract infection were common (Table 1). Sixty-three percent of patients had a documented fever upon presentation, and 35% of patients presented with gastrointestinal complaints of vomiting, diarrhea, and/or abdominal pain (always in combination with one or more respiratory symptoms). Sixty-one (40%) patients were treated as outpatients (mild illness), while 92 (60%) patients were hospitalized for a median length of stay of 3 days. In the hospitalized group, 43 (28%) met our definition of severe illness. When we evaluated presenting symptoms and illness severity according to PIV type or PIV genus (respiroviruses or rubulaviruses), PIV type did not predict disease severity, however we found that supplemental oxygen treatment was more likely to be given to patients with respirovirus infection when compared to those with rubulavirus infection ($p<0.05$). We were surprised to find that gastrointestinal symptoms were also more commonly reported in those patients with respirovirus infection. Of the 125 (82%) patients who had a chest radiograph performed, 74 (59%) had abnormalities identified by the radiologist including infiltrates, consolidation or effacement of the cardiac border. Almost half of the patients (71 (46%)) were treated with antibiotics although none had a documented bacterial infection.

Table 2 summarizes the final clinical diagnoses of our patients by PIV type and genus. Seventy (46%) patients had an upper respiratory tract infection, 21 (14%) of whom had LTB. Sixty-seven (44%) patients had a LRTI. Apnea was described for 6 (4%) patients (three of whom also had other respiratory symptoms), and non-respiratory diagnoses included fever with no source (6 patients), dehydration (1), and meningoencephalitis (1). Two patients died. When we evaluated the final clinical diagnosis according to PIV type or genus, we noted that LTB was most often caused by PIV 1 and 2 with only a single case caused by PIV 3. Thirty-two of thirty-seven (86%) cases of bronchiolitis occurred during infection with respiroviruses, while 21 of the 30 (70%) cases of pneumonia were caused by PIV 3. We identified only 8 patients infected with PIV 4. Four of these patients had a lower respiratory tract infection, 3 had an upper respiratory tract infection, and one presented with apnea alone.

Of the 26 inflammatory mediators assayed from the nasal wash fluid, six were present in higher concentrations from PIV-infected patients when compared to uninfected controls, specifically IL-6, CXCL8, (IL-8), CCL3 (MIP-1α), CCL4 (MIP-1β), CXCL9 (MIG)) and CCL5 (RANTES) (Figure 2A). When we compared the concentrations of inflammatory mediators detected in patients infected with respiroviruses with those infected with rubulavirses, three mediators (IL-1β, IL-1RA, and CXCL8) were present in significantly

higher concentrations among those infected with respiroviruses (Figure 2B). Finally, when we compared mediator concentrations among the four PIV types, the only consistent finding was a statistically higher concentration of CXCL8 in patients infected with PIV 3 (Figure 2C).

When we evaluated inflammatory mediator concentrations in relationship to illness severity we found that nasal wash CXCL8 concentrations were elevated in patients with LRTI when compared to those with URI (Figure 3A). A similar comparison between patients with mild illness (not hospitalized) and moderate or severe illness combined (by study definition, hospitalized), revealed that CXCL8 concentrations were likewise higher in the latter group (Figure 3B). CXCL10 was also detected at elevated concentrations in nasal wash samples from patients with moderate and severe illness. Moderate and severe respirovirus infections were associated with higher nasal wash concentrations of IL-1β, CXCL8, and CXCL10 when compared to mild respirovirus infections, and similarly, CXCL8 and CXCL10 concentrations were higher in samples from patients diagnosed with moderate to severe rubulavirus infections when compared to those with mild rubulavirus infections. Finally, we observed higher nasal wash concentrations of CXCL8, IL-1RA and IL-1β in patients with moderate and severe respirovirus infection compared to those with moderate and severe infection with rubulaviruses (Figure 3C).

Discussion

In this study, we describe the clinical manifestations, infecting PIV types, and nasal wash cytokine profiles from 153 children with acute PIV infection. The median age of our cohort was six months, and 59% were males. The seasonal distribution of the infections showed two peaks, one in the spring from PIVs 1 and 3, and another in the fall from PIV 1. As expected, PIV 3 was the most common virus type isolated, while PIV 4 infections were not commonly documented. Almost all patients had some respiratory symptoms, the majority had fever, and a large proportion also had documented vomiting, diarrhea, and/or abdominal pain (54/153)[30]. Forty percent of the patients included in the study were treated as outpatients. Of those hospitalized, 47% required oxygen and/or ICU admission. There were two deaths, one with PIV 1 and one with PIV 2. We found that respiroviruses (PIV 1 and 3) were more likely to cause lower respiratory tract infections when compared to rubulaviruses (PIV 2 and 4), and PIV associated pneumonia was most common with PIV 3, consistent with our observation that supplemental oxygen was more commonly used in the respirovirus group. PIV 1 and 2 infections accounted for 20 of 21 cases of LTB (95%). In our cohort, 31% of the patients infected with PIV 2 required admission to the intensive care unit. Nearly half of the children included in our study received antibiotic therapy either in the emergency department or during their hospitalization, although none of them had a documented bacterial infection.

This is the first published study to present a systematic evaluation of the pro-inflammatory cytokine profile from PIV-infected children and to examine this in relation to disease severity. Compared to healthy subjects, PIV-infected children produce and release a profile of pro-inflammatory cytokines into their airway including IL-6, CCL3, CCL4, CCL5, CXCL8 and CXCL9. Most of the published literature in the area of virus induced chemokine expression, and localized innate immune responses during acute respiratory viral infection has focused on cytokine profiles obtained form RSV-infected children. These studies have shown that CCL2, CCL3, CCL5 and CXCL8 concentrations each correlated with RSV disease severity [24–26, 28]. Among these reports, Henrickson and colleagues showed that higher concentrations of IL-6, CXCL8 and IFN-γ were detected in nasopharyngeal aspirates collected from wheezing children with RSV and influenza A infections, but CXCL8 concentrations did not correlate with illness severity [6]. Gern and colleagues evaluated nasal

wash samples from infants with acute viral infections and found elevated levels of CXCL8 from infants with RSV, parainfluenza and rhinovirus infections, but did not find a significant correlation between nasal CXCL8 concentrations and symptom scores; of note PIV virus typing and disease severity were not evaluated independently [31].

The innate immune responses to infection with RSV and related pneumoviruses have also been explored in animal models of infection. In the mouse model of RSV, elevated concentrations of CCL2, CCL3, and CCL5 are detected in lung tissue following virus challenge [32,33]. Similarly, in the cotton rat model, RSV challenged rodents show elevated pulmonary concentrations of growth-regulated protein, CCL2, CCL5, IL-1β, IL-6, interferons α and γ, and CXCL10 [34]. The rodent specific pneumovirus, pneumonia virus of mice (PVM), induces airway expression and release of several mediators that correlate with illness severity and pulmonary function abnormalities including CCL2, CCL3, and the human CXCL8 orthologues CXCL2 (MIP-2) and KC [35].

A role for CXCL8 in PIV pathogenesis is likely given our observations in infected children. First, CXCL8 concentrations were higher in the nasal wash samples from PIV-infected patients compared to the control, uninfected patients. In addition, we noted that CXCL8 concentrations were higher in patients with LRTI when compared to URI, and in patients with moderate and severe illness compared to mild illness. When we stratified our patients by disease severity, we found a significant difference in CXCL8 concentrations in severe cases when compared to non-severe cases. The mechanism(s) by which PIV induces host CXCL8 production and release *in vivo* is not known, however PIV 4 has been shown to enhance CXCL8 release from airway epithelial cells (NCI-H292) via both transcriptional and post transcriptional means[36].

Conclusion

Our finding that nasal wash CXCL8 concentrations were higher in patients with severe PIV disease, together with our previously published observation that hydrocortisone reduces PIV-induced CXCL8 release in PIV-infected cultured epithelial cells[16] may offer a biochemical explanation for why glucocorticoids are effective at reducing the severity of clinical PIV disease in children [37–40]. Specifically, reducing CXCL8 (and other chemokine) production via glucocorticoid administration may result in diminished recruitment of pro-inflammatory leukocytes resulting in a diminished inflammatory response with symptom amelioration.

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Abbreviations

CCL and CXCL	chemokines ligand (s) as numbered
CHARGE syndrome	Coloboma of the eye, Heart defects, Atresia of the choanae, Retardation of growth and/or development, Genital and/or urinary abnormalities, and Ear abnormalities and deafness
IFN	interferon
IL	interleukin
IL-1RA	interleukin-1 receptor antagonist

IP-10	inducible protein 10
LRTI	lower respiratory tract infection
LTB	laryngotracheobronchitis
MCP	macrophage chemotactic protein
MIG	monokine induced by gamma interferon
MIP	macrophage inflammatory protein
PIV	parainfluenza virus
RANTES	regulated upon activation, normal T-cell expressed and secreted
URI	upper respiratory tract infection

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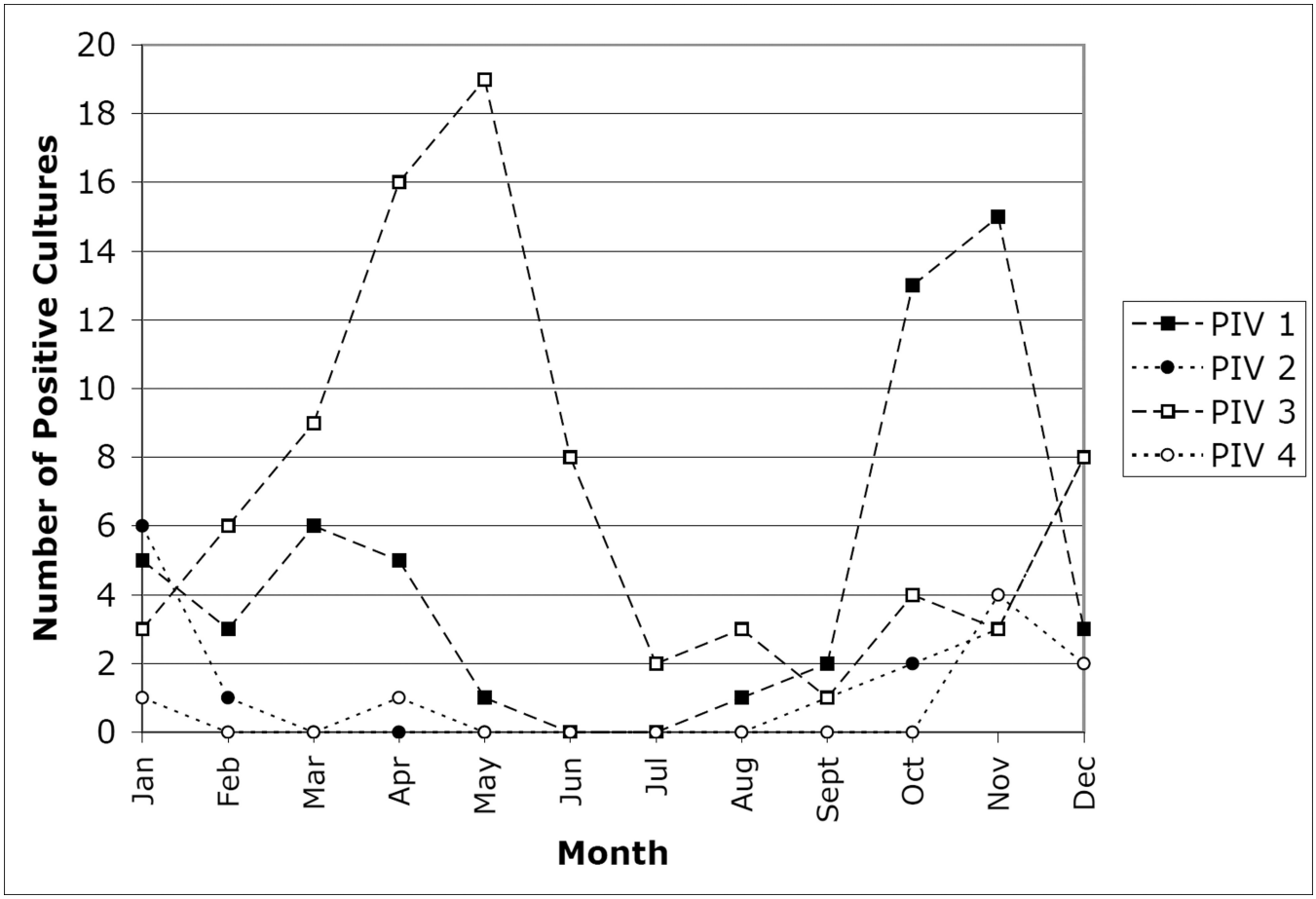
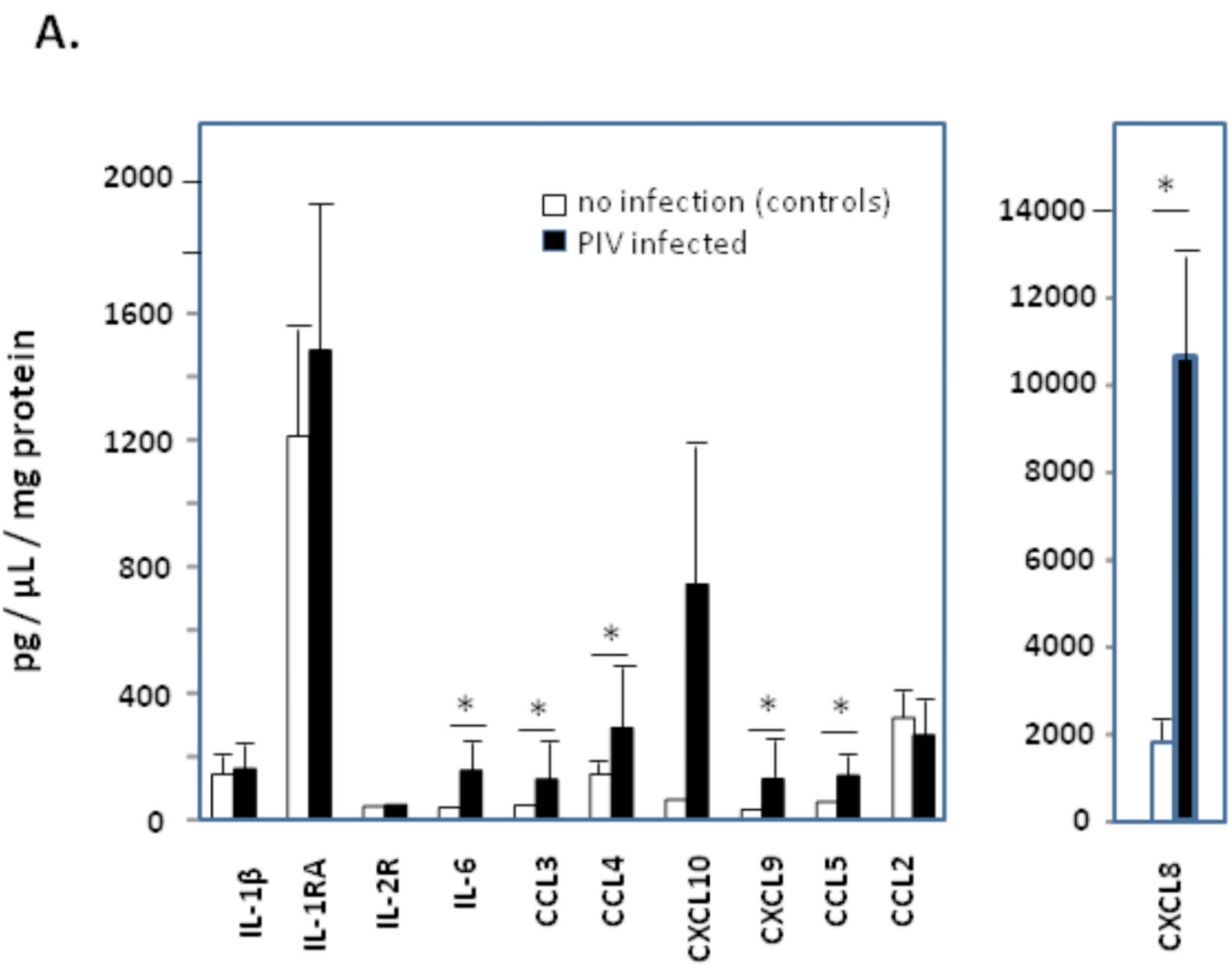
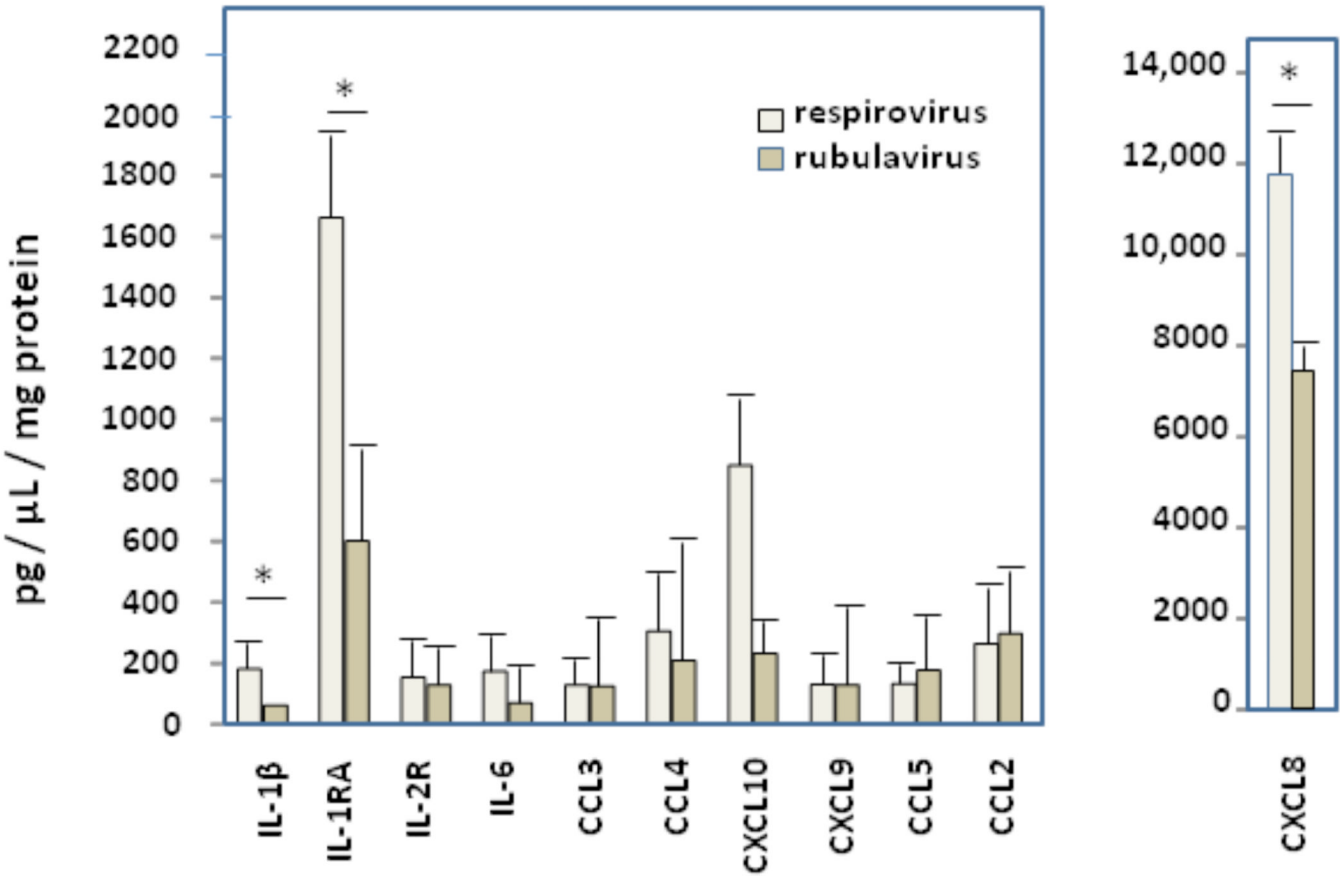


Figure 1.
Seasonal distribution of PIV infections: Shown are the number of positive parainfluenza virus cultures, by virus type and by month 1998–2008 inclusive.



B.



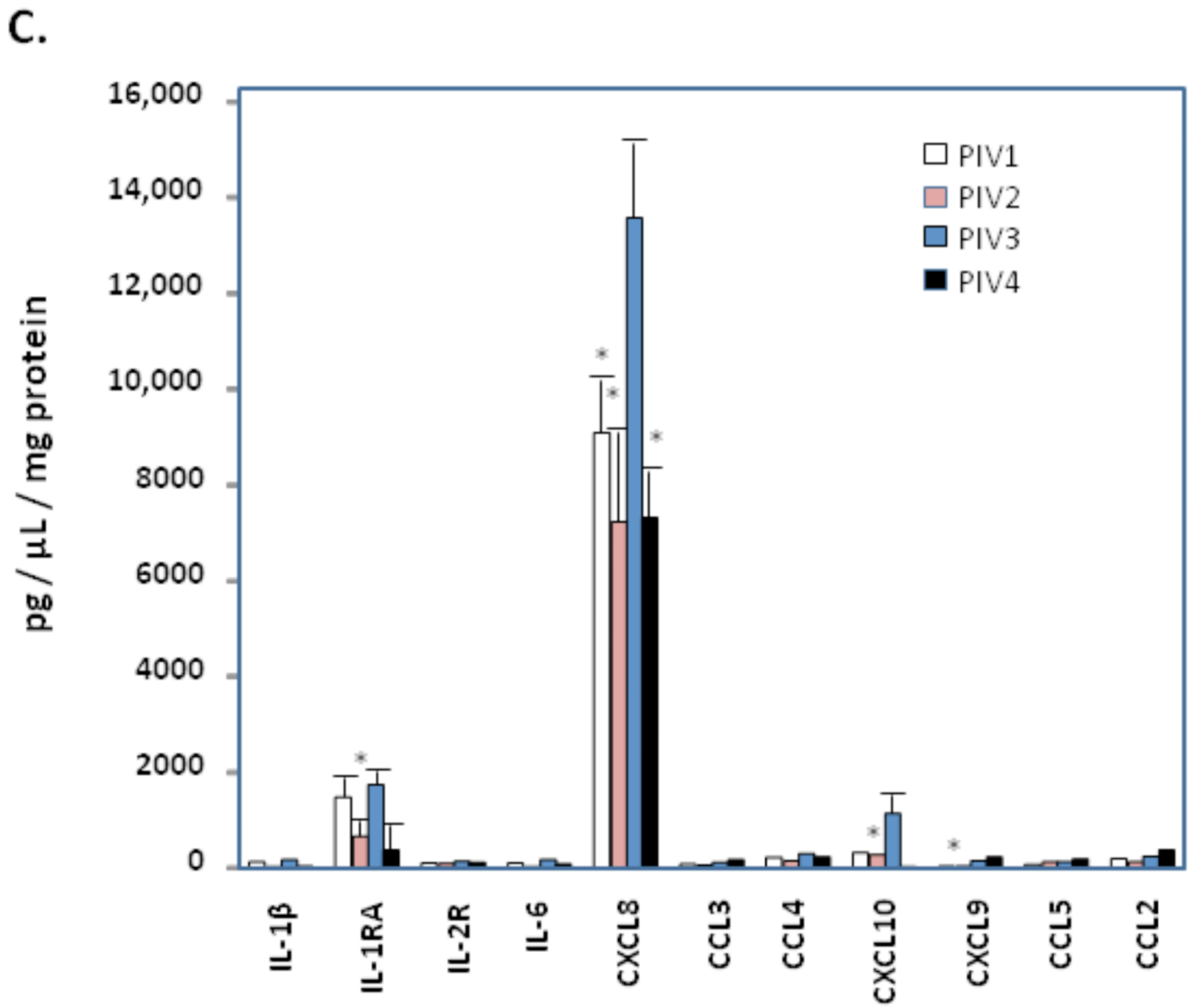


Figure 2.

Nasal wash concentrations (mean plus SD) of proinflammatory mediators comparing results obtained from (A) uninfected control patients to all PIV-infected patients (*$p<0.05$), (B) respirovirus-infected patients to rubulavirus-infected patients (*$p<0.05$), and (C) patients infected with each of the four PIV types ($p<0.05$ when compared to PIV 3-infected patients).

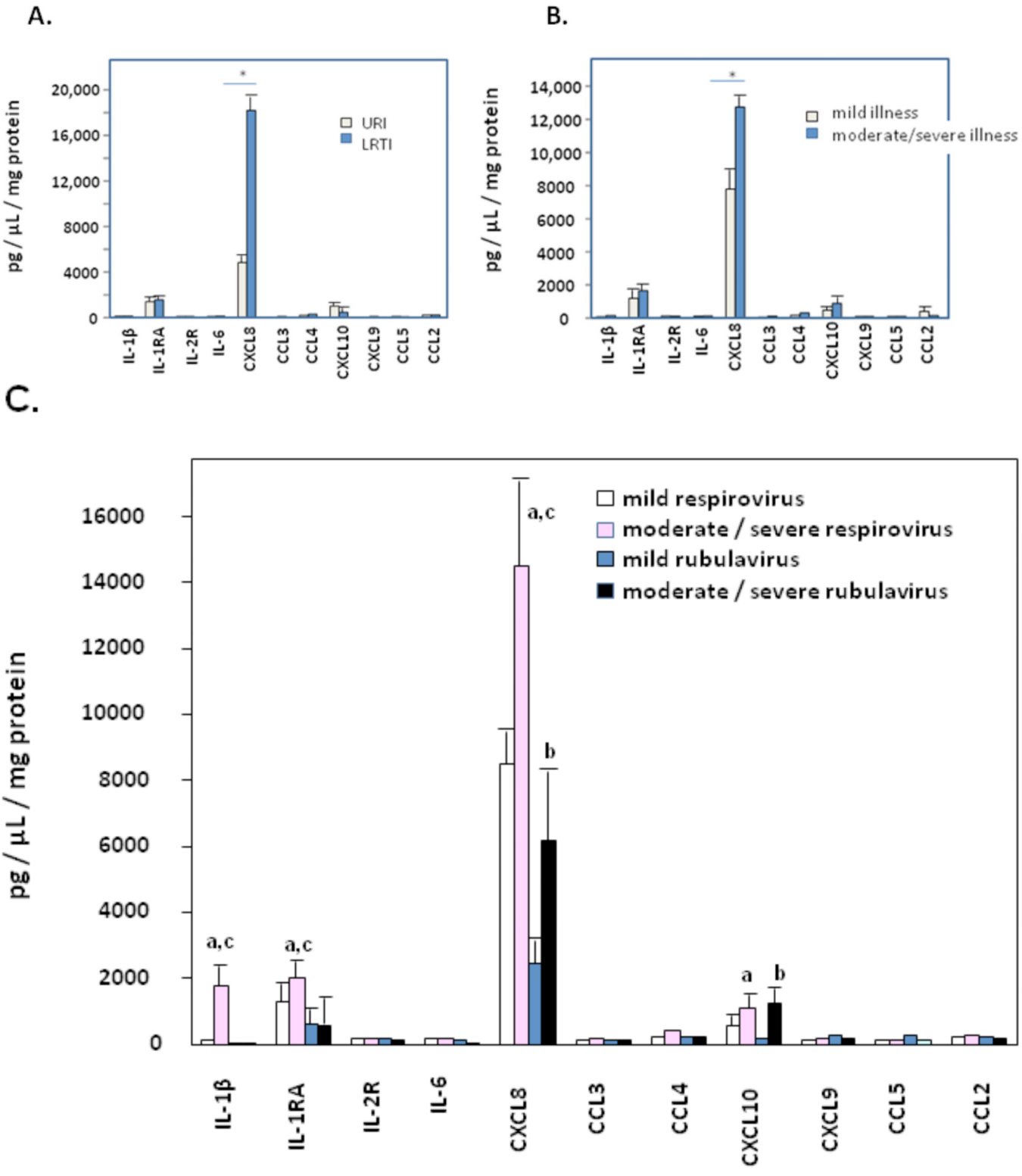


Figure 3.

Nasal wash concentrations (mean plus SD) of proinflammatory mediators comparing results obtained from (A) patients with URI compared to LRTI (*$p<0.05$), (B) mild illness compared to moderate and severe illness ($p<0.05$), and (C) mild respirovirus, mild

rubulavirus, moderate/severe respirovirus, and moderate/severe rubulavirus infection (‘a’ indicates $p<0.05$ compared to mild respirovirus infection, ‘b’ indicates $p<0.05$ compared to mild rubulavirus infection, and ‘c’ indicates $p<0.05$ compared to moderate to severe rubulavirus infection.

Table 1

Presenting symptoms, illness severity and parainfluenza virus types

	PIV1	PIV3	Respirovirus (PIV1+3)	PIV2	PIV4	Rubulavirus (PIV2 + 4)	Total
Numbers of patients n (%)	52 (34)	74 (48)	126 (82)	19 (12)	08 (05)	27 (18)	153 (100)
Female n (%)	24 (46)	24 (32)	48 (38)	10 (53)	04 (50)	14 (52)	62 (41)
Male n (%)	28 (54)	50 (67)	78 (62)	09 (47)	04 (50)	13 (48)	91 (59)
Median age (mos)	06	06	06	12	03.5	06	06
Presenting Symptoms							
Fever n (%)	36 (69)	46 (62)	82 (65)	12 (63)	03 (37)	15 (55)	97 (63)
Nasal symptoms n (%)	39 (75)	42 (57)	81 (64)	09 (47)	07 (87)	16 (59)	97 (63)
Cough n (%)	41 (79)	58 (78)	99 (78)	12 (63)	07 (87)	19 (70)	118 (77)
Shortness of breath n (%)	25 (48)	41 (55)	66 (52)	12 (63)	05 (62)	17(63)	83 (54)
Tachypnea n (%)	14 (27)	29 (39)	43 (34)	08 (42)	01 (12)	09 (33)	52 (34)
Wheezing n (%)	21 (40)	32 (43)	53 (42)	04 (21)	02 (25)	06 (22)	60 (39)
Stridor n (%)	07 (13)	02 (03)	09 (07)	07 (37)	01 (12)	08 (30)	17 (11)
Crackles n (%)	06 (11)	06 (08)	12 (09)	00 (00)	01 (12)	01 (04)	13 (08)
Gastrointestinal symptoms n (%)	19 (36)	30 (40)	49 (39)	04 (21)	01 (12)	05 (18)	54 (35)
Illness Severity							
Mild n (%)	24 (46)	30 (41)	54 (43)	03 (16)	04 (50)	07 (26)	61 (40)
Moderate n (%)	16 (31)	21 (28)	37 (29)	09 (47)	03 (38)	12 (44)	49 (32)
Severe n (%)	12 (23)	23 (31)	35 (28)	07 (37)	01 (12)	08 (30)	43 (28)
Oxygen requirement n (%)	09 (17)	14 (19)	23 (18)	01 (05)	00 (00)	01 (04)	24 (16)
ICU admission n (%)	03 (06)	09 (12)	12 (09)	06 (31)	01 (12)	07 (26)	19 (12)

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Table 2

Final clinical diagnosis

	PIV1	PIV3	Respirovirus (PIV1+3)	PIV2	PIV4	Rubulavirus (PIV2 + 4)	Total
Numbers of patients	52	74	126	19	08	27	153
Upper respiratory tract infection n (%)	30 (58)	26 (35)	56 (37)	11 (58)	03 (37)	14 (52)	70 (46)
1. LTB n (%)	11 (21)	01 (01)	12 (09)	09 (47)	00 (00)	09 (33)	21 (14)
2. Other n (%)	19 (36)	25 (34)	44 (35)	02 (10)	03 (37)	05 (18)	49 (32)
Lower respiratory Tract infection n (%)	18 (35)	40 (54)	v58 (46)	05 (26)	04 (50)	09 (33)	67 (44)
1. Bronchiolitis n (%)	13 (25)	19 (26)	32 (25)	03 (16)	02 (25)	05 (18)	37 (24)
2. Pneumonia n (%)	05 (10)	21 (28)	26 (21)	02 (10)	02 (25)	04 (15)	30 (20)
Fever, no source n (%)	02 (04)	02 (03)	04 (03)	02 (10)	00 (00)	02 (07)	06 (04)
Apnea n (%)	00 (00)	05 (07)	05 (04)	00 (00)	01 (12)	01 (04)	06 (04)
Other n (%)	02: death, dehydration	01: meningoencephalitis	03 (02)	01: death	00 (00)	01 (04)	04 (03)