Adenovirus and respiratory syncytial virus-adenovirus mixed acute lower respiratory infections in Chilean infants

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Background. **In Chile respiratory syncytial virus (RSV) and adenovirus (AD) are the principal viruses detected in acute lower respiratory infections (ALRI) in infants. An overview of AD pneumonia in Chile to detect annual trends and to compare the severity of single AD or mixed RSV-AD infections is presented.**

Methods. **Surveillance in 4927 infants hospitalized for ALRI has been performed from 1989 to 2001 using immunofluorescence assay (IFA) and viral isolation. Clinical features in 117 infants with single genotyped AD and 81 infants with mixed RSV-AD infections were analyzed.**

Results. **Adenovirus cases declined from 20% annually in the early 1990s to ~5% in the 2000 decade. Genotype 7h showed increasing prevalence in hospitalized cases. The mean annual burden of hospitalizations caused by AD in Santiago was estimated to be 0.6%. No difference was observed in duration of fever, oxygen requirement and hospital stay between groups. Lung consolidation was more frequent in AD cases than mixed cases ($P < 0.01$); interstitial pattern and hyperinflation prevailed in the mixed cases ($P < 0.01$). No child died. AD diagnosis was confirmed on admission by IFA in 17% of cases of RSV-AD and in 43% of cases of single AD ALRI. AD cases diagnosed early by IFA had worse clinical outcome than those diagnosed later by virus isolation ($P < 0.05$).**

Conclusions. **AD cases declined since 1989. Mixed RSV-AD infections were not more severe than single AD etiology. AD cases admitted with positive IFA had worse prognoses than AD infections diagnosed later by virus isolation.**

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INTRODUCTION

Acute lower respiratory tract infections (ALRI) are the main cause of hospitalization of infants, particularly during the cold seasons, and they are the leading cause of death in children between 1 month and 4 years of age.[1, 2] In Chile respiratory viruses are the leading cause of acute lower respiratory tract infection in children <2 years of age.[3] Epidemiologic studies of ALRI requiring hospitalization in Chile have detected respiratory syncytial virus (RSV) in 50% and adenovirus (AD) in 12.6% of cases.[3–5] Whereas RSV is detected mainly during the cold season, AD is isolated all year long.[4, 5] AD infection has been associated with severe pneumonia, wheezing bronchitis, prolonged hospital stay, admission to intensive care units, need for respiratory support, nosocomial infections and death.[6–9] AD serotypes 3, 7 and 21 have been associated with ALRI,[7] and in Chile serotypes 7, 2 and 1 are the most frequently detected.[4] Epidemiologic studies in Chile and in the south cone of South America demonstrated in 1984 the emergence of the AD 7h genomic variant, which has enhanced pathogenic potential.[9–13] The implementation of genome typing techniques with the use of restriction enzymes has allowed the cross-matching of clinical and epidemiologic aspects with the AD strains isolated.[10–13]

Viral mixed infections are detected in the surveillance program of Roberto del Río Children's Hospital in ~10% of cases, mainly during cold seasons. The most frequent association is RSV-AD infections (LF Avendaño and MA Palomino, personal communication). Bacterial coinfection is difficult to prove in these cases. It remains unclear whether infections by mixed agents result in more severe illness than those caused by AD alone. Immunofluorescence assay (IFA) has less sensitivity than viral isolation for AD detection.[4]

The aim of this report is to present an overview of hospitalization of Chilean infants ALRI caused by adenovirus infections during a period long enough to detect multiyear trends and to compare the severity of

ALRI associated with single AD or mixed RSV-AD infection.

MATERIALS AND METHODS

Study design. Epidemiologic surveillance of ALRI in infants <2 years of age admitted to the Roberto Del Río Children's Hospital in Santiago, Chile, was performed from 1989 to 2001. Cases were evaluated prospectively. Clinical criteria for the diagnosis of ALRI included two or more of the following signs: fever; tachypnea; wheezing; cough; dyspnea; rales; cyanosis or respiratory distress; abnormal chest roentgenograms; and hypoxemia (O_2 saturation, <94%). Nasopharyngeal aspirates (NPA) were obtained within 72 h of admission and processed for IFA and viral isolation. Clinical and virologic data were entered in Excel. All surveillance cases were included in the study. For clinical comparison of single AD and mixed RSV-AD infections, only patients <1 year of age were selected retrospectively, including all mixed RSV-AD infections. We selected the single AD cases with the isolates already genotyped.

Clinical severity assessment. Data registered during the study period were analyzed (diagnosis, age, sex, underlying illness, full blood counts and chest radiographs). Severity markers included rectal temperature >38°C and days of hospital stay, oxygen requirements and mechanical ventilation. For the aims of the clinical severity comparison, exclusion criteria were children >12 months of age, history of prematurity, underlying pulmonary, cardiac or neurologic disease, children who required previous hospitalization for any reason and those with a previous history of wheezing. the selection criteria for analysis were met by 81 of the 110 cases with mixed infection and 117 of the 230 genotyped cases of AD infection. Both groups were comparable for age and sex.

Respiratory viruses diagnosis. *Specimen collection.* A nasopharyngeal aspirate was routinely obtained for each patient within 72 h of admission for RSV, influenza (FLU), parainfluenza (PI) and AD detection.

IFA. For indirect detection of viral agent, smears were prepared in triplicate and fixed in cold acetone. IFA was run for RSV, AD, FLU A and B and PI 1 to 3 as described elsewhere, with the use of monoclonal antibodies. RSV, AD and PI monoclonal antibodies were kindly provided by Dr. L Anderson (CDC, Atlanta, GA), and PI 3 and FLU A and B were kindly provided by Dr. P Pothier (Dijon, France). We used commercial conjugates (Sigma-Aldrich).[3, 5, 14]

Viral isolation. Each specimen was processed as described by Avendaño et al.[3] Briefly samples were inoculated into HEp-2 and Madin-Darby canine kidney cells. Cultures were observed every other day for development of cytopathic effect for 1 week, after which confirmatory IFA were performed in both cultures, with and without cytopathic effect.

Adenovirus genome typing. Each strain was propagated on HEp-2 or A-549 cells, and viral DNA was extracted according to the method of Shinagawa et al.[15] and further studied with different endonucleases.[10–13] Isolates belonging to subgenus B were typed by digestion with the endonucleases *Bam*HI and *Sma*I and for further characterization *Xho*I. For subgenus C isolates the endonucleases used were *Bam*HI, *Sma*I, *Bgl*II and *Hin*dIII. The profiles obtained were compared with international reference patterns to assign serotypes and genotypes to the strains analyzed.[10–13, 16]

Statistical analysis. The statistical analysis was done with Epi-Info 6.04[17] and using χ^2 for linear trends for the epidemiologic data. For the comparison of proportions and means, χ^2 or Fisher's exact test, with a 5% statistical significance, was computed. For the comparison of central tendency measurements with an abnormal distribution (days of supplementary oxygen, hospitalization and fever), a median test was used.

RESULTS

Adenovirus surveillance. The Roberto del Río Children's Hospital serves ~26 000 children <2 years of age. During the 13 years of the survey, an annual mean of 1800 children <2 years old were admitted for ALRI in this hospital. In our surveillance system AD was detected on admission by IFA and/or cell culture isolation in 9.3% of the enrolled patients. (Table 1). According to this information the AD ALRI admissions rate in children <2 years of age was 1.3% from 1989 through 1992 and 0.3% from 1994 through 2001. Therefore the total burden of AD ALRI hospitalizations in this area of Santiago de Chile is estimated to be 0.6% per year.

Adenoviruses were detected all year round. From 1989 through 1992 an increase in AD detection took

TABLE 1. Adenovirus surveillance on infants hospitalized for acute respiratory infections in Santiago, Chile: annual distribution 1989 to 2001

Yr	No. of Hospitalized Patients Enrolled	Adenovirus-positive Cases*	
		No.	%
1989	270	60	22
1990	260	69	27
1991	502	96	19
1992	421	58	14
1993	315	20	6
1994	452	22	5
1995	448	37	8
1996	326	10	3
1997	337	11	3
1998	448	24	5
1999	445	15	3
2000	389	16	4
2001	309	19	6
Total	4927	457	9.3

* χ^2 for linear trend: $P < 0.01$.

place during the winter, and more cases with mixed RSV-AD infections were observed. There were high AD detection rates from 1989 through 1992 (20%), the peak being in May 1991 (56%). A decline was observed afterward to an annual level of ~5% (Fig. 1). During 1989 and 1990 AD subgenus C serotypes 2 and 1 were predominant, but from 1991 forward, subgenus B emerged as more frequent, with genotype 7h the most prevalent (Table 2).

With respect to age infants younger than 6 months of age accounted for 64% of all ALRI-enrolled cases, 516 cases (10%) being younger than 1 month of age. AD detection was more common in infants >6 months of age: 240 of 1751 = 13.7% *vs.* 217 of 2176 = 6.8% in infants <6 months of age ($P < 0.01$).

Pneumonia and/or wheezing bronchitis were the more frequent diagnoses on admission (97.5%). Fifty-one percent of the single AD infections had clinical signs of bronchial obstruction. In this series of previously healthy children no fatalities associated with AD infection were observed.

Mixed RSV-AD *vs.* single AD infections. AD diagnosis was confirmed by rapid test (IFA) in 17% of the mixed RSV-AD cases and in 43% of those cases with single AD ALRI, all of them having positive viral isolation. In a large proportion of the cases, positive AD culture reports were available with 1 or 2 weeks delay, and patients were considered negative for AD at the time of discharge. The average age for the cases with pure AD or mixed RSV-AD infections were 5.4 and 2.6 months, respectively ($P < 0.01$). Male sex accounted for 60 and 58% of cases, respectively.

Significant differences in clinical outcomes with regard to oxygen requirement, hospital stay and fever between both groups were not observed; patients with AD infection required supplementary oxygen and mechanical ventilation as frequent as those with mixed infection (Table 3). The presence of lung consolidation was significantly more frequent in AD infection as a single agent ($P < 0.01$). An interstitial and hyperinflation pattern prevailed in the mixed RSV-AD cases (Table 4). Atelectasis was similar in both groups, 7 and 10%, respectively. The analyses of the 81 patients with a full blood count with AD alone and 37 with mixed infection did not exhibit any difference with respect to leukocyte counts, left deviation or average erythrocyte sedimentation rate.

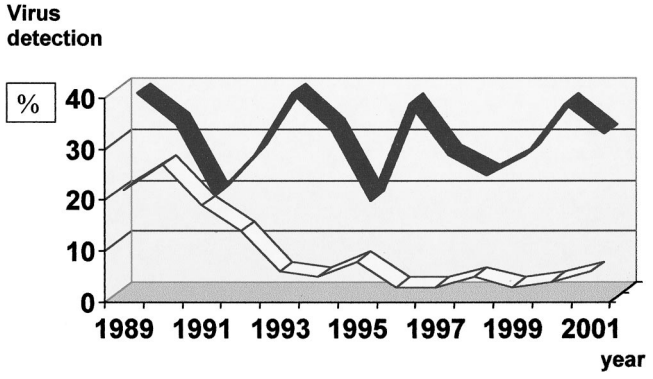

FIG. 1. RSV and adenovirus surveillance in infants hospitalized for acute lower respiratory infections in Chile. 1989–2001. □, adenovirus; ■, RSV.

Immunofluorescence assay positivity on admission as a severity marker of adenovirus infection. In the group of 117 AD patients, the cases diagnosed by rapid IFA on admission had significantly worse clinical outcomes than those diagnosed later by viral isolation alone ($P < 0.05$). In the group diagnosed by IFA on admission, genotype 7h was more frequent (Table 5).

DISCUSSION

Adenovirus is the second most frequent respiratory viral agent detected in pediatric cases admitted for ALRI in Chile.[3, 4] Its frequency has been decreasing during the past decade, but it represents an important cause of nosocomial diseases, which could have high lethality and may produce severe pulmonary sequelae.[6, 9] The emergence of a virulent adenovirus strain, genotype 7h, in the south cone of Latin America during the 1980s has been recognized in Chile.[10–13] The prevalence of this strain is illustrated in this survey. We have no explanation for the decrease in frequency of adenovirus detection from 1990 to 2003. Because AD infection is not very common in the community, increasing herd immunity to the prevalent AD 7h strain would hardly explain the phenomenon. Studies of the AD genome type prevalence in outpatients and of neutralizing antibody titers in the community are very difficult to perform to elucidate this point.[18] A major problem of severe AD infection in Latin America is nosocomial transmission where children with underlying disease are frequently infected.[9,19] Chilean health authorities are aware of the problem of adenovirus infections and have improved both the diagnostic techniques and the hospital facilities by increasing the number of individual isolation rooms. The routine application of rapid respiratory viral diagnosis in hospitals has shortened the hospital stay, minimized the unnecessary use of antibiotics and prevented the spread of nosocomial infection.[2–4] In contrast to other descriptions of AD severe prognosis, where children with underlying illness were included,[19, 20] in this report previously healthy children hospitalized for ALRI had good outcomes, and no death occurred. The clinical picture of an ALRI on admission does not allow differentiating viral or bacterial etiology or defining the type of viruses involved.[3, 21] Some clinical features like wheezing are expected in a viral etiology. In AD the presence of high fever, severe wheezing and progressive consolidation in the chest radiograph are common.[7] In the present study, interstitial pneumonia and

TABLE 2. Genome typing of adenovirus strains

No. of Samples		Subgenus B			Subgenus C		
Yr	Genome typed	Ad-3	Ad-7	Other	Ad-1	Ad-2	Ad-5
1989	33	1	7	0	3	20	2
1990	62	1	28	1*	11	21	0
1991	32	1	28	0	2	1	0
1992	34	0	27	1†	1	4	1
1993	10	0	4	1†	1	2	2
1994	16	2	3	0	3	7	1
1995	15	3	11	0	0	1	0
1996	9	0	8	0	0	0	1
Total	211	8	116‡	3	21	56	7

* Ad-16.
† Ad-11.
‡ 54.9% ($P < 0.01$).

TABLE 3. Severity of adenovirus or mixed RSV-adenovirus acute lower respiratory infections*

Severity Markers	*N*	% of Cases with Supplementary O_2	% of Cases with Mechanical Ventilation	Median Days of Hospital Stay	Median Days of Fever >38°C
AD ALRI	117	63	1.7	7	1
RSV-AD ALRI	81	54	4.93	6	1

* *P*, not significant.

TABLE 4. Chest radiographs in single adenovirus or mixed RSV-adenovirus acute lower respiratory infections

	N	% Consolidation	% Interstitial Pattern	% Hyperinflation
Only AD ALRI	102	84.31	60.78	24.5
RSV + AD ALRI	78	43.58	79.48	48.71
P		0.000	0.007	0.000

TABLE 5. Severity of adenovirus acute lower respiratory infections according to the positivity of immunofluorescence on admission

	N	% Supplementary Oxygen	% Mechanical Ventilation	Median Days of Hospitalization	% Fever >38°C	% Genotype B 7h
IFA-positive	53	51	3.7	8	74	75
IFA-negative	64	25	0	5	52	50
P		0.003	0.046*	0.044	0.014	0.04

* Fisher's exact test.

hyperinflation were more relevant in those cases with mixed RSV-AD infection than in single AD infections in which consolidation was more frequent. To predict the severity of the infection, some objective and easily measured markers such as days of hospital stay, mechanical ventilation or supplementary oxygen requirement were chosen. The results showed that the severity in the group with a single AD infection *vs.* mixed RSV-AD infections was the same. In the group of patients with mixed infections, AD was diagnosed with delay by viral isolation.

In previous reports we demonstrated that IFA was positive for >4 days in severe cases as compared with the milder infections. In the current prospective study, the cases with single AD infection diagnosed by IFA on admission had more severe outcome than those diagnosed only by viral isolation. The single AD cases more frequently diagnosed by IFA on admission than those with a mixed RSV-AD infection argue in favor of the hypothesis that IFA would easily detect the more severe cases. The delay in the AD diagnosis in the mixed RSV-AD cases because of negative IFA on admission might presume a less severe AD infection. In fact, in the group of mixed RSV-AD detection, we believe that the diagnosis and the clinical picture were determined mainly by the fact that the RSV infection and the AD isolation were laboratory findings. Viral isolation is a slow procedure and restricted to a few specialized laboratories. In this respect rapid viral isolation techniques in shell vials, although less sensitive than classic tissue culture isolation, is more sensitive than IFA and should be considered.

In conclusion, in this long surveillance in previously healthy children, the AD infection had better outcomes than previously reported, and no fatal cases were found. Furthermore mixed RSV-AD infections, mainly detected during the winter RSV epidemics, were not more severe than single AD ones. Because IFA is less sensitive than isolation for AD diagnosis, a positive IFA on admission may point to a more severe outcome. This study highlights the importance of the viral isolation for the detection of less severe and subclinical infections, which may constitute a source for nosocomial infection. The increasing number of children hospitalized with chronic illnesses forces the need for development of rapid and more sensitive adenovirus diagnostic techniques and set up of individual isolation wards to prevent nosocomial spread of adenoviruses.

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REFERENCES

1. Kaempffer AM, Medina E. Análisis de la mortalidad infantil y factores condicionantes. Chile 1998. Rev Chil Pediatr 2000; 71:405–12.
2. Girardi G, Astudillo P, Zúñiga F. El programa IRA en Chile: hitos e historia. Rev Chil Pediatr 2001;72:292–300.
3. Avendaño LF, Larrañaga C, Palomino MA, et al. Community and hospital acquired respiratory syncytial virus infection in Chile. Pediatr Infect Dis J 1991;10:564–8.
4. Larrañaga C, Kajon A, Villagra E, Avendaño LF. Adenovirus surveillance on children hospitalized for acute lower respiratory infections in Chile (1988–1996). J Med Virol 2000;60: 342–6.
5. Avendaño LF, Palomino MA, Larrañaga C. Surveillance for respiratory syncytial virus in infants hospitalized for acute lower respiratory infection in Chile (1989 to 2000). J Clin Microbiol 2003;41:4879–82.
6. Wu E, Martínez V, Alvarez A, Larrañaga C, Vela H. Casos fatales de infección por adenovirus. Rev Chil Pediatr 1990; 61:177–84.
7. Horwitz MS. Adenoviruses. In: Knipe DM, Howley PM, eds. Fields virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins, 2001:2301–26.
8. Singh-Naz N, Brown M, Ganeshananthan M. Nosocomial adenovirus infection: molecular epidemiology of an outbreak. Pediatr Infect Dis J 1993;12:922–5.
9. Palomino MA, Larrañaga C, Avendaño LF. Hospital acquired adenovirus 7h infantile respiratory infection in Chile. Pediatr Infect Dis J 2000;19:527–53.
10. Kajon A, Vicente M. Molecular epidemiology of adenovirus isolated from hospitalized children with severe lower acute respiratory infection in Santiago, Chile. J Med Virol 1990;30: 294–7.
11. Kajon A, Mistchenko A, Videla C, Hortal M, Wadell G, Avendaño LF. Molecular epidemiology of adenovirus acute lower respiratory infections of children in the South Cone of South America (1991–1994). J Med Virol 1996;48:151–6.
12. Kajon A, Vicente M, Suarez M, Avendaño LF, Wadell G, Hortal M. Genome type analysis in South America adenovirus of subgenus C collected over a seven-year period. Arch Virol 1993;32:29–35.
13. Kajon A, Larrañaga C, Suarez M, et al. Genome type analysis of Chilean adenovirus strains isolated in a Children's Hospital between 1988 and 1990. J Med Virol 1994;44:16–21.
14. Larrañaga C, Avendaño LF, Gaggero A, et al. Diagnóstico de infección por adenovirus y VRS en lactantes: comparación entre aislamiento e inmunofluorescencia indirecta. Rev Chil Infect 1990;7:167–71.
15. Shinagawa M, Matsuda A, Ishiyama T, Goto H, Sato G. A rapid and simple method for preparation of adenovirus DNA from infected cells. Microbiol Inmunol 1983;27:817–22.
16. Adrian Th, Wadell G, Hierholzer J, Wignad R. DNA restriction analysis of adenovirus prototypes 1 to 41. Arch Virol l986;91:277–90.
17. Epi-Info Computer Program. Version 6.04. Atlanta: Centers for Disease Control and Prevention, 1998.
18. Papic Z, Rodriguez L, Larrañaga C, Avendaño LF. Virus respiratorios en lactantes con infecciones respiratorias altas y bajas. Rev Chil Pediatr 1992;63:256–61.
19. Mistchenko AS, Robaldo JF, Rosman FC, Koch ER, Kajon AE. Fatal adenovirus infection associated with new genome type. J Med Virol 1998;54:233–6.
20. Carballal G, Siminovich M, Murtagh P, et al. Etiological, clinical and histopathological analysis of 32 fatal cases of acute respiratory infection in children under 5 years old. Rev Infect Dis 1990;12:S1074–80.
21. Palomino MA, Larrañaga C, Montaldo G, Suárez M, Díaz A, Avendaño LF. Infección extra e intrahospitalaria por virus sincicial respiratorio en lactantes. Rev Chil Pediatr 1994;65: 11–16.