Article

Not peer-reviewed version

High Copy Number from Pharyngeal Swab Is Not Associated with Different Presenting Features in 100 Children with Acute Adenovirus Infection from a Cluster in Italy

Anthea Mariani , Federica Cavallo , Saverio La Bella , Giusi Graziano , Martina Passarelli , Carlo Crescenzi , Daniela Trotta , Maurizio Arico' *

Posted Date: 10 October 2023

doi: 10.20944/preprints202310.0565.v1

Keywords: adenovirus; C-reactive protein; copy number

Article

High Copy Number from Pharyngeal Swab Is Not Associated with Different Presenting Features in 100 Children with Acute Adenovirus Infection from a Cluster in Italy

Anthea Mariani [1], Federica Cavallo [1], Saverio La Bella [2], Giusi Graziano [3], Martina Passarelli [2], Carlo Crescenzi [4], Daniela Trotta [1] and Maurizio Aricò [1]

[1] Pediatrics, S. Spirito Hospital, Azienda Sanitaria Pescara, Italy
[2] Postgraduate School of Pediatrics, University of Chieti-Pescara, Italy
[3] CORESEARCH (Center for Outcomes Research and Clinical Epidemiology) Pescara, Italy
[4] Clinical Microbiology and Virology, Spirito Santo Hospital, Pescara, Italy
* Correspondence: maurizio.arico@asl.pe.it;

Abstract: Adenoviruses are associated with respiratory tract, gastrointestinal or, less frequently, other involvement. Epidemics of adenovirus infections occur globally, in communities and in closed or crowded settings. In our institution, an outbreak of Adenovirus infection in infants and children was recently observed. Two main patterns of clinical presentation were observed: 68 patients had mainly respiratory symptoms (pharyngitis n=67, cough n=44; tonsillar exudate n=17; other respiratory signs n=4) while 26 patients showed prevalent gastrointestinal involvement (diarrhea n=26, vomiting n=8). Patients with respiratory symptoms had a significantly higher count of WBC, PMN and platelets, while CRP level approached statistical significance (p=0.07) for higher values in the patients with diarrhea. In order to explore the impact of selected presenting features, possible association between level of CRP and presence of pharyngeal exudate, cough, vomiting, diarrhea, duration of fever, number of neutrophils, and administration of antibiotics was analyzed. Patients falling in the tertile with more elevated CRP values had significantly more often tonsillar exudate and diarrhea, while those in the lower tertile had a 4.4 days duration of fever, vs. ≥5.0 days in the remaining patients. Antibiotic therapy was administered more frequently to patients with higher values of CRP (p=0.006). Duration of hospitalization was not associated with the CRP level. The median time from the receipt of positive adenovirus PCR test result to patient discharge was 1 day in 73% of cases. The number of copies of adenovirus detected by PCR ranged between 47 million and 15/ L. Falling in the highest terzile of copy number was significantly associated with pharyngitis. The 24 patients with evidence of viral coinfection had no difference in the demographics or presenting features, with the only exception of a significantly higher leukocyte count. Rapid turn-around of results of molecular testing of adenovirus genome on pharyngeal swab allowed us to rapidly diagnose adenovirus infection, allowing stopping antibiotic therapy and immediate discharge, with reduced discomfort for the families and more appropriate use of hospital beds. A high copy number of adenovirus from pharyngeal swab should not be taken as an indicator of worse prognosis, thus allowing preferential use of qualitative rather than quantitaive assay.

Keywords: adenovirus; C-reactive protein; copy number

1. Introduction

Adenoviruses are most frequently associated with respiratory tract and gastrointestinal symptoms, while ophthalmologic, genitourinary, and neurologic involvement is far less frequent. Adenoviral disease is usually self-limiting, although fatal outcome may occur in

 doi:10.20944/preprints202310.0565.v1

immunocompromised hosts and occasionally in healthy children and adults [1–4]. Adenoviruses have a worldwide distribution, cause 5 to 10 percent of all febrile illnesses in infants and young children, throughout the year without seasonality [5]. Serologic evidence of prior adenoviral infection is usually acquired by the age of 10, and by nearly all adults [6]. Transmission of adenovirus can occur via aerosol droplets, the fecal-oral route, and by contact with contaminated fomites. Epidemics of adenovirus infections occur globally, in communities and in closed or crowded settings [7,8]. Adenoviruses febrile respiratory illness usually lasts five to seven days, occasionally up to two weeks. Pharyngitis and coryza are common presentations but in many cases, exudative tonsillitis and cervical adenopathy may be present, mimicking streptococcal infection [9,10]. In a study of 2,638 hospitalized children with pneumonia, adenoviruses were detected in 15 percent of children younger than five years of age compared with 3 percent of older children [11]. Pneumonia is more severe in infants than older children, and may be associated with lethargy, diarrhea, and vomiting. Meningoencephalitis, hepatitis, myocarditis, nephritis, neutropenia, and disseminated intravascular coagulation [12,13] may complicate infection. In early 2022, an outbreak of acute hepatitis was identified among young children (most <5 years) in the United Kingdom and Ireland, and other clusters with similar characteristics were subsequently reported in at least 35 countries, including the United States [14–19].

In our institution, an outbreak of adenovirus infection in infants and children was recently observed. Main presenting features, the number of adenovirus copies, the role of CRP for differential diagnosis with bacterial infection and assessment of the need for hospitalization and antibiotic therapy were analyzed in a consecutive series of 100 cases.

2. Materials and Methods

We analyzed the clinical records of 100 children with adenovirus infection, admitted between January 1, 2023 and May 18, 2023. During the same time interval, a total of 5,080 children aged less than 18 years were seen at our pediatric emergency room, and of them 385 (7.5%) were admitted to our Pediatric ward.

2.1. Viral Genome Quantification by Real-Time PCR (q-PCR)

The presence of adenovirus genome was investigated on pharingeal swab in patients with respiratory or gastro-intestinal symptoms suggesting adenovirus infection.

The sample is extracted in about 1 hour and 30 minutes according to a diagnostic protocol that involves the use of the QIAsynphony extraction tool (Qiagen GmbH, Hilden Germany) and the QIAsynphony DSP Virus/Patogen Kit (Qiagen GmbH, Hilden Germany). Subsequently, the extracted genetic material is amplified, in about 2 hours, according to a genomic amplification protocol (Adenovirus ELITe MGB Kit, Nanogen Advanced Diagnostics, S.p.A. Buttigliera Alta, Torino, Italy), using the CFX96 Real-Time System instrument (Bio-Rad Laboratories Inc., Hercules, CA, USA).

For the quantification of viral DNA copies in different samples, the following formula was used: number of copies = Ve x Quantity

$$Vc \times Va \times Ep \quad (1)$$

Ve = Total volume obtained from extraction (110µL/Various Materials - 165µL/Blood) (2)

Vc = quantity of sample used in the extraction, 400/ µL Various materials, 200/ µL Blood- (3)

Va = eluate volume used in amplification (4)

a) Blood: 20 µL of eluate + 20 µL of amplification mix (5)

b) Various Materials: 20 µL of eluate + 20 µL of amplification mix (6)

Ep = Procedure Efficiency (100% Various Materials; 98.95% Blood) (7)

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 October 2023 doi:10.20944/preprints202310.0565.v1

The quantitative result is calculated in copies/mL based on a comparison with a straight line created by amplifying four amplification standards of known titer.

2.2. Clinical Data Collection

To characterize the pattern of clinical manifestations of adenovirus infection, the following fully anonymized data were collected from patient medical charts: age and gender; duration of hospital stay; physical exam findings; leukocyte and neutrophil counts and C-reactive protein (CRP) levels on admission; use of antibiotic therapy. Anonymized data were collected in a specific Excel data-base.

2.3. Statistical Analysis

The socio-demographic and clinical characteristics were summarized as mean and standard deviation (SD) and as frequencies and percentages depending on the nature of each variable. The comparison between the two groups of interest (presence/absence of diarrhea) was made through the t-test or Mann-Whitney test in case of continuous variables and through the Chi-squared test or Fisher exact test in case of categorical variables. For the purpose of this study, the patients were also grouped according to CRP tertiles and were formally compared with the ANOVA test or the non-parametric Kruskal-Wallis test and with the Chi-squared test or the Fisher exact test, as appropriate. The statistical significance was reached if p-values <0.05. All the analyses were carried out with SAS software (release 9.4; Cary, NC, USA).

Informed consent for data analysis for scientific purposes was obtained from the parents or legal guardians for all patients. The study was conducted in accordance with the Declaration of Helsinki Ethical Principles and Good Clinical Practices. IRB approval was waived due to study design.

3. Results

During the first 4.5 months of the current year 2023, 100 children were hospitalized and then released with a diagnosis of adenovirus infection. They had a mean age of 4 years, with a preponderance of males.

Respiratory symptoms such as pharyngitis and cough were most frequent; one quarter of the patient also had tonsillar exudate. Conversely, about one quarter of the patients had prevalent gastrointestinal involvement, with diarrhea or, less frequently, vomiting. The frequency of individual symptoms is summarized in Table 1.

Table 1. Main features of 100 children with Adenovirus infection requiring hospital admission.

Variable	N	Mean±sd or n (%)
Demographics		
Gender (Female / Male)	100	41/59
Age (years)	100	4.0±3.5
Clinical manifestation		
Pharyngitis	100	87 (87.0)
Cough	100	55 (55.0)
Diarrhea	100	26 (26.0)
Tonsillar exudate	99	25 (25.3)
Vomiting	99	18 (18.2)
Days of fever	70	5.4±2.6
Laboratory		
WBC (cell count/uL)	99	14,690±7,985
PMN (cell count/uL)	99	9,593±7,136
Hb (gr/dL)	100	11.4±1.4
Platelet (cell countx10^3/uL)	99	334±134

 doi:10.20944/preprints202310.0565.v1

CRP (mg/L)	99	85±74
ALT (IU/L)	99	44±100
AST (IU/L)	99	49±66
GGT (IU/L)	98	21±42
Adenovirus diagnostics		
Fecal antigen	100	
Negative*		24 (24.0)
Positive		6 (6.0)
PCR detection on pharyngeal swab (n. of copies, million/ml)	99	31.1 ± 78.7
Viral co-infection**	100	24 (24%)
Treatment		
Antibiotic therapy	100	75 (75.0)
IVIG	100	3 (3.0)
Hospital days	100	5.1±3.4

* 1 case PCR positive, 3 uncertain; ** see text for detail.

The mean values for leukocyte and polimorphonuclear cell count were 14.690/uL and 9.593/uL, respectively, while hemoglobin and platelet count remained normal. Values of transaminases and gamma-GT were moderately elevated.

Evidence of adenovirus genome was obtained by PCR on pharyngeal swab, with a mean copy number exceeding 30 million. Among 30 patients with some gastrointestinal involvement and thus investigated for adenovirus antigen on stools, six were positive. Twenty-four patients had evidence of viral co-infection: HHV6 (n=11), EBV (n=4), norovirus (n=4), influenza B (n=2), rotavirus, RSV, CMV (one each).

Three quarters of the patients received empiric antibiotic therapy. Mean duration of hospitalization was 5 days. None of them required admission to ICU.

Two main patterns of clinical presentation were observed: one group of 68 patients had mainly respiratory symptoms (pharyngitis n=67, cough n= 44; tonsillar exudate n=17; other respiratory signs n=4) and a second group of 26 patients showed prevalent gastrointestinal involvement (diarrhea n=26, vomiting n=8). Patients with respiratory symptoms had a significantly higher count of WBC, PMN and platelets, while CRP level approached statistical significance (p=0.07) for higher values in the patients with diarrhea (Table 2).

Table 2. Distribution of presenting features by clinical subgroups.

	Gastrointestinal symptoms*	**Respiratory symptoms****	**p-value§**
Number	26	68	
Days of fever	5.5±2.3	5.5±2.7	0.8614
Laboratory			
WBC (cell count/uL)	11,160±6,316	16,234±8,364	**0.008**
PMN (cell count/uL)	6,385±5,037	11,046±7,610	**0.007**
Hb (gr/dL)	11.3±1.5	11.4±1.4	0.9563
Platelets (cell countx10^3/uL)	290±136	355±128	**0.0332**
CRP (mg/L)	112±82	80±70	0.0715
ALT (IU/L)	22±17	54±120	0.5702
AST (IU/L)	34±13	57±79	0.2701
GGT (IU/L)	15±13	21±42	0.9723

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 10 October 2023 doi:10.20944/preprints202310.0565.v1

Antibiotic therapy	17 (65.4)	53 (77.9)	0.2117
IVIG	2 (7.7)	1 (1.5)	0.1843
Hospital days	5.2±3.1	5.1±3.6	0.8356

*Diarrhea with or without vomiting, **Fever, cough, pharyngitis, pharyngeal exudate, no diarrhea, § P-values derived from t-test or Mann-Whitney test (for continuous variables) and Chi-squared test or Fisher exact test (for categorical variables). Values in bold are statistically significant.

Furthermore, six additional patients had fever and limited respiratory symptom but in the presence of another acute, defined clinical condition: urinary tract infection (n=2), localized skin cellulitis (n=2), immune thrombocytopenia, neonatal lupus. These six patients with non-characteristic adenovirus infection clinical picture shared low or very low copy numbers at adenovirus genome detection by PCR: 752±987 copies/ml.

In order to explore the impact of selected presenting features, possible association between level of CRP and presence of pharyngeal exudate, cough, vomiting, diarrhea, duration of fever, number of neutrophils, and administration of antibiotics was analyzed (Table 3). Patients falling in the tertile with more elevated CRP values had significantly more often tonsillar exudate and diarrhea, while those in the lower tertile had a 4.4 days duration of fever, vs. ≥5.0 days in the remaining patients.

Table 3. Distribution of the presenting features of 100 children with Adenovirus infection according to CRP tertiles.

	CRP value (mg/L)			
	0-39	**39-105**	**>=105**	**p-value§**
Number	32	33	34	
Demographics				
Gender (Female / Male)	14 (43.8)	13 (39.4)	13 (38.2)	0.8917
Age (years)	3.4±3.4	3.7±3.1	4.8±4.1	0.2465
Clinical manifestation				
Tonsillar exudate	5 (15.6)	5 (15.6)	15 (44.1)	**0.0087**
Pharyngitis	24 (75.0)	30 (93.8)	31 (91.2)	0.067
Cough	15 (46.9)	21 (65.6)	18 (52.9)	0.3055
Diarrhea	7 (21.9)	4 (12.5)	15 (44.1)	**0.0112**
Vomiting	5 (15.6)	4 (12.5)	9 (26.5)	0.3036
Days of fever	4.3±3.2	6.0±2.1	6.0±2.1	0.0627
Laboratory				
WBC (cell count/uL)	12,700±7399	16,134±8,879	15,176±7542	0.1879
PMN (cell count/uL)	7,809±6710	10,559±8,038	10,434±6494	0.1495
Hb (gr/dL)	11.8±1.3	11.3±1.5	11.1±1.4	0.1408
Platelets (cell countx10³/uL)	325±127	361±124	310±146	0.2134
ALT (IU/L)	77±147	35±89	22.1±16.4	0.1337
AST (IU/L)	73±101	42±48	35.3±17.6	0.1387
GGT (IU/L)	23±42	26±58	16.4±12.7	0.289
Adenovirus diagnostics				
Fecal antigen Negative*	8 (25.0)	7 (21.2)	9 (26.5)	0.8723
Positive	3 (9.4)	1 (3.0)	2 (5.9)	

 doi:10.20944/preprints202310.0565.v1

PCR detection on pharyngeal swab (million of copies/ml)	47.551±117.495	28.283±55.827	19.926±49.220	0.2633
Treatment				
Antibiotic therapy	18 (56.3)	27 (81.8)	30 (88.2)	**0.0062**
IVIG	2 (6.3)	0 (0.0)	1 (2.9)	0.3164
Hospital days	4.4±1.9	5.0±2.2	5.3±3.1	0.4904

* 1 case PCR positive, 3 uncertain; § p-values derived from ANOVA or Kruskal-Wallis test (for continuous variables) and Chi-squared test or Fisher exact test (for categorical variables). Values in bold are statistically significant.

Antibiotic therapy was administered more frequently to patients with higher values of CRP (p=0.006). Duration of hospitalization was not associated with the CRP level.

The median time from the receipt of positive Adenovirus PCR test result to patient discharge was 1 day in 73% of cases.

The number of copies of adenovirus detected by PCR ranged between 47 million and 15/µL (Table 4). Falling in the highest terzile of copy number was significantly associated with pharyngitis and a non-significant trend to having pharyngeal exudate; patients with higher copy numbers had lower values of gamma-GT, while blood cell count was not affected.

Table 4. Distribution of the presenting features of 100 children with Adenovirus infection by tertiles of Adenovirus copy number/ml.

		Copies			p-value§
		≤954	**954-9,490,618**	**>9,490,618**	
Number		32	33	34	
Demographics					
Gender	Female	12 (37.5)	12 (36.4)	17 (50.0)	0.4534
	Male	20 (62.5)	21 (63.6)	17 (50.0)	
Age (years)		4.6±4.6	3.5±3.6	3.8±2.2	0.474
Clinical manifestation					
Tonsillar exudate		5 (15.6)	7 (21.2)	13 (39.4)	0.0702
Pharyngitis		25 (78.1)	28 (84.8)	34 (100.0)	**0.0093**
Cough		15 (46.9)	18 (54.5)	22 (64.7)	0.3425
Diarrea		7 (21.9)	9 (27.3)	9 (26.5)	0.8644
Vomiting		5 (15.6)	7 (21.2)	6 (18.2)	0.8439
Days of fever		5.3±3.1	5.1±2.8	5.9±1.9	0.3799
Laboratory					
WBC (cell count/uL)		14781±8508.3	14935±8260	14117±7386	0.8512
PMN (cell count/uL)		9409±7822.8	9694±7342	9422±6395	0.8746
Hb		11.5±1.6	11.3±1.4	11.3±1.3	0.6926
Platelets (cell countx10³/uL)		345±161	332±137	329±103	0.9376
ALT (IU/L)		82±162	29±51	24±38	0.0536
AST (IU/L)		75±109	38±24	38±24	0.8209

 doi:10.20944/preprints202310.0565.v1

GGT (IU/L)		30±49	24±54	11±5	**0.0002**
Fecal antigen	Negative*	8 (25.0)	9 (27.3)	7 (20.6)	0.5752
Antibiotic therapy		24 (75.0)	24 (72.7)	27 (79.4)	0.8097
IVIG	No	30 (93.8)	32 (97.0)	34 (100.0)	0.2089
Hospital days		6.2±5.2	5.1±2.4	4.2±1.4	0.1425

* 1 case PCR positive, 3 uncertain; § p-values derived from ANOVA or Kruskal-Wallis test (for continuous variables) and Chi-squared test or Fisher exact test (for categorical variables). Values in bold are statistically significant.

The 24 patients with evidence of viral coinfection had no difference in the demographics or presenting features, with the only exception of a significantly higher leukocyte count. The number of copies of Adenovirus and ALT values showed a trend to higher values, but those data did not reach statistical significance (Table 5).

Table 5. Distribution of the presenting features of 100 children with Adenovirus infection according to detection of viral coinfection.

		No Viral Coinfection	Viral Coinfection	p-value
Demographics				
Gender (Female / Male)	F	29 (38.2)	12 (50.0)	0.3038
	M	47 (61.8)	12 (50.0)	
Age (years)		3.7±3.3	4.8±4.1	0.1719
Clinical manifestation				
Pharyngitis	No	10 (13.2)	3 (12.5)	1*
	Yes	66 (86.8)	21 (87.5)	
Cough	No	31 (40.8)	14 (58.3)	0.132
	Yes	45 (59.2)	10 (41.7)	
Diarrhea	No	54 (71.1)	20 (83.3)	0.2318
	Yes	22 (28.9)	4 (16.7)	
Tonsillar exudate	No	57 (76.0)	17 (70.8)	0.6121
	Yes	18 (24.0)	7 (29.2)	
Vomiting	No	62 (82.7)	19 (79.2)	0.7631*
	Yes	13 (17.3)	5 (20.8)	
Days of fever		5.4±2.5	5.3±3.1	0.8605
Laboratory				
WBC (cell count/uL)		15,528±8,263	12,070±6,521	0.11
PMN (cell count/uL)		10,590±7,191	6,477±6,102	**0.0077**
Hb (gr/dL)		11.3±1.4	11.7±1.5	0.2036
Platelet (cell countx10³/uL)		334.7±137.7	333±125	0.8674
ALT (IU/L)		24±35	110±186	0.0877
AST (IU/L)		36±19	94±124	0.1334
GGT (IU/L)		19±36	31±57	0.711
Adenovirus diagnostics				
PCR detection on pharyngeal swab (million of copies/ml)		31.455±72.373	30.242±97.816	0.0926
Treatment				
Antibiotic therapy	No	18 (23.7)	7 (29.2)	0.5887
	Yes	58 (76.3)	17 (70.8)	

 doi:10.20944/preprints202310.0565.v1

Hospital days	4.8±2.5	6.2±5.2	0,1173

* Fisher test.

4. Discussion

On the tail of the annual epidemic of bronchiolitis, during late winter rapid spread of adenovirus infection made it the main reason for admission of children in our pediatric ward in central Italy. Children with persistent fever and upper respiratory symptoms came at the pediatric emergency room for initial evaluation, often after 2-3 days of oral antibiotic therapy. In keeping with the observation made by Jain et al. in their study of 2,638 hospitalized children [11], their mean age was of 4 years.

Based on first level laboratory work-up, children with neutrophilia and higher values of CRP more often received empiric antibiotic therapy, also considering possible invasive bacterial infection. Not surprisingly, fever did not subside, and the option of a higher level of antibiotic coverage was often on the table.

It is interesting to note that the differential diagnosis between adenovirus infection and other, partially overlapping conditions, remains a focus of research. In a recent report, Fabi et al. developed a scoring system based on five clinical and one laboratory parameters, to differentiate Kawasaki disease from Multisystem Inflammatory Syndrome in Children (MIS-C) from Adenovirus infection. By using a multivariable logistic regression analysis, they report accuracy in recognizing Kawasaki disease from the other overlapping conditions, including 30 cases of Adenovirus infection. Neutrophilia appears the laboratory parameter, which contributes to differentiate Adenovirus infection from potentially mis-recognized MIS-C [20]. We asked the microbiology lab to start a fast-lane for adenovirus PCR testing. Positive results were thus received within 1-2 days in the ward and this was associated with immediate stop of antibiotic therapy followed by rapid discharge of children. Reassuring the parents on the identification of the etiology as another case of the ongoing Adenovirus epidemic allowed them to agree on immediate discharge despite some persisting fever. Overall, this translated into fewer hospital days, less antibiotics administered and a more appropriate use of hospital beds. The expense of a higher number of molecular testing was thus largely compensated even under the mere economical point of view, regardless the social advantage for the families.

Positive testing was supported by the detail of the copy number of Adenovirus. How far is this information useful in the clinical practice? In a recent study, Goichman et al. investigated the correlation between Adenovirus viral load in clinical respiratory samples and the respiratory disease severity in pediatric patients. Adenovirus load in respiratory samples, as measured by Ct values, was found to be negatively correlated with respiratory disease severity in hospitalized patients aged under 9 years [21]. We tried to analyze possible correlation of copy number with the presenting feature, and we have not been able to identify any specific feature predictive for a higher copy number, except for report of pharyngitis an inverse relationship with a moderate elevation of gamma-GT.

In a virology surveillance study of 18,603 children seen at seven US sites, adenovirus was detected in 1,136; of them, 6.1% had co-detection with at least one other respiratory virus (human rhinovirus/enterovirus, respiratory syncytial virus, parainfluenza, influenza, and human meta-pneumovirus), and greater disease severity compared to those with adenovirus alone [22]. In our series, 24 patients had evidence of viral coinfection, in 15/24 HHV6 or EBV. Their presenting features were not different from the remaining ones, with the only exception of a higher leukocyte count; yet adenovirus copy number and ALT level showed a border-line trend toward higher values in the presence of coinfection. Overall, the disease course in patients with viral coinfection was not more severe.

During a cluster of adenovirus infection in an urban area, persistent fever drove many families to our pediatric emergency room, and one quarter of admissions to the pediatric ward consisted of patients with adenovirus infection. At initial evaluation, neutrophilia and higher values of CRP induced the attending physician to cautiously prescribe empiric antibiotic therapy. Rapid turn-around of results of molecular testing of adenovirus genome on pharyngeal swab allowed us to

rapidly diagnose adenovirus infection, allowing stopping antibiotic therapy and immediate discharge, with reduced discomfort for the families and more appropriate use of hospital beds. A high copy number of adenovirus from pharyngeal swab should not be taken as an indicator of worse prognosis, thus allowing preferential use of qualitative rather than quantitaive assay.

Author Contributions: Conceptualization, M.A. and D.T..; methodology, M.A., D.T., G.G.X.X.; software, S.L.B., M.P.; formal analysis, G.G.; investigation, C.C..; data curation, F.C., A.M., M.P.; writing—original draft preparation, M.A., D.T., F.C., A.M..; writing—review and editing, M.A., D.T..; supervision, F.C., A.M.; All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki. Ethical review and approval were waived for this study due to current bylaws for observational studies in Italy.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: No new data were created.

Acknowledgments: The authors are grateful to Dr. Enrico Valletta (Department of Pediatrics, G. B. Morgagni - L. Pierantoni Hospital, AUSL Romagna, Forli, Italy) for reading the manuscript and helpful discussion.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Finianos, M., Issa, R., Curran, M. D., Afif, C., Rajab, M., Irani, J., Hakimeh, N., Naous, A., Hajj, M. J., Hajj, P., El Jisr, T., & El Chaar, M. (2016). Etiology, seasonality, and clinical characterization of viral respiratory infections among hospitalized children in Beirut, Lebanon. *Journal of medical virology*, *88*(11), 1874–1881. https://doi.org/10.1002/jmv.24544
2. Sandkovsky, U., Vargas, L., & Florescu, D. F. (2014). Adenovirus: current epidemiology and emerging approaches to prevention and treatment. *Current infectious disease reports*, *16*(8), 416. https://doi.org/10.1007/s11908-014-0416-y
3. Jain, S., Williams, D. J., Arnold, S. R., Ampofo, K., Bramley, A. M., Reed, C., Stockmann, C., Anderson, E. J., Grijalva, C. G., Self, W. H., Zhu, Y., Patel, A., Hymas, W., Chappell, J. D., Kaufman, R. A., Kan, J. H., Dansie, D., Lenny, N., Hillyard, D. R., Haynes, L. M., … CDC EPIC Study Team (2015). Community-acquired pneumonia requiring hospitalization among U.S. children. *The New England journal of medicine*, *372*(9), 835–845. https://doi.org/10.1056/NEJMoa1405870.
4. Xie, L., Zhang, B., Xiao, N., Zhang, F., Zhao, X., Liu, Q., Xie, Z., Gao, H., Duan, Z., & Zhong, L. (2019). Epidemiology of human adenovirus infection in children hospitalized with lower respiratory tract infections in Hunan, China. *Journal of medical virology*, *91*(3), 392–400. https://doi.org/10.1002/jmv.25333.
5. Fox, J. P., Hall, C. E., & Cooney, M. K. (1977). The Seattle Virus Watch. VII. Observations of adenovirus infections. *American journal of epidemiology*, *105*(4), 362–386. https://doi.org/10.1093/oxfordjournals.aje.a112394.
6. Pathogenesis, epidemiology, and clinical manifestations of adenovirus infection - UpToDate
7. Birenbaum, E., Linder, N., Varsano, N., Azar, R., Kuint, J., Spierer, A., & Reichman, B. (1993). Adenovirus type 8 conjunctivitis outbreak in a neonatal intensive care unit. *Archives of disease in childhood*, *68*(5 Spec No), 610–611. doi.org/10.1136/adc.68.5_spec_no.610.
8. Centers for Disease Control and Prevention (CDC). Adenovirus-associated epidemic keratoconjunctivitis outbreaks--four states, 2008-2010. MMWR Morb Mortal Wkly Rep 2013; 62:637.
9. Edwards, K. M., Thompson, J., Paolini, J., & Wright, P. F. (1985). Adenovirus infections in young children. *Pediatrics*, *76*(3), 420–424.
10. Pacini, D. L., Collier, A. M., & Henderson, F. W. (1987). Adenovirus infections and respiratory illnesses in children in group day care. *The Journal of infectious diseases*, *156*(6), 920–927. https://doi.org/10.1093/infdis/156.6.920.
11. Jain, S., Williams, D. J., Arnold, S. R., Ampofo, K., Bramley, A. M., Reed, C., Stockmann, C., Anderson, E. J., Grijalva, C. G., Self, W. H., Zhu, Y., Patel, A., Hymas, W., Chappell, J. D., Kaufman, R. A., Kan, J. H.,

Dansie, D., Lenny, N., Hillyard, D. R., Haynes, L. M., … CDC EPIC Study Team (2015). Community-acquired pneumonia requiring hospitalization among U.S. children. *The New England journal of medicine*, *372*(9), 835–845. https://doi.org/10.1056/NEJMoa1405870.

12. Similä, S., Jouppila, R., Salmi, A., & Pohjonen, R. (1970). Encephaloningitis in children associated with an adenovirus type 7 epidemic. *Acta paediatrica Scandinavica*, *59*(3), 310–316. https://doi.org/10.1111/j.1651-2227.1970.tb09009.x
13. Wadell, G., Varsányi, T. M., Lord, A., & Sutton, R. N. (1980). Epidemic outbreaks of adenovirus 7 with special reference to the pathogenicity of adenovirus genome type 7b. *American journal of epidemiology*, *112*(5), 619–628. https://doi.org/10.1093/oxfordjournals.aje.a113034.
14. Center for Infectious Disease Research and Policy. Acute pediatric hepatitis cases raise flags in UK, US, Spain. 2022. Available at: Acute pediatric hepatitis cases raise flags in UK, US, Spain | CIDRAP (umn.edu) (Accessed on October 8, 2023).
15. Baker, J. M., Buchfellner, M., Britt, W., Sanchez, V., Potter, J. L., Ingram, L. A., Shiau, H., Gutierrez Sanchez, L. H., Saaybi, S., Kelly, D., Lu, X., Vega, E. M., Ayers-Millsap, S., Willeford, W. G., Rassaei, N., Bullock, H., Reagan-Steiner, S., Martin, A., Moulton, E. A., Lamson, D. M., … Kirking, H. L. (2022). Acute Hepatitis and Adenovirus Infection Among Children - Alabama, October 2021-February 2022. *MMWR. Morbidity and mortality weekly report*, *71*(18), 638–640. https://doi.org/10.15585/mmwr.mm7118e1.
16. Center for Infectious Disease Research and Policy. Global number of unexplained hepatitis cases in kids climbs to 450. 2022. Available at: https://www.cidrap.umn.edu/news-perspective/2022/05/news-scan-may-11-2022 (Accessed on October 04, 2023).
17. Centers for Disease Control and Prevention: Technical Report: Acute Hepatitis of Unknown Cause (6/16/22). Available at: https://www.cdc.gov/ncird/investigation/hepatitis-unknown-cause/technical-report.html (Accessed on October 04, 2023).
18. World Health Organization. Severe acute hepatits of unknown aetiology in children - multicountry. https://www.who.int/emergencies/disease-outbreak-news/item/2022-DON400 (Accessed on October 04, 2023).
19. Aricò, M., & Caselli, D. Acute, Severe Hepatitis of Unknown Origin: Should We Really Be Afraid of Another Obscure Enemy of Our Children? Pediatric Reports, 2022; 14, 217–219.
20. Fabi, M., Dondi, A., Andreozzi, L., Frazzoni, L., Biserni, G. B., Ghiazza, F., Dajti, E., Zagari, R. M., & Lanari, M. Kawasaki disease, multisystem inflammatory syndrome in children, and adenoviral infection: a scoring system to guide differential diagnosis. European journal of pediatrics, 2023; 10.1007/s00431-023-05142-6.
21. Goikhman, Y., Drori, Y., Friedman, N., Sherbany, H., Keller, N., Mendelson, E., Pando, R., & Mandelboim, M. (2020). Adenovirus load correlates with respiratory disease severity among hospitalized pediatric patients. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases*, *97*, 145–150. https://doi.org/10.1016/j.ijid.2020.06.010.
22. Probst, V., Spieker, A. J., Stopczynski, T., Stewart, L. S., Haddadin, Z., Selvarangan, R., Harrison, C. J., Schuster, J. E., Staat, M. A., McNeal, M., Weinberg, G. A., Szilagyi, P. G., Boom, J. A., Sahni, L. C., Piedra, P. A., Englund, J. A., Klein, E. J., Michaels, M. G., Williams, J. V., Campbell, A. P., … Halasa, N. B. Clinical Presentation and Severity of Adenovirus Detection Alone vs Adenovirus Co-detection With Other Respiratory Viruses in US Children With Acute Respiratory Illness from 2016 to 2018. Journal of the Pediatric Infectious Diseases Society, 2022 11, 430–439