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A Clinical Comparison of Hospitalized Pediatric Patients with Influenza A(H1N1)Pdm09 Virus Infection of the Years 2009/10 and 2012/13

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Abstract

Background: The A(H1N1)pdm09 virus is a major pathogen causing acute respiratory diseases (ARD) in children worldwide, leading to high rates of ambulatory consultations and hospitalizations. The seasonal influenza of 2012/2013 in Germany was unusually severe, leading to higher numbers of hospitalization rates as compared with previous seasons. This trend was even notable in our tertiary care Children's Hospital at the University of Bonn, Germany.

Patients and Methods: Data of 74 hospitalized children (≤ 18 years) from the pandemic season in 2009/10 and the post pandemic season 2012/13 with ARD and A(H1N1)pdm09 virus infection were analyzed. A retrospective patient's chart analysis was performed.

Results: During the pandemic more children with detection of A(H1N1)pdm09 virus need to be hospitalized (48 vs. 65%). Children were younger in the post-pandemic season 2012/13 than 2009/10 (6.5 vs. 2.1 years, $p < 0.01$) and the hospital length of stay was longer (5.1 vs. 10.8 days, $p < 0.01$). Proportionally more children in 2012/13 presented with pneumonia (22 vs. 47%, $p = 0.05$), suffered under an airway obstruction (25 vs. 73%, $p < 0.01$) or tachypnea (7 vs. 33%, $p < 0.01$). Needs for oxygen therapy (12 vs. 47%, $p < 0.01$) and intravenous fluid replacement (24 vs. 73%, $p < 0.001$) were augmented.

Conclusion: The clinical course of German children with seasonal A(H1N1)pdm09 virus infections in 2012/13 appeared to be more severe than during the pandemic 2009/10. Influencing factors for this difference are probably multifactorial. Physicians need to focus especially on infants and preschool children, because these patients are at higher risk for severe clinical outcome and prolonged hospital stay.

Keywords

A (H1N1) pdm09, Children, Influenza, In-house admissions

Introduction

Influenza virus infections contribute largely to in-house admissions in German hospitals. In 2009 and 2010 the A(H1N1) pdm09 pandemic increased the influenza virus induced hospital admissions dramatically and raised the awareness in the general population and among health care providers tremendously [1]. In the season of 2012/13 we noted an increased occurrence of ARD in our tertiary care Children's Hospital at the University of Bonn, Germany, and an increased number of in-house admissions with A(H1N1)pdm09 virus infections. In accordance with our observation the influenza surveillance group of the Robert Koch Institute (RKI) reported an increased number of A(H1N1)pdm09 virus induced infections in the season 2012/13 compared to previous post-pandemic seasons in Germany [2].

In recent years, several studies were conducted to identify predictors for a severe clinical course of A(H1N1)pdm09 virus induced infections, in order to optimize patient´s treatment and the assessment of clinical symptoms [3-5].

The present study investigated the clinical course of pediatric in patients with A(H1N1)pdm09 virus infection comparing the season 2012/13 with the pandemic of 2009/10 in our Children's Hospital, based on clinically tested predictors.

Methods

Patient cohort

We conducted a retrospective cohort study on 74 in-house patients treated in our Children's Hospital at the University of Bonn during the seasons 2009/10 and 2012/13, diagnosed with A(H1N1) pdm09 virus infections (see below).

Inclusion criteria were: age ≤ 18 years, hospitalization, symptoms of ARD at time of admission and detection of A(H1N1)pdm09 virus in nasopharyngeal swab or pharyngeal aspirate, the testing

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being performed either immediately in the emergency room or after admission of patients.

Data collection

Data on the clinical course and symptoms were obtained from the hospital's electronic medical record system ORBIS® (AGFA HealthCare GmbH, Germany), the laboratory system Lauris® (Swiss lab GmbH, Germany) and from patient´s charts, including all departments of our Children's Hospital (General Pediatrics and Pediatric Intensive Care Unit, Pediatric Oncology, Pediatric Cardiology and Neonatology).

The presence of the following symptoms and diagnoses were evaluated: airway obstruction, pneumonia, fever (temp. > 38.5°C), acute otitis media (AOM), conjunctivitis, rhinitis, cough, tachypnea, chest retractions, vomiting, diarrhea, cephalgia and arthralgia. An airway obstruction was defined as the presence of an acute obstructive bronchitis, laryngotracheitis with in- or expiratory stridor and the need for a treatment with inhaled salbutamol (albuterol), ipratropriumbromid or epinephrine.

Diagnosis of pneumonia on chest X-rays required the presence of recent emerged infiltrates. All radiographs of the chest were re-read by two investigators for the study (pediatric radiologist and pediatrician).

The following parameters were evaluated for clinical diagnostics and treatment: carrying out a chest X-ray, need of antibiotic therapy, use of a neuraminidase inhibitor, oxygen demand due to respiratory failure and the need for intravenous fluid replacement. Oxygen administration was performed if pulse oximetry oxygen saturation was below < 94%.

The following laboratory parameters were evaluated: C-reactive protein (CRP), leukocyte count (absolute), neutrophils (absolute and relative), lymphocytes (absolute and relative), creatinine, glutamate pyruvate transaminase (GPT) and glutamate oxaloacetate transaminase (GOT). The decision to perform laboratory diagnostic, including complete blood count, CRP and liver enzymes, was at the decision of the attending physician.

Virological diagnosis

Nasopharyngeal swabs and pharyngeal aspirate were used for virological diagnostics. The swabs were collected with Copan Flocked Swaps (Copan Diagnostics, Brescia, Italy) and were eluted in the laboratory in 0.4 milliliters (ml) of sterile phosphate-buffered saline. Viral nucleic acid was prepared from 140 microliters (µl) of sample by the QIA amp Viral RNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions and eluted in 60 µl. The reverse transcription/real-time polymerase chain reaction (RT-PCR) for the detection of influenza A viruses was performed according to the manufacturer's instructions with the Real Star® influenza S & T RT-PCR assay system (astra/Altona diagnostics, Hamburg, Germany) [6,7]. The assay was based on primers amplifying an 87-base-pair region of the viral matrix (M) gene (influenza genome segment 7) and two specific hydrolysis probes for discrimination of influenza A(H1N1)pdm09 virus and influenza A virus as described elsewhere [7,8]. This assay contained 10 µl RNA sample in a total reaction volume of 25 µl. A heterologous amplification system (internal control) identified possible RT-PCR inhibition on the sample matrix. For detection of other influenza A viruses (not A(H1N1)pdm09 type), sub typing was performed by H (H1, H3) and N (N1, N2) with H and N subtype-specific primer pairs and fluorophore-labeled probes (4 reverse transcription/real-time PCR: FAM for Influenza strain A(H1N1)pdm09, Cy5 for seasonal human Influenza A, ROX for Influenza B, JOE for the target of the internal control) [9].

The RT-PCR for Influenza B viruses was carried out as described elsewhere [10], except that the probe was marked at its 5'-end with the fluorophore YAK and at the 3'-end with the Black Hole Quencher 1 (BHQ1). After implementation of the influenza B virus PCR in the commercial PCR system, the influenza B virus diagnosis was performed with the Real-Star® system. All amplifications were performed with an ABI Prism 7500 real-time thermocycler® (Applied Biosystems, Weiterstadt, Germany).

Statistics

Data are presented as mean or percentage. First, univariate analysis was performed to compare demographic and clinical data. Comparison between two groups (1) Influenza season 2009/10 and 2) 2012/13) was performed with a t-test analysis or Wilcoxon rank sum tests for continuous variables and chi-square test for categorical variables. Odds ratios (ORs) were calculated. Both groups were further divided into six subgroups for evaluation of patients with preexisting chronicmedical conditions (pulmonary, cardiac, neurological, hematology-oncology pre-existing disorders; metabolic disorders; no pre-existing disorders). A p-value < 0.05 was considered significant. Statistical analysis was performed using statistical software (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Results

Epidemiological data

During the 2009/10 pandemic, A(H1N1)pdm09 virus infections

Table 1: Epidemiological data of patients with A(H1N1)pdm09 virus infection.

Variables	Influenza Season		odds ratio	p-value
	2009/10	2012/13		
Male sex	30 (51)	7 (47)	1.0 (0.6-2.0)	> 0.05
Age, y	6.5	2.1		< 0.01
- < 1 y	5 (8)	3 (20)	0.4 (0.1-1.6)	> 0.05
- 1-5 y	26 (44)	11 (73)	0.6 (0.4-0.9)	< 0.05
- 5-10 y	9 (15)	1 (7)	2.3 (0.3-16.7)	> 0.05
- 10-15 y	18 (31)	0		
- > 15 y	1 (2)	0		
In-house admissions	59 (48)	15 (65)		> 0.05
Hospital length of stay, d	5.1	10.8		< 0.01
PICU treatment	2 (3)	2 (13)	0.3 (0.04-1.7)	> 0.05
Underlying chronical medical conditions				> 0.05
None	40 (68)	12 (80)	0.8 (0.6-1.2)	> 0.05
Respiratory disease	3 (5)	1 (7)	0.8 (0.1-6.8)	> 0.05
Metabolic defect	0	1 (7)		
Cardiac disease	3 (5)	1 (7)	0.8 (0.1-6.8)	> 0.05
Neurodevelopment disorder	5 (9)	0		
Hematooncology disease	8 (14)	0		

Values are absolute numbers (and % of the respective collective), except as indicated.

d: Days, PICU: Pediatric Intensive Care Unit, y: Year.

were detected in a total of 124 patients in our Children's Hospital, of which 59 patients (~48%) needed to be hospitalized. Two inpatients were excluded from the study (> 18 years of age at the time of admission). Infections with other influenza A (e.g. H3N2) or influenza B viruses in 2009/10 could not be detected. In the 2012/13 season a total of 23 patients were tested positive for A(H1N1)pdm09 virus infections, 15 patients (~65%) required hospitalization. Three patients were excluded from the study (> 18 years of age at the time of admission). Furthermore, eight patients in 2012/13 presented with an influenza A(H3N2) virus infection and in samples of five patients influenza B virus was detected. In the department of neonatology, there was no evidence of influenza virus infections in either period.

In 2012/13, the rate of in-house admissions for A(H1N1)pdm09 virus infections was about 1.4 times higher compared to 2009/10 (Table 1). During both seasons, gender distribution of patients was equal. In house patients in 2012/13 were significantly younger than during the pandemic season 2009/10 (4.4 years (1.6-7.2 years), $p < 0.01$, Table 1). In addition, during the 2012/13 season, none of the affected patients were older than 10 years of age. In the 2012/13 season, the hospital length of stay was significantly longer than during the season 2009/10 ($p < 0.01$, Table 1). During both seasons, there was no difference when looked at the need for PICU admissions (days admitted to PICU).

When comparing the respective subgroups regarding preexisting chronical medical conditions, no significant difference was found ($p > 0.05$, Table 1).

Clinical parameters

More patients were found to have pneumonia, based on chest X-ray, in 2012/13 ($p = 0.05$, Table 2). A significant difference was found respective the appearance of an acute airway obstruction between both seasons ($p < 0.01$, Table 2). During the 2012/13 season, patients presented more frequently with tachypnea ($p < 0.01$, Table 2). Patients in 2012/13 tended to present more frequently with inter costal retractions compared to 2009/10, but without statistical significance (5 vs. 20%, $p > 0.05$, Table 2).

In 2012/13 significant more patients required oxygen supply compared to 2009/10 ($p < 0.01$, Table 3). Likewise, intravenous fluid replacement was required more frequently in 2012/13 compared to 2009/10 ($p < 0.001$, Table 3). Looking for the treatment with antibiotics and neuraminidase inhibitors and for the performance of chest X-ray, no significant difference was found between both seasons.

When comparing laboratory parameter values from both periods, no significant difference was found (Table 3). Values of complete blood count and creatinine were almost equal in both groups. Nevertheless, the CRP value in 2012/13 tended to be higher compared to the 2009/10 season, but without statistical significance (17 vs 34 mg/l, $p > 0.05$, Table 3). Vice versa, the transaminases tended to be higher in infected patients during the pandemic, but even without statistical significance (GPT: 99 vs 19 and GOT: 220 vs. 31 U/l, $p > 0.05$, Table 3).

Discussion

In this retrospective study, we compared data of children requiring hospital treatment due to A(H1N1)pdm09 virus infections from the seasons 2009/10 and 2012/13 in a German tertiary care University Children's Hospital. Our analysis suggests that the clinical course of A(H1N1)pdm09 virus infections was more severe among children of the 2012/13 influenza wave, compared to data of the pandemic in 2009/10. In particular, infants and preschool children were affected. This is reflected by several parameters, such as higher rates of children with radiologically detectable pneumonia, acute airway obstruction, tachypnea and increased thoracic retractions, as well as a higher mean CRP values, a higher rates of oxygen requirement and higher rates of intravenous fluid replacements. On the other hand, more children during the pandemic in 2009/10 had need for in-house treatment and

Table 2: Clinical signs of patients with A(H1N1)pdm09 virus infection.

Symptoms	Influenza Season 2009/10	Influenza Season 2012/13	odds-ratio	p-value
pneumonia	13 (22)	7 (47)	0.5 (0.2-1.0)	= 0.05
airway obstruction	15 (25)	11 (73)	0.3 (0.2-0.6)	< 0.01
temp. > 38.5°C	51 (88)	15 (100)	0.9 (0.8-1.0)	> 0.05
acute otitis media	12 (21)	2 (13)	1.6 (0.4-6.3)	> 0.05
conjunctivitis	3 (5)	2 (13)	0.4 (0.1-2.2)	> 0.05
rhinitis	9 (16)	5 (33)	0.5 (0.2-1.2)	> 0.05
cough	42 (74)	10 (77)	1.1 (0.7-1.6)	> 0.05
tachypnea	4 (7)	5 (33)	0.2 (0.1-0.7)	< 0.01
chest retractions	3 (5)	3 (20)	0.3 (0.1-1.1)	> 0.05
vomiting	17 (30)	3 (20)	1.5 (0.5-4.4)	> 0.05
diarrhea	10 (18)	4 (27)	0.7 (0.2-1.8)	> 0.05
cephalgia	10 (18)	0		
arhtralgia	5 (9)	0		

Values are absolute numbers (and % of the respective collective), except as indicated. d: Days, PICU: Pediatric Intensive Care Unit, temp: Temperature.

Table 3: Diagnostic and therapeutic parameters of patients with A(H1N1)pdm09 virus infection.

Variables	Influenza Season 2009/10	Influenza Season 2012/13	odds-ratio	p-value
Diagnostic and therapy				
chest X-ray	33 (56)	10 (67)	0.8 (0.5-1.3)	> 0.05
antibiotics	42 (71)	13 (87)	0.8 (0.6-1.1)	> 0.05
NA-inhibitor[1]	31 (53)	8 (53)	1.0 (0.6-1.7)	> 0.05
oxygen demand	7 (12)	7 (47)	0.3 (0.1-0.6)	< 0.01
fluid replacement i.v.	14 (24)	11 (73)	0.3 (0.2-0.6)	< 0.001
Laboratory parameters				
CRP (mg/l)	17 ± 22	34 ± 49		> 0.05
leukocytes abs. (10^3/µl)	7556 ± 4133	8217 ± 4005		> 0.05
neutrophilesabs (10^3/µl)	5092 ± 3373	5123 ± 3907		> 0.05
neutrophiles rel. (%)	59 ± 21	56 ± 25		> 0.05
lymphocytes abs. (10^3/µl)	1812 ± 1480	2070 ±1315		> 0.05
lymphocytes rel. (%)	25 ± 17	32 ± 26		> 0.05
creatinine (mg/dl)	0.52 ± 0,20	0.42 ± 0,26		> 0.05
GPT (U/l)	99 ± 469	17 ± 6		> 0.05
GOT (U/l)	220 ± 993	31 ± 8		> 0.05

Values are absolute numbers ± SD (and % of the respective collective), except as indicated.

abs: Absolute, CRP: C - Reactive Protein, GPT: Glutamate- Pyruvate Transaminase, GOT: Glutamate-oxalacetate-transaminase, i.v.: Intravenous, NA: Neuraminidase, rel.: Relative, [1]: In all patients oseltamivir.

fewer A(H1N1)pdm09 virus related hospitalizations were seen in the post-pandemic season 2012/13. The disease awareness during the pandemic influenza wave and high rates of ambulatory consultations probably resulted in higher rates of testing for the A(H1N1)pdm09 virus. Therefore, this circumstance could have influenced the A(H1N1)pdm09 related hospitalizations among our cohort and the reduced number of patients in 2012/13 need to be interpreted with caution.

However, there are limiting factors influencing the statistical power of our study. The low number of cases, in particular, the small number of patient's in 2012/13, could have led to limitations in comparing the retrospective data. Moreover, we analyzed data of just one single tertiary care Children's Hospital. The extension of the study to all ages and inclusion of non-pediatric patients could contribute to an increase in the power of the test. Thus, there are limitations to the generalizability of our results to other populations.

Data from the Germans public health institute RKI demonstrated that in the 2012/13 season the frequency, of a doctor's consulted by patients with ARD, was unusually high and showed an increased rate of patients tested positive for A(H1N1)pdm09 virus infections [11]. This is in accordance with the observed increase of in-house admissions by A(H1N1)pdm09 virus infections at our Children's Hospital in the season 2012/13. In terms of when the first positive

A(H1N1)pdm09 virus samples were detected at our laboratory, the pandemic began much earlier in the season 2009/10 (July 2009 vs January 2013) and lasted much longer (six vs. three months). This is in accordance to other German studies and to the influenza surveillance results of the Germans public health institute RKI [2,11,12].

Altmann et al. and Lehners et al. demonstrated that the clinical course of patients with A(H1N1)pdm09 virus infections in the post-pandemic season 2010/11 was severe, and in some cases more pronounced than during the 2009/10 pandemic [2,13]. In both studies, the authors noted an age-shift towards affection of younger age. Our analysis of the 2012/13 season showed continuation of this trend. Lehners et al. noted a more severe clinical course of patients in 2010/11, compared with the season 2009/10, based on an extended in patient course and a higher mortality rate. Viasus et al. observed comparable figures, however, they exclusively investigated adult patients [14]. In a study on pediatric patients Stripeli et al. found no significant difference in the clinical presentation of pediatric patients, comparing the 2009/10 season with the post-pandemic season 2011/12, however, mean hospital stay in the post-pandemic season was significantly longer [15]. A recent analysis on pediatric patients in a Children's Hospital in Colorado reported that children with A(H1N1)pdm09 virus infections in the post-pandemic years appeared to have less severe disease, as compared to data of the pandemic, which is in contrast to our findings [16]. But the authors even reported for a higher proportion of younger children in the post-pandemic years, which is similar to our findings.

Dalziel et al. identified six risk factors and warning signs, which are predictors for a severe course of A(H1N1)pdm09 virus infections, comprising the presence of (i) pre-existing chronic lung disease, (ii) cerebral palsy/developmental delay, (iii) requirement of oxygen therapy/decreased oxygen saturation, (iv) tachycardia, (v) chest retractions and (vi) signs of dehydration [5]. For two of the mentioned risk factors, we noted a significant difference (significantly increased oxygen demand and signs of dehydration 2012/13, Table 3). Likewise, for the risk factor chest retractions we also noted a distinct difference, but not with statistical significance. Attending physicians need to focus on these risk factors, to prevent deterioration of clinical course, respiratory failure and subsequent ICU admissions.

Funaki et al. used the extent of radiological pulmonary findings as a predictor for the severity of the clinical course in A(H1N1) pdm09 virus infections [17]. The more pronounced the radiological findings, the more complicated the clinical course. The higher rate of radiologically detectable pneumonia in 2012/13 in our cohort supports this observation.

The trend to higher mean CRP values among our patients of the 2012/13 season could suggest a more severe course of the infection. The difference in the CRP values did not reached statistical significance, perhaps due to a small sample size in 2012/13. Chen et al. identified a CRP value > 3 mg/dl (or 30 mg/l) as an independent risk factor for a severe clinical course of A(H1N1)pdm09 virus infections [4]. In accordance with this, other studies indicate a positive correlation between CRP values and the severity of the clinical course, when during an A(H1N1)pdm09 virus infection [18-21]. Several studies showed an association between the severity of the clinical course of an A(H1N1)pdm09 virus infection and pre-existing chronic disease [3,5,21-24]. Contrary to this, we found 32% of our patients to have a chronic pre-existing disease in the season 2009/10, compared to only 20% of patients in the 2012/13 season. Probably there was a shift to higher vaccination rates among chronically ill patients in the post-pandemic seasons and the season 2012/13. Due to missing and incomplete data of vaccination status a univariate analysis between both seasons wasn't carried out.

A possible cause for differing disease severity observed between both seasons could be the influence of antigenic drift in influenza viruses. Multiple studies reported about mutations of the hemagglutinin and neuraminidase genes among A(H1N1)pdm09 isolates in post-pandemic seasons in Germany and worldwide [11,25-31]. Though, no significant changes in sequencing analysis were reported for the A(H1N1)pdm09 strain for the post pandemic seasons [11,31]. Interestingly, Lindermann et al. reported for mutations in a region of a hemogglutinin targeted by antibiodies, which could be potential explanation for higher rates of infection in middle-aged adults in the 2013-2014 season [28]. But, if there is a significant association between antigenic drift and disease severity still remains unclear.

There was no difference found for treatment with oseltamivir, comparing both seasons in our cohort. However, timing of treatment with oseltamivir or missing treatment could be a contributing factor to differences in the clinical presentation of patients with an A(H1N1)pdm09 virus infection. A recent study investigated the association between treatments with neuraminidase inhibitors (NAI) on influenza-related pneumonia (IRP) [32]. The authors reported that early initiation of NAI treatment (≤ 48 h after illness onset) led to a significant reduction in IRP. Furthermore, the authors reported that NAI treatment had significant impact on the clinical outcome, in particular, early NAI treatment lowered morbidity and the needs for ventilatory support. More detailed information about exact timing of NAI treatment in our study are missing, because onset of NAI treatment was at the decision of the attending physician and no formal protocol for assessment of medication treatment was used in this retrospective study. Therefore, it remains unclear whether NAI treatment and timing of NAI treatment contributed to differences in the clinical outcome of the patients between both seasons.

Conclusion

In conclusion, the clinical presentation of hospitalized German children with seasonal A(H1N1)pdm09 virus infection in 2012/13 was more pronounced than during the pandemic influenza season. Influencing factors for the difference in disease severity are likely multifactorial. However, treatment of seasonal A(H1N1)pdm09 virus infections in children still remain a challenge for the attending physicians and the health care system. In particular, infants and preschool children are at higher risk for severe clinical outcome and prolonged hospital stay, and physicians need to focus in examination on early clinical sings for respiratory insufficiency.

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