Viral Kinetics and Resistance Development in Children Treated with Neuraminidase Inhibitors: The Influenza Resistance Information Study (IRIS)

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Summary:

This study demonstrates that children aged 1–5 years shed a 1.04- to 1.24-fold higher total quantity of influenza virus compared with older children associated with an increased risk of acquiring resistance mutations following antiviral treatment.

Abstract

Background

The effect of age, baseline viral load, vaccination status, antiviral therapy and emergence of drug resistance on viral shedding in children infected with influenza A or B virus was studied.

Methods

Samples from children (aged ≤13 years) enrolled during the 7 years of the prospective Influenza Resistance Information Study (IRIS; NCT00884117) were analyzed by polymerase chain reaction to determine the influenza virus(sub-)type, viral load and resistance mutations. Disease severity was assessed; clinical symptoms were recorded. The association of age with viral load and viral clearance was examined by determining the area under the curve for viral RNA shedding using logistic regression and Kaplan-Meier analyses.

Results

A total of 2131 children infected with influenza (683 A/H1N1pdm09; 825 A/H3N2; 623 influenza B) were investigated. Age did not affect the mean baseline viral load. Children aged 1–5 years, infected with A/H1N1pdm09, A/H3N2 or influenza B virus had prolonged viral RNA shedding (±1-2 days) compared with older children (aged >5 years) and up to 1.2-fold higher total viral burden. Besides older age (odds ratio [OR] 1.08; confidence interval [CI]: 1.05-1.12), prior vaccination status (OR 1.72; CI: 1.22-2.43) and antiviral treatment (OR 1.74; CI: 1.43-2.12) increased the rate of viral clearance. Resistance mutations were detected in 49 children infected with influenza A virus (34 A/H1N1pdm09; 15 A/H3N2) treated with oseltamivir, most of whom were aged <5 years (n = 39).

Conclusions

Children aged 1–5 years had a higher total viral burden with prolonged virus shedding and had an increased risk of acquiring resistance mutations following antiviral treatment.

Key words:

Influenza, pediatrics, Influenza Resistance Information Study, viral load, resistance mutations

Introduction

Children are more likely to be infected with influenza virus during epidemics than adults [1]. Annually, pediatric infections are associated with a high number of emergency room visits and hospitalizations [2–5]. This susceptibility of children to influenza virus infection largely results from the absence of pre-existing acquired immunity [6,7]. Thus, children can serve as a major reservoir for further prolongation of outbreaks in the community [8–10]. Although several studies report prolonged influenza virus replication in children compared with adults, this finding is not consistently reported and remains to be elucidated [1,8–14].

To gain further insight into the drivers of pediatric viral replication and antiviral resistance, studies with sufficient numbers of patients are needed to compare kinetics between the different age strata. The Influenza Resistance Information Study (IRIS) provided a unique opportunity to capitalize on the knowledge of influenza viral kinetics and the incidence of antiviral resistance in children [15–17]. With more than 2000 children included, it was possible to analyze the effect of age, baseline viral load, antiviral usage, emergence of resistance, and vaccination status of children on viral shedding and clearance for influenza A (A/H1N1pdm09, A/H3N2) and influenza B viruses.

Methods

Study design

IRIS (NCT00884117) was a prospective, multicenter, non-randomized study undertaken from 2008 to 2015. Study participants were enrolled from Europe, United States, China (Hong Kong), Australia and South Africa. The study was implemented in compliance with the principles of the Declaration of Helsinki and its amendments, and in accordance with Good Clinical Practice. Written informed consent was obtained from all study participants and/or their legal guardians. Local ethics committees and institutional review boards approved the study protocol and amendments.

Inclusion criteria and clinical assessment

A detailed description of the study procedures has been previously published [15,17]. In brief, during the first 5 years of IRIS (December 2008 to March 2013), both adults and children aged ≥1 year were eligible for enrollment when positive for influenza virus by rapid test and/or upon display of influenza-like signs and symptoms within 48 hours (≤96 hours for hospitalized adults, no time limit for hospitalized children) [15]. Since resistance mutations were more prevalent in children during the first 5 years of the study, the inclusion criteria for the last 2 years of the study (March 2013 to September 2015) were modified to recruit only children aged ≤13 years on antiviral treatment. Clinical management, including prescription of antivirals was at the discretion of the healthcare provider.

For clinical assessment, patients were evaluated on days 1 (study enrollment), 6 and 10. Influenza signs and symptoms were assessed by investigators at each center using a 4-point scale [15]. Temperature (oral or tympanic) and adverse events (AE) were recorded daily on diary cards. In the last 2 years of the study, baseline and follow-up symptom assessments were conducted by the study physicians, but diary cards were not obtained.

Virological assessment

Qualitative, quantitative, and mutation specific real-time reverse transcriptase polymerase chain reaction (RT-PCR) was performed on collected nasal and throat swabs on days 1 (study enrollment), 3, 6 and 10 to determine the influenza virus(sub-)type, viral load and resistance mutations in the neuraminidase (NA) gene (H275Y, R292K, E119V, R150K, D197N, N294S) [15,17]. Viral RNA loads (RNA copies [$\log_{10}$/ml]) were determined by converting the cycle threshold value to viral particle counts (viral RNA copies), by processing electron-microcopy-counted influenza A/Puerto Rico/8/34 and B/Lee/40 virus stocks (Advanced Biotechnologies Inc., Maryland, USA) in parallel to the patient samples.

Statistical analysis

Data from all children enrolled during the entire 7-years of IRIS were evaluated. Patients who received more than one NA inhibitor treatment or other antivirals were excluded. Children were stratified into age groups according to presumed immunity development [6,7]: ≤6 months; 6 months–1 year; 1–3 years; 3–5 years; 5–10 years and 10–13 years.

Continuous data, such as viral load, were summarized as means and standard deviations (SD), medians and ranges were calculated/reported to one decimal point. Categorical data were summarized as frequency and percentage of the appropriate study population. The area under the curve (AUC) of the virus load was determined using the trapezoid rule. Student's t-test was used to compare AUC values between age groups. Logistic multivariable analyses were performed to explore the association between duration of viral RNA detection and baseline viral load, age (years), antiviral treatment, virus subtype, influenza virus vaccination status during the previous 4 months and emergence of resistance in the post-baseline samples. The dependent variable was "cleared" or "not cleared" depending on RNA detection on day 6. The association of emergence of resistance mutations with baseline viral load, age (years) and influenza virus subtype was analyzed by a regression analysis. Results of the regression analyses are shown as odd ratios (OR) and confidence intervals (CI) with significance determined by chi-squared (χ^2) test.

Kaplan-Meier plots for time to non-detection of viral RNA by quantitative RT-PCR and for time to symptom resolution were generated for various age strata using the recorded symptoms diary cards of children age ≥1 year. Data were censored at the date of the last available sample, if patients were lost to follow-up, if samples were inadequate or if RNA was still detected at the final visit. Wilcoxon and log-rank tests were used to compare outcomes between the age groups and influenza virus (sub-)type.

Results

Patients characteristics and demographics

A total of 2131 children, aged ≤13 years, tested positive for a single influenza virus (sub-)type by RT-PCR (see Supplementary Figure 1 for study flow chart). Baseline characteristics stratified by age are summarized in Table 1 and Supplementary Tables 1 and 2 [6]. Relatively few infants (aged <1 year) were included in IRIS and were positive for influenza virus (N = 23). A total of 683 children were infected with A/H1N1pdm09, 825 with A/H3N2 and 623 with influenza B virus. No infants aged 6 months–1 year had influenza B virus infection. Gender was distributed similarly across all age groups. The majority of children received antivirals (61%). Pulmonary (13.4%) and cardiovascular (0.8%) comorbidities were relatively uncommon, as was influenza vaccination coverage in the previous 4 months (8.3%).

Virological kinetics

Baseline viral RNA load

The mean viral RNA loads (RNA copies [$\log_{10}$/ml]) at the day of study enrollment (baseline) of all children infected with A/H1N1pdm09 and A/H3N2 virus were comparable, except in the small group of infants infected with A/H3N2 virus (Figure 1). Children aged 10–13 years (6.3 $\log_{10}$/ml) infected with influenza B virus had a significantly higher baseline viral load compared with children aged 1–10 years (range: 5.8-6.0 $\log_{10}$/ml; *0.05>*P*>0.01).

Viral RNA clearance

The change in viral RNA load over time relative to the baseline viral load was calculated for all children to determine the rate of viral RNA clearance (Table 2). In most cases, children aged 10–13 years cleared viral RNA faster than younger children. For A/H1N1pdm09 virus, the higher rate of viral load reduction in older children was only significant at day 6. For A/H3N2 and influenza B virus, this effect remained significant until day 10 post-baseline.

When corrected for the date of symptom onset, children aged 10–13 years infected with A/H1N1pdm09 and A/H3N2 cleared virus faster than younger children if they were enrolled on the day that symptoms first occurred (n = 270) (Supplementary Figure 2A). A/H3N2 virus infected children aged 10 to 13 years showed a similar trend even if they were enrolled ≥3 days after symptom onset (Supplementary Figure 2D and E). At longer time periods between study enrollment and disease onset, the difference in viral RNA load reduction was no longer observed between the different age groups (Supplementary Figure 2B–E).

Total quantified viral RNA load in time

The mean AUC of the viral load was calculated over the course of infection (Figure 2). A/H1N1pdm09 infected infants (aged <1 year, 37.3 $\log_{10}$/ml*time) and young children (aged 1–5 years, 26.2 $\log_{10}$/ml*time) had significantly higher mean AUCs of 1.60- and 1.12-fold, respectively, than older children (aged >5 years, 23.3 $\log_{10}$/ml*time) (**0.01>*P*>0.001 and ***P<0.001). The same trend was observed for influenza B virus infection with a 1.37- and 1.04-fold larger total mean quantity of virus detected in infants (aged <6 months; 39.6 $\log_{10}$/ml*time) and young children (aged 1–5 years, 29.5 $\log_{10}$/ml*time), respectively, compared with older children (aged >5 years, 28.4 $\log_{10}$/ml*time). Infants (aged <1 year, 16.2 $\log_{10}$/ml*time) infected with A/H3N2 virus shed a lower amount of virus than older children (*0.05>*P*>0.01). The mean AUCs of A/H3N2 virus infected in children aged 1–5 years (28.3 $\log_{10}$/ml*time) were 1.24-fold higher compared with older children (22.8 $\log_{10}$/ml*time) (**0.01>*P*>0.001 and ***P<0.001). When corrected for treatment status, these age-related effects on the total quantity of viral RNA load persisted (Supplementary Figure 3).

Time to non-detection of viral RNA

The median time to A/H1N1pdm09 virus RNA clearance was longest for young children (aged <5 years (N = 320), median range: 9.9-11.5 days) compared with older children (aged

>5 years (N = 363), median range: 7.2-9.0 days) (Figure 3A). Viral RNA clearance in A/H3N2 virus infected older children (aged >5 years [N = 451], median range: 8.7-8.9 days) was faster compared with younger children (aged 1–5 years [N = 364], median range: 10.0-10.6 days). Infants (aged 6 months–1 year) cleared the A/H3N2 virus faster than all other children (Figure 3B). Older children infected with influenza B virus also tended to clear the virus faster than younger children, however, these differences were not statistically significant (Figure 3C).

Variables associated with viral RNA clearance

Logistic regression analyses confirmed the relationship between duration of virus shedding and older age (OR 1.08 [CI: 1.05-1.12], *P*<0.0001). Additionally, vaccination (OR 1.72 [CI: 1.22-2.43], *P*=0.0017) and antiviral treatment (OR 1.74 [CI: 1.43-2.12], *P*<0.0001) were associated with shorter duration of virus shedding. High baseline viral loads (OR 0.57 [CI: 0.52-0.62], *P*<0.0001) and the emergence of resistance mutations (OR 0.05 [CI: 0.01-0.20], *P*<0.0001) were independently associated with longer duration of virus shedding. Infection with A/H3N2 (OR 0.71 [CI: 0.57-0.90], *P*=0.01) or influenza B virus (OR 0.78 [CI: 0.61-0.99], *P*=0.01) decreased the odds of viral RNA clearance compared with infection with A/H1N1pdm09 virus.

Clinical symptoms

Clinical signs were mild and complications were relatively rare. A total of 185 (8.7%) patients reported AEs. Of these, 117 (9.0%) received antiviral therapy and 68 (8.2%) were untreated (Supplementary Tables 3 and 4). The incidence of AEs was the highest for young children (aged <5 years). Serious AEs (SAEs) were reported in 14 (0.7%) children, of whom 10 (0.8%) received oseltamivir treatment and 4 (0.5%) were untreated (Supplementary Tables 5 and 6). Two children were admitted to the intensive care unit (ICU), one 5-month old infant and one 8-year old child. Both were treated with oseltamivir and recovered.

The duration of symptoms of older children infected with A/H1N1pdm09 and A/H3N2 (aged >5 years, median range: 4-5 days) was shorter compared with younger children (aged <5 years, median range: 5-6 days; *0.05>*P*>0.01 and **0.01>*P*>0.001) (Supplementary Figure 4). This age-related difference in symptom duration was less pronounced when infected children were stratified according to their antiviral treatment status (Supplementary Figure 5 and 6). There was no significant observed difference in symptom resolution between the age groups of all children infected with influenza B virus (Supplementary Figures 4–6).

Emergence of resistance

Neuraminidase inhibitor associated resistance mutations were detected in the NA gene of the post-baseline samples in 49 oseltamivir-treated children (2.3%), including 34 (1.6%) A/H1N1pdm09 viruses with the H274Y mutation and 15 (0.7%) A/H3N2 viruses with the R292K mutation (Table 3). No resistance mutations were detected in influenza B viruses. The emergence of resistance mutations was equally distributed over the entire 7-years of IRIS (Table 4). The prevalence of resistance was higher in children aged <5 years (n = 39) compared with children aged >5 years (n = 10) (Table 3).The children infected with viruses that acquired resistance mutations had a higher viral load at day 3 and/or 6 compared with children infected with wild-type A/H1N1pdm09 and A/H3N2 virus (Supplementary Figures 7 and 8). This difference was not observed at day 10, since most children had cleared the virus.

Variables associated with emergence of resistance

Logistic regression analyses demonstrated that older age was associated with reduced odds of acquiring resistance mutations (OR 0.70 [CI: 0.62-0.81], *P*<0.0001). A high baseline viral load was associated with the development of resistance mutations (OR 1.50, *P*=0.005). Influenza A/H3N2 virus was less likely to become resistant compared with A/H1N1pdm09 virus (OR 0.38, *P*=0.0016).

Discussion

The viral load at baseline and the rate of viral clearance both contribute to the total viral RNA load in patients with influenza virus infection. The presented data demonstrate that, over the duration of their infection, children aged 1–5 years shed a 1.04- to 1.24-fold higher total quantity of influenza virus compared with older children. This higher total viral burden in young children was observed in both untreated and oseltamivir-treated children's age groups.

Similar to previous data, no age-related differences were observed in the baseline viral load of infected children in this study (excluding infants aged <1 year infected with A/H3N2 virus), indicating that baseline viral load did not have a direct effect on the high total quantity of virus shedding detected in the upper respiratory tract of infected young children [13,14]. The relatively low rate of viral RNA clearance observed in children aged 1–5 years may have led to their high viral burden and it most likely resulted from their immature immune response and/or the absence of prior exposure to influenza A viruses [6]. In contrast to the data presented here, several studies reported no difference in viral burden between children of different age groups [12–14,18]. However, those studies did not take the timing of symptom onset into account. The influenza virus load depends on the time when a sample is obtained in relation to disease onset [12,19]. When corrected for the date of symptom onset, this study showed that viral RNA shedding was in general still prolonged in young children compared with older children. Ultimately, this age-related viral load difference was no longer observed for children that were included ≥2 days after symptom onset. Therefore, by not correcting for the date of disease onset, previous studies may have failed to detect differences between age groups [12–14,18].

Surprisingly, infants infected with A/H3N2 virus had a low baseline viral load and cleared viral RNA faster compared with older children, whereas the opposite was observed for A/H1N1pdm09 virus. Technical reasons for the observed low baseline viral load in these infants were ruled out first, since the same sampling and virological assays were used for all patients included in this study. Secondly, parents with sick infants tend to seek professional care earlier upon symptom onset compared with parents with older children [20]. This early sampling may have resulted in very low viral loads, since virus production has only just started. However, in this study, the time from symptom onset to study baseline in infants was either similar or higher than older children. As infants were not included during the first 5 years of IRIS, only a small number of young children, primarily from the United States, were included in this study. This small sample size and the observed substantial variation in the detected viral loads makes it difficult to draw any solid conclusion from this group.

Age-related differences in the viral kinetics in children infected with influenza B virus were not as prominent as those observed in influenza A virus-infected children. According to available surveillance data to date, most influenza epidemics were dominated by influenza A virus infections [21,22]. Correspondingly, due to the absence of pre-existing immunity, influenza B virus infection might affect both young and older children equally [23].

Interestingly, despite the low observed vaccination coverage, in accordance with previous data, the present study showed that both vaccination and antiviral treatment reduced the duration of virus shedding [11,24,25]. In addition, antiviral treatment seemed to have a beneficial effect on symptom resolution. However, IRIS was a non-randomized clinical study that was not designed to determine the efficacy of antiviral treatment. Therefore, no conclusions were drawn regarding oseltamivir usage with clinical symptoms. Previous studies have suggested that antiviral therapy is associated with the development of resistance mutations [11,15,26–28]. Similarly, in this study only children infected with A/H1N1pdm09 and A/H3N2 who received oseltamivir treatment acquired resistance mutations. The prevalence of resistance in this study was highest in children younger than 5 years and almost absent in the older ones, which may suggest that the protracted viral RNA shedding in young children allowed for the influenza viruses to evolve and acquire resistance mutations upon selective pressure of antiviral therapy. Similar to a previous study, emergence of resistant viruses did not affect symptom resolution in influenza A-infected children (data not shown) [16].

The emergence of resistance can also delay viral clearance [16]. This may be a contributing factor to prolonged shedding in young children prone to the developed antiviral resistance. However, when children who acquired antiviral resistance were excluded from the analysis, the probability of age to increase viral clearance remained the same (data not shown).

Young children are at higher risk of acquiring severe influenza [1,4,29]. In this study, serious complications were rare, with only two ICU admissions and influenza-related symptoms that lasted for approximately 1 week. Since antiviral therapy was at the discretion

of the physician, it is not known whether the observed AEs were related to the antiviral usage or a reflection of the severity of influenza virus infection.

In conclusion, this study showed that the baseline nasopharyngeal influenza virus loads were comparable between all age groups. However, over time, viral RNA shedding was protracted in young children (aged 1–5 years) compared with older children. This could explain why young children are more likely to develop antiviral resistance upon antiviral therapy and serve as spreaders of influenza virus in the community.

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Potential conflict of Interest

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JSN-V-T is currently seconded to the Department of Health and Social Care (DHSC), England. The views expressed in this manuscript are not necessarily those of DHSC.

RW receives support from the US NIH and is on the Board of Directors of Gilead Science

AO reports personal fees from Hoffman La Roche and GSK, is the founder, CSO and minor shareholder of Viroclinics Biosciences BV and C202, and also reports EU and CEPI grants, outside the submitted work.

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BC reports employment with stock from Roche Products Limited.

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All other authors have no Potential Conflicts of Interest.

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Figure legends

Figure 1: Baseline viral RNA load of children infected with A/H1N1pdm09, A/H3N2 and influenza B virus.

The mean baseline viral RNA load of children infected with A/H1N1pdm09 (A), A/H3N2 (B) and Influenza B virus (C) are depicted as mean ± standard deviation. Influenza B virus was not detected in infants aged 6 months–1 year. Asterisks represents significant p-values (*0.05<*P*<0.01 and **0.01>*P*>0.001). Abbreviations: n, total number of patients; y, years.

Figure 2: Total viral RNA load of children infected with A/H1N1pdm09, A/H3N2 and influenza B virus.

The total amount of viral RNA shedding in children infected with A/H1N1pdm09, A/H3N2 and influenza B virus was determined by calculating the area under the curve (AUC). Influenza B virus was not detected in infants aged 6 months–1 year. The bar graphs depict the mean ± standard deviation. Asterisks represent significant p-values (*0.05<*P*<0.01, **0.01>*P*>0.001, ***P<0.001). Abbreviations: n, total number of patients; y, years.

Figure 3: Kaplan-Meier plots for time to viral RNA clearance of children infected with H1N1pmd09, A/H3N2 and influenza B virus.

Censored patients are illustrated as plus signs. The median time to viral RNA clearance in each age group is depicted next to the Kaplan-Meier plots. Influenza B virus was not detected in infants aged 6 months–1 year. Asterisk represents significant p-values (*0.05<*P*<0.01, and **0.01>*P*>0.001). Abbreviation: N, total number of patients; CI, confidence interval.

Tables

Table 1: Clinical characteristics of children with laboratory confirmed influenza at baseline

Patients characteristics	Total (N = 2131)	Age groups <6 months (N = 12)	6 months–1 year (N = 11)	1–3 years (N = 369)	3–5 years (N = 473)	5–10 years (N = 936)	10–13 years (N = 330)
Virus(sub-)type							
A/H1N1pdm09	683 (32.1%)	4 (33.3%)	7 (63.6%)	151 (40.9%)	158 (33.4%)	270 (28.8%)	93 (28.2%)
A/H3N2	825 (38.7%)	6 (50.0%)	4 (36.4%)	150 (40.7%)	214 (45.2%)	335 (35.8%)	116 (35.2%)
Influenza B	623 (29.2%)	2 (16.7%)	0 0	68 (18.4%)	101 (21.4%)	331 (35.4%)	121 (36.7%)
Country							
France	396 (18.6%)	0	0	60 (16.3%)	88 (18.6%)	186 (19.9%)	62 (18.8%)
Germany	41 (1.9%)	0	0	4 (1.1%)	15 (3.2%)	15 (1.6%)	7 (2.1%)
Norway	3 (0.1%)	0	0	0 0	1 (0.2%)	2 (0.2%)	0 0
Poland	573 (26.9%)	0	0	139 (37.7%)	169 (35.7%)	210 (22.4%)	55 (16.7%)
United States	695 (32.6%)	12 (100%)	10 (90.9%)	88 (23.8%)	111 (23.5%)	336 (35.9%)	138 (41.8%)
China (Hong Kong, (SARPCR))	369 (17.3%)	0 0	0 0	61 (16.5%)	76 (16.1%)	170 (18.2%)	62 (18.8%)

South Africa	22 (1.0%)	0 0	1 (9.1%)	6 (1.6%)	5 (1.1%)	8 (0.9%)	2 (0.6%)
Australia	32 (1.5%)	0(0.0)	0(0.0)	11 (3.0)	8 (1.7)	9 (1.0)	4 (1.2)
Gender							
Female	1031 (48.4%)	7 (58.3%)	5 (45.5%)	183 (49.6%)	236 (49.9%)	455 (48.6%)	145 (43.9%)
Male	1100 (51.6%)	5 (41.7%)	6 (54.5	186 (50.4%)	237 (50.1%)	481 (51.4%)	185 (56.1%)
Antiviral treatment							
No	831 (39.0%)	0	0	140 (37.9%)	210 (44.4%)	360 (38.5%)	121 (36.7%)
Yes	1300 (61.0%)	12 (100%)	11 (100%)	229 (62.1%)	263 (55.6%)	576 (61.5%)	209 (63.3%)
Febrile [a]							
No	827 (38.8%)	9 (75.0%)	5 (45.5%)	137 (37.1%)	165 (34.9%)	366 (39.1%)	145 (43.9%)
Yes	1303 (61.1%)	3 (25.0%)	6 (54.5%)	232 (62.9%)	308 (65.1%)	570 (60.9%)	184 (55.8%)
Cardiovascular disease							
No	2115 (99.2%)	12 (100%)	11 (100%)	365 (98.9%)	468 (98.9%)	931 (99.5%)	328 (99.4%)
Yes	16 (0.8%)	0	0	4 (1.1%)	5 (1.1	5 (0.5%)	2 (0.6%)
Pulmonary disease							
No	1846 (86.6%)	12 (100%)	10 (90.9%)	339 (91.9%)	424 (89.6%)	791 (84.5%)	270 (81.8%)
Yes	285 (13.4%)	0	1 (9.1%)	30 (8.1%)	49 (10.4%)	145 (15.5%)	60 (18.2%)

Vaccinated in previous 4 months [b]							
No	1952 (91.6%)	12 (100%)	7 (63.6%)	333 (90.2%)	451 (95.3%)	851 (90.9%)	298 (90.3%)
Yes	177 (8.3%)	0	4 (36.4%)	35 (9.5%)	22 (4.7%)	85 (9.1%)	31 (9.4%)
Time from symptom onset to study baseline, d, mean (SD)	1.2 (0.77)	1.6 (0.90)	1.4 (0.67)	1.1 (0.75%)	1.2 (0.94)	1.2 (0.70)	1.2 (0.68)

Abbreviations: SARPRC, Special Administrative Region of the People's Republic of China; d, day; SD, standard deviation

[a] Total N = 2130, febrile status of 1 patient was not reported.

[b] Total N = 2129, vaccination status of 3 patients were not reported.

Table 2: Post-baseline mean viral load (RNA copies log_{10}/ml) change from baseline

Influenza virus (sub)-type	Time post-baseline enrollment (day)	Total [a] (N = 2131)	Age groups <6 months (N = 4)	6 months–1 year (N = 7)	1–3 years (N = 151)	3–5 years (N = 158)	5–10 years (N = 270)	10–13 years (N = 93)
A/H1N1pdm09	3	650	-1.04 (1.37)	-1.00 (1.52)	-1.52 (1.80)	-1.50 (1.70)	-1.70 (1.75)	**-2.08 (1.74)**
	6*	662	-1.35 (1.58)	-3.12 (1.18)	-3.36 (2.25)	-3.48 (2.13)	-3.93 (1.95)	**-4.18 (2.12)**
	10	654	-3.77 (1.70)	**-5.74 (0.85)**	-5.01 (1.66)	-4.85 (1.66)	-5.06 (1.50)	-5.07 (1.57)
A/H3N2	3**	789	-0.97 (2.42)	-1.32 (0.92)	-1.77 (1.79)	-1.59 1.85)	-2.11 (1.77)	**-2.18 (1.86)**
	6***	792	-3.49 (0.98)	**-4.04 (2.01)**	-3.11 (2.09)	-3.08 2.09)	-3.97 (1.85)	-3.85 (2.07)
	10*	785	-3.97 (0.85)	-4.04 (2.01)	-5.21 (1.45)	-4.82 1.69)	-5.10 (1.50)	**-5.26 (1.46)**
Influenza B[b]	3*	601	-0.93 (0.64)		-1.05 (1.53)	-1.23 (1.88)	-1.31 (1.85)	**-1.83 (1.94)**
	6**	599	-3.39 (1.07)		-3.25 (2.22)	-3.52 (2.14)	-3.43 (2.18)	**-4.25 (1.94)**
	10**	592	**-6.25 (0.16)**		-4.87 (1.89)	-5.20 (1.64)	-5.08 (1.80)	-5.76 (1.34)

Data depicted are the mean viral load (RNA copies log_{10}/ml) and in brackets the standard deviation.

Reduction in the virus RNA loads (RNA copies log_{10}/ml) are shown as minus values. The highest reduction in mean viral RNA load relative to baseline of children infected with A/H1N1pdm09 virus, A/H3N2 virus and influenza B virus are marked in bold.

[a] The total N swabs collected at each time point

[b] Influenza B virus was not detected in infants aged 6 months–1 year.

[*]Asterisk depicts the post-baseline sample day where the analysis of variance within the age groups was significant (*0.05<*P*< 0.01, **0.01>*P*>0.001 and ***$P<0.0001$)

Table 3: Emergence of resistance to neuraminidase inhibitors in children with laboratory confirmed A/H1N1pdm09 and A/H3N2 influenza virus at baseline.

	Age groups					
Influenza (sub-)type [a]	**<6 months** N = 10	**6 months–1 year** N = 11	**1–3 years** N = 301	**3–5 years** N = 372	**5–10 years** N = 605	**10–13 years** N = 209
A/H1N1pdm09	2/4 (50.0)	2/7 (28.6)	15/151 (9.9)	9/158 (5.7)	6/270 (2.2)	0/93 (0.0)
A/H3N2	0/6 (0.0)	0/4 (0.0)	5/150 (3.3)	6/214 (2.8)	4/335 (1.2)	0/116 (0.0)

Data are the fraction of resistant (%).

[a] Data from children infected with influenza B are not shown, since no resistance mutations were detected in these patients.

Table 4: Emergence of resistance mutations over the IRIS years [a]

IRIS year	Total	Age groups					
		<6 months	6 months–1 year	1–3 years	3–5 years	5–10 years	10–13 years
	N = 1508	N = 10	N = 11	N = 301	N = 372	N = 605	N = 209
2009	7/700 (1.0%)	0	0	0/1	0/2	0/3	0/1
2009/2010	1/293 (0.3%)	0	0	1/45 (2.2%)	0/56	0/131	0/61
2010/2011	14/274 (5.1%)	0	0	9/64 (14.1%)	3/72 (4.2%)	2/97 (2.1%)	0/41
2011/2012	5/131 (3.8%)	0	0	1/23 (4.4%)	2/39 (5.1%)	2/53 (3.8%)	0/16
2012/2013	15/400 (3.8%)	0	0	6/94 (6.4%)	7/113 (6.2%)	2/144 (1.4%)	0/49
2013/2014	7/151 (4.7%)	2/4 (50%)	2/8 (25%)	0/30	2/40 (5.0%)	1/62 (1.6%)	0/7
2014/2015	7/252 (2.8%)	0/6	0/3	3/44 (6.8%)	1/50 (2.0%)	3/155 (2.6%)	0/34
All years combined	49/1508 (3.3%)	2/10 (20%)	2/11 (18.2%)	20/301 (6.6%)	15/372 (4.0%)	10/605 (1.7%)	0/209

[a] Data are the fraction of resistant (%), unless specified otherwise. Children infected with influenza B are excluded, since no resistance mutations were detected in these patients. The denominator for percentages is the total number of A/H1N1pdm09 and A/H3N2 patients by age group enrolled in the respective years. Children <1 year of age were not recruited during years 1 to 5 of IRIS.

Figure 1

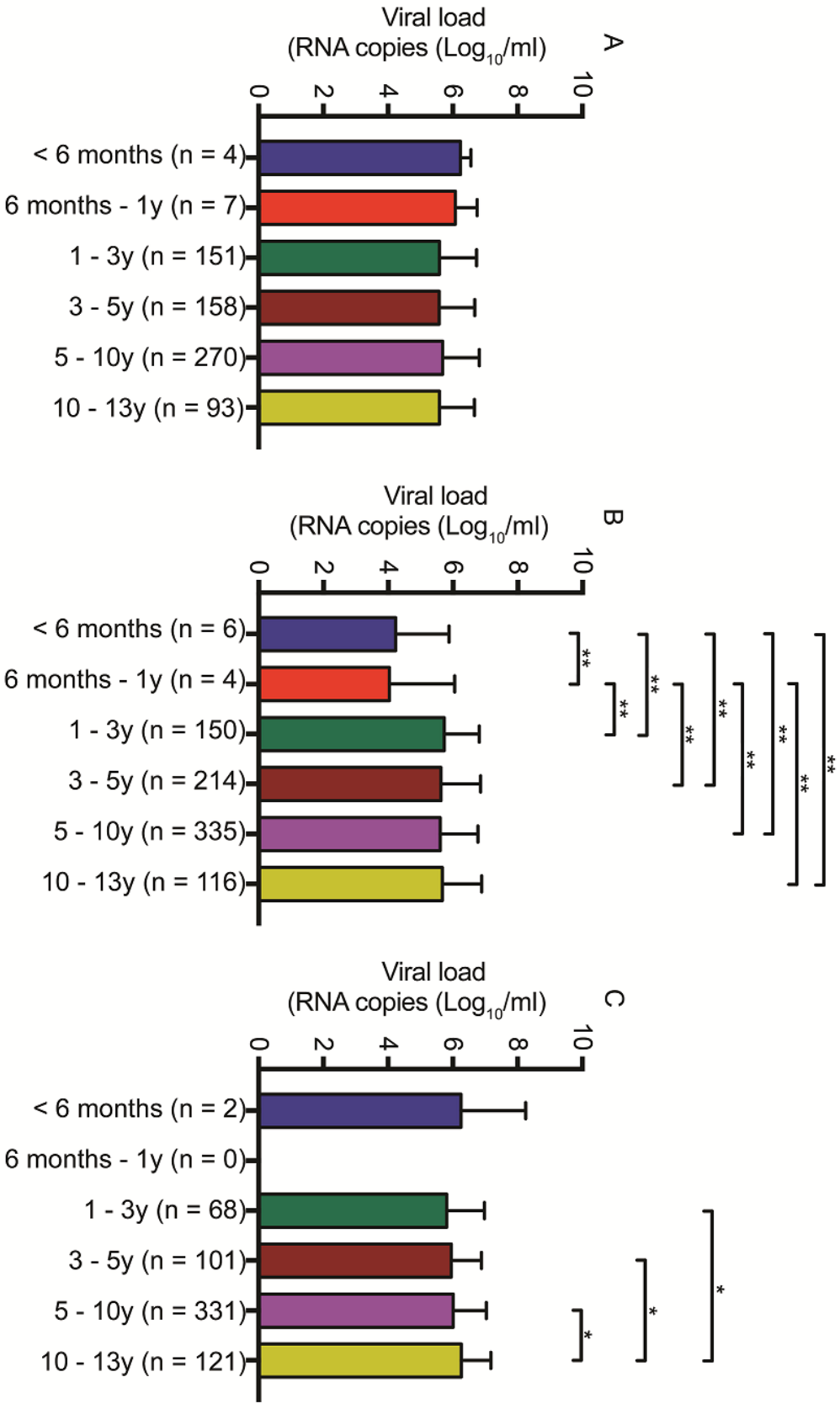


Figure 2

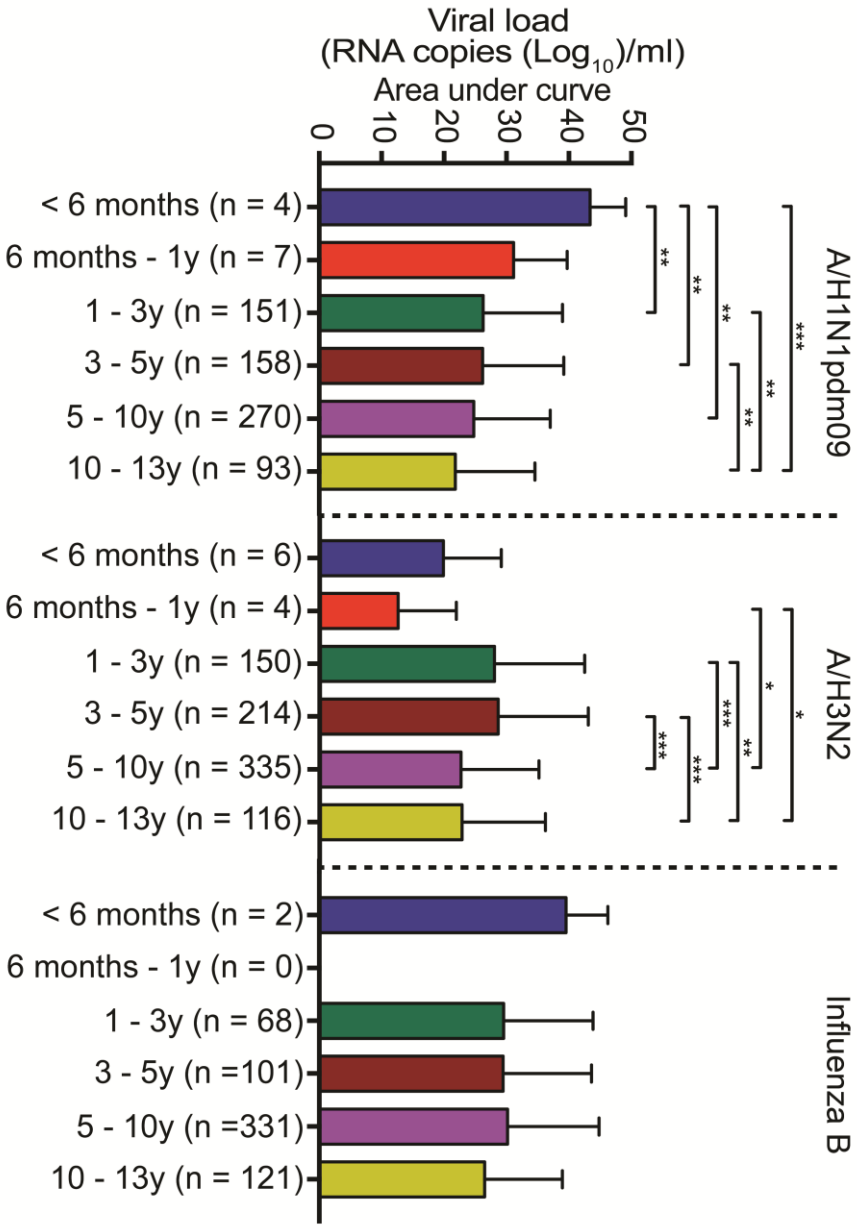
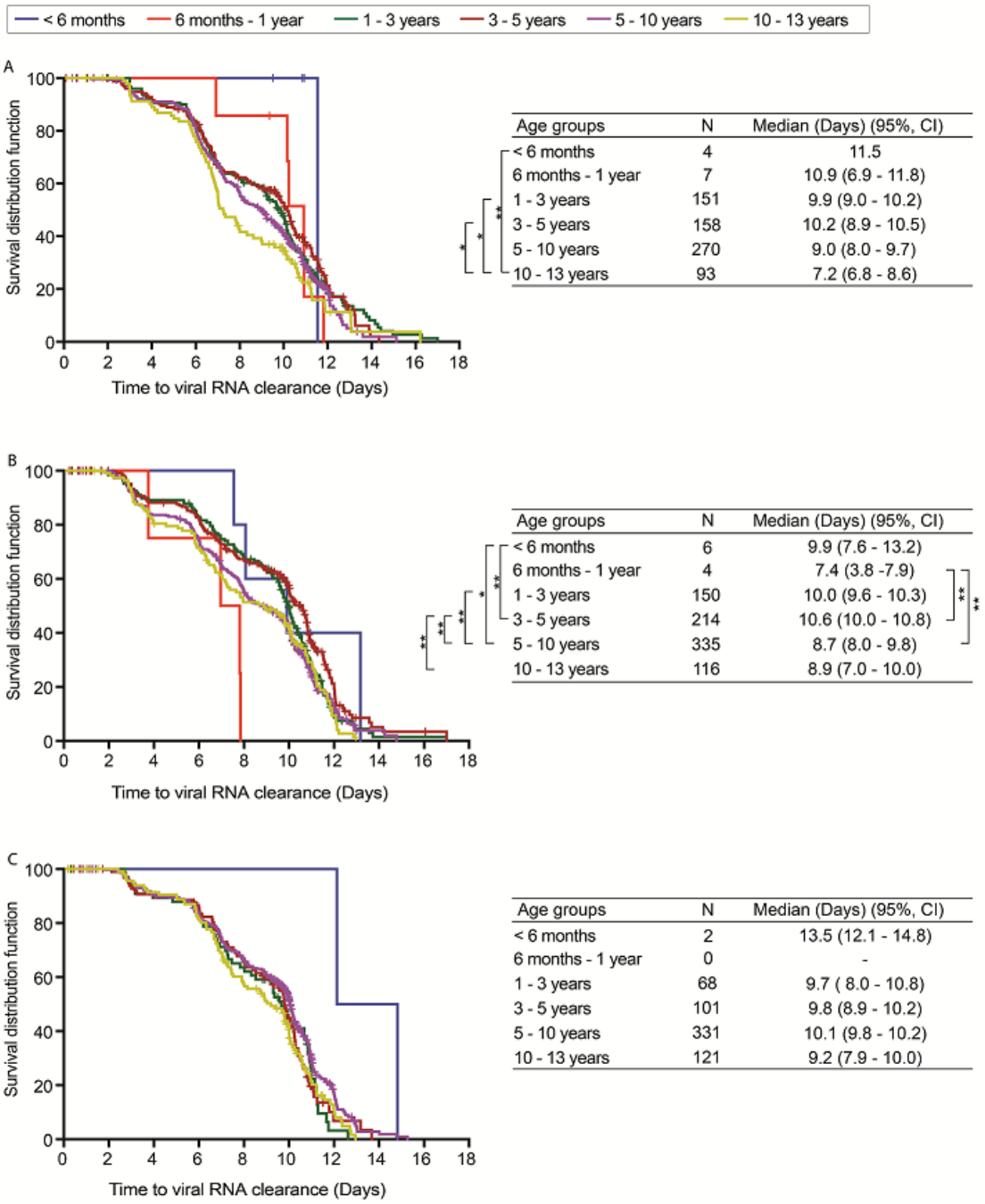


Figure 3



Age groups	N	Median (Days) (95%, CI)
< 6 months	4	11.5
6 months - 1 year	7	10.9 (6.9 - 11.8)
1 - 3 years	151	9.9 (9.0 - 10.2)
3 - 5 years	158	10.2 (8.9 - 10.5)
5 - 10 years	270	9.0 (8.0 - 9.7)
10 - 13 years	93	7.2 (6.8 - 8.6)

Age groups	N	Median (Days) (95%, CI)
< 6 months	6	9.9 (7.6 - 13.2)
6 months - 1 year	4	7.4 (3.8 -7.9)
1 - 3 years	150	10.0 (9.6 - 10.3)
3 - 5 years	214	10.6 (10.0 - 10.8)
5 - 10 years	335	8.7 (8.0 - 9.8)
10 - 13 years	116	8.9 (7.0 - 10.0)

Age groups	N	Median (Days) (95%, CI)
< 6 months	2	13.5 (12.1 - 14.8)
6 months - 1 year	0	-
1 - 3 years	68	9.7 (8.0 - 10.8)
3 - 5 years	101	9.8 (8.9 - 10.2)
5 - 10 years	331	10.1 (9.8 - 10.2)
10 - 13 years	121	9.2 (7.9 - 10.0)