

Increased H1N1 Infection Rate in Children with Asthma

Online Data Supplement

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METHODS

Recruitment and Inclusion/Exclusion Criteria

The study population was recruited from the general population in Wisconsin via primary care physicians, allergy and asthma specialists and advertisements in the community. Our goal in this study was to enroll a diverse population from the Wisconsin area. Any child with or without asthma, ages 4-12 years, was enrolled as long as they did not have a history of prematurity, complications at birth, respiratory problems at birth or any other significant medical illness. In addition, they could not be enrolled in another respiratory study.

H1N1 RT-PCR assay.

A semi-nested PCR protocol was used to amplify the target from the cDNA sample using three primers targeting the 2009 H1N1 Flu-specific HA (hemagglutinin) gene: HA-G2F1 (AATGGAACGTGTTACCCAG), HA-G2R1 (TTCTTTCCTTTATCATTAATGTAGG) and HA-G2R7 (TGCTGAGCTTTGGGTATG). These primers were selected within conserved regions present in all (n=462) HA genes available in Genbank in Sept 2009. Primers HA-G2F1 (forward) and HA-G2R1 (reverse) were used in the first PCR 'touchdown' cycle. Its product was further amplified in a second regular PCR with HA-G2F1 (forward) and HA-G2R7 (reverse). Final PCR products were then analyzed in a 1.5% agarose gel for the presence of the predicted 228-base PCR fragment (corresponding to nts 319-547 of HA gene). The sensitivity of the assay was 10 copies of cDNA template per reaction. The assay was validated with 50 H1N1 positive and 50 negative samples that were tested with the CDC novel H1N1 real-time PCR detection kit by the Wisconsin State Lab of Hygiene. Our assay detected H1N1 in all 50 positive samples and in 1 negative sample. Compared to the standard CDC assay, it has 100% sensitivity and 98% specificity, and the sample that tested negative by CDC assay and positive in our assay had H1N1 by cloning and sequencing.

Table E1. Definition of Cold and Asthma Scores

		Cold Symptoms	Asthma Symptoms
0	Absent	None	None
1	Mild	Mild stuffy or runny nose but does not affect daily activity	Occasional cough or wheeze but does not affect daily activity
2	Moderate	Moderate stuffy or runny nose and reduced activity but does not affect sleep	Frequent cough or wheeze with some shortness of breath and reduced activity but not affecting sleep
3	Severe	Cannot breathe through the nose and not able to sleep well because of symptoms	Unable to sleep well because of symptoms

Figure E1. Subjects included in analysis

