# Pandemic influenza A (H1N1) and other respiratory pathogens:

clinical insights - from epidemiology to treatment

Pandemic influenza A (H1N1) and other respiratory pathogens: clinical insights - from epidemiology to treatment
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# Pandemic influenza A (H1N1) and other respiratory pathogens: clinical insights - from epidemiology to treatment

# Pandemische influenza A (H1N1) en andere respiratoire pathogenen: klinische inzichten - van epidemiologie tot behandeling (met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof.dr. G.J. van der Zwaan, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op maandag 11 juni 2012 des ochtends te 10.30 uur

door

Patrick Martijn Smit

geboren op 3 april 1980
te Amsterdam

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## Preface

Influenza (the "flu") is an acute, mostly self-limiting illness that is predominantly characterized by fever and cough, and is caused by infection with influenza type A or B virus that occurs in outbreaks almost every winter.[1] These outbreaks or epidemics were described by Hippocrates in as early as the 5th century B.C. and have been reported repeatedly throughout the Middle Ages. The etymological origin of the word influenza derives from the late 14th century Medieval Latin īn-fluentia, an astrological term meaning "streaming ethereal power from the stars acting upon character or destiny of men". In 1933, the viral etiology of influenza was ultimately proved by a breakthrough attempt to isolate human influenza virus through experiments on ferrets.[2] The modern understanding of influenza was then founded.

One of the unique and most remarkable features of influenza virus is antigenic variation.[1] This is the mechanism by which changes in several viral proteins (most noticeably hemagglutinin and neuraminidase) causes the introduction of virus variants to which little or no resistance is present in the population. Minor antigenic changes are called antigenic drift, which occurs every year or every few years preceding a new influenza season, and major antigenic changes are called antigenic shifts, leading to completely new virus variants with pandemic potential. The most dramatic example of such a pandemic is the 1918-1919 "Spanish flu" pandemic, the greatest influenza pandemic in recorded history with over 20 million deaths worldwide. The persistent legacy of the 1918 influenza virus contributed to the development of the recent 2009 novel influenza A (H1N1) pandemic.[3] This emergence of yet another global health threat has been the consequence of a series of evolutionary and epidemiological events that led to the genetic reassortments between two influenza A (H1N1) swine viruses, which were actually the product of at least four independent avian-to-mammalian cross-species transmissions.[4] The clinical experience that was gained during the 2009 H1N1 pandemic gave rise to the very origin of this thesis.

Compared to influenza, other viruses, such as rhinovirus, adenovirus and coronavirus, are characteristically associated with a mild clinical syndrome of infection of the respiratory tract. However, mainly because of the high attack rate, respiratory tract infections are associated with significant morbidity and mortality, and thus are a substantial burden to society.[5] Therefore, the aim of this thesis is to provide insight into the clinical impact of respiratory pathogens, most importantly from 2009 pandemic influenza A (H1N1), but also from other common respiratory pathogens. Various studies, that were designed to cover a selection of clinically relevant facets of a range of key topics of interest, are described

in different chapters. In **Chapter 2**, the clinical-epidemiological aspects of the outbreak of 2009's pandemic influenza virus are considered, including the share of other circulating respiratory pathogens. In addition, this chapter elaborates on the value of screening for respiratory pathogens in neonates. **Chapter 3** describes how occupational transmission of pandemic influenza is prevented in a setting with an elaborated containment plan. **Chapter 4** gives details on two diagnostic modalities to rapidly determine whether one has influenza or not and to differentiate respiratory viral from bacterial infection, respectively. The risk of cardiovascular complications from respiratory tract infection and influenza vaccination is discussed in **Chapter 5**. Finally, **Chapter 6** concludes this thesis by summarizing the effectiveness and safety of influenza antiviral drug treatment and prophylaxis.

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# Chapter 1

## Introduction

# 1.1

# Lessons learnt from the influenza outpatient clinic

Patrick M Smit, Jan W Mulder, Chris Roggeveen, Dees PM Brandjes

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## SUMMARY

- The influenza outpatient clinic at the Slotervaart Hospital was established as a separate diagnostic facility during the novel influenza A/H1N1 pandemic.
- A total of 1161 patients visited the outpatient clinic, 242 of whom tested positive for the influenza virus (21%).
- Restricting the diagnostic procedure to only asking about influenza-like signs and symptoms seems insufficient.
- The use of oseltamivir could be discontinued in 48 of 86 cases after RT-PCR test results came back as negative.

*The influenza outpatient clinic at the Slotervaart Hospital, which was established during the 2009 influenza A/H1N1 pandemic, evaluated its findings. The rapid test seemed not to work, as opposed to what the manufacturer had promised, and most patients could discontinue the use of oseltamivir promptly.*

In April 2009, officials at the United States Centers for Disease Control and Prevention reported the first cases of infection with a novel swine influenza virus in California and Mexico.[1,2] This 'flu', also known as 'Mexican flu', turned out to be a new variant of an influenza A/H1N1 virus with genetic components from human, avian, as well as swine influenza viruses.[3,4] A subsequent worldwide human-to-human spread of this novel virus led the World Health Organization to declare an actual influenza pandemic on June 11, 2009.[5,6]

Anticipating a predicted extensive epidemic of the novel influenza A/H1N1 virus, on August 12, 2009, the Slotervaart Hospital opened a separate facility for diagnosis and treatment of patients with an influenza-like illness. This influenza outpatient clinic was established as a subunit of the Diagnostic Center for Infectious Diseases, which is an outpatient department of the Slotervaart Hospital from which febrile illness diagnostics are performed.

**Bridging the gap**

During the announced code red alert, the Dutch national government had advised on vaccination and in-hospital strategies, but not on outpatient procedures.[7] The influenza outpatient clinic bridged this gap by providing adequate diagnostics and treatment. However, The National Institute for Public Health and the Environment (RIVM) released a modified advice on August 15, 2009: laboratory confirmation of infection was no longer necessary. Because the rationale underlying this change was not clearly funded, we decided to continue our then used testing policy: every patient with influenza-like signs and symptoms was tested for the presence of the pandemic influenza virus and was for that purpose examined at the outpatient clinic during office hours.

**The influenza outpatient clinic**

The influenza outpatient clinic was organized as consultation hours from Monday through Friday from 9 am till 5 pm. Outside office hours, patients with suspected influenza infection were able to present themselves at the emergency ward. General preventive measures were taken according to the RIVM guidelines.[7]

The outpatient clinic was separated from the remainder of the hospital by using an isolated room with a separate entrance. Immediately after entry, the visitor was requested to wear a

respiratory device for the full duration of the visit. The patient was first seen by a nurse and subsequently by a medical doctor who performed a directed patient history and physical examination. When indicated, additional laboratory and imaging tests were performed.

At the end of the outpatient consultation, the medical doctor concluded whether there was an influenza-like illness (ILI) or not. The diagnosis of ILI was based on clinical criteria that were developed by the Dutch Organization of Medical Assistance for Accidents and Disasters (GHOR). Those criteria are: body temperature ≥38.5 °C, two or more influenza-like signs and symptoms in the preceding week (cough, rhinorrhea, sore throat, headache, myalgia, malaise, chills), and an acute onset of experienced complaints. From each patient a throat swab was collected for detection of the influenza virus. Within 24 hours influenza virus RNA was amplified and detected by means of real-time reverse-transcriptase-polymerase-chain-reaction-assay (RT-PCR).[8]

If the medical doctor ascertained an influenza-like illness based on the abovementioned criteria, he advised to stay at home until definitive test results were known. This was the next weekday. In case of a positive test result he subsequently advised to stay at home for a minimum of five days counting from the onset of complaints, and to return to our outpatient clinic if the complaints would aggravate; in case of a negative result the earlier advise was withdrawn. Treatment with oseltamivir (Tamiflu®; F. Hoffmann-La Roche Ltd, Basel, Switzerland) was initiated according to the RIVM recommendations.[9] The antiviral drug was only prescribed for 'high-risk' patients and/or patients with a complicated course. In case of a positive test result, thus a confirmation of the clinical suspicion, it was advised to finish the entire five-day course; in case of a negative test result the use of oseltamivir could be discontinued.

**Characteristics**

In the period from August 12, 2009 through December 31, 2009, a total of 1161 patients, both adults and children, visited the influenza outpatient clinic and took the influenza diagnostic test. H1N1 infection was demonstrated in 242 cases (21%). The time course of the number of H1N1-positive samples showed similarities to the Nivel Continuous Morbidity Registration (CMR) results.[10] Almost 40 percent of the patients signed in at the outpatient clinic after being referred by their general physician, the others on their own initiative or after referral by other healthcare providers.

Demographical characteristics were highly comparable to data from other European countries.[11-13] For example, infection with the novel influenza A/H1N1 virus affected

mainly the young. Our patient population consisted for almost one third of children and the percentage of H1N1-positive cases among children was noticeably higher than among adults (35% and 18% respectively). Clinical characteristics such as fever and most influenza-like signs and symptoms were significantly more frequently present among the group of patients with a confirmed influenza infection.

A recently published epidemiological study from China describes comparable clinical characteristics among 426 individuals with novel influenza A/H1N1 infection.[14]

**Unnecessary long**

Both the sensitivity and specificity of the used clinical criteria according to the GHOR triage system was 70 percent in our total population. Taking into account the entire period, it is an interesting finding that we noticed a positive predictive value of merely 40 percent for the use of these criteria. With these numbers, the restriction of the diagnostic procedure to only asking about influenza-like signs and symptoms seemed insufficient.

One other interesting finding concerns the use of oseltamivir. Because we performed RT-PCR assays for the detection of influenza in every single patient, the use of oseltamivir could be discontinued after one weekday in 48 of a total of 86 cases (56%). Without laboratory confirmation these patients would have been treated with the antiviral drug for an unnecessary long time, and, consequently, would have been subjected to an unnecessary increased risk of adverse events, which concerns especially children. In addition to oseltamivir, ambulant antibiotics were prescribed to 11 patients with and 25 patients without confirmed influenza infection.

**Rapid test**

In the beginning, the QuickVue® rapid test (Quidel Corporation, San Diego, USA) was applied to nose swab samples, collected and processed by trained nurses conform the manufacturer's instructions. The rapid test was used in the first 187 consecutive patients. Test results were negative for all 187 patients, including 16 patients in whom the RT-PCR was positive for novel influenza A/H1N1.

These findings had led to the decision to quit the use of the rapid test. To exclude problems with manipulation and storage of the test kit contents, as verification we applied the rapid test to nine highly positive H1N1 virus containing nose washes of children in whom a rapid test was not performed initially. The rapid test did show positive results in five of these nasal washes. The extremely low sensitivity that was established in our patient population was in

conflict with the package leaflet of the manufacturer claiming a high sensitivity. Based upon our observations we discourage the use of this rapid test in an outpatient setting during a next influenza epidemic.

**Hospitalizations**

In total, we have had 28 hospitalizations that were related to H1N1 infection, of which 22 concerned children and 6 adults. The hospitalizations largely took place in the month October. One adult patient had to be admitted to the intensive care unit because of respiratory insufficiency. In addition to these H1N1 related hospitalizations, another three adults and fifty children were hospitalized via the influenza outpatient clinic with other diagnoses, ranging from airway infection caused by a different viral pathogen to a space-occupying lesion suspected for lung carcinoma.

**Personnel**

From all 26 healthcare workers working at the influenza outpatient clinic weekly throat swab samples were collected. RT-PCR was H1N1-positive in only one of all tested samples. The affected nurse had developed influenza-like signs and symptoms and was not allowed to work at the hospital for one week; she recovered completely and without complications. The entire season no other infections through contamination occurred. We can therefore state that working at the influenza outpatient clinic was highly safe for the involved personnel.

On December 28, 2009, the RIVM concluded that the mildly ensued influenza epidemic in the Netherlands appeared to have come to an end.[10] The influenza outpatient clinic closed its doors on December 31, 2009. Owing to our outpatient clinic, H1N1 infection could be diagnosed rapidly. This meant clarity for the patient and adequate medical care, with the use of oseltamivir at the same time being limited to a minimum.

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# Chapter 2

## Epidemiology

# 2.1

# Adult outpatient experience of the 2009 H1N1 pandemic: clinical course, pathogens, and evaluation of case definitions

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## ABSTRACT

**Objectives:** The aim was to describe causative agents and clinical characteristics in adult outpatients with upper airway symptoms during the 2009 H1N1 pandemic and to evaluate case definitions that are used in clinical practice.

**Methods:** From August through December 2009, 964 symptomatic adult outpatients were included. RT-PCR was used to detect the following pathogens: influenza A (H1N1) and B, parainfluenza 1-4, adenovirus, respiratory syncytial virus, human rhinovirus, human metapneumovirus, human coronavirus (OC43, 229E, NL63), *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* species. The Dutch GHOR, American CDC and WHO, and British HPA case definitions were evaluated.

**Results:** A respiratory pathogen was detected in 41% of tested patient samples; influenza A (H1N1) and human rhinovirus were both detected in 16%. Clinical presentation of influenza cases was significantly more serious when compared to rhinovirus or negative-tested cases. Test characteristics were almost similar for all 4 case definitions, with an average sensitivity of 66%, specificity of 70%, positive predictive value of 34% and negative predictive value of 90%.

**Conclusions:** Influenza A (H1N1) and human rhinovirus were the major pathogens responsible for respiratory disease. The 2009 H1N1 pandemic in Amsterdam followed a mild course. Test characteristics of 4 different clinical case definitions seemed comparable but rather useless.

## INTRODUCTION

In April 2009, officials at the Centers for Disease Control and Prevention (CDC) confirmed two cases of swine influenza in children living in neighboring counties in California, after several cases had already been reported in Mexico.[1,2] This event led to the proclamation of a serious global health threat caused by a new influenza A (H1N1) virus.[3,4] Several surveillance studies have shown a moderate severity of the pandemic, with an overall relatively mild illness in those infected with the virus.[5-7] Nevertheless, the virus spread globally and almost all countries had reported cases, with more than 17,700 deaths among those that were laboratory confirmed.[8] The actual impact of the pandemic, however, is not really known because the number of laboratory-confirmed infected cases is undoubtedly a significant underestimation of the true number of infected cases.

Acute respiratory tract infections are the most common illnesses in all individuals over the world.[9] Whereas influenza has always been an important causative agent in this regard, rhinoviruses have generally been associated with the greatest number of illnesses.[10] However, influenza viruses produce more severe symptoms and, when there is a major influenza outbreak, they may be identified at a greater frequency when compared to other common causative viral agents.[11] Interactions between viruses causing respiratory infections are known to cause an interference between successive outbreaks in the community.[12-14] It has been postulated that, during the 2009 H1N1 pandemic, the interaction between novel influenza A (H1N1) virus and rhinoviruses has caused a delay in the circulation of respiratory syncytial viruses in France.[15]

Molecular methods, and in particular the development of polymerase-chain-reaction (PCR) technology, has proved invaluable in our understanding of the epidemiology of influenza and other respiratory viruses and has enabled rapid and sensitive diagnostic tests influencing patient management.[16] Clinicians have always been identifying patients with influenza-like illness mainly based on clinical findings, despite them not being particularly useful for confirming or excluding a true diagnosis of influenza.[17] Studies evaluating several clinical case definitions have demonstrated moderate sensitivity, poor specificity and extremely divergent predictive values, with positive predictive values ranging from 27% to 87% and negative predictive values ranging from 39% to 91%.[18,19] Of all signs and symptoms, a pooled analysis of eight double-blind, placebo-controlled studies showed both cough and fever to be most predictive of influenza infection in patients with influenza-like illness.[20]

The aim of this study is to describe clinical characteristics of adults with flu-like symptoms visiting an influenza outpatient clinic in Amsterdam during the 2009 H1N1 pandemic. Furthermore, we aim to provide an overview of the distribution and possible mutual interferences of different viral agents causing respiratory infections in our outpatient population. Another secondary objective is to evaluate the practical usefulness of several existing clinical case definitions attempting to predict influenza virus infection by comparing their predictive values.

## PATIENTS AND METHODS

### Study design and population

The data that have been collected were studied in order to provide an epidemiological overview of the causative viral respiratory pathogens and general characterization of the 2009 flu pandemic in a population of adults. All adults aged ≥18 years presenting with any flu-like signs and symptoms at the Slotervaart Hospital from August 12, 2009, until December 31, 2009, were included for our analysis. Patients could be referred by their general practitioner or other (para-) medical, but so-called 'self-referred' patients were also welcome to sign-up for a consultation, and those were included in our analysis as well. The Slotervaart Hospital is a general, 410-bed, teaching hospital that provides basic care for the Western region of Amsterdam, serving a low- to middle-income urban population of about 140,000 inhabitants; the population consists for 49% of ethnic minorities, most of them from Moroccan and Turkish origin. Ethical approval and informed consent were not required since this study solely describes findings resulting from regular patient care in our hospital.

### Laboratory confirmation of infection

Influenza virus RNA was amplified and detected by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR) performed on oropharyngeal aspirates.[16] A generic PCR (directed against the matrix gene) was used to detect influenza virus type A or B and an H1N1-specific PCR was applied to the H1 gene. Next to influenza, the presence of the following pathogens was also detected by RT-PCR: parainfluenza-1, parainfluenza-2, parainfluenza-3, parainfluenza-4, adenovirus, respiratory syncytial virus (RSV), human rhinovirus, human metapneumovirus, human coronavirus OC43, human coronavirus 229E, human coronavirus NL63, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* species.

**Clinical presentation and case definitions**

Upon presentation at our hospital, every patient with any flu-like signs or symptoms was submitted to a structured patient history and physical examination by one of our internal medicine residents. An oropharyngeal swab sample was collected by a trained nurse for diagnostic purposes. When indicated, additional laboratory and imaging tests were performed to exclude influenza-related complications or any suspected differential diagnoses.

Test results with regard to presence or absence of influenza virus RNA were mostly available within 24 hours. Adults with suspected influenza infection according to the case definition that was used in our hospital (GHOR – Dutch organization of Medical Assistance for Accidents and Disasters – website www.ghor.nl), were always given the strict advice to stay at home until the definitive influenza test results were known. If the test turned out positive, the advised period to stay at home was extended until at least five days after the onset of complaints; if the test turned out negative, the strict staying-at-home advice was undone.

Table 1 describes 4 influenza case definitions that are applied worldwide. The national GHOR case definition has been used in our clinical practice, aiding in our prediction of a clinical diagnosis and the initiation of proper medical management. The remaining case definitions have been used for analytical purposes in order to compare associated predictive values between different definitions.

**Table 1.** Clinical case definitions for influenza diagnosis.

| Abbreviation | Explanation/country of origin | Definition |
|---|---|---|
| *GHOR* | Medical Assistance for Accidents and Disasters<br>*The Netherlands* | fever ≥38.5 degrees Celsius AND two or more acute-onset 'flu' complaints: cough, rhinorrhea, sore throat, headache, myalgia, malaise, chills |
| *WHO* | World Health Organization<br>*international United Nations system* | fever >38 degrees Celsius, AND cough or sore throat<br>*(in the absence of other diagnoses)* |
| *CDC* | Centers for Disease Control and Prevention<br>*United States* | fever >100 degrees Fahrenheit (>37.8 degrees Celsius), AND cough or sore throat |
| *HPA* | Health Protection Agency<br>*United Kingdom* | fever ≥38 degrees Celsius AND two or more of following: cough, sore throat, rhinorrhea, limb or joint pain, headache, vomiting or diarrhoea; OR severe and/or life-threatening illness suggestive of an infectious process |

**Medical management**

Treatment with the antiviral drug oseltamivir was started in suspected influenza cases conform national guidelines (www.rivm.nl/en). Oseltamivir was prescribed only in high-risk patients (age ≥60 years, pregnancy in 3rd trimester or suffering from a specified chronic medical condition) and in patients with a complicated course. If the clinical suspicion was confirmed by a positive test result, patients were supposed to finish the five-day course with the antiviral; in case of a negative test result the use of oseltamivir was immediately discontinued. Antibiotics were prescribed at the discretion of the responsible physician. Hospitalization would follow in case of a complicated course and/or instability of the patient's medical condition.

**Statistical analysis**

Statistical analysis was performed using the SPSS software package (version 18.0, SPSS Inc. Chicago, Illinois). Continuous variables were summarized as means and for categorical variables percentages of adults in each group were calculated. Demographic and clinical characteristics were compared between groups using a Student's t-test or non-parametric test for continuous variables and Chi-square or Fisher's exact test for categorical variables, as appropriate. Stepwise logistic regression analysis was performed in order to determine which independent variables contributed significantly to the prediction of the outcome variable. In general, a p-value of less than 0.05 was considered statistically significant.

## RESULTS

**Study population**

From August 12, 2009, until December 31, 2009, a total of 964 adults visited the influenza outpatient clinic of our hospital. The overall mean age was 36 years and 43% were male. General characteristics as well as the presence of certain risk groups and way of referral are summarized for patients with and without any detected respiratory pathogens in Table 2.

**RT-PCR influenza and other pathogens**

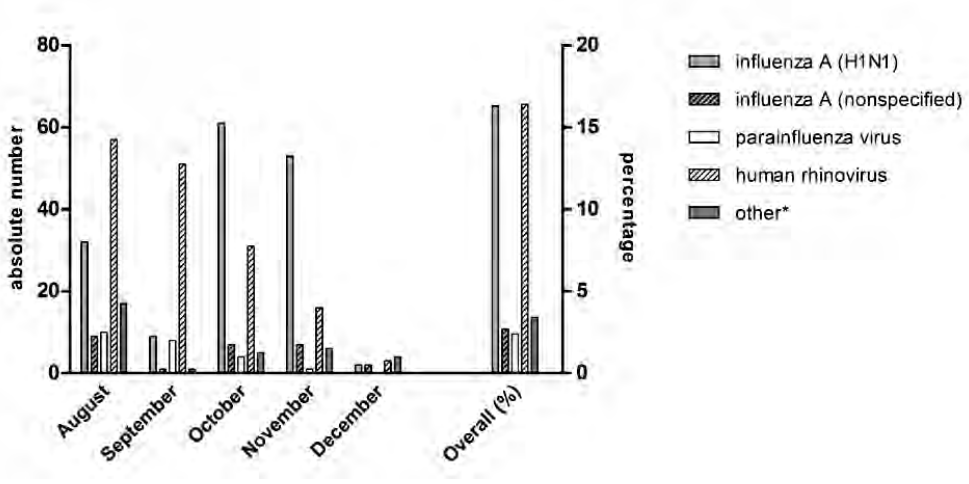
Figure 1 demonstrates detected respiratory pathogens in oropharyngeal samples collected from all 964 adult patients, given as absolute numbers per month and percentage overall distribution. Major responsible pathogens causative of respiratory disease were influenza A (H1N1) and human rhinovirus, both overall contributing in 16% of the patient samples that had been tested. Diagnosis of infection with influenza A (H1N1) virus peaked in October (corresponding prevalence 30%). Human rhinovirus infection showed its peak in August

and September (average corresponding prevalence 20%). Furthermore, in December RSV prevalence reached 7%, equalling the influenza A (H1N1) prevalence in that month. Of particular last note is that infections with influenza B and *Legionella* species were not seen at all.

**Table 2.** General characteristics of symptomatic adults visiting an influenza outpatient clinic during the 2009 H1N1 pandemic.

| | | any confirmed pathogen | no confirmed pathogen |
|---|---|---|---|
| **number of adults** | | 379 | 585 |
| **age (mean, range)** | | 34 (18-78) | 38 (18-88) |
| **sex** | **male** | 43% | 43% |
| | **female** | 57% | 57% |
| **risk groups** | **airway disorder** | 13% | 12% |
| | **cardiological disorder** | 3% | 5% |
| | **diabetes mellitus** | 4% | 3% |
| | **immunocompromised** | 3% | 3% |
| | **pregnancy 3rd trimester** | 3% | 2% |
| | **age ≥60 years** | 3% | 7% |
| **referral** | **general practitioner (GP)** | 35% | 28% |
| | **self-referral** | 54% | 55% |
| | **referral by (para-) medical other than GP** | 11% | 17% |

**Figure 1.** Detected respiratory pathogens in 964 symptomatic adult outpatients during the 2009 H1N1 pandemic.




**Other includes: adenovirus, respiratory syncytial virus, human metapneumovirus, human coronavirus, Mycoplasma pneumoniae and Chlamydia pneumoniae*

Two concurrent pathogens were demonstrated in 18 individuals of which 5 suffered from at least one comorbid condition. The following double infections were seen: influenza A (H1N1) with human rhinovirus (n=8); influenza A (H1N1) with parainfluenza virus (n=2); influenza A (H1N1) with adenovirus (n=2); influenza A (H1N1) with human metapneumovirus (n=1); influenza A (H1N1) with human coronavirus (n=1); parainfluenza virus with *Chlamydia pneumoniae* (n=1); RSV with human rhinovirus (n=1); human rhinovirus with human metapneumovirus (n=1); and human rhinovirus with human coronavirus (n=1).

**Clinical presentation and case definitions**

Clinical characteristics of patients with the two most frequently detected pathogens (i.e. influenza A (H1N1) and human rhinovirus) and of those without any detected pathogens are summarized in Table 3. Influenza A (H1N1)-positive patients, compared with patients in whom we did not detect any pathogen, reported more fever ≥38.5 °C (65% versus 32%; $p<0.001$), showed significantly worse vital parameters, and suffered more from most general flu complaints. In patients with RT-PCR confirmed human rhinovirus infection, less statistically significant differences, when compared again with patients in whom we did not detect any pathogen, were seen. When comparing influenza A (H1N1) cases with human rhinovirus cases, a history of fever ≥38.5 °C was reported more frequently by H1N1-positive cases (65% versus 34%; $p<0.001$) and those patients suffered more cough, less rhinorrhea, more myalgia and more subjective chills than those infected with rhinovirus.

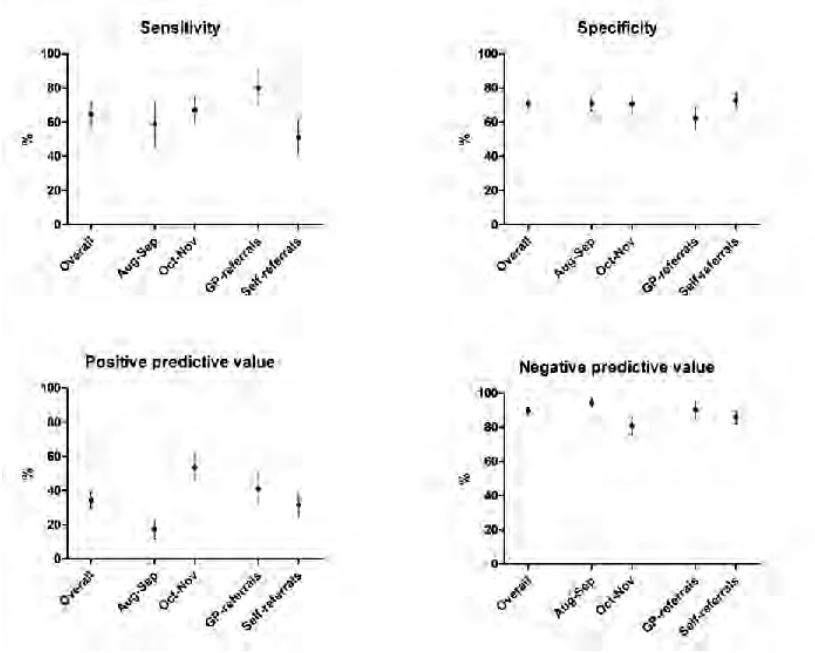
Table 1 explains the influenza case definitions that were used for this study. In general, no statistically significant and relevant differences between the different criteria sets were demonstrated for each individual performance characteristic. Figure 2 therefore shows the performance characteristics (i.e. sensitivity, specificity, positive predictive value and negative predictive value) of only the GHOR case definition, which was used in our outpatient practice. Overall performance characteristics are shown, as well as performance characteristics in August−September (period of relatively low incidence of pandemic flu) and October−November (period of relatively high incidence of pandemic flu), and also for patients with and without having been referred by their general practitioner (GP). Overall, sensitivity and specificity were 64% and 71%, respectively; the positive predictive value was 35% and the negative predictive value 89%.

**Table 3.** Clinical characteristics.

| | | Influenza A (H1N1) | Human Rhinovirus | | None |
|---|---|---|---|---|---|
| Number of adults | | 157 | 158 | | 585 |
| Age (mean, range) | | 32 (18-67)*** | 36 (18-78) | ## | 38 (18-88) |
| Sex | Male | 46% | 41% | | 43% |
| | Female | 54% | 59% | | 57% |
| History of fever ≥38.5 °C | | 65%*** | 34% | ### | 32% |
| Highest temperature by history | (Mean ± SD) | 39.0 ± 0.61* | 38.8 ± 0.59 | # | 38.8 ± 0.74 |
| Vital parameters (mean ± SD) | Temperature (tympanic) | 37.6 ± 0.97*** | 37.1 ± 0.61 | ### | 37.0 ± 0.61 |
| | Heart rate | 92 ± 17.1*** | 80 ± 14.5 | ### | 81 ± 14.7 |
| | Systolic blood pressure | 120 ± 17.1*** | 123 ± 17.3 | | 127 ± 19.0 |
| | Diastolic blood pressure | 73 ± 11.0*** | 76 ± 11.1** | # | 79 ± 11.8 |
| | Peripheral O2 saturation | 98 ± 1.3** | 99 ± 1.4 | ## | 99 ± 1.2 |
| General flu-complaints | Cough | 97%*** | 88%*** | ## | 72% |
| | Rhinorrhea | 70%*** | 82%*** | # | 52% |
| | Sore throat | 74% | 83%** | | 68% |
| | Headache | 83%* | 76% | | 74% |
| | Myalgia | 81%*** | 68% | ## | 66% |
| | Malaise | 90%** | 87%* | | 79% |
| | Subjective chills | 80%*** | 59% | ### | 58% |
| Complicated flu-complaints | Subjective dyspnea | 42% | 42% | | 39% |
| | Productive cough | 59%*** | 54%** | | 39% |
| | Chest pain | 34%*** | 23% | | 20% |
| | Otalgia | 27% | 26% | | 23% |
| Chest radiograph | Performed | 4% | 4% | | 3% |
| | Abnormal result | 0% | 0% | | 1% |
| Laboratory investigation | Performed | 4%* | 2% | | 1% |
| | Abnormal result | 2% | 1% | | 1% |
| Influenza-like illness (GHOR) | | 68%*** | 33% | ### | 27% |
| Oseltamivir prescription (initiated) | | 14%*** | 6% | ### | 6% |
| Antibiotics prescription | | 5% | 5% | | 4% |

**** p < 0.001, ** p < 0.01, * p < 0.05: Statistical significance level of difference between group of individuals with detected virus (influenza A (H1N1) or human rhinovirus) and group of individuals with none detected.*
*### p < 0.001, ## p < 0.01, # p < 0.05: Statistical significance level of difference between group of individuals with detected influenza A (H1N1) virus and human rhinovirus.*

**Figure 2.** Performance characteristics (including 95% confidence intervals) of GHOR influenza case definition.




Statistically significant differences in positive and negative predictive values were seen within all criteria sets when comparing the period August–September with the period October–November. With regard to the GHOR case definition, positive predictive value increased from 17% to 53% and negative predictive value decreased from 94% to 81%. Additional statistically significant differences were seen within criteria sets when comparing patients that had been referred by their GP with patients who visited the outpatient clinic without having been referred by their GP. For all criteria sets sensitivity was significantly higher for GP referrals than self-referrals (80% versus 51% in Figure 2). Specificity for the GHOR case definition showed a borderline significant lower value of 62% among GP referrals (compared with 73% among self-referrals); for the other case definitions the lower specificity that was demonstrated among GP referrals did reach true statistical significance ($p<0.05$).

Finally, besides fever and cough, stepwise logistic regression analysis did not result in the discovery of any additional statistically significant and clinically relevant predictor variables.

**Medical management**

Oseltamivir therapy was initiated according to national guidelines in 23 patients (13%) with confirmed influenza A infection (either H1N1 subtype or non-specified) and in 38 patients (5%) without confirmed influenza A infection. Treatment with oseltamivir was discontinued

(mostly within 24 hours) in all of the 38 influenza A negative patients. Antibiotics were prescribed on average in 4% of all patients visiting the outpatient clinic; no statistically significant difference in antibiotics prescription rate was demonstrated between groups based on RT-PCR result. Hospitalization for complicated infection was seen in 7 adult patients. Four patients had to be hospitalized because of a complicated course of infection with the pandemic H1N1 virus; for two of these patients, admission to the intensive care unit was required because of respiratory insufficiency and the need for mechanical ventilation. The other three adults were hospitalized for other diagnoses than viral respiratory infection: bronchial carcinoma, colonic peri-diverticulitis and community acquired bacterial pneumonia. No influenza-related deaths occurred during the entire study period.

## DISCUSSION

This observational study describes interesting epidemiological findings in a population of 964 symptomatic adults visiting an influenza outpatient clinic during the 2009 H1N1 pandemic from August 12 until December 31, 2009. Influenza A (H1N1) and human rhinovirus were the major pathogens responsible for respiratory disease among our patients, both having been detected in 16% of the tested throat swab samples. Overall, any respiratory viral pathogen was detected in 41% of tested patient samples. Infections with influenza B and *Legionella* species were not observed at all. Double infections were seen in 18 patients. These findings are reasonably comparable to a published report describing prevalence rates of respiratory viruses that were identified annually from 1967-1981 in Tecumseh, Michigan.[10,11] In contrast, due to the pandemic nature of the influenza A outbreak last year, we observed a much higher number of cases with a confirmed influenza A diagnosis than from previous influenza seasons that were observed in the Tecumseh studies. The incidence of confirmed H1N1-positive cases over the course of time, however, does correspond fairly well with numbers reported during the past 2009 H1N1 pandemic by sentinel stations for influenza surveillance in the Netherlands and Europe.[21,22]

During the course of the pandemic, different pathogens were dominating the etiologic picture of upper airway infection at different periods. In August and September, human rhinovirus infection peaked among symptomatic patients, with a corresponding 20% of throat swab samples having tested positive in those months. Novel swine-origin influenza A (H1N1) virus infection encountered its highest peak prevalence of 30% in the month October. RSV, a less frequently seen viral cause of airway disease in adults, did reach a 7% prevalence rate in December. The consecutive outbreaks of human rhinovirus, influenza A

(H1N1) and RSV followed its usual pattern. Unlike postulations from French investigators, the interaction between the pandemic flu and rhinoviruses, did not cause a delay in the circulation of respiratory syncytial viruses in our population.[15]

Clinical features of the 157 cases of 2009 pandemic influenza A (H1N1) were different from cases that had not been diagnosed with any respiratory viral infection. A history of fever was reported twice as much by the H1N1-positive cases. Most flu-signs and -symptoms were reported significantly more often and vital parameters were slightly, but significantly worse than in the cases in which no respiratory virus could be demonstrated. When comparing the H1N1-positive cases with patients with human rhinovirus infection, less statistically significant differences were seen, but on average the 'flu' patient could be regarded as being sicker than the 'common cold' patient. Our study is the first to compare clinical features of patients in an outpatient setting in relation to different pathogens that have been detected during the 2009 H1N1 pandemic. A Chinese observational study described clinical features of 426 persons infected with the 2009 pandemic influenza A (H1N1) virus, using thermal scanners installed at airports and ports of entry to China to include travellers and subsequently their close contacts.[6] Furthermore, a report from the United States summarized clinical findings from hospitalized patients only with 2009 H1N1 influenza infection.[23]

In the Netherlands, with a population size of approximately 16.5 million people, the pandemic followed a relatively mild course. Until the end of December 2009, a total of 2156 hospitalizations of cases with laboratory confirmed influenza A (H1N1) were reported of which 10% required intensive care, and in total 53 patients had died. In our population, the low number of H1N1-related hospitalizations, and the fact that only two adults had to be admitted to the ICU because of respiratory insufficiency, all confirmed the mild course of the pandemic. An important finding from our study is that treatment with oseltamivir could be discontinued in 38 of 61 patients after the test results had become available and turned negative for influenza virus. These adults, had they not been tested by a throat swab and RT-PCR, would have been treated for an unnecessarily long time with the antiviral drug and, although clinically probably not very serious, subjected to an unnecessary risk of adverse effects.[24]

Four different case definitions for influenza-like illness were evaluated. The corresponding criteria sets were derived from the World Health Organization (WHO), the United States Centers for Disease Control and Prevention (CDC), the United Kingdom Health Protecting Agency (HPA), and the Dutch organization of Medical Assistance for Accidents and Disasters (GHOR). Overall, performance characteristics were rather poor and similar for the different

criteria sets. Positive predictive value increased and negative predictive value decreased when comparing the lower-prevalence period August–September with the higher-prevalence period October–November. This finding is not surprising since the predictive value is determined by the prevalence of disease in the population being tested.[25] Further statistically significant differences were seen within criteria sets when comparing patients that had been referred by their GP with patients that visited the outpatient clinic without having been referred. For all criteria sets, sensitivity was significantly higher for GP referrals than self-referrals, and specificity was significantly lower. This could be explained by some kind of selection bias, i.e. general practitioners referring those patients who were more ill and therefore might meet case definition criteria more easily.

In the daily practice of our influenza outpatient clinic, we needed a case definition with a high sensitivity, since missing influenza infection might have had important consequences with regard to uncontrolled spread of the virus and the risk for a complicated course of the infection if treatment would not have been initiated. Unfortunately, neither the GHOR case definition that has been used by us, nor the other case definitions that were studied, have been very helpful in that regard. Even though a maximum positive predictive value of 53%, which was seen in October and November at the peak of the epidemic, is still rather useless when wishing to confirm the diagnosis being sought, a maximum negative predictive value of 90% (among patients that had been referred by their GP) might be of quite some value to our practicing clinicians, who also want to be confident that a negative case definition rules out infection with influenza virus. In a report evaluating clinical case definitions in France during the 1995-1996 influenza epidemic, 12 case definitions were associated with positive predictive values of 27%–40% and negative predictive values of 80%–91%.[19] These findings are comparable to findings from our population.

Limitations of this observational study should be mentioned. First, a selection bias of so-called ‘worried well’, mostly self-referred, patients is very likely to have influenced our results. National surveillances, however, have demonstrated comparable low rates of hospitalization and ICU admission. Another limitation is that we restricted the RT-PCR analyses to 8 viral and 3 ‘bacterial’ pathogens. Although we have been detecting the most common pathogens during a normal influenza season, we might have missed some notable causative bacterial organisms like *Streptococcus pneumoniae* and *Haemophilus influenzae*. Sputum and blood samples have been collected from few adults for bacterial cultures in our microbiology laboratory; none did result in the detection of any bacterial pathogens.

In conclusion, from August through December 2009, influenza A (H1N1) and human rhinovirus were the major pathogens responsible for respiratory disease in a population of 964 symptomatic adult outpatients. The clinical presentation of influenza cases was significantly more serious when compared to rhinovirus cases or cases that tested negative for any respiratory pathogen. Overall, viewed from an outpatient setting, we can conclude that the 2009 H1N1 pandemic in Amsterdam followed a mild course. Test characteristics of 4 different clinical case definitions seemed comparable but rather useless, with the exception of a relatively high negative predictive value that might be of value in clinical practice when ruling out a diagnosis of influenza infection is of importance to the practicing clinician.

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# 2.2

# Characterization of 2009 H1N1 pandemic influenza in a population of Dutch children with influenza-like signs and symptoms

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## ABSTRACT

**Aim:** To determine causative respiratory pathogens and describe epidemiological and clinical characteristics in a pediatric population with influenza-like illness during the 2009 H1N1 pandemic.

**Methods:** Observational study of 412 children visiting an outpatient clinic of a Dutch teaching hospital.

**Results:** From August to December 2009, 412 children were tested at the clinic; 32% proved H1N1-positive, confirmed by reverse-transcriptase-polymerase-chain-reaction (RT-PCR). Pathogens were detected in 65% of samples. Influenza A (H1N1) (n=132), human rhinovirus (n=55), respiratory syncytial virus (n=45) and adenovirus (n=34) were mostly identified. Co-infections were seen in 34 children (8.3%). Mean age was 6.8 and 4.2 years in H1N1-positive and -negative cases, respectively ($p<0.01$). H1N1-positive outpatient children reported fever, cough and rhinorrhea more frequently than their H1N1-negative counterparts. Of 72 hospitalized children 31% proved H1N1-positive; all showed a relatively mild clinical illness. None of the children had been admitted to an intensive care unit or died. Oseltamivir treatment was initiated in 72 children and discontinued in 42 (63%) when RT-PCR results turned negative.

**Conclusion:** The 2009 H1N1 pandemic showed a mild clinical course in a Dutch pediatric outpatient clinic population. Respiratory pathogens were detected in the majority of children with influenza-like illness and influenza A (H1N1) virus was identified in one third. Testing symptomatic children during an influenza pandemic has effectively limited the use of oseltamivir.

## INTRODUCTION

In April 2009, officials at the Centers for Disease Control and Prevention (CDC) confirmed two cases of swine influenza infection in children living in neighboring counties in California, after several cases had already been reported in Mexico.[1,2] These observations announced the emergence of a serious global health threat caused by a new influenza A (H1N1) virus.[3] The first case in the Netherlands was detected in a three-year old child, approximately two weeks after the first cases had been detected in the United States.[4] By the time that the World Health Organization (WHO) declared an actual influenza pandemic in June 2009, epidemiological observations had led to the conclusion that swine-origin influenza virus infection was associated with less favorable outcomes in children.[5,6] Of 345 children hospitalized with 2009 influenza A (H1N1) in California two thirds had comorbidities, most commonly chronic pulmonary disease, underlying neurologic disorders, and immunosuppression.[7]

To our knowledge, published articles have mainly focused on the inpatient clinical experience of the H1N1 pandemic in children, describing merely hospitalized cases, and lacking a detailed description of the respiratory viruses involved other than the pandemic influenza A (H1N1) virus. This observational study provides a description of both the inpatient and outpatient clinical experience of the 2009 influenza pandemic. Our aim was to characterize a population of pediatric in- and outpatients with influenza-like signs and symptoms, to determine how many children had influenza A (H1N1) infection or other specific respiratory viral infections, and to observe dual infections in this population.

## PATIENTS AND METHODS

### Study design and population

The design of the study was observational; data were prospectively collected. On August 12, 2009, the Slotervaart Hospital, a 410-bed teaching hospital in Amsterdam, the Netherlands, opened the doors of a special influenza outpatient clinic. The hospital serves a low- to middle- income urban population of approximately 140,000 inhabitants, of which 18% are children and 49% are ethnic minorities, mostly of Moroccan and Turkish origin. The influenza outpatient clinic operated as a separate facility for diagnosis and management of patients with suspicion of influenza virus infection. Children with any of the following influenza-like signs and symptoms were accepted to the clinic: fever ≥38 °C, cough, rhinorrhea, sore throat, headache, myalgia, malaise, chills, vomiting and/or diarrhea. Patients were referred either

directly by the responsible family physician or other healthcare provider, or by the children's parents/caregivers. From August 12, 2009, to December 31, 2009, all children aged 0-17 years presenting at the Slotervaart Hospital, with at least one of the abovementioned signs or symptoms, were included in this observational study. Ethical approval and informed consent were not required according to Dutch law, since this study solely describes findings resulting form regular patient care provided at our hospital.

**Provided care delivered at the influenza outpatient clinic**

Every patient with any of the before mentioned influenza-like signs or symptoms was welcome to sign up for a consultation at the influenza outpatient clinic, even in the absence of a physician referral note (i.e. 'self-referred'). Children presenting outside of office hours and on weekends were cared for at the Emergency Department (ED) of our hospital. A complete medical history was obtained using a unified case report form and a physical examination was performed, followed by either an oropharyngeal swab or a nasal wash, depending on the child's age and ability to cooperate. Test results were usually available within 24 hours. Additional laboratory and imaging tests were performed at the discretion of the supervising pediatrician.

Appropriate medical management was determined based on clinical findings and local and (inter)national protocols. Treatment with the antiviral drug oseltamivir was initiated in suspected influenza cases in accordance with national guidelines at that time (www.rivm.nl/en): oseltamivir was prescribed only in high-risk patients, i.e. children less than two years of age or suffering from a specified chronic medical condition, and in patients with a complicated course of infection. If the clinical suspicion of H1N1 infection was confirmed by a positive test result, patients were advised to finish the five-day treatment with the antiviral drug; in the case of a negative test result, the use of oseltamivir was discontinued.

**Laboratory confirmation of infection**

Influenza virus ribonucleic acid was amplified and detected by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR), performed on oropharyngeal swabs or nasal washes; this method has been found to be 95% sensitive and 98% specific.[8] A generic PCR (directed against the matrix gene) was used to detect influenza virus type A or B and an H1N1-specific PCR was applied to the H1 gene.[9] In addition, we also used RT-PCR to detect the following pathogens: parainfluenza-1, parainfluenza-2, parainfluenza-3, parainfluenza-4, adenovirus, respiratory syncytial virus (RSV), human rhinovirus, human metapneumovirus, human coronavirus OC43, human coronavirus 229E, human coronavirus NL63, *Chlamydia pneumoniae, Mycoplasma pneumoniae* and *Legionella* species. The selected pathogens

were detected by using a multiplex PCR that combined the most prevalent pathogens in children as well as adults.

**Statistical analysis**

Statistical analysis was performed using the SPSS software package (version 17.0, SPSS Inc. Chicago, Illinois). Continuous variables were summarized as means (and standard deviation), and for each categorical variable, the percentage of children in each group was calculated. Demographic and clinical characteristics were compared between groups using a Student's t-test or non-parametric test for continuous variables, and Chi-square or Fisher's exact test for categorical variables, as appropriate. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

**Study population and characteristics**

From August to December 2009, a total of 423 children with one ore more influenza-like signs or symptoms presented to our hospital and valid RT-PCR test results were ultimately obtained for 412 of those. Three hundred and twenty cases were managed through our influenza outpatient clinic (78%) and 92 presented through the ED (22%). Five children had an additional visit which was more than two weeks apart from the first, and therefore regarded as independent from the initial visit and included in the analysis. There were no differences in demographic characteristics and clinical presentation between patients presenting to the outpatient clinic as compared to the ED.

H1N1 virus was detected in 132 of 412 outpatients (32%). Table 1 outlines general and clinical characteristics of both H1N1-positive and -negative children who visited the outpatient clinic. Mean age of H1N1-positive children was significantly higher (6.8 and 4.2 years for H1N1-positives and -negatives, respectively). In total 137 children (33%) were less than 2 years of age and 14 were infants younger than 3 months (3%). H1N1-positive outpatient children did report fever, cough and rhinorrhea significantly more frequently than their H1N1-negative counterparts. There was no significant difference with regard to these clinical symptoms for H1N1-positive children who were above or below 2 years of age (data not shown). The most frequently mentioned comorbid condition was asthmatic bronchitis, which was reported in 27% and 18% of H1N1-positives and -negatives, respectively.

**Table 1.** Characteristics of children visiting the outpatient influenza clinic.

| | | RT-PCR ***positive*** for H1N1 virus infection ***n=132*** | RT-PCR ***negative*** for H1N1 virus infection ***n=280*** |
|---|---|---|---|
| **Sex *(n, %)*** | **male** | 70 (53%) | 137 (49%) |
| | **female** | 62 (47%) | 143 (51%) |
| **Age *(mean, SD)*** | | 6.8 (4.7) | 4.2 (4.1)* |
| **History of fever *(n, %)*** | | 115 (87%) | 202 (72%)* |
| **Cough *(n, %)*** | | 123 (93%) | 230 (82%)* |
| **Rhinorrhea *(n, %)*** | | 102 (77%) | 179 (64%)* |
| **Duration of symptoms at presentation *(days; mean, SD)*** | | 3.0 (1.7) | 3.2 (2.1) |
| **Diagnosis of otitis media *(n, %)*** | | 7 (5%) | 17 (6%) |
| **Underlying comorbidity *(n, %)*** | **asthmatic bronchitis** | 36 (27%) | 49 (18%) |
| | **neurological disorder** | 5 (4%) | 4 (1%) |
| | **cardiac dysfunction** | 2 (2%) | 4 (1%) |
| | **diabetes mellitus** | 2 (2%) | 3 (1%) |
| | **chronic renal condition** | 1 (1%) | 2 (1%) |
| | **laryngotracheomalacia** | 2 (2%) | 2 (1%) |
| | **other**[1] | 0 (0%) | 4 (1%) |

[1] *Other comorbidity includes observations for: rheumatoid arthritis (n=1), brain tumor (n=1), 22q11 deletion (n=1) and HIV infection (n=1).*

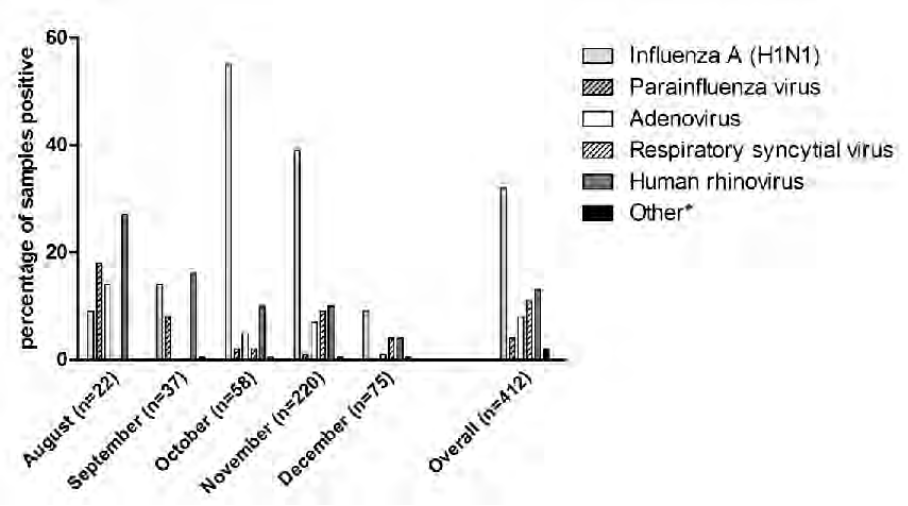
* *p<0.05 for difference between groups.*

**RT-PCR influenza and other pathogens**

Pathogens were detected in 65% of 412 patient samples. The sampling method used was an oropharyngeal swab in 343 children (83%) and a nasal wash in 69 (17%); mean age of children in these groups was 4.4 and 1.4 years, respectively. Figure 1 points out the relative distribution of all detected pathogens over the entire study period and also per month. Influenza A (H1N1) showed a maximum prevalence in October, rhinovirus in August, and RSV in November. Influenza B*,* human coronavirus*, Mycoplasma pneumoniae* and *Legionella* species were not detected at all.

In 14 H1N1-confirmed cases the following pathogens were concurrently detected: adenovirus in 6, rhinovirus in 6, parainfluenza in 1, and *Chlamydia pneumoniae* in 1. The following co-infections were seen in 20 H1N1-negative children: rhinovirus-RSV in 6, rhinovirus-adenovirus in 5, rhinovirus-parainfluenza in 3, RSV-adenovirus in 3, rhinovirus-metapneumovirus in 1, RSV-*Chlamydia pneumoniae* in 1, and rhinovirus-RSV-parainfluenza in 1.

**Figure 1.** Detected respiratory pathogens in a symptomatic pediatric outpatient population (n=412) during the 2009 H1N1 pandemic.




**Other includes human metapneumovirus and Chlamydia pneumoniae*

**Clinical management**

*Hospitalizations*

A total of 72 children with influenza-like symptoms were hospitalized, all had been presented before to either the outpatient clinic or ED. None had to be transferred to another hospital for intensive care support. Relevant characteristics of clinically admitted patients with and without H1N1 infection are described in Table 2. Mean age of hospitalized H1N1-positive children was significantly higher (5.5 versus 1.9). More antibiotics were initially prescribed and administered in H1N1-positive children than in H1N1-negatives (42% and 18% respectively). Fourteen blood samples that had been collected for bacterial cultures did not result in the detection of any bacterial pathogens.

*Imaging*

In 57 (13 H1N1-positive and 44 H1N1-negative) of 412 children (14%) with a suspected lower airway infection, a chest radiograph was performed to exclude pulmonary abnormalities. The resulting radiographs were evaluated by the hospital's attending radiologists. Forty-three out of 57 radiographs identified abnormalities, of which enhanced peribronchial cuffing was observed in 27 (47%). Pulmonary infiltrates were seen in 16 children (10 inpatients and 6 outpatients), all without H1N1-infection.

**Table 2.** Characteristics of hospitalized pediatric patients.

| | | RT-PCR *positive* for H1N1 virus infection *n=22* | RT-PCR *negative* for H1N1 virus infection *n=50* |
|---|---|---|---|
| **Age (*years; mean, SD*)** | | 5.5 (5.2) | 1.9 (2.0)* |
| **Duration of admission (*days; mean, SD*)** | | 3 (2.4) | 4 (2.7) |
| **Presence of any comorbid condition *(n, %)*** | | 11 (50%) | 22 (44%) |
| **Temperature >38 degrees Celsius *(n, %)*** | | 17 (77%) | 40 (80%) |
| **In need of oxygen suppletion *(n, %)*** | | 3 (14%) | 18 (36%)# |
| **Clinically dehydrated *(n, %)*** | | 10 (45%) | 21 (42%) |
| **Chest X-ray *(n, %)*** | **number performed** | 5 (23%) | 26 (52%)* |
| | **pulmonary infiltrate** | 0 (0%) | 10 (20%) |
| | **peribronchial cuffing** | 3 (14%) | 12 (24%) |
| **Antibiotics treatment *(n, %)*** | | 4 (18%) | 21 (42%)# |
| **Oseltamivir treatment *(n, %)*** | **initiated** | 20 (91%) | 17 (34%)* |
| | **continued** | 10 (45%) | 0 (0%)* |

* *p<0.05 for difference between groups.*

\# *p=0.05 for difference between groups.*

*Oseltamivir treatment*

Oseltamivir treatment was initiated in 72, both in- and outpatient, children (17.5%), who were eligible for antiviral therapy according to national guidelines (data not shown for outpatient children; see Table 2 for inpatient children). No statistically significant difference in overall oseltamivir initiation rate was demonstrated with regard to H1N1 status. In accordance with the same guidelines, 4 infants less than three months of age were hospitalized because of the requirement to clinically monitor the administration of the antiviral drug. Only one of those infants tested H1N1-positive and in the remaining 3 infants oseltamivir administration was discontinued the next day. Overall, antiviral therapy was discontinued early in 42 out of 72 cases (63%) because RT-PCR test results had turned negative the next day.

## DISCUSSION

This observational study describes the responsible respiratory pathogens and clinical characteristics of 412 Dutch children with influenza-like signs and symptoms who visited an influenza outpatient facility during the 2009 H1N1 pandemic. An extensive range of different pathogens had been detected in 65% of the tested samples. Infection with pandemic influenza A (H1N1) virus was diagnosed in 132 pediatric outpatients (32%). The majority of infected children showed a mild clinical picture.

H1N1 infected children reported fever, cough and rhinorrhea more frequently than those who were uninfected. Despite this finding, we have continued to advocate the use of specific virologic diagnostics instead of clinical differentiation. In a recently published study that was performed among adults with symptoms of respiratory infection, it was concluded that clinical differentiation between patients with and without influenza infection based solely on influenza-like signs and symptoms is rather ineffective.[10] It is very plausible that this also holds true for a pediatric population. Furthermore, our results have shown that H1N1-positive children, both in- and outpatients, were significantly older than their H1N1-negative counterparts. The lower age of H1N1-negative children is probably best explained by a selection bias of relatively healthy infants and young children, brought to the hospital by parents who were concerned by the pandemic. Finally, from our results it seems that certain pre-existing comorbid conditions in children, particularly asthmatic bronchitis, had not been a risk factor for a more complicated course of the infection, unlike findings from other observational studies.[6,11,12]

The H1N1-positivity rate started to increase from October 2009. In the course of that month the prevalence rate exceeded 50%. The Health Council of the Netherlands had advised the vaccination of children from 'traditional' medical risk groups in that same month.[13] In the course of the next month, the relative H1N1-positivity rate declined; however, the absolute number of visiting patients increased dramatically. In November 2009 the Health Council advice was changed, and it was recommended that all children from the ages of 6 months to 4 years be vaccinated, regardless of risk group. A mass vaccination program was therefore organized in November and December, after which a definite decline in the number of visiting patients was seen. In Amsterdam and surrounding area, this had resulted in a total vaccination coverage rate in children of approximately 40%.[14] Based on this coverage rate, and taking into account that mass vaccination was started after the peak of the epidemic, it is estimated that maximally 10-20% of our outpatient children had received the vaccine.

In our population of symptomatic children we detected influenza A (H1N1), human rhinovirus, RSV and adenovirus most commonly, in order of descending frequency. In a community study of considerable size, Monto et al. observed rhinovirus as the most frequently identified viral isolate in children, with parainfluenza, RSV and influenza A being the next most common pathogens, in order of descending frequency.[15-17] The differences can for most part be explained by the fact that our study period, and not Monto's, was encompassed by a true influenza pandemic. Furthermore, advanced diagnostic techniques that are used currently, such as RT-PCR, were not available during that time period. Our population did not show any influenza A (H1N1)-RSV co-infections, which is opposite to

findings from Poehling et al.[18] We also found that the consecutive outbreaks of rhinovirus, influenza and RSV one after the other followed its usual pattern.[19,20] This is in contrast to findings from a French influenza research team suggesting a delayed circulation of RSV.[21]

Clinical course in 72 hospitalized children was relatively uncomplicated. There were no influenza-related deaths or transferrals to other hospitals because of pediatric intensive care unit (PICU) requirement. In the Netherlands, with over 16 million inhabitants, 56 H1N1-related PICU admissions were seen (approximately 25% of all H1N1-related intensive care admissions) and 15 children had died from influenza infection.[22] For comparison, the United States total for the entire pandemic period was 344 influenza-associated pediatric deaths,[23] yet the clinical course in the majority of cases of 2009 pandemic influenza A (H1N1) in children had been mild.[24] We had found that peribronchial cuffing was the most commonly described radiological abnormality, and serious imaging abnormalities were not seen, consistent with findings from a previous study.[25] Oseltamivir treatment was initiated in 72 children of whom 42 discontinued the drug because RT-PCR results were negative for influenza virus the next day. In a randomized controlled trial, Heinonen et al. proved that early oseltamivir treatment in children with influenza A infection decreases the incidence of acute otitis media and time to resolution of illness.[26] Without laboratory confirmation, however, some children would have been treated for an unnecessarily long time and, as a consequence, would have been exposed to an unnecessary high risk of adverse effects.[27]

A few limitations of this study should be mentioned. First, our hospital lacks a PICU facility. Severe cases of complicated influenza infection might therefore directly have been referred to one of Amsterdam's 2 PICU-containing university hospitals. A second limitation is that we restricted the RT-PCR analyses to 8 viral and 3 'bacterial' pathogens, and we did not routinely test for notable bacterial organisms, such as *Group A Streptococcus*. Finally, two different sampling methods had been used. Oropharyngeal swabs were performed in the majority of our children; in a minority nasal washes were performed. Comparative studies have shown that the sensitivity for the identification of respiratory viruses is lower for oropharyngeal swabs.[28-30] There is therefore a possibility that our results are an underestimation of the real situation, particularly in older children in whom oropharyngeal swabs were preferably collected.

In conclusion, this is the first observational study that focuses on the pediatric outpatient clinical experience and on the circulation of other respiratory pathogens in addition to the influenza A virus during the 2009 H1N1 pandemic. Causative respiratory pathogens were detected in a majority of children visiting our clinic. In one third of the outpatients we

identified pandemic influenza A (H1N1). Of all children, the vast majority showed a mild clinical illness. The use of oseltamivir was discontinued in high-risk children who ultimately proved H1N1-negative, which is considered a valuable advantage of testing symptomatic outpatient children.

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# 2.3

# RT-PCR detection of respiratory pathogens in newborn children admitted to a neonatal medium care unit

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## ABSTRACT

**Objective:** Primary objective was to determine the prevalence of respiratory pathogens among a population of neonates admitted to a neonatal medium care unit (NMCU). Secondary objective was to identify clinical predictors for the presence of those pathogens.

**Patients and methods:** During a 1-year period, a prospective observational study was performed of neonates admitted to the NMCU of a teaching hospital in Amsterdam, the Netherlands. Nasopharyngeal samples were collected from all neonates for detection of a range of respiratory pathogens by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR). Univariate as well as logistic regression analysis was performed to identify predictors for the presence of respiratory pathogens.

**Results:** From October 2010 through September 2011, a total of 334 neonates (median age 1.3 days, 53.6% male) were included. Undifferentiated perinatal infection was diagnosed in 79 newborns (23.7%) and antibiotics were given to 108 (32.3%). Overall, 37 respiratory pathogens were detected in 34 children (10.2%). The following pathogens were found: parainfluenza-1 (n=9), human rhinovirus (n=7), parainfluenza-3 (n=6), respiratory syncytial virus (n=6), *Streptococcus pneumoniae* (n=3), adenovirus (n=2), human coronavirus (n=2), influenza A (n=1) and bocavirus (n=1). After adjustment for confounding, two variables significantly contributed to the risk of detection of respiratory pathogens: age (OR 1.21 for each day older; 95% 1.12-1.30) and rhinorrhea (OR 6.71; 95% CI 1.54-29.21).

**Conclusions:** Respiratory pathogens were present in one of ten neonates admitted to an NMCU. Increasing age and symptoms of rhinorrhea were significantly associated with an increased risk of detection of any respiratory pathogen.

## INTRODUCTION

Infections in neonates (newborn children aged until 28 days post partum) have been known to cause significant mortality and long-term morbidity.[1] One of the most important infectious diseases syndromes in newborns is neonatal sepsis, with an estimated incidence of 1.5% during the first 72 hours of life.[2] Most infants with sepsis present with non-specific signs and symptoms. The most common of these vague signs are temperature instability, lethargy, apnea, and poor feeding.[3,4] Neonates with viral respiratory tract infection can present with a clinical picture that is consistent with sepsis, in addition to more classical symptoms such as tachypnea and hypoxemia.[5]

The outcome of neonatal infections may be improved if illness is recognized early and appropriate antimicrobial agents are administered promptly.[6] If sepsis cannot be reasonably excluded on clinical grounds, blood cultures should be obtained and empiric antibiotics administered. Unfortunately, laboratory investigations are not always helpful, and cultures of blood or other tissues are often negative or not possible to perform. The early detection of a viral respiratory tract infection could be useful since it might reduce the use of antibiotics.[7]

Common viruses causing respiratory illness in newborns are: respiratory syncytial virus (RSV), influenza virus, adenovirus, rhinovirus and parainfluenza viruses.[2,3] Less common pathogens, including the more recently identified bocavirus, have also been detected in infants with acute respiratory infection.[7,8] However, the prevalence of infection with respiratory pathogens in neonates, and therefore the extent of the clinical problem in this fragile pediatric population, remains largely unknown. Therefore we aimed to determine the prevalence of respiratory pathogens among a population of neonates admitted to a neonatal medium care unit (NMCU) during a 1-year period. Secondary objectives were to compare the clinical course of neonates with and without respiratory pathogens and to identify risk factors and predictors for the presence of those pathogens.

## METHODS

### Study design and population

The design of the study was observational; data were prospectively collected from October 1, 2010 until September 30, 2011. During the study period, all neonates (i.e. both early and late newborn children aged until 28 days post partum) that were admitted to the neonatal medium care unit (NMCU) of the Slotervaart Hospital were consecutively included. The

Slotervaart Hospital is a 410-bed teaching hospital in Amsterdam, the Netherlands, which serves a low- to middle- income urban population of about 140,000 inhabitants that consists for 18% of children and for 49% of ethnic minorities, most of them from Moroccan and Turkish origin. The study has been approved by the institutional medical ethics committee.

**Data collection**

Epidemiological and clinical data were collected by using an extensive standardized form. Electronic patient files were used to provide the required information. Data included demographic and general characteristics, pregnancy and delivery characteristics and complications, presenting symptoms, physical examination signs, diagnoses, and treatment. Additional laboratory and radiology investigations, including microbiology blood cultures, were performed at the full discretion of the responsible pediatrician, and the results of those investigations, if applicable, were also included in the database.

**Respiratory pathogens**

From all neonates a diagnostic nasopharyngeal aspirate was collected as soon as possible after NMCU admission. Total nucleic acids were extracted from the clinical samples using the automated MagnaPureLC Isolation platform (Roche Applied Science, Penzberg, Germany). One step real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) was performed on extracted samples for detection of respiratory pathogens. Multiplex RT-PCRs were performed for parainfluenza 1-4, human coronavirus OC43/229E/NL63, influenza A and B virus, adenovirus, human rhinovirus, *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*. Monoplex RT-PCRs were performed for respiratory syncytial virus (RSV), human metapneumovirus, *Legionella* species, bocavirus and *Streptococcus pneumoniae*. PhHV and EAV virus were spiked to the clinical samples and used as internal controls to monitor nucleic acid isolation and inhibition of the RT-PCR.[9] All pediatricians and pediatric residents that were involved with delivered patient care were blinded to the RT-PCR results.

**Statistical analysis**

Statistical analysis was performed using the SPSS software package (version 18.0, SPSS Inc. Chicago, Illinois). Continuous variables were summarized as means or medians and for categorical variables percentages were calculated. To determine which independent variables significantly contributed to the prediction of the outcome of RT-PCR positive for respiratory pathogens, a univariate analysis was performed: demographic and clinical characteristics were compared by using a Student's t-test or non-parametric test for continuous variables and Chi-square or Fisher's exact test for categorical variables, as appropriate. If significant contribution was found for any of the independent factors in the univariate analysis, they

were added as variables to a logistic regression model. Crude odds ratios were adjusted for age and sex. In general, a p-value of less than 0.05 was considered statistically significant.

## RESULTS

### Patient population and characteristics

From October 1, 2010, until September 30, 2011, a total of 334 neonates were admitted to the NMCU of our hospital and screened by nasopharyngeal aspirate for the presence of respiratory pathogens. Median age at hospitalization was 1.3 days, 53.6% were male and the median duration of admission was 4 days. Eighty eight children (26.3%) were delivered preterm (i.e. <37 weeks) and 99 (29.6%) were born after prolonged rupture of membranes (i.e. >24 hours). Undifferentiated perinatal infection was diagnosed in 79 newborns (23.7%), whereas a diagnosis of perinatal sepsis was established in 5 (1.5%); antibiotics were given to 108 (32.3%). These and other relevant characteristics are summarized in Table 1. Of notice, 8 of 138 performed blood cultures (5.8%) were positive for bacterial pathogens: *Staphylococcus aureus* (n=2), *Staphylococcus epidermidis* (n=2), *Micrococcus lylae* (n=1), *Escherichia coli* (n=1), *Streptococcus sanguis* (n=1) and *Staphylococcus capitis* (n=1).

**Table 1.** Characteristics of neonates (n=334) admitted to a medium care unit (October 2010 - September 2011).

| | |
|---|---|
| **Demographic and general characteristics:** | |
| Male sex, n (%) | 179 (53.6) |
| Age, days, mean (range) | 1.3 (0-28) |
| Ethnicity | |
| Caucasian, n (%) | 166 (49.7) |
| Mediterranean, n (%) | 120 (35.9) |
| Other, n (%) | 47 (14.4) |
| Admission 0-72 hrs post partum, n (%) | 309 (92.5) |
| Duration of admission, days, median (IQR) | 4 (2-7) |
| **Pregnancy and delivery characteristics:** | |
| pregnancy duration, days, mean (SD) | 270 (17.2) |
| first gravidity, n (%) | 152 (45.5) |
| birth weight (grams), mean (SD) | 3167 (711.0) |
| preterm delivery (<37 weeks), n (%) | 88 (26.3) |
| caesarean section, n (%) | 80 (24.0) |
| epidural anesthesia, n (%) | 91 (27.2) |

**Table 1.** continued

| | |
|---|---|
| **Pregnancy and delivery complications:** | |
| maternal fever during delivery, n (%) | 28 (8.4) |
| prolonged rupture of membranes (>24 hours), n (%) | 99 (29.6) |
| Group B Streptococcus (GBS) colonization, n (%) | 25 (7.5) |
| Antibiotic treatment/prophylaxis, n/n (%) | 17/25 (68.0) |
| **Presenting symptoms:** | |
| dyspnea, n (%) | 23 (6.9) |
| nasal flaring, n (%) | 28 (8.4) |
| rhinorrhea, n (%) | 17 (5.1) |
| wheezing, n (%) | 10 (3.0) |
| **Physical examination signs:** | |
| O2 saturation (pulse oximetry) <90%, n (%) | 19 (5.7) |
| tachypnea >60 breaths/min, n (%) | 34 (10.2) |
| fever >37.8 °C, n (%) | 29 (8.7) |
| hypothermia <36.5 °C, n (%) | 67 (20.1) |
| inter-/subcostal retractions, n (%) | 33 (9.9) |
| **Laboratory and radiology results:** | |
| CRP >10 mg/dL, n/n (%) | 64/204 (31.4) |
| leukocyte count, mean (SD) | 17.2 (6.83) |
| blood culture positive, n/n (%) | 8/138 (5.8) |
| neonatal GBS colonization, n/n (%) | 8/279 (2.9) |
| abnormal chest X-ray, n/n (%) | 19/47 (40.4) |
| **Diagnosis of neonatal infection:** | |
| neonatal sepsis, n (%) | 5 (1.5) |
| pneumonia, n (%) | 11 (3.3) |
| meningitis, n (%) | 6 (1.8) |
| diagnosis of undifferentiated perinatal infection, n (%) | 79 (23.7) |
| **Other diagnoses:** | |
| Infant Respiratory Distress Syndrome (IRDS), n (%) | 10 (3.0) |
| hyperbilirubinemia, n (%) | 56 (16.8) |
| hematological disorder (anemia, thrombocytopenia, polycythemia), n (%) | 32 (9.6) |
| **Treatment:** | |
| antibiotics, n (%) | 108 (32.3) |
| oxygen suppletion, n (%) | 51 (15.3) |
| CPAP, n (%) | 17 (5.1) |
| mechanical ventilation, n (%) | 6 (1.8) |

Not mentioned in the table are details regarding antibiotic duration and type. An extended course of antibiotics (i.e. >3 days) was administered to 77 of 108 neonates (71.3%) who received antibiotic treatment. Antibiotic treatment was guided by local protocols. Initial antibiotic treatment in case of presumed sepsis or undifferentiated perinatal infection consisted of amoxicillin combined with gentamicin, followed by a switch from gentamicin to cefotaxim if antibiotic treatment had to be continued for more than 3 days. In case of a diagnosis of meningitis being considered, flucloxacillin was added to the abovementioned regimen. The antibiotic used in case of community-acquired pneumonia was amoxicillin, and cefotaxim was used if the pneumonia was hospital-acquired. Obviously, the choice of antibiotics to be used was guided by the results of available positive microbiology culture reports.

**Respiratory pathogens**

Table 2 demonstrates the number of respiratory pathogens that were identified via RT-PCR on nasopharyngeal aspirates in all newborn children that were admitted during the one-year study period to the NMCU department of our hospital. Overall, 37 pathogens were detected in 34 children, which comprises 10.2% of the total population. Most respiratory pathogens (20.8%) were identified in the month December, with parainfluenza viruses being detected in 5 of 10 positive samples. Regarding the entire study period, parainfluenza-1 (n=9), human rhinovirus (n=7), parainfluenza-3 (n=6) and RSV (n=6) were most frequently detected. Human rhinovirus was the most frequently detected pathogen in October (3 out of 4), parainfluenza viruses comprised almost half of the identified pathogens in November and December (7 out of 15) and RSV showed its peak activity in January (4 out of 6). Although *Streptococcus pneumoniae* (n=3) has been detected as a bacterial pathogen by RT-PCR, blood cultures remained negative. Influenza B, parainfluenza-2, parainfluenza-4, human metapneumovirus, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae* and *Legionella* species were not detected at all. The two coronaviruses that had been detected were human coronavirus OC43 and NL63. The simultaneous presence of two pathogens was observed in 3 children: all were positive for RSV combined with either human rhinovirus, human coronavirus OC43 or *Streptococcus pneumoniae*.

**Table 2.** Respiratory pathogens identified in neonates admitted to a medium care unit.

| year | month | *n* | INFA | PIV1 | PIV3 | ADV | RSV | hRhV | hCoV | Boca | Spneu | Total pathogens | Total neonates with respiratory pathogens |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **2010** | **October** | *29* | | 1 | | | | 3 | | | | 4 | 4 (13.8%) |
| | **November** | *28* | | 2 | | 1 | | 1 | | | | 4 | 4 (14.3%) |
| | **December** | *48* | 1 | 2 | 3 | 1 | 2 | | 1 | | 1 | 11 | 10 (20.8%) |
| **2011** | **January** | *31* | | | | | 4 | 1 | | | 1 | 6 | 4 (12.9%) |
| | **February** | *24* | | | | | | 1 | | | 1 | 2 | 2 (8.3%) |
| | **March** | *23* | | 1 | 1 | | | | 1 | | | 3 | 3 (13.0%) |
| | **April** | *21* | | | | | | | | 1 | | 1 | 1 (4.8%) |
| | **May** | *19* | | | | | | | | | | 0 | 0 (0.0%) |
| | **June** | *25* | | | 1 | | | | | | | 1 | 1 (4.0%) |
| | **July** | *19* | | 2 | | | | | | | | 2 | 2 (10.5%) |
| | **August** | *38* | | | | | | 1 | | | | 1 | 1 (2.6%) |
| | **September** | *29* | | 1 | 1 | | | | | | | 2 | 2 (6.9%) |
| **Entire study period 2010-2011** | | *334* | 1 (0.3%) | 9 (2.7%) | 6 (1.8%) | 2 (0.6%) | 6 (1.8%) | 7 (2.1%) | 2 (0.6%) | 1 (0.3%) | 3 (0.9%) | 37 | **34 (10.2%)** |

*Both absolute numbers as well as percentages of respiratory samples that were positive are provided per month and per detected pathogen.*

*INFA - influenza A; PIV1 - parainfluenza 1; PIV3 - parainfluenza 3; ADV - adenovirus; RSV - respiratory syncytial virus; hRhV - human rhinovirus; hCoV - human coronavirus; Boca - bocavirus; Spneu – Streptococcus pneumoniae*

**Analysis of variables associated with detection of respiratory pathogens**

Demographic and clinical characteristics from Table 1 were compared between neonates with RT-PCR positive for any respiratory pathogen (n=34) and those with a negative RT-PCR result (n=300). From this univariate analysis we identified 7 statistically significant ($p<0.05$) predictor variables, which were subsequently added to a logistic regression model (Table 3). In addition to these significant contributing variables, some non-significant differences were observed with regard to other variables from the univariate analysis. Comparing the RT-PCR-positive group with the RT-PCR-negative group, the proportion of male participants was higher (67.6% versus 52.0%; p=0.083), median duration of admission was shorter (3 days versus 4 days; p=0.089), mean birth weight was higher (3366 grams versus 3144 grams; p=0.089), and mean leukocyte count was lower ($14.7 \times 10^9$/L versus $17.5\ 10^9$/L; p=0.066). The remaining characteristics from Table 1 were statistically comparable between the two groups, including diagnosis of undifferentiated perinatal infection (23.5% versus 23.7%) and pneumonia (2.9% versus 3.3%). The positive blood culture results (n=8) were restricted to the group of neonates of whom nasopharyngeal samples were RT-PCR-negative for respiratory pathogens.

In addition to comparing variables between neonates with and without a positive RT-PCR for respiratory pathogens, another comparison was made based on presenting signs of respiratory infection (dyspnea, nasal flaring, rhinorrhea and wheezing). Of 39 neonates with respiratory symptoms, 12 (30.8%) had a positive RT-PCR for respiratory pathogens, whereas of 295 neonates without respiratory symptoms, 22 (7.5%) had a positive RT-PCR. The difference in proportion was statistically significant ($p<0.001$).

Table 3 summarizes the results of the logistic regression analysis. Odds ratios were calculated and adjusted for age and sex to estimate the risk of a positive RT-PCR for respiratory pathogens per included variable. After adjustment, two variables were identified as significant contributors to the model: age (OR 1.21 for each day older; 95% 1.12-1.30) and symptoms of rhinorrhea (OR 6.71; 95% CI 1.54-29.21). For rhinorrhea we calculated the predictive characteristics of this symptom within our population: we determined a positive predictive value of 58.8% and negative predictive value of 92.4%.

**Table 3.** Risk of RT-PCR positive for respiratory pathogens per selected variable.

| Variable | RT-PCR positive (N=34) *mean/n* | RT-PCR negative (N=300) *mean/n* | Crude OR (95% CI) | Adjusted OR (95% CI)* |
|---|---|---|---|---|
| **Age, days** | 7.4 | 0.6 | 1.21 (1.12-1.30) | 1.21 (1.12-1.30) |
| **Late newborn admission (≥72 hrs post partum)** | 12 | 13 | 12.04 (4.91-29.51) | 0.40 (0.032-5.00) |
| **first gravidity** | 7 | 145 | 0.28 (0.12-0.66) | 0.50 (0.19-1.27) |
| **preterm delivery (<37 weeks)** | 4 | 84 | 0.34 (0.12-1.00) | 0.62 (0.20-1.90) |
| **epidural anesthesia** | 4 | 87 | 0.33 (0.11-0.95) | 0.53 (0.17-1.60) |
| **rhinorrhea** | 10 | 7 | 17.44 (6.09-49.93) | 6.71 (1.54-29.21) |
| **wheezing** | 4 | 6 | 6.53 (1.75-24.45) | 4.34 (0.84-22.34) |

*The selected variables were included if from univariate analysis a statistically significant difference between groups with positive and negative RT-PCR for respiratory pathogens was demonstrated.*

**Adjusted for age and sex.*

*OR - odds ratio; CI - confidence interval*

## DISCUSSION

This prospective observational study describes a population of 334 neonates who have been admitted to the NMCU of our hospital in the period October 1, 2010, until September 30, 2011. The median age at hospitalization was 1.3 days, 53.6% were male, and 35.9% were classified as of Mediterranean ethnicity (i.e. mostly from Turkish or Moroccan origin). Any respiratory pathogen was detected by RT-PCR on nasopharyngeal aspirates in a total of 34 newborn children (10.2%). Parainfluenza-1 (n=9), human rhinovirus (n=7), parainfluenza-3 (n=6) and RSV (n=6) were most frequently detected.

There are few publications reporting on the epidemiology of respiratory pathogens among newborn children (i.e. from birth until 28 days post partum). Verboon-Maciolek et al. performed a retrospective analysis of 5396 infants that had been admitted to a university neonatal intensive care unit (NICU) in the Netherlands over a period of 12 years.[8] Viral infection, determined by positive culture, was detected in only 1% of the total

population. In addition to a range of non-respiratory pathogens, RSV (n=15), adenovirus (n=2), parainfluenza (n=2) and rhinovirus (n=1) were the respiratory pathogens that had been detected. However, as opposed to our study in which all neonates were screened for the presence of respiratory pathogens by RT-PCR, the investigators of the 12-year study performed viral cultures only in 'clinically ill' infants, and this was probably decided at the discretion of the responsible pediatrician. Another study, performed by Vieira et al., prospectively determined the prevalence of respiratory viruses among a selected population of 90 neonates with bronchiolitis or pneumonia that were admitted at a Brazilian NICU.[10] Nasopharyngeal aspirates were subjected to rapid evaluation by antigen detection through immunofluorescence (IF) assay and viral culture in case of a negative IF result. PCR methods had not been used. A respiratory virus was identified in 80% of newborns: RSV (n=45), influenza A (n=25), parainfluenza-3 (n=3), adenovirus (n=3) and parainfluenza-1 (n=1). In an older prospective study that was published in 1984, a relatively unselected population of 280 NICU admitted infants were screened to establish the incidence of chlamydial, mycoplasmal and several viral infections.[11] Nasopharyngeal aspirates were collected for the detection of respiratory pathogens. Culture and IF were the diagnostic methods that were used. Respiratory viral infection was detected in 14 of 280 babies (5.0%): *Mycoplasma* (n=6), RSV (n=6), parainfluenza-1 (n=1) and parainfluenza-3 (n=1).

To our knowledge, this is the first study that determines the prevalence of respiratory pathogens among neonates admitted to a medium care unit. The above cited studies of newborn children have been performed in neonatal intensive care units,[8,10,11] and are therefore not entirely comparable to our medium care unit population, which is generally considered to be a milder neonatal patient category. Other studies are even less comparable to our study because they have focused on selected pediatric populations of older children (up until 5 years of age) with an acute respiratory infection.[12-14] Overall pathogen detection rates in those studies ranged from 61% to 86%, and most common respiratory pathogens were RSV, human rhinovirus, adenovirus, and bocavirus. Whereas in our population parainfluenza virus is the number one identified pathogen (15 out of 37 detected pathogens), most other studies have in common that RSV is the most frequently detected virus (which was 6 out of 37 detected pathogens in our study).[8,10,11,13,14] Besides the study populations not being entirely comparable, another explanation for this discrepancy might be the fact that we had noticed a mild RSV epidemic in the winter of 2010-2011 among children in our hospital in general. Nonetheless, it is known that RSV infection is associated with substantial morbidity and even mortality in young children,[15,16] and there is also a significant burden of parainfluenza viruses among those who are hospitalized.[17]

Furthermore, whereas RT-PCR has been used as the solitary diagnostic method in our study to detect the presence of respiratory pathogens, this method was not used by the three NICU population studies.[8,10,11] In a study comparing multiplex PCR assays and conventional techniques for the diagnostic of respiratory virus infections in children admitted to the hospital with an acute respiratory illness, PCR was more sensitive, had the advantage of a shorter delay in specific diagnosis, and a lower cost than IF and culture.[18] Another study concluded that RT-PCR for respiratory viruses was found to be a sensitive and reliable method in pediatric intensive care unit (PICU) patients with lower respiratory tract infection, with a two-fold increase in the diagnostic yield compared to conventional methods.[19] However, a limitation of the use of PCR assays has been acknowledged by studies showing that asymptomatic carriage of a respiratory virus occurs frequently in young children,[20] and the presence of viral nucleic acids may not always reflect an association with infectious virus production.[21] In general, the cycle threshold (Ct) value may be of help in differentiating symptomatic from asymptomatic infections. This limitation most likely holds true for newborns as well, as is reflected by our findings showing that respiratory pathogens have been detected in 7.5% of neonates without respiratory symptoms. However, our study design does not really allow us to draw conclusions regarding that aspect, because all the neonates from our population were hospitalized and therefore we did not have a true 'asymptomatic' comparison group. Moreover, we could not retrieve any articles that specifically focus on the capacity of a positive PCR predicting clinically relevant infection in a population of newborns until 28 days post partum.

We compared demographic and clinical characteristics between neonates with a positive RT-PCR for any respiratory pathogen with those with a negative RT-PCR result, and found that only 7 variables were statistically significant different and therefore potentially associated with the risk of a positive RT-PCR result for respiratory pathogens: age, late newborn admission, first gravidity, preterm delivery, epidural anesthesia, rhinorrhea and wheezing. After adjustment for confounding, only age (OR 1.21 for each day older; 95% 1.12-1.30) and rhinorrhea (OR 6.71; 95% CI 1.54-29.21) remained significant contributors to an increased risk of RT-PCR positive for respiratory pathogens. The risk factors for infection have been most thoroughly characterized for RSV. Prematurity and young age have been shown to be independent risk factors for RSV-associated hospitalizations.[15] Birth at the onset of the RSV season and other environmental factors, such as the presence of (school-aged) siblings and day care attendance, have also been designated as significant risk factors for severe RSV infection.[22,23] Similar to the results from our unadjusted univariate analysis, a study in which newborns infected with a range of respiratory viruses were compared with newborns without infection, showed that wheezing was one of the symptoms that was significantly more frequently noticed in the presence of respiratory virus infection.[10]

For neonates with a non-specific syndrome of infection with unknown origin, there are currently no guidelines for the use of respiratory pathogen diagnostics. In clinical practice, these cases are usually regarded as possible sepsis and, after the collection of blood samples for bacterial culture, empirical antibiotics are then initiated conform guidelines.[24] However, antibiotics in neonatal infections, especially the frequent use of aminoglycosides, can lead to serious toxicity.[25] In addition, from a cost-effectiveness study it has been shown that treatment of children aged 0 to 36 months with lower respiratory tract infection causes a considerable economic burden, which is for the most part caused by (duration of) hospitalization.[26] Studies on the value of RT-PCR diagnostics in children have shown conflicting results as to whether the introduction of this method as a diagnostic tool for neonatal infection might be of benefit.[27,28] The results of our study, in which all pediatricians were blinded for the RT-PCR results, show that median duration of clinical admission was one day shorter among those neonates with a positive result (non-significant difference; p=0.089). Other variables, relating to clinical course and outcome, were comparable between both groups. Therefore, for neonates hospitalized with non-specific signs and symptoms, the possible advantage of performing RT-PCR assays for the detection of respiratory pathogens remains to be elucidated as well. Of notice here, we did show that positive blood cultures, which are considered to be the ultimate proof of bacterial infection, were not seen at all among neonates with a nasopharyngeal aspirate being positive for respiratory pathogens as detected by RT-PCR. This might be a cautious argument in support of withholding antibiotics or decreasing its use when respiratory viruses as the etiological agents are detected using RT-PCR assays on respiratory samples.

There are some limitations of our study that need to be discussed. First, although we are the first to describe a neonatal population in a medium care setting, by that we have restricted ourselves to milder cases as compared to neonates admitted to an intensive care setting. It would be interesting and useful to repeat our research among unselected neonates who are hospitalized, irrespective of the intensity of the provided care. Another limitation is that we have identified only 34 neonates with a respiratory pathogen. Even though this comprises 10.2% of the total population, the absolute numbers that were used for the analyses are relatively small, especially when considering the detected pathogens separately. To increase the power to detect any relevant difference between groups, a longer follow-up period would be of added value. A last limitation is that our study design does not yet allow us to draw solid conclusions on the usefulness of RT-PCR, and therefore its role in clinical practice. Only a randomized controlled trial would give us definite answers with that regard.

In conclusion, this study has demonstrated that respiratory pathogens were present in one of ten unselected neonates admitted to an NMCU. Parainfluenza viruses, rhinovirus and RSV were most important with regard to frequency of detection. Increasing age and symptoms of rhinorrhea significantly contributed to an increased risk of detection of any respiratory pathogen. There was no significant difference regarding use of antibiotics or duration of hospital stay; however, pediatricians were blinded for RT-PCR results. Respiratory pathogens seem to play a role in neonates admitted to a medium care unit. The question whether clinical management will be influenced by the knowledge of respiratory viruses being present in respiratory samples of newborn children, remains to be answered.

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# Chapter 3

## Prevention

# 3.1

# Low attack rate of novel influenza A (H1N1) virus infection among healthcare workers: a prospective study in a setting with an elaborated containment plan

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## ABSTRACT

**Purpose:** This study aimed to determine incidence rates of novel influenza A (H1N1) infection among healthcare personnel with different exposure risks during the 2009 H1N1 pandemic.

**Methods:** From August 2009 until April 2010, 66 healthcare workers from a 410-bed teaching hospital in Amsterdam were monitored. Three different exposure groups were created: a high- (n=26), intermediate- (n=20) and low-risk group (n=20). Throat swabs were collected each week and analyzed by real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) in order to detect the H1N1 virus. Blood was drawn at study enrolment and once monthly thereafter, and serum specimens were tested with an H1N1-specific hemagglutination-inhibition serologic assay. Influenza-like signs and symptoms were assessed weekly.

**Results:** One of 26 high-risk group participants proved H1N1-positive once by RT-PCR. This corresponds to an incidence rate in the high-risk group of 5.7/1,000 person weeks (95% CI 0-17/1,000). None of the intermediate- and low-risk group participants proved H1N1-positive by RT-PCR. Significant antibody titer rises in convalescent sera were demonstrated in three participants: one was a confirmation of the case that had proved H1N1-positive by RT-PCR; the others occurred in two asymptomatic participants belonging to the low- and high-risk group. An influenza-like illness was assumed in four participants from the high- (n=1), intermediate- (n=1) and low-risk (n=2) groups; these findings were not confirmed by positive results from either diagnostic test.

**Conclusions:** This study demonstrates a low incidence rate of influenza A (H1N1) infection among healthcare workers during the 2009 H1N1 pandemic in a setting with high hygiene standards.

## INTRODUCTION

On April 17, 2009, officials at the Centers for Disease Control and Prevention (CDC) confirmed two cases of swine influenza in children living in neighboring counties in California.[1] This event led to the emergence of a serious global health threat caused by a new influenza A (H1N1) virus.[2]

Soon after the identification of this novel influenza A (H1N1) virus in the United States in mid-April 2009, the CDC recommended strategies to reduce the risk for transmission in healthcare settings. These strategies included administrative controls, provision of infection-control resources, training in infection-control practices, correct use of personal protective equipment, identification of all ill healthcare personnel, and exclusion of ill healthcare personnel from work.[3]

The novel influenza A (H1N1) pandemic also forced the Dutch government to take its precautions as part of infection control in the Netherlands.[4] On August 12, 2009, the Slotervaart Hospital, a 410 bed teaching hospital in Amsterdam, opened the doors of an influenza outpatient clinic as part of its Diagnostic Center for Infectious Diseases (DCI). This outpatient clinic operated as a separate facility for the diagnosis and management of patients and healthcare personnel with suspected influenza virus infection. From August 12 until December 31, 2009, 964 adults with influenza-like signs and symptoms were tested, of whom 157 proved H1N1-positive.[5] However, soon after the opening of this influenza outpatient clinic, the Dutch Department of Health and Human Services recommended that individuals were not to be tested in outpatient settings. An important reason provided by the national government for this change in policy was to constrain the risk of novel influenza A (H1N1) infection between patients and healthcare personnel by limiting crowding of patients with influenza-like signs and symptoms in healthcare settings.

Initial evidence suggested that healthcare workers were not overrepresented among reported cases of novel influenza A (H1N1) virus infection. Approximately 4% of proven and probable novel influenza A (H1N1) cases have occurred under healthcare workers.[3,6] However, few articles reporting on prospective studies of influenza transmission risk in hospital settings have been published to date.[7] It has been commonly accepted that contact and respiratory precautions, such as frequent hand washing and wearing a respiratory device, will reduce transmission of the virus.[8,9] While these precautions will significantly slow transmission, vaccination of healthcare personnel, as soon as a tailored influenza vaccine is available, seems to provide the best preventive strategy in the long term.[8] At the

influenza outpatient clinic of the Slotervaart Hospital, strategies as recommended by the CDC to prevent transmission to healthcare personnel had been strictly applied.[10]

The aim of this prospective study was to describe the incidence rate of infection with novel influenza A (H1N1) virus among healthcare personnel at risk working at our hospital. We wanted to compare the incidence rate in three groups of healthcare personnel with a high-, intermediate- and low-risk of H1N1 virus exposure through patient contact.

## METHODS

**Study design and study population**

This prospective study was carried out among healthcare personnel from several departments of the Slotervaart Hospital in Amsterdam, the Netherlands. In the period from mid-August 2009 until the beginning of 2010, healthcare personnel from three different exposure categories were prospectively followed. There were no specific exclusion criteria for participation in this study.

Three approximately equally sized groups were created from healthcare personnel aged 18 years and older according to estimated novel influenza A (H1N1) virus exposure risk. The high-risk group consisted of resident physicians, nurses and doctor's assistants with an expected overexposure to influenza patients, i.e. those working at the DCI in its separated influenza outpatient clinic. This group was studied from 17 August 2009 until 6 April 2010. Participants were classified as intermediate-risk group healthcare workers when their professional exposure to influenza patients was considered to be average; corresponding participants were doctors, nurses and doctors' assistants from the internal medicine, cardiology and pulmonary diseases outpatient clinics. Absence of any elevated risk for occupational contact with (influenza) patients in the hospital defined the low-risk group, with the following professionals belonging to that category: pharmaceutical technicians, managers, ICT workers, assistants and secretaries from clinical pharmacology, ICT, management division and the Human Resources department. Inclusion of participants from the intermediate- and low-risk groups started as of 2 November 2009, just in the middle of the peak of the H1N1 epidemic among patients presenting at our hospital. The study period for these two groups officially ended on 8 January 2010.

All study participants self-collected throat swabs for H1N1 virus detection and filled out questionnaires every week while working. For each returned throat swab we counted

one studied person week for analytical purposes, irrespective of vaccination status; both 'unvaccinated' and 'vaccinated' person weeks, defined as until and from the calendar week of the first vaccination against novel influenza A (H1N1) respectively, were calculated. Self-collection of throat swabs was taught to each participant in a short training session by the primary researcher himself at study inclusion. If participants were absent because of flu-like illness, they were asked to contact the primary researcher who would arrange for a throat swab to be sent to their homes. In this way, weekly self-collection of throat swabs was possible despite absence from work. If participants were absent because of scheduled time off, throat swabs were not collected and the lost weeks did not count as valid patient weeks in our analysis. Blood was withdrawn for serological analysis at study enrolment and approximately once a month thereafter.

The study was approved by the local Ethical Review Committee and was conducted in accordance with the principles of the Declaration of Helsinki and with Dutch regulatory requirements; informed consent was given by each participant prior to study inclusion.

**Preventive strategies**

In accordance with national and international recommendations and guidelines on preventive strategies, the Slotervaart Hospital applied several measures to protect its healthcare personnel from infection with novel influenza A (H1N1) virus.[10,11] The DCI's outpatient facility for diagnosis and management of influenza infection was separated from the rest of the hospital with a separate entrance next to the emergency department. The room was cleaned several times each day. Healthcare personnel working at the DCI department were required to wear respiratory protection (FFP2 mask) and examination gloves during the entire shift. The cleaning of hands with water, soap and alcohol was mandatory after treating each patient. Patients themselves were also required to wear respiratory protection during their visit to the DCI facility.

Personnel working in the hospital and presenting to Occupational Health Services with influenza-like signs and symptoms were immediately referred to the outpatient DCI facility for diagnosis and, if necessary, the administration of oseltamivir phosphate (i.e. in case of age ≥65 years, pregnancy in 3$^{rd}$ trimester, suffering from a specified chronic medical condition, or complications in the course of the infection). All personnel with possible influenza infection were excluded from work until definitive test results were available after one day on average. Personnel with proven influenza infection were required to stay at home for seven days from the first day of symptoms.

The last preventive measure to be carried out was to offer the influenza A (H1N1) 2009 vaccine (Focetria©) to all of the hospital›s healthcare workers as soon as it became available, irrespective of their exposure risk. An easily accessible vaccination programme was organised in the hospital in November and December 2009.

**Laboratory measurements**

Molecular biological testing by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assay for influenza on all pharyngeal swab samples was performed in the molecular biology laboratory of our hospital. Blood samples for paired serological analysis were collected at study enrolment and once monthly thereafter; the assays were performed at the virology department of the Erasmus Medical Center in Rotterdam, the Netherlands. Both these diagnostic methods have been described before by Wallace et al.[12]

*RT-PCR influenza*:

Influenza virus RNA was amplified and detected by RT-PCR performed on the self-collected pharyngeal swabs. A generic PCR (directed against the matrix gene) was used to detect influenza virus type A and an H1N1-specific PCR was applied to the H1 gene. The assay involved CDC protocols with the swine H1 forward-reverse primer set and probe.[13] Samples from high-risk group participants were tested as soon as possible (mostly within 24 hours) after sample collection. If a sample tested positive, the individual concerned was immediately excluded from work, and antiviral treatment was started when indicated. Samples from intermediate- and low-risk group participants were stored at -80 degrees Celsius before being tested together. However, when influenza-like signs and symptoms were reported by an intermediate- or low-risk group participant, the RT-PCR was performed as soon as possible in order to conduct rapid and proper management as described above.

*Serological samples*:

Blood was drawn from each participant and collected in 8 ml serum gel containers once monthly by the investigator for serological confirmation of influenza infection. Serum specimens were stored at -20 degrees Celsius before being tested with a hemagglutination inhibition assay for antibody responses to the pandemic virus A/California/007/09 using four hemagglutinating units of virus and turkey erythrocytes. We defined serologically confirmed influenza A (H1N1) virus infection or successful vaccination as a fourfold rise or more in convalescent serum antibody titers to pandemic influenza A virus, as compared with titers at baseline.

**Assessment of influenza-like signs and symptoms**

All study participants filled out a weekly clinical questionnaire. Influenza-like signs and symptoms were scored repeatedly every week. Influenza-like illness was clinically assumed when meeting the following criteria: temperature ≥38.5 degrees Celsius and two or more acute-onset 'flu' complaints (cough, runny nose, sore throat, headache, myalgia, malaise, chills) in the last seven days. These criteria are in accordance with the Dutch GHOR (Medical Assistance for Accidents and Disasters) guidelines, available at http://www.ghor.nl.

**Statistical analysis**

Statistical analysis was performed using the SPSS software package (version 17.0, SPSS Inc., Chicago, Illinois). The incidence rate of H1N1 infection was calculated by dividing the number of positive RT-PCR's by the number of studied person weeks and was subsequently displayed as a proportion with its corresponding 95% confidence interval. Continuous variables were summarized as mean values (± standard deviation or range) and for categorical variables percentages of participants in each predefined group were calculated. Demographic and clinical characteristics were compared between groups using one-way ANOVA for continuous variables and Chi-square or Fisher's exact test for categorical variables as appropriate. A p-value of less than 0.05 was considered statistically significant.

## RESULTS

**Study population**

Data from a total of 66 individual healthcare workers were used for this analysis. In the high-risk group we observed a total dynamic population of 26 different individuals working at the DCI influenza outpatient department for various time periods. In both the intermediate- and low-risk groups we observed a cohort of 20 individuals in each group. During the follow-up of the study, three healthcare workers had withdrawn from further participation and for two individuals a throat swab was sent to their home because of absence from work due to influenza-like illness. In total, 470 throat swabs were returned by the participants to the molecular biology laboratory. The corresponding total of 470 studied person weeks (mean 7.1 ± 3.0 weeks) consisted of 332 'unvaccinated' (mean 5.0 ± 2.9 weeks) and 138 'vaccinated' person weeks (mean 2.1 ± 2.4 weeks). In the high-risk group we studied 178 person weeks, subdivided into 152 'unvaccinated' (mean 5.9 ± 3.1 weeks) and 26 'vaccinated' (mean 1.0 ± 1.9 weeks); in the intermediate-risk group we studied 149 person weeks, subdivided into 87 'unvaccinated' (mean 4.4 ± 2.3 weeks) and 62 'vaccinated' (mean 3.1 ± 2.4 weeks); and in the low-risk group we studied 143 person weeks, subdivided into 93 'unvaccinated' (mean 4.7 ± 2.9 weeks) and 50 'vaccinated' (mean 2.5 ± 2.6 weeks).

Table 1 outlines the baseline characteristics of the three different exposure groups. The majority of study participants were female and mean age for all participants was 40 years. Overall, 6% reported seasonal influenza vaccination in the preceding months, 52% received the first 2009 H1N1 vaccination in November, and 36% received the second 2009 H1N1 vaccination in December. On average, participants from the high-risk group worked at the DCI influenza outpatient clinic for two days per week; the remaining working days were mainly spent in Accident and Emergency or various nursing wards.

**Table 1.** Baseline characteristics of healthcare personnel from three different risk groups.

| | | high-risk group *n=26* | intermediate-risk group *n=20* | low-risk group *n=20* |
|---|---|---|---|---|
| **Person weeks** | ***total*** | 178 | 149 | 143 |
| | ***mean (± SD)*** | 6.9 (±4.2) | 7.5 (±1.4) | 7.2 (±2.5) |
| **Sex** | ***male (%)*** | 6 (23%) | 20 (4%) | 8 (40%) |
| | ***female (%)*** | 20 (77%) | 16 (80%) | 12 (60%) |
| **Age** | ***mean (range)*** | 37 (23-61) | 44 (27-63) | 40 (23-57) |
| **Vaccination** | ***seasonal influenza (%)*** | 1 (4%) | 2 (10%) | 1 (5%) |
| | ***H1N1 1st (%)*** | 11 (42%) | 13 (65%) | 10 (50%) |
| | ***H1N1 2nd (%)*** | 5 (19%) | 9 (45%) | 10 (50%) |
| **Exposure influenza outpatient clinic** | | | | |
| *(mean days per week per person ± SD)* | | 2.0 ± 1 | | |

**Laboratory measurements**

*RT-PCR influenza*

From a total of 470 weekly collected pharyngeal swabs, one proved influenza A (H1N1)-positive in October. The corresponding nurse belonged to the high-risk group and did report some influenza-like signs and symptoms in the previous days. Immediate exclusion from work for the duration of one week was imposed; she recovered completely and without any complications. With this finding, the calculated incidence rate of novel influenza A (H1N1) infection in the high-risk group was 5.7/1,000 (95% CI 0.0-17/1,000) person weeks. Incidence was null in the intermediate- and low-risk groups. The difference in incidence rate between groups did not reach any level of statistical significance.

*Serological samples*

Two or more paired serological samples were available from 63/66 participants. Table 2 gives an overview of ≥4-fold rises in antibody titers among participants from the different risk groups. First, a total of 25 of 31 vaccinated individuals (81%) showed a rise in antibody titer by a factor of four or more in convalescent sera taken after vaccination compared with

before. None of the vaccinated individuals showed significant rises in paired sera before the introduction of the vaccine. Second, the table also demonstrates that a rise in antibody titer by a factor of four or more occurred in three unvaccinated individuals from two different risk groups: two participants from the high-risk group and one participant from the low-risk group. One serologically confirmed infection in the high-risk group corresponded to the nurse that proved positive for influenza A (H1N1) by RT-PCR. The other serologically confirmed infections occurred in two asymptomatic individuals. The difference in the serologically confirmed infection rate was not statistically significant between the three risk groups.

**Table 2.** Hemagglutination inhibition assay results: ≥4-fold increase in antibody titer from convalescent sera.

| | high-risk group | | intermediate-risk group | |
|---|---|---|---|---|
| | **vaccinated** | **non-vaccinated** | **vaccinated** | **non-vaccinated** |
| | *n=8* | *n=15* | *n=13* | *n=7* |
| **≥4-fold increase in antibody titer (%)** | 5 (63%) | 2 (13%) | 12 (92%) | 0 (0%) |

| | low-risk group | | overall | |
|---|---|---|---|---|
| | **vaccinated** | **non-vaccinated** | **vaccinated** | **non-vaccinated** |
| | *n=10* | *n=10* | *n=31* | *n=32* |
| **≥4-fold increase in antibody titer (%)** | 8 (80%) | 1 (10%) | 25 (81%) | 3 (9%) |

*Data from three vaccinated high-risk group subjects are missing because no serological samples were available from them during follow-up.*

### Influenza-like signs and symptoms

Table 3 points out the clinical characteristics for each risk group. Flu complaints were reported with a varying frequency within and between groups. The most frequently mentioned complaints were cough, rhinorrhea, sore throat and headache. No statistically significant differences for individual complaints between groups were demonstrated. An influenza like-illness according to the used criteria was assumed in one participant from the high-risk group, one participant from the intermediate-risk group and two participants from the low-risk group. No influenza-like illness was confirmed in any of these four participants, neither by a RT-PCR positive throat swab, nor by a significant antibody titer rise in convalescent sera. The participant from the high-risk group with the RT-PCR proven H1N1 infection did not meet the criteria used to determine an influenza-like illness. However, she did report cough, headache and malaise in the days before the infection was confirmed.

**Table 3.** Clinical characteristics of healthcare personnel from three different risk groups.

| | high-risk group *n=26* | intermediate-risk group *n=20* | low-risk group *n=20* |
|---|---|---|---|
| **Flu complaints (%)** | | | |
| *Fever* | 2 (8%) | 1 (5%) | 6 (30%) |
| *Cough* | 13 (50%) | 5 (25%) | 8 (40%) |
| *Rhinorrhea* | 13 (50%) | 6 (30%) | 8 (40%) |
| *Sore throat* | 12 (46%) | 9 (45%) | 8 (40%) |
| *Headache* | 13 (50%) | 10 (50%) | 8 (40%) |
| *Myalgia* | 9 (35%) | 4 (20%) | 4 (20%) |
| *Malaise* | 7 (27%) | 3 (15%) | 6 (30%) |
| *Chills* | 3 (12%) | 3 (15%) | 2 (10%) |
| *Dyspnea* | 2 (8%) | 2 (10%) | 2 (10%) |
| *Productive cough* | 5 (19%) | 2 (10%) | 5 (25%) |
| *Chest pain* | 0 (0%) | 1 (5%) | 0 (0%) |
| *Otalgia* | 1 (4%) | 2 (10%) | 0 (0%) |
| **Influenza-like illness (%)** | 1 (4%) | 1 (5%) | 2 (10%) |
| **PCR influenza A (H1N1)-positive (%)** | 1 (4%) | 0 (0%) | 0 (0%) |

## DISCUSSION

This prospective follow-up study compared the incidence of novel influenza A (H1N1) virus infection in three groups of healthcare workers with different estimated exposure risks during the 2009 H1N1 pandemic. During the entire study period, only one of the 26 participants working at the influenza outpatient clinic proved to be influenza A (H1N1)-positive by a weekly PCR on pharyngeal swab. Serologically, we found significant antibody titer rises in three unvaccinated participants: one of those was a confirmation of the case that proved H1N1-positive by RT-PCR; the others occurred in two asymptomatic participants belonging to the low- and high-risk group.

The discrepancy between H1N1 incidence as detected by RT-PCR and serological analysis can be explained in several ways. First, RT-PCR is usually insensitive in detecting a virus if it is replicating at a fairly low level.[12] Furthermore, it is known that influenza virus shedding lasts up to five days on average.[14,15] This means that viral RNA might not be detected in asymptomatic individuals if screening by RT-PCR is only conducted in intervals of seven days.

In a surveillance study of influenza A (H1N1) in a University Hospital in Thailand, one of 54 screened healthcare workers exposed to influenza patients tested H1N1-positive by PCR.[7] Although the number of studied patient weeks in the Thai study is comparable to our study, their screening program was carried out in an earlier and less serious phase of the pandemic under different circumstances. Notwithstanding, the overall H1N1 incidence rate that we found matches the incidence among Thai healthcare workers fairly well.

In addition to the laboratory investigations that have been discussed so far, we also gathered weekly questionnaires on influenza-like signs and symptoms from all study participants. Flu-like complaints were present at a much higher frequency than the laboratory confirmed H1N1-incidence seems to prove, without any significant differences demonstrated between the risk-groups. An explanation for this phenomenon, as we think, is that influenza-like signs and symptoms can very well be caused by other viral, and even bacterial, pathogens that have been circulating during the H1N1-pandemic; we did not test for those pathogens in this study.

The low incidence rate of H1N1 infection among our healthcare personnel suggests that, if accurately applied, the recommended preventive strategies seem very efficacious. The healthcare workers from our study who worked at the influenza outpatient clinic did consistently follow the CDC recommendations and national published guidelines.[10,11] Unlike the situation in our hospital, suboptimal use of personal protective equipment and suboptimal hand-washing techniques have been mentioned in the available literature.[16-18] Interestingly, our results are consistent with findings from a randomized trial by Cowling et al., concluding that wearing examination gloves, the use of face masks and strict hand hygiene seemed to effectively prevent influenza transmission between index patients and their contacts.[19]

Influenza vaccination appears to be the best measure available to prevent transmission of the virus.[8] Some have even proposed mandatory vaccination of healthcare workers, despite ethical and legal objections.[20] In our hospital we offered influenza vaccination to all our personnel on voluntary grounds. From our total study population of 66 healthcare workers, 34 (52%) received at least one vaccination with the 2009 H1N1 Focetria© vaccine. The vaccination coverage was not significantly different between the high-, intermediate- and low-risk groups. Compared with previous seasonal influenza vaccination coverage rates among healthcare workers in Europe, varying from as low as 6.4% in Poland to a maximum of 25.4% in Portugal and Spain, the current 2009 H1N1 vaccination coverage rate among our hospital personnel was fairly high.[21] This is in contrast with data from the United States that

imply a much higher vaccination rate among healthcare workers during regular influenza seasons.[22] Among the vaccinated study participants, 81% showed a rise in antibody titer by a factor of four or more in convalescent sera from before and after vaccination. This finding is confirmative of a general effective response to vaccination.[23]

Our study has some limitations that should be taken into account. Firstly, the three groups that were compared differed in more than just the risk of exposure. The follow-up period was shorter for the intermediate- and low-risk group, because the required ethical review procedures regarding the volunteers from these groups without excessive exposure was time consuming. In addition, participants from the high-risk group were exposed for a mean of two days per week at the influenza outpatient clinic. The periods before and after vaccination differed between the groups, with most 'unvaccinated' patient weeks having been observed in the high-risk group. In line with this it should be noted that the possibility of influenza virus replication is probably low but not zero after vaccination, considering the antibody response that we found in our population.

Secondly, although we have studied a total of 470 patient weeks, the sample size of 66 healthcare workers is still quite small. Of course the number of healthcare workers in the outpatient clinic was limited (n=26). Therefore, the statistical analyses have been performed rather to generate hypotheses than to prove them.

Lastly, despite regular reminders, we might have missed some pharyngeal swab collections due to absence from work or ordinary forgetfulness of participants. However, as a kind of control mechanism for missed influenza infection, we did analyze the paired serological samples and, as mentioned before, we found no significant difference in seroconversion rate between the groups.

A positive aspect of our study is the prospective follow-up of 66 healthcare workers in three groups with a different estimated influenza exposure risk, which has not been done before with regard to the past 2009 H1N1 pandemic. Because individual throat swab samples were collected weekly, we managed to calculate a true incidence rate of H1N1 infection for healthcare personnel during the 2009 pandemic. The incidence rate of H1N1 infection in asymptomatic healthcare workers has never been published by national survey systems and international publications on this subject are scarce.

In conclusion, we have demonstrated a low incidence rate of infection with novel influenza A (H1N1) virus among healthcare workers excessively exposed to patients with possible

influenza infection visiting our influenza outpatient clinic. These results prove that having a separate facility for diagnosis and management of influenza patients in an outpatient hospital based setting can be considered safe from an occupational health viewpoint. Preventive measures such as the use of proper facemasks, examination gloves and optimal hand hygiene are probably very effective, even with suboptimal vaccination coverage among our healthcare personnel. Screening for influenza infection by PCR or serology might not be very useful in asymptomatic healthcare workers, but can be regarded a useful tool from an occupational health research perspective.

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# Chapter 4

## Diagnostic testing

# 4.1

# Clinical performance of rapid-test for 2009 pandemic influenza A (H1N1) virus

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## ABSTRACT

**Background:** The outbreak of the 2009 influenza A (H1N1) pandemic had caused an urgent need for rapid and accurate diagnostic methods. Several rapid-influenza-diagnostic-tests (RIDT) had been approved for clinical use under these circumstances.

**Objectives:** To evaluate the clinical performance of a commonly used RIDT for the detection of novel influenza A (H1N1) virus.

**Patients and methods:** During the outbreak of the pandemic, consecutive patients who visited an influenza outpatient clinic in Amsterdam, the Netherlands, were included in this study. Rapid-test results were compared to RT-PCR results as the "gold" standard. The RIDT was also applied to 9 strongly RT-PCR H1N1-positive samples. Cycle threshold (Ct) values were provided for each H1N1-positive RT-PCR result.

**Results:** One-hundred-and-eighty-seven outpatients were included. Mean age was 34 years (range 2-84 years) and 87 were male (46.5%). RT-PCR results were positive for novel influenza A (H1N1) in 16 of 187 patients (9%). Rapid-test results were negative in all these patients, but did show positive results in 5 of 9 strongly RT-PCR H1N1-positive samples. Comparing true positive RIDT results (n=5) with false negative RIDT results (n=20), the RIDT-positive samples contained significantly higher viral loads (mean Ct-value 23.62 versus 32.12; $p<0.001$). Overall sensitivity of the rapid-test was 20% (95% CI 6.8%-40.7%).

**Conclusions:** This study has demonstrated poor sensitivity of a rapid-test for the detection of 2009 pandemic influenza A (H1N1) virus among a population of outpatients. Although the test was highly specific due to absence of false positive results, the predictive capacity to diagnose pandemic influenza infection was disappointingly low.

## INTRODUCTION

The 2009 novel swine-origin influenza A (H1N1) virus has been the first influenza virus to cause a true pandemic since the 1968 'Hong Kong flu'.[1] The initial cases of human respiratory infection that were caused by this new virus had been detected in Mexico in April 2009.[2,3] Soon after this event, the virus spread to the United States and from there to the rest of the world.[4] The highest incidence was observed among children and young adults, and, in retrospect, severity of disease caused by the pandemic virus has generally been mild.[5] Nevertheless, the World Health Organization (WHO) had reported more than 18,000 fatal cases worldwide.[6] Obviously, the outbreak of the pandemic had caused an urgent need for rapid and accurate diagnostic methods to support clinical decision making, but also for surveillance purposes and to guide public health interventions.

The United States Food and Drug Administration (FDA) had approved several point-of-care rapid-influenza-diagnostic-tests (RIDT) for clinical use during the 2009 influenza A (H1N1) pandemic.[7] However, these commercially available tests did not discriminate between influenza A subtypes, i.e. pandemic H1N1, seasonal H1N1, seasonal H3N2 or avian H5N1. Nonetheless, under the new 'pandemic' circumstances there was no time to profoundly validate the tests in advance and before use in clinical practice. Practical considerations thus lead to the widespread use of these RIDTs. Reports evaluating the performance of many of those tests started to appear in July 2009 and have even been published until recently.[8] Although initially the analytical sensitivity of 5 commercially RIDTs was reported to be comparable for pandemic and seasonal influenza A (H1N1),[9] approximately at the same time data on performance characteristics of two RIDTs with very poor sensitivities were published (overall sensitivity 17.8%).[10]

This article describes our own experience with the use of a point-of-care rapid-influenza-diagnostic-test and places our findings in perspective to what is known today regarding the performance of these tests during the 2009 H1N1 pandemic. Clinical performance characteristics of the test were obtained based on results coming from a population of patients attending an influenza outpatient clinic at our hospital.

## METHODS

At the Diagnostic Center for Infectious Diseases, an outpatient infectious diseases clinic of the Slotervaart Hospital in Amsterdam, the Netherlands, we have been testing all patients

with influenza-like signs and symptoms for infection with the pandemic influenza A (H1N1) virus since August 2009 until the end of that year. The Slotervaart hospital is a 410-bed teaching hospital serving a low- to middle-income urban population of about 140,000 inhabitants that consists for 18% of children and for 49% of ethnic minorities, most of them of Moroccan and Turkish origin. During the pandemic outbreak, though, individuals from all over the country visited the outpatient clinic. Both children and adults were welcome to sign-up for a consultation at the influenza outpatient clinic. Referral was arranged for either directly by the responsible family physician or by patients or their parents/caregivers themselves.

In addition to a medical history and physical examination, two respiratory samples were collected from each patient. A sterile swab was inserted in one of the nostrils (preferably the one which had more mucus) to obtain a nasal swab specimen for the point-of-care rapid-test diagnostics. The RIDT that was used was the QuickVue Influenza A+B test (Quidel®; San Diego, USA), which is a lateral-flow immunoassay able to detect both influenza A and B antigens in about 10 minutes; however, the assay does not discriminate between different influenza subtypes. Prior to use in clinical practice, positive results had been ascertained by laboratory technicians from our hospital on viral lysates. Right after collection, the rapid-test was immediately performed on-site by trained nurses and physicians in accordance with instructions in the package insert that was provided by the manufacturer. Test results were intended to help the physician in clinical decision making, mainly with regard to initiation of oseltamivir (Tamiflu®) treatment.

Another sterile swab was used to obtain an oropharyngeal specimen for molecular detection of pandemic influenza A (H1N1) virus. Immediately after collection, each swab was transported in a distinct container to the molecular biology laboratory at our hospital. Influenza virus RNA was amplified and detected by real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR), performed on oropharyngeal swabs or nasal washes, within 24 hours. Earlier, this method has been found to be 95% sensitive and 98% specific for detection of type A influenza virus,[11] and has therefore been used in our study as the “gold” standard. A generic PCR (directed against the matrix gene) was used to detect influenza virus type A or B and an H1N1-specific PCR, according to CDC protocol, was applied to the H1 gene.[12] For study purposes, cycle threshold (Ct) values were provided for each H1N1-positive RT-PCR result in order to give an estimate of the amount of virus in the sample; low Ct-values are correlated with high viral loads.

To exclude false negative results due to problems with handling and storage of test kit contents, we also applied the QuickVue rapid-test to 9 strongly RT-PCR influenza A (H1N1)-positive samples from hospitalized children obtained by nasal wash. These samples had been collected previous to our study period and had been stored since then at -70 °C at the molecular biology laboratory.

QuickVue rapid-test results were compared to RT-PCR results. Performance characteristics (i.e. sensitivity, specificity, positive predictive value and negative predictive value), including 95% confidence intervals (CI), were calculated if possible based both on the clinical samples from our outpatient population as well as on the retrieved laboratory samples of the previously confirmed influenza A (H1N1)-positive children. Rapid-test performance was evaluated against demographic and clinical characteristics and Ct-values. SPSS (version 18.0, SPSS Inc. Chicago, Illinois) was used to obtain descriptive statistics and to test for differences in means, medians or proportions, if applicable, using a Student's t-test or non-parametric test for continuous variables and Chi-square or Fisher's exact test for categorical variables, as appropriate.

## RESULTS

Between August 12, 2009, and December 31, 2009, 1376 outpatients with airway symptoms were tested using RT-PCR assay on oropharyngeal swabs to detect pandemic influenza A (H1N1) virus. Results regarding the entire population of both pediatric and adult patients have been described elsewhere.[13,14] The QuickVue rapid-antigen-detection-test was applied to nasal swabs of the first 187 consecutive patients visiting our outpatient clinic from August 12, 2009, to August 17, 2009; however, we gave up on that test soon after we detected an extremely low sensitivity in our population. Mean age in this subpopulation of patients, in whom both the rapid-test as well as RT-PCR was executed, was 34 years (range 2-84 years), 87 were male (46.5%) and mean time since onset of symptoms was 4 days (SD 2.1 days).

RT-PCR on oropharyngeal aspirates was positive for novel influenza A (H1N1) in 16 of 187 patients. This implied a prevalence of 9% in our outpatient clinic at that time. Mean Ct-value in the H1N1 positive samples was 32.79 (range 26.50 to 37.12). The QuickVue rapid-antigen-test was negative in all 187 patients, including the 16 patients in whom the RT-PCR was positive for influenza A (H1N1). The rapid-test did show positive results in 5 of the 9 strongly RT-PCR influenza A (H1N1)-positive nasal washes from hospitalized children aged from 0 to 7 years (mean Ct-value 26.37; range 21.60 to 30.00). Mean Ct-value was significantly lower

among the nasal washes with a positive RIDT result than among those with a negative result (23.62 versus 29.80; p=0.007).

Performance characteristics of the QuickVue RIDT in both populations (outpatients and confirmed H1N1-positive pediatric nasal washes) are demonstrated in Table 1. There were no false positive results at all. RIDT sensitivity was significantly higher when used among strongly H1N1-positive nasal washes compared to the studied outpatient population (55.6% versus 0%; p<0.05). Overall, comparing the true positive pediatric nasal washes (n=5) with all false negative RIDT results (n=20), the RIDT-positive samples contained significantly higher viral loads (mean Ct-value 23.62 versus 32.12; p<0.001). Furthermore, although not statistically significant, the RIDT-positive samples belonged to younger patients (median age 0 years versus 21 years; p=0.061). Since there were no RIDT-positive results among the samples of our outpatient clinic study population, rapid-test performance could not be evaluated against other relevant clinical variables.

**Table 1.** Performance of QuickVue rapid-influenza-diagnostic-test (RIDT) among a population of outpatients with influenza-like signs and symptoms (n=187) and RT-PCR confirmed pandemic influenza A (H1N1)-positive nasal washes (n=9).

| **Outpatients with influenza-like signs and symptoms** | | | | |
|---|---|---|---|---|
| | PCR + | PCR - | Sensitivity | 0/16 = 0% (95% CI 0.0%-20.6%) |
| RIDT + | 0 | 0 | Specificity | 171/171 = 100% (95% CI 97.9%-100%) |
| RIDT - | 16 | 171 | Positive predictive value | 0/0 = NAN |
| | | | Negative predictive value | 171/187 = 91.4% (95% CI 86.5%-95.0%) |
| **RT-PCR confirmed pandemic influenza A (H1N1)-positive nasal washes** | | | | |
| | PCR + | PCR - | Sensitivity | 5/9 = 55.6% (95% CI 21.2%-86.3%) |
| RIDT + | 5 | | Specificity | NA |
| RIDT - | 4 | | Positive predictive value | NA |
| | | | Negative predictive value | NA |
| **Overall combined** | | | | |
| | PCR + | PCR - | Sensitivity | 5/25 = 20.0% (95% CI 6.8%-40.7%) |
| RIDT + | 5 | 0 | Specificity | 171/171 = 100% (95% CI 97.9%-100%) |
| RIDT - | 20 | 171 | Positive predictive value | NA |
| | | | Negative predictive value | NA |

*NA - not applicable*

*NAN - not a number*

## DISCUSSION

The results of this observational study have shown an extremely poor sensitivity of the QuickVue rapid-influenza-antigen-detection test. The overall sensitivity of the test was 20%, but ranged from 0% among 187 outpatients with influenza-like signs and symptoms to 55.6% among 9 stored laboratory samples highly positive for novel influenza A (H1N1) virus. There were no false positive results at all, implying a 100% specificity of the rapid-test. With a novel influenza A (H1N1) prevalence of 9% in our outpatient population, positive predictive value of the rapid-test was, obviously, very low, and negative predictive value reasonably high.

According to Quidel's package leaflet, a previous validation of nasal swab specimens from 122 patients, tested in QuickVue Influenza A+B and in cell culture, had revealed a rapid-test sensitivity of 94% and specificity of 90% for the detection of influenza A virus. However, there had also been discussion over the utility of the test for routine application in the clinical setting, due to a relatively poor sensitivity of the test in the 2007 and 2008 influenza seasons.[15] Anyhow, when we used the test, a caution note was inserted in the leaflet: "Although this test has been shown to detect the 2009 H1N1 virus cultured from a positive human respiratory specimen, the performance characteristics of this device with clinical specimens that are positive for the 2009 H1N1 influenza virus have not been established". Therefore, data were urgently needed on the clinical performance of these and other RIDTs to detect novel influenza A (H1N1) virus in different specimens. Our findings have important implications for clinical practice. A negative rapid-test result did absolutely not exclude infection with the pandemic influenza A virus. These data highlight the need to carefully interpret rapid-test results in case of the development of a novel, whether or not pandemic, influenza strain.

Following clinical experience with influenza rapid-tests during the 2009 H1N1 pandemic, other observational studies that evaluated performance characteristics of the QuickVue rapid-test in patients with influenza-like signs and symptoms have demonstrated sensitivities ranging from as low as 18.2% to as high as 75%.[16-27] Specificity was mostly high and more consistent between the studies (range 80%-100%). Other approved RIDTs had also been evaluated. A meta-analysis of 14 published studies evaluating 7 rapid-tests showed that the pooled overall sensitivity and specificity was 67.5% (95% CI 66.2%-68.9%) and 80.7% (95% CI 80.0%-81.4%), respectively.[28] The QuickVue rapid-test was significantly more sensitive than two other frequently used RIDTs. However, this meta-analysis was limited by heterogeneity of results. Another meta-analysis of 17 published studies evaluating the same rapid-tests

with the exception of one, demonstrated an overall sensitivity of 51% (95% CI 41%-61%) and specificity of 98% (95% CI 94%-99%).[29] In addition, the authors had calculated positive and negative predictive values conditional to an assumed H1N1 prevalence of 30%, and found them to be 94% and 82%, respectively. Furthermore, from this meta-analysis it also became clear that the QuickVue rapid-test had a higher overall sensitivity compared to other rapid-tests.

Compared to the other reports, we observed an even poorer sensitivity of the used RIDT. There are several factors that might have decreased the performance of the QuickVue rapid-test in our population that should be taken into account. Although we did use one of the recommended clinical samples (nasal swabs) according to the manufacturer's instructions, it remains questionable whether the quality of the specimen was adequate. The nurses and physicians who did use the swabs on patients were trained how to do this accurately prior to use in practice. Because oropharyngeal sampling, intended for the RT-PCR assays, had led to positive results, this sampling method must therefore have been accurate. However, the rapid-tests were performed on nasal swabs and not oropharyngeal swabs. As a result, we cannot verify correctness of nasal sampling intended for the rapid-test. However, in a study in which both nasal and oropharyngeal swabs were collected, there was a high agreement between the obtained RT-PCR results (kappa 0.932).[19] In line with this, one might doubt if sampling from different anatomical locations (i.e. nasal vestibules or oropharynx) could be associated with different performance characteristics. Then again, this is stated not to be the case for the QuickVue rapid-test according to Quidel's package insert. On the other hand, studies that used solely nasal swabs to detect pandemic influenza with the same rapid-test have demonstrated an average sensitivity of 63%,[19,21,23] which is moderately lower than QuickVue's pooled sensitivity of 74% when taking all sampling methods together.[28]

Other factors that might have influenced test performance are age and time from onset of illness to specimen collection. It has been shown that the sensitivity of the QuickVue rapid-test for pandemic influenza A (H1N1) virus was greatest for children less than 3 years of age.[19] Drexler et al., who evaluated a different commercially available rapid-test during the 2009 H1N1 pandemic, concluded that the paramount feature of rapid-test positive samples was high virus concentration, and that the overall sensitivity among (young) children can be explained by them having higher influenza virus shedding than adults.[30] Our findings confirm this: RIDT-positive samples belonged to younger individuals and contained significantly higher viral loads. Performance of the QuickVue rapid-test has also been shown to vary by day of presentation, with a higher sensitivity for samples from children presenting one or more days post-symptom onset.[23] Again here, the paramount feature is probably viral

load. Influenza viral shedding increases sharply between 0.5 and 1 day after experimental challenge and consistently peaks on day 2, which is fairly coincident with the development and course of symptoms.[31] Mean time since onset of symptoms was 4 days (i.e. after the peak of viral shedding) in our study population of outpatients with influenza-like signs and symptoms, which might explain why we did not have any positive rapid-test results among them. In addition, from our results it was shown that mean Ct-value, which is inversely correlated to viral load, in the RIDT-positive group was significantly lower compared with the RIDT-negative group (mean Ct-value 23.62 versus 32.12; $p<0.001$). From the other reports that evaluated performance of the QuickVue rapid-test during the novel influenza A (H1N1) pandemic, increased viral loads were indeed clearly associated with a higher sensitivity and better performance of the test.[8,20,21,32]

An important limitation of our study is the limited population size, because we stopped using the test soon after we detected an extremely low sensitivity in our population. If we would have continued using the QuickVue rapid-test, we might have been able to obtain some actual positive results among the outpatient population that attended our clinics and with that a more accurate estimate of the true performance characteristics of the test. It was not our intention, however, to design a performance evaluation study. This is merely a description of our clinical experience, based on which we swiftly decided to discontinue using the rapid-test. Another limitation is that we have used the test in a population of patients with a relatively long mean time since onset of symptoms. We could, therefore, have missed the peak of viral shedding with decreased performance of the rapid-test as a consequence. Nevertheless, our results are a true reflection of real clinical practice.

In conclusion, this study has demonstrated limited performance of the QuickVue rapid-test for the detection of 2009 pandemic influenza A (H1N1) virus among a population of outpatients with influenza-like signs and symptoms. Although the test was highly specific, the predictive capacity to diagnose pandemic influenza infection was disappointingly low. Our findings are of major concern to physicians dealing with outpatients with influenza-like signs and symptoms. Based on these results we do not recommend the use of rapid-tests in an outpatient based setting in case of the development of a novel (pandemic) influenza strain, when there has not been any time to adequately validate the test in advance.

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# 4.2

# Procalcitonin in children with suspected influenza A (H1N1) infection

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## ABSTRACT

**Aims and methods:** To gain insight into the acute phase response and to investigate the value of procalcitonin (PCT) in children with suspected novel influenza A (H1N1) infection, 30 patients with suspected H1N1 infection were included.

**Results:** Twenty-five patients (83.3 %) were diagnosed with a viral illness, including 9 patients with H1N1 infection. Median PCT levels, CRP levels and leukocyte counts in the subgroup with proven viral infections were 0.153 ng/mL with IQR 0.094–0.261 ng/mL; 19.0 mg/L with IQR 5.6–28.3 mg/L; and 13.1 giga/L with IQR 9.5–16.0 giga/L, respectively. PCT levels were below the lower cut-off value in 22 of 25 children (88%) with H1N1 infection and other viral diseases, whereas CRP levels and leukocyte counts were shown to exceed the lower cut-off value in 6 and 5 patients, respectively.

**Conclusion:** Low PCT values seem to be of added value to support a diagnosis of viral infection during the diagnostic procedure in children with flu-like symptoms.

## INTRODUCTION

In June 2009, the first patients with Novel Influenza A (H1N1) were diagnosed in The Netherlands. Children under 5 years of age were particularly at risk for hospitalization, with a total of 574 hospitalized children under 5 years of age until December 2009, according to The National Institute for Public Health and the Environment.

Rapid diagnosis of children with flu-like symptoms is of great clinical importance, in particular for the guidance of clinical management. In case of a suspected influenza infection, there might be an indication to prescribe neuraminidase inhibitors, whereas in case of a suspected bacterial infection, the patient should be treated with antibiotics. Both therapies are associated with possible side-effects, which urges the development of clinical and laboratory markers to distinguish between viral and/or bacterial infection.[1,2] It has been shown that the discrimination of influenza from other viral and bacterial infections in children during an influenza epidemic, based on clinical signs and symptoms, is unreliable.[3] As testing for influenza by means of nucleic acid amplification techniques in practice takes one day or more, children with flu-like symptoms will often initially get treated with double-therapy until the final diagnosis is clear.

Earlier studies showed that the differentiated white blood cell count (WBC) and the C-reactive protein (CRP) may exceed the upper limits of normal in children with adenovirus infection or influenza, sometimes mimicking findings as can be observed during bacterial infection.[4,5] Discriminating between bacterial and viral infections, based on these parameters, might not be sufficiently adequate. In a number of pediatric studies, circulating levels of procalcitonin (PCT), the 116 amino acid pro-form of calcitonin, were mainly raised in the early stage of severe bacterial infection and only mildly raised in the absence of bacteria.[6-8] As a lower cut-off point for the exclusion of bacterial infection in children, values between 0.53-0.9 ng/mL have been suggested.[9-11] We hypothesize that PCT might be a useful tool for the discrimination of children with bacterial infection from children with viral infection during an influenza pandemic.

To our knowledge, the acute phase response in children with H1N1 infection has not been described before. To be able to evaluate the value of PCT in children with suspected viral infection during the H1N1 pandemic, more information on the acute phase response is needed. This study was performed to gain insight into the acute phase response in children with suspected H1N1 infection. Furthermore, the value of PCT in children with suspected H1N1 infection was investigated.

## MATERIALS AND METHODS

### Patients

From October until December 2009, 30 consecutive children with suspected novel influenza A (H1N1) infection (i.e. patients with fever (rectal temperature >38.2 °C or tympanic temperature >38 °C) and at least two of the following complaints: cough, rhinorrhea, myalgia, sore throat, headache, chills, malaise) who underwent venapuncture for routine diagnostic purposes, were included at the freely accessible (i.e. no referral needed) influenza outpatient clinic of the Slotervaart Hospital, Amsterdam, The Netherlands. As no extra blood for specific research purposes was needed and no additional patient discomfort was caused, informed consent was not deemed necessary.

### Diagnostic procedures

Standard blood tests included C-reactive protein (CRP) and differentiated white blood cell count (WBC). Quantitative PCT levels were measured using a specific immunometric assay (BRAHMS Ag, Hennigsdorf/Berlin, Germany) according to the protocol. From all patients a standard pharyngeal swab was obtained and, using primers as advised by the World Health Organization (WHO), real-time one-step reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays were used to detect the following pathogens: influenza A and subtype pH1N1, influenza B, rhinovirus, adenovirus, parainfluenza virus 1-4, enterovirus, human coronavirus, human metapneumovirus, respiratory syncytial virus, *Legionella* species, *Mycoplasma pneumoniae* and *Chlamydia pneumoniae*. Blood and sputum cultures, lumbar punctures and radiologic examinations were routinely ordered at the discretion of the treating physician.

### Statistical analysis

Values are presented as numbers with percentages or as medians with interquartile ranges (IQR). Correlations between CRP, leukocytes and PCT were assessed using Spearman's correlation test. Student's t-test was used for inter-group comparison. A p-value of less than 0.05 was considered significant. Statistical calculations were performed with SPSS software (version 17.0, SPSS Inc., Chicago, Illinois).

## RESULTS

Thirty consecutive patients were included. Patient characteristics are shown in Table 1. Twenty-five patients (83.3%) were diagnosed with RT-PCR confirmed viral illness, three

patients were diagnosed with a - clinically suspected - viral upper airway infection without positive RT-PCR result, one patient was diagnosed with a *Mycoplasma pneumoniae* infection and one patient was diagnosed with a non-confirmed bacterial pneumonia (double-sided infiltrates on the thoracic X-ray and a good response to antibiotics; cultures negative). Overall, 9 cases of H1N1 (30%), 11 cases of rhinovirus (36.7%), 5 cases of respiratory syncytial virus (16.7%), 4 cases of adenovirus (13.3%) and one case of human metapneumovirus (3.3%) were diagnosed (Table 2). A total of 18 children (60%) were hospitalized. Patients with respiratory syncytial virus, rhinovirus and H1N1 were most frequently hospitalized (hospitalization rate 100%, 54.5% and 44.4%, respectively).

**Table 1.** Patient characteristics.

| **Characteristics** | Total *n*=30 |
|---|---|
| Female sex, n (%) | 19 (63.3) |
| Age (months), mean ± SD | 24 ± 29.8 |
| Body temperature (°C), mean ± SD | 38.1 ± 1.01 |
| Heart rate (bpm), mean ± SD | 140 ± 24.5 |
| Oxygen saturation (peripheral), mean ± SD | 97.2 ± 3.2 |
| Hospitalized, n (%) | 18 (60) |

**Table 2.** Detected viruses by means of RT-PCR in children with suspected Novel Influenza A (H1N1) (n=30) during the H1N1 pandemic.

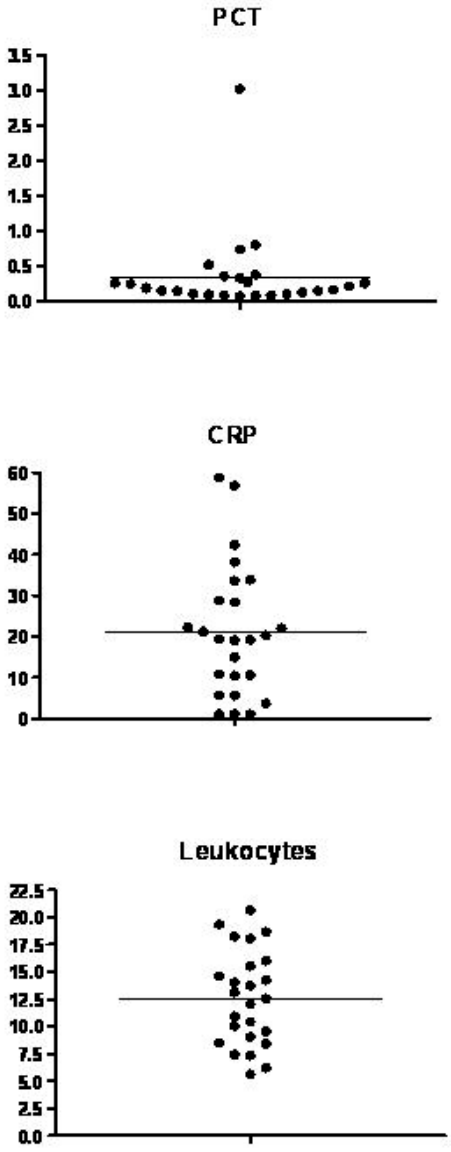
| **Virus** | ***n*** | **%** |
|---|---|---|
| Influenza A (H1N1) | 9 | 30.0 |
| Rhinovirus | 11 | 36.7 |
| Adenovirus | 4 | 13.3 |
| Respiratory syncytial virus | 5 | 16.7 |
| Human metapneumovirus | 1 | 3.3 |

*Sum of percentages for each virus exceeds the percentage of children diagnosed with a confirmed viral illness (83.3%) due to double infections.*

Median PCT value in the subgroup with confirmed viral infections was 0.153 ng/mL with IQR 0.094–0.261 ng/mL, median CRP value was 19.0 mg/L with IQR 5.6–28.3 mg/L and median leukocyte count was 13.1 giga/L with IQR 9.5–16.0 giga/L (Figure 1). Combining this subgroup with the three cases with suspected, but non-confirmed viral infection, did not significantly influence the results (median 0.155 ng/mL, IQR 0.09–0.262 ng/mL; median 19.05 mg/L, IQR 5.53–28.53 mg/L; median 12.55 giga/L, IQR 9.13–15.88 giga/L for PCT, CRP and leukocyte count, respectively). The one case with non-confirmed bacterial pneumonia

showed a marked acute phase response, with a PCT value of 32.17 ng/mL and a CRP value of 444.1 mg/L. The one case with metapneumovirus infection also exhibited elevated PCT and CRP (3.02 ng/mL and 42.3 mg/L), with normal leukocyte counts. The sensitivity of PCT for viral infection was 88%, at a cut-off <0.71 ng/mL - the earlier described lower cut-off point in healthy neonates -[10], whereas the sensitivity of CRP was 28% at a cut-off <10 mg/L and 76% at a cut-off <30 mg/L.

**Figure 1**. PCT levels (ng/mL), CRP levels (mg/L) and leukocyte counts (giga/L) in patients (n=25) with a laboratory confirmed (RT-PCR) viral infection at presentation.



Subgroup analysis per virus of PCT, CRP and leukocyte counts did not show any significant difference (data not shown), although a trend towards significance could be observed in leukocyte counts, with lower counts in patients with H1N1 compared to the other viral infections (p=0.07).

In the overall cohort, a significant correlation between PCT and CRP (ρ 0.531, p=0.003), between CRP and leukocyte count (ρ 0.445, p=0.016), but not between PCT and leukocyte count (ρ 0.041, p=0.83) could be observed. Exclusion of the one case with suspected bacterial infection still resulted in a significant correlation between PCT and CRP (ρ 0.479, p=0.010), between CRP and leukocyte count (ρ 0.383, p=0.044), and not between PCT and leukocyte count (ρ -0.065, p=0.74).

## CONCLUSIONS

Our study was aimed to characterize the acute phase response and to assess the value of PCT in children with suspected novel influenza A (H1N1) infection during the pandemic outbreak. Based on the results of this small cohort study, we can conclude that the acute phase response in children with H1N1 infection and other viral infections is mild, with low levels of CRP, PCT and leukocyte count. For CRP and leukocyte count, this is in conjuncture with earlier studies in children and adults.[4,5,12,13]

We show that values of PCT are mostly below 0.71 ng/mL - the earlier described lower cut-off point in healthy neonates -[10] in children with viral infection of any cause. One patient with a human metapneumovirus infection had a PCT value of 3.02 ng/mL. The reason for this finding is unclear; one explanation might be that this patient was suffering from an undetected bacterial co-infection.

Levels of CRP were considerably low in the subgroup with viral infections and showed a significant correlation with PCT levels. However, in addition to the one patient with human metapneumovirus infection (CRP 42.3 mg/L), 5 other patients exhibited CRP values ≥30 mg/L, whereas the generally accepted lower cut-off point is <10 mg/L.[14] In this cohort, in 5 patients with a viral infection the leukocyte count was elevated (i.e. >17.5 giga/L). A correlation with CRP, but not with PCT could be found.

In this cohort, PCT measurement in children with suspected H1N1 infection seems to be of added value during the diagnostic process. Low circulating levels of PCT appear to correlate

with the presence of viral infection and/or the absence of bacterial infection. Although circulating levels of CRP show a correlation with PCT and are generally low in patients with viral infection, a considerable amount of patients had moderately elevated levels of CRP. The same holds true for leukocyte counts.

Given this findings, low PCT levels seem to be of added value to support a diagnosis of viral infection during the diagnostic procedure in children with flu-like symptoms.

This study has some limitations. Apart from the small cohort size, a major drawback lies in the fact that we almost uniformly included patients with viral infections. To be able to analyze the prognostic properties of PCT, CRP and leukocyte counts in differentiating between viral and bacterial infections, a group of children with bacterial infection from the same cohort is needed. During the study period, we were only able to include one patient with a - non-proven - bacterial infection. However, many earlier studies have shown that values of PCT during bacterial infection in children can be elevated.[6-8] Because of the small sample size, analysis of the acute phase response between patients infected with different viruses is difficult. A future study should be powered to discover possible differences in levels of biomarkers between various viral infections. Another limitation is that diagnostic possibilities for bacterial infection in children are limited. To obtain culture materials may be difficult; moreover, a negative culture result does not exclude the presence of bacteria.[15] Although we confirmed the presence of virus in a large majority of our patients, a concomitant bacterial infection may be present in some. However, all of these patients recovered without antibiotics, suggesting that this possible presence of bacterial co-infection is of little clinical importance.

The clinical presentation of children with flu-like symptoms is aspecific. There is a need for accurate discriminative diagnostic tools to guide therapeutic management. Besides optimalization of microbiological and molecular diagnostic tests, the role and the use of a selection of biomarkers may be helpful in the clinical setting. PCT in combination with clinical symptoms and routinely used markers may be of use in this context, directing the clinician in choosing antiviral or antimicrobial therapy, but also guiding the selection of those patients at risk of developing severe disease and need for intensive monitoring.

In children with flu-like symptoms, a rapid and adequate diagnosis is of great importance. Reliable biomarkers that are readily available may enhance and quicken the diagnostic process. Based on our findings, PCT may be of added value when the etiology of flu-like symptoms is unclear and the treating physician is uncertain whether to initiate or withhold antibiotics.

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# Chapter 5

## Cardiovascular complications

# 5.1

# Respiratory virus infection and risk of acute coronary syndrome

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Submitted for publication

## ABSTRACT

**Background**: Previous studies have shown that signs and symptoms of respiratory tract infections are associated with a 2-5 fold increased risk of acute myocardial infarction. The purpose of this pilot study was to investigate whether laboratory proven recent viral respiratory tract infections are associated with an increased risk of acute coronary syndrome (ACS).

**Methods:** We designed a case-control study which was performed at the Slotervaart Hospital in Amsterdam, the Netherlands. Cases were patients with ACS admitted to the coronary care unit; matched controls were selected from the internal medicine outpatient clinic. Laboratory confirmation of infection was established by serological assays and real-time reverse-transcriptase-polymerase-chain-reaction. We performed logistic regression analysis to obtain an odds ratio for a diagnosis of ACS in relation to recent respiratory tract infection, adjusted for potential confounding factors.

**Results:** From January 7, 2008, until January 30, 2009, 41 cases and 41 matched controls were included: 7 (17%) cases and 6 (15%) controls had laboratory evidence of a recent respiratory viral infection. The adjusted odds ratio of ACS for patients who had laboratory proven recent respiratory viral infection was 1.1 (95% CI 0.3-4.0). None of 3 participants with influenza-like illness (3 cases, 0 controls) had a proven infection.

**Conclusions:** In this pilot study we did not find evidence for an increased risk of acute coronary syndrome in patients with respiratory viral infection, as proven by laboratory techniques. A diagnosis of respiratory tract infection based merely on signs and symptoms did not predict laboratory proven infection at all.

## INTRODUCTION

Atherosclerosis is considered to be an inflammatory disease.[1] It has been shown that systemic inflammation and infections accelerate atherogenesis in animals.[2] Inflammation does not only play a role in early atherogenesis, but may also be an important factor in causing instability of atherosclerotic plaques and, as a consequence, thrombotic complications such as myocardial infarction and ischemic stroke.[3,4] In humans, there is evidence that infections increase the risk of coronary artery disease: studies have shown that there is an association between the incidence of atherosclerosis and the presence of herpes virus and cytomegalovirus,[5] and inflammatory markers seem to predict the outcome in acute vascular events.[6] Furthermore, systemic factors such as infection seem to cause instability of atherosclerotic plaques. However, whether infectious agents are really important factors in the pathogenesis of atherosclerosis and acute ischemic events in humans is still controversial.[7]

Strong evidence for a role of acute respiratory infections in the pathogenesis of acute vascular events in humans comes from clinical studies.[8] Observational studies have demonstrated that both mortality and hospitalizations due to cardiovascular and cerebrovascular causes are increased during influenza epidemics.[9-11] Other studies suggested that protection from respiratory viral infection by vaccination against influenza virus reduces cardiovascular complications.[12,13] In case-control studies it has been shown that recent respiratory infections are associated with a 2-5 fold increased risk of acute myocardial infarction and atherothrombotic stroke.[14-17] However, none of these studies had validated the clinical diagnosis of respiratory tract infection by laboratory confirmation.

The purpose of this pilot study was to investigate whether laboratory proven recent viral respiratory tract infections are associated with an increased risk of acute coronary syndrome (ACS) and to evaluate whether the presence of clinical signs of respiratory tract infection could be confirmed by laboratory tests. Respiratory tract infections were evaluated both serologically and by means of real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) in a prospective case-control study in which patients with a proven ACS and matched control persons were included.

## METHODS

### Study design and population

This prospective case-control study was carried out at the Coronary Care Unit (CCU) and the Internal Medicine outpatient clinic of the Slotervaart Hospital in Amsterdam, the Netherlands, from January 7, 2008, until January 30, 2009. Epidemiological and clinical data were collected according to a standardized case record form. Blood samples were obtained at study inclusion and at a follow-up visit two weeks after inclusion; throat swab samples were collected once at study inclusion. The study was approved by the Institutional Ethical Review Board and all patients provided informed consent.

Consecutive patients that were admitted to the CCU with a proven ACS, either myocardial infarction or unstable angina, were included as cases. A diagnosis of myocardial infarction or unstable angina was considered proven when the definitions for measuring outcome in acute coronary syndromes of the American College of Cardiology were met.[18] We restricted the study to patients who were 80 years of age or younger at the date of inclusion. Exclusion criteria for cases were: a diagnosis of stable angina at admission; an ACS provoked by cardiac intervention; having had a coronary artery bypass graft or a percutaneous coronary intervention within 3 months before inclusion; blood samples not obtained within 72 hours after admission; non-Dutch speaking; and absence of written informed consent.

For the control group we included patients that visited the internal medicine outpatient clinic. We matched one control to each case by age and sex. We controlled for calendar time, and thereby for confounding by seasonal fluctuations in the circulation of respiratory viruses, by including control subjects within 7 days from inclusion of the matching case patient. Exclusion criteria for controls were: primary diagnosis of respiratory tract infection, history of coronary artery disease or cerebrovascular disease, and having chronic disorders for which yearly seasonal influenza vaccination is recommended (e.g. diabetes mellitus, HIV infection, and chronic obstructive pulmonary disease). In the Netherlands, seasonal influenza vaccination is also recommended for all adults aged 60 years and older; we did include participants until 80 years of age, and therefore senior individuals who might have been vaccinated could still be included.

A detailed clinical questionnaire was taken from both cases and controls at study inclusion in order to collect a proper history of recent and current signs and symptoms of respiratory tract infection. For each participant we determined whether criteria for an influenza-like illness (ILI) in the preceding two weeks before study inclusion were met. We used a case

definition that has been recommended by the United States Centers for Disease Control and Prevention (CDC): ILI was defined as fever >38 °C, and cough or sore throat.[19]

**Laboratory confirmation of infection**

Confirmation of infection was established by serological tests performed on paired blood samples two weeks apart, and nucleic acid amplification techniques performed on throat swab samples, both running in a routine laboratory setting at Erasmus Medical Center, Rotterdam, the Netherlands. Laboratory confirmed recent respiratory tract infection was defined as a positive result from the serological and/or molecular biological test. Analyses and determination of positive results were done blindly with regard to case/control status.

Presence of antibodies against respiratory pathogens was established using enzyme immunoassays (EIA) from Virion/Serion® (Würzburg, Germany). Antibody titers were determined and quantified for the following respiratory pathogens: influenza A virus, influenza B virus, parainfluenza virus type 1- 3, respiratory syncytial virus (RSV), adenovirus (all IgG and IgA); and *Mycoplasma pneumoniae* (IgG and IgM). Serologically confirmed recent respiratory tract infection was considered proven if the paired blood samples showed an increase in antibody titer (either IgG, IgA or IgM) of 4-fold or more, and/or if seroconversion from negative (below cut-off value) to positive (above cut-off value) was demonstrated.

Throat swab samples were tested by means of multiple validated duplex real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays. Nucleic acids of the following respiratory viruses were amplified and detected: influenza A virus, influenza B virus, parainfluenza virus types 1-4, human rhinovirus, human coronavirus 229E, human coronavirus OC43, human coronavirus NL63, human metapneumovirus, RSV-A, RSV-B, adenovirus, *Mycoplasma pneumoniae*, and bocavirus. Total nucleic acids were routinely isolated at the MagnaPureLC Isolation Station (Roche Applied Science, Penzberg, Germany). A universal internal control virus was used to monitor the whole process from nucleic acid isolation until real-time detection.[20]

In addition to these virological tests, high sensitivity C-reactive protein (hs-CRP) was measured using the Synchron LX system (Beckman Coulter, Fullerton, USA) according to the manufacturer's guidelines.

**Power considerations**

Based on the literature by Spodick et al.,[14] we assumed a difference of 13% in the occurrence of respiratory viral infections (controls 15% and cases 28%). Following this assumption, at

least 155 cases and 310 matched controls should be included to detect a significant difference between the study groups (power 0.90, statistical significance level at 0.05). However, these assumed prevalence rates can only be derived from incidence rates coming from previous studies that use signs and symptoms and not laboratory tests to confirm infection. Since we are planning a larger study to further investigate the association between laboratory proven respiratory viral infection and ACS, we designed this pilot study to have a more accurate estimate of the prevalence of positive serology or throat swabs. Therefore, we aimed to include 40 cases and 40 controls in this pilot design.

**Statistical analysis**

Statistical analysis was performed using the SPSS software package (version 18.0, SPSS Inc. Chicago, Illinois). Continuous variables were summarized as means and standard deviations (SD) or medians and interquartile range (IQR), and for categorical variables absolute numbers were used and percentages were calculated. We performed logistic regression analysis to obtain an odds ratio and the corresponding 95% confidence interval (CI) for a diagnosis of ACS in relation to recent respiratory tract infection. The influence of possible confounding factors (age, sex, smoking status, body mass index (BMI), physical activity, diabetes mellitus, previous influenza vaccination) was analyzed in a univariate model. If significant contribution ($p<0.10$) was found for any of the independent factors, these variables were added as covariates to the multivariate logistic regression model in order to obtain an adjusted odds ratio.

## RESULTS

**Study population**

From January 7, 2008, until January 30, 2009, 41 patients with an acute coronary syndrome and 41 matched controls were included. Throat swab samples were available for all participants; paired blood samples were available for 37 cases and 38 controls. Characteristics for cases and controls are summarized in Table 1. Matching ensured that age and gender distribution was comparable between cases and controls. Also, BMI did not differ between groups. As expected, cases were more likely to show less physical activity, more current smoking, and higher median hs-CRP levels. Influenza vaccination coverage was higher among cases than controls. Three cases and none of the controls met criteria for ILI.

**Table 1.** Patient characteristics.

| | | | Cases (*n=41*) | Controls (*n=41*) |
|---|---|---|---|---|
| **Age (years)** | | *mean (SD)* | 66 (10) | 66 (10) |
| **Sex** | ***Male*** | *n (%)* | 30 (73%) | 30 (73%) |
| | ***Female*** | *n (%)* | 11 (27%) | 11 (27%) |
| **BMI (kg/m²)** | | *mean (SD)* | 27.4 (4.7) | 26.8 (3.7) |
| **hs-CRP (mg/L)** | | *median (IQR)* | 16.4 (5.9-82.4) | 1.7 (0.9-3.3) |
| **Physical activity** | ***<15 minutes/day*** | *n (%)* | 15 (37%) | 7 (17%) |
| | ***15-30 minutes/day*** | *n (%)* | 10 (24%) | 4 (10%) |
| | ***>30 minutes/day*** | *n (%)* | 16 (39%) | 30 (73%) |
| **Smoking status** | ***Non-smoker*** | *n (%)* | 10 (24%) | 13 (32%) |
| | ***Past smoker*** | *n (%)* | 16 (39%) | 19 (46%) |
| | ***Current smoker*** | *n (%)* | 15 (37%) | 9 (22%) |
| **Primary diagnosis** | ***Myocardial infarction***[a] | *n (%)* | 40 (98%) | X |
| | ***Unstable angina*** | *n (%)* | 1 (2%) | X |
| | ***Yearly check-up*** | *n (%)* | X | 13 (32%) |
| | ***Hyperthyroidism*** | *n (%)* | X | 5 (12%) |
| | ***Hypertension*** | *n (%)* | X | 3 (7%) |
| | ***General malaise*** | *n (%)* | X | 2 (5%) |
| | ***Sleep apnea*** | *n (%)* | X | 2 (5%) |
| | ***Other***[b] | *n (%)* | X | 16 (39%) |
| **Relevant comorbidity** | ***Diabetes mellitus*** | *n (%)* | 6 (15%) | 0 (0%) |
| | ***Hypertension*** | *n (%)* | 18 (44%) | 13 (32%) |
| | ***Dyslipidemia*** | *n (%)* | 20 (50%) | 9 (22%) |
| | ***History of CVD*** | *n (%)* | 19 (46%) | 0 (0%) |
| **Influenza vaccination**[c] | | *n (%)* | 27 (66%) | 19 (46%) |
| **Influenza-like illness**[d] | | *n (%)* | 3 (7%) | 0 (0%) |
| **Confirmed recent respiratory viral infection** | | *n (%)* | 7 (17%) | 6 (15%) |

*Abbreviations: BMI - body mass index; hs-CRP - high sensitivity C-reactive protein*

[a]*Of all myocardial infarctions, 39 were typed as STEMI and 1 as NSTEMI.*

[b]*Other primary diagnoses include: hypothyroidism (n=1), abdominal complaints (n=1), thrombocytopenia (n=1), hypercholesterolemia (n=1), anemia (n=1), Lyme disease (n=1), cold extremities (n=1), chronic fatigue (n=1), hematuria (n=1), unspecified (n=7).*

[c]*Influenza vaccination in the (preceding) year of inclusion.*

[d]*Influenza-like illness according to CDC case definition.*

**Laboratory confirmation of infection**

Among the 41 cases and 41 matched controls, 7 (17%) cases and 6 (15%) controls had laboratory evidence of a recent respiratory viral infection (Table 1). None of these 13 patients met the criteria for ILI, and of the 3 cases who did meet criteria for ILI none had a proven respiratory viral infection. Table 2 summarizes the laboratory confirmation of all proven recent respiratory viral infections.

**Case-control analysis**

The crude odds ratio of ACS for patients who had any laboratory proven recent respiratory viral infection (compared with patients who had no such infection) was 1.2 (95% CI 0.4-3.9). In univariate analysis physical activity and previous influenza vaccination were identified as influential confounding factors ($p<0.10$, see methods). Adjustment for these potential confounders did not substantially alter the odds ratio (adjusted OR 1.1; 95% CI 0.3-4.0).

**Table 2.** Laboratory confirmed recent respiratory viral infections.

| Case / Control | RT-PCR result | Serology result |
|---|---|---|
| Case | positive for ADV | *negative* |
| Case | positive for RSV-a | *borderline IgG seroconversion for RSV* |
| Case | *negative* | IgG seroconversion for ADV |
| Case | *negative* | IgM seroconversion for HMPV; IgG seroconversion for PIV-2 |
| Case | *negative* | IgA seroconversion for ADV |
| Case | *negative* | IgG and IgA seroconversion for ADV; IgG seroconversion for HMPV |
| Case | *negative* | IgA seroconversion for PIV; IgA seroconversion for ADV |
| Control | positive for PIV-4 and HuCoV229E | *negative*[a] |
| Control | positive for RSV-a and Boca | *negative*[b] |
| Control | positive for RSV-a | *negative*[c] |
| Control | positive for RSV-a | *second blood sample not available for paired analysis* |
| Control | *negative* | IgG seroconversion for PIV-1 and PIV-2; IgA seroconversion for RSV |
| Control | *negative* | IgA seroconversion for PIV |

*ADV - adenovirus; RSV-a - respiratory syncytial virus, type a; RSV - respiratory syncytial virus, unspecified; HMPV - human metapneumovirus; PIV-2 - parainfluenza virus, type 2; PIV-4 - parainfluenza virus, type 4; PIV- parainfluenza virus, unspecified; HuCoV229E - human coronavirus, type 229E; Boca – bocavirus*

[a]*Serological tests for PIV-4 and HuCoV229E are not commercially available.*

[b]*Serological test for Boca is not commercially available.*

[c]*In both (paired) samples RSV IgG as well as IgA antibody titers were above cut-off values.*

## DISCUSSION

The findings of this prospective pilot study did not confirm an association between recent respiratory tract infection and a higher risk of ACS. Our results suggest approximately equal risks of ACS for patients with and without a laboratory proven recent respiratory viral infection, but the confidence intervals are wide. There was no relationship between clinical signs of respiratory tract infection and confirmative laboratory investigations.

This is the first prospective case-control study that uses both serology and RT-PCR to objectively determine recent respiratory tract infection. In previous studies an evident association between preceding symptoms of a respiratory tract infection and a higher risk of cardiovascular disease (CVD) has been demonstrated.[14-17] Even though these studies were well conducted with large sample sizes, a major drawback is that most were retrospective and none used laboratory confirmation of infection. There is a potential risk for misdiagnosis in these studies: prodromal symptoms of acute coronary syndrome might have been misdiagnosed as acute respiratory infection. PCR alone, or in combination with serology, has been shown to be an adequate method for respiratory virus surveillance.[21] One Chinese study group actually assessed antibodies to a limited range of respiratory pathogens: a strong association was found between acute myocardial infarction and previous influenza and respiratory syncytial virus infection.[22,23] However, follow-up blood samples in the convalescent phase, that are obviously needed in order to really demonstrate seroconversion and prove recent infection, were lacking and RT-PCR had not been performed.

In our population we found that 17% of ACS patients had laboratory evidence of an acute respiratory tract infection. In a well-designed case control study of considerable size by Meier et al. the absolute risk of acute myocardial infarction in association with acute respiratory tract infections was low; only 4% of acute myocardial infarction cases in their study population had an acute infection based upon a history of clinical signs and symptoms.[17] The difference in absolute risk between Meier's study and ours can be explained by a difference in sensitivity of the different diagnostic methods: compared with laboratory confirmation clinical case definitions that are based only on signs and symptoms have showed poor sensitivity when attempting to diagnose acute respiratory tract infection in clinical practice.[24] From our results it can also be concluded that the use of ILI case definitions is not specific in this situation: none of the 3 patients with a positive ILI-score corresponded to any of the 13 laboratory confirmations in our population. This is yet another argument for the risk of misdiagnosis bias when using clinical signs and symptoms only to diagnose recent respiratory tract infection in a population of patients with ACS. On the other hand,

one can question whether laboratory evidence of viral infection necessarily means clinical infection. However, in this setting we can never disentangle whether a positive laboratory test translates to clinical disease or merely corresponds to an asymptomatic carrier status.

Although we have not been able to confirm an association between respiratory viral infection and acute coronary syndrome, we did notice a difference between cases and controls: 5 adenovirus infections were found exclusively in patients with ACS. In vivo experiments have demonstrated that adenovirus and other respiratory viruses are capable of infecting cultured human endothelial cells and causing a shift from anticoagulant to procoagulant activity associated with the induction of tissue factor expression.[25] Keller et al. have previously demonstrated an increase in systemic levels of hemostatic proteins in elderly people after infection with influenza, parainfluenza, respiratory syncytial virus and human coronavirus.[26] It would be interesting to find more evidence from future studies for a pathophysiological role of adenovirus in particular and other potential candidate respiratory viruses that are less frequently studied.

An important limitation of this study is the moderate sample size. Due to the limited power as a consequence, we have not been able to detect statistically significant results, if any. However, we regard this study as a pilot study and we encourage future prospective case-control studies of considerably larger size that make use of laboratory confirmation of a recent respiratory viral infection. Another limitation is that we tested the most relevant pathogens only, and that certain respiratory pathogens may have been missed. Nevertheless, our multiple duplex RT-PCR assays did cover a wide range of respiratory pathogens. Furthermore, we did take into account seasonal fluctuations in the circulation of respiratory viruses by having a study period of one full year. It should also be mentioned that no laboratory proven influenza infections were found in our study participants. The absence of influenza infections can be explained by a low epidemic status during the study period. In addition, the relatively high vaccination coverage among cases and controls, which is mostly a consequence of the national recommendations to vaccinate all individuals aged 60 years or older, is another explanation for not having detected any influenza infections. Lastly, there is some disconcordance between the results of serology and PCR in our study: 7 patients with positive serology had a negative PCR and one patient with positive PCR truly had a negative serology result. It has been shown that RT-PCR for influenza diagnosis is 95% sensitive and 98% specific, relative to culture.[27] Serology, on the other hand, is known for its difficult interpretation, especially at low titers.[21] Nevertheless, these diagnostic tests have been developed for use in clinical situations and are not designed for asymptomatic study populations. The combination of serology and nucleic acid amplification techniques to

definitely prove infection is probably the most sensitive method for research purposes and we consider this a major strength of our study.

In summary, in this pilot study no evidence was found for an increased risk of developing acute coronary syndrome in patients with recent respiratory viral infection, as proven by laboratory techniques; however, confidence intervals were wide. There was no clear relationship between signs of respiratory tract infection in these patients and proven infection by confirmative laboratory investigations. A larger scaled prospective study using confirmative laboratory tests in order to diagnose respiratory tract infection with more reliable diagnostic specificity is warranted.

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# 5.2

# Influenza vaccination and hemostasis: no sustainable procoagulant effects from 2009 H1N1 influenza vaccination in healthy healthcare workers

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## ABSTRACT

**Background***:* Epidemiological observations have demonstrated an association between influenza epidemics and cardiovascular mortality. It remains unclear whether influenza vaccination reduces or increases the risk of acute coronary syndromes. We hypothesized that influenza vaccination induces coagulation abnormalities through a vaccine-induced inflammatory response.

**Methods***:* Healthy healthcare workers receiving an adjuvanted 2009 H1N1 vaccine (Focetria®; Novartis, Basel, Switzerland) were monitored for two weeks. Just before administering the vaccine and 14 days later blood was taken for routine hematology, hemagglutination-inhibition antibody response to pandemic virus A/California/007/09, and the following inflammatory and hemostatic markers: hs-CRP, PT, aPTT, VWF, FVIII, APC-sr, F1+2, ETP, and D-dimer.

**Results**: Ninety four participants, with a mean age of 37 (range 18-59) years were included for our analysis. Protective antibody titers were seen in 22 (23%) and 90 (97%) participants before and after vaccination, respectively; a fourfold rise or more in antibody titer was demonstrated in 80 participants (85%). Levels of leukocytes declined significantly after vaccination (p-value 0.012); PT increased from 11.8 to 11.9 seconds (p=0.006); D-dimer decreased from 0.30 to 0.26 μg/L (p=0.037); and VWF decreased from 98.6% to 92.2% (p<0.001). All other laboratory measurements did not change significantly. After adjustment for variation of hemostatic factors according to a circadian rhythm by estimating a linear regression model, only the decrease in D-dimer remained statistically significant different (adjusted difference -0.051 μg/L; 95% CI -0.090 to -0.011).

**Conclusion***:* This study failed to demonstrate any sustainable procoagulant effects of 2009 H1N1 influenza vaccination in healthy healthcare workers. The humoral immune response following influenza vaccination does not seem to be clinically important with regard to any speculative procoagulant changes in the hemostatic system.

## INTRODUCTION

The 2009 novel influenza A (H1N1) virus has been the first influenza virus to cause a true pandemic since the 1968 'Hong Kong flu'.[1] The initial cases of human respiratory infection that were caused by this new virus had been detected in Mexico in April 2009.[2,3] Soon after this event, the virus spread to the United States and from there to the rest of the world.[4] Severity of disease caused by the pandemic virus has generally been mild, and the highest incidence was observed among children and young adults.[5] Worldwide, more than 18,000 fatal cases have been reported to the World Health Organization (WHO).[6]

Preliminary and postmarketing evidence showed that 2009 H1N1 vaccines were immunogenic and safe with mild-to-moderate vaccine-associated reactions.[7-9] In August 2009, even though vaccines had not yet been licensed, the Centers for Disease Control and Prevention (CDC) recommended to vaccinate as many persons as quickly as possible, as soon as vaccines would become available.[10] This recommendation was also adopted by the Dutch government, leading to mass vaccination of certain target groups, among them healthcare personnel.

Epidemiological observations have demonstrated an association between influenza epidemics and cardiovascular mortality.[11] It has been reported that influenza vaccination reduces the risk of acute coronary syndromes as a complication of influenza infection.[12-14] Other reports, however, suggest the opposite.[15] For example, during the swine influenza vaccination program in the United States in 1976, sudden "cardiac deaths" in elderly people were reported. Combining all the available evidence, the net effect remains unclear: according to a 2008 Cochrane review there are not enough data to evaluate the effect of vaccination on coronary heart disease.[16]

The linkage between inflammation and coagulation is a well-known concept in the field of infectious diseases.[17] We hypothesized that influenza vaccination induces coagulation abnormalities through a vaccine-induced immunogenic, mild inflammatory response. To investigate this hypothesis we performed laboratory measurements reflecting coagulation, fibrinolysis and endothelial activation in healthy healthcare workers who received a 2009 H1N1 vaccine.

## METHODS

### Study design

Healthcare workers receiving an adjuvanted 2009 H1N1 vaccine (Focetria®; Novartis, Basel, Switzerland) were monitored for two weeks from the moment of vaccination. Clinical information and laboratory samples were collected both at study inclusion and at study ending. The study was performed at the Slotervaart Hospital in Amsterdam, The Netherlands, between November 16, 2009, and December 2, 2009. The study has been approved by the institutional medical ethics committee. All participants had provided written informed consent to participate.

### Participants and study procedures

Healthcare workers, aged 18 years or older, that had already signed up for the local voluntary influenza vaccination program were asked to participate in this study. Persons with cardiovascular disease, using anticoagulant therapy or with a febrile illness in the two weeks preceding vaccination were not included.

Information on general and relevant medical characteristics was gathered by asking the participants to fill out a short questionnaire both before and two weeks after vaccination. Within one hour before administering the vaccine (t=0) and 14 days later (t=1) blood was taken for routine hematology, influenza A (H1N1) specific serology, and inflammatory and coagulation markers.

### Laboratory assays

At both visits a total of 33 ml of blood was collected for the proposed laboratory assays. Citrated blood was centrifuged, and the supernatant re-centrifuged for 15 minutes at 2500 x g at 15 °C to obtain platelet-poor plasma within two hours after blood withdrawal; the remaining plasma was aliquoted and stored at -80 °C until further use. EDTA blood and serum blood was used to assess levels of hemoglobin, platelets, differentiated leukocytes and high-sensitivity CRP (hs-CRP); these assays were performed on the day of blood sampling using commercially available assays. Reference values are shown in Table 2. Serum blood was obtained for serological assays and stored at -20 °C until further use.

Coagulation and fibrinolysis tests were performed batch-wise after all participants had completed the study. Prothrombin time (PT), activated partial thromboplastin time (aPTT) and a functional assay of coagulation factor VIII (FVIII:C) were performed using an automated coagulation analyzer (Behring Coagulation System XP, Siemens Healthcare Diagnostics,

Marburg, Germany) with reagents and protocols of the manufacturer. D-dimer (Innovance, Siemens Healthcare Diagnostics) was also determined on the Behring Coagulation System. For the measurements of von Willebrand factor antigen (VWF), we used in-house enzyme linked immunosorbent assays (ELISA) with antibodies from DAKO (Glostrup, Denmark). Prothrombin fragment 1+2 (Enzygnost monoclonal F1+2, Siemens Healthcare Diagnostics) was determined by ELISA. Endogenous thrombin potential (ETP) was determined with a calibrated automated thrombogram (CAT). The CAT assays the generation of thrombin in clotting plasma using a microtiter plate reading fluorometer (Fluoroskan Ascent, ThermoLab systems, Helsinki, Finland) and Thrombinoscope software (Thrombinoscope BV, Maastricht, The Netherlands) as previously described.[18] Three parameters were derived from the thrombin generation curve: lag time, peak height, and ETP (area under the curve). Resistance to activated protein C (APC) was determined by testing the effect of APC on the ETP. The sensitivity of APC (APC-SR) of each plasma sample was determined in both the presence and absence of approximately 4 nM APC (f.c., Enzyme Research Laboratories). The APC concentrations used were adjusted to maintain a residual thrombin generation activity of approximately 10% in normal pooled plasma. Normal pooled plasma was run in parallel on each plate. The normalized APC-SR was determined by dividing the APC-SR of an individual by the APC-SR of the pooled plasma. A normalized APC-SR >1.0 reflects an APC resistant phenotype. Reference values for coagulation and fibrinolytic parameters are shown in Table 2.

The serum blood samples were also tested batch-wise for the presence of antibodies to 2009 H1N1 virus by performing a hemagglutination-inhibition assay using four hemagglutinating units of influenza virus A/California/007/09 and turkey erythrocytes. Antibody titers of 40 or higher were considered protective and a fourfold rise in antibody titer or greater in paired samples was considered a successful response to vaccination.

**Statistical analysis**

The main outcome of the study was the difference in levels of coagulation and fibrinolysis measurements before and 14 days after vaccination. We expected vaccination to cause a worsening hemostatic profile and, as a consequence, a corresponding change in coagulation measurements. To detect a standardized difference of at least 0.6 in the mean of coagulation parameters before and after vaccination, 88 participants were needed to obtain a power of 80% and significance level of 0.05. Taking into account a drop-out percentage of 10%, we aimed to include approximately 100 participants.

Statistical analysis was performed using the SPSS software package (version 18.0, SPSS

Inc. Chicago, Illinois). We used a paired samples t-test to test for statistical significance of the mean difference of the concerning laboratory measurements. Multivariate analysis was performed and, if necessary, a regression model was estimated to adjust for possible confounding factors. A probability value less than 0.05 was regarded statistically significant.

## RESULTS

### Participants

Characteristics of participants are shown in Table 1. One hundred and two participants were enrolled at study inclusion. Of those, 94 participants, 19 men and 75 women, with a mean age of 37 (range 18-59) years completed the study and had a second blood sample taken 14 days after vaccination. Only those participants that completed the study were included for our analysis.

**Table 1.** Characteristics of 94 healthy volunteers receiving pandemic influenza A (H1N1) vaccination.

| | | | | |
|---|---|---|---|---|
| **Age (years)** | *mean* | *(range)* | 37.1 | (18-59) |
| **Male** | *n* | *(%)* | 19 | (20.2) |
| **Female** | *n* | *(%)* | 75 | (79.8) |
| **OAC use** | *n* | *(%)* | 22 | (29.3) |
| **HAR-titer t=0** | *median* | *(range)* | 5.0 | (5-160) |
| **HAR-titer t=1** | *median* | *(range)* | 560 | (5-2560) |
| **≥4-fold rise** | *n* | (%) | 80 | (85.1) |

*HAR = hemagglutination-inhibition antibody response to pandemic influenza virus A/California/007/09*

### Laboratory assays

Table 1 demonstrates antibody responses to pandemic influenza virus A/California/007/09 in the 94 participants that were included. From one participant the hemagglutination-inhibition assay, performed on the second serum sample taken 14 days after vaccination (t=1), failed due to technical problems in the laboratory. Therefore, the rise in antibody titer between samples of each individual was determined in 93 participants. Protective antibody titers (i.e. ≥40) were seen in 23% and 97% before and after vaccination, respectively. Eighty (85%) showed a fourfold rise or more in antibody titer.

Table 2 demonstrates the mean values and standard deviation of the measured laboratory parameters. Since for each patient the parameters were measured before and after influenza vaccination, and the estimates of the means generally followed a normal distribution, these related measurements were compared using a paired samples t-test. The resultant

p-values are shown in the table. Levels of leukocytes declined significantly after vaccination (p-value 0.012); all other hematology and inflammation laboratory measurements did not change significantly. Small, but statistically significant changes in the mean values of three coagulation and fibrinolysis parameters were seen: PT increased from 11.8 to 11.9 seconds (p=0.006); D-dimer decreased from 0.30 to 0.26 µg/L (p=0.037); and VWF decreased from 98.6% to 92.2% (p<0.001).

**Table 2.** Labaratory parameters measured before (t=0) and after (t=1) influenza vaccination (all participants, n=94).

| | | t=0 | | t=1 | | p-value paired |
|---|---|---|---|---|---|---|
| **laboratory parameter** | *(reference value)* | **mean** | **SD** | **mean** | **SD** | **samples t-test** |
| *Inflammation* | | | | | | |
| **hs-CRP** | *(<8 mg/L)* | 2.49 | 4.18 | 3.61 | 8.91 | 0.189 |
| *Hematology* | | | | | | |
| **hemoglobin** | *(7.8 - 10.4 mmol/L)* | 8.53 | 0.66 | 8.45 | 0.65 | 0,065 |
| **leukocytes** | *(4.5 - 10.9 x 10E9/L)* | 7.11 | 2.18 | 6.71 | 1.99 | 0,012 |
| **platelets** | *(150 - 400 x 10E9/L)* | 275 | 61 | 277 | 59 | 0,663 |
| *Coagulation* | | | | | | |
| **PT** | *(10.7 - 12.9 sec)* | 11.8 | 0.7 | 11.9 | 0.7 | 0,006 |
| **aPTT** | *(25.0 - 38.0 sec)* | 32.9 | 3.9 | 33.0 | 3.9 | 0,562 |
| **VWF** | *(50 - 150 %)* | 98.6 | 33.1 | 92.2 | 28.0 | <0,001 |
| **fVIII** | *(63 - 173 %)* | 119.9 | 33.4 | 116.7 | 31.9 | 0,123 |
| *Anticoagulation* | | | | | | |
| **APC-SR** | *(<1.6 NR)* | 2.08 | 1.24 | 2.14 | 1.28 | 0,152 |
| *Thrombin generation* | | | | | | |
| **F1+2** | *(52 - 271 pmol/L)* | 162 | 79 | 204 | 293 | 0,147 |
| **lagtime ETP** | *(1.5 - 3.2 min)* | 2.80 | 0.69 | 2.82 | 0.68 | 0,482 |
| **peak ETP** | *(194 - 503 nM)* | 332.4 | 77.7 | 325.0 | 70.9 | 0,064 |
| **ETP (AUC)** | *(1155 - 2606 nM.min)* | 1731 | 376 | 1723 | 366 | 0,649 |
| *Fibrinolysis* | | | | | | |
| **D-dimer** | *(<1.00 µg/L FEU)* | 0.30 | 0.26 | 0.26 | 0.22 | 0,037 |

**Subgroup and multivariate analysis**

The following 6 subgroups were studied: men (n=19), women (n=75), women with systemic contraceptive use (n=22), women without systemic contraceptive use (n=53), individuals with ≥1 local or systemic adverse reaction after vaccination (n=47), and individuals with a ≥4-fold rise in antibody titer (n=80). VWF was the only parameter that consistently showed a statistically significant decrease 14 days after vaccination in all subgroups (data not shown). PT only increased significantly among male participants and D-dimer did not change significantly in any of the subgroups.

Hemostatic factors for an individual are known to vary according to a circadian rhythm.[19] To explore whether results were influenced by difference in timing of sampling, we performed a multivariate analysis; the difference in timing did indeed contribute significantly. We can adjust for this by estimating a linear regression model of the difference in the mean values of the performed laboratory measurements (after minus before) on the timing of both blood samples per patient introduced as dummy variables (morning or afternoon). Having the blood samples taken both in the morning or both in the afternoon is taken as reference group.

Table 3 summarizes the outcome of the linear regression analysis. Results are interpreted as follows: the intercept estimates the expected difference in mean values for the reference group, but also for the other groups after adjusting for the difference in blood sampling timing. For patients who had their samples taken at different times, the corresponding estimated coefficient (beta) should be added to the intercept. If the intercept is significantly different from zero we reject the hypothesis that mean levels of laboratory measurements before and after influenza vaccination are the same. As can be inferred from the table, only D-dimer shows a significant change from baseline (mean change -0.051 µg/L, p-value 0.013) after adjustment for timing of blood sampling. PT and VWF did not change significantly after adjustment.

**Table 3.** Linear regression results for the difference in hemostatic parameters associated with timing of blood sampling.

| | | *n=* | intercept (B) | 95% CI (B) | p-value | beta (β) | 95% CI (β) | p-value |
|---|---|---|---|---|---|---|---|---|
| **PT** | reference group* | *55* | 0.053 | -0.048; 0.154 | 0.302 | | | |
| | 1st sample morning; 2nd afternoon | *19* | | | | 0.253 | 0.053; 0.452 | 0.013 |
| | 1st sample afternoon; 2nd morning | *20* | | | | 0.037 | -0.158; 0.233 | 0.706 |
| **VWF** | reference group* | *55* | -3.6 | -7.5; 0.2 | 0.065 | | | |
| | 1st sample morning; 2nd afternoon | *19* | | | | -15.5 | -23.1; -7.9 | <0.001 |
| | 1st sample afternoon; 2nd morning | *20* | | | | 1.7 | -5.8; 9.1 | 0.657 |
| **D-dimer** | reference group* | *55* | -0.051 | -0.090; -0.011 | 0.013 | | | |
| | 1st sample morning; 2nd afternoon | *19* | | | | 0.010 | -0.069; 0.088 | 0.807 |
| | 1st sample afternoon; 2nd morning | *20* | | | | 0.075 | -0.002; 0.152 | 0.055 |

**Reference group = both blood samples taken in the morning or both in the afternoon*

## DISCUSSION

Our study showed that administering an adjuvanted 2009 H1N1 influenza vaccine in healthy healthcare workers did not result in a hypercoagulable state 14 days after intramuscular injection. These findings demonstrate that administration of this vaccine did not cause a sustainable procoagulant effect.

Most studies have investigated the long-term protective effect of both primary and secondary influenza vaccination with regard to clinical endpoints, such as cardiovascular disease and stroke.[12-14,20] Other studies have focused on the inflammatory and coagulation response in plasma within the first twenty-four hours after vaccination.[15,21] In the present study we focused for the first time on medium-term changes in coagulation and fibrinolysis in healthy volunteers.

Although the results of our study show that 2009 H1N1 influenza vaccination did not induce a hypercoagulable state, the small increase in PT and concomitant decreases in VWF and D-dimer were unexpected findings. When we adjusted for timing of blood sampling only D-dimer remained significantly different. Since D-dimer is generated from cross-linked fibrin, an elevated plasma concentration of D-dimer indicates recent or ongoing intravascular blood coagulation.[22] We observed a decrease in D-dimer concentration in our study subjects after vaccination, which may indicate impaired endogenous fibrinolysis after influenza vaccination.[23] However, we do not think that the D-dimer decrease that we found is clinically relevant. The absolute change is very small (adjusted difference -0.051 μg/L), and the value of plasma fibrinolysis activation markers in predicting cardiovascular disease is known to be limited.[24] In addition, since we tested multiple variables this finding could even be due to chance only.

Circadian variations in markers of coagulation and fibrinolysis are important sources of heterogeneity that may bias results of studies that include measurements of such markers.[19] We have been taking into account the circadian variation by statistically adjusting for this potential confounder. The results of the multivariate linear regression analysis that we performed, suggest that coagulation is affected by diurnal variation. Unlike our findings, several studies that have looked at circadian patterns of hemostatic systems have mostly shown a lower fibrinolytic activity in the early morning, and little or absent variation in VWF and certain coagulation factors.[25-28] Despite these contradictory findings between our results and the results of others, we think that timing of blood sampling should always be accounted for in studies on coagulation and fibrinolysis.

A limitation of our study is that we have collected laboratory samples from only two different points in time. The only post vaccination sample was taken after 14 days, coinciding with the peak humoral antibody response. We can only speculate about the dynamical course of the measured parameters earlier after vaccination. If we had collected blood samples more frequently during the study period of two weeks, it would have been possible to provide legitimate information about these dynamics. Rivard and Potier demonstrated spontaneous platelet aggregation soon after incubation of swine influenza vaccine with normal human platelet rich plasma.[15] In an other clinical study investigators have measured the inflammatory response within the first 24 hours after influenza vaccination.[21] These two studies imply that the first 24 hours after vaccination are an interesting period to look at with regard to the acute dynamics of inflammation and coagulation.

Another limitation is that we have included healthy volunteers only. Including a group of patients with cardiovascular disease would have been of considerable added value. Carty et al., for example, included men with and without carotid artery disease and they did find a difference in the acute phase response between the groups.[21] Because of this limitation, extrapolations of our results to patients with a high risk of cardiovascular morbidity and mortality should be made cautiously. Strengths of our study are the relatively large sample size and the extensive set of specific laboratory measurements that we used.

In conclusion, our study failed to demonstrate any sustainable procoagulant effects of 2009 H1N1 influenza vaccination in healthy healthcare workers. The humoral immune response following influenza vaccination does not seem to be clinically important with regard to any speculative procoagulant changes in the hemostatic system. Vaccination of individuals without significant comorbidity should therefore be considered safe with regard to the risk of cardiovascular disease.

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# Chapter 6

## Treatment

# 6.1

# Oseltamivir for treatment and prophylaxis of influenza: a systematic review of the evidence for clinical effectiveness and safety

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Submitted for publication

## ABSTRACT

**Objectives:** With this review we aimed to systematically evaluate and grade the evidence for clinical effectiveness and safety of oseltamivir treatment and prophylaxis in otherwise healthy individuals and individuals from recommended 'high-risk' populations.

**Methods:** Using predefined search terms combined with Cochrane search strategies for comparative studies, a PubMed search was performed. Data were extracted and a level of evidence was assigned to each study. Recommendations were subsequently graded.

**Results:** In total, 66 relevant articles were included in this review. In otherwise healthy individuals treatment reduces duration of illness by 0.5-1.5 days and secondary complications by 40-50% (level AI). Results regarding treatment effectiveness among those who are hospitalized or have severe, complicated, or progressive illness are conflicting (level CII). There is little evidence for treatment effectiveness among those who are at higher risk of complications (level CI-III), except for those with chronic pulmonary or cardiovascular disease in whom oseltamivir treatment significantly reduces secondary complications (RR 0.17-0.65; level BI). Oseltamivir prophylaxis offers a 64-75% protection rate (level AI). Nausea and vomiting are the most important adverse events. Incidence rate for oseltamivir resistance is currently 2.6% and resistance is associated with a 4.2 times increased risk of developing pneumonia (p=0.02).

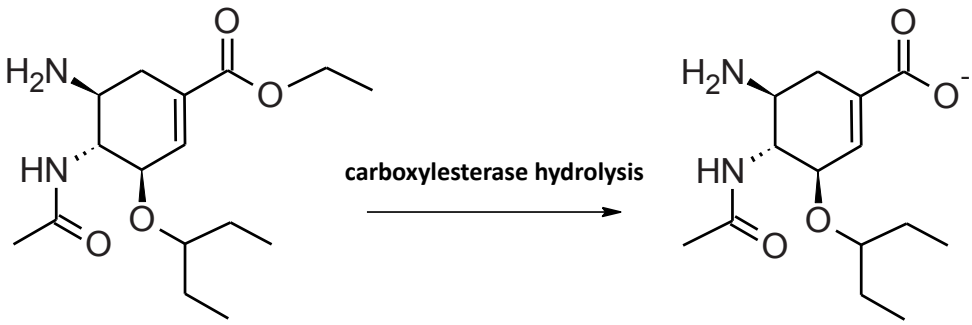
**Conclusions:** For otherwise healthy individuals, there is good, high-quality evidence that oseltamivir treatment reduces illness duration by approximately 1 day and nearly halves the risk of secondary complications and that prophylaxis is effective and safe; however, its use is not indicated for uncomplicated cases. There is moderate evidence to support the therapeutic use of oseltamivir among individuals with chronic pulmonary or cardiovascular disease. Evidence for individuals from other recommended 'high-risk' populations is scarce.

## INTRODUCTION

Influenza is an acute, mostly self-limiting illness that is predominantly characterized by fever and cough, and is caused by infection with influenza type A or B virus that occurs in outbreaks almost every winter. Although the course of influenza infection is usually mild, seasonal epidemics are associated with increased morbidity and mortality.[1] In case of the development of a novel, pandemic strain, excess morbidity and mortality may be expected.

Oseltamivir (Tamiflu®; F. Hoffmann-La Roche Ltd, Basel, Switzerland) is the most widely used antiviral medication to treat and protect against influenza. After intake, oseltamivir is readily absorbed from the gastrointestinal tract and rapidly hydrolyzed by liver carboxylesterase 1A1 into the active metabolite oseltamivir carboxylate (Figure 1).[2] Oseltamivir and oseltamivir carboxylate are almost fully excreted renally through glomerular filtration and active tubular secretion.[3] Together with zanamivir, which is administered by inhalation only, and the unregistered intravenous drug peramivir, oseltamivir belongs to the group of neuraminidase inhibitors.[4,5] Another group of drugs with activity against influenza A virus are the so-called adamantanes (amantadine and rimantadine). However, due to substantial resistance to this group of drugs, they are no longer recommended for antiviral treatment.[6]

**Figure 1.** After intake, oseltamivir is readily absorbed from the gastrointestinal tract and rapidly hydrolyzed by the liver carboxylesterase 1A1 into the active metabolite oseltamivir carboxylate.




**Oseltamivir** **Oseltamivir Carboxylate**

Antiviral drugs are effective for the prevention of influenza and, when used for treatment, can reduce the duration and severity of illness.[1] Several observational studies have identified patients who are at increased risk of developing complications from influenza infection.[7-12] The influenza-related complications in those 'high-risk' patients could be prevented by timely administration of antiviral medication. Therefore, expert advisory groups from different institutional settings from all over the world have been providing

rather similar recommendations on antiviral prophylaxis and treatment of influenza in certain 'high-risk' populations, particularly since their updates in relation to 2009's influenza A/H1N1 pandemic.[6,13-16] However, the level of the scientific evidence is on which those recommendations are built remains unclear.

With this review we aim to provide a systematic overview of the evidence for effectiveness of oseltamivir for treatment of influenza in otherwise healthy individuals and individuals from recommended 'high-risk' group populations, and for effectiveness of oseltamivir prophylaxis in the general population. Furthermore we aim to summarize the evidence for safety of oseltamivir by systematically describing reported adverse effects and giving a comprehensive overview of (clinically relevant) oseltamivir resistance. In addition, we scored the obtained evidence according to a distinguished system for grading recommendations in evidence based practice guidelines.

## METHODS

A systematic literature review was performed to answer the research question: "What is the evidence, underlying current guidelines, for clinical effectiveness and safety of oseltamivir for treatment and prophylaxis of influenza in otherwise healthy individuals and individuals from diverse 'high-risk' populations? ". The risk group classification that was used originates from a recently updated report by the United States Center for Disease Control's (CDC) Advisory Committee on Immunization Practices (ACIP) on the use of antiviral medication.[6]

**Search strategy and selection criteria**

A computer-assisted search of the PubMed electronic database up to September 2011 was performed to identify published studies on the clinical effectiveness and safety of oseltamivir for prophylaxis and treatment of influenza. The following search terms (text words and MeSH) were used: oseltamivir, Tamiflu, GS 4104, GS 4071 combined with highly sensitive Cochrane search strategies for systematic reviews and randomized controlled and clinical trials. No language restrictions were initially applied to the search strategy. Reference lists of included studies were searched for additional eligible studies. When data concerning relevant 'high-risk' populations were completely lacking after this first search strategy, a directed literature search using specific search terms was performed. With regard to oseltamivir resistance data a directed literature search only was performed.

Main inclusion criterion was that the paper had to compare oseltamivir treatment with placebo, control antivirals (e.g. zanamivir), or no antiviral treatment. Therefore, only comparative studies, i.e. either systematic reviews or meta-analyses of randomized controlled trials, randomized controlled trials (RCT), non-randomized clinical trials, and comparative observational studies, were included. Non-systematic reviews and non-human studies were excluded. No attempt was made to translate papers written in a language other than English.

**Data abstraction**

The following data were extracted from the included studies: year of publication, study population, number of participants, number of included studies (for systematic reviews and meta-analyses only), method of diagnosis of influenza infection (clinical or laboratory confirmed), influenza type and subtype if known, intervention, comparison, outcome, absolute risk of outcome, relative risk of outcome, mean or median duration of outcome, absolute difference in duration of outcome, and level of evidence. Outcome measures for treatment were: death, hospital admission, length of hospital stay, incidence of complications, antibiotic use, time to resolution of symptoms, time to return to normal activity, symptom severity, and relevant physiologic measurements (i.e. lung function tests). Outcome measures for prophylaxis were: laboratory confirmed influenza or influenza-like illness. Outcome measures for adverse events were: incidence and severity of adverse effects and treatment discontinuations or study-withdrawals as a consequence.

**Levels of evidence and grading of recommendations**

Levels of evidence were assigned to each contributing comparative study by the primary investigator and ranged from 1++ (high-quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias) to 4 (expert opinion). This scoring method was adopted from the Scottish Intercollegiate Guidelines Network (SIGN) system developed for grading recommendations in evidence based guidelines.[17] Based on the individual levels of evidence, for each 'recommended' population both a strength as well as a quality label of the evidence was provided. Recommendations were graded by using the Infectious Diseases Society of America (IDSA)−United States Public Health Service Grading System for ranking recommendations in clinical guidelines.[18] A summarized overview of the grading systems that were used can be found in Table 1.

**Table 1.** Methods of grading of recommendations.

| | |
|---|---|
| Levels of evidence[1] | |
| 1++ | High quality meta-analyses, systematic reviews of RCTs, or RCTs with a very low risk of bias |
| 1+ | Well conducted meta-analyses, systematic reviews of RCTs, or RCTs with a low risk of bias |
| 1- | Meta-analyses, systematic reviews of RCTs, or RCTs with a high risk of bias |
| 2++ | High quality systematic reviews of case-control or cohort studies *or* |
| | High quality case-control or cohort studies with a very low risk of confounding, bias, or chance and a high probability that the relationship is causal |
| 2+ | Well conducted case-control or cohort studies with a low risk of confounding, bias, or chance and a moderate probability that the relationship is causal |
| 2- | Case-control or cohort studies with a high risk of confounding, bias, or chance and a significant risk that the relationship is not causal |
| 3 | Non-analytic study, e.g. case reports, case series |
| 4 | Expert opinion |
| Strength of recommendation[2] | |
| A | Good evidence to support a recommendation for use |
| B | Moderate evidence to support a recommendation for use |
| C | Poor evidence to support a recommendation for use |
| D | Moderate evidence to support a recommendation against use |
| E | Good evidence to support a recommendation against use |
| Quality of evidence[2] | |
| I | Evidence from ≥1 properly randomized, controlled trial |
| II | Evidence from ≥1 well-designed clinical trial, without randomization; from cohort or case-controlled analytic studies (preferably from >1 center); |
| | from multiple time-series; or from dramatic results from uncontrolled experiments |
| III | Evidence from opinions of respected authorities, based on clinical experience, descriptive studies, or reports of expert committees |

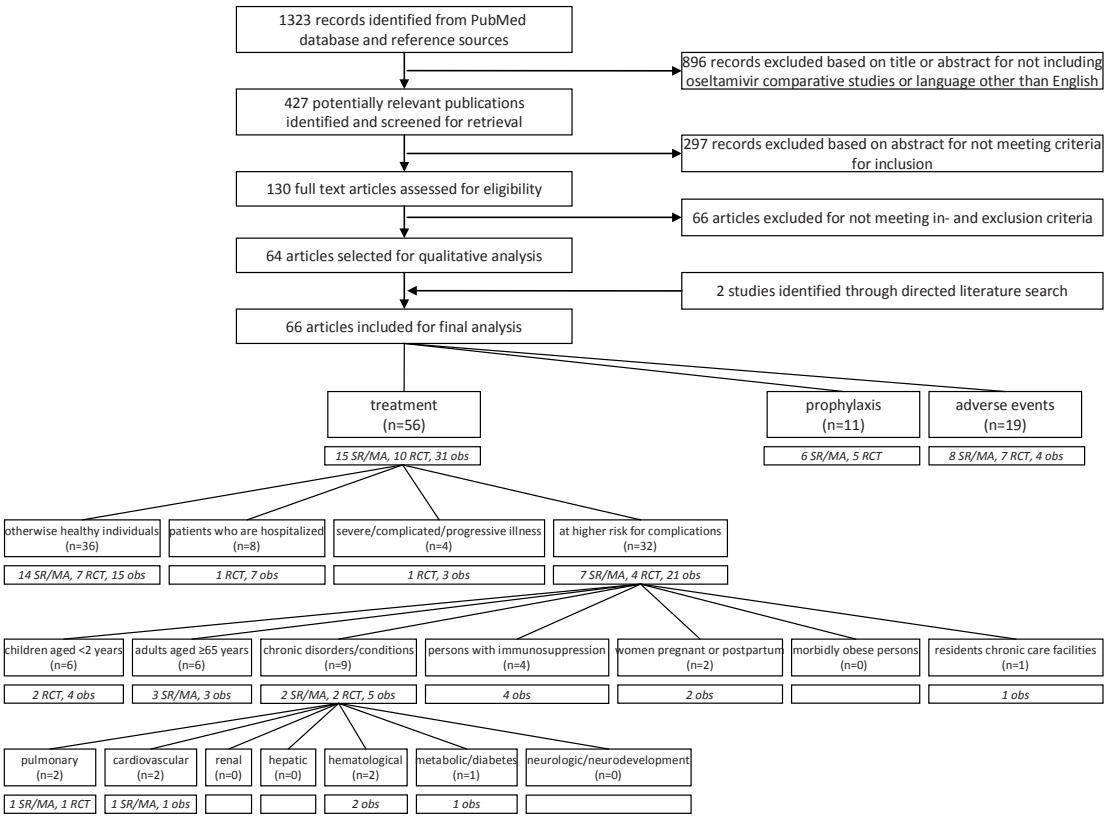
[1]*From: The Scottish Intercollegiate Guidelines Network (2001); A new system for grading recommendations in evidence based guidelines*

[2]*From: Infectious Diseases Society of America-United States Public Health Service (2001); Grading system for ranking recommendations in clinical guidelines*

## RESULTS

In total, 1323 citations from the database and reference sources were identified, of which 427 publications were considered potentially relevant (Figure 2). After intensive screening, 64 studies were carefully selected for inclusion in this review. Another 2 studies were identified through a directed literature search after the first strategy had failed at yielding any publications regarding certain 'high-risk' populations, rendering a total of 66 included studies.

**Figure 2.** Flow chart of search strategy and selection, including categorization of included studies.




*Because of considerable overlap between studies being categorized under different headings, and because some studies could not be classified any further downstream, the numbers of articles in the subdivided categories do not necessarily add up to the total number of articles in the embracing category.*

*Abbreviations:*
*SR/MA – systematic review or meta-analysis of randomized controlled trials*
*RCT – randomized controlled trial*
*obs – observational study*

**TREATMENT EFFECTIVENESS**

This section describes the evidence for treatment effectiveness of oseltamivir for otherwise healthy individuals and for the indicated 'high-risk' populations according to CDC's ACIP (i.e. hospitalized patients with confirmed or suspected influenza; patients with confirmed or suspected influenza who have severe, complicated, or progressive illness; children aged <2 years; adults aged ≥65 years; chronic disorders or conditions; persons with immunosuppression; women who are pregnant or postpartum; morbid obesity; residents of nursing homes and other chronic-care facilities).[6]

**Otherwise healthy individuals**

A total of 36 of the 66 studies concerned otherwise healthy individuals, both adults and/or children, as their sole or main investigated population. The highest level of evidence for a favorable effect of treatment with oseltamivir derives from 14 systematic reviews/meta-analyses and from 7 published RCTs. Six RCTs are not described separately here, because they have already been included in most of the meta-analyses summarized below.[19-24]

Three important papers can be considered as having the highest impact with regard to grade of evidence (level of evidence 1+/1++). Two Cochrane reviews have been included.[25,26] The other paper is a systematic review that has been included in the Health Technology Assessment program,[27] part of the United Kingdom National Institute for Health Research (NIHR), which is actually an update of the 2003 version.[28]

The Cochrane review and a preceding version of this paper by Jefferson et al. (level of evidence 1++) showed that healthy adults who were treated with oseltamivir were 20% more likely to have all symptoms alleviated than those on placebo by a given time point.[25,29] In addition, all types of complications were significantly reduced for those who were treated with oseltamivir from 6.3% to 3.4% (OR 0.39; 95% CI 0.28-0.55). These results were based on 3 RCTs including a total of 1797 participants.[19,20,24] The Cochrane review by Matheson et al. (level of evidence 1+) summarized data from two treatment trials,[22,23] and showed that among 452 children with laboratory proven influenza infection, treatment with oseltamivir significantly reduced median time to resolution of illness from 137 to 101 hours ($p<0.0001$).[26] Time to return to normal activity was reduced from 112 to 67 hours ($p<0.0001$). The overall rate of secondary complications was significantly lower among those who were treated with oseltamivir as compared to placebo (16.6% versus 27.7%), which was mainly caused by a decreased incidence of acute otitis media (RR 0.56; 95% CI 0.36-0.87).

The systematic review by Burch et al. (level of evidence 1++) studied an extensive range of outcome variables among adults and children.[27] Pooled results of 6 trials (1182 adult participants with laboratory confirmed influenza), including 3 RCTs that had also been included in Jefferson's Cochrane review,[19,20,24] showed a weighted median difference, comparing oseltamivir with placebo, of -22 hours (p=0.004) for time to alleviation of symptoms, of -63 hours (p=0.0006) for time to return to normal activity, and of -25 hours (p<0.0001) for time to alleviation of fever. Secondary complications were not significantly reduced (absolute risk 7.2% and 9.4% for those receiving oseltamivir and placebo, respectively). Another pooled analysis was performed with regard to children, based on two studies with a total of 631 participants.[23,30] With oseltamivir treatment, median time to alleviation of symptoms was significantly reduced (weighted median difference -29 hours; 95% CI -44 to -14), as well as median time to return to normal activity (weighted median difference -32 hours; 95% CI -47 to -17). However, as opposed to Matheson's Cochrane review,[26] this pooled analysis did not show a statistically significant treatment benefit with regard to secondary complications in children.

The remaining 9 systematic reviews and meta-analyses of RCTs (level of evidence 1+/1-) all showed in some way or the other a favorable effect of oseltamivir treatment regarding reduction in median time to alleviation of symptoms (range 0.6-1.5 days),[28,31-34] reduction of influenza-related complications (overall reduction by 40%),[31,35] antibiotic use for secondary complications (reduced by 50-70%),[28,36,37] and median time to return to baseline health scores.[38] The only (non-blinded) randomized trial that has not been incorporated by any systematic review, was performed by Wang et al. (level of evidence 1-).[39] This is the only trial that included individuals with laboratory proven pandemic 2009 influenza A/H1N1. Three hundred eight otherwise healthy adults were randomized to receive oseltamivir, placebo, or the Chinese traditional therapy maxinshigan-yinqiaosan. Oseltamivir use, compared with placebo, was associated with a 54% relative reduction in antibiotic use (RR 0.46; 95% CI 0.27-0.78).

Finally, 15 papers remain that are based on data from observational studies. Of these, 7 large retrospective observational studies (level of evidence 2+/2-) have compared complications in patients with a clinical diagnosis of influenza infection who were treated with oseltamivir with those who received no antiviral. Results were inconsistent: 3 studies demonstrated a decreased hospitalization rate,[40-42] whereas 2 did not;[43,44] 4 studies showed a decreased incidence of pneumonia diagnosis,[41-43,45] whereas 2 did not;[40,44] and 2 studies reported a decrease in antibiotic use,[42,43] whereas antibiotic use was not mentioned by the others. Two papers provided data on cardiovascular outcome and showed a reduced risk of stroke or

TIA and major cardiovascular outcome in favor of oseltamivir treatment.[45,46] Eight smaller studies (level of evidence 2-) have reported on diverse outcome variables and, in general, most of the results pointed towards a favorable effect for the use of oseltamivir.[47-54]

**Hospitalized with confirmed or suspected influenza**

Eight papers were retrieved that included hospitalized patients, all with laboratory confirmed influenza. Only one small RCT, appraised as having a relatively high risk of bias (level of evidence 1-), was found; the remaining 7 papers were observational studies, all being considered as having a high risk of bias as well (level of evidence 2-).

In the RCT hospitalized children with either seasonal influenza A or B (approximately 20 children in each group) were randomized to receive oseltamivir or no antiviral.[55] Among those infected with influenza A virus, duration of fever was significantly shorter for those treated with oseltamivir as compared to no antiviral (1.8 days versus 2.5 days; $p<0.05$). The same applies to those infected with influenza B virus (2.0 days versus 2.6 days; $p<0.05$). Duration of viral shedding did not significantly differ.

In 3 observational studies oseltamivir treatment was directly compared with no antiviral treatment. All three studies included hospitalized patients with laboratory confirmed seasonal influenza infection. Hanshaoworakul et al. reported a statistically significant reduction in overall death rate from 13% to 1.6% in favor of oseltamivir treatment,[56] whereas McGeer et al. reported a roughly comparable, but non-significant reduction in 15-day mortality rate from 10.1% to 3.9%.[57] Lee et al. found that viral shedding one week after symptom onset was significantly reduced in favor of oseltamivir treatment (RR 0.44; 95% CI 0.28-0.68).[58]

In 3 other observational studies of patients infected with 2009 pandemic influenza A/H1N1, early treatment with oseltamivir, i.e. ≤48h after symptom onset, was compared to late treatment, i.e. >48h after symptom onset. Higuera Iglesias et al. demonstrated a statistically significant decreased relative risk of developing severe pneumonia for adult patients in whom treatment was started early (RR 0.11; 95% CI 0.03-0.44).[53] Rodriguez et al. observed critically ill patients and showed a statistically significant shorter hospital and ICU length of stay (mean difference 6.8 and 4.3 days, respectively), and a significantly lower ICU mortality rate for those who received early treatment (22% versus 34%; $p=0.03$).[59] Hiba et al. demonstrated that the risks for severe complications, ICU admission and mechanical ventilation were significantly reduced in favor of early treatment; however, 30-day mortality rate was not significantly reduced.[60] From another observational study by Viasus et al., who

included 538 hospitalized patients with laboratory confirmed 2009 pandemic influenza A/H1N1, a multivariate analysis showed that for each day increase in time from onset of symptoms to oseltamivir administration, duration of fever, mortality, and hospital length of stay all significantly increased.[61]

**Confirmed or suspected influenza with severe, complicated, or progressive illness**

Only 4 comparative studies, including 1 RCT (level of evidence 1-) and 3 observational studies with a high risk of bias (level of evidence 2-), have been identified that report on oseltamivir effectiveness in patients with influenza who have severe, complicated, or progressive illness. Because these patients are usually hospitalized, all 4 included papers have already been reviewed under the previous section. In summary, oseltamivir treatment significantly reduced duration of fever by approximately half a day,[55] death rate was significantly lower,[56] and clinical outcomes were significantly better with early versus late oseltamivir treatment.[53,59]

**At higher risk for influenza complications**

A total of 32 of 66 comparative studies included individuals who were at higher risk for influenza complications. Most of these papers reported their outcomes for individual, more or less specific 'at risk' populations, and therefore they are summarized under the corresponding subheadings below. However, 9 papers could not be classified under one or more of the restricted populations since mixed 'at risk' populations were described which could not be any further differentiated. These papers are summarized in the next paragraph.

Four systematic reviews produced meta-analyses with regard to combined 'at risk' subgroups. A systematic review of high quality (level of evidence 1++) pooled the results of in total 907 individuals coming from combined 'at risk' populations: the weighted median difference for time to alleviation of symptoms and for time to return to normal activity was not significantly lower for those being treated with oseltamivir as compared to placebo; secondary complications were not significantly reduced, however, antibiotic use was (OR 0.57; 95% CI 0.33-0.98).[27] Burch et al. meta-analyzed 6 RCTs in a well conducted systematic review (level of evidence 1+), including a total of 1472 individuals from different 'at risk' groups, and demonstrated a non-significant pooled difference of 0.74 days in time to symptom alleviation in favor of oseltamivir treatment.[31] Cooper et al. pooled the results of 5 individual trials (level of evidence 1-), and they also did not demonstrate a significant difference regarding time to symptom alleviation.[34] Kaiser et al. performed a meta-analysis (level of evidence 1-) for a combined subgroup of elderly individuals, patients with chronic obstructive airway disease, and patients with chronic cardiac disease. Six RCTs were

included with a total of 769 individuals: compared to placebo, oseltamivir was associated with a reduced risk for all lower respiratory tract complications leading to antibiotic use (RR 0.66; 95% CI 0.47-0.93).[36] Finally, several observational studies that included mixed 'at risk' populations have shown that oseltamivir treatment was significantly associated with reduced viral shedding,[58] and that early treatment initiation was significantly associated with fewer secondary complications,[53,60] a shorter hospital length of stay,[59,61] and a decreased mortality rate.[59,61]

***Children aged <2 years***

Six papers could be retrieved that specifically focus on the 'high-risk' population of children younger than 2 years of age. Two RCTs have studied children with laboratory confirmed influenza infection who were randomized to receive either oseltamivir or placebo. Whitley et al. provided subgroup results for 94 children <2 years of age (level of evidence 1+).[23] Median duration of illness in the oseltamivir treatment arm was 5.8 days compared to 6.7 days in the placebo arm (non-significant). A more recently published RCT by Heinonen et al., appraised as having a rather high risk of bias (level of evidence 1-), included 98 children aged 0-3 years.[62] Time to resolution of illness was significantly reduced from 5.7 days in the placebo group to 4.3 days in the oseltamivir treatment group (p=0.004); incidence of acute otitis media was not significantly reduced. Gums et al. performed a large, well designed, retrospective observational study (level of evidence 2+) of 7653 children (3804 oseltamivir, 3849 no antiviral) aged 0-5 years.[40] Acute otitis media was the only secondary complication with a significantly lower incidence rate among those who received oseltamivir (RR 0.81; 95% CI 0.72-0.92). From subgroup analyses of the remaining observational studies (all had level of evidence 2-) it was shown that the duration of fever was significantly shorter and the incidence of secondary complications was reduced with the use of oseltamivir.[41,43,48]

***Adults aged ≥65 years***

Six studies have been published that focus on oseltamivir treatment effectiveness in elderly individuals (aged ≥65 years). In two subsequent versions of the same well-designed, high-quality meta-analysis (level of evidence 1++), subgroup analyses were performed for elderly individuals, all with laboratory confirmed seasonal influenza infection, who were randomized to receive oseltamivir treatment or placebo.[27,28] In the most recent version, data from a total of 477 elderly patients from 3 RCTs were combined and it was shown that oseltamivir treatment reduced time to return to normal activity by 3.1 days, which was borderline significant (p=0.06). Based on data from one RCT only, time to alleviation of symptoms was reduced by one day, although this was not statistically significant. In addition, a non-significant reduction in incidence rate of pneumonia, antibiotic use, and complications

requiring hospitalization was demonstrated.[27] Another meta-analysis of reasonable quality (level of evidence 1+) performed a subgroup analysis among 917 individuals aged >50 years and also found a similar non-significant decrease in time to alleviation of symptoms.[33] Three comparative observational studies of elderly patients with influenza remain. Bowles et al. found that oseltamivir treatment was associated with statistically significant reductions in secondary complications and hospitalization,[63] whereas Blumentals et al. did not find any statistically significant reductions.[44] Madjid demonstrated a statistically significant decreased risk of stroke and TIA up until 6 months among elderly individuals who were treated with oseltamivir.[46]

***Chronic disorders or conditions***

Chronic disorders or conditions that are associated with a higher risk for influenza are further subdivided according to organ system, i.e. pulmonary, cardiovascular, renal, hepatic, hematological, metabolic, and neurologic/neurodevelopmental disorders. One study described a mixed population of individuals with diverse chronic medical conditions corresponding to different organ systems, which will be summarized first. Two treatment studies focused on a combined population of individuals with pulmonary and cardiovascular disorders. Six treatment studies specifically focused on pulmonary, cardiovascular, hematological or metabolic disorders. No evidence could be found for treatment effectiveness in specified populations of patients suffering from any other chronic disease.

First of all, Piedra et al. retrospectively studied a mixed population of 5355 children aged 1-17 years with diverse chronic medical conditions, i.e. cardiac disease, chronic lung disease, diabetes mellitus, immunocompromised, renal dysfunction, central nervous system disorder/disease, and neuromuscular disease (level of evidence 2-).[64] Compared to no antiviral treatment, pneumonia and respiratory illness other than pneumonia were not significantly reduced; however, otitis media and its complications and hospitalization did show statistically significant reductions (HR 0.61; 95% CI 0.47-0.79 and HR 0.56; 95% CI 0.32-0.98, respectively).

Turner et al. performed a combined subgroup analysis (level of evidence 1++) for laboratory proven influenza infected patients with known chronic obstructive airway disease or chronic cardiac disorders.[28] Combining the results of 10 separate trials (n=769), they found a statistically significant decreased risk for lower respiratory tract complications requiring the use of antibiotics, with incidence rates of 12% and 19% for those on oseltamivir and placebo, respectively (OR 0.62; 95% CI 0.40-0.94). Another study, which is a randomized trial appraised as having a rather high risk of bias due to an open-label design (level of evidence

1-), also combined a population of 56 individuals with chronic respiratory or cardiac disease with laboratory confirmed influenza.[65] Comparing oseltamivir with symptomatic treatment, it was shown that time to remission of all influenza symptoms was significantly lower (4.6 days versus 7.3 days; p=0.0479), as well as the incidence of secondary complications (11% versus 45%), and antibiotic use (37% versus 69%).

The reasonably conducted meta-analysis by Singh et al. provided data from a subgroup of influenza infected patients with chronic obstructive airway disease (level of evidence 1+/-).[33] It was shown that median time to alleviation of febrile illness was significantly shorter for oseltamivir treatment as compared to placebo (1.6 and 2.2 days respectively, p=0.004). Johnston et al. performed an RCT in which they had included children with asthma (level of evidence 1-).[30] A total of 179 children with laboratory confirmed influenza infections were randomized to receive oseltamivir or placebo treatment. Mean time to return to normal health and activity, mean time to alleviation of all symptoms, and mean total symptom score was not statistically significant different between groups. However, the improvement in FEV1 values between study entry and day 6 was greater among oseltamivir recipients than among placebo recipients (median improvements from entry of 10.8% and 4.7%, respectively; p=0.01). In line with this, asthma exacerbations, as measured by pulmonary function at day 7, were significantly reduced from 50% to 32% (RR 0.65; 95% CI 0.45-0.95), in favor of oseltamivir treatment.

The same meta-analysis by Singh et al. also performed a subgroup analysis of patients with laboratory confirmed influenza infection with chronic cardiac disease (level of evidence 1+/-).[33] Median time to alleviation of febrile illness was 1.8 days among those who received oseltamivir and 2.7 days among those who received placebo (p=0.026). In addition, a large retrospective observational study provided interesting data on cardiovascular outcomes of over 30.000 individuals with known cardiovascular disease (level of evidence 2-).[66] Within 30 days after a clinical diagnosis of influenza any recurrent cardiovascular outcome, angina pectoris, heart failure, myocardial infarction, sudden cardiac death, and stroke were all significantly reduced in favor of oseltamivir treatment, with relative risks ranging from 0.17 to 0.51.

With regard to hematological disorders, two observational studies were retrieved (level of evidence 2-). The first included 111 hematopoietic stem cell recipients with laboratory confirmed influenza and showed a significantly decreased incidence rate of pneumonia, which was 10% among those who were treated with oseltamivir and 42% among those who were not (RR 0.24; 95% CI 0.09-0.62).[67] The other study included 62 hematopoietic stem cell

recipients with laboratory confirmed influenza and did not show any statistically significant difference with regard to progression to pneumonia.[68]

Finally, one study remains which is the only one that focuses on patients with metabolic disease, all with diabetes mellitus.[69] Orzeck et al. performed a retrospective observational study of 9090 diabetics with a clinical diagnosis of influenza infection (level of evidence 2-). The investigators showed that pneumonia and otitis media and its complications occurred with an equal frequency among those treated with oseltamivir and those who did not receive antiviral treatment. However, other respiratory illness and hospitalization for any reason was slightly but significantly lower among those treated with oseltamivir.

***Persons with immunosuppression***

Only 4 publications (level of evidence 2-) were retrieved that reported on oseltamivir effectiveness in persons with immunosuppression (including that caused by medication or HIV infection). Of those, two included hematopoietic stem cell recipients and these have already been described above.[67,68] Kumar et al. compared early versus late oseltamivir treatment in a population of 115 recipients of solid organ transplants, all with laboratory confirmed pandemic 2009 influenza A/H1N1 infection.[70] The ICU admission rate was significantly lower for those in whom oseltamivir treatment was initiated within 48 hours after symptom onset. One observational study was found that included 30 HIV positive patients with confirmed 2009 influenza A/H1N1 virus infection.[71] It was demonstrated that delayed administration of oseltamivir was significantly associated with mortality (p=0.0022).

***Women who are pregnant or postpartum***

Evidence for a beneficial effect of oseltamivir treatment in women who are pregnant or postpartum came from two observational studies of moderate quality (level of evidence 2-). The first study included 220 pregnant women with laboratory confirmed pandemic 2009 influenza A/H1N1 infection and compared early antiviral treatment with no antiviral treatment.[72] However, only approximately one third of the women who were treated early used oseltamivir and most other women were treated with inhaled zanamivir. ICU admission rate was 9.2% in the early antiviral treatment group versus 38.2% in the group without early antiviral treatment; the odds for ICU admission were significantly reduced (OR 0.16; 95% CI 0.08-0.34). The other observational study, of 62 pregnant women with laboratory-confirmed 2009 H1N1 influenza, showed that only 3.3% of pregnant women who received oseltamivir treatment within 2 days of symptom onset had severe illness compared to 21.4% and 44.4% for those in whom treatment was started 3-4 days and ≥5 days after symptom onset, respectively (p=0.002 for trend).[73]

***Morbid obesity***

No comparative studies were retrieved.

***Residents of nursing homes and other chronic-care facilities***

Only one comparative observational treatment study (level of evidence 2-) could be retrieved that focuses specifically on residents of nursing homes. Bowles et al. studied 73 elderly nursing home residents with laboratory confirmed influenza infection.[63] Compared with no treatment, early oseltamivir was significantly associated with less antibiotic therapy, fewer serious complications, fewer hospitalizations, and a significantly smaller death rate.

**PROPHYLAXIS**

According to CDC's ACIP recommendations, postexposure chemoprophylaxis should be used only when antivirals can be started within 48 hours of the most recent exposure, and preexposure chemoprophylaxis should be used only for persons who are at very high risk (e.g. severely immunosuppressed patients) for influenza-related complications, who cannot otherwise be protected during times when a high risk of exposure exists.[6] In the event of concern about potential shortage of antiviral medications, CDC or other health authorities might recommend prioritizing prophylactic treatment of certain 'high-risk' populations. Eleven publications were identified regarding oseltamivir prophylaxis, of which 6 were systematic reviews and meta-analyses and 5 RCTs. All of the retrieved articles have a high level of evidence (1+ or 1++).

Pooled results of the Cochrane review by Jefferson et al. showed that oseltamivir prophylaxis for healthy adults was associated with a significantly, approximately 75% decreased relative risk of obtaining laboratory confirmed influenza, as compared to placebo.[25] An earlier publication by the same author describes an almost identical meta-analysis with similar results.[29] Another systematic review showed that once daily oseltamivir 75 mg prophylaxis was associated with significantly decreased odds of acquiring laboratory confirmed influenza for otherwise healthy adults (OR 0.26; 95% CI 0.08-0.84), elderly individuals (OR 0.08; 95% CI 0.01-0.61), and adults with comorbid conditions (OR 0.10; 95% CI 0.03-0.34).[28] Khazeni et al. pooled the results from 4 RCTs and proved that 6 weeks duration oseltamivir chemoprophylaxis was associated with a comparable statistically significant risk reduction of obtaining symptomatic influenza (RR 0.24; 95% CI 0.14-0.39).[74] Shun-Shin et al. performed a systematic review, and based their results with regard to prophylaxis effectiveness in children on one RCT solely.[75] They showed a statistically significant relative risk reduction of 65% in the incidence of symptomatic influenza with oseltamivir chemoprophylaxis.[32] The

Cochrane review by Matheson et al. included the same prevention RCT on which they based their results.[75] Likewise they showed a statistically significant protective efficacy of 64%.[26]

Hayden et al. published 3 oseltamivir prophylaxis RCTs. In one trial 476 household contacts of index cases with confirmed influenza infection were randomized to receive 10 days of pre-exposure prophylaxis or expectant treatment (i.e. treatment with oseltamivir for 5 days if illness occurs).[75] Influenza infections were seen among 1.8% in the pre-exposure prophylaxis arm versus 11.3% in the expectant treatment arm, which is a statistically significant risk reduction. In another trial 1559 individuals were randomized to receive oseltamivir or placebo for a duration of 6 weeks.[76] Significantly less laboratory confirmed influenza-like illnesses were found among those receiving oseltamivir treatment compared to those receiving placebo (1.3% and 4.8%, respectively). The third RCT by Hayden et al. included 33 healthy individuals who were infected with experimental influenza virus.[21] No symptomatic influenza infections were observed among 21 individuals who received oseltamivir treatment, whereas two thirds of 12 individuals who did not receive oseltamivir developed influenza-like illness. Welliver et al. performed an RCT in which 415 household contacts of influenza index cases were randomized to oseltamivir 75 mg once daily prophylaxis or placebo.[77] The proportion of individuals diagnosed with laboratory confirmed clinical influenza was 1.4% and 12.6% among those who received oseltamivir and placebo, respectively, which was a statistically significant relative risk reduction of 83%. The last RCT was performed among a special population of elderly, frail individuals who received either 75 mg of oseltamivir once daily for 6 weeks or placebo.[78] Laboratory confirmed clinical cases were significantly less frequently observed among oseltamivir recipients (0.4%) than among placebo recipients (4.4%).

**ADVERSE EVENTS**

Adverse events have been reported among the outcomes in 19 of the in total 66 included comparative trials. Two high-quality Cochrane reviews (level of evidence 1+/1++), of which the safety results have been summarized by Jones et al., have concluded that the odds for developing vomiting were significantly increased when using oseltamivir for treatment in children (OR 1.68; 95% CI 1.15-2.47), and that the odds for developing nausea were significantly increased when using oseltamivir for prophylaxis in adults (OR 1.79; 95% CI 1.10-2.93).[25,26,79]

Regarding adverse effects of oseltamivir treatment, two meta-analyses (level of evidence 1+/-) demonstrated a 1.5-2-fold statistically significant increased risk of nausea and vomiting

with the therapeutic use of oseltamivir.[33,37] However, from the results of several randomized placebo-controlled trials, no statistically significant increased rate of any relevant adverse event could be demonstrated,[21,23,24,30] which is also confirmed by Burch's high-quality meta-analysis (level of evidence 1++).[27] No significantly increased discontinuation rate because of adverse events has been demonstrated.[23,33] Furthermore, although from one observational study a non-significant increased risk of all grade 3-4 adverse events was shown with oseltamivir treatment,[80] others oppositely reported slightly but significantly decreased risks for a variety of diverse adverse events in favor of oseltamivir therapy.[41,45,81] Finally, neuropsychiatric side effects were not reported to be a significant side effect of oseltamivir treatment by most studies,[30,37,41,45,80,81] with the exception of headache which was reported as 1.5-fold significantly increased by one meta-analysis.[37]

Several meta-analyses of prophylaxis trials and the corresponding RCTs themselves have demonstrated conflicting results. The risk for nausea was consistently approximately 2-fold significantly increased with the prophylactic use of oseltamivir.[77,82] Although the overall rate of adverse events was reported not to be significantly increased by two studies,[74,78] a 1.87 statistically significant increased relative risk was reported by one RCT.[83]

**RESISTANCE**

The prevalence of oseltamivir resistance had been relatively low until 2007, and emerged in only 1 to 5 percent of ambulatory care patients.[84,85] However, in the 2008-2009 influenza season, the US CDC reported a prevalence of oseltamivir resistance of 97% among seasonal influenza A/H1N1 viruses. With the onset of the 2009-2010 influenza A/H1N1 pandemic, in which genetic reassortments had caused an antigenic shift resulting in the emergence of a new pandemic influenza virus, the predominantly oseltamivir-resistant seasonal H1N1 strain was replaced by a predominantly oseltamivir-susceptible pandemic strain. Oseltamivir resistance has been observed rarely among influenza B viruses.[86] The majority of cases of oseltamivir resistance have not resulted in cross-resistance to zanamivir.[87]

The increased resistance to oseltamivir among influenza A virus strains has been associated with a specific mutation that results in an amino acid substitution in the conserved active site of the neuraminidase (H274Y). This mutation is sometimes described as "H275Y" using a different numbering system.[88] The observed IC50 values for virus strains with this specific point mutation are usually 200-400 fold higher, thus dramatically decreasing susceptibility of the virus to oseltamivir.[89-91] Other mutations that confer resistance to oseltamivir have been described, although these have not caused widespread resistance to date.[92]

A recently published systematic review of influenza resistance to the neuraminidase inhibitors meta-analyzed the results of 15 studies and yielded a pooled incidence rate for oseltamivir resistance of 2.6% (95% CI 0.7-5.5%).[93] Data comparing the clinical manifestations of infections with resistant and susceptible strains of the same influenza virus are limited. However, a meta-analysis of 4 studies reported an association between oseltamivir resistance and pneumonia and yielded a statistically significant risk ratio of 4.2 (95% CI 1.3-13.3, p=0.02).[93] Oseltamivir resistance was not statistically significantly associated with other clinical complications and symptoms.

## SUMMARY OF EVIDENCE AND GRADING OF RECOMMENDATIONS

This review provides an overview of the evidence for clinical effectiveness of oseltamivir treatment in otherwise healthy individuals and individuals from relevant 'high-risk' populations, and for effectiveness of oseltamivir prophylaxis. Together with a summary of our findings and the assigned levels of evidence, a grade of recommendation for the therapeutic use of oseltamivir in each specified patient population and for prophylactic use of oseltamivir can now be given (see Table 2).

The evidence for treatment effectiveness is highest among otherwise healthy individuals. A worthy number of important systematic reviews (level of evidence 1+/1++) have demonstrated a favorable effect of oseltamivir treatment, both with regard to duration of illness as well as secondary complications.[25-28,35] This results in the highest grade of recommendation (AI-II) for this population. Regarding patients who are hospitalized with influenza, the evidence for effectiveness of oseltamivir treatment is scarce. Only one RCT (level of evidence 1-) showed a significant reduction in duration of fever,[55] and from observational studies, all with high risk of bias (level of evidence 2-), there might possibly be a reduction in secondary complications.[53,60] However, the results with regard to mortality are more conflicting.[56,57,59-61] Because of this poor evidence, the grade of recommendation for those who are hospitalized is rather poor (CII). Based on only 4 comparative studies with moderate to poor quality (level of evidence 1-/2-), the evidence for treatment effectiveness is even scarcer for those with severe, complicated or progressive illness. Although there is consistency of results demonstrating an overall treatment benefit for oseltamivir with regard to illness duration and complications,[53,55,56,59] the grade of recommendation for the use of oseltamivir in this subgroup of patients is also of poor strength (CII).

**Table 2.** Oseltamivir treatment and prophylaxis: grading of the evidence for use in diverse patient populations.

| Patient population | Grade of recommendation** |
|---|---|
| **Treatment - otherwise healthy individuals** | AI-II |
| **Treatment - any patient with confirmed or suspected influenza who*:** | |
| is hospitalized | CII |
| has severe, complicated, or progressive illness | CII |
| is at higher risk for influenza complications: | CI |
| children aged <2 years | CI-II |
| adults aged ≥65 years | CI |
| the following chronic disorders/conditions: | |
| *pulmonary disorders (including asthma)* | BI |
| *cardiovascular disorders (excluding hypertension alone)* | BI |
| *renal disorders* | CIII |
| *hepatic disorders* | CIII |
| *hematological disorders (including sickle cell disease)* | CII |
| *metabolic disorders (including diabetes mellitus)* | CII-III |
| *neurologic and neurodevelopment conditions*[1] | CIII |
| persons with immunosuppression[2] | CII |
| women who are pregnant or postpartum (within 2 weeks after delivery) | CII |
| persons who are morbidly obese (i.e., body-mass index ≥40) | CIII |
| residents of nursing homes and other chronic-care facilities | CII-III |
| **Prophylaxis** | AI |

**The included patient populations are derived from CDC's ACIP recommended populations for which oseltamivir treatment is indicated (from: CDC MMWR Recommendations and Reports (2011); Antiviral Agents for the Treatment and Chemoprophylaxis of Influenza).*

***The grades of recommendation have been assigned based upon the results of our own systematic literature review.*

*[1]Including disorders of brain, spinal cord, peripheral nerve and muscle.*

*[2]Including that caused by medication or HIV infection.*

Those who are at higher risk for influenza complications are children aged <2 years, elderly/nursing home residents, and those suffering from certain chronic disorders or conditions. In general, when taking into account meta-analyses that have included unspecified mixed high-risk populations, the only real evidence for clinical effectiveness of antiviral treatment comes from one systematic review (level of evidence 1-) suggesting that secondary complications are reduced with oseltamivir treatment.[36] However, the possible effectiveness of oseltamivir treatment is contradicted by other meta-analyses (level of evidence ranging from 1- to 1++),[27,31,34] and therefore the general strength of recommendation is poor (CI). Observing the comparative research that specifically included children <2 years of age, the duration of

illness is reduced by approximately one day with oseltamivir treatment, which is considered statistically significant by only one of two available RCTs (level of evidence 1+).[23,62] From observational studies (level of evidence 2+/2-) the risk for secondary complications in children <2 years seems to be significantly reduced,[40,41,43,48] but from randomized controlled research (level of evidence 1-) this risk seems to be no different with or without oseltamivir treatment.[62] These conflicting results for oseltamivir treatment effectiveness among young children have resulted in a poor strength of recommendation (CI-II). For elderly individuals aged 65 years or older, there is actually only one meta-analysis (level of evidence 1++) that demonstrated a borderline significant benefit for oseltamivir treatment with regard to time to return to normal activity.[27] Taking into account the evidence from all 3 meta-analyses there seems to be no significant reduction in time to alleviation of symptoms and secondary complications,[27,28,33] and the results from observational studies are conflicting.[44,46,63] Therefore, the evidence for treatment effectiveness of oseltamivir among elderly individuals is of poor strength, but sufficient quality (CI).

The best evidence for treatment effectiveness of oseltamivir among those with chronic disorders is found for those with pulmonary and cardiac disease. One meta-analysis (level of evidence 1++) and one RCT (level of evidence 1-) could be retrieved that included a mixed population of patients with either COPD or chronic cardiac disease, and from both these studies it seems that secondary complications are significantly reduced with oseltamivir treatment.[28,65] Data concerning children with asthma from one RCT only (level of evidence 1-) have shown that, although time to alleviation of symptoms does not seem to be significantly reduced with oseltamivir treatment, measured asthma exacerbations were significantly lower.[30] In contrast, regarding adults with COPD or chronic cardiac disease, median time to alleviation of symptoms does seem to be significantly reduced (level of evidence 1+/-).[33] Cardiovascular outcome, among those with a history of chronic cardiac disease, was significantly better with oseltamivir treatment in a large observational study (level of evidence 2-).[66] Combining the evidence, there is a moderate support for recommending the therapeutic use of oseltamivir among those with chronic pulmonary or cardiovascular disorders (BI).

The only other, scarce evidence for treatment effectiveness of oseltamivir among individuals with chronic disorders has been described for hematological and metabolic disease. Two observational studies (level of evidence 2-) have shown that the incidence of pneumonia is likely reduced with the therapeutic use of oseltamivir in hematopoietic stem cell recipients, resulting in a rather poor grade of recommendation for this risk group (CII).[67,68] The evidence for metabolic disease is limited to one study that included diabetics (level of evidence

2-).[69] It remained questionable whether secondary complications are reduced with the therapeutic use of oseltamivir (CII-III). For renal disorders, hepatic disorders, and neurologic and neurodevelopment conditions no comparative studies on oseltamivir treatment effectiveness were found. Therefore, the recommendation for the use of oseltamivir among these risk groups is probably based on expert opinions only (CIII).

If any, the evidence for treatment effectiveness in those with immunosuppression is limited. Three observational studies with organ transplant recipients (level of evidence 2-) and one with HIV-infected individuals (level of evidence 2-) have shown conflicting results with regard to secondary complications.[67,68,70,71] Therefore, the evidence to support this recommendation is rather poor (CII). For women who are pregnant or postpartum, only two observational studies were identified (level of evidence 2-).[72,73] There seems to be a trend towards less severe illness with (earlier) oseltamivir treatment (strength and quality of recommendation: CII). There is no evidence for any treatment effect from comparative studies in persons who are morbidly obese. The recommendation to use oseltamivir for treatment in this group is therefore weak (CIII). For the category 'residents of nursing homes and other chronic-care facilities' only one observational study was retrieved (level of evidence 2-) which showed less serious complications among nursing home residents who received oseltamivir treatment(CII-III).[63]

In addition, oseltamivir prophylaxis appears to be really quite effective in reducing the risk for someone to develop influenza (relative risk reduction ranging from 64% to 75%).[25,28,74-78] The strong support from meta-analyses of various RCTs (level of evidence 1+/1++) have led to a recommendation of high strength and quality (AI). Nausea and vomiting are the most important adverse events.[25,26,33,37,77,79,82] And, although oseltamivir resistance seems to be less of a problem than in the previous decade, resistance does seem to be associated with relevant secondary complications.[93]

## DISCUSSION

This systematic literature review and qualitative analysis of the evidence for oseltamivir effectiveness and safety adds relevant information to the systematic review articles that have been published before.[25-28,31-38] These previous publications have limited their investigated populations mostly to healthy adults, children, or elderly individuals. To our knowledge, a comprehensive systematically performed review article that combines data from the variety of risk-groups for which antiviral treatment is currently recommended has not been published before.

Our grading of the evidence for oseltamivir treatment effectiveness in patient populations for which treatment is actually indicated has led us to the conclusion that, although for some populations the quality of the supporting evidence is reasonable, the strength of evidence to support a recommendation is in general poor. Only for otherwise healthy individuals there is good and high quality evidence that oseltamivir treatment reduces illness duration by approximately 1 day and nearly halves the risk of secondary complications; however, the absolute risk of secondary complications in this population is relatively low and the complications that do develop are usually mild. Furthermore, treatment is generally not indicated for healthy individuals according to current guidelines.[6,13-16] And, according to the one of the newest meta-analyses by the Cochrane collaboration, the safety and effectiveness of oseltamivir remains unclear, which will be discussed below.[94] The only 'high-risk' populations for which there is moderate evidence to support a recommendation for the therapeutic use of oseltamivir, are those with chronic pulmonary and cardiovascular disorders. Nonetheless, the authors of the only Cochrane review of neuraminidase inhibitors for the treatment of influenza infection in people with a specific pulmonary condition (cystic fibrosis), were unable to identify any evidence for effectiveness.[95] There is poor evidence to support a recommendation for therapeutic use of oseltamivir in other patients who are considered to be at higher risk for influenza complications. Oseltamivir prophylaxis, on the other hand, does seem to be effective in preventing influenza infections.

Of significant importance, two new Cochrane reviews appeared in January 2012,[94,96] of which one has led to a broad discussion in the media.[97] Because of the upfront established time frame for inclusion in our systematic review (up to September 2011), these reports have obviously not been included in our study. However, a discussion of the new findings seems of added value here.

In the first review "Neuraminidase inhibitors for preventing and treating influenza in children", executed by Wang et al. on behalf of the Cochrane collaboration, a new search was performed and the content of the previous version by Matheson et al. was updated.[26,96] On top of four included trials in the previous version (3 treatment and 1 prophylaxis), three additional treatment trials were included and two additional prophylaxis trials. This has led to a subtle change in the author's conclusions: oseltamivir now appears to have only a modest benefit in reducing duration of illness in children with influenza and preventing the transmission of influenza in households. Furthermore, it is suggested that larger high-quality trials are needed with sufficient power to determine the efficacy of neuraminidase inhibitors in preventing serious complications of influenza, particularly in 'at risk' groups. This suggestion is in concordance with our conclusion.

The other review "Neuraminidase inhibitors for preventing and treating influenza in healthy adults and children" has caused discussion among clinicians and scientists, but probably also among regulators and members of public health advisory boards.[94] The previous version by Jefferson et al. was actually withdrawn from the Cochrane Library in 2011 because the authors were unable to verify the data underlying manufacturer and government claims about the effectiveness of oseltamivir: 8 of 10 manufacturer-funded clinical trials had never been published and their complete data sets were not available from either the authors or the manufacturers.[25,98] Hence it was decided to update and combine the previous reviews and to perform an analysis based on original clinical study reports and regulatory information, rather than on single published or unpublished studies. Despite promises of the manufacturer and multiple requests by the Cochrane collaboration, the authors have been unable to obtain the full set of clinical study reports or obtain verification of data.[99] The new Cochrane report concludes that there has been a high risk of publication and reporting biases in the trial program of oseltamivir and that there were substantial problems with the design and conduct from many of the trials. As a result, the authors decided not to proceed with a meta-analysis of all the oseltamivir data as was intended. The only conclusion that could be made was that there was a significant beneficial effect on duration of symptoms of around 21 hours and there was no effect on hospitalizations; other outcomes could not be assessed.

The scope of our systematic review, however, is more widespread and different from that of the Cochrane collaborators. We performed a systematic literature search of evidence as published in peer-reviewed scientific journals, with a special focus on 'high-risk' populations. Our qualitative analysis of the overall level of evidence behind the CDC's ACIP recommendations for the use of oseltamivir treatment shows that there is a relative paucity of recommendations supported by level I quality of evidence and there is no evidence at all to support a high strength A recommendation. These findings might have implications for clinical practice, and therefore a discussion of the limited evidence is necessary. In a recently published paper, an analysis was performed of the overall level of evidence underlying general recommendations in 41 guidelines from the Infectious Diseases Society of America (IDSA).[100] Comparable to our findings, the investigators of that article found that the majority of the current recommendations of the IDSA are based on level II and III evidence only. However, as opposed to 1796 (43%) of 4218 individual IDSA recommendations that were designated as strength A (highest strength of recommendation), our analysis showed no strength A recommendations for the use of oseltamivir among those at higher risk for influenza complications at all.

There are some limitations to this review. First of all, there is a striking discrepancy between the relatively large number of systematic reviews and meta-analyses and the number of RCTs for the studies that were retrieved, the former clearly outweighing the latter. There are two explanations for this phenomenon. First, the excessive amount of available meta-analyses roughly all use the same RCTs that have been considered as important in the field. Second, for a large part, unpublished or unavailable data have been used in the meta-analyses. There is a substantial amount of references to conference abstract publications, and reasonable amounts of unpublished data have been used that were provided in confidence by the manufacturer of oseltamivir itself. Understandably this is an important point of discussion, as is reflected by the widespread prominent dialogue around the latest Cochrane report and its negative conclusions.

In addition, there is also the conflicting role of the funding source. Of the 66 studies that have been included in this review, 34 (52%) carry with them a potential conflict of interest because the studies are either directly sponsored by or because the investigators are involved with commercial activities of the manufacturer. Conflict of interest statements have not been made by 7 papers, therefore possible involvement of the manufacturer in that research can not be excluded. From our results, the ratio between published research with a positive outcome (i.e. in favor of oseltamivir) and with a negative outcome, does not really seem to be different for studies that have a conflict of interest and studies that are free of such a conflict of interest. This is in contrast with the results of an interesting systematic literature review in which investigators concluded that sponsored studies were more likely to have conclusions favoring the intervention.[101]

## CONCLUSION

The conclusion of this systematically performed literature review is that there is little evidence to strongly support the use of oseltamivir among individuals from various 'high-risk' populations. In persons with influenza who are otherwise healthy and present with an uncomplicated febrile illness, the duration of illness does seem to be effectively reduced by 0.5-1.5 days and major and minor secondary complications by approximately 40-50% if antiviral treatment can be initiated within 48 hours of illness onset.[25-28,35] Likewise, oseltamivir prophylaxis has shown to offer a 64-75% protection rate.[25,26] Despite these apparently effective reductions in disease burden, treatment and prophylaxis are considered not to be necessary for outpatients with uncomplicated influenza according to current guidelines. The inconsistency between the rather strong scientific evidence for effectiveness

on the one hand, and the use of oseltamivir among otherwise healthy individuals not being recommended by clinical guidelines on the other hand, possibly relates to a delicate cost-benefit balance. When weighing the advantages against the disadvantages for the use of oseltamivir, various factors should be taken into account: individual patient factors, such as the risk of development of severe or progressive disease, risk of development of secondary complications, time since onset of illness, but also risk of developing adverse drug effects; epidemiological factors, i.e. whether there is an outbreak of seasonal influenza in the community or even a pandemic threat caused by a zoonotic influenza A virus, but also the emergence and circulation of drug resistant isolates; and economical factors, such as cost-effectiveness of antiviral drug use and stockpiling issues.

Although current recommendations on the use of oseltamivir can for the most part be considered weak because of the lack of good supporting evidence, guidelines ought to be a reflection of the best available evidence, and following guidelines is not always 'best medicine'. Clinical judgment on whether to use oseltamivir or not in an individual patient is based on multiple factors and can never completely be covered by clinical guidelines. The results of this study are relevant to clinical practice, not only because of the recent 2009 influenza A/H1N1 pandemic during which much attention was given to the use of oseltamivir, but also because each year again clinicians have to deal with seasonal influenza epidemics and the decision whether to treat 'high-risk' patients. Hopefully, this article has provided clinicians a clear knowledge of the available evidence underlying the recommended use of oseltamivir.

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# Conclusions and perspectives

This thesis aimed to provide clinical insights into several aspects of 2009 pandemic influenza A (H1N1) virus and other respiratory pathogens. Here, we evaluate the major conclusions deriving from the studies that have been described in this work, and they are discussed from a broader perspective.

## CONCLUSIONS

The work that has lead to this thesis started with the clinical experience that was gained at the Slotervaart Hospital influenza outpatient clinic, which was established during the 2009 influenza A (H1N1) pandemic. After the first findings from this special clinic had been evaluated, several clinical studies followed covering aspects from epidemiology to treatment with reference to pandemic influenza A (H1N1), but also other respiratory pathogens.

### Epidemiology

Clinical-epidemiological observations are often an important source of hypothesis-generating research. Most certainly, during the rather abrupt outbreak of a novel influenza virus, that spread from Mexico to Southern California and from there to the rest of the world, causing over 18,000 fatal cases worldwide, the recording of observational studies in scientific papers was of utmost importance to scientists and clinicians who needed to gain insight into relevant clinical aspects. This work has attempted to contribute to some extent to this important gain of insight.

In a rather large population of symptomatic adult outpatients, it was demonstrated that human rhinovirus played just as important a role as novel influenza A (H1N1) virus did with regard to frequency of detection. Although the H1N1 pandemic seemed to follow a mild course in the observed population, the clinical presentation of patients with 2009 H1N1 influenza was significantly more serious than of patients with human rhinovirus infection. This conclusion did not come as a surprise, but it is a confirmation of widespread assumptions about the clinical behavior deriving from other epidemiological studies that have been performed elsewhere and not always in similar settings. In addition, the opportunity was taken to evaluate case definitions that are frequently used in clinical practice to diagnose influenza-like illness. It was thought that these case definitions could aid clinicians with limited access to laboratory resources in the diagnosis of pandemic influenza. However, the performance of these case definitions seemed moderate to poor after a proper evaluation. For exactly those patients in whom one might have thought that confirmative laboratory diagnostics are not always necessary, i.e. symptomatic outpatients, drawing on case definitions seemed rather useless.

Pandemic influenza A (H1N1) virus infection had been associated with less favorable outcomes in children. However, there were few observational studies focusing on the pediatric outpatient experience. The experience that was gained at the influenza outpatient clinic in Amsterdam did not confirm the association: the vast majority of children infected with the pandemic virus showed a mild clinical illness, there were no intensive care requirements and none had died. Data from studies in hospitalized children could not just be extrapolated to outpatient settings. Furthermore, it was concluded that using a highly specific laboratory test to diagnose pandemic influenza in a pediatric outpatient population, as opposed to a clinical diagnosis based on signs and symptoms only, had effectively reduced the use of the antiviral drug oseltamivir, which on its turn might have reduced exposure to significant side effects.

Perhaps the seemingly non-serious clinical aspect and the fact that infection caused by respiratory viruses is very common, have led to clinicians frequently taking respiratory viral infections for granted. However, the impact of respiratory pathogens should not be underestimated when looking at certain specific populations of patients who are at higher risk. An important example of one such fragile patient population is newborn children admitted to a neonatal care unit. One of the clinically most relevant studies in this work demonstrated that a 1-year screening program, during which physicians were blinded for screening results, yielded respiratory pathogens in one of ten neonates admitted to a neonatal medium care unit. It was also demonstrated that approximately one in four neonates were diagnosed with undifferentiated perinatal infection, i.e. strong suspicion of infectious disease without a clear focus for the infection, and that antibiotics were given to one third of the total population. These findings all reflect the clinical importance of this and, hopefully, future related studies. Antibiotics, always administered intravenously in this population, can lead to serious toxicity and extended duration of hospital stay. A role for respiratory viral pathogens, for which antibiotic treatment is usually not considered to be necessary, has now been proven, which should direct future studies.

**Prevention**

In order to contain an influenza epidemic, various preventive measures are recommended, which was also the case during the outbreak of 2009 pandemic influenza A (H1N1). It has been commonly accepted that contact and respiratory precautions, such as frequent hand washing and wearing a respiratory device, will immediately reduce transmission of the virus, while vaccination of healthcare personnel seems to provide the best preventive strategy in the long term to significantly slow in-hospital transmission of the virus. At the Slotervaart influenza outpatient clinic, a setting with high hygiene standards, these and other preventive

strategies, as recommended by national and international disease control centers, were strictly applied. However, 2009 H1N1 vaccines did not become available until after the peak of the epidemic. Although there had been much concern over the safety of having a separate outpatient facility for diagnosis and management of patients with influenza, an extremely low attack rate was demonstrated among healthcare workers with a relatively high occupational exposure risk. It could be concluded that having an influenza outpatient clinic during a pandemic outbreak can be considered safe if an adequate containment plan is present and practiced.

**Diagnostic testing**

The continuous evaluation of current diagnostic tests that have been validated under different circumstances, and the development of novel diagnostic methods to aid in clinical diagnosis and management, are important aspects of applied medicine research in this thesis. One such example has been the use of rapid-antigen-detection-tests for the diagnosis of pandemic influenza A (H1N1). Although most of these tests had shown to detect pandemic H1N1 virus cultured from a positive human respiratory specimen, the performance characteristics under real clinical conditions had not been established. Albeit giving rapid results, usually within 10 minutes, and being available for point-of-care use, soon there were strong doubts with regard to the sensitivity of these easy-to-use tests for pandemic influenza. One frequently employed and approved rapid-test had been used during the initial phases of the pandemic outbreak at the influenza outpatient clinic in Amsterdam. The test was given up upon after an extremely low sensitivity was noticed in practice. A subsequent evaluation of its performance did show a high specificity, but the capacity to diagnose pandemic influenza infection among symptomatic outpatients was disappointingly low. These data have highlighted the need to interpret test results always carefully if the circumstances are substantially different from those under which the test has originally been validated.

In addition to a need for (rapid) influenza diagnosis, differentiating between bacterial and viral infection in case of febrile illness is of great clinical importance, especially during influenza pandemics at which time the demand for adequate clinical care might get close to exceeding the limits of the healthcare system's capacity. The decision whether or not to initiate antibiotics in children with influenza-like symptoms is usually based on clinical findings and standard laboratory tests; however, discriminating viral from bacterial infection based on these parameters during an influenza epidemic has been shown to be unreliable. Procalcitonin is a promising biomarker which does have such discriminative potential. In a number of pediatric studies circulating levels of procalcitonin were raised in bacterial

infection and only mildly raised in the absence of bacteria, i.e. for example in case of a respiratory viral infection. From a small population of children with febrile illness who had presented to the influenza outpatient clinic during the 2009 H1N1 pandemic outbreak, it was demonstrated that levels of procalcitonin, as opposed to leukocyte count and C-reactive protein, was mostly below the lower cut-off value in a subgroup of children with confirmed viral infection. These results show that the use of procalcitonin, a novel biomarker that could be readily available, may be of added value under circumstances during which the practicing clinician is uncertain whether to withhold or initiate antibiotics in case of suspicion of infection without clear evidence of its microbiological cause. Further prospective validation studies on the use of procalcitonin in diverse clinical settings are currently ongoing.

**Cardiovascular complications**

The linkage between inflammation and coagulation is a well-known concept in the field of infectious diseases medicine. Previous observational studies have clearly demonstrated an association between influenza epidemics and cardiovascular morbidity and mortality. It has also been shown by several well-designed case-control studies that symptoms of recent respiratory tract infection are associated with a 2-5 fold increased risk of acute myocardial infarction and ischemic stroke. However, none of these studies had validated the clinical diagnosis by laboratory confirmation, and therefore carry with them a potential risk of misdiagnosis bias. Hence, a case-control study was designed, taking into account the risk of misdiagnosis when merely signs and symptoms are used, to determine recent respiratory tract infection in patients with and without an acute coronary syndrome. Interestingly, it was shown that risks of developing an acute coronary syndrome were approximately equal for patients with and without laboratory evidence of a recent infection. Of important further notice, there was no relation between clinical signs of respiratory tract infection and confirmative laboratory investigations. These results emphasize the important need to remain always critical when interpreting the results of case-control studies, even when bias and confounding seem unlikely at first sight. Future studies on the association between respiratory tract infection and cardiovascular pathology should at least include reliable diagnostic tests to reinforce the diagnosis of exposure.

While it has been suggested that protection against respiratory virus infection reduces cardiovascular complications, it remains unclear whether influenza vaccination reduces or increases the risk of acute coronary syndromes. Preliminary and post-marketing evidence had shown that the available 2009 H1N1 vaccines were generally immunogenic and safe. However, controversy over the safety of the vaccine rose after some fatal cases had been reported following vaccination in Japan. Of notice, an important share of these fatal

events had occurred under individuals who already had underlying cardiovascular disease. The hypothesis that influenza vaccination induces coagulation abnormalities through a vaccine-induced immunogenic response was tested by observing the hemostatic changes in healthy volunteers following the injection of an adjuvanted 2009 H1N1 vaccine. A small but significant decrease in D-dimer was observed, which may indicate impaired fibrinolysis after influenza vaccination. Other measurements, reflecting various components of the coagulation and anticoagulation system, did not change significantly. Clinically relevant sustainable procoagulant effects were not seen, and therefore the hypothesis was rejected. However, whether these findings hold true for individuals who already have underlying cardiovascular disease, remains to be elucidated.

**Treatment**

Oseltamivir belongs to the group of neuraminidase inhibitors and is the most widely used antiviral drug to treat and protect against influenza. Its effectiveness and safety have been well established for otherwise healthy individuals; however, the absolute risk of secondary complications in this population is relatively low and the complications that do develop are usually mild. Furthermore, antiviral treatment and prophylaxis is precisely recommended for individuals from various 'high-risk' populations (e.g. hospitalized patients, individuals at age extremes, patients with certain chronic disorders or conditions, women who are pregnant or postpartum). With the exception of children and elderly individuals, systematic reviews have never really focused on those 'high-risk' patient populations. The systematic literature review in this chapter had a different approach. In addition to focusing on the evidence for individuals from recommended 'high-risk' populations, a qualitative analysis of the overall evidence behind current recommendations from prominent guidelines for the use of oseltamivir was performed. Little evidence for effectiveness resulted from the studies that did focus on 'high-risk' groups. Only for individuals with chronic pulmonary or cardiovascular disease there appears to be moderate evidence to support the use of oseltamivir. In conclusion, grading the recommendations from current guidelines, there is a relative paucity of recommendations supported by level I quality of evidence, which is the highest quality status according to an acknowledged grading system. Moreover, there is no evidence at all to support a strength A recommendation, which is the highest rank in its category. Although intuitively it seems very likely that the use of oseltamivir can prevent secondary complications among certain 'at risk' individuals, clinicians should be aware that clinical guidelines supporting the use of oseltamivir are based on rather weak evidence.

## PERSPECTIVES

The ultimate goal of clinical studies on the impact of respiratory pathogens is to share the gained scientific experience with clinicians caring for patients who develop respiratory tract infection. Because respiratory tract infection is so common, the professionals to whom this research might be of concern come from a variety of specialties: from general practitioners to internal medicine specialists and pediatricians, and from medical microbiologists to clinical epidemiologists.

Especially during the recent 2009 influenza A (H1N1) pandemic, much attention was given to various kinds of research relating to the pandemic virus. Despite a huge clinical impact due to numerous transmissions and an extensive worldwide spread, fortunately, the severity of disease caused by this virus had generally been mild. Although it had been a challenge to develop tailored vaccines protecting against infection with the pandemic virus, soon after they became available many individuals were vaccinated. Furthermore, oseltamivir had been stockpiled in many countries, for large-scale use in prioritized groups. However, even during a pandemic, the continuous role played by other respiratory viruses contributing to influenza-like illness should not be forgotten. Even though for most of these other pathogens there are not really any prevention or treatment options - with the exception of passive vaccination for children at high-risk for severe respiratory syncytial virus infections -, the influence exerted by these pathogens on daily clinical practice must not be underestimated. Acute respiratory tract infections account for the number one illness in developed countries with regard to frequency of doctor visits. While these infections are predominantly caused by viruses and not bacteria, antibiotics are frequently prescribed. Further studies will give indications whether more frequent testing for respiratory viral pathogens can reduce the use of antibiotics.

In conclusion, the study of respiratory pathogens is an interesting and useful scientific activity. Clinicians and researchers should never stop exploring various aspects of the clinical impact that they have, even though common colds and influenza-like illnesses are often taken for granted. Whether we are dealing with an impressive and ‘hot’ pandemic outbreak or the usual seasonal fluctuation of common respiratory viruses, the ultimate goal should be kept in mind: learning about respiratory pathogens might possibly lead to improvements in the clinical practice of an undeniably significant healthcare problem.

# Appendix

## Summary

This thesis contains studies on the clinical impact of 2009 pandemic influenza A (H1N1) virus and other respiratory pathogens. It consists of six chapters. The first chapter is an introductory chapter describing the very origin of this thesis: the influenza outpatient clinic which was set up at the Slotervaart Hospital during the 2009 Mexican flu pandemic (**Chapter 1.1**). The second chapter gives an epidemiological overview of the role of respiratory pathogens in both adults and children. The third and fourth chapters involve aspects of prevention and diagnostic testing of infection with the pandemic influenza virus, respectively. The fifth chapter focuses on cardiovascular complications of respiratory virus infection and influenza vaccination. In the sixth and last chapter, a comprehensive overview is provided regarding influenza antiviral treatment effectiveness and safety.

**Chapter 2 Epidemiology**

**Chapter 2.1** aims to describe causative agents and clinical characteristics in adult outpatients with upper airway symptoms during the 2009 influenza A (H1N1) pandemic. In addition, case definitions, which are commonly used in clinical practice to aid in the diagnosis of influenza-like illness, were evaluated. During the outbreak of the pandemic in the Netherlands, a total of 964 symptomatic adults had signed up for a consultation at the influenza outpatient clinic of the Slotervaart Hospital. Real-time reverse-transcriptase-polymerase-chain-reaction (RT-PCR) assays had been used to identify the pandemic virus and a whole range of other common respiratory pathogens. The results of this observational study showed that a respiratory pathogen was detected in 41% of the tested patient samples. Pandemic influenza A (H1N1) and human rhinovirus were most frequently detected with a similar percentage of 16%. Although the 2009 H1N1 pandemic in Amsterdam had followed a mild course, the clinical presentation of patients with confirmed influenza A (H1N1) virus infection was significantly more serious than of those patients with rhinovirus infection or without any detected pathogen. Test characteristics of 4 case definitions were fairly similar: sensitivity was on average 66%, specificity 70%, and positive and negative predictive values were 34% and 90%, respectively. It was concluded that the value of case definitions to predict pandemic influenza infection is rather poor, with the exception of a relatively high negative predictive value that might be of use in clinical practice when ruling out a diagnosis of influenza infection is of importance to the practicing clinician.

Similar to the preceding chapter, **Chapter 2.2** characterizes the 2009 H1N1 influenza pandemic, but this time in children with influenza-like signs and symptoms. Previous observations from other investigators had led to the conclusion that novel influenza A (H1N1) virus infection

was associated with less favorable outcomes in children. Therefore, the causative respiratory pathogens were determined and epidemiological and clinical characteristics were described in a population of 412 children who had visited the Slotervaart influenza outpatient clinic. One third of the population proved positive for pandemic influenza A (H1N1), as confirmed by RT-PCR. In another one third various other pathogens were detected, with human rhinovirus (13%), respiratory syncytial virus (11%) and adenovirus (8%) most often being identified. Mean age of H1N1-positive cases was significantly higher than of H1N1-negative cases (6.8 and 4.2 years, respectively). H1N1-positive outpatient children reported fever, cough and rhinorrhea more frequently than their H1N1-negative counterparts. Of 72 children hospitalized with respiratory infection, 31% proved H1N1-positive; all showed a relatively mild clinical illness. None of the children had been admitted to an intensive care unit or died. Oseltamivir treatment was initiated in 72 children and discontinued in 42 (63%) when RT-PCR results turned negative. In conclusion, the findings of this observational study showed a mild clinical course of the 2009 H1N1 pandemic in a Dutch pediatric outpatient population.

The last part of this section, **Chapter 2.3**, elaborates on the role of respiratory pathogens in a subpopulation of children for whom a diagnosis of viral respiratory infection is probably clinically most relevant. Neonates or newborn children aged up until 28 days post-partum can be hospitalized with respiratory illness caused by common viruses; yet, the prevalence of these viruses is largely unknown. Differentiation from other important infectious diseases syndromes is of utmost importance because this could possibly lead to reductions in the duration of expensive hospital stays and potentially toxic antibiotic use. This physician-blinded study was designed to determine the prevalence of respiratory pathogens among a population of newborns admitted to a neonatal medium care unit (NMCU) during a 1-year period. Risk factors and clinical predictors for the presence of those pathogens were identified. A total of 334 neonates were screened for a range of common pathogens by RT-PCR on nasopharyngeal aspirates. Undifferentiated perinatal infection was diagnosed in 79 newborns (23.7%) and antibiotics were given to 108 (32.3%). Overall, 37 respiratory pathogens were detected in 34 children, which comprises 10.2% of the total population. Parainfluenza, human rhinovirus and respiratory syncytial virus were most frequently detected. After adjustment for confounders, two variables were identified as significantly contributing to the risk of a respiratory sample being positive for any respiratory pathogen: age (OR 1.21 for each day older; 95% CI 1.12-1.30) and symptoms of rhinorrhea (OR 6.71; 95% CI 1.54-29.21). There was no significant difference regarding use of antibiotics or duration of hospital stay; however, pediatricians were blinded for RT-PCR results. The conclusion is that respiratory pathogens seem to play a role in neonates admitted to a medium care unit.

The question whether clinical management of newborn children will be influenced by the knowledge of respiratory viruses being present, remains to be answered.

**Chapter 3 Prevention**

**Chapter 3.1** describes a prospective study of three groups of hospital workers with different occupational exposure risks to pandemic influenza A (H1N1) virus. There were major concerns that crowding of patients with influenza-like symptoms in healthcare settings would lead to a significant risk of transmission of the virus to their employees. The Slotervaart Hospital's influenza outpatient clinic took care of over 1,000 patients during the 2009 H1N1 pandemic. Sixty-six healthcare workers were carefully monitored for study purposes. From 26 high-risk influenza outpatient clinic employees and from 20 intermediate-risk and 20 low-risk employees, weekly throat swabs were collected for RT-PCR detection of pandemic influenza A (H1N1). Monthly blood samples were obtained for serological confirmation. In addition, influenza-like signs and symptoms were assessed regularly. Influenza vaccination was offered to all healthcare workers through a local vaccination program; however, 2009 H1N1 vaccines did not become available until after the peak of the epidemic. One of 26 high-risk group participants proved H1N1 positive by RT-PCR. This corresponds to an incidence rate in the high-risk group of 5.7/1,000 person weeks (95% CI 0-17/1,000). None of the intermediate-risk and low-risk group participants proved H1N1-positive by RT-PCR. Significant antibody titer rises in convalescent sera were demonstrated in three participants: one was a confirmation of the case that had proved H1N1-positive by RT-PCR; the others occurred in two asymptomatic participants belonging to the low- and high-risk groups. This study demonstrated a low incidence rate of influenza A (H1N1) virus infection among healthcare workers during the 2009 H1N1 pandemic in a setting with high hygiene standards.

**Chapter 4 Diagnostic testing**

In **Chapter 4.1**, the experience with a point-of-care rapid-influenza-diagnostic-test during the 2009 H1N1 pandemic is described. The outbreak of the pandemic had caused an urgent need for rapid and accurate diagnostic methods. Several immunoassays were available that were able to detect both influenza A and B antigens within 10 minutes. However, these assays do not discriminate between influenza subtypes (e.g. pandemic H1N1 or seasonal H1N1/H3N2) and had not been validated in clinical settings during the pandemic outbreak. Performance of a commonly used rapid-test (QuickVue® Influenza A+B test; Quidel, San Diego, USA) was evaluated after it had been used in the first 187 patients who visited the Slotervaart Hospital influenza outpatient clinic to aid in clinical decision making. Rapid-test results were compared to RT-PCR as the "gold" standard. Mean age of patients was 34

years (range 2-84 years), 87 were male (46.5%) and mean time since onset of symptoms was 4 days (SD 2.1 days). RT-PCR was positive for novel influenza A (H1N1) in 16 of 187 patients, which implied a prevalence of 9% at that time. The rapid-test was negative in all 187 patients. To exclude false negative results due to problems with handling and storage of test kit contents, the rapid-test was also applied to 9 stored strongly pandemic H1N1-positive samples. Rapid-test results did turn positive for 5 of these 9 samples. Overall, the sensitivity of the test was 20%, but ranged from 0% among 187 outpatients with influenza-like signs and symptoms to 55.6% among 9 stored highly H1N1-positive laboratory samples. There were no false positive results at all, implying a 100% specificity of the rapid-test. Of notice, the RT-PCR confirmed H1N1-positive samples of which rapid-test results were also positive contained significantly higher viral loads than those with negative rapid-test results. In conclusion, this study has demonstrated limited clinical performance of the used rapid-test for the detection of 2009 pandemic influenza A (H1N1) virus. Although the test was highly specific, the predictive capacity to diagnose pandemic influenza infection was disappointingly low.

**Chapter 4.2** aims to gain insight into the acute phase response and to investigate the value of procalcitonin (PCT), a novel biomarker of inflammation, in children with suspected 2009 pandemic influenza A (H1N1) virus infection. During the pandemic outbreak, rapid diagnosis of children with influenza-like signs and symptoms was of great clinical importance. In addition to deciding whether or not to initiate oseltamivir, differentiation between viral and bacterial (super-) infection was of significant need in these children. From previous studies it has been shown that a differentiated white blood cell count and measuring plasma C-reactive protein (CRP) level might not be sufficiently helpful in this regard. PCT, on the other hand, could be a useful tool to discriminate bacterial from viral infection, because circulating levels of this biomarker are mainly raised in the early stage of severe bacterial infection and only mildly raised in the absence of bacteria. Therefore, 30 children with influenza-like signs and symptoms were included in this prospective observational study that was carried out during the 2009 H1N1 pandemic. Of these, 25 patients were diagnosed with a viral respiratory illness by RT-PCR, including 9 with pandemic influenza A (H1N1) infection. Blood samples were obtained from all children to study the acute phase response. Median PCT levels, CRP levels and leukocyte counts in the subgroup with proven viral infections were 0.153 ng/mL (IQR 0.094-0.261 ng/mL), 19.0 mg/L (IQR 5.6-28.3 mg/L) and 13.1 giga/L (IQR 9.5-16.0 giga/L), respectively. PCT levels in children with H1N1 infection and other viral diseases were below the cut-off value of <0.71 ng/mL in 22 of 25 children (88%), whereas CRP levels and leukocyte counts were shown to exceed the cut-off value in 6 and 5 patients, respectively. These results suggest that PCT seems to be of added value in the diagnostic approach of

children with suspected pandemic influenza infection in whom differentiating viral from bacterial (super-) infection is clinically highly relevant.

**Chapter 5 Cardiovascular complications**

In **Chapter 5.1** the association between respiratory virus infection and acute coronary syndrome is investigated. Previous studies had demonstrated an increased risk of acute myocardial infarction for patients with symptoms of respiratory tract infection. To confirm this relation, a case-control study was designed in which cases with an acute coronary syndrome were compared to matched controls without a history of coronary events. This study aimed to provide a risk estimate of acute coronary syndrome for patients with laboratory confirmed recent viral respiratory tract infection. Laboratory confirmation was obtained by RT-PCR and serology for a range of respiratory pathogens. From January 7, 2008 until January 30, 2009, 41 cases and 41 matched controls were included: 7 cases (17%) and 6 controls (15%) had laboratory evidence of a recent respiratory viral infection. The adjusted odds ratio of acute coronary syndrome for patients who had laboratory proven recent respiratory viral infection was 1.1 (95% CI 0.3-4.0). None of 3 participants with symptoms of an influenza-like illness (3 cases, 0 controls) had a proven infection. The findings of this prospective study suggest approximately equal risks of acute coronary syndrome for patients with and without a laboratory proven recent respiratory viral infection. Furthermore, there was no relation between clinical signs of respiratory tract infection and confirmative laboratory investigations. This underlines the risk of misdiagnosis bias when using clinical symptoms only to diagnose recent respiratory tract infection in studies investigating the association between respiratory virus infection and acute coronary syndrome.

**Chapter 5.2** explores the hemostatic changes after influenza vaccination in healthy volunteers. During the initial phases of the 2009 influenza A (H1N1) pandemic it was recommended to vaccinate as many persons as quickly as possible, as soon as vaccines would become available. Preliminary evidence showed that 2009 H1N1 vaccines were immunogenic and safe with mild-to-moderate vaccine-associated reactions. However, it has always remained unclear whether influenza vaccination reduces or increases the risk of acute coronary syndromes. This study investigated the hypothesis that influenza vaccination induces coagulation abnormalities through a vaccine-induced inflammatory response. Healthy healthcare workers receiving a registered 2009 H1N1 vaccine (Focetria®; Novartis, Basel, Switzerland) were followed for two weeks. Just before administering the vaccine and 14 days later, blood was taken to determine the antibody response to pandemic influenza A (H1N1) virus and to assess several parameters reflecting coagulation and fibrinolysis. Ninety four participants, with a mean age of 37 years (range 18-59 years) were included

for the analysis. An adequate antibody response, i.e. a fourfold rise or more in antibody titer, was demonstrated in 80 participants (85%). Prothrombin time (PT) increased from 11.8 to 11.9 seconds, D-dimer decreased from 0.30 to 0.26 µg/L and von Willebrand factor (VWF) decreased from 98.6% to 92.2%; all these changes were statistically significant. After adjustment for variation of hemostatic factors according to a circadian rhythm, only the decrease in D-dimer remained statistically significant. D-dimer is a plasma fibrinolysis activation marker and therefore the observed decrease may indicate impaired fibrinolysis after influenza vaccination. However, since the absolute change is very small and the value of plasma fibrinolysis activation markers in predicting cardiovascular disease is known to be limited, this finding is probably not clinically relevant. This study concluded that there were no sustainable procoagulant effects from 2009 H1N1 influenza vaccination in healthy healthcare workers.

**Chapter 6 Treatment**

Finally, **Chapter 6.1** contains a systematic review of the evidence for clinical effectiveness and safety of oseltamivir for treatment and prophylaxis of influenza. Oseltamivir is an antiviral drug that is considered to be effective for the prevention and treatment of influenza. Therefore, expert advisory groups recommend the use of the antiviral drug in patients who are at high risk of secondary complications related to influenza infection. Previous systematic review articles on this topic had limited their investigated populations mostly to healthy adults, children or elderly individuals. This chapter provides a comprehensive review that combines data from the variety of risk-groups for which antiviral treatment is currently recommended, in addition to a qualitative analysis of the evidence for effectiveness among healthy individuals. Using predefined search terms combined with Cochrane search strategies for comparative studies, 66 studies were finally included after a thorough search of the PubMed database. For otherwise healthy individuals there is good and high-quality evidence that treatment reduces duration of illness by 0.5-1.5 days and (mostly mild) secondary complications by 40-50%. Results regarding treatment effectiveness among those who are hospitalized or have severe, complicated, or progressive illness are conflicting. There is little evidence for treatment effectiveness among individuals who are at higher risk of complications, except for moderate evidence among those with chronic pulmonary or cardiovascular disease in whom oseltamivir treatment significantly reduces secondary complications (relative risk range 0.17-0.65). Overall, oseltamivir prophylaxis offers a 64-75% protection rate against infection. In conclusion, there is moderate evidence to support the therapeutic use of oseltamivir among individuals with chronic pulmonary or cardiovascular disease and evidence for individuals from other recommended ‘high-risk’ populations is scarce.

## Samenvatting

Dit proefschrift bevat studies over de klinische invloed van het 2009 pandemische influenza A (H1N1) virus en andere respiratoire pathogenen. Het bestaat uit zes hoofdstukken. Het eerste hoofdstuk is een inleidend hoofdstuk dat de feitelijke aanleiding van dit proefschrift beschrijft: de grieppolikliniek die werd opgezet in het Slotervaartziekenhuis ten tijde van de Mexicaanse griep pandemie in 2009 (**Hoofdstuk 1.1**). Het tweede hoofdstuk geeft een epidemiologisch overzicht van de rol die respiratoire pathogenen spelen, zowel bij volwassenen als bij kinderen. De derde en vierde hoofdstukken hebben betrekking op aspecten van preventie en diagnostiek van infectie met het pandemische influenza virus, respectievelijk. Het vijfde hoofdstuk richt zich op cardiovasculaire complicaties van infectie met respiratoire virussen en influenza vaccinatie. In het zesde en laatste hoofdstuk wordt een overzicht gegeven betreffende de effectiviteit en veiligheid van antivirale behandeling tegen influenza.

**Hoofdstuk 2 Epidemiologie**

**Hoofdstuk 2.1** heeft tot doel het beschrijven van de verwekkers en klinische kenmerken bij volwassen ambulante patiënten met bovenste luchtwegklachten ten tijde van de 2009 influenza A (H1N1) pandemie. Daarnaast werden zogenoemde "case definitions" geëvalueerd, die in de klinische praktijk regelmatig worden gebruikt om te helpen bij de vaststelling van de diagnose influenza-achtige ziekte. Gedurende de uitbraak van de pandemie in Nederland hadden in totaal 964 symptomatische volwassenen zich aangemeld voor het grieppolikliniek spreekuur in het Slotervaartziekenhuis. Real-time reverse-transcriptase-polymerase-ketting-reactie (RT-PCR) bepalingsmethoden werden gebruikt om het pandemische virus en een heel scala aan andere veel voorkomende respiratoire pathogenen aan te tonen. De resultaten van deze observationele studie lieten zien dat een respiratoir pathogeen werd aangetoond bij 41% van het geteste patiëntenmateriaal. Pandemische influenza A (H1N1) and humaan rhinovirus werden het meest frequent aangetoond met een gelijk percentage van 16%. Alhoewel het beloop van de 2009 H1N1 pandemie in Amsterdam mild was geweest, was de klinische presentatie van patiënten met een bevestigde influenza A (H1N1) virus infectie toch significant ernstiger dan die van patiënten met rhinovirus infectie of zonder enig aangetoond pathogeen. De test karakteristieken van 4 "case definitions" waren redelijk vergelijkbaar: de sensitiviteit was gemiddeld 66%, specificiteit 70%, en de positief en negatief voorspellende waarden waren 34% en 90%, respectievelijk. Er werd geconcludeerd dat de waarde van "case definitions" om pandemische influenza infectie te voorspellen nogal matig was, met uitzondering van een relatief hoge negatief voorspellende

waarde, wat van pas zou kunnen komen in de klinische praktijk wanneer het uitsluiten van een influenza infectie diagnose van belang is voor de praktiserend arts.

Vrijwel identiek aan het voorgaande hoofdstuk, karakteriseert **Hoofdstuk 2.2** de 2009 H1N1 influenza pandemie, maar deze keer bij kinderen met influenza-achtige verschijnselen en symptomen. Eerdere observaties van andere onderzoekers hadden geleid tot de conclusie dat infectie met het nieuwe influenza A (H1N1) virus was geassocieerd met minder gunstige uitkomsten bij kinderen. Daarom werden bij een populatie van 412 kinderen, die de grieppolikliniek in het Slotervaartziekenhuis hadden bezocht, de verantwoordelijke pathogenen vastgesteld en epidemiologische en klinische karakteristieken beschreven. Eén derde van de populatie testte positief op pandemische influenza A (H1N1), zoals bevestigd met RT-PCR. Bij nog eens eén derde werden verscheidene andere pathogenen aangetoond, waarbij humaan rhinovirus (13%), respiratoir syncytieel virus (11%) en adenovirus (8%) het vaakst werden gevonden. De gemiddelde leeftijd van H1N1-positieve gevallen was significant hoger dan van H1N1-negatieve gevallen (6.8 en 4.2 jaar, respectievelijk). H1N1-positieve kinderen die de polikliniek bezochten, rapporteerden vaker koorts, hoesten en een loopneus dan hun H1N1-negatieve tegenhangers. Van 72 met respiratoire infectie in het ziekenhuis opgenomen kinderen bleek 31% H1N1-positief; allen toonden een relatief mild klinisch ziektebeeld. Geen van de kinderen werd opgenomen op een intensive care afdeling of overleed. Behandeling met oseltamivir werd gestart bij 72 kinderen en gestopt bij 42 (63%) van hen op het moment dat de RT-PCR resultaten bekend en negatief waren. Concluderend toonden de bevindingen van deze observationele studie een mild klinisch beloop van de 2009 H1N1 pandemie in een poliklinische populatie van Nederlandse kinderen.

Het laatste deel van deze sectie, **Hoofdstuk 2.3**, wijdt uit over de rol van respiratoire pathogenen bij een subpopulatie van kinderen voor wie een diagnose van virale respiratoire infectie waarschijnlijk het meest klinisch relevant is. Neonaten of pasgeboren kinderen met een leeftijd tot en met 28 dagen na de geboorte kunnen in het ziekenhuis worden opgenomen met respiratoire ziekte veroorzaakt door veel voorkomende virussen; toch is de prevalentie van deze virussen grotendeels onbekend. Het onderscheiden van andere belangrijke infectieziekten syndromen is van groot belang, want dit zou mogelijk kunnen leiden tot een reductie in de duur van kostbare ziekenhuisopnames en potentieel toxisch antibiotica gebruik. Deze dokter-geblindeerde studie werd opgezet om de prevalentie van respiratoire pathogenen vast te stellen in een populatie van pasgeborenen, opgenomen op een neonatale medium care afdeling (NMCU), gedurende een periode van een jaar. Risicofactoren en klinische voorspellers voor de aanwezigheid van deze pathogenen werden geïdentificeerd. In totaal werden 334 neonaten gescreend op een scala aan veel voorkomende respiratoire

pathogenen door middel van RT-PCR op nasofaryngeale aspiraten. Ongedifferentieerde perinatale infectie werd gediagnosticeerd bij 79 pasgeborenen (23.7%) en antibiotica werden gegeven aan 108 (32.3%). Er werden in totaal 37 pathogenen aangetoond bij 34 kinderen, wat 10.2% van de totale populatie behelst. Parainfluenza, humaan rhinovirus en respiratoir syncytieel virus werden het meest frequent aangetoond. Na correctie voor verstorende variabelen werden twee variabelen gevonden die significant bijdroegen aan het risico dat een respiratoir monster positief was voor een - om het even welk - respiratoir pathogeen: leeftijd (OR 1.21 voor elke dag ouder; 95% BI 1.12-1.30) en symptomen van een loopneus (OR 6.71; 95% BI 1.54-29.21). Er was geen significant verschil met betrekking tot antibiotica gebruik of duur van de ziekenhuisopname; echter, de kinderartsen waren geblindeerd voor de RT-PCR resultaten. De conclusie is dat respiratoire pathogenen een rol lijken te spelen bij neonaten die worden opgenomen op een medium care afdeling. De vraag of het klinisch beleid ten aanzien van pasgeboren kinderen zal worden beïnvloed door kennis over de aanwezigheid van respiratoire virussen, zal nog moeten worden beantwoord.

**Hoofdstuk 3 Preventie**

**Hoofdstuk 3.1** beschrijft een prospectieve studie van drie groepen van ziekenhuismedewerkers met verschillende beroepsmatige blootstelling aan het pandemische influenza A (H1N1) virus. Er bestonden belangrijke bedenkingen dat het samenbrengen van patiënten met influenza-achtige symptomen in gezondheidszorg instellingen zou kunnen leiden tot een significant risico op transmissie van het virus naar de medewerkers. De grieppolikliniek van het Slotervaartziekenhuis heeft zorg gedragen voor meer dan 1,000 patiënten gedurende de 2009 H1N1 pandemie. Zesenzestig gezondheidszorg medewerkers werden nauwgezet gecontroleerd voor studie doeleinden. Wekelijkse keeluitstrijken werden verzameld van 26 hoog-risico medewerkers op de grieppolikliniek en van 20 middel-risico en 20 laag-risico medewerkers ten behoeve van RT-PCR detectie van pandemische influenza A (H1N1). Maandelijkse bloedmonsters werden verkregen voor serologische confirmatie. Daarnaast werden influenza-achtige verschijnselen en symptomen op regelmatige basis beoordeeld. Influenza vaccinatie werd aangeboden aan alle gezondheidszorg medewerkers via een lokaal vaccinatie programma: echter, de 2009 H1N1 vaccins waren pas na de piek van de epidemie beschikbaar. Eén van 26 hoog-risico deelnemers testte H1N1 positief door middel van RT-PCR. Dit komt overeen met een incidentiecijfer in de hoog-risico groep van 5.7/1,000 persoonsweken (95% BI 0-17/1,000). Geen van de middel- en laag-risico deelnemers testte positief door middel van RT-PCR. Significante antistof titer stijgingen in convalescente sera werden aangetoond in drie deelnemers: één was een bevestiging van het geval dat H1N1-positief was getest met RT-PCR; de andere werden waargenomen bij twee asymptomatische deelnemers die behoorden tot de middel- en hoog-risico groepen. Deze studie toonde een

lage incidentie van influenza A (H1N1) virus infectie aan onder gezondheidszorg medewerkers ten tijde van de 2009 H1N1 pandemie in een instelling met een hoge hygiënische standaard.

**Hoofdstuk 4 Diagnostiek**

In **Hoofdstuk 4.1** wordt de ervaring die gedurende de 2009 H1N1 pandemie is opgedaan met een "point-of-care" influenza-sneltest beschreven. De uitbraak van de pandemie had een dringende behoefte aan snelle en accurate diagnostische methoden veroorzaakt. Verschillende immunoassays waren beschikbaar die in staat waren om binnen 10 minuten zowel influenza A als influenza B antigenen aan te tonen. Maar deze bepalingsmethoden discrimineren niet tussen influenza subtypen (bijvoorbeeld pandemisch H1N1 of seizoens H1N1/H3N2) en ze zijn niet gevalideerd geweest onder klinische omstandigheden tijdens de pandemische uitbraak. De prestatie van een vaak gebruikte sneltest (QuickVue® Influenza A+B test; Quidel, San Diego, VS) werd geëvalueerd, nadat het was gebruikt op de eerste 187 patiënten die de grieppolikliniek van het Slotervaartziekenhuis bezochten om te helpen bij de klinische besluitvorming. De resultaten van de sneltest werden vergeleken met RT-PCR als de "gouden" standaard. De gemiddelde leeftijd van de patiënten was 34 jaar (bereik 2-84 jaar), 87 waren man (46.5%) en de gemiddelde tijdsduur vanaf het ontstaan van symptomen was 4 dagen (SD 2.1 dagen). RT-PCR was positief voor nieuwe influenza A (H1N1) bij 16 van 187 patiënten, wat een prevalentie van 9% in die periode impliceerde. De sneltest was negatief bij alle 187 patiënten. Om vals negatieve resultaten vanwege manipulatie en opslag van de inhoud van de testkits uit te sluiten, werd de sneltest ook nog eens toegepast op 9 opgeslagen monsters die sterk positief waren voor pandemische H1N1. De sneltest resultaten werden positief voor 5 van de 9 monsters. Over het geheel beschouwd, was de sensitiviteit van de test 20%, maar deze varieerde van 0% onder 187 ambulante patiënten met influenza-achtige verschijnselen en symptomen tot 55.6% onder de 9 opgeslagen, sterk H1N1-positieve laboratorium monsters. Er waren helemaal geen vals positieve resultaten, wat een specificiteit van de sneltest van 100% impliceert. Opmerkelijk is dat de door middel van RT-PCR bevestigde H1N1-positieve monsters waarvan de sneltest resultaten ook positief waren significant hogere virale ladingen bevatten dan die waarvan de sneltest resultaten negatief waren. Concluderend heeft deze studie een beperkte klinische prestatie aangetoond van de gebruikte sneltest voor het aantonen van het 2009 pandemische influenza A (H1N1) virus. Alhoewel de test in hoge mate specifiek bleek, was de voorspellende waarde om pandemische influenza infectie te diagnosticeren teleurstellend laag.

**Hoofdstuk 4.2** heeft tot doel om inzicht te geven in de acute fase respons en om de waarde van procalcitonine (PCT), een nieuwe biomarker voor ontsteking, te onderzoeken bij kinderen met een verdenking op 2009 pandemische influenza A (H1N1) virus infectie.

Gedurende de uitbraak van de pandemie was een snelle diagnose bij kinderen met griepachtige verschijnselen en symptomen van groot klinisch belang. Naast de beslissing om wel of niet te starten met oseltamivir, was het differentiëren tussen virale en bacteriële (super-) infectie erg belangrijk bij deze kinderen. Uit eerdere studies is aangetoond dat het gedifferentieerde leukocyten aantal en het meten van "C reactive protein" (CRP) in plasma mogelijk niet voldoende hulp zou kunnen bieden in dit verband. Aan de andere kant zou PCT een handig instrument kunnen zijn om te discrimineren tussen bacteriële en virale infectie, want het circulerende niveau van deze biomarker is met name verhoogd tijdens de vroege fase van ernstige bacteriële infectie en slechts mild verhoogd in de afwezigheid van bacteriën. Daarom werden 30 kinderen met influenza-achtige verschijnselen en symptomen geïncludeerd in deze prospectieve observationele studie die werd uitgevoerd tijdens de 2009 H1N1 pandemie. Hiervan werden 25 kinderen gediagnosticeerd met een virale respiratoire ziekte door middel van RT-PCR, inclusief 9 met pandemische influenza A (H1N1) infectie. Bloedmonsters werden verkregen van alle kinderen om de acute fase respons te bestuderen. Mediane PCT concentratie, CRP concentratie en leukocyten aantal in de subgroep met bewezen virale infectie was 0.153 ng/mL (IQR 0.094-0.261 ng/mL), 19.0 mg/L (IQR 5.6-28.3 mg/L) en 13.1 giga/L (IQR 9.5-16.0 giga/L), respectievelijk. De PCT concentratie bij kinderen met H1N1 infectie en andere virale ziekte was onder de afkwapwaarde van 0.71 ng/mL in 22 van de 25 kinderen (88%), terwijl werd gezien dat voor de CRP concentratie en het leukocyten aantal de afkapwaarde werd overschreden door 6 en 5 patiënten, respectievelijk. Deze resultaten suggereren dat PCT van toegevoegde waarde lijkt te zijn voor de diagnostische benadering van kinderen met een verdenking op pandemische influenza infectie, bij wie het klinisch zeer relevant is om een onderscheid tussen virale en bacteriële (super-) infectie te maken.

**Hoofdstuk 5 Cardiovasculaire complicaties**

In **Hoofdstuk 5.1** wordt de associatie tussen respiratoire virus infectie en acuut coronair syndroom onderzocht. Eerdere studies hadden aangetoond dat patiënten met symptomen van een luchtweginfectie een verhoogd risico op een acuut hartinfarct hebben. Om deze relatie te bevestigen werd een patiënt-controle onderzoek opgezet waarbij patiënten met een acuut coronair syndroom werden vergeleken met "gematchte" controle patiënten zonder enige voorgeschiedenis van coronairlijden. Het doel van deze studie was om een schatting van het risico te geven op acuut coronair syndroom voor patiënten met een laboratorium bevestigde recente virale luchtweginfectie. Laboratorium bevestiging werd verkregen door middel van RT-PCR en serologie op een scala aan respiratoire pathogenen. Van 7 januari 2008 tot en met 30 januari 2009 werden 41 patiënten met een acuut coronair syndroom en 41 "gematchte" controle patiënten geïncludeerd: laboratorium bewijs voor een

recente respiratoire virale infectie werd gevonden bij 7 patiënten met een acuut coronair syndroom (17%) en 6 controle patiënten (15%). De gecorrigeerde odds ratio op een acuut coronair syndroom voor patiënten die een laboratorium bevestige recente respiratoire virale infectie hadden, was 1.1 (95% BI 0.3-4.0). Geen van de drie deelnemers met symptomen van een influenza-achtig ziektebeeld (3 patiënten met een acuut coronair syndroom, 0 controle patiënten) had een bewezen infectie. De bevindingen van deze prospectieve studie suggereren dat het risico op een acuut coronair syndroom vergelijkbaar is voor patiënten met en zonder een laboratorium bewezen recente respiratoire virale infectie. Bovendien was er geen relatie tussen klinische verschijnselen van een luchtweginfectie en bevestigende laboratorium testen. Dit onderstreept het risico op misdiagnose-bias wanneer alleen maar symptomen worden gebruikt voor het diagnosticeren van een recente luchtweginfectie in studies die de associatie tussen respiratoire virus infectie en acuut coronair syndroom onderzoeken.

**Hoofdstuk 5.2** exploreert de hemostatische veranderingen na influenza vaccinatie in gezonde vrijwilligers. Gedurende de beginfases van de 2009 influenza A (H1N1) pandemie werd aanbevolen om zoveel mogelijk personen zo snel als mogelijk te vaccineren zodra de vaccins beschikbaar zouden komen. Preliminair bewijs had aangetoond dat de 2009 H1N1 vaccins immunogeen en veilig waren met mild-tot-matige vaccin-geassocieerde reacties. Het is echter altijd onduidelijk gebleven of influenza vaccinatie het risico op een acuut coronair syndroom verlaagt of verhoogt. Deze studie onderzocht de hypothese dat influenza vaccinatie stollingsafwijkingen veroorzaakt via een door het vaccin geïnduceerde ontstekingsreactie. Gezonde gezondheidszorg medewerkers die een geregistreerd 2009 H1N1 vaccin kregen (Focetria®; Novartis, Basel, Zwitserland), werden twee weken lang gevolgd. Net voor toediening van het vaccin en 14 dagen later werd bloed afgenomen om de antistof respons tegen het pandemische influenza A (H1N1) virus te bepalen en om verschillende parameters te meten die verband houden met stolling en fibrinolyse. Vierennegentig deelnemers met een gemiddelde leeftijd van 37 jaar (bereik 18-59 jaar) werden geïncludeerd voor de analyse. Een adequate antistof respons, d.w.z. een viervoudige stijging of meer van de antistof titer, werd aangetoond bij 80 deelnemers (85%). Protrombinetijd (PT) nam toe van 11.8 naar 11.9 seconden, D-dimeer nam af van 0.30 naar 0.26 µg/L en von Willebrand factor (VWF) daalde van 98.6% naar 92.2%; al deze veranderingen waren statistisch significant. Na correctie voor variatie van de hemostatische factoren volgens een circadiaan ritme bleef alleen de daling in D-dimeer statistisch significant. D-dimeer is een plasma fibrinolyse activatie marker en daarom zou de waargenomen daling een gestoorde fibrinolyse na influenza vaccinatie kunnen betekenen. Maar omdat de absolute verandering heel klein is en omdat het bekend is dat de waarde van plasma fibrinolyse activatie markers om cardiovasculaire ziekte te

voorspellen beperkt is, is deze bevinding waarschijnlijk niet klinisch relevant. Deze studie concludeerde dat er geen blijvende procoagulante effecten waren van 2009 H1N1 vaccinatie onder gezonde gezondheidszorg medewerkers.

**Hoofdstuk 6 Behandeling**

Ten slotte bevat **Hoofdstuk 6.1** een systematisch literatuur onderzoek naar bewijzen voor klinische effectiviteit en veiligheid van oseltamivir voor de behandeling en profylaxe van influenza. Oseltamivir is een antiviraal middel waarvan wordt aangenomen dat het effectief is voor preventie en behandeling van influenza. Om die reden bevelen deskundige adviesgroepen aan om het antivirale geneesmiddel te gebruiken voor patiënten die een hoger risico lopen op secundaire complicaties gerelateerd aan influenza infectie. Eerdere, gepubliceerde systematische literatuur onderzoeken over dit onderwerp hadden hun onderzochte populaties meestal beperkt tot gezonde volwassenen, kinderen of ouderen. Dit hoofdstuk geeft een veelomvattende beschouwing waarbij gegevens worden gecombineerd van de verscheidenheid aan risicogroepen waarvoor antivirale behandeling tegenwoordig wordt aanbevolen, naast een kwalitatieve analyse van het bewijs voor effectiviteit bij gezonde vrijwilligers. Gebruik makende van vooraf gedefinieerde zoektermen gecombineerd met Cochrane zoekstrategieën voor vergelijkende studies, werden, na een uitgebreide zoektocht van de PubMed database, uiteindelijk 66 studies geïncludeerd. Voor normaalgesproken gezonde volwassenen is er goed bewijs van hoge kwaliteit dat behandeling de ziekteduur verkort met 0.5-1.5 dagen en (meestal milde) secundaire complicaties met 40-50%. De resultaten met betrekking tot effectiviteit van behandeling voor diegenen die zijn opgenomen in een ziekenhuis of die ernstige, gecompliceerde of progressieve ziekte hebben, zijn tegenstrijdig. Er is weinig bewijs voor effectiviteit van behandeling voor individuën die een hoog risico op complicaties hebben, behalve matig bewijs voor diegenen met chronische pulmonale of cardiovasculaire aandoeningen, bij wie behandeling met oseltamivir secundaire complicaties significant lijkt te verminderen (relatief risico bereik 0.17-0.65). Oseltamivir profylaxe geeft in het algemeen een bescherming van 64-75% tegen infectie. Concluderend is er matig bewijs om het therapeutische gebruik van oseltamivir voor individuën met chronische pulmonale en cardiovasculaire aandoeningen te ondersteunen en het bewijs voor individuën uit andere aanbevolen hoog-risico populaties is schaars.

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## Curriculum vitae

Patrick Smit was born on April 3, 1980 in Amsterdam, the Netherlands. He graduated from secondary school (Jan van Egmond College, Purmerend) in 1998. Next he attained an undergraduate degree in psychology at VU University in Amsterdam in 1999 and, subsequently, an undergraduate degree in dance at the Amsterdam School of the Arts in 2001. Patrick continued his educational pursuits by attending medical school at the Academic Medical Center in Amsterdam in 2002 and obtained his medical degree *cum laude* from the University of Amsterdam in 2008. After working as a junior medical doctor at Slotervaart Hospital in Amsterdam, he commenced his PhD training there in April 2009, under the supervision of Dr. D.P.M. Brandjes, Dr. J.W. Mulder and Prof. Dr. J.H. Beijnen. During this time, he practiced as a medical doctor at the hospital's outpatient clinic responsible for addressing the healthcare needs of HIV patients. In addition, he concurrently completed a program offered by the Dutch Society of Clinical Pharmacology & Biopharmacy to become a clinical pharmacologist. In May 2012, Patrick will start his internal medicine residency at Gelre Ziekenhuizen in Apeldoorn and the University Medical Center in Utrecht.

Patrick Smit werd geboren op 3 april 1980 in Amsterdam, Nederland. Hij slaagde hij voor zijn middelbare school opleiding (Jan van Egmond College, Purmerend) in 1998. Daarna behaalde hij een propedeuse psychologie aan de Vrije Universiteit in Amsterdam in 1999 en vervolgens een propedeuse uitvoerende theaterdans aan de Amsterdamse Hogeschool voor de Kunsten in 2001. Patrick zette zijn educatieve bezigheden voort door geneeskunde te gaan studeren in het Academisch Medisch Centrum in Amsterdam in 2002 en behaalde zijn artsenbul *cum laude* aan de Universiteit van Amsterdam in 2008. Nadat hij ervaring had opgedaan als ANIOS (arts niet in opleiding tot specialist) op de afdeling interne geneeskunde van het Slotervaartziekenhuis, begon hij in april 2009 aan zijn promotie traject in hetzelfde ziekenhuis onder begeleiding van Dr. D.P.M. Brandjes, Dr. J.W. Mulder en Prof. Dr. J.H. Beijnen. Hij combineerde zijn promotie onderzoek met het werken als HIV behandelaar op de polikliniek interne geneeskunde van het ziekenhuis. Daarnaast volgde hij tegelijkertijd een opleiding tot klinisch farmacoloog, aangeboden door de Nederlandse Vereniging voor Klinische Farmacologie en Biofarmacie. In mei 2012 zal hij starten met zijn medische specialisatie interne geneeskunde in Gelre ziekenhuizen te Apeldoorn en het Universitair Medisch Centrum in Utrecht.