Thesis

Development of a clinical score for diagnosis of Influenza A/ H1N1 infection and differentiation from influenza-like illnesses

Comparison of laboratory values between PCR confirmed H1N1 influenza and PCR negative influenza-like illnesses

submitted by

Manuela Drescher

Date of birth: February 6, 1990

in order to attain the academic degree

Doctor of Medicine, MD

(Dr. med. univ.)

at the

Medical University of Graz

performed at the

Section of Infectious Diseases

under the supervision of

Priv. Doz. Dr. Martin Hönigl

Ao. Univ. Prof. Dr. Robert Krause

Graz, 30.10.2013 Unterschrift

Principal Investigators:

Cand. med. Drescher Manuela

Priv. Doz. Dr. Martin Hönigl

Ao. Univ.Prof.Dr. Robert Krause

OA Holger Flick

Section of Infectious Diseases
Department of Internal Medicine
Medical University of Graz
Auenbruggerplatz 20
8036 Graz
Phone +43 316 385 81796
Fax +43 316 385 4622
robert.krause@meduni-graz.at

Eidesstattliche Erklärung

Ich erkläre ehrenwörtlich, dass ich die vorliegende Arbeit selbstständig und ohne fremde Hilfe verfasst habe, andere als die angegebenen Quellen nicht verwendet habe und die den benutzten Quellen wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe.

Graz am 30.10.2013 *Unterschrift*

Declaration of Originality

I hereby declare that this thesis and the work reported here in was composed by and originated entirely from me. Information derived from the published and unpublished work of others has been acknowledged in the text and references are given in the list of sources.

Graz, 30.10.2013 *Signature*

Preamble

A poster of this work was presented at the 6th congress of the Austrian society of infectious diseases and tropical medicine (OEGIT), April 2012, Saalfelden/ Austria- and won a poster prize (second place).

Acknowledgements

First and foremost I owe my deepest gratitude to Priv.Doz.Dr. Martin Hönigl. None of this would have been possible without his advice and support during the preparation. I want to thank him for the offer to take part in this research project. Furthermore I want to thank him for his patience and helpfulness.
I like to express greatest thanks for encouragement, cheerfulness and instructions to Dr. Holger Flick and Prof. Dr. Robert Krause.
Last but not least my sincere thanks go to my family for their care, their never- ending encouragement and their support.

Abstract

Aim

During the 2009 influenza A /H1N1 pandemic more than 18000 people lost their lives worldwide. Reliable and rapid diagnosis is essential to initiate appropriate antiviral therapy in influenza as treatment is most effective when initiated within 48 to 72 hours after the onset of symptoms. PCR is the gold standard to confirm H1N1 but is expensive and results usually are not available in time for therapeutic or preventive decisions within 72h after onset of symptoms. The currently available antigen tests are fast but display low sensitivity.

The development of a clinical score for H1N1 influenza may facilitate the decision whether or not to initiate antiviral treatment, improve outcome and reduce morbidity and mortality. As a possible component of a proposed score in the future we retrospectively analyzed the differences in laboratory values between patients with PCR confirmed H1N1 influenza and patients with clinical suspicion but negative PCR for influenza (=influenza-like illness [ILI]).

Methods

During Oct 2009 and Jan 2010 H1N1 PCR was performed in 1681 patients with clinical suspicion of influenza. 624 patients had a positive and 1057 patients had a negative H1N1 PCR result. Laboratory data sets were available from 221/624 (35%) H1N1 patients and 312/1057 (30%) ILI patients.

Differences in laboratory values between both groups and correlation of laboratory values with underlying disease were calculated using the Mann-Whitney U test , and $p<0.05$ was considered significant.

Results

At initial presentation the median of the total white blood cell count (WBC) in the H1N1 influenza group was 6.7 G/l (IQR, 5- 8.6 G/l) and therefore significantly lower than in the ILI group (median 9.86 G/l [IQR, 7.35- 13.74 G/l]); $p < 0.001$). A similar pattern was seen for day 3 and 5 values of leukocytes during hospitalization. Also thrombocyte count at admission was significantly lower in patients with PCR confirmed H1N1 infection than in patients with ILI (201 G/l [IQR, 156.5- 225 G/l] versus 232 G/l [IQR, 182- 288.5 G/l]; $p < 0.001$).

In the distribution of the WBC count only the relative eosinophil count showed significantly lower values in patients with confirmed H1N1 infection ($p= 0.006$).

Comparison of C- reactive protein (CRP) values between PCR confirmed H1N1 influenza and PCR negative ILI demonstrated that CRP was significantly lower in patients with H1N1 (11 mg/l [IQR, 5- 64 mg/l] versus 23 mg/l [IQR, 7- 100.85 mg/l]; $p < 0.001$). Furthermore, also gamma-Glutamyl-Transferase (GGT) was significantly lower in patients with confirmed H1N1 infection ($p< 0.05$).

Creatine kinase (CK) values were significantly higher in patients with PCR confirmed H1N1 infection (113 U/l [IQR, 61.5- 192 U/l] versus 72 U/l [IQR, 48.5-116.5 U/l]; p< 0.001).

Conclusion

The results revealed that there were significant differences in laboratory admission values between people with PCR confirmed H1N1 influenza and those with PCR negative influenza-like illnesses. Total white blood cell count, relative and absolute eosinophil count, thrombocytes, CRP and GGT were significantly lower in patients with confirmed H1N1 infection, while creatine kinase values were significantly higher.

The results of this study might contribute to the implementation of a clinical score for a more efficient and faster clinical diagnosis of a H1N1 infection.

Zusammenfassung

Zielsetzung

Während der Schweinegrippe-Pandemie 2009 (Influenza A/ H1N1) starben laut Angaben der WHO mehr als 18000 Menschen an den Folgen der neuen Grippe. Eine verfügbare und schnelle Diagnostik ist in solch einer Zeit essentiell, um eine adäquate antivirale Therapie einleiten zu können. Diese sollte spätestens 72 Stunden nach dem Auftreten der ersten Symptome eingeleitet werden. Die PCR stellt zwar den Goldstandard dar, um eine H1N1 Infektion zu bestätigen, aber sie ist teuer und die Wartezeit bis zum Erhalt der Resultate nimmt zu viel Zeit in Anspruch, um sie für therapeutische oder präventive Entscheidungen nutzen zu können. Es gibt auch Antigen- Tests, aber diese zeigen eine sehr niedrige Sensitivität.

Die Entwicklung eines klinischen Scores für H1N1 Influenza könnte die Entscheidung erleichtern, ob man eine antivirale Therapie einleiten sollte, oder nicht. Außerdem könnte dadurch das Outcome der Patienten verbessert werden, und sowohl die Mortalität als auch die Morbidität reduziert werden.

Eine mögliche Komponente dieses Scores könnten Unterschiede in den Laborparametern sein. Deshalb analysierten wir die Unterschiede in den Laborwerten von Patienten mit einer PCR gesicherten H1N1 Infektion mit den Laborwerten von Patienten, die wegen des klinischen Verdachtes einer H1N1 Infektion kamen, aber bei denen die PCR negativ ausfiel (Influenza-like illness [ILI]).

Methoden

Von Oktober 2009 bis Januar 2010 wurden bei insgesamt 1681 Patienten, welche den klinischen Verdacht auf eine Influenza A/H1N1 Infektion hatten, eine PCR durchgeführt.

Von diesen 1681 Patienten hatten 624 ein positives PCR Ergebnis bezüglich einer H1N1 Infektion; sprich sie hatten tatsächlich eine „Schweinegrippe“. 1057 Patienten wiesen ein negatives PCR Ergebnis auf (ILI). Anschließend wurden die Laborwerte von den PCR positiven und den PCR negativen Patienten ermittelt und verglichen. Bei der PCR positiven Gruppe konnten von 35% (221/624) der Patienten die Labordaten verwendet werden und von der PCR negativen Gruppe 30% (312/1057). Bei den restlichen Patienten waren die Labordaten unvollständig.

Die Unterschiede bei den Laborparametern wurden mit dem Statistik Programm SPSS analysiert. Die Berechnung erfolgte mittels Mann-Whitney U Test. Ein $p < 0,05$ wurde als statistisch signifikant erachtet.

Resultate

Bei der Erstpräsentation betrug der Median der Leukozyten bei den Patienten mit H1N1 6,7 G/l (IQR, 5- 8,6 G/l) und war somit signifikant niedriger als bei den ILI

Patienten (Median 9,86 G/l [IQR, 7,35- 13,74 G/l]; p< 0,001). Ein ähnliches Bild zeigte sich bei den Leukozytenwerten am 3. und 5. Tag während des Krankenhausaufenthaltes. Auch die Thrombozytenwerte waren am Aufnahmetag signifikant niedriger bei den Patienten mit einer H1N1 Infektion als bei den Patienten mit einer ILI (201 G/l [IQR, 156,5- 225 G/l] versus 232 G/l [IQR, 182-288,5 G/l]; p<0.001).
Im Differentialblutbild waren die eosinophilen Granulozyten signifikant niedriger bei den Patienten mit einer H1N1 Infektion (p=0,006). Der Vergleich des CRP zwischen der H1N1 Influenza Gruppe und der ILI Gruppe zeigte sigifikant niedrigere Werte in der H1N1 Gruppe (11 mg/l [IQR, 5- 64 mg/l] versus 23 mg/l [IQR, 7- 100,85 mg/l]; p<0,001). Des Weiteren war die GGT signifikant niedriger bei den Patienten mit gesicherter H1N1 Infektion (p<0.05).
Die Kreatinkinase war in der H1N1 Influenza Gruppe signifikant höher als in der ILI Gruppe (113 U/l [IQR, 61,5- 192 U/l] versus 72 U/l [IQR, 48,5- 116,5 U/l]; p< 0.001).

Diskussion

Die Ergebnisse zeigten statistisch signifikante Unterschiede bei den Laborwerten zwischen Patienten mit PCR gesicherter H1N1 Infektion und Patienten mit klinischem Verdacht, aber negativer H1N1 PCR. Bei der Erstpräsentation im Krankenhaus zeigten Patienten mit einer gesicherten H1N1 Infektion signifikant niedrigere Werte bei den Leukozyten, bei den eosinophilen Granulozyten, bei den Thrombozyten, beim CRP und bei der GGT als Patienten mit einem negativen PCR Ergebnis.
Die Kreatinkinase war in der Gruppe mit den H1N1 infizierten Patienten signifikant höher als in der ILI Gruppe.
Diese Daten könnten bei der Implementierung eines klinischen Scores helfen, damit eine H1N1 Infektion schneller diagnostiziert und behandelt werden kann.

List of contents

Glossary and abbreviations

ALT	alanine aminotransferase
AST	aspartate aminotransferase
CDC	Centers for Disease Control
CK	creatine kinase
CRP	C- reactive protein
DNA	Deoxyribonucleic acid
ECDC	European Centre for Disease Prevention and Control
GGT	gamma-Glutamyl-Transferase
H or HA	hemagglutinin
ILI	influenza-like illness
INR	International Normalized Ratio
IQR	interquartile range
LDH	lactate dehydrogenase
N or NA	neuraminidase
PA	Polymerase acid
PB1	Polymerase basic 1
PB2	Polymerase basic 2
PCR	Polymerase Chain Reaction
RIDT	Rapid Influenza Diagnostic Test
RNA	Ribonucleic acid
RTPCR	Real time Polymerase Chain Reaction
SPSS	Statistic Package for Social Sciences
WHO	World Health Organisation

1 Introduction

Influenza has been one of the most important infectious diseases of the last century. The word “influenza” roots back to the Latin word “influere” and means “to flow into”. There are three types of influenza viruses: A, B and C (1). Influenza A viruses are the perpetual cause of perseverative human influenza pandemics.
In 2009 a new strain of influenza A appeared and spread all over the world. Pandemic influenza A /H1N1 (better known as „swine flu“) lasted for approximately one year and was responsible for over 18000 death cases worldwide (2).

1.1 Structure of the virus

Influenza A viruses are negative-sense, single-stranded RNA viruses and belong to the family of the Orthomyxoviridae (3).

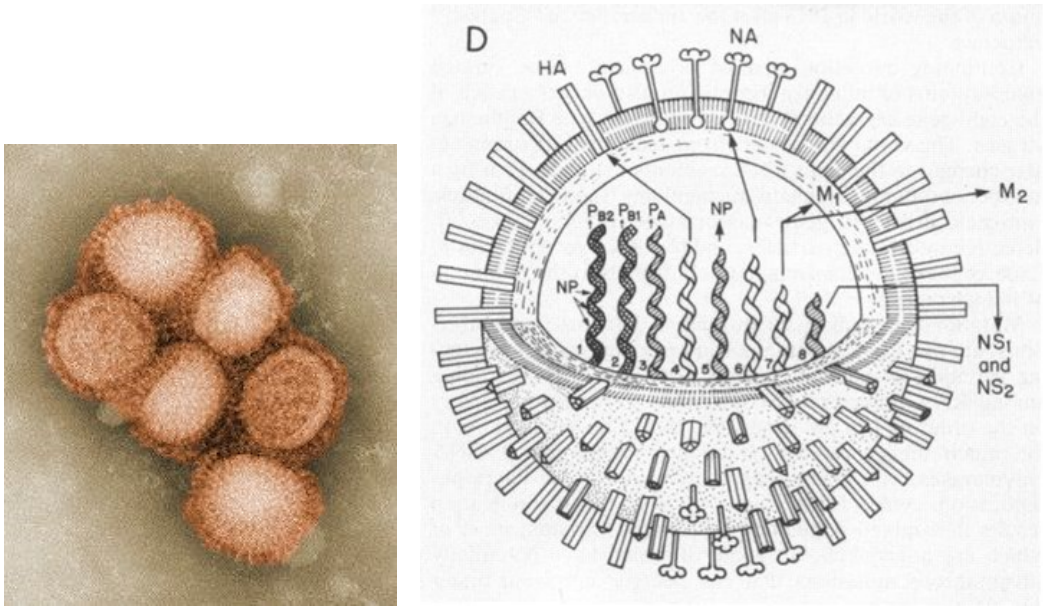

Figure 1: Structure of the virus: The virus is built up of a lipid bilayer envelope, a inner coat of matrix protein and nucleocapsids of viral genome at the centre (adapted from [3, 4])

On the surface of the viruses are two different proteins -a hemagglutinin molecule (HA) and a neuraminidase molecule (NA). Based on these proteins the viruses are categorized into different subtypes. There are 16 hemagglutinin subtypes (H1-H16) and 9 neuraminidase subtypes (N1- N9). These proteins are responsible in

large part for human infectivity, pathogenicity and transmissibility- but they are admittedly poorly understood (5).

Another integral part of the viral envelope is the matrix protein (M2). Inside the viral envelope the matrix protein M1 forms a coat and binds the viral RNA (6). In the core there are the genome segments. Each of the eight nucleocapsid segments consists of a genomic RNA, a nucleoprotein (N), and three polymerase poly peptides (PB1, PB2 and PA) (1, 7). The polymerase poly peptides are responsible for the transcription and replication of the viral RNA genome in infected cells (8).

Influenza A viruses are pleomorphic and small (80 to 120nm in diameter) particles that later become spherical.

Furthermore influenza A viruses are able to infect a number of host species, including pigs, birds, horses, sea mammals and humans (3).

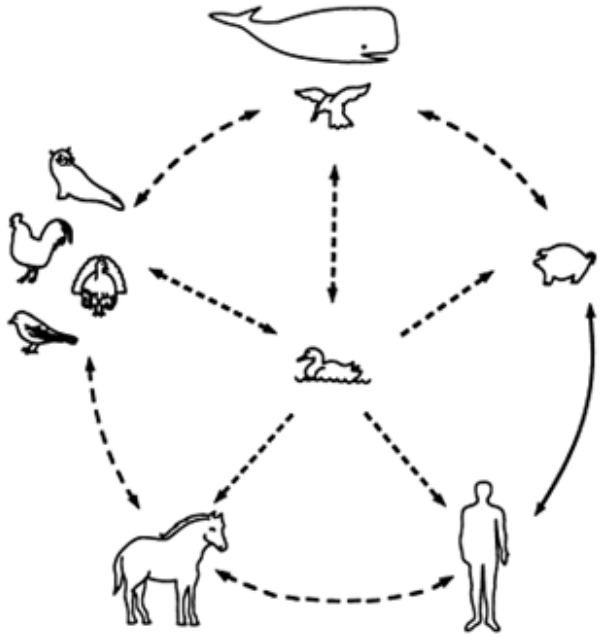

Figure 2: The figure shows the reservoir of influenza A viruses. Wild aquatic birds are the primordial reservoir of all influenza viruses (adapted from [3])

Influenza A viruses are capable to provoke pandemics[1] (3, 9). Pandemics mostly arise when new virus strains appear and people do not yet have any immunity (10).

[1] A pandemic is the global spread of a disease (10).

1.2 Historical background

1.2.1 Emergence of Influenza A/ H1N1

The history of swine influenza A (H1N1) began in 1918. During this year the human influenza A (H1N1) (better known as “Spanish Influenza”) killed about 40-50 million people all over the world. At the same time herds of swine in Iowa/ United States developed similar clinical symptoms like people who suffered from human influenza A (H1N1) (11).

Only in 1931 the veterinarian Robert Shope discovered with his experiments that swine influenza virus had his roots in the 1918 pandemic strain (12).

The Asian Flu (1957 pandemic A [H2N2]) and the Hong Kong Flu (1968 pandemic A [H3N2]) resulted from a reassortment event, generating new subtypes (13).

In 1976 influenza A (H1N1) was isolated in European pigs for the first time. The first case of a human infected with swine influenza A (H1N1) was reported in 1998 in Wisconsin. It was a 17 year old who had been exposed to pigs in a slaughterhouse before.

Between 2005 and 2009 11 cases of an infection with swine influenza A (H1N1) were recognized. Most of them had contact with pigs before (11).

The 2009 influenza A (H1N1) virus strain was a mixture of gene segments that has never been seen in human or swine influenza viruses before.

It was a mixture of influenza viruses from pigs, humans and birds but it affected only humans (11, 14).

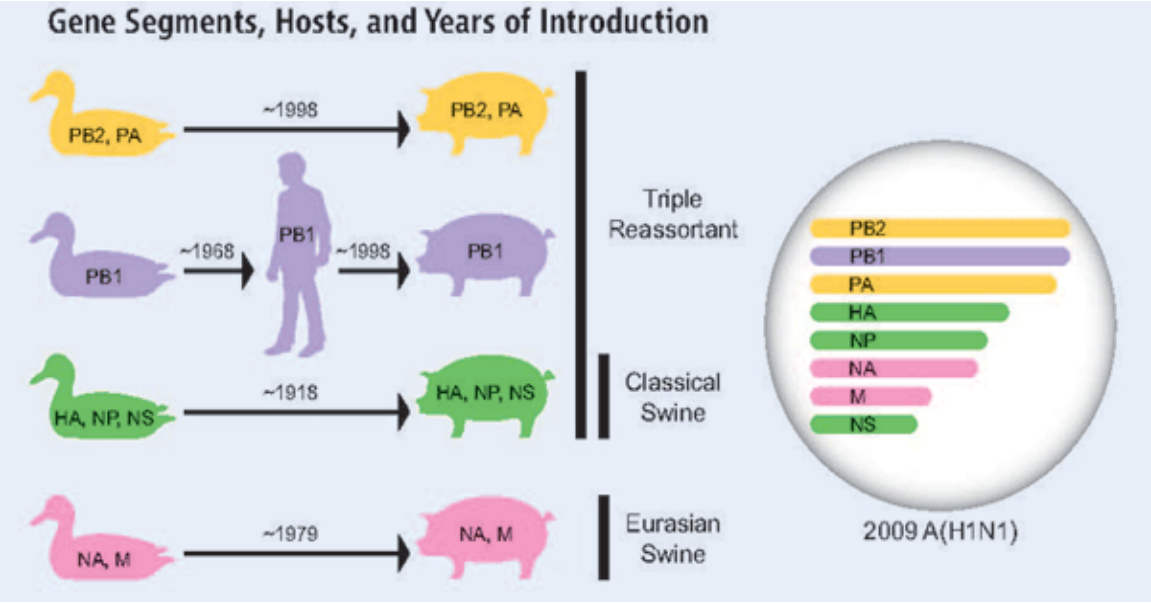

Figure 3: Origins for the gene segments of the pandemic H1N1 virus (adapted from [7])

1.2.2 Pandemic outbreak in 2009

On 17 April 2009 Centers for Disease Control and Prevention (CDC) reported the outbreak of a novel triple -reassortant swine-origin influenza A (H1N1) among two children in California.

Both of them had onset of fever and cough, but none of the children had direct exposure to pigs. The transmission of the virus was unknown (15).

At the same time as the events with the two children in California became public, the Mexican Ministry of Health notified an increased number of hospitalizations on the basis of severe pneumonia and a heaped number of unclear death cases.

An increased number of laboratory confirmed influenza was found. This was very unusual because in Mexico seasonal influenza occurs normally from October to March and especially elderly people are affected. Oddly, particular young people between the ages 20 to 40 years showed symptoms.

Consequently, the ministry of health intensified the notification of people who were hospitalized due to severe pneumonia.

During March 24 and April 29, 2009 8817 nasopharyngeal specimens were tested for influenza A. 3664 of them were positive (42%) (16).

In 2009, 6945 cases of confirmed H1N1 infections were reported in Mexico between April 28 and July 31 (17).

In late April 2009 the World Health Organisation (WHO) officially proclaimed the appearance of a novel influenza A (H1N1) virus and raised the flu pandemic alert level to five which meant human-to-human transmission of a novel influenza strain in one WHO region of the world (18).

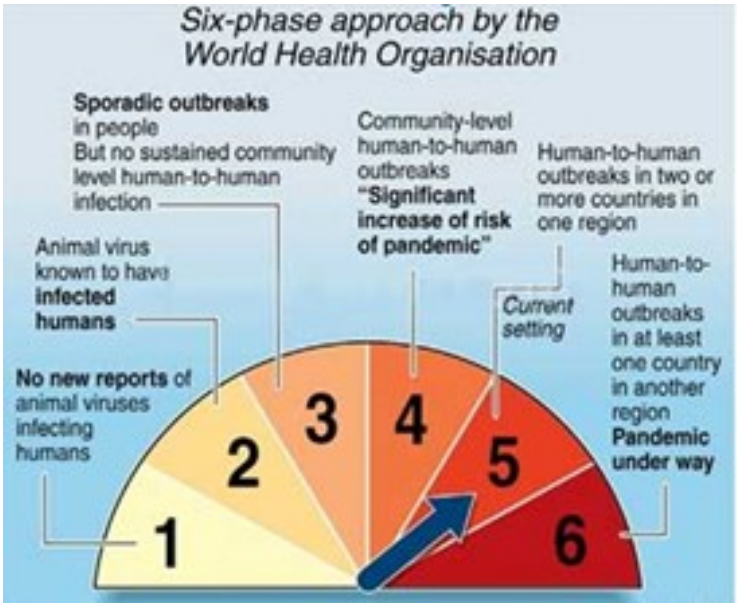

Figure 4: WHO flu alert system (adapted from [14])

On 11 June 2009, WHO Director-General Dr. Margaret Chan made a press statement about this special H1N1 strain. Up to this date there were reports from almost 30000 confirmed H1N1 infections in 74 countries.

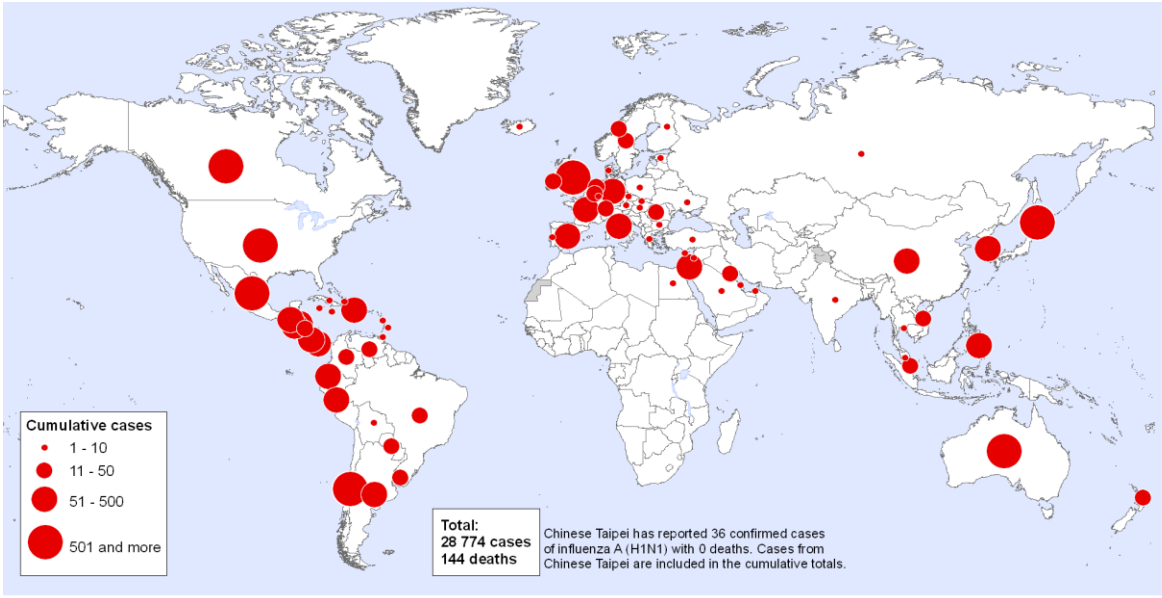

Figure 5: Number of laboratory confirmed cases of influenza A (H1N1) infections; Status as of 11 June 2009 (adapted from [19])

According to experts and leading virologists criteria for a pandemic have been met. WHO declared that “the world is now at the start of the 2009 influenza pandemic”. WHO elevated the level of the new influenza in their alert system to phase 6 (20). This meant that there were human-to-human outbreaks in different WHO regions (18).

The new influenza infection differed from the seasonal infection with regards to the fact that the majority of cases of severe infections were seen in people between the age of 30 and 50. It was a warning sign that half of the cases of severe and fatal H1N1 infections concerned healthy young and middle aged people (20).

1.2.3 Geographic spread of the pandemic

The rapid spread of the new influenza strain is depicted in Figures 6 and 7. On the 27th of July 2009 a total of 134503 cases of influenza A (H1N1) virus infections were reported.

In only one month the number of confirmed cases had more than quadrupled.

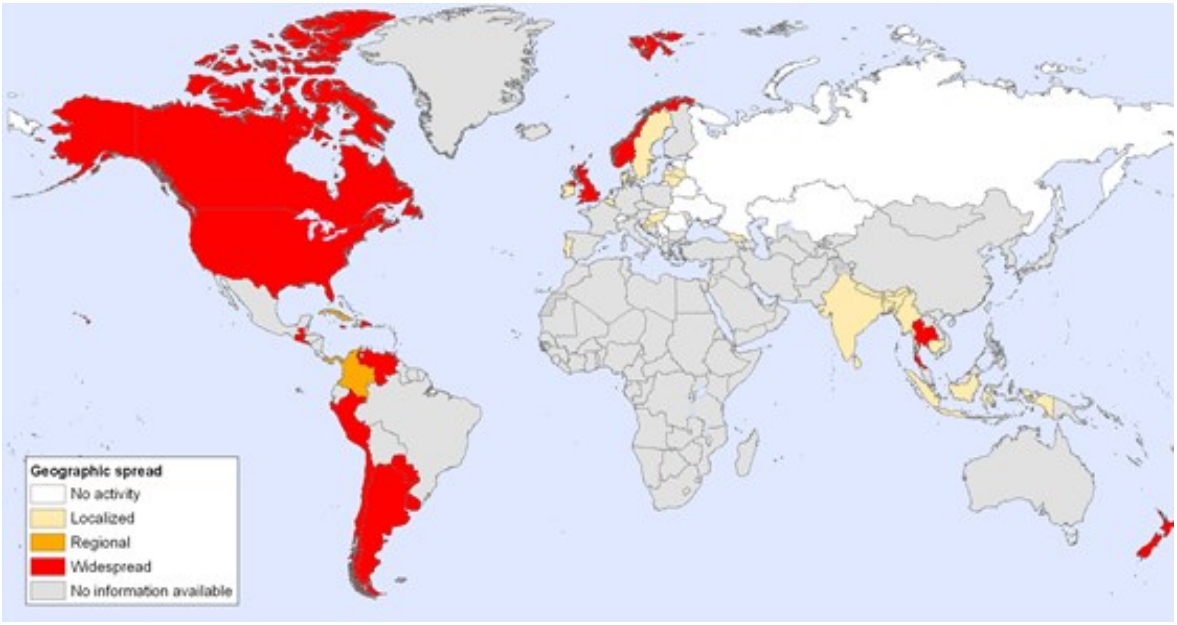

Figure 6: The figure shows the initial spread of influenza activity; status as of 20- 26 July 2009 (adapted from [21])

On the 31st July 2009 WHO announced that 168 countries had reported at least one laboratory confirmed case of H1N1 infection. At that time the pandemic influenza had already affected all continents. Only four days after the last WHO

update of the new influenza another 30000 new cases of confirmed H1N1 infections were reported (22).

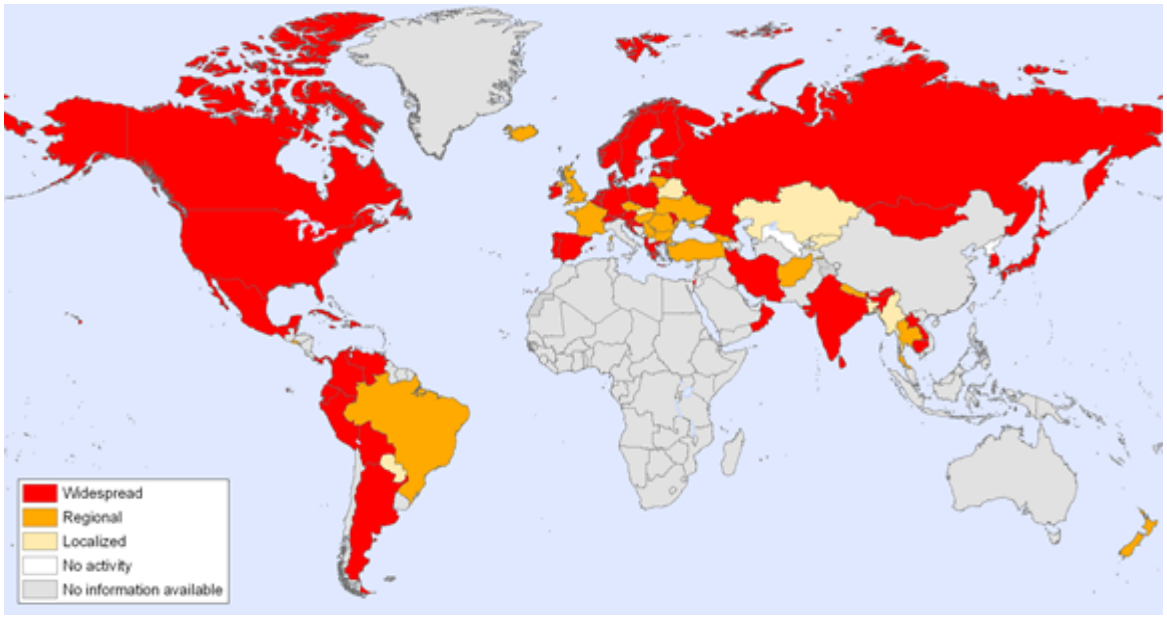

Figure 7: In November 2009 (week 47) the influenza virus already emerged in the entire continent of America, in Europe and in Asia (adapted from [23])

Controlling the spread of the pandemic became a demanding task for governments worldwide. The propagation of the virus was enormous. In November 2009 a total of 622482 confirmed cases of pandemic influenza A were reported from the WHO (24).

In reality the number of cases that have occurred was considerably higher than the officially case count. However, many countries even stopped counting individual cases, especially those that were not that serious (25).

In April 2010, one year after the outbreak of the pandemic more than 213 countries had reported laboratory confirmed cases of influenza A/ H1N1 infections. At this time Southeast Asia, West Africa, and the tropical zone of the Americas were the most affected areas of the pandemic. In Europe and in the northern moderate climate zones of Americas a slowdown of the virus circulation was already observed (26).

In summer 2010, pandemic influenza activity declined to very low levels, but persisted in areas of the tropics, especially in Southeast Asia and the Caribbean (27).

On the 10th of August 2010 WHO Director General Dr. Margaret Chan declared that the influenza A/H1N1 virus had moved into the post-pandemic period, which meant that the virus had spread over the world and because people were immune to it. However, there were localized outbreaks of H1N1 infections in different parts of the world.

Although the H1N1 influenza virus is now in the post pandemic period, the WHO with its global influenza program will continue to register the activity of the influenza A (H1N1) virus all over the world (28).

.

1.2.4 Situation in Europe during the pandemic

In Europe, the 2009 influenza A (H1N1) pandemic lasted for 68 weeks (from April 2009 to August 2010).

According to the European Centre for Disease Prevention and Control (ECDC) the influenza A (H1N1) virus entered Europe around week 16, 2009. Travellers arriving from Mexico brought the virus to different European countries. The first case describing an influenza A (H1N1) infection in Europe was reported on April 19th, 2009 (29).

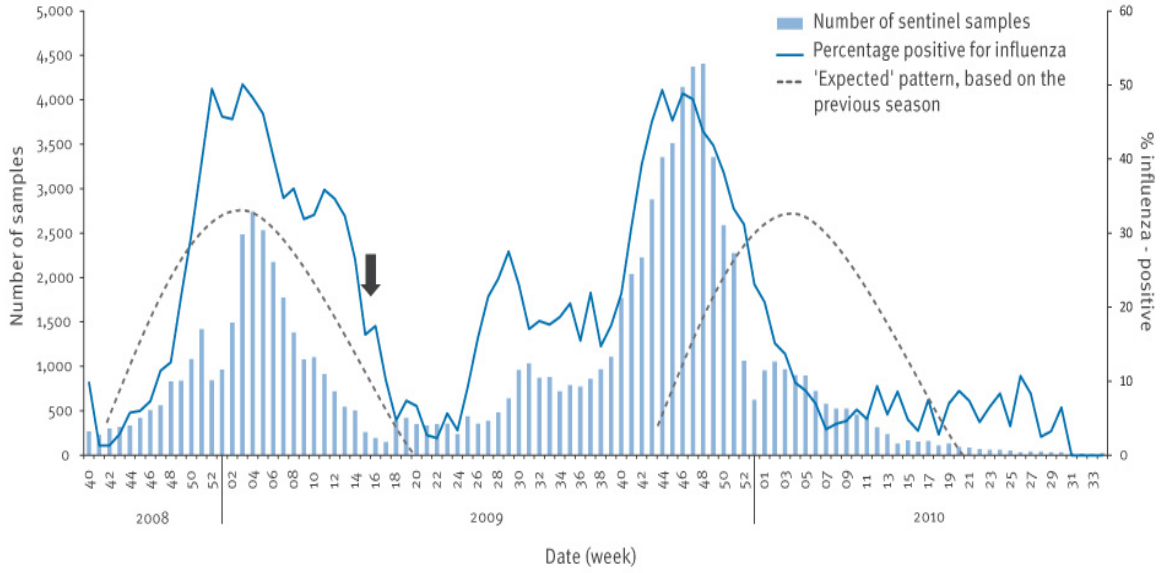

Figure 8: The division of the number of sentinel samples submitted and the percentage found positive for influenza. The arrow marks the beginning of the pandemic in Europe (adapted from [29])

In November 2009 very high activity of the pandemic was seen in Sweden, Norway, Moldova and Italy.
In Europe, over 99% of subtyped influenza A viruses transpired as H1N1 (24).

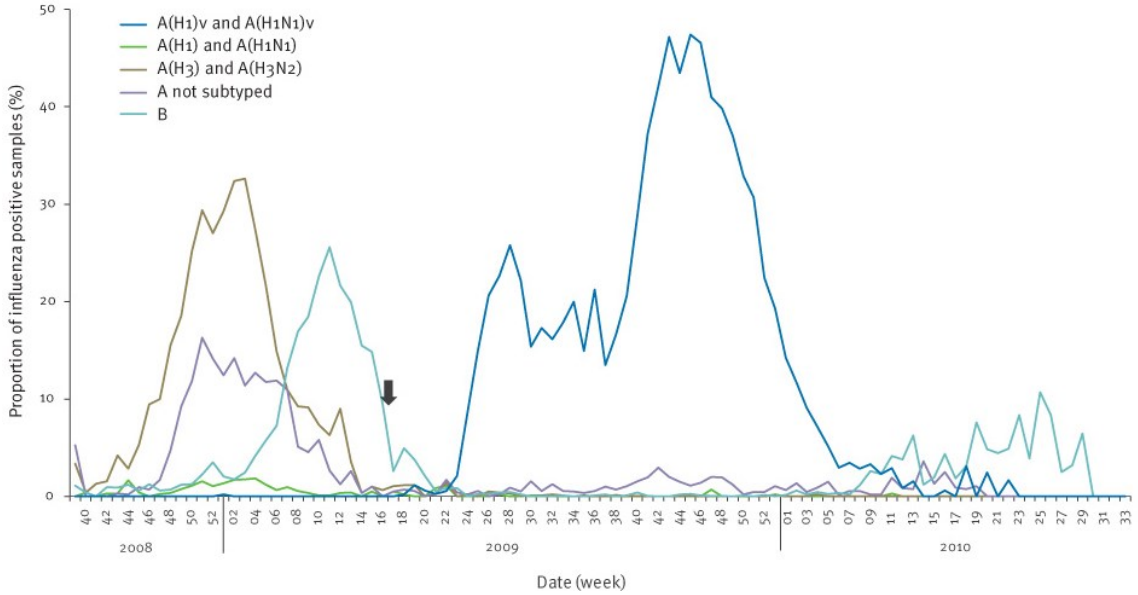

Figure 9: Distribution of virus subtypes during the pandemic in Europe (adapted from [29])

In December 2009 the pandemic activity already declined in North America, but peaked in western and northern Europe (25). In April 2010 pandemic activity declined in Europe and the virus circulated only across limited areas (26). The H1N1 pandemic was responsible for almost 5000 deaths in Europe (30).

1.2.5 H1N1 associated Deaths

At the end of April 2009 WHO reported the first eight death cases, seven of them in Mexico (31).
In July 2009 there were already reports of over 1000 deaths associated with H1N1 influenza (22).

Region	H1N1 associated deaths
WHO Regional Office for Africa	0
WHO Regional Office for the Americas	1008
WHO Regional Office for the Eastern Mediterranean	1

WHO Regional Office for Europe	41
WHO Regional Office for South-East-Asia	65
WHO Regional Office for the Western Pacific	39
Total	**1154**

Table 1: Reported deaths worldwide; July 2009 (adapted from [22])

The number of deaths doubled within one month. In August 2009 already 2000 death cases were reported to WHO.

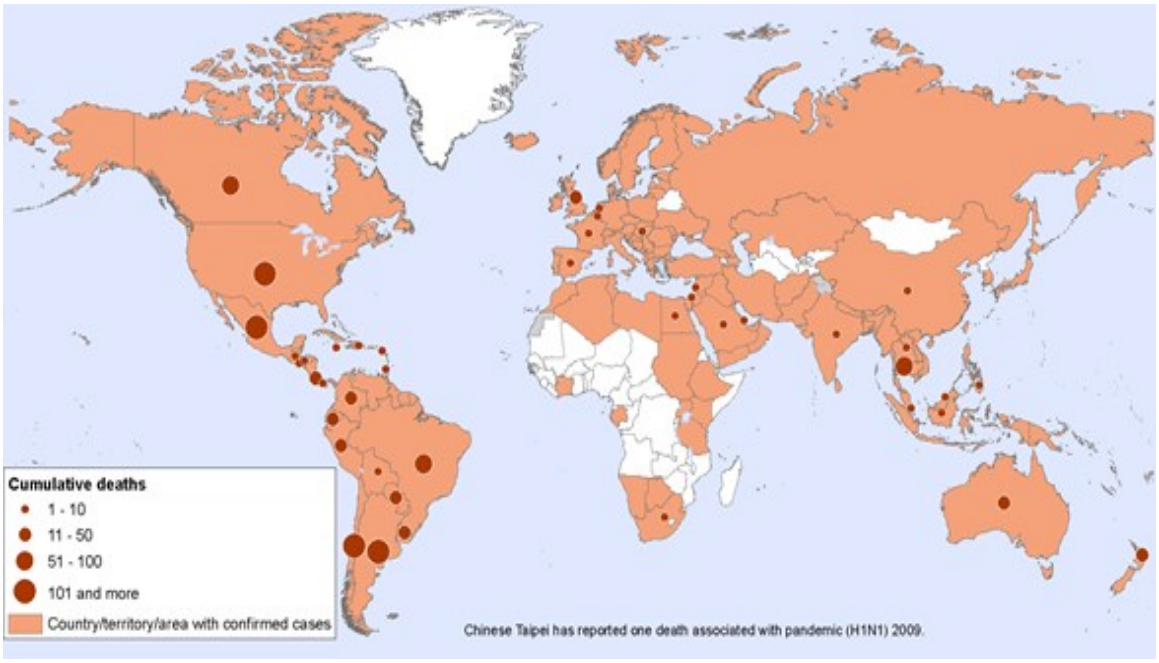

Figure 10: Number of deaths as reported to WHO in August 2009 (adapted from [23])

When comparing figure 10 and figure 11 the development of H1N1 influenza mortality within one year becomes evident. According to the WHO all in all over 18000 people died during that time (2, 30).

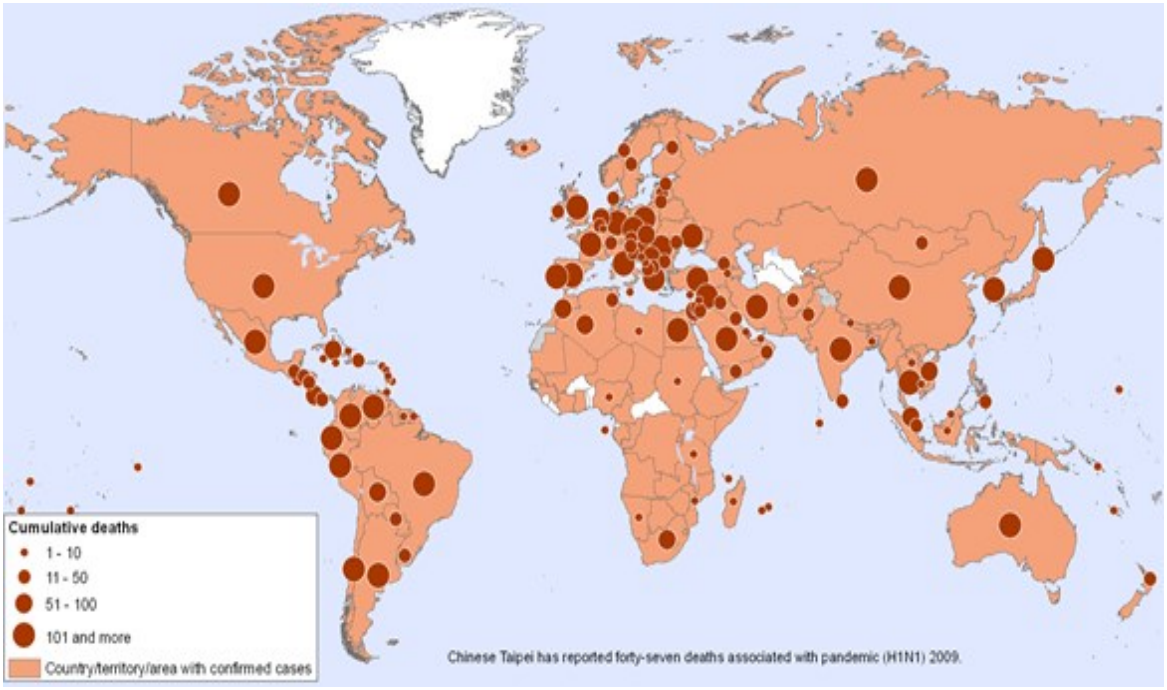

Figure 11: Reported death cases published by the WHO in August 2010 (adapted from [23])

Region	**H1N1 associated deaths**
WHO Regional Office for Africa	168
WHO Regional Office for the Americas	At least 8532
WHO Regional Office for the Eastern Mediterranean	1019
WHO Regional Office for Europe	At least 4879
WHO Regional Office for South-East-Asia	1945
WHO Regional Office for the Western Pacific	1855
Total	**At least 18398**

Table 2: Reported death cases with confirmed H1N1 infection during the pandemic (adapted from [30])

1.3 Clinical characteristics

Symptoms of pandemic influenza A /H1N1 include (32, 33):

- Fever
- Cough
- Sore throat
- Runny nose
- Body aches
- Headache
- Chills
- Fatigue
- Nausea
- Diarrhea
- Vomiting

Especially young people were affected by the pandemic influenza A -young people with chronic conditions as well as healthy young people (10). The Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team 2009 showed in a study with 642 patients with confirmed H1N1 infection that 40 % of them were between the ages of 10 and 18 years; only 5% were older than 51. The most frequent symptoms included: fever, cough, sore throat, diarrhea and vomiting (34, 35).

Radiological findings were common in pulmonary manifestations, but showed a wide variety of patterns and were not specific for the diagnosis (36). Exsudative diffuse alveolar damage with alveolar and interstitial edema, alveolar fibrinous exudate and reactive pneumocytes were the most frequent findings (37, 38).

1.3.1 High risk patients

In most cases, pandemic influenza A infections led to rather weak infections. Patients recovered within a week and did not require antiviral drugs or medical care (39). Severe illness and fatal outcome were associated with underlying medical conditions, but did occur in previously healthy people too.

Some patient groups, however, had a higher risk for developing severe complications from H1N1 influenza A (40-45):

- Children younger than 5, in particular < 2 years
- Pregnant women
- Persons aged 65 years and older
- Persons with different medical conditions including
    - chronic pulmonary diseases (e.g. COPD)
    - asthma
    - chronic cardiac diseases (e.g. coronary artery disease, congestive heart failure)
    - endocrine disorders (e.g. diabetes mellitus)
    - chronic renal diseases
    - chronic hepatic diseases
    - immunosuppression (e.g. HIV, immunosuppressive medication, malignancy)
    - metabolic disorders
    - certain neurological conditions (e.g. seizure disorders)
    - blood disorders
- People (<19 years) receiving long-term aspirin therapy

1.3.2 H1N1 related complications and bacterial coinfections

The most frequent H1N1 related complications included: Pneumonia, neurological symptoms like seizures or confusion, respiratory failure, septic shock, renal failure, worsening of chronic conditions, such as asthma or a heart disease (16, 33, 39, 46, 47).

Bacterial coinfections were not as rare as believed -especially older people were affected. The coinfecting agents isolated most frequently were *Streptococcus pneumoniae, Staphylococcus aureus, Streptococus pyogenes, Streptococcus mitis and Haemophilus influenzae.* People with bacterial coinfections were more likely to develop the already mentioned complications (48, 49).

Also fungal infections were observed in patients with H1N1 infection. The virus seemed to predispose immunosuppressed patients to develop invasive pulmonary

aspergillosis. Studies showed that the use of corticosteroids in critically ill patients was a risk factor for fungal superinfection (50-52).

1.4 Transmission

1.4.1 Human-to-human transmission

The transmission of the pandemic influenza A /H1N1 virus occurred over droplets from person to person through coughing, sneezing, talking or physical contact, usually of the hands.
Sometimes people got infected by touching surfaces with virus on it and then touching their own mucosal surfaces (e.g. mouth, nose) (32, 53).

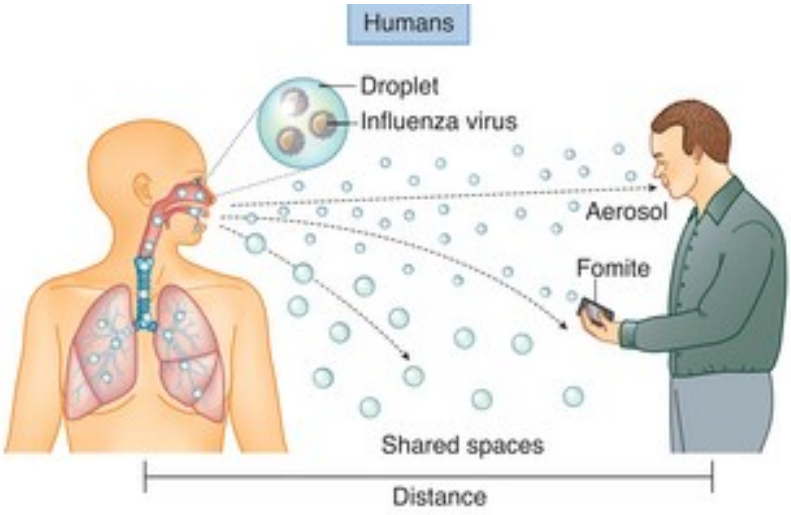

Figure 12: Droplet transmission (adapted from [54])

Shedding duration (i.e. = time in which a person might be infectious to another person) of influenza A /H1N1 seemed to be similar to seasonal influenza viruses. During the first three days of illness viral shedding had its peak. Virus shedding started a day before symptoms onset and lasted approximately for five to seven days (32, 55).

	PCR Median (range)	**Culture Median (range)**
Overall	6 (1-13)	5 (1-7)

Age group		
< 5 yr	6 (4-13)	5 (4-6)
5-9 yr	6 (2-10)	6 (2-6)
10-18 yr	5 (1-11)	5 (2-7)
19-50 yr	4 (1-10)	4 (1-5)

Table 3: Viral shedding duration of pandemic influenza A (adapted from [55])

1.5 Diagnosis

Rapid confirmation of a H1N1 infection is important for appropriate management of patients and is essential to implement infection control strategies. For detecting H1N1 pandemic flu there are different laboratory diagnostic tests available.

1.5.1 Real-time polymerase chain reaction

With polymerase chain reaction (PCR) it is possible to amplify DNA sequences in vitro. The DNA is separated into two strands and incubated with oligonucleotide primers and DNA polymerase. PCR is able to amplify a specific sequence of DNA by as many as one billion times (56).

Real-time PCR (RTPCR) includes oligonucleotide primers and dual-labeled hydrolysis probes. With this assay an in vitro detection of influenza A (H1N1) virus in respiratory specimens from people with suspicion of swine influenza A infection is possible.
There are different primers, e.g. the InfA primer for universal detection of type A influenza viruses; the swInfA primer for the detection of all swine influenza A viruses; and the swH1 primer for the specific detection of swine H1 influenza (57).
According to CDC protocol acceptable specimens for PCR -testing include (57):

- broncheoalveolar lavage
- tracheal aspirates
- sputum
- nasopharyngeal or oropharyngeal aspirates or washes, and

- nasopharyngeal or oropharyngeal swabs

Respiratory specimens should be collected as soon as possible after the onset of symptoms. Specimens should be kept no longer than for four days and at 4° C (58).
Reverse-transcriptase polymerase chain reaction (RT- PCR) is the gold standard to confirm an influenza A (H1N1) infection. In 2009 CDC appealed clinicians to test people with suspicion of H1N1 infection, especially those with severe illness.
A confirmed case of influenza A (H1N1) infection was a person with influenza-like illness plus a positive real-time PCR or a positive viral culture (58).
Despite a relatively high sensitivity a negative H1N1 PCR does not exclude influenza A (H1N1) diagnosis (59).

PCR has a high sensitivity and specificity, but it is expensive and needs trained expertise. During the pandemic 2009/2010 there were an enormous number of requests for RT-PCR testing. Resources have been exhausted which meant that in some countries - not including Austria - only patients with severe illness were tested (60, 61).

1.5.2 Rapid Antigen Test

Rapid influenza diagnostic tests (RIDTs) are tests for detecting influenza viral nucleoprotein antigen.
The advantage is that the test is simple to perform and requires little time. Test results are available within 30 minutes which is important for further clinical decisions.
Available RIDTs may either:

- detect and distinguish between influenza A and B viruses or
- detect both influenza A and B viruses but cannot distinguish between the two different types of virus
- detect only influenza A viruses

A handicap is that common RIDTs cannot distinguish between influenza A virus subtypes (e.g. seasonal influenza A vs. pandemic influenza A/ H1N1) (62).

Another disadvantage of the tests is the low sensitivity when compared with RT-PCR or viral culture (62-64).

On the other hand, the tests show high specificity and may include useful information for the patients care. The figure below demonstrates an algorithm to support the interpretation of RIDTs results:

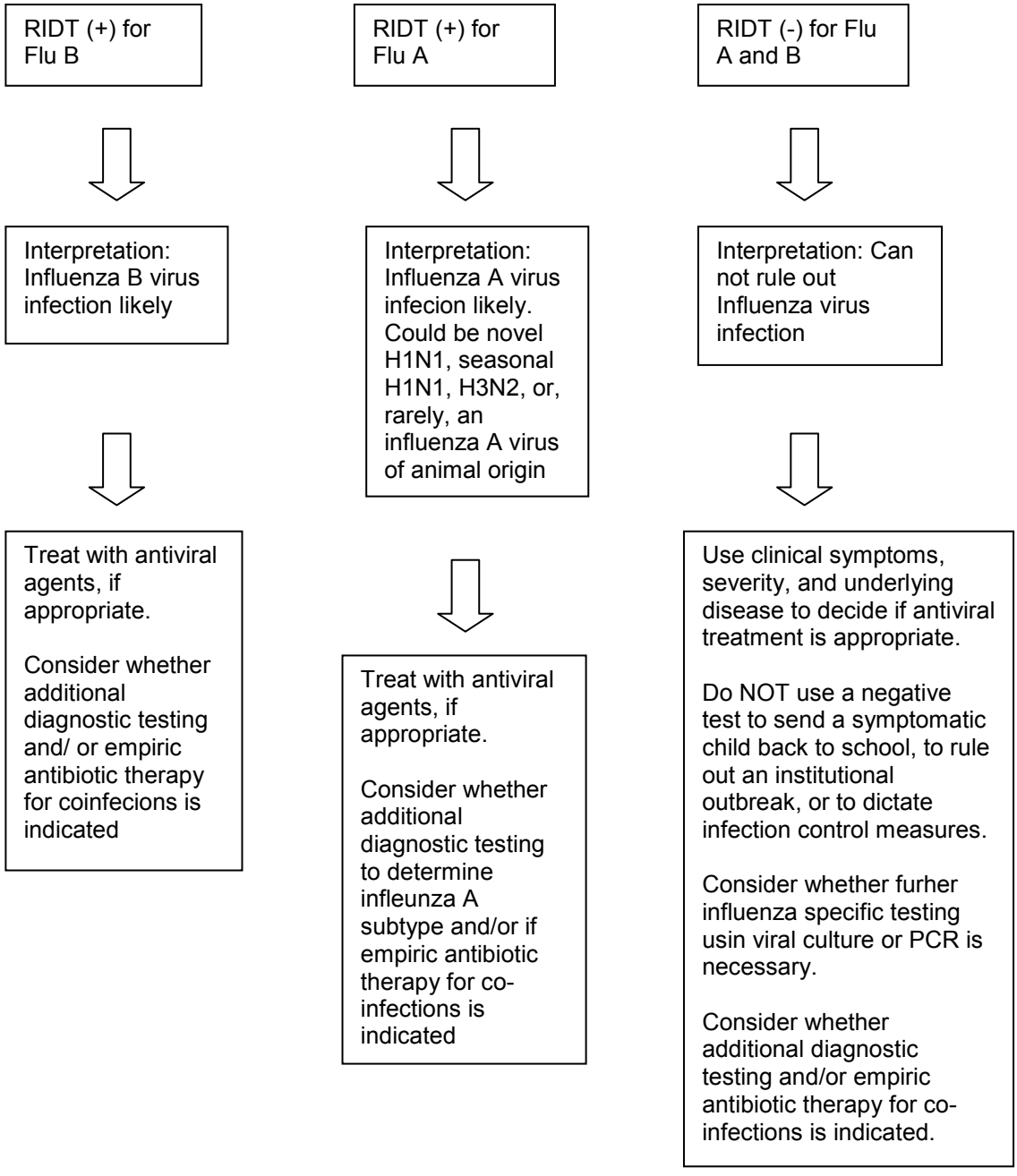

Figure 13: RIDT and interpretation (adapted from [62])

Vasoo et al compared three different antigen tests with the LuminexxTAGRVP RT-PCR and concluded that the sensitivity of rapid antigen tests for diagnosis of influenza A (H1N1) was low to moderate. All tests showed excellent specificity.

Test	Sensitivity, %	Specificity, %
BD Directigen EZ Flu A+B	46.7 (34.6-59.1)	100 (86.2- 100)
BinaxNOW Influenza A&B	38.3 (27.1-51.0)	100 (86.2- 100)
QuickVue Influenza A+B Test	53.3 (40.9- 65.4)	100 (86.2- 100)

Table 4: Analytic Performance of Rapid Influenza Antigen Tests, Compared with the Luminex xTAG RVP (Luminex) Reverse-Transcriptase Polymerase Chain Reaction (adapted from [61])

Overall, the sensitivity of RIDTs for detection of influenza A/ H1N1 varies between 10% and 70% (65). Therefore patients with clinical suspicion of a H1N1 infection but a negative result in a rapid antigen test should undergo further laboratory testing (61, 66).

1.5.3 Viral culture

A viral culture can also be used for the confirmation of an influenza A/ H1N1 infection (58).

In this diagnostic test a fluid or a tissue sample of a contaminated person is collected and put in a growing medium (= a container with a cell type the virus is able to infect). If the virus grows the cells show changes which can be seen under a microscope. Via this means, the virus can be identified. The test requires three to ten days for results, however. That means that it takes too long for having a clinical impact and is therefore done mostly for study purposes (58, 67, 68).

An advantage is that it is possible to assign the virus to the different strains of influenza that are circulating in a community and therefore to identify the antiviral agents that are effective in the treatment of the virus (67).

1.5.4 Immunofluorescence (Direct- or indirect fluorescent antibody tests)

These tests may distinguish between influenza A and B viruses, but they cannot differentiate between pandemic influenza A and seasonal influenza A viruses.
Another drawback is the fact that the sensitivity and specificity of the test are unknown for the new virus. Furthermore, the test result depends on the quality of the specimen and on the operator skills. The test is usually performed in a laboratory and not in a doctor's office because special equipment is needed (58, 67).

1.6 Therapeutic options

1.6.1 Antiviral drugs and the H1N1 pandemic

The 2009 influenza A (H1N1) virus was susceptible to the neuraminidase inhibitors oseltamivir and zanamivir, but showed resistance to the M2 inhibitors amantadine or rimandatine (41, 69).
Oseltamivir and zanamivir were the drugs of choice for treatment of 2009 H1N1 influenza in both children and adults (70). These drugs inhibit the virus -encoded neuraminidase (71).

Oseltamivir (Tamiflu ®): The neuraminidase inhibitor is formulated as capsules or oral suspension (70). When administered early oseltamivir reduces the duration of symptoms, the duration of viral shedding and the severity of influenza- related symptoms (72, 73).

Zanamivir (Relenza ®): Zanamivir is formulated for oral inhalation (70).

Who should be treated with antivirals?
Antiviral treatment is approved for people with suspected or confirmed influenza who had additionally:

- risk factors
- severe or progressive illness and/or
- illness requiring hospitalization (70)

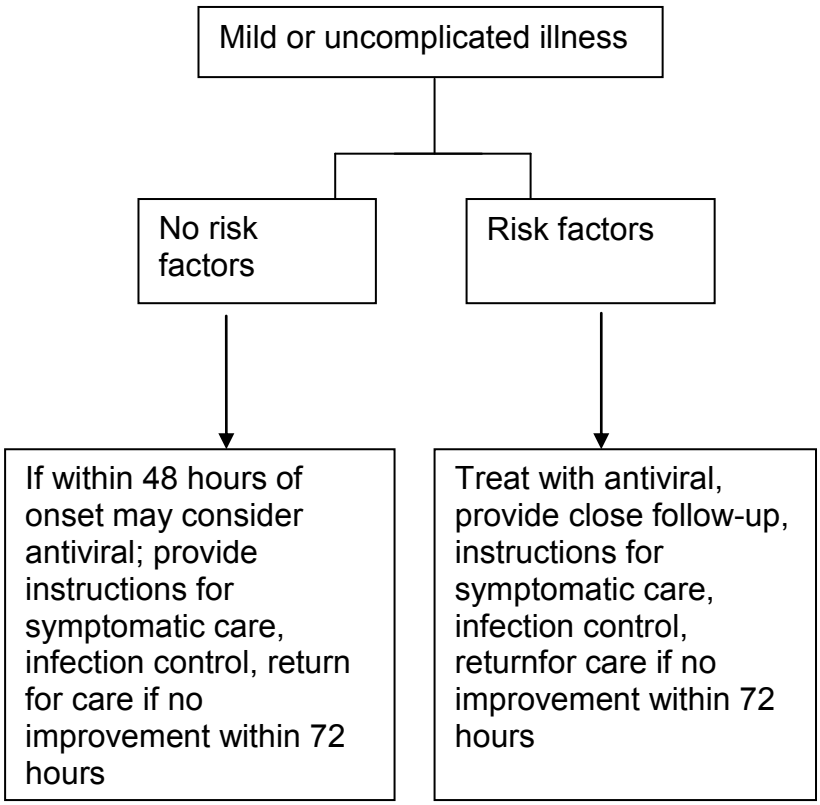

Figure 14: Clinical algorithm for consideration in the assessment of persons with mild or uncomplicated influenza illness (adapted from [70])

Timing was also an important point in the treatment of 2009 H1N1 influenza infection: Patients with severe and/ or progressive clinical symptoms should be treated with oseltamivir as soon as possible (74).

Clinical trials show that antiviral treatment was most effective when initiated within 48 hours after the onset of typical influenza-like symptoms (33, 74, 75). Treatment should therefore be started empirically before definitive test results from PCR or viral culture are available (70).

Population	**Pandemic influenza A (H1N1) 2009 and other Seasonal influenza viruses**	**Influenza viruses known or suspected to be oseltamivir resistant**
Uncomplicated clinical presentation		
Patients in higher risk	Treatment with	Treatment with zanamivir

groups	oseltamivir or zanamivir as soon as possible	as soon as possible
Severe or progressive clinical presentation		
All patients (including children and adolescents)	Treatment with oseltamivir as soon as possible (zanamivir should be used if oseltamivir unavailable)	Treatment with zanamivir as soon as possible
Patients with severe immunosuppression	Treatment with oseltamivir as soon as possible. Consider higher doses and longer duration of treatment	Treatment with zanamivir as soon as possible

Table 5: Therapeutical options in the case of a H1N1 infection (adapted from [74])

When oseltamivir resistance is suspected or proven zanamivir should be the treatment of choice (74). Overall oseltamivir resistance was, however, rare during the pandemic. Most patients with oseltamivir resistance had a severe immunocompromising condition (e.g. immunosuppressive treatment, chemotherapy, hematologic malignancies) (76).

1.6.2 Antibiotics

As mentioned before influenza A/ H1N1 infections were associated with a higher risk for bacterial confections- especially with *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. In case of bacterial superinfection initiation of antibacterial therapy is necessary.

1.7 Prevention

1.7.1 Everyday steps

With the outbreak of H1N1 public health care organisations like the WHO or the CDC showcased orders how everyone can protect himself or herself against the virus.

Figure 15: Everyday steps to protect the health and to limit the spread of the virus (adapted from [77])

CDC advices contained:

- You should frequently wash the hands with soap and water
- You should avoid touching your mouth, eyes or nose
- You should avoid contact with sick people
- If you are sick you should stay at home for approximately 24 hours after fever is gone
- When you cough or sneeze you should cover your nose with a tissue
- You should avoid crowds
- You should follow public health advice (78)

1.7.2 Vaccination

Vaccination was the best tool available to protect people from developing severe disease from pandemic influenza A /H1N1 infection.
There were two main types of H1N1 vaccines. On the one hand a H1N1 “flu shot”. This was a vaccine that contained killed virus and that was given with a needle in the upper arm or in the thigh. On the other hand, there was a nasal spray flu vaccine. This type of vaccine used living viruses.
After vaccination, antibodies developed in the body which protected against H1N1 infection (79, 80).

CDC advised vaccination especially to all high- risk individuals, like:

- Pregnant women
- Household contacts and caregivers for children younger than 6 months of age
- Healthcare and emergency medical services personnel
- All people from 6 month through 24years of age
- Persons aged 25 through 64 years who have health conditions associated with higher risk of medical complications from influenza (81)

Some people should not get influenza vaccination or rather should consult a physician before getting flu vaccination. For example, people with a severe allergy to chicken eggs, children younger than six month, people with a severe reaction to a flu vaccination before or people who have an illness with fever (they should wait until they recover) (79).
Like any vaccination, these H1N1 pandemic vaccinations could cause side-effects. The most common side- effects were (79):

- Fever
- Aches
- Soreness or swelling where the shot was given
- Headache
- Cough
- Vomiting

If side effects occurred they were usually mild and lasted one to two days (79).

Name, producer	Product description	Culture medium	Haemagglutinin content	Adjuvant emulsion	Number of doses
Celvapan, Baxter	Inactivated, whole wild-type virus A/California/7/2009 (H1N1)v	Cell-culture	7.5µg	None	All> 6 month 2x 0.5 ml
Pandemrix,GSK	Inactivated, split-influenza, reassortant, A/California/7/2009 (H1N1)v- like strain	Egg-culture	3.75µg (per adult dose) 1.87µg (per pediatric dose)	AS03	>10 years 2x 0.5ml 6 month- 9 years 2x0.25ml
Focetria, Novartis	Inactivated, surface- influenza antigens (haemagglutinin and neuraminidase), reassortant, A/California/7/2009 (H1N1)v- like strain	Egg-culture	7.5µg	MF59	All> 6 month 2x 0.5ml
Fluval P. Omninvest	Inactivated, whole reassortant virus A/California/7/2009 (H1N1)v- like strain	Egg-culture	6 µg (per adult dose) 3 µ (per pediatric dose)	Aluminium phosphate	Adults and adolescents > 12 years 1x 0.5 ml Children 3-12 years 1x0.25 ml Children 6 month – 3* years 1x 0.25ml (*decision pending)

Table 6: Overview of vaccines against pandemic influenza A (H1N1) available in the European Union in October 2009 (adapted from [82])

An important fact was that the H1N1 pandemic vaccination did not protect against other influenza viruses, such as the seasonal influenza (80).

1.8 Influenza-like illness

"Influenza-like illness" is a clinical definition that is very general but when combined with the information about circulating viruses it gives a good overview of influenza activity (83).

According to WHO's definition someone with "Influenza-like illness" is a person with sudden onset of fever of >38 °C and cough or sore throat in the absence of other diagnoses (84).

According to CDC ILI is defined as a person with following symptoms: temperature > 37.8°C and either a cough or sore throat without a known cause other than influenza.

In Europe there are also many different case definitions for the term "Influenza-like illness":

Surveillance networks	**Influenza-like illness**
Belgium	Sudden onset of fever, with respiratory symptoms and systemic symptoms
Cyprus	Sudden onset of fever (38.5°C) of at least 2 days duration, symptoms from the upper respiratory system, weakness, muscular pains, and headache.
Denmark	Sudden onset of disease with fever, myalgia and symptoms of respiratory infection
England	No case definition
Estonia	Clinical picture compatible with influenza, e.g. sudden onset of disease, cough, fever > 38°C, muscular pain and/or headache.
Greece	Acute onset of illness, cough, fever >38ºC, muscle ache and/or headache.
Ireland	Sudden onset of symptoms with a temperature of 38°C or more in the absence of any other disease with at least 2 of the following: headache, myalgia, sore throat, dry cough
Italy	Sudden onset of symptoms, with temperature >38°C, plus at

	least 1 systemic symptom and at least 1 respiratory symptom
Latvia	Every illness characterized by sudden onset of fever (>38°C) with respiratory symptoms (dry cough and sore throat), headache and/or myalgia
Lithuania	Sudden onset of fever (> 38°C), cough, myalgia and/or headache.
Luxembourg	Sudden onset of fever (> or equal to 38°C), myalgia and respiratory symptoms (e.g. cough or pharyngitis).
Malta	Fever (>38°C) with cough and headache and/or muscular pain
The Netherlands	Pel criteria [§]
Northern Ireland	An acute respiratory illness accompanied by variable fever and myalgia
Norway	A patient with clear general symptoms, primarily acute fever >38°C, headache, muscle ache, and in addition a dry cough
Poland	No case definition
Portugal	ICHPPC-2-D definition[§§]
Romania	Every illness characterized by sudden onset, fever, myalgia and respiratory symptoms (cough, coryza).
Scotland	No case definition
Slovak Republic	Sudden onset and fever with (1) at least 1 respiratory symptoms; cough, rhinitis, sore throat, and (2) at least 1 general symptoms: headache, joint ache, chills, malaise
Slovenia	Sudden onset of fever (>38°C) with general weakness, muscle and joint pain, dry cough and symptoms of upper respiratory tract affection
Spain	ICHPPC-2-D case definition[2]
Sweden	No case definition
Switzerland	Respiratory illness with fever >38°C, myalgia, general pain, chills, anorexia. (optional symptoms are: cough, rhinitis and arthralgia)
Wales	Upper respiratory tract symptoms, fever, chills, myalgia, cough

[§]: Pel criteria: An acute onset (i.e. at most a prodromal stage of three to four days), accompanied by a rise in rectal temperature of >38°C, and at least 1 of the following symptoms: cough, coryza, sore throat, frontal headache, retrosternal pain, myalgia.

$$: International Classification of Health Problems in Primary Care

ILI: at least one of the following characteristics:

1. Influenza virus culture positive or serological evidence of influenza virus infection
2. Context of influenza epidemic, plus 4 of the criteria in 3.
3. 6 of the following criteria: sudden onset (within 12 hours), cough, fever, chills, prostration and weakness, myalgia or general pain, rhinitis, pharyngitis, contact with a case.

Table 7: Different case definitions in Europe (adapted from [85])

During the influenza A (H1N1) pandemic in 2009 other respiratory viruses have co -circulated in many countries and provoked respiratory infections and influenza-like illness. The two most common viruses that were responsible for influenza-like illness were the human rhinovirus and the respiratory syncytial virus (86). But also other viruses can be responsible for ILI -e.g. adenoviruses, enteroviruses and parainfluenza viruses (87-89).

Especially during a pandemic it is important to differentiate between infections caused by influenza viruses from those caused by other viruses.

1.9 Usefulness of a clinical score

PCR is the gold standard to confirm H1N1 infection, but usually is time- consuming and expensive. Moreover, PCR assays are not available in every clinical setting.

The currently available antigen tests are fast, but display low sensitivity. The advantage is that the test is simple to perform and requires little time. However, patients with clinical suspicion of H1N1 infection, but with a negative result in a rapid antigen test should undergo further laboratory testing (61, 63, 66, 90-92).

The development of a clinical score for H1N1 influenza could be useful for a more efficient and faster clinical diagnosis. This may help to improve decision making regarding antiviral therapy, outcome, reduce mortality and morbidity (93).

A clinical score could be used in clinical settings where diagnostic resources are limited. Particularly for resident doctors a clinical score for detecting H1N1 influenza A could be very useful. Depending on the test results they can decide faster whether a patient needs antiviral treatment or not. The main antivirals for the treatment against H1N1 influenza A infection are oseltamivir and zanamivir (71).

Nevertheless there are reports of oseltamivir resistance (94, 95). Particularly among patients with severe immunocompromising conditions (chemotherapy or immunosuppressive treatment) oseltamivir resistance was observed frequently during the pandemic (76, 96, 97).

The incidence of oseltamivir resistance is higher among patients with H1N1 subtype than among patients with the seasonal flu (95).

1% to 1.5% of patients are resistant against oseltmamivir (98).

Although oseltamivir is a successful drug there are reports of possible serious harms especially in people younger than 20 years. These adverse effects include abnormal behaviour, convulsion, delirium, hallucinations, diarrhea, nausea, headache and many others (99).

Therefore, it may be very important to avoid unnecessary treatment with oseltamivir.

Besides a clinical score may facilitate and accelerate the decision whether a patient has to be transferred into hospital or not.

A clinical score would be easy to use and quick to carry out. Another economic advantage is that a highly sensitive and specific score may help to save costs.

2 Materials and Methods

2.1 Study objectives

- To compare the laboratory values between patients with PCR confirmed H1N1 infection and those with ILI in which PCR turned out negative.

- To analyze the development of laboratory values during the first seven days of disease of PCR confirmed H1N1 influenza cases and cases with ILI.

2.2 Study Design

This is a retrospective survey of patients with PCR confirmed H1N1 infection or PCR negative influenza-like illness.
During 1st October 2009 and 19th January 2010 polymerase chain reaction (PCR) assays were performed in clinical samples from 1681 patients with clinical suspicion of influenza A, H1N1 infection presenting to hospitals of south east Austria (Styria). 624 of them had a positive H1N1 PCR test result which confirmed H1N1 influenza.
1057 people had negative H1N1 PCR result (ILI). From these 1681 people laboratory data was searched and completed with the use of the database "MEDOCS" which include patient's records. This is the electronic network at the Medical University Hospital Graz and other Hospitals of South East Austria. Finally, we compared the laboratory values between patients with confirmed H1N1 influenza A infection and those with clinical suspicion of H1N1 infection, but in which PCR turned out negative.
The study was initiated and conducted by the Section of Infectious Diseases, Medical University of Graz.
Participating hospitals were the University Hospital Graz and the State Hospitals of Leoben, Bruck, Graz-West, Feldbach, Judenburg, Hörgas-Enzenbach, Rottenmann, Deutschlandsberg, Wagna and Bad Aussee.
The Institute for Hygiene, Microbiology and Environmental Medicine, Medical University of Graz and the Institute for Hospital Hygiene and Microbiology, University Hospital of Graz carried out the RT-PCR testing for the study.

Local ethic committee granted to conduct the study also because of the non-interventional study design.

2.3 Data collection

Data was collected by reviewing medical reports within the database "MEDOCS". All the information was collected anonymously in an electronic database. Data was collected between October 2011 and January 2012.
Laboratory data sets were available from 221/624 (35%) patients with PCR confirmed H1N1 infection and 312/1057 (30%) of patients with clinical suspicion of H1N1 infection, but in which PCR turned out negative.

2.4 Inclusion criteria

All patients tested for influenza A, H1N1 by PCR of respiratory tract specimens at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz and the Institute for Hospital Hygiene and Microbiology, University Hospital of Graz from October 2009 to January 2010 were included in the study.
Laboratory values had to be available for at least one day. At given time points (first day, second or third day, fourth or fifth day, sixth or seventh day) some laboratory values were not available for all patients.
Influenza A (H1N1) virus infection was confirmed with a positive PCR result. Specimens were taken from nasopharyngeal swab, pharyngeal lavage or bronchoalveolar lavage.

2.5 Analysis

We collected the laboratory values from the first seven days of clinical admission and analyzed them.
Laboratory values, analyzed from the first day, second or third day, fourth or fifth day and sixth or seventh day included:

- White Blood Cell count (WBC)

- Thrombocyte count
- Neutrophils
- Eosinophils
- Absolute eosinophil count
- Monocytes
- Lymphocytes
- C-reactive protein
- Procalcitonin

Hemoglobin and hematocrit were analyzed only on the first day.
Creatinine, GGT, aspartate aminotransferase (AST), alanine aminotransferase (ALT), CK, lactate dehydrogenase (LDH), Troponin, International Normalised Ratio (INR), Bilirubin and Lipase were analyzed from the first day of hospitalization. Additionally the maximum of these laboratory values was notified during the disease.
For time calculation, the day of presenting to the emergency unit/ hospitalization was considered to be day 1.

Laboratory values have been calculated using the standard values of the Clinical Institute of Medical and Chemical Laboratory Diagnostics, Medical University Hospital of Graz, Austria (Table 8).

Laboratory values	**Normal range**
WBC	4.4 – 11.3 G/l
Thrombocyte count	140- 440 G/l
Haemoglobin	12.0- 15.3 g/dl
Haematocrit	35.0- 45.0 %
Absolute eosinophil count	-0.7 G/l
Neutrophils	50- 75 %
Eosinophils	-5.0 %
Monocytes	2.0- 12.0 %
Lymphocytes	20.0- 40.0 %

Absolute lymphocyte count	1.0- 4.8 G/l
C- reactive protein	-5.0 mg/l
Procalcitonin	-0.5 ng/ml
Creatinine	0.5- 1.00 mg/dl
GGT	-38 U/l
AST	-30 U/l
ALT	-35 U/l
Creatine kinase	-145 U/l
LDH	120-240 U/l
INR	-
Bilirubin	0.10-1.20 mg/dl
Lipase	- 60 U/l

Table 8: Laboratory standard values

2.6 Statistical analysis

We calculated the medians and interquartile ranges (IQR) of the different laboratory values for PCR confirmed H1N1 cases and the cases with clinical suspicion but negative PCR result.

Analysis was carried out with the use of the statistical program SPSS (Statistical Package for the Social Sciences).

We compared the laboratory values between patients with PCR confirmed H1N1 virus infection and those with clinical suspicion but negative PCR result. Differences in laboratory values between both groups and correlation of laboratory values with underlying disease were calculated using Mann-Whitney U test.

P-value <0.05 was considered statistically significant.

The study protocol has been approved by the local ethics committee, Medical University Graz, Austria.

3 Results

3.1 Demographic data

During the study period 624 patients in South East Austria had positive and 1057 patients had negative H1N1 PCR. Laboratory data sets were available from 221 patients with PCR confirmed H1N1 infection and 312 patients with ILI. Influenza A/H1N1 was more repeatedly diagnosed in men (58.4%) than in women (41.2%). Gender distribution in patients with PCR negative ILI was close-to- balance. 51.3% were male and 48.7 % were women.

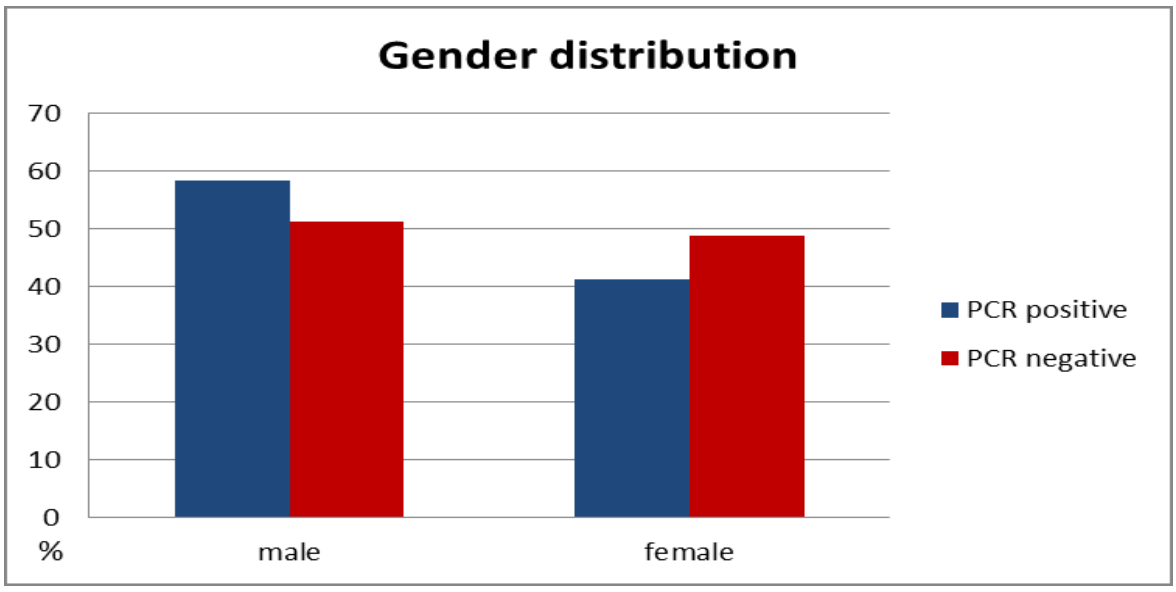

Figure 16: Gender distribution in patients with H1N1 infection and in patients with ILI

The age of patients with confirmed H1N1 infection ranged from one month to 79 years. The mean age was 23 years. In patients with ILI age ranged from one day to 92 years. The mean age was 33 years.

Children and young adults between 0 and 20 years of age showed the highest number of confirmed H1N1 cases (139 out of 221). Half of patients with ILI were between 0 and 30 years of age.

During the pandemic 2 out of 207 (1%) of H1N1 cases were confirmed in October, 116/207 (56%) in November, 84/207 (40.6%) in December and 5/207 (2.4%) in January. The situation for patients with ILI looked similar. 17/311 (5.5%) of ILI patients were confirmed in October, 166/311 (53.4%) in November, 100/311 (32%)

in December and 21/311 (6.8%) in January. The peaks of PCR confirmation were in November and December 2009 for both groups.

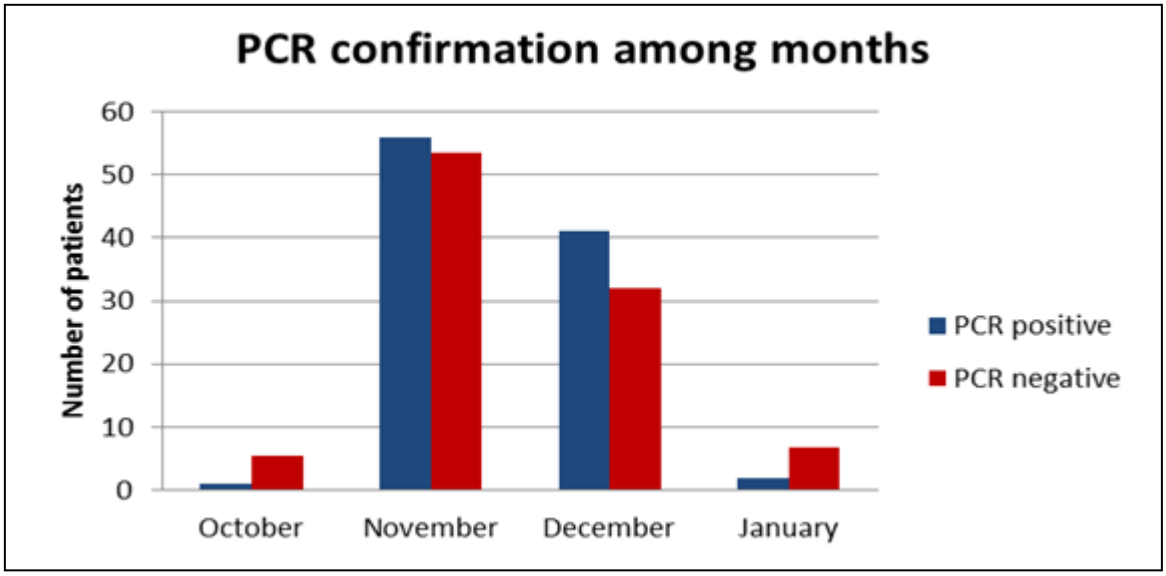

Figure 17: Epidemiologic curve of PCR confirmed H1N1 cases and cases with ILI from October, 2009 to January,2010

3.2 White Blood Cell count at presentation

At the time of admission 203 leukocyte measurements were available from patients with PCR confirmed influenza A/ H1N1 infection (n=221).

The median of this group was 6.7 G/l (IQR, 5- 8.6 G/l). 24 out of 203 (11.8%) patients who were tested had leucocytosis and 32 out of 203 (15.7%) presented with leukopenia.

In the group of patients with ILI in which PCR turned out negative initial WBC count was available from 283 data sets. Median was 9.86 G/l (IQR, 7.4- 13.7 G/l). 105/283 (37%) patients with ILI had leucocytosis and 20/283 (7%) presented with leukopenia.

Comparison of the PCR positive and the PCR negative group showed that total white blood cell count was significantly lower in patients with PCR confirmed H1N1 infection ($p < 0.001$).

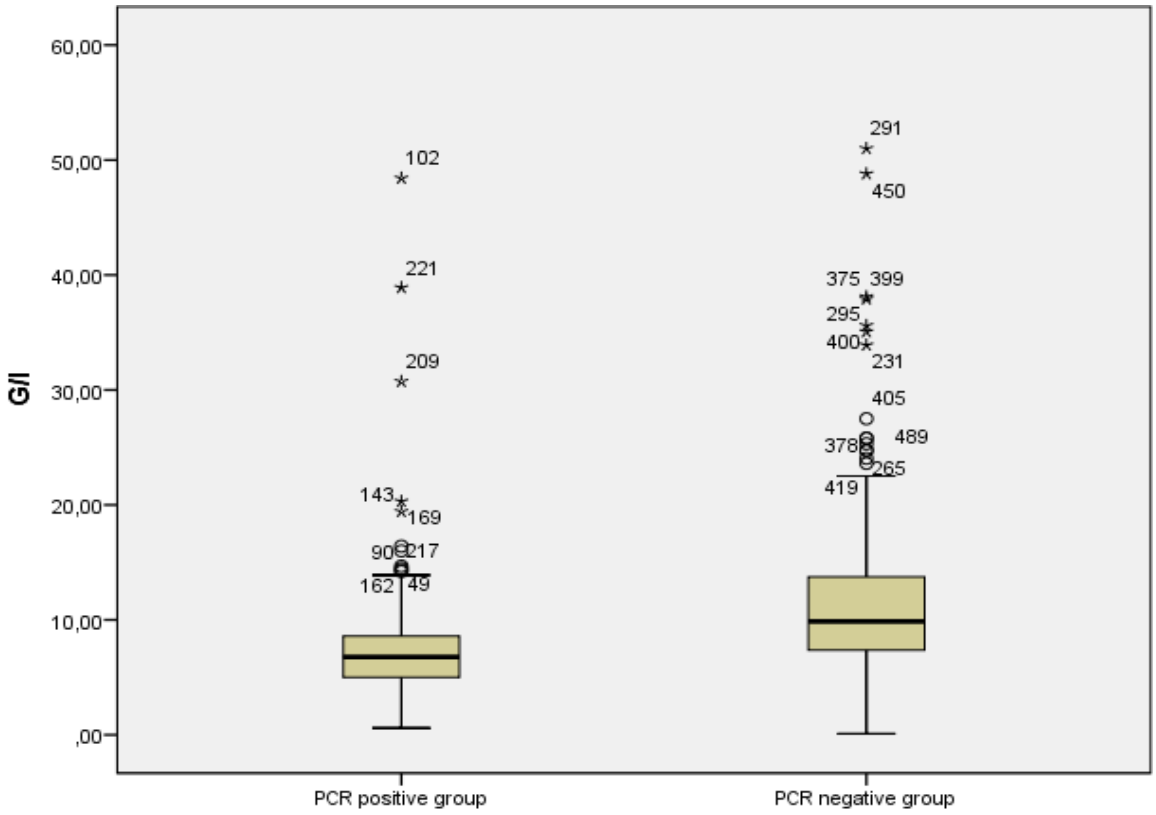

Figure 18: WBC count on admission

The number of patients where laboratory values were available (ni) decreased during the first week. On day 4/5 of hospitalization no more than 60 data sets from the PCR positive group and 154 data sets from the PCR negative group were available.

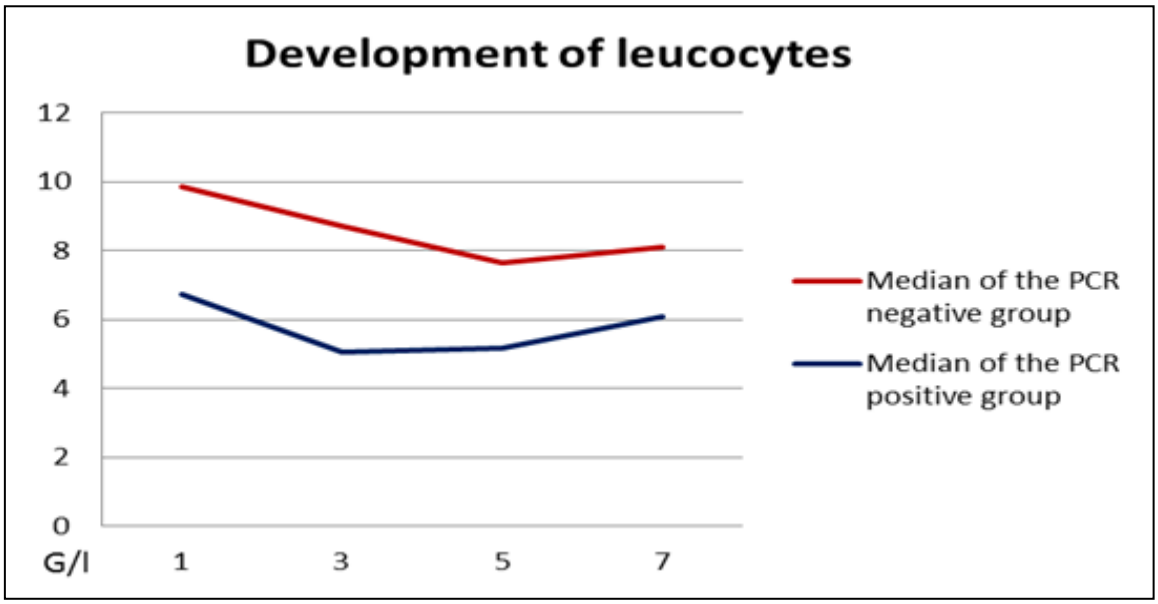

Figure 19: Development of WBC count

In the first seven days of hospitalization WBC decreased slowly but there was constantly a significant difference between the two groups.
WBC count was always significant lower in patients with PCR confirmed H1N1 infection- p value was permanently lower than 0.001.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
WBC count (G/l) day 1	**6.74** * (ni=203)	**9.86** * (ni=283)	*p< 0.001*
WBC count (G/l) day 2/3	**5.05** (ni=103)	**8.72** (ni=185)	*p <0.001*
WBC count (G/l) day 4/5	**5.18** (ni=60)	**7.66** (ni=154)	*p <0.001*
* Median displayed			
ni= number of patients where laboratory values were available			

Table 9: WBC development during hospitalization

3.3 Thrombocytes

Normal range of thrombocyte count was between 140 G/l and 440 G/l.
On admission to hospital 175 out of 201 patients with H1N1 infection (87%) and 234 out of 281 patients with ILI (83%) had normal levels of platelets.
22 out of 201 (11%) patients with H1N1 infection and 28 out of 281 (10%) patients with ILI suffered from thrombopenia. 4/201 (2%) H1N1 patients and 19/281 (6.8%) ILI patients presented with thrombocytosis.

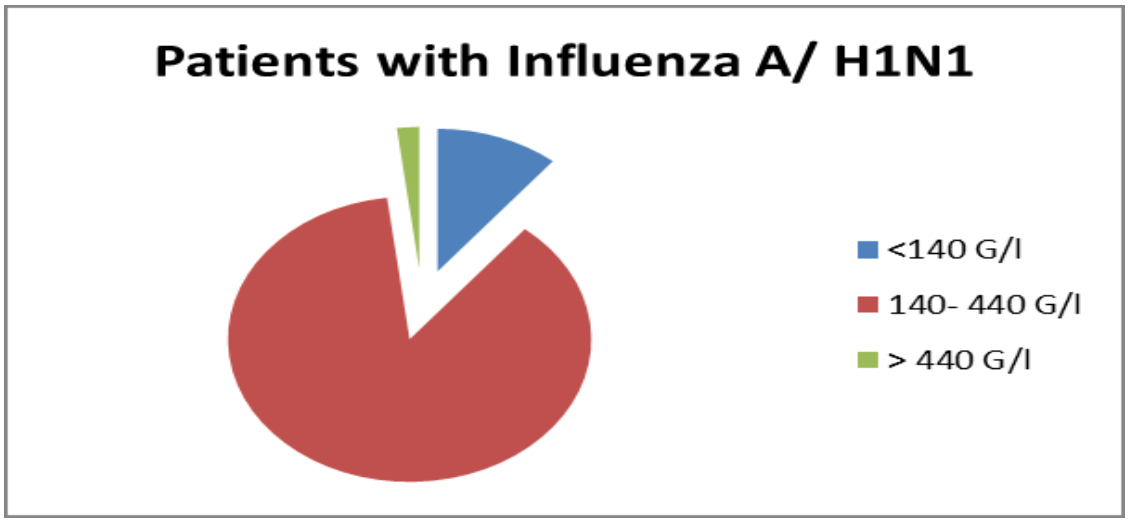

Figure 20: Platelet levels in patients with H1N1 infection at time of admission

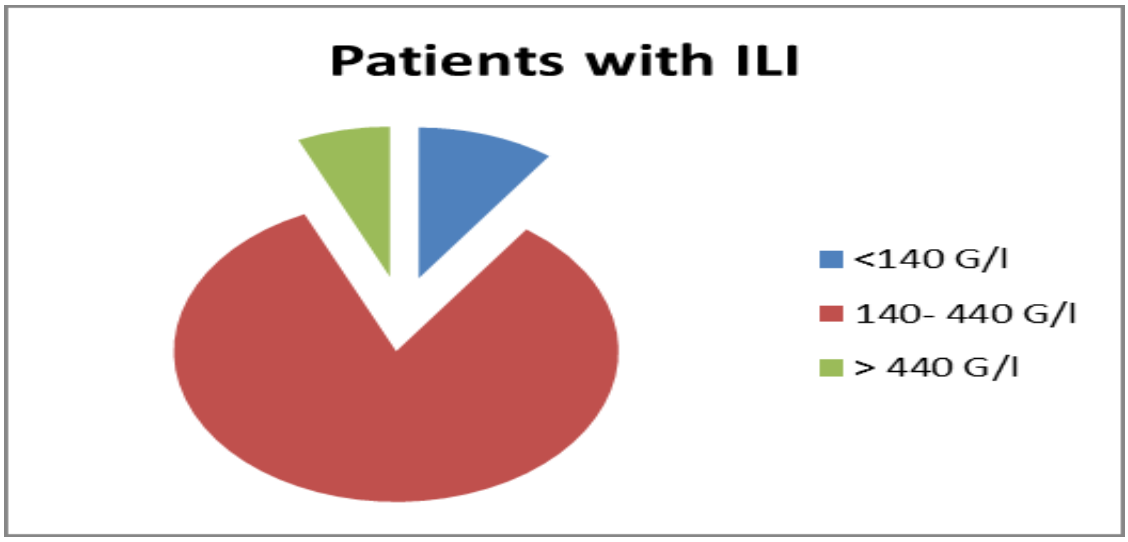

Figure 21: Platelet levels in patients with ILI at time of admission

At admission median thrombocyte count was 201 G/l (IQR, 156.5- 225 G/l) in patients with PCR confirmed H1N1 infection. In patients with ILI median was 232 G/l (IQR, 182- 288.5 G/l).

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
Thorombocyte count (G/l) day 1	201* (ni=201)	232* (ni=281)	p <0.001
Thorombocyte count (G/l) day 2/3	181 (ni=102)	228 (ni=186)	p <0.001

Thorombocyte count (G/l) day 4/5	182.5 (ni=61)	213 (ni=154)	p <0.001
Thorombocyte count (G/l) day 6/7	173.5 (ni=47)	244 (ni=101)	p =0.001
* Median displayed			
n_i= number of patients where laboratory values were available			

Table 10: Thrombocyte counts

Similar to WBC, comparison of thrombocyte counts demonstrated that they were significantly lower in patients with PCR confirmed H1N1 infection than in patients with ILI ($p< 0.001$).

Furthermore the development of thrombocyte levels during the first seven days showed significant lower values in patients with H1N1 infection.

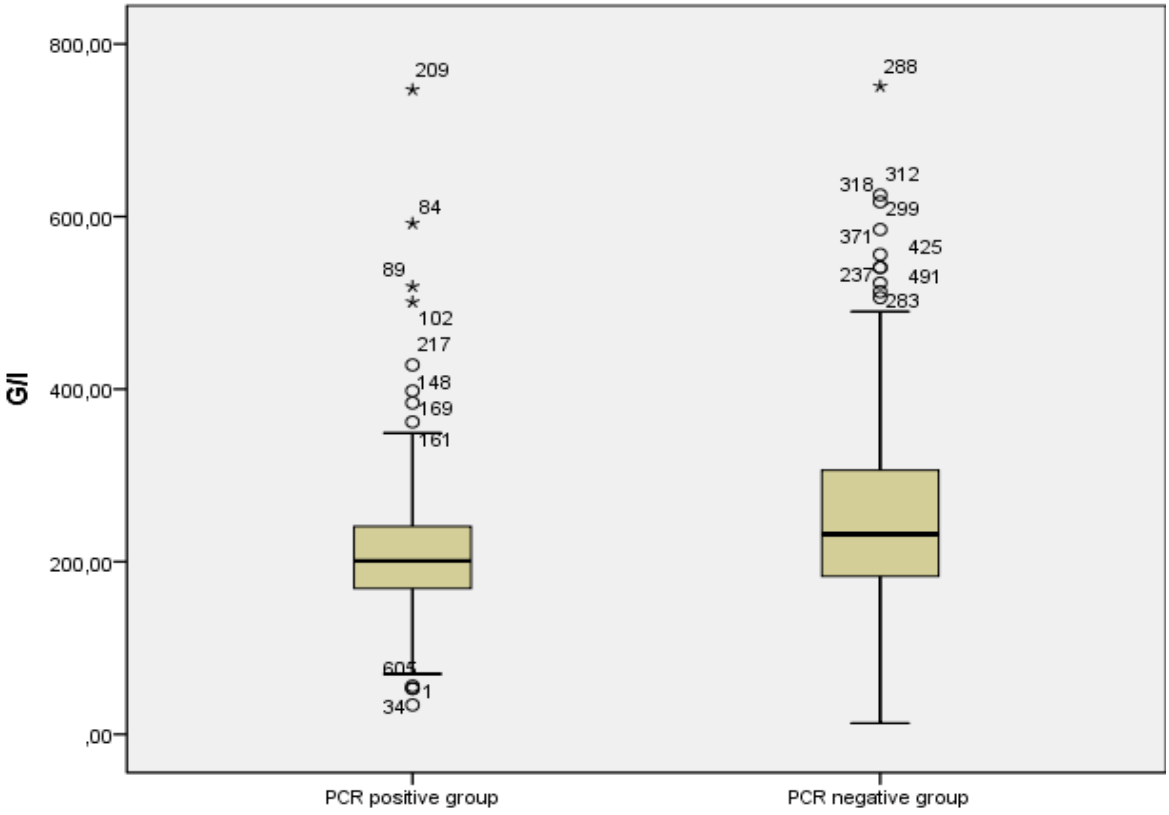

Figure 22: Thrombocyte counts on the first day

3.4 Eosinophils

At first presentation to the hospital the median of the absolute eosinophil count was 0 G/l (IQR, 0- 0.04 G/l) in the PCR positive group. In contrast the median in the PCR negative group was 0.015 G/l (IQR, 0- 0.125 G/l).

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
Eosinophils day 1 (G/l)	0* (ni=185)	0.015* (ni=256)	p = 0.002
* Median displayed			
ni= number of patients where laboratory values were available			

Table 11: Eosinophils day 1

Eosinophils were significant lower in patients with PCR confirmed H1N1 infection than in patients with ILI.

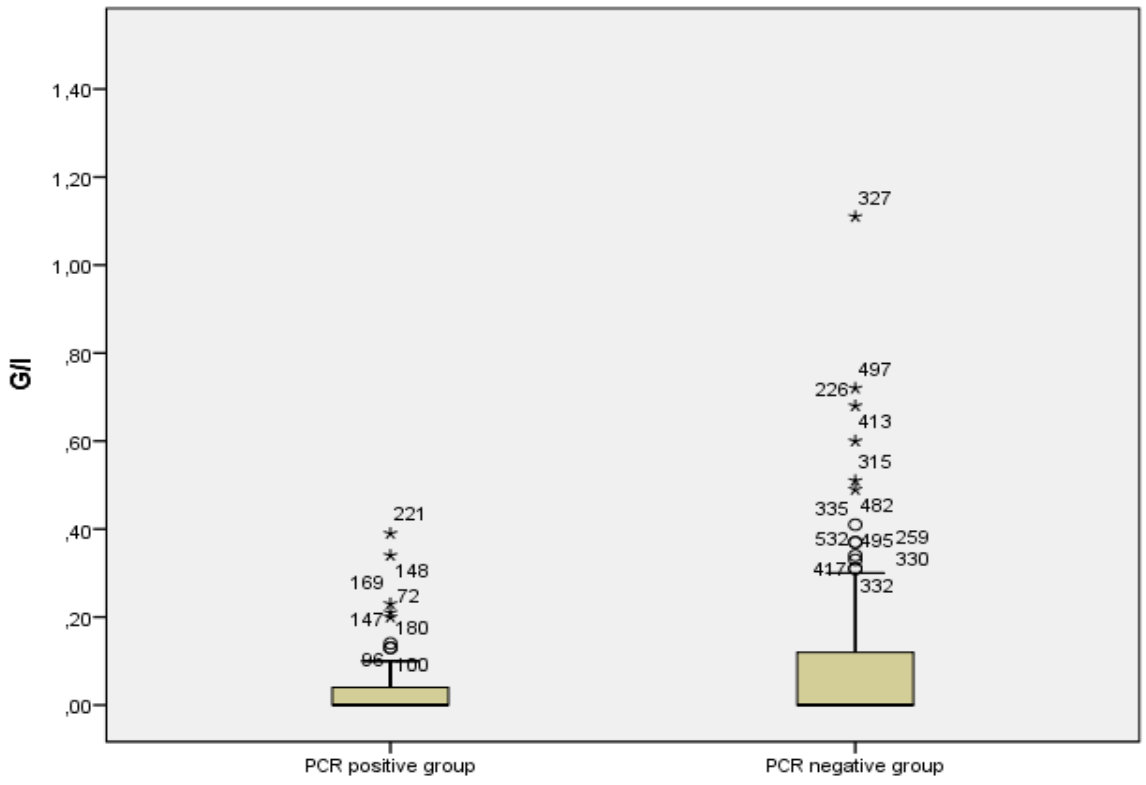

Figure 23: Eosinophils on the first day

3.5 Distribution of the White Blood Cells

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
Neutrophil % day 1	73* (ni=187)	74* (ni=252)	
Eosinophil % day 1	0 (ni=185)	0.2 (ni=256)	p= 0.006
Eosinophil % day 2/3	0 (ni=94)	1 (ni=151)	
Eosinophil % day 4/5	0.13 (ni=52)	2 (ni=117)	
Monocyte % day 1	9.1 (ni=186)	8 (ni=260)	
Monocyte % day 2/3	10 (ni=95)	8 (ni=151)	
Lymphocyte % day 1	16 (ni=192)	15 (ni=261)	
* Median displayed			
ni= number of patients where laboratory values were available			

Table 12: Distribution of the White Blood Cells

The table above shows the distribution of the White Blood Cells in patients with H1N1 infection and patients with ILI. At the first presentation in hospital the median of the relative neutrophil count was 73% (IQR, 60-81%), of the relative eosinophil count 0 % (IQR, 0-1%), of the relative monocyte count 9.1 % (IQR, 7-13%) and of the relative lymphocyte count 16% (IQR, 9-26%).

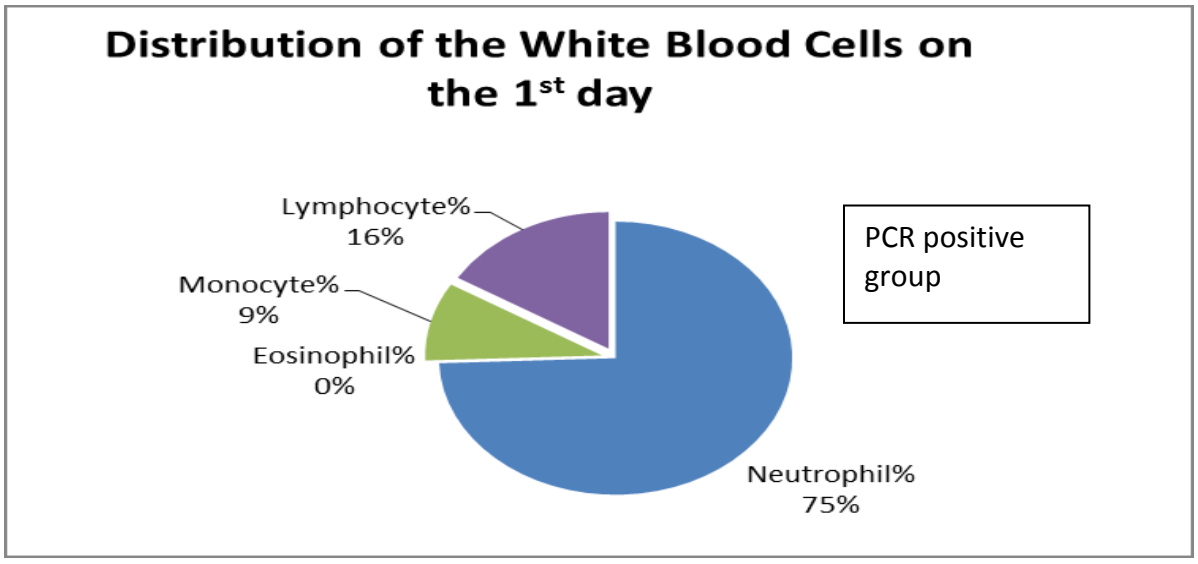

Figure 24: Distribution of the White Blood Cells in the PCR positive group

In patients with ILI the distribution looked similar. At time of admission the median of the relative neutrophil count was 74% (IQR, 63.3- 84%). The median of the relative eosinophil count was 0.2% (IQR, 0-2%), of the relative monocyte count 8% (IQR, 7-13%) and of the relative lymphocyte count 15% (IQR, 9-26%).

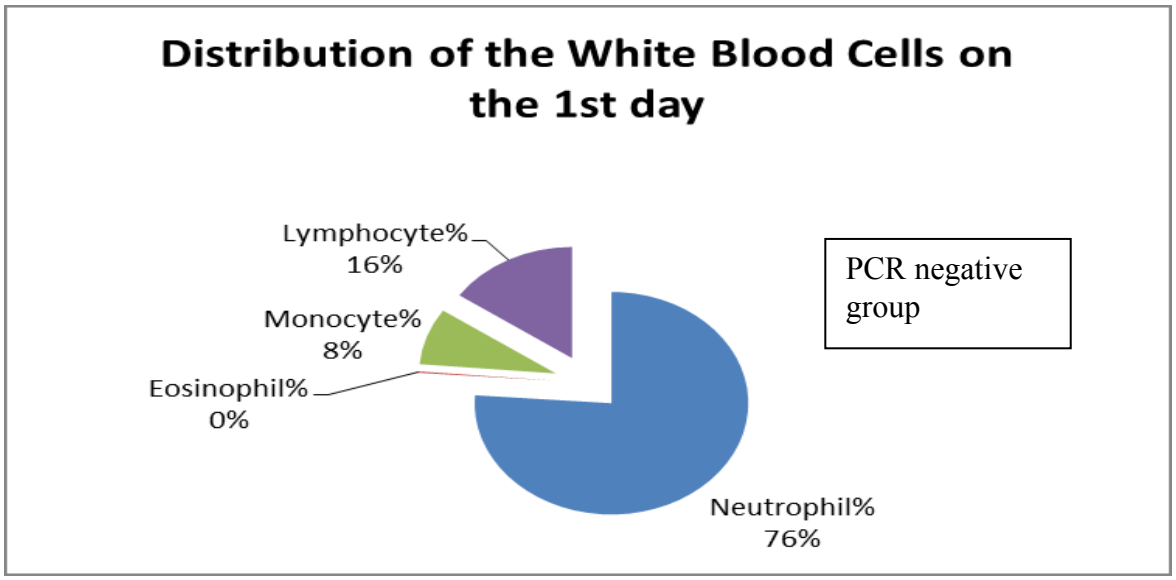

Figure 25: Distribution of the White Blood Cells in the PCR negative group

In the distribution of the WBC count only the relative eosinophil count showed significantly lower values in patients with confirmed H1N1 infection (p= 0.006).

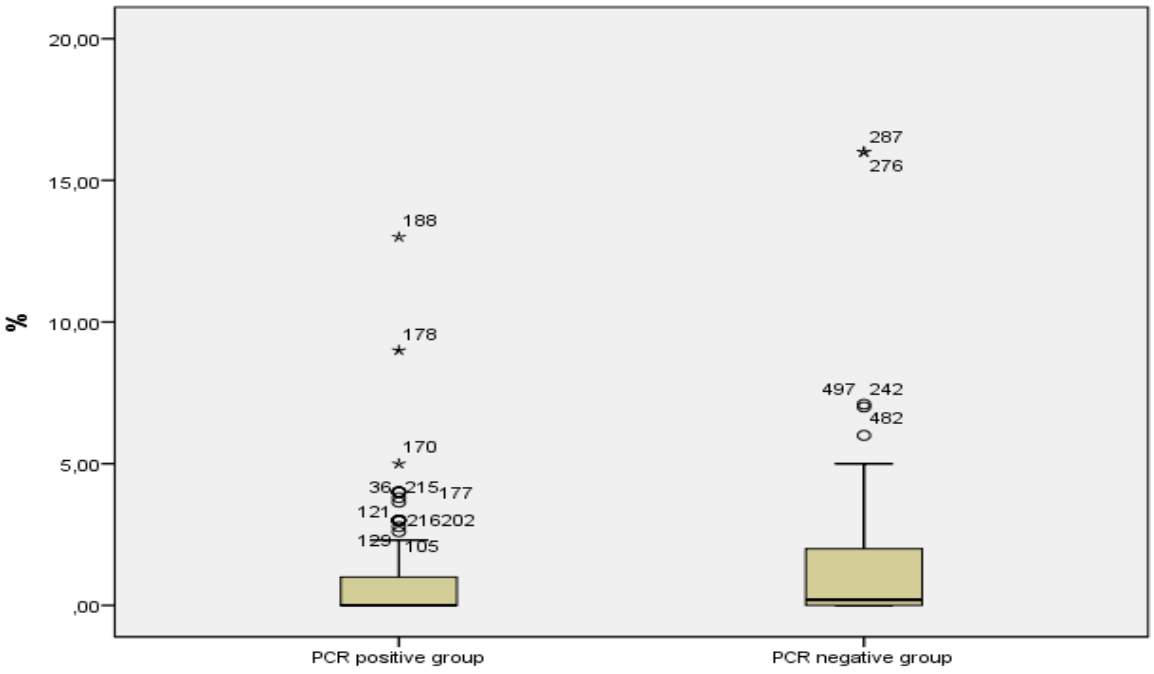

Figure 26: Comparison of eosinophils % on the 1st day

3.6 C-reactive protein

At first presentation to hospital CRP was elevated in both groups, in patients with PCR confirmed H1N1 infection and in patients with ILI.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
C-reactive protein (mg/l) day 1	11* (ni=199)	23* (ni=280)	p <0.001
* Median displayed			
ni= number of patients where laboratory values were available			

Table 13: C-reactive protein

Patients with ILI had higher CRP values than patients with H1N1 infection.
The median in the PCR positive group was 11 mg/l (IQR, 5- 64 mg/l). In the PCR negative group the median was 23mg/l (IQR, 7-101 mg/l).

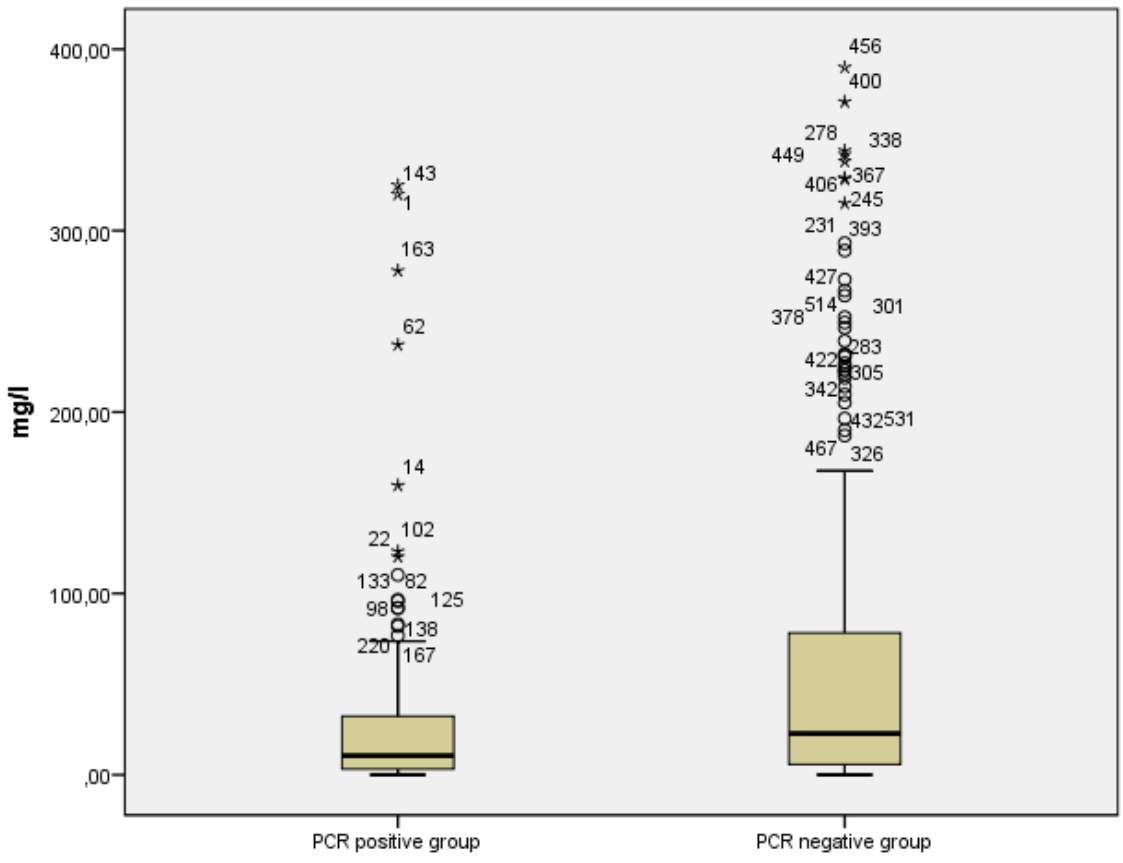

Figure 27: CRP level on the first day

Comparison between PCR confirmed H1N1 influenza and PCR negative ILI demonstrated significantly lower CRP values in patients with H1N1 infection (p< 0.001).

3.7 Creatine kinase at presentation

At the time of admission 37/91 (41%) of patients with H1N1 infection and 33/166 (20%) of ILI patients had elevated creatine kinase levels.

The median in patients with PCR confirmed H1N1 influenza was 113 U/l (IQR, 61.5- 192 U/l) and 72 U/l (IQR, 48.5- 116.5 U/l) in patients with ILI.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
Creatine kinase (U/l) day 1	113 (ni=90)	72 (ni=166)	p <0.001
* Median displayed			
ni= number of patients where laboratory values were available			

Table 14: Creatine kinase

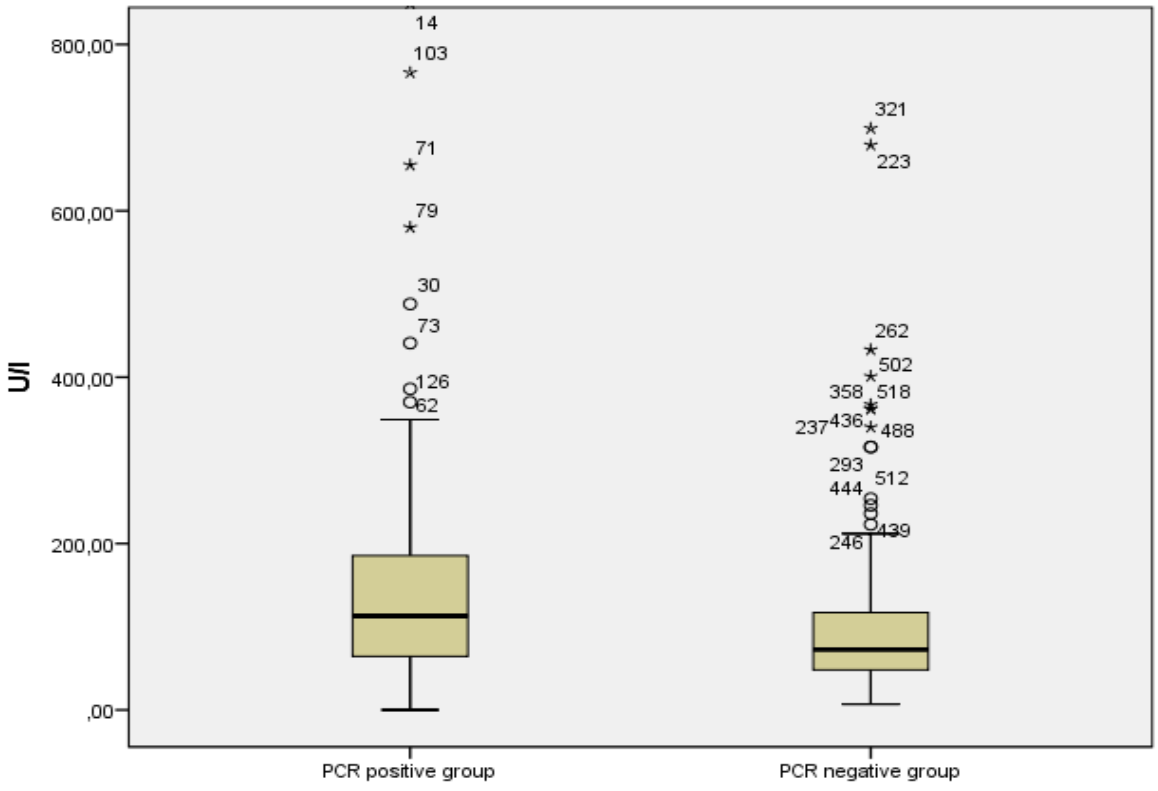

Figure 28: Creatine kinase at time of admission

Comparison showed that creatine kinase values were significantly higher in patients with PCR confirmed H1N1 infection (p< 0.001).

3.8 Liver parameters at presentation

3.8.1 AST and ALT at time of admission

On admission 58/121 (48%) of patients with H1N1 infection and 106/226 (46%) of patients with ILI had elevated levels of AST. 27/121 (22%) of patients with H1N1 infection and 69/227 (30%) of patients with ILI showed elevated levels of ALT.
Levels of AST and ALT were comparable in the PCR positive group as in the PCR negative group. There were no statistically significant differences in laboratory values between both groups.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
GGT (U/l) day 1	23* (ni=119)	30* (ni=225)	p < 0.05
AST (U/l) day 1	29 (ni=121)	29 (ni=226)	
ALT (U/l) day 1	19 (ni=121)	22 (ni=227)	
* Median displayed			
ni= number of patients where laboratory values were available			

Table 15: Liver parameters at presentation

3.8.2 GGT at presentation

39/119 (33%) of patients with H1N1 infection who were tested when presenting to the hospital had increased GGT counts ranging from 39 U/l to 552 U/l.
Patients with ILI 94/225 (42%) had increased GGT counts ranging from 39 U/l to 2319 U/l.
On admission to the hospital the median of GGT was 23 U/l (IQR, 15- 56.5 U/l) in patients with H1N1 influenza and 30 U/l (IQR, 14.5- 68.5 U/l) in patients with ILI.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
GGT (U/l) day 1	23* (ni=119)	30* (ni=225)	p < 0.05
* Median displayed			
ni= number of patients where laboratory values were available			

Table 16: GGT at time of admission

Comparison of the median between PCR confirmed H1N1 influenza and PCR negative ILI illustrated that GGT was significantly lower in patients with confirmed H1N1 infection (p< 0.05).

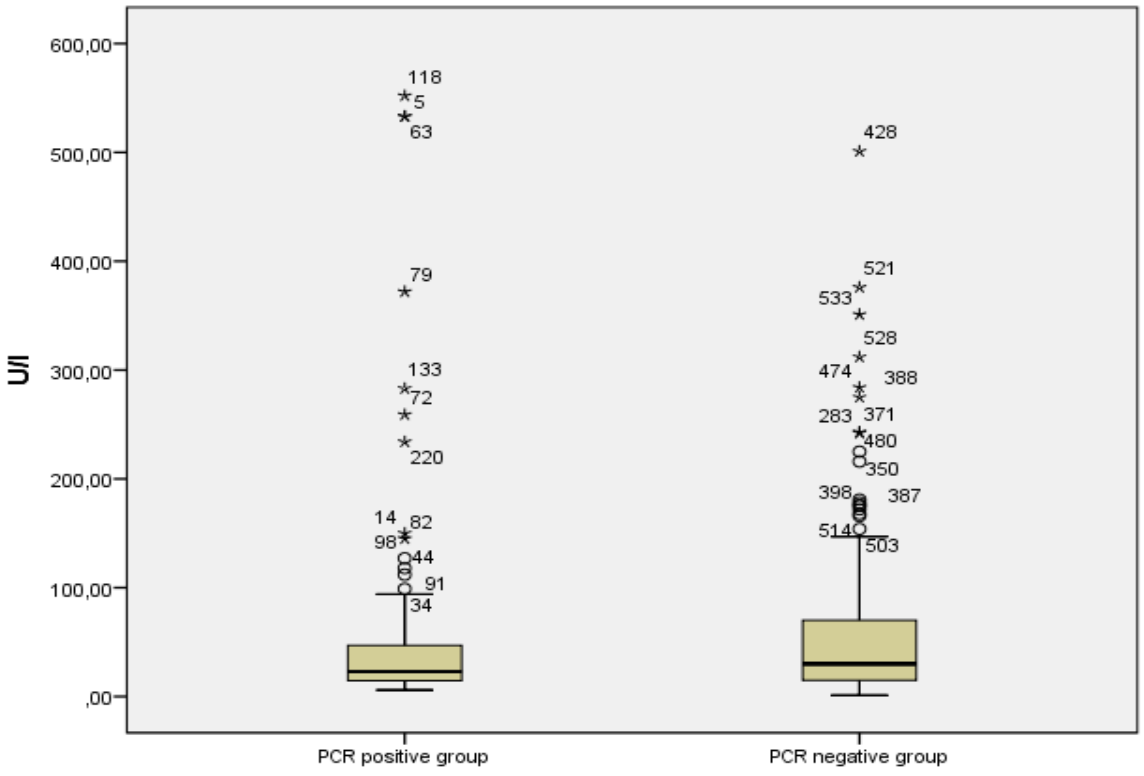

Figure 29: GGT on 1st day

3.9 Creatinine at presentation

Normal range of creatinine is between 0.5 mg/dl and 1 mg/dl. At presentation half of patients with H1N1 infection showed normal creatinine levels. 46/123 (37%) had increased counts with highest level of about 4.87 mg/dl. 15/123 (12%) presented with decreased levels.

In patients with ILI 112/234 (48%) showed normal creatinine levels. 91/234 (39%) had increased counts with highest level of about 17.51 mg/dl. 31/234 (13%) presented decreased levels.

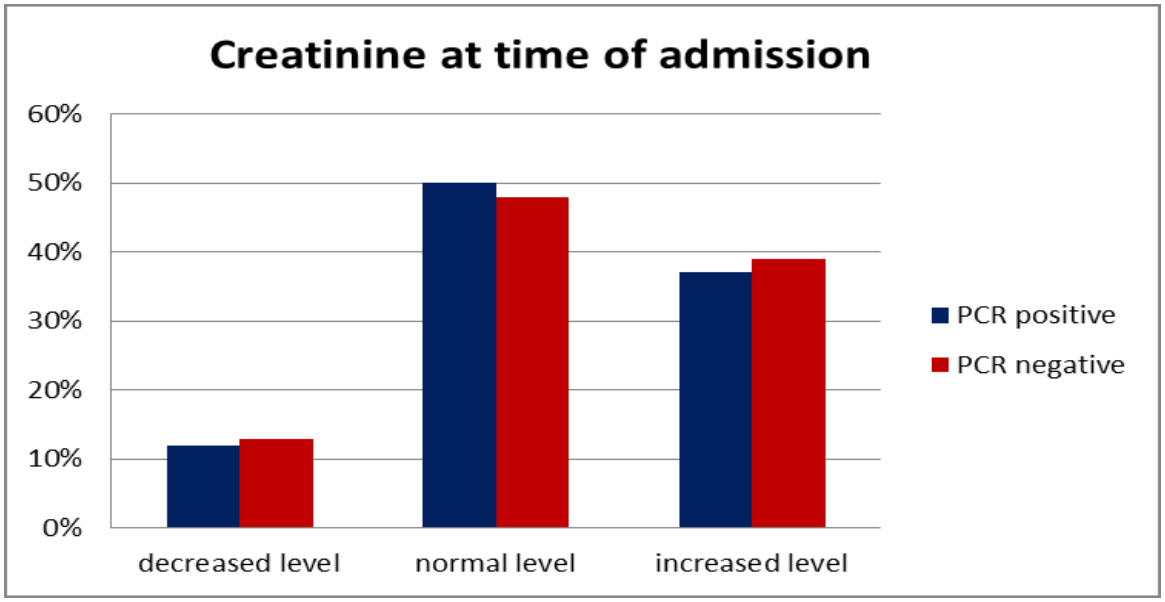

Figure 30: Creatinine at time of admission

Median was 0.87 mg/dl both in the PCR positive group (IQR, 0.6- 1.2 mg/dl) and in the ILI group (IQR, 0.6- 1.3 mg/dl).

Comparison between the two groups revealed no statistically significant differences.

	PCR positive group (n=221)	PCR negative group (n=312)	p-value
Laboratory values			
Creatinine (mg/dl) day 1	0.87* (ni=123)	0.8 * (ni=234)	
* Median displayed			
ni= number of patients where laboratory values were available			
§ statistically significant difference, p<0.05			

Table 17: Creatinine at time of admission

The other laboratory values we analyzed (procalcitonin, hemoglobin, hematocrit, LDH, troponin, INR, bilirubin and lipase) showed no statistically significant differences between the two groups.

4 Discussion

During the 2009 influenza A/ H1N1 pandemic more than 18000 people lost their lives worldwide (30).

During a pandemic, but also during seasonal influenza reliable and rapid diagnosis is essential to initiate appropriate antiviral therapy in influenza.

Therefore, the implementation of a clinical score for the rapid identification of H1N1 cases could be helpful.

Up to the present published clinical diagnostic criteria for influenza A (H1N1) infection revealed, however, low to moderate sensitivity and specificity (100).

WHO	CDC	HPA
Sudden onset of fever > 38°C and cough or sore throat in absence of other diagnoses	Fever (greater than 37,8°C) plus one or more of the following : rhinorrhoea or nasal congestion; sore throat; cough	Fever (≥38°C) or a history of fever , and influenza-like illness (two or more of the following symptoms: cough; sore throat; rhinorrhoea; limb or joint pain; headache; vomiting or diarrhea) or severe and/or life-threatening illness suggestive of an infectious process

Table 18: Clinical diagnostic criteria for patients with suspected influenza A (H1N1) (adapted from [100])

A study from Scotland revealed that most clinically diagnosed H1N1 infections in 2009/2010 did in fact not suffer from H1N1. The West of Scotland Specialist Virology Centre in Glasgow tested more than 16,000 samples from patients who were clinically diagnosed with H1N1 influenza using real time reverse transcriptase polymerase chain reaction (rtRT-PCR) assays. Only 9% of the 16,000 samples were confirmed as H1N1 infection. The rest was misdiagnosed as H1N1/2009.

This finding has serious consequences. Many people were unnecessarily treated with oseltamivir. Health costs were higher and people may have been exposed to the side- effects of antiviral treatment (101).

Other studies showed that during the pandemic accurate clinical diagnosis of H1N1 infections was difficult and not improved significantly (102).
A score that combines clinical and laboratory parameters may overcome these limitations and exhibit higher sensitivity and specificity than the currently proposed case definitions.
Until now there are only a few studies that deal with this topic and compare the clinical symptoms together with laboratory values between people with influenza A/H1N1 and people with ILI.
Implementation of a clinical score for diagnosing H1N1 infection could also help to reduce unnecessary antibiotic use.
Overuse of antibiotics is a problem worldwide. It is costly and especially broad-spectrum antibiotics rear bacterial resistance. In many countries antibiotics are prescribed for viral illnesses. Hebert et al. demonstrates in a study that during the H1N1 pandemic 2009 physicians were less likely to prescribe antibiotics for patients with febrile respiratory illness than in other years. Instead of that, they were more likely to prescribe antivirals. The conclusion of the study was that epidemiologic context and number of cases of febrile respiratory illnesses a physician had recently seen were associated with the number of prescriptions of antibiotics (103). Thus, the prescription of antibiotics and antivirals during a pandemic is not dependent on the random occurrence, it is important that especially resident doctors have the necessary information about the clinical symptoms and the typical laboratory changes.

As a possible component of a proposed score in the future we retrospectively analyzed the laboratory values of patients with PCR confirmed H1N1 influenza and patients with clinical suspicion, but negative PCR in South East Austria. We found that there are statistically significant differences in laboratory values between patients with PCR confirmed H1N1 infection and patients with ILI.
Total white blood cell count, relative and absolute eosinophil count, thrombocytes, C-reactive protein and GGT were significantly lower in patients with confirmed H1N1 infection.
On the other hand, creatine kinase values were significantly higher in patients with PCR confirmed H1N1 infection.

Just as in our study Zarogoulidis et al. as well as Karplus et al. were able to show that patients with confirmed H1N1 infection had significantly lower white blood cell count, absolute lymphocyte counts and C-reactive protein levels on admission to hospital than patients with clinical suspicion but negative H1N1 PCR (104, 105).

Furthermore, Kumar et al. demonstrated in a survey that creatine kinase levels were elevated over the first week of illness in patients with severe H1N1 infection (106).

In an interesting study comparing hospitalized patients with H1N1 infection and those infected by other viruses Chan and colleagues revealed that patients with H1N1 were more likely to have lower WBC counts, potassium and platelet counts, but higher hemoglobin, hematocrit and albumin levels (107).

A study from Mexico which compared clinical characteristics on admission between patients who were positive for H1N1 and those who were negative showed that tested patients with H1N1 infection had elevated lactate dehydrogenase levels and increased creatine kinase levels. Further they were more likely to have lymphopenia, thrombocytopenia and elevated creatinine levels (35). In contrast to these two previous publications we did not observe higher levels of hemoglobin, hematocrit, lactate dehydrogenase or creatinine in patients with H1N1.

A study by Hoenigl et al. demonstrated the clinical differences between PCR confirmed H1N1 influenza and PCR negative ILI. Interestingly there were significant clinical differences between the two groups.

Fever above 38°C, cough, fatigue, rapid onset, rhinitis, wheezing and pharyngitis were significantly more frequent in patients with confirmed H1N1 infection compared to patients with ILI (108).

	PCR positive group (n=221)	PCR negative group (n=312)
Age §	23.1 *	32.88 *
Hospitalization (days) §	6.68	9.9
Days onset symptoms to hospitalization	1.74	4.18
Prodrome before fever onset §	23/215 (11%)	50/300 (17%)
Fever>38°C at home §	191/196 (97%)	226/306 (74%)
Dyspnea	44/206 (21%)	59/307 (19%)
Rhinitis §	48/209 (23%)	44/307 (14%)
Cough §	155/216 (72%)	158/307 (51%)

Pharyngitis §	70/209 (33%)	63/300 (21%)
Fatigue §	124/219 (57%)	69/307 (22%)
Headache §	66/209 (30%)	71/307 (23%)
Wheezing §	29/209 (14%)	17/299 (6%)
Chronic renal insufficiency §	10/209 (5%)	38/206 (12%)

* Mean displayed, § statistically significant difference, $p < 0.05$

Table 19: Clinical differences between patients with confirmed H1N1 infection and patients with ILI (adapted from [108])

Perez-Padilla et al. demonstrated that patients with H1N1 infection presented more likely fever, with temperatures higher than 38°C, cough, dyspnea or respiratory distress (35). Also other studies present similar results (109-111).
Our study has several limitations. Data sets were incomplete for 65% of patients with confirmed influenza A/H1N1 infection and for 70% of patients with ILI.
We analyzed only patients who required medical care at hospitals. This means that laboratory data may not be representative for all patients, as those with mild illness may not have sought medical care.

To conclude, we found differences in laboratory values between patients with H1N1 infection and patients with ILI which may help to develop a simply and quick to perform clinical score for rapid diagnosis of suspected H1N1 infection.
Our study also may contribute a better understanding about influenza infections and may be beneficial for appropriate pandemic management in the future.

5 References

1. Genetics of the influenza virus | learn science at scitable [Internet].; cited 6/20/2013]. Available from: http://www.nature.com/scitable/topicpage/genetics-of-the-influenza-virus-716.
2. WHO | pandemic (H1N1) 2009 - update 100 [Internet].; cited 6/13/2013]. Available from: http://www.who.int/csr/don/2010_05_14/en/.
3. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. Microbiol Rev. 1992 Mar;56(1):152-79.
4. CDC newsroom image library disease agents [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/media/subtopic/library/diseases.htm.
5. Morens DM, Taubenberger JK, Fauci AS. The persistent legacy of the 1918 influenza virus. N Engl J Med. 2009 Jul 16;361(3):225-9.
6. Sha B, Luo M. Structure of a bifunctional membrane-RNA binding protein, influenza virus matrix protein M1. Nat Struct Biol. 1997 Mar;4(3):239-44.
7. Garten RJ, Davis CT, Russell CA, Shu B, Lindstrom S, Balish A, et al. Antigenic and genetic characteristics of swine-origin 2009 A(H1N1) influenza viruses circulating in humans. Science. 2009 Jul 10;325(5937):197-201.
8. Engelhardt OG, Fodor E. Functional association between viral and cellular transcription during influenza virus infection. Rev Med Virol. 2006 Sep-Oct;16(5):329-45.
9. Neumann G, Noda T, Kawaoka Y. Emergence and pandemic potential of swine-origin H1N1 influenza virus. Nature. 2009 Jun 18;459(7249):931-9.
10. WHO | what is a pandemic? [Internet].; cited 6/14/2013]. Available from: http://www.who.int/csr/disease/swineflu/frequently_asked_questions/pandemic/en/index.html.
11. Zimmer SM, Burke DS. Historical perspective--emergence of influenza A (H1N1) viruses. N Engl J Med. 2009 Jul 16;361(3):279-85.
12. Shope RE. Swine influenza : Iii. filtration experiments and etiology. J Exp Med. 1931 Jul 31;54(3):373-85.
13. Nicholls H. Pandemic influenza: The inside story. PLoS Biol. 2006 Feb;4(2):e50.
14. Pandemic: NO PANIC! [Internet].; cited 6/6/2013]. Available from: http://www.expatica.com/nl/health_fitness/healthcare/Pandemic_-NO-PANIC__14214.html.
15. Centers for Disease Control and Prevention (CDC). Swine influenza A (H1N1) infection in two children--southern california, march-april 2009. MMWR Morb Mortal Wkly Rep. 2009 Apr 24;58(15):400-2.
16. Chowell G, Bertozzi SM, Colchero MA, Lopez-Gatell H, Alpuche-Aranda C, Hernandez M, et al. Severe respiratory disease concurrent with the circulation of H1N1 influenza. N Engl J Med. 2009 Aug 13;361(7):674-9.
17. Echevarria-Zuno S, Mejia-Arangure JM, Mar-Obeso AJ, Grajales-Muniz C, Robles-Perez E, Gonzalez-Leon M, et al. Infection and death from influenza A

H1N1 virus in mexico: A retrospective analysis. Lancet. 2009 Dec 19;374(9707):2072-9.
18. WHO | current WHO phase of pandemic alert for pandemic (H1N1) 2009 [Internet].; cited 6/6/2013]. Available from: http://www.who.int/csr/disease/swineflu/phase/en/.
19. GlobalSubnationalMasterGradSym_20090611_1600.png (PNG-grafik, 2027 × 1358 pixel) - skaliert (47%) [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/GlobalSubnationalMasterGradSym_20090611_1600.png.
20. WHO | world now at the start of 2009 influenza pandemic [Internet].; cited 5/28/2013]. Available from: http://www.who.int/mediacentre/news/statements/2009/h1n1_pandemic_phase6_20090611/en/index.html.
21. Global_geographicSpreadH1N1A_week30.png (PNG-grafik, 2027 × 1358 pixel) - skaliert (47%) [Internet].; cited 6/20/2013]. Available from: http://www.who.int/csr/don/Global_geographicSpreadH1N1A_week30.png.
22. WHO | pandemic (H1N1) 2009 - update 60 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2009_08_04/en/index.html.
23. WHO | global health observatory | map gallery [Internet].; cited 3/2/2013]. Available from: http://gamapserver.who.int/mapLibrary/app/searchResults.aspx.
24. WHO | pandemic (H1N1) 2009 - update 76 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2009_11_27a/en/index.html.
25. WHO | pandemic (H1N1) 2009 - update 77 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2009_12_04/en/.
26. WHO | pandemic (H1N1) 2009 - update 95 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2010_04_09/en/index.html.
27. WHO | pandemic (H1N1) 2009 - update 104 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2010_06_11/en/index.html.
28. WHO | pandemic (H1N1) 2009 [Internet].; cited 11/3/2012]. Available from: http://www.who.int/csr/disease/swineflu/en/index.html.
29. Amato-Gauci A, Zucs P, Snacken R, Ciancio B, Lopez V, Broberg E, et al. Surveillance trends of the 2009 influenza A(H1N1) pandemic in europe. Euro Surveill. 2011 Jun 30;16(26):19903.
30. WHO | pandemic (H1N1) 2009 - update 111 [Internet].; cited 11/9/2012]. Available from: http://www.who.int/csr/don/2010_07_30/en/index.html.
31. WHO | influenza A(H1N1) - update 6 [Internet].; cited 7/1/2013]. Available from: http://www.who.int/csr/don/2009_04_30_a/en/index.html.
32. CDC H1N1 flu | interim guidance on infection control measures for 2009 H1N1 influenza in healthcare settings, including protection of healthcare personnel [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm.
33. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized patients with 2009 H1N1 influenza in the united states, april-june 2009. N Engl J Med. 2009 Nov 12;361(20):1935-44.

34. Novel Swine-Origin Influenza A (H1N1) Virus Investigation Team, Dawood FS, Jain S, Finelli L, Shaw MW, Lindstrom S, et al. Emergence of a novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009 Jun 18;360(25):2605-15.
35. Perez-Padilla R, de la Rosa-Zamboni D, Ponce de Leon S, Hernandez M, Quinones-Falconi F, Bautista E, et al. Pneumonia and respiratory failure from swine-origin influenza A (H1N1) in mexico. N Engl J Med. 2009 Aug 13;361(7):680-9.
36. Marchiori E, Zanetti G, D'Ippolito G, Verrastro CG, Meirelles GS, Capobianco J, et al. Swine-origin influenza A (H1N1) viral infection: Thoracic findings on CT. AJR Am J Roentgenol. 2011 Jun;196(6):W723-8.
37. Mauad T, Hajjar LA, Callegari GD, da Silva LF, Schout D, Galas FR, et al. Lung pathology in fatal novel human influenza A (H1N1) infection. Am J Respir Crit Care Med. 2010 Jan 1;181(1):72-9.
38. Guo HH, Sweeney RT, Regula D, Leung AN. Best cases from the AFIP: Fatal 2009 influenza A (H1N1) infection, complicated by acute respiratory distress syndrome and pulmonary interstitial emphysema. Radiographics. 2010 Mar;30(2):327-33.
39. CDC H1N1 flu | people at high risk of developing flu-related complications [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/h1n1flu/highrisk.htm.
40. CDC H1N1 flu | information for specific groups [Internet].; cited 3/1/2013]. Available from: http://www.cdc.gov/h1n1flu/groups.htm.
41. WHO | clinical management of human infection with pandemic (H1N1) 2009: Revised guidance [Internet].; cited 2/13/2013]. Available from: http://www.who.int/csr/resources/publications/swineflu/clinical_management/en/index.html.
42. Yu H, Feng Z, Uyeki TM, Liao Q, Zhou L, Feng L, et al. Risk factors for severe illness with 2009 pandemic influenza A (H1N1) virus infection in china. Clin Infect Dis. 2011 Feb 15;52(4):457-65.
43. Jain S, Kamimoto L, Bramley AM, Schmitz AM, Benoit SR, Louie J, et al. Hospitalized patients with 2009 H1N1 influenza in the united states, april-june 2009. N Engl J Med. 2009 Nov 12;361(20):1935-44.
44. Libster R, Bugna J, Coviello S, Hijano DR, Dunaiewsky M, Reynoso N, et al. Pediatric hospitalizations associated with 2009 pandemic influenza A (H1N1) in argentina. N Engl J Med. 2010 Jan 7;362(1):45-55.
45. Campbell A, Rodin R, Kropp R, Mao Y, Hong Z, Vachon J, et al. Risk of severe outcomes among patients admitted to hospital with pandemic (H1N1) influenza. CMAJ. 2010 Mar 9;182(4):349-55.
46. Swine flu: Complications - MayoClinic.com [Internet].; cited 6/4/2013]. Available from: http://www.mayoclinic.com/health/swine-flu/DS01144/DSECTION=complications.
47. Khandaker G, Zurynski Y, Buttery J, Marshall H, Richmond PC, Dale RC, et al. Neurologic complications of influenza A(H1N1)pdm09: Surveillance in 6 pediatric hospitals. Neurology. 2012 Oct 2;79(14):1474-81.

48. Centers for Disease Control and Prevention (CDC). Bacterial coinfections in lung tissue specimens from fatal cases of 2009 pandemic influenza A (H1N1) - united states, may-august 2009. MMWR Morb Mortal Wkly Rep. 2009 Oct 2;58(38):1071-4.
49. Dhanoa A, Fang NC, Hassan SS, Kaniappan P, Rajasekaram G. Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza A (H1N1) 2009 infections: The effects of bacterial coinfection. Virol J. 2011 Nov 3;8:501.
50. Lat A, Bhadelia N, Miko B, Furuya EY, Thompson GR,3rd. Invasive aspergillosis after pandemic (H1N1) 2009. Emerg Infect Dis. 2010 Jun;16(6):971-3.
51. Wauters J, Baar I, Meersseman P, Meersseman W, Dams K, De Paep R, et al. Invasive pulmonary aspergillosis is a frequent complication of critically ill H1N1 patients: A retrospective study. Intensive Care Med. 2012 Nov;38(11):1761-8.
52. Garcia-Vidal C, Barba P, Arnan M, Moreno A, Ruiz-Camps I, Gudiol C, et al. Invasive aspergillosis complicating pandemic influenza A (H1N1) infection in severely immunocompromised patients. Clin Infect Dis. 2011 Sep;53(6):e16-9.
53. CDC - seasonal influenza (flu) - how flu spreads [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/flu/about/disease/spread.htm.
54. Lakdawala SS, Subbarao K. The ongoing battle against influenza: The challenge of flu transmission. Nat Med. 2012 Oct;18(10):1468-70.
55. Bhattarai A, Villanueva J, Palekar RS, Fagan R, Sessions W, Winter J, et al. Viral shedding duration of pandemic influenza A H1N1 virus during an elementary school outbreak--pennsylvania, may-june 2009. Clin Infect Dis. 2011 Jan 1;52 Suppl 1:S102-8.
56. Polymerase chain reaction - definition of polymerase chain reaction by the free online dictionary, thesaurus and encyclopedia. [Internet].; cited 6/4/2013]. Available from: http://www.thefreedictionary.com/polymerase+chain+reaction.
57. WHO | CDC protocol of realtime RTPCR for influenza A (H1N1) [Internet].; cited 2/13/2013]. Available from: http://www.who.int/csr/resources/publications/swineflu/realtimeptpcr/en/index.html.
58. CDC H1N1 flu | interim guidance on specimen collection, processing, and testing for patients with suspected novel influenza A (H1N1) (swine flu) virus infection [Internet].; cited 2/13/2013]. Available from: http://www.cdc.gov/h1n1flu/specimencollection.htm.
59. Rello J, Rodriguez A, Ibanez P, Socias L, Cebrian J, Marques A, et al. Intensive care adult patients with severe respiratory failure caused by influenza A (H1N1)v in spain. Crit Care. 2009;13(5):R148.
60. Poon LL, Chan KH, Smith GJ, Leung CS, Guan Y, Yuen KY, et al. Molecular detection of a novel human influenza (H1N1) of pandemic potential by conventional and real-time quantitative RT-PCR assays. Clin Chem. 2009 Aug;55(8):1555-8.
61. Vasoo S, Stevens J, Singh K. Rapid antigen tests for diagnosis of pandemic (swine) influenza A/H1N1 Clin Infect Dis. 2009 Oct 1;49(7):1090-3.

62. Harper SA, Bradley JS, Englund JA, File TM, Gravenstein S, Hayden FG, et al. Seasonal influenza in adults and Children—Diagnosis, treatment, chemoprophylaxis, and institutional outbreak management: Clinical practice guidelines of the infectious diseases society of america Clinical Infectious Diseases. 2009;48(8):1003 <last_page> 1032.
63. Drexler JF, Helmer A, Kirberg H, Reber U, Panning M, Muller M, et al. Poor clinical sensitivity of rapid antigen test for influenza A pandemic (H1N1) 2009 virus. Emerg Infect Dis. 2009 Oct;15(10):1662-4.
64. Blyth CC, Iredell JR, Dwyer DE. Rapid-test sensitivity for novel swine-origin influenza A (H1N1) virus in humans. N Engl J Med. 2009 Dec 17;361(25):2493.
65. CDC H1N1 flu | influenza diagnostic testing during the 2009-2010 flu season [Internet].; cited 6/5/2013]. Available from: http://www.cdc.gov/h1n1flu/diagnostic_testing_public_qa.htm.
66. Sutter DE, Worthy SA, Hensley DM, Maranich AM, Dolan DM, Fischer GW, et al. Performance of five FDA-approved rapid antigen tests in the detection of 2009 H1N1 influenza A virus. J Med Virol. 2012 Nov;84(11):1699-702.
67. Influenza tests: The test [Internet].; cited 6/6/2013]. Available from: http://labtestsonline.org/understanding/analytes/flu/tab/test.
68. Viral culture [Internet].; cited 6/6/2013]. Available from: http://www.webmd.com/hw-popup/viral-culture.
69. Centers for Disease Control and Prevention (CDC). Update: Drug susceptibility of swine-origin influenza A (H1N1) viruses, april 2009. MMWR Morb Mortal Wkly Rep. 2009 May 1;58(16):433-5.
70. CDC H1N1 flu | updated interim recommendations for the use of antiviral medications in the treatment and prevention of influenza for the 2009-2010 season [Internet].; cited 2/25/2013]. Available from: http://www.cdc.gov/H1N1flu/recommendations.htm.
71. Dimmock NJ, Dove BK, Meng B, Scott PD, Taylor I, Cheung L, et al. Comparison of the protection of ferrets against pandemic 2009 influenza A virus (H1N1) by 244 DI influenza virus and oseltamivir. Antiviral Res. 2012 Oct 4.
72. Bardsley-Elliot A, Noble S. Oseltamivir. Drugs. 1999 Nov;58(5):851,60; discussion 861-2.
73. Tullu MS. Oseltamivir. J Postgrad Med. 2009 Jul-Sep;55(3):225-30.
74. WHO | WHO guidelines for pharmacological management of pandemic (H1N1) 2009 influenza and other influenza viruses [Internet].; cited 2/13/2013]. Available from: http://www.who.int/csr/resources/publications/swineflu/h1n1_use_antivirals_20090820/en/index.html.
75. CDC H1N1 flu | quick facts for clinicians on antiviral treatments for 2009 H1N1 [Internet].; cited 2/25/2013]. Available from: http://www.cdc.gov/h1n1flu/antivirals/facts_clinicians.htm.
76. Graitcer SB, Gubareva L, Kamimoto L, Doshi S, Vandermeer M, Louie J, et al. Characteristics of patients with oseltamivir-resistant pandemic (H1N1) 2009, united states. Emerg Infect Dis. 2011 Feb;17(2):255-7.

77. Swine flu: How the virus works - telegraph [Internet].; cited 6/4/2013]. Available from: http://www.telegraph.co.uk/health/swine-flu/5258873/Swine-flu-how-the-virus-works.html.
78. CDC H1N1 flu | H1N1 flu and you [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/h1n1flu/qa.htm.
79. CDC H1N1 flu | key facts about 2009 H1N1 flu vaccine [Internet].; cited 3/2/2013]. Available from: http://www.cdc.gov/h1n1flu/vaccination/vaccine_keyfacts.htm.
80. WHO | use of the pandemic (H1N1) 2009 vaccines [Internet].; cited 6/4/2013]. Available from: http://www.who.int/csr/disease/swineflu/frequently_asked_questions/vaccine_preparedness/use/en/index.html.
81. CDC H1N1 flu | novel H1N1 vaccination recommendations [Internet].; cited 6/4/2013]. Available from: http://www.cdc.gov/h1n1flu/vaccination/acip.htm.
82. Johansen K, Nicoll A, Ciancio BC, Kramarz P. Pandemic influenza A(H1N1) 2009 vaccines in the european union. Euro Surveill. 2009 Oct 15;14(41):19361.
83. CDC - seasonal influenza (flu) - overview of influenza surveillance in the united states [Internet].; cited 2/13/2013]. Available from: http://han.medunigraz.at/han/pubmed/www.cdc.gov/flu/weekly/overview.htm.
84. WHO | human infection with pandemic (H1N1) 2009 virus: Updated interim WHO guidance on global surveillance [Internet].; cited 11/9/2012]. Available from: http://han.medunigraz.at/han/pubmed/www.who.int/csr/resources/publications/swineflu/interim_guidance/en/index.html.
85. EuroFlu - case definitions [Internet].; cited 2/13/2013]. Available from: http://www.euroflu.org/html/case_definitions.html.
86. Navarro-Mari JM, Perez-Ruiz M, Galan Montemayor JC, Marcos Maeso MA, Reina J, de Ona Navarro M, et al. Circulation of other respiratory viruses and viral co-infection during the 2009 pandemic influenza. Enferm Infecc Microbiol Clin. 2012 Oct;30 Suppl 4:25-31.
87. Bellei N, Carraro E, Perosa A, Watanabe A, Arruda E, Granato C. Acute respiratory infection and influenza-like illness viral etiologies in brazilian adults. J Med Virol. 2008 Oct;80(10):1824-7.
88. Laguna-Torres VA, Gomez J, Ocana V, Aguilar P, Saldarriaga T, Chavez E, et al. Influenza-like illness sentinel surveillance in peru. PLoS One. 2009 Jul 1;4(7):e6118.
89. Ren L, Gonzalez R, Wang Z, Xiang Z, Wang Y, Zhou H, et al. Prevalence of human respiratory viruses in adults with acute respiratory tract infections in beijing, 2005-2007. Clin Microbiol Infect. 2009 Dec;15(12):1146-53.
90. Uyeki TM, Prasad R, Vukotich C, Stebbins S, Rinaldo CR, Ferng Y, et al. Low sensitivity of rapid diagnostic test for influenza Clinical Infectious Diseases. 2009;48(9):e89 <last_page> e92.
91. Cunha BA, Syed U, Mickail N, Strollo S. Rapid clinical diagnosis in fatal swine influenza (H1N1) pneumonia in an adult with negative rapid influenza diagnostic tests (RIDTs): Diagnostic swine influenza triad. Heart Lung. 2010 Jan-Feb;39(1):78-86.

92. Lucas PM, Morgan OW, Gibbons TF, Guerrero AC, Maupin GM, Butler JL, et al. Diagnosis of 2009 pandemic influenza A (pH1N1) and seasonal influenza using rapid influenza antigen tests, san antonio, texas, april-june 2009. Clin Infect Dis. 2011 Jan 1;52 Suppl 1:S116-22.
93. Lee CY, Chuang YF, Huang WY, Cheng SH, Pei JS. Epidemiology, clinical features, treatment, and outcomes of cases of influenza a infection during the 2009 influenza pandemic in northern taiwan. Pediatr Neonatol. 2012 Aug;53(4):257-63.
94. Dapat C, Saito R, Kyaw Y, Myint YY, Oo HN, Oo KY, et al. Delayed emergence of oseltamivir-resistant seasonal influenza A (H1N1) and pandemic influenza A(H1N1)pdm09 viruses in myanmar. Influenza Other Respi Viruses. 2012 Nov 5.
95. Thorlund K, Awad T, Boivin G, Thabane L. Systematic review of influenza resistance to the neuraminidase inhibitors. BMC Infect Dis. 2011 May 19;11:134.
96. Harvala H, Gunson R, Simmonds P, Hardie A, Bennett S, Scott F, et al. The emergence of oseltamivir-resistant pandemic influenza A (H1N1) 2009 virus amongst hospitalised immunocompromised patients in scotland, november-december, 2009. Euro Surveill. 2010 Apr 8;15(14):19536.
97. Tramontana AR, George B, Hurt AC, Doyle JS, Langan K, Reid AB, et al. Oseltamivir resistance in adult oncology and hematology patients infected with pandemic (H1N1) 2009 virus, australia. Emerg Infect Dis. 2010 Jul;16(7):1068-75.
98. Pizzorno A, Abed Y, Boivin G. Influenza drug resistance. Semin Respir Crit Care Med. 2011 Aug;32(4):409-22.
99. Jefferson T, Jones M, Doshi P, Del Mar C. Possible harms of oseltamivir--a call for urgent action. Lancet. 2009 Oct 17;374(9698):1312-3.
100. Gerrard J, Keijzers G, Zhang P, Vossen C, Macbeth D. Clinical diagnostic criteria for isolating patients admitted to hospital with suspected pandemic influenza. Lancet. 2009 Nov 14;374(9702):1673.
101. Gunson RN, Carman WF. During the summer 2009 outbreak of "swine flu" in scotland what respiratory pathogens were diagnosed as H1N1/2009? BMC Infect Dis. 2011 Jul 13;11:192.
102. Mahony AA, Cheng AC, Olsen KL, Aboltins CA, Black JF, Johnson PD, et al. Diagnosing swine flu: The inaccuracy of case definitions during the 2009 pandemic, an attempt at refinement, and the implications for future planning. Influenza Other Respi Viruses. 2012 Jun 19.
103. Hebert C, Beaumont J, Schwartz G, Robicsek A. The influence of context on antimicrobial prescribing for febrile respiratory illness: A cohort study. Ann Intern Med. 2012 Aug 7;157(3):160-9.
104. Zarogoulidis P, Glaros D, Kioumis I, Terzi E, Porpodis K, Tsiotsios A, et al. Clinical differences between influenza A (H1N1) virus and respiratory infection between the two waves in 2009 and 2010. Int J Gen Med. 2012;5:675-82.
105. Karplus R, Weinberger M, Zaidenstein R, Goldshtein L, Natif N, Gayer G. The role of readily available clinical, laboratory and radiologic findings in distinguishing a/H1N1/2009 influenza from other causes of acute febrile respiratory illness under pandemic conditions. Isr Med Assoc J. 2012 Oct;14(10):613-9.

106. Kumar A, Zarychanski R, Pinto R, Cook DJ, Marshall J, Lacroix J, et al. Critically ill patients with 2009 influenza A(H1N1) infection in canada. JAMA. 2009 Nov 4;302(17):1872-9.
107. Chan PA, Mermel LA, Andrea SB, McCulloh R, Mills JP, Echenique I, et al. Distinguishing characteristics between pandemic 2009-2010 influenza A (H1N1) and other viruses in patients hospitalized with respiratory illness. PLoS One. 2011;6(9):e24734.
108. Hoenigl M, Drescher M, Prattes J, Tovilo K, Kessler H, Vander K, et al. Clinical characteristics and laboratory admission values for differentiation between H1N1 influenza and influenza like illnesses: A case control study. APR 27-30, 2013.
109. The role of readily available clinical, laboratory and radiologic findings in distinguishing a/H1N1/2009 influenza from other causes of acute febrile respiratory illness under pandemic conditions. . 2012 Oct;14(10):613-9.
110. Midha T, Nath B, Kumari R, Rao YK, Lekhwani S, Vaswani ND, et al. Clinical predictors of influenza A(H1N1) in kanpur, india. J Indian Med Assoc. 2012 Jan;110(1):22, 24, 39.
111. Lee IK, Liu JW, Wang L, Yang KD, Li CC, Eng HL. 2009 pandemic influenza A (H1N1): Clinical and laboratory characteristics in pediatric and adult patients and in patients with pulmonary involvement. Influenza Other Respi Viruses. 2012 Nov;6(6):e152-61.

6 List of tables

7 List of figures

8 Curriculum vitae

NAME: MANUELA DRESCHER

Address	Elisabethstaße 19, 8010 Graz, Austria
E-Mail	manuela.drescher@stud.medunigraz.at
Nationality	Austrian
Civil Status	Single
Date of birth	6th February 1990, Wolfsberg im Lavanttal
Present Status	Student

EDUCATIONAL HISTORY

October 2008- present	Medical University of Graz
2000-2008	Stiftsgymnasium der Benediktiner zu St. Paul (Austria), Matura (A- Levels Equivalent) with excellent success

SCIENCE WORK

April 2012	Participant at the **6th congress of the Austrian society of infectious diseases and tropical medicine** (OEGIT) Saalfelden, Austria; presenting a poster

(Comparison of laboratory values between PCR confirmed H1N1 influenza and PCR negative Influenza-like illnesses)

2013 **Case report:** *Successful Management of Nosocomial ventriculitis and meningitis caused by extensive drug resistant Acinetobacter baumannii, Austria;* published in the **Canadian Journal of Infectious Diseases and Medical Microbiology**

Coauthor of the paper: *Predictors of H1N1 influenza in the emergency department: proposition for a modified H1N1 case definition* published in the **Clinical Microbiology and Infection (CMI)**

AWARDS

2012 Poster price (second place*);* at the 6th congress of the Austrian society of infectious diseases and tropical medicine (OEGIT), April 2012, Saalfelden, Austria

WORK EXPERIENCE/ CLERKHIPS

February 2010 University Hospital Graz, Department of Surgery/ General Surgery

July 2010	Krankenhaus der Elisabethinen Graz, Department of Internal Medicine
August 2010	Wolfsberg Regional Hospital, Department of Anesthesia
July 2011	Graz West Regional Hospital, Deparment of Internal Medicine
August 2011	University Hospital Graz, Division of Oncology
February 2012	University Hospital Graz, Department of Neurology
September 2012	University Hospital Graz, Department of surgery/ vascular surgery
February 2013	University Hospital Graz, Department of Radiology
2013/2014	**Practical year:**
	6 weeks general surgery at the Helios clinic Schwerin/ Germany
	10 weeks internal medicine (emergency ward) at the University Hospital Graz, Austria

3 weeks at the Helios clinic Schwerin, Germany, Department of Paediatrics

5 weeks general medicine, Dr. Johannes Hipfl, Carinthia (Austria)

SPECIAL ABILITIES/ ELECTIVE COURSE

Basic Medical English I
Sonographie in der Inneren Medizin
Sonographische Diagnostik in der Akutaufnahme

LANGUAGES

German (native)
English (spoken and written)
Italian (basics)

COMPUTER SKILLS

ECDL Ceritficate (European Computer Driving Licence)

OTHER INTERESTS

Volleyball, Tennis

Travelling, Literature