

Diplomarbeit

Comparison of demographic data, clinical presentation, risk factors and outcome between PCR confirmed H1N1 influenza and PCR negative influenza-like illnesses

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Eidesstattliche Erklärung

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1 Abbreviations

ASA = American Society of Anaesthesiologists

BAL = Bronchoalveolar lavage

BPM = Beats per minute

CDC = Centers for Disease Control and Prevention

COPD = Chronic obstructive pulmonary disease

DAD = Diffuse alveolar damage

DFA = Direct immunofluorescence assay

HA = Hemagglutinin

ICU = Intensive care unit

ILI = Influenza-like illness

LKH = Landeskrankenhaus

M = Matrix gene

NA = Neuraminidase

NP = Nucleoprotein

NS = Non structure gene

PA = Polymerase acid

PB1 = Polymerase basic 1

PB2 = Polymerase basic 2

PCR = Polymerase chain reaction

RIAT = Rapid influenza antigen test

RT-PCR = Reverse transcription polymerase chain reaction

vRNP's = viral ribonucleoprotein particles

WHO = World Health Organisation

95% CI = 95% confidence interval

2 Abstract

2.1 Aim

In April 2009, Mexico was the first country where an increase of pneumonia caused by H1N1 influenza was observed. This was the beginning of the 2009/2010 influenza pandemic. As of August 2010, over 18000 deaths among laboratory-confirmed cases had been reported to the WHO. One of the major diagnostic problems is the poor sensitivity of currently available rapid influenza antigen tests (RIATs) for H1N1 influenza detection, and the inability to differentiate with RIATs between H1N1 and other influenza A strains. On the other hand, PCR, the gold standard for H1N1 influenza diagnosis and confirmation, has a long turn-around time and is also expensive. Timely diagnosis is important, however, as antiviral therapy - if started within 48 hours after infection - may reduce duration of hospitalization as well as the duration of illness, and lowers the risk of intensive care unit (ICU) admission. For these reasons, it seems to be important to understand the differences in first clinical presentation between PCR confirmed influenza and influenza-like illnesses (ILI), in order to improve the outcome and reduce health care costs. Therefore, knowledge of the differences between H1N1 influenza and ILI may help to develop a clinical score that could help to decide whether antiviral therapy should be started before PCR results are available.

2.2 Methods

We retrospectively reviewed 1681 patients with PCR confirmed H1N1 influenza or influenza-PCR negative ILI from 1st October 2009 to 19th January 2010. H1N1 influenza PCR was performed at the Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz or at the Institute of Hospital Hygiene, at the state hospital Graz. Data sets were obtained from MEDOCS, the electronic hospital database. Participating hospitals were the University Hospital Graz, and the state hospitals of Graz-West, Leoben, Bruck, Judenburg, Hörgas-Enzenbach, Feldbach, Deutschlandsberg, Rottenmann, Wagna and Bad Aussee. Data sets were available from 209/624 (31%) patients with PCR confirmed H1N1 and from 310/1057 (30%) patients with ILI. The vast majority of the remaining 1162 (69%) patients presented to their family doctor only,

resulting in incomplete data sets and exclusion from further investigation. In some cases not all data were available. Therefore the number of included patients varies in the different categories of the results.

We compared patients with H1N1 influenza and ILI with respect to demographic data, clinical presentation, treatment, preexisting underlying illnesses and outcome.

2.3 Results

The mean age in the H1N1 influenza group was 23.88 years (95% CI 20.95-26.8) versus 32.88 years in the ILI group (95% CI 29.92-35.85) ($p<0.001$). The mean time from onset of symptoms to first clinical presentation was lower in the H1N1 influenza group compared to the ILI group [1.78 days (95% CI 1.43-2.13) versus 4.18 days (95% CI 3.59-4.78); $p<0.001$]. Likewise, the mean time from onset of symptoms until PCR results were available was 3.46 days (95% CI 3-3.92) for H1N1 patients and 6.7 days (95% CI 5.85-7.54) for ILI patients ($p<0.001$). No significant difference could be observed in either number of patients who needed ICU admission (8% H1N1 influenza versus 5% ILI) nor in the mean age of patients who needed intensified care (44.4 years versus 43.8 years). Rapid influenza antigen tests (BinaxNow, Inverness Medical, Maine, U.S.) turned out to be unreliable because of their low sensitivity. In 89.3% of tests in H1N1 influenza patients, a false negative result was observed. H1N1 influenza patients were more likely to present with fever (92.2% versus 73.9%; $p<0.001$), cough (72.7% versus 51.5%; $p<0.001$), rhinitis (23% versus 14.3%; $p=0.012$), fatigue (59.1% versus 22.5%; $p<0.001$) and headache (31.6% versus 23.1%; $p=0.033$) and had a significant higher initial heart rate (107.3 bpm versus 98.2 bpm; $p=0.008$). However H1N1 influenza patients presented less often with abdominal pain (5.7% versus 10.7%; $p=0.048$). Furthermore H1N1 influenza patients had significantly fewer underlying co-morbidities (coronary heart disease 4.3% versus 10.2%, $p=0.015$; cardiac insufficiency 2.9% versus 8.8%, $p=0.007$; arterial hypertension 12% versus 23.5%, $p<0.001$; liver disease 2.9% versus 12.7%, $p<0.001$; chronic renal failure 4.8% versus 12.4%; $p=0.003$). Case fatality rate did not differ between groups. 1.9% of H1N1 influenza and 3.2% of ILI patients died ($p=0,424$).

2.4 Conclusion

Our data has shown that H1N1 influenza patients presented 1.78 days after onset of symptoms, and PCR results needed 1.68 days on average until they were available, after

first clinical presentation. Furthermore it was found that the rapid influenza antigen tests were unreliable. In our study, cough, rhinitis, fatigue and fever turned out to be significant clinical predictors for H1N1 influenza. A lack of fever may be used as exclusion criteria for H1N1 influenza. Presented data may help to develop a clinical score for early treatment decisions in H1N1 influenza.

3 Zusammenfassung

3.1 Ziel

Im April des Jahres 2009 wurde in Mexiko ein Anstieg von Pneumonie Fällen, verursacht durch das H1N1 Influenza Virus, beobachtet. Dies war der Beginn der H1N1 Influenza Pandemie 2009/2010. Alleine bis Ende August 2010 wurden der WHO über 18.000 H1N1 Influenza Todesopfer bestätigt. Eines der größten Probleme bei der Diagnose der H1N1 Influenza ist die niedrige Sensitivität der Influenza Schnelltests, sowie deren Unvermögen verschiedene Influenza A Stämme zu unterscheiden. Die PCR, als anerkannter Goldstandard zur Influenza Diagnostik, wiederum ist in den meisten Fällen nicht immer sofort verfügbar. Des Weiteren sind Wartezeiten bis zum Erlangen des Ergebnisses der PCR unumgänglich und es handelt sich um eine sehr kostenintensive Diagnostik. Jedoch kann eine antivirale Therapie die Aufenthaltsdauer in einem Spital, die Krankheitsdauer sowie das Risiko intensivmedizinische Versorgung zu benötigen, nur dann reduzieren, wenn sie innerhalb von 48 Stunden nach Auftreten der ersten Symptome eingeleitet wird. Aus diesem Grund ist es wichtig die klinischen Unterschiede zwischen einer H1N1 Influenza und einer Influenza-like illness (ILI) zu erkennen, um schlussendlich die Krankheitsdauer dieser Patienten zu verringern, sowie Kosten im Gesundheitssystem zu reduzieren. So könnte das bessere Verstehen der klinischen Unterschiede zwischen den beiden Erkrankungen zur Entwicklung eines klinischen Scores führen, welcher zur Entscheidung ob eine antivirale Therapie induziert ist oder nicht beitragen könnte, selbst wenn die PCR Ergebnisse noch ausständig sind.

3.2 Methoden

Es wurden retrospektiv die Daten von 1681 Patienten mit einer PCR bestätigten H1N1 Influenza oder einer ILI im Zeitraum vom 1.Oktober 2009 bis 19.Januar 2010 überprüft. Sämtliche eingeschlossene Patienten wurden mittels PCR auf H1N1 Influenza getestet. Die PCR Untersuchungen wurden am Institut für Hygiene, Mikrobiologie und Umweltmedizin, Medizinische Universität Graz und am Institut für Krankenhaushygiene, Landeskrankenhaus Graz durchgeführt. Schlussendlich wurden die Daten von 209/624 (31%) Patienten mit PCR bestätigter H1N1 Influenza und von 310/1057 (30%) Patienten

mit einer ILI analysiert. Die Daten wurden dabei aus der elektronischen Krankenhausdatenbank, dem MEDOCS, entnommen. Teilnehmende Krankenhäuser waren die Universitätsklinik Graz und die Landeskrankenhäuser Graz-West, Leoben, Bruck an der Mur, Judenburg, Hörgas-Enzenbach, Feldbach, Deutschlandsberg, Rottenmann, Wagna und Bad Aussee. Die restlichen 1162 (69%) wurden aufgrund von unvollständigen oder fehlenden Datensätzen aus der Analyse ausgeschlossen. Die meisten dieser Patienten waren nur bei ihrem Hausarzt und nicht im Krankenhaus vorstellig. Des weiteren variieren die Zahlen der analysierten Patienten in den einzelnen Kapiteln der Resultate, da von einigen Patienten einzelne Daten nicht erhoben werden konnten.

Es wurden die Daten bezüglich Demographie, klinischer Präsentation, Therapie, Grunderkrankungen und Überleben analysiert.

3.3 Resultate

Der Mittelwert des Alters in der H1N1 Influenza Gruppe betrug 23.88 Jahre (95% CI 29.92-35.85) und 32.88 Jahre (95% CI 29.92-35.85) in der Gruppe der ILI Patienten ($p < 0.001$). Auch der Mittelwert der Zeitspanne vom Beginn der Symptome bis zur klinischen Vorstellung war signifikant niedriger in der H1N1 Influenza Gruppe [1.78 (95% CI 1.43-2.13) Tage versus 4.18 (95% CI 3.59-4.78) Tage; $p < 0.001$]. Der Mittelwert der Zeitspanne vom Beginn der Symptome bis das Ergebnis der PCR vorhanden war betrug 3.46 Tage (95% CI 3-3.92) für die H1N1 Influenza Patienten und 6.7 Tage (95% CI 5.85-7.54) für die ILI Patienten ($p < 0.001$). Kein Unterschied konnte beobachtet werden hinsichtlich der Anzahl der Patienten welche eine Behandlung auf einer Intensivstation benötigten (8% für H1N1 Influenza Patienten und 5% für ILI Patienten), sowie hinsichtlich des Alters dieser Patienten (Mittelwert 44.4 Jahre versus 43.8 Jahre). Die Unzuverlässigkeit der Influenza-Schnelltests (BinaxNow, Inverness Medical, Maine, U.S.) aufgrund ihrer niedrigen Sensitivität bei H1N1 Influenza konnte ebenfalls bestätigt werden. 89.3% aller bei H1N1 Influenza Patienten durchgeführte Schnelltest zeigten ein falsch negatives Ergebnis. H1N1 Influenza Patienten präsentierten sich häufiger mit Fieber (92.2% versus 73.9%; $p < 0.001$), Husten (72.7% versus 51.5%; $p < 0.001$), Schnupfen (23% versus 14.3%; $p = 0.012$), Abgeschlagenheit (59.1% versus 22.5%; $p < 0.001$) und Kopfschmerzen (31.6% versus 23.1%; $p = 0.033$) und zeigten eine signifikant höhere initiale Herzfrequenz (107.3/min versus 98.2/min; $p = 0.008$). Auf der anderen Seite präsentierten sich H1N1 Influenza Patienten weniger häufig mit Bauchschmerzen (5.7%

versus 10.7%; $p=0.048$). Außerdem war die Anzahl von diversen Begleiterkrankungen in der H1N1 Influenza Gruppe deutlich niedriger (Koronare Herzerkrankungen 4.3% versus 10.2%, $p=0.015$; Herzinsuffizienz 2.9% versus 8.8%, $p=0.007$; arterielle Hypertonie 12% versus 23.5%, $p<0.001$; Lebererkrankungen 2.9% versus 12.7%, $p<0.001$; chronische Niereninsuffizienz 4.8% versus 12.4%, $p=0.003$). Die Todesrate unterschied sich nicht in beiden Gruppen. 1.9% aller H1N1 Influenza Patienten und 3.2% aller ILI Patienten verstarben ($p=0.424$).

3.4 Zusammenfassung

Unsere Daten zeigten dass H1N1 Influenza Patienten im Schnitt 1.78 Tagen nach Auftreten der Symptome ärztliche Hilfe aufsuchen. Bis die PCR Resultate vorliegen dauert es im Schnitt jedoch weitere 1.68 Tage. Des Weiteren erwies sich der Influenza Schnelltest im klinischen Alltag als unzuverlässig. Husten, Schnupfen, Abgeschlagenheit und Fieber waren Symptome welche signifikant häufiger bei H1N1 Influenza Patienten auftraten. Das Fehlen von Fieber könnte als Ausschlusskriterium für eine H1N1 Influenza herangezogen werden. Unsere Daten können somit zur Entwicklung eines klinischen Scores, zur frühen und sicheren Detektion von H1N1 Influenza verwendet werden.

4 The Influenza Viruses

Almost every winter a new kind of influenza virus spreads out over our planet and causes millions of infections. Usually influenza is an acute, self-limited, febrile illness that is caused by one of the two main influenza strains: either influenza A or B (1). Severity of symptoms varies widely, always depending on the virus strain and underlying risk factors. The most common symptoms that occur after an incubation period of 1-5 days (2), are fever, fatigue, cough, rhinitis, sore throat, headache, myalgia and arthralgia (1,2). Pulmonary complications like primary viral pneumonia or secondary bacterial pneumonia are the most important and common risk factors for mortality (1).

4.1 About the Influenza Viruses

There are three different influenza subtypes that are classified on the basis of antigenetic differences: influenza A, B and C. All three belong to the family of orthomyxoviridae. Common to these influenza viruses is their host-cell derived envelope, envelope glycoproteins responsible for virus entry and egress from host cells and a segmented genome of negative sense single stranded RNA. Influenza A and B viruses contain 8 gene segments, whereas influenza C contains only 7 (1,2). Differences between influenza A,B and C are displayed in Table 1.

Table 1 Differences between influenza A, B and C (adapted from (2))

	Influenza A	Influenza B	Influenza C
Genetics	8 gene segments	8 gene segments	7 gene segments
Host range	Humans, swine, equine, avian, marine animals	Humans only	Humans and swine
Clinical features	Pandemics with high mortality in young patients	No pandemics; Severe diseases in older and high-risk patients	Mild diseases

In contrast to influenza B, influenza A viruses have been associated with pandemics and high mortality in particular in young adults.

The viral envelope of influenza A virus is a double lipid layer derived from the plasma membrane of the host cell (3). The virions are covered with three transmembrane viral proteins: hemagglutinin (HA), neuraminidase (NA) and an ion channel protein (M2) (4). The so-called M1 matrix protein is located on the internal layer. It is an integral component for virus morphogenesis as it is supposed to bind to all other virus components like the viral ribonucleoprotein particles (vRNP's) and the membrane proteins (3).

NAs and HAs activity are used to differentiate among the influenza subtypes (e.g., H1N1 or H3N2). At least 16 divergent HAs (H1-H16) and 9 distinct NAs (NA1-NA9) have been described in influenza A viruses. The standard nomenclature regarding influenza viruses includes the influenza type, place of initial isolation, strain designation and year of first isolation. For example, influenza A/Puerto Rico/8/34 was isolated by Francis in 1934 in Puerto Rico (1).

4.2 History of Influenza

During the last 400 years, influenza viruses have caused recurrent epidemics of respiratory diseases worldwide.

In 1892, Richard Pfeiffer isolated a microbiological agent which he thought was the causative agent for influenza. He called it *Bacillus influenzae* (today known as *Haemophilus influenzae*) and believed it to be a bacterium. Only few of his colleagues doubted his discovery, because bacteria at this time were responsible for a lot of different diseases. Nearly 40 years later a young physician from Iowa started his researches in swine influenza. His name was Richard Shope (5). Shope's breakthrough in his researches came in 1931 when he extracted virus-contaminated secretions from influenza-infected pigs and transmitted the infectious agent into healthy animals (6). Smith, Andrews and Laidlaw also confirmed infectivity.

Smith and associates coined the modern understanding of influenza as they isolated a virus from ferrets in 1933, and shortly afterwards from humans and named it 'Influenza A' (1,7). In 1939, Francis isolated the influenza B virus and Taylor isolated influenza C virus in 1950. In 1936, Burnet discovered that influenza viruses could be cultured in embryonated hen eggs, which led to extensive researches on the characteristics of the virus and enabled research for vaccines against influenza (1). The first evidence of the effectiveness of

vaccines with inactivated viruses for influenza A and B were published in 1945/1946 by T. Francis et al (8,9).

In 1941 Hirst discovered the phenomenon of hemagglutinin, which enabled a relatively simple and inexpensive way of measurement of the virus load and specific antibodies (1,10).

4.3 Influenza Pandemics before H1N1 2009

4.3.1 1918-1919: The Spanish H1N1 Influenza

The first well-documented influenza pandemic occurred during 1918 and 1919. The virus spread all over the planet, causing infections in nearly one third of the world's population (≈500 million people). Case fatality rate was overly high (>2.5%) compared to former influenza pandemics (<0.1%) (6,11).

Even today, the impact of the 1918 influenza is still felt. Nearly all influenza A pandemics since 1918, except for human infections from avian viruses, have been caused by descendants of the 1918 virus. But none of these descendants approached the pathogenicity of the 1918 virus. Even today's H1N1 and H3N2 viruses (these are the two major descendant lineages from 1918 which still persist naturally) have been associated with substantially lower rates of illness and death (11). This fact continues to lack a satisfactory explanation even today.

One hypothesis declares that the reason for the high death rate among the 1918 pandemic influenza was the high rate of secondary bacterial pneumonia, which was caused by highly prevalent pneumopathogenic bacteria, like *streptococci* (primarily *pneumococci*) and *staphylococci*. In support of this hypothesis it should be mentioned that antibacterial vaccines administered during 1918-1919 seemed to prevent post-influenza deaths (12).

The vast majority of pandemics before and after 1918 had their origin in Asia and spread over the planet thereafter. Regarding the 1918 H1N1 influenza pandemic a geographic point of origin has to date not been verified as it has spread nearly simultaneously in 3 waves during a one-year period in Europe, Asia and North America (11). This was just one of the unique features of the 1918 influenza.

4.3.2 1957-1958: The Asian H2N2 Influenza

In 1957, H1N1 influenza suddenly disappeared from humans and was replaced by a new reassortant virus that combined genes from the 1918 H1N1 virus with an avian virus. This new strain was called Asian influenza or H2N2 (6). Even though this pandemic occurred in the era of influenza virology, the host of the reassortment events as well as the timeline are still unknown.

The 1957 pandemic started in Southeast Asia and spread all over the world thereafter. However, its speed of expansion and mortality rate was not as dramatic as the 1918 influenza. Nevertheless, clinical presentation was similar to the 1918 influenza. Within two years, influenza H2N2 became endemic and disappeared within 11 years after it was first detected. Instead, it was supplanted by the Hong Kong (H3N2) subtype and until today, has never again been detected (7,13).

4.3.3 1968-1969: The Hong Kong H3N2 Influenza

In 1968 a new influenza pandemic occurred. The origin of the 1968 pandemic was again Southeast Asia and it was called Hong Kong influenza (7). The H3N2 virus can be considered an update from the 1957 H2N2 influenza virus. The H3N2 virus obtained new genes coding for a new HA (H3) antigen by reassortment with an avian virus. The PB1 polymerase gene segment had also been replaced. The result was a new virus that spread out over the world and caused a modest pandemic. In some areas, the mortality in the 1968 pandemic was even lower than in non-pandemic years. Like the 1957 virus, the Hong Kong virus quickly became endemic and sporadic. It still continues to globally circulate today (7,13).

5 Influenza H1N1 2009

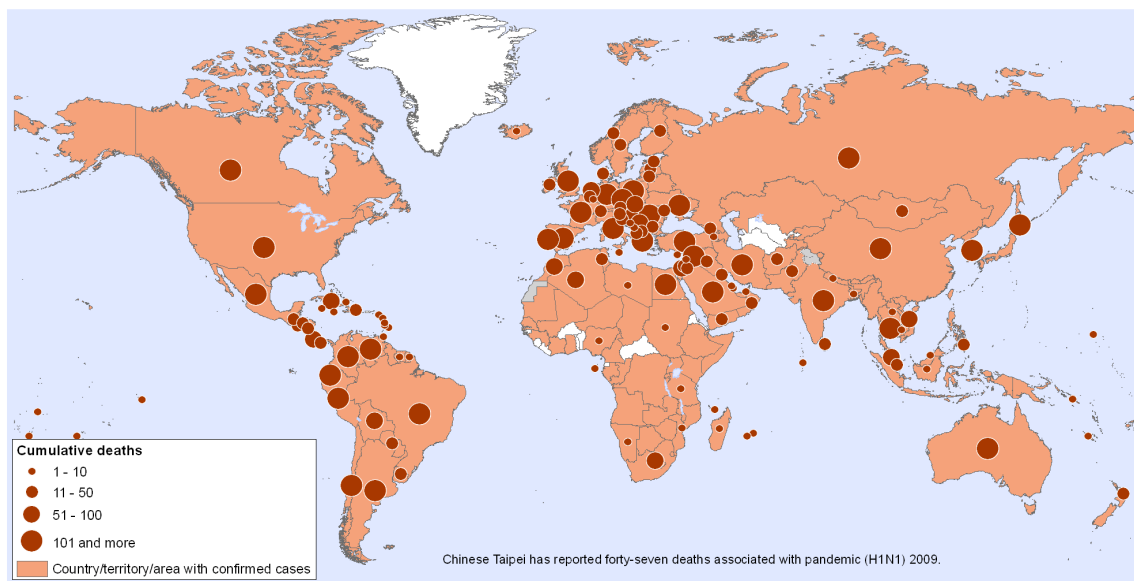
5.1 H1N1 Influenza Pandemic Timeline

The first confirmed infection of what the WHO later named pandemic H1N1 2009 virus occurred in a 6-month-old girl from San Luis Potosi State in Mexico, on 24th February

2009 (14). This was the origin of the 2009 H1N1 influenza pandemic that resulted in millions of infections and over 18000 deaths up to August 2010 (15).

Two months later, two cases of acute respiratory illnesses occurred in children in California, caused by an influenza A virus that showed a unique combination of gene segments that had never been reported before among swine or human influenza viruses. On 15th April, the virus was identified as a novel influenza A (H1N1) virus of swine origin by the Centers of Disease Control and Prevention (CDC). It had been obtained from a 10-year-old boy in California. Just two days later, a second case of H1N1 influenza, without epidemiological link to the first patient, was confirmed by the CDC (16). By 27th April 2009, seven countries had reported confirmed cases of H1N1 influenza infections: Canada, Israel, Mexico, New Zealand, Spain, the UK and the USA. Two days later, the alert was raised to phase 5 (human-to-human transmission of the virus in at least two countries in one WHO region). As of 29th May 2009 Mexico alone had reported 4910 cases and 85 deaths caused by swine-origin H1N1 influenza (17) and finally the WHO raised the pandemic alert to the highest phase, phase 6 (community-level outbreaks in at least one other country in a different WHO region in addition to the criteria defined in phase 5) on 11th June 2009 (14). In the end, more than 214 countries worldwide had reported confirmed H1N1 influenza infections (displayed in Figure 1) (15).

Figure 1 Territories with confirmed H1N1 influenza cases and death up to 15th August 2009 (adapted from (15))



In October 2009, the pandemic had passed its peak and the number of infections began to decline (14). The end of the pandemic and the start of the post-pandemic period was announced by the WHO on 10th of August 2010 (18).

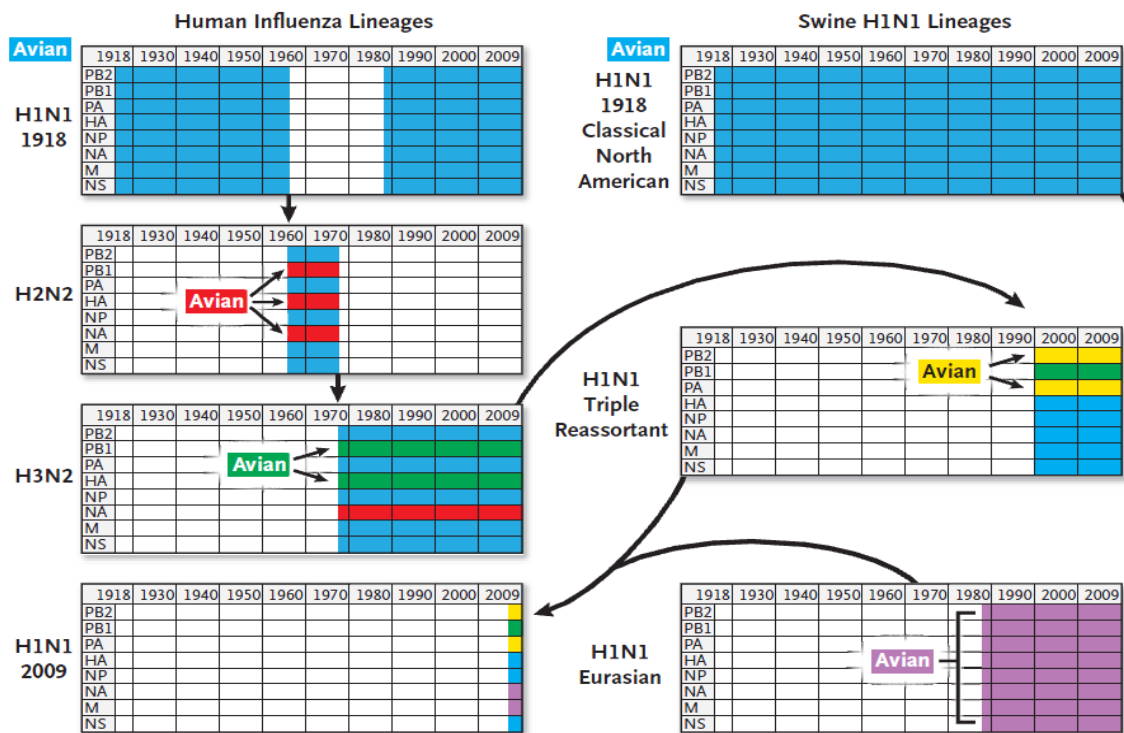
5.2 The H1N1 Influenza Virus Characteristics

Since the first H1N1 pandemic in 1918, descendants of this virus have been circulating in humans, causing seasonal influenza every year except during 1957 and 1977 (6). However, influenza pandemics only occur when an influenza virus gains a new hemagglutinin (HA) and emerges in humans and transmits from human-to-human. When there is no or little immunity against this new HA in people, a new influenza pandemic can occur, and this was the case in 2009 (19).

The 2009 H1N1 pandemic virus obtained 2 gene segments from the Eurasian swine genetic lineage (NA, M) (6) and 6 gene segments from the North American triple reassortant virus (HA, NP, NS, PA, PB1 and PB2). NA and M gene segments in the Eurasian swine lineage where originally obtained from the avian influenza virus around 1979. The triple reassortant swine influenza was first isolated in a North American swine population in 1998. Analysis of these viruses showed a fairly complex genetic makeup. The triple reassortant virus obtained the HA, NP, NA, M and NS gene segments from the classical North American swine virus A/H1N1; the polymerase PB2 (PB2) and polymerase (PA) segments from avian influenza viruses and the polymerase PB1 (PB1) from human influenza A viruses (19).

The M protein has the closest homology to the M protein in the Eurasian lineage of swine influenza viruses. But analysis revealed a serine 31-to-asparagine mutation that leads to a resistance to M2 blockers like amantadine and rimantadine (adamantanes) (20). Figure 2 gives an overview about the history of human and swine influenza lineages.

Figure 2 History of human and swine influenza lineages (adapted from (6))



5.3 Pathogenesis of H1N1 Influenza

Studies of hemagglutinin-receptor binding characteristics have shown that the 2009 pandemic H1N1 influenza virus is able to bind to α 2-6-linked sialyl sequences (as do seasonal influenza viruses) but is also able to bind to α 2-3 linked sialyl sequences. Seasonal influenza binds exclusively to α 2-6-linked sialyl sequences; even in high concentrations no binding to α 2-3 receptors was detected. The ability to bind to α 2-3 receptors may be associated with the ability of H1N1 influenza to infect the lower respiratory tract, as there is a higher proportion of α 2-3 than α 2-6 receptors. The capability of the 2009 H1N1 influenza virus to bind to both, α 2-3 and α 2-6 receptors, is therefore in part responsible for the stronger virulence compared to seasonal influenza viruses (21).

Furthermore a higher ex vivo replication in human bronchial tissue was obtained in 2009 H1N1 influenza compared to seasonal influenza and also a higher replication and an increase of pathological changes in the lungs of nonhuman primates was observed. These are important observations to explain the 2009 H1N1 influenza's ability to cause severe viral pneumonia and other complications (22).

Studies have also shown that virus replication and shedding may be prolonged in 2009 H1N1 influenza patients, when compared to seasonal influenza patients. Usually,

nasopharyngeal viral RNA loads have their peak on the day of onset of symptoms and decline afterwards. On day 8 after onset of symptoms, still 13% of 2009 H1N1 infected people were shedding replicating viruses (23). This may be a reason why the 2009 influenza pandemic spread rapidly.

5.4 Epidemiology

5.4.1 Demographic Data

Reported rates of infections, hospitalizations, case fatalities, and complications have been varying strongly during the 2009 H1N1 influenza pandemic. Nevertheless certain demographic data coincided among worldwide published data.

Frequently coherence between age and attack rates as well as case fatalities was obtained. Children and young adults had the highest attack rates and hospitalization rates overall (20,22,24-27), while adults older than 60 years were rarely infected (20,24). This may be the result of a lack of neutralizing cross-reactive antibodies in young children (26). Studies have shown that people born after 1980 had very little evidence of cross-reactive antibody titers to the 2009 H1N1 influenza strain. In contrast the highest concentration of antibodies against 2009 H1N1 influenza was obtained in patients born before 1930. This may be explained by the fact, that this group of people was probably exposed to the 1918 H1N1 influenza virus or other antigenically related influenza strains and had therefore previously established antibodies. In contrast, the highest case fatality rate among hospitalized patients was obtained among people of 50 years of age or older (28). So it seems that a lack of neutralizing antibodies in higher age groups is related with a higher degree of severity of disease. ICU admission was necessary in 9 to 31% of hospitalized patients and 14 to 46% died on ICU (22).

5.4.2 Transmission

The person-to-person transmission of the 2009 H1N1 virus appears to be similar to those of seasonal influenza viruses. The relative contributions of small-particle aerosols, large droplets and contaminated droplets, however, have not been completely explored (22). In

contrast to seasonal influenza, a fecal viral shedding and therefore a potential fecal-oral transmission should be considered in the 2009 H1N1 virus (20).

Household transmission appeared to be lower than seen in past pandemics. Most susceptible for household transmitted infection were people under 18 years of age. The lowest risk for household transmission was obtained in adults 50 years of age or older. The average rates of secondary outbreaks in households were between 10% and 28% (29,30). Later on increased rates for secondary outbreaks were obtained in schools, day-care facilities, camps and hospitals (31,32).

5.4.3 Risk Groups

In November 2009 the WHO published certain risk groups for severe 2009 H1N1 disease. Those risk groups were similar to risk groups for seasonal influenza virus infection (33):

- Infants and young children, in particular <2 years of age
- Pregnant women
- Persons of any age with chronic pulmonary disease (e.g. COPD, asthma)
- Persons of any age with chronic cardiac disease
- Persons with metabolic disorders (e.g. diabetes mellitus)
- Persons with chronic renal disease, chronic hepatic disease, certain neurological conditions, hemoglobinopathies and immunosuppression
- Children receiving chronic aspirin therapy
- Persons aged 65 years or older

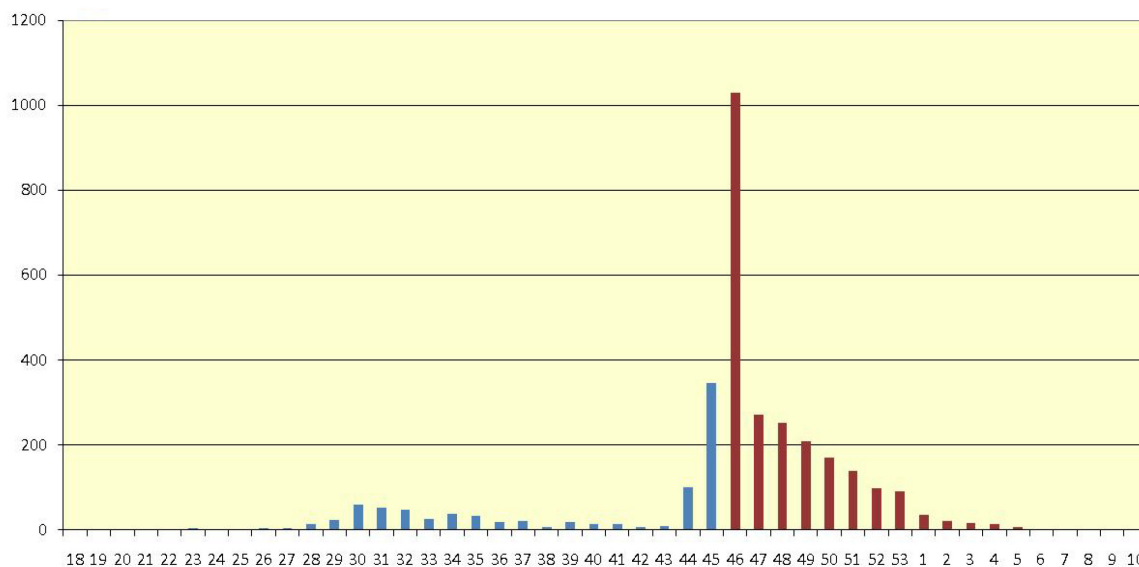
Nonetheless nearly one quarter up to one half of all patients who were hospitalized or died in cases of 2009 H1N1 infection had no or just mild (ASA score 1 or 2) underlying clinical conditions (24,34,35). The most common underlying illnesses included obesity, chronic lung diseases, chronic heart diseases, hypertonia, diabetes, neurological disorders and immunosuppression of any kind (22,25-27,34-38)

5.4.4 H1N1 in Austria

In late April 2009, the H1N1 influenza pandemic reached Austria. The first case of H1N1 influenza was notified on 29th of April 2009 (38). The Austrian Ministry of Health reacted

and implemented containment measures, similar to most other European nations. This strategy included active case finding, airport entry controls, isolation and antiviral treatment of patients as well as antiviral prophylaxis for contacts (39). Especially in the initial phase of the H1N1 pandemic in Austria, in all cases an association to traveling could be observed (38). As published data showed the moderate severity of the new H1N1 virus, Austria switched from containment to mitigation strategy on 10th August 2009. Therefore, airport entry controls were dropped and hospitalization of all H1N1 patients was no longer recommended by healthcare institutions. In November 2009, a rapid increase of H1N1 cases occurred, as typically found in seasonal influenza at this time of the year. As a consequence, “Mitigation 2” was proclaimed on November 11th 2009. Laboratory diagnostics and report to the Austrian Ministry of Health were compulsory for hospitalized patients only (39). Numbers of H1N1 infections by date are shown in Figure 3.

Figure 3 Numbers of PCR confirmed H1N1 cases in Austria by week number in 2009/2010 (adapted from (39))



On 27th August 2009, the first vaccination campaign among healthcare workers started in Austria. After this initial phase a nationwide vaccination campaign was launched on 9th November with the bivalent vaccine “Celvapan”. Despite the fact that there were multiple campaigns by public health authorities only 300,000 people (3.6% of Austrian citizens) were vaccinated against the H1N1 pandemic influenza during the 2009-2010 influenza season (40).

A total number of 350,000-400,000 people were infected by the pandemic influenza virus during the 2009-2010 influenza season in Austria. This number is fairly similar to the

numbers observed in the non-pandemic influenza seasons. From the estimated number of 1569 people who were hospitalized, 40 people died (39). The 2009 pandemic H1N1 influenza A stream was the dominant influenza stream in the 2009-2010 season in Austria. Just two cases of influenza B had been reported throughout this season (38).

5.5 Clinical Features

The incubation period of H1N1 influenza 2009 was similar to those of seasonal influenza viruses, typically ranging from 1.5 to 3 days. In some cases, the incubation period may extend up to 7 days (29,32,41).

The H1N1 virus showed a very broad spectrum of clinical symptoms. The most common symptoms were fever, cough, sore throat, rhinorrhea, headache, fatigue and myalgia/arthralgia. Gastrointestinal symptoms like nausea, vomiting or diarrhea occurred more often than in seasonal influenza (between 3 and 25%) (17,20,25-27,38,42).

5.5.1 Complications

In influenza infection occurrence of certain signs and symptoms is frequently associated with progression to more severe disease (33):

- Signs and symptoms suggesting *oxygen impairment or cardiopulmonary insufficiency*
 - Shortness of breath, cyanosis, difficulty in breathing, sputum with blood or chest pain
- Signs and symptoms suggesting *central nervous system complications*
 - Altered mental status, unconsciousness, confusion, seizures, severe weakness or paralysis
- Sustained *virus replication* or invasive secondary *bacterial infection*
- *Severe dehydration*, manifested as decreased activity, dizziness, oliguria, anuria and lethargy

It is very important to recognize such warning signals, because progression from an initial uncomplicated influenza to a severe disease can be very rapid (i.e. within 24 hours) (33).

5.5.2 Radiographic Findings

Typical radiographic findings include diffuse mixed alveolar and interstitial infiltrates. These either present as bilateral infiltrates, infiltrates limited to one lobe, or multilobar infiltrates in one lung. These findings were more common in patients with bacterial co-infection (17,25,35). Computed tomography has also shown pathological changes in patients' lungs such as ground glass opacities, air bronchograms and alveolar consolidations (27,35).

5.5.3 Laboratory Findings

Most patients with H1N1 infection presented with normal or low leukocyte counts. The white blood cells returned to normal range within 6 to 9 days after onset of symptoms (17,27,35,37,43,44). High leukocyte counts were found in critically ill patients around day 7 up to day 14 of hospitalization (27). An elevation of alanine aminotransferase, aspartate aminotransferase, C-reactive-protein and creatinine was observed in different studies (17,27,35).

5.5.4 Pathological Findings

Histopathological changes in fatal cases of H1N1 range from tracheitis and bronchitis to diffuse alveolar damage (DAD) with hyaline membrane and hemorrhage (45-47). In nearly all patients with fatal H1N1 infection inflammation of the trachea and bronchi was observed. These were the most common findings. Necrosis and hemorrhage of the epithelium were less frequently observed. Usually inflammation was mild and consisted predominantly of mononuclear cells (46). Tracheitis and bronchitis were, however, unspecific predictors for the progress of disease. Pathological changes in the lower respiratory tract again were more often combined with fatal cases of severe H1N1 infection. Lung tissues showed a wide spectrum of histopathological changes of DAD. Viral pneumonia associated with hyaline membranes, pulmonary edema and acute hemorrhage were also found in many patients. These changes often corresponded to the duration of illness. Thromboembolic changes similar to those of the 1918 pandemic were also observed in several patients (46,47).

In contrast to seasonal influenza viruses, the H1N1 virus is able to target lower respiratory tract cells (type 1 and 2 pneumocytes) resulting in respiratory distress syndrome. Viral

antigens were detected - predominantly in lung parenchyma, tracheobronchial epithelium and submucosal glands- in around 66% of all people who died within 10 days after the onset of symptoms (46,47).

Confirmed bacterial co-infection was observed in 26% to 55% (46,47).

5.6 Diagnosis

5.6.1 Clinical Signs

Depending on whether the influenza infection occurs sporadically or during an outbreak, like the 2009 H1N1 pandemic, the accuracy of diagnosis based on clinical symptoms can vary. When a pandemic outbreak is known, patients presenting with symptoms of uncomplicated influenza can be diagnosed on clinical features and on an epidemiological basis. Symptoms of an uncomplicated influenza include (33):

- Fever
- Cough
- Sore throat
- Rhinorrhea
- Headache
- Muscle pain
- Malaise

Shortness of breath and other difficulties in breathing are signs of progressive disease and should be evaluated by adequate diagnostic facilities.

Nonetheless, diagnosis on clinical and epidemiological grounds have sometimes led to misdiagnosis due to the wide spectrum of clinical symptoms and the tendency of pandemic 2009 H1N1 to overlap with clinical symptoms of other common infections (e.g. legionellosis, meningococemia, dengue or malaria) (22). Overall clinical signs and symptoms in the 2009 H1N1 pandemic differed markedly from those seen in seasonal influenza.

5.6.2 Diagnostic Tests

Several different diagnostic tests are available for influenza detection. These tests differ in their sensitivity and specificity for influenza detection, processing time, and ability for differentiation among the influenza subtypes (48).

Diagnostic tests for influenza should be considered for following patients (48):

- Hospitalized patients with suspected influenza
- Patients for whom the confirmation of influenza will affect clinical care, infection control, or management of contacts
- Patients who died from suspected influenza

5.6.2.1 Polymerase Chain Reaction (PCR)

Real-time reverse transcriptase polymerase chain reaction (RT-PCR) or conventional PCR has shown the highest sensitivity for influenza A detection (33,48,49). Thus, PCR still remains the Gold Standard for influenza A detection. Nevertheless, PCR is often not immediately available, takes one to several days for test results to become available, test performance depends on individual PCR assay, and also false negative cases can occur (48). Furthermore, the results of all diagnostic tests depend upon several factors like specimen type, specimen collection, timing of collection, storage, and transport conditions. Negative results in a single testing therefore cannot rule out 2009 H1N1 infection completely and clinicians should consider repeat testing when clinical suspicion is high.

Suitable specimens are nasopharyngeal aspirates or swabs taken early after the onset of symptoms. Because of the ability of the 2009 H1N1 influenza virus to infect the lower respiratory tract, samples taken from lower respiratory tract (tracheal and bronchial aspirates) are also advised for PCR performance. These specimens showed even higher diagnostic yields than samples from the upper respiratory tract (33). For example, Blyth et al. tested 21 patients for H1N1 infection by RT-PCR in bronchoscopic samples. In all specimens, the 2009 H1N1 virus was detected. In contrast, only 17 (81%) of those patients had a positive RT-PCR result in upper respiratory tract specimens (49).

5.6.2.2 Rapid Influenza Antigen Test and Direct Immunofluorescence Assays

Rapid influenza Antigen Tests (RIATs) are widely available, have a high specificity, are cheap, easy to handle, and results are available within half an hour. However, RIATs show

a low sensitivity for 2009 H1N1 influenza detection (10% - 70%) and cannot differentiate between different influenza A subtypes. Therefore, negative RIATs results should not be used as reason to withhold therapy if clinical suspicion for influenza infection is high. Instead, further diagnostic should be performed (33,48,49). In contrast to pandemic influenza, sensitivity of RIATs for seasonal influenza has shown to be much higher. Therefore RIATs are still an important component in rapid and reliable detection of seasonal influenza.

Like the RIATs, direct immunofluorescence assays (DFAs) are also widely available and have a high specificity ($\geq 96\%$) but are less sensitive (range 47% - 93%) among PCR tests. DFAs are also able to distinguish between influenza A and B but cannot differentiate between influenza A subtypes (48).

5.6.2.3 Serological Assays

Serological assays including complement fixation, microneutralization and hemagglutination inhibition, can be used to detect an increased antibody titer and therefore provide a retrospective diagnosis. It is recommended to always test paired serum specimens including an acute serum and a convalescent serum collected 10 to 20 days later. Singular serum testing would not guarantee an adequate result because most people have been infected with influenza at some point in their lives (1,22).

5.6.2.4 Viral Isolation in Tissue Cell Cultures

2009 H1N1 pandemic influenza A virus can replicate in different cell types similar to seasonal influenza and the H5N1 influenza (50). However isolation usually takes several days (22). Suitable specimens for viral isolation are nasal swabs, throat swabs, nasal washes, combined nose and throat swab and also sputum samples (1). Characteristics of H1N1 tests are shown in the Table 2.

Table 2 Summary of available H1N1 tests and characteristics (adapted from (48))

	Method	Availability	Average Processing Time	Sensitivity to 2009 H1N1 influenza	Ability to distinguish 2009 H1N1 from other influenza A subtypes?
Rapid influenza antigen tests	Antigen detection	Wide	30 minutes	10 - 70%	No
Direct and indirect immunofluorescence assays	Antigen detection	Wide	2-4 hours	47 – 93%	No
Viral isolation in tissue cell cultures	Virus isolation	Limited	2-10 days	-	Yes
Nucleic acid amplification tests (Including RT-PCR)	RNA detection	Limited	48-96 hours	86 – 100%	Yes

5.7 Treatment

5.7.1 General Treatment Considerations

Patients suffering from potentially severe diseases like the 2009 H1N1 pandemic need individualised and optimal therapy. Therefore, the WHO published some general treatment considerations that should guarantee an adequate and well-adapted treatment for every patient (51):

- Patients presenting with an uncomplicated self-limiting illness, like most 2009 H1N1 infected people did, can be treated with supportive care when needed. Supportive care includes, for example, antipyretics and fluid rehydration.
- Patients presenting with a mild or uncomplicated illness but with high risk for disease progression or complications, should receive antiviral treatment early on.
- Patients presenting with severe or complicated illness should be treated with antiviral drugs such as oseltamivir as soon as possible.

5.7.2 Antiviral Therapy

Antiviral therapy, if necessary, should always be started as soon as possible. Waiting on laboratory confirmation is not recommended (33). Evidence from different trials suggests that therapy started within 48 hours after the onset of symptoms brings the greatest benefit for patients (51,52). On the other hand, a prospective cohort study by McGeer et al. indicated a reduction in mortality even if oseltamivir therapy was initiated more than 48 hours after the onset of symptoms (53). Therefore, antiviral therapy should be started in hospitalized patients with 2009 H1N1 influenza, even if such therapy is initiated more than 48 hours after the onset of illness (35).

Agents of choice in antiviral therapy are neuraminidase inhibitors like oseltamivir and zanamivir. The 2009 H1N1 influenza virus is almost always resistant to M2 inhibitors like amantadine and rimatadine (51,54).

5.7.3 Neuraminidase Inhibitors

The 2009 H1N1 influenza virus shows susceptibility to neuraminidase inhibitors like oseltamivir (Tamiflu[®]) or zanamivir (Relenza[®]) (20,33,51,54). Oseltamivir is the first line agent for a 2009 H1N1 infection. It is available orally or intravenously and suitable for persons of all ages, including children and neonates. Patients who are 13 years of age or older and weigh over 40kg should be treated with a twice daily dose of 75mg oseltamivir for 5 days. For children under 40kg bodyweight, oseltamivir doses should be fitted to their bodyweight (30mg, 45mg and 75mg capsules are available). In some critically ill adults, doses of 150mg twice daily have been administered for an increased period of time (up to 10 days). For children who are not able to take capsules, a oseltamivir suspension is available. For critically ill patients who are not able to swallow oral medication, oseltamivir should be administered by a nasogastric or orogastric tube (33,51).

In case of oseltamivir resistance, a switch to other neuraminidase inhibitors like zanamivir should be performed. All oseltamivir resistant specimens have the same H275Y mutation that leads to oseltamivir but not zanamivir resistance. But there is no evidence for a reassortment of those oseltamivir resistant 2009 H1N1 viruses with other influenza viruses like seasonal influenza. Most cases of resistance were sporadic, but a higher rate of resistance was observed in immunosuppressed patients and in patients who received oseltamivir chemoprophylaxis (55,56). In these cases, zanamivir is the agent of choice. Zanamivir can be inhaled or administered intravenously (33,51).

5.7.4 Antibiotic Therapy

One of the major complications of the 2009 pandemic H1N1 infection were secondary bacterial infections. Secondary bacterial pneumonia was in most cases caused by *Staphylococcus aureus* (including *multi resistant Staphylococcus aureus*), *Streptococcus pneumoniae* and *Streptococcus pyogenes*. In such cases, an early therapy with empiric antibiotics for community acquired pneumonia should be initiated promptly to improve outcome (33).

5.7.5 Oxygen Therapy and Corticosteroids

Moderate to high doses of corticosteroids are not suitable for patients with a H1N1 infection and are potentially harmful.

Oxygen should be provided to patients presenting with hypoxemia. Those patients should always be monitored with pulse oximetry. In case of pneumonia, the WHO recommends to maintain oxygen saturation above 90% (33).

5.7.6 Vaccination

As antiviral chemoprophylaxis is generally not recommended for the 2009 H1N1 influenza (51), vaccination is the most effective way of influenza prevention. When the 2009 H1N1 pandemic occurred, many people had already received seasonal influenza vaccine. But seasonal influenza vaccines did not protect from the 2009 H1N1 influenza virus infection. The first vaccines against the 2009 H1N1 influenza were launched in September 2009. Because the supply of the new vaccine was not enough to vaccinate the whole population, the CDC's Advisory Committee on Immunization Practices (ACIP) recommended five groups as primary targets for vaccination (57):

- Pregnant women
- Persons who live with or provide care for infants under 6 years of age
- Health care and emergency medical services personnel
- Persons aged 6 months to 24 years (children under 6 years should not be vaccinated)
- Persons aged 25 – 64 years who have medical conditions that put them at higher risk for complications

The basic idea behind this vaccination strategy was 1.) to protect the integrity of the national health care service and infrastructure; 2.) to reduce mortality and morbidity and 3.) to reduce transmission of the virus within communities.

The WHO had selected the A/California/07/2009 H1N1 strain for vaccine manufacture. The vaccine is available as live-attenuated and inactivated monovalent formulation. Three manufacturers produced inactivated vaccines and one manufacturer produced a live attenuated vaccine. These live attenuated vaccines were only indicated in persons aged 2-49 years who were not pregnant and had no underlying medical conditions corresponding with a higher risk of influenza complications. Persons of 10 years of age or older should receive just one dose. Children between 6 months and 9 years of age on the other hand should receive two doses of vaccine separated by approximately 4 weeks, to guarantee an adequate increase of antibody titer.

Greenberg and colleagues analyzed the safety and effectiveness of the 2009 H1N1 vaccine in adults. They showed that 95% of all patients who received 15 μ g doses and 89.1% of all patients who received 30 μ g doses had an antibody titer of 1:40 or more. A second dose of vaccine did therefore not result in an adequate antibody increase. Nearly all side effects were mild to moderate and comparable with seasonal influenza vaccines (58).

6 Methods

6.1 Objectives

- To determine the differences in first clinical presentation between patients with PCR confirmed H1N1 influenza infection and patients with influenza-like illness and negative PCR results for H1N1 influenza.
- To describe the incidence of PCR confirmed H1N1 influenza infections in Styria during the 2009/2010 pandemic.
- To determine risk factors such as underlying medical conditions which may be associated with a higher risk for complications, and to compare these factors with the PCR negative influenza-like illness group.
- To describe the clinical management and treatment in confirmed H1N1 influenza cases.
- To describe sensitivity of rapid influenza antigen tests for H1N1 influenza.
- To describe differences concerning the outcome between H1N1 influenza patients and ILI patients.

6.2 Study Design

This study was performed as a retrospective survey of patients with PCR confirmed H1N1 influenza infection or influenza PCR negative influenza-like illness. It was a multicenter survey and patients tested by PCR in Styria between 1st October 2009 and 21st January 2010 were included. Participating hospitals were University Hospital of Graz and the state hospitals of Graz-West, Leoben, Bruck and der Mur, Judenburg, Hörgas-Enzbenbach, Feldbach, Deutschlandsberg, Rottenmann, Wagna and Bad Aussee. We analyzed 624 patients with PCR confirmed H1N1 influenza and 1057 with influenza-like illness and negative influenza PCR results. Data sets were available from 209/624 (31%) H1N1 infected patients and from 310/1057 (30%) patients with influenza-like illness. In some cases not all data were available. Therefore the number of included patients varies in the different categories of the results. The reasons for the exclusion of 69% respectively 70% of the patients are listed in “6.3.2 Exclusion Criteria”.

The study was conducted at the Section of Infectious diseases of the Medical University Graz. Patients' data were collected in the Case Report Form, by reviewing medical reports in the MEDOCS database. Data was collected anonymously.

Our local ethic commission granted the research ethic board approval. Because of the non-interventional character of the study, a priori patient consent was not required.

6.3 Patient Definition

6.3.1 Inclusion Criteria

- All patients who were tested for H1N1 influenza by PCR at the Institute for Hygiene, Karl-Franzens-Universität Graz or at the Institute for Hospital Hygiene, state hospital Graz, using respiratory tract specimens (nasopharyngeal swabs, bronchoalveolar lavage), were included in this study.

6.3.2 Exclusion Criteria

- 695/1057 (65%) of PCR negative patients and 407/624 (65%) of PCR positive patients were excluded from the survey because not all needed or no data could be obtained from the MEDOCS system. In the majority of cases, the reason was that most patients did not submit to a hospital and just presented to their family doctor. Therefore, no data was available in the MEDOCS system.
- 8/624 (1.3%) PCR positive and 51/1057 (5%) of PCR negative patients were excluded because of their severe underlying medical conditions (mainly malignant diseases in an advanced stage).
- 1/1057 (0.1%) of PCR negative patients was excluded because two PCR testing on the same day showed different results. The PCR performed on the Institute of Hospital Hygiene and Microbiology found a positive test result, while the PCR performed on the Institute for Hygiene, Karl-Franzens-University ended in a negative test result. This patient was therefore included in the H1N1 cohort only.

6.3.3 Group Stratification

- Stratification was performed according to the following definitions:
 - PCR negative influenza-like illness (ILI) was defined as acute respiratory and/or febrile illness where influenza was clinically suspected, but influenza PCR resulted negative.
 - H1N1 influenza illness was defined as acute respiratory and/or febrile disease which was confirmed by positive H1N1 influenza PCR.

6.4 The Case Report Form

The case report form contained:

- PCR result
- Demographic data
- Date of PCR testing
- Date of onset of symptoms
- Date of first clinical presentation
- Care unit
- Symptoms on first presentation
- Clinical findings
- Rapid antigen test results
- Antiviral and antibiotic treatment
- Radiological data
- Microbiological findings
- Underlying medical conditions
- Vaccination status
- Outcome

Furthermore, we calculated the time from the onset of symptoms to presentation at the hospital, the duration of hospitalization, the period from onset of symptoms to PCR result, the period from first presentation to PCR results and the duration from onset of symptoms to start of antibiotic and/or antiviral therapy.

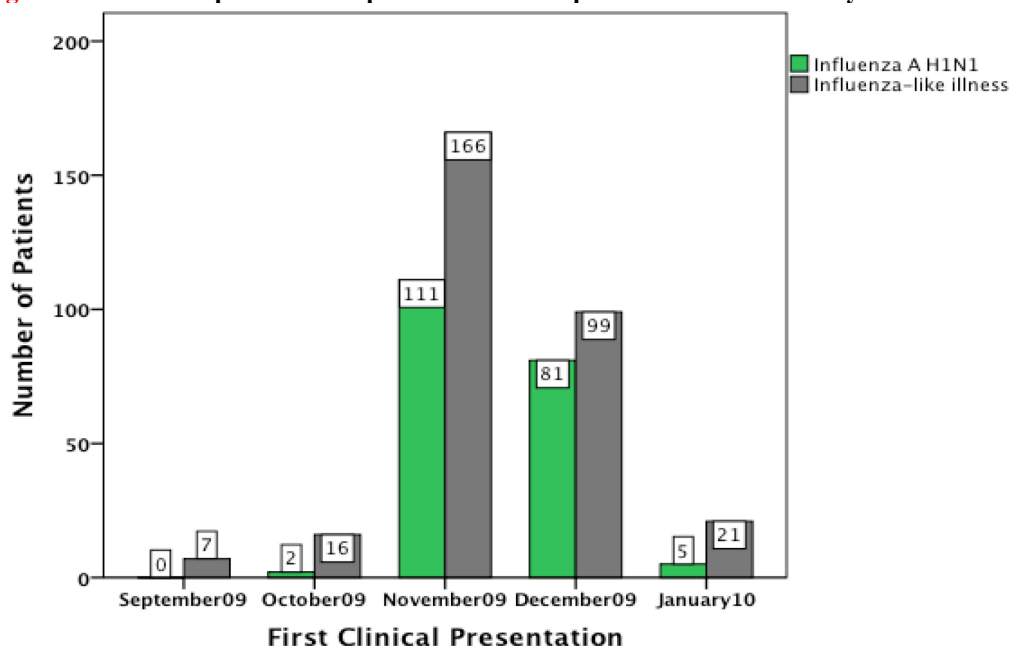
6.5 Analysis

Statistical analysis was performed using “IBM SPSS Statistics 19”. Data from H1N1 influenza patients and ILI patients was compared using Chi-squared or Fishers exact test. Continuous data is displayed as means [95% confidence interval (CI 95%)] and/or median [interquartile range 25-75 (IQR 25-75)]. A p-value less than 0.05 was considered as statistically significant.

7 Results

We reviewed data from patients who presented to clinics in Styria between 01st September 2009 and 19th January 2010. The first positive PCR result for H1N1 influenza in our study was observed on 28th October 2009 and the last on 19th January 2010. In this time period, 209 patients tested positive for H1N1 influenza. 2/209 (1%) of H1N1 influenza cases were confirmed in October 2009, 111/209 (53%) in November 2009, 81/209 (39%) in December 2009 and 5/209 (2%) in January 2010. In 10/209 (5%) cases, we were unable to find out the date of PCR confirmation. From the group of patients who suffered from influenza-like illness 7/310 (2%) presented in September 2009, 16/310 (5%) in October 2009, 166/310 (54%) in November 2009, 99/310 (32%) in December 2009 and 21/310 (7%) in January 2010. Thus, a peak of infections could be observed in both groups during November 2009 (Displayed in Figure 4).

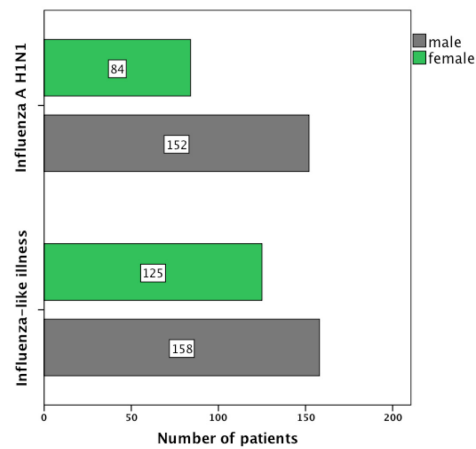
Figure 4 Number of patients who presented from September 2009 to January 2010



7.1 Demographic Data

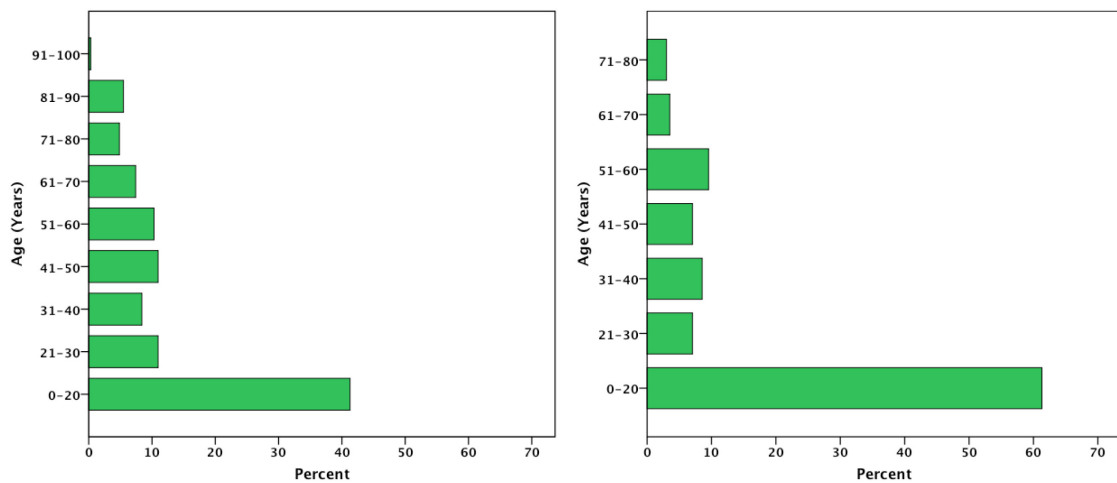
During the 2009 influenza pandemic, H1N1 influenza was more often diagnosed in men than in women. Among the 209 included cases, 125 (59.8%) patients were male and 84 (40.2%) were female. Gender distribution was observed to be more even in the ILI group. Among the 310 patients who suffered from ILI, 158 (51%) were male and 152 (49%) were female (See Figure 5).

Figure 5 Gender distribution in H1N1 and ILI patients



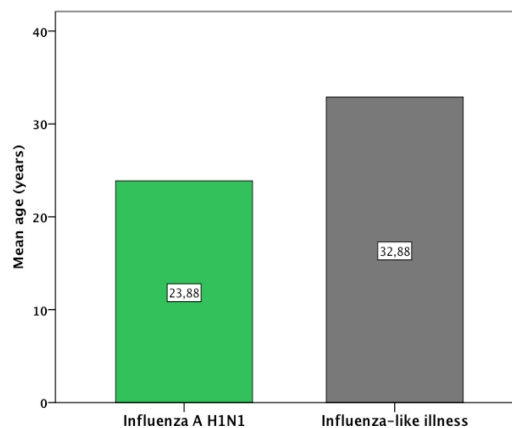
The age of H1N1 influenza patients ranged from 0 to 79 years, that from ILI patients from 0 to 92 years. 61% of all H1N1 influenza patients were younger than 21 years, compared to 41% in the ILI group ($p=0.047$) (See Figure 6).

Figure 5 Age distribution in H1N1 influenza and ILI patients



Patients who suffered from H1N1 influenza (mean age 23.88 years; 95% CI 20.95-26.80 years) were significantly younger than patients who suffered from influenza-like illness (mean age 32.88 years; 95% CI 29.92-35.85 years) ($p < 0.001$). Patients under the age of 21 had the highest risk for an H1N1 influenza infection in our study cohort (See Figure 7).

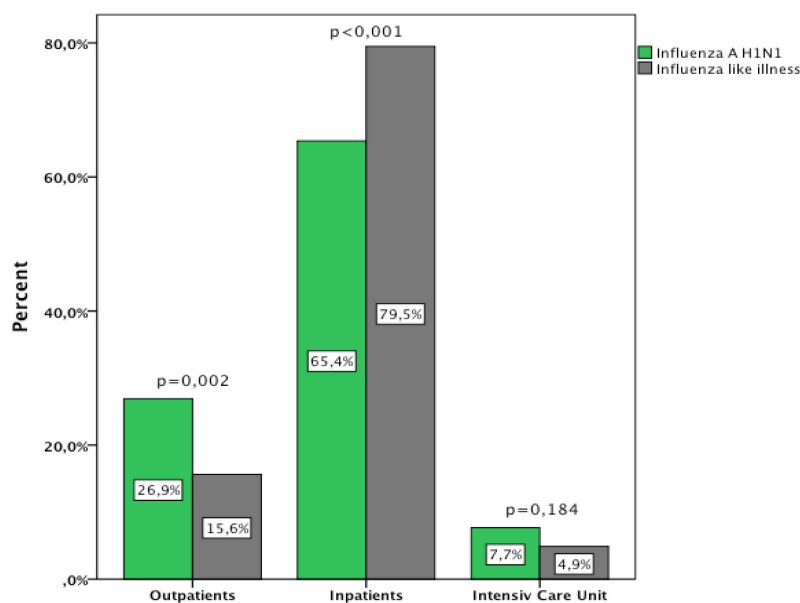
Figure 6 Mean age in H1N1 and ILI patients



7.2 Hospitalization

Among all patients with confirmed H1N1 influenza, 56/209 (26.9%) presented to outpatient clinics only, compared to 48/310 (15.6%) patients from the ILI group ($p = 0.002$). Patients with ILI on the other hand were more frequently admitted to normal wards compared to H1N1 influenza patients [244/310 (79.5%) versus 136/209 (65.4%); $p < 0.001$]. No difference between the H1N1 influenza patients and the patients from the ILI group was found in respect to ICU admission. 16/209 (7.7%) respectively 15/310 (4.9%) ($p = 0.184$) needed intensified care (See Figure 8).

Figure 8 Amount of patients who presented as outpatients, inpatients or on ICUs



The mean duration of hospitalization of H1N1 influenza patients was 6.01 days (95% CI 4.64-7.39) and significantly ($p < 0.001$) lower than in patients of the ILI group (9.9 days; 95% CI 8.34-11.46) (See Figure 9). We observed a range of hospitalization time for H1N1 influenza patients from 1 to 60 days and 1 to 78 days for ILI patients. Median duration of hospitalization was 4 days (IQR 2-6) for H1N1 influenza patients and 6 days (IQR 3-11) for ILI patients.

The mean duration on the ICU was 15.57 days (range: 1-51 days) for H1N1 influenza patients and 12.6 (range: 1-41 days) days for ILI patients (See Figure 9). This difference was not statistically significant ($p = 0.717$). Median duration on ICU was 7 days (IQR 6-29) for H1N1 influenza patients and 4.5 days (IQR 2.75-3.75) for ILI patients. The mean age of H1N1 influenza patients who needed ICU admission was 44.4 years and 43.8 years for ILI patients. Length of stay on general wards and on ICUs are displayed in Figures 10 and 11.

Figure 9 Mean duration of hospitalization and mean duration on ICUs

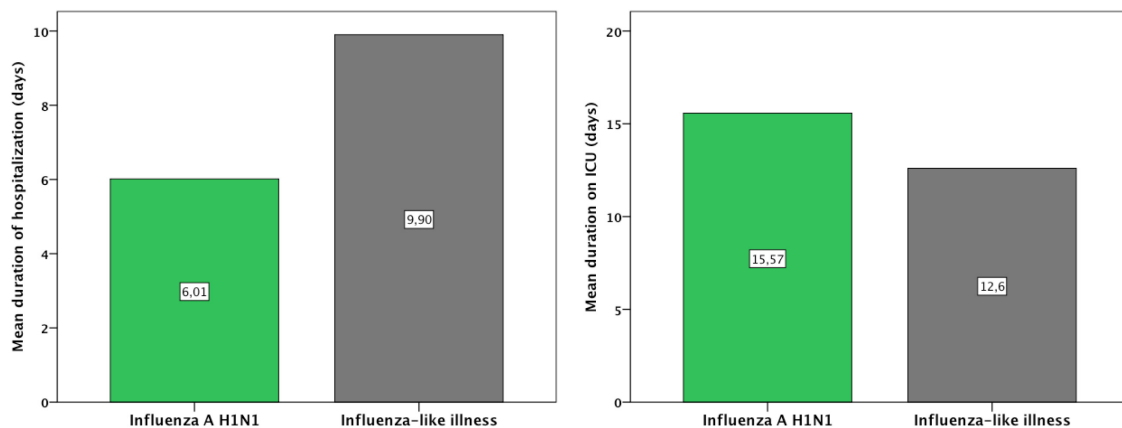


Figure 10 Length of stay on general wards among H1N1 and ILI patients

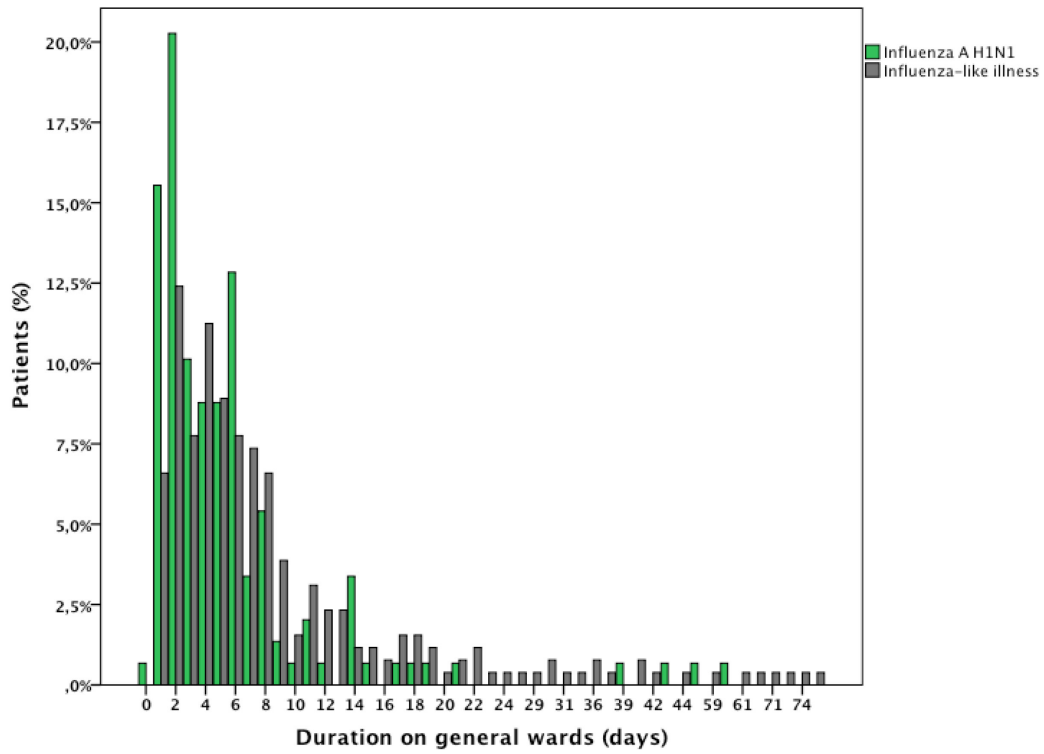
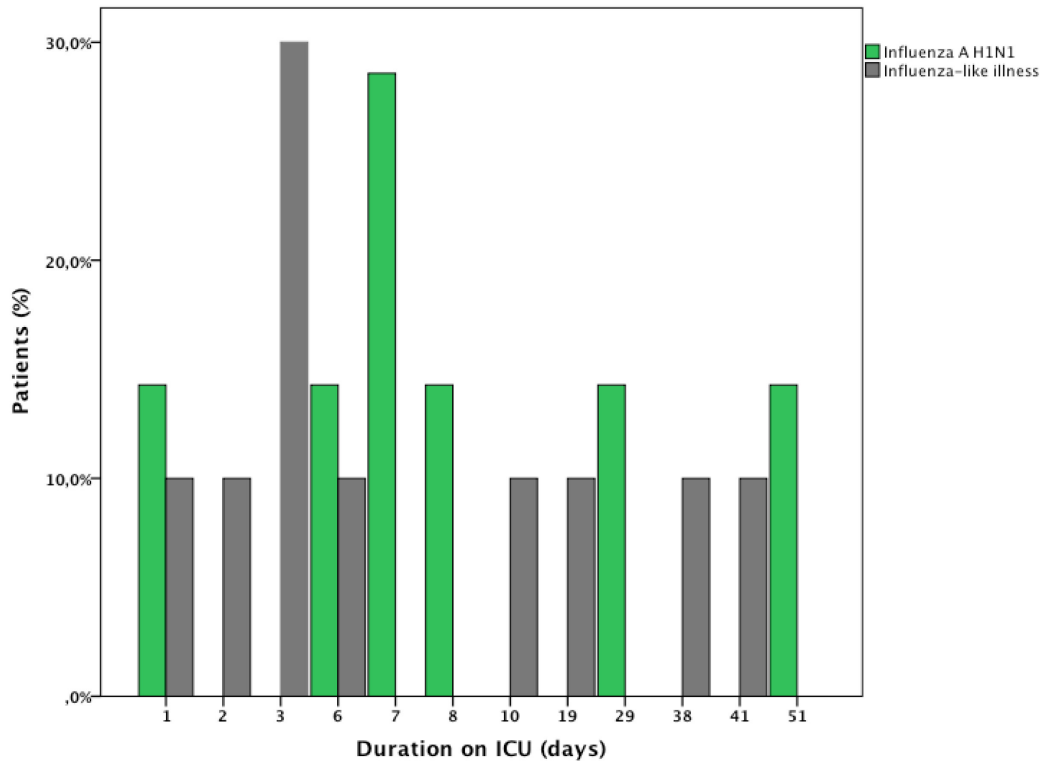
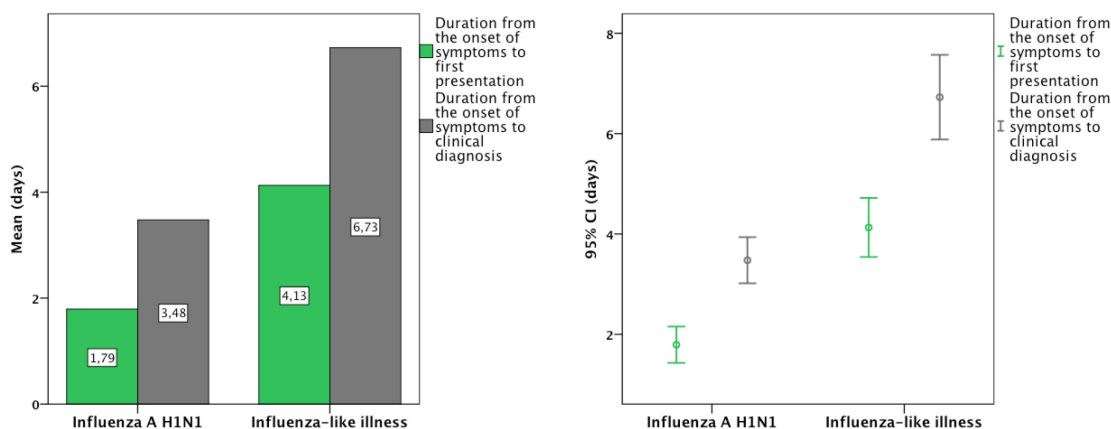


Figure 11 Length of stay on ICU among H1N1 and ILI patients



Regarding the time period from onset of symptoms to first clinical presentation, we found a significant difference between patients with H1N1 influenza and ILI. The mean time for patients with H1N1 influenza was 1.78 days [95% CI 1.43-2.13 (Median 1; IQR 0-2)] and for patients with ILI 4.18 days [95% CI 3.59-4.78 (Median 3; IQR 1-6)] ($p < 0.001$). In accordance with these results, we also observed a significant difference in the mean duration from onset of symptoms to PCR result. In the H1N1 influenza group, the mean time was 3.46 days [95% CI 3-3.92 (Median 2; IQR 2-5)] compared to 6.7 [95% CI 5.85-7.54 (Median 5; IQR 2-8)] days in the ILI group ($p < 0.001$). No significant difference could be observed regarding the time period from first clinical presentation to PCR result. Mean time for H1N1 influenza patients was 2.17 days [95% CI 1.27-3.06 (Median 1; IQR 1-2)] and for ILI patients 2.59 days [95% CI 2.04-3.14 (Median 1; IQR 1-3)] ($p = 0.399$). Mean duration from the onset of symptoms to first presentation and diagnosis, as well as 95% CIs are displayed in the Figure 12.

Figure 12 Mean duration from the onset of symptoms to first presentation and diagnosis



7.3 Case History

A sudden onset of symptoms was observed in 165/191 (86.3%) H1N1 influenza patients and in 267/306 (87.3%) ILI patients ($p = 0.780$).

17/209 (8.1%) H1N1 influenza patients confirmed previous contact with H1N1 infected persons. Similarly, 14/304 (4.6%) ILI patients also confirmed previous contact with H1N1 infected people ($p = 0.099$) (See Table 3).

Table 3 Disease onset and previous contact to H1N1

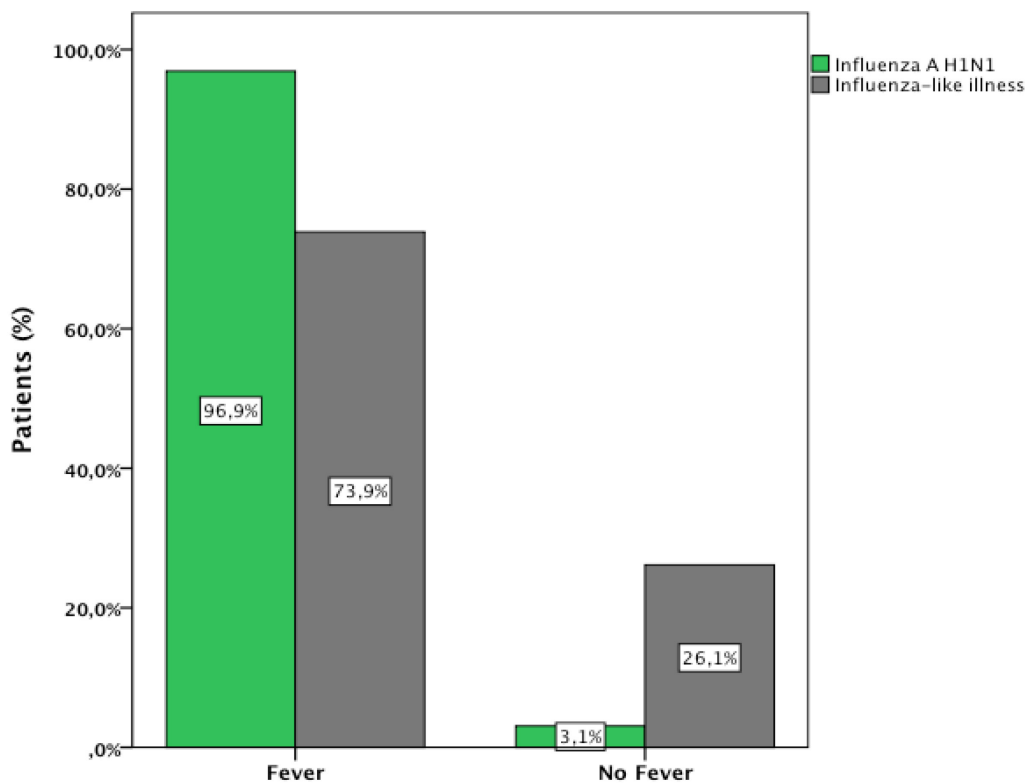
	H1N1 influenza	Influenza-like illness	p-value
Sudden onset of symptoms	165/191 (86.3%)	267/306 (87.3%)	0.780
Previous contact to H1N1 influenza	17/209 (8.1%)	14/304 (4.6%)	0.099

7.4 Fever

Fever was the most common symptom observed in both groups. Fever was defined as temperature over 37.2° Celsius intraauricular or axillar, over 37.8° Celsius sublingual and/or over 38.3° Celsius rectal.

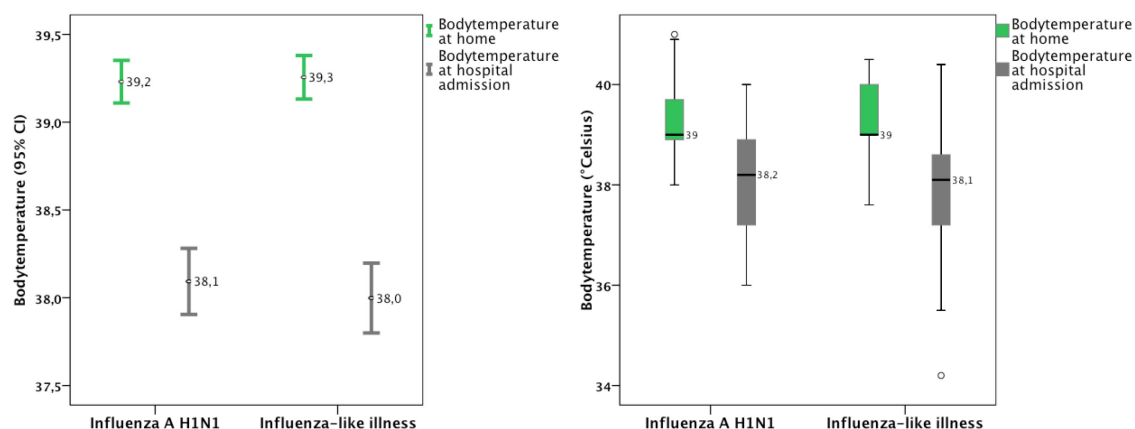
189/205 (92.2%) patients from the H1N1 influenza group and 226/306 (73.9%) patients from the ILI group ($p < 0.001$) had fever at home and/or at the time of hospital admission (See Figure 13).

Figure 13 Percentage of H1N1 and ILI patients who presented with fever



The mean maximal temperature patients had measured at home was 39.2°Celsius (Median 39.0) for H1N1 influenza patients and 39.2°Celsius (Median 39.0) for ILI patients ($p=0.545$). We could not find a significant difference in mean temperature measured at presentation in hospital [38.1°Celsius (Median 38.2) for H1N1 influenza patients versus 38.0°Celsius (Median 38.1) for ILI patients; $p=0.183$]. So at the time of first hospital admission the patients' temperature was on average 1.1°Celsius lower for patients from the H1N1 influenza group and 1.2°Celsius lower for patients from the ILI group. 95% CIs and boxplots are displayed in Figure 14.

Figure 14 Mean and median body temperature at home and at hospital admission



22/204 (10.8%) H1N1 influenza patients and 50/300 (16.7%) ILI patients reported prodromes (including: coughing, rhinitis, strep throat, nausea, vomiting, diarrhea, stomach ache, obstipation, headache, dyspnea, fatigue, myalgia, arthralgia) before they caught fever.

113/207 (54.6%) H1N1 patients and 172/304 (56.6%) ILI patients took anti-inflammatory and/or antipyretic drugs before hospital admission. This may explain the reduction of body temperature at time of presentation (See Table 4).

Table 4 Fever, prodromes and premedication in H1N1 and ILI patients

	H1N1 influenza	Influenza-like illness	p-value
Fever, n/N (%)	189/205 (92.2)	226/306 (73.9)	<0.001

Temperature at home (°Celsius), Mean/Median	39.2/39.0 {39.1-39.3} [38.9-39.7]	39.2/39.0 {39.1-39.3} [38.6-40.0]	0.545
Temperature at hospital admission (°Celsius), Mean/Median	38.1/38.2 {38.0-38.2} [37.2-38.9]	38.0/38.1 {37.7-38.1} [37.0-38.8]	0.183
Difference in body temperature (home-hospital), Mean	1.1	1.2	-
Prodromes, n/N (%)	22/204 (10.8)	50/300 (16.7)	0.064
Premedication, n/N (%)	113/207 (54.6)	172/304 (56.6)	0.657
{95% CI} [IQR25-75]			

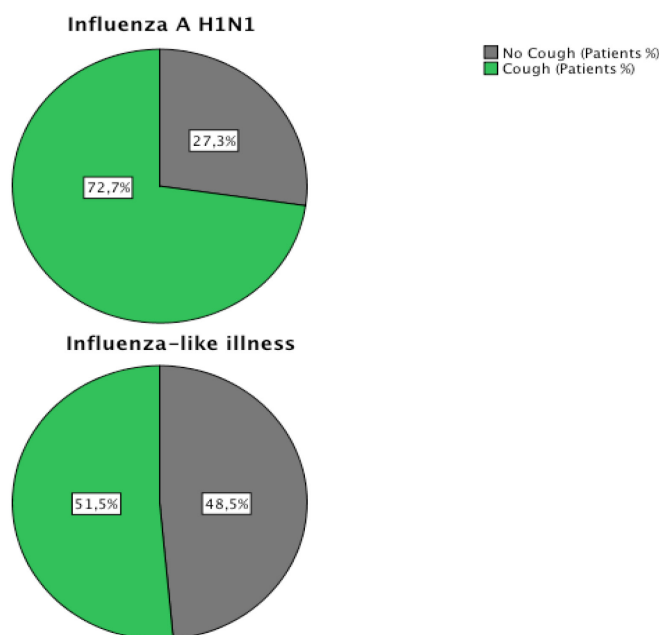
7.5 Symptoms at First Clinical Presentation

7.5.1 Cough

A statistically significant difference between our two groups was observed in respect to cough. 152/209 (72.7%) patients suffering from H1N1 influenza presented with cough compared to 158/307 (51.5%) patients from the ILI group ($p < 0.001$).

7 out of those 152 H1N1 influenza patients and 1 out of those 158 ILI patients presented with hemoptysis (4.7% versus 0.6%; $p = 0.027$) (Displayed in Figure 15).

Figure 15 Proportion of H1N1 and ILI patients who presented with cough



7.5.2 Dyspnea and Chest Pain

Proportions of patients who presented with dyspnea and/or chest pain were similar in both groups. 44/209 (21.1%) patients with H1N1 influenza had dyspnea at first clinical presentation compared to 59/307 (19.2%) patients suffering from ILI ($p=0.609$). Chest pain was observed in 17/209 (8.1%) respectively 36/307 (11.7%) patients ($p=0.187$).

7.5.3 Gastrointestinal Symptoms

Nausea and vomiting were the most frequent gastrointestinal symptoms observed. 49/209 (23.4%) influenza patients and 86/307 (28.0%) ILI patients presented with nausea and/or vomiting at home and/or at hospital admission ($p=0.246$). We also reviewed patients' data in respect to diarrhea. In 21/209 (10.0%) H1N1 influenza patients and 39/307 (12.7%) ILI patients, diarrhea was reported ($p=0.356$). Stomachache was the only gastrointestinal symptoms where a difference between our two groups was observed. Stomachache was more frequent in the ILI group than in the influenza group [33/307 (10.7%) versus 12/209 (5.7%); $p=0.048$].

7.5.4 Other Symptoms

Patients from the H1N1 influenza group presented more often symptoms of rhinitis than patients from the ILI group. 48/209 (23.0%) H1N1 influenza patients presented with rhinitis compared to 44/307 (14.3%) ILI patients ($p=0.012$). H1N1 influenza patients were also more likely to present with headache and fatigue. 66/209 (31.6%) had headaches compared to 71/307 (23.1%) patients from the ILI group ($p=0.033$) and 123/209 (59.1%) H1N1 influenza patients presented with fatigue compared to 69/307 (22.5%) ILI patients ($p<0.001$).

No differences could be found related to eczema [6/209 (2.9%) H1N1 influenza versus 18/307 (5.9%) ILI; $p=0.113$], myalgia/arthralgia [49/209 (23.4%) H1N1 influenza versus 65/307 (21.3%) ILI; $p=0.541$] and sore throat [23/209 (11.0%) H1N1 influenza versus 29/307 (9.4%) ILI; $p=0.564$]. Table 5 gives an overview about clinical symptoms at first presentation.

Table 5 Symptoms at first clinical presentation

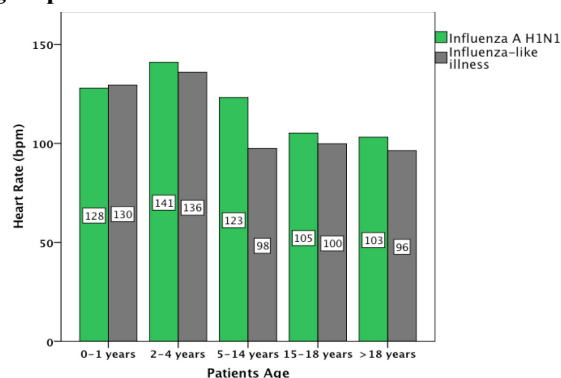
	H1N1 influenza N=209	Influenza-like illness N=307	p-value
Cough	152/209 (72.7)	158/307 (51.5)	<0.001
Hemoptysis	7/152 (4.7)	1/158 (0.6)	0.027
Dyspnea	44/209 (21.1)	59/307 (19.2)	0.609
Chest pain	17/209 (8.1)	36/307 (11.7)	0.187
Nausea/Vomiting	49/209 (23.4)	86/307 (28.0)	0.246
Diarrhea	21/209 (10.0)	39/307 (12.7)	0.356
Stomachache	12/209 (5.7)	33/307 (10.7)	0.048
Rhinitis	48/209 (23.0)	44/307 (14.3)	0.012
Sore throat	23/209 (11.0)	29/307 (9.4)	0.564
Headache	66/209 (31.6)	71/307 (23.1)	0.033
Fatigue	123/209 (59.1)	69/307 (22.5)	<0.001

Eczema	6/209 (2.9)	18/307 (5.9)	0.113
Myalgia/Arthralgia	49/209 (23.4)	65/307 (21.3)	0.541
n/N (%)			

7.6 Vital Parameters

A significant higher initial heart rate was observed in the H1N1 influenza group versus the ILI group. The mean heart rate in the influenza group was 107 (range 60-207) beats per minute (bpm) versus 98 (range 51-200) beats per minute in the ILI group ($p=0.008$). In both groups, children aged 2-4 years showed the highest heart rates and patients older than 18 years the lowest (See Figure 16).

Figure 16 Mean heart rate among different age groups



No difference regarding systolic (127.4mmHg for H1N1 influenza group versus 126.3mmHg for the ILI group), diastolic (76.0mmHg versus 75.6mmHg) and mean arterial blood pressure (93.1mmHg versus 92.5mmHg) was observed between our two groups at first clinical presentation (See Table 6).

Table 6 Vital parameters in different age groups

	H1N1 influenza	Influenza-like illness	p-value
Heart Rate bpm/Mean (SD)	107.3 (24.9)	98.2 (21.4)	0.008
0-1 years	128 (14.7)	129,5 (32.5)	0.944
2-4 years	141 (16.9)	136 (13.1)	0.706
5-14 years	123.2 (26.3)	97.5 (12.7)	0.022
15-18 years	105.2 (33.9)	99.9 (12.7)	0.702
>18 years	103.2 (22.2)	96.4 (20.8)	0.032
Blood pressure systolic mmHG/Mean (SD)	127.4 (20.9)	126.3 (23.2)	0.716
0-1 years	119 (1.4)	105.7 (45.6)	0.722
2-4 years	110 (-)	89 (6.6)	-
5-14 years	119.3 (23.5)	113.8 (19.2)	0.677

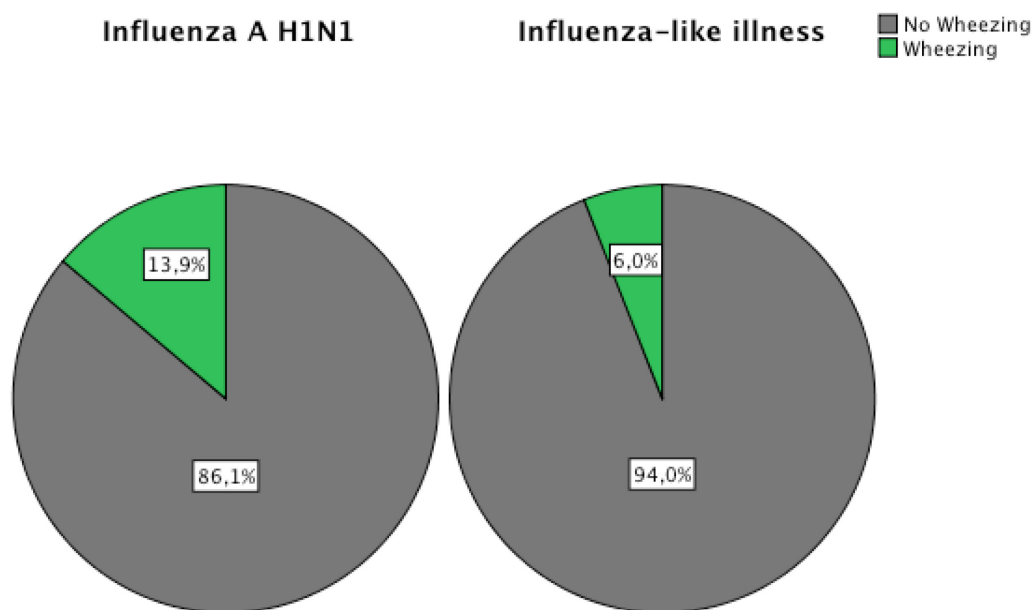
15-18 years	121.5 (17)	114.4 (17.2)	0.384
>18 years	129 (20.9)	128.9 (22.1)	0.976
Blood pressure diastolic mmHg/Mean (SD)	76 (13.2)	75.6 (14.6)	0.841
0-1 years	62.5 (5)	74 (32.6)	0.670
2-4 years	50 (-)	55 (11.1)	-
5-14 years	67.6 (9.5)	66.4 (6.8)	0.815
15-18 years	67.6 (6.9)	66.5 (7.2)	0.740
>18 years	78.6 (13.1)	76.3 (14.1)	0.410
Mean arterial pressure mmHg/Mean (SD)	93.1 (14.2)	92.5 (16.1)	0.755
0-1 years	81.5 (3.5)	84.6 (36.7)	0.917
2-4 years	70 (-)	66.3 (8.8)	-
5-14 years	84.7 (13.1)	82.2 (10.5)	0.732
15-18 years	85.6 (6.3)	82.5 (9.8)	0.412
>18 years	95.4 (14.2)	94.3 (15.3)	0.632

7.7 Physical Examination

An increased frequency of heart murmurs and pathological heart tones as a sign of heart pathologies like valve insufficiency or valve stenosis was found in influenza-like illness patients. In 8/209 (3.8%) H1N1 influenza patients, a pathological heart sound was observed compared to 25/300 (8.2%) ILI patients ($p=0.042$).

In contrast, the amount of patients presenting with signs of obstructive lung diseases, like wheezing, was significantly higher in patients with H1N1 influenza. 29/209 (13.9%) H1N1 influenza patients presented with signs of obstructive lung diseases at first clinical presentation compared to 18/300 (6%) ILI patients ($p=0.003$) (See Figure 17). Crackles were reported in 35/209 (16.7%) H1N1 versus 69/300 (23%) ILI patients ($p=0.085$).

Figure 17 Proportion of H1N1 and ILI patients who presented with wheezing



Signs of pharyngitis were more frequently found in the H1N1 influenza group than the ILI group [70/209 (33.5%) versus 63/300 (21%); $p=0.002$]. No difference regarding pathological findings in the abdominal examination was observed between the groups. In 15/209 (7.2%) respectively 24/300 (8%) patients from either group pathological abdominal findings were observed. Also no significant differences in rates of conjunctivitis were observed (3.8% in the H1N1 influenza group and 1.7% in the ILI group). Table 7 gives an overview about clinical findings at physical examination.

Table 7 Findings at physical examination

	H1N1 influenza N=209	Influenza-like illness N=300	p-value
Heart murmurs	8/209 (3.8)	25/300 (8.2)	0.042
Wheezing	29/209 (13.9)	18/300 (6)	0.003
Crackles	35/209 (16.7)	69/300 (23)	0.085
Pharyngitis	70/209 (33.5)	63/300 (21)	0.002

Abdominal Findings	15/209 (7.2)	24/300 (8)	0.731
Conjunctivitis	8/209 (3.8)	5/300 (1.7)	0.128
n/N (%)			

7.8 Rapid Influenza Antigen Test

In 66/519 (12.7%) patients, Rapid influenza Antigen Test (“BinaxNow”, Inverness Medical, US) was performed. 29 tests were performed in the H1N1 influenza group and 37 in the ILI group. Out of the 29 Rapid influenza Antigen Tests in H1N1 patients, 26 (89.7%) turned out to be false negative. Similar, out of the 37 test that were performed in the ILI group, 34 showed a negative result and 3 were false positive (8.8%) (See Table 8).

Table 8 Results of rapid influenza antigen tests

Rapid Influenza Antigen Test	H1N1 influenza N=209	Influenza-like illness N=310
Performed	29 (13.9)	37 (12)
Not performed	180 (86.1)	273 (88.1)
Positive	3 (10.3)	-
False Negative	26 (89.7)	-
Negative	-	34 (91.2)
False Positive	-	3 (8,8)
n (%)		

7.9 Antibiotic Therapy

The majority of H1N1 influenza patients did not receive systemic antibiotic treatment. 91/209 (43.5%) patients received antibiotic agents. On the other hand 216/310 (69.7%) ILI patients were treated with antibiotics ($p<0.001$). So overall, 307/519 (59.2%) patients who were suspected of having an H1N1 influenza infection received systemic antibiotic treatment. A detailed list of antibiotic agents is shown in the Table 9.

Table 9 Detailed list of prescribed antibiotic agents in H1N1 and ILI patients

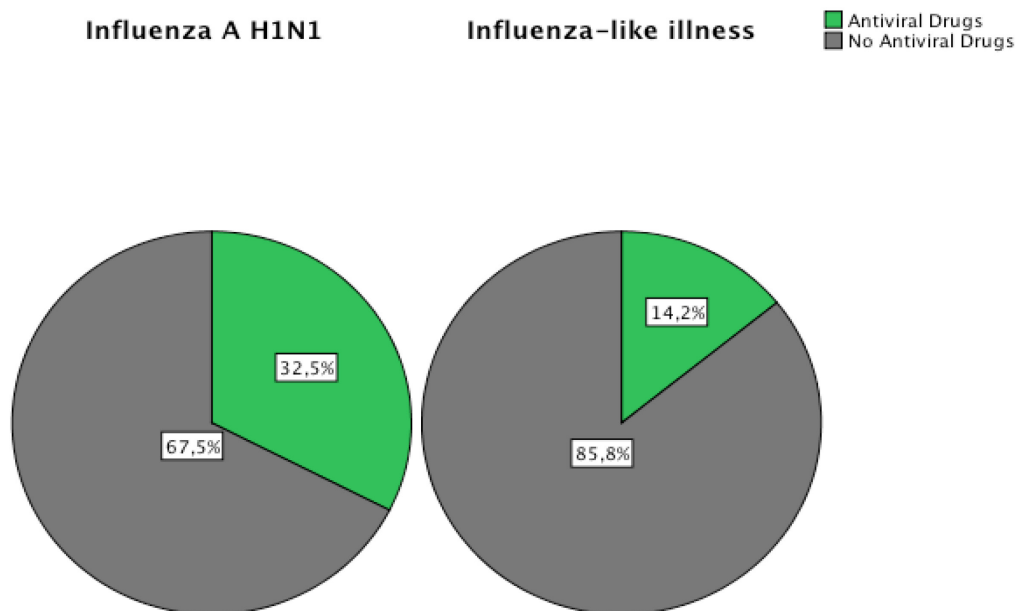
Antibiotic agent	H1N1 influenza N=91	Influenza-like illness N=216
Penicillins		
Phenoxymethylpenicillin	1	4
Amoxicillin + Clavulanic acid	29	66
Amoxicillin	1	5
Ampicillin	1	-
Piperacillin + Tazobactam	4	11
Piperacillin	1	2
Cephalosporins		
Cefalexin	1	1
Cefuroxin-Axetil	3	11
Cefaclor	2	6
Cefotaxim	2	1
Cefepim	4	3
Ceftriaxon	4	7
Cefpodoxim	1	3
Cefixim	1	5
Macrolides		
Azithromycin	4	7
Clarithromycin	17	38
Josamycin	1	3
Roxithromycin	1	1
Fluorchinolones		
Moxifloxacin	12	38
Ciprofloxacin	2	10
Levofloxacin	-	8
Carbapenems		
Imipenem	2	3
Meropenem	1	9
Doripenem	1	-
Glycopeptides		
Vancomycin	1	-
Teicoplanin	-	1

Tetracyclines			
	Doxycyclin	1	2
Nitroimidazoles			
	Metronidazol	1	3
Lincosamides			
	Clindamycin	2	2
Diaminpyrimidines			
	Trimethoprim	1	1

7.10 Antiviral Therapy

A significant difference regarding antiviral drug administration was observed between our two groups. 32.5% (68 patients) of H1N1 influenza patients received antiviral drugs compared to just 14.2% (44 patients) of patients from the ILI group ($p < 0.001$) (See Figure 18).

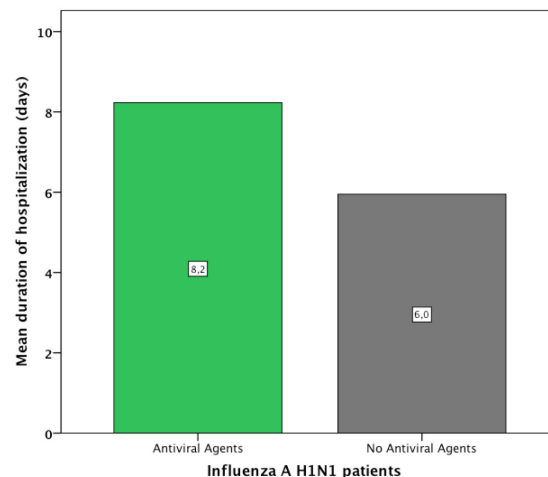
Figure 18 Percentage of H1N1 and ILI patients who received antiviral agents



All 68 H1N1 influenza patients who were treated with antiviral agents received Tamiflu® (Oseltamivir). In the ILI group one patient received Zovirax® (Aciclovir) because of an infection with Varicella Zoster virus, 39 patients received Tamiflu® and in 4 patients we were unable to find information concerning their specific antiviral treatment.

Within the H1N1 influenza group we found no significant difference in respect to their length of hospitalization, as we compared patients who received an antiviral treatment and patients who did not. The mean duration of hospitalization of patients who received antiviral agents in our H1N1 influenza group was about the same (8.2 days) than the mean hospitalization time in patients who did not receive antiviral agents (6 days) ($p=0.258$) (See Figure 19).

Figure 7 Mean duration of hospitalization among H1N1 patients who received antiviral agents and who did not



7.11 Weight, Height and BMI

The mean body weight was significantly lower in the H1N1 influenza group compared to the ILI group (47kg versus 56.5kg; $p=0.008$). A fact that is not really surprising as the mean age of patients in the influenza group was also significantly lower than in the ILI group. Also, the body height was lower in the influenza group but without statistical significance (147.41cm versus 152.34cm; $p=0.256$).

If only patients who are 18 years old or older were compared, patients from the H1N1 influenza group are slightly heavier and taller than patients from the ILI group (80.2kg versus 77.6kg; $p=0.487$ and 172.1cm versus 170cm; $p=0.361$), but without statistic significance.

The same was found regarding the Body Mass Index. The mean BMI of influenza patients ≥ 18 years was just slightly higher than the mean BMI of the ILI patients ≥ 18 years (27.1 versus 26.4; $p=0.535$). A summarization about weight, height and BMI, is displayed in Table 10.

Table 10 Weight, height and BMI in H1N1 and ILI patients

	H1N1 influenza	Influenza-like illness	p-value
Weight kg (SD) Patients ≥18 years	80.2 (29.5)	77.6 (32.2)	0.487
Height cm (SD) Patients ≥18 years	172.1 (37.8)	170 (37.6)	0.361
BMI (SD) Patients ≥18 years	27.1 (5.9)	26.4 (5.8)	0.535

7.12 Medical Imaging

Chest x-rays were ordered significantly more frequently in the group of ILI patients. In 225/307 (73.3%) ILI patients a chest x-ray was ordered, compared to just 119/209 (56.9%) patients of the H1N1 influenza group ($p < 0.001$) (See Figure 20). Also, radiological findings in chest x-rays (primarily pulmonary infiltrates) were more frequent in ILI patients. 44% of performed chest x-rays in ILI patients showed pulmonary infiltrates compared to 33.6% of chest x-rays in H1N1 influenza patients ($p < 0.001$) (See Figure 21).

Figure 20 Proportion of H1N1 patients who received chest X-Ray and in whom infiltrates could be observed

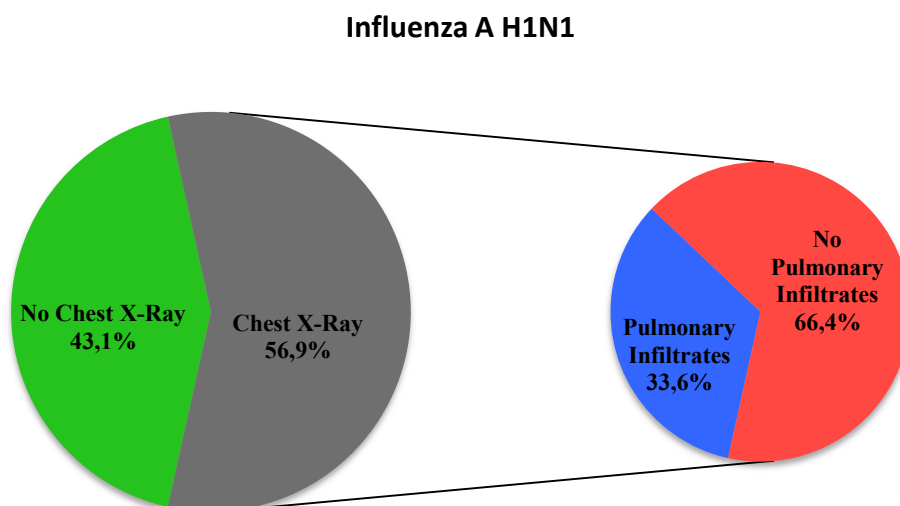
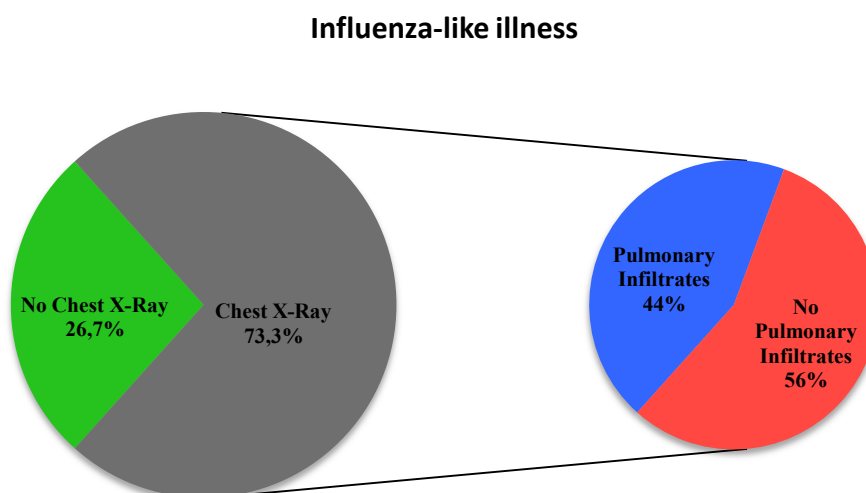


Figure 21 Number of ILI patients who received chest X-Ray and in whom infiltrates could be observed



In 16/204 (7.8%) H1N1 influenza patients and 50/305 (16.4%) ILI patients computed tomography of the thorax was ordered ($p=0.005$).

In addition, the amount of echocardiograms performed in the ILI group was significantly higher than in the H1N1 influenza group 24.2% (74/306 patients versus 11.5% 24/209 patients; $p<0.001$).

No difference between our two groups could be observed in respect to the frequency of abdominal x-rays. In 6/208 (2.9%) influenza patients compared to 12/305 (3.9%) ILI patients abdominal x-ray imaging was ordered. 5.3% (11/208 patients) of all H1N1 influenza patients received abdominal ultrasound but without any pathological findings. Furthermore, in 5.3% (11/208) H1N1 patients ultrasound was conducted and pathological findings observed. In 12.3% (37/302 patients; $p=0.008$) of our ILI patients, an ultrasound without findings was performed and in 11.9% (36/302 ILI patients; $p=0.011$) pathological findings were observed. Abdominal computed tomography was considered performed in 1% (2/206) of H1N1 influenza patients and in 5.6% (17/305) ILI patients ($p=0.006$). Table 11 gives an overview about results of medical imaging in H1N1 and ILI patients.

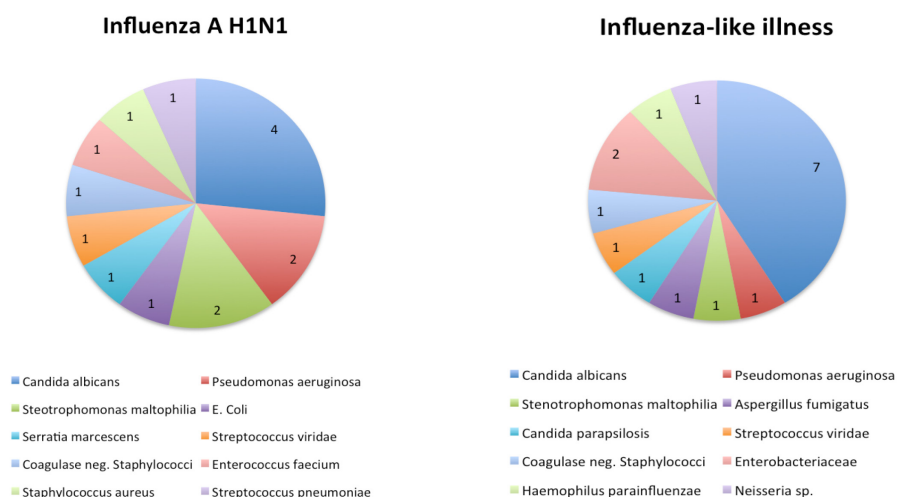
Table 11 Results of medical imaging in H1N1 and ILI patients

	H1N1 influenza	Influenza-like illness	p-value
Chest imaging			
Chest x-ray with infiltrates	40/119 (33.6)	99/225 (44)	<0.001
Chest x-ray without infiltrates	79/119 (66.4)	126/225 (56)	<0.001
Thorax-CT	16/204 (7.8)	50/305 (16.4)	0.005
Echocardiography	24/209 (11.5)	74/306 (24.2)	<0.001
Abdominal imaging			
Ultrasound with pathological findings	11/208 (5.3)	36/302 (11.9)	0.011
Ultrasound without pathological findings	11/208 (5.3)	37/302 (12.3)	0.008
Abdominal-CT	2/206 (1)	17/305 (5.6)	0.006
n/N (%)			

7.13 Microbiological Examinations

In 39 patients (14 H1N1 influenza patients and 25 ILI patients) sputum samples were collected and cytology was performed. Abnormal bacterial/fungal growth in sputum samples was observed in 10/209 (4.8%) influenza patients and in 19/309 (6.1%) ILI patients ($p=0.508$). The most common pathogen found in both groups was *Candida albicans* (found in 5 influenza and 7 ILI patients) followed by *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* (each found in 1 influenza and 2 ILI patients) (See Figure 22).

Figure 22 Detailed list of pathogens found in sputum samples



In 8/209 (3.8%) H1N1 influenza patients a bronchoalveolar lavage (BAL) was performed. In 6 of these 8 cases, culture grew potential pathogens. *Candida albicans* was also the dominant in BALs. 4 out of those 6 BALs showed colonization with *Candida albicans*.

Bronchoscopy with BAL was performed in 13/309 (4.2%) ILI patients. Potential pathogens were found in 9 cases. Like in the H1N1 influenza group, *Candida albicans* was also dominant in the ILI group. In 5 patients a positive BAL result for *Candida albicans* was observed.

Legionella rapid antigen tests were performed in 13/209 (6.2%) H1N1 and 22/310 (7.1%) ILI cases. All antigen tests in both groups resulted negative. Rapid antigen tests were conducted for *pneumococci* in 10/209 (4.8%) H1N1 influenza patients and 25/310 (8.1%) ILI patients. In 1 (0.5%) H1N1 influenza patient and 2 (0.6%) ILI patients, the antigen tests resulted positive. This H1N1 influenza patient with a positive rapid antigen test for *pneumococci* died as a consequence of secondary *pneumococcal* pneumonia and consecutive sepsis with multi organ failure.

Blood cultures were obtained in 36/209 (17.2%) H1N1 influenza patients and in 79/309 (25.6%) ILI patients. Out of these 36 blood cultures in H1N1 influenza patients, 30 cultures turned out to be negative and 6 showed a positive result. In the ILI group, 59 out of 79 bacterial cultures showed no growth and 20 resulted positive. Details about the number of performed blood cultures, as well as pathogens found in blood cultures, are shown in Table 12 and 13.

Table 12 Number of performed, positive and negative blood cultures

	H1N1 influenza	Influenza-like illness	p-value
Blood culture performed	36/209 (17.3)	79/309 (25.6)	0.025
Blood culture positive	6/209 (2.9)	21/309 (6.5)	0.065
Blood culture negative	30/209 (14.4)	58/309 (19.1)	0.161
n/N (%)			

Table 13 Pathogens found in blood cultures

	H1N1 influenza	Influenza-like illness
<i>Staphylococci coagulase negative</i>	2	6
<i>Staphylococcus aureus</i>	1	2
<i>Streptococcus pneumoniae</i>	-	1
<i>Streptococcus group A</i>	-	1
<i>Streptococcus viridans</i>	-	1
<i>Escherichia coli</i>	1	5
<i>Salmonella typhimurium</i>	1	-
<i>Corynebacterium species</i>	1	-
<i>Chlamydia pneumoniae</i>	-	1
<i>Stenotrophomans maltophilia</i>	-	1
<i>Pseudomonas aeruginosa</i>	-	1
<i>Campylobacter species</i>	-	1
<i>Bacteroides stercoris</i>	-	1

7.14 Past Medical History

7.14.1 Lung Diseases

A history of Asthma bronchiale was found in 22/209 (10.5%) H1N1 patients and in 20/306 (6.5%) patients from the ILI group ($p=0.104$).

Also no significant difference between those two groups was observed in respect to the amount of patients suffering from chronic obstructive pulmonary disease (COPD). 14/209 (6.7%) H1N1 influenza patients and 20/306 (6.5%) ILI patients had a history of COPD ($p=0.942$). A detailed list of patients suffering from COPD in respect to the GOLD classification, is shown in the Table 14.

Table 14 Number of H1N1 and ILI patients in different COPD stages (GOLD classification)

	H1N1 influenza	Influenza-like illness
COPD GOLD 1	6	14
COPD GOLD 2	4	3
COPD GOLD 3	3	1
COPD GOLD 4	1	2

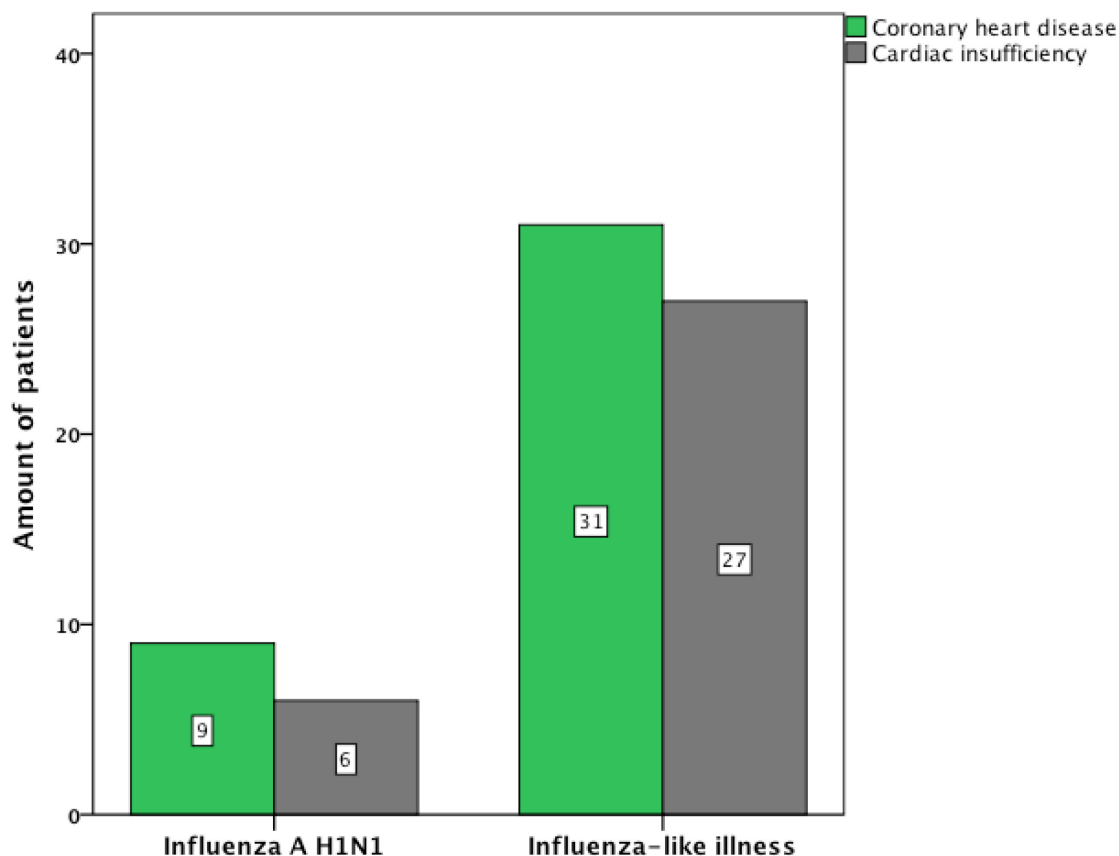
Other lung diseases we observed in patients from our influenza group were chronic bronchitis (n=3) and lung emphysema (n=1). In our ILI group we observed 5 patients with chronic bronchitis, 3 with lung emphysema, 1 with lung atelectasis and 1 patient with pulmonary arterial hypertension.

7.14.2 Cardiovascular Diseases

The amount of patients suffering from chronic coronary heart diseases was significantly higher in the group of ILI patients. 31/305 (10.2%) ILI patients have had a history of coronary heart disease compared to 9/209 (4.3%) H1N1 influenza patients ($p=0.015$).

We also observed a higher number of patients suffering from cardiac insufficiency in the ILI group than in the H1N1 influenza group (27/306 (8.8%) ILI patients versus 6/209 (2.9%) H1N1 patients; $p=0.007$) (See Figure 23).

Figure 23 Number of patients with coronary heart disease or cardiac insufficiency



No differences were observed with regard to the amount of patients suffering from cardiac arrhythmia or valve diseases. In our H1N1 influenza group 8/209 (3.8%) patients had cardiac arrhythmias and 6/209 (2.8%) have had some kind of valve disease, compared to 21/306 (6.9%; $p=0.140$) ILI patients who had cardiac arrhythmias and 9/306 (3%; $p=0.958$) patients with valve disease.

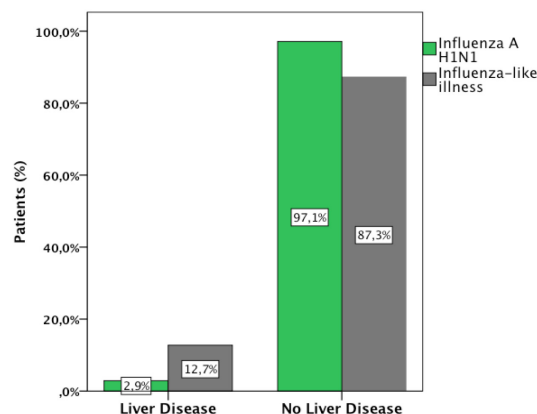
The amount of patients with arterial hypertension was significantly higher in the ILI group. In this group 72/306 (23.5%) patients have had arterial hypertension compared to 25/209 (12%) patients in our H1N1 influenza group ($p<0.001$). Contrariwise no difference was observed regarding the number of patients who have had any history of peripheral arterial disease, central arterial disease or stroke.

7.14.3 Gastrointestinal-, Liver Disease and Diabetes Mellitus

The number of patients suffering from chronic gastrointestinal diseases was about the same in both groups. 20/209 (9.6%) H1N1 patients had a history of gastrointestinal diseases compared to 42/306 (13.7%) patients in the ILI group ($p=0.155$).

On the other hand, the amount of patients who have had chronic liver diseases (especially Steatosis hepatitis) was distinctly higher in the ILI group. 39/306 (12.7%) patients from this group had chronic liver diseases compared to 6/209 (2.9%) patients from the H1N1 influenza group ($p<0.001$) (See Figure 24).

Figure 24 H1N1 and ILI patients with chronic liver disease



No difference between those two groups were observed in respect to Diabetes mellitus. 11/209 (5.3%) H1N1 influenza patients had diabetes mellitus compared to 26/306 (8.5%) patients out of the ILI group ($p=0.160$).

7.14.4 Other Diseases

The number of patients suffering from chronic renal insufficiency was significantly higher in the ILI group. 38/306 (12.4%) ILI patients and 10/209 (4.8%) H1N1 influenza patients had been suffering from chronic renal insufficiency ($p=0.003$) (See Table 15).

Table 15 Stages of chronic renal insufficiency based on the kidney disease outcomes quality initiative (KDOQI) guidelines

	H1N1 influenza	Influenza-like illness
Stage 1; no.	2	8
Stage 2; no.	1	5
Stage 3; no.	5	11
Stage 4; no.	0	3
Stage 5; no.	2	11

No difference in the frequency of malignancies was observed. 10/209 (4.8%) H1N1 influenza patients and 18/306 (5.9%) ILI patients had a malignant disease in their history ($p=0.590$).

20/209 (9.6%) influenza patients and in 41/306 (13.4%) ILI patients had a history of neurological disease ($p=0.182$).

7.15 Pregnancy

10/209 (4.8%) H1N1 influenza patients were pregnant. None of them had severe complications or a fatal outcome. In the ILI group 6/306 (2%; $p=0.070$) patients were pregnant. Like in the influenza group, none of those patients had any complications. A summarization about co-morbidities in H1N1 and ILI patients is given in Table 16.

Table 16 List of co-morbidities in H1N1 and ILI patients

	H1N1 influenza	Influenza-like illness	p-value
Bronchial asthma	22/209 (10.5)	20/306 (6.5)	0.104
COPD	14/209 (6.7)	20/306 (6.5)	0.942
Coronary heart disease	9/209 (4.3)	31/305 (10.2)	0.015
Cardiac insufficiency	6/209 (2.9)	27/306 (8.8)	0.007
Cardiac arrhythmia	8/209 (3.8)	21/306 (6.9)	0.140
Valve disease	6/209 (2.8)	9/306 (3)	0.958
Arterial hypertension	25/209 (12)	72/306 (23.5)	<0.001
Peripheral arterial disease	2/209 (1)	7/306 (2.5)	0.323
Central arterial disease	1/209 (0.5)	3/306 (1)	0.650
Stroke	5/209 (2.4)	9/306 (2.9)	0.789
Gastrointestinal disease	20/209 (9.6)	42/306 (13.7)	0.155
Liver disease	6/209 (2.9)	39/306 (12.7)	<0.001
Diabetes mellitus	11/209 (5.3)	26/306 (8.5)	0.160
Chronic renal failure	10/209 (4.8)	38/306 (12.4)	0.003
Malignancies	10/209 (4.8)	18/306 (5.9)	0.590
Neurological diseases	20/209 (9.6)	41/306 (13.4)	0.182
Pregnancy	10/209 (4.8)	6/306 (2)	0.070
n/N (%)			

7.16 H1N1 Influenza Vaccination

A significant difference between the two groups was observed in respect to the amount of patients who received H1N1 influenza vaccination. In the H1N1 influenza group just one (0.5%) patient had received a vaccination against H1N1 influenza. This patient was vaccinated at the day of symptoms onset. In the ILI group 15 (4.9%) patients had received a vaccination against H1N1 influenza ($p=0.004$).

7.17 Outcome

With regard to fatal outcome no difference was observed between our groups. 4/206 (1.9%) H1N1 influenza patients died, compared to 10/310 (3.2%) ILI patients ($p=0.424$) (See Table 17).

All 4 H1N1 influenza patients died as a consequence of pneumonia. 3 out of 4 patients with H1N1 influenza died within 9 days after hospitalization. All three had developed primary pneumonia. One patient with H1N1 infection died due to multiorgan failure 2 weeks after he had been admitted. The patient had developed secondary pneumonia due to *Streptococcus pneumoniae* and *Aspergillus fumigatus*. The latter had also been cultured from peripheral blood. 4/10 ILI patients also died because of pneumonia, 2 died as a consequence of bronchitis/bronchiolitis, 1 died due to acute renal failure, 1 due to acute exacerbation of COPD, 1 as a result of acute endocarditis and 1 as a consequence of sepsis with *enterococci*.

Table 17 Outcome of H1N1 and ILI patients

	H1N1 influenza N=206	Influenza-like illness N=310
Died	4 (1.9)	10 (3.2)
0-10 years	0	2
11-20 years	0	0
21-30 years	1	0
31-40 years	1	0
41-50 years	0	0
51-60 years	1	3
>60 years	1	5
Mean age (years)	23.7	32.9
Male	4	6
Female	0	4
Survived	202 (98.1)	300 (96.8)
n (%)		

8 Discussion

We retrospectively analyzed differences in demographic data, clinical presentation, treatment, preexisting medical conditions and outcome of patients with PCR confirmed H1N1 influenza and patients with influenza-like illness (ILI), but with a negative influenza PCR result in the 2009/2010 pandemic influenza season. One goal of our study was to evaluate clinical characteristics of H1N1 influenza and differentiate between H1N1 and influenza-like illness to facilitate early detection and guarantee an efficient, targeted and early treatment in influenza infections.

As published data have previously shown (22), we can confirm that the use of Rapid antigen tests is ineffective because of its low sensitivity. In our study we observed that 89.7% of all conducted Rapid antigen tests have resulted falsely negative. Another setback is the not perfect specificity. Just as tests resulted falsely positive in 8.8% of ILI patients.

32.5% of all influenza patients received antiviral agents. The number of patients who received antiviral treatment was therefore significantly higher in the H1N1 group when compared to the ILI group, but can be considered low if we compare it to data from the United States (35) and Australia (59), where antiviral treatment was initiated in 75% to 83%. Furthermore, nearly 30% (n=20) of our H1N1 influenza patients received specific antiviral therapy more than 48 hours after onset of symptoms. In 4/4 patients who died as a consequence of H1N1 influenza infection, treatment with oseltamivir was initiated more than 48 hours after the onset of symptoms. Earlier identification of influenza patients would help to initiate specific antiviral therapy within 48 hours after the onset of symptoms, as recommended by the WHO (51). On average, H1N1 influenza patients presented to a medical doctor 1.78 days after the onset of symptoms. Although those patients presented early enough to receive antiviral drugs within this 48 hour period, there is not much time left after first contact to medical institutions for diagnosis and initiation of targeted treatment. As rapid antigen tests have been shown to be unreliable and PCR results need too much time until they are available, diagnosis based on clinical findings and laboratory values may be of great clinical value. Furthermore, reliable and fast diagnosis may help to reduce healthcare costs as well as side effects of unneeded drugs.

All H1N1 influenza patients and the vast majority of ILI patients who received antiviral agents were treated with Oseltamivir, in accordance to the WHO recommendations of

pharmacological treatment for H1N1 influenza (51). No resistances of H1N1 influenza virus against Oseltamivir was observed in our study. The most common antibiotic agents in both groups were amoxicillin/clavulanic acid, followed by clarithromycin and moxifloxacin.

As previously published data has shown, we found that H1N1 influenza virus is more likely to infect younger people and spars the elderly (17,24,26,32,35,38,60). 61% of all H1N1 influenza infected patients were 21 years or younger compared to 41% of all ILI patients. The lack of neutralizing antibodies in patients less than 21 years may be an explanation for this result (26). Also because of higher mortality in this age group, young adults and children are more likely to be tested than elderly patients. Furthermore, young adults and children are under a higher viral exposure through other infected people in kindergarten, schools or other communities. Therefore, patients under 21 years of age seem to have the highest risk for H1N1 influenza infection. In contrast to other studies (22,35,61,62), we could not observe a significant higher infection or mortality rate during pregnancy in our H1N1 influenza group, mainly due to low numbers.

The mean time from onset of symptoms to first clinical presentation was significantly lower in our H1N1 influenza cohort. The mean time in this group was 1.78 days compared to 4.18 days in our ILI group. So the onset of symptoms seems to be more rapid in influenza patients and therefore can be used as an indicator for an actual influenza infection. Surprisingly the mean time of hospitalization, regarding to H1N1 patients who received antiviral treatment and who did not, did not differ (8.2 days versus 6 days; $p=0.258$). A possible reason may have been, that patients with severe Influenza were more likely to be treated with antiviral agents also >72h after onset of symptoms than those with a milder course.

Out of our study population, 8% of all influenza patients and 5% of all ILI patients needed intensified care at an ICU. Especially for influenza patients, this number can be considered low if we compare it to numbers published by Jain S. et al. (25%) (35). Maybe the rapid onset of symptoms and therefore the short period of time from onset of symptoms to clinical presentation and treatment can be considered as an explanation for this. The median time on ICU in our H1N1 influenza study population (median 7 days; range 0-51) was nearly the same compared to data from Australia (34). For ILI patients we found a median time on ICU of 4.5 days (range 1-41). So it seems that H1N1 influenza virus caused more severe infections than other common viruses in 2009/2010 and therefore time on ICU was prolonged in this group.

Chan et al. published that individuals with H1N1 influenza were more likely to present with fever, cough, sore throat, myalgia, chest pain, headaches and gastrointestinal symptoms like nausea, vomiting and diarrhea (60). However, we found that the vast majority (92.2%) of H1N1 influenza patients presented with fever at home and/or at first clinical presentation. Similarly 73.9 % of all ILI patients presented with fever. Therefore we cannot use fever as an indicator for a H1N1 influenza infection but we can use absence of fever as an exclusion criteria for an actual influenza infection.

In accordance to the data of Chan et al., we also observed that coughing was one of the most frequent symptoms in our H1N1 influenza group. In addition, a significant higher number of influenza patients presented with fatigue, rhinitis and headache. Overall, fever, cough and fatigue were the strongest predictors in our study, regarding clinical symptoms of an H1N1 influenza infection. On the other hand, we did not observe that a higher number of H1N1 influenza patients compared to ILI patients presented with any kind of gastrointestinal symptoms like nausea, vomiting or diarrhea, as published by different authors (17,20,27,35,42,44,60).

According to findings at the first physical examination we noticed that H1N1 influenza patients had a higher initial heart rate than ILI patients. The biggest difference between those two groups was observed in patients at the age of 5-14 years. In contrast, findings like pathological heart tones and murmurs and signs of obstructive lung disease were more often found in ILI patients. This may be explained by the fact that the mean age in our ILI group was significantly higher and co-morbidities are more likely at a higher age.

We observed that ILI patients had more co-morbidities in general compared to influenza patients. Especially a significant higher amount of coronary heart disease, heart insufficiency, arterial hypertension, liver diseases and chronic renal failure was observed in ILI patients. Only bronchial asthma was more often observed in influenza patients. Again the higher mean age in ILI patients can be considered a reason for those differences.

Vaccination seems to be a powerful prophylaxis for H1N1 influenza. Just one patient from our influenza cohort fell ill with H1N1 influenza despite having received a vaccination. This patient showed first clinical symptoms at the same day he had received vaccination. Possibly, this patient was infected by H1N1 influenza, even before he was vaccinated.

Case fatality rate among our study cohort did not differ between the two groups. 1.9% (n=4) of H1N1 influenza patients and 3.2% (n=10) of ILI patients had a fatal outcome. This is a lower case fatality rate than published in other reports (17,61). We did also not find as many co-morbidities in H1N1 influenza patients who died, as published in previous

reports (24,35,61). One patient was a former smoker without any other co-morbidity. But all 4 H1N1 influenza patients who died suffered from secondary bacterial pneumonia. Likewise, in 4/10 ILI patients with a fatal outcome, bacterial pneumonia could be observed.

Our study has several limitations. We only collected data from patients who presented to hospitals in Styria. We did not review data from outpatients. Therefore, data sets were complete in just 519/1681 (31%) cases. The remaining 1162 (69%) patients were excluded from further investigation. Thus, data may not be representative for patients with mild illness, who did not seek medical care in hospitals. So our data cannot give any information about mortality and outcome for outpatients and therefore may not be representative for those patients. But we suggest a low case fatality rate as patients with severe symptoms and severe underlying medical conditions would have sought medical advice in hospitals. Furthermore, we only evaluated data from patients who presented during the first pandemic H1N1 season from September 2009 through January 2010 and other studies have reported differences in clinical presentation between the first to the second wave (63,64).

In conclusion, studies like this seem to be an essential part in developing a clinical score for reliable and rapid H1N1 influenza diagnosis and early initiation of efficient antiviral treatment. According to our study, coughing, rhinitis, fatigue and fever are the most powerful clinical signs for differentiation between H1N1 influenza and influenza-like illness.

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11 Curriculum Vitae



Personal Data

Name	Jürgen Prattes
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Civil status	Single
Present status	Student

Educational History

2005	Graduated from BORG Monsbergergasse, Graz
2007 - present	Medical University Graz

Clinical Rotations

2010	LKH Graz, Department of Pneumology
2010	LKH Graz, Intensive Care Unit – Internal Medicine
2010	LKH Graz, Department of Thorax Surgery
2011	Krankenhaus der Elisabethinen Graz, Department of Radiology
2011	LKH Graz, Department of Neurology
2012	NOVA Southeastern University Fort Lauderdale/Florida, Department of Osteopathic Principles and Practice

Practical Year

2012	LKH Graz, Department of Pediatrics and Adolescents, Division of General Pediatrics (Pediatric Intensive Care Unit); 5 Weeks
2012	General Medicine, Dr. Elisabeth Krainer, 5 Weeks
2012 – 2013	LKH Graz, Department of Orthopedic Surgery; 10 Weeks

2013 University Hospital Zurich/Switzerland; Department of Internal Medicine; 12 Weeks

Science Work

2011 – present Research Associate at the Clinical Research Center Graz, Department of Endocrinology and Metabolism

2011 – present Diploma Thesis “Comparison of demographic data, clinical presentation, risk factors and outcome between PCR confirmed H1N1 influenza and PCR negative influenza-like illnesses” at the Department of Infectious Diseases, Medical University Graz

2012 Poster Award (2nd Place) at the Annual Meeting from the Austrian Society of Infectious Diseases and Tropical Medicine (co-author)

Grants

2012 Grant for International Rotations at the NOVA Southeastern University Fort Lauderdale/Florida USA

2012 Merit Allowance for the years 2008-2012 by the Medical University of Graz

Languages

German (Mother tongue)

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Special Abilities

2010 Basic Medical English Course 1

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2011 Good Clinical Practice Training, Trainer Dr. Stefan Korsatko

