Diplomarbeit

PCR confirmed Influenza A H1N1 in South East Austria, season 2009/2010: Epidemiology, laboratory characteristics and outcome

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List of contents

1 Abbreviation

LKH= Landeskrankenhaus

H or HA= hemagglutinin

N or NA= neuraminidase

RNA= ribonucleic acid

NP= nucleoprotein

PB1= polymerase basic 1

PB2= polymerase basic 2

PA= polymerase acid

S-OIV= swine-origin influenza virus

CDC= Centre for Disease Control

WHO= World Health Organization

UK= United Kingdom

COPD= chronic obstructive pulmonary disease

RIDTs= rapid influenza diagnostic tests

RT- PCR= real-time reverse - polymerase-chain-reaction

CRP= C-reactive protein

PCT= procalcitonin

ALT= alanine aminotransferase

AST= aspartate aminotransferase

GGT= gamma-glutamyl transferase

LDH= lactate dehydrogenase

CK= creatine kinase

ECMO= extracorporeal membrane oxygenation

CFR= case fatality rate

2 Summary

2.1 Aim

During spring 2009 novel pandemic influenza A (H1N1) virus emerged in Mexico and spread rapidly globally for almost one year till the announced end of the pandemic resulting in millions of laboratory confirmed cases and over 18000 deaths worldwide. Compared to previous non-pandemic influenza seasons, epidemiology of the H1N1 pandemic in 2009-2010 differed significantly. As sensitivity of currently available rapid antigen tests has shown to be low, PCR is the recommended test for diagnosis and confirmation of infection.
We retrospectively analyzed cases of PCR confirmed H1N1 influenza in South East Austria, and describe epidemiologic characteristics, laboratory values and outcome of hospitalized patients.

2.2 Methods

This is a retrospective survey of patients with PCR-proven influenza H1N1 infection in South East Austria. 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. PCR testing for the whole region of South-East Austria was done at the Institute for Hygiene, Microbiology and Environmental Medicine, Medical University of Graz and at the Institute for Hospital Hygiene and Microbiology, University Hospital of Graz. 624 cases of H1N1 influenza were detected during the surveillance period from October, 1, 2009 to January, 19, 2010. Complete data sets were available from 197/624 (32%) of patients who were hospitalized or presented to outpatient clinic only at one of the participating hospitals. Data from 402/624 (64%) of patients could not been surveyed as they were, in the vast majority of cases, not submitted to hospital and presented to their family doctor only and therefore no data was available in MEDOCS, the electronic data base.

2.3 Results

Out of 197 cases (117 male, 80 female, mean age 25 years) 17 (9%) required intensified medical care, 132 (67%) care at normal wards, and 48 (24%) presented to

outpatient clinic only. 1% (2/197) had a history of vaccination against H1N1 influenza while 99% had not. Rapid antigen test (BinaxNow, Inverness Medical, Maine, U.S.) was negative in the vast majority of cases (89%). The most consistent laboratory characteristics in our study were total leukocyte count within normal limits, lymphocytopenia, increased lactate dehydrogenase levels, increased creatinine and CRP levels and elevated AST, ALT and GGT counts. 65/197 (33%) of cases received oseltamivir treatment and in a majority of these patients (44/65, 68%) oseltamivir was administered within 48 hours. Antibacterial therapy was administered in 88/197 (45%) of cases. Complications occurred in 51/197 (26%) of cases. 33/197 (17%) of patients developed pneumonia, 8/33 of patients had bacterial pneumonia and 25/33 viral pneumonia due to H1N1. 4/51 of patients showed neurological disorders, 13/51 cases developed respiratory insufficiency and 4/51 cases developed bacteraemia/fungaemia. 5/197 cases (2.5%) had a fatal outcome, 97% survived. Mean age of patients with a fatal outcome was 39.4 years (ranging from 15 to 69 years of age); 2 out of 5 patients with fatal outcome had a history of mild chronic obstructive pulmonary disease.

2.4 Conclusion

During the surveillance period influenza A (H1N1) virus caused a high number of hospitalizations. In accordance with previously published data median age of PCR confirmed cases in South East Austria was comparably low and a high rate of lower respiratory disease associated with influenza H1N1 infection was observed. Laboratory findings were comparable with previous reports. In summary, although the majority of the surveyed cases in our study had mild illness in terms of clinical presentation and mortality was comparatively low, severe and even fatal illness was identified also among healthy young adults.

3 Zusammenfassung

3.1 Ziel

Beginnend im Frühjahr 2009 verbreitete sich der neue pandemische Influenza A (H1N1) Virus von Mexiko ausgehend innerhalb kürzester Zeit weltweit, resultierend in Millionen von bestätigten Infektionen und über 18.000 Todesfällen. Die Pandemie unterschied sich deutlich von der saisonalen Influenza Erkrankung bezüglich der Epidemiologie. Die PCR erwies sich aufgrund der ausgesprochen niedrigen Sensitivität der vorhandenen Influenza Schnelltests als empfehlenswerteste Methode zur Diagnostik. Wir analysierten retrospektiv durch PCR bestätigte Influenza A (H1N1) 2009 Fälle in Süd-Ost-Österreich und beschrieben Epidemiologie, klinische Ergebnisse, Laborwerte und Ergebnisse von hospitalisierten Personen.

3.2 Methoden

Es handelt sich um eine retrospektive Untersuchung von Patienten mit bestätigter Influenza A (H1N1) Infektion in Süd-Ost-Österreich. Teilnehmende Krankenhäuser waren das Universitätsklinikum Graz, sowie das LKH Leoben, LKH Bruck, LKH Judenburg, LKH Hörgas- Enzenbach, LKH Feldbach, LKH Deutschlandsberg, LKH Rottenmann, LKH Wagna LKH Bad Aussee und das LKH Graz-West. 624 Fälle wurden mittels PCR in einem Untersuchungszeitraum von 01.10.2009 bis 19.01.2010 diagnostiziert. Die PCR-Untersuchungen wurden am Institut für Krankenhaushygiene und Mikrobiologie, Medizinische Universität Graz und am Institut für Hygiene, Mikrobiologie und Umweltmedizin, Medizinische Universität Graz durchgeführt. Anhand von vollständig dokumentierten Krankengeschichten wurden Daten von 197/624 Patienten (32%), welche in den teilnehmenden Krankenhäusern stationär oder ambulant vorstellig waren, erhoben. Die Daten von 402/624 (64%) der Patienten konnten aufgrund des fehlenden Zugangs im MEDOCS, der elektronischen Datenbank, nicht erhoben werden. In den meisten Fällen waren diese 402 Patienten ausschließlich im Niedergelassenen Bereich vorstellig.

3.3 Resultate

Von 197 Fällen (117 Männer, 80 Frauen, mittleres Alter 25 Jahre) waren 17 Patienten (9%) in intensiv-medizinischer Behandlung, 132 (67%) wurden auf

Normalstationen behandelt und 48 (24%) der Patienten wurden ambulant versorgt. Bei 1% der Patienten (2/197) war anamnestisch eine Impfung gegen den H1N1 Virus bekannt, wohingegen 99% nicht geimpft waren. Die Ergebnisse der Influenza Schnelltests (BinaxNow, Inverness Medical Maine, U.S.)) waren in den meisten Fällen falsch negativ (89%). Die Laborergebnisse unserer Studie ergaben in den meisten Fällen normale Leukozyten-Werte, Lymphozytopenie, erhöhte Laktatdehydrogenase-Werte, gesteigertes Kreatinin und CRP sowie erhöhte Werte von ALT, AST und GGT. In 65/197 (33%) der Fälle wurde eine antivirale Therapie verschrieben und im Großteil der Fälle (44/65,68%) wurde Oseltamivir innerhalb der ersten 48 Stunden verabreicht. Eine antibiotische Behandlung wurde in 88/197 (45%) der Fälle durchgeführt. In 51/197 der Fälle (26%) traten Komplikationen auf. 33/197 (17%) der Patienten entwickelten eine Pneumonie, davon in 8/33 der Fälle bakteriell und in 25/33 der Fälle viral durch H1N1 bedingt. 4/51 der Patienten entwickelten neurologische Komplikationen, 13/51 eine respiratorische Insuffizienz und 4/51 der Fälle entwickelten eine Bakteriämie/Fungämie. 5/197 der Patienten (2.5%) verstarben, 97% haben überlebt. Das mittlere Alter der der verstorbenen Patienten war 39.4 Jahre (von 15 bis 69 Jahren); 2 der verstorbenen Patienten hatten eine bekannte COPD.

3.4 Zusammenfassung

Der Influenza A (H1N1) 2009 Virus verursachte eine Vielzahl von Krankenhausaufenthalten in dem von uns beschriebenen Zeitraum. Vergleichbar mit anderen Studien zeigte sich ein sehr niedriges mittleres Alter und eine erhöhte Anzahl an Infektionen der unteren Atemwege in Verbindung mit dem H1N1 Virus. Die evaluierten Laborergebnisse folgen den bisherigen Publikationen. Obwohl der Großteil der untersuchten Fälle einen milden Verlauf aufwies und die Mortalität vergleichbar niedrig war, waren schwere Fälle sowie fataler Ausgang auch unter gesunden jungen Erwachsenen anzutreffen.

4 Influenza Viruses

Influenza-virus infection is an acute usually self-limited illness. The virus can present in a wide spectrum of clinical syndromes, depending on the virus strain but also on certain underlying risk conditions. Clinical manifestation after an incubation period of 1 to 2 days includes fever, headache, cough, sore throat, nasal congestion, sneezing, and body aches. Typical complications are the primary influenza viral pneumonia and the secondary bacterial pneumonia. Usually influenza proceeds epidemical und seasonally (1).

4.1 History

Influenza viruses lead to a large number of respiratory diseases, morbidity and mortality in human population every year worldwide for at least the past 400 years.

In 1930 Rockefeller Institute investigator Richard Shope first isolated the influenza virus A/Swine/Iowa/30.The first isolation of the human virus in ferrets was reported in 1933 by Wilson Smith, Sir Christopher Andrews and Sir Patrick Laidlaw and lead to our modern understanding of the influenza virus. Smith and associates vaccinated ferrets intranasally with nasopharyngeal washes from an influenza patient. The inoculation leaded to an influenza-like disease in ferrets after two days. During the experiments one of their junior colleagues was infected with the virus. The virus was consecutively isolated for the first time in a human and named ‘influenza A’ (2).

In 1939 ‘Influenza B’ was recovered by Francis and in 1950 ‘Influenza C’ by Taylor. In 1936 Burnet published that influenza virus could be cultivated in embryonated hen eggs. This discovery afforded a better understanding of the virus but also opened the door for development of inactivated vaccines (1). In 1940 the efficiency of inactivated vaccines against influenza A disease was recognized by Francis (3) and in 1941 Hirst discovered the phenomenon of hemagglutinin (4).

4.2 Virology

The influenza viruses belong to the family Orthomyxoviridae that encode a segmented RNA genome. There are three different types of influenza: influenza A, influenza B and influenza C. The three types differ in host, range, genetic, pathogenicity and clinical manifestation (1).

Table 1

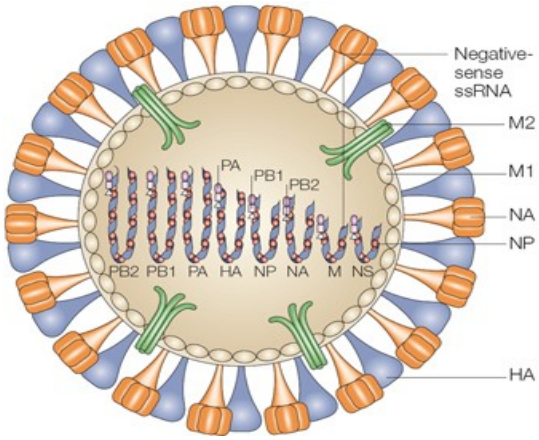
Differences among Influenza viruses (adapted from (1))

Influenza type	Genetics	Host range	Clinical features
Influenza A	8 gene segments	humans, swine, avian, equine, marine mammals	severe disease to young persons
Influenza B	8 gene segments	humans only	severe disease to older adults or high risk patients
Influenza C	7 gene segments	humans and swine	mild diseases, without seasonality

Influenza A is an enveloped virus that contains the hemagglutinin (H or HA), the neuraminidase (N or NA) and the matrix proteins (M2) projecting from the surface. Interior is the matrix protein M1, which plays an important rule for virus assembly. Within the virion there are eight nucleocapsid segments, each consisting of a single segment of genomic RNA, the nucleoprotein (NP) and the three polymerase proteins PB1 (polymerase basic 1), PB2 (polymerase basic 2) and PA (polymerase acid) (5).

Figure 1

Schematic model of influenza A virus (adapted from (6))



Influenza A viruses are further divided into subtypes based on their hemagglutinin and neuraminidase activity. At least 16 HA subtypes (H1-H16) and 9 NA (N1-N9) subtypes are described. The standard nomenclature includes the influenza type, the place of initial isolation, strain number and year of isolation. Influenza A viruses evolve by the mechanisms of antigenic drift and shift (1, 5).

4.3 Pandemics

Intermittently new virus strains emerge in humans and induce global pandemics. Pandemics are characterized by rapid transmission through all parts of the world, the emergence of new virus strains and the resultant absence of immunity, high attack rates especially in young age groups, an outbreak out of usual seasonality, high mortality rate und multiple waves of diseases (1). There have been pandemics in 1889, 1918, 1957, 1968, 1977 and 2009. The worst pandemic in recorded history was the so-called ‘Spanish influenza’ which occurred from 1918 to 1919.

4.3.1 The Spanish Influenza, H1N1 (1918-1919)

The 1918 pandemic was the most lethal influenza pandemic in human history and killed an estimated 50 million people worldwide (7). Almost one third of the world’ population were clinically infected during the pandemic that may have lead to an earlier end of World War I. The pandemic occurred in three waves within one year. Because of the near-simultaneous emergence of influenza in March and April 1918 in North America, Europe and Asia the place of origin continues to be unknown (8).

Analysis on fixed and frozen lung tissues of 1918 influenza victims determined the complete genomic sequence of the virus. These findings propose that the virus contains genes derived from avian-like virus strains which adapted to humans and support the fact that the 1918 virus is the progenitor of human and classical swine H1N1 influenza viruses (9)(8).

The clinical manifestation was similar to seasonal influenza. However, there was a higher rate of severe pneumonic complications and most people died of secondary bacterial pneumonia (10)(11). The pandemic differed especially in epidemiologic aspects. The case mortally rate in the United States was several times higher than the current rate. Furthermore the mortality was concentrated on a younger

population. Almost half of the influenza-related deaths in 1918 were young adults aged 20 to 40 and nearly 99% of lethal outcomes were people younger than 65 years of age (12).

4.3.2 The Asian Influenza, H2N2 (1957-1958)

The pandemic virus emerged in February 1957 from the Southern Chinese province of Guizhou and spread globally. The virus, a linear descendent of the 1918 H1N1 pandemic virus, was isolated in May 1959 in Japan. Analysis showed that the virus developed from reassortment between human and avian viruses (2). The HA and NA protein segments were replaced by avian-like H2 and N2 subtypes (13). The gene segment encoding the PB1 polymerase was also exchanged by an avian-like gene segment (14).

The H2N2 pandemic didn't proceed as dramatic as the pandemic from 1918 but was similar in its clinical and pathological appearance. An estimated one million people were killed worldwide. Within two years the virus became seasonally endemic, exhaled and has not returned till now (2, 10).

4.3.3 The Hong Kong Influenza (1968-1969)

In summer of 1968 the 'Hong Kong flu' emerged from Southern Asia and spread around the world during winter. The pandemic was relatively innocuous, so that certain areas showed fewer influenza deaths than in non-pandemic years (15). The virus was an 'update' from the formerly circulating virus. Molecular analysis showed that the H2 was replaced by an avian-like H3 and the PB1 polymerase gene segment again by an avian-like PB1(13)(14). The mildness of the pandemic may base on a present immunity to former circulating NA viruses. The pandemic soon became endemic und circulates globally up to now.

5 Influenza H1N1

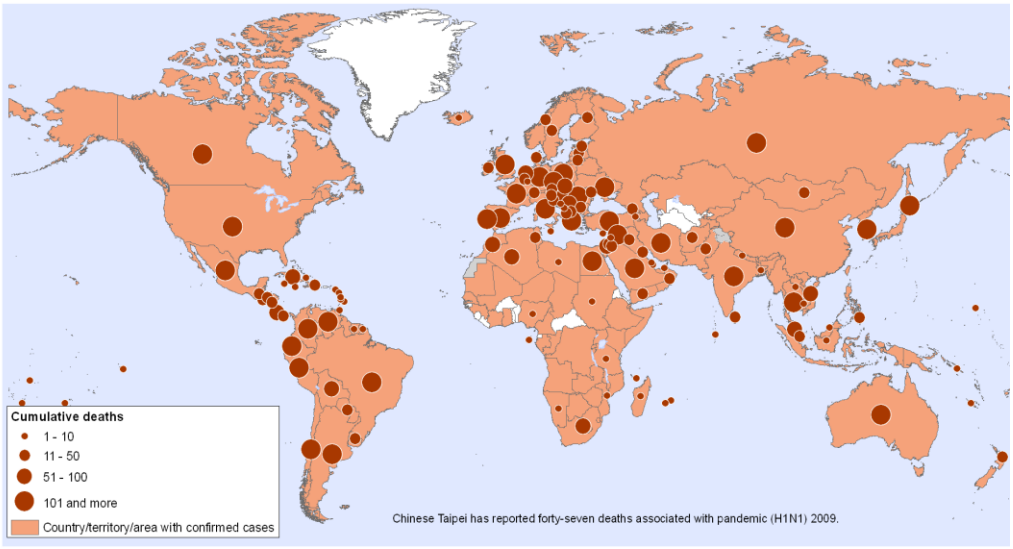
5.1 Pandemic Timeline

In April 2009 a novel influenza A H1N1 virus of swine origin emerged from Mexico (16) and rapidly spread over the world for almost one year till the announced end of the pandemic.

Early April, 2009, the Mexican Ministry of Health noted an increasing number of young people affected by severe pneumonia and requiring hospitalization, unusually high laboratory confirmed influenza cases outside the season and series of deaths from several cities (17). On April 15 and April 17, 2009, the Centre for Disease Control (CDC) identified a novel swine-origin influenza A (H1N1) virus (S-OIV) obtained from two infected patients in the United States (18). A few weeks later other cases of S-OIV were identified in Mexico, Canada, New Zealand, the UK and other countries and the virus rapidly spread globally (19). On April 29, 2009, WHO raised the pandemic alert to phase 5 (human-to-human transmission in at least two countries in one WHO region), and on June 11, 2009, to phase 6 (additionally outbreaks in at least one other country in a different WHO region) (19). As on August 6 2010 worldwide more that 214 countries had reported laboratory-confirmed cases of pandemic H1N1 influenza 2009 and over 18449 deaths had been accounted (20).

Figure 2

Areas with confirmed H1N1 cases and number of deaths by August 15 2010 (adapted from (20)**)**



By October 2009 the virus had passed its peak and incidence declined. On August 10 2010 the WHO Director-General Chan announced the end of the influenza pandemic and declared the H1N1 2009 influenza virus in the post-pandemic phase (21).

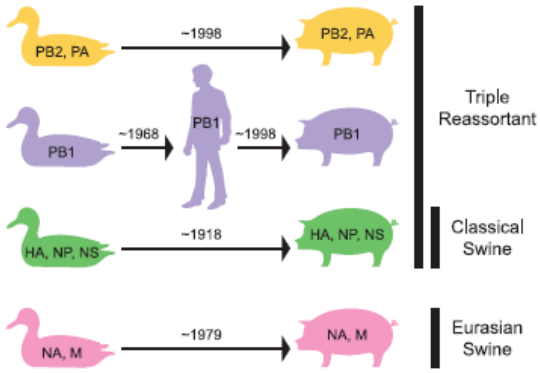
5.2 Viral Characteristics

In April 2009 a new virus gene combination was described that previously has not been seen in swine or human influenza viruses. The 2009 H1N1 pandemic virus derives genes from North American swine triple-reassortant lineage viruses (HA, NP, NS, PB2, PA, PB1) and genes from Eurasian swine virus lineages (M, NA). That M and NA gene segments were originated from an avian influenza virus found in 1979 and not have been described outside Eurasia. HA, NP and NS derived from the classic swine lineage and PB2, PB1 and PA are in the swine triple reassortant (22)

Like the pandemic viruses before pandemic influenza A (H1N1) 2009 is a descendant of the 1918 virus (23).

Figure 3

Influenza A (H1N1) virus reassortant (adapted from (22)



Studies indicate that the virus is binding to both α2,6-linked cellular receptors and 2,3-linked receptors, which are existent in the conjunctivae, distal airways and alveolar pneumocytes. This receptor specificity is an important determinant of establishment and impact of the 2009 pandemic (24).

5.3 Epidemiology

5.3.1 Demographic Characteristics

Varying case fatality, hospitalization and attack rates have been reported .Reasons for varying results are amongst others different capabilities, denominators, and criteria defined for the studies and analyses from several countries. However, there were certain common and comparable demographic characteristics.

Focusing on age distribution highest attack rate but lowest case fatality rate was recorded in different reports among children and young adults (25-31). Hospitalization rates were reported to be 2-3 times higher in children under the age of 5 years than in other age groups (32). Probable clarification for this phenomenon include that children are under a higher exposure in kindergartens and schools with an increased potential for transmission, have a possible higher level of susceptibility to the virus and the fact that children are more likely to be tested. Furthermore, there had been a sparing of the over 65 years olds, which showed the lowest attack rate (25,28,30,33,34) that could be explained by a possible existing immunity to the influenza A (H1N1) 2009 virus strain in people from previous infections or vaccinations of comparable viruses earlier in life (31). In contrast to that, diverse studies recorded the highest case fatality rate among hospitalized patients within the age group of 50 years and more (16, 25-30).

5.3.2 Risk Groups and Conditions

The WHO published risk groups (35) which are similar to those of seasonal influenza:

- infants and young children in particularly < 2 years
- pregnant women
- persons of any age with chronic pulmonary disease (e.g. asthma, COPD)
- persons of any age with chronic cardiac disease (e.g. congestive heart failure)
- persons with metabolic disorders (e.g. diabetes)
- persons with chronic renal disease, chronic hepatic disease,
- persons with certain neurological conditions
- persons with hemoglobinopathies or immunosuppression

- children receiving chronic aspirin therapy
- persons aged 65 years and older

Children under age of 5 years or with certain chronic disease belonged to an increased risk group for complicated influenza infection and therefore treatment in this age group was optimised (36). Furthermore patients with severe obesity (body-mass index>35) or morbidly obesity (body-mass index > 40) showed higher risk for severe illness (27, 29, 30, 37).

A certainly disadvantaged group presented by the indigenous population of North America and Australia showed a several time higher rate of severe infections (27,29,30,38). This fact could be explained by underlying conditions in that group including crowding, higher prevalence of medical disorders, alcoholism, smoking, delayed seeking of health care or even genetic disorders (38).

An estimated one half of the patients who were admitted to hospital had one or more underlying medical conditions (35). The most common reported coexisting illnesses associated with influenza complications or hospitalization included obesity, chronic lung disease, cardiac disease, diabetes mellitus, hypertension, hyperlipidemia, neurologic disease and renal disease (16, 25-30, 39-43).

5.3.3 Transmission

The 2009 pandemic virus followed the mechanisms of person to person transmission through infected respiratory secretions. Transmission mode seems to be similar to seasonal influenza with an uncertain contribution through small particle aerosols, large droplets and possibly contaminated fomites but is not completely understood. Furthermore a possibly fecal-oral transmission has been considered because of the frequently reported accompanied gastrointestinal symptoms (34).

Increased rates of secondary outbreaks were recorded in households, camps, and especially in schools (32, 44-46). S-OIV 2009 presented in a more rapid spread than is characteristic of seasonal influenza (47).

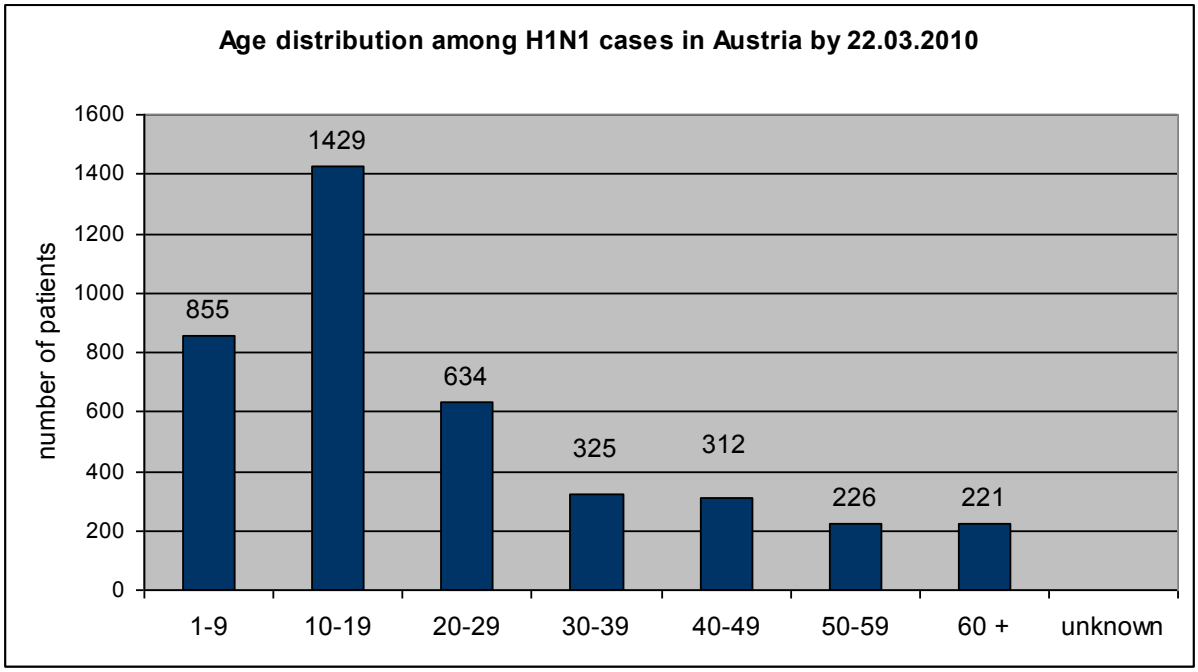
5.3.4 Situation in Austria

In Austria, the first case of infection with pandemic H1N1 virus was notified on April, 29, 2009. Between week 18/2009 and week 11/2010 4017 cases with influenza A (H1N1) 2009 were confirmed and 1569 patients hospitalized. Attack rate was highest

among persons aged between 10-19 years with 1429 cases and lowest among persons over 60 years of age with 221 cases. (48) The estimated number of patients infected with S-OIV in the season 2009/2010 was comparable to previous seasonal influenza seasons with a total of 350.000 to 400.000 cases. 40 persons had a fatal outcome (49).

Figure 4

Age distribution among H1N1 cases in Austria (adapted from (48))



As of week 17/2010 the European Centre for Disease Prevention and Control had registered a total of 2900 fatal cases in EU and EFTA countries. (50)

5.4 Clinical Features

The incubation period is similar to seasonal influenza between 1.5 and 3 days, in few cases even up to seven days (32).

The virus infection presented in a broad spectrum of symptoms ranging from self-limited to severe illness. Most patients showed a typical influenza-like illness including fever, cough, soar throat, wheeze, headache and myalgias. More commonly than in seasonal influenza some cases were accompanied by gastrointestinal symptoms like diarrhea, vomiting and nausea (34,39) (16,26-29,40,46,51) .

5.4.1 Complications

Most common clinical syndromes that led to severe illness, respiratory failure or death were primary viral pneumonia, secondary bacterial pneumonia mostly caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus. pyogenes* and exacerbation of underlying chronic conditions, including exacerbation of COPD or Asthma bronchiale (26, 27, 29, 30, 34, 40). An Australian study (43) reported rather unusual complications including febrile seizures, rhabdomyolysis and Guillain–Barré syndrome.

5.4.2 Radiographic findings

Diffuse mixed interstitial and alveolar infiltrates unifocal or multifocal were the most common diagnostic finding (16, 26, 27, 29, 30, 39, 43). Lobar and multilobar parts were affected in cases with accompanied bacterial coinfection (26). Chest computer tomography showed ground-glass opacities, air bronchograms, bronchovascular margins and alveolar consolidation consistent with bacterial pneumonia (28,42).

5.4.3 Laboratory Findings

Most of the 2009 influenza A (H1N1) cases presented with normal or low leukocyte counts, lymphocytopenia and elevated levels of serum aminotransferases, lactate dehydrogenase, creatine kinase and creatinine (29,39). An observational study of critical ill patients in Mexico reported frequently elevated counts of creatine kinase (40). Results from investigations in China demonstrated common low normal leukocytes and lymphopenia (28). Jain and associates reported in an evaluation of 246 hospitalized patients with S-OIV 2009 in the United States leukopenia in about 20%, leukocytosis in 18%; of 234 patients 14% had thromobocytopenia and 9% thrombocytosis; elevated aminotransferases were confirmed in around 40% of 131 patients and anemia in 37% of a total number of 238 (26). A prospective analyses of critical ill patients with S-OIV 2009 in Canada presented moderately elevated creatine kinase and normal leukocyte count (29).

5.5 Pathological Features

Histopathological analyses on lung tissues presented alveolar damages with hyaline membranes, alveolar septal edema, alveolar hyperplasia, tracheitis, necrotizing bronchiolitis, alveolar hemorrhage and bronchopneumonia (42, 52-54). Primary

infected were alveolar lining cells pneumocytes type I and II but also upper respiratory and tracheobronchial epithelial cells and mucosal glands (52, 53). Other postmortem autopsy studies found hemophagocytosis, thromboemboli, hemorrhage and myocarditis (55).

Figure 5

Alveolar with hyaline membranes (54)

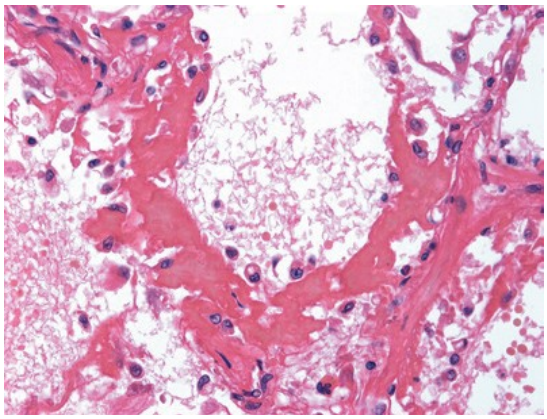
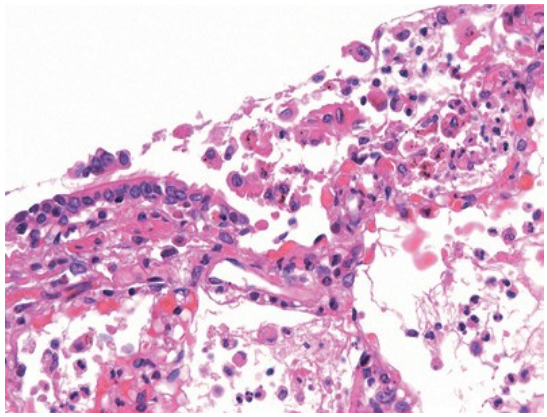


Figure 6

Necrotizing bronchilotis (54)



Patients who died or had acute respiratory distress syndrome presented higher plasma levels of interleukin-6, interleukin-10, interleukin-15, granulocyte-colony-stimulating factor, interleukin 1α, inteleukin-8, interferon-inducible-protein and tumor necrosis factor α (55).

5.6 Diagnoses

The WHO published case descriptions for diagnosis:

- Influenza-like illness: fever and soar throat, coat, or both in the absence of another known cause
- Probable case: influenza-like illness with positive test for influenza A but negative for H1 and H3 by real- time reverse transcriptase-polymerase chain reaction test (rRT-PCR)
- Confirmed cases: influenza-like illness with confirmed H1N1 influenza infection based on rRT-PCR or viral culture

5.6.1 Clinical Signs

A diagnosis may further be made on clinical and epidemiological grounds when a patient shows influenza-like symptoms and virus circulation is known in a community or area. Influenza-like illness includes fever, soar throat, cough, rhinorrhea, headache, muscle pain and mailaise (35). Patients could show one or all symptoms. However, because of the wide spectrum of clinical signs several misdiagnoses were confirmed.

5.6.2 Diagnostic Tests

Numerous diagnostic tests with different sensitivity and specificity are available to detect influenza. Furthermore the use depends on clinical settings, commercial disposal and time.

5.6.2.1 Rapid Influenza Diagnostic Tests (RIDT)

There are different rapid tests available to identify influenza. The tests are easy to perform, offer results within 30 minutes and are therefore practical for clinical management. Depending on which test is used, RIDT differentiate between influenza A and influenza B or detect both but do usually not distinguish between influenza A subtypes (1). Rapid influenza tests show sensitivities of 10-70% (56) and missed many infections with pandemic influenza A (H1N1) (35) and should therefore not be used primary for detection of S-OIV 2009.

5.6.2.2 RT-PCR

The viral RNA detection with real-time reverse transcriptase-polymerase-chain-reaction seemed to be the best method for diagnosis of S-OIV 2009 (35). RT-PCR tests are most sensitive and specific for influenza (56). Specimen can be taken from nasopharyngeal aspirates and swabs of endotracheal and bronchoscopic aspirates, for detection lower respiratory illness. The testing requires more technical demands and equipment and results can take several days. However, during pandemic influenza A (H1N1) 2009 the RT-PCR was the golden standard for diagnosis and in most cases available through state laboratories.

5.6.2.3 Virus Isolation

Influenza samples can be inoculated onto cell cultures of rhesus monkey kidneys, cynomolgus monkey kidneys or Madin-Darby canine kidneys. Furthermore less

shared virus can be injected and cultured on embryonated eggs. Results can be provided within 3 days (1).

5.6.2.4 Immunofluorescence Tests

Direct or indirect immunofluorescence tests detect influenza antigens but are less sensitive than RT-PCR tests (57). Processing time lasts from 2 to 4 hours.

5.6.2.5 Serology

Serology assays including microneutralisation, fixation and hemagglutination inhibition can retrospectively detect influenza infections. The testing identifies antibody levels in paired serum samples. The method is not appropriate and common for testing of acute illness because increased antibody levels can originate from former influenza infections (1).

Table 2

Available diagnostic tests adapted from (56))

Influenza Diagnostic Tests	Rapid influenza diagnostic tests	Direct and indirect immunofluorescence assays	Viral isolation in tissue cell culture	Nucleic acid amplification tests
Method	Antigen detection	Antigen detection	Virus isolation	RNA detection
Availability	Wide	Wide	Limited	Limited
Tipical Processing Time	30 minutes	2 – 4 hours	2 -10 days	48 – 96 hours
Sensitivity for S-OIV	10-70%	47–93%	-	86 – 100%
Distinguishes S-OIV from other influenza A viruses?	no	No	Yes	Yes

5.7 Treatment

WHO published guidelines for treatment of S-OIV 2009 (35):

- Patients with mild illness and not at high risk can be treated symptomatically and no antivirals have to be given.
- Patients with mild or uncomplicated illness but at high risk for developing complications should be early treated with antiviral drugs.
- All patients presenting severe and complicated illness should be treated with oseltamivir as soon as possible including pregnant women, young children under age of 2 and neonates. In case of no responding oseltamivir should be

given in increased dose (up to 150 mg twice a day) and for longer duration (10 d). Patients with resistance to oseltamivir should be treated with zanamivir.

Decision for treatment should not await laboratory confirmation. Patients with severe illness or progression of symptoms for more than 48 hours should be treated empirically with antiviral drugs immediately even if confirmation is not available. In case of negative results therapy should be continued until an alternative diagnose is indicated.

5.7.1 Antiviral Therapy

The effect of the currently available antiviral drugs depends on an early initiation and best result is shown by treatment within the first 48 hours. Data indicate that pandemic virus is susceptible to the neuraminidase inhibitors oseltamivir and zanamivir but almost always resistant to M2 inhibitors amantadine or rimantadine (34,58). Several studies suggest that an early antiviral therapy in severe virus infection with oseltamivir causes a reduction of duration of hospitalization and number of deaths among hospitalized patients (26,40).

5.7.1.1 Neuraminidase Inhibitors

Oseltamivir (Tamiflu) and Zanamivir (Relenza) take effect by inhibiting the enzyme neuraminidase. Oseltamivir is available orally and is indicated for patients one year of age and older. Resistance to oseltamivir based on a His276 Tyr mutation in viral neuraminidase was reported (59)(34). Most resistant cases were sporadic but there was a higher rate of resistance in patients with immunosuppressive therapy. Furthermore reassortment of novel S-OIV 2009 and seasonal influenza A (H1N1) viruses suggested higher resistance prevalence. Cases associated with resistance have also been reported in patients without treatment with oseltamivir but there is no evidence for community transmission (59,60). Zanamivir (Relenza) is not orally bioavailable and considered the alternative treatment for patients with resistance to oseltamivir.

Table 3

Available treatment (adapted from (1))

	Oseltamivir	Zanamivir
Protein target	Neuraminidase	Neuraminidase
Activity	A and B	A and B
Side effect	Gastrointestinal	Bronchospasm
Metabolism	hepatic	none
Drug interactions	Probenecid	none
Dose adjustment needed	CrCL < 30 ml/min severe liber dysfunction	none
Contraindications	none	underlying airway disease
Therapy of S-OIV	adults and children > 3 months of age	adults and children > 7 years of age
Prophylaxis S-OIV	adults and children > 1 year of age	

5.7.2 Antibiotic Therapy

As mentioned the pandemic influenza A (H1N1) virus 2009 was associated with an increased risk of secondary bacterial pneumonia in most cases caused by *Staphylococcus aureus*, *Streptococcus pneumoniae* and *S. pyogenes*. In these cases antibiotics should be prescribed immediately.

5.8 Vaccination

Vaccination is the primary prevention and most effective control of influenza-caused morbidity and mortality. When the novel swine influenza A (H1N1) 2009 virus emerged in spring 2009 the seasonal influenza vaccine production was already in process. The annual vaccine did not protect against S-OIV and therefore new vaccines have been developed. The vaccine was available in both live-attenuated and inactived formulations (61). Live-attenuated vaccines were only indicated for nonpregnant persons aged between 2 – 49 years, and with an absence of chronic medical conditions or immunosuppressant therapy. Furthermore live vaccines were not licensed for children under age of five with asthma. A single dose is adequate for persons aged less than 10 years of age, children aged 6 months to 9 years should require two doses of influenza A (H1N1) 2009 monovalent vaccines(61).

The CDC's Advisory Committee on Immunization Practice defined priority groups and an order for receiving vaccination: pregnant women, caregivers or cohabitants of infants younger than 6 months, health care workers, healthy children and young adults aged from 6 months to 24 years, persons aged 25-64 years who have medical conditions that put them at higher risk for influenza-related complications (62).

Safety of novel 2009 H1N1 vaccine was tested in studies and showed similar results to seasonal influenza vaccine (63).

6 Materials and Methods

6.1 Study objectives

To determine the number of confirmed pandemic H1N1 influenza cases in South East Austria during pandemic 2009/2010.

To describe demographic data, baseline characteristics and patients outcome of confirmed pandemic H1N1 influenza cases.

To describe clinical management and treatment procedures in confirmed pandemic H1N1 influenza cases.

To describe the development of laboratory values in the first seven days of disease of confirmed pandemic H1N1 influenza cases.

6.2 Study Design

This is a retrospective survey of patients with PCR-proven influenza H1N1 infection in South East Austria confirmed between October 1, 2009 and January 19, 2010. 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. PCR confirmed 624 cases of H1N1 influenza. Complete data were obtained from 197/624 (32%) of patients. Reasons for missing 68% of patients are described below (see 6.3).

RT-PCR testing for the whole region of South-East Austria was done at the Institute for Hygiene, Microbiology and Environmental Medicine, Medical University of Graz and at the Institute for Hospital Hygiene and Microbiology, University Hospital of Graz.

The study was conducted at the Section of Infectious Diseases, Division of Pulmonology, Medical University of Graz. The Case Report Forms were completed with the use of MEDOCS, the electronic data base of patients at the Medical University Hospital Graz and other Hospitals of South-East Austria.

Research ethic board approval was granted by the local ethic committee. The need for a priori informed consent was waived because of the noninterventional study design.

6.3 Data collection

We retrospectively studied data from 197 patients with confirmed S-OIV who were hospitalized or presented to outpatient clinic only by reviewing medical charts and laboratory findings. The data collection started in the beginning of May 2010 and ended in December 2010.

Eligible patients included all individuals admitted to participating hospitals in South East Austria with PCR- confirmed 2009 influenza A (H1N1) infection with complete data. Complete data included demographic data, clinical characteristics and outcome and laboratory values. 25/624 (4%) of patients have been excluded from the study because not all information has been available. Data from 402/624 (64%) of patients could not been surveyed as they were, in the vast majority of cases, not submitted to hospital and presented to their family doctor only and therefore no data was available in MEDOCS, the electronic data base. At given time points some laboratory values were not available for all 197 patients.

All of the 197 patients had influenza-like illness and laboratory confirmed S-OIV infection. H1N1 influenza infection was diagnosed by a positive polymerase chain reaction (PCR) result from respiratory samples obtained via a nasopharyngeal-swab specimen or bronchoalveolar lavage samples performed during the admission episode.

We reviewed the first seven days of clinical admission.

The following data were extracted from medical records:

• Demographic data (age, gender)

• date of PCR-confirmation

• date und time of admission to the hospital

• Rapid influenza diagnostic test results

• Care unit

• History of vaccination

• Laboratory values from the first seven days

• Complications

• Antiviral treatment

• Antibiotic treatment

• Outcome

We calculated the duration of hospitalization, time spent in intensive care units (ICUs) as well as time from symptom onset to treatment.

In patients who were admitted, development of laboratory values including white blood cells, platelets, serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), bilirubin, GGT, lactate dehydrogenase (LDH), PCT, C-reactive protein (CRP), creatine kinase, Troponin T and lipase were analyzed for the first seven days of disease.

6.4 Definitions

A case was defined as a person with confirmed influenza A (H1N1) 2009 infection by a positive result of a real-time reverse-transcriptase–polymerase-chain-reaction (RT-PCR) assay that was hospitalized or presented to outpatient clinic only.

For time calculation, the day of admission to hospital was considered to be day 1.

We categorized patients according to the age groups: 0 to 10 year of age, 11 to 20 years, 21 to 30 years, 31 to 40 years, 41 to 50 years, 51 to 60 years, 61 to 70 years, 71 to 80 years, 81 to 90 years and 91 of age or older.

Laboratory values have been calculated based on the standard values in accordance to the local area and are depicted in table 4.

Table 4

Laboratory standard values

Values	Normal
Leukocytes	4400 – 11300 mm³
Thrombocytes	140 – 440 G/l
Neutrophil granulocytes	1800 – 7700 mm
Lymphocytes	1000 – 4800 mm
Monocytes	200 – 1000 mm
Eosinophil granulocytes	- 7000 mm
CRP	- 8.0 mg/l
PCT	-0.50 ng/ml
Creatinine	0.50 – 1.00 mg/dl
ALT	- 35 U/l
AST	- 30 U/l
Bilirubin	0.10 – 1.20 mg/dl
GGT	- 38 U/l
LDH	120 – 240 U/l
Creatine kinase	- 145 U/l
Troponin T	- 0.030 mg/ml
Lipase	- 60 U/l

6.5 Analysis

Descriptive statistics included frequency analyses (percentage) for categorical variables and continuous variables were reported as means and medians.

7 Results

7.1 Demographic data

During the study period a total number of 624 patients in South East Austria were confirmed to have S-OIV infection. Complete data sets were available from 197 patients. Influenza A (H1N1) 2009 was more frequently diagnosed in men than in woman, among the 197 patients 117 (59.4%) were male and 80 (40.6%) female. The mean age was 24.6 years, median was 16 years. Highest number of confirmed cases was among children and young adults. (0 to 20 years of age). The age of patients with confirmed S-OIV infection ranged from 1 month to 79 years. Demographic data are depicted in table 4, figure 7 and figure 8.

Table 5

Demographic Data of study population

Characteristics	All Patients of study group n=197 (%)
age	
1-10	62 (31.6)
11-20	56 (28.4)
21-30	14 (7.1)
31-40	17 (8.6)
41-50	14 (7.1)
51-60	20 (10.2)
>60	14 (7.1)
mean age	24.6
male	117 (59.4)
female	80 (40.6)

Figure 7

Gender distribution in total number and percentage

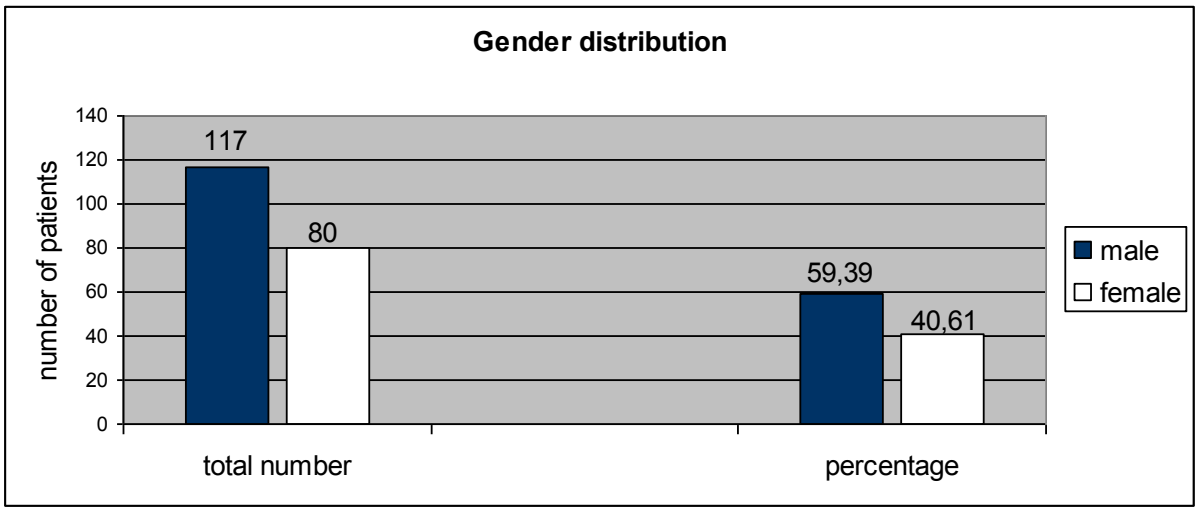
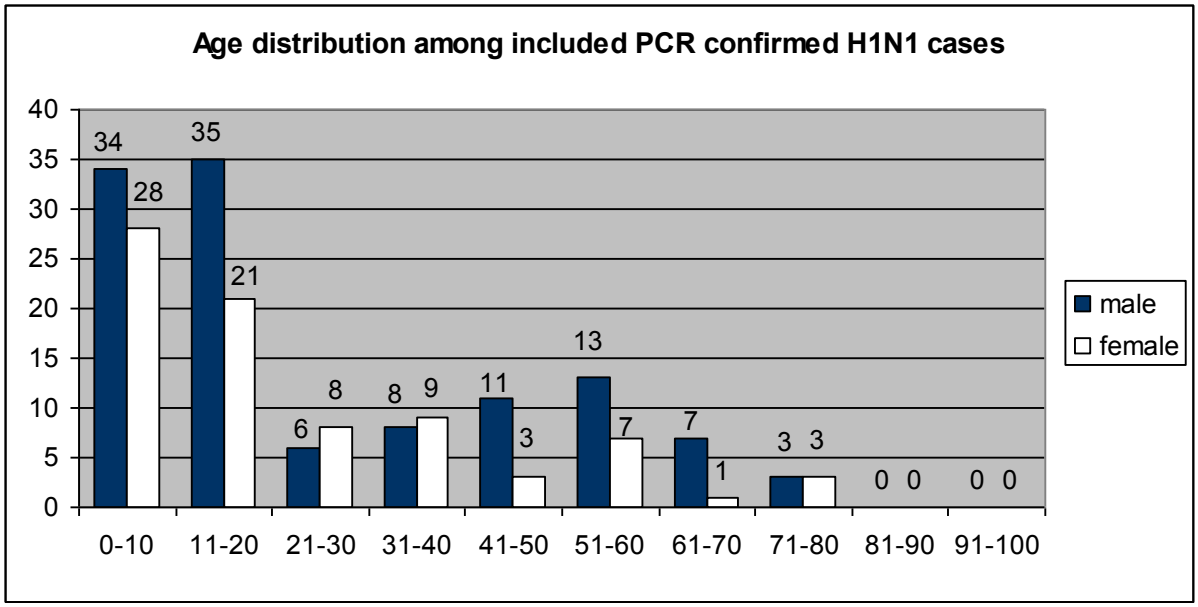


Figure 8

Age distribution among female and male



During the pandemic in South East Austria 3/197 (1.5%) of cases were confirmed in October, 109/197 (55.3%) in November, 81/197 (41.1%) in December and 4/197 (2.0%) in January. In our study the first case was confirmed on October 28th 2009 the last on January 18th 2010. Epidemiologic curve presented four peaks with over 10 confirmed cases per day on November, 11, 2009, November, 16, 2009, November 23, 2009 and November, 30, 2010. Distribution in detail is described in Figure 9.

Figure 9

Epidemiologic curve of confirmed case of infection with S-OIV from October, 2009 to January, 2010 among study population in months

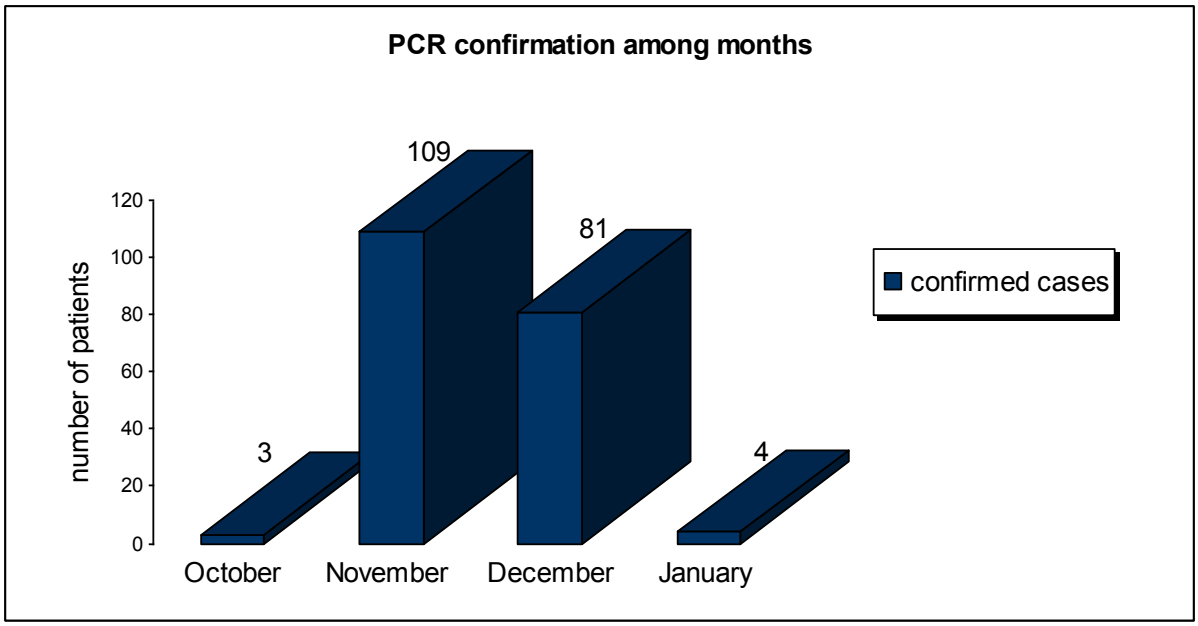
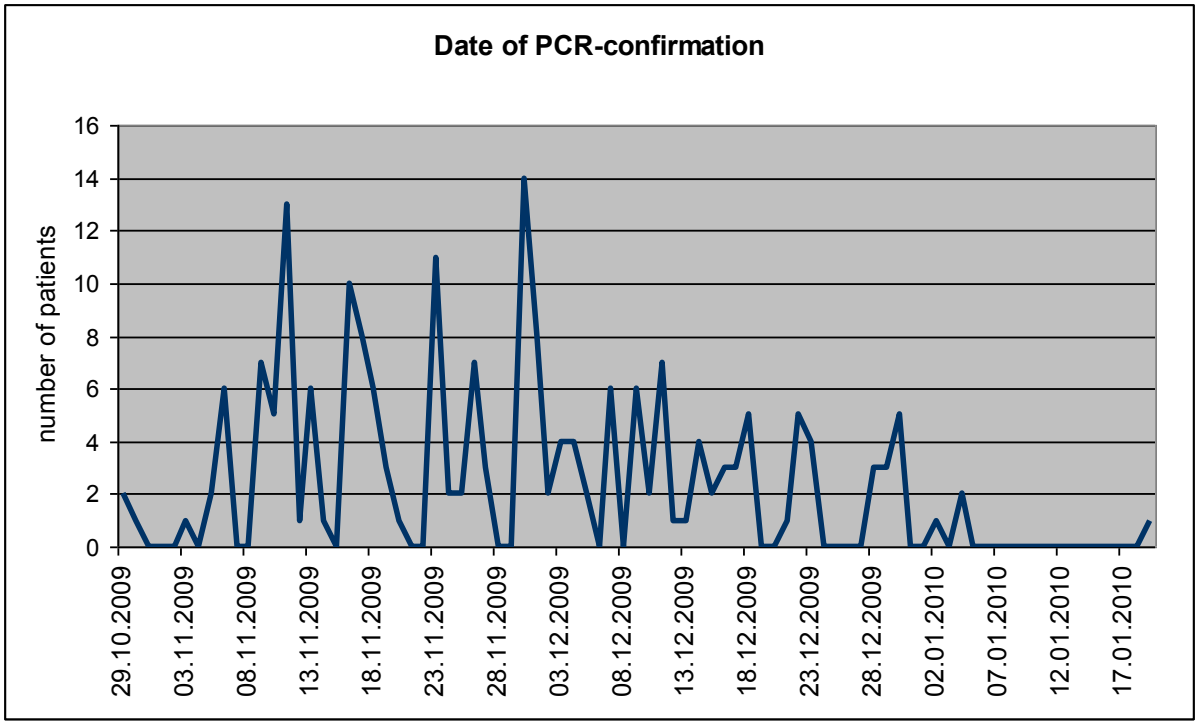


Figure 10

Epidemiologic curve of date of confirmation



7.2 Rapid influenza diagnostic test

Rapid antigen test ("BinaxNow", Inverness Medical; Maine, U.S.) was conducted in 26/197 (13.2%) of cases. Sensitivity for the detection of pandemic influenza A (H1N1) 2009 was low with test results being falsely negative in 23/26 (88.5%) of cases.

Table 6

Sensitivity of rapid influenza diagnostic test

Rapid influenza test	All Patients of study group n=197 (%)
not conducted	171 (86.8)
conducted	26 (13.2)
positive	3 (11.5)
false negative	23 (88.5)

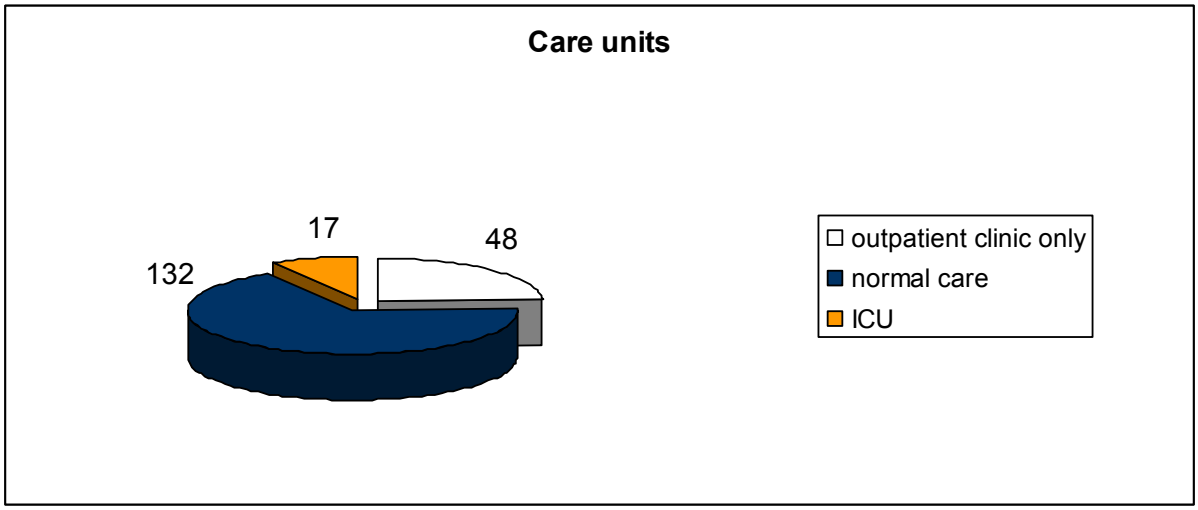
7.3 Hospitalization

Among the study population 17/197 (8.6%) of patients required intensified medical care, 132/197 (67%) were admitted to normal wards and 48/197 (24.4%) presented to outpatient clinic only. Age of patients who required intensive medical care ranged

from 9 to 69 years (median 47 years and mean 42.4 years). 13/17 (76%) of patients at ICU were male and 4/17 (24%) female. Time from symptom onset to presentation at hospital ranged from 0 to 14 days with average duration of 1.6 days and a median of 1 day.

Figure 11

Care units

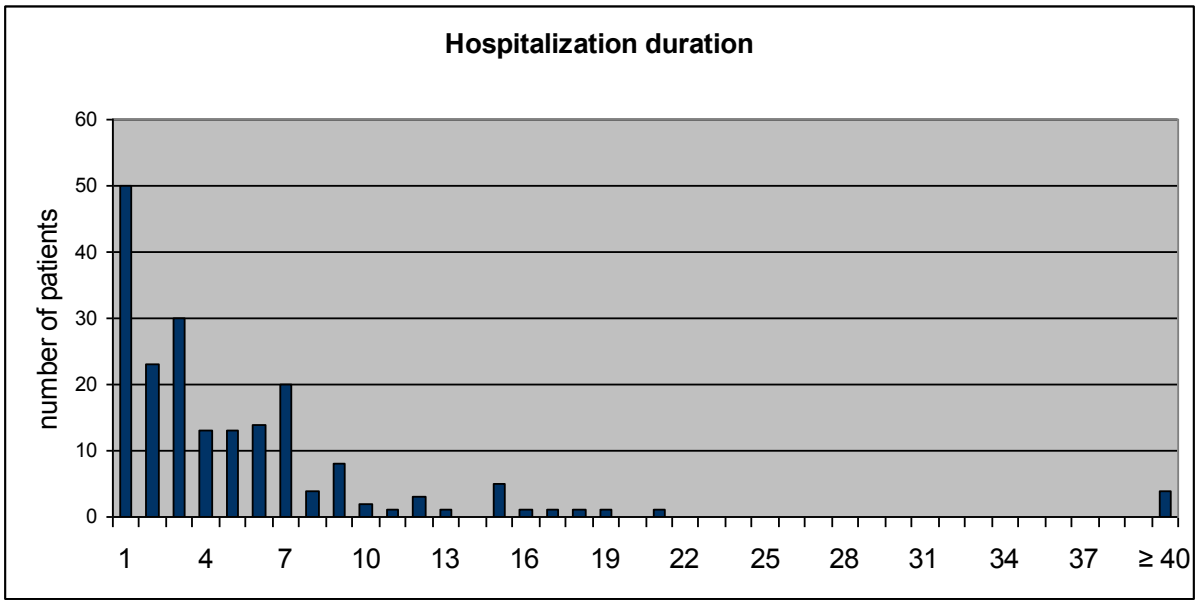


7.3.1 Duration of hospitalization

The duration of hospitalization ranged from 1 to 60 days with a median of 3 days and average time of 5.5 days. Four patients presented duration time of about 40 days or more. Among these patients all required intensified medical care and survived Duration in detail is depicted in figure 12.

Figure 12

Duration of hospitalization among 196 patients with available data.



7.3.2 Duration at ICU

17 cases (8.6%) were admitted to intensive care. Duration at ICU ranged from 1 to 50 days with a median of 8 days and average duration of 12.2 days. Out of the 17 patients 5 died at ICU and 12 were discharged.

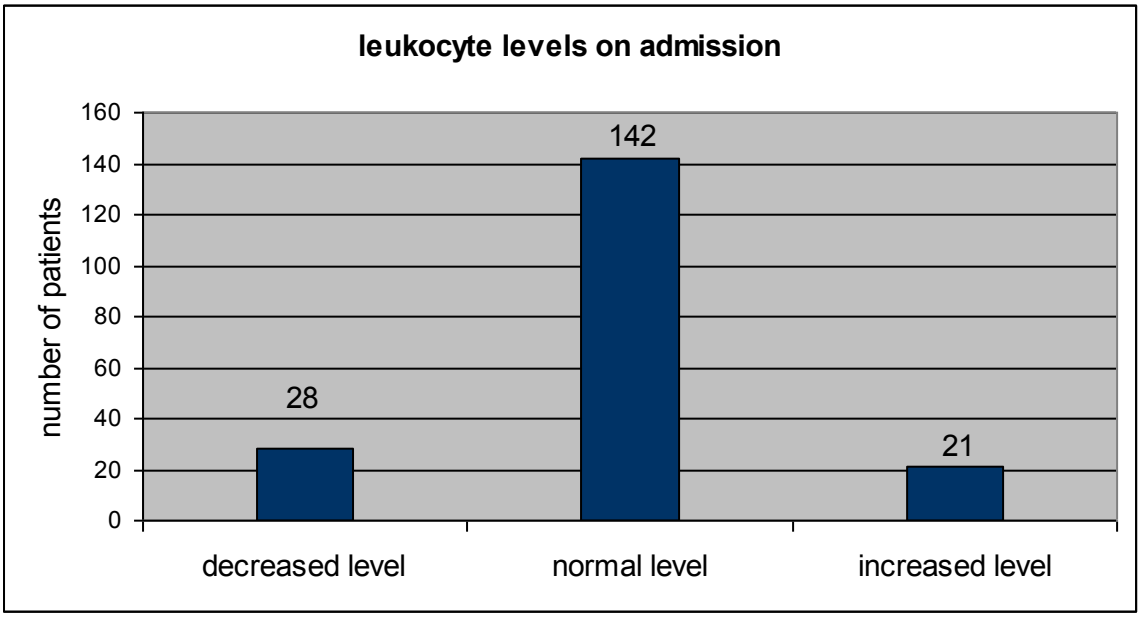
7.4 Laboratory values

7.4.1 Leukocytes at presentation

At the time of admission 28/191 (15%) of patients who were tested had leukopenia, 142/191 (74%) had normal leukocyte counts and 21/191 (11%) presented with leukocytosis. Leukopenia ranged from 4.3 G/l to 0.6 G/l and leukocytosis ranged from 11.4 G/l to 48.4 G/l.

Figure 13

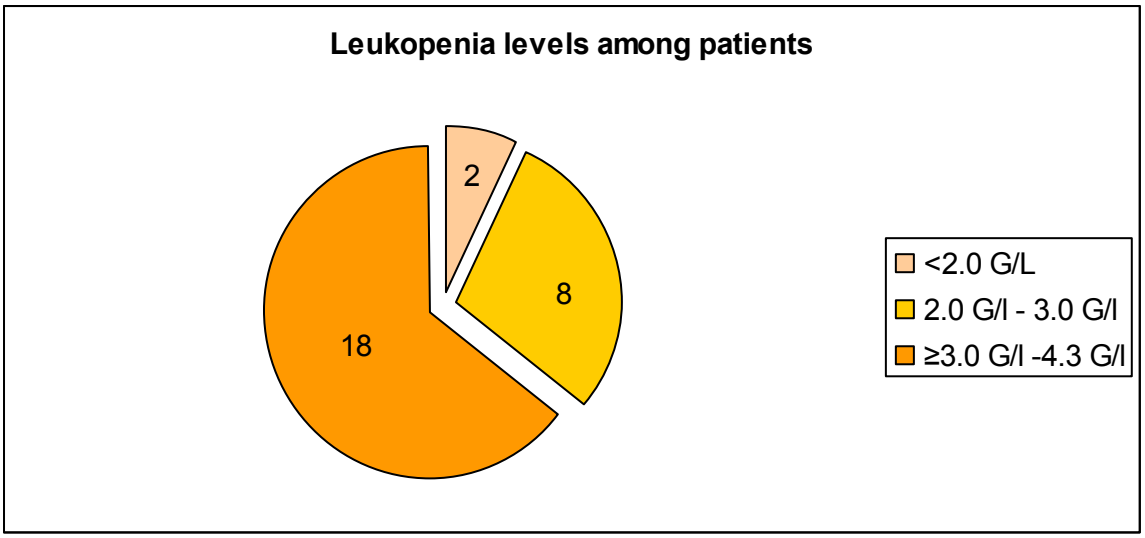
Leukocyte levels on admission among 191 patients with data available.



Out of 28 patients with decreased levels of leukocytes at presentation 18 (64%) patients had counts ranging from 3.0 G/l to 4.3 G/l, 8 (29%) between 2.0 G/l and 3.0 G/l and 2 (7%) less than 2.0 G/l.

Figure 14

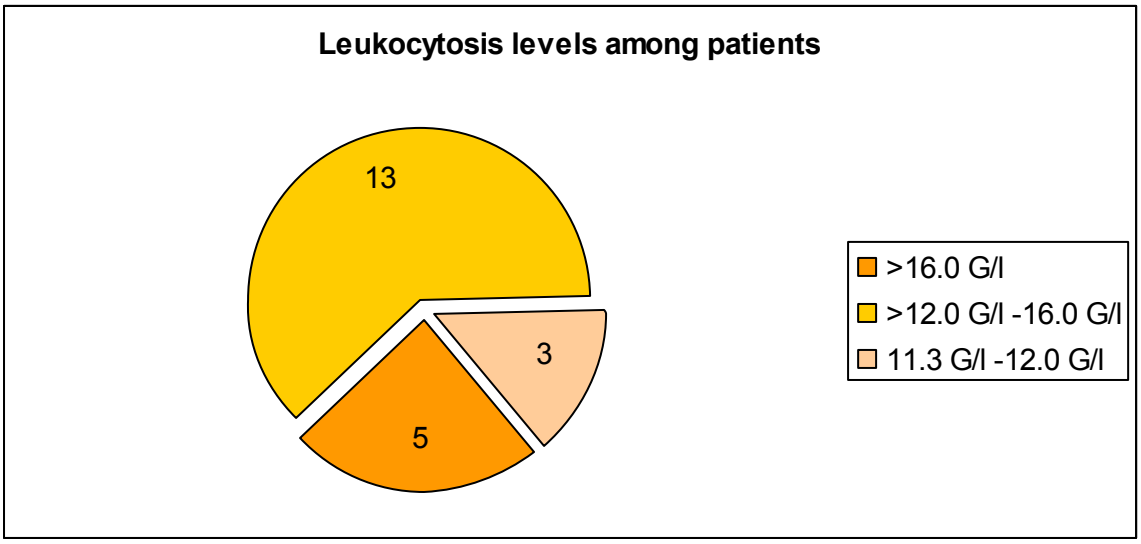
Leukopenia levels at presentation among 28 patients.



Out of 21 patients with increased levels of leukocytes at presentation 3 (14%) patients had counts between 11.3 G/l to 12.0 G/l, 13 (62%) counts between 12.0 G/l and 16.0 G/l and 5 (25%) counts of more than 16.0 G/l.

Figure 15

Leukocytosis levels at presentation among 21 patients.



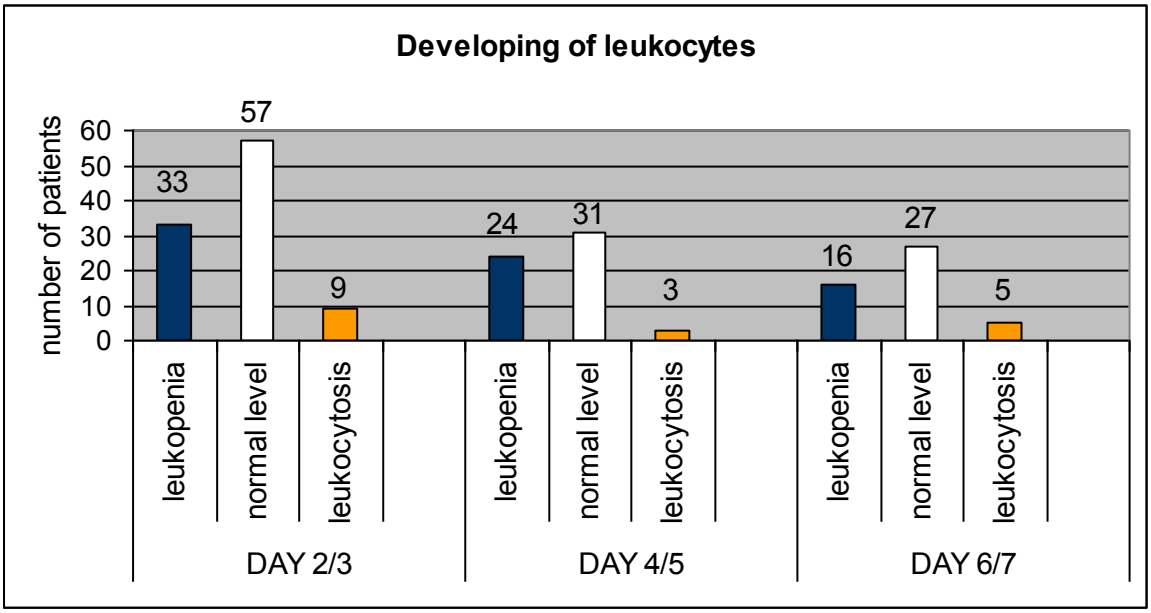
7.4.2 Development of leukocytes in the first seven days

At day 2/3 the number of patients with leukopenia elevated from 28 to 33 out of 99 patients with available results with counts ranging from 4.3 G/l to 0.35 G/l and number of patients with leukocytosis declined from 21 to 9 ranging from 11.7 G/l to 20.1 G/l. At day 4/5 24 patients among 58 who were tested had decreased leukocyte counts ranging from 4.3 G/l to 0.93 G/l and 3 patients increased counts ranging from

12.9 G/l to 30.7 G/l. At day 6/7 16 patients among 48 who were tested had decreased leukocyte counts ranging from 4.2 G/l to 0.86 G/l and 5 patients increased counts ranging from 13.4 G/l to 40.7 G/l.

Figure 16

Development of leukocytes levels in the first seven days.

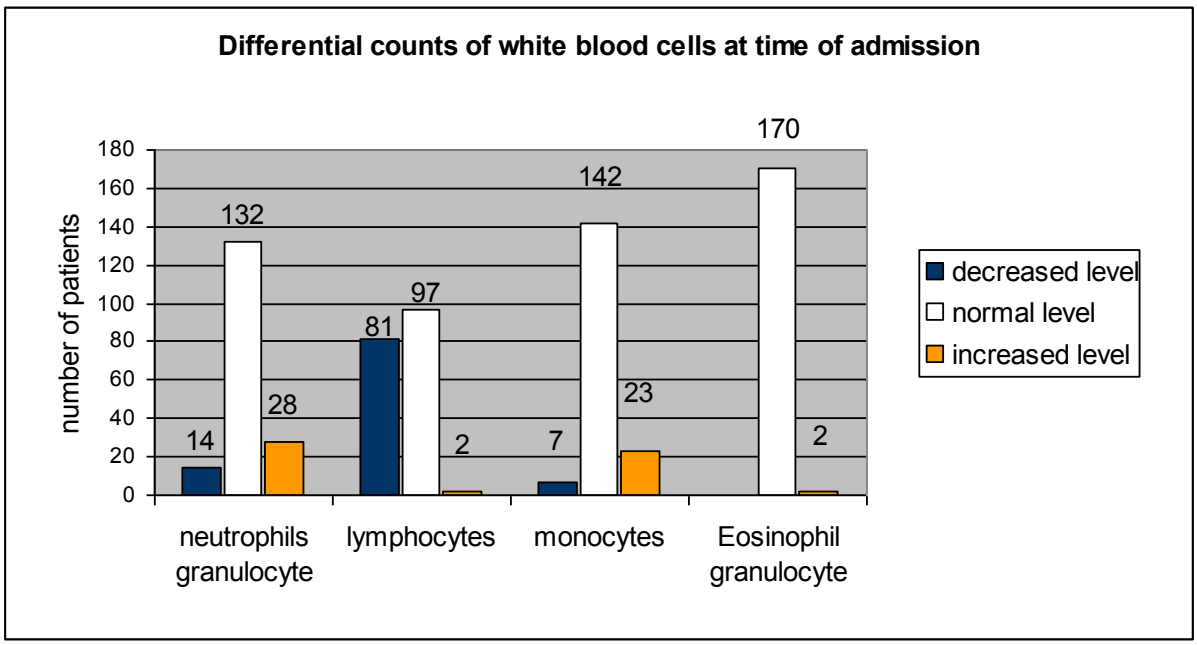


7.4.3 Differential counts of white blood cells at presentation

Levels of neutrophil granulocytes, lymphocytes, monocytes and eosinophile granulocytes have been analysed. Majority of patients showed normal differential counts at presentation. 14 patients had decreased (ranging from 1.80 G/l to 0.40 G/l) and 28 increased (ranging from 7.80 G/l to 42.10 G/l) levels of neutrophil granulocytes. At the time of admission 81/180 (45%) patients presented with lymphocytopenia (ranging from 0.99 G/l to 0.14 G/l) and 2 patients had lymphocytosis (with 5.86 G/l and 6.24 G/l). 7 patients showed monocytopenia and 23 patients had monocytosis (ranging from 1.10 G/l to 2.90 G/l). At presentation only two patients had an increased level of eosinophil granulocytes (0.79 G/l and 164 G/l).

Figure 17

Differential counts of white blood cells at time of admission.



Levels of neutrophil granulocytes, lymphocytes, and monocytes and eosinophils in detail at the time of admission are described in figure 18 to 21.

Figure 18

Neutropenia at time of admission.

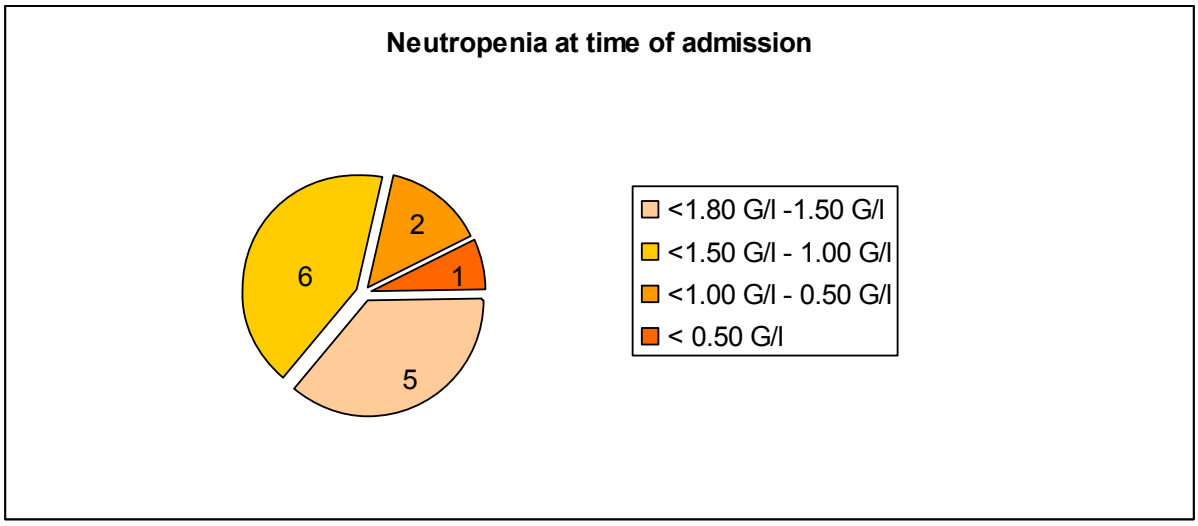


Figure 19

Neutrophilia at time of admission.

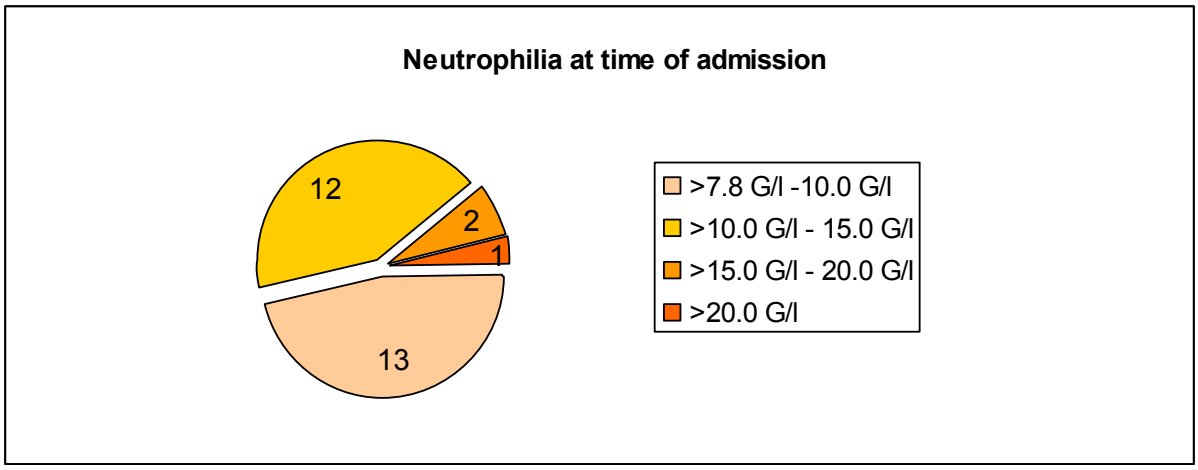


Figure 20

Lymphocytopenia at time of admission.

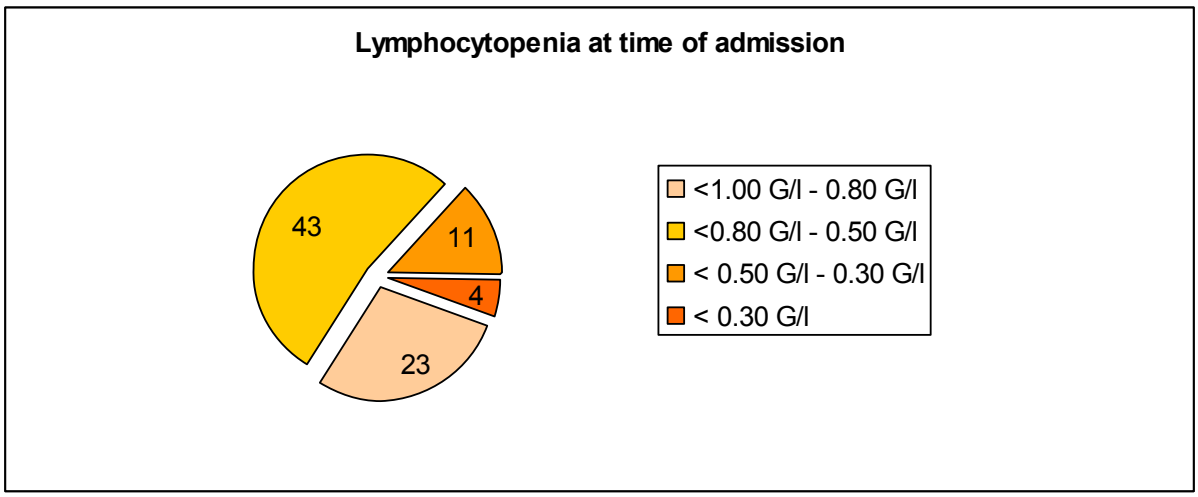
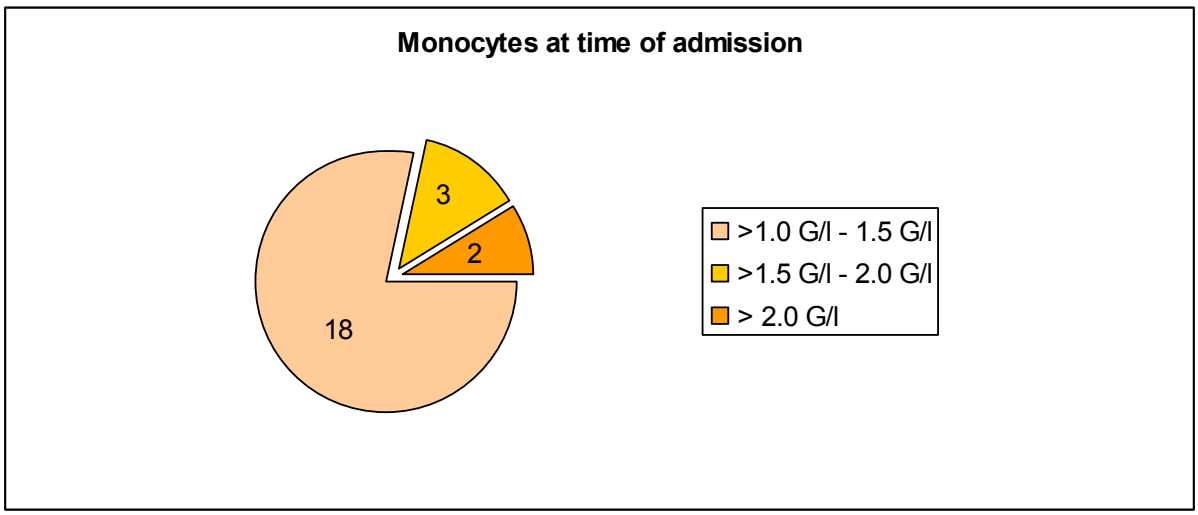


Figure 21

Monocytes at time of admission.



7.4.4 Development of differential blood counts in the first seven days

In the course the majority of patients had normal differential blood counts. None of the patients had increased eosinophil granulocytes between days 2 to day 7. Results are shown in figure 22 to 24.

Figure 22

Development of neutrophil granulocyte levels in the first seven days.

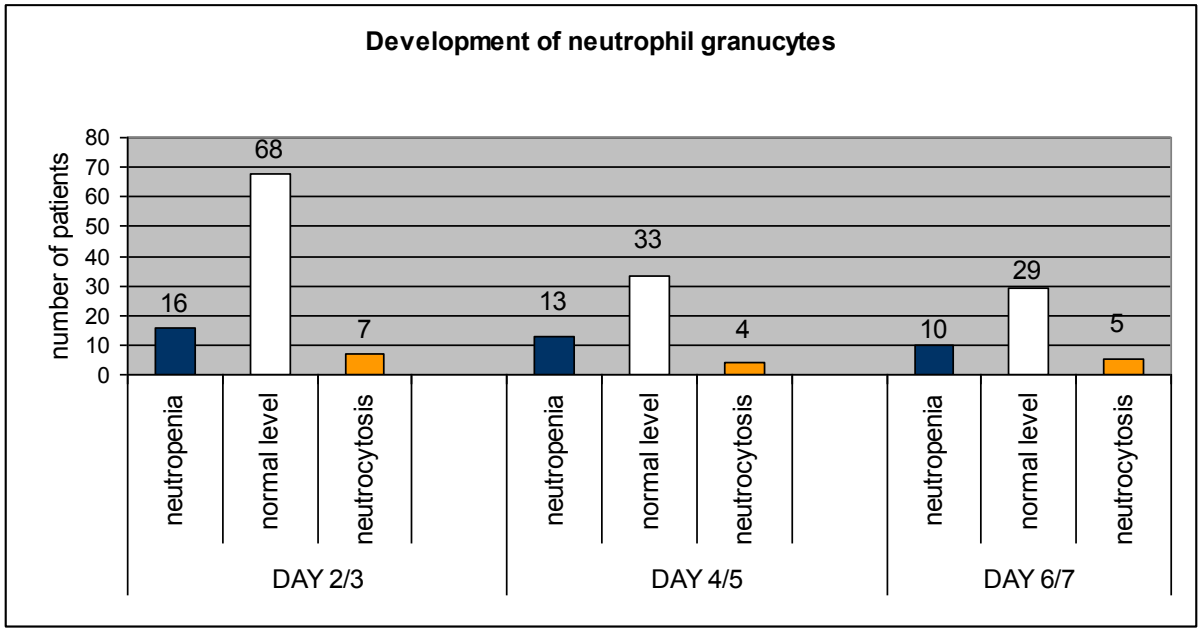


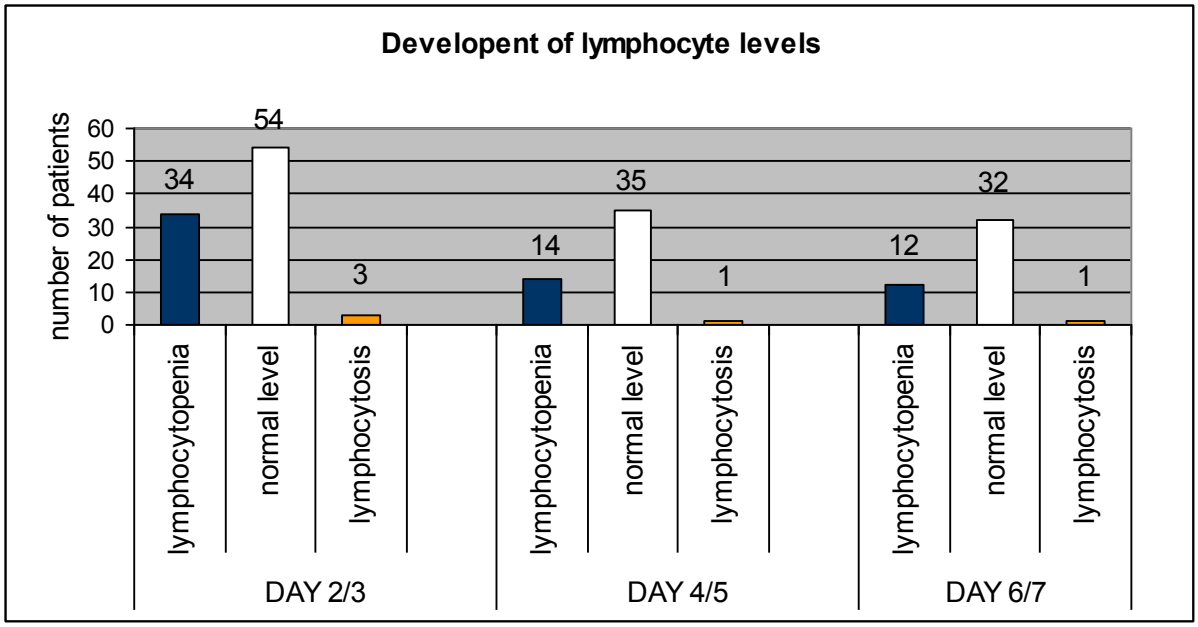
Table 7

Neutropenia and neutrophilia in the first seven days.

	neutropenia range	neutrophilia range
day 2/3	1.70 G/l to 0.30 G/l	8.1 G/l to 16.7 G/l
day 4/5	1.70 G/l to 0.05 G/l	7.9 G/l to 27.6 G/l
day 6/7	1.70 G/l to 0.60 G/l	9.1 G/l to 37.4 G/l

Figure 23

Development of lymphocyte levels in the first seven days.



Lymphocytopenia ranged on all days from 0.90 G/l to 0.10 G/l. At day 2/3 three patients had lymphocytosis ranging from 5.1 G/l to 6.0 G/l, at day 4/5 one patient with a level of 6.2 G/l and at day 6/7 one patient with a level of 5.6 G/l.

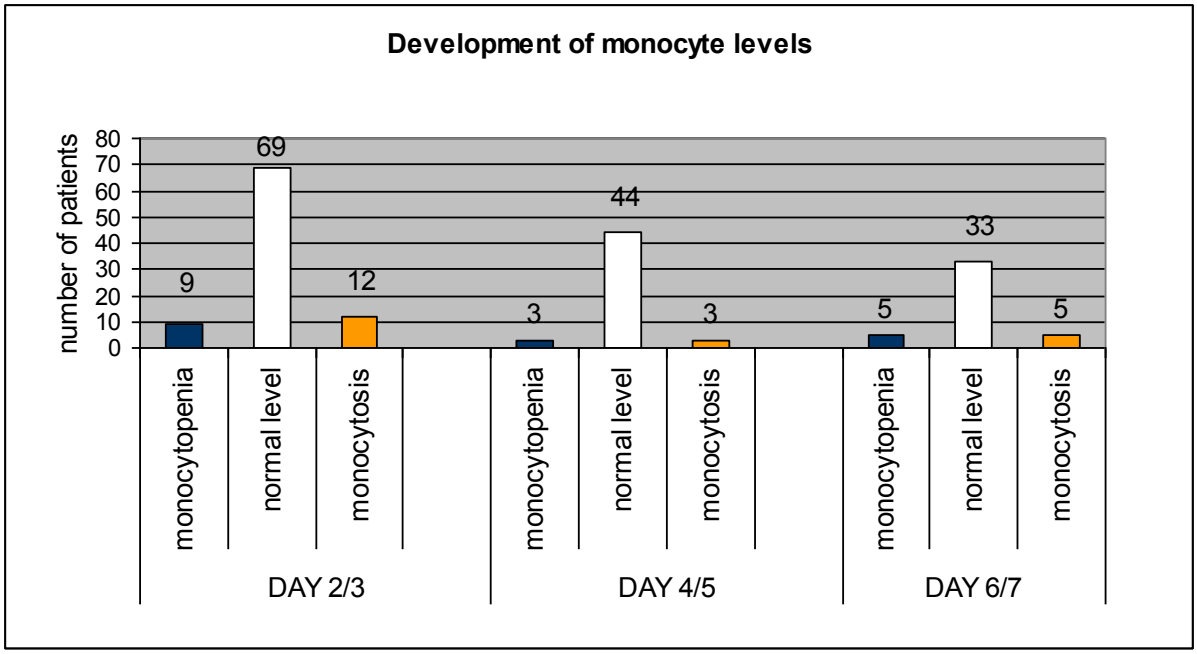
Table 8

Lymphocytopenia and lymphocytosis range in the first seven days.

	lymphocytopenia range	lymphocytosis range
day 2/3	0.90 G/l to 0.10 G/L	5.1 G/l to 6.0 G/l
day 4/5	0.90 G/l to 0.10 G/L	6.2 G/l
day 6/7	0.90 G/l to 0.10 G/L	5.6 G/l

Figure 24

Development of monocyte levels in the first seven days.



At day 2/3 monocytosis ranged to 1.6 G/L at day 3/4 and 6/7 ranged to 1.5 G/l.

7.4.5 C-reactive protein at presentation

On admission 106/193 (54.9%) of patients with laboratory tests had increased CRP levels, while 87/193 (45.1%) patients had normal level. Increased CRP levels ranged from 8.7 mg/l to 325 mg/l.

Figure 25

CRP levels at time of admission.

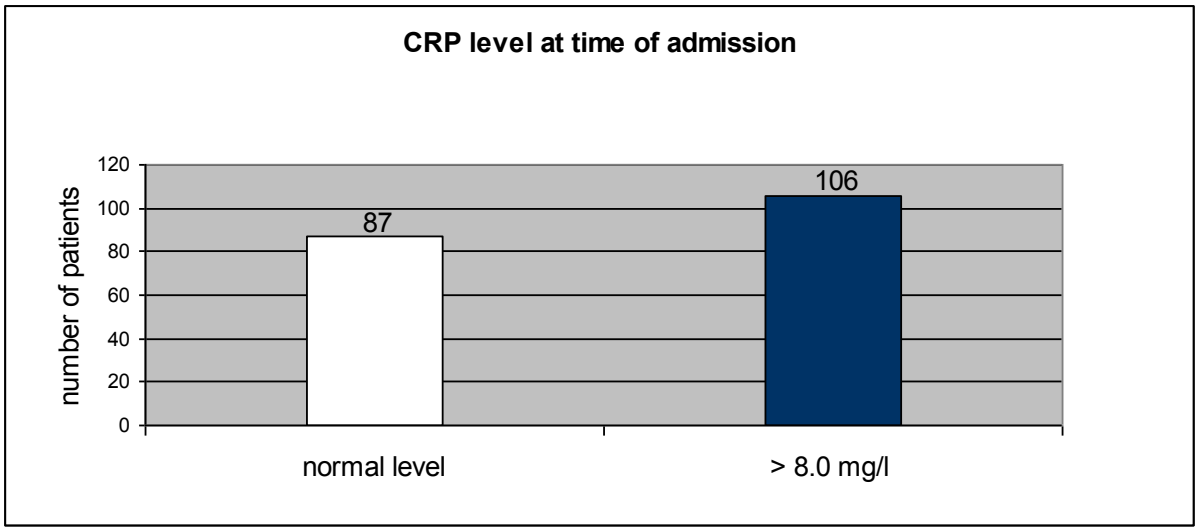
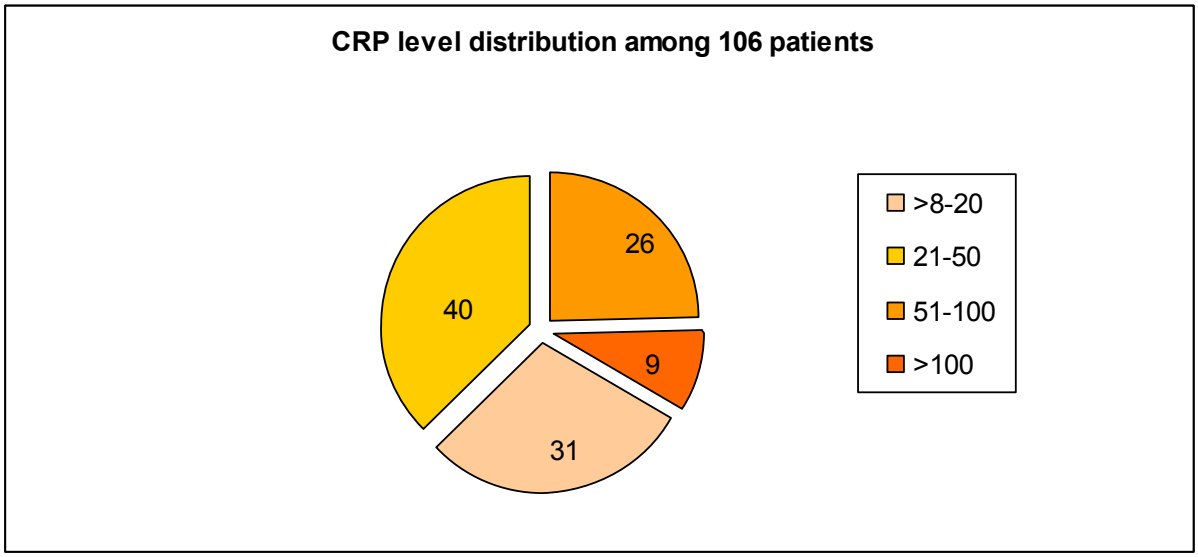


Figure 26

CRP among patients with increased levels.

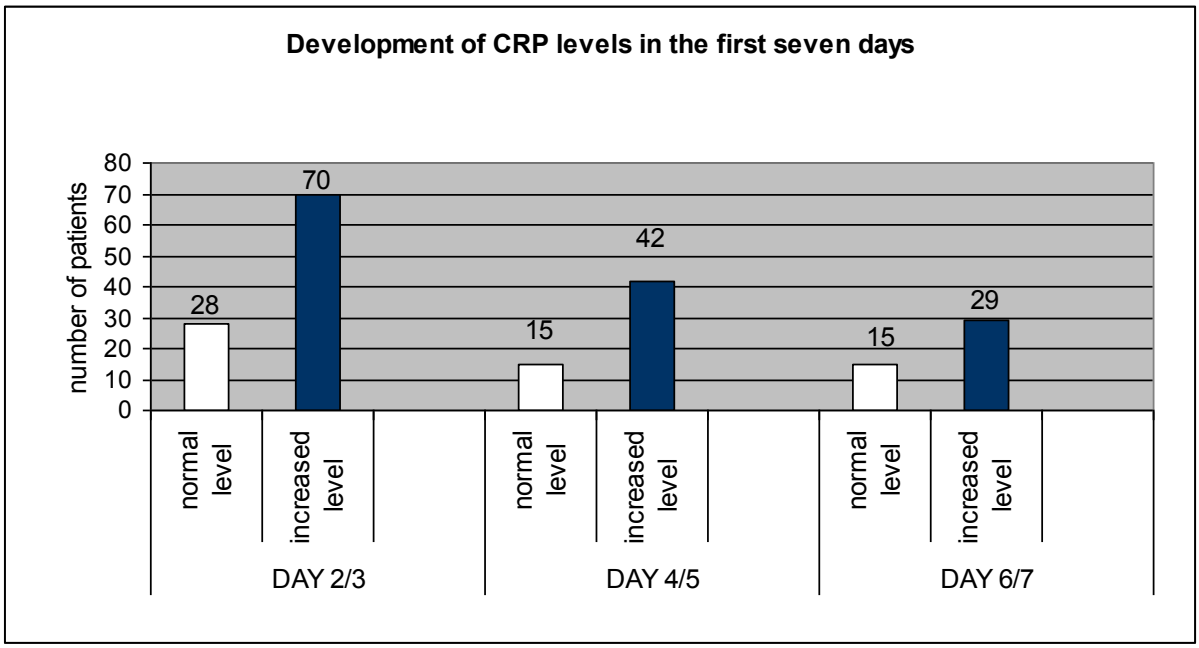


7.4.6 Development of CRP in the first seven days

During the first seven days of disease the majority of patients tested had increased CRP levels. At day 2/3 CRP levels ranged from 8.3 mg/l to 386 mg/l, at day 4/5 from 8.1 mg/l to 396 mg/l and at day 6/7 from 9.4 mg/l to 286 mg/l.

Figure 27

Development of CRP levels in the first seven days.



7.4.7 Platelet counts at presentation

165 out of 189 patients (87%) had normal levels of platelets at time of presentation to hospital. 21/189 (11%) of patients showed thrombopenia ranging from 136 G/l to 34 G/l and 3/189 (2%) of patients presented thrombocytosis with counts of about 501 G/l, 519 G/l and 592 G/l.

Figure 28

Platelet levels at time of admission.

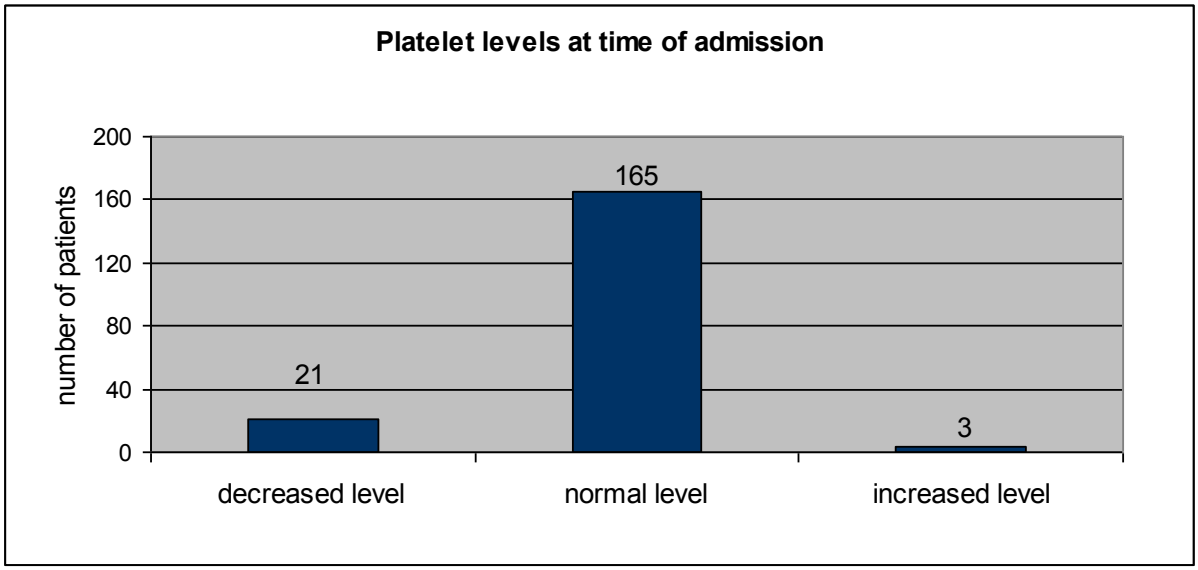
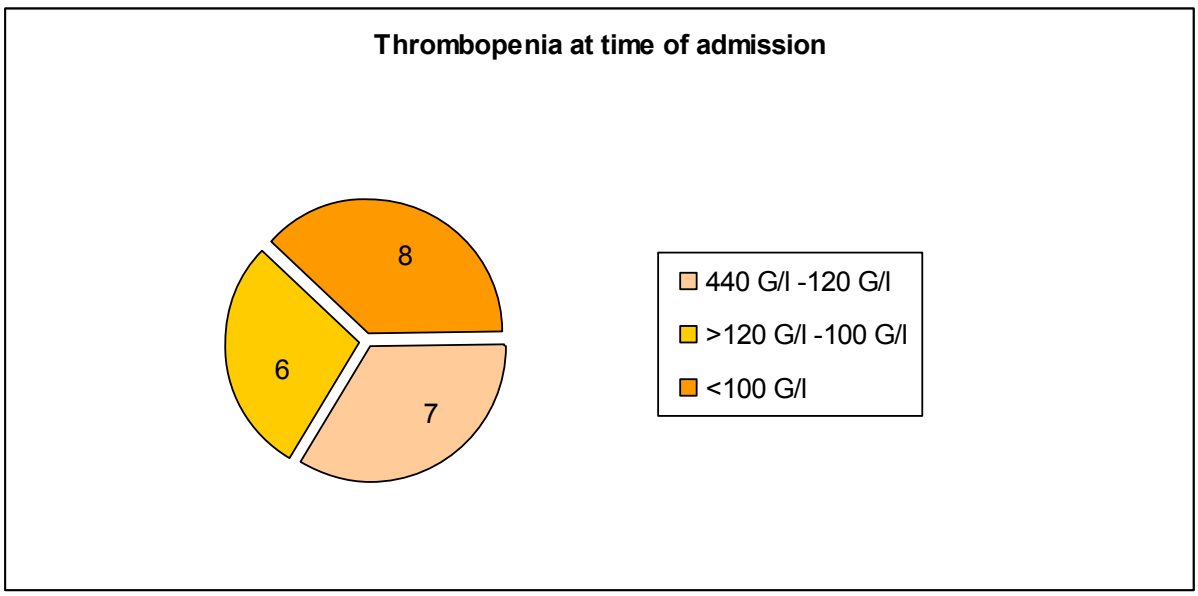


Figure 29

Thrombopenia at time of admission.

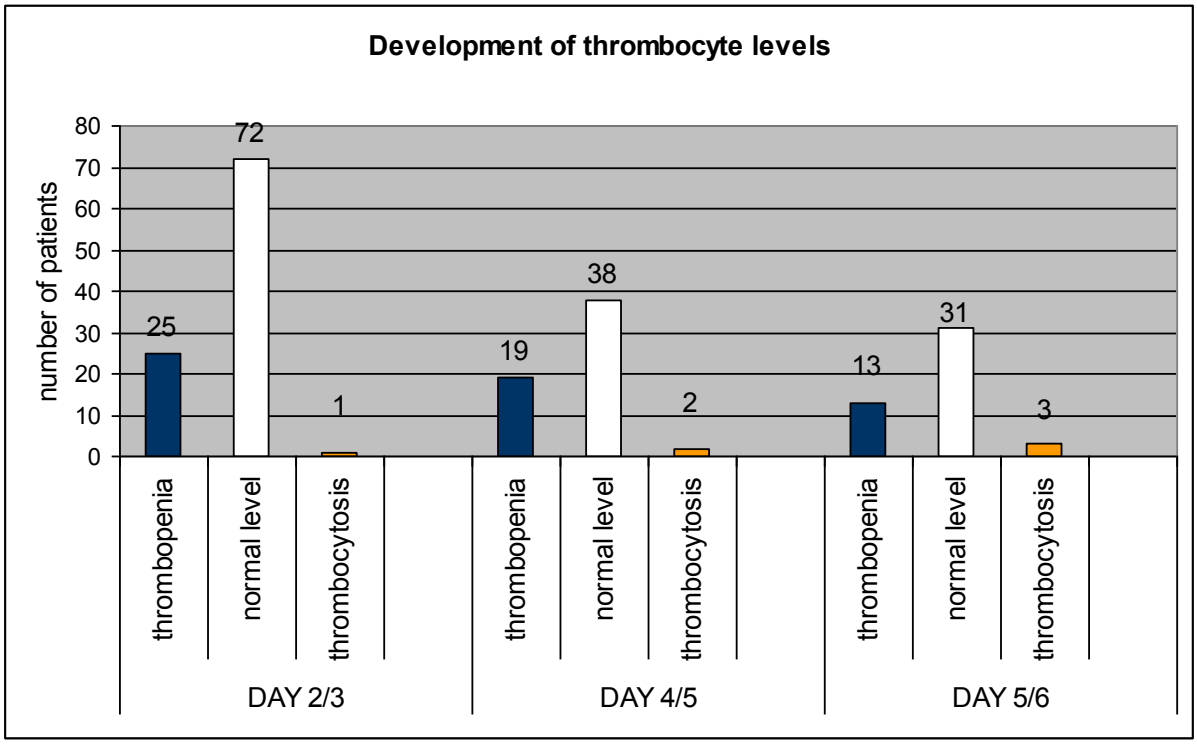


7.4.8 Development of platelet counts in the first seven days

In the 7 day course most patients had normal platelet levels. The number of patients with thrombopenia elevated at day 2/3 to 25/98 (26%) of cases and than decreased to 19/59 (32%) cases at day 4/5 and 13/47 cases (28%) at day 6/7.

Figure 30

Development of thrombocyte levels in the first seven days.



Thrombopenia ranged at day 2/3 from 139 G/l to 28 G/l, at day 4/5 from 137 G/l to 32 G/l and at day 6/7 from 133 G/l to 31 G/l.

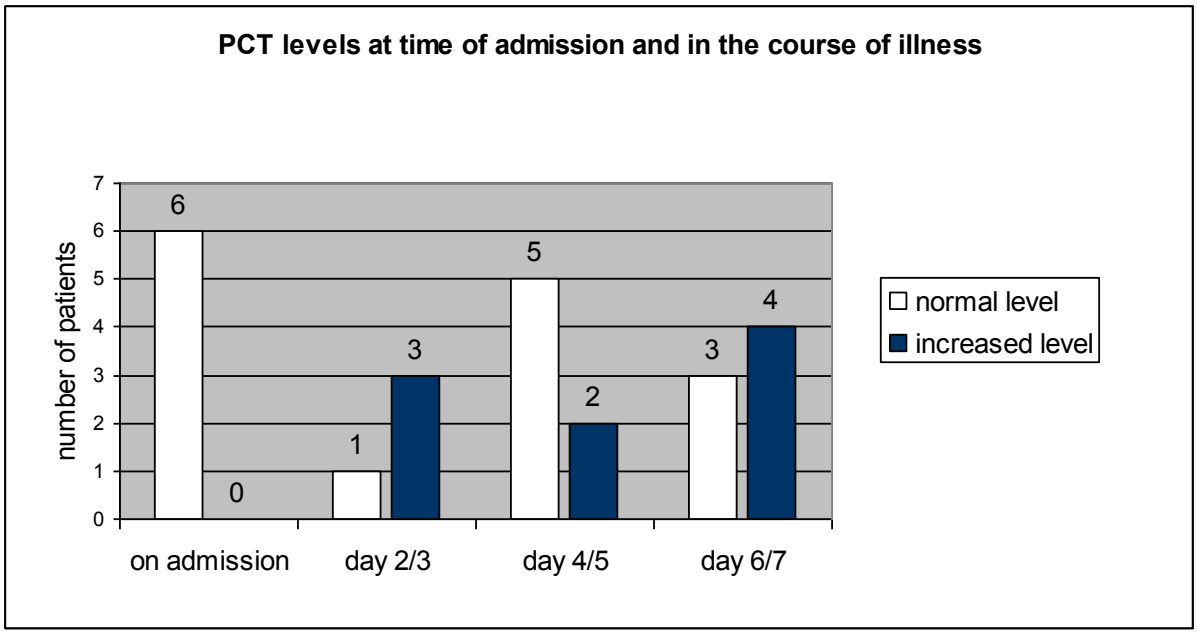
At day 2/3 one patient had thrombocytosis with about 524 G/l, at day 4/5 two patients with 509 G/l and 514 g/l and at day 6/7 three patients with 480 G/l, 483 G/l and 514 G/l.

7.4.9 PCT at presentation and in the course of illness

Values of PCT have been determined in just a few cases. On admission 0/6 patient had increased levels. At day 2/3 3/4 of patients who have been tested had increased PCT levels ranging from 0.92 ng/ml to 49.2 ng/ml, at day 4/5 2/7 of patients with levels ranging from 9.58 ng/ml to 100 ng/ml and at day 6/7 4/7 of patients with levels ranging from 1.89 ng/ml to 34.5 ng/ml. 6/7 of patients with increased PCT were admitted to ICU. 4/7 (57%) of patients with high levels of PCT had bacterial superinfection. 2 out of 7 patients with increased PCT levels had a fatal outcome.

Figure 31

PCT levels at time of admission and in the course of illness.



7.4.10 Creatinine at presentation

Half of patients who were tested showed normal creatinine levels. 46/117 (40%) had increased counts with highest level of about 4.87 mg/dl. 12/117 (10%) presented decreased levels.

Figure 32

Creatinine at time of admission

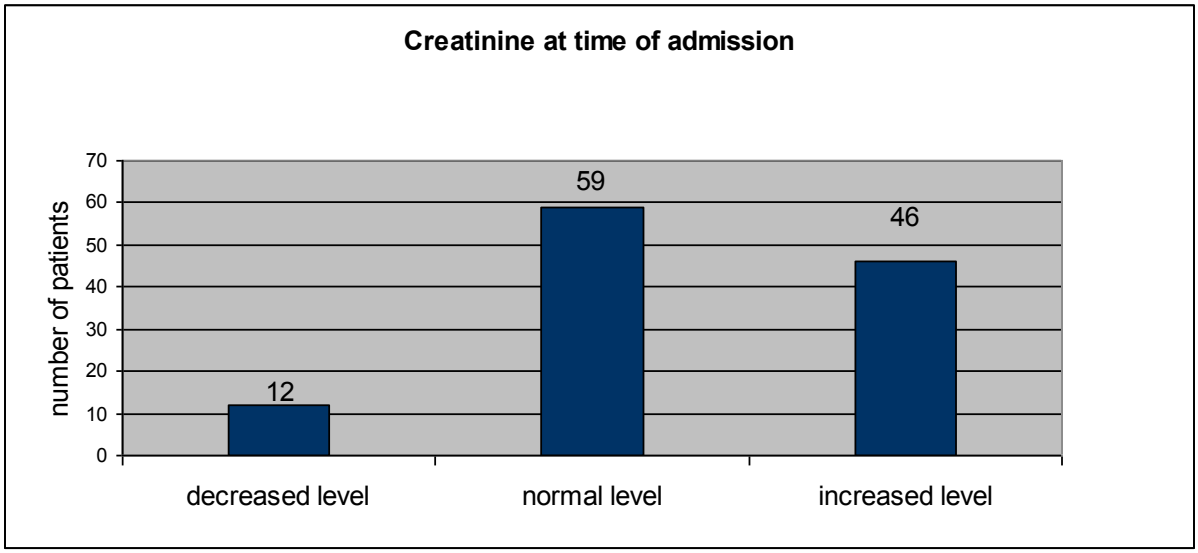
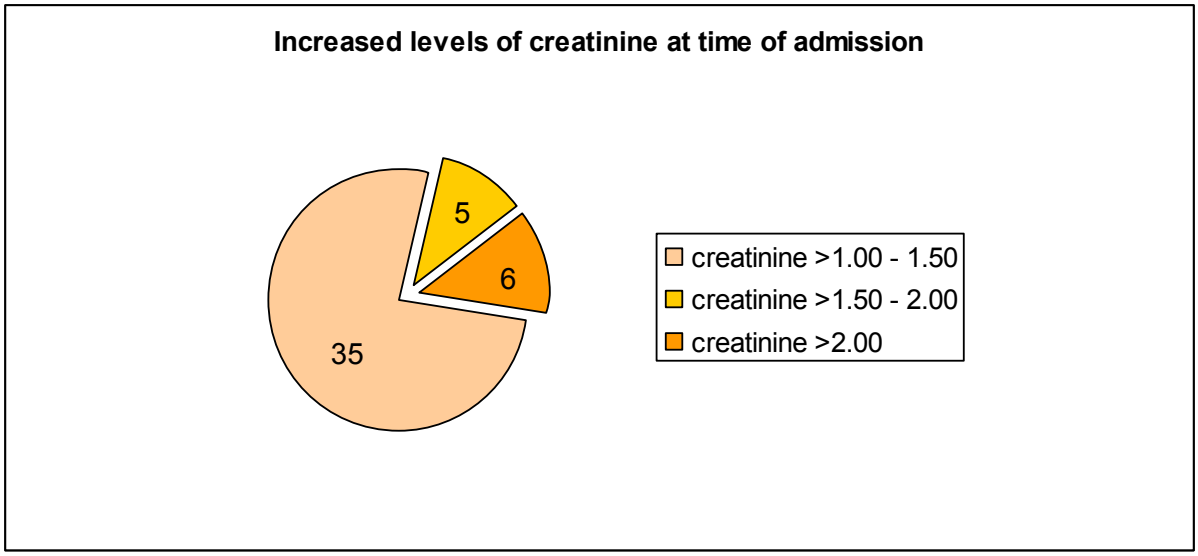


Figure 33

Increased creatinine levels at time of admission.

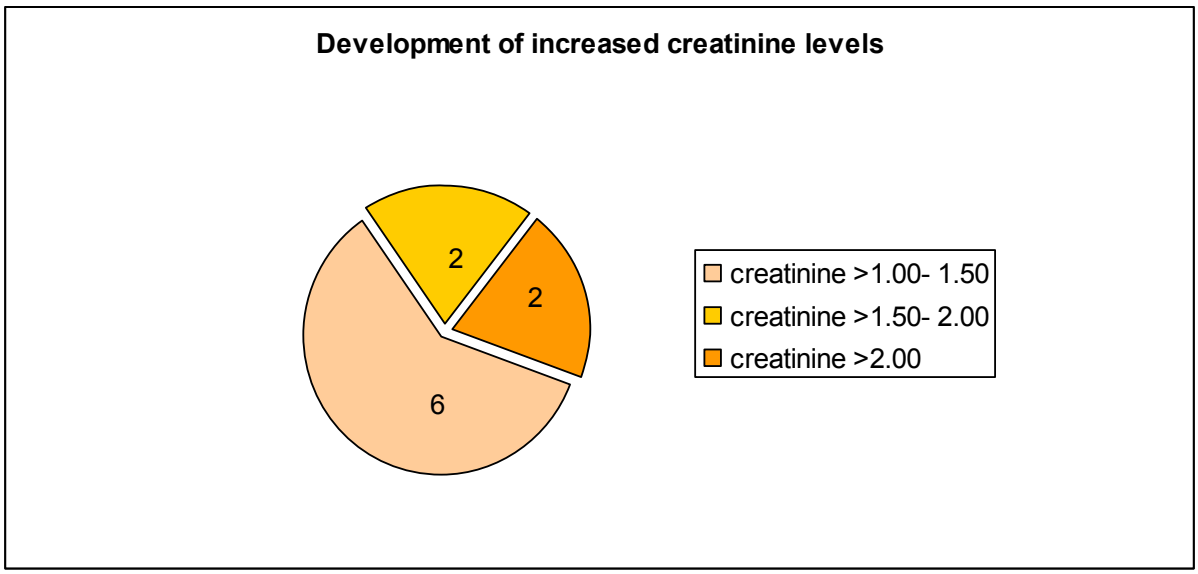


7.4.11 Development of creatinine levels in the first seven days

During the first seven days 10 out of 20 patients tested showed an elevation of creatinine with counts ranging from 1.03 to 6.88.

Figure 34

Development of increased creatinine levels in the first seven days.

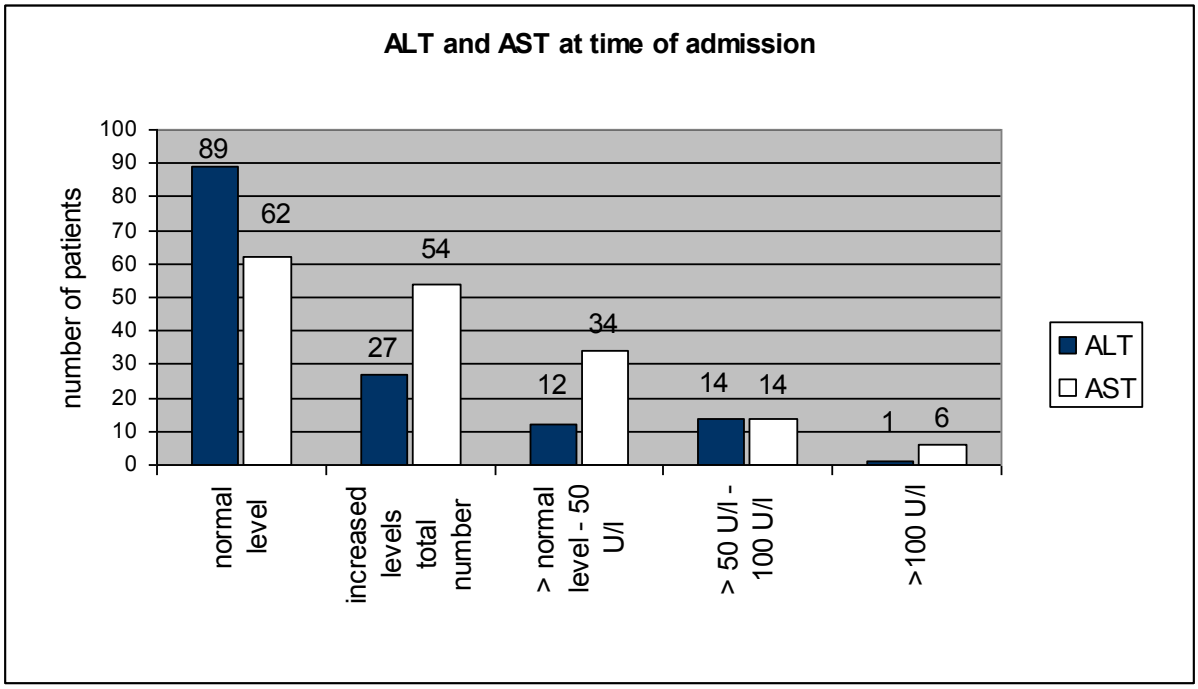


7.4.12 ALT and AST at presentation

On admission 27/116 (23%) of patients had elevated levels of ALT ranging from 26 U/l to 103 U/l and 54/117 (46%) of patients elevated levels of AST ranging from 31 U/l to 258 U/l.

Figure 35

ALT and AST at time of admission. Additional differentiation of increased levels is shown on the left sight.

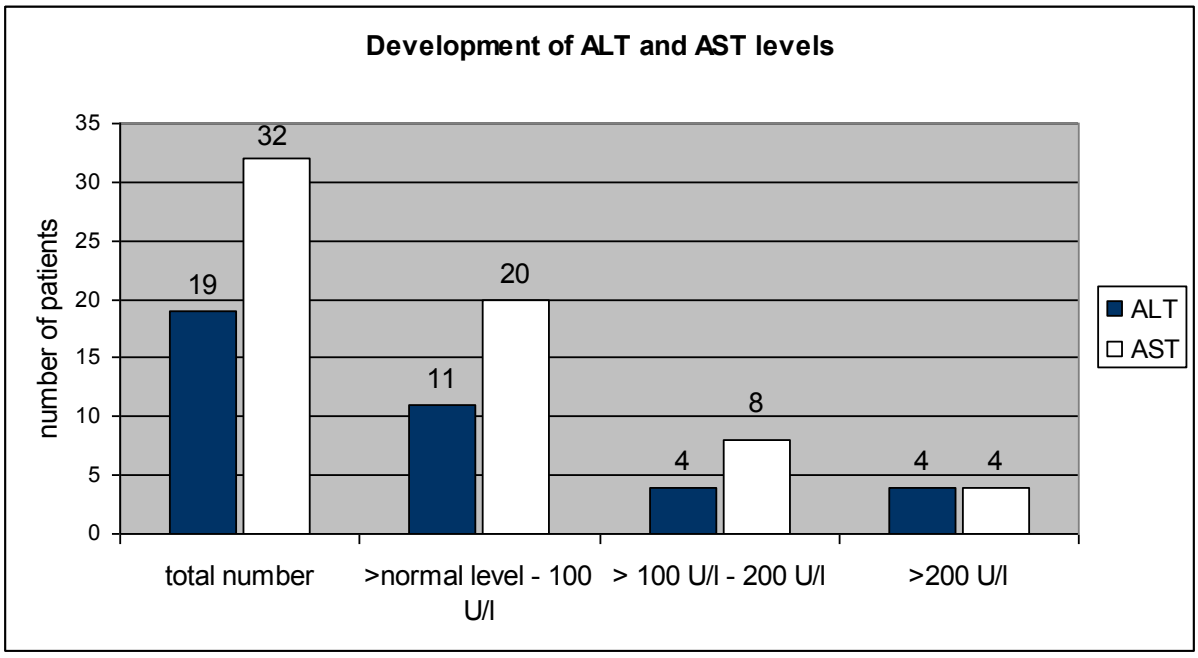


7.4.13 ALT and AST development during hospitalization

In the course 19 patients had increased ALT levels ranging from 36 U/l to 801 U/l and 32 patients presented with increased AST levels from 33 U/l to 1655 U/l.

Figure 36

Development of ALT and AST levels in the first seven days. Additional differentiation of increased levels is shown on the left sight.

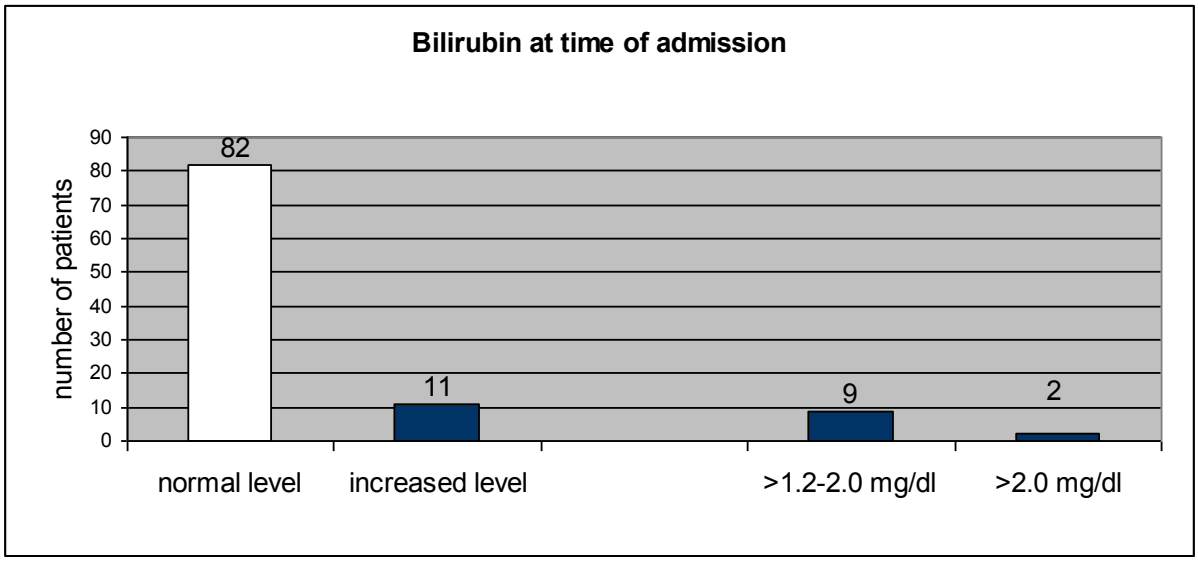


7.4.14 Bilirubin at presentation

11/93 (12%) of patients who have been tested presented elevated bilirubin levels at time of admission to hospital ranging from 1.23 to 7.92 mg/dl.

Figure 37

Bilirubin levels at time of admission. Additional differentiation of increased levels is shown on the left sight.

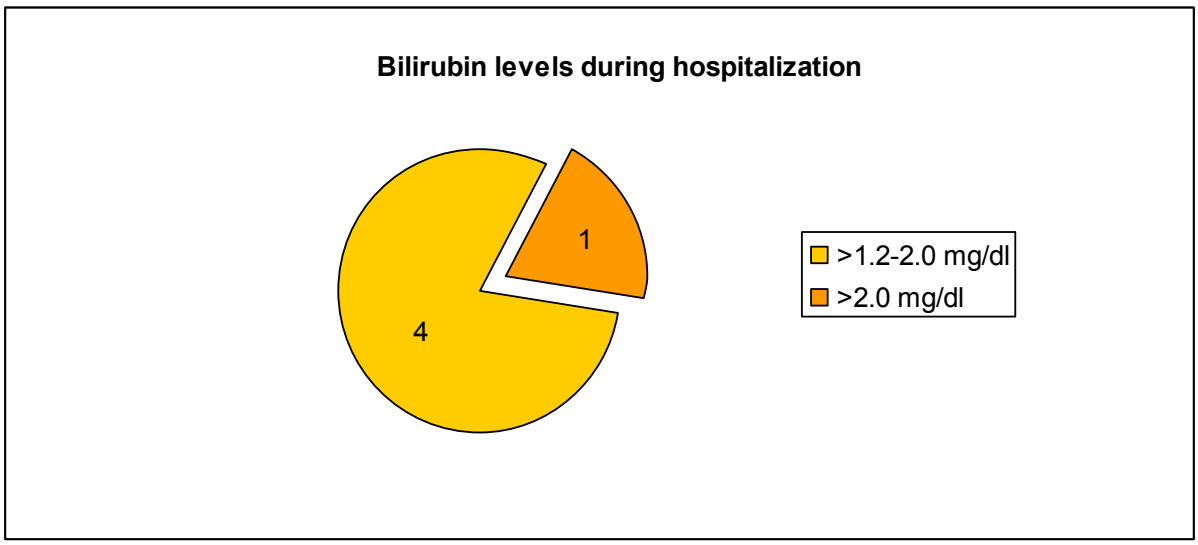


7.4.15 Bilirubin development during hospitalization

5 out of 11 patients with available laboratory results showed an elevation of bilirubin during their hospitalization ranging from 1.36 mg/dl to 13.57 mg/dl.

Figure 38

Bilirubin levels during hospitalization.

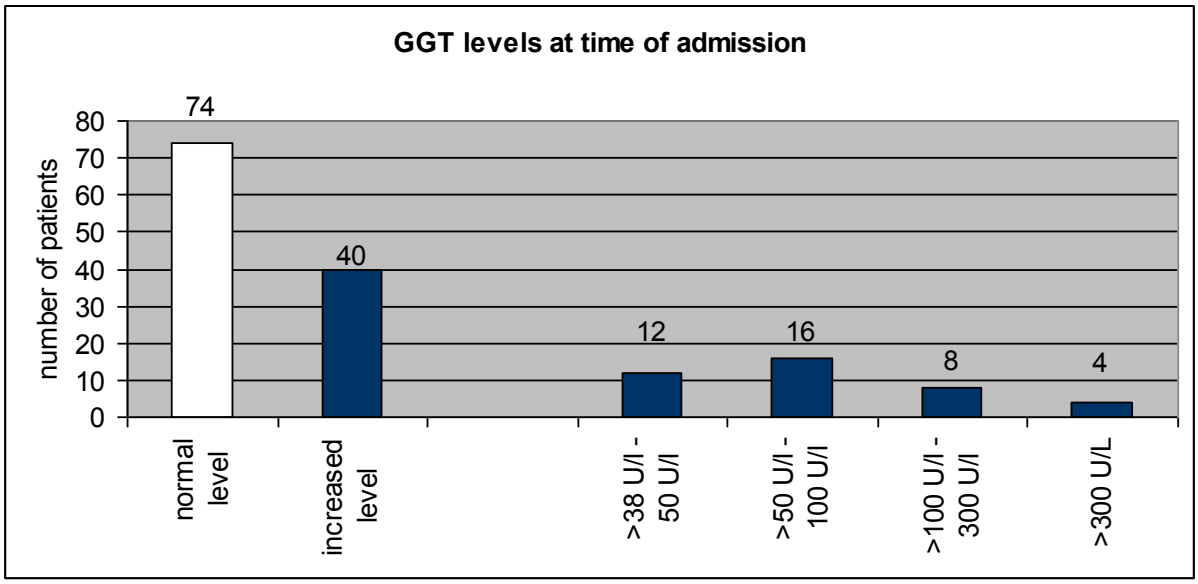


7.4.16 GGT at presentation

40/114 (35%) of patients who were tested at first day in hospital had increased GGT counts ranging from 39 U/l to 552 U/l.

Figure 39

GGT levels at time of admission. Additional differentiation of increased levels is shown on the left sight.

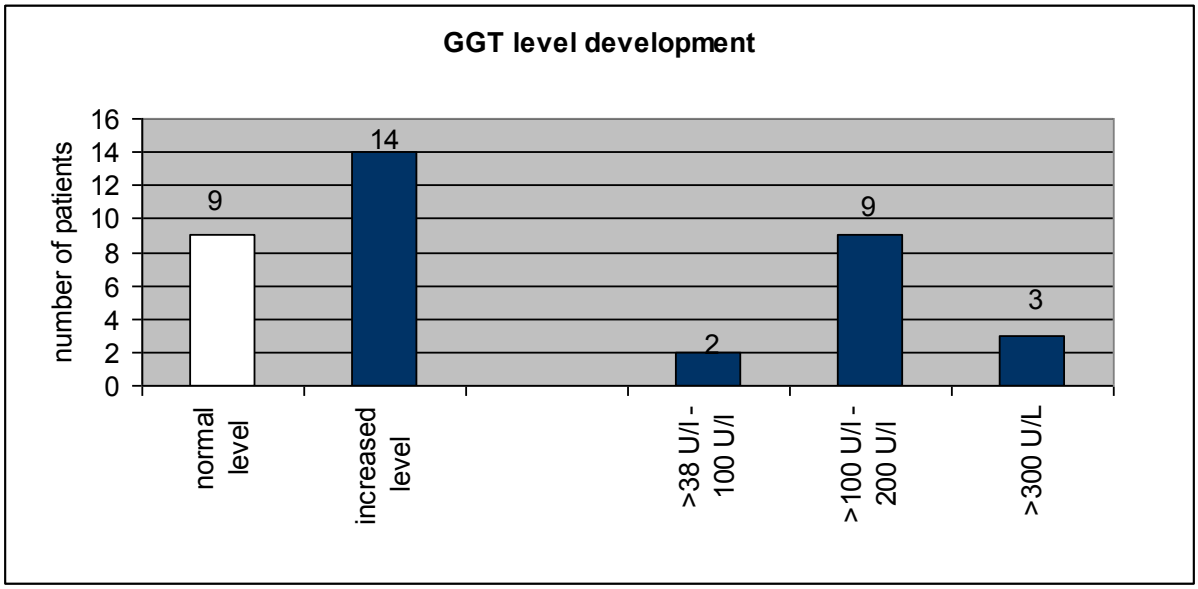


7.4.17 GGT development during hospitalization

During hospitalization 14 patients out of 23 patients (61%) showed elevated GGT counts ranging from 67 U/l to 847 U/l.

Figure 40

GGT level development. Additional differentiation of increased levels is shown on the left sight.

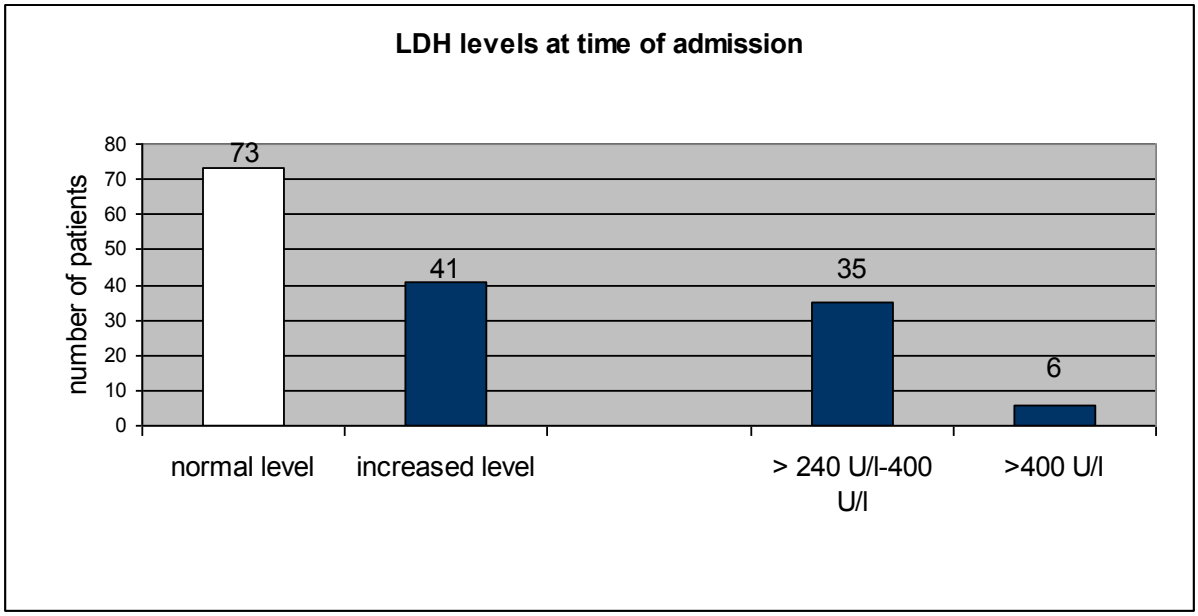


7.4.18 LDH at presentation

At time of presentation to hospital 41/114 (36%) of patients had increased LDH counts while 73/114 (64%) had not. Increased levels ranged from 241 U/l to 1469 U/l.

Figure 41

LDH at time of admission. Additional differentiation of increased levels is shown on the left sight.

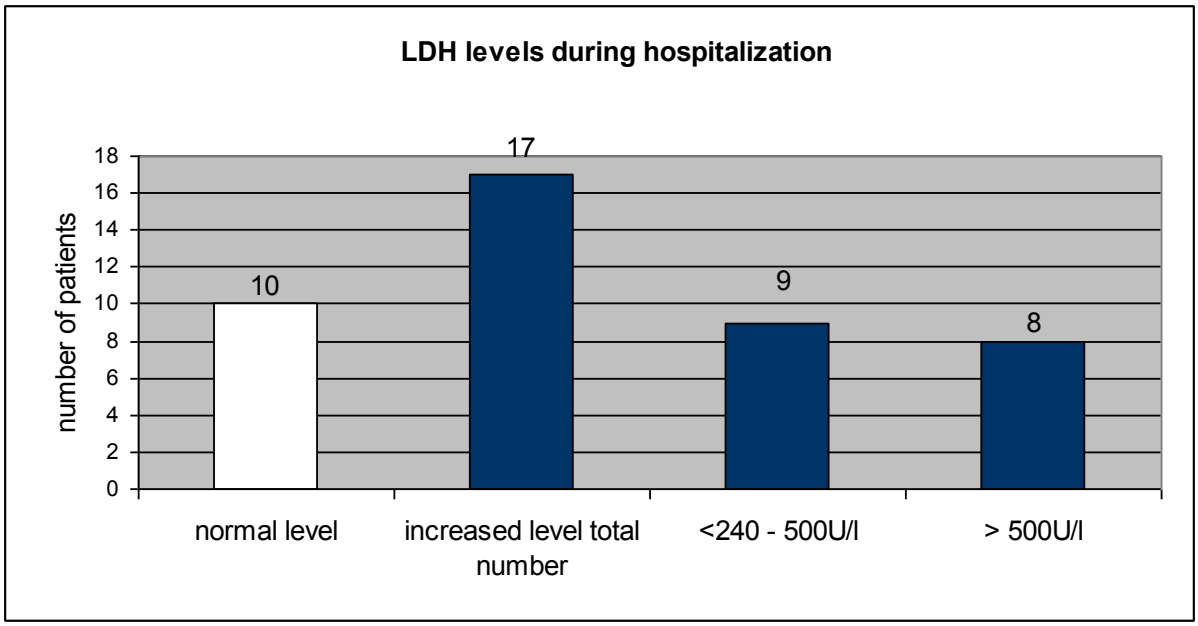


7.4.19 LDH development during hospitalization

Majority of patients tested in the course showed an elevation of LDH ranging from 269 U/l to 2224 U/l.

Figure 42

LDH levels during hospitalization. Additional differentiation of increased levels is shown on the left sight.

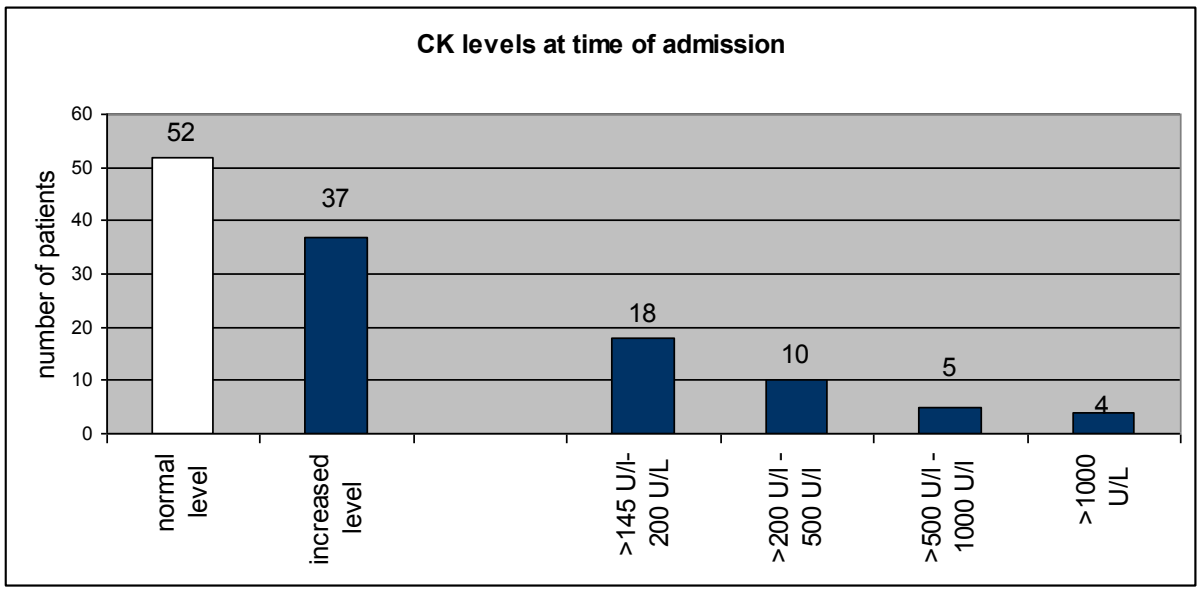


7.4.20 Creatine kinase at presentation

37/89 (42%) of cases had elevated creatine kinase levels ranging from 121 U/l to 4938 U/l.

Figure 43

CK levels at time of admission. Additional differentiation of increased levels is shown on the left sight.

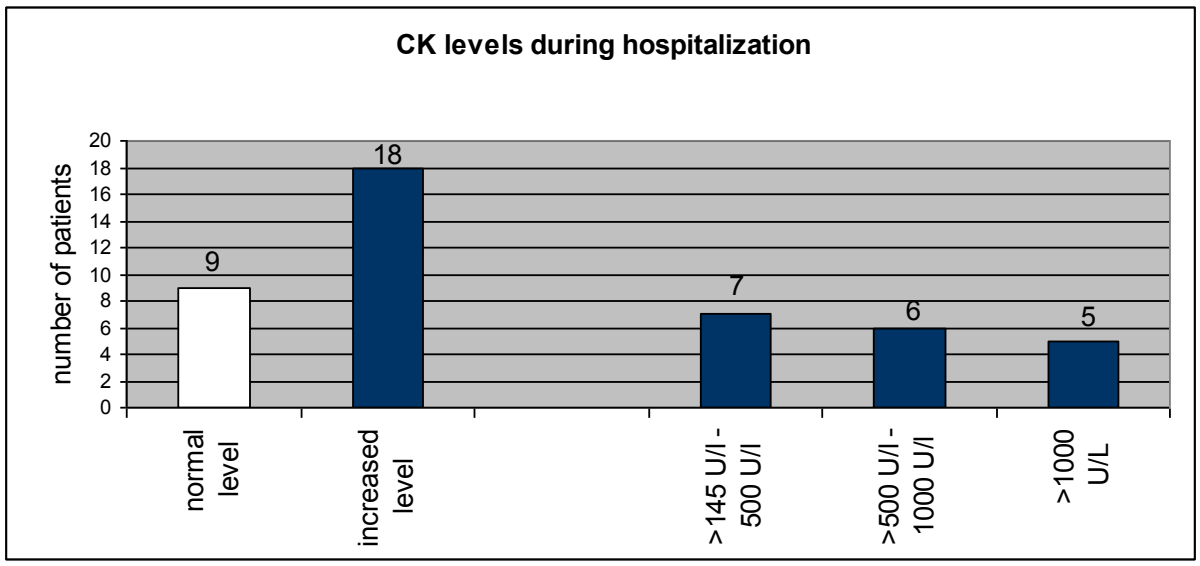


7.4.21 CK development during hospitalization

18/27 patients presented elevated creatine kinase counts in the course during the first seven days ranging from 177 U/l to 2638 U/l.

Figure 44

CK levels during hospitalization. Additional differentiation of increased levels is shown on the left sight.

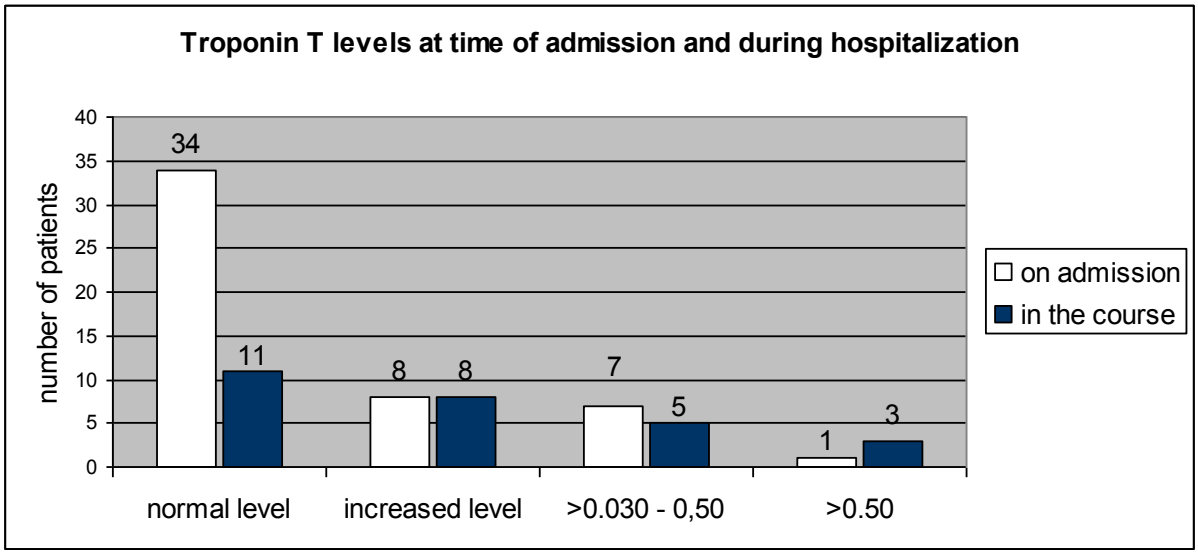


7.4.22 Troponin T at presentation and during hospitalization

On admission 34/42 patients showed elevated troponin t levels ranging from 0,035 to 1175 while in the course 11 patients had increased counts ranging from 0.035 to 1.17.

Figure 45

Troponin T levels at of admission and during hospitalization. Additional differentiation of increased levels is shown on the left sight.

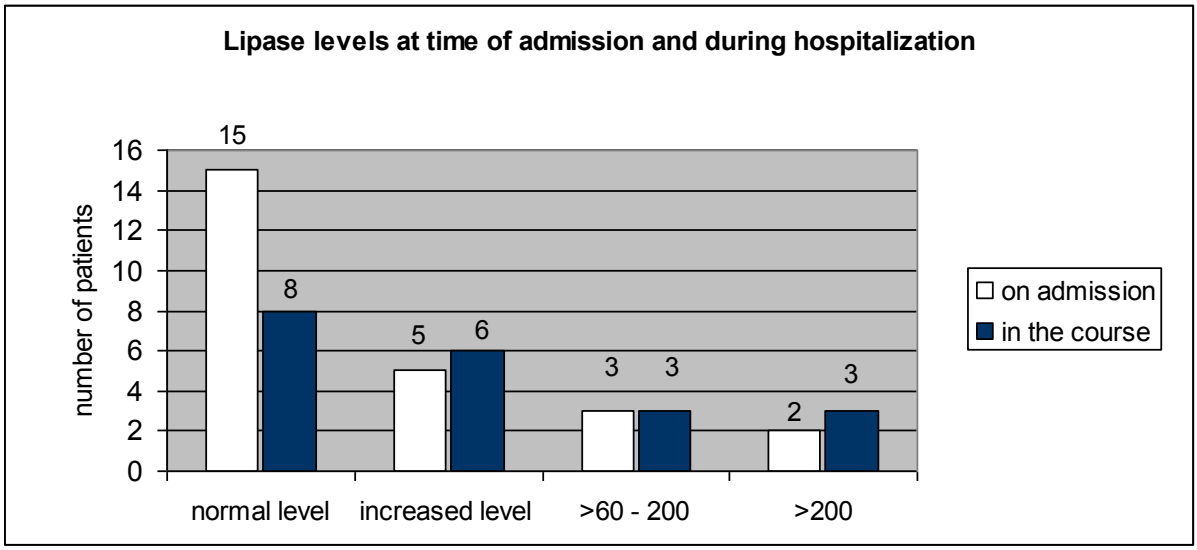


7.4.23 Lipase at presentation and during hospitalization

At time of presentation to hospital 15 out of 20 (75%) patients had elevated levels and in the course 8 out of 14 (57%) patients had elevated levels ranging from 66 to 409.

Figure 46

Lipase levels at time of admission and during hospitalization. Additional differentiation of increased levels is shown on the left sight.

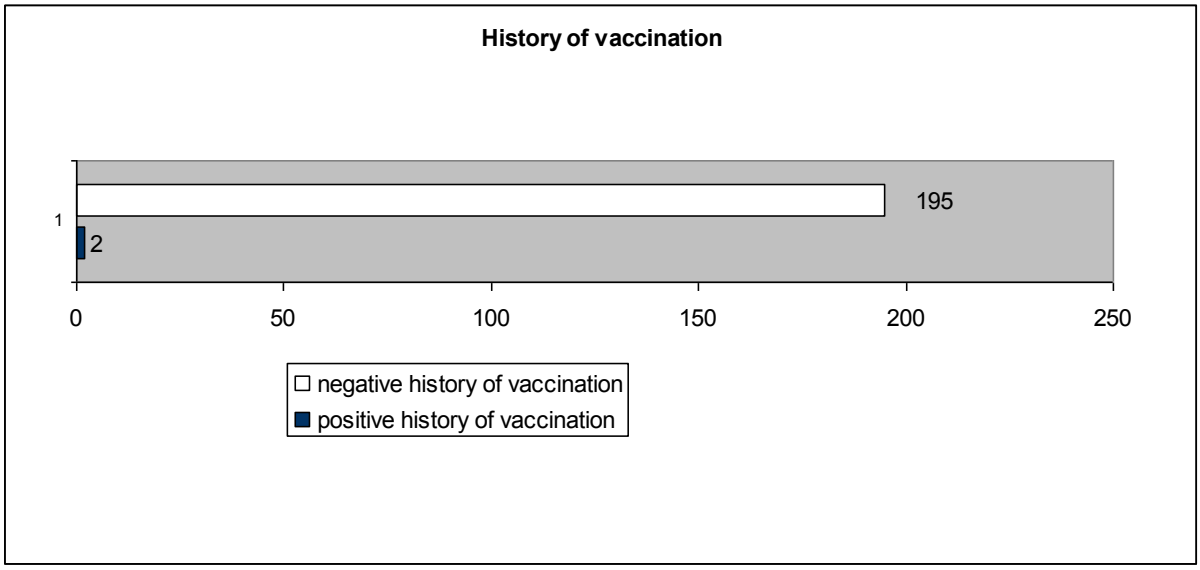


7.5 Vaccination

2/197 (1%) patients had a history of vaccination against H1N1 influenza while 99% had not. One of the 2 patients was vaccinated at day of symptom onset and five days before his H1N1 PCR confirmation.

Figure 47

History of vaccination.



7.6 Antiviral Treatment

Of the 197 cases with available information, 65 (33%) received antiviral treatment with neuraminidase inhibitor oseltamivir (Tamiflu^{TM}). The mean time from onset of symptoms to initiation of antiviral therapy was 2.5 days (ranging from 0-34 days) and median time was 1 day. 44/65 (68%) of patients received treatment within 48 hours after the onset of symptoms. 4/5 of patients with fatal outcome received oseltamivir during their hospitalization, but none of them within the first 48 hours from symptom onset.

Table 9

Antiviral treatment among study population.

Tamiflu	All Patients of study group n=197
Received	65 (33%)
Not received	132 (67%)
<48 hours	44
>48 hours	17
mean time to onset	2.5 days
mean age	36.3 years
median age	39 years
male	42 (65%)
female	23 (35%)

7.7 Complications

Complications occurred in 51/197 (26%) of cases. 33/197 (17%) of patients had pneumonia, 25/33 developed pneumonia due to H1N1 and 8/33 of patients had bacterial pneumonia. 4/197 of patients showed neurological disorders, 13/197 cases developed respiratory insufficiency and 4/197 cases developed bacteraemia/fungaemia. 2 out of 5 patients with fatal outcome had a history of mild chronic obstructive pulmonary disease.

In 44/51 (86%) of cases with complications patients have not been treated with Oseltamivir at all or not within the first 48 hours from illness onset.

7.7.1 Antibiotic treatment

Among study population 88 (45%) received antibiotic therapy. 2 out of the 89 patients received the medication before presentation to hospital. The mean time from onset of symptoms to initiation of antibiotic therapy in hospital was 2.2 days (ranging from 0-10 days) and median time was 1 day.

Table 10

Antibiotic treatment among study population.

Antibiotic therapy	All Patients of study group n=197
Received	88 (45%)
Not received	109 (55%)
mean time to onset	2.5 days
mean age	34.4 years
median age	37.5 years
male	55 (63%)
female	33 (37%)

21/88 of patients received more than one antibiotic during hospitalization. Antibiotics used are depicted in table 11.

Table 11

Antibiotics among study population.

Antibiotic therapy	Among 88 patients who received antibiotics
Amoxicillin/Clavulanic acid	31
Moxifloxacin	15
Other Quinolones	4
Carbapenem	4
Clarithromycin/Azithromycin	22
Piperacilllin/Tazobactam	4
Vancomycin	1
Clindamycin	1
Cephalosporin	
1. generation	1
2. generation	17
3. generation	8
4. generation	4
Penicillin	1
Amoxicillin	1
Tetracyclin	1
Others	5

7.7.2 Extracorporeal membrane oxygenation

One patient has been treated 19 days with ECMO.

7.8 Outcome

Of 197 cases with PCR- confirmed influenza A (H1N1) 2009 5 (2.5%) patients had a fatal outcome, 97.5% survived. 4/5 of fatal cases were male and 1/5 female. Mean age of patients with a fatal outcome was 39.4 years (ranging from 15 to 69 years of age).

Results are described in table 12.

Table 12

Outcome among study population.

Outcome	All Patients of study group n=197
Died	5 (2.5%)
1-10	0
11-20	1
21-30	1
31-40	1
41-50	0
51-60	1
>60	1
mean age	39.4
median age	33
male	4
female	1
Survived	192 (97.5%)

8 Discussion

The 2009/2010 influenza pandemic resulted in millions of laboratory confirmed cases and over 18000 deaths worldwide (20). We retrospectively evaluated epidemiology and clinical characteristics of S-OIV 2009 infection in South East Austria. Between October 1st 2009 and January 19th 2010, 624 cases of H1N1 virus infections were confirmed by RT-PCR. We surveyed 197 confirmed influenza cases who presented to participating hospitals within this period. During the study period most cases (55%) were confirmed in November 2009. To date, it has been difficult to define the true number of influenza infections during the pandemic 2009-2010 because data were collected only on patients seeking emergency medical care and being confirmed by RT-PCR diagnostic. Therefore the delivered number of cases may underestimate the real number of people infected with S-OIV 2009 during the pandemic.

Compared to previous non-pandemic influenza seasons epidemiology of the H1N1 pandemic in 2009-2010 differed significantly. In accordance with previously published data from the United States (26,34), Australia and New Zealand (30) and China (28) median age of PCR confirmed cases in South East Austria was with 16 years which was comparably low to previous influenza seasons caused by non-H1N1 virus (i.e. H3N2). Furthermore our study showed a lower morbidity among persons who were 60 years of age or older. Of the 197 patients 92.9% were under 60 years of age. Highest number of confirmed infections was among children and young adults between 0 to 20 years of age. This phenomenon has tried to be explained by the theory about a possible existing immunity to the influenza A (H1N1) 2009 virus strain in people aged 60 years or older from previous infections or vaccinations to similar strains (31,64). Another reason however may be the fact that children and young adults seem more likely to be tested and are under a higher exposure in schools and other communities. Furthermore the 2009 influenza A (H1N1) virus differed markedly from seasonal influenza were hospitalization rate is highest among persons aged 65 years and older and children under 5 years of age (65)..

In the present study rapid influenza tests resulted falsely negative in 88.5% of cases. Our results are in line with the growing body of literature about low sensitivity of rapid tests for pandemic 2009 influenza A (H1N1) infection (56,66). Compared to a report from California (27) of 34% falsely negative cases rate among our study population was notably high. The high rate of false negatives may have lead to a delayed

diagnosis of H1N1 infection and therefore to a delayed onset of antiviral therapy, which should be preferable prescribed within the first 48 hours after symptom onset.

Time from onset of symptoms and admission to hospital was lower than reported in studies from Mexico (39) and the United States (26)(median time in our study collective 1 day vs. 6 days and 3 days, respectively).

Among our study population 8.6% required intensified medical care. This rate may be considered low when compared to rates ranging from 25% to 31% reported from the United states (26,27), a rate of 13% reported from the United Kingdom (67) and a rate of 27% reported from Australia (43). These results may be explained by the early presentation to hospital and treatment with antiviral drugs within 48 hours which was noted through our evaluation. Compared to the total study population median age of patients at ICU was considerable higher (median 47 years vs. median 16 years). This data suggest that severe illness was more common in older persons. A cohort study from Australia and New Zealand (30) described 722 patients who required intensified medical care. Median time at ICU was reported with 7 days, which was comparable to another report from Australia (43). Our results are in the line with the growing body of literature with a median duration time at ICU of about 8 days (ranging from 1 to 50 days).

There are few existing reports on the development of laboratory values of patients with S-OIV influenza infection. Laboratory findings in most 2009 influenza A (H1N1) reports showed normal or low normal leukocyte counts, lymphocytopenia and higher levels of serum aminotransferases, lactate dehydrogenase, creatine kinase and creatinine (29,39,40,68). In our present study laboratory findings were comparable with this literature. Majority of patients tested showed at presentation and in the course normal leukocyte levels and differential counts of white blood cells. The most consistent laboratory characteristics in our study were total leukocyte count within normal limits, lymphocytopenia, increased lactate dehydrogenase levels, increased creatinine and CRP levels and elevated AST, ALT and GGT counts. Similarly to an observational study of critical ill patients in Mexico and another report on patients admitted to intensive care units in Spain (68) the rate of patients presenting with elevated creatinine counts was notably high among our study collective (40). Among patients tested number of patients with increased CRP- levels was remarkably high, especially on day 1 and day 2/3 of disease. Elevated CRP values are frequent in

patients with bacterial superinfections infections and are reported to be associated with serious outcome (67) .

The virus infection presented in a broad spectrum of illness ranging from self-limited to severe illness. In accordance to publications from the Unites States (26,34), Australia and New Zealand (30) and Mexico (40), bacterial pneumonia mostly caused by *Staphylococcus aureus* and *Streptococcus pneumoniae* was a common clinical characteristic of bacterial superinfection in our study population. Lower respiratory disease among our study population was diagnosed as viral pneumonia due to H1N1 in 75% of cases, in 8 cases bacterial pneumonia was diagnosed. Not all of our study patients, however, have been tested for bacterial superinfection.

In our study population, 33% of patients received antiviral treatment and in a majority of patients oseltamivir was administered within 48 hours. Compared to studies on 272 hospitalized patients in the United States (26) and reports from Australia (43) where antiviral therapy was initiated in 75% and 83% of patients, number of patients treated with antiviral drugs was low in our study collective. In 44/51 (86%) of cases complication occurred in patients who have not been treated with Oseltamivir at all or not within the first 48 hours from illness onset. 4/5 of patients with fatal outcome received oseltamivir, but none of them within 48 hours. In accordance to treatment guidance for the use of antiviral medications for S-OIV published from the CDC (69) patients seemed to benefit from antiviral therapy when prescribed within the first 48 hours after illness onset.

As of August, 6, 2010 the World Health Organization accounted over 18449 deaths caused through S-OIV 2009 worldwide (20). Case fatality ratio among our study population was 2.5%. To compare our findings, a survey on hospitalized patients in the United States (26) presented a case fatality rate of about 7%; another study on hospitalized patients in California (27) reported an overall fatality rate of about 11% and evaluations on patients in Australia and New Zealand (30,43) reported a rate of 14% and 2.7%. Analysis by the epidemic intelligence team at the French Institute for public health surveillance on 503 fatal cases worldwide (41) calculated a ‘computed CFR’ (number of reported deaths per number of reported cases) of about 0.6% with a broad range from 0.1 to 5.1% depending on the country. The definition of a true case fatality rate (CRF) seems to be difficult, because it is almost impossible to detect every case of pandemic influenza (H1N1) infection in a community (32). However,

reports from the United States (70), the United Kingdom (25) and New Zealand (71) estimated the true CFR under 0.05%, another modelling from New Zealand suggests CFR in developed countries from 0.0004% to 0.06%. However, all methods and estimates have possible restrictions.

Our study has several limitations. Our study population represented 32% of total number of S-OIV infections that were confirmed at local laboratories in South East Austria during the surveillance period. Data sets were incomplete for 68% of patients. Furthermore we evaluated only PCR-confirmed 2009 H1N1 infections in patients seeking medical care at hospitals. Finally not all information was available from all patients. Therefore, data may not be representative for all S-OIV infections during the pandemic, such as mild illness in patients who did not require medical care. Although we are not able to give evidence about outcome and mortality of patients not presenting to hospital, we suggest the numbers low assuming that people with severe disease would have sought medical advice anyway.

In summary, although the majority of the surveyed cases in our study were mild in terms of clinical presentation and mortality was comparatively low, severe and even fatal illness was identified also among healthy young adults.

For improvements in management and treatment guidance of possible upcoming influenza pandemics, a basic understanding of the clinical characterisations and risk factors for severe disease in different populations is necessary. Furthermore the early detection and therefore early treatment with antiviral drugs has to be focused. In consideration of the fact that among our study population only two of the patients had received influenza H1N1 vaccination in fall 2009 prevention by seasonal influenza vaccination has to be promoted and is of utmost importance in the handling of future pandemics.

The 2009/2010 influenza pandemic has caused a global impact on public health especially through the media. No one predicted the emergency of the S-OIV and its global impact. In fact nobody really knew how the pandemic would evolve and result. Studies and analyses on the S-OIV 2009 pandemic may lead to a better understanding about influenza virus infections and help to guide pandemic planning in the future.

Scientists (72) suggest that we are still unable to predict coming pandemics, but we are able to prepare and improve our management, available capacity and knowledge.

"We regret very much the fact that an influenza virologist is unable to live say 200 years, so that he himself would be able to see what has developed from his earlier assumptions."

J. Mulder and J.F.P Hers, *Influenza* (1972)

9 References

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10 Tables and Figures

11 Curriculum vitae

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EDUCATIONAL HISTORY

October 2004 – present	**Medical University of Graz**
June 2004	**Bundesrealgymnasium Akademiestraße, Salzburg (Austria)** Matura (A-Levels Equivalent) with good success

WORK EXPERIENCE/ CLERKSHIPS

September 2006	University Clinic of Internal Medicine I, Gastroenterology, Nephrology and Metabolic Disorders, County Hospital of Salzburg (Austria)
September 2007	University Clinic of Internal Medicine I, Gastroenterology, Nephrology and Metabolic Disorders, County Hospital of Salzburg (Austria)
Februar 2009	University Clinic of Pneumology, County Hospital of Salzburg (Austria)
August 2009	University Clinic of Internal Medicine II, Cardiology and Internal Intensive Care Medicine including cardiac catheterization laboratory with invasive cardiology; County Hospital of Salzburg (Austria)
Februar 2010	University Clinic of Psychiatry and Psychotherapy I, - Special Assignment Unit for Adolescent Psychiatry, Christian Doppler Clinic Salzburg (Austria)

WORK EXPERIENCE / CLERKSHIPS

2010/11	**Practical year:** 10 weeks surgery at the University Hospital Graz, Department for Thorax Surgery and Hyperbaric Medicine, Medical University of Graz (Austria) 10 weeks internal medicine at the University Hospital Graz, Department of Endocrinology and Metabolism, Medical University of Graz (Austria) 5 weeks general medicine, Dr. Elisabeth Krainer, Graz (Austria) 5 weeks at the University Hospital Berlin Charité, Department of Neonatology, Berlin (Germany)

SCIENCE WORK

February 2011	Participant at the **International Meeting on Emerging Diseases and Surveillance** in Vienna, presenting a Case Report (Epidemiology of PCR confirmed H1N1 Influenza in South-East Austria)
May 2010 – present	**Dissertation** **'**PCR confirmed Influenza A H1N1 in South East Austria, season 2009/2010, Epidemiology, laboratory characteristics and outcome**'** **Ao, Univ. Prof. Dr. Robert Krause** **Ass. Dr. Martin Hönigl** Division of Infectious Diseases, Department of Pulmonary Medicine, University Clinic of Internal Medicine, Medical University Graz

SPECIAL ABILITIES

- Basic Medical English I
- Basic Medical English II

LANGUAGES

- German (native)
- Croatian (native)
- English (fluent in spoken and written)
- Italian (advanced)
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OTHER INTERESTS

- Travelling
- Literature
- History
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