Diploma thesis

PCR confirmed influenza A and B infections treated at the intensive care unit of the Department of Internal Medicine of the Medical University of Graz between 2009 and 2020

Epidemiological, clinical and laboratory characteristics, risk factors for cardiovascular/pulmonary complications, bacterial/fungal infections and death

by

Matthias Funck

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Division of Pulmonology

Under supervision of

OA Dr. med. Holger Flick (primary supervisor)

Univ. FA Priv.-Doz. Dr.med.univ. Dr.scient.med. Jürgen Prattes (secondary supervisor)

Graz, July 21, 2022

Affirmation in lieu of an Oath

I hereby declare that I have written the submitted thesis independently and without any illegitimate assistance from third parties. Furthermore, I confirm to not have used any other than the declared sources for the preparation of this academic work. All used sources have been indicated as such and acknowledged by means of complete references in the text.

Graz, July 21, 2022 *Matthias Funck eh.*

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Table of contents

1 Abbreviations

ARDS	Acute respiratory distress syndrome
AST	Aspartate transaminase
ALT	Alanine transaminase
BMI	Body mass index
BAL	Bronchoalveolar lavage
BM2	Matrix protein 2
Bpm	Beats per minute
CAOD	Central Arterial occlusive disease
CHD	Coronary heart disease
CMP	Cardiomyopathy
COPD	Chronic obstructive pulmonary disease
CK	Creatine kinase
CRP	C-reactive protein
DFA	Direct immunofluorescence assay
ECG	Electrocardiogram
ECMO	Extracorporeal membrane oxygenation
EDTA	Ethylene diamine tetraacetic acid
EU	European Union
FiO2	Fraction of inspired oxygen
HA	Hemagglutinin
HAP	Hospital aquired pneumonia
ICU	Intensive care unit
IFA	Indirect immunofluorescence assay
iNAT	Influenza nucleic acid amplification test
IQR	Interquartile range
LB2	Blocklabor 2
LDH	Lactate dehydrogenase
LKH	Landeskrankenhaus
MAP	Mean arterial pressure
MRSA	Methicillin-resistant Staphylococcus aureus
M2	Matrix protein 2
MUG	Medical University Graz

NA	Neuraminidase
NEP	Nuclear export protein
NP	Nucleoprotein
NS1	Nonstructural protein 1
NS2	Nonstructural protein 2
NT-pro-BNP	N-terminal prohormone of brain natriuretic peptide
OECD	Organisation for Economic Cooperation and Development
ÖVIH	Austrian Association of Vaccine Manufacturers
PA	Polymerase acidic protein
PAOD	Peripheral arterial occlusive disease
PB1	Polymerase basic protein 1
PB2	Polymerase basic protein 2
PCR	Polymerase chain reaction
PCT	Procalcitonin
PPV	Positive predictive value
RNA	Ribonucleic acid
RIDT	Rapid influenza diagnostic test
RSV	Respiratory syncytial virus
RT-PCR	Reverse transcription polymerase chain reaction
SAPS	Simplified acute physiology score
VAT	Ventilator associated tracheobronchitis
WHO	World Health Organization

2 Tables and Figures

3 Zusammenfassung in Deutsch

3.1 Ziel

Österreichs Influenza-Impfrate ist im Vergleich zu anderen europäischen Ländern niedrig, dies gilt vor allem bei Patient*innen mit chronisch kardiopulmonalen Erkrankungen. Diese sollten daher häufiger an einer schweren Influenza-Infektion erkranken. Verlaufsdaten österreichischer Intensivstationen über die Belastung durch schwere Influenza-Infektionen existieren jedoch bisher nicht.

Ein wichtiges Ziel dieser Studie war es daher, epidemiologische klinische Eigenschaften und Laborparameter von an der ICU behandelten PCR-bestätigten Influenzainfektionen im Verlauf zu analysieren. Wir nahmen an, dass diese Patient*innen meist über 50 Jahre sein und zudem kardiovaskuläre oder pulmonale Vorerkrankungen haben würden. Außerdem erwarteten wir bei mehr als 50% kardiovaskuläre oder pulmonale Komplikationen sowie sekundäre bakterielle oder Pilz-Infektionen.

Zudem sollten die Belastung der Intensivstationen durch Influenza-Patient*innen in der Steiermark und die Charakteristika dieser Patient*innen untersucht werden.

3.2 Methoden

In einer retrospektiven Studie wurden klinische Daten und Laborparameter von Intensivpatienten mit PCR-bestätigten Influenza-Infektionen evaluiert. Erfasst wurden Patient*innen, die zwischen 2009 und 2020 auf der Intensivstation der MUG wegen Influenza behandelt wurden. Insgesamt wurden Daten von 111 Patient*innen aus fünf Influenza-Saisons (2009/2010, 2016/17, 2017/18, 2018/19, 2019/20) untersucht und in einer deskriptiven Analyse dargestellt.

3.3 Ergebnisse

Das mediane Alter der 111 Patient*innen lag bei 69 Jahren. Die Patientenpopulation enthielt mehr Männer als Frauen, wobei die Patientinnen signifikant älter waren (p=0.037). Die mediane Aufenthaltsdauer auf der Intensivstation betrug 6 Tage, 39,1 % verstarben. Eine respiratorische Insuffizienz lag bei 83,2% vor, bei 43,5% eine Hyperkapnie und bei 43,3% eine Lungenschädigung.

19,8 % entwickelten während der Intensivbehandlung kardiale Komplikationen, 82,9 % zeigten pulmonale Verschattungen (u.a. vereinbar mit pulmonalen Infiltraten) und 37,8 % hatten Sekundärinfektionen.

Patient*innen mit vorbestehenden kardiovaskulären Erkrankungen wie Herzinsuffizienz oder Herzklappenerkrankungen entwickelten signifikant häufiger kardiale Komplikationen, auch Herzrhythmusstörungen und koronare Herzkrankheiten traten bei diesen Patient*innen gehäuft auf (nicht signifikant). Patient*innen mit kardialen Komplikationen waren häufiger männlich und älter. Sie wiesen eine höhere Körpertemperatur auf und waren eher tachykard, hatten eine erhöhte Leukozytenzahl im peripheren Blut, höhere PCT- und NT-pro-BNP-Werte sowie einen höheren Horowitz-Index und deutlich seltener eine Hyperkapnie.

Die Gruppe der Patient*innen ohne kardiale Komplikationen hatte häufiger Vorerkrankungen wie arterielle Hypertonie, Asthma, periphere oder zerebrale arterielle Verschlusskrankheiten, Diabetes mellitus, gastrointestinale Erkrankungen und Malignome sowie einen höheren BMI. Bei der klinischen Untersuchung konnten zudem vermehrt Giemen und Rasselgeräusche festgestellt werden. Im Einklang damit hatten diese Patient*innen häufiger Lungenentzündungen und mussten eher mechanisch beatmet werden. Die Laboranalyse ergab höhere CRP-Werte. Außerdem kam es vermehrt zu Sekundärinfektionen, dabei traten vor allem bakterielle nosokomiale Pneumonien signifikant häufiger auf.

Zusammengefasst fällt in unserer Patientenpopulation die hohe Rate an Komplikationen sowie eine hohe Sterblichkeitsrate von 39.1 % auf.

3.4 Diskussion

Basierend auf unserer Analyse unterscheiden wir zwischen dem kardialen und dem pulmonalen Phänotyp einer schweren Influenza-Infektion. Der kardiale Phänotyp war älter, eher männlich, hatte signifikant häufiger eine kardiale Vorerkrankung (Herzinsuffizienz $p < 0.001$, Herzklappenerkrankungen $p = 0.031$) und eine höhere Mortalität. Der pulmonale Phänotyp musste häufiger invasiv beatmet werden, er hatte häufiger Pneumonien. Außerdem traten hier eher sekundäre Infektionen auf und signifikant häufiger waren bakterielle nosokomiale Pneumonien ($p = 0.014$).

4 Abstract in English

4.1 Aim

Austria's influenza vaccination rate is low compared to other European countries, this is especially true for patients with chronic cardiopulmonary diseases. These should therefore be more likely to develop a severe influenza infection. However, follow-up data of Austrian ICUs on the burden of severe influenza infections do not exist yet.
Therefore, an important aim of this study was to analyze epidemiological clinical characteristics and laboratory parameters of PCR-confirmed influenza infections treated in the ICU during their course. We assumed that these patients would mostly be over 50 years of age and would also have previous cardiovascular or pulmonary disease. We also expected more than 50% to have cardiovascular or pulmonary complications and secondary bacterial or fungal infections.
In addition, we aimed to investigate the burden of influenza patients in intensive care units in Styria and the characteristics of these patients.

4.2 Methods

In a retrospective study, clinical data and laboratory parameters of ICU patients with PCR-confirmed influenza infections were evaluated. Patients treated for influenza in the MUG ICU between 2009 and 2020 were included. In total, data from 111 patients from five influenza seasons (2009/2010, 2016/17, 2017/18, 2018/19, 2019/20) were examined and presented in a descriptive analysis.

4.3 Results

The median age of the 111 influenza patients was 69 years. The patient population contained more males than females, with female patients being significantly older than male patients (p=0.037). The median length of stay in the ICU was 6 days, 39.1% of died. Respiratory insufficiency was present in 83.2%, hypercapnia in 43.5%, and lung injury in 43.3%.
19.8% developed cardiac complications during their ICU stay, 82.9% developed increased airspace shadowing (compatible with pulmonary infiltrates) , and 37.8% had secondary infections.

Patients with preexisting cardiovascular disease, such as heart failure or valvular heart disease, developed significantly more cardiac complications; arrhythmias and coronary artery disease were also more common in these patients (not significant). In addition, the patients who developed cardiac complications were more likely to be male and older. They had higher body temperature and were more likely to be tachycardic, had a higher leukocyte count, higher PCT and NT-pro-BNP levels, a higher Horowitz index, and were less likely to develop hypercapnia.

The group of patients without cardiac complications more often had preexisting conditions such as arterial hypertension, asthma, PAOD and CAOD, diabetes mellitus, gastrointestinal disease, and malignancies. In addition, these patients had a higher BMI. Clinical examination also revealed increased wheezing and crackles. Consistent with this, these patients had a higher incidence of pneumonia and required more often mechanical ventilation. Laboratory analysis revealed higher CRP levels. In addition, secondary infections were more frequent, with bacterial nosocomial pneumonia in particular occurring significantly more frequently.

In summary, the high rate of complications in our patient population is striking, especially a high mortality rate of 39.1%.

4.4 Discussion

Based on our analysis, we distinguish between the cardiac and pulmonary phenotypes of severe influenza infection. The cardiac type is older, more often male, significantly more often has previous cardiac disease (heart failure $p < 0.001$, valvular heart disease $p = 0.031$) and higher mortality. The pulmonary type more often required invasive ventilation, had more frequent pneumonias. In addition, secondary infections were more frequent, bacterial nosocomial pneumonia was significantly more frequent ($p = 0.014$).

5 Introduction

In intensive care units, we repeatedly see very seriously ill patients whose lives are nevertheless sometimes barely salvageable despite a great deal of personal and professional effort. One disease that can lead to such severe and sometimes life-threatening complications is infection with influenza viruses types A and B (1, 2). According to data from the Austrian Ministry of Social Affairs, the average influenza mortality rate in Austria, which fluctuates from year to year, is over 15 deaths per 100,000 inhabitants, i.e. over 1,000 deaths per year (3).

In two studies conducted at the University Hospital Graz during the 2009/10 influenza season by Hoenig, Flick et al., parameters could be recorded that should enable the treating physicians to start a targeted therapy in a prompt manner (4, 5). So far, however, there is no longitudinal data on several influenza seasons in Styria. A central aim of this work is therefore to close this research gap by collecting longitudinal data about the burden of influenza patients treated in intensive care unit (ICU) and patient characteristics over a longer period of time. For this study, patient data from the years 2009 to 2020 were evaluated and the particular symptomatology of these patients investigated.

It is important to distinguish infections with the influenza virus (A and B) from seasonal colds caused by other respiratory viruses. The subject of this paper is the polymerase chain reaction (PCR)-proven influenza virus (A and B) infection.

The symptoms and course of the disease are influenced by immunological, virus-specific and individual characteristics. This is why, for example, the Austrian Ministry of Social Affairs as well as the permanent vaccination commission of the German Robert Koch Institute recommend the vaccination of people who are particularly at risk (3, 6). People with cardiovascular, pulmonary or other chronic diseases are particularly at risk of severe disease. People over 60 years of age and especially over 65 years of age also have a high risk of severe disease progression (7-10).

6 Influenza Virus

6.1 General

Influenza virus is a virus of the Orthomyxoviridae family, which are enveloped viruses with single-stranded RNA (11). Three different influenza subtypes can cause influenza in humans: Influenza A, influenza B and influenza C. The three subtypes are similar in terms of host cell-derived envelope, segmented genome with single-stranded RNA, and envelope glycoproteins, which are critical for virus entry and exit into host cells. As shown in the table below, there are differences in structure, genetics, host range and clinical manifestation (12).

Influenza A and B each contain 8 gene segments, and influenza C contains 7 gene segments (12). The most common influenza illnesses are caused by influenza A and B viruses. Influenza C virus is significantly less often responsible for illnesses (13).

Table 1 Differences Among Influenza A, B and C Virus, adapted from (12)

Virus Subtype	Influenza A	Influenza B	Influenza C
Structure	10 viral proteins	11 viral proteins	9 viral proteins
Genetics	8 gene segments	8 gene segments	7 gene segments
Host range	Humans, swine, equine, birds, marine mammals	Humans only	Humans and swine
Clinical manifestations	pandemics with significant mortality in young persons	No pandemics, but severe disease to high risk patients and older persons	Mild disease, no seasonality

Several specific proteins are expressed on the surface of the virion. The influenza A subtype is characterized by the proteins hemagglutinin (HA), neuraminidase (NA) and M2. Influenza A is sub differentiated by several HA and NA proteins. In total, there are at least 18 different HAs (H1-H18) and at least 11 different NAs (N1-N11) (14, 15). While the hemagglutinins are important for host cell entry, the M2 protein is an ion channel that is

relevant for virus uncoating. The neuraminidase is responsible in the final stage for the shedding of the virus offspring (12).

The influenza B subtype also has HA and NA expressed on the surface but with a lower genetic diversity. However, there is a sub-differentiation into two distinct strains: the Victoria strain and the Yamagata strain, both circulating in the human population (16). Other proteins on the surface of influenza B are BM2 and NB. While BM2 is thought to have a similar mechanism and structure to M2, the utility of the NB protein is not yet fully understood. Hatta and Kawaoka were able to show that the protein is not a total requirement for replication of influenza B in vitro but aids in replication of influenza B in vivo (17).

Inside the virion is the M1 protein which has a structuring effect. In the core are the ribonucleoprotein particles which are composed of viral RNA segments, polymerase proteins (PB1, PB2 and PA) nucleoprotein (NP). In addition, there are two other proteins: nonstructural protein 1 (NS1) and nonstructural protein 2 (NS2) or also called nuclear export protein (NEP) (12, 14).

For the nomenclature of the different influenza strains, the type, the species from which the virus was isolated, the place of isolation, the number of isolation, the year of isolation and for influenza type A the hemagglutinin (H) and the neuraminidase (N) are used. For example influenza A/chicken/Hong Kong/220/97(H5N1) was isolated from chickens in Hong Kong in 1997 and is the 220th isolation of influenza A subtype H5N1 (14).

6.2 History

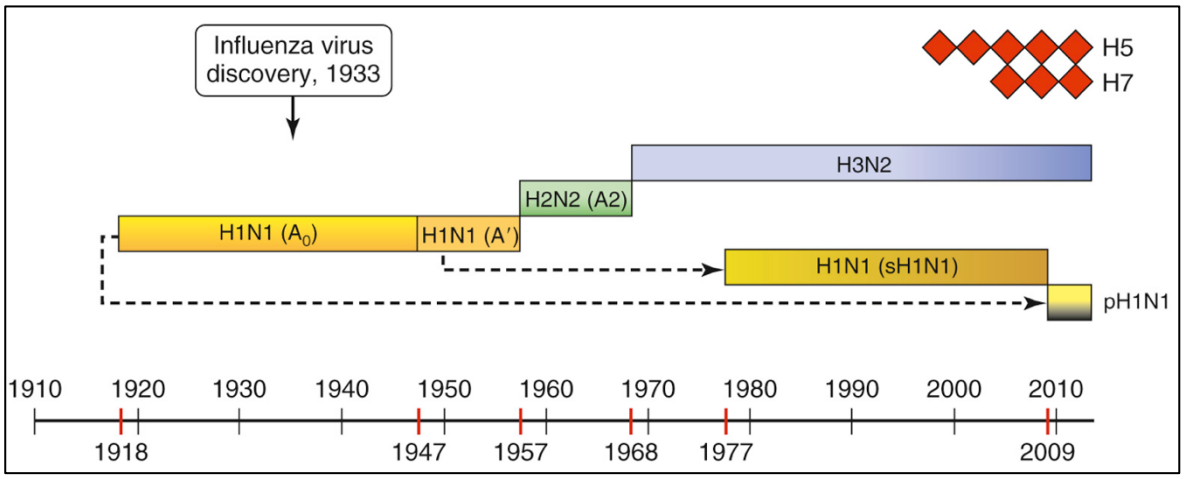
The Greek physician Hippocrates was the first to describe the disease influenza in 412 BC (18). However, the older descriptions of influenza outbreaks have many uncertainties. In 1173, an epidemic was reported for the first time that was probably actually caused by influenza. Other reports date from the 14th and 15th centuries (19). As the causes of influenza were still unclear at that time, only the typical manifestations, such as the frequency of coughing, sudden onset of a 3-day fever, a fatigue that did not match the severity of the other symptoms, and the epidemic character with a high attack rate, could help identify earlier outbreaks. There have been well-documented accounts of influenza and its outbreaks by scientists and lay people since at least 1650 (12, 19). In 1892, Robert Pfeiffer discovered a bacterium that he mistakenly believed to be the causative agent of influenza. It was *Bacillus influenzae*, known today as *Hemophilus influenzae*, which is not responsible for influenza but can often cause secondary infections (20, 21). During the

Spanish flu of 1918, Peter K. Olitsky and Frederick L. Gates, researchers at the Rockefeller Institute for Medical Research, provided evidence against the bacterial hypothesis by showing that the influenza agent could not be eliminated by Berkefeld V and N filters, which filter out bacteria (22). In 1930, Richard Shope of the Rockefeller Institute succeeded in identifying and isolating a virus as the causative agent of swine flu: Influenza virus A/Swine/Iowa/30 (23, 24). Then 3 years later in 1933, the first human virus was isolated in ferrets by Smith, Andrewes and Laidlaw, which finally provided evidence for a virus as the causative agent of influenza (influenza virus A) (25). In 1940, a different virus in terms of antigenicity was isolated by Francis and was called influenza type B: Influenza B/Lee/40 (23, 26). Influenza virus type C was first described by Taylor in 1950 (12). In 1936, Burnet demonstrated that influenza virus could be cultured in embryonic chicken eggs. This enabled extensive research into the virus, thereby opening up the field of inactivated vaccine development (12). In addition, the efficacy of inactivated influenza vaccines (containing inactivated influenza virus types A and B) was successfully confirmed by Francis, Salk, Pearson, and Brown in a human trial in 1942/43 (27). Francis, Salk, Pearson and Brown measured and compared antibody titers in their study, which was only due to the effect of hemagglutination discovered by Hirst in 1941 (28).

6.3 Pandemics

As mentioned in chapter 5.1, the influenza virus presents with different surface proteins. The immune defense in humans is directed against the surface proteins HA and NA, with HA playing a more important role (29). However, the influenza virus is subject to a high mutation rate which leads to frequent changes in the antigens HA and NA. This phenomenon is known as antigenic drift and leads to a poorer immune response from a host that has been vaccinated or previously infected with this subtype. As a result, influenza epidemics occur regularly. As stated earlier, there are only two distinct strains of influenza B, whereas influenza A is derived from at least 18 different HAs and at least 11 different NAs. New viral strains can emerge in influenza A through an antigenic shift, that can cause worldwide pandemics (12). Figure 1 shows the occurrence of influenza A subtypes and pandemics in humans over the last 100 years. Several pandemics have already occurred, among them in 1889-1893, 1918-19, 1957-58, 1968-69, 1977-78, and 2009-2010 (30).

Figure 1 **Circulating Influenza A Subtypes and Recent Pandemics, Adapted from (12)**



6.3.1 1889-1894 Russian Flu 1

The Russian flu was the first pandemic in which influenza was considered an easily diagnosed disease. During this time, Robert Pfeiffer misidentified *Bacillus influenza* as the causative agent of influenza (see chapter 5.2). Russian flu, also called Asian flu, spread from east to west along railroads and other travel routes. In total, about one million people died, among them primarily the elderly and the very young (18, 30, 31). The suspected influenza A subtype was first H2, then H3 (32, 33), although influenza as the causative agent of Russian influenza is now being questioned, as recent studies also suggest an origin from coronaviruses (34).

6.3.2 1918-1919 Spanish Flu H1N1

The Spanish flu was a major influenza pandemic in 1918-1919. The pandemic spread in three waves across Asia, North America and Europe within a year, infecting around 500 million people, about a third of the world's population. The pandemic's high mortality rate was particularly notable, with a case fatality rate of over 2.5%, resulting in an estimated 50 million deaths, with some estimates as many as 100 million deaths (35). While in other pandemics the case fatality rate was less than 0.1% and in previous years mainly older and very young people died, the case fatality rate for 20-39 year olds was 1-2%, even though people of this age rarely die of influenza. Other risk groups were young children, especially those under one year of age, and pregnant women (18, 35). Remarkably, there were significantly fewer deaths in the over-50 age group and especially in the over-80 age group (36). One explanation for the extraordinarily high mortality of the Spanish flu could

be the increased occurrence of secondary bacterial infections (mainly *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Staphylococcus aureus* and *Hemophilus influenzae*) for which antibiotic treatment was not yet available at that time (18). In their study, McAuley et al. demonstrated an association between PB1-F2 protein and the frequency and severity of secondary bacterial infections in mice (37). The Spanish flu is now known as the mother of all pandemics, as not only all influenza pandemics but also all influenza A cases since then have originated from the original H1N1 subtype that caused the Spanish flu. Only the human infections of avian influenza (subtypes H5N1 and H7N) are not derived from it (35). The initial location of the Spanish flu outbreak remains unclear. The places of origin discussed are China, Northern France and America, with the latter currently considered the most probable (38).

6.3.3 1957-1958 Asian Flu H2N2

In February 1957, a new pandemic began in Guizhou Province, Southern China and quickly expanded to Hunan Province, Singapore, and Hong Kong (23). The causative agent was a new influenza A virus of subtype H2N2. It resulted from an antigenic shift of 3 gene segments from an avian influenza virus into the subtype influenza A H1N1. The 3 gene segments encode PB1, hemagglutinin and neuraminidase (18). Within 6 months, the virus spread throughout the world, infecting about 40-50% of people, particularly the young, the elderly, and patients with cardiac abnormalities (18) with a total of over one million deaths. Again, the main cause of death was secondary bacterial infection resulting in death from bacterial pneumonia (19). Two years after the pandemic, the virus became endemic and finally disappeared after 11 years and has not been isolated from humans since (39).

6.3.4 1968-1969 Hong Kong Flu H3N2

The mechanism for the emergence of Hong Kong influenza was the same as for Asian influenza: an antigenic shift of two genes (encoding hemagglutinin and PB1) from an avian influenza virus into influenza A subtype H2N2 created a new influenza A H3N2 subtype (39). The new virus first appeared in Southeast Asia and spread from there. In total, about 1 million people died from the pandemic, most of them older than 65 years. The virus circulated around the globe until at least 2019 (40). Compared to other pandemics, Hong Kong flu was relatively mild, probably because only hemagglutinin and PB1 changed, but not neuraminidase (39).

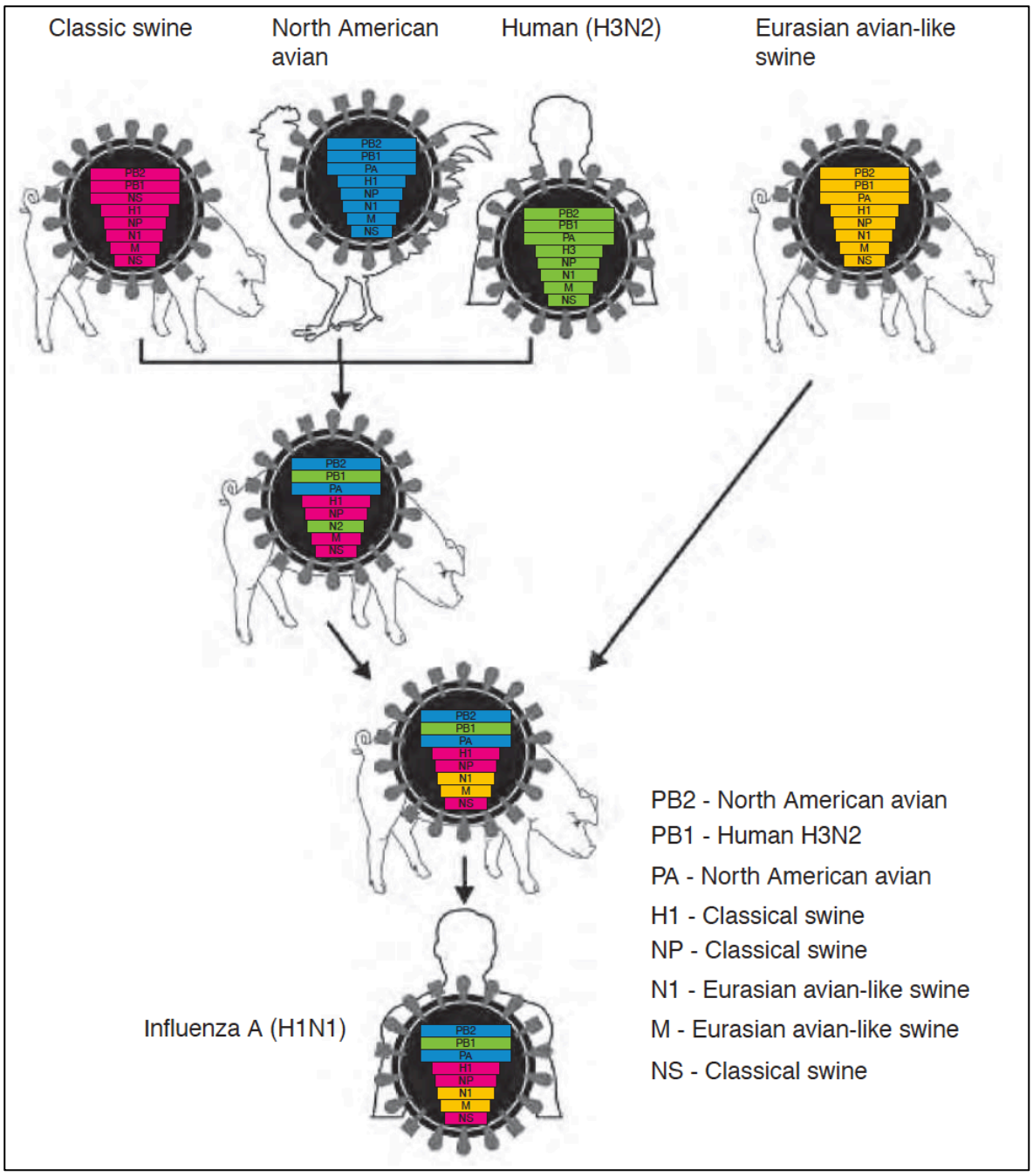
6.3.5 1977-1978 Russian Flu 2 H1N1

In 1977, there was an outbreak with a new influenza virus in Tianjin, China, which spread from there to the Soviet Union. The interesting thing about this outbreak was that the previously circulating virus (influenza A H3N2) was not replaced by the new virus, as had been the case in previous pandemics. (see chapter 5.3.3 and chapter 5.3.4). In addition, the new virus (an influenza A H1N1) was very similar to the H1N1 virus of the early 1950s. Due to the population's immunity to the H1N1 virus of the 1950s, mainly people under 25 years of age were infected. Because the virus was so similar to the 1950s H1N1 virus, the current assumption is that a laboratory accident led to the pandemic. Since 1977, both influenza A H1N1 and influenza A H3N2 have occurred regularly (18, 23).

6.3.6 2009-2010 Influenza pdm09H1N1

In 2009, H1N1 emerged on a pandemic scale. It was first detected in California in April 2009, but the initial outbreak could be traced to Mexico (18, 23). Due to its antigenic relationship to the H1N1 virus that caused the Spanish flu (18), initial reports of a very high mortality, especially among younger people (41), and rapid global spread, pandemic status was quickly declared (42). What was special about this pandemic was that despite a seasonal H1N1 virus in previous years, an H1N1 pandemic occurred at all, which no one had expected. The pandemic occurred because a new virus (the pdm09H1N1) was created by "quadruple reassortment" of both pigs and birds as shown in Figure 2. The H1 originated from the classical swine flu and bore a strong resemblance to the H1 of the 20th century human influenza (12). Therefore, this pandemic more often affected younger age groups that did not yet have cross-immunity with the 20th century H1N1 virus (12, 43). The 2009 pandemic with the pdm09H1N1 virus resulted in 18,500 laboratory-confirmed deaths. In their study, Dawood et al. suggest a death toll of approximately 284,500, with 83,300 deaths from cardiac causes and the remainder from respiratory causes. Overall, about 80% of the deaths were younger than 65 years (44). Because influenza deaths tend to occur primarily in the over-65 age group, the 2009 pdm09H1N1 pandemic is considered mild compared to previous pandemics. In terms of years of life lost, the pandemic is comparable to the 1968 H3N2 pandemic because primarily younger people died (18, 45). Since 2009 until at least 2018, the pdm09H1N1 virus circulated regularly in humans (46).

Figure 2 **Quadruple Reassortment of the Influenza A H1N1 pdm09 Virus adapted from (23)**



6.4 Clinical Features

Influenza virus infections are common worldwide, with temporal clustering during the winter months (southern and northern hemispheres) and year-round in tropical areas (47). The influenza incubation period is about 2 days and is usually within the range of one to four days (2). The severity of influenza ranges from asymptomatic courses to very severe

illnesses with fatal outcomes (13). About one-third of influenza infections result in a course with fever, one-third in a mild course without fever, and one-third in an asymptomatic course (47). A normal course of the disease with fever and without further complications lasts about a week. It is characterized by sudden onset of illness with high fever, chills, loss of appetite, arthralgias, myalgias, sore throat, headache, and dry cough. Elderly people usually do not have such a high fever; in young children, gastrointestinal symptoms such as abdominal pain, vomiting, and diarrhea dominate along with the fever (48). Lung function may be impaired for up to a month or more after the acute illness, and the cough may persist for several days even after other symptoms have resolved (49).

6.4.1 Complications

Despite the fact that influenza is self-limited and harmless in most cases, complications regularly occur and can be severe or even fatal. The most common severe complications of influenza involve the respiratory tract. Influenza can cause primary influenza pneumonia, which is characterized by severe illness, dyspnea, cyanosis, wheezing, and crackles. Pathologically, necrotizing bronchitis, hyaline membranes, interstitial inflammation, and intraalveolar hemorrhage can be detected. The mortality of primary influenza pneumonia is estimated to be 6-29% (1). Another important cause of severe to fatal complications is also secondary bacterial superinfection, which can lead to secondary bacterial pneumonia. Typical pathogens are *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus influenzae* and further Gram-negative rods. Secondary bacterial pneumonia caused by MRSA has a mortality of about 33%. Secondary bacterial superinfections play a major role, especially in influenza, as neuraminidase favors the adherence and spread of bacteria. In addition, the virus weakens the mucociliary clearance of the lungs and causes epithelial damage, allowing bacteria to invade more easily (1, 50). Just like the typical pathogens of a bacterial superinfection, the atypical pathogens can also cause a superinfection. These include *Chlamydia pneumoniae*, *Legionella pneumophila*, *B-hemolytic Streptococci* and *Aspergillus species*. Secondary Aspergillus infections in particular have a high mortality rate (1, 51). In addition, influenza infection can exacerbate pre-existing COPD or asthma (52). In their study, Rello et al. described exacerbated COPD and asthma as the most common co-morbidity of influenza patients with severe respiratory failure (53). Influenza infection can also cause rapidly worsening severe illness associated with severe ARDS, shock, and multiorgan failure (54). Regarding the non-respiratory complications of influenza, rhabdomyolysis and myositis are rare but severe complications that

predominantly affect children (55, 56). Neurological complications can also occur with influenza infection. It is estimated that 1.2 neurological complications occur per 100,000 symptomatic H1N1 infections. The majority (about three-quarters) of complications occur in children. The most common neurologic complications are encephalopathies and febrile convulsions, and less commonly Guillain-Barré syndrome (57). In terms of cardiac complications, influenza infection is often associated with an abnormal electrocardiogram (ECG). Ison et al. showed in their study that in most young patients with influenza infection and no previous cardiac disease, ECG abnormalities were seen without an increase in cardiac markers, reduced ejection fraction or wall motion abnormalities (58). However, it must be stated that influenza infection is associated with a significantly increased risk of an acute cardiac event, such as acute heart failure, acute ischemic heart disease, cardiogenic shock, acute myocarditis, acute pericarditis, and pericardial tamponade. Approximately 12% of influenza patients develop an acute cardiac event according to the study by Chow et al. (59). Influenza infection increases heart attack risk 6-fold compared to uninfected individuals (48). This is supported by the study of Kwong et al. who also found that the risk of myocardial infarction increases approximately 5-fold with influenza A, while with influenza B the risk of myocardial infarction increases approximately 10-fold (8).

6.4.2 Patients at Risk for a Severe Course

According to the vaccination recommendation of the Austrian Ministry of Social Affairs, there are several risk groups that have an increased risk for a severe course. These include infants from the age of 6 months, young children, and people over the age of 60. In addition, chronic diseases such as lung and cardiovascular diseases, as well as neurological diseases, kidney diseases, immunodeficiencies and metabolic diseases increase the risk for a severe course. Furthermore, pregnant women, the third degree obese (BMI ≥40), HIV-infected individuals, and patients on immunosuppressive therapy have a significantly increased risk (3).

6.5 Diagnosis

Influenza can be diagnosed in several ways, either clinically or by diagnostic tests. Clinically, influenza is usually manifested by weakness, myalgias, cough, and nasal congestion, but at least weakness and myalgias are also common in patients with other respiratory infections. Monto et al. demonstrated in their study that patients who presented

with fever and cough had a PPV of 79% for influenza (60). According to the Infectious Diseases Society of America's 2018 guideline, clinical diagnosis should be verified by diagnostic testing during the influenza season if the patient is hospitalized, if the test would change treatment, or if the patient has atypical symptoms or influenza-associated complications. Outside of influenza seasons, testing is appropriate for high-risk patients and immunocompromised patients, as well as for patients to be hospitalized with acute respiratory illness and contact with influenza patients or if they come from regions with unknown influenza activity (61). There are different diagnostic tests for influenza diagnosis: rapid influenza tests, rapid molecular assays, direct and indirect immunofluorescence assays, RT and multiplex PCR, rapid cell culture (shell vial and cell mixtures) and viral cultures (61, 62). Influenza PCR is accepted as the gold standard for influenza diagnostics for which Zambon et al. showed a sensitivity of 92% and a specificity of 84% in their study (63). Since PCR analysis, including laboratory transport, takes a lot of time, faster tests are needed. Rapid influenza diagnostic tests (RIDTs) are available that show a result within 30 minutes. The RIDTs have a high specificity of approximately 99.4% for influenza A and 99.8% for influenza B, but only a low sensitivity of approximately 54.4% for influenza A and 53.2% for influenza B. In 2011, rapid molecular assays were introduced that can also provide a result within 30 minutes. These assays are based on the nucleic acid amplification method and have a significantly higher sensitivity of approximately 91.6% for influenza A and 95.4% for influenza B and a similarly high specificity of approximately 99.2% for influenza A and 99.4% for influenza B (64). Due to the significantly superior sensitivity of rapid molecular assays over RIDTs, the Infectious Diseases Society of America recommends rapid molecular assays as the primary test in its 2018 guideline (61). Other diagnostic tests are direct and indirect immunofluorescence assays (DFA and IFA). Both tests require laboratory expertise and thus either a hospital-based laboratory or a reference laboratory. The sensitivity of DFA ranges from 47 to 93% for influenza A (H1N1)pdm09. For IFA, Nutter et al. demonstrated a sensitivity of 71% for influenza A (H1N1)pdm09 and thus no advantage of IFA over RIDT (65). Rapid cell culture (shell vial and cell mixtures) and viral cultures used to be relevant in diagnostics with a sensitivity of more than 90% within three days and an even better result after 5 to 7 days. But this diagnostic period is too long to influence the treatment (12). Serological tests are not useful in routine diagnostics due to the non-interpretability of a single serum sample, but are rather used for research purposes (62).

6.5.1 Differential Diagnosis

Influenza has numerous differential diagnoses that can cause similar symptomatology. The most common are acute lower and upper respiratory tract infections such as acute bronchitis (including the so called common cold), pertussis, and pneumonia. The causative agents of bronchitis and pneumonia are frequently rhinoviruses, adenoviruses, parainfluenza, respiratory syncytial viruses (RSV), coronaviruses, *Streptococcus pneumoniae, Hemophilus influenzae,* and in comorbid patients also enterobacteriaceae and *Staphylococcus aureus* (48). Other differential diagnoses include non-infectious asthma or COPD exacerbations

6.6 Treatment

The Infectious Diseases Society of America's 2018 guideline recommends antiviral treatment as soon as possible for suspected or confirmed influenza infection in hospitalized patients, high-risk patients, patients with severe illness, children younger than 2 years, adults older than 65 years, and pregnant women up to two weeks postpartum (61). Possible antiviral medications include neuraminidase inhibitors such as oseltamivir, zanamivir, and peramivir; M2 inhibitors such as amantadine and rimantadine; and cap-dependent endonuclease inhibitors such as baloxavir (12). According to the 2018 Infectious Diseases Society of America guideline, neuraminidase inhibitors are the antiviral agent of choice (61). The neuraminidase inhibitors have a better effect the sooner they are applied, preferably within 48 hours (61, 66). Which emphasizes the importance of a rapid diagnosis. Oseltamivir is the most common neuraminidase inhibitor and is administered orally. It reduces the risk for influenza complications, as well as the risk for hospitalization, and reduces the time to symptom relief (67). Zanamivir is a neuraminidase inhibitor that is inhaled and peramivir is administered intravenously. Peramivir appears to have slightly higher efficacy than other neuraminidase inhibitors (68), but was only approved in the EU from 2018 to 2020 and is currently withdrawn for commercial reasons (69). M2 inhibitors are only effective against influenza A. Today, however, circulating influenza viruses are resistant to amantadine and rimantadine, which is why they are not currently administered (12). Baloxavir has been approved as a new antiviral drug in the EU since January 2021 (70). This is a cap-dependent endonuclease inhibitor and seems to work best when administered within 24 hours of symptom onset. Hayden et al. were able to show in their study that baloxavir has a better antiviral effect than oseltamivir, although with a similar time to symptom relief (71). In addition to antiviral treatment, the Infectious

diseases society of America's 2018 guideline recommends empiric antibiotic therapy for patients with severe disease including complications, such as respiratory failure, pneumonia, hypotension, and in patients who first improve and then worsen again on antiviral therapy (61).

6.7 Vaccination

As already described in chapter 5.2, the effectiveness of inactivated influenza vaccines was proven as early as 1942/43 by Francis et al. (27). Today, influenza vaccination is considered the best option for the control and prevention of influenza infections (72). According to the vaccination recommendation of the Austrian Ministry of Social Affairs, vaccination is recommended for all persons (from 7 months of age). In particular, vaccination is recommended for risk groups (see chapter 5.4.2). Due to frequent changes in the antigenic structure of the virus (see chapter 5.3), vaccines must be adjusted annually (3). The WHO recommends influenza vaccine composition annually in February for the upcoming influenza season in the northern hemisphere. For quadrivalent vaccines, the following influenza strains were recommended for the 2021/22 influenza Season: (73)
For egg-based vaccines:

- A/Victoria/2570/2019 (H1N1)pdm09-like virus
- A/Cambodia/e0826360/2020 (H3N2)-like virus
- B/Washington/02/2019 (B/Victoria lineage)-like virus
- B/Phuket/3073/2013 (B/Yamagata lineage)-like virus.

For cell or recombinant-based vaccines:

- A/Wisconsin/588/2019 (H1N1)pdm09-like virus
- A/Cambodia/e0826360/2020 (H3N2)-like virus
- B/Washington/02/2019 (B/Victoria lineage)-like virus
- B/Phuket/3073/2013 (B/Yamagata lineage)-like virus

In Austria, only quadrivalent vaccines are licensed for the 2021/2022 influenza season. The Austrian vaccination strategy aims to reduce the spread of influenza viruses in order to relieve the burden on the health care system, especially due to the still ongoing COVID-19 pandemic (3).

7 Materials and Methods

The method chosen for this study is explained below. First, study objects, study design and data collection are outlined. Then case report and finally statistical analyses used are explained.

7.1 Study Objectives

Influenza has turned out to be a disease with sometimes very serious complications. In the following, patients treated in intensive care units will be examined with regard to the following characteristics in order to better assess the individual patient risk:

- To determine the epidemiological, clinical and laboratory characteristics of PCR proven influenza infections treated on the ICU.
- To determine the rate of cardiovascular and pulmonary complications.
- To determine secondary bacterial / fungal infections during the treatment on the ICU.

7.2 Study Design

This is a retrospective study with PCR-proven influenza infections, treated on ICUs in Styria (mainly but not exclusively at the Medical University of Graz). The study was conducted by the division of pulmonology in cooperation with the devision of cardiology, the division of infectious diseases, and the division of intensive care medicine at the department of internal medicine, Medical University of Graz.
It included the years between 2009 and 2020. Altogether five influenza seasons were covered, which are 2009/10, 2016/17, 2017/18, 2018/19 and 2019/20.
During this period, 111 patients with PCR-confirmed influenza infections and their treatment on ICU were analyzed.

7.3 Data Collection

The patient data were collected anonymously. An ethic approval was granted by the local ethic committee and based on the EK 23-049 ex 10/11 and associated amendments (Date 19.05.2020).
Due to the non-interventional study design, prior informed consent was not required.
While the patient data from the 2009/10 influenza season are derived from the data collection diploma thesis "Comparison of demographic data, clinical presentation, risk

factors and outcome between PCR confirmed H1N1 influenza and PCR negative influenza-like illnesses" Jürgen Prattes, the data from the 2016/17 and 2017/18 influenza seasons are based on a systematic direct search in MEDOCS (admissions to the ICU from 15.12. to 30.04.) and were matched with iNAT lists from LB2 of the University Hospital Graz. Since 2018 influenza-patients have been continuously documented by the department of infectious diseases. Influenza ICU patients were filtered out from this list.

7.4 Case Report Form

In order to record the patient characteristics and symptomatology in a structured way, a case report form was created that contained the following information:

- Demographic data
- Date of PCR testing and PCR result
- Date of onset of symptoms
- Date of first clinical presentation
- Hospital(s)
- Radiologic data
- Outcome
- Date of ICU admission
- Clinical Presentation at ICU admission (temperature, symptoms, findings)
- Laboratory data (+/-1 Day from ICU admission)
- Microbiological findings
- Antibiotic and antiviral treatment
- Preexisting underlying diseases
- Invasive mechanical ventilation
- SAPS 2/3 score
- Complications (cardiovascular / pulmonary)

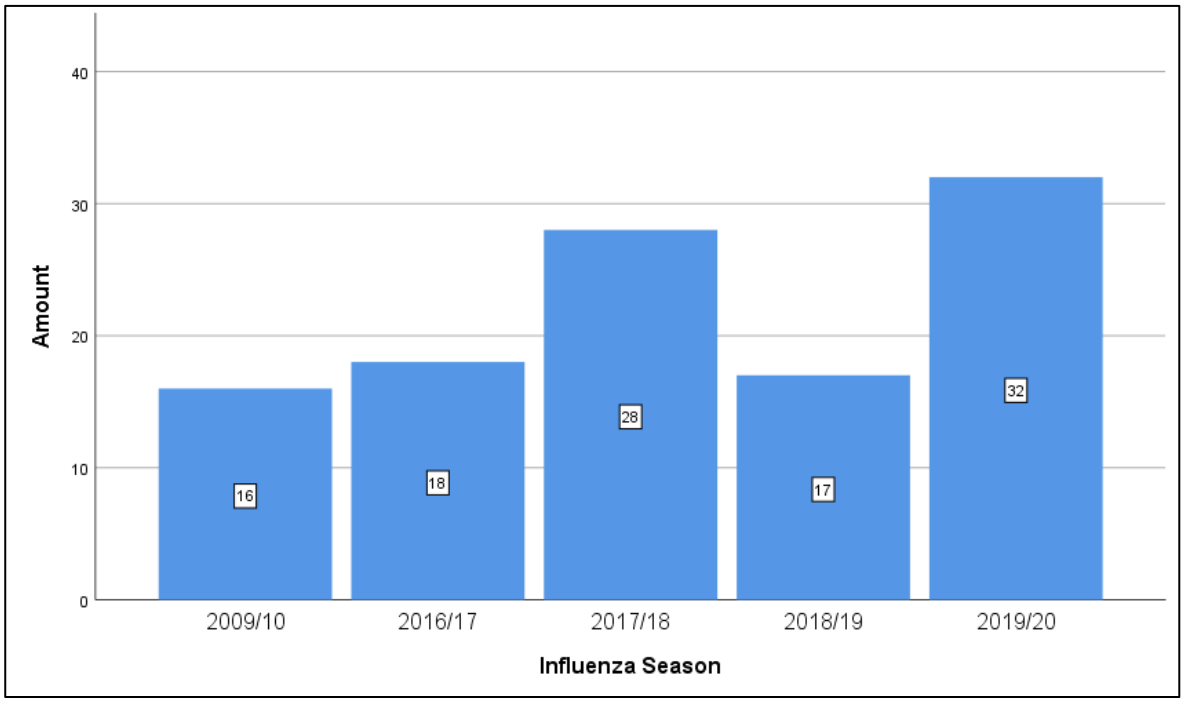
7.5 Statistical Analysis

Statistical analysis was carried out using "IBM SPSS Statistics Version 26" and the data from the different subgroups were compared using the chi-square test or the Fisher exact test. Data are presented as medians (interquartile range 25-75: IQR 25-75) or as absolute and relative counts. A p-value less than 0.05 was assumed to be significant.

8 Results

In total, patient data from 01 September 2009 to 01 May 2020 were evaluated. All these patients were treated in an intensive care unit of the LKH network in Styria and were PCR-tested for influenza. During the observation period, data could be collected from 111 patients. By including data from the 2009/2010 season collected as part of a previous thesis (see chapter 6.3.), this study starts with data from an influenza-positive patient admitted to the hospital on 8 November 2009. No patient data were collected during the 2010/11, 2011/12, 2012/13, 2013/14, 2014/15, 2015/16 influenza seasons, and the data collection of this study starts with the 2016/2017 season and ends with an influenza-positive patient admitted to the hospital on 12 March 2020. Of the total 111 patients, 16 (14.4%) were tested positive for influenza in the 2009/10 influenza season, 18 (16.2%) in 2016/17, 28 (25.2%) in 2017/18, 17 (15.3%) in 2018/19 and 32 (25.8%) in 2019/20. Most patients were observed in the two seasons 2017/18 and 2019/20 (Figure 3).

Figure 3 **Number of ICU Patients between 2009 and 2020**

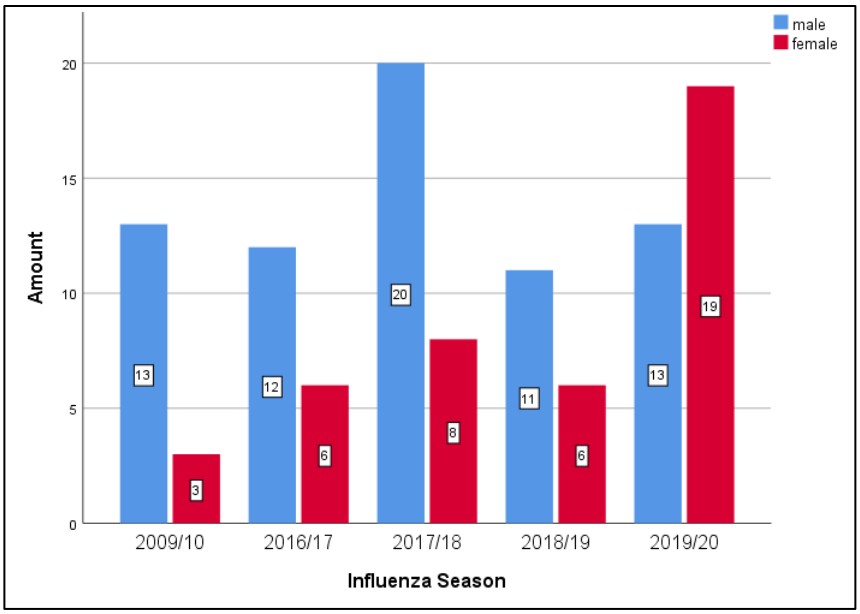


Our main objective was to observe differences between patients with development of cardiac complications during their ICU hospitalization in contrast to patients without development of cardiac complications. For this purpose, we divided the patients into a group of patients with development of cardiac complications and one group of patients

without development of cardiac complications. In the following, the data of all patients are always given first, followed by the data of the patients with development of cardiac complications, and the data of the patients without development of cardiac complications. Based on their symptomatology, of the total 111 patients, 22/111 (19.8%) patients were assigned to the group with development of cardiac complications, while 89/111 (80.2%) were assigned to the group without development of cardiac complications.

8.1 Demographic Data

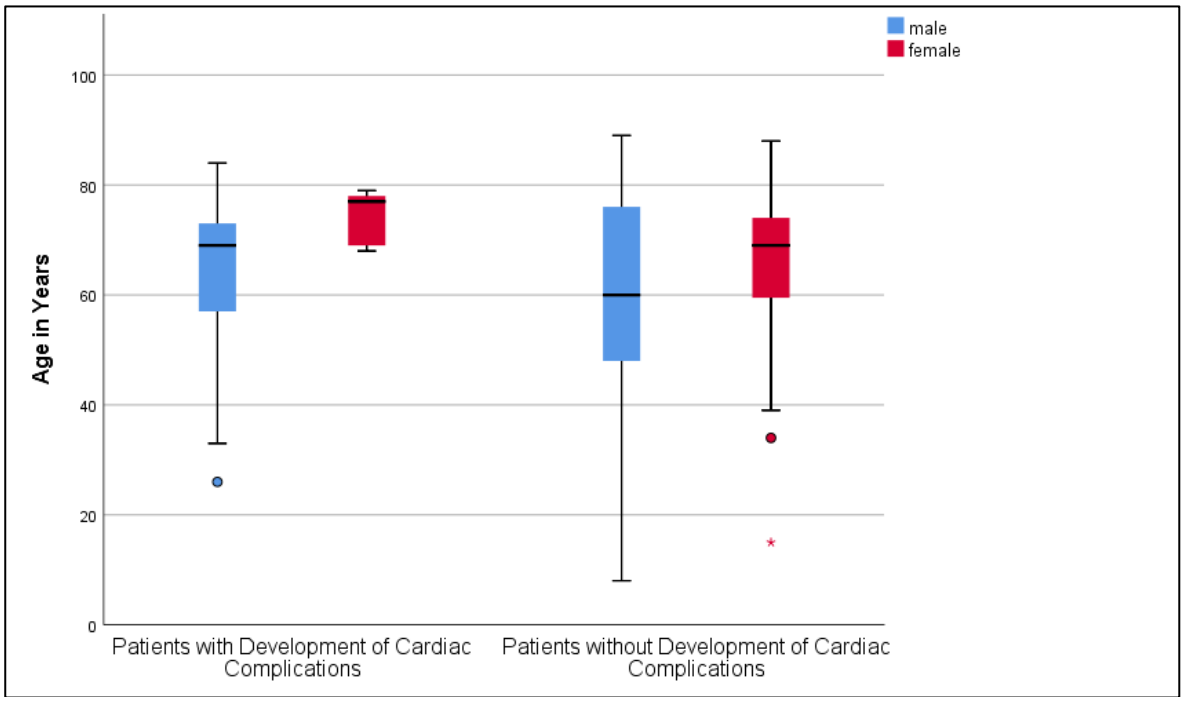
Figure 4 **Gender Distribution during Influenza Seasons**



Data show that patients with influenza illness who were treated in the ICU were more likely to be men than women. During the five influenza seasons influenza was diagnosed in 69 men (62.2%) and 42 women (37.8%). However, the gender distribution varied across the five influenza seasons (Figure 4): Men were more likely to fall ill severely enough to require treatment in intensive care units in 2009/2010 (81.3%), 2016/2017 (66.7%), 2017/2018 (71.4%) and 2018/2019 (64.7%). Only in the 2019/2020 influenza season were women affected more frequently (59.4%).

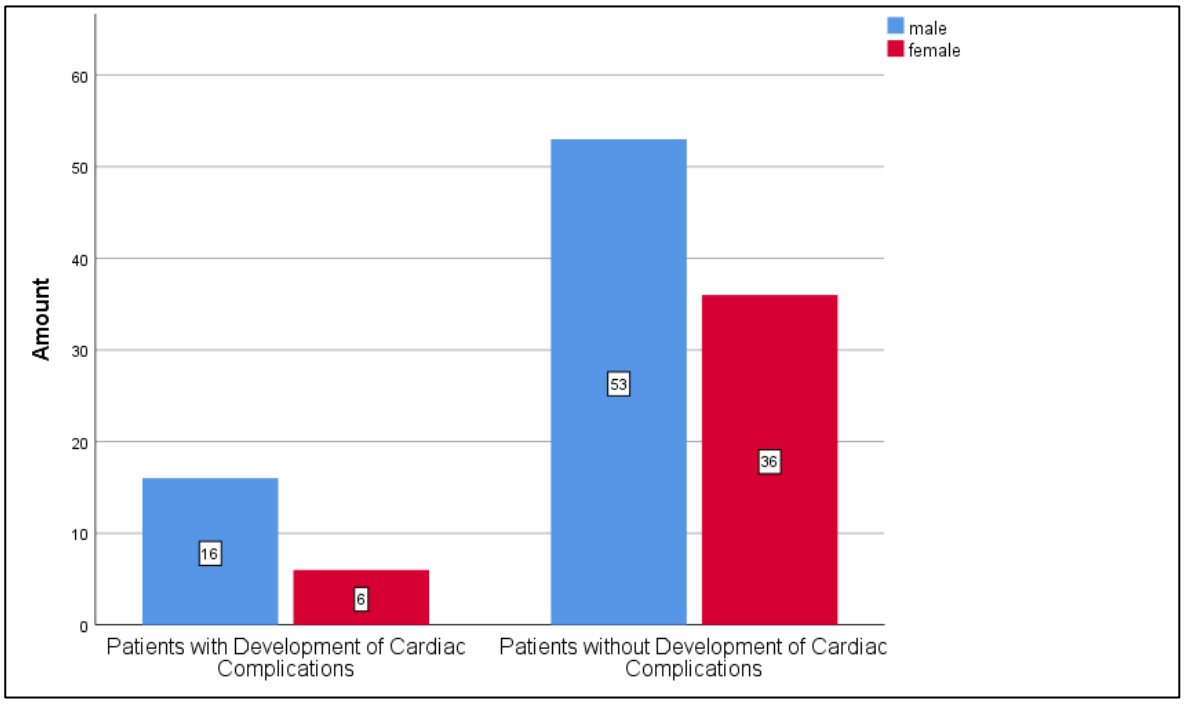
An age distribution was observed from 8 to 89 years with a median age of 66 (IQR 56-76) which also differed among men and women (Figure 5). The male patients had an age distribution from 8 to 89 years with a median age of 61 (IQR 50-74) while the female patients showed an age distribution from 15 to 88 years with a median age of 69 (IQR 60-76). The female patients were significantly older than the male patients. ($p = 0.037$). In addition, patients with development of cardiac complications were older (71 years, IQR 65-76 years) than patients without development of cardiac complications (63 years, IQR 54-75 years).

Figure 5 **Age Distribution**



There were also gender differences with regard to the development of cardiological complications (Figure 6). In the group of patients with development of cardiac complications 16/22 (72.7%) patients were men and 6/22 (27.3%) patients were women. In the group of patients without development of cardiac complications the gender distribution was more balanced. 53/89 (59.6%) patients were men and 36/89 (40.4%) patients were women.

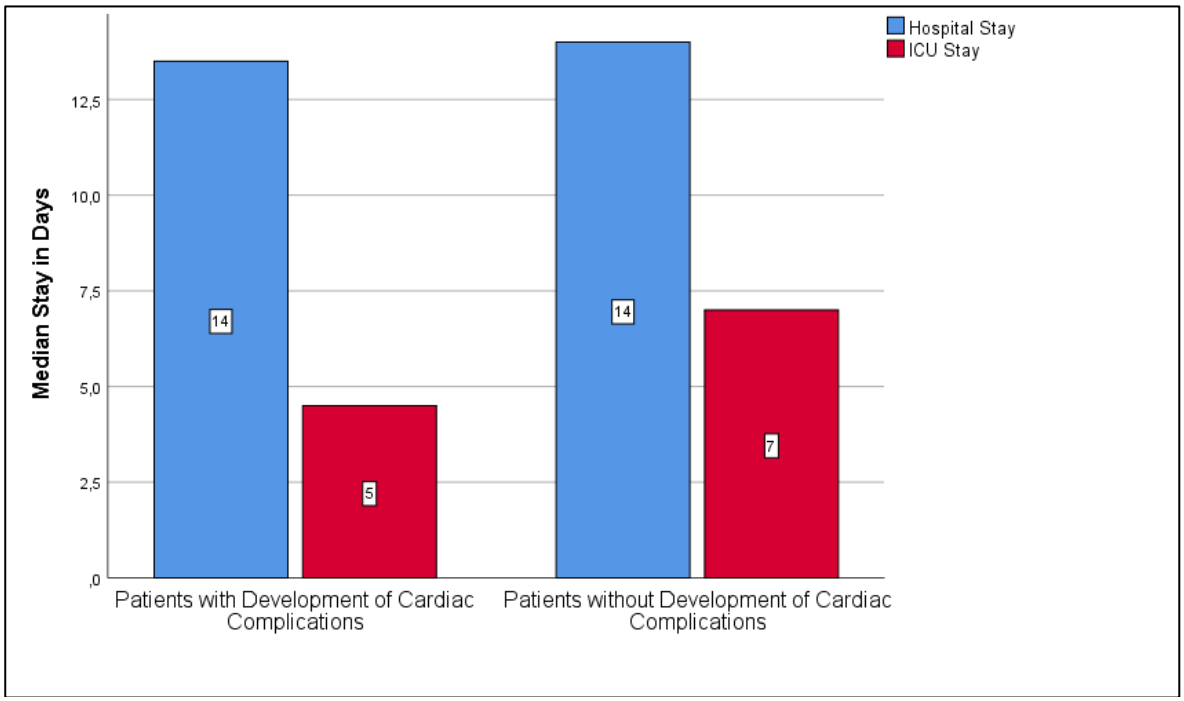
Figure 6 **Gender Differences in Development of Cardiac Complications**



8.2 Hospitalization

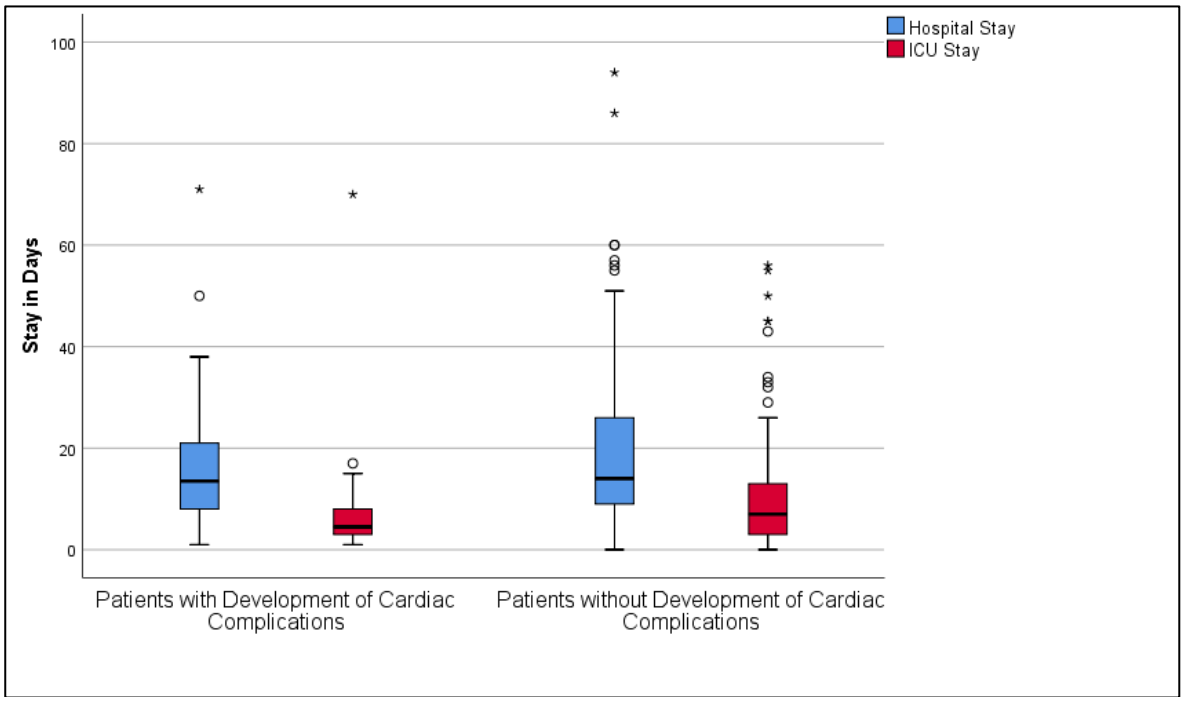
All patients in this study were hospitalized and temporarily treated in the intensive care unit. The mean hospital stay was 14 days (IQR 8-26 days) and the mean ICU stay was 6 days (IQR 3-13 days). There were no significant differences in median hospital length of stay between patients with and without development of cardiac complications (Figure 7). Patients with development of cardiac complications stayed in hospital for a total of 14 days (IQR 8-21 days), of which 5 days (IQR 3-8 days) were spent in the ICU. Patients without development of cardiac complications were also hospitalized for 14 days (IQR 9-26 days), of which 7 days (IQR 3-13 days) were spent in the ICU.

Figure 7 **Median Hospital and ICU Stay**



The length of hospitalization for all patients ranged from 0 to 94 days, patients with development of cardiac complications were hospitalized from 1 to 71 days, and patients without development of cardiac complications were hospitalized from 0 to 94 days. The duration of treatment in the ICU for all patients ranged from 0 to 70 days, the patients with development of cardiac complications were treated in the ICU between 1 and 70 days, and the patients without development of cardiac complications were treated in the ICU for 0 to 56 days (Figure 8).

Figure 8 **Differences in Hospital and ICU Stay**



8.3 Clinical Presentation

8.3.1 Shortness of Breath

At the time of ICU admission, shortness of breath was the most common symptom among all patients, with 79/111 (71.2%) suffering of it (Table 2). In the group of patients with development of cardiac complications 15/22 patients (68.2%) had shortness of breath and there was no significant difference to the group without development of cardiac complications: 64/89 (71.9%).

8.3.2 Chest Pain

In total 7/111 patients (6.3%) had chest pain during ICU admission (Table 2). There were slightly more patients in the group with development of cardiac complications who presented with chest pain at the ICU admission (2/22, 9.1%) but there was not a significant difference to the group of patients without development of cardiac complications (5/89, 5.6%).

8.3.3 Myalgia / Arthralgia

7/111 (6,3 %) patients showed myalgia or arthralgia at the time of ICU admission. No patient (0/22, 0%) in the group of patients with development of cardiac complication

showed myalgias or arthralgias during ICU admission, while 7/89 patients (7.9%) in the group without development of cardiac complications showed myalgia or arthralgia at the ICU admission (Table 2).

8.3.4 Diarrhea

Of all patients 6/111 patients (5.4%) had diarrhea during ICU admission. 0/22 (0%) of patients with development of cardiac complications had diarrhea during ICU admission. In the group of patients without development of cardiac complications 6/89 (6.7%) had diarrhea at the time of ICU admission (Table 2).

8.3.5 Nausea / Vomiting

Nausea and or vomiting occurred in 11/111 patients (9.9%). These symptoms were shown quite similar between the two groups. 2/22 (9.1 %) patients with development of cardiac complications and 9/89 (10.1 %) patients without development of cardiac complications presented with nausea and or vomiting during ICU admission (Table 2).

8.3.6 Headache

Headache was reported in 2/111 patients (1.8%). There were no patients with development of cardiac complications who presented with headache (0/22, 0%). In the group of patients without development of cardiac complications 2/89 (2.2%) suffered from headache during hospital admission (Table 2).

8.3.7 Sore Throat

1/111 (0,9 %) patient had a sore throat at the time of ICU admission. None of the patients with development of cardiac complications had a sore throat 0/22 (0%). Only 1/89 (1.1%) of patients without development of cardiac complications had a sore throat during ICU admission (Table 2).

8.3.8 Abdominal Pain

There was no abdominal pain in either group of patients: neither in the 22 patients with development of cardiac complications nor in the 89 patients without development of cardiac complications (Table 2).

8.3.9 Fatigue

8/111 patients (7,2 %) described fatigue on ICU admission. No patient in the group with development of cardiac complications complained of fatigue (0/22, 0%) during hospitalization whereas 8/89 patients (9%) in the group of patients without development of cardiac complications presented with fatigue at the time of ICU admission (Table 2).

Table 2 **Clinical Presentation**

Symptoms	**Total N = 111**	**Patient group with development of cardiac complications N = 22**	**Patient group without development of cardiac complications N = 89**
Shortness of Breath	79 (71.2)	15 (68.2)	64 (71.9)
Chest Pain	7 (6.3)	2 (9.1)	5 (5.6)
Myalgia / Arthralgia	7 (6.3)	0 (0)	7 (7.9)
Diarrhea	6 (5.4)	0 (0)	6 (6.7)
Nausea / Vomiting	11 (9.9)	2 (9.1)	9 (10.1)
Headache	2 (1.8)	0 (0)	2 (2.2)
Sore Throat	1 (0.9)	0 (0)	1 (1.1)
Abdominal pain	0 (0)	0 (0)	0 (0)
Fatigue	8 (7.2)	0 (0)	8 (9)
n/N (%)			

8.4 Vital Parameters

8.4.1 ICU Admission Temperature

Temperature was observed 101/111 patients (91.0%). Temperature measurement was absent in 10 patients: it was absent in 3 patients in the group with development of cardiac

complications (3/22) and in 7 patients in the group without development of cardiac complications (7/89).

For all patients, the median temperature was 37.5 °C (IQR 36.9-38.3 °C) at the time of admission to the ICU (Table 3). There was a non-significant difference in temperature between the two patient groups: in the group with development of cardiac complications the median ICU admission temperature was 38.3 °C (IQR 37-39.1 °C) whereas in the group without development of cardiac complications the median ICU admission temperature was 37.3 °C (IQR 36.8-38.2 °C).

8.4.2 Blood Pressure

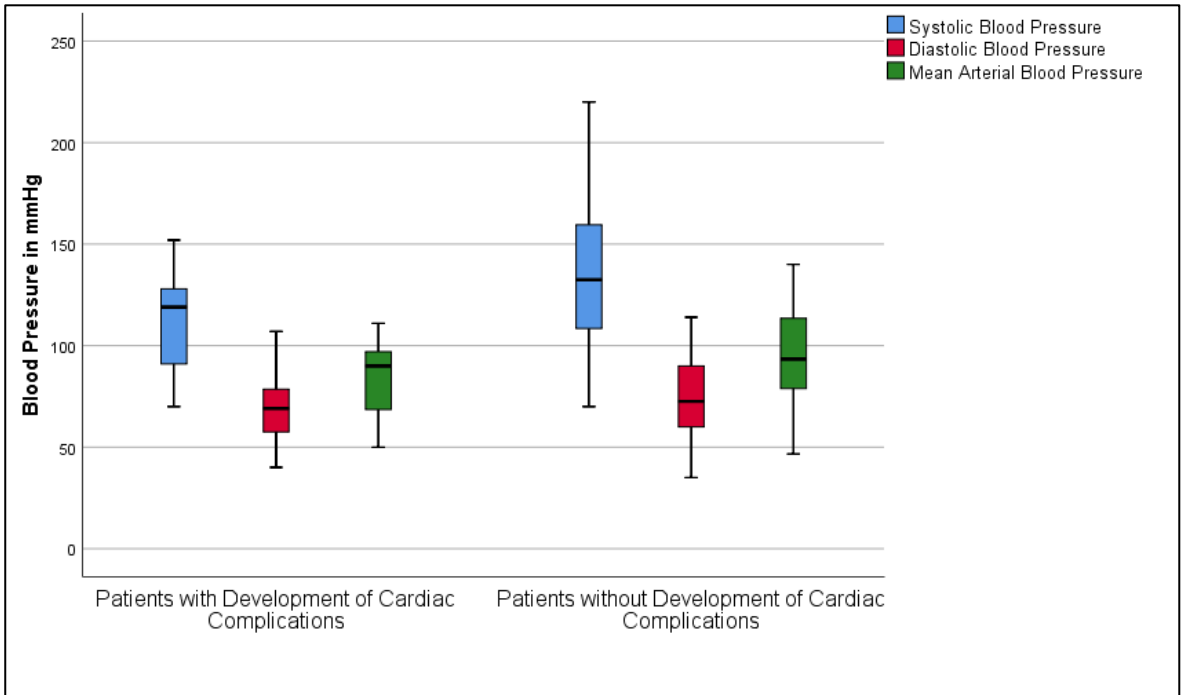
Systolic blood pressure was observed in 108/111 patients (97.3%). It was absent in only 3 patients in the group of patients without development of a cardiac complication. The median systolic blood pressure for all patients was 120 mmHg (IQR 100-150 mmHg) (Table 3). There was no significant difference between both patient groups (Figure 9). In the group with development of cardiac complications the median systolic blood pressure was 120 mmHg (IQR 92-133 mmHg). In the group without development of cardiac complications the median systolic blood pressure was 120 mmHg (IQR 101-150 mmHg). Diastolic blood pressure was observed in 51/111 (49.5%) patients. In the group of patients with development of cardiac complications, the values were missing in 11/22 patients and in the group of patients without development of cardiac complications, 49/89 values were missing. For all patients the median diastolic blood pressure was 70 mmHg (IQR 60-87 mmHg). There was a difference in diastolic blood pressure between the two patient groups, but it was not significant. While the median diastolic blood pressure was 69 mmHg (IQR 53-79 mmHg) in the patient group with development of cardiac complications, it was 73 mmHg (IQR 60-90 mmHg) in the patient group without development of cardiac complications. The mean arterial pressure MAP was calculated using the following formula:

$$MAP = diastolic\ blood\ pressure + \frac{1}{3} \times (systolic\ blood\ pressure - diastolic\ blood\ pressure)$$

It could be calculated in 51/111 patients (45.9%). In the group of patients with development of cardiac complications the MAP could be calculated in 11/22 (50%) patients and in the group of patients without development of cardiac complications the MAP could be calculated in 40/89 (44.9%) patients. The median MAP for all patient was 92 mmHg (IQR 74-103 mmHg). There was only a small difference between the two

groups, but it was not significant. In the group of patients with development of cardiac complications, the median MAP was 90 mmHg (IQR 65-100 mmHg) and in the group of patients without development of cardiac complications, the median MAP was 93 mmHg (IQR 79-113 mmHg).

Figure 9 **Blood Pressure**



8.4.3 Heart Rate

Heart rate was observed in 107/111 patients. Data were missing from 1/22 in the group of patients with development of cardiac complications and from 3/89 in the group of patients without development of cardiac complications. For all patients the median heart rate was 102 bpm (IQR 87-120 bpm). There was a median difference in initial heart rate measurements of 9 bpm between the two groups but this was not significant (Table 3). In the group of patients with development of cardiac complications the median initial heart rate was 111 bpm (IQR 80-129 bpm) while the median initial heart rate in the group of patients without development of cardiac complications was 102 bpm (IQR 90-120).

Table 3 **Vital Parameters**

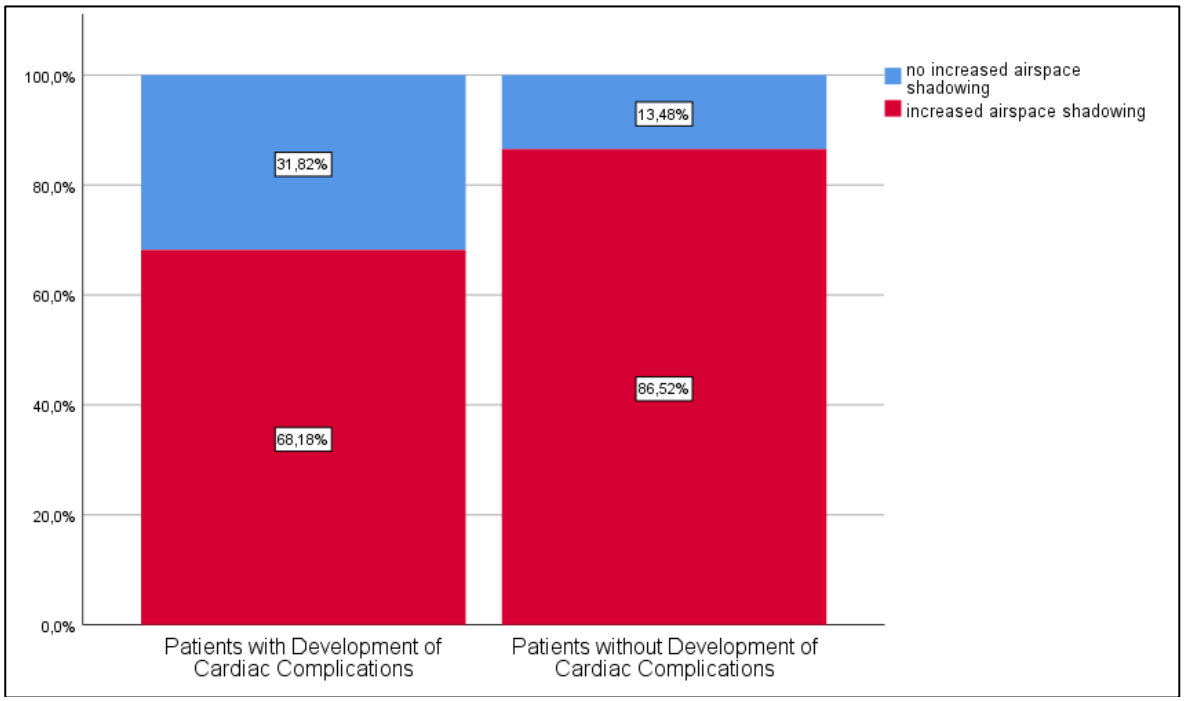
Vital Parameters	Total	Patient group with development of cardiac complications	Patient group without development of cardiac complications
ICU Admission Temperature °C	37.5 (36.9-38.3)	38.3 (37-39.1)	37.3 (36.8-38.2)
systolic Blood Pressure mmHg	120 (100-150)	120 (92-133)	120 (101-150)
diastolic Blood Pressure mmHG	70 (60-87)	69 (53-79)	73 (60-90)
mean Arterial Pressure (MAP) mmHG	92 (87-120)	90 (65-100)	93 (79-113)
Heart rate	102 (87-120)	111 (80-129)	102 (90-120)
Median (IQR 25-75)			

8.5 Radiological Results And Physical Examination

8.5.1 Lung Infiltrates

In total 92/111 (82.9%) patients had increased airspace shadowing (diffuse or lobal consolidations or ground glass opacities, compatible with pulmonary infiltrates) in chest x-ray or computed tomography . There was a difference between the two patient groups but it was not significant. In the group of patients with development of cardiac complications we observed increased airspace shadowing in 15/22 (68.2%) patients. Whereas in the group of patients without development of cardiac complications increased airspace shadowing were found in 77/89 (86.5%) patients (Figure 10).

Figure 10 **Increased airspace shadowing**



8.5.2 Pathologic Heart Auscultation

Overall, we observed a pathological heart auscultation in 15/111 (13.5%) patients. There was a percentage but not significant difference between the two patient groups.
In the group of patients with development of cardiac complications, 4/22 (18.2%) had a pathological heart auscultation, while in the group of patients without development of cardiac complications, only 11/89 (12.4%) had a pathological heart auscultation.

8.5.3 Wheezing

Wheezing was found in 14/111 (12.6%) patients. In the group of patients with development of cardiac complications no wheezing was observed (0/22, 0%), whereas in the group of patients without development of cardiac complications, wheezing occurred in 14/89 (15.7%).

8.5.4 Crackles

Patients in both groups had relatively equal frequency of crackles. In total, crackles were observed in 55/111 (49.5%) patients. In the group of patients with development of cardiac complications, crackles occurred in 9/22 (40.9%), and in the group of patients without development of cardiac complications, crackles occurred in 46/89 (51.7%).

8.5.5 Tachypnea

Overall, 39/111 (35.1%) patients experienced tachypnea. In the group of patients with development of cardiac complications 8/22 (36.4%) had tachypnea, while in the group of patients without development of cardiac complications, 31/89 (34.8%) had tachypnea. The difference between the two groups was not significant.

8.5.6 Pathologic Abdominal Examination

In total, only 4/111 (3.6%) patients had a pathological examination of the abdomen: 1/22 (4.5%) in the group of patients with development of cardiac complications and 3/89 (3.4%) in the group of patients without development of cardiac complications.

Table 4 **Physical Examination**

Physical Examination	**Total N = 111**	**Patient group with development of cardiac complications N = 22**	**Patient group without development of cardiac complications N = 89**
Increased airspace shadowing	92 (82.9)	15 (68.2)	77 (86.5)
Pathologic Heart Auscultation	15 (13.5)	4 (18.2)	11 (12.4)
Wheezing	14 (12.6)	0 (0)	14 (15.7)
Crackles	55 (49.5)	9 (40.9)	46 (51.7)
Tachypnea	39 (35.1)	8 (36.4)	31 (34.8)
Pathologic Abdominal Examination	4 (3.6)	1 (4.5)	3 (3.4)
n/N (%)			

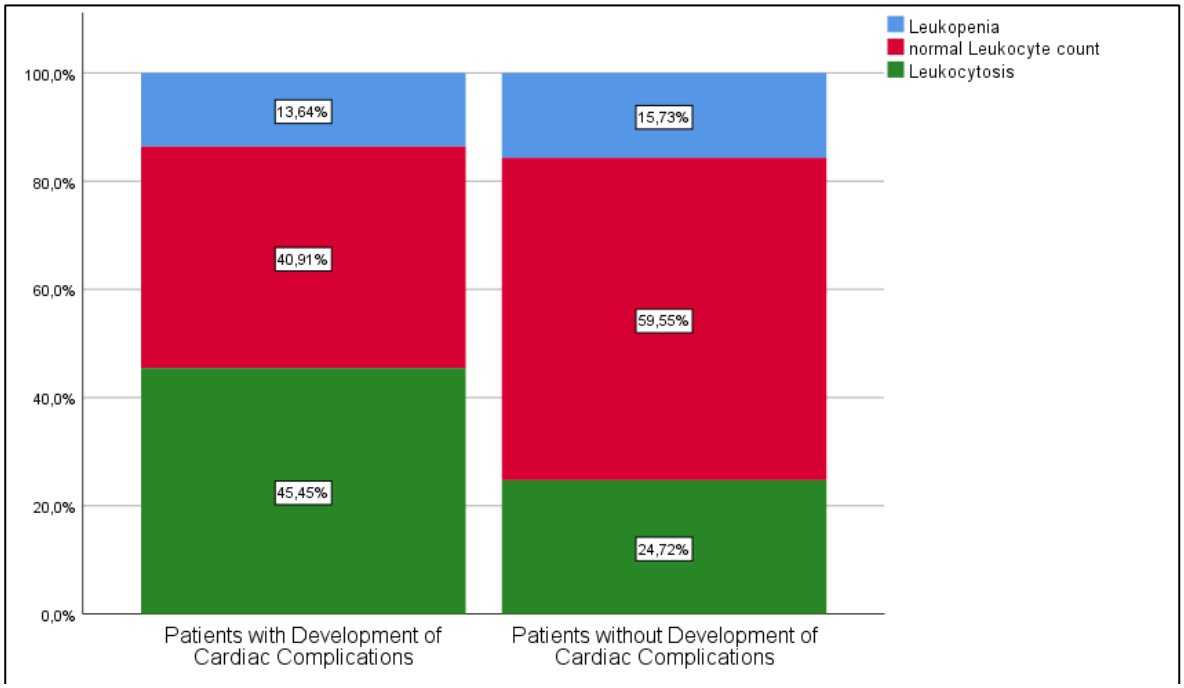
8.6 Laboratory Values

Laboratory values were obtained on the day of admission to the ICU with an additional window of +/- one day if no laboratory values were available on the day of ICU admission.

8.6.1 Leukocytes

We observed leukocyte values in all patients. The leukocyte counts were considered normal within a range of 4.4 to 11.3 x10^9/L. Counts below 4.4 x10^9/L were considered Leukopenia and counts above 11.3 x10^9/L were considered Leukocytosis. The median leukocyte count was 8.52 x10^9/L (IQR 6.10-12.09 x10^9/2). There was a difference between the two patient groups, but it was not significant. In the group of patients with development of cardiac complications the median leukocyte count was 9.96 x10^9/L (IQR 6.90-13.48 x10^9/L) and in the group of patients without development of cardiac complications the median leukocyte count was 7.91 x10^9/L (IQR 5.99-10.9 x10^9/L). Overall, we found 17/111 (15.3%) of patients with leukopenia, 62/111 (55.9%) with normal leukocyte count and 32/111 (28.8%) with leukocytosis. In the group of patients with development of cardiac complications 3/22 (13.6%) had leukopenia, 9/22 (40.9%) had normal leukocyte count and 10/22 (45.5%) had leukocytosis. In the group of patients without development of cardiac complications 14/89 (15.7%) had leukopenia, 53/89 (59.6%) had normal leukocyte count and 22/89 (24.7%) had leukocytosis.

Figure 11 **Leukocytes**



8.6.2 Differential Counts of Leukocytes

Levels of neutrophile granulocytes, lymphocytes and eosinophile granulocytes were measured at 109/111 patients. The values were missing in each case in the same 2 patients. For neutrophile granulocytes values between 50 to 75% were considered normal neutrophile granulocytes count, values below 50% were considered neutropenia and values above 75% were considered neutrocytosis. For lymphocytes values between 20 to 40% were considered normal, values below 20% were considered lymphopenia and values above 40% were considered lymphocytosis. For eosinophile granulocytes values from 0 to 5% were considered normal and values above 5% were considered eosinophilia.

Of all patients 4/109 (3.7%) had neutropenia, 16/109 (14.7%) had normal neutrophiles count and 89/109 (81.7%) had neutrocytosis.

Many 94/109 (86.2%) patients had lymphopenia, 12/109 (11.0%) had normal lymphocyte count and 3/109 (2.8%) had lymphocytosis.

Regarding eosinophile granulocytes almost all patients (106/109 (97.2%)) had normal eosinophile counts and 3/109 (2.8%) had eosinophilia.

There were only minor differences between the two groups of patients with and without the development of cardiac complications, as shown in the table below.

Table 5 **Differential Counts of Leukocytes**

Differential counts of leukocytes		**Total N = 109**	**Patient Group without development of cardiac complications N = 87**	**Patient Group with development of cardiac complications N = 22**
Neutrophiles	Neutropenia	4 (3.7)	3 (3.4)	1 (4.5)
	normal Neutrophiles Count	16 (14.7)	13 (14.9)	3 (13.6)
	Neutrocytosis	89 (81.7)	71 (81.6)	18 (81.8)
Lymphocytes	Lymphopenia	94 (86.2)	75 (86.2)	19 (86.4)
	normal Lymphocyte count	12 (11.0)	10 (11.5)	2 (9.1)
	Lymphocytosis	3 (2.8)	2 (2.3)	1 (4.5)
Eosinophiles	normal Eosinophiles Count	106 (97.2)	84 (96.6)	22 (100)
	Eosinophilia	3 (2.8)	3 (3.4)	0 (0)
n/N (%)				

*corrected N due to exclusion of missing Data

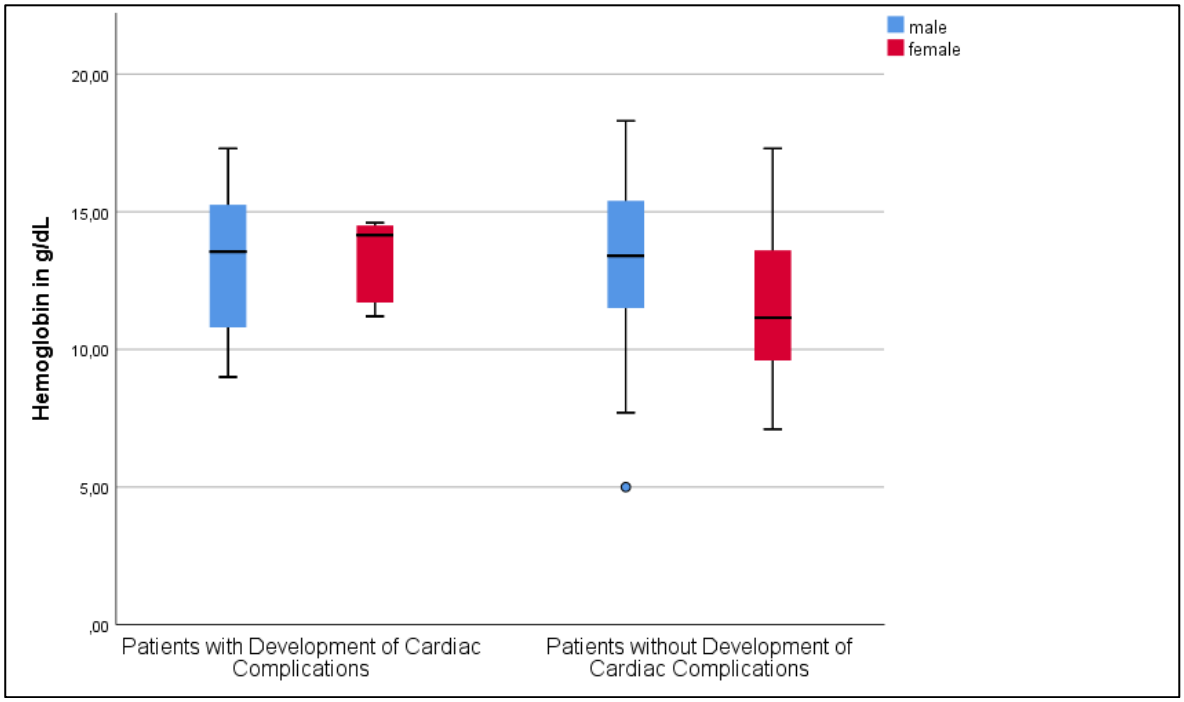
8.6.3 Hemoglobin and Hematocrit

Hemoglobin and hematocrit counts were measured in all patients.

Hemoglobin counts between 13.0-17.5 g/dL for men and between 12.0-15.3 g/dL for women were considered normal. Counts below 13.0 g/dL in men and below 12.0 g/dL in women were considered anemic. Counts above 17.5 g/dL in men and above 15.3 g/dL in women were considered elevated hemoglobin levels. Of all patients, 55/111 (49.5%) were anemic, 53/111 (47.7%) had normal hemoglobin levels and 3/111 (2.7%) had elevated hemoglobin levels. There were some differences between the two groups: In the group of patients with development of cardiac complications 8/22 (36.4%) were anemic, 14/22 (63.6%) had normal hemoglobin levels and 0/22 (0%) had increased hemoglobin levels. In the group of patients without development of cardiac complications 47/89 (52.8%) were anemic, 39/89 (43.8%) had normal hemoglobin levels and 3/89 (3.4%) had elevated

hemoglobin levels. The patients with development of cardiac complications were less anemic than those without development of cardiac complications. However, these differences were not significant.

Figure 12 **Hemoglobin Levels**



Hematocrit levels between 40-50% in men and between 35-45% in women were considered normal. Levels below 40% in men and below 35% in women were considered low hematocrit levels and levels above 50% in men and above 45% in women were considered high hematocrit levels.

Of all patients, 61/111 (55%) had low hematocrit levels, 44/111 (39.6%) had normal hematocrit levels and 6/111 (5.4%) had high hematocrit levels. There were differences between the two groups, as there were less patients with low hematocrit levels in the group of patients with development of cardiac complications as in the group without development of cardiac complications, but these differences were not significant. In the group of patients with development of cardiac complications 9/111 (40.9%) had low hematocrit levels, 13/22 (59.1%) had normal hematocrit levels and 9/22 (0%) had high hematocrit levels, while in the group of patients without development of cardiac complications, 52/89 (58.4%) had low hematocrit levels, 31/89 (34.8%) had normal hematocrit levels and 6/89 (6.7%) had high hematocrit levels.

Table 6 **Hemoglobin and Hematocrit Counts**

		Total N = 111	Patient Group with development of cardiac complications N = 22	Patient Group without development of cardiac complications N = 89
Hemoglobin	Low	55 (49.5)	8 (36.4)	47 (52.8)
	Normal	53 (47.7)	14 (63.6)	39 (43.8)
	High	3 (2.7)	0 (0)	3 (3.4)
Hematocrit	Low	61 (55.0)	9 (40.9)	52 (58.4)
	Normal	44 (39.6)	13 (59.1)	31 (34.8)
	High	6 (5.4)	0 (0)	6 (6.7)
n/N (%)				

8.6.4 Platelet Counts

Platelet counts between 140-440 x10^9/L were considered normal. Counts below 140 x10^9/L were considered thrombocytopenia, while counts above 440 x10^9/L were considered thrombocytosis. Platelet counts were available for all patients. 32/111 (28.8%) had thrombocytopenia, 76/111 (68.5%) had normal platelet counts and 3/111 (2.7%) had thrombocytosis. There were only minor differences without significance between the two patient groups with and without cardiac complications. In the group of patients with development of cardiac complications 5/22 (22.7%) had thrombopenia, 17/22 (77,3%) had normal platelet counts and 0/22 (0%) had thrombocytosis. In the group of patients without development of cardiac complications 27/89 (30.3%) had thrombocytopenia, 59/89 (66.3%) had normal platelet counts and 3/89 (3.4%) had thrombocytosis.

Table 7 **Platelet Counts**

		Total N = 111	Patient Group with development of cardiac complications N = 22	Patient Group without development of cardiac complications N = 89
Platelet counts	Thrombopenia	32 (28.8)	5 (22.7)	27 (30.3)
	Normal	76 (68.5)	17 (77.3)	59 (66.3)
	Thrombocytosis	3 (2.7)	0 (0)	3 (3.4)
n/N (%)				

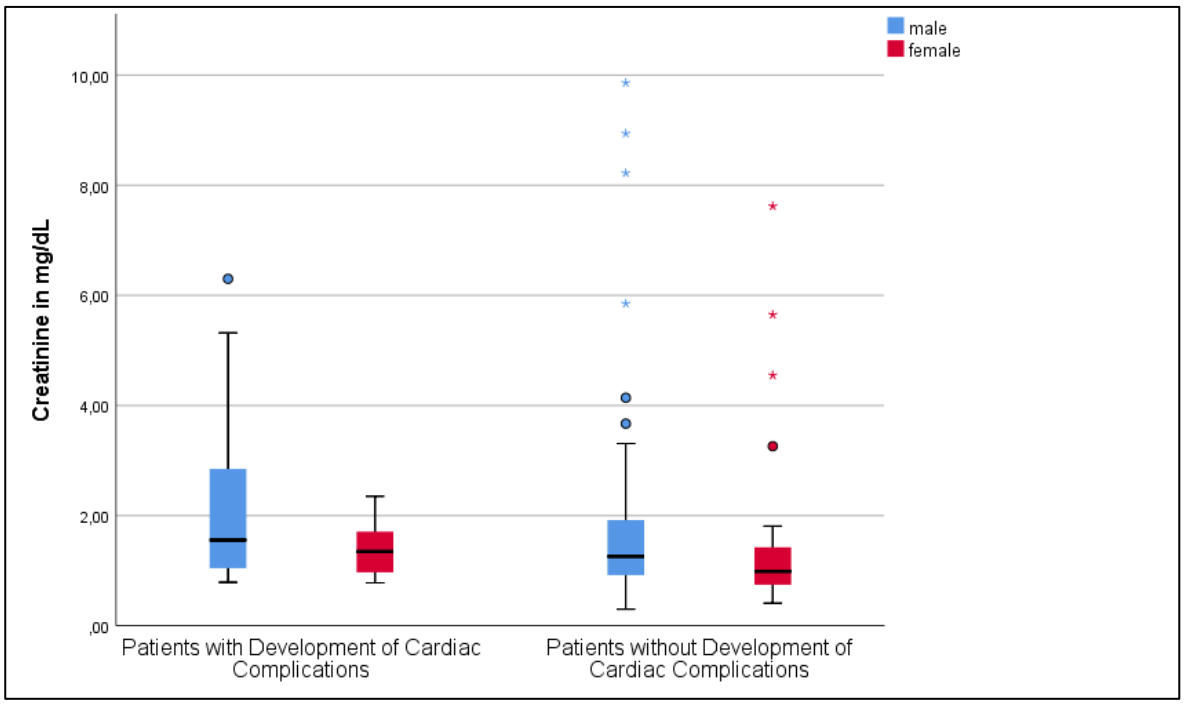
8.6.5 Creatinine Levels

Creatinine levels were obtained in all patients. Levels up to 1.20 mg/dL in men and up to 1.00 mg/dL in women were considered normal creatinine levels. Levels above 1.20 mg/dL in men and above 1.00 mg/dL in women were considered elevated creatinine levels. Of all patients, 52/111 (46.8%) had normal creatinine levels and 59/111 (53.2%) had elevated creatinine levels. There were differences between the two patient groups, as more patients (relatively) in the group with development of cardiac complications had elevated creatinine levels. These differences were not significant. 8/22 (36.4%) in the group of patients with development of cardiac complications had normal creatinine counts, while 14/22 (63.6%) had elevated creatinine levels. The mean creatinine level in this group was 1.56 mg/dL (IQR 1.02-2.35 mg/dL). In the group of patients without development of cardiac complications 44/89 (49.4%) had a normal creatinine level and 45/89 (50.6%) had an elevated creatinine level in this group the mean creatinine level was 1.15 mg/dL (IQR 0.84-1.67 mg/dL).

Table 8 **Creatinine Levels**

		Total N = 111	Patient Group with development of cardiac complications N = 22	Patient Group without development of cardiac complications N = 89
Creatinine levels	Normal	52 (46.8)	8 (36.4)	44 (49.4)
	Elevated	59 (53.2)	14 (63.6)	45 (50.6)
n/N (%)				

Figure 13 **Creatinine Levels**

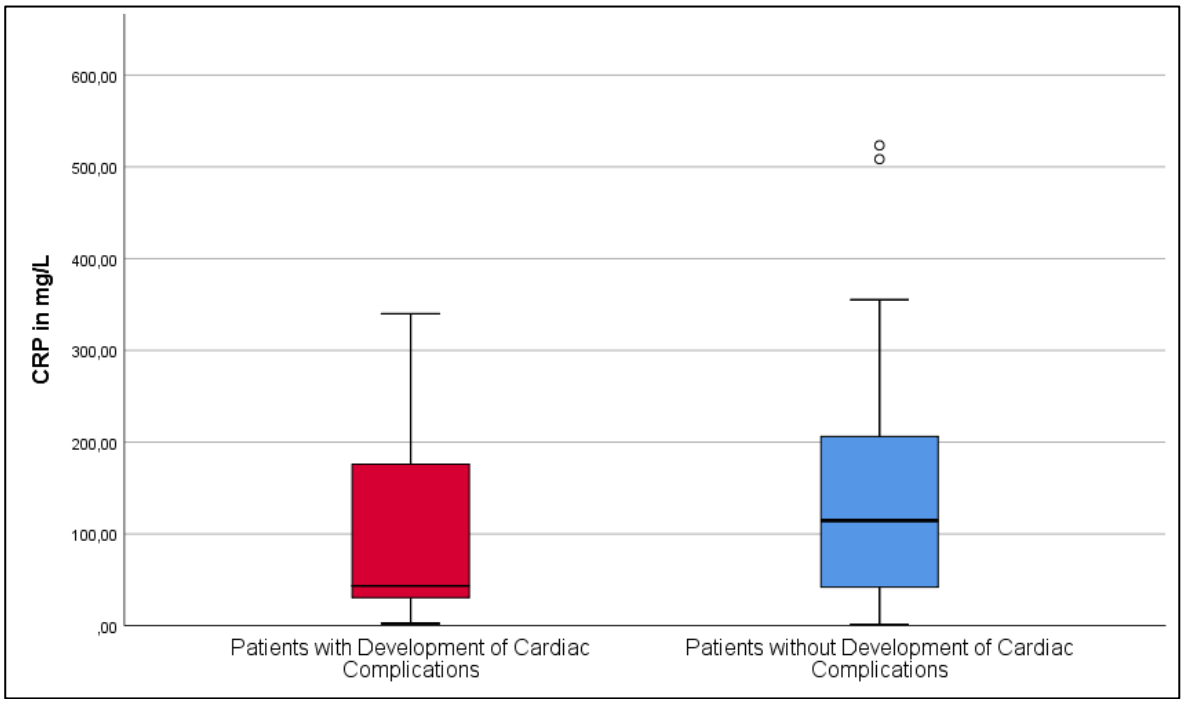


8.6.6 C-reactive Protein Levels

C-reactive protein (CRP) levels were present in all patients. Levels up to 5 mg/L were considered normal, levels above 5 mg/L were considered elevated. Increased CRP levels ranged from 6.9 mg/l up to 523.4 mg/L. In all patients, the median CRP level was 93.5 mg/L (IQR 34-206.2 mg/L). Only 6/111 (5.4%) had normal CRP levels, while 105/111 (94.6%) had elevated CRP levels. In the group of patients with development of cardiac complications 1/22 (4.5%) had normal CRP levels and 21/22 (95.5%) had elevated CRP levels. 5/89 (5.6%) of patients in the group without development of cardiac complications had normal CRP levels and 84/89 (94.4%) had elevated CRP levels. There was a difference

between the two groups, but it was not significant. In the group of patients with development of cardiac complications, the median CRP level was 43.45 mg/L (IQR 30.7-176.0 mg/L), while in the group of patients without development of cardiac complications the median CRP level was higher: 114.5 mg/L (IQR 42.0-206.2 mg/L).

Figure 14 **C-Reactive Protein Levels**

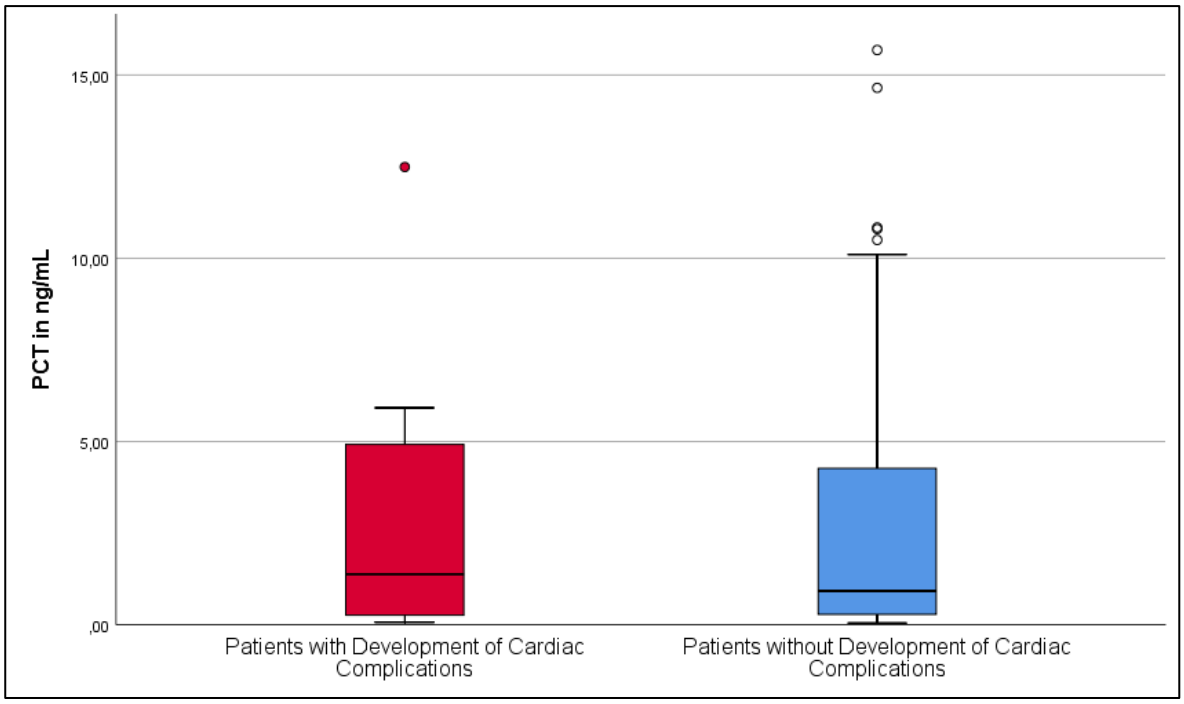


8.6.7 Procalcitonin Levels

Procalcitonin (PCT) levels up to 0.5 ng/mL were considered as normal and levels above 0.5 ng/mL were considered elevated. PCT levels were measured at 87/111 patients. In the group of patients with development of cardiac complications, PCT levels were measured in 15/22 patients. In the group of patients without development of cardiac complications PCT levels were measured in 72/89 patients. Among all patients 35/87 (40.2%) had normal PCT levels and 52/87 (59.8%) had elevated PCT levels. The median PCT level of all patients was 0.97 ng/mL (IQR 0.27-4.39 ng/mL). There were only few differences between the two patient groups without any significance. In the group of patients with development of cardiac complications 6/15 (40.0%) had normal PCT levels and 9/15 (60.0%) had elevated PCT levels. The median PCT level in this group of patients was 1.38 ng/mL (IQR 0.26-5.92 ng/mL). In the group of patients without development of cardiac complications, 29/72

(40.3%) had normal PCT levels and 43/72 (59.3%) had elevated PCT levels. In this group of patients the median PCT level was 0.93 ng/mL (IQR 0.29-4.27 ng/mL).

Figure 15 **PCT Levels**



8.6.8 AST and ALT Levels

AST and ALT levels were available for 110/111 patients. AST levels were considered normal up to a 50 U/L in men and up to 35 U/L in women. Levels above 50 U/L in men and levels above 35 U/L in women were considered elevated. Overall, 41/110 (37.3) patients had normal AST levels and 69/110 (62.7%) patients had elevated AST levels. In the group of patients with development of cardiac complications 7/22 (31.8%) had normal AST levels and 15/22 (68.2%) had elevated AST levels. In the group of patients without development of cardiac complications 34/88 (38.6%) had normal AST levels and 54/88 (61.4%) had elevated AST levels. In both groups, the median AST level was 48 U/L (IQR 33-95 U/L). In the group of patients with development of cardiac complications, the median AST level was 59.5 U/L (IQR 35-114 U/L) and in the group of patients without development of cardiac complications the median AST level was 48 U/L (IQR 32.5-86.5 U/L). The normal and elevated levels for ALT were the same as for AST levels. Overall, 81/110 (73.6%) patients had normal ALT levels whereas 29/110 (26.4%) patients had elevated ALT levels. In the group of patients with development of cardiac complications

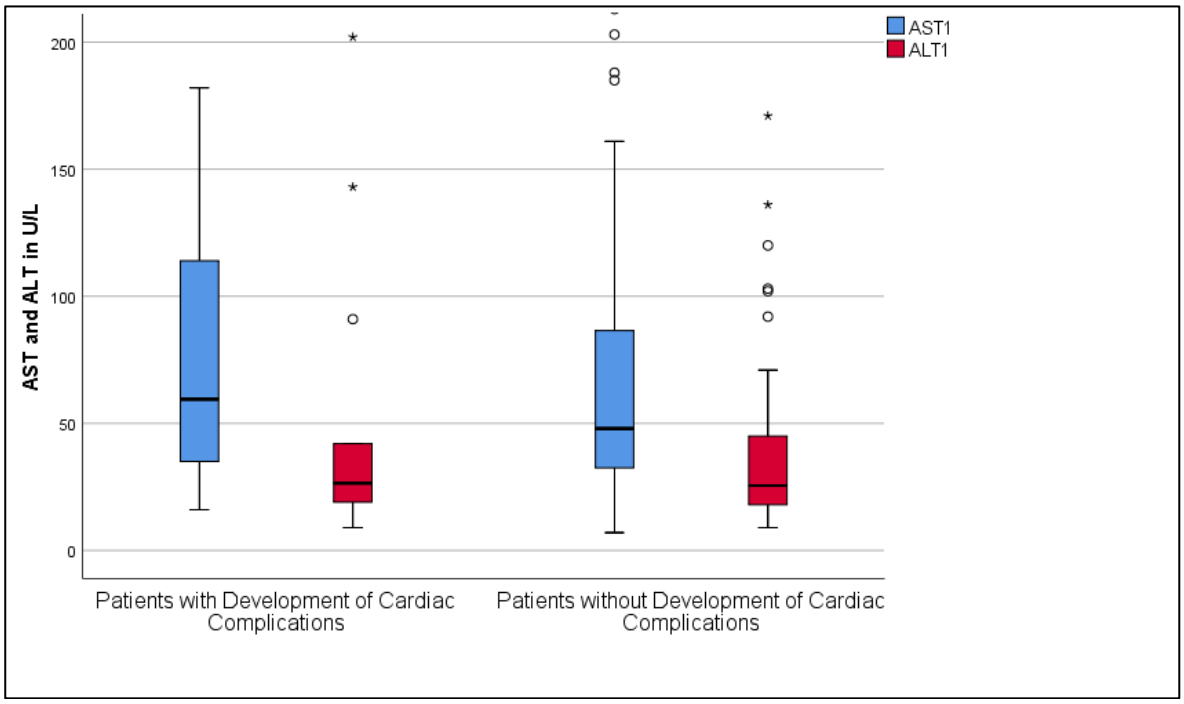
16/22 (72.7%) patients had normal ALT levels and 6/22 (27.3%) had elevated ALT levels. In the group of patients without development of cardiac complications 65/88 (73.9%) had normal ALT levels and 23/88 (26.1%) had elevated ALT levels. The median ALT level in both groups was 26 U/L (IQR 18-44 U/L). In the group of patients with development of cardiac complications the median ALT level was 26.5 U/L (IQR 19-42 U/L) and in the group of patients without development of cardiac complications, the median ALT level was 25.5 U/L (IQR 18-45 U/L). All differences were not significant.

Table 9 **AST and ALT Levels**

		Total N = 110	**Patient Group <u>with</u> development of cardiac complications N = 22**	**Patient Group <u>without</u> development of cardiac complications N = 88**
AST levels	Normal	41 (37.3)	7 (31.8)	34 (38.6)
	Elevated	69 (62.7)	15 (68.2)	54 (61.4)
ALT levels	Normal	81 (73.6)	16 (72.7)	65 (73.9)
	Elevated	29 (26.4)	6 (27.3)	23 (26.1)
n/N (%)				

*corrected N due to exclusion of missing data

Figure 16 **AST and ALT Levels**



8.6.9 Bilirubin

Bilirubin levels were measured in 107/111 patients. Counts in a range from 0.10 – 1.20 mg/dL were considered normal Bilirubin levels. Levels above 1.20 mg/dL were considered hyperbilirubinemia. Of all patients, 91/107 (85.0%) had normal bilirubin levels and 16/107 (15.0%) had hyperbilirubinemia. In the group of patients with development of cardiac complications 14/22 (63.6%) had normal bilirubin levels and 8/22 (36.4%) had elevated bilirubin levels. In the group of patients without development of cardiac complications 77/85 (90.6%) had normal bilirubin levels and 8/85 (9.4%) had elevated bilirubin levels. The median bilirubin level for both patient groups was 0.47 mg/dL (IQR 0.28-0.88mg/dL). In the group of patients with development of cardiac complications the median bilirubin level was 0.9 mg/dL (IQR 0.44-1.71 mg/dL) and in the group of patients without development of cardiac complications the median bilirubin level was 0.4 mg/dL (IQR 0.27-0.67 mg/dL). The difference between the two groups shown in the table below was significant (P value < 0.001).

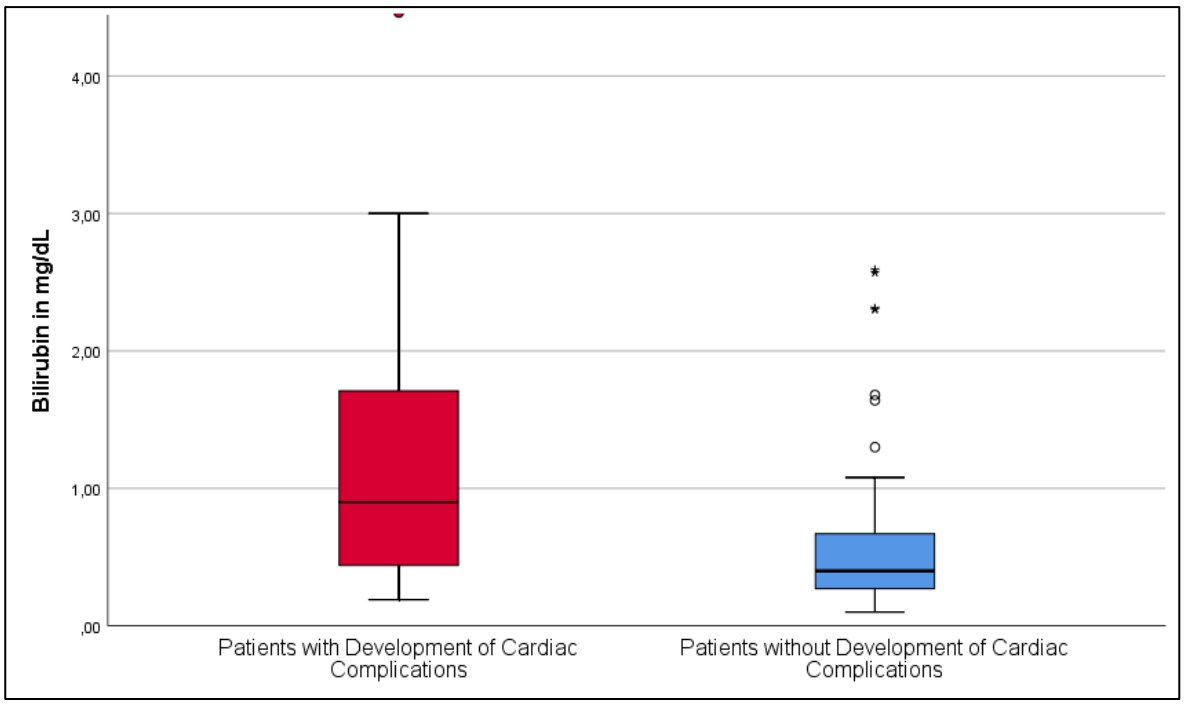
Table 10 **Bilirubin Levels**

		Total N = 107	Patient Group with development of cardiac complications N = 22	Patient Group without development of cardiac complications N = 85
Bilirubin levels**	Normal	91 (85.0)	14 (63.6)	77 (90.6)
	Elevated	16 (15.0)	8 (36.4)	8 (9.4)
n/N (%)				

*corrected N due to exclusion of missing data

**p value <0.001

Figure 17 **Bilirubin Levels**



8.6.10 LDH Levels

We considered normal LDH levels to be between 120 and 240 U/L. Levels above 240 U/L were considered elevated LDH levels. LDH levels were available for 104/111 patients. Overall, 24/104 (23.1%) patients had normal LDH levels and 80/104 (76.9%) patients had elevated LDH levels. The median LDH level was 316 U/L (IQR 245.5-490.5 U/L). In the group of patients with development of cardiac complications 7/19 (36.8%) had normal

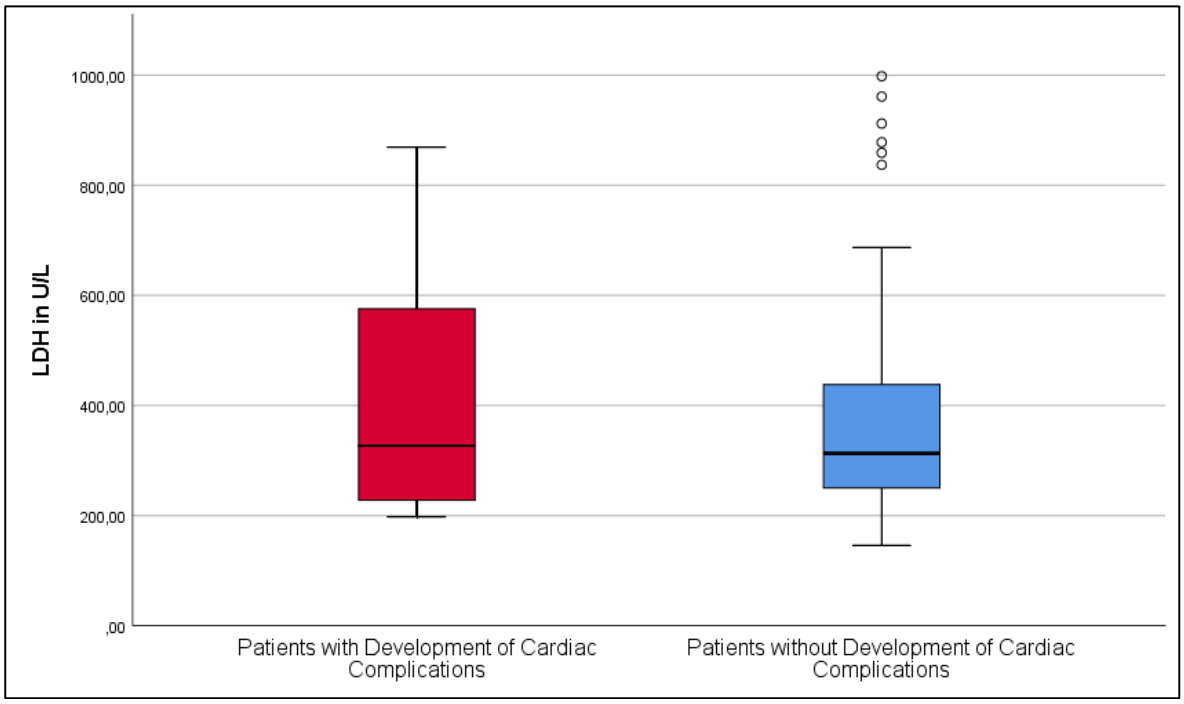
LDH levels and 12/19 (63.2%) had elevated LDH levels. The median LDH level for this group was 327 U/L (IQR 222-632 U/L). In the group of patients without development of cardiac complications 17/85 (20%) had normal LDH levels and 68/85 (80%) had elevated LDH levels. In this group the median LDH level was 313 U/L (IQR 250-438 U/L). The differences between the two groups shown in the table below were not significant.

Table 11 **LDH Levels**

		Total N = 104	**Patient Group <u>with</u> development of cardiac complications N = 19**	**Patient Group <u>without</u> development of cardiac complications N = 85**
LDH levels	Normal	24 (23.1)	7 (36.8)	17 (20.0)
	Elevated	80 76.9)	12 (63.2)	68 (80.0)
n/N (%)				

*corrected N due to exclusion of missing data

Figure 18 **LDH Levels**



8.6.11 Myoglobin

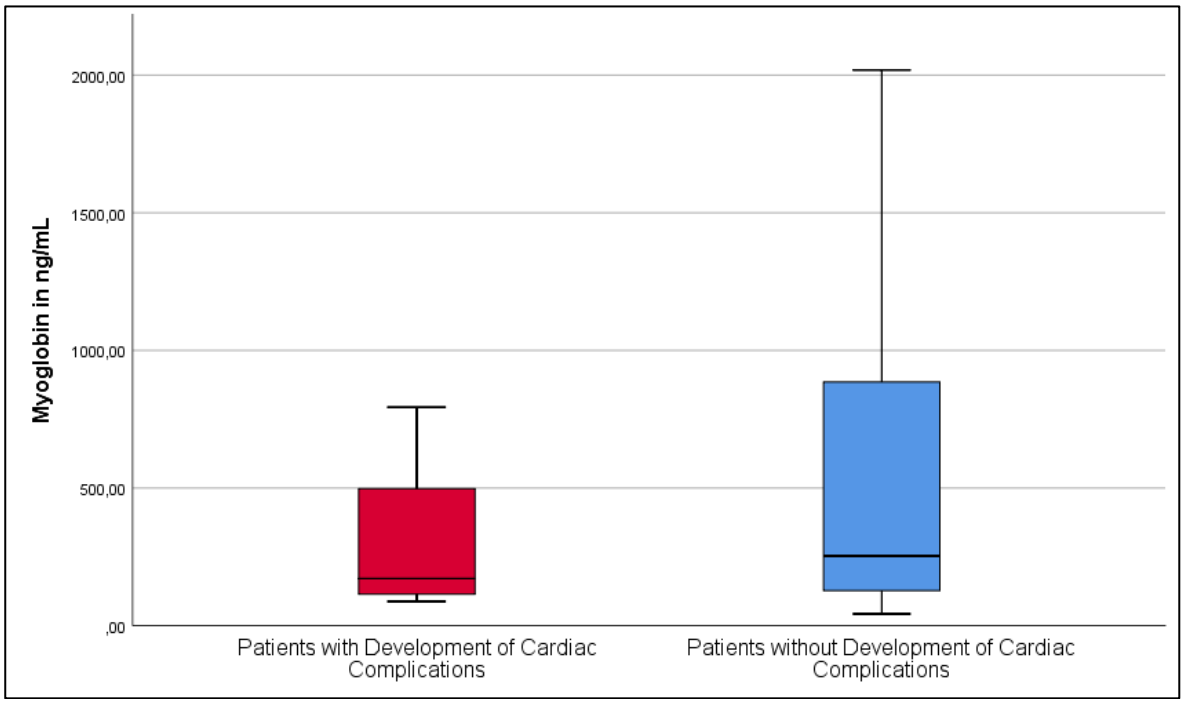
Myoglobin levels were considered normal up to a level of 60 ng/mL in women and up to 80 ng/mL in men. Myoglobin levels above 60 ng/mL in women and above 80 ng/mL in men were considered elevated. Myoglobin levels were measured in 30/111 patients. Of all patients 3/30 (10.0%) had normal myoglobin levels and 27/30 (90.0%) had elevated myoglobin levels. The median myoglobin level was 213.7 ng/mL (IQR 127.7-794.0 ng/mL). In the group of patients with development of cardiac complications 0/4 (0%) had normal myoglobin levels and 4/4 (100%) had elevated myoglobin levels. In the group of patients without development of cardiac complications 3/26 (11.5%) had normal myoglobin levels and 23/26 (88.5%) had elevated myoglobin levels. Median myoglobin levels were 171,6 ng/mL (IQR 114.85-497.80 ng/mL) in the group of patients with development of cardiac complications and 253.4 ng/mL (IQR 127.7-885.4 ng/mL) in the group of patients without development of cardiac complications.

Table 12 **Myoglobin Levels**

		Total N = 30	**Patient Group <u>with</u> development of cardiac complications N = 4**	**Patient Group <u>without</u> development of cardiac complications N = 26**
Myoglobin levels	Normal	3 (10.0)	0 (0)	3 (11.5)
	Elevated	27 (90.0)	4 (100)	23 (88.5)
n/N (%)				

*corrected N due to exclusion of missing Data

Figure 19 **Myoglobin Levels**



8.6.12 Troponin T Levels

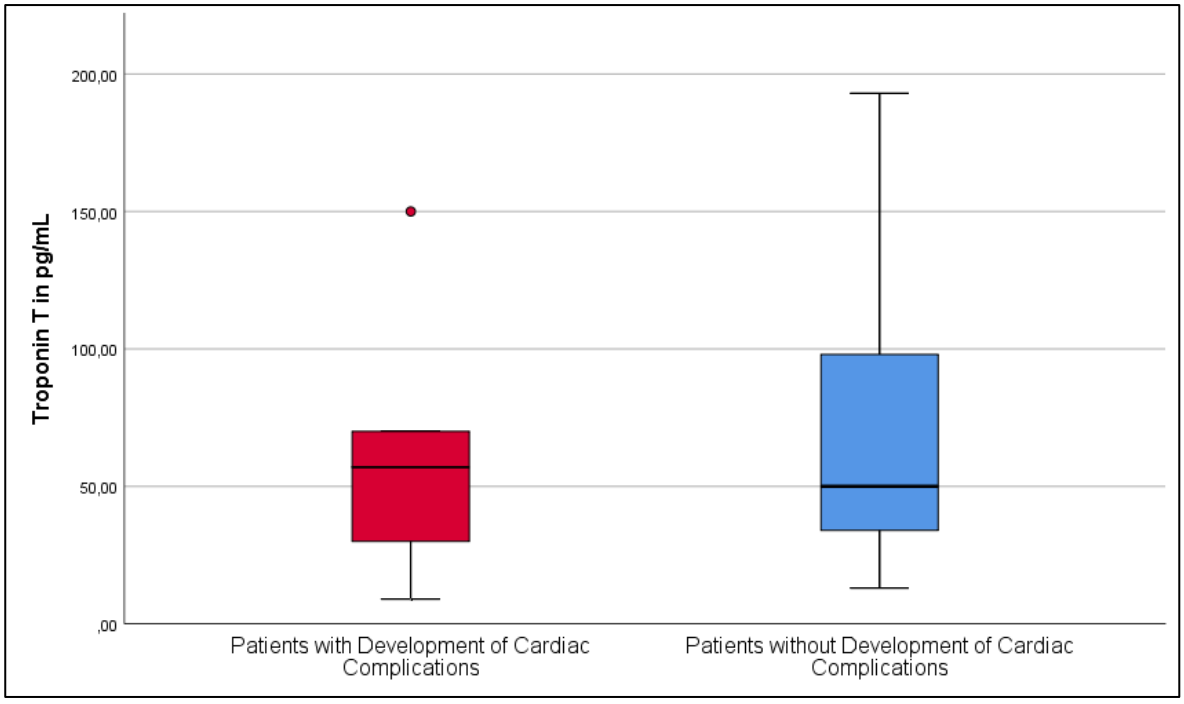
We considered Troponin T levels up to 14 pg/mL as normal. Levels above 14 pg/mL were considered to be elevated Troponin T levels. Troponin T levels were available from 50/111 patients. 4/50 (8%) patients had normal Troponin T levels whereas 46/50 (92%) had elevated Troponin T levels. The median Troponin T level of all patients was 50 pg/mL (IQR 30-98 pg/mL). 2/13 (15.4%) patients with development of cardiac complications had normal Troponin T levels, 11/13 (84.6%) had elevated Troponin T levels. The median Troponin T level for this group of patients was 57 pg/mL (IQR 30-70 pg/mL). In the group of patients without development of cardiac complications 2/37 (5.4%) had normal Troponin T levels, while 35/37 (94.6%) patients had elevated Troponin T levels. 50 pg/mL (IQR 34-98 pg/mL) was the median Troponin T level for this patient group.

Table 13 **Troponin T Levels**

		Total N = 50	Patient Group with development of cardiac complications N = 13	Patient Group without development of cardiac complications N = 37
Troponin T levels	Normal	4 (8.0)	2 (15.4)	2 (5.4)
	Elevated	46 (92.0)	11 (84.6)	35 (94.6)
n/N (%)				

*corrected N due to exclusion of missing Data

Figure 20 **Troponin T Levels**



8.6.13 NT-pro-BNP Levels

NT-pro-BNP levels were measured in 43/111 patients. Levels up to 150 pg/mL in women and up to 100 pg/mL in men were considered normal NT-pro-BNP levels. Levels above 150 pg/mL in women and above 100 pg/mL in men were considered to be increased NT-pro-BNP levels. 3/43 (7.0%) of all patients had normal NT-pro-BNP levels and 40 (93.0%) had elevated NT-pro-BNP levels. The median NT-pro-BNP level was 2713 pg/mL (IQR 693-13442 pg/mL). In the group of patients with development of cardiac complications 0/14 (0%) had normal NT-pro-BNP levels and 14/14 (100%) had elevated NT-pro-BNP

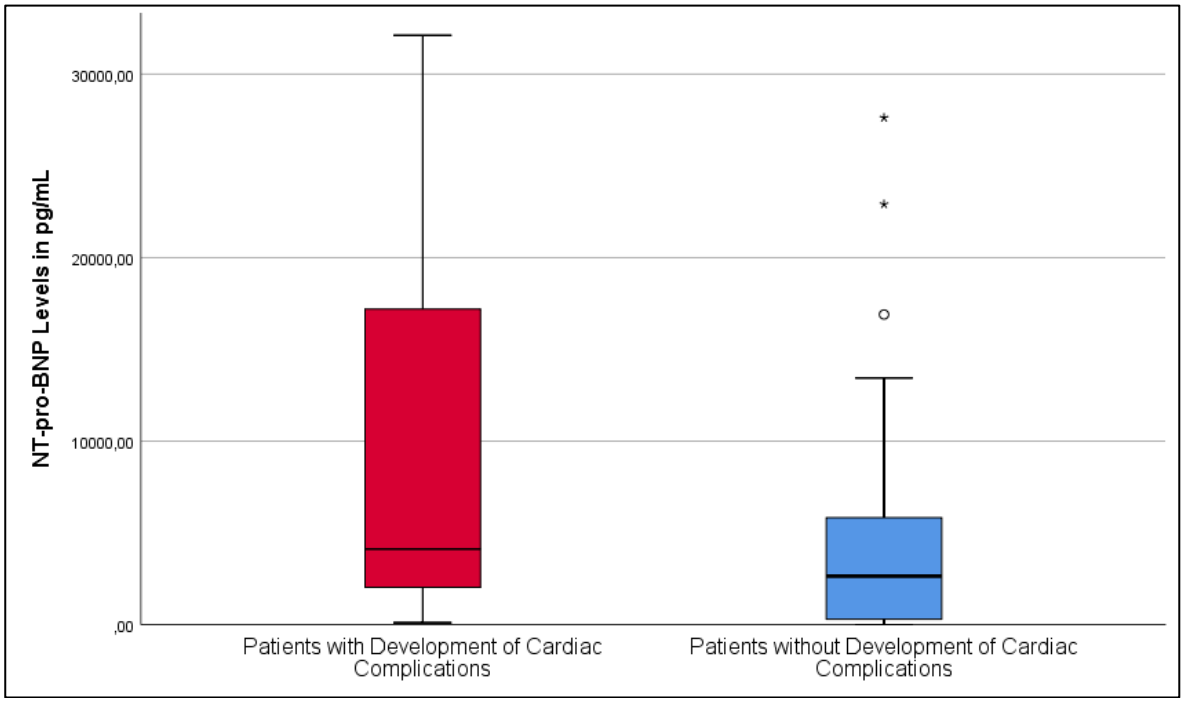
levels. The median NT-pro-BNP level in this group was 4119.5 pg/mL (IQR 2041-17199 pg/mL). 3/29 (10.3%) patients in the group without development of cardiac complications had normal NT-pro-BNP levels and 26/29 (89.7%) had elevated NT-pro-BNP levels. In this group the median NT-pro-BNP level was 2648 pg/mL (IQR 297-5830 pg/mL). The higher NT-pro-BNP level in the group of patients with development of cardiac complications was without significance.

Table 14 **NT-pro-BNP Levels**

		Total N = 43	**Patient Group <u>with</u> development of cardiac complications N = 14**	**Patient Group <u>without</u> development of cardiac complications N = 29**
NT-pro-BNP levels	Normal	3 (7.0)	0 (0.0)	3 (10.3)
	Elevated	40 (93.0)	14 (100.0)	26 (89.7)
n/N (%)				

*corrected N due to exclusion of missing data

Figure 21 **NT-pro-BNP Levels**



8.6.14 Creatine kinase Levels

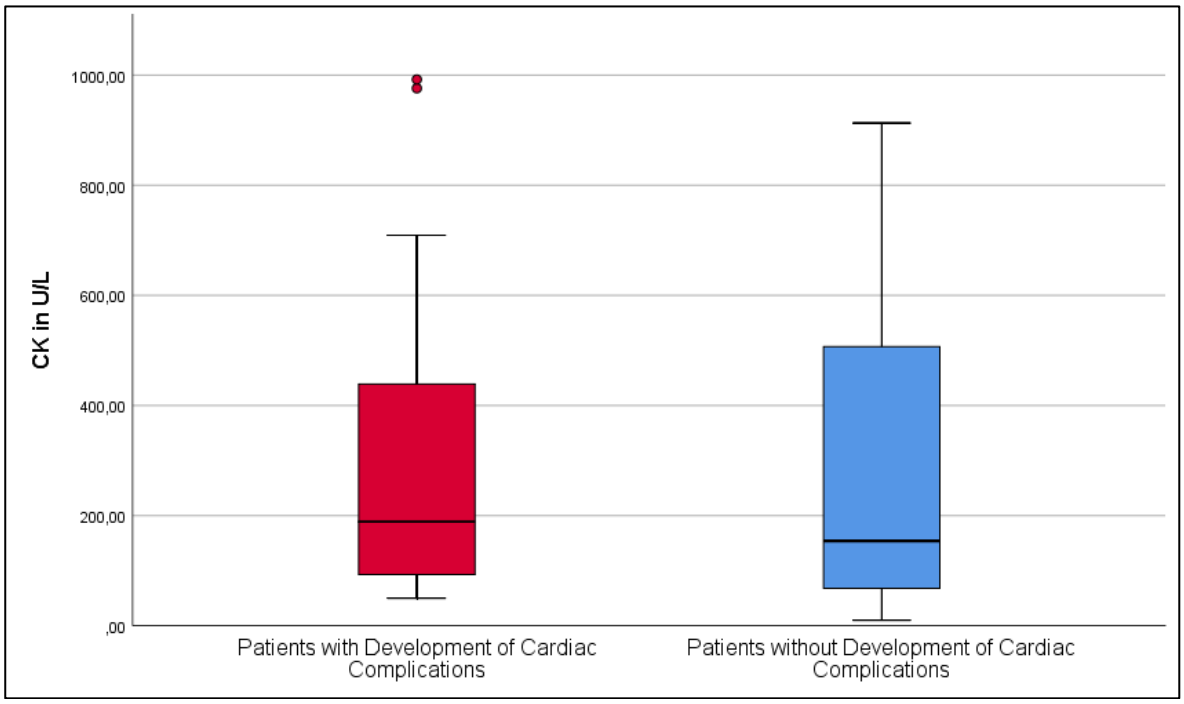
In men a creatine kinase (CK) level up to 170 U/L was considered normal, in women up to 145 U/L. CK Levels above 170 U/L in men and above 145 U/L in women were considered as elevated CK levels. CK levels were available for 107/111 patients. Of all patients, 54/107 (50.5%) had normal CK levels and 53/107 (49.5%) had elevated CK levels. The median CK level for all patients was 162 U/L (IQR 77-504 U/L). 9/21 (42.9%) of patients with development of cardiac complications had normal CK levels and 12/21 (57.1%) had elevated CK levels. 189 U/L (IQR 93-439 U/L) was the median CK level for this group of patients. In the group of patients without development of cardiac complications 45/86 (52.3%) had normal CK levels and 41/86 (47.7%) had elevated CK levels. In this group of patients, the median CK level was 154 U/L (IQR 68-507 U/L).

Table 15 **CK Levels**

		Total N = 107	**Patient Group <u>with</u> development of cardiac complications N = 21**	**Patient Group <u>without</u> development of cardiac complications N = 86**
CK levels	Normal	54 (50.5)	9 (42.9)	45 (52.3)
	Elevated	53 (49.5)	12 (57.1)	41 (47.7)
n/N (%)				

*corrected N due to exclusion of missing data

Figure 22 **CK Levels**



8.6.15 D-Dimer Levels

Normal D-Dimer levels were up to 50 mg/L, levels above 50 mg/L were considered elevated D-Dimer levels. D-Dimer levels were measured in 22/111 patients. The median D-Dimer level for all patients was 2.29 (IQR 1.18-8.12 mg/L). 19/22 (86.4%) had normal D-Dimer levels and 3/22 (13.6%) had elevated D-Dimer levels. 2/2 (100%) patients in the group with development of cardiac complications had normal D-Dimer levels and 0 (0%) had elevated D-Dimer levels. The median D-Dimer level for this group was 1.58 mg/L (IQR 1.34-1.81 mg/L). In the group of patients without development of cardiac complications 17/20 (85%) had normal D-Dimer levels and 3/20 (15%) had elevated D-Dimer levels. For this group the median D-Dimer level was 3.26 mg/L (IQR 1.16-9.39 mg/L).

8.6.16 Blood Gas Analysis

For blood gas analysis we took levels of arterial pO2, arterial PCO2, pH, HCO3 and the Horovitz index was calculated: $Horovitz\ index = \frac{arterial\ pO2}{FiO2}$

Levels between 71 and 104 mmHg were considered normal for arterial pO2. Levels below 71 mmHg were considered hypoxemic and levels above 104 mmHg hyperoxemic. Arterial

pO2 levels were available from 108/111 patients. Of all patients, 39/108 (36.1%) were hypoxemic, 38/108 (35.2%) were normoxemic and 31/108 (28.7%) were hyperoxemic. The median arterial PO2 level was 82.15 mmHg (IQR 62.80-110.50 mmHg). In the group of patients with development of cardiac complications 4/22 (18.2%) were hypoxemic, 13/22 (59.1%) were normoxemic and 5/22 (22.7%) were hyperoxemic. For this group the median arterial pO2 was 85.05 mmHg (IQR 71.80-104.00 mmHg). 35/86 (40.7%) patients in the group without development of cardiac complications were hypoxemic, 25/86 (29.1%) were normoxemic and 25/86 (30.2%) were hyperoxemic. The median arterial pO2 for this group was 78.85 mmHg (IQR 60.50-112.00 mmHg). Patient without development of cardiac complications had significantly more frequent hypoxia (p= 0,026).
Arterial pCO2 levels in the range of 32-43 mmHg in women and 35-46 in men were considered normocapnic. Levels below 32 mmHg in women and below 35 mmHg in men were considered as hypocapnic and levels above 43 mmHg in women and above 46 mmHg in men were considered hypercapnic. Arterial pCO2 levels were missing in 3 patients. Of all patients, 32/108 (29.6%) were hypocapnic, 29/108 (26.9%) were normocapnic and 47/108 (43.5%) were hypercapnic. Of all patients, the median arterial pCO2 level was 41.95 mmHg (IQR 32.35-56.20 mmHg). 9/22 (40.9%) patients in the group with development of cardiac complications were hypocapnic, 7/22 (31.8%) were normocapnic and 6/22 (27.3%) were hypercapnic. In this group the median arterial pCO2 level was 38.45 mmHg (IQR 31.00-47.50 mmHg). In the group of patients without development of cardiac complications 23/86 (26.7%) were hypocapnic, 22/86 (25.6%) were normocapnic and 41/86 (47.7%) were hypercapnic. 45.05 mmHg (IQR 32.60-57.00 mmHg) was the median arterial pCO2 level for this patient group.
For arterial pH, levels in the range from 7.370-7.450 were considered normal. Levels below 7.370 were considered acidosis and levels above 7.450 were considered alkalosis. pH levels were available from 109/111 patients. Of all patients, 53/109 (48.6%) had acidosis, 26/109 (23.9%) had normal pH and 30/109 (27.5%) had alkalosis. The median pH for all patients was 7.378 (IQR 7.278-7.458). In the group of patients with development of cardiac complications, 8/22 (36.4%) had acidosis, 8/22 (36.4%) had normal pH levels and 6/22 (27.3%) had alkalosis. For this group, the median pH was 7.414 (IQR 7.295-7.458). 45/87 (51.7%) patients in the group without development of cardiac complications had acidosis, 18/87 (20.7%) had normal pH levels and 24/87 (27.6%) had alkalosis. 7.354 (IQR 7.273-7.462) was the median pH level for this group.

HCO3 levels were considered normal in a range between 21 and 26 mmol/L. Levels below 21 mmol/L were considered decreased HCO3 levels and levels above 26mmol/L were considered elevated HCO3 levels. HCO3 levels were available from 102/111 patients. From all patients 23/102 (22.5%) had decreased HCO3 levels, 39/102 (38.2%) had normal HCO3 levels and 40/102 (39.2%) had elevated HCO3 levels. The median HCO3 level of all patients was 24.6 mmol/L (IQR 21.20-27.90 mmol/L). In the group of patients with development of cardiac complications 6/21 (28.6%) had decreased HCO3 levels, 8/21 (38.1%) had normal HCO3 levels and 7/21 (33.3%) had elevated HCO3 levels. The median HCO3 level in this group was 23.20 mmol/L (IQR 20.60-27.20 mmol/L). 17/81 (21.0%) patients in the group of patients without development of cardiac complications had decreased HCO3 levels, 31/81 (38.3%) had normal HCO3 levels and 33/81 (40.7%) had elevated HCO3 levels. 24.90 mmol/L (IQR 21.50-28.10 mmol/L) was the median HCO3 level in this group.

The Horowitz index was calculated by dividing the arterial pO2 by the fractions of oxygen in the inhaled air (FiO2). Levels above 300 were considered normal, levels between 200 and 300 were considered mild ARDS, levels between 100 and 200 were considered moderate ARDS, and levels below 100 were considered severe ARDS, according to the Berlin definition (74). The Horowitz index could be calculated for 107/111 patients. Of all patients, 15/107 (14%) had severe lung injury, 42/107 (39.3%) had moderately severe lung injury, 32/107 (29.9%) had mild lung injury and 18/107 (16.8%) had a normal Horowitz index. In all patients, the median Horowitz index was 193.4 (IQR 126.8-272.5). In the group of patients with development of cardiac complications 2/22 (9.1%) had severe lung injury, 8/22 (36.4%) had moderately severe lung injury, 9/22 (40.9%) had mild lung injury and 3/22 (13.6%) had normal Horowitz index. For this group, the median Horowitz index was 205.46 (IQR 152.40-232.86). 13/85 (15.3%) of patients in the group without development of cardiac complications had severe lung injury, 34/85 (40.0%) had moderately severe lung injury, 23/85 (27.1%) had mild lung injury and 15/85 (17.6%) had normal Horowitz index. 181.08 (IQR 125.00-287.00) was the median Horowitz index for this patient group.

Figure 23 **Blood Gas Analysis**

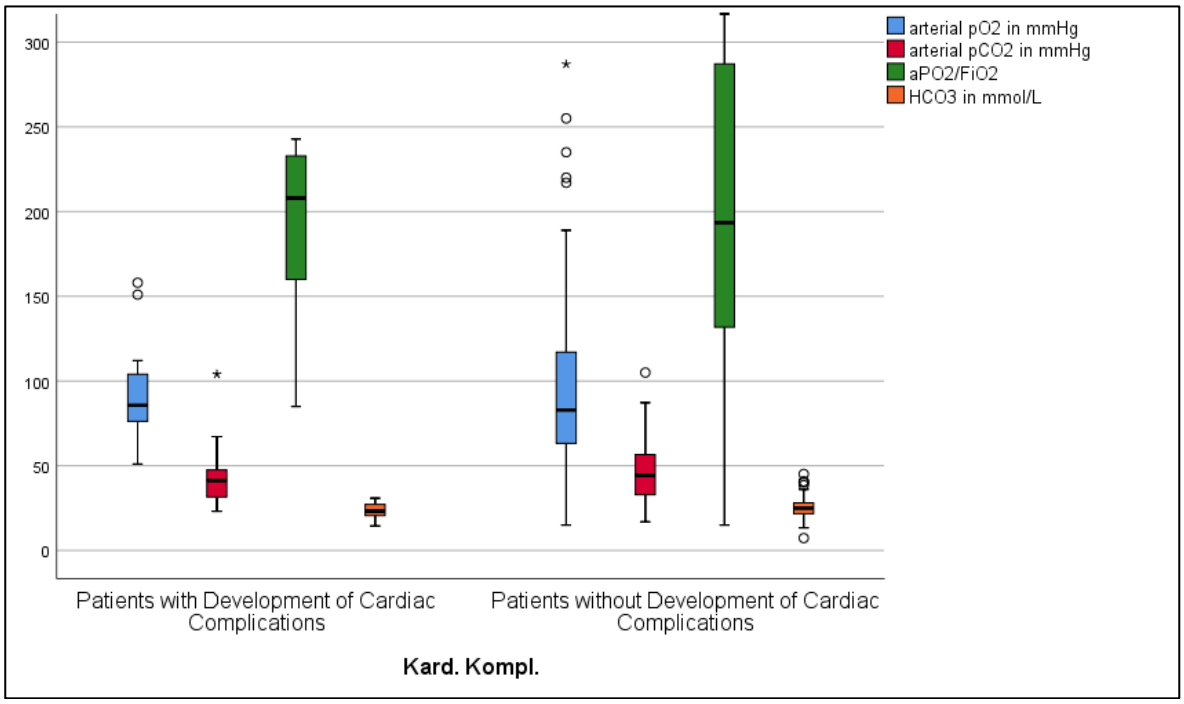


Figure 24 **pH**

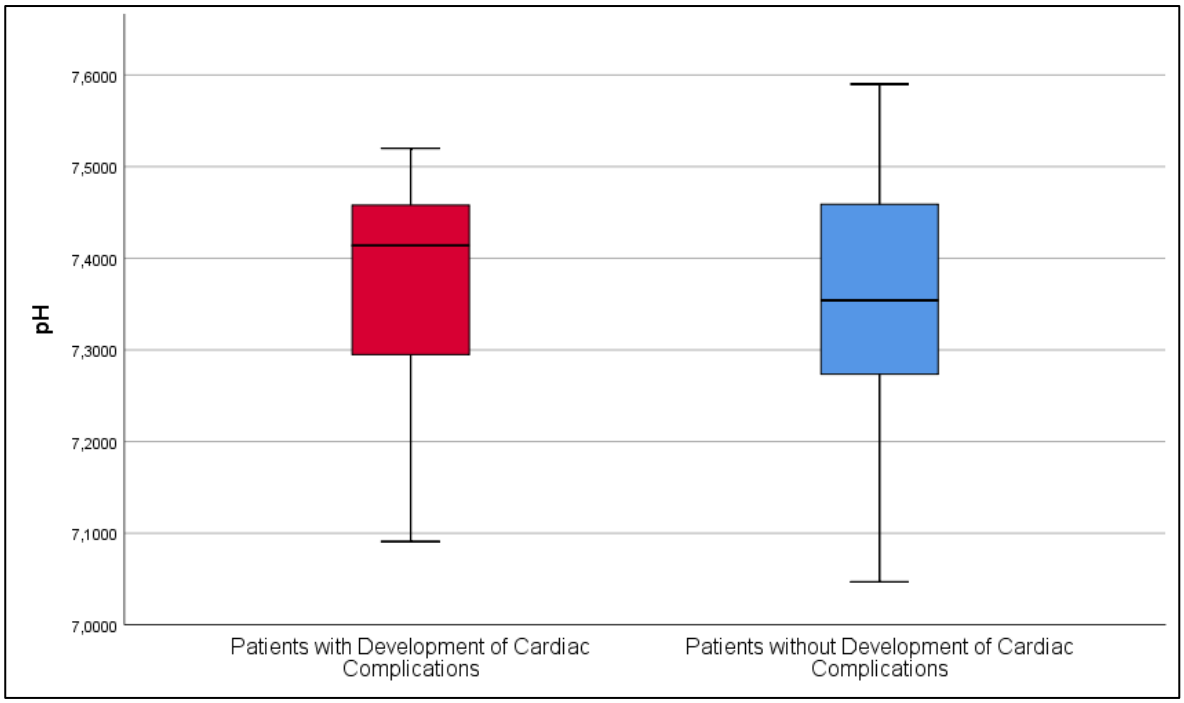


Table 16 **Blood Gas Analysis**

		Total	Patient Group <u>with</u> development of cardiac complications	Patient Group <u>without</u> development of cardiac complications
arterialPO2**		**N = 108**	**N = 22**	**N = 86**
	Hypoxemic	39 (36,1)	4 (18,2)	35 (40,7)
	Normoxemic	38 (35,2)	13 (59,1)	25 (29,1)
	Hyperoxemic	31 (28,7)	5 (22,7)	26 (30,2)
arterialCO2	Hypocapnic	32 (29,6)	9 (40,9)	23 (26,7)
	Normocapnic	29 (26,9)	7 (31,8)	22 (25,6)
	Hypercapnic	47 (43,5)	6 (27,3)	41 (47,7)
pH		**N = 109**	**N = 22**	**N = 87**
	Acidosis	53 (48,6)	8 (36,4)	45 (51,7)
	Normal	26 (23,9)	8 (36,4)	18 (20,7)
	Alkalosis	30 (27,5)	6 (27,3)	24 (27,6)
arterialHCO3		**N = 102**	**N = 21**	**N = 81**
	Decreased	23 (22,5)	6 (28,6)	17 (21,0)
	Normal	39 (38,2)	8 (38,1)	31 (38,3)
	Elevated	40 (39,2)	7 (33,3)	33 (40,7)
Horowitz Index		**N = 107**	**N = 22**	**N = 85**
	Severe Lung Injury	15 (14,0)	2 (9,1)	13 (15,3)
	Moderately severe Lung Injury	42 (39,3)	8 (36,4)	34 (40,0)
	Mild Lung Injury	32 (29,9)	9 (40,9)	23 (27,1)
	No Lung injury	18 (16,8)	3 (13,6)	15 (17,6)
n/N (%)				

*Corrected N due to missing data.
**p value = 0,026

8.7 BMI

The BMI was calculated using the following formula: $BMI = \frac{Body\ weight}{Height^2}$. A BMI below 18.5 was considered underweight. In the range of 18.5 to 24.9, a BMI was considered normal weight, in the range of 25 to 29.9 overweight, and a value of 30 or higher obese. Due to missing data the BMI could be calculated in 85/111 patients. Of all patients, 3/85 (3.5%) were underweight, 30/85 (35.3%) had normal weight, 24/85 (28.2%) were overweight and 28/85 (32.9%) were obese. The median BMI for all patients was 27.3 (IQR 23.5-31.2). In the group of patients with development of cardiac complications 0/20 (0%) were underweight, 11/20 (55.0%) had normal weight, 3/20 (15.0%) were overweight and 6/20 (30.0%) were obese. The median BMI in this group of patients was 24.4 (IQR 22.4-31.1). 3/65 (4.6%) patients in the group without development of cardiac complications were underweight, 19/65 (29.2%) had normal weight, 21/65 (32.3%) were overweight and 22/65 (33.8%) were obese. In this group of patients, the median BMI was 27.7 (IQR 23.5-32.1). The differences as shown in the table below between the two groups of patients were not significant.

Figure 25 **BMI**

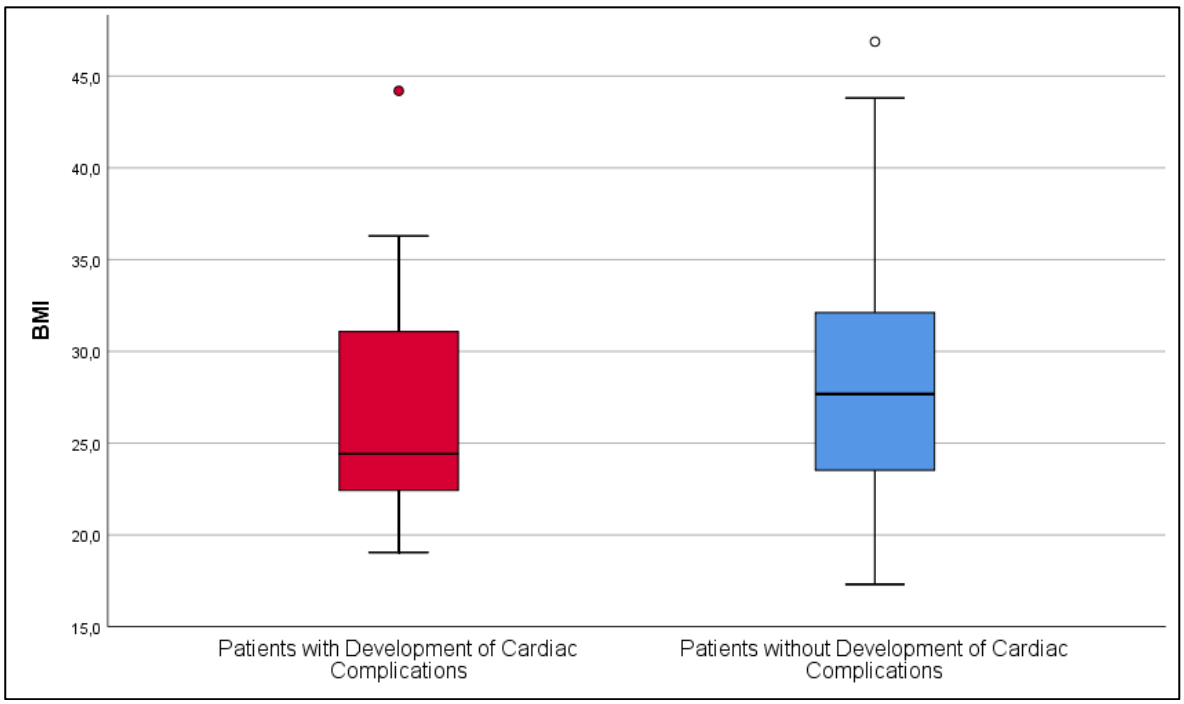


Table 17 **BMI**

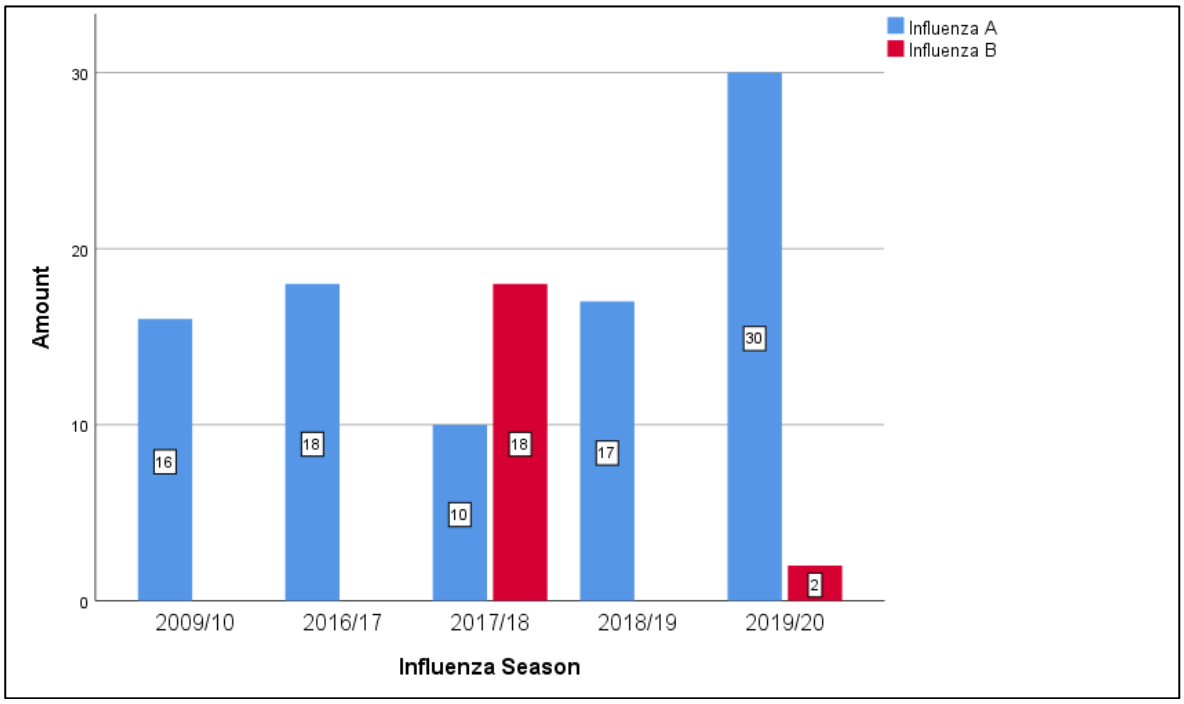
		Total N = 85	Patient Group with development of cardiac complications N = 20	Patient Group without development of cardiac complications N = 65
BMI	Underweight	3 (3.5)	0 (0)	3 (4.6)
	Normal Weight	30 (35.3)	11 (55.0)	19 (29.2)
	Overweight	24 (28.2)	3 (15.0)	21 (32.3)
	Obese	28 (32.9)	6 (30.0)	22 (33.8)
n/N (%)				

*Corrected N due to missing data

8.8 Influenza A + B

When looking at the influenza subtype, it was noticeable that influenza A occurred more frequently than influenza B. Of all Patients 91/111 (82,0%) had influenza A, while 20/111 (18.0%) patients had influenza B. Regarding differences between the two groups of patients, 16/22 (72.7%) in the group of patients with development of cardiac complications had influenza A and 6/22 (27.3%) patients had influenza B, while in the group of patients without development of cardiac complications, 75/89 (84.3%) had influenza A and 14/89 (15.7%) had influenza B. The difference between the two groups of patients was not significant. Looking at the differences between influenza seasons, it is noticeable that in 2009/10 (16 cases), 2013/14 (18 cases), and 2018/19 (17 cases) there were only influenza A cases. In 2017/18, there were more influenza B cases (18 cases) than influenza A cases (10 cases), and in 2019/20, there were only 2 influenza B cases but 30 influenza A cases.

Figure 26 **Influenza A and B Distribution**



8.9 Secondary Infections

For secondary infections, only the relevant causative agents were considered, all others were excluded beforehand. During their ICU stay, secondary infections (bacterial and/or fungal and/or viral) were diagnosed in 48/111 (43.2%) patients. Secondary bacterial infections occurred in 40/111 (36.0%) patients. Secondary fungal infections were detected in 14/111 (12.6%) patients and viral infections other than influenza virus infections were detected in 14/111 (12.6%) patients. The most common causative agents were *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Candida albicans* and *Aspergillus fumigatus* as shown in table 18. For further analysis of secondary infections, they were divided according to the sites of infection. A secondary infection was classified as nosocomial respiratory tract infections (including pneumonia and tracheobronchitis) if a pathogen could be detected in a sputum culture, a tracheal secretion culture, or in a culture from a bronchoalveolar lavage (BAL). Bloodstream infections were considered confirmed if they could be detected by positive blood cultures. Pleural infections were detected by cultures of pleural punctates, urinary tract infections by urine cultures, and other infections by cultures of biliary punctate, stool cultures, swab cultures from central venous catheter, PCR analysis of nasopharyngeal swabs, and from EDTA blood analysis.

20/111 patients (18.0%) developed nosocomial bacterial pneumonia or tracheobronchitis (HAP/VAT) due to a secondary pathogen.
In the group of patients with development of cardiac complications, no patient (0%) developed nosocomial bacterial pneumonia, whereas 20/89 (22.5%) in the group of patients without development of cardiac complications developed nosocomial bacterial pneumonia. This difference was significant (p value = 0.014). Nosocomial fungal pneumonia affected 7/111 (6.3%) patients. In the group of patients with development of cardiac complications, no patient (0%) developed nosocomial fungal pneumonia while 7/89 (7.9%) of patients without development of cardiac complications developed nosocomial fungal pneumonia.
Bacterial bloodstream infections affected 21/111 (18.9%) patients. 1/22 (4.5%) patients in the group with development of cardiac complications developed bacterial bloodstream infection and 20/89 (22.5%) patients in the group without development of cardiac complications developed bacterial bloodstream infection. Only one patient (1/111, 10.9%) got a fungal bloodstream infection. This patient belonged to the group of patients without development of cardiac complications (1/89, 1.1%).
In 3/111 (2.7%) patients, an infection was detected in the pleura. All these three patients were from the group without development of cardiac complications (3/89, 3.4%). Fungal infections could not be detected in the pleura.
10/111 (9.0%) patients developed a bacterial urinary tract infection. 3/22 (13.6%) in the group of patients with development of cardiac complications developed a bacterial urinary tract infection while 7/89 (7.9%) of patients without development of cardiac complications developed a bacterial urinary tract infection. A fungal urinary tract infection was found in a total of 5/111 (4.5%) patients, all of whom were from the group of patients without development of cardiac complications (5/89, 5.6%).
4/111 (3.6%) patients developed another bacterial infection. All 4 patients came from the group of patients without development of cardiac complications (4/89, 4.5%). Among the other fungal infections, a total of 3 infections were detected (3/111, 2.7%). 1/22 (4.5%) patients developed another fungal infection while 2/89 (2.2%) developed another fungal infection.
Viral infections were detected in 14/111 (12.6%) of the patients. In the group of patients with development of cardiac complications, 1/22 (4.5%) developed a viral infection. In the group of patients without development of cardiac complications, 13/89 (14.6%) developed a viral infection.

All differences are shown in Table 19 and were not significant except for nosocomial bacterial pneumonia.

Table 18 **Causative Agents of Secondary Infections**

Secondary Infections		Causative Agent	Amount
HAP/VAT	**Bacterial Infections**	*Staphylococcus aureus*	8
		Pseudomonas aeruginosa	7
		Streptococcus pneumoniae	4
		Escherichia coli	2
		Hemophilus parainfluenzae	2
		Klebsiella oxytoca	2
		Stenotrophomonas maltophilia	2
		Citrobacter diversus	1
		Enterobacter aerogenes	1
		Enterobacter cloacae	1
		Klebsiella pneumonia	1
		Proteus vulgaris	1
		Pseudomonas oleovorans	1
	Fungal Infections	*Aspergillus fumigatus*	6
		Aspergillus flavus	1
		Pneumocystis jirovecii	1
Bloodstream Infections	**Bacterial Infections**	*Staphylococcus epidermidis*	6
		Staphylococcus aureus	5
		Staphylococcus hominis	3
		Escherichia coli	2
		Staphylococcus capitis	2
		Corynebcaterium jeikeium	1
		Coagulase negative Staphylococci	1
		Klebsiella ornitholytica	1
		Staphylococcus haemolyticus	1
		Streptococcus anginosus	1
		Streptococcus pneumoniae	1
	Fungal Infections	*Candida glabrata*	1
Pleural Infections	**Bacterial Infections**	*Staphylococcus epidermidis*	1
		Staphylococcus saccharolyticus	1
		Streptococcus salivarius	1
Urinary tract Infections	**Bacterial Infections**	*Enterococcus faecium*	3
		Escherichia coli	3
		Klebsiella pneumonia	3
		Klebsiella oxytoca	1
		Pseudomonas species	1
	Fungal Infections	*Candida albicans*	5

Other Infections	**Bacterial Infections**	*Clostridium difficile*[1]	1
		Enterococcus faecium[2]	1
		Escherichia coli[3]	1
		Micrococcus luteus[3]	1
	Fungal Infections	*Candida albicans*[4]	3
	Viral Infections	HSV-1	9
		Adenovirus	2
		HHV-6	2
		Bocavirus	1
		CMV	1
		EBV	1
		HSV-2	1
		RSV	1
		SARS-CoV-2	1

[1]Stool culture, [2]Bile puncture culture, [3]Swab culture from central venous catheter, [4]1x Swab culture from central venous catheter 2x Bile puncture culture

Table 19 **Frequencies of Secondary Infections**

Secondary Infections		**Total N = 111**	**Patient Group <u>with</u> development of cardiac complications N = 22**	**Patient Group <u>without</u> development of cardiac complications N = 89**
HPA/VAT*	Bacterial	20 (18.0)	0 (0)	20 (22.5)
	Fungal	7 (6.3)	0 (0.0)	7 (7.9)
Bloodstream Infection	Bacterial	21 (18.9)	1 (4.5)	20 (22.5)
	Fungal	1 (0.9)	0 (0.0)	1 (1.1)
Pleural Infections	Bacterial	3 (2.7)	0 (0.0)	3 (3.4)
	Fungal	0 (0.0)	0 (0.0)	0 (0.0)
Urinary Tract Infections	Bacterial	10 (9.0)	3 (13.6)	7 (7.9)
	Fungal	5 (4.5)	0 (0.0)	5 (5.6)
Other Infections	Bacterial	4 (3.6)	0 (0.0)	4 (4.5)
	Fungal	3 (2.7)	1 (4.5)	2 (2.2)
	Viral	14 (12.6)	1 (4.5)	13 (14.6)

n/N (%)
***** p value = 0.014

8.10 Antiviral Treatment

Of all patients, 100/111 (90.1%) were treated with a neuraminidase inhibitor. In almost all cases 98/100 (98%) the drug was oseltamivir (Tamiflu™). One patient was treated with peramivir (Rapivab™) and one patient was given peramivir (Rapivab™) and oseltamivir (Tamiflu™). In the group of patients with development of cardiac complications, 21/22 (95.5%) were treated with a neuraminidase inhibitor, while 79/89 (88.8%) in the group of patients without development of cardiac complications received a neuraminidase inhibitor. The differences between the two groups of patients were not significant. Other antiviral medications administered were aciclovir in 9 patients, ganciclovir in one patient, and valaciclovir in 2 patients.

8.11 Antibiotic Treatment

A total of 106/111 (95.5%) patients were treated with antibiotics. The most common antibiotics were piperacillin/tazobactam, meropenem, and moxifloxacin. Some patients (21/111 (18.9%)) were also treated with antifungals. The most common antimycotic agent was caspofungin. The mean start of antibiotic therapy was 2 (IQR 0-6) days after disease onset. There were no differences between the two groups of patients regarding the frequency of antibiotic therapy: 21/22 (95.5%) in the group of patients with development of cardiac complications received antibiotic therapy, 85/89 (95.5%) in the group of patients without development of cardiac complications received antibiotic therapy. Regarding the initiation of antibiotic therapy, the mean starting time in the group of patients with development of cardiac complications was 2 (IQR 0-4) days, whereas in the group of patients without development of cardiac complications, the mean starting time of antibiotic therapy was also 2 (IQR 0-6) days. The differences between the two patient groups were not significant.

Table 20 **Antibiotics Administered**

Antibiotics		**Number of patients treated**
Acylaminopenicillins	Piperacillin/Tazobactam	60
Aminoglycosides	Amikacin	1
	Gentamicin	1
Aminopenicillins	Amoxycillin/Clavulanic Acid	17
	Ampicillin/Sulbactam	12
Ansamycines	Rifampicin	2
Carbapenems	Doripenem	1
	Imipenem	3
	Meropenem	31
Cephalosporin 2. Gen	Cefuroxime	3
Cephalosporin 3. Gen	Cefotaxime	1
	Ceftazidime	1
	Ceftriaxone	6
Cephalosporin 4. Gen	Cefepime	19
	Cefpirome	1
Cyclic lipopeptides	Daptomycin	5
Fluoroquinolones	Ciprofloxacin	3
	Levofloxacin	13
	Moxifloxacin	31
Glycopeptides	Vancomycin	5
Glycylcylines	Tigecycline	1
Isoxazolyl-antibiotics	Flucloxacillin	3
Macrolides	Azithromycin	19
	Clarithromycin	12
	Erythromycin	4
	Roxithromycin	1
Oxazolidinones	Linezolid	13
Sulfonamides	Sulfamethoxazole/Trimethoprim	1
	Sulfametrole/Trimethoprim	1
Tetracyclines	Doxycycline	3
Tuberculostatics	Isoniazid (Rifampicin see above)	1
Antimycotics		
	Amphotericin B	2
	Anidulafungin	1
	Clotrimazole	1
	Caspofungin	9
	Fluconazole	2
	Isavuconazole	2
	Voriconazole	4

8.12 Past Medical History – Underlying Disease

8.12.1 Cardiovascular Diseases

Pre-existing cardiovascular diseases recorded were hypertension, arrhythmias, heart failure, CHD, valvular heart disease, PAOD, and CAOD. Hypertension was the most common cardiovascular disease with 60/111 patients (54.1%). In the group of patients with development of cardiac complications, 10/22 (45.5%) had hypertension. 50/89 patients (56.2%) had hypertension in the group without development of cardiac complications. Another common previous cardiovascular condition was arrhythmias. Of all patients, 32/111 (28.8%) already suffered from arrhythmias such as atrial fibrillation. 9/22 patients (40.9%) in the group with development of cardiac complications had arrhythmias while 23/89 patients (25.5%) without development of cardiac complications had arrhythmias. The differences between the two patient groups were not significant. Heart failure was a similarly common pre-existing condition. In total, 30/111 patients had heart failure. Patients with development of cardiac complications had heart failure significantly (p value < 0.001) more often (13/22, 59.1%) than those without development of cardiac complications (17/89, 19.1%). CHD was preexisting in 25/111 patients (22.5%). In the group of patients with development of cardiac complications, 6/22 (27.3%) had CHD. With a small, non-significant difference, 19/89 patients (21.3%) in the group without development of cardiac complications had CHD. Valvular heart diseases were identified as a pre-existing condition in 21/111 (18.9%) of all patients. Valvular heart diseases occurred significantly more frequently (p value = 0.031) in the group of patients with development of cardiac complications (8/22, 36.4%). In the group of patients without development of cardiac complications, valvular heart diseases were identified in 13/89 patients (14.6%). Pre-existing arterial occlusive disease occurred less frequently. 10/111 patients (9.0%) had a PAOD and 9/111 patients (8.1%) had a CAOD. In the group of patients with development of cardiac complications, 1/22 patient (4.5%) had a PAOD and no patient had a CAOD (0/22, 0.0%). In the group of patients without development of cardiac complications, 9/89 patients (10.1%) had a PAOD and 9/89 (10.1%) had a CAOD. The differences between the two groups of patients were not significant.

8.12.2 Lung Diseases

The most common pre-existing pulmonary disease was COPD. A total of 38/111 patients (34.2%) had COPD. In the group of patients with development of cardiac complications,

7/22 patients (31.8%) had COPD. 31/89 patients (34.8%) in the group without development of cardiac complications had COPD. Previously known asthma was identified in 3/111 patients (2.7%). No patient in the group with development of cardiac complications had asthma (0/22, 0.0%) while 3/89 patients (3.4%) in the group without development of cardiac complications had asthma. The differences between the two groups were not significant.

8.12.3 Other Diseases

Chronic renal failure was a common pre-existing condition with a total of 29/111 patients (26.1%). In the group of patients with development of cardiac complications, 5/22 patients (22.7%) had chronic renal failure while 24/89 patients (27.0%) in the group without development of cardiac complications had chronic renal failure. Malignancies were another common pre-existing condition. Of all patients, 20/111 (18.0%) had a malignant disease. In the group of patients with development of cardiac complications, 3/22 patients (13.6%) had a malignancy. 17/89 patients (19.1%) in the group without development of cardiac complications had malignant disease. Gastrointestinal diseases such as sigmoid diverticulitis or gastroesophageal reflux could be identified as pre-existing disease in a total of 19/89 patients (17.1%). 2/22 patients (9.1%) in the group with development of cardiac complications suffered from gastrointestinal disease while 17/89 patients (19.1%) in the group without development of cardiac complications had gastrointestinal disease as a pre-existing condition. Diabetes mellitus was a pre-existing condition that occurred in 18/111 patients (16.2%). 2/22 patients (9.1%) in the group without development of cardiac complications had diabetes mellitus. In the group of patients without development of cardiac complications, diabetes mellitus occurred in 16/89 patients (18.0%). Liver diseases such as cirrhosis occurred in a total of 16/111 patients (14.4%). In the group of patients with development of cardiac complications, liver disease occurred in 3/22 patients (13.6%). Liver disease was similarly frequent in patients without development of cardiac complications (13/89, 14.6%). Immunosuppression was present in a total of 15/111 patients (13.5%). 2/22 patients (9.1%) had immunosuppression in the group with development of cardiac complications. In the group of patients without development of cardiac complications, immunosuppression was present in 13/89 patients (14.6%). Neurological diseases such as polyneuropathies were identified as pre-existing conditions in 14/111 patients (12.6%). In the group of patients with development of cardiac complications, 3/22 (13.6%) suffered from a neurological disease. 11/89 patients (12.4%)

in the group without development of cardiac complications had a neurological disease. Stroke was a known prior condition in 7/111 patients (6.3%). In the group of patients with development of cardiac complications, stroke was known in 2/22 patients (9.1%). 5/89 patients (5.6%) in the group without development of cardiac complications had stroke as a known prior condition. The differences between the two groups of patients were not significant. The table below summarizes the pre-existing conditions.

Table 21 **Underlying Diseases**

		Total N = 111	Patient Group with development of cardiac complications N = 22	Patient Group without development of cardiac complications N = 89
Cardio-vascular Diseases	Hypertension	60 (54.1)	10 (45.5)	50 (56.2)
	Arrhythmia	32 (28.8)	9 (40.9)	23 (25.8)
	Heart failure*	30 (27.0)	13 (59.1)	17 (19.1)
	CHD	25 (22.5)	6 (27.3)	19 (21.3)
	Valvular heart disease**	21 (18.9)	8 (36.4)	13 (14.6)
	PAOD	10 (9.0)	1 (4.5)	9 (10.1)
	CAOD	9 (8.1)	0 (0.0)	9 (10.1)
Lung diseases	COPD	38 (34.2)	7 (31.8)	31 (34.8)
	Asthma	3 (2.7)	0 (0.0)	3 (3.4)
Other diseases	Kidney failure	29 (26.1)	5 (22.7)	24 (27.0)
	Malignancies	20 (18.0)	3 (13.6)	17 (19.1)
	Gastrointestinal disease	19 (17.1)	2 (9.1)	17 (19.1)
	Diabetes mellitus	18 (16.2)	2 (9.1)	16 (18.0)
	Liver disease	16 (14.4)	3 (13.6)	13 (14.6)
	Immunosuppression	15 (13.5)	2 (9.1)	13 (14.6)
	Neurologic disease	14 (12.6)	3 (13.6)	11 (12.4)
	Stroke	7 (6.3)	2 (9.1)	5 (5.6)

n/N (%)
*p value < 0.001
**p value = 0.031

8.13 Cardiac complications

Of all patients, 22/111 (19.8%) developed cardiac complications during their ICU stay. We have analyzed in more detail what the cardiac complications are: 9/22 (40.9%) developed a CMP, 9/22 (40.9%) developed arrhythmogenic complications such as atrial fibrillation,

2/22 (9.1%) developed ischemic complications, 1/22 (4.5%) developed valvular complications and 1/22 (4.5%) developed a not categorizable complication.

8.14 SAPS II & III

To compare the severity of disease between the two groups of patients, we used the Simplified Acute Physiology Score (SAPS) score. The SAPS score is a tool for assessing a patient's expected mortality in the ICU (75). Due to the long duration of the study period, changes in the SAPS score occurred. The SAPS III score is a further development of the SAPS II score, as the latter estimated the expected mortality to be rather too high (76). In the influenza season 2009/10 the SAPS II score was calculated while in the following years the SAPS III was calculated. In the 2009/10 influenza season, the SAPS II score could be calculated in a total of 13 patients. The mean SAPS II score for these 13 patients was 26 (IQR 18-37). Regarding the two patient groups, the mean SAPS II was 17 (IQR 17-17) in the group of patients with development of cardiac complications and 27 (IQR 22-39) in the group of patients without development of cardiac complications.
The SAPS III score was calculated for 95 patients and the mean SAPS III score for all patients was 51 (IQR 44-67). In the group of patients with development of cardiac complications, the mean SAPS III score was 51 (IQR 46-68) while in the group of patients without development of cardiac complications the mean SAPS III score was 50 (IQR 42-63). The differences between the two patient groups regarding the SAPS II score as well as the SAPS III score were not significant.

8.15 Mechanical Ventilation

Of all patients, 50/111 (45.0%) required mechanical ventilation. In the group of patients with development of cardiac complications, 8/22 (36.4%) patients required mechanical ventilation, while in the group of patients without development of cardiac complications 42/89 (47.2%) patients required mechanical ventilation. There was a difference in the frequency of requirement for mechanical ventilation within the two patient groups, but this difference was not significant. As shown in the table below, there was a difference in mortality rates between patients who required mechanical ventilation and those who did not. This difference was significant ($p < 0.001$). Overall, the mortality rate among mechanically ventilated patients was 60%, as 30/50 died. Mechanically ventilated patients with development of cardiac complications had a mortality rate of 75% (6/8 died) while

patients without development of cardiac problems had a mortality rate of 57.1%, as 24/42 died.

Table 22 **Mechanical Ventilation**

Mechanical Ventilation		**Total**	**Patient Group <u>with</u> development of cardiac complications**	**Patient Group <u>without</u> development of cardiac complications**
Yes	Survivor	20/50 (40.0)	2/8 (25.0)	18/42 (42.9)
	Death	30/50 (60.0)	6/8 (75.0)	24/42 (57.1)
No	Survivor	47/61 (77.0)	10/14 (71.4)	37/47 (78.7)
	Death	14/61 (23.0)	4/14 (28.6)	10/47 (21.3)
n/N (%)				

8.16 Outcome

Out of a total of 111 patients 67/111 (60.4%) survived while 44/111 (39.6%) died. Among those who died were 28 (63.6%) men and 16 (36.4%) women. The median age of the deceased patients was 69 (IQR 59-76) years within a range from 15 to 89 years.
In the group of patients with development of cardiac complications, 10/22 (45.5%) died. The median age of the deceased patients was 72 (IQR 66-76) within a range from 26 to 79 years. In the group of patients without development of cardiac complications, 34/89 (38.2%) died. The median age of patients with a fatal outcome was 69 (IQR 58-76) within a range from 15 to 89 years. The differences, as shown in the table below between the two patient groups were not significant.

Table 23 **Outcome**

		Total N = 111	Patient Group <u>with</u> development of cardiac complications N = 22	Patient Group <u>without</u> development of cardiac complications N = 89
Survivor	Amount	67/111 (60.4)	12/22 (54.5)	55/89 (61.8)
	Age	63 (48-74)	70 (61-76)	60 (47-74)
Death	Amount	44/111 (39.6)	10/22 (45.5)	34/89 (38.2)
	Age	69 (59-76)	72 (66-76)	69 (58-76)
n/N (%) mean (IQR)				

9 Discussion

Influenza is a common disease causing a severe seasonal burden on the health care system. In fact, influenza is the greatest burden in terms of incidence and mortality among epidemic and endemic infectious diseases (77). As our study shows, influenza can lead to extremely severe courses.

Retrospectively, we analyzed a total of 5 influenza seasons and described the epidemiological, clinical, and laboratory parameters of patients with confirmed severe influenza infection in the intensive care unit. We also explored the incidence of cardiovascular and pulmonary complications, as well as the rate of secondary bacterial viral and fungal infections.

Our main objective was to identify differences between patients with development of cardiac complications and patients without development of cardiac complications. Because the study is a retrospective data analysis, information bias in the data analysis cannot be excluded.

In total, we analyzed the parameters of 111 ICU patients. Our patient population consisted of more men (62.2%) than women (37.8%), the mean age was 69 years with women (69 years, IQR 60-76 years) being significantly older than men (61 years, IQR 50-74 years, $p = 0.037$). 103/111 patients (92.8%) had some type of previous disease.

Approximately half of the patients (45.0%) required high-end respiratory support with mechanical ventilation (36.4% in the group of patients with development of cardiac complications and even more in the group without cardiac complications (47.2%)).

22/111 patients (19.8%) developed a cardiac complication in association with their severe influenza infection (40.9% acute CMP, 40.9% acute arrhythmogenic complications, 9.1% ischemic complications, 4.5% valvular complications), 92/111 patients (82.9%) increased airspace shadowing compatible with pulmonary infiltrates (68.2% in patients with development of cardiac complications vs. 86.5% in other patients), and 48/111 patients (43.2%) had secondary bacterial, fungal and/or viral infections.

The influenza infection was fatal in 44/111 patients (39.1%) with a significantly higher mortality rate in the mechanically ventilated group (60.0% vs. 23.0%, $p < 0.001$). The highest mortality rate was seen in mechanically ventilated patients with the development of cardiac complications (75.0%).

Based on our analysis, we differentiate between two different pattern of severe influenza infection: The cardiac versus the pulmonary phenotype of severe influenza infection (see table 24).

The cardiac type of severe influenza is characterized by the group of patients with development of cardiac complications. Our study demonstrated that these were older (71 vs. 63 years), more often male (72.7% vs. 59.6%), significantly more likely to have already suffered from heart failure (59.1% vs. 19.1%, $p < 0.001$) or valvular heart disease (36.4% vs. 14.6%, $p = 0.031$) and had a higher mortality (45.5% vs. 38.2%).
In addition, patients with development of cardiac complications were significantly less likely to have hypoxia ($p = 0{,}026$) and had a significantly higher bilirubin level (0.9 mg/dL compared to 0.5 mg/dL in patients without development of cardiac complications, $p < 0.001$). Bilirubin could be elevated due to the more common heart failure in this patient group. Both central venous congestion and lower cardiac output with resulting reduced perfusion of the liver can lead to elevated bilirubin levels (78). No other significant laboratory differences were found, probably due to the unequal composition of the patient groups: we compared a group of 22 patients (with development of cardiac complications) with a group of 89 patients (without development of cardiac complications). However, patients with development of cardiac complications showed some laboratory trends (e.g. more often leukocytosis, higher creatinine-level). Of special interest is the marked but non-significant elevated NT-pro-BNP (4119.5 pg/mL vs. 2648 pg/mL), which is related to the acute cardiac complications, the more common pre-existing cardiac diseases and in part probably also to the slightly more reduced renal function in this group of patients.
Another interesting point was that although considerably fewer secondary infections occurred in the patients with development of cardiac complications, leukocytosis was still observed more frequently, as well as higher PCT.

Differentiation into influenza A and B showed that patients with development of cardiac complications were more frequently infected with influenza B compared to patients without development of cardiac complications. This observation may be explained by the fact that influenza B virus has the ability to directly infect cardiomyocytes and thus may lead to cardiac complications more frequently (79). Furthermore, patients with development of cardiac complications presented with a slightly better oxygenation rate (Horowitz index) and considerably less frequent hypercapnia.

In contrast, patients without development of cardiac complications (the pulmonary phenotype of severe influenza) showed more often wheezing, more often they had to be mechanically ventilated, which may be explained by the fact that lung infiltrates, and thus pneumonia, could be identified much more frequently. In addition, a slightly worse Horowitz Index was found. Laboratory parameters showed a higher CRP, a higher myoglobin and a higher D-dimer in these patients. With regard to secondary infections, bacterial, fungal and viral infections occurred more frequently. Especially bacterial and fungal nosocomial pneumonias were observed considerably more frequently. The difference in secondary bacterial nosocomial pneumonia was significant (p value = 0.014). Among the preexisting conditions, asthma, PAOD, CAOD, hypertension, diabetes mellitus, gastrointestinal, and malignancies occurred more frequently in patients without development of cardiac complications.

Table 24 ***Differences between cardiac and pulmonary type of severe influenza***

Patient Group with development of cardiac complications N = 22 (19.8%) • acute CMP (40.9%) • acute arrhythmogenic complications (40.9%) • acute ischemic complications developed (9.1%)	**Patient Group without development of cardiac complications N = 89 (80.2%)**
Less often influenza A (72.7%)	**More often influenza A (84.3%)**
More often influenza B (27.3%)	Less often influenza B (15.7%)
Older age (71 years)	Less older (63 years)
More often men (72.7%)	Less often men (59.6%)
More often pre-existing heart failure (59.1%, p value < 0.001)	Less pre-existing heart failure (19.1%)
More often pre-existing valvular heart diseases (36.4%, p value = 0.031)	Less pre-existing valvular heart diseases (14.6%)
More often pre-existing arrhythmias (40.9%)	Less often pre-existing arrhythmias (25.8%)
Less often pulmonary infiltrates (68.2%)	**More often pulmonary infiltrates (86.5%)**

No wheezing (0.0%)	**Wheezing (15.7%)**
More Leukocytosis (45.5%)	Less often Leukocytosis (24.72%)
Lower median CRP (43.45 mg/L)	**Higher median CRP (114.5 mg/L)**
Higher PCT-level (1.38 ng/mL)	Lower PCT-level (0.93 ng/mL)
Less often hypercapnic (27.3%)	**More often hypercapnic (47.7%)**
Less often acidaemic (36,4%)	**More often acidaemic (51.7%)**
Less often hypoxic (18.2%)	**More often hypoxic (40.7%, p value = 0,026)**
Higher median bilirubin (0.9 mg/dL, p value < 0.001)	Lower median bilirubin (0.4 mg/dL)
Higher median NT-pro-BNP (4119.5 pg/mL)	Lower median NT-pro-BNP (2648 pg/mL)
No bacterial HAP/VAT (0.0%)	**More often bacterial HAP/VAT (22.5%, p value = 0.014)**
No fungal HAP/VAT (0.0%)	**More often fungal HAP/VAT (7.9%)**
Less often bacterial bloodstream infections (4.5%)	**More often bacterial bloodstream infections (22.5%)**
Less non-influenza nosocomial viral infections (4.5%)	**More often non-influenza nosocomial viral infections (14.6%)**
Less often MV (36.4%)	**More often MV (47.2%)**
Higher mortality if MV (75.0%)	Lower mortality if MV (57.1%)
Higher general mortality (45.5%)	Lower general mortality (38.2%)

A remarkable result of our data analysis was the high mortality of the patients. A total of 44/111 patients died, resulting in a mortality rate of 39.6%. In the literature, ICU mortality rates tend to be considerably lower. It is found to range from 16.7% to 41.2% with a majority of studies reporting a mortality rate below 32% and some even below 20% (80-84) with only two studies exceeding the mortality rate we observed (85, 86).

There could be several explanations for this high mortality: Since our hospital is a university hospital, it regularly happens that patients who are very severely ill are transferred from surrounding hospitals for intensified therapy (for example, ECMO therapy). In our observation period, a total of 17/111 patients (15.3%) were transferred from surrounding hospitals to the University Hospital Graz. Another explanation could be that in Austria the influenza vaccination rate is very low in the overall population, but also

among high-risk patients. In fact, the influenza vaccination coverage rate in Austria is one of the lowest in the EU. The Austrian Health Survey 2019 showed a vaccination rate of 8.8% for the 2018/19 influenza season (87), which is in line with the calculated vaccination coverage rate of the Austrian Vaccine Manufacturers Association (ÖVIH), which was able to calculate an annually fluctuating vaccination coverage rate between 5 and 12% for the observation period of our study (88). Regarding patients older than 65, who have a significantly higher risk of a severe course, the OECD reported a vaccination coverage rate of 18.3% for Austria for the year 2019. Only three other EU countries had a worse vaccination coverage rate than Austria (89). There are probably several reasons for the poor vaccination coverage rates in Austria. Kunze et al. complain that there is still confusion between influenza and influenza like illness in parts of the population. In addition, influenza vaccination should be free of charge as a matter of principle, and promotional campaigns for education, effectiveness, and tolerability should take place among the general public (90, 91). This is in line with the recommendations of the Influenza Task Force in its December 2020 statement, which also calls for increased inclusion of preventive and vaccination medicine in health professions education curricula (92). In addition, in a new study, Fröbert et al. showed that influenza vaccination within 72 hours of a heart attack can reduce cardiovascular mortality by 41% within a year. This effect is even stronger than current post-myocardial infarction therapy. In this context, it is discussed that influenza vaccination not only prevents or attenuates influenza infections, but also has an anti-inflammatory and plaque-stabilizing effect due to the immunomodulatory effect (93, 94).

However, a fundamental problem also remains the inadequate data collection of vaccination coverage rates in Austria. In retrospect, it was not possible for us to determine whether the patients in our study had been vaccinated or not. In the future, the vaccination status of patients should be collected in a structured and consistent manner. As we have seen with the COVID-19 pandemic, it is useful to know exactly which patient groups have been vaccinated in order to be able to target vaccine education and vaccination offers as well as to assess the risk potential of patients at risk. In addition, further studies will be needed to verify the effectiveness of measures and make adjustments where necessary. In summary, we can only emphasize that vaccination is the best way to prevent severe disease progression and thus reduce the burden on the health care system. This is especially true for older patients, pregnant women and patients who are at significantly higher risk due to

cardiac, pulmonary or other pre-existing conditions. In general, it should be noted that influenza vaccination is a recommended measure for all individuals. In conclusion, it has been shown that in Austria both a fundamentally higher vaccination rate should be aimed at and better documentation of the vaccination status in intensive care units should be carried out in order to be able to better evaluate their impact in the future.

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