The Protective Effect of Serum Antibodies in Preventing SARS-CoV-2 Virus Entry Into Cardiac Muscle

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Summary

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been associated with significant cardiovascular complications, including myocardial infection and pulmonary embolism. This study aims to elucidate the relationship between the presence of SARS-CoV-2 RNA in the myocardium of the left ventricle and the levels of IgG and IgM antibodies against the SARS-CoV-2 virus in deceased COVID-19 patients. We conducted a post-mortem examination on 91 individuals who succumbed to COVID-19-related complications. The presence of SARS-CoV-2 RNA in the myocardium of the left ventricle was analyzed reverse transcription real time PCR (RT-qPCR) (EliGene® COVID19 UKV/SAV RT kit, Elisabeth Pharmacon), and antibody levels in serum were analyzed by serological assays (VIDAS SARS-COV-2 IgM and VIDAS SARS-COV-2 IgG II tests, BioMérieux). Of the heart tissue samples, 44 % tested positive for SARS-CoV-2 RNA. Our findings indicate that any detectable level of IgG antibodies against SARS-CoV-2 reduces the risk of viral penetration into the myocardium by more than fourfold. Specifically, individuals with detectable levels of IgG and IgM antibodies exhibited a significantly reduced presence of SARS-CoV-2 RNA in cardiac tissues ($p<0.0001$ for IgG and $p<0.001$ for IgM). Notably, all patients who died from pulmonary embolism had elevated levels of IgG antibodies. The study underscores the protective role of IgG and IgM antibodies in preventing SARS-CoV-2 penetration into cardiac tissues. However, high antibody titers were associated with fatal outcomes such as pulmonary embolism, pointing to the intricate balance of immune response in COVID-19 pathology.

Key words

SARS-CoV-2 • Antibody • IgG • IgM • Cardiac damage • qPCR • Pneumonia • Pulmonary embolism • Heart failure

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Introduction

SARS-CoV-2, a single-stranded RNA virus from the *Coronaviridae* family, triggered a global pandemic that resulted in millions of deaths. This crisis presented unprecedented public health challenges and caused substantial economic impacts, profoundly affecting healthcare systems and global economies [1]. The reported cases of SARS-CoV-2 infection likely represent only a fraction of the actual total, as many cases of COVID-19 went unreported. This underreporting is attributed to a substantial number of infected individuals who were either asymptomatic or presymptomatic, or who experienced only mild and nonspecific symptoms [2]. This

suggests that the virus poses a health risk to a much broader population than official statistics indicate, encompassing not only the immediate health risks of acute infection but also long-term health implications resulting from the disease.

SARS-CoV-2 primarily affects the respiratory tract, especially the lungs, but can also have a severe impact on other tissues such as the heart, brain, liver, kidneys, and testicles among others [3,4]. The ability to invade various tissues is facilitated by the presence of cells expressing ACE2 protein, which serves as a primary gateway for SARS-CoV-2 [5]. Cells containing not only ACE2 but also TMPRSS2, another protein involved in virus entry, are at higher risk [4,6]. The virus enters the host cell *via* surface ACE2 receptors using the spike (S) protein as the binding component. This fusion results in TMPRSS2-mediated cleavage of the S2 spike protein unit, leading to conformational rearrangement and ensuring full compatibility between receptor and virus [7]. After entry, the ACE2 receptor is functionally removed from the external membrane site, leading to a reduction in ACE2 function and further imbalance of the renin-angiotensin-aldosterone system (RAAS) [4,8], which can manifest as chronic inflammation and degenerative processes [9].

The infection and replication of SARS-CoV-2 in the human body can lead to different symptoms and vary from asymptomatic to severe disease, followed by death [10]. Risk factors for severe COVID-19 generally include chronic comorbidities such as cardiovascular disease, diabetes, hypertension, obesity, renal failure, cancer, or a history of smoking [4,11,12]. Studies have shown that some of these factors are related to the expression of ACE2, which has been found to be increased in the tissues of patients suffering from diabetes, obesity, or heart failure. These findings are consistent with the understanding of how SARS-CoV-2 virus entry into host cells contributes to worse outcomes [13]. Additionally, the higher risk of severe COVID-19 in obese patients may also be related to the expression of ACE2 in adipocytes, which express high levels of ACE2 themselves [14].

In addition to the respiratory tract, where the highest level of the virus is usually detected, SARS-CoV-2 also often replicates in non-respiratory tissues such as the heart, where it can persist for several months [15]. The incidence of cardiac invasion, and therefore cardiac complications, is approximately 10 % in patients with COVID-19, although this specific percentage varies likely due to individual comorbidities and the severity of illness. For instance, Pillarisetti *et al.* reported a 4.6 % incidence of heart failure, whereas Zhou *et al.* reported a 23 % incidence [16,17], with such differences potentially caused by genetic variants of the SARS-CoV-2 virus [18]. Heart damage associated with SARS-CoV-2 infection may occur both during the acute phase and as a post-acute sequela of the disease [4,19-21]. The mechanisms of post-acute damage have not yet been fully elucidated, but potential mechanisms such as damage caused by persistent viral reservoirs followed by the induction of a chronic inflammatory response have been discussed [19,20]. The presence of the SARS-CoV-2 RNA in heart tissue has been observed in individuals with both symptomatic and asymptomatic outcomes [7,22,23]. There is a significant association between the incidence of cardiac complications and higher mortality in individuals suffering from SARS-CoV-2 infection, with the risk of death described as nearly seven times higher than in individuals without COVID-19 [16].

At the tissue level, characteristic heart damage often manifests as myocarditis, pericarditis, myocardial damage, or arrhythmias [8], with the most affected cells in the heart typically being cardiomyocytes and pericytes. Damage to cardiomyocytes is very diverse and includes both disturbances in cell morphology and abnormal electrical function. The structural and functional changes are closely related and lead to reduced contraction, which impairs the heart's ability to pump blood effectively as the generation and propagation of electrical signals are altered [4,7,24,25]. Structural dysfunction is characterrized primarily by disintegration of sarcomeres and myofibrils [7,26]. Due to the fusogenic property of the viral S protein, SARS-CoV-2 can also induce cell-to-cell fusion, resulting in altered syncytia formation [4,26]. In COVID-19 tissue samples, widened intercalated discs have also been detected. Consequent intercellular space widening and reduced action potential velocity can contribute to susceptibility to arrhythmia [19,27]. This is closely related to the DSG2 protein and its function, which affects cell-to-cell contact, necessary for cardiomyocyte adhesion [19]. The increase in DSG2 levels and the production of anti-DSG2 antibodies appear to be specific to individuals suffering from severe COVID-19 and could therefore potentially be used as a marker of cardiac damage in the post-acute state and in long-term COVID-19 [19].

The diagnosis of COVID-19 is primarily based on the detection of viral RNA in various clinical samples using reverse transcription real time PCR (RT-qPCR)

[28]. For basic diagnostics, samples are taken from nasopharyngeal or oral swabs. This methodology allows for the high-sensitivity detection of viral RNA in tissue and quantification of its amount. In living individuals, the detection of the virus in various clinical materials is limited to samples from mucosal swabs, urine, feces, semen, and blood [2], which restricts the ability to monitor the presence of the virus in other important clinical materials such as samples from other organs. Autopsy material obtained from individuals who died from COVID-19 enables the monitoring of the virus's presence in various tissues [29]. RT-qPCR analysis is one of the methods that can identify the presence of the virus in tissue. Adhering to the rules for protecting samples from cross-contamination within an autopsied body, RT-qPCR from various tissues can provide important insights into the dynamics of infection across different tissues within the same body.

In addition to detecting viral RNA using RT-qPCR in clinical samples, the presence of antibodies in the serum of patients can also be monitored. As a protective immune response to infection, individuals suffering from COVID-19 produce immunoglobulins specific to the S (spike) or N (nucleocapsid) subunit of the virus. Production typically begins around day 5 after the onset of the disease, with IgM antibody levels peaking around day 35-45 and then declining rapidly, while IgG antibody levels peak around day 45 and persist for several months [30,31]. Due to the presence of elevated IgG and IgM antibodies in both symptomatic and asymptomatic individuals, monitoring this phenomenon can help to describe the course and burden of the disease in the community [32]. More importantly, the detection of IgG and IgM antibodies can be useful for the diagnosis and prognosis of infected individuals [31]. Considering their key role in neutralizing the virus, antibodies could protect cells from virus-related damage. The level of protection depends on the type and amount of antibodies, where anti-S-IgG were found to be more effective than others [33]. According to these findings, it seems that a high level of IgG antibodies could provide protection against COVID-19. A high level of antibodies associated with a worse course of the disease has also been described [34]. Thus, a high level of IgG antibodies can play a dual role in the pathogenesis of COVID-19, and the course of the disease will depend on other factors.

Therefore, the aim of this paper is to investigate the associations between antibodies against SARS-CoV-2 and the presence of the virus in the heart tissue of individuals for whom COVID-19 was fatal.

Material and Methods

Sample collection

The subjects were individuals who unexpectedly died from unknown causes. Forensic autopsies were conducted at the Department of Forensic Medicine at the Medical Faculty and St. Anne's Faculty Hospital in Brno to establish the causes of death. These included tests for the presence of SARS-CoV-2 RNA. Between 2020 and 2022, 98 individuals (91 included for further analysis) who succumbed to COVID-19 complications underwent these autopsies. These individuals had previously tested positive for COVID-19 *via* RT-qPCR tests. The causes of death in tested group of persons were viral pneumonia confirmed by light-microscopic examination, pulmonary embolism, heart failure and others. Demographic and clinical characteristics of the subjects are presented in Table 1. Special measures were taken to prevent cross-contamination during the sample collection process.

Table 1. Demographic and clinical characteristics of *post-mortem* COVID-19 samples.

	Males (n=76)	Females (n=15)	Total (n=91)
Age (years)	65.6 ± 11.8	68.8 ± 17	66.1 ± 12.8
RNA positive heart tissue	30 (39.5 %)	10 (66.7 %)	40 (44 %)
IgG positive	42 (55.3 %)	4 (26.7 %)	46 (50.5 %)
IgM positive	43 (56.6 %)	5 (33.3 %)	48 (52.7 %)
Pneumonia	46 (60.5 %)	8 (53.3 %)	54 (59.3 %)
Pulmonary embolism	11 (14.5 %)	2 (13.3 %)	13 (14.3 %)
Heart failure	5 (6.6 %)	3 (20 %)	8 (8.8 %)
Others	14 (18.4 %)	2 (13.3 %)	16 (17.6 %)

Note: Table presents the individuals categorized by gender. The data include age, RNA positivity, immunoglobulin G (IgG) and M (IgM) positivity, and the incidence of pneumonia, pulmonary embolism, heart failure, and other causes of death.

RNA analysis

The left ventricular myocardium was exposed through a precise incision. The myocardium surface was then swabbed using flocking swabs (Copan Italia, Italy). This technique allowed for the collection of cellular material and potential viral particles from the tissue.

The swabs were immediately placed into 2 ml screw-cap tubes containing 600 µl of Lysis Buffer (EliGene® Viral DNA/RNA FAST Isolation Kit, Elisabeth Pharmacon, Czech Republic) to prepare swab suspensions. The lysis buffer lysed any viral particles, thereby preserving the viral RNA at room temperature. This step was crucial to maintain RNA integrity during transport to the laboratory for analysis. Samples were processed and analyzed the following day. RNA isolation was carried out using the EliGene® Viral DNA/RNA FAST Isolation Kit (Elisabeth Pharmacon) as per the manufacturer's recommendations.

An aliquot of 400 µl of swab suspension was mixed with 200 µl of Lysis Buffer with added Solution M and 5 µl of IAC (internal amplification control) RNA. DNA was eluted using 50 µl of Elution Buffer. The qPCR analysis was performed by *in vitro* diagnostic CE certified EliGene® COVID19 UKV/SAV RT kit (Elisabeth Pharmacon, Czech Republic), detecting British and South African variants. From November 2021, samples were additionally tested using the EliGene® COVID19 Omicron RT kit (Elisabeth Pharmacon), which allows the simultaneous detection of the COVID-19 Omicron variant, Respiratory Syncytial Virus (RSV), and Influenza A and B. The qPCR analyses were performed on a CFX Touch qPCR instrument (Bio-Rad, USA) following the manufacturer's protocol. The quantity of viral particles was determined using a 3-point standard curve derived from a Positive Control included in the qPCR detection kit. The quantity of SARS-CoV-2 was expressed as the number of detection targets in a qPCR reaction. Each isolated RNA was analyzed in triplicate.

Serological testing

Serum was prepared by centrifuging blood collected from the veins of the left lower extremity at 2,000× g for 10 min. A 100 µl sample of serum was used to estimate levels of IgM and IgG antibodies using the Enzyme Linked Fluorescent Assay (ELFA). The VIDAS SARS-COV-2 IgM and VIDAS SARS-COV-2 IgG II tests were performed on the MINI VIDAS® instrument (BioMérieux, France) as per the instruction manuals. The instrument measures Relative Fluorescence Values (RFV), and a test value greater than 1 indicates a positive result. According to the manufacturer, the analytical sensitivity was evaluated using a dilution series of the first WHO International Standard for antibodies against the SARS-CoV-2 virus (NIBSC, code 20/136). The cut-off value for the VIDAS® SARS-COV-2 IgG II test (index=1.00) was estimated to be equivalent to a value of 20.33 BAU/ml (binding antibody units per milliliter) of the first WHO International Standard for antibodies against the SARS-CoV-2 virus (NIBSC, code 20/136).

Statistical analysis

Based on the DNA analysis results, the subjects were categorized into two groups: 1) those with SARS-CoV-2 RNA detected in the heart, and 2) those without SARS-CoV-2 RNA detected in the heart. Differences in IgG and IgM antibody levels between these groups were analyzed using the Mann-Whitney U test. The Fisher exact test assessed variations in the incidence of SARS-CoV-2 RNA in the heart depending on the presence of IgG and IgM antibodies. All statistical analyses were conducted using R software [35].

Results

A total of 98 deceased individuals who succumbed to COVID-19 complications were tested for the presence of viral RNA and antibodies against SARS-CoV-2 using RT-qPCR and serological assays, respectively. In seven cases, the serological assays failed, likely due to a high degree of blood cell degradation from ongoing post-mortem changes. These cases were excluded from the statistical analysis, leaving 91 patients (76 males and 15 females) in the study. Of these individuals, 54 died from pneumonia, 13 from pulmonary embolism, and 24 from other causes (e.g. heart failure, bleeding to gastrointestinal tract etc.).

Of the 91 heart tissue samples, 40 tested positive for SARS-CoV-2 viral RNA, while 51 were virus-negative. Individuals with SARS-CoV-2 RNA-negative heart tissue mostly had detectable levels of both IgG and IgM antibodies, as indicated in Table 2. This was in stark contrast to those with SARS-CoV-2 RNA-positive heart tissue, most of whom lacked detectable IgG and IgM antibodies. The data suggest that individuals with IgG and IgM antibodies were significantly less likely to have the virus penetrate the heart tissue ($p<0.0001$ for

IgG and p<0.001 for IgM), as detailed in Tables 3 and 4. In addition, in individuals with detectable levels of IgG and IgM antibodies, those with SARS-CoV-2 RNA-positive heart tissue exhibited significantly lower levels of antibodies, correlating with a higher risk of viral penetration into the heart tissue, as shown in Figure 1 (p<0.05 for IgG and p<0.01 for IgM).

In individuals who died from pneumonia, the risk of virus presence in the heart tissue was similar to that observed in the overall dataset, approximately four times lower in individuals with detectable IgG antibodies (Table 5) and 59 % lower in individuals with detectable IgM antibodies (Table 6) than in those without antibodies. Notably, results from those who died from embolism revealed that all had IgG antibodies (Table 7), and almost all also had IgM antibodies (Table 8). Only one individual in this group had the virus in their heart tissue, indicating a lower risk of viral transmission to the heart tissue in this subgroup compared to those who died from other causes.

Among the set of 91 heart samples, the Alpha variant (B.1.1.7), previously known as the UK variant, was detected in 38 cases, and the Omicron variant in 8 cases. No samples tested positive for the Beta variant (B.1.351), formerly known as the South African variant. In the remaining samples, other variants were present, or the viral load was too low to successfully determine the variant.

Table 2. The distribution of IgG and IgM antibodies in the samples.

	RNA neg. (n=51)	**RNA pos. (n=40)**
No detectable IgG and IgM antibodies	9	25
IgG antibodies only	7	2
IgM antibodies only	3	8
Both IgG and IgM antibodies detected	32	5

Note: number of individuals are given. RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Table 3. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgG antibodies.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**RR**	**Odd**	**OR (95 % CI)**	**p value**
IgG neg.	33	12	0.73		2.75		
IgG pos.	7	39	0.15	0.21	0.18	0.07 (0.02-0.2)	**<0.0001**

Note: p value was calculated using the Fisher exact test. CI – confidence interval, IgG neg. – individuals without detectable IgG antibodies, IgG pos. – individuals with detectable IgG antibodies, N – number of subjects, RR – risk ratio, OR – odds ratio, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Table 4. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgM antibodies.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**RR**	**Odd**	**OR (95 % CI)**	**p value**
IgM neg.	27	16	0.63		1.69		
IgM pos.	13	35	0.27	0.43	0.37	0.22 (0.09-0.53)	**0.0008**

Note: p value was calculated using the Fisher exact test. CI – confidence interval, IgM neg. – individuals without detectable IgM antibodies, IgM pos. – individuals with detectable IgM antibodies, N – number of subjects, RR – risk ratio, OR – odds ratio, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

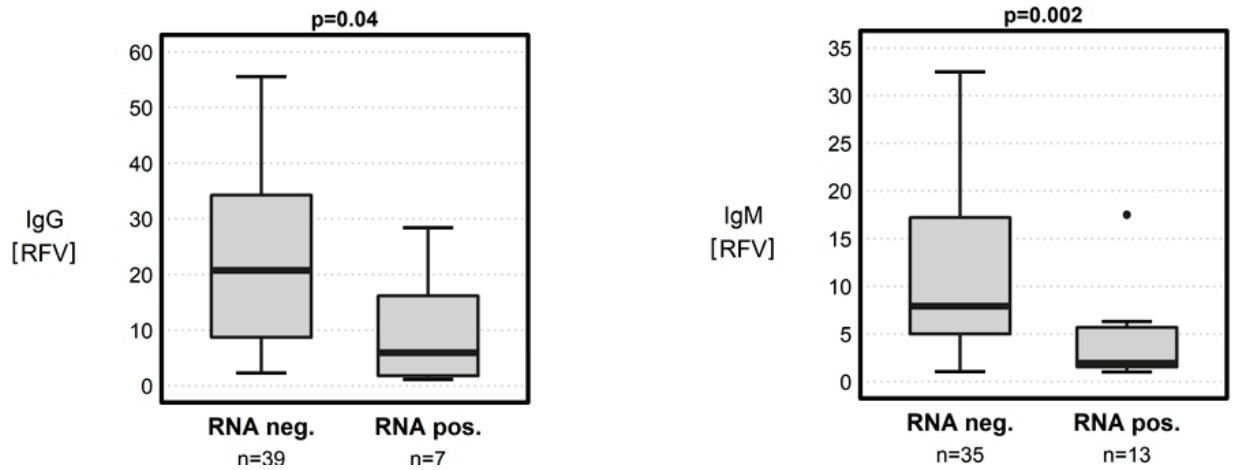

Fig. 1. Comparison of SARS-CoV-2 RNA presence in heart tissue and corresponding serum levels of IgG and IgM antibodies in deceased individuals. RFV are Relative Fluorescence Values which reflect the fluorescence intensity measured by the instrument.

Table 5. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgG antibodies in individuals who succumbed to pneumonia.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**RR**	**Odd**	**OR (95 % CI)**	**p value**
IgG neg.	24	8	0.75		3		
IgG pos.	4	18	0.18	0.24	0.22	0.07 (0.02-0.27)	**0.0001**

Note: p value was calculated using the Fisher exact test. CI – confidence interval, IgG neg. – individuals without detectable IgG antibodies, IgG pos. – individuals with detectable IgG antibodies, N – number of subjects, RR – risk ratio, OR – odds ratio, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Table 6. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgM antibodies who succumbed to pneumonia.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**RR**	**Odd**	**OR (95 % CI)**	**p value**
IgM neg.	19	6	0.76		3.17		
IgM pos.	9	20	0.31	0.41	0.45	0.14 (0.04-0.47)	**0.0012**

Note: p value was calculated using the Fisher exact test. CI – confidence interval, IgM neg. – individuals without detectable IgM antibodies, IgM pos. – individuals with detectable IgM antibodies, N – number of subjects, RR – risk ratio, OR – odds ratio, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Table 7. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgG antibodies in individuals who succumbed to pulmonary embolism.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**Odd**
IgG neg.	0	0	-	-
IgG pos.	1	12	0.08	0.08

Note: IgG neg. – individuals without detectable IgG antibodies, IgG pos. – individuals with detectable IgG antibodies, N – number of subjects, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Table 8. The occurrence of the SARS-CoV-2 RNA in heart tissue depending on the presence of IgM antibodies who succumbed to pulmonary embolism.

Group	**N** RNA pos.	**N** RNA neg.	**Risk**	**Odd**
IgM neg.	0	2	-	-
IgM pos.	1	10	0.09	0.1

Note. IgM neg. – individuals without detectable IgM antibodies, IgM pos. – individuals with detectable IgM antibodies, N – number of subjects, RNA neg. – individuals with SARS-CoV-2 RNA negative heart tissue, RNA pos. – individuals with SARS-CoV-2 RNA positive heart tissue.

Discussion

In our study, we analyzed 98 individuals who died from SARS-CoV-2 infection. We discovered *post-mortem* that the presence of anti-SARS-CoV-2 antibodies in the serum significantly reduces the risk of viral penetration into the left ventricular tissue. Specifically, the presence of IgG antibodies reduces this risk by more than fourfold. The protective effect of IgG antibodies against viral invasion into cardiac muscle increases with higher serum levels, as demonstrated in Figure 1 ($p<0.05$). A higher level of IgM antibodies was found to have an even greater protective effect ($p<0.01$). Additionally, all patients who died from pulmonary embolism had IgG antibodies present in their serum, and nearly all had IgM antibodies. Except for one individual, none of the patients who died from pulmonary embolism had detectable viral RNA in their heart tissue.

COVID-19 can cause fatal cardiac damage leading to death [4,36]. Tahan *et al.* [36] reported that the incidence of new heart failure during COVID-19 was higher in patients with IgG levels exceeding 100 AU/ml; these patients showed a significant increase in IgG levels regardless of their previous medical history or underlying cardiovascular diseases. Moreover, an increased level of IgG was observed in individuals with a history of ischemic heart disease and hypertension, suggesting that heightened IgG levels may indicate worsening cardiac conditions during COVID-19 [36]. It is important to note that our study is based on individuals deceased from COVID-19. Therefore, our findings should not be extrapolated to predict the prognosis of cardiovascular diseases. While higher antibody levels indicate that the virus did not penetrate the cardiac tissue, they might also suggest a more severe progression of COVID-19, possibly linked to a cytokine storm that could have impacted cardiac functions without direct viral invasion of the heart tissue. These insights are discussed in the review by Šerý et Dziedzinska [4].

The results of our study demonstrated a significant negative correlation between the levels of IgG and IgM antibodies in serum and the presence of SARS-CoV-2 in heart tissue, supporting the hypothesis of a protective function of these antibodies in preventing direct viral invasion of the heart. Numerous studies have indicated that higher levels of IgG and IgM antibodies against SARS-CoV-2 might play a protective role in COVID-19. Li *et al.* [37] observed that individuals with higher IgG antibody levels died less frequently compared to other hospitalized COVID-19 patients [37]. Radziejewska *et al.* [38] linked the presence of IgG and IgM antibodies to a better prognosis in COVID-19 patients [38]. The potential protective nature of IgG and IgM antibodies was also observed in animal models, where IgM antibodies showed higher neutralization potency, while IgG antibodies were more effective in preventing the disease [33].

One of the common complications connected to SARS-CoV-2 infection is pulmonary embolism with incidence ranging from 3 to 26 % [39]. In our cohort, 14 % of the patients died from pulmonary embolism. Pulmonary embolism can occur as a result of a very strong response by the body's defense system following SARS-CoV-2 infection, which causes inflammation and damage to the inside of blood vessels, activating blood platelets to stick together and form clots. Due to reduced oxygen saturation, thrombi cause damage to vital organs such as the heart and brain. This blockage further causes high blood pressure in the lungs, making it harder for the heart to pump blood and leading to heart failure [40]. Some studies demonstrated higher levels of IgG antibodies and severe inflammation in severe COVID-19 cases, suggesting there is a relation between them. Nevertheless, despite high levels of IgG and increased defense reaction of the host to the infection, the disease remains very serious and can lead to death [41]. These

findings are consistent with the results of our study and demonstrate the crucial role of inflammation in pulmonary embolism, where almost all tested individuals had SARS-CoV-2 RNA negative heart tissue and had high levels of IgG antibodies at the same time (Fig. 2). These results assume, that the primary cause of death in this set of deceased may be exaggerated immune response.

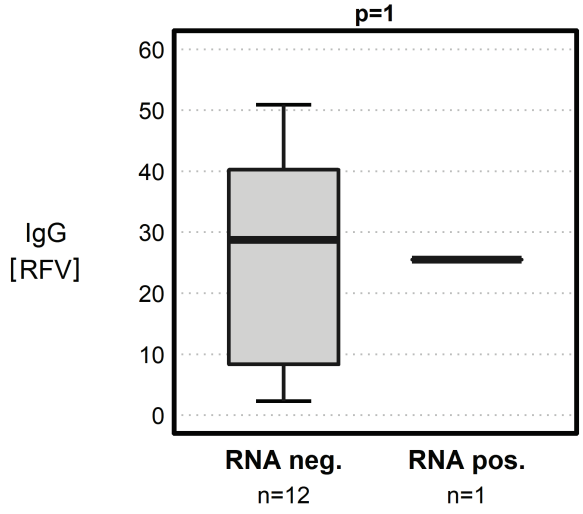

Fig. 2. Comparison of SARS-CoV-2 RNA presence in heart tissue and corresponding serum levels of IgG antibodies in deceased individuals died on pulmonary embolism. RFV are Relative Fluorescence Values which reflect the fluorescence intensity measured by the instrument.

A common cause of death from SARS-CoV-2 infection is COVID-19-related pneumonia, characterized by the viral invasion of the lungs, which can cause fatal damage manifesting as a disrupted ability to transfer oxygen into the blood and subsequently to other tissues and organs. SARS-CoV-2 infects alveoli, causing swelling in the lungs. Additionally, the spaces around the alveoli are affected, showing that COVID-19 lung infection often combines aspects of both alveolar and interstitial pneumonia[42]. Unlike typical pneumonia, COVID-19-related pneumonia starts in localized pockets before spreading, making it more resilient and more likely to progress to acute respiratory distress syndrome [42]. Studies have also shown that there is a link between the severity of COVID-19 pneumonia and inflammation resulting from the lungs' reaction to the virus, with higher inflammation levels potentially leading to a more severe disease [43,44]. Our results also demonstrate potential associations between the incidence of COVID-19-related pneumonia and the role of the immune system in death due to SARS-CoV-2 infection, showing a significant difference between the levels of antibodies against SARS-CoV-2 in individuals with viral RNA-positive and RNA-negative heart tissue.

The mechanisms of heart failure in COVID-19 involve various pathways, including virus-induced infiltration of inflammatory cells, pro-inflammatory cytokines causing myocardial necrosis, endothelial injury with micro-thrombosis damaging the endocardium, and respiratory distress-induced severe hypoxia [26]. COVID-19 also exacerbates heart failure by inducing myocardial injury through direct viral damage, cytokine storms, hypercoagulation, inflammation, and endothelial dysfunction [4,45]. The variability in the causes ultimately leading to heart failure could explain the large variability in the results of our set of tested individuals.

Conclusions

This study aimed to find associations between the presence of SARS-CoV-2 RNA in the left ventricle heart tissue of patients who died from COVID-19 and the IgG and IgM antibody levels in the serum of these individuals. From the sample set consisting of 91 individuals, 40 samples of heart tissue tested positive for SARS-CoV-2, while 51 were negative. Our study found that higher levels of both IgG and IgM antibodies against SARS-CoV-2 significantly protect the myocardium of the left ventricle from viral penetration. On the other hand, the scientific literature has described that higher levels of antibodies against the coronavirus may indicate a worse progression of the disease with cardiac complications. This highlights the dual role of antibodies against the coronavirus, which not only protect important organs such as the heart from attack by the coronavirus and damage, but also higher levels of antibodies may indicate a worse disease course, such as the occurrence of a cytokine storm. As our study showed, individuals who died from pulmonary embolism all had higher levels of IgG antibodies.

Although our results are statistically significant, they were derived from research on only 91 individuals, conducted *post-mortem*. Even though an increased level of antibodies against the coronavirus protected them from the penetration of the virus into the cardiac tissue, it did not protect them from death. Our results would benefit from validation in further studies that should analyze samples collected during the SARS-CoV-2 coronavirus pandemic.

Conflict of Interest

There is no conflict of interest.

Acknowledgements

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