COMORBIDITY AND CAUSE OF DEATH IN THE DIFFERENT VARIANTS OF SARS-COV-2 VIRUS, WITH CONTRIBUTION OF 20 AUTOPSY CASES

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ABSTRACT

Analyzing the clinical and epidemiological data of COVID-19 suggests that specific comorbidities increase the risk of infection leading to worse lung injury and an even higher risk of death. The most common comorbidities reported up till now are hypertension, cardiovascular diseases, and diabetes.

Aim to study: The design of the study includes comorbidity of patients and cause of death in alfa, delta, and omicron variants of SARS-CoV-2 virus, histological changes in the lungs, thrombotic complications of coronavirus infection and laboratory tests concerning thrombotic changes.

Materials and Methods: We systematically evaluated 20 autopsies of patients deceased by COVID-19 infection. Collecting data was from February 28th, 2020, until June 2022. The cases were diagnosed with a PCR (Polymerase Chain Reaction) test and a rapid antigen test. 10 of the deceased patients were from the first, second, third, and fourth wave (I group) infected predominantly with the alfa and delta variants of coronavirus (from March 2020 until October 2021), and 10 patients infected after that date with predominantly the omicron variant (II group).

Results:

Most patients were over 50 years of age with multiple co-morbidities (28-88, average 63.9). Post-mortem case studies have shown Arterial hypertension in 80% (I/II gr), 60%/90% of patients with chronic ischemic heart disease, chronic and acute ischemic brain disease in 30%/10%, atherosclerosis, 60%/90%, diabetes mellitus 30%/40%, obesity stage III, 100%/95%)**.** Clinical laboratory studies, in connection with thrombotic complications, revealed the increased value of creatine kinase, fibrinogen, D-dimers, and CRP. Lymphopenia was observed in 60%.

All of the cases with COVID-19 viral desquamative pneumonia, at different stages, developed vascular thrombosis in medium-sized pulmonary vessels. Two patients developed pulmonary thromboembolism. We established 5 patients with generalized thrombosis. Three patients were complicated by infarcts in the brain, kidney, and spleen.

Conclusion: The autopsies revealed a consistent pattern of pulmonary alveolar damage and generalized vascular/thrombotic disease in patients with frequent co-morbidities. The high frequency of generalized thrombotic complications was observed in predominant alfa and delta variants of the infection, while in the group with the omicron prevailing variant, the lung lesions were dominant, without extrapulmonary thrombotic complications, which we explain by the effective antithrombotic therapy. Major complications in these patients were secondary bacterial infection, sepsis, and respiratory distress syndrome.

Keywords: COVID-19, SARS-CoV-2, Infarction, thrombosis, co-morbidities

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INTRODUCTION

In December 2019 a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was identified in Wuhan, China from a cluster of severe pneumonia cases (1). In February 2020 the pandemic reached European countries including Bulgaria and the first deaths due to coronavirus were announced at the

beginning of March. Not more than 5 autopsies were performed in Bulgaria. For safety reasons, autopsies were forbidden since there was not enough data on the infectivity of the virus following the death of a patient. Strict quarantine measures were introduced which reduced the morbidity to 30-40 cases a day, while Italy and Spain confirmed unprecedented numbers of morbidity and mortality. The situation changed in September, a month after opening schools and kindergartens. Morbidity rose to 4000 cases a day and mortality - to 150 cases and Bulgaria took the first place in morbidity per 100 000 people. To date, in Bulgaria, the infected are 1 281 155, deceased 37 916 (3.9%). For this period in *Plovdiv* 113 656 patients fell ill, and 2996 (5.7%) patients passed away.

Indeed, while COVID-19 has been shown to impact a variety of organ systems (2,3,4), respiratory system pathology predominates with mortality linked primarily to acute respiratory distress syndrome (ARDS) (5). Patients experience marked abnormalities in the coagulation system, particularly elevated D-dimer levels, which predispose them to thrombotic disease (6).

Initial autopsies from COVID-19 patients showed microthrombi in the lung vasculature (10). The report of heparin having a mortality benefit in a subgroup of patients drew attention to the thrombosis prevalent in COVID-19 (26). Most of these studies demonstrate venous thromboembolism and microthrombi in arterioles and venules. There are numerous reports of patients with COVID-19 presenting with both arterial (stroke, myocardial infarction) and venous thrombosis (deep vein thrombosis, pulmonary thromboembolism, venous sinus thrombosis). Many of these patients had traditional risk factors for thrombosis. Perhaps the most important risk factors in the context of COVID-19 are obesity and poorly controlled diabetes mellitus which may aggravate physiological processes such as pregnancy and result in venous and arterial thromboses (27).

Aim: Since the first autopsies showed generalized thrombotic changes in all organs, we aimed to study histological and immunohistochemical changes in the lungs and organs based on autopsy cases, thrombotic complications of coronavirus infection, suggesting that it is not only a respiratory but also a generalized disease. The design of the study also includes laboratory tests concerning thrombotic changes, comorbidity of patients, and cause of death in alfa, delta, and omicron variants of SARS-CoV-2.

MATERIALS AND METHODS

Autopsy procedures:

This is a prospective study of 20 consecutive COVID-19 autopsies performed at "St. George" University Hospital, Plovdiv, Bulgaria. Collecting data was from February 28th, 2020 until June 2022. For statistical purposes, 10 of the deceased patients were from the first, second, third, and fourth wave (group I) infected predominantly with the alfa and delta variants of coronavirus (from March 2020 until October 2021), and 10 patients infected after that date with predominantly the omicron variant (group II).

Autopsies: For security reasons, 4 autopsies were in-situ dissection in patients 3, 4, 5, and 7, the skull was not open, and the other 16 autopsies were complete. Samples were taken from both lungs, from the central and peripheral parts of the lobes. From each lung, 4 blocks were released for routine HE testing. To detect the size of the vessels affected by thrombosis, small arteries were defined as <1.0 mm; the term "medium-sized pulmonary vessels" was used for those larger than 1.0 mm, often grossly visible, but not include pulmonary emboli of the main pulmonary artery. In the complete autopsies, the organs were examined macroscopically and microscopically, carefully looking for signs of thrombosis and infarcts.

Genetic testing. Eighteen of the cases were diagnosed with a PCR (Polymerase Chain Reaction) test (AccuPower® SARS-CoV-2 Real-Time RT-PCR Kit (Bioneer, Korea)). Two cases were confirmed with a rapid antigen test. All patients were unvaccinated with any dose.

Histological examination

The autopsy material was fixed in 10% neutral buffered formalin and submitted for standard processing with hematoxylin and eosin staining. Additionally, the lungs were stained according to Van Giezon for initial collagen formation and fibrous changes.

Immunohistochemistry

Immunohistochemical staining was performed on formalin-fixed, paraffin-embedded 5-µm sections

following citrate pH 6.0 antigen retrieval, endogenous biotin, and peroxidase block. Dako's immunosteiner is used.

Immunohistochemically, both lungs were examined with CK 7, and CKAE1/AE3 to detect changes in alveolocytes. Antibody binding to the cells in sections was detected using the horseradish peroxidase (HRP) reaction kits (DAKO, Carpinteria, CA, USA) according to the manufacturer's protocol instructions. Images were visualized and captured with a digital camera mounted on a Nikon Eclipse 80i microscope using NIS-Elements Advanced Research Software version 4.13 (Nikon Instruments; Tokyo, Japan).

Ethical statement

The study was exempt from institutional review board approval as it did not meet the criteria for living human research.

RESULTS

Clinical Data

Out of 20 presented patients, 9 were women, and 11 were men. Severe acute respiratory syndrome developed in 15 patients as a complication of COVID-19 pneumonia. Most patients developed the complication after 14 days of illness, 13 patients were on mechanical ventilation, 3 died in the emergency department, and the complication developed within hours. The earliest development of the syndrome was on days 7-10 of the onset of the disease. Two patients developed pulmonary thromboembolism. Six out of ten patients (I gr. 60%) and 10/10 (II gr) developed SARS-CoV-2 accompanied by respiratory syndrome and lethal outcome.

Most patients were over 50 years of age with multiple comorbidities (28-88, average 63.9). Only one 61-year-old patient did not report any concomitant diseases. Two patients were under 40 years of age (37, 28), but with arterial hypertension (AH): left ventricular hypertrophy of the heart (LVH) and obesity stage III. Most patients presented with high blood pressure and LVH - 16/20 (80%), chronic ischemic heart disease (CIHD) - 15/20 (75%), chronic and acute ischemic brain disease (CIBD) - 20%, atherosclerosis - 15/20 (75%), diabetes mellitus - 5/20 (25%), obesity stage III - 19/20 (95%) **(Figure 1**).

The comparative analysis between the two groups revealed a constant frequency of patients with obesity, arterial hypertension, and diabetes. The patients from the last wave (group II) had more pronounced signs of atherosclerosis complicated by myocardial infarctions, liver diseases, and one patient with oncological disease. The number of patients with

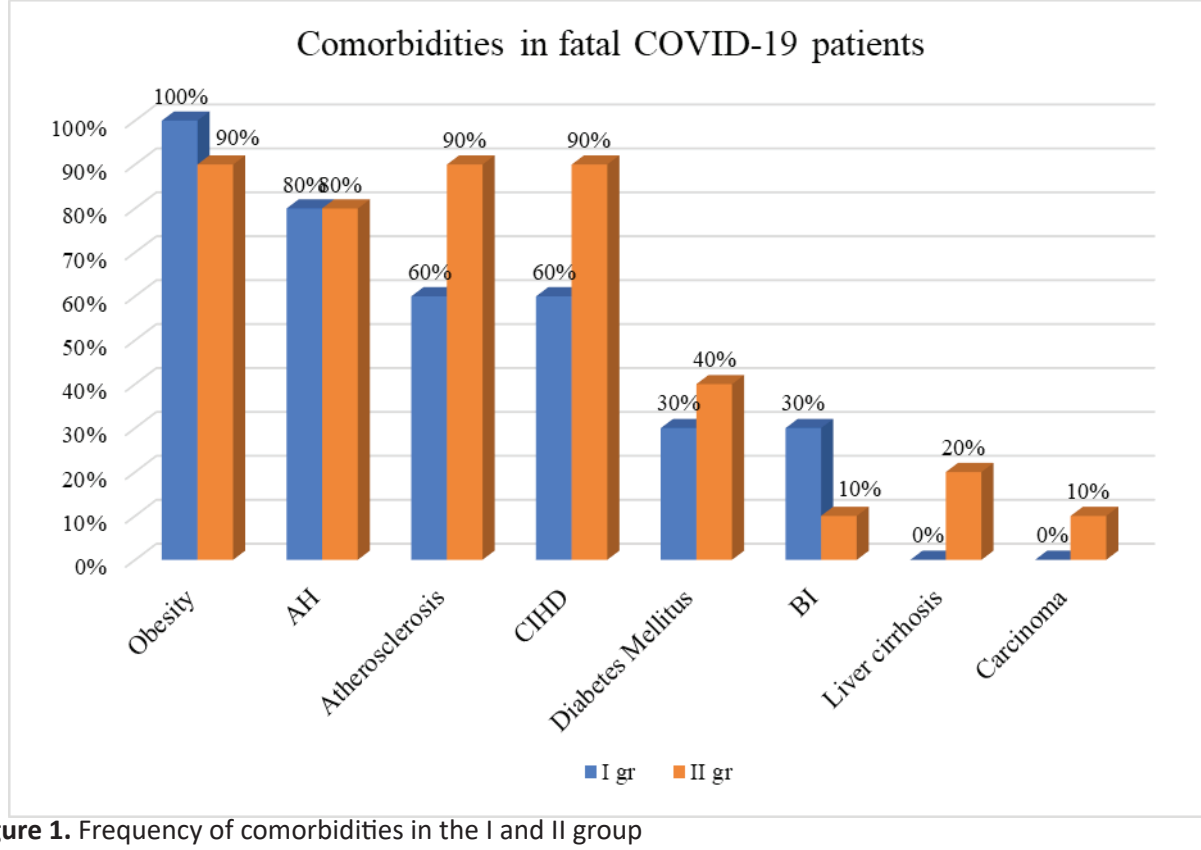

Figure 1. Frequency of comorbidities in the I and II group

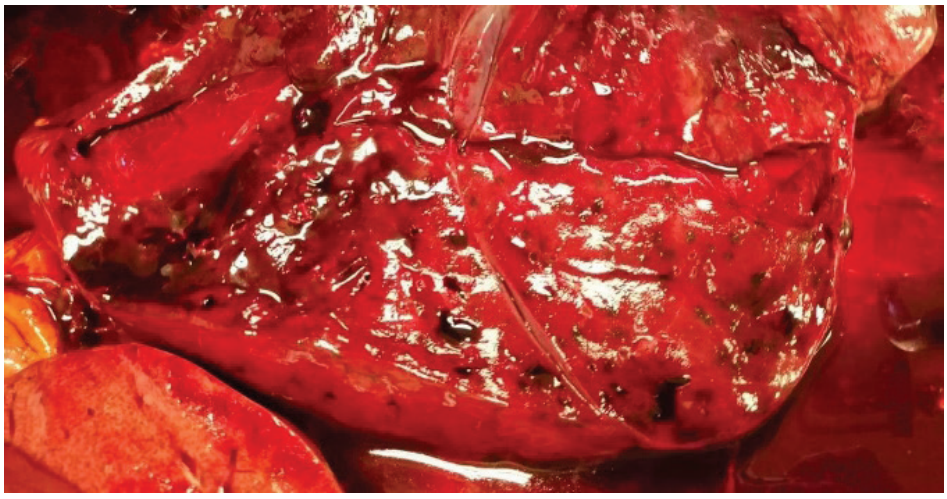

Figure 2. COVID-19 pneumonia. Enlarged, heavy lungs with a brownish-red color cut surface, and scattered, nodular areas

brain thrombosis and brain infarctions was higher in the first group. Patients with diabetes developed severe arterial and venous thrombosis of the lower extremities and diabetic lower limb gangrene. Patients 3 and 8 developed secondary phlegmon and gangrene at the site of surgical wounds. Patient 3 was on antibiotic therapy due to phlegmon in the area of the operative wound which led to ulcerative-necrotic, fibrinous-purulent colitis accompanied by local pelvic fibrinous-purulent peritonitis. Other patients have had concomitant diseases such as macronodular cirrhosis of the liver, steatosis, constrictive pericarditis, paranoid schizophrenia on therapy with Flupenthixol, Clozapine, and one patient with low-grade adenocarcinoma of the lung.

Pulmonary findings: Lung tissue was obtained from autopsies with a postmortem interval ranging from 1 to 5 days between February 2020 and June 2022.

Macroscopically: The lungs were enlarged bilaterally, heavy, with hemorrhages (600-800 g). The cut surface was with greatly reduced elasticity and increased density, with a brownish-reddish color and firm nodular areas **(Figure 2).**

Microscopical pictures described in the previous paper identified four stages in the development of COVID-19 pneumonia (34). *Severe respiratory syndrome* develops most commonly in 7-14 days after initial symptoms. Patients who died in the stage of fibrosis revealed collagen formation, which was more pronounced in the periphery of the lobes.

Brain examination was performed in 15 patients. Patients (1, 2, 6) from the first group developed brain infarction because of thrombosis in medium-sized cerebral vessels. Focal hemorrhages on the meninges and thrombosis in meningeal vessels were found in patient 8 (26.7%)**.**

All of the cases with COVID-19 viral desquamative pneumonia at different stages developed vascular thrombosis in medium-sized pulmonary vessels (20/20). Two patients developed Pulmonary thromboembolism, (patients 3 and 4). We established 5 patients with generalized thrombosis. In patients, 1, 2, and 8 thromboses were complicated by infarcts in the brain, kidneys, and spleen **(Figure 3).**

Cardiac involvement: I gr. Patients 8 and 9 developed acute myocardial infarction, because of complicated atheromatous plaque with occlusive thrombosis of the left coronary artery, and patient 2 developed abacterial thrombotic endocarditis of the mitral and aortic valves.

Patients infected with the omicron prevailed variant (group II) did not show extrapulmonary thrombotic complications. Complications were associated with secondary bacterial bronchopneumonia resulting from prolonged respiratory ventilation in 2/10 (20%) patients, with one case developing bacterial sepsis. One of the patients has been presented with a dissecting aneurysm (type A) of the abdominal aorta, rupture of the abdominal aortic aneurysm wall, and abdominal hemorrhage. In this group, most patients have at least 3 comorbidities. 3/10 have CIHD: Extensive post-infarct cicatrices on the anterior wall of the left heart chamber. Three patients with diabetes mellitus have been complicated by

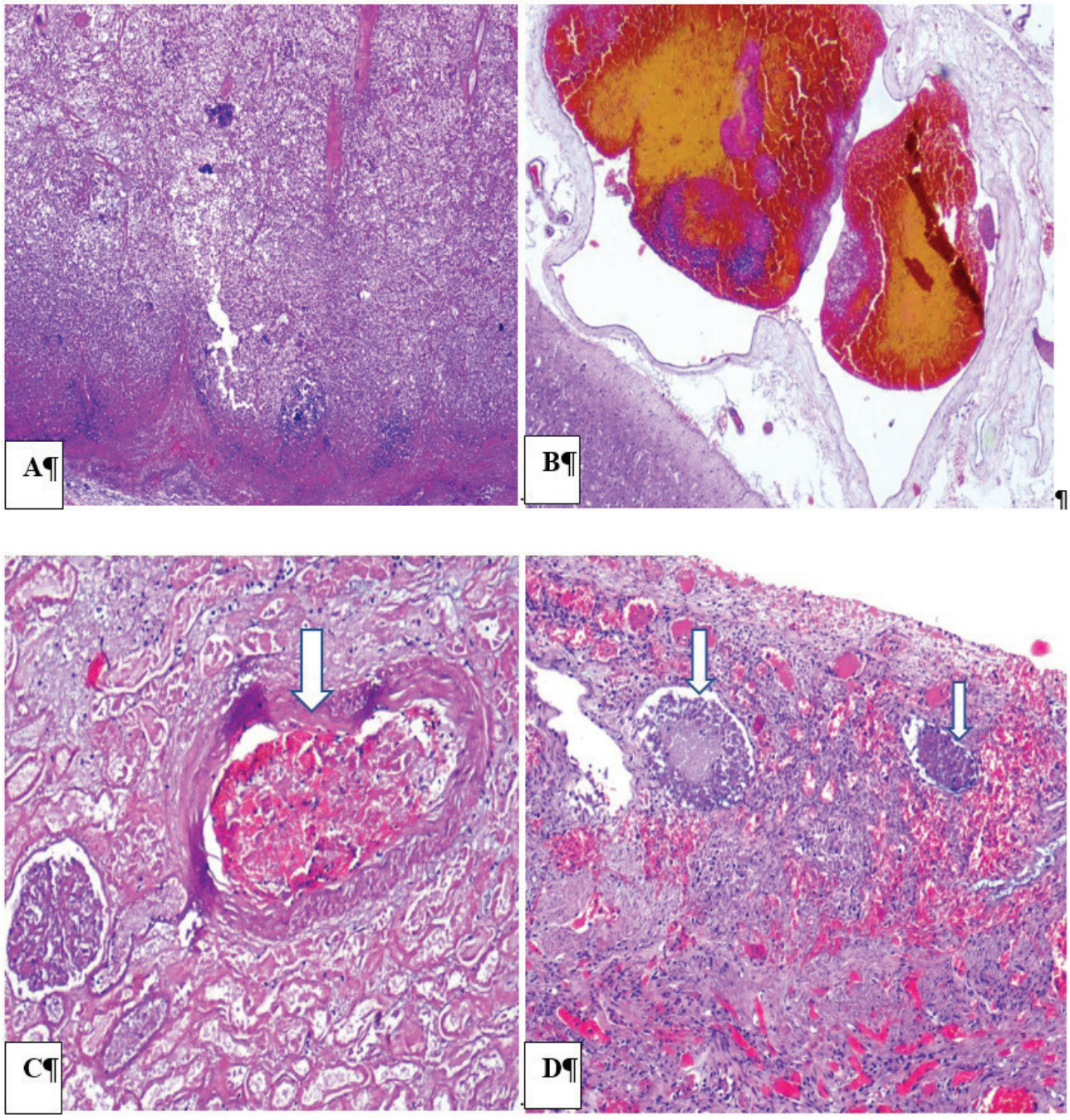

Figure 3: A. Spleen infarction (HE x4). B. Thrombosis in brain artery (HE x4). C. Kidney thrombosis and infarction (HE x10). D. Uterus thrombosis and infarction (HE x10)

gangrene and amputation of a limb. 2/10 patients had brain conditions after micro stroke, 2 had chronic pyelonephritis, a 28-year-old woman and a 72-year-old man had steatosis of the liver, and 1 patient had an oncological disease (**Figure 4**).

LABORATORY TESTS

The laboratory results were taken from the hospital database of the gamma-multi-star system. Clinical laboratory studies, in connection with thrombotic complications, showed an increased value of Creatinkinase in 17/20 (85%) patients. The highest value was in patient 8 - (3362 U / l) with thrombosis of the coronary and cerebral artery and infarction in the spleen, right kidney, and heart. Fibrinogen was elevated in 8/20 patients, while D-Dimers were elevated in all patients, but were highest in patient 8 (> 35.2 µg / ml). Lymphopenia was observed in 12/20 (60%). CRP was elevated in all patients, but was highest in patient 9 (490mg/l) The results of the 10 patients of the first group are summarized in **Table 1.**

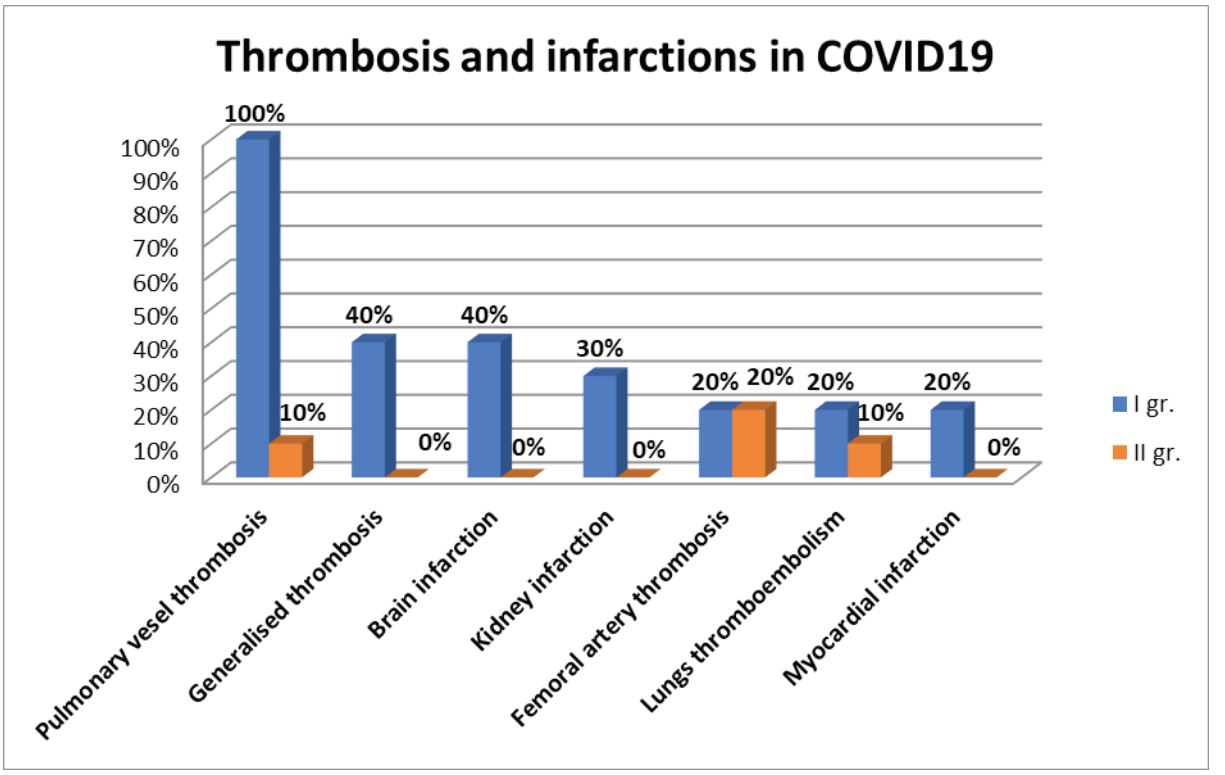

Figure 4: The most common thrombotic complications in COVID-19

DISCUSSION

According to the WHO, Johns Hopkins University & Medicine, Coronavirus resource center (2023), by the end of the first week of October, the disease had already infected 67 660 995 people worldwide. Of these, 6 881 955 died, amounting to approximately 10.17% case-fatality ratio of the total. The region with the highest number of infections and deaths was the Americas, and Southeast Asia is second in the number infected, followed by Europe (28). Bulgaria ranks second in the world (after Peru) in the number of deaths per one million inhabitants.

Since the beginning of the COVID-19 pandemic, a sufficient number of autopsy cases have constantly shed light on the first wave caused by SARS-CoV-2, based on single cases described at the beginning of the pandemic, such as a meta-analysis (10), a series of 14 cases in Washington State (7), large study, 68 autopsies, in two hospitals multi-institutional autopsy cohort from Italy and New York (8). Our modest contribution is 20 autopsy cases of patients with PCR and a rapid antigen test, established COVID-19 (in alfa, delta, and omicron cases), and we tried to systematize the age, sex, comorbidity of patients, hypercoagulability, based on laboratory parameters and cause of death. Extensive studies of lung damage and extrapulmonary involvement of SARS-CoV-2 during severe infection have previously been described by Deshmukh *et al*. (9).

In the first and second groups of the COVID-19 pandemic, 55% of the affected patients were men and 45% were women. All patients had comorbidity, and for one patient at the age of 61, there was no anamnestic data, as he died in the emergency department. 80% of patients had arterial hypertension and left ventricular hypertrophy. 60% of patients had CIHD (Chronic Ischemic Heart Disease), and 25% had diabetes. The highest percentage of patients (90%) had an obesity III degree. The disease course was most severe and accompanied by complications in patients with diabetes mellitus such as arterial and venous thrombosis of the lower extremities and gangrene of the lower limbs (7.6%). Patients 3 and 8 developed secondary phlegmon and gangrene at the site of surgical wounds. In patient 3, antibiotic therapy on the occasion of a phlegmon in the area of the operative wound resulted in ulcerative-necrotic, fibrinous-purulent colitis with local pelvic fibrinous-purulent peritonitis. Patients 2, 4, and 5 suffered from concomitant diseases such as macronodular cirrhosis of the liver, constrictive pericarditis, paranoid schizophrenia, and

Table 1. Patient laboratory data, comorbidities, and cause of death, I gr:

№	Age	Sex	Day of symp-toms	Creatine kinase NR(22-198 U/l)	Fibrinogen NR(2-4g/l)	D-Dimers NR (0–0.5μg/ml)	Lymph NR(1.3-3.9 10^9/l)	CRP NR (0-8mg/l)	Comorbidities	Cause of death
1.	55	f	21	712	4.15	2.96	0,49	88	AH; Obesity III st.	SARS-CoV-2; DAD; RV; Generalized thrombosis, BI
2.	63	f	21	630	7.74	1.23	0.31	398	AH; Atherosclerosis; Obesity III st.	COVID-19; DAD; RV; Generalized thrombosis: Infarction of brain, kidney, spleen
3.	56	f	14	740	3.23	2.32	1.3	264.0	AH; CIHD; DM; Atherosclerosis; Obesity III st.	DAD; Pulmonary thromboembolism
4.	62	m	7	735	3.74	2.4	1.1	149	Cirrhosis liver; AH; CIHD; DM; Obesity III st.	SARS-CoV-2; DAD; Pulmonary thromboembolism
5.	56	m	8	540	7.82	10.9	0.5	70.9	CIHD. Constrictive pericarditis	DAD, III st; RV; CAD
6.	69	m	30	no	no	no	no	no	Old BI; AH; CIHD; Atherosclerosis; Obesity III st.	SARS-CoV-2; BI
7.	37	m	14	37	2.28	2.5	1.7	64	AH; Obesity III st.	SARS-CoV-2; DAD
8.	81	f	18	6579	3.39	>35.2	0.6	67	AH; CIHD; Atherosclerosis; DM; Obesity III st.	COVID-19; DAD; Generalized thrombosis: Infarction of kidney, spleen; CAD
9.	70	m	7	800	7.36	5.54	1.49	490	AH; CIHD; Old MI; Atherosclerosis	SARS-CoV-2; DAD; CAD: MI
10.	61	m	14	34	4.34	1.22	0.36	107	Obesity III st.	SARS-CoV-2; DAD; RV; Pulmonary thrombosis.

Legend: NR - normal range; AH - arterial hypertension; CIHD – chronic ischemic heart disease; CAD - coronary arteria disease; MI -myocardial infarction; DAD – diffuse alveolar damage; DM - diabetes mellitus; BI - brain infarction; RV - respiratory ventilation.

therapy with Flupenthixol and Clozapine. 4 out of 20 (22.8%) patients - 1, 2, 6, in whom the brain was examined, developed cerebral infarction as a result of thrombosis in medium-sized vessels.

All patients who developed COVID-19 viral desquamative pneumonia in different stages exhibit vascular thromboses in the lungs. A total of two patients, in the Ist gr, developed pulmonary thromboembolism. Five patients suffered from generalized thrombosis (38%). The thromboses have been complicated by infarctions in the brain, heart, kidneys, and spleen (patients 1, 2, and 8). Two patients developed acute myocardial infarction due to complicated atheromatous plaque with occlusive thrombosis of the left coronary artery.

In many of the reported COVID-19 autopsy cases, thrombosis in large pulmonary vessels has been reported in 15% (10). The reported rate of thrombosis has been higher in SARS-CoV-2, with 28% of cases describing thrombi in medium or large vessels (11). On average, patients with COVID-19 developed diffuse, bilateral, viral desquamative pneumonia on day 14 from the onset of the disease. SARS-CoV-2 and thromboses have been observed on days 7-10 at the earliest. Most of the studies demonstrate venous thromboembolism and microthrombi in arterioles and venules. There are numerous reports of patients with COVID-19 presenting with both arterial (stroke, myocardial infarction) and venous thrombosis (deep vein thrombosis, pulmonary thromboembolism, venous sinus thrombosis). Many of these patients had traditional risk factors for thrombosis. They have been summarized in 2 meta-analyses by Ahmed *et al.* (12) and Deshmukh *et al.* (9). COVID-19 can be viewed as a prothrombotic disease. Endothelial dysfunction, activation of the renin-angioten-sin-aldosterone system (RAAS) with the release of procoagulant plasminogen activator inhibitor (PAI-1), and hyperimmune response with activated platelets seem to be significant contributors to thrombogenesis in COVID-19. Stratifying the risk of COVID-19 thromboses should be based on age, presence of comorbidities, D-dimer, CT scoring, and various blood cell ratios (12).

Diffuse Alveolar Damage (DAD), the histologic correlate of ARDS, is the predominant histopathologic pattern identified in lung pathology from patients with COVID-19, H1N1 influenza, and SARS-CoV-2. Microthrombi were reported more frequently in both patients, with COVID-19 and SARS-Cov-2, as compared with H1N1 influenza. DAD lies on the severe end of the ALI spectrum (13), and is the histopathologic pattern typically associated with clinical ARDS. DAD is caused by endothelial and alveolar lining cell injury which leads to fluid and cellular exudation culminating in physical disruption of the blood-air barrier and interstitial loose fibrosis with chronic inflammatory infiltrates, and intra-alveolar loose fibrous plugs. In most foci is seen intra-alveolar organizing fibrin and injured epithelial cells desquamated into the alveolar spaces (14, 18). Zhi Zeng et al (17) have identified intracytoplasmic viral-like inclusions in a few types II pneumocyte-like cells and macrophage-like cells. Thus, the results demonstrated the presence of SARS-CoV-2 in the lung tissue and confirmed that the patient was infected with SARS-CoV-2.

Diffuse alveolar damage was seen in 87% of cases. Later phases of DAD were less frequent and correlated with a longer duration of disease (8). However, a few studies, together with previous observations, suggests an alternative to traditional models of the injury response, whereby inflammatory and repair mechanisms occur in parallel rather than in series (15, 20).

Vascular thrombosis and macro thrombosis are frequently observed in DAD, even in the absence of a systemic hypercoagulable state, and they are thought to result from local inflammation (16). We did not find inflammatory cells in any of the vessels in the different organs, suggesting active replication and release of the virus in epithelial cells. Endothelial cells may cause the host cell to undergo pyroptosis (pro-inflammatory apoptosis) and release damage-associated molecular patterns (DAMPs), activating oxidant stress, and generating pro-inflammatory cytokine and chemokine release. This mechanism leads to the activation of both the extrinsic and intrinsic coagulation pathways to make thrombosis.

Post-mortem case studies have shown pulmonary, renal, and small vessel injury, with particles resembling viruses observed in the kidney by electron microscopy [5,19,20,21). Bradley et al. (7) found broad tropism for SARS-CoV-2 with coronavirus-like

particles identified in the pulmonary system, kidneys, and gastrointestinal tract. This series reported an association with diabetes, hypertension, obesity, and vascular disease, and was also male predominant in its involvement.

Already, at the beginning of the pandemic, have been reported a wider spectrum of histological lesions involving both the epithelial and vascular components in lungs and other organs. Extensive sampling, thrombi, and vascular injury of both small- and middle-sized pulmonary vessels are common alterations. Many thrombi are platelet-rich, confirmed by CD61 immunohistochemistry. While vascular thrombi, both fibrin and platelet types, are reported as a component of DAD and would be expected to some extent given the damage to the alveolar-capillary barrier and subsequent activation of repair mechanisms and the coagulation cascade, it does appear that the frequency and severity of this finding are higher in COVID-19. Large vessel thrombi have been seen in 42% of cases but platelet and/or fibrin microthrombi have been presented at least focally in 84%. Ultrastructural, small vessels showed basal membrane reduplication and significant endothelial swelling with cytoplasmic vacuolization. The virus has been seen in airway epithelium and type 2 pneumocytes (8).

Infection of *endothelial cells* by SARS-CoV-2 is hypothesized to cause dysregulation of the clotting system, which particularly affects vessels and leads to pulmonary microthrombi and altered ventilatory patterns in intubated patients. Marini et al. (21) reported 12 cases focusing on thromboembolic events. Deep venous thrombosis was seen in 58% of patients with pulmonary embolus as the cause of death in four and DAD in eight (22). These observations lend support to direct pulmonary epithelial and endothelial injury as well as vascular/endothelial complications.

Cardiac injury in COVID-19 is common, although our results do not provide direct evidence of myocardial injury by SARS-CoV-2 in the acute stage. The damage in the severe stage is rather the result of thrombotic vascular complications. We detected two patients with acute myocardial infarction due to complicated atheromatous plaque with occlusive thrombosis of the coronary artery and abacterial thrombotic endocarditis of the mitral and aortic valves. In a study that documented the clinical course of patients in the intensive care unit in Kirkland, WA, 33% of patients had cardiomyopathy of unclear cause (12). Previous post-mortem examinations have detected viral RNA in cardiac tissue from a single patient, although histopathological evidence of myocarditis was not present (23). Cardiovascular diseases, which occupy the third position among comorbidities in patients with COVID-19, they currently represent one of the most serious public health problems because, besides their high prevalence, they are also among the leading causes of mortality worldwide (29).

Thromboses in medium-sized *renal arteries*, accompanied by anemic renal infarcts, have been observed in two patients. Tubular epithelial cells, endothelial cells, and podocytes express ACE2, making kidneys a candidate target for SARS-CoV-2 infection. Direct infection of kidney cells by the virus has been proposed as a mechanism for acute kidney injury observed during SARS-CoV-2 infection. Two studies reported SARS-CoV-2 virus infection in tubular epithelial cells and podocytes (2, 24). As SARS-CoV-2 has been detected in urine and stool of non-severe COVID-19 cases, there is evidence suggesting productive infection of non-pulmonary sites (25).

The systematic review of the literature for observational studies published between December 2019 and September 2020, based on the protocol for a systematic review registered in the International prospective register of systematic reviews (PROSPERO): In the 31 studies analyzed, 10.8% of infected with COVID-19 patients died. The 3 most prevalent comorbidities were hypertension, diabetes mellitus, cardiovascular diseases, respiratory diseases, renal disease, and malignancy (29).

Obesity is one of the factors to be evaluated in patients infected by COVID-19, who died. Our study also revealed that 90% of the patients, including I gr and II gr, who died of COVID-19, were in obesity stage III.

In *Diabetes Mellitus* there is a higher risk of severe pro-inflammatory conditions, hypercoagulation, and impairment of the immune system because diabetes is a condition associated with several macrovascular and microvascular complications.

Moreover, inadequate glycemic control in patients with diabetes mellitus can further compromise the immune system's response capacity. In this sense, proper glycemic control is an important factor in diabetic patients diagnosed with COVID-19 (30).

One in five people worldwide is estimated to be at higher risk of adverse COVID-19 outcomes based on the prevalence of chronic conditions. The risk also increases with age and with a greater number of underlying conditions. Compared with someone younger than 40 years, the risk of death increases fourfold for people aged 50-64, and more than 10-fold for those aged over 85. Upon analyzing for an association between sex and comorbidities in deaths, men were found to represent 65.5% of the total, markedly exceeding the 34.5% proportion for women. (29). Similarly, compared with people with no underlying conditions, the risk of death is 1.5 and 3.8 times higher for those with one comorbidity and over 10 comorbidities, respectively. Various studies have found that 14% of adults who had had COVID-19 developed new clinical conditions within six months. Clinical sequelae included interstitial lung disease, respiratory failure, congestive heart failure, arrhythmia, and type 2 diabetes (31, 32, 33). In this study, the deceased patients of the IInd group, registered with the omicron prevailed variant, had three or more accompanying diseases, some with severe complications such as old brain infarction; old myocardial infarction; gangrene with amputation of a limb; liver disease; oncological disease.

CONCLUSION

Overall, our series of twenty autopsies show a consistent pattern of alveolar injury and pulmonary and generalized vascular/thrombotic disease in patients with frequent co-morbidities such as hypertension, atherosclerosis, and obesity. Conversely, in patients with diabetes mellitus, the course of COVID-19 is more severe and with more extrapulmonary thrombotic complications. As the disease affects alveolar zones, acute viral-associated alveolar damage with hyaline membrane lung injury was seen; these foci persist well into week 2 of the disease, and on occasion longer, end with fibrotic changes in the lungs. The results show that the presence of one or more comorbidities is an aggravating risk factor for complications and death after COVID-19 infection.

One important manifestation is the high frequency of platelet thrombi, which was observed more in the alfa and delta prevailed variants of the infection, while in the group with the omicron prevailed variant the lung lesions were dominant, without extrapulmonary generalized thrombotic complications, which we explain by the effective antithrombotic therapy. The late major complications in these patients were secondary bacterial infection, sepsis, and respiratory distress syndrome. However, despite effective anticoagulant therapy, in patients who died in the acute phase of the disease, in some cases, the risk of pulmonary thromboembolism, pulmonary thrombosis, and thrombotic complications in diabetic patients remains high.

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CONFLICT OF INTERESTS:

The authors declare no conflict of interest.

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