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"Natural forces within us are the true healers of disease" Hippocrates

Dear colleagues

The Governing Board of The Medical Society of Western Greece and Peloponnesus (IEDEP) honored me with the duty to serve this journal. As the new Editor in Chief of 'Achaiki Iatriki', first of all, I would like to thank Professor N. Kounis for his contribution to the journal. He carefully and diligently endeavored to establish the journal's uninterrupted presence with consistency, validity and scientific prestige. My effort will be to continue his important work and I rely on his help.

The journal has an excellent editorial board consisting of internationally renowned scientists. But its course depends on your own support. Please send us clinical notes, case reports, reviews and original articles and we will be happy to accept them following a peer review process. We address all healthcare professionals in both the private and public sector. Our goal is to present high-quality reliable data, thus disseminating novel, valuable knowledge on the one hand and providing clinicians with an important tool for their patients' everyday management on the other hand. This journal will be published every three months in the English language.

Dear colleagues, Covid-19 is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with global spread, currently characterized as a pandemic by the World Health Organization (WHO). In the current issue you will find emerging new data on the novel coronavirus SARS-CoV-2. The editorial by Tsounis et al., describes the potential mechanisms that contribute to the development of SARS-CoV-2-related liver damage, the association of the virus with liver diseases and the relation of the ongoing therapeutic strategies of COVID-19 with the potential induction of hepatotoxicity. The review by Anastasopoulou et al., summarizes up-to date information on the biological basis of the coronavirus SARS-CoV-2, including genomic differences between the pre-existing coronaviruses and the novel virus and a detailed description of its structure and the mechanisms it uses to adhere and enter the host cell. Lastly, the review by Syrokosta et al., summarizes current knowledge about the virology, pathophysiology and clinical characteristics of the COVID-19 infection.

In addition, this issue includes an editorial by Kounis et al. that focuses on cardio-oncoimmunology as an emerging medical discipline, discussing the cardiovascular effects of chemotherapeutics, and especially of chemotherapy-induced cardiac toxicity and hypersensitivity. The third editorial of this issue by Ntouvas et al., concerns the intermittent claudication in young adults and discusses the most common causes of this condition beyond atherosclerosis and its differential diagnosis.

Two original studies are also included in this first issue. The first study by Karaivazoglou et al., aims to determine the presence of the broad autism phenotype in parents of children with developmental disorders including autism spectrum disorder and developmental language and speech disorders and detect potential associations. The second study by Tourkochristou et al., aims to estimate the prevalence of anti-HCV antibodies in prisoners at a detention center in Southwestern Greece. The introduction of direct-acting antiviral agents revolutionized the treatment of hepatitis C virus (HCV) infection. The strategy is now being adapted into national hepatitis elimination plans and our country is on track to reach the targets. Treating HCV infection in prisoners can play a pivotal role in the implementation of the HCV National Plan and in the efforts to reach the goal of HCV elimination. Lastly, this issue includes an interesting case report by Konstantakis et al., presenting a rare cause of abdominal pain which was attributed to a foreign body in the biliary tract.

Dear colleagues, Covid-19 is a disease that makes no exemptions. Solidarity is an important weapon to fight this enemy and move forward. A system based on solidarity largely relies on participants' responsible

behavior. Nonetheless, we should not focus exclusively on solidarity within the healthcare setting. Citizens are entitled to high-quality healthcare services. Providing healthcare efficiently requires financial resources to be properly balanced. Importantly, both categories of the fundamental health system inputs, namely human resources and consumables, should be provided. Many of you are finding your academic and clinical duties affected by the virus. But crisis moments also represent opportunities; for example current circumstances call for a more sophisticated and flexible use of technology and virtual meetings in standard clinical practice. Our country is struggling with the pandemic and healthcare professionals are at the forefront of this fight. All together we will overcome the crisis. May this situation be an opportunity for our country's health system to come out stronger.

I wish you all the best and invite you all to support our Journal.

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COVID-19 and the Liver

Efthymios P Tsounis[1], Stelios F Assimakopoulos[2], Christos Triantos[1]

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) with global spread, currently characterized as a pandemic by World Health Organization (WHO). SARS-CoV-2 shares approximately 82% genome sequence homology to already known SARS-CoV responsible for the 2002-2004 SARS outbreak epidemic [1]. There is solid evidence that the new virus is not a laboratory construct or a purposefully manipulated virus but has evolved as a result of natural-selection [2]. Confirmed routes of transmission include respiratory droplets and close contact, but recent data reveal the fecal-oral as an alternative way, underlining the role of gastrointestinal system in the viral pathogenesis [3]. Common symptoms of COVID-19 include fever, dry cough, fatigue, myalgia and dyspnea. A subgroup of patients progresses into severe COVID-19 characterized by cytokine storm syndrome associated with acute respiratory distress syndrome (ARDS), multiple organ failure and increased mortality [4].

Abnormal liver function is a frequent extra pulmonary finding in hospitalized patients. It is documented that 14-53% of them present abnormal serum liver enzyme levels, mainly elevated aminotransferases, with mild bilirubin increase [4-12]. Decreased serum albumin on hospital admission appears to be an indicator of the disease's severity [7,10]. Whether laboratory test alterations are a sign of pre-existing occult or apparent well decompensated liver disease (alcoholic, viral, non-alcoholic steatohepatitis) remains unclear at this point due to a shortage of appropriate studies. Possibly, patients with advanced liver disease are more prone to developing a severe form of the illness due to cirrhosis associated immune dysfunction [5]. In a large multicenter cohort study including 1099 patients, critically ill subjects had higher rates of liver dysfunction when compared to non-severe cases [4]. Therefore, the incidence of liver impairment is speculated to be associated with the severity of the infection. In deceased patients liver injury is reported to be as high as 78% [12]. Acute liver failure has been described in one critically ill patient with serum ALT and AST levels rising to 7590 U/l and 1445 U/l respectively [9]. Nevertheless, in mild cases hepatic injury is temporary and no specific treatment is necessary. Lactate dehydrogenase (LDH) levels were found to be an independent risk factor for severe COVID-19 [7]. Nonetheless, increased serum LDH levels may be promoted by non-hepatic sources like muscles or red blood cells.

The pathophysiology of SARS-CoV-2 related hepatic damage has not been fully elucidated, albeit a variety of mechanisms is proposed. First, a direct virus-induced cytopathic effect in hepatocytes is possible [5]. In autopsies of SARS cases, virus particles were detected in hepatocytes and endothelial cells [6,13]. Postmortem liver biopsy in a COVID-19 patient showed microvesicular steatosis and mild lobular and portal activity [14]. Besides, SARS-CoV-2, in similarity to SARS-CoV, binds to the target cells via angiotensin converting enzyme 2 (ACE 2) [15]. Novel data reveal that, ACE-2 is expressed in hepatocytes and bile duct cells in a level comparable to that of alveolar type 2 cells in the lungs [16]. However, markers of cholangiocytes' injury, namely alkaline phosphatase (ALP) and gamma-glutamyl transferase (γ-GT), are not usually elevated in COVID-19 cases [7,16].

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Second, liver injury may be induced indirectly through a cytokine-mediated mechanism [5,6]. Previous studies have demonstrated elevated inflammatory biomarkers (C-Reactive Protein, procalcitonin, ferritin) and increased levels of inflammatory cytokines and chemokines (IL-1, IL-6, IL-8, IL-10) in severe COVID-19 patients [4,7,8,17]. In addition, activation of coagulation and fibrinolytic cascades with reduced platelet count and elevated D-dimers has been also shown [4,8]. Furthermore, lymphopenia with decreased CD4+ T cells and increased neutrophils-lymphocytes ratio (NLR) are described [7,8,17]. These findings suggest derangement in immunomodulation and a hyperinflammatory response that may exert injurious effects in liver parenchyma possibly by oxidative stress-related mechanisms [18].

Patients with chronic liver diseases and cirrhosis constitute a potential high-risk group for liver injury from SARS-CoV-2. It is reported that 2-11% of COVID-19 patients have liver comorbidities, even though the dynamic evolvement of the pandemic does not allow accurate estimations [4,5,8,10]. Previous data on SARS-CoV patients with coexisting HBV and/or HCV infection showed increased susceptibility to hepatic impairment, possibly as a consequence of amplified replication of HBV/HCV viruses [6]. Given the homology between the two coronaviruses, clinicians should be more alert when a patient with chronic viral hepatitis is presented with COVID-19. Serologic testing for HBV and HCV is recommended in cases with laboratory findings of liver injury. Furthermore, given the abundant expression of ACE-2 in cholangiocytes, SARS-CoV-2 infection might aggravate cholestasis in patients with cholestatic disease [16]. Although data on this topic are scarce, patients with primary biliary cholangitis and primary sclerosing cholangitis should be closely monitored with meticulous evaluation of ALP and γ-GT measurements. Reasonably, patients with hepatocellular carcinoma or cirrhosis are at increased risk for severe infection by SARS CoV-2 due to immunosuppression. A concern has been raised regarding the manipulation of immunosuppressive treatments in patients with COVID-19. Discontinuation of immunosuppressive regimens could exacerbate autoimmune hepatitis or could trigger an acute rejection in a post-liver-transplant patient. American Association for the study of Liver Diseases (AASLD) does not advise preventive modification of therapy for chronic liver disease patients during this outbreak [19]. In case of infection, it is recommended to reduce the dosage of high-dose prednisone with caution to avoid adrenal insufficiency (at least 10mg/day is recommended). Similar adjustments are required for azathioprine, mycophenolate or calcineurin inhibitors [19]. Obviously, since our background knowledge on such cases is insufficient, individualized approach is necessary.

A series of therapeutic agents have already been used in hospitalized COVID-19 patients. Therefore, drug induced hepatotoxicity is a possible factor of liver damage [10,11]. A variety of antivirals (remdesivir, lopinavir/ritonavir), antimicrobials (macrolides, quinolones, beta-lactams, chloroquine), biological agents (tolicizumab) and antipyretics (paracetamol), used in SARS-2-CoV, have the potential to induce liver injury [4,6,7]. Up to now, such a causality has been demonstrated only for the anti-retroviral drug combination of lopinavir/ritonavir [7]. Abnormal liver function test results should not discourage the use of investigational or off-label therapeutics according to the AASLD [19]. Undoubtedly, all hospitalized SARS-CoV-2 patients should be submitted in regular testing of liver biochemistries, especially those under treatment with tolicizumab or remdesivir, regardless of baseline values. Finally, in critically ill COVID-19 cases liver dysfunction might be associated with mechanical ventilation. Application of positive end expiratory pressure (PEEP) results to high right atrial pressure with liver congestion. The absence of counterbalancing arterial vasodilation and the high surrounding tissue pressure lead to decreased arterial flow as well [20].

In conclusion, COVID-19 may induce a multifactorial liver injury with unresolved pathophysiology. Direct viral-induced hepatotoxicity or indirect mechanisms associated with hyperinflammation, have been hypothesized. Careful monitoring of serum hepatic enzymes is imperative, especially in hospitalized patient or those with liver comorbidities.

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Cardio-oncoimmunology: An emerging medical discipline

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The two main causes of mortality worldwide are currently cardiovascular diseases and various types of cancer. Whereas cancer constitutes the major cause of death among adults up to 74 years of age, after this age, cardiovascular disease surpasses cancer as the primary cause of mortality [1]. Recent advancements in cancer treatment and diagnosis have contributed to the presence of nearly 14.5 million American cancer survivors in 2014, that are anticipated to reach 18 million by the current year 2020 [2].

However, despite these therapeutic advancements, practicing physicians often face problems, during both chemotherapy and radiotherapy, related to cardiovascular dysfunction and cardiac function deterioration. Cardiovascular deterioration can be manifested as acute and chronic cardiac events as shown in the table 1. Chemotherapeutic drugs can affect the cardiovascular system either through direct effects to cardiac myocytes resulting in cardiomyopathy, or indirect effects, such as hypertension, subsequently increasing the risk of cardiac disease [3].

Radiation therapy can induce heart failure that may become evident months or years after radiotherapy completion. Structural abnormalities such as valvular heart disease, circulatory problems including coronary artery disease, carotid artery disease, pericarditis, pericardial effusion and myocardial infarction associated with further electrical abnormalities like rhythm and conduction disturbances may follow radiotherapy [4].

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As far as the pathophysiology of chemotherapy-related cardiovascular dysfunction is concerned, there is clinical and laboratory evidence that acute coronary syndromes, myocarditis and cardiac arrhythmias are not induced

Table 1. *Potential acute and chronic cardiac events during chemotherapy.*

Acute conditions	Acute myocardial infarction
	Cardiac arrhythmias (bradycardia, tachyarrhythmias, atrio-ventricular blocks, QT prolongation, torsades de pointes)
	Coronary spasm
	Heart failure
	Hypotension
	Myocardial infarction
	Myocarditis
	Pericardial effusion
	Pericarditis
	Stroke
	Thromboembolism
Chronic conditions	Cardiomyopathy
	Congestive cardiac failure
	Coronary artery disease
	Diastolic left ventricular dysfunction
	Hypertension
	Peripheral vascular disease
	Pulmonary hypertension
	Systolic left ventricular dysfunction
	Valvular heart disease

by cardiac toxicity, but mainly by hypersensitivity and especially by coronary hypersensitivity and Kounis syndrome [5,6].

CHEMOTHERAPY-INDUCED CARDIAC TOXICITY

Cardiac toxicity during chemotherapy, generally, refers to a dose-dependent cardiovascular adverse reaction depending on the quantity of substance to which the organism is exposed and the route of exposure, for example skin absorption, mouth ingestion, or respiratory tract inhalation, that persists despite the discontinuation of the causative treatment. The final outcome of cardiac toxicity is a fibrotic response that should be confirmed histologically, a procedure that has not been undertaken until now [7]. Cardiac toxicity can be acute involving deleterious consequences through a single or short-term exposure. Subchronic toxicity is the ability of a toxic substance to cause effects lasting for more than one year but less than the lifetime of the exposed organism, usually upon repeated or continuous exposure, sometimes lasting for the entire life of the exposed organism. Chronic toxicity is referred as the ability of a substance or mixture of substances to exert their harmful effects over an extended period. However, the definition, characterization and pathophysiology of cardiac dysfunction during chemotherapy have not been completely elucidated. There are several discrepancies among the medical societies regarding the term cardiac toxicity leading to a lack of consensus especially when this term is used to characterize the acute adverse effects of chemotherapeutic monoclonal antibodies.

The National Cancer Institute defines cardiovascular dysfunction as "toxicity that affects the heart" [8]. The American Society of Echocardiography and the European Association of Cardiovascular Imaging define cardiovascular dysfunction as a decrease of left ventricular ejection fraction of 10% that is confirmed on a repeat study within 2–3 weeks [9]. The National Comprehensive Cancer Network defines cardiovascular dysfunction as "cardiac toxicity referred to the heart damage by harmful chemicals" [10]. Finally, the most accurate definition so far has been formulated [11] by the Cardiac Review and Evaluation Committee supervising trastuzumab clinical trials and includes one or more of the following:

1. Heart failure symptoms
2. Cardiac signs, including audible third heart sound associated with gallop rhythm, tachycardia, or both
3. Global or more severe septal cardiomyopathy with reduced left ventricular ejection fraction
4. Left ventricular ejection fraction reduction from baseline that is in the range of ≤5% to ≤55%, with accompanying signs or symptoms of heart failure, or a reduction in left ventricular ejection fraction in the range of ≤10% to ≤55%, without accompanying signs or symptoms. The Committee has concluded that an ideal definition is still lacking given that the above definition does not include subclinical cardiovascular damage that may occur early in response to some chemotherapeutic agents.

CHEMOTHERAPY-INDUCED CARDIAC HYPERSENSITIVITY

Chemotherapeutic agents, in several occasions, have been associated with the development of serum antibodies able to induce a variety of cardiovascular, cutaneous, gastrointestinal, muscular, neurological and respiratory hypersensitivity reactions [12]. It seems that cardiac hypersensitivity is an appropriate term that should be used along with cardiac toxicity in order to describe the adverse events elicited by several chemotherapeutic agents including monoclonal antibodies.

Cardiovascular hypersensitivity, in particular, refers to an inflammatory response that is not dose-dependent, may arise at any time during treatment, even with minimal drug concentrations and is accompanied by anti-drug antibodies. Anti-drug antibodies are the most often encountered antibodies which belong to the IgG isotype, but a proportion of hypersensitivity reactions also involve IgE antibodies [13]. Several chemotherapeutic drugs including platinum agents, taxanes, chimeric monoclonal antibodies and others have been incriminated to induce IgE-mediated hypersensitivity reactions.

Platinum agents, such as cisplatin, carboplatin, oxaliplatin inhibit DNA replication and suppress cancer cells' division and proliferation. Cisplatin's hypersensitivity prevalence ranges from 5 to 20%, carboplatin's from 9 to 27%, and oxaliplatin's from 10 to 19% [14]. Cardiac hypersensitivity reactions to platinum agents can induce acute myocardial infarction such as Kounis syndrome [5,6,15], cardiac arrest and even death [16].

Taxanes, that are commonly known as microtubule inhibitors, mitotic inhibitors, and mitotic poisons including paclitaxel, docetaxel and others inhibit cell division, chromatid separation and growth events that may lead to cell death. Hypersensitivity reactions are common in patients receiving taxanes, ranging from mild to severe or even lethal, not responding to premedication therapy, and their prevalence is estimated to reach 30%

[17]. Immunoglobulin E-mediated anaphylaxis with increased tryptase levels, direct mast cell and/or basophil activation and complement activation are some of the mechanisms potentially underlying these reactions [18].

Chimeric monoclonal antibodies, (the name originates from the Greek mythological monstrous fire-breathing hybrid chimera composed of parts of several animals e.g. a lion body with a goat head of and a tail end with a snake's head, offspring of Typhon and Echidna and sibling of Cerberus and Lernaean Hydra) are used for the treatment of systemic inflammatory, neoplastic or hematological diseases. These antibodies bind to the epidermal growth factor receptor and block receptor dependent signal transduction pathways such as anti-apoptosis, angiogenesis, and tumor metastasis. Other antibodies act against tumor necrosis factor TNF-α and are used for the treatment of chronic inflammatory diseases including rheumatoid arthritis, ankylosing spondylitis, Crohn's disease and systemic vasculitis. These chimeric monoclonal antibodies have been incriminated to underlie acute or chronic cardiac hypersensitivity reaction, including chest pain, hypotension, severe life-threatening anaphylaxis and Kounis hypersensitivity-associated acute coronary syndrome [13].

Other chemotherapeutic agents, such as capecitabine which is an orally available pro-drug converted to 5-fluorouracil within the neoplastic tissue used for the treatment of metastatic colorectal and breast cancer, may also induce hypersensitivity reactions. Cardiac manifestations such as angina, acute coronary syndrome, arrhythmias, myocarditis, and heart failure are known/common side effects induced by 5-fluorouracil use. In a report of capecitabine-induced cardiac arrest from ventricular fibrillation, immunological markers indicated a type I Kounis hypersensitivity-associated syndrome as the underlying pathophysiological mechanism [19].

The majority of chemotherapeutic drugs are able to induce hypersensitivity reactions primarily of anaphylactic type I but also of types II, III, and IV [1]. Severe and lethal reactions have also occurred [1]. In addition, there are reports indicating specific allergic tests, especially in atopic and susceptible patients, and several colleagues have already performed such tests successfully [20].

THE CARDIO-ONCOIMMUNOLOGY DISCIPLINE

All of the above suggest that an emerging medical discipline termed cardio-oncoimmunology should encompass all scientific knowledge regarding the cardiovascular effects of chemotherapeutic. In this context the interdisciplinary cooperation among cardiologists, oncologists, hematologists, cardiac imaging specialists, immunologists, pathologists, allergists together with other medical professionals involved in cancer care seems to be of paramount importance. Furthermore, the need to incorporate several tests, measures, and actions before, during and long after chemotherapy in order to monitor for cardiac adverse events should be pursued. Several disciplines should be integrated in order to identify, diagnose, prevent, and treat cardiovascular complications associated with chemotherapy. We believe that cardio-oncology, onco-cardiology, immuno-oncology, and onco-immunology should had already been merged into a single discipline, that of cardio-oncoimmunology.

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Intermittent Claudication (IC) in young adults

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Intermittent claudication (IC) is a symptom defined as reproducible fatigue, discomfort, or pain that occurs mainly in calf due to ischemia. It is a common symptom in older patients suffering from atherosclerotic peripheral vascular disease. However, intermittent claudication in young patients should prompt a search for causes other than atherosclerosis. The differential diagnosis includes well-known entities such as popliteal artery entrapment syndrome, cystic adventitial disease, fybromuscular dysplasia, Takayasu arteritis, Buerger disease and arterial compression by tumor.

Popliteal artery entrapment syndrome (PAES) is the most common cause of intermittent claudication in young patients. Its overall incidence ranges from 0.17% to 3.5% in the general population. Furthermore, the majority of patients (85%), are males, with almost 60% of cases occurring in young athletes during the third decade of life. In about 30% of patients, the disease has a bilaterally symptomatic presentation [1,2]. The term popliteal artery entrapment refers to popliteal artery compression caused by an abnormal anatomical relationship between the vessel and nearby musculotendinous structures or surrounding muscle hypertrophy. This arterial compression which initially causes intermittent claudication, might lead to chronic vascular microtraumas of the arterial wall with possible intramural hematoma or thrombus, distal embolization, aneurysm, dissections and thrombosis with acute distal ischemia [3]. PAES is further classified into six different types based on the relationship of the medial head of the gastrocnemius muscle with the popliteal artery [4]:

- Type I: An aberrant medial course of the popliteal artery around a normally positioned medial head of the gastrocnemius muscle (MHG)
- Type II: MHG attaches abnormally and more laterally on the femur causing the popliteal artery to pass medially and inferiorly
- Type III: Abnormal fibrous band or accessory muscle arising from the medial or lateral condyle encircling the popliteal artery
- Type IV: Popliteal artery lying in its primitive deep or axial position within the fossa, becoming compressed by the popliteus muscle or fibrous bands
- Type V: Entrapment of both the popliteal artery and vein due to any of the causes mentioned above
- Type VI: Muscular hypertrophy, resulting in a functional compression of both the popliteal artery and vein

The diagnosis of popliteal artery entrapment syndrome requires not only the depiction of the arterial changes but also the identification of the abnormal anatomic structures responsible for the entrapment. Although arterial compression can be shown on conventional angiography or ultrasonography with provoked maneuvers, such us plantar flexion or dorsiflexion, the underlying anatomic abnormality cannot be identified on either modality. Tailored MRI and MR angiography can reveal the abnormal muscular or fibrous attachment and the arterial findings necessary for diagnosis and surgical planning.

Treatment of PAES requires surgical release of the popliteal artery occasionally followed by a by-pass through the interposed saphenous vein graft in case that the popliteal artery is affected.

Cystic adventitial disease (CAD) is a rare vascular disorder. CAD predominantly affects the arteries,

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although rare reports of CAD of the veins have also been described. The majority (85%) of the cysts in CAD is found in the popliteal artery, but the disease may affect the external iliac, femoral, radial, ulnar, brachial, and axillary arteries as well. Bilateral disease has also been described [5]. CAD predominantly occurs in young to middle-aged population; however, the age of presentation ranges from 11 to 70 years old. CAD has a male predisposition, with a male-to-female ratio of 5:1 [6]. Various theories have been proposed for the origin of CAD, including a systemic disorder, repetitive trauma, and a persistent embryonic synovial track. Symptoms are caused by compression of the arterial lumen by a cystic collection of mucinous material inside the adventitia of the artery. The pain insists for as long as 20 minutes after the cessation of activity, in contrast to the rapid relief of pain that most patients with PAD-associated limb discomfort experience. CAD-related intermittent claudication may vary in clinical presentation or even disappear for several months and reappear without any clear inaugural event. On physical examination, peripheral pulse may or may not be absent at rest. The Ishikawa's sign can be seen in CAD, which is the disappearance of the foot pulses with flexion of the knee. This differentiates CAD from the popliteal entrapment syndrome, where the pulse would disappear with contraction of the gastrocnemius muscle during active plantar flexion or passive dorsiflexion of the foot. Conventional angiography typically reveals a smoothly tapered narrowing of the mid-popliteal artery with otherwise normal-appearing lower extremity arteries. Ultrasonography, CT, or MRI may also show the cystic structure in the arterial wall. A number of techniques exist for the treatment of CAD, including surgical intervention, percutaneous aspiration, and percutaneous endovascular intervention. However, the decision to treat should be based on clinical and radiological presentations. Conservative treatment of CAD should be considered first, as the cysts in CAD may resolve spontaneously, or the patient may not experience significant discomfort to prompt for more invasive treatment options [7].

Endofibrosis of the iliac artery is another rare cause of arterial stenosis and intermittent claudication in young adults. It is reported most often in highly functioning and competitive cyclists and runners [8]. It is considered to result from repetitive trauma, predominantly of the external iliac artery. Symptoms include IC and a sensation of swelling or paresthesia in the proximal lower limb at the time of maximum exercise. Physical examination may be normal at rest, although a bruit may be heard over the ipsilateral pelvic fossa or inguinal region [9].

Fibromuscular dysplasia (FMD) is a noninflammatory, nonatherosclerotic arterial disease that occurs most commonly in women 20 to 60 years of age. The true prevalence of FMD is unknown. The renal arteries and the extracranial carotid arteries are mostly affected. However, less commonly, FMD may affect the iliac, femoral, or popliteal arteries. Lower-extremity involvement may result in IC, microembolisms, or (rarely) critical limb ischemia via dissection or rupture of the artery [10]. FMD is divided into several types according to which arterial layer is affected and by the arteriographic pattern of disease: medial fibroplasia, intimal fibroplasia, and adventitial (periarterial) hyperplasia. Medial fibroplasia is the most common type [11]. Angiographic studies will show a string-of-beads appearance reflecting multiple adjacent stenosis and focal aneurysm. Treatment of FMD depends on the location and the extension of the stenosis and includes by-pass surgery or endovascular treatment.

Takayasu arteritis, most commonly seen in young Asian women, is a chronic inflammatory arteritis affecting large vessels, predominantly the aorta and its main branches. Vessel inflammation leads to wall thickening, fibrosis, stenosis, and thrombus formation. Intermittent claudication as a result of aorta involvement, is a common symptom. The disease commonly emerges in the 2nd or 3rd decade of life [12]. Although the disease mostly affects the female population, the female:male ratio seems to decline from Eastern Asia to the West. Conventional angiography is the standard imaging tool in the evaluation of Takayasu's arteritis, showing nonspecific focal stenoses. Whereas CT angiography, MRI, and MRA may show mural thickening in addition to narrowing of the lumen. Steroids are the mainstay of treatment with acceptable results. Surgery is recommended when the disease is in remission to avoid complications, which include restenosis, anastamotic failure, thrombosis, haemorrhage and infection. Endovascular treatment is another option [13,14].

Thromboangiitis obliterans or Buerger's disease is a segmental occlusive inflammatory condition affecting small and medium sized arteries and veins of the upper and lower-extremities in heavy smokers [15]. The onset of Buerger's disease occurs between 40 and 45 years of age, and men are most affected. Several different criteria have been proposed for the diagnosis of thromboangiitis obliterans. The most accepted are the criteria of Olin [16]:

- age under 45 years
- current or recent history of tobacco use
- the presence of distal-extremity ischemia indicated by claudication, pain at rest, ischemic ulcers or gangrenes and documented by non-invasive vascular testing
- exclusion of autoimmune diseases, hypercoagulable states and diabetes mellitus
- exclusion of a proximal source of emboli by echocardiography or arteriography
- consistent arteriographic findings in the clinically involved and non-involved limbs

Conventional angiography shows multilevel occlusions and segmental narrowing of the lower extremity arteries with extensive collateral flow, which has a characteristic corkscrew or "tree root" appearance. The only effective treatment of intermittent claudication in Buerger's disease is smoking cessation.

Extrinsic artery compression by a tumor such us an osteochondroma, lipoma etc. is an extremely rare cause of intermittent claudication. Most reported cases of extrinsic compression are in veins, which have a thinner muscle layer in comparison to arteries and are consequently less resistant to vascular collapse. A radiograph of the affected extremity to exclude a bone lesion causing extrinsic compression of the nearby artery is of importance in patients with intermittent claudication but no signs of intrinsic vascular disease. Other imaging tests (CT or MRI) could complete the investigation [17].

In conclusion, intermittent claudication in young adults remains a diagnostic challenge. As it is mentioned above, many nosological entities may be responsible for this condition. In any case, the combination of thorough clinical examination and targeted imaging evaluation could lead to the cause of the intermittent claudication.

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Broad autism phenotype traits in parents of children with developmental disorders

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Abstract

Background: Autism spectrum disorder (ASD) is a chronic neurodevelopmental disorder characterized by high heritability and increased genetic and clinical heterogeneity. Research has shown that parents of ASD probands, commonly exhibit qualitatively similar yet milder manifestations of ASD, a set of traits which are known as the broad autism phenotype. The aim of the present study was to determine the presence of the broad autism phenotype in parents of children with developmental disorders including ASD and developmental language and speech disorders and detect potential associations.

Methods: Parents of children diagnosed with ASD, developmental language or speech disorder consecutively entered the study. The broad autism phenotype was assessed with the Broad Autism Phenotype Questionnaire (BAPQ).

Results: 60 parents were enrolled, 22 (36.7%) males, with a mean age of 40.23 years. Forty-six (76.7%) participants had a child with ASD, while 14 (33.3%) participants had a child with a developmental language or speech disorder. 21.7% of parents of developmentally impaired children reported clinically significant characteristics of the broad autism phenotype. Parents of children with developmental language and speech disorders scored significantly higher at the pragmatics sub-scale of the BAPQ (p=0.042) compared to parents of children with ASD. Furthermore, in the whole sample, there was a borderline tendency of fathers to report higher levels of social aloofness (p=0.082) compared to mothers.

Conclusion: The broad autism phenotype is present in over 20% of parents with children with developmental disorders. Parents of children with language or speech delay exhibit greater difficulties in pragmatic language skills compared to parents of ASD children, while fathers of developmentally impaired children appear more socially impaired.

Key words: *Broad autism phenotype; parents; developmental disorders*

INTRODUCTION

Autism spectrum disorder is a chronic neurodevelopmental disorder which is characterized by deficits in social communication and interaction and restricted, circumscribed behaviors and interests. There is significant variation in ASD clinical manifestations and autistic-like behaviors can be conceptualized as part of a continuum between neurotypicality and the full syndrome of infantile autism [1]. In a similar vein, underlying this clinical heterogeneity, research has revealed that ASD is a highly hereditable condition with a strong genetic component and multiple genetic loci have been implicated in its pathogenesis [2]. Due to this genetic heterogeneity, studying the genetic background of the disorder represents a challenging, almost impossible task and researchers have turned to the quest of endophenotypes in order to obtain a better understanding of ASD genetic basis [1]. Endophenotypes are

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stable, heritable, measurable traits lying in the middle between disease phenotype and its underlying genetics. They may include neurophysiological, biochemical, neuroanatomical, endocrinological, cognitive or neuropsychological markers which are more prevalent in unaffected relatives of diagnosed patients and their presence reflects increased genetic vulnerability [4].

Research and clinical practice have shown that autism runs in families and sub-clinical autistic-like symptoms are highly prevalent in first-degree relatives of autistic probands. This constellation of behavioral, cognitive and linguistic characteristics represent milder but qualitatively similar to ASD symptoms manifestations and are known with the term broad autism phenotype (BAP). BAP manifestations include social communication deficits, deviant cognitive processing, persistent interests, rigid and aloof personality. BAP is highly prevalent in first-degree relatives of ASD patients, affecting 14-23% of parents of ASD children, while in the general population BAP prevalence hardly reaches 9% [5].

In this context, the aim of the current study was to determine the presence of BAP in a sample of parents with developmentally impaired children, including children with ASD, developmental language or speech disorders, and detect potential associations.

MATERIALS AND METHODS

The current cross-sectional controlled study was conducted at the Day Centre for Children with Autism Spectrum and other Developmental Disorders in Messolonghi, Greece, between January 2019 and July 2019. Parents of children referred to the Day Centre were approached and invited to participate after being thoroughly informed about the aim and methodology of the study. All children had undergone detailed evaluation by the centre's child-psychiatrist and had been diagnosed with autism spectrum disorder (ASD), developmental language or speech disorder according to the DSM-V diagnostic criteria. All participants provided their informed consent prior to study entry.

The presence of BAP was evaluated with the Broad Autism Phenotype Questionnaire (BAPQ) which is a Likert-like dimensional scale with 36-items. It is a reliable screening tool that assesses particular personality and language characteristics along three dimensions - aloof personality, rigid personality, and pragmatic language skills [6]. The questionnaire was translated in the Greek language by two members of the research team. All parents were asked to complete the self-report version of the BAPQ. In addition, participants' demographic data were collected through interview.

Statistical analysis was performed with the SPSS package (RELEASE 17.0). Well-validated cut-off scores [7] were used to detect clinically significant traits of the BAP and chi-square tests were calculated to detect potential differences in the percentage of participants reporting clinically significant BAP characteristics between the two parental groups and between males and females.

RESULTS

Sixty (60) parents provided their consent and entered the study, 22 (36.7%) males, with a mean age of 40.23 years (range:26-55). Forty-six (76.7%) participants had a child with ASD, while 14 (33.3%) participants had a child with a developmental language or speech disorder. Table 1 provides total BAPQ and sub-scales scores and percentages of clinically significant BAP traits for the whole sample and by disease and gender group. 21.7% of all participants reported clinically significant characteristics of the broad autism phenotype.

A greater percentage of parents of children with developmental language and speech disorders scored above the clinical cut-off at the pragmatics sub-scale of the BAPQ (19% vs 50%, p=0.042) compared to parents of children with ASD (Figure 1). In contrast, no significant differences were observed in the percentage of parents who scored above the clinical cut-off at the total BAPQ scale (22.2% vs 20.0%, p=0.880) and the aloof (23.3% vs 14.3%, p=0.475) and rigid (41.5% vs 46.2%, p=0.766) sub-scales (Figure 2). Subsequently, comparisons between mothers and fathers in the whole sample failed to reveal any significant differences in the percentage of parents who scored above the clinical cut-off at the total BAPQ scale (22.2% vs 21.4%, p=0.949) and at the rigid (52.4% vs 36.4%, p=0.246) and pragmatics (15.0% vs 31.2%, p=0.188) sub-scales (Figure 3). However, there was a borderline tendency of fathers to score above the clinical cut-off at the social aloofness sub-scale at a higher percentage (33.3% vs 13.9%, p=0.082) compared to mothers (Figure 4).

DISCUSSION

The present study's findings suggest that over 20% of parents of children with developmental disorders exhibit clinically relevant characteristics of the broad autism phenotype. This finding corroborates existing literature given that several studies have shown that BAP traits can be detected at 14-23% of parents of children with ASD

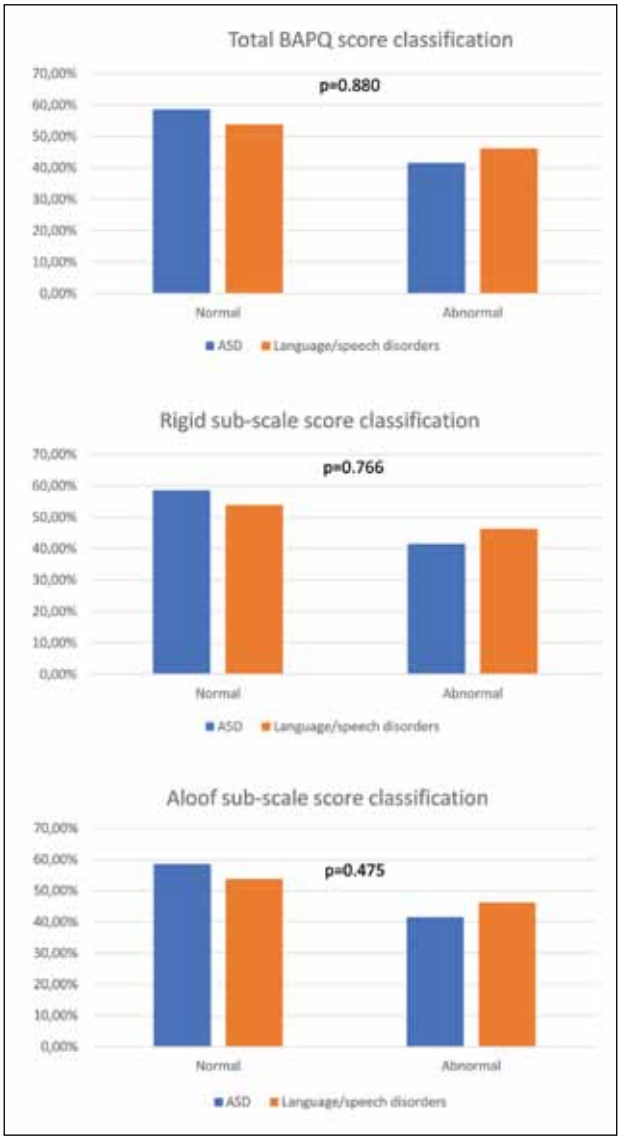

Figure 1. Comparison of the percentage of participants scoring above the clinical cut-off at the total BAPQ, aloof and rigid sub-scales between the two parental groups.

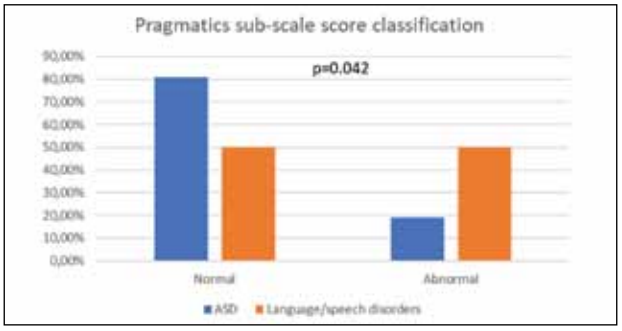

Figure 2. Comparison of the percentage of participants scoring above the clinical cut-off at the pragmatics sub-scale between the two parental groups.

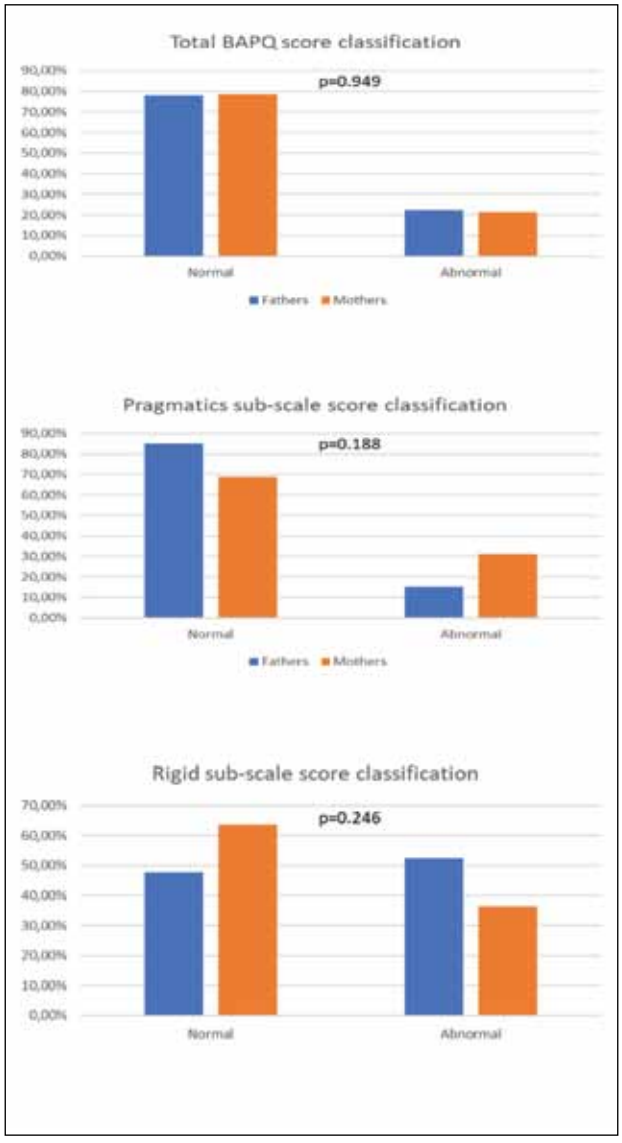

Figure 3. Comparison of the percentage of participants scoring above the clinical cut-off at the total BAPQ scale and the pragmatics and rigid sub-scales between mothers and fathers.

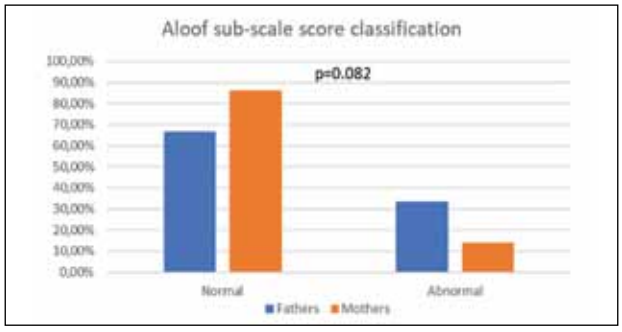

Figure 4. Comparison of the percentage of participants scoring above the clinical cut-off at the aloof sub-scale between mothers and fathers.

Table 1. *Total BAPQ and sub-scales scores and percentages of clinically significant BAP traits for the whole sample and by disease and gender group.*

		All participants	ASD parents	Language/speech disorders parents	Fathers	Mothers
N		60	46	14	22	38
Total BAPQ	Mean, (SD)	2.42 (0.62)	2.40 (0.63)	2.49 (0.60)	2.43 (0.57)	2.41 (0.66)
	% of clinical cases	21.7	22.2	20.0	22.2	21.4
Aloof sub-scale	Mean, (SD)	2.39 (0.76)	2.41 (0.79)	2.32 (0.69)	2.50 (0.84)	2.32 (0.71)
	% of clinical cases	21.1	23.3	14.3	33.3	13.9
Pragmatics sub-scale	Mean, (SD)	2.12 (0.70)	2.04 (0.66)	2.46 (0.79)	1.98 (0.63)	2.22 (0.73)
	% of clinical cases	25.0	19.0	50.0	15.0	31.3
Rigid sub-scale	Mean, (SD)	2.76 (0.69)	2.72 (0.74)	2.85 (0.54)	2.92 (0.67)	2.65 (0.69)
	% of clinical cases	42.6	41.5	46.2	52.4	36,4

[5]. In addition, our analysis revealed that parents of ASD children reported similar levels of social difficulties and rigid personality compared to parents of children with developmental language and speech disorder, while the latter parental group exhibited greater impairment in pragmatic language skills. To our knowledge, most relevant studies focusing on the BAP, have compared parents of ASD children with parents either of typically developing children or of children with Down syndrome [5]. Only two studies have included a group of parents whose children had been diagnosed with specific language impairment. An earlier study [8] showed that parents of ASD children had better language skills compared to parents of children with specific language impairment, a finding which is in our accordance with the present study's results. In contrast, another study reported that parents of ASD children demonstrate greater deficits in pragmatic language compared to parents of children with specific language impairment [9]. Research and clinical practice have shown that there may be significant overlap between ASD and developmental language disorders and that there are yet unspecified genetic and phenotypic associations between these two diagnostic categories [10,11]. Our findings provide support to the concept of a shared genetic background between ASD and language impairment and raise the question of whether pragmatic language deficits constitute part of the autism endophenotype or represent a broader developmental vulnerability. The associations between parental BAP traits and specific types of developmental delays are too far from being conclusive and further studies on the issue are warranted.

Moreover, the present analysis revealed that fathers of developmentally impaired children have the tendency to suffer from greater social difficulties compared to mothers, corroborating earlier findings regarding the presence of sexual bimorphism in BAP characteristics [12,13]. Baron-Cohen et al [12] formulated and empirically supported the extreme male brain theory in order to explain autism pathogenesis. There is evidence suggesting that fathers of ASD children are more socially aloof while mothers display more rigid personality characteristics [13]. In a similar vein, a recent study revealed that fathers of ASD children report higher scores in the aloof sub-scale of the BAPQ compared to mothers, while no other significant between-gender differences were observed [6].

The current findings should be treated with caution, taking into consideration certain limitations, namely the small study sample and the absence of informant-report data given that the BAPQ was not administered to spouses or partners.

In conclusion, the present investigation detected clinically relevant BAP characteristics in a significant proportion of parents with developmentally impaired children including ASD and language and speech impairment and provided useful information regarding BAP's associations with the type of developmental delay and parental gender. Studying these distinctive behavioral traits may provide valuable information regarding the genetic background of developmental disorders and could also reveal parental behavioral and cognitive vulnerabilities which should be addressed through counseling and psychoeducation.

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RESEARCH ARTICLE ACHAIKI IATRIKI | 2020; 39(1):23–28

Prevalence of anti-HCV antibodies and risk factors among prison inmates in Southwestern Greece

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Abstract

Introduction: Hepatitis C virus (HCV) infection is a major issue of public health worldwide. Prisoners constitute a social group with a high risk of HCV infection. In our study, we aimed to estimate the prevalence of anti-HCV antibodies in prisoners at a detention center in Southwestern Greece.

Materials and Methods: Participants, recruited from Detention Center of Patras, Greece, were tested for HCV antibodies using Line ImmunoAssay (Inno-LIA HCV Ab) and filled out a questionnaire with demographic data and risk factors for HCV infection. Prison inmates who were anti-HCV positive, participated in the study.

Results: In total, 535 of all eligible prisoners consented to participate. The prevalence of anti-HCV positive prisoners was 14.95% (80/535). Anti-HCV positive prisoners had a mean age of 40.33 years (range 22-56). Risk factors, associated with HCV transmission among prisoners were intravenous drug use, tattooing and unprotected sexual intercourse.

Conclusion: A high prevalence of anti-HCV antibodies was observed in adult prisoners in Southwestern Greece and the majority of prisoners had developed behaviors of high HCV transmission risk prior to incarceration. Promotion of HCV screening and therapeutic interventions will improve management and prevention of HCV infection in prisons.

Key words: *Hepatitis C; infection; prevalence; prisoners*

INTRODUCTION

Hepatitis C virus infection (HCV) is a major public health issue, distributed in different countries worldwide. A systematic review of HCV epidemiology by Petruzziello et al. showed that the global prevalence of HCV is 2,5%, which refers to 177,5 million of HCV infected adults, and a global viraemic rate of 67%, which represents HCV chronically infected individuals out of all HCV infected individuals [1]. According to the 2017 WHO report, more than 70 million people globally were infected by the HCV in 2015 and developed chronic hepatitis C. HCV prevalence varies in different regions with higher rates reported in the Eastern Mediterranean Region (2,3%)

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and the European Region (1,5%) [2]. Moreover, a great increase in HCV incidence has been observed between 2005 (10.000 cases per year) and 2016 (40.000 cases per year) [3]. However, other systematic epidemiological studies, including data from different time periods and geographic regions, reported a lower HCV prevalence in high-income compared to low-income countries [1, 4]. Regarding the proportion of HCV infected individuals in Greece, it has been reported that almost 74,000-134,000 Greek patients have chronic hepatitis C [5]. A study about the epidemiology of the HCV infection in Greece showed significant changes in HCV genotype distribution and mode of HCV transmission during the last three decades. In particular, a shift from genotype 1 to genotype 3 has been recorded, accompanied by an increased HCV prevalence in intravenous drug users [6].

Hepatitis C is a severe infection, given that a significant proportion of HCV infected patients may develop chronic liver diseases such as hepatitis, liver cirrhosis, hepatocellular carcinoma (HCC) and face increased mortality risk [7]. Approximately 399.000 HCV-related deaths were recorded by WHO in 2016, the majority of which were associated with chronic liver diseases [2]. Progressive, HCV-related liver disease has also been a major indication for liver transplantation [8]. In this context, chronic HCV infection represents a great health and economic burden, as regular monitoring and medical interventions are required. Although new antiviral agents have been developed, demonstrating high efficacy in eliminating HCV [9], further research on novel therapeutic strategies, interventions, vaccination and diagnosis is still ongoing. A WHO report regarding HBV and HCV elimination by 2030, highlights the need of universal access to diagnosis and treatment, combination of treatment with prevention, treatment of HBV and HCV coinfection among HIV infected individuals, as well as improvement of safety policies and prevention (injection and blood safety, vaccination) [10].

There are certain social groups which constitute high-risk populations, taking into account the transmission route of the virus (drug users, babies born to HCV mothers, sexual partners of HCV or HIV patients, tattoos and piercings) [11]. Prisoners run an increased risk for the HCV infection compared to other populations, probably due to their living conditions which predispose to behaviors that favor HCV transmission [12, 13]. According to a meta-analysis of HCV prevalence in prisons worldwide, for the time period 2005-2015, HCV prevalence was higher (2,4%) among prisoners in Central Brazil compared to the general population (1,38%) and HCV exposure was significantly higher in males (2,7%) than females (0,6%) [14, 15]. Another systematic review reported that globally 2,2 million prisoners are anti-HCV positive and a significant increase in the prevalence of infection was observed in detainees who have been drug users [12]. Taking into consideration that more than 10 million people are being held at detention centers worldwide, the study of prevalence of infectious diseases, like hepatitis C in this social group is of high epidemiological importance [16]. In parallel, improvement of HCV management in prisons is quite difficult due to the limited data regarding HCV prevalence and the number of HCV-infected prisoners that need treatment [17].

Prisoners' attitude regarding the knowledge and treatment of HCV-related liver diseases constitutes an important barrier to HCV management. Specifically, a major proportion of detainees have no knowledge about the HCV or the transmission route of the virus and they are afraid of screening, treatment or being stigmatized [18]. In our study, we aimed to estimate the prevalence of anti-HCV antibodies in prisoners in Southwestern Greece and record patients' demographic characteristics and lifestyle habits.

MATERIALS AND METHODS

Study participants

In this prevalence study, a total of 535 male adults, held at Detention Center of Patras, Greece were initially enrolled to be examined for anti-HCV antibodies. Prisoners' recruitment and baseline examination were performed at the University Hospital of Patras (Greece). After getting baseline values and recording the complete medical history of each participant, the prisoners were tested for the presence of HCV antibodies (anti-HCV). Management of study participants was conducted in accordance with the Declaration of Helsinki. Participants gave written informed consent and the study protocol and all relevant procedures were approved by Ethical Committee of the University Hospital of Patras.

Data Collection

Data regarding participants' demographic characteristics, lifestyle habits, HCV transmission risk factors and incarceration-related information were collected through the administration of a detailed questionnaire. Detection of serum anti-HCV antibodies was performed by Line ImmunoAssay (Inno-LIA HCV Ab).

Statistical analysis

Data analysis was performed by the statistical program IBM®SPSS Statistical Software® version 20, using descriptive statistics.

RESULTS

A total of 535 prisoners were recruited to participate in the study and were evaluated for the presence of HCV antibodies. 80 (14.95%) prisoners were anti-HCV positive and 63 [median age 40.33 years (22-56)] eventually agreed to participate in the study. The remaining 17, either refused to participate in the study or were released or transferred to other detention centers, hence being excluded from the study. Forty-five (45) prisoners (71,4%) had the Greek nationality, 26 prisoners (41,3%) attended secondary education and 31 prisoners (49,2%) had full-time employment prior to incarceration. All participating prisoners had been admitted to detention center more than once in the past. The major causes of incarceration were burglary (n=26, 41.3%) and drug possession/trafficking (n=41, 65.1%). 30 prisoners (47.6%) had received tattoos prior to incarceration and 34 prisoners (54%) had tattooed after incarceration (Table 1).

Unprotected sexual intercourse was reported by 62 prisoners (98.40%) and 8 (12.7%) prisoners had unprotected intercourse with HCV positive partners. Needle sharing was reported by 27 prisoners (42.9%) and 21 (33.30%) had a history of alcohol abuse prior to incarceration. Two (2) prisoners (3.2%) reported that they engaged in homosexual relationships while 3 (4.8%) reported a history of blood transfusion (Figure 1).

Table 1. *Allocation of demographic characteristics among participants.*

Demographic Characteristics	n (%)
Nationality	
Greek	45 (71.4%)
Other	18 (28.6%)
Level of Education	
Secondary Education	26 (41.3%)
Other	37 (58.7%)
Cause of incarceration	
Burglary	26 (41.3%)
Drug possession/trafficking	41 (65.1%)
Tattooing	
Prior to incarceration	30 (47.6%)
After incarceration	34 (54%)

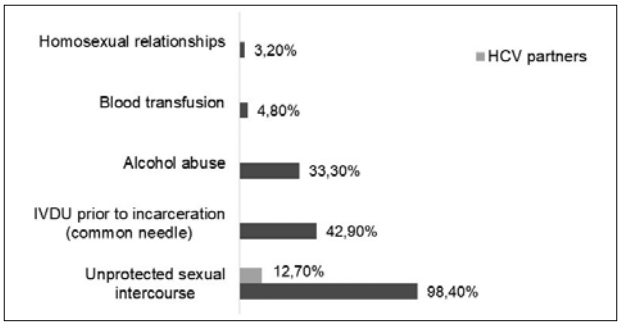

Figure 1. Rates of lifestyle characteristics among participants

DISCUSSION

Great variance in HCV prevalence among specific sub-populations has been reported in several European countries. Many studies have focused on estimating HCV prevalence rates in the general population, but there is limited information about the frequency of HCV infection among certain groups of people, like prisoners, and potential risk factors [19, 20]. The highest prevalence rates of anti-HCV antibodies have been recorded in drug users, followed by prisoners, HIV positive men who have sex with men, pregnant women and first-time blood donors [21].

In our study, we aimed to estimate the prevalence of anti-HCV antibodies in prisoners at Detention Center in Southwestern Greece and record patients' demographic characteristics and lifestyle habits. Determining the prevalence of anti-HCV antibodies in prisoners is of high epidemiological importance as prisons are characterized by behaviors that promote HCV transmission (tattooing, unprotected sexual intercourse, homosexual relationships) and the number of prisoners is significantly increased worldwide [16]. In parallel, many prisoners may have adopted behaviors of high HCV risk prior to incarceration, a fact that favors HCV transmission. The majority of the 1.8 million prisoners in the United States were HCV infected prior to incarceration [22] and HCV infected prisoners in a prison state of Mexico continued to engage in high-risk behaviors at a higher frequency compared to non-infected prisoners during incarceration [23].

Taking into account the existence of various barriers to the control of HCV infection in prison settings, further investigation of HCV prevalence among prisoners needs to be conducted. Some of the factors which may impede early therapeutic intervention and HCV infection control in prison are short prison sentences, regular interprison transfers that interrupt treatment, prisoners'

behavior, lack of knowledge regarding HCV screening, therapy and disease transmission and economic issues including the high cost of available treatment and low healthcare resources in detention centers [24]. In general, low socioeconomic status has been highlighted as a risk factor for HCV infection and has been associated with poor prognosis [25]. Thus, determining the prevalence of anti-HCV antibodies in prisoners will provide useful guidance for more efficient management of HCV infection in this environment.

The prevalence of anti-HCV antibodies in prisoners was estimated at 14.95% in our study, an extremely higher percentage than the observed rates of 0.83%-1.79% and 2.5% in the Greek and global general population respectively [1, 5]. This finding corroborates an earlier study by Moradi et al. regarding the worldwide prevalence of HCV in prisons, which was estimated at 13.22%. The highest HCV prevalence rates, 26.4% and 24.26%, were recorded in Australia and the Southeast Asia region, respectively [15].

Regarding the prisoners' demographic characteristics and lifestyle behaviors, the majority of participants reported behaviors that promote HCV transmission, as they were incarcerated due to drug possession/trafficking, almost half of them had tattooing prior to incarceration and a small trend of increase was observed in the rate of tattooing after incarceration. A high rate of unprotected sexual intercourse was reported by prisoners in our study and a small percentage of those had HCV partners. Almost half of prisoners were drug users (sharing needles) prior to incarceration and 1/3 of participants reported alcohol abuse. A small percentage of prisoners had a history of homosexual relationships and blood transfusion.

The present findings are in line with those reported by previous epidemiological studies. In particular, intravenous drug use has been shown to be an independent prognostic factor for HCV infection among prisoners at prison of Brazil [26]. Soholm et al. also found that intravenous drug use, along with older age (>40 years) and long duration of incarceration (>10 years) constitute risk factors of HCV infection among prisoners at Danish prisons. An observed decline in overall incidence of HCV infection during the last 20 years has been associated with a decrease in the prevalence of intravenous drug use among Danish prisoners, especially those of younger age (<35) [27]. Exposure to HCV was high among prisoners of Iran who had history of drug use, age over 30 and tattooing [28]. Older age was a main characteristic in our study sample, as the mean age of participated prisoners was 40.33 years. A cross sectional, anonymous survey conducted by Ramamoorthy et al. in prison inmates in India reported a high rate of unprotected sexual intercourse (81%), as a main characteristic among prisoners, which is consistent with the high rate (98,4%) reported in our study. Ramamoorthy et al. also showed that significant risk factors among HCV positive prisoners were intravenous drug use, frequent commercial sex worker visitor and homosexual behavior [29].

We must consider some limitations in estimating the prevalence of anti-HCV positive prisoners in combination with recorded demographic and other characteristics in our study. Specifically, the research was limited to only one prison in a wider region of Southwestern Greece. Study on gender distribution among prisoners was not possible as all participants were males and the duration prior to anti-HCV positive diagnosis was unknown. Thus, further cross-sectional surveys and epidemiological studies need to be conducted in the field of HCV prevalence in prisons, including data derived from many prisons in every studied geographical region. Significant efforts should be made on investigating the association of risk factors with HCV prevalence in order to design effective screening and intervention strategies in prisoner groups.

A high prevalence of anti-HCV positive prisoners was recorded in Southwestern Greece. The majority of prison inmates had developed behaviors of high HCV transmission risk prior to incarceration, a finding that highlights the need for regular monitoring of prisons regarding the HCV transmission risk. Investigation of HCV epidemiology and risk factors among prisoners will provide valuable information to set up the appropriate policy and clinical guidance, in targeting HCV infection in prisons. Promotion of HCV screening and therapeutic interventions, characterization of prisoners according to their demographic features and their lifestyle will improve management of HCV infection in prisons. Understanding the epidemiology and all the characteristics that may affect HCV transmission and infection in prison could be one crucial step in designing prevention and treatment interventions, leading to better disease control.

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The biology of SARS-CoV-2 and the ensuing COVID-19

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Abstract

Recently there has been a worldwide concern about the novel coronavirus SARS-CoV-2 which originated in China but rapidly spread internationally posing a global health emergency. As a Betacoronavirus (βCoV), SARS-CoV-2 is a positive single-stranded RNA virus and infects the respiratory tract. The ensuing disease has been named COVID-19. Genome structural analyses of SARS-CoV-2 have revealed genomic similarities but low evolutionary relationship to existing SARS. The S glycoprotein is vital for cell adhesion and virus entrance to host cells. Cell entry depends on ACE2, as already described for βCoVs, but recent studies proposed a newly discovered receptor, CD147. Genome RNA translation encodes structural and unstructural proteins starting with ORF1a and ORF1ab which produce non-structural proteins (nsps) with different functions. Although nsps are conserved among βCoVs, mutations in nsp2 and nsp3 may play an important role in viral transmission and cell and tissue tropism. Currently, no vaccine or specific antiviral treatments are available for COVID-19. As a result, preventive measures are the main strategy to limit the spread of the virus.

Key words: *COVID-19; SARS-CoV-2; genome structure; viral entry; ACE2, therapy*

INTRODUCTION

The 2019 novel coronavirus (2019-nCoV) was recently named SARS-CoV-2 by the World Health Organization (WHO). The disease caused by SARS-CoV-2 has been named COVID-19 [1]. The origin of the novel coronavirus is not known with certainty. There are those who claim that SARS-CoV-2 originated through laboratory manipulations. However, the study of coronaviruses implicates the use of reverse genetic systems using BACs, in vitro ligation or vaccinia virus vectors to study virus RNA replication [2]. Genetic and structure analyses among sequences derived from different existing CoVs indicate that SARS-CoV-2 is a novel coronavirus which originated due to natural selection either in an animal host before zoonotic transfer or following zoonotic transfer [3]. Mostly, it is believed that bats and palm civets are the natural reservoirs for SARS-CoV, and it was reported that SARS-Cov-2 infected humans in an animal market [4].

Coronaviruses cause respiratory and intestinal infections in humans. Human-to-human transmission of SARS-CoV-2 has been confirmed. Its sequence (MN908947) that was released by GenBank, is 96% identical to a bat coronavirus [5-7]. RNA viruses have an average evolutionary range of 10^{-4} nucleotide substitutions per site per year. This means that alterations to genome sequence arise during each replication site [8]. Coronaviruses are enveloped positive single-stranded RNA viruses ((+)RNA) with a genome of 27-32

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kb. They are classified into four genera: Alphacoronavirus (αCoVs), Betacoronavirus (βCoVs), Gammacoronavirus (γCoVs) and Deltacoronavirus (δCoVs), that share common ancestors and genomic structures. Evolutionary analyses have shown that bats and rodents are the gene sources of most αCoVs and βCoVs whereas avian species are the gene sources of most δCoVs and γCoVs [4]. SARS-CoV-2 belongs to the same family of viruses as the well-known severe acute respiratory syndrome coronavirus (SARS-CoV) and the Middle East respiratory syndrome coronavirus MERS-CoV and more precisely to Betacoronavirus, lineage b [6, 9, 10]. Virions have average diametres of 80-160 nm. Structural analysis has revealed genomic similarity between the three virus genomes. Genome phylogenetic analyses have revealed a greater similarity of 2019-nCoV to SARS-CoV than MERS-CoV [11]. Nevertheless, because SARS-CoV and MERS-CoV showed approximate similarities of 79% and 50% with SARS-CoV-2, respectively, that are considered low evolutionary relationships, SARS-CoV-2 is considered a novel human Betacoronavirus [8]. Currently, diagnosis is based on robust molecular techniques such as Real-Time PCR by detecting amplicons for the most preserved viral genes [12]. Although, to date, there is no available treatment for COVID-19 or a vaccine against the virus SARS-CoV-2, anti-viral and anti-inflammatory therapies used in other diseases are being tried. Drugs that have been designed for other viruses, such as Ebola or HIV-1, and treatment regimens for other diseases such as malaria, are being tested in COVID-19 patients with promising results [13-16].

GENOME STRUCTURE

SARS-CoV-2 genome consists of a single positive-strand RNA of almost 29,900 nucleotides and 38% G+C content, encoding 9,860 amino acids. Genome characterization showed two flanking untranslated regions, the 5'UTR -265 nucleotides- and the 3' UTR -358 nucleotides-long [4]. The genomic RNA has a 5' cap and a poly-A 3' tail and numerous open reading frames (ORFs). The virus replicase is encoded by two large ORFs, ORF1a and ORF1b. This RNA codes for both structural and unstructural proteins with different functions. Structural proteins are encoded by the 3'-terminus and include envelope glycoprotein spike (S), envelope (E), membrane (M) and nucleocapsid (N) protein [17, 18]. The 5'-terminal of the genome consists of accessory genes that are species-specific and encode polyproteins pp1a and pp1b. Polyprotein pp1a is further divided into nonstructural proteins that participate in genome transcription and replication [4, 6, 19]. Genome structure analysis revealed a main difference between lineage A β-CoVs and SARS-CoV-2 because the latter lacks the hemagglutinin-esterase gene in the 3'-terminus [4]. Structural proteins M, N, and E of SARS-CoV-2 showed a similarity over 90% to the known coronaviruses. However, the reference sequence-based analysis confirmed a reduced genetic similarity of the S protein [8]. Although great genome similarities are observed throughout the Betacoronaviruses, it is known that the gene that encodes for the spike glycoprotein is the least conserved, with genome identity reaching 74-83%. Full-length genome analysis showed that the S gene of SARS-CoV-2 is longer than in other SARSr-CoVs. There are three short insertions in the N-terminal domain and alterations in four of five of the residues in the receptor-binding motif. The role of the spike glycoprotein is to form spikes on the surface of coronaviruses and is responsible for the entrance of the viruses into the host cells [6, 18].

CORONAVIRUS SARS-COV-2 STRUCTURE AND MAIN STRUCTURAL PROTEINS

Coronaviruses are spherical with spikes on the surface. Recently it has been argued that not all coronaviruses encode for the same structural proteins for a complete virion [20]. SARS-CoV-2 in comparison to other viruses of the same family lacks hemagglutinin-esterase (HE) protein. The protein that has been studied most is the S-spike glycoprotein. It is a type I transmembrane protein and consists of a large ectodomain, a single-pass transmembrane anchor and a short C-terminal intracellular tail. The role of that protein is well defined. S-spike glycoprotein is crucial for cell adherence and entry to the host cell [1, 8, 21]. However, the entry requires priming of the S protein. SARS-CoV-2 needs serine protease TMPRSS2 to cleave S into S1/S2 and S'; this was deduced because an inhibitor for TMPRSS2 could block viral entry [22]. The envelope (E) is a small transmembrane protein involved in the life cycle of the virus. It consists of three domains and functions as an ion-channeling viroporin. E contains a binding motif which acts as a protein-protein interaction module and is involved in host cell processes and SARS-CoV pathogenesis [20]. The M protein is a membrane glycoprotein that supports the viral envelope and is the most abundant structural protein. M consists of three transmembrane domains, can adopt two conformations and plays a key role in virion shape and size. M plays a key role in organizing

coronavirus assembly and interacts with some of the major structural proteins [20, 23]. Finally, the N protein binds to the RNA genome forming the helically symmetric nucleocapsid. Phosphorylation of protein residues by glycogen synthase kinase 3 (GSK3) activates the N protein. Inhibition of GSK3 in cells infected with SARS-CoV, resulted in a restriction of viral replication [17, 21].

ATTACHMENT AND ENTRY TO THE HOST CELL

Infection starts when the virus attaches to its cellular receptor on the surface of the host cell and is endocytosed. The trimeric S transmembrane protein is responsible for the virus attachment and entrance and consists of two subunits, S1 and S2, which have distinct roles. S1-ectodomain binds to the receptor and initiates a structural change to the S2 subunit, which is essential for membrane fusion between the virus envelope and the host membrane. The activation of the S protein requires sequential cleavage by endosomal proteases. For example, cysteine protease cathepsin L activates the S protein in SARS-CoV [9, 21]. The S1/S2 cleavage of coronavirus S protein is mediated by host proteases at the time of infection. The SARS-CoV S1-protein contains a receptor binding domain (RBD) and implicates 14 amino acids, 9 of which recognize the angiotensin-converting enzyme 2 (ACE2) [24, 25]. The S2-protein consists of fused peptides, internal fusion peptides and a second proteolytic site S2' which is furin-like, that are totally preserved in SARS-CoV-2 compared to SARS-CoV. It is possible that since furin is highly expressed in lungs, an enveloped virus such as SARS-CoV-2, that infects the respiratory tract, could take advantage of this convertase to activate the S protein and enter the host cell [9], although previous experimental data suggest that insertion of a furin cleavage site at the S1/S2 domain enhances cell to cell fusion without affecting viral entry [26]. To note, ACE2 in not exclusively expressed by lung cells; it is therefore possible that SARS-CoV-2 could also spread to other cells and organs, as is the case with SARS-CoV [27].

ROLE OF HOST ACE2 IN VIRAL ENTRY - OTHER HOST RECEPTORS

The recognition of the host receptor is vital for viral entry. As previously mentioned, the S1 protein of the SARS-CoV recognizes the angiotensin-converting enzyme 2 ACE2. Many studies include structural analyses that predict that SARS-CoV-2 also identifies ACE2 as a host receptor [4, 25, 28]. The spike glycoprotein structure analysis of SARS-CoV has revealed an RBD with a core structure and a receptor-binding motif (RBM) that binds to ACE2. When analyzing the RBM of both SARS-CoV and SARS-CoV-2, neither deletions nor insertions were found. The only alteration predicted was a one-residue insertion away from the binding domain; other coronaviruses that do not use ACE2 lack these residues [22, 25, 29]. In vitro experiments have also determined ACE2 as a functional receptor for SARS-CoV-2 and additionally showed that the novel virus does not use aminopeptidase N (APN) or dipeptidyl peptidase 4 (DPP4) to enter the cell, as is the case for MERS-CoV [12, 18, 23]. In addition to ACE2, it is likely that other receptors exist for S binding on the host cell. CD209L, which is expressed in human lung alveolar and endothelial cells, was previously reported as a putative cell receptor for SARS-CoV [30], and could be tested for SARS-CoV-2. A recent study reported that the S protein can bind to the CD147 receptor on the host cell, based on in vitro experiments that demonstrated that an anti-CD147 humanized antibody significantly inhibited SARS-CoV-2 entry into the cells [31]. The identification of all the receptors and the exact mechanism SARS-CoV-2 uses for cell entry will aid the quest for antiviral targets.

ssRNA REPLICATION AND VIRION SECRETION

Once the virus manages to enter the host cell, the viral nucleocapsid is released to the cytoplasm. Due to the nature of their genome, viruses have to exploit the host's replication machinery. Single stranded RNA positive sense is the same sense as mRNA. Sense strand contains the exact nucleotide sequence to mRNA, which encodes for a functional protein. The genomic RNA serves as a template and polyproteins pp1a and pp1ab are at first encoded and cleaved by virally produced chymotrypsin-like protease (3CLpro) or main protease (Mpro) and one or two papain-like proteases to form 16 nsps which participate in minus-strand RNA synthesis, genome replication and subgenomic RNA [21, 32, 33]. A number of these nsps, the N protein, host proteins and the endoplasmic reticulum (ER), compose coronavirus replicative structures where viral RNA synthesis takes place [34]. The role of each nsp is well defined. For example, the RNA-dependent RNA polymerase is encoded in nsp12 [35], nsp3 participates in polyprotein processing as a protease, nsp8 and nsp9 bind to cis-acting elements of the viral RNA [36, 37]. Although genome replication and transcription are catalyzed by the viral replicase, in some cases the host machinery is implicated. Full-length gRNA is replicated using a complementary

negative sense RNA molecule as an intermediate, while smaller RNA molecules (subgenomic RNA) produce structural and accessory proteins. New virions form in the ER and mature virions are secreted [21]. Obviously, the nsps play an important role in genome replication and virus formation. The ORF1ab of SARS-CoV-2 was recently analyzed to investigate possible alterations in genome structure caused by selective pressure on the virus. The analysis predicted alterations in position 321 of the nsp2 protein and positions 192 and 543 of the nsp3 protein. These alterations may be related to the differences between SARS-CoV-2 from SARS-CoV, and to SARS-CoV-2 contagion [38].

COVID-19 THERAPEUTIC STRATEGIES

No specific treatment is available for COVID-19, and investigations are under way to test whether existing treatment regimens for other viruses are also effective for COVID-19. The most notable examples include remdesivir (GS-5734), a nucleotide analog prodrug currently in clinical trials for treating Ebola, that inhibited the replication of SARS-CoV and MERS-CoV in tissue cultures and displayed efficacy in non-human animal models, and a combination of HIV-1 protease inhibitors lopinavir/ritonavir and IFN-β (LPV/RTV-INFb) that were shown to be effective in animal models and patients infected with SARS-CoV [13]. Additionally, chloroquine phosphate, an approved malaria drug, is being used in certain patients with COVID-19. Previous studies showed its therapeutic activity against viruses, including in vivo and in vitro experiments with existing human coronavirus OC43 and SARS-CoV [39]. Results from 100 Chinese patients demonstrated that treatment with chloroquine inhibited the exacerbation of pneumonia and shortened the disease course; it was proposed that chloroquine exerts anti-viral and anti-inflammatory activities by interfering with the glycosylation of cellular receptors for SARS-CoV-2 [40]. In addition to antiviral therapies, immunological therapies are also under consideration for COVID-19 treatment, mainly to lower the elevated cytokine levels observed in COVID-19 patients. Tocilizumab, a humanized antibody against the IL-6 receptor was recently used with encouraging results [15]. Baricitinib, fedratinib and ruxolitinib, which are selective JAK inhibitors approved for rheumatoid arthritis and myelofibrosis treatmen, are also likely to be effective against the consequences of the elevated levels of proinflammatory cytokines in COVID-19 patients [16]. The antibiotic teicoplanin, used to treat Gram-positive bacterial infections, was previously shown to inhibit cellular entry of SARS-CoV and MERS-CoV by inhibiting the activity of cathepsin-L; a recent study suggested that teicoplanin also inhibits the entry of SARS-CoV-2 into the host cells [41]. Another proposed method to treat COVID-19 is to transfuse patients with plasma from patients that have recovered from it; this method was used in the past with Ebola and H1N1 outbreaks. Plasma from recovered COVID-19 patients should include specific antibodies against SARS-CoV-2 capable of viremia suppression [14].

INFECTION, PATHOPHYSIOLOGY AND EPIDEMIOLOGY

COVID-19 is acquired by inhalation through droplets by symptomatic patients as well as from asymptomatic people and even by touching contaminated surfaces. Some of the most common symptoms include fever, cough, fatigue, pneumonia which can develop to acute respiratory distress syndrome, metabolic acidosis, liver, kidney and heart failure [42]. All ages are susceptible and certain groups of patients are at a higher risk [43]. Available epidemiological data show that most patients are 30 to 79 years old and the case-fatality rate increases in patients aged 70 years and older [44].

Hypertension has been studied and approved as a host risk factor associated with severe COVID-19 [45]. Other studies suggest several underlying comorbidities such as diabetes mellitus, cardiovascular diseases and respiratory system diseases [46].

Particular attention is being paid to patients with underlying cardiovascular diseases and other inflammatory disorders. ACE2, the functional receptor for SARS-CoV-2, exists as a membrane-bound enzyme (98%) and in a soluble state in blood and other body fluids (2%). In pathological cases, the concentration of soluble ACE2 is increased. Although there are conflicting reports about the role of ACE inhibitors in the treatment of cardiovascular diseases, to date there are no supporting evidence that ACE inhibitors or angiotensin II type 1 receptor blockers enhance coronavirus entry by increasing ACE2 expression [43, 47].

Factors that affect the disease severity are not fully investigated. Previous studies have revealed the cytokine profiles of SARS-CoV infected patients. Lymphopenia and CD4 and CD8 T-cell lymphocyte depletion, often encountered in viral infections, are probably associated with the disease. Recently, routine complete blood counts of COVID-19 patients confirmed the low levels

of lymphocytes [42]. Specific cytokines (IL-1β, IL-6, IL-10) showed an increase in COVID-19 patients; however, the plasma concentrations of Th1 cytokine IL-2 and Th2 cytokine IL-4 did not show a significant increase [48].

CONCLUDING REMARKS

There is no vaccine or effective treatment for COVID-19 at present. The epidemiological characteristics of the novel coronavirus SARS-CoV-2 differ dramatically from those of the previous coronavirus outbreaks, SARS-CoV in 2002-2003 and MERS-CoV in 2012. SARS-CoV-2 is more transmissible from human to human and the mortality rate is higher. Anti-retroviral therapies such as LPV/RTV-INFb and remdesivir have shown some hope. Another proposed method to treat COVID-19 is to transfuse patients with plasma from patients that have recovered from it; this method was used in the past with Ebola and H1N1 outbreaks [14]. The use of chloroquine, used for malaria treatment, as well as other anti-inflammatory drugs and especially Baricitinib may also help [16]. However, the need for direct and effective treatment is emerging and great efforts are made to develop a vaccine against SARS-CoV-2.

Since the COVID-19 epidemic outbreak, several genetic structure analyses have been conducted that revealed similarities among genomes extracted from existing coronaviruses. Notably, the feature of the genome that differs most in the novel coronavirus, is the S gene that encodes for the S protein that is responsible for viral attachment and entry to the host cell. An obvious target of a protein-based vaccine is therefore the S protein, which binds to the ACE2 receptor on the host cell, and especially the S1-subunit which plays a pivotal role in the adherence of the virus to the cell [12, 18, 22, 27, 29]. Another approach is to mask ACE2 by delivering an excessive soluble form of ACE2 as a competitor for the virus [49, 50]; a similar strategy may be tried for the CD147 receptor on the host cells [31]. In addition to targeting the receptor or its ligand, another approach could be the inhibition of the proteases that participate in S cleavage. TMPRSS2, a transmembrane protease serine may do this, thus inhibiting the priming of S protein and subsequent viral entry to the host cells [22, 50].

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Coronavirus Disease 2019 (COVID-19): A Review of the Current Literature

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Abstract

An acute respiratory disease caused by a novel coronavirus, the coronavirus disease -19 (COVID-19) has spread throughout China and to more than 200 countries worldwide and has received global attention. The World Health Organization (WHO) has characterized COVID-19 as a pandemic. Coronaviruses (CoV), named so due to their "crown-like" appearance, constitute a large family of viruses that spread from animals to humans. The new coronavirus is highly contagious with a reproduction number (R0) between 1.4-2.5 patients and recent epidemiological data indicate that it may affect up to 30-40% of the population. Its high transmissibility in combination with the presence of a large percentage of asymptomatic carriers and the current lack of an effective vaccine render the new virus a major threat for people's health, especially for older age individuals and chronic disease patients. Although there is significant variation in case fatality rate for COVID-19, it is estimated to reach 2%.

Key words: *SARS-CoV-2, COVID-19, coronaviruses, infection, acute respiratory disease*

CLASSIFICATION AND HISTORICAL PERSPECTIVE: FROM URBANI-SARS TO MERS AND THE COVID-19 PANDEMIC

Coronaviruses (CoV) are a group of large enveloped non-segmented positive-sense single-stranded RNA viruses belonging to the family of Coronaviridae [1]. All CoVs are pleomorphic RNA viruses containing crown-shape peplomers. They belong to a large family of viruses broadly affecting humans and other mammals [1-5]. They can be classified into four genera, namely alpha, beta, gamma and delta CoVs of thirty-eight unique species [6].

Previously identified human CoVs infections include the alpha CoV hCoV-NL63, hCoV-229E and the beta CoVs HCoV-OC43 and HKU1 that cause self-limiting common cold-like illnesses [1,4,5]. The two previous beta coronaviruses fatal infections [6], namely the severe acute respiratory syndrome coronavirus (SARS-CoV) [7] and the Middle East respiratory syndrome coronavirus (MERS-CoV), [8] had pandemic potential and caused more than 10.000 cumulative cases during the past two decades, with mortality rates reaching 10% for SARS-CoV and 37% for MERS-CoV [6-8].

Several studies have shown that bats are the natural hosts of both SARS-CoV and MERS-CoV [9,10], while palm civets (*Pagumalarvata*) [11] and dromedary camels have served as intermediate hosts playing an important part in the transmission of these viruses from bats to humans [11-13]. During 2002-2003, SARS-CoV initially emerged in China and rapidly spread to a number of other countries, causing over 8,000 infections and approximately 800 deaths worldwide [7]. In 2012, MERS-CoV was first identified in the Middle East and then spread through the globe [8]. 2,494 MERS cases with 858 related deaths have

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been recorded in 27 countries [6,8]. Notably, new cases of MERS-CoV infections continue to be reported [13-15].

In December 2019, Wuhan State in Hubei Province, China became the center of global attention due to the outbreak of a pneumonia epidemic of unknown cause with characteristics similar to those of viral pneumonia [16]. Influenza viruses and all known coronaviruses were ruled out by laboratory testing which revealed the existence of a novel coronavirus [17]. This virus was named 2019-nCoV by WHO on January 12 (2019-nCoV) and was classified in the betacoronavirus 2b lineage [16,18]. It bares similarities to bat coronaviruses, and it has been postulated that bats constitute the primary source [17]. Although 2019-nCoV and SARS-CoV belong to the same beta coronavirus subgroup, genomic similarity is less than 79.5%, and the novel group has been found to show genetic differences from SARS-CoV [17,18]. The presence of high-risk animal contact in the medical histories of these patients suffering from a viral-like pneumonia has strengthened the likelihood of an infection transmitted from animals to humans [3,4].While the origin of the 2019-nCoV is still under investigation, relevant studies have shown that the outbreak is likely to have started at Huanan, a large Seafood Market where live wild animals' trade takes place [19]. Although the outbreak is likely to have started as a zoonotic transmission [3], it soon became clear that human-to-human transmission was also occurring [19].

The Coronavirus Study Group of the International Committee on Taxonomy of Viruses on February 11, formally named the novel virus SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2) and the relevant disease COVID-19 (Coronavirus Disease-2019) [20,21]. SARS-CoV-2 represents the seventh coronavirus known to cause human diseases. Population genetic analyses of SARS-CoV-2 genomes indicated that these viruses evolved into two major types, L and S. This probably explains why initial reports from Wuhan described a higher mortality than several more recent case series [22]. On March 11, 2020, COVID-19 was declared as global pandemic by WHO, based on reports of more than 118,000 cases in over 110 countries and the sustained risk of further spread worldwide [23].

GENOMIC STRUCTURE AND PROTEINS

Coronaviruses' genomic structure has been extensively studied. Two-thirds of the viral RNA encodes viral polymerase (RdRp), RNA synthesis materials, and two large nonstructural polyproteins not involved in modulating host-virus interaction (ORF1a-ORF1b). The remaining genome encodes four structural proteins [spike (S), envelope (E), membrane (M) nucleoprotein (N)], hematogglutinin-esterase (HE) and other helper proteins [6,18,24-27]. Viral infections initiate with the binding of viral particles to host surface cellular receptors. Receptor recognition constitutes an important determinant of the virus preference for specific cells and tissues (viral tropism). In addition, the gain-of-function of a virus to bind to the receptor-counterparts in other species is also a prerequisite for inter-species transmission [24]. Recently, the human angiotensin converting enzyme 2 (hACE2) has been reported as an entry receptor for SARS-CoV-2 [25].

VIRAL REPLICATION

The first step in viral infection is the interaction of human cells with viral spike protein (S). SARS-CoV-2 binds to the hACE2, located at the surface of type II alveolar cells and epithelium cells of the ileum and colon [16-18]. The S protein is subject to cleavage by host proteases into the S1 and S2 subunits that are responsible for receptor recognition and membrane fusion, respectively. S1 can be further divided into an N-terminal domain (NTD) and a C-terminal domain (CTD), which are called receptor binding domains (RBD) due to their receptor-binding properties [25]. SARS-CoV-2 uses the S1 CTD to bind to hACE2 in order to enter target cells and there is evidence of a specific region in ACE2 which is recognized by the virus's spike (S) glycoprotein [25].

Following the virus's entry into the cell through receptor binding and fusion of the viral envelope with the cell membrane, the incoming viral genome is translated to produce a polyprotein precursor. Subsequently, this large polyprotein is cleaved by proteases to release RNA-dependent RNA polymerase (RdRp). The RdRp is integrated into a membrane-associated viral enzyme complex which guides negative-strand RNA (–RNA) production resulting in intracellular viral replication. At the next step of this process, the newly-produced viral copies (virions) are released through exocytosis damaging the infected cell and potentially invading adjacent cells [28,29]. SARS-CoV-2 can infect kidney cells, liver cells, intestinal cells, T lymphocytes, and lung cells causing main symptoms and signs [25].

IMMUNITY

The entry of the virus into the host cell is accompanied by a rapid response of the innate and adaptive immune

system characterized by the release of proinflammatory cytokines and the activation of CD4 and CD8+ T cells [12, 29]. Innate immune response is mediated by antigen presentation to dendritic cells, macrophage maturation and the subsequent activation of the type I IFN pathway. SARS-CoV-2 N (nucleocapsid) protein interferes with IFN signaling and synthesis [28]. In this way, the innate immune system plays a key part in controlling viral replication and promoting adaptive immune response, which is initiated by T cells proliferation and a further cascade of cytokine production including IL-1, IL-6, IL-8, IL-21 and tumor necrosis factor β (TNF-β). CD4+ and CD8+ T cells play a significant antiviral role by balancing the fight against the virus and the risk of developing autoimmunity or overwhelming inflammation. CD4+ T cells promote the production of virus-specific antibodies by activating T-dependent B cells. CD8+ T cells eliminate virus-infected cells and secrete cytokines such as TNF-α and IFN-γ which induce protective immunity against intracellular micro-organisms. In addition, NK cells are recruited in in the host's defense against the virus. They recognize infected cells in an antigen-independent manner, exert cytotoxic activities and rapidly produce large amounts of IFN-γ [28,29].

CLINICAL MANIFESTATIONS

Spectrum of illness

Incubation time ranges between 2-14 days and is divided into two stages. The first stage is characterized by non-specific symptoms, fever, headache, myalgia and to a lesser extent diarrhea. The second stage includes the respiratory phase of the disease and occurs after the seventh day with productive cough, dyspnea and chest pain [30-33]. It may progress into acute respiratory distress that requires intubation and mechanical ventilation [34,35]. In this phase, jaundice is high and there may be multi-organ involvement [35]. There is an increase in inflammatory markers, including CRP and ferritin. Procalcitonin is usually not elevated, unless there is a bacterial co-infection. CRP levels have prognostic value [34,36,37]. In severely ill patients, particularly those with strong inflammatory reactions, cytokine storm may contribute to multiorgan failure and eventually death [32].

It is emphasized that due to the unpredictability of the disease's clinical course, treatment is needed in the early stages [33]. Corticosteroids were used both in SARS and MERS cases [14,15] but initial results regarding corticosteroid treatment of COVID-19 were rather disappointing and other solutions are being investigated, such as IL-6 inhibitors or anti-TNF agents. Colchicine is presently used in patients with a history of heart disease [28]. It has been noted that the virus shows tropism for the myocardium, causing myocarditis. Initial troponin measurement is mandatory and increased levels also constitute an adverse prognostic factor [36].

Symptoms and signs

SARS-CoV-2 causes mild influenza-like symptomatology with nasal congestion, sore throat, cough and fever, but it may progress into severe pneumonia, ARDS, sepsis or septic shock, requiring hospitalization in intensive care units (ICUs), with a fatal outcome in 2.9 % of laboratory confirmed cases [33]. Huang et al (2020) reported that the most common clinical finding was fever (98%), followed by cough (76%) and myalgia / fatigue (44%) [33]. Headache, sputum production, and diarrhea were less common. In many cases in Europe, acutely emerged anosmia and taste disturbance were reported as early symptoms [38]. Leukopenia and lymphopenia were common in the early stages affecting almost 66% of patients [31,32,36,37]. According to WHO situation report on February 12th 2020, among 44,730 confirmed cases in Chine, 8,204 (18%) cases were recorded as severe infections [39]. People with chronic illnesses, including cardiovascular disorders, diabetes, liver disorders, respiratory diseases, cancer and the elderly appear more prone to serious illness [31,34,36]. Children are usually asymptomatic or have mild symptoms but co-infection with bacterial pneumonia is common. Children with comorbidities are more vulnerable [40].

Risk Factors

Mortality risk factors have not been well described. In-hospital death risk can be predicted by older age, higher Sequential Organ Failure Assessment (SOFA) score, higher breathing rate, elevated lymphocyte count, creatinine, lactate dehydrogenase, creatine kinase, elevated d-dimer, high-sensitive cardiac troponin I, serum ferritin and interleukin-6 (IL-6) [34,36]. Sepsis was a common complication, which might be causally related to COVID19, but further research is needed to clarify its pathogenesis [34]. Cardiac complications, including arrhythmia, myocardial infarction or heart failure are frequently encountered in patients suffering from pneumonia [34]. 28-day mortality in septic patients is strongly associated with high d-dimer levels through systemic pro-inflammatory cytokine responses which mediate

local inflammation and plaque rupture, induction of procoagulant factors, and haemodynamic alterations further contributing to ischemia and thrombosis [37].

Infections with the pre-existing coronaviruses have been shown to be linked to severe complications during pregnancy. Given that SARS-CoV2 has the potential for similar behavior, systemic screening for any suspected infection during pregnancy is recommended [33,34]. At present, there are no conclusive findings regarding the risk of vertical mother-baby transmission. Limited data suggest that there is not an increased risk of intrauterine infection in women who were infected during late pregnancy. In addition, there are no sufficient data on the perinatal outcome when women are infected during the first or second trimester, however these pregnancies should be closely monitored [41].

DIAGNOSTIC TOOLS

Diagnostic tests are powerful tools to combat COVID-19 and include molecular methods, serology and viral culture. There are two types of SARS-CoV-2 tests: those that detect the virus and those that detect the host's response to the virus. Molecular methods including RT-PCR (reverse transcription) or real-time PCR, which use RNA from respiratory samples such as oropharyngeal swabs, sputum, nasopharyngeal aspirate, deep tracheal aspirate, or bronchoalveolar lavage, constitute the most common diagnostic methods [42]. Serological assays can also be useful, but they do not address the same questions [43,44]. The sensitivity of antibody detection is generally lower compared to that of molecular methods and is mostly used in retrospective diagnosis. Serological testing may help identify patients who are asymptomatic or immunized to SARS-CoV-2.

PCR seems to have a sensitivity of ~75% [45]. Sensitivity is even lower if the sample is derived from the upper respiratory tract, or it is obtained relatively early in the course of the disease and a negative result does not preclude infection. When there is strong clinical suspicion of serious illness, repetition of diagnostic testing using samples from the lower respiratory tract is strongly recommended. In addition, in cases of high clinical suspicion, the detection of another pathogenic microorganism does not exclude the presence of the new virus, as data on the role of coinfection have so far been limited [43]. If RT-PCR is negative but there is a high clinical suspicion of COVID-19, it is recommended that the individual remains isolated testing should be repeated at a later time. Specificity seems to be high (although contamination can cause false-positive results) [45].

One question that arises in the case of patients with positive CT scan and negative RT-PCR, is which of these diagnostic tools is more sensitive for early diagnosis. PCR sensitivity is around 75% [45] due to difficulties in sampling and other technical issues. CT scan appears to show earlier lesions specific to COVID-19, namely bilateral ground glass lung opacities [43,45]. It is preferable, in order to have patients detected and quarantined as early as possible, to perform CT scan to confirm cases of COVID-19, in spite of a negative PCR [45].

TREATMENT AND PROTECTION

There is currently no specific treatment or effective preventive measure for COVID-19. Numerous antiviral agents, immunotherapies and vaccines are being researched and developed as potential therapies. Finding effective treatments for COVID-19 infection is a complex process

Vaccines

There is currently no vaccine to prevent COVID-19 infection. Vaccines studies are conducted worldwide but it is estimated that a clinically useful product will not be available before 2021 [46]. Wide-range collaboration between scientists from multiple disciplines and policy makers will enhance and even accelerate vaccine development and testing. Furthermore, social and ethical issues associated with COVID-19 vaccine distribution should be carefully addressed in order to implement a global and successful disease prevention strategy.

Development of a COVID-19 vaccine will not only have protective implications in the present day, but also advance preparedness for future coronavirus outbreaks.

Treatment

No specific antiviral treatment is recommended for the 2019-nCoV infection [47]. Patients should receive supportive care to help relieve symptoms. Vital organs' function should be supported in severe cases. [47]. A large number of researchers and clinicians worldwide are currently focusing on finding a treatment for COVID-19 [48]. There are almost 60 clinical trials around the world regarding effective treatment. Most of them focus on existing drugs which are known to be safe for human use and need to be tested for effectiveness against the SARS-CoV-2 virus [48].

Antiviral Agents

Favipiravir has been approved in Japan and China for influenza and is currently under testing for potential use in COVID-19. Preliminary results of antiviral effect on COVID-19 are satisfactory [47]. Remdesivir (GS-5734) is another broad-spectrum antiviral agent. This drug, which appeared rather ineffective in Ebola infection, appears to be effective in treating COVID-19 [47,48]. To this end, 5 clinical trials are currently underway in China and the US. Remdesivir is a nucleotide analog pre-drug which interrupts the replication of the SARS-CoV-2 virus without damaging the human cell, so it has a targeted effect. Positive results have been reported. Prophylactic and therapeutic remdesivir treatment improved pulmonary function and reduced viral loads and lung histopathological lesions in experimental models. Clinical trials with remdesivir are ongoing, but so far it is the only antiviral agent that according to WHO may display real efficacy. Other antivirals being tested against COVID-19 are arbidol, darunavir and various protease-inhibitor combinations in trials in China and Thailand [47,48]. The WHO has just launched the SOLIDARITY trial, a randomized, multicenter trial of antiviral medications, currently involving 45 countries [49].

Recently, anti-malaria drugs have received attention as potential COVID-19 treatments. Chloroquine and hydroxychloroquine are substances that have been officially approved for the treatment of malaria, systemic lupus erythematosus and rheumatoid arthritis. It appears, from the results of clinical trials, that they may be effective in COVID-19 [47,48]. In France and other countries, an experimental protocol of a hydroxychloroquine and azithromycin combination has been used for potential prophylaxis or treatment for COVID-19 [50,51]. At 6 days, among patients given combination therapy, the percentage of cases still carrying SARS-CoV-2 was no more than 5%. Azithromycin was added due to its effectiveness against bacterial pneumonia but also because it has been shown to be effective in vitro against a large number of viruses. Chloroquine and hydroxychloroquine help to neutralize acids, making the environment less friendly for the virus. Another possibility is that these drugs have subtle effects on a wide variety of immune cells. Hydroxychloroquine was found to be more potent than chloroquine in vitro. Hydroxychloroquine's therapeutic effect is evident in relatively low doses and the authors recommend a loading dose of 400 mg PO BID, followed by 200 mg BID for 4 days. In this dosage, hydroxychloroquine reduced viral load, symptoms duration and severe cardiac or psychiatric side effects, pain or fever were less common [50]. The main complication (retinal toxicity) is rare and is evident after at least 5 years of continuous use. Both hydroxychloroquine and azithromycin are listed as definite causes of torsade de pointes (TdP) and for this reason there are specific recommendations regarding their use. These include monitoring cardiac rhythm and QT interval, sustaining potassium levels above 4 mEq/L and magnesium levels above 2 mg/dL and avoiding other QTc-prolonging agents when possible. In addition, these drugs are contraindicated in patients with baseline QT prolongation (e.g., QTc of at least 500 msec) or with congenital long QT syndrome [47,50,51].

Immunotherapy

Immunomodulators and other investigational drugs

Researchers are investigating treatment agents that boost the immune system to fight the virus [47-49]. Drugs like *interferons* have already been used to treat COVID-19 cases in China, but their effectiveness as monotherapy has not been established. Interferons combined with other drugs could be more effective [49]. Therapeutic antibodies can be administered to patients and help their immune system counteract right away. A set of premade antibodies can be used prophylactically to prevent infection as well as therapeutically to treat the disease [47,48]. Recently scientists have worked to develop a convalescent serum therapy to treat COVID-19 using blood plasma from recovered patients. Convalescent plasma antibody-rich products are collected from recovered COVID-19 patients. Cellular therapy, using mesenchymal stem cells, has been shown to reduce inflammation and trigger tissue regeneration and is being evaluated in patients with acute respiratory distress syndrome (ARDS). There are ongoing clinical trials is to determine the safety and efficacy of mesenchymal stem cells (MSCs) therapy for severe COVID-19 [52]. As COVID-19 can lead to overreaction of the immune system and activation of the inflammation cascade known as cytokine storm leading to detrimental effects, immunosuppressive agents are tested [28,47]. By reducing the excessive inflammatory reaction, it is hoped that the body's immune system will be able to fight the coronavirus, and prevent the complications of pneumonia, organ failure and death.

IL-6 inhibitors

IL-6 inhibitors may prevent severe pulmonary dam-

age caused by cytokine release in COVID-19 patients. Several studies have indicated that clinical worsening is attributed to a "cytokine storm" with release of IL-6, IL-1, IL-12, and IL-18, along with TNF-α and other inflammatory mediators. The increased pulmonary inflammatory response may result to increased alveolar-capillary gas exchange, making oxygenation difficult for patients with severe illness [28,29].

Tocilizumab, an IL-6 inhibitor, is currently used in the treatment of rheumatoid arthritis. Patients with severe COVID-19 received a single intravenous dose of 400mg tocilizumab with satisfying results. In general, patients improved with lower oxygen requirements, lymphocyte counts returned to normal, and they were discharged with a mean hospitalization duration of 15.5 days [47,53].

On March 16, 2020, the initiation of a phase 2/3 trial of the IL-6 inhibitor *sarilumab* was announced [53]. The ODYSSEY study is another clinical trial focusing on the efficacy and safety of tradipitant (85 mg PO BID) in cases of lung injury associated with severe COVID-19 infection. Tradipitant is a neurokinin-1 (NK-1) receptor antagonist. Substance P binds to the NK-1 receptor and triggers neuroinflammatory processes which lead to severe lung injury [54].

Rintatolimod is a toll-like receptor 3 (TLR-3) agonist which has been used in chronic fatigue syndrome, HIV and influenza. It is currently evaluated as a potential treatment for COVID-19 by the National Institute of Infectious Diseases (NIID) in Japan and the University of Tokyo [55]. Recently, colchicine has been authorized to enter clinical trials to determine whether short-term treatment reduces mortality and lung complications related to COVID-19 in heart disease patients. Colchicine is an anti-inflammatory agent used to treat gout, Behcet's syndrome and Familial Mediterranean Fever. In addition, it is regularly prescribed as a short-term treatment of pericarditis. It is supported that colchicine may moderate the overproduction of immune cells and their activating compounds thus reducing the likelihood of heart complications such as myocardial injury observed in COVID-19 patients [24].

WHO has proposed a therapeutic algorithm for COVID-19 in February 2020, however, due to the constantly growing research on this new infection, WHO's guidelines are frequently updated [48].

TRANSMISSION ROUTES

The virus is transmitted mainly through respiratory droplets produced during coughing or sneezing or by direct contact with infected hands, surfaces or objects. Airborne spread is not considered a major transmission route except during certain aerosol-generating procedures which are exclusively conducted in health-care facilities. Fecal shedding has been demonstrated from some patients, and viable virus has been detected in feces in a few case reports. Nevertheless, the fecal-oral route does not appear to be a major cause of COVID-19 transmission; its role and significance for COVID-19 spread remains to be determined [56,57].

Incubation time is usually 5-6 days, although it can range from 2 to 14 days [30-33]. For this reason, people who may have contacted a confirmed case are asked to limit themselves for a period of 14 days [32]. Most cases of COVID-19 appear to be spread by people with symptoms. A small number of people may be infected before their symptoms have been developed [57,58]. The virus can remain in the air for 30' to 3 hours. It can survive for 24 hours on cardboard, for 2 days on metals, and has a longer survival time on plastic (at least 3 days) [59]. According to recent research, the novel coronavirus was most stable on plastic and stainless steel, with some virus remaining viable up to 72 hours [59]. Coronaviruses have the potential to contaminate cutlery, plates or other surfaces, through sneezing or coughing, and can survive on these surfaces for some time. An epidermal infection could theoretically be possible if the virus is transmitted by cutlery or hands to the mucosa of the mouth, throat or eyes. However, no contamination with SARS-CoV-2 has been proven through this transmission pathway [56,57].

ARE THERE ANY MEASURES TO PREVENT ITS TRANSMISSION?

Like all encapsulated viruses in which the genetic material is coated with a layer of fat, coronaviruses are susceptible to fat-soluble substances, [59] such as alcohol or surfactants, contained in soaps and dish detergents [2]. These substances are thought to damage the surface of the virus thus rendering it inactive. This is especially true if dishes are washed and dried in a dishwasher at 60°C or higher. Antiseptic alcoholic solutions neutralize the new coronavirus. Personal hygiene measures are therefore very important: good hand washing, with soap and water, for at least 20 seconds, according to the WHO guidelines. Good hygiene practice and social distancing remain the best means to prevent viral spread. [59].

INSTRUCTIONS FOR HEALTH PROFESSIONALS

Prior to patients' arrival [59]

Screen all patients for new respiratory infection symptoms before non-urgent care or elective visits. Ask about cough, shortness of breath, and fever. Explore alternatives to face-to-face triage and visits to reduce risk of transmission and spare personal protective equipment (PPE) supplies. Consider limiting facility points of entry and establishing triage stations outside the facility to screen patients before entering. Ensure rapid and safe triage of patients with symptoms of suspected COVID-19. Display signs on all entrances about COVID-19 symptoms. Ask symptomatic patients to inform triage personnel of symptoms upon arrival. Provide them with respiratory hygiene supplies, including masks, hand sanitizers, and tissues. Consider installing a barrier, such as a glass or plastic window, to limit contact between triage personnel and patients. Isolate symptomatic patients in an examination room as soon as possible. If not available, identify a separate, well-ventilated place where patients can be separated by 6 feet. Provide easy access to hygiene supplies.

DURING PATIENTS VISIT

Reserve airborne infection isolation rooms (AIIR) for aerosol-generating procedures. Healthcare facilities should provide healthcare workers with respirators during aerosol-generating procedures performed on suspected or confirmed COVID-19 patients [59]. Use PPE, according to guidance from your facility. This includes clean, non-sterile gloves, gowns and eye protection, like goggles or eye shields. If there are shortages, the existing equipment should be reserved for aerosol-generating procedures and close-contact care or anticipated splashes or sprays. Recruit specific staff to care exclusively for patients with suspected or confirmed COVID-19. Limit personnel in patient rooms to essential staff, and limit aimless patients' wandering. Basic precautions must be applied systematically and in all cases. Additional precautions for contact and droplets should be applied as long as the patient is symptomatic [59].

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A rare cause of abdominal pain

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An 82-year-old woman was referred to our hospital because of generalized weakness and vertigo, accompanied by epigastric pain. She complained of progressively worsening shortness of breath on light exertion during the last month. She also reported frequent episodes of (non – debilitating) epigastric pain / discomfort that impacted on her quality of life. The abdominal symptoms varied in frequency and severity but had grown progressively more frequent and severe in the last several months (at least 4 months). Her caretaker (daughter of patient) that provided patient history information reported a complicated cholecystectomy (for gallbladder stone disease) more than 25 years ago and atrial fibrillation (AFib) on dabigatran. Findings from physical examination were mostly nonspecific: Non-tender non-firm smooth hepatomegaly (1-2cm), a systolic 3/6 (functional) murmur and mild epigastric tenderness on deep palpation (Murphy's, McBurney and Rovsing's sign negative). Digital rectal examination was also unremarkable. The patient appeared pale, non-icteric and afebrile. The only interesting finding was the scar from an "extended" Kocher incision to the abdomen. Initial laboratory examinations revealed decreased hematocrit (26,5 %), hemoglobin levels with depleted iron supplies (low levels MCV, MCH, iron and Ferritin), while the rest of the blood exams (including amylase, bilirubin, liver function tests) appeared unremarkable. An ultrasonographic examination of the abdomen, only managed to confirm the cholecystectomy. The patient was hospitalized, transfused and was administered intravenous iron supplementation. An esophagogastroduodenoscopy (EGD) was ordered, based on chief complains. Findings from the stomach were unremarkable during the EGD. However, inspection of the duodenum bulb revealed a diverticulum – like "pocket" with bile coming out of the orifice. On further examination, this was confirmed to be the opening of a biliary enteric anastomosis (BEA). What was also interesting was that inside the distal end of the BEA we found a long chicken bone (Figure 1), which was retrieved with the use of grasping forceps (Figure 2). The patient was shortly after discharged with instructions for further investigation (of anemia). On follow – up, more than 8 weeks later, patient disclosed complete resolution of epigastric symptoms.

DISCUSSION

Foreign bodies in the biliary tract are a rare finding. They are mainly associated with altered biliary – enteric anatomy, most commonly presence of choledochoduodenostomy / choledochojejunostomy [1, 2]. Surgical variation of the biliary anatomy permits regurgitation of the duodenal content inside the bile duct. An especially wide BEA opening can facilitate the introduction of a sizable foreign body [1]. Moreover, a choledochoduodenostomy would probably provide easier access to a foreign body, given the linear axis connecting the pyloric sphincter with the anterior duodenal bulb. However, there have been case reports of foreign bodies even in patients with a native papilla [3]. In these patients, loss of function of the sphincteric mechanism, permitting reflux from the alimentary tract is hypothesized [3]. Patient symptoms have been attributed mainly to bouts of cholangitis [1] and formation of bile stones [3]. Foreign bodies in the biliary tract (bone, toothpick, phytobezoar) act as nidus for the formation of stones. These foreign bodies are usually amendable to com-

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Figure 1. Endoscopic imaging of the biliary enteric anastomosis located in the duodenum of the patient. A long foreign body is easily identified.

Figure 2. The foreign body after retrieval in the endoscopic suite. It appears to be a long chicken bone.

mon endoscopic procedures [endoscopy and possibly endoscopic retrograde cholangio – pancreatography (ERCP) [2]. Our patient did not exhibit clinical (Charcot's triad) or laboratory (elevated liver function tests) evidence of cholangitis. Moreover, ERCP or magnetic resonance cholangio - pancreatography (MRCP) was not performed. The cause of pain might be only mechanical due to transient obstruction or distention of the opening of the bile duct or the duodenum (resulting from retrogression of the foreign body).

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COVER LETTER

A submission letter to the Editor should accompany the manuscript and contain the following:

- The manuscript has not been published previously, and is not under consideration for publication elsewhere.
- Acknowledgment of grants or financial support.
- The manuscript has been approved by all authors.

INFORMATION ABOUT ARTICLE TYPES

The Editors will consider and publish the following:

1. Original research articles
2. Narrative Reviews
3. Systematic Reviews and Meta-analyses
4. Editorials
5. Letters to the Editor
6. Case Reports

Original research articles

The maximum length of the main text is 3,500 words excluding the abstract, references, tables, and figure legends. A maximum of 6 tables and/or figures is allowed. References should not exceed a maximum of 100.

Narrative Reviews / Systematic Reviews / Meta-analyses

These manuscripts are solicited and unsolicited manuscripts that feature an organized and detailed review of the scientific literature about a particular topic. This section is peer-reviewed and acceptance for publication is not guaranteed. The maximum length of the main text is 5,000 words excluding the abstract, references, tables, and figure legends. A maximum of 6 tables and/or figures to summarize critical points is highly desirable. References should not exceed a maximum of 150.

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- Title Page
- Abstract and Key Words
- Main Text
- Acknowledgements
- References
- Tables
- Figures

Margins should be not less than 2.5 cm. Pages should be numbered consecutively.

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Do not use non-standard abbreviations. The use of abbreviations in the title and abstract should be avoided. Abbreviations should be defined on their first appearance in the text; those not accepted by international bodies should be avoided.

Title page

The title page should include:

- Title of the manuscript
- Short title which will be used as a running head
- Full name of each author
- Full location of department and institution where work was performed
- Name and address for correspondence, including fax number, telephone number, and e-mail address.
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For Original Articles, structured abstracts should be 250 words or less and include the following sections: Background, Methods, Results and Conclusion. Review articles should carry an unstructured abstract which should not exceed 200 words.

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The abstract should be followed by a list of 3–5 keywords which will assist the cross-indexing of the article and which may be published separated by semicolons.

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For the main body of the text, the recommended structure of

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- Materials and Methods
- Results
- Discussion

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State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

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Provide sufficient detail to allow the work to be reproduced. Methods already published should be indicated by a reference. This includes a description of the design, measurement and collection of data, type and source of subjects, inclusion and exclusion criteria and measures of outcome, number of subjects studied and why this number was chosen. Any deviation from the study protocol should be stated. Randomized controlled trials should adhere to the CONSORT guidelines that can be found at: http://www.consort-statement.org. Observational studies should also adhere to Strobe statement: http://www.strobe-statement.org/. Diagnostic accuracy studies should follow the Stard statement: http://www.stard-statement.org/. Systematic Reviews and Meta-Analyses should adhere to the PRISMA statement: http://www.prisma-statement.org/.

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The statistical methods used should be relevant and clearly stated. Special or complex statistical methods should be explained and referenced. Describe statistical methods with enough detail to enable a knowledgeable reader with access to the original data to verify the reported results. When possible, quantify findings and present them with appropriate indicators of measurement error or uncertainty (such as confidence intervals). Avoid relying solely on statistical hypothesis testing, such as P values, which fail to convey important information about effect size. Define statistical terms, abbreviations, and symbols. Specify the software used.

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Follow internationally accepted rules and conventions: use the internal system of units (SI).

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Results should be clear and concise. Results should be explained and illustrated by using Tables and Figures. Do not duplicate information contained in tables and figures.

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Discussion should directly relate to the results of the study and should explore their significance. Do not provide a general review of the topic.

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The conclusions should provide a summary of the key results and discuss the appropriateness and impact of this original work.

Acknowledgements

Collate acknowledgements in a separate section at the end of the article before the references. Acknowledgements should be made only to those who have made a substantial contribution to the study. Authors are responsible for obtaining written permission from people acknowledged by name in case readers infer their endorsement of data and conclusions.

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Ensure that every reference cited in the text is also present in the reference list (and vice versa). References should be numbered in the order they appear in the text. Manuscripts should follow the style of the Vancouver agreement detailed in the International Committee of Medical Journal Editors' revised 'Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Writing and Editing for Biomedical Publication', as presented at http://www.ICMJE.org/. In the text, references should be cited using Arabic numerals enclosed in square brackets [1]. The last names and initials of all authors should be referred to if they are up to six, otherwise only the first six are referred, with et al following. References should also include full title and source information. Journal names should be abbreviated according to the standard in the Index Medicus. No periods should be placed at the end of abbreviations of the journal.

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Journal article, more than 6 personal author(s):

Example: Liaw S, Hasan I, Wade, V, Canalese R, Kelaher M, Lau P, et al. Improving cultural respect to improve Aboriginal health in general practice: a multi-perspective pragmatic study. Aust Fam Physician. 2015;44(6):387-92.

Journal article/ Issue with a supplement

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Electronic journal article:

Example: Poling J, Kelly L, Chan C, Fisman D, Ulanova M. Hospital admission for community-acquired pneumonia in a First Nations population. Can J Rural Med [Internet]. 2014 Fall [cited 2015 Apr 27];19(4):135-41. Available from: http://www.srpc.ca/14fal.html by selecting PDF link in table of contents.

Book, personal author(s):

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Book, editor(s):
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Poster presentation/session presented at a meeting or conference:
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