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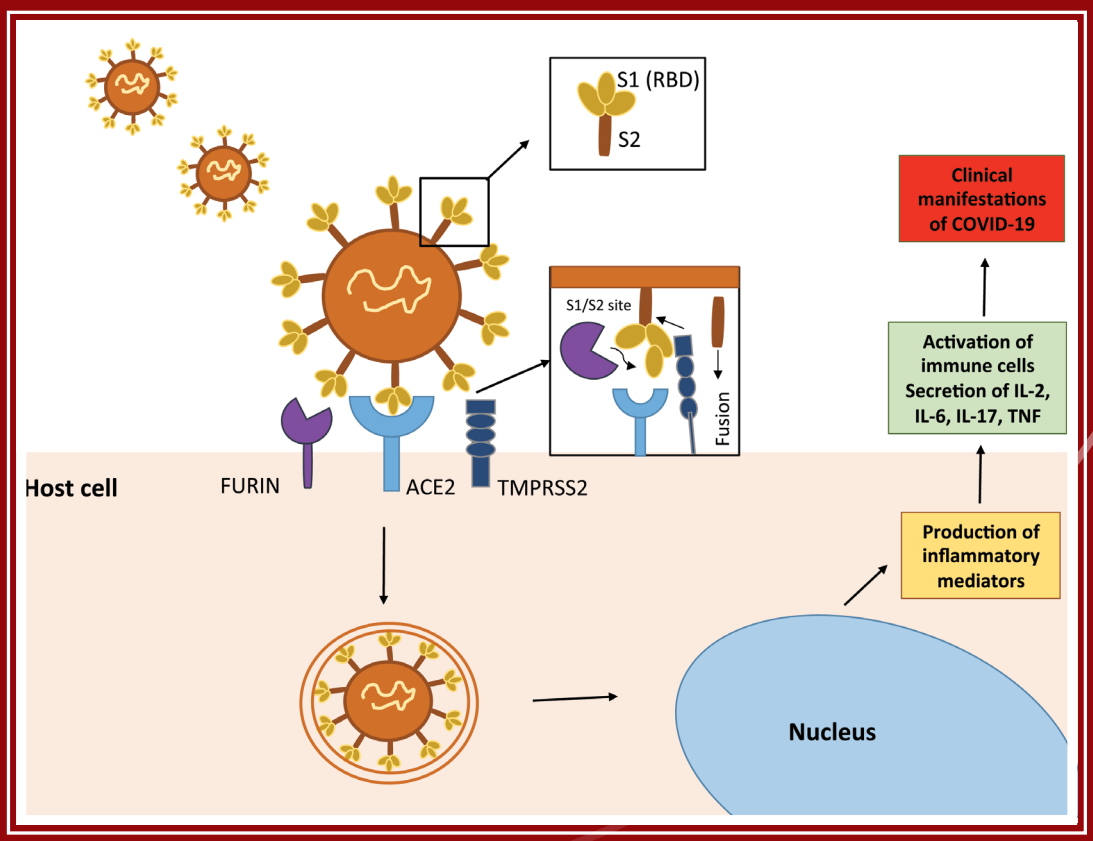

Proposed mechanism of SARS-CoV-2 transmission to the digestive tract.

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CONTENTS

Dear colleagues,

In the current issue, the editorial by Tourkochristou et al. addresses the effects of SARS-CoV-2 infection on the gastrointestinal tract. In particular, it focuses on clinical studies on this topic and presents all evidence regarding SARS-CoV-2 transmission and COVID-19 pathophysiology in the digestive system.

The original article by Konstantakis et al. evaluates the feasibility and efficacy of endoscopic treatment of biliary complications in patients undergoing cholecystectomy. Another original study by Lagadinou et al. investigates the knowledge of healthcare professionals in regards to adult vaccination and its safety; in parallel the article explores the proportion of health care professionals who have been vaccinated and the correlation of vaccination rates to educational level and work type.

Moreover, this issue includes three reviews. The first review, by Moulakakis et al. investigates the pathogenesis, clinical presentation and treatment of secondary aorto-enteric fistula, an uncommon and life-threatening clinical complication following both open and endovascular repair of abdominal aortic aneurysms. Papasotiriou et al. describes current clinical data on the efficacy and safety of oral anticoagulation in patients with atrial fibrillation and established chronic kidney disease. Lastly, the review by Dara et al. discusses the non-invasive functional and morphological assessment of brain circulation and microstructure in patients with systemic lupus erythematosus and neuropsychiatric lupus erythematosus.

Dear friends, on behalf of our editorial team, I wish you a pleasant and safe summer. A summer that will mark the end of this pandemic.

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COVID-19 and the Digestive System - Pathophysiological Aspects

Evanthia Tourkochristou[1,2], Athanasia Mouzaki[1,3]

INTRODUCTION

The COVID-19 pandemic has posed major public health challenges worldwide. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is transmitted primarily through the respiratory tract and causes typical flu-like symptoms of mild to moderate severity. However, evidence is accumulating that SARS-CoV-2 can also affect the gastrointestinal (GI) tract. An analysis of clinical data from 4,434 COVID-19 patients showed that the pooled prevalence of GI manifestations was 11.51% of infected patients. The most common symptom was diarrhea (7.78%), followed by nausea/vomiting (3.57%), loss of appetite (2.39%), and abdominal pain/discomfort (0.78%) [1]. In addition, autopsy of COVID-19 patients and imaging studies revealed microscopic and macroscopic changes and abnormalities of gastrointestinal tissues, including segmental dilatation and stenosis of the small intestine combined with mucosal detachment and necrosis, and colitis/enteritis characterized by inflammatory infiltrates and interstitial edema [2]. Mild and transient liver injury characterized by abnormal levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, and mildly elevated serum bilirubin was noted in COVID-19 patients, with a greater extent of liver injury observed in patients with severe disease. Pathological liver tissue findings observed include macrovesicular and microvesicular steatosis, histiocytic hyperplasia, mild hepatic lobular and portal tract inflammatory infiltrates, increased platelet-fibrin microthrombi in the hepatic sinusoids, central or portal vein, rare megakaryocytes in the sinusoids, and hepatic necrosis of the ischemic type [3]. The prolonged presence (mean 11 days) of SARS-CoV-2 mRNA in fecal samples in more than half of COVID-19 patients after clearance of respiratory samples underscored the need for further investigation of the GI tract as another potent route of viral transmission [4].

SARS-COV-2 TRANSMISSION IN THE GI TRACT

A proposed mechanism of SARS-CoV-2 transmission in the digestive tract is shown in Figure 1. SARS-Cov-2 invades host cells using the spike (S) glycoprotein, which consists of an S1 receptor-binding domain (RBD) and an S2 domain. Fusion of viral and host cell membranes depends on activation of the S protein mediated by the proteases furin and transmembrane serine 2 (TMPRSS2), which cleave the S protein at two sites (S1/S2 and S2) and stimulate the release of the fusion peptide from the virus. Furin-mediated cleavage at the S1/S2 site can cause conformational changes in the viral protein, making the RBD and S2 domains accessible. The S protein binds to angiotensin converting enzyme 2 (ACE2) via the S1 receptor binding domain. Removal of the S1 domain from the viral surface allows the S2 domain to fuse with the host cell membrane, allowing viral entry [5].

Upon infection of ACE2-expressing cells, SARS-CoV-2 is thought to stimulate the production of inflammatory mediators, which in turn activate immune cells. The release of inflammatory cytokines (IL-2, IL-6, IL-17, TNF) by activated immune cells contributes to the

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Key words: *SARS-CoV-2; gastrointestinal tract; liver; ACE2; cytokines; inflammation*

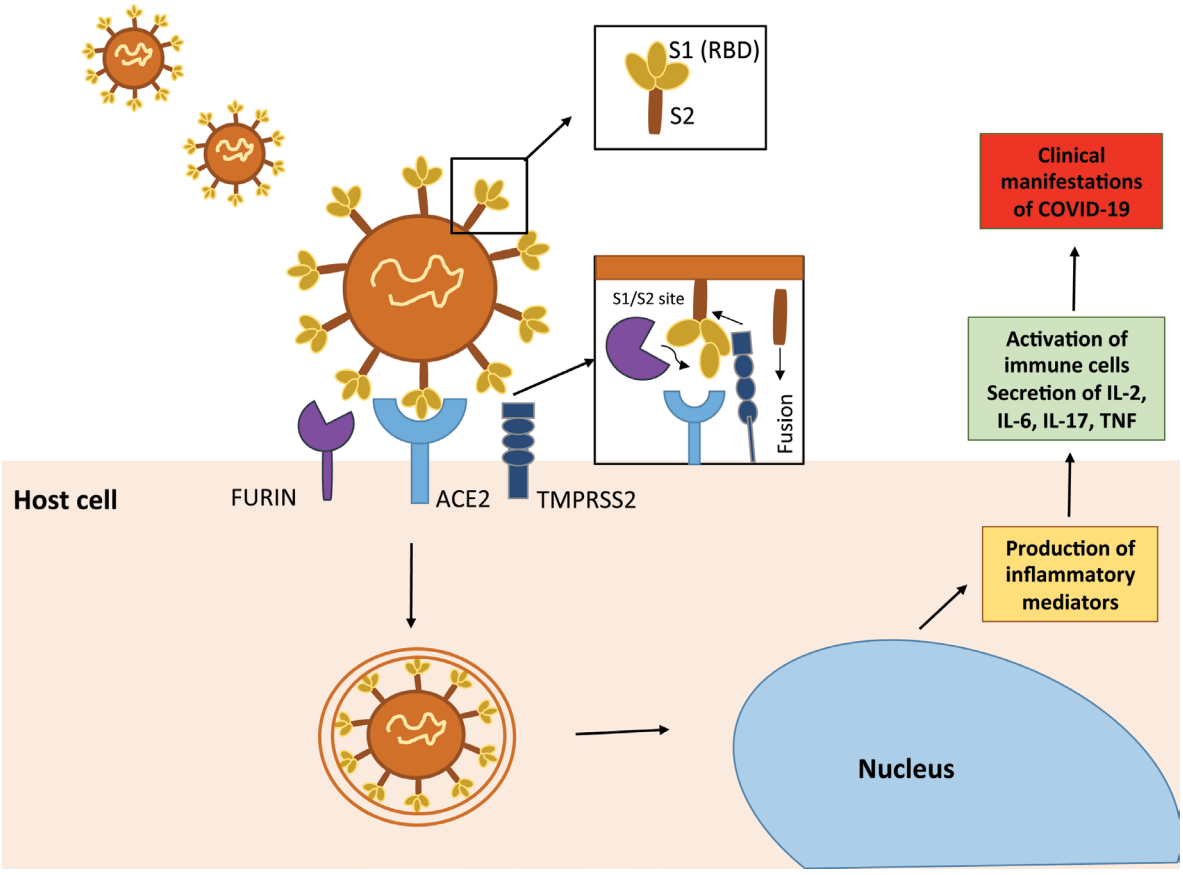

Figure 1. A proposed mechanism of SARS-CoV-2 transmission to the digestive tract. SARS-CoV-2 invades host cells using the spike (S) glycoprotein, which consists of an S1 receptor-binding domain (RBD) and an S2 domain. Fusion of viral and host cell membranes depends on activation of the S protein mediated by the proteases furin and transmembrane serine 2 (TMPRSS2), which cleave the S protein at two sites (S1/S2 and S2) and induce release of the fusion peptide (S2) from the virus. The S protein binds to a metallopeptidase, angiotensin converting enzyme-2 (ACE2), via the S1 receptor binding domain. When the S1 domain is removed from the viral surface, the S2 domain can fuse with the host cell membrane, allowing entry of the virus. Upon infection of ACE2-expressing cells, SARS-CoV-2 is thought to stimulate the production of inflammatory mediators, which in turn activate immune cells. The release of inflammatory cytokines (IL-2, IL-6, IL-17, TNF) by activated immune cells contributes to the clinical manifestations of COVID-19.

clinical manifestations of COVID-19 [6]. ACE2 is highly expressed in upper esophageal epithelial cells and small intestinal enterocytes, and higher expression of ACE2 was found in absorptive enterocytes of the ileum and colon compared to lung tissue. Single-cell transcriptome studies have shown that ACE2 and TMPRSS2 are highly co-expressed in upper esophageal cells, upper epithelial and glandular cells, and enterocytes of the ileum and colon, allowing viral invasion of the digestive tract [7].

COVID-19 PATHOPHYSIOLOGY OF THE DIGESTIVE SYSTEM

Effect of SARS-CoV-2 on the GI tract

The relatively high expression of viral receptors in cells of the GI tract makes the digestive system susceptible to SARS CoV-2 infection. The mechanism underlying the manifestation of GI symptoms in COVID-19 is not clear and several theories have been proposed. Most human coronaviruses have evolved through the presence of structural similarities and interspecies immunological cross-reactivity between animal and human coronaviruses, some of which can inherently cause gastroenteritis and maintain their enteric infectivity through interspecies recombination events, suggesting the possible gastrointestinal activity of SARS-CoV-2 [8], which can exert its effect on enterocytes by binding to ACE2. SARS-CoV-2 is thought to contribute to intestinal inflammation by interfering with the ACE2-dependent absorption of tryptophan, a major component of antimicrobial peptides. Decreased absorption of tryptophan

due to ACE2 occupation by SARS-CoV-2 may lead to decreased absorption of antimicrobial peptides, promoting disruption of gut microbiota homeostasis and triggering of inflammation [9]. Alteration of the gut microbiota has been reported in COVID-19 patients, with dysbiosis of the gut microbiota persisting after resolution of the disease. This could reinforce the presence of intestinal symptomatology, considering the potent role of the gut microbiome in modulating immune responses and an association between gut microbiota composition and levels of cytokines and inflammatory markers found in patients with COVID-19 [10]. A possible direct viral attack on enterocytes may lead to cellular dysfunction and increased permeability, which could be responsible for malabsorption and diarrhea. Symptoms of nausea and vomiting could be the result of a similar mechanism, considering the ability of SARS-CoV-2 to invade the upper GI glandular epithelium [7]. Damage to the GI tract could be part of the observed acute systemic inflammatory response and multi-organ failure in COVID-19, with dysregulation of cytokine levels and abnormal immune responses exacerbating the severity of the disease. High serum levels of pro-inflammatory cytokines and chemoattractant molecules, including IFN-γ, IL-6, IFN-γ-inducible protein-10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), human granulocyte-macrophage colony-stimulating factor (GMCSF), are found in COVID-19 patients. In addition, activated T cells were found in the peripheral blood of a COVID-19 patient, showing high cytotoxicity with increased levels of cytotoxic granules granulysin and perforin, indicating a possible association of pathogenic T cells with the manifestation of systemic inflammation [11,12].

The distribution of viral RNA and nucleocapsid protein in the gastric, duodenal, and rectal epithelium of COVID-19 patients was also observed [13], suggesting that the virus can replicate and persist in the GI tract. A higher prevalence of GI symptoms, hypoxia-induced necrosis, and cellular injury leading to enterocyte dysfunction has been associated with various drugs administered to COVID-19 patients, including antiviral agents, antibiotics, and immunomodulators [2]. It is not yet clear whether the intestinal lesions in COVID-19 occur after systemic inflammation as a result of a secondary reaction or are caused by a primary intestinal infection or a combination of the above factors, and further analysis of intestinal samples from biopsy and autopsy should be performed. It should be noted that fecal calprotectin, which is a biomarker of intestinal inflammation in inflammatory bowel disease and infectious colitis, was found elevated in hospitalized COVID-19 patients who had resolved or persistent diarrhea compared with patients without diarrhea. This is a finding that should be considered when treating patients with preexisting gastrointestinal disease who have already developed intestinal inflammation [14].

Effect of SARS-CoV-2 on the liver

Liver injury could be due to either immune response-related injury from direct viral infection of hepatocytes or to strong ACE2-dependent viral invasion into cholangiocytes, which could lead to dysregulation of liver function or drug-induced hepatotoxicity [15]. ACE2 is highly expressed in cholangiocytes and hepatocytes and physiologically contributes to the reduction of liver injury caused by the renin-angiotensin system by degrading angiotensin Ang II to Ang 1-7 [16]. The liver also contains a large number of immune cells, and the antibody-dependent enhancement of infection (ADE) and systemic inflammatory response/cytokine storm might be related to the immune capacity of the liver and enhance the deleterious effects of abnormal immune responses and inflammation in COVID-19. ADE is induced by antibodies produced against SARS-CoV-2 spike protein and promotes SARS-CoV-2 entry into immune cells and immune-mediated tissue damage [17]. Patients with severe COVID-19 show swelling and steatosis of hepatocytes, hyperplasia of Kupffer cells, mild proliferation of hepatic sinusoidal cells, and lymphocyte infiltration, as well as increased levels of the immune activation markers IL-2 receptor and IL-6, which have been correlated with disease severity [18]. Hypoxia and hypotension associated with acute respiratory syndrome could also contribute to liver injury or even liver failure in critically ill patients, as hypoxia is associated with an oxidative stress response and increased release of reactive oxygen species, which can trigger the production of various pro-inflammatory factors that cause liver injury [19].

Pancreatic injury characterized by amylase or lipase abnormalities is another GI manifestation of COVID-19, considering that ACE2 is highly expressed in pancreatic islet cells. SARS-CoV-2 could likely cause islet cell damage, leading to an increased risk of diabetes. Similar to the mechanisms of liver injury, the direct cytopathic effect of SARS-CoV-2 or the acute systemic inflammatory response and subsequent immune cell response and cytokine storm as well as COVID-19 medication

could be responsible for pancreatic tissue damage and enzyme abnormalities [20].

CONCLUSIONS

COVID-19 disease presents with a wide spectrum of clinical manifestations in the digestive system, combined with abnormal laboratory and imaging findings. The ability of SARS-CoV-2 to invade host cells of the digestive system and the persistence of the virus in fecal samples from COVID-19 patients even after respiratory symptoms have resolved should not be overlooked, as this indicates possible oral-fecal transmission of the virus. The pathophysiological mechanisms of SARS-CoV-2 infection that underlie gastrointestinal symptoms have not yet been elucidated, highlighting the need for further studies, including the analysis of patients' clinical samples (autopsies, biopsies) and imaging findings during different phases of the disease. In addition, experimental studies will lead to a better understanding of the cellular and molecular biology of the interaction of the virus with host cells and its effects on the immune system. From a clinical point of view, attention should also be paid to the monitoring of patients with pre-existing pathology in the digestive system to prevent exacerbation of the disease and severe complications.

Conflict of interest disclosure: *None to declare.*

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ERCP for the treatment of biliary complications following cholecystectomy

Christos Konstantakis[1], Georgios Theocharis[1], Georgios Skroubis[2], Ioannis Kehagias[2], Christos Triantos[1], Konstantinos Thomopoulos[1]

Abstract

Background: The purpose of this study is to evaluate the feasibility and efficacy of endoscopic treatment of biliary complications in patients undergoing cholecystectomy, as represented by a decade of data.

Methods: During the 01/2010 - 12/2019 period, 4465 ERCPs were performed in our department. We selected and studied retrospectively cases with complications after cholecystectomy. We evaluated mainly patients with post-operative biliary leak and biliary stenosis. Patients with choledocholithiasis found after cholecystectomy were not included in the study unless coexisting with the above conditions. All data were retrieved from patients´ files and electronic records.

Results: A total of 86 ERCPs (1.9%) were performed in 65 unique patients (31 male) for biliary complications following cholecystectomy. Patients range in age from 27 to 90. Forty-eight patients (73.8%) presented with Amsterdam type A injuries (leakage from cystic duct, duct of Luschka or peripheral biliary radicals), 8 (12.3%) type B (major bile duct leakage), 5 (7.6%) type C (an isolated ductal stricture) and 4 (6.1%) type D (complete transection of the bile duct). In one patient (0.65%) selective canulation of the bile duct was not possible. In 60 out of the 65 patients' permanent resolution of the biliary injury was achieved giving an overall success rate of 92.3 % following one to five procedures.

Conclusion: Endoscopic treatment of postoperative complications of cholecystectomy is both feasible and highly effective, accompanied by a very high success rate. However, multiple sessions / hospitalizations are often required, and in cases such as complete duct transection the solution remains surgical.

Key words: *ERCP; biliary complications; biliary leak; biliary stenosis; management; cholecystectomy; guidelines*

INTRODUCTION

Cholecystectomy (CH) is considered the gold standard for the treatment of symptomatic cholelithiasis among other indications (acalculous cholecystitis, gallbladder polyps, porcelain gallbladder). It has an excellent safety profile. In population-based studies, post-operative mortality after CH for gallstone disease ranged between 0.1% - 0.7%. Complications following CH are rare but not insignificant. They can be identified intra-operatively or present in the immediate or late post-operative period. Surgical site infection, haemorrhage, bowel perforation, post cholecystectomy syndrome, cystic duct remnant, residual and recurrent bile duct stones and iatrogenic bile duct injuries (bile leak and biliary strictures) are among the most common.

Biliary injuries (BI) are perhaps the most feared complications following cholecystectomy. Less than 40% of these injuries are recognized during operation. The majority of BI will present during the 1st post – operative week (leaks) but can present years after index CH as biliary stenosis. Patients with BI are at an increased 1y mortality (4% vs. 1%) with a hazard ratio of 1.92 (95% CI

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= 1.24–2.97) when compared with uncomplicated CH. The introduction of laparoscopic cholecystectomy (LC) has not changed the frequency of complications, as it was comparable in a systematic review between LC and open cholecystectomy (OC). Despite increasing experience with laparoscopy, an unchanging incidence of bile duct injury between 0.42 and 1.1% has been reported in earlier studies. Although, true incidence may not be known due to underreporting bias [1-4].

Endoscopic retrograde cholangiopancreatography (ERCP) has been safely and effectively used for the diagnosis and management of postsurgical biliary leaks and stenoses [1]. Several endoscopic techniques have been implemented in the management of bile leaks. Biliary sphincterotomy (BS) alone, biliary stenting with or without sphincterotomy, and nasobiliary drainage with or without sphincterotomy have all been tried. The main principle of these methods is to reduce the pressure gradient between the biliary tree and the duodenum, thus facilitating bile flow into the duodenum instead of extravasation via the leak site. The principal treatment of bile duct stricture, with or without bile leak, is gradual dilatation of the strictured segment with pneumatic balloon dilators and stenting, with stents being exchanged every 3 months, till the site of narrowing disappeared. Stenting is essential for the preservation of the dilatation effect and usually multiple endoscopic sessions are required. Both multiple plastic stents and single fully covered metallic stent have been used to achieve this [3-5].

There are many classifications of post - CH BI. The Amsterdam classification is widely accepted because it links directly BI and treatment options, which is quite practical to use for endoscopic purposes (Table 1) [6].

The purpose of this study was to evaluate the feasibility and efficacy of endoscopic treatment of biliary complications in patients undergoing CH, as illustrated by a decade of data.

MATERIALS AND METHODS

All patients who underwent ERCP in our department for BI complications following both LC and OC between 01/2011 and 12/2020 were identified and included in our study. The ethics board of our hospital approved the performance of this study.

All data (table 1) regarding the baseline patients' demographics, the characteristics of the biliary injury, the type of endoscopic management, the presence of common bile duct stones, the diagnostic and therapeutic endoscopic interventions and follow-up (resolution of BI / adverse events / surgical treatment) were retrospectively retrieved from patients´ files and electronic records.

Follow-up: In our hospital (tertiary centre) alone, there are more than 500 cholecystectomies performed annually. Furthermore, our hepatobiliary unit acts as regional referral centre. The likelihood of patients being referred to another unit would be truly improbable. On the contrary, BI patients are referred to our department from other hospitals too. That means that most outcomes (including any long – term outcomes on biliary strictures) could be recovered from our records (especially those requiring further endoscopic evaluation / intervention).

Patients with postoperative biliary leaks and/or

Table 1. *Patient characteristics: baseline demographics, type of biliary injury, type of endoscopic management, presence of common bile duct stones and follow-up data (complications, success).*

No of patients	65
Mean age ± SD in years (range)	61.7 ± 12 (27 - 90)
Male / Female	31/34
No of ERCPs (total)	86
Type of Biliary injury N (%)	
Type A	46 (70.7%)
Type B	5 (7.6%)
Type C	4 (6.1%)
Type D	4 (6.1%)
Underlying choledocholithiasis	7 (10.7%)
Sphincterotomy only	4 (6.1%)
Placement of fully covered self-expandable stent	3
Placement of nasobiliary drain	1
Sphincterotomy and stent placement	59 (%)
Failed cannulation of the bile duct in the first ERCP	1
More than one ERCP for the initial management	5 (7.8 %)
Additional ERCP for patients with biliary strictures	21
Post ERCP complications	7
Success	60 (92.2%)

biliary strictures were included. Patients with choledocholithiasis found after cholecystectomy were not included in the study unless coexisting with BI.

These patients were referred by the attending surgeon for persistent *bilious*-looking *drainage* from the surgical *drain* or following investigation of clinically relevant post-operative symptoms (abdominal pain, fever, nausea, vomiting, jaundice, gut distension).

Suspected bile duct injuries were investigated with the use of imaging modalities, like abdominal computed tomography (CT), abdominal ultrasound (US), and/or magnetic resonance cholangiopancreatography (MRCP) and were confirmed by fluoroscopy during ERCP. Patients with strong clinical suspicion underwent ERCP without prior investigation.

The type of the biliary leak / injury was classified by reviewing the cholangiography acquired during the endoscopic session, and it was graded according to the Amsterdam criteria [6].

Type A: Leakage from cystic duct or peripheral radicals / aberrant ducts.

Type B: Common bile duct injury, leakage (with or without stricture)

Type C: Bile duct stricture, without leakage

Type D: Complete transection or excision of the common bile duct with inability to visualize the proximal biliary radicles and failure of passage of guide wire proximally.

The endoscopic therapeutic interventions performed for the management of complication were recorded and classified into sphincterotomy, stenting or combination therapy. All procedures were performed by experienced pancreatobiliary endoscopists. The performed technique in each case was mainly based on endoscopist's preference. Usually, a 10 Fr plastic stent was inserted proximal to the site of leakage or stricture to eliminate the transpapillary biliary-duodenal pressure gradient. The stents were removed after four weeks in patients with leaks. For patients with bile duct stricture, dilatation with 6-10 mm pneumatic biliary balloons and placement of a gradually increased number of 10Fr plastic stents was performed, with three-month intervals, until stricture resolution.

If residual bile duct stones were identified, they were also removed by balloon extraction, following sphincterotomy.

Success of endoscopic management was defined as:

- In the immediate post - ERCP period: Resolution of symptoms, normalization of liver enzymes and significant reduction / cessation of drainage output in patients with a percutaneous drain.
- On follow-up: No adverse outcomes recorded following a) removal of the drain in patients with a leak, b) resolution of the stricture in cholangiography when the stents were removed if a stricture was present.

ERCP failure was defined as the need for salvage biliary surgery. Note that need for repeat ERCP in a given patient was sometimes necessary and is not considered a failure if the objectives stated above were eventually achieved.

RESULTS

A total of 86 out of 4465 ERCPs (1.9%) were performed during the study period for biliary complications following cholecystectomy in 65 unique patients. Mean age was 61.7 (27 – 90), with relatively equal gender distribution (31 men, 34 women).

Out of the 65 patients, 48 patients (73.8%) presented with Amsterdam type A injuries (leakage from cystic duct, duct of Luschka or peripheral biliary radicals), 8 (12.3%) with type B (major bile duct leakage), 5 (7.6%) with type C (an isolated ductal stricture) and 4 (6.1%) with type D (complete transection of the bile duct) (Table 1).

In the 4 patients with type D injuries, ERCP contributed strictly diagnostic input, confirming the diagnosis of a complete CBD transection. All patients in this group were referred for surgical treatment. In one patient, presenting with jaundice attributed to a post-operative biliary stricture (type C BI), selective canulation of the bile duct wasn't possible, even after two attempts. This patient was also treated surgically.

Seven (7) out of the 65 patients (10.7%) had underlying choledocholithiasis (undetected at baseline evaluation) and bile duct clearance of stones was performed following sphincterotomy.

Four (4) patients (6.1%) underwent only sphincterotomy (1 patient was eventually classified as type D) and 57 patients (87%) a combination of sphincterotomy and stent placement. Plastic stents were used in the majority, while in three patients total, a fully covered self-expandable metallic stent was placed in the first (one patient) or second ERCP session (in two patients – rescue therapy).

In five patients (7.6%) more than one ERCP was performed. One patient required repeat ERCP due to bile duct cannulation failure. The rest four patients due to failure to completely resolve the problem at first attempt.

Additionally, patients with bile duct stricture required 3 to 5 sessions, performed at 3-month intervals, were treated with insertion of an increasing number of stents (max number of sessions was 5 and maximum number of simultaneous stents was 4).

In 60 out of the 65 patients, permanent resolution of the BI was achieved giving an overall success rate of 92.3%.

Safety profile: There were 4 cases of post-ERCP pancreatitis (mild and moderately severe), three cases of infectious complications / cholangitis but no case of bleeding, perforation, or mortality related to endoscopic treatment.

DISCUSSION

Endoscopic management of post cholecystectomy biliary injuries is highly successful. In our study 92.3 % of injuries were treated successfully following one or more endoscopic sessions. Only 4 out of the 65 patients eventually required surgical intervention. Surgery is required in patients with type D injuries according to the Amsterdam classification [6]. In cases with complex biliary injury restoration of the continuity of the biliary tree is nearly impossible with an endoscopic / radiologic approach (only few case reports) and these patients usually require operative management. Timing and type of surgery depends on patient condition and local expertise. The most common type of repair surgery is the (Roux en Y) hepaticojejunostomy.

Although the role of endoscopic management of post cholecystectomy bile duct injuries has been established as the preferred option, it still remains unclear which is the optimal endoscopic approach [7-9]. Endoscopic sphincterotomy, stenting, and combination therapy have all been used. In our study group the majority of patients were managed with both techniques.

The effectiveness of both endoscopic stenting and sphincterotomy alone in the treatment of biliary leaks after cholecystectomy has been a topic of research for many authors. It is an established fact that ES carries an additional risk of complications (pancreatitis, bleeding, perforation). Besides these drawbacks it is a very attractive option because it:

a) obviates the need for repeat endoscopy (as opposed to stent insertion) and thus
b) reduces the cost of the procedure. However, this must be weighed against potential severe and costly ES complications.
c) finally, cannulation of the papilla is easier following sphincterotomy, thus facilitating easier stent insertion especially in cases of guide wire loss.

However, several studies report that ES is inferior to only stenting in all kind of leaks [9-12] with the exception maybe of Amsterdam A leaks where ES was reported to be non-inferior to combination treatment (ES plus stent) [3]. In other studies, endoscopic sphincterotomy alone has been found effective in treating bile leak patients [13,14]. Sandha et al., [14] showed in their study of 207 bile leak patients that ES alone is an effective treatment for the low-grade (LG) leaks. For high-grade leaks, they still recommended biliary stenting. Aksoz et al., [12] showed in their 31-patient study that ES is an effective treatment (87% success rate) when treating LG biliary leak. They recommend stenting as the primary treatment for high-grade leaks and for LG leaks only in the event that ES fails. Mavrogiannis et al., in a prospective study found that small-diameter biliary stent alone can be as effective and safe as endoscopic sphincterotomy followed by insertion of a large-diameter stent in bile leak patients [15]. Different results between studies may be influenced by the extent of sphincterotomy. If the ES is the only treatment, it has to be done properly. In our unit, ES is performed to cut the sphincter completely by extending the incision to the superior margin of the intramural bile duct. Smaller ES may not be effective enough when treating leaks, and this may have been the reason of poorer outcomes in previous studies. Moreover, extensive ES in these patients was carried out in our unit by expert endoscopists. The above represent the experience from our centre and in no way constitute a general recommendation. ES (especially extended) carries a non-negligible risk for serious complications even in the hands of the most experienced endoscopist.

Endoscopic (European) society guidelines (ESGE) advocate the use of stent insertion. Stent diameter (7-Fr vs 10-Fr) does not seem to be an issue [16].

Despite frequent sphincterotomies the risk of pancreatitis, bleeding, and perforation has been low in our study and similar results have been reported in previous studies [10-14].

Even following endoscopic sphincterotomy and stenting, some leaks cannot be ameliorated and these patients need additional intervention. Placement of a fully covered self-expandable metallic stent may reduce extravasation [17,18]. Nasobiliary drainage may be helpful in some patients [19].

Second look ERCP and cholangiography is not required for removal of the stent, provided that there is

no sign of clinical, biochemical or radiological abnormality persisting. Stents can be removed with normal upper endoscopy using either an end- or side-viewing endoscope without performing fluoroscopy - ERCP, and this could help reduce costs and adverse events [20].

Endoscopic management has the advantage that the interval between BI and index ERCP for treatment does not seem to affect the outcome [16]. With that in mind, we believe that ERCP must be performed as soon as possible in order to prevent peritonitis and/or sepsis. Drain placement (of bilomas) either during the primary operation in case of injury suspicion or radiologically post-surgery is beneficial and it is recommended if there are difficulties with the anatomy of the biliary tree during the operation.

Our study has several limitations which mainly arise from its retrospective design. Several methodological issues regarding patient follow-up and data collection exist. Furthermore, the overwhelming majority of these patients are diagnosed and referred from other departments or even hospitals. All this further hinders acquisition of useful input.

In conclusion, ERCP is an effective procedure for the treatment of post cholecystectomy biliary complications.

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Considerations regarding adult vaccines by health care professionals. The experience of a university hospital in western Greece

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Abstract

Background: Vaccination issues cause increasing concern both to the research community and the population, basically due to financial reasons and vaccination-related side effects. However, no one can dispute the contribution of vaccination to the reduction of epidemics. The aim of this study was to investigate the knowledge of healthcare professionals in a Mediterranean University Hospital in regards to adult vaccination and its safety. We also evaluated the proportion of health care professionals who have been vaccinated and the relation of vaccination rates to the level of education and type of work.

Methods: We prospectively conducted a survey in order to identify possible reasons for the adherence to the national recommendations for adults' vaccines among health care workers in western Greece.

Results: From a total number of 1080 Healthcare Workers, 384 were randomly selected and participated in our study. More than half of participants recognized the efficacy (58%) of vaccines. 63.7% of the responders have received the vaccines proposed by the National Vaccination Program for children and adults. Their main sources of information were printed materials (43.1%) and internet resources (29.1%). Several participants believed that vaccination can harm the health of the person being vaccinated (33.7%), and is generally unhelpful (24%). A significant proportion of the sample (20.7%) believed that vaccination is recommended because it serves pharmaceutical companies' interests, demonstrating a general mistrust against health care system. The limited amount of knowledge that health care professionals have about some types of vaccine (herpes zoster, diphtheria-tetanus vaccine every 10 years) has emerged, which is linked to their concern that vaccines can cause inactivation or can threaten their health.

Conclusion: A more intensive worldwide survey among health care professionals is warranted in order to depict health care professionals' thoughts and beliefs in regards to adult vaccines. Broadening and improving health care workers' knowledge regarding vaccination will reduce any anxiety, and will also probably increase their vaccination coverage.

Key words: *Vaccines; healthcare workers; vaccination safety*

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INTRODUCTION

The introduction of vaccinations improved global health dramatically, decreasing the spread of infectious diseases and their related consequences [1]. Furthermore, it is overall accepted that vaccination contributed worldwide in the reduction of pandemics [2]. The issue of vaccination, is however of great concern to both the

research community and the general population. Healthcare workers (HCWs) are at increased risk of contracting infections and further transmitting them to colleagues and patients [3]. Vaccinations of HCWs constitute a key measure of occupational medicine and infection control programs within healthcare facilities [4]. Although the vast majority of HCWs endorse vaccination, negative attitudes towards vaccination can be found among them as well. According to a recent systematic review, HCWs with lower confidence in the benefits and safety of vaccines are less willing to recommend vaccines to their patients and less likely to accept vaccinations for themselves [1]. Today a number of health care workers is sceptic towards vaccination, which results from both external factors (internet / media) and factors related to the human nature itself [5]. At the same time, research shows lack of knowledge among them in regards to vaccination and vaccines [6].

The aim of this study was to investigate the thoughts and beliefs of health care workers in the largest university hospital of western Greece regarding vaccination. Specifically, key points of the research were the investigation of the believed efficacy and necessity of vaccines, potential fear of side effects and the sources of information. In addition, we aimed to investigate the impact of participants' demographic characteristics (gender, age, type of work) on their views and attitudes towards vaccination.

PATIENTS AND METHODS

A cross-sectional study was conducted for a period of three months in 2019, among 384 randomly selected health care workers employed by the University Hospital of Patras, Greece. Study participants were selected by means of simple random sampling from the list of physicians, nurses, and other paramedical and nonmedical personnel (internal medicine, obstetrics, surgery, intensive care, pediatrics, microbiology department) and services (administrative and technical).

All participating HCWs completed a self-administered questionnaire that contained 33 questions regarding demographic characteristics (age, gender, level of education, field of work), their knowledge about the efficacy and safety of vaccination and specific vaccines for adults (Varicella Zoster Virus - VZV, Diphtheria-Tetanus-Pertussis – DTP, Pneumococcus - Pneumo, Hepatitis B - HepB, and Influenza vaccines) and beliefs regarding adverse effects of vaccines. The questionnaire was anonymous and the principles of research ethics were also implemented. The Ethics Committee of the University Hospital approved the study protocol.

DATA ANALYSIS

The SPSS was used for the statistical analysis of data obtained from the questionnaires. The statistical analysis included two levels. In the beginning, frequencies, mean values and standard deviations were calculated, in order to draw initial conclusions about participants' views on vaccination. Secondly, between group comparisons were performed to detect potential correlations between demographic characteristics and outcome measures. The following statistical criteria were used: the Pearson correlation, the t-test for 2 independent samples and the ANOVA test for more than 2 independent samples. Statistical significance was set at p=0.05.

RESULTS

Demographic characteristics

From the 384 enrolled participants, 67.5% were females and 32.5% were males. 65.1% were between 40-67 years old and 34.9% were between 20-40 years old. In regards to their educational level, 67.2% had higher educational level (university degree), 28.1% had middle educational level (high-school graduates) and 4.72% had received basic education. Most of the participants were nurses (46.4%), followed by paramedics (22.7%), administration workers (18,9%) and doctors (11.7%).

Participants' opinion regarding vaccines

In regards to vaccines' safety, 58% of the participants considered vaccines mostly not safe, while only 40.4% considered vaccines very safe. Interestingly, 6 of them (1.6%) considered vaccines totally not safe. Regarding vaccines' efficacy, 57.7% considered them very efficient, while 39.2% believed that vaccines are mostly effective. Only 1.6% considered vaccines mostly ineffective and 1.6% considered them totally ineffective. The high level of efficacy and safety attributed to vaccines seems to be in accordance with the level of acceptance of the participants towards the vaccines suggested by the Greek National Vaccination Program (NVP). A significant majority of participants (63.7%) had already been vaccinated according to the NVP while 31.1% had not.

The most frequent reasons for not being vaccinated were: "Negligence", followed by the "Fear of risks and side effects". 81.1% of participants believe that "vaccines are likely to cause side effects". When asked about the

time the side effects may occur, 72.8% of participants answered that side effects occur during the "first few days", followed by 18.2% stating "during the first 5 years", and 9% believing "after the first 5 years". A significant proportion of participants were skeptical towards vaccination, 33% believed that vaccines are not safe, 24% believed that they are unnecessary, while 20.7% believed that "vaccination serves pharmaceutical companies' interests".

43.1% of the participants obtained information about vaccines mostly from medical books/manuals, while 29.1% got information from internet resources and 18.3% drew information from social conducts. Concerning the necessity of vaccination for high and low-risk population, 66% of participants stated the need for vaccination even in the low-risk population and 91.3% answered that vaccination is extremely necessary for the group of high-risk patients, even those who had not been ill previously. When asked about which medical specialty is considered the most suitable to administer vaccines, 39.5% answered internal medicine specialist, 33.5% answered general practitioner and 26.8% answered a Health Care Visitor. 89.2% of the participants answered that the free administration of vaccines by the National Health System (NHS) will increase vaccination rates.

Participants' views on specific vaccine categories

Most of healthcare workers seem well-informed about the Influenza vaccine, followed by the Hepatitis B and the *Pneumococcus* vaccines. Specifically, 97.4% knew about the Influenza vaccine, 92.9% about the HepB vaccine and 86.9% about the Pneumo vaccine. Extremely few participants knew about the Dipheritis–Tetanus-Pertussi (DTP) and the Varicella Zoster Virus (VZV) vaccines (69.4% and 51.6% respectively). Concerning the source of information for each vaccine, medical staff (Internal Medicine Specialists, Infectious Diseases specialists) and media were the main sources of information.

Correlations

Further analysis showed statistically significant correlation between sex and beliefs regarding the complications of vaccinations proposed by the National Vaccination Program ($p = 0.000$). A significant correlation was also found between sex and skepticism regarding vaccination-related adverse effects, with 57,2% of females and 24.3% of males answering that vaccines are likely to cause side effects. Interestingly, we found a statistically significant difference between gender and the reasons for not being vaccinated. 25.4% of females answered that vaccines are not safe and 31.9% of males answered that vaccines are unnecessary. Gender differentiated participants' responses regarding the factors that may prevent vaccination ($x2 = 10,074$, $p = 0.018$). More precisely, women believe that vaccination can be dangerous to health and should therefore be avoided (25.37%), and men believe that it is useless (16.92%). Moreover, the correlation between the age and NVP completion showed that 38.2% of participants aged 40-67 years old had completed vaccination.

Statistically significant correlation was observed between the type of work and the implementation of all vaccines proposed by the National Vaccination Program. Specifically, it appeared that physicians (9.43%) and nurses (33.15%) had received these vaccines to a greater extent than other specialties in the sample. Doctors seemed to differentiate from the rest healthcare workers, believing that vaccines are safe and efficient. As for the most suitable specialty to administer vaccines, doctors supported the Internist (7.36%), while nurses preferred Health Care Visitor at a rate of 17.4%.

Finally, the type of work appeared to influence participants' responses to the information they have about specific vaccine categories. Particularly, both physicians and nurses were found to have higher levels of knowledge about the shingles vaccine than administrators, paramedics / others.

DISCUSSION

Health care providers is a special group of workers, exposed to several viruses. They belong to a population, who is more likely to get sick, and are at increased risk of transmitting diseases to patients whose health is already compromised [4]. To prevent this risk, it is necessary to carry out the vaccines proposed by National Vaccination Programs.

In our study vaccination rate was 63.7%. Our results are similar to the study by Maltezou et al, where approximately two thirds (63%) of the study group favored mandatory vaccinations for HCWs. Similar acceptance rates were noted in a German study but significantly higher in an Australian study (68.4% and 83%, respectively) [7].

Moreover, we reported that employees agree regarding the view that vaccines can bring positive results to a large extent (57.7%). A small proportion seemed

to have a negative attitude regarding vaccines safety and the vaccination process. This can be attributed to the increasing recognition of the importance of the condition of the patients over the years and to the further recommendation of adult vaccination by primary caregivers [8]. There are many factors that influence vaccination rates. In our study, we observed that vaccination rates were related to social and demographic characteristics of participants (age, gender, education, occupation). Women, were found to be highly vaccinated. This finding is in accordance to the existing literature, where it is recognized that the acceptance of vaccines by healthcare workers and their subsequent implementation depends on factors such as gender, age or the department in which they work [9].

On the other hand, the proportion of non-vaccinated personnel observed in this study raises the issue of a small yet significant percentage of individuals who despite the availability of effective prevention remain at great risk to get infected [10]. Immunization of HCWs has been associated with improvements in patient safety and decreased morbidity and mortality in hospitals and other health care facilities. Moreover, vaccination of HCWs can reduce workplace absences, deliver economic benefits for healthcare systems, and provide cost savings for healthcare organizations [11].

In many studies, the most common reasons for not vaccinating are the lack of suggestions for vaccination and the fear for serious complications [8]. In our study most of employees reported negligence as the main reason for non-vaccination. This is a finding that might reflect practical difficulties in obtaining access to vaccination services or a less cautious and responsible attitude of Greek health care workers towards their personal health and safety [10]. However, according to our study, healthcare workers were well-informed regarding the influenza vaccine compared to other vaccines, an observation which is compatible with findings of previous studies [12]. In our study, knowledge of influenza, hepatitis B and pneumonococcus was 97.4%, 92.7% and 86.9% respectively.

Vaccination implementation, moreover, is considered necessary for individual health, the improvement of quality of living and of course the promotion of public health [13]. This is a necessity recognized by health care professionals and as long as they work in the field of health-care they can promote both their own health and the health of people in their immediate environment [14,15].

Our study has some limitations. First limitation of the research concerns the limited time available. This time constraint also affected the sample, which, as already mentioned, is a sample of convenience and comes from employees in a single hospital, making it impossible to generalize the results to the population. In this study, it was observed that some of the questions were not answered, while there is no way for the researcher to control the degree to which the participants' answers are true or false.

Our study demonstrated that vaccines are generally accepted by people who work in a hospital, an acceptance that affects both their safety and efficacy. This seems to be consistent with the extent to which they have accepted the National Vaccination Program. This high degree of acceptance and vaccination may stem from the sense of responsibility that participants have, their respect for the patients with whom the participants meet every day, their desire to protect them from various diseases and perhaps their effort to set a "good example" for patients [16].

Another major finding is that a significant percentage of healthcare workers believe that vaccines are unnecessary. If voluntary vaccine uptake fails to achieve the desired rates, mandatory policies should be considered, provided that benefits outweigh harm for HCWs, patients' welfare is enhanced, and fair rules and exemptions are defined [17].

Nosocomial transmission of vaccine-preventable diseases can be avoided thanks to immunization. The ideal coverage is dynamic for each disease, depending on the effective reproductive rate, which itself varies with the level of contacts. Improving vaccine coverage among HCWs is challenging, but benefits patients who might face contagious HCWs as well as HCWs who provide care to contagious patients [18].

CONCLUSIONS

This cross-sectional epidemiological survey from a Mediterranean tertiary centre boosts already known facts from previous surveys, further highlighting the issue of vaccination of health-care professionals. The recognition of the efficacy and necessity of vaccines across almost all participants is noteworthy. We showed that although vaccines are widely recognized by health authorities and the medical community as a major tool for promoting public health, for many individuals (even for health care workers), this is not a sufficient basis. They doubt the benefits of vaccines, worry over their safety

and question their necessity, an attitude we refer to as vaccine hesitancy. The question that arises is what can happen especially to the general population who do not have special knowledge and experience. A similar survey should be conducted in the general population too.

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Management of secondary aortoenteric fistulas occurring as complications after open and endovascular repair of abdominal aortic aneurysms

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Abstract

Secondary aortoenteric fistula (SAEF) is an uncommon and life-threatening clinical complication of both open and endovascular abdominal aortic aneurysm repair surgery. The most common site of SAEF is the duodenum, especially the 3rd part. Aortoenteric fistula may initially present with transient and self-limited gastrointestinal bleeding episodes, followed by a later catastrophic life-threatening hemorrhage. Endoprosthesis excision followed by extra-anatomic by-pass grafting or in situ aortic replacement procedure is the gold standart treatment. In unstable patients with severe comorbidities endovascular intervention can serve as a bridging procedure to optimize patient's status for aortic reconstruction.

Key words: *Aortoenteric fistula; secondary; aneurysm; endovascular; open repair*

INTRODUCTION

Aortoenteric fistula is defined as a communication between the aorta and the gastrointestinal (GI) tract. Secondary aortoenteric fistula (SAEF) is an uncommon and life-threatening clinical condition that can complicate aortic reconstructive surgery [1]. It is a devastating complication of both open and endovascular abdominal aortic aneurysm (AAA) repair surgery, may be related to endoprosthesis infection and can result in gastrointestinal bleeding [2,3].

The first report of SAEF was made in 1953 when Brock described a fistula of a proximal anastomosis of an aortic homograft and the duodenum. SAEFs may occur between 2 weeks and 10 years after open repair while an annual incidence of 0.6% to 2% has been reported [3]. Aortoenteric fistula after endovascular repair (EVAR) of abdominal aortic aneurysm occurs in approximately 0.36% of cases [1,4].

SAEFs can be classified into two forms: the direct abnormal communication between the aorta and bowel lumen and the aortoparaprosthetic-enteric fistula due to intestinal erosion [5].

The purpose of this review article is to investigate the pathogenesis, clinical presentation and treatment of this frequently fatal disease.

PATHOGENESIS OF SAEF AFTER OPEN AND ENDOVASCULAR REPAIR

Pulsating mechanical pressure of the graft on the bowel wall or a pseudoaneurysm due to perigraft bacterial infection or due to a contaminated perigraft he-

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matoma are thought to be the causative mechanisms leading to this catastrophic complication [6]. SAEF most commonly occurs between the proximal aortic suture line and the duodenum after open abdominal aortic surgery.

The pathogenesis of secondary aortoenteric fistulae after EVAR is controversial with a number of different mechanisms proposed for its occurrence. It develops months to years after EVAR, although early occurrence has been also described. A strong hypothesis is that endograft infection could be secondary to the grafting procedure (bacterial inoculation during endovascular procedure) or due to a pre-existing mycotic or inflammatory aneurysm. Endograft infection could result in intestinal necrosis and fistula formation between the aneurysm sac and the intestinal wall [4]. Other causes of aortoenteric fistula include stent migration, erosion of the aorta and the duodenum by embolization coils, fabric rupture, erosion of the aorta by the hooks and barbs, Crohn's disease or other septicemic conditions that result to secondary endograft inoculation [4]. Endoleak and even endotension may also lead to aortoenteric fistula formation. Authors have suggested that this condition may result in pressure necrosis of the aneurismal sac against the intestinal wall.

CLINICAL PRESENTATION AND DIAGNOSIS

The most common site of secondary AEF is the duodenum (73%), especially the 3rd part [7]. Due to the low incidence of this condition and the nonspecific signs and symptoms, the diagnosis requires a high index of suspicion and a careful review of patient's history.

The main clinical manifestations are gastrointestinal hemorrhage (70%), septic complications (16%) or a combination of both (12%). Typically, the aortoenteric fistula may initially be presented with transient and self-limited gastrointestinal bleeding episodes ("herald bleeding"), followed by a later catastrophic life-threatening hemorrhage. Other presenting symptoms are unexplained fever, abdominal or back pain, chronic anemia, shock, or symptoms associated with compression of adjacent structures [8].

Computed Tomographic Angiography (CTA) is the preferred imaging modality for the diagnosis of SAEF. CT angiography has a relatively high sensitivity (94%) and specificity (85%) for the diagnosis of SAEF [9,10]. Imaging findings of SAEF include increased perigraft soft tissue, pseudoaneurysm formation, presence of gas or fluid around the graft, close proximity of the graft to the adjacent bowel wall and extravasation of contrast agent into the bowel lumen [11,12]. If the imaging findings of CTA are not specific and the gastrointestinal (GI) bleeding persists, it is then appropriate to proceed with esophagogastroduodenoscopy (EGD) to further investigate for the disease or seek other potential causes of GI bleeding. A typical endoscopic finding in the case of SAEF is the observation of adherent clots or bleeding at the fistula opening and the identification of the vascular graft or stent into the bowel lumen.

MANAGEMENT AND TREATMENT

OPEN REPAIR

Patient's clinical status, hemodynamic stability and the presence of preoperative sepsis are the most important determinants for the choice of the surgical strategy. Operative strategies that have been used include graft excision accomplished with extra-anatomic by-pass or in situ aortic replacement [13,14]. Extra-anatomic revascularization consists of staged or concomitant axillo-bifemoral bypass and graft explantation with aortic stump closure [13,14]. In situ reconstruction using homografts, prosthetic grafts or vein grafts- the "neo-aortoiliac system" procedure - is another open repair option [15]. Bowel repair is of great importance. Excision of the eroded part of the duodenum or the bowel and interposition of the omentum, eliminates a septic source and decreases the risk for recurrence of infection. These procedures are demanding and associated with high mortality and morbidity rates, especially when undertaken in unstable, septic patients with severe comorbidities [8,15].

ENDOVASCULAR TREATMENT

An additional treatment option has been added to our inventory, first described by Deshpande et al, who used endovascular repair for a SAEF in a high-risk patient [16]. The advantages of endovascular approach are the rapid control of hemorrhage and the avoidance of an intervention in a hostile abdomen or in patients with severe comorbidities, unfit for open surgery. In unstable patients with severe sepsis endovascular intervention can serve as a bridging procedure to open repair offering immediate control of hemorrhage and time to improve the patient's clinical status. On the other hand, endovascular approach has great limitations as bowel defect is not repaired, infection if present persists and retroperitoneum debridement is not feasible [4].

A review study on outcome after endovascular repair of SAEF showed that endovascular approach is associated with a high incidence of persistent/recurrent/new infection or recurrent bleeding which significantly limits patient's survival. Preoperative evidence of sepsis was found to be an indicating factor for unfavorable outcome [2]. Another study showed that endovascular repair was associated with lower morbidity and in-hospital mortality rates compared with open repair. However, there was a trend for worse recurrence-free, sepsis-free, re-operation-free and AEF-related death-free rates after endovascular repair. The early survival advantage of EV-AEFR was lost after two years. Preoperative sepsis was associated with worse two-year overall survival [17]. A more recent meta-analysis concluded that endovascular surgery is associated with better early survival than open surgery for secondary AEFs but most of this benefit is lost during long-term follow-up. The authors recommended that the method can be used as bridging to early conversion using in situ vein grafting [18].

As a bridging method, endovascular repair of SAEF has demonstrated promising results, but as a definitive therapy for SAEF it should be considered only in high-risk patients unfit for open repair, where sepsis or systemic infection is not present. These patients should remain under rigorous follow-up for recurrence of infection or bleeding.

CONCLUSIONS

Secondary aortoenteric fistula (SAEF) is a life-threatening complication of prior aortic reconstructive surgery. Endoprosthesis excision followed by extra-anatomic by-pass grafting or in situ aortic replacement procedure is the gold standard treatment. In unstable patients with severe comorbidities, endovascular intervention can serve as a bridging procedure to optimize patient's status for aortic reconstruction. In high-risk and elderly non-septic patients, endovascular repair can be a permanent solution requiring however close surveillance and long-term antibiotic therapy.

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Oral anticoagulants in patients with Chronic Kidney Disease. A friend or foe?

Marios Papasotiriou, Paraskevi Pavlakou, Theodoros Ntrinias, Dimitrios S. Goumenos, Evangelos Papachristou

Abstract

Anticoagulant treatment of atrial fibrillation (AF) in patients with chronic kidney disease (CKD) is a common clinical problem as the prevalence of AF increases as kidney function deteriorates. Nevertheless, the risk-benefit ratio of anticoagulant treatment, especially warfarin, in patients with CKD and AF is unclear. Data analysis in patients with CKD stage III or lower, showed that well-adjusted doses of warfarin reduce the risk of ischemic stroke and systemic embolism. Regarding the use of DOACs, their administration is not inferior in efficacy for the prevention of ischemic stroke and thromboembolic events compared to warfarin in this particular group, while their safety profile is superior as they have been associated with a significant reduction in the risk of intracranial hemorrhage. In patients with end stage kidney disease on hemodialysis, warfarin administration has not been associated with a reduced risk of ischemic stroke. Moreover, there is a significant increase in the risk of hemorrhagic stroke. Regarding the use of DOACs in patients on hemodialysis and AF, treatment with DOACs has not been associated with a lower risk of new stroke or thromboembolic events. Concluding, in patients with AF and mild to moderate CKD without the need for renal replacement therapy, oral anticoagulation efficiently reduces the risk of ischemic stroke, while in those with advanced stage CKD or on hemodialysis the risk benefit ratio is still unidentified.

Key words: *Anticoagulants; direct oral anticoagulants; chronic kidney disease; hemodialysis*

INTRODUCTION

Anticoagulant treatment of atrial fibrillation (AF) in patients with chronic kidney disease (CKD) is a common and important daily problem that concerns both the cardiology and nephrology community as well as the general physician. The prevalence of AF (paroxysmal and permanent) increases as kidney function deteriorates and reaches up to 40% of patients with end-stage CKD (ESKD). In addition, these patients have an increased risk of both ischemic stroke - 1.5 times compared to patients without CKD - but also for severe bleeding episodes - 2 times higher than patients without CKD [1]. According to the revised 2019 AHA guidelines, patients with AF and CHA_2DS_2-VASc score, above 2 for men and above 3 for women, should receive oral anticoagulant therapy with one of the available agents (Warfarin, Dabigatran, Rivaroxaban, Apixaban or Edoxaban) [2].

Though, the prevalence of AF in patients with CKD is increased, the risk-benefit ratio of anticoagulant treatment, especially warfarin, in patients with CKD and AF is unclear. This is because initiation of such treatment, in this group of patients, greatly increases the risk of bleeding while at the same time controlling the therapeutic levels of warfarin (internal normalized ratio, INR) is difficult and its use is associated with increased risk of vascular calcification and cases of calciphylaxis es-

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pecially among those with ESKD [3]. Considering these data, in recent years, there has been an increase in the clinical use of newer direct oral anticoagulants (DOACs) in patients with CKD of all stages with concomitant AF without, however, sufficient data from randomized trials that confirm the safety and efficacy of these drugs in this group of patients. This uncertainty in the safety and efficacy of anticoagulant treatment in patients with CKD and especially ESKD is depicted in prescribing trends which show inter-country variation, as well as significant within-country variation between facilities ranging from 0 to 45% of the dialysis patients in the use of an oral anticoagulant, mostly warfarin (85%) [4]. Nevertheless, more recent data from the United States and Canada, report that just 24% to 46% of those with AF are prescribed warfarin while the use of DOACs is increasing with an average 23.5% of CKD patients taking them regularly for stroke prevention [5,6]. In line with these data, a Danish study with over 1500 patients with AF and CKD, describes that in recent years and especially since 2017 the rate of DOACs administration has exceeded that of coumarin anticoagulants, with apixaban being the most common substance [7]. Clinical ambiguity is further emphasized by a survey of Canadian nephrologists treating dialysis patients with nonvalvular AF, which revealed that warfarin was more likely to be recommended to patients with high stroke risk and low bleeding risk and less likely to be prescribed to patients with moderate stroke risk and high bleeding risk [8].

Moreover, despite such an increased incidence of AF in patients with CKD, the risk assessment for stroke is incomplete and problematic. The most commonly used tool for ischemic stroke risk assessment which is validated for patients with CKD (all stages) is the CHAD-VASC score which, however, does not show an acceptable accuracy in distinguishing high from low risk patients for stroke in those with established CKD (C statistic, 0.6 CKD-III, 0.7 CKD-IV / V and CKD-V Dialysis) [9]. Concerning bleeding risk assessment, the use of the HAS-BLED, ORBIT and ATRIA tools is not recommended by most of the published guidelines [1]. Thus, the aim of this review is to show the contemporary clinical data on the efficacy and safety of oral anticoagulation in patients with AF and established CKD.

METABOLISM OF ORAL ANTICOAGULANTS

Vitamin K antagonists (VKA) are still the most widely used anticoagulants for stroke prevention in patients with nonvalvular atrial fibrillation. From this group of drugs, warfarin is the one for which the majority of clinical evidence has been obtained. Warfarin is extensively metabolized by CYP2C9 in the liver, has a peak concentration (Cmax) at 2–6 h after administration, a t1/2 of 42 h, is protein bound by 97–99% and has a bioavailability of 99%. Other drugs that interact with warfarin and increase its anticoagulant effect include: amiodarone, verapamil, diltiazem, fluconazole, voriconazole, tigecycline, fluoroquinolones, NSAIDs, and SSRIs while drugs that decrease its effects include: rifampin, phenobarbital and carbamazepine [10]. Although the guidelines do not recommend VKA dose adjustment in CKD, clinical studies reveal an increased hemorrhagic risk, particularly within the first 3 months after initiation of treatment, most of them gastrointestinal [11]. For this reason, to reduce the risk of hemorrhage requires an average reduction of warfarin doses by 10% in patients with eGFR between 30 and 59 mL/min/1.73m^2 and by 19% in those with eGFR < 30 mL/min/1.73m^2, in order to maintain INR ≤ 4 [11]. Dose adjustment is also necessary in case of liver damage as then the renal clearance is enhanced. Warfarin administration is even more complicated in patients with ESKD as it is associated with an increased risk of thromboembolic events and hemorrhage.

All DOACs are excreted by the kidneys, so the presence of CKD greatly affects their metabolism. More specifically, renal involvement in the excretion of these drugs ranges from 27% for apixaban and can reach up to 80% for dabigatran. Table 1 details the hepatic and renal involvement of warfarin and DOACs metabolism [6]. The integrity of renal function in the pharmacokinetics of substances excreted by the kidneys is crucial. Renal excretion of drugs occurs primarily by glomerular filtration and occasionally by tubular secretion. When the glomerular filtration rate (GFR) and tubular function are reduced, the clearance of drugs eliminated by these mechanisms decreases and consequently the plasma half-life of these drugs is extended. This leads to increased exposure to drugs, as quantified by the area under the curve (AUC). In such cases, without proper dose adjustment, repeated dosing of a drug leads to bioaccumulation over time and toxicity [6]. The basic metabolic pathways of oral anticoagulants are presented in Table 1.

Therefore, as all DOACs show renal excretion, the presence of renal impairment will inevitably lead to their eventual accumulation. An additional problematic point in trying to adjust the appropriate therapeutic dosage of these drugs comes from the way renal function is assessed. It is noteworthy that all randomized

Table 1. *Basic metabolic pathways of oral anticoagulants.*

Substance	Kidney excretion (%)	Hepatic or other form of metabolism	Dialyzable
Warfarin	-	Predominantly via cytochrome P450 type 2C9 (CYP2C9)	No
Apixaban	27	CYP-3A4/5 P-glycoprotein	Partially (small)
Rivaroxaban	36	CYP-3A4/5 and CYP-2J2	No
Dabigatran	80	Metabolized by esterases	Yes
Edoxaban	50	CYP-3A4	No

trials (RCTs) using DOACs used eCrCl assay according to the Cockcroft-Gault formula to assess renal function. However, there are clinically significant dose deviation of DOACs based on this equation, especially considering that the proposed equation for the assessment of renal function is CKD-EPI and not the Cockcroft-Gault (CG) formula. These discrepancies are particularly significant for DOACs with greater dependence on renal clearance (dabigatran, rivaroxaban) and among elderly patients with dose discrepancies of up to 30%. In particular, mild to moderate CKD occurs in ~ 54% of patients with long-term anticoagulant therapy and approximately 25% of these patients develop severe CKD [12].

More specifically, compared to the CG equation, the MDRD and CKD-EPI equations in many cases overestimate eGFR and it is precisely this overestimation that can lead to an increased total dose of DOACs. By using the CKD-EPI or MDRD equations instead of CG, dosing discrepancies are higher with substances whose metabolism is more dependent on renal function such as dabigatran and rivaroxaban than with apixaban. Especially in patients with impaired renal function as calculated by the CG equation <60 ml / min and in elderly patients (> 75 years), the discrepancy between dabigatran and rivaroxaban doses is higher than in the general population (from 13.2% to 30.4%). In contrast, the dose mismatch of apixaban from the use of different equations to calculate eGFR is less than 5% [13]. The frequency of overdose of DOACs has also been seen in a large U.S. administrative database with over 14,000 patients with AF. In this, more than 40% of patients received a higher dose (for a given eGFR) than they normally should. More specifically, the proportion of patients receiving higher than the recommended dose was 48.5% for apixaban, 39.4% for dabigatran and 41.3% for rivaroxaban. Characteristically, the use of standard, but not appropriately reduced, doses of DOACs in patients with severe kidney impairment has been associated with a doubling of the risk of bleeding without any reduction in the risk of stroke [14]. DOACs dose adjustment according to CKD stage is presented in Table 2.

MONITORING OF THE ANTICOAGULATION EFFECT

Among patients treated with warfarin, INR is the most common test used to monitor warfarin response which should be determined at least weekly during initiation of anticoagulant therapy and at least monthly when anticoagulation (INR) is stable in order to keep the therapeutic range of an INR between 2.0 and 3.0 [2,10]. Clinical practice guidelines do not recommend dosage reduction for CKD or ESKD. Nevertheless, there are studies which show that dose reduction between 10-19% is required in patients with eGFR <60 ml/min/1.73m^2 and <30 ml/min/1.73m^2, respectively. It has not been established whether different dialysis procedures and methods result in changes in warfarin exposure and pharmacodynamics [10]. In contrast to VKAs, DOACs do not require routine monitoring as they have shown a predictable pharmacokinetic profile in patients though without high grade CKD. Monitoring of these agents, especially in CKD for assessing drug accumulation, relies on measurement for dabigatran, of thrombin time (TT) or ecarin clotting time (ECT), while for the factor Xa inhibitors, anti-Xa activity should be assessed [3]. There is a strong correlation between anti-Xa activity and factor Xa inhibitor concentration, however, it should be highlighted that there are no FDA-approved kits for universal standardization of the anti-Xa activity assay [15].

Reversal of the antithrombotic effect of oral anticoagulants is achieved using fresh frozen plasma, prothrombin complex concentrate, recombinant factor VIIa, factor VIII inhibitor by passing activity or a specific antidote. When warfarin is used, in patients with INR

Table 2. *DOACs dose adjustment according to CKD stage.*

CKD stage	eGFR (ml/min)	Apixaban	Rivaroxaban	Dabigatran	Edoxaban
I & II	> 60	Normal dose	Normal dose	Normal dose	Normal dose
III	30 - 59	Normal dose	reduced dose (eGFR < 49)	Reduced dose (eGFR < 49)	Reduced dose (eGFR < 49)
IV	15 - 29	normal or reduced dose (in patients with at least 2 of: age≥80, s. creatinine ≥1.5 mg/dl, weight ≤60 kg)	reduced dose	EMA: contraindicated FDA: reduced dose	reduced dose
V	< 15	EMA: contraindicated FDA: normal or reduced dose (in patients with at least 2 of: age≥80, s. creatinine ≥1.5 mg/dl, weight ≤60 kg)	contraindicated	contraindicated	contraindicated

value over 9, even with no major bleeding events, a single oral dose of vitamin K (2.5-5 mg) is needed, while in major bleeding regardless of INR value, vitamin K (10 mg) should be administered parenterally along with fresh frozen plasma, prothrombin complex concentrates or recombinant factor VIIa that shows a rapid effect. For dabigatran, the specific antidote Idarucizumab is indicated in case of life-threatening bleeding [16]. Idarucizumab has a rapid effect after a single dose of 5 g i.v. with no dose modification needed in CKD, though its efficacy and safety in ESKD has not been tested. For rivaroxaban, apixaban and edoxaban, FDA has recently approved Andexanet alfa as a reversal agent [10,11,16].

ANTICOAGULATION IN PATIENTS WITH NONVALVULAR AF AND ESTABLISHED CKD STAGE III (EGFR: 60 – 30 ML/MIN/1.73 M^2)

Patients' data from studies on VKAs primarily refer to warfarin and most of the evidence comes from extrapolation analysis of AF patient subgroups within larger groups of patients. There is only one randomized control study (Stroke Prevention in Atrial Fibrillation III study) that included patients with CKD stage III and patients with normal kidney function [17]. In this trial, high-risk participants were assigned to adjusted-dose warfarin (target INR 2 - 3) versus aspirin (325 mg) plus fixed, low-dose warfarin while low-risk participants received 325mg aspirin daily. Data analysis in CKD subgroup showed that well-adjusted doses reduce the risk of ischemic stroke and systemic embolism by 76% and 67%, respectively, without statistically significant differences in major bleeding rates [17]. Other data on warfarin derive from observational studies that include CKD subgroups, but overall, the results are consistent in terms of effectiveness in reducing the risk of stroke and thromboembolic episodes. A Cochrane database systematic review also favored the efficient and safe use of warfarin in patients with CKD stage III [18]. Finally, in a meta-analysis by Dahal et al, it was shown that the use of warfarin in non-dialysis dependent CKD reduces the risk of ischemic stroke and systemic embolism by 30% but at the cost of an insignificant 15% increase in major hemorrhages compared to the group not receiving warfarin [19].

Regarding the use of DOACs, the results of RCTs as well as their meta-analyses, have shown that their administration is not inferior in efficacy for prevention of ischemic stroke and thromboembolic events compared to warfarin in patients with estimated eCrCl (CG) 30-50 ml/min (apixaban, 25–50 ml/min). Recent evidence that derives from a meta-analysis of 8 RCTs and 46 observational studies, indicate the superiority of DOACs over warfarin in thromboembolic events prevention (HR 0.86, 95% CI 0.78-0.95) and bleeding risk reduction (HR

0.81, CI 0.66-0.99) in non-dialysis CKD population. In the same meta-analysis apixaban, in an eGFR accordingly adjusted dose, presents an advantage in thromboembolic events prevention compared to edoxaban [20]. However, there are insufficient data on the use of one particular DOAC over others, as there are no head-to-head studies in this CKD population. Although their efficacy is not inferior to warfarin, the safety profile of DOACs is superior. In all major RCTs, DOACs have been associated with a significant reduction (approximately 50%) in the risk of intracranial hemorrhage compared with warfarin [1]. More specifically, there was not any significant difference between DOACs and warfarin in reducing ischemic stroke in patients with moderate CKD, except for dabigatran (150 mg) and apixaban, which were superior in reducing the risk of ischemic stroke. In addition, in patients with moderate CKD, edoxaban and apixaban had a significantly reduced risk of bleeding compared to warfarin, while rivaroxaban and dabigatran showed no difference [21].

ANTICOAGULATION IN PATIENTS WITH NONVALVULAR AF AND ESTABLISHED CKD STAGE IV-V (EGFR: < 30 ML/MIN/1.73 M^2)

To date, there are no RCTs to explore the use of warfarin or other coumarin anticoagulants in patients with ESKD and AF, thus clinical practice is largely determined by retrospective studies. In a meta-analysis of 14 observational studies with 20,398 patients on hemodialysis, warfarin administration was not associated with a reduced risk of ischemic stroke (HR, 0.77; 95% CI, 0.55 to 1.07) compared with no warfarin use. In contrast, there was a significant increase in the risk of hemorrhagic stroke (HR, 1.93; 95% CI, 0.93 to 4.00) and gastrointestinal bleeding (HR, 1.19; 95% CI, 0.8 to 1.76) compared with no warfarin use [22,23]. In order to investigate the effectiveness of warfarin in the prevention of ischemic strokes and thromboembolic events in patients with ESKD and AF, a multicenter open label RCT (NCT02886962 - AVKDIAL) is in development, in which patients will be randomized to warfarin or no treatment. The results from this trial are expected in early 2023 and they will largely determine the effectiveness of warfarin treatment in patients with ESKD and AF.

Regarding the use of DOACs in patients with ESKD (on hemodialysis) and AF, in a retrospective study by the US renal data system covering the period between 2012–2015, patients receiving apixaban (521) were compared with 1561 patients with the same characteristics without treatment. In this study, treatment with apixaban was not associated with a lower risk of new stroke or thromboembolic events. However, there was a tendency to reduce ischemic strokes (insignificant) but this was accompanied by more hemorrhagic strokes when using apixaban at a dose of 5 mg bid in comparison to non-administration. At the 2.5 mg bid dose of apixaban, the incidence of intracranial hemorrhage was not significantly higher in comparison to the control group, however, at this dose, significantly more cases of ischemic strokes or thromboembolic events were observed [24]. In a meta-analysis of retrospective studies on the use of DOACs versus warfarin, the results showed that there was no difference in the prevention of ischemic strokes or arterial embolism. In particular, this result applied to all DOACs (apixaban, dabigatran and rivaroxaban) versus warfarin, in patients with ESKD on hemodialysis. However, the studies on which these results were based had significant limitations as they either involved a small sample size or a short follow-up [21]. It is noteworthy, however, that the same meta-analysis showed that patients in this group had a higher risk of major bleeding and higher mortality due to bleeding with dabigatran or rivaroxaban compared with warfarin. In contrast, administration of apixaban did not appear to increase the risk of bleeding in comparison to the use of VKA [21]. The only completed RCT to date which was designed to investigate the effectiveness of DOACs in patients with ESKD on hemodialysis, is RENAL - AF. Initially, this study, was planned to randomize 760 patients with nonvalvular AF on dialysis, to be treated with either warfarin or apixaban 5 mg twice daily or 2.5 mg twice daily in selected patients. The main endpoints included the risk of major and clinically significant bleeding, the risk of stroke, pulmonary embolism and death. The study was terminated prematurely due to insufficient patient participation. Finally, 154 patients were randomized (82 received apixaban and 72 warfarin). Apixaban appeared to be associated with fewer bleeding events than warfarin, but the difference was not significant. Therefore, even this initially well-designed study failed to be completed and bring clear results on the effectiveness of DOACs in patients with AF and ESKD.

IS THERE ANY BENEFIT FROM ORAL ANTICOAGULANT TREATMENT IN PATIENTS WITH AF AND CKD?

In patients with mild to moderate CKD (stage I to III) without the need for renal replacement therapy, oral anticoagulation treatment efficiently reduces the risk of ischemic stroke and thromboembolic events.

Moreover, all major prospective RCTs of different DOACs have shown that these agents are equally effective or even better in preventing ischemic stroke and thromboembolic events in comparison to warfarin [18,21]. Most importantly, the administration of these drugs reduces the risk of serious bleeding complications [18]. In addition, the ease of DOACs administration at predetermined doses which do not need to be adjusted should not be overlooked. On the contrary, VKA dose must be adjusted following frequent INR testing.

Overall, anticoagulant therapy increases the risk of bleeding by at least 20% in patients with advanced CKD or on dialysis, while the extent to which warfarin and DOACs reduce the risk of ischemic stroke in patients of this group remains unclear [11,21]. RCTs in the general population have shown warfarin to reduce the risk of ischemic stroke by 64% in patients with AF compared with placebo. However, there is evidence that anticoagulant therapy does not lead to a similar risk reduction in patients with advanced CKD and ESKD. There are several reasons for the ineffectiveness of VKA in patients with CKD-IV or V [21,25]. On the one hand, uremic induced platelet dysfunction may protect against thrombosis. On the other hand, increased comorbidity may reduce the chance to show a benefit (patients with ESKD have reduced life expectancy and follow-up time for stroke events) [6]. Furthermore, it should not be overlooked that all patients undergoing a chronic hemodialysis program are already receiving intravenous anticoagulation either with unfractionated heparin or more commonly with low molecular weight heparin which has a 24-hour effect and offers potential protection from thromboembolic events or may increase the risk of bleeding with concomitant administration of another anticoagulant.

WHAT ARE THE GUIDELINES RECOMMENDATIONS?

Cardiologists tend to extrapolate guideline recommendations for patients with CKD from studies and data of patients without CKD, while nephrologists are reticent in this respect. The latest 2011 KDIGO (Kidney Disease: Improving Global Outcomes) guidelines state that only nephrologists should recommend oral anticoagulants as primary prevention in ESKD and dialysis patients, based

Table 3. *Summary of guidelines about anticoagulation for stroke prevention in patients with established CKD and non-valvular AF.*

Association or approving authority	Summary of guidelines
Kidney Disease Improving Global Outcomes	"Team-based, multidisciplinary active communication, particularly involving the nephrologist, cardiologist (or cardiac electrophysiologist), primary care physician, and when possible, clinical pharmacist, may be useful to evaluate the risk-benefit of any decision regarding choice of VKA or a DOAC" [1]
American Heart Association	Dabigatran 150 mg twice daily in patients with CrCl > 30 mL/min Rivaroxaban 20 mg od for patients with CrCl > 50 mL/min Apixaban 5 mg twice daily for patients with no more than 1 of the following characteristics: age ≥ 80 years, serum creatinine ≥ 1.5 mg/dL, or body weight ≤ 60 kg Apixaban 2.5 mg twice daily for patients with at least 2 of the following: ≥ 80 years, body mass ≤ 60 kg, or serum creatinine ≥ 1.5 mg/dL74 CHA_2DS_2-VASc score ≥ 2 in men or ≥ 3 in women and eCrCl < 15 mL/min or on dialysis, reasonable to prescribe warfarin (INR 2.0-3.0) or apixaban For moderate to severe CKD (serum creatinine ≥ 1.5 mg/dL [apixaban], CrCl 15-30 mL/min [dabigatran], CrCl 15-50 mL/min [rivaroxaban], or CrCl 15-50 mL/min [edoxaban]) with an elevated CHA_2DS_2-VASc score, reduced doses of direct thrombin or factor Xa inhibitors should be considered [2]
European Society of Cardiology	Rivaroxaban 15 mg od if CrCl 30-49 mL/min Apixaban 2.5 mg twice daily if Cr ≥ 1.5 mg/dL, and age ≥ 80 years or weight ≤ 60 kg Edoxaban 30 mg daily if CrCl < 50 mL/min In dialysis patients: no consensus; controlled studies of anticoagulants (VKAs and NOAC) in AF patients receiving dialysis are needed [27]

on a strictly individualized algorithm [26]. The American Heart Association guidelines recommends warfarin even in dialysis patients with CHA_2DS_2-VASc score ≥ 2 for men and ≥ 3 for women and an INR target between 2 and 3 (class IIa, level of evidence B) and does not allow the use of dabigatran, edoxaban and rivaroxaban in ESKD and patients on dialysis [2]. For patients with AF who have a CHA_2DS_2-VASc score of 2 or greater in men or 3 or greater in women and who have end-stage chronic kidney disease (CKD; creatinine clearance [CrCl] <15 mL/min) or are on dialysis, it might be reasonable to prescribe warfarin (INR 2.0 to 3.0) or apixaban for oral anticoagulation [2]. The ESC guideline recommends that for patients with moderate or moderate-to-severe CKD (eGFR ≥ 15 ml/min/1.73 m^2) anticoagulation can be safely used in AF while for patients with ESKD on dialysis controlled studies of anticoagulants (both VKAs and NOACs) are needed [27]. These guidelines are summarized in Table 3.

ONGOING TRIALS

Currently ongoing studies include: the Strategies for the Management of Atrial Fibrillation in Patients Receiving Hemodialysis (SAFE HD; ClinicalTrials.gov identifier NCT03987711) trial, comparing warfarin, apixaban, and no anticoagulation (with a planned enrollment of 150 patients) and an estimated completion date on December 31, 2021; and the Compare Apixaban and Vitamin-K Antagonists in Patients with Atrial Fibrillation and End-Stage Kidney Disease (AXADIA; ClinicalTrials.gov identifier NCT02933697), comparing phenprocoumon and apixaban (with a planned enrollment of 222 patients). A total of 222 patients will be randomized in an open-labelled, 1:1 design to receive either apixaban 2.5 mg twice daily or dose-adjusted vitamin K antagonist therapy (target INR 2.0–3.0). All patients will be treated and followed up for a minimum of 6 months up to a maximum of 24 months. The primary outcome is major or clinically relevant, non-major bleedings or death of any cause. Secondary outcomes include stroke, cardiovascular death and other thromboembolic events, thus exploring the efficacy of apixaban. The estimated completion date of AXADIA trial is in July 2023.

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Non-invasive assessment of brain circulation and microstructure in systemic lupus erythematosus

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Abstract

Neuropsychiatric symptoms are expressed in approximately 40% of SLE patients. The underlying alterations in the microstructure of the brain in systemic lupus erythematosus (SLE) patients are caused by the activation of pathogenic pathways, such as antibody-mediated and cytokine-induced neurotoxicity or vasculopathy caused by antiphospholipid antibodies. Neuropsychiatric involvement in SLE manifests through a diverse range of symptoms, none of which are pathognomonic signs of SLE. The wide variety of neurologic symptoms and confounding disorders, in addition to the uncertainty surrounding their aetiopathogenesis, makes it difficult to establish their connection to the underlying disease and to clinically diagnose neuropsychiatric lupus eythematosus (NPSLE). Conventional magnetic resonance imaging (MRI) is considered the gold standard for diagnosing CNS involvement, by detecting small and large vessel disease and inflammatory-type lesions, whereas computer tomography (CT) is used to establish acute complications, such as hemorrhage or large infarcts and to assess differential diagnoses. However, since one in two NPSLE patients will have normal MRI findings upon examination, especially when they are presented with diffuse disorders, such as headache, mood alterations and psychiatric disease, it is becoming increasingly evident that more advanced MRI techniques should be integrated in a multimodal diagnostic strategy aiming to detect microstructural brain damage in early disease stages. Magnetization transfer imaging (MTI), diffusion tensor imaging (DTI), positron emission tomography with fluorine-18 fluorodeoxyglucose [(18) F-FDG-PET], single-photon emission computed tomography (SPECT), arterial spin labeling magnetic resonance imaging (ASL-MRI), as well as dynamic susceptibility contrast-enhanced perfusion magnetic resonance imaging (DSC-MRI) for the evaluation of cerebral blood flow, are all complementary non-invasive methods discussed in the present article that could contribute to the functional and morphological assessment of brain's circulation and microstructure in SLE and NPSLE patients.

Key words: *Systemic lupus erythematosus; neuropsychiatric systemic lupus erythematosus; cerebral small vessel disease; cerebral blood flow; non-invasive imaging techniques.*

INTRODUCTION

Systemic Lupus Erythematosus is an inflammatory autoimmune disease with several phenotypes and diverse clinical presentation, ranging from mild manifestations to multi-organ and severe central nervous system involvement [1]. Vascular disease is a common occurrence in SLE patients, either as an acute/subacute manifestation of the disease in the context of antiphospholipid syndrome or lupus vasculitis, or as an accompanying co-morbidity due to steroid-related atherosclerosis or accelerated atherosclerosis cause by a pro-inflammatory environment

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[2]. Cerebral small vessel disease (CVD) is an intrinsic disorder of the brain's perforating arterioles and it is one of the most common and severe manifestations of the aforementioned vascular pathology [3]. An important mechanism that could be held responsible for CVD is an increase in pro-inflammatory cytokine production that might disintegrate the blood-brain barrier, which in turn facilitates the entrance of neurotoxic antibodies into the CNS [4,5]. Neuroinflammation, microangiopathy, chronic diffuse ischemia, thromboembolism and atherosclerosis also take place [6,7].

Moreover, activation of the microglia by circulating auto-antibodies, IFN-α and other immune reactants, augments the inflammatory response worsening neuronal damage. Inflammation, cell infiltration in the perforating arteriolar walls, microglial activation in the perivascular tissue, alterations in brain perfusion and metabolism, vasculopathy and neuronal impairment all take place in neuropsychiatric systemic lupus erythematosus (NPSLE) [8,9]. NPSLE manifestations vary from mild disturbances such as headaches, mood disorders and cognitive dysfunction to more severe events such as myelitis, seizures and stroke [10]. As a matter of fact, stroke is a primary cause of morbidity, mortality and disability in SLE patients, who appear to have a greater risk of stroke compared to healthy subjects. Especially, young SLE patients appear to have a ten-fold increase in the risk of stroke compared to age-matched controls [11]. Interestingly enough, while the overall prognosis of SLE has improved, mortality rates due to cerebrovascular events remain unchanged, accounting for 15% of deaths in SLE [12,13]. Strokes that are ascribed to systemic inflammation, endothelial activation or an affiliation for thrombosis due to aPL usually occur close to the time of diagnosis, while those attributed to atherosclerosis take place later on [14,15].

NPSLE poses a formidable diagnostic challenge due to the multifarious neurological and psychiatric manifestations that characterize it, which are usually not pathognomonic of the disease. These symptoms are often overlooked despite their connection with increased mortality and morbidity [16]. Suspicion of disease arises primarily from clinical observation and experience due to the heterogeneity of NPSLE and the absence of etiological insight [17]. A noteworthy progress in the diagnosis of NPSLE was made in 1999, when the ACR Research Committee presented a uniform classification and a standardized methodology for recognizing NPSLE patients [18]. This classification includes 19 neuropsychiatric syndromes in SLE, which can be divided into CNS and PNS manifestations. These criteria enable a better case definition, through a detailed exclusion method. Even though no clear physiological and pathological mechanisms are explained under this categorization, it provides rheumatologists with a useful tool for the identification of neurological involvement in SLE. Inspired by this classification, Hanly et al. developed a model for determining the correlation between NP events and SLE, that assessed three parameters; firstly, the temporal relationship between NP symptoms and SLE diagnosis, secondly, the type of NP event that occurred, and lastly, a comprehensive list of exclusions and associations consistent with ACR nomenclature [19,20,21]. Bortoluzzi et al. proposed two additions to this algorithm; a careful evaluation of several risk factors that could aid the attribution process, as well as, the assignment of a numerical score to each selected item and the establishment of a global score; the greater the score, the higher the probability that the NP symptoms can be credited to SLE [22]. Ultimately however, the attribution of an NP event to this specific underlying disease remains to this day a challenge based on clinical judgment and expert opinion.

Early diagnosis of NPSLE, as well as close monitoring of disease progression, are of paramount importance to better patient management and the prevention of more severe CNS manifestations. Non-invasive imaging techniques that could contribute to a multimodal diagnostic algorithm are discussed below.

RESEARCH STRATEGY

A MedLine and Embase search was carried out according to published guidance on narrative reviews using the following terms: systemic lupus erythematosus, neuropsychiatric systemic lupus erythematosus, cerebral small vessel disease, cerebral blood flow, non-invasive imaging techniques [23]. Original research papers and review articles registered until the end of December 2020 were selected to be included in this review. Publications not in English and data from ongoing research were excluded.

NON-INVASIVE IMAGING TECHNIQUES

Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) is the standard radiologic imaging modality for NPSLE diagnosis, with the most frequent findings being hyper intensive lesions in the white matter (WMHI lesions) on T2 and FLAIR weighted sequences. However, these alterations were also observed in non NPSLE patients [24,25]. On

the other hand, 45% of NPSLE patients had no visible MRI abnormalities [26]. A study of 83 SLE patients with brain MRIs, obtained from the National University of Malaysia Medical Centre, indicated that the presence of these WMHI lesions was associated with high SLE activity, cerebral infarcts and aPL positivity. Inflammation and ischaemia were suggested as the underlying pathologies [27].

A different study by Nystedt et al. examined alterations in white matter microstructure in SLE patients with and without neuropsychiatric symptoms [28]. For this purpose, structural MRI and DTI were performed in 64 female SLE patients and 21 healthy controls. Results suggested that the alteration of white matter microstructure was not limited to the NPSLE subgroup and that it appeared to be related to disease duration and fatigue. Ainiala et al. performed a study on 43 SLE patients, who were found to have increased volumes of both T1- and T2- weighted lesions and increased cerebral atrophy, findings that were also related to specific NP manifestations [29].

Takahashi et al., reported the case of a 28-year-old woman, diagnosed with SLE, who suddenly developed dyplopia, unconsciousness and general convulsions [30]. Asymmetrical, multifocal, high signal intensity lesions on T2-weighted images and low signal intensity lesions on T1-weighted images were observed especially in subcortical white matter and the overlying cerebral cortex. The significance of this case report was that lesions were described using MRI but also when using both apparent diffusion coefficient imaging (ADCI) and diffusion weighted images (DWI) in a SLE patient with symptoms from the CNS.

The above-mentioned data suggests that conventional MRI may detect T1- and T2- weighted lesions, signs of cerebral atrophy and other findings indicative of inflammatory microangiopathy and ischemic changes. However, its inability to detect alterations in a significant number of NPSLE patients suggests that a multimodal diagnostic algorithm would significantly increase sensitivity and specificity for CNS involvement in SLE.

Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is used for measuring specific neuronal activity in regional brain structures during cognitive tasks by detecting the differences in the ferromagnetic properties of oxygenated and deoxygenated blood [31]. A systematic review of fMRI studies leads to the conclusion that disturbances in working memory and executive function brain regions are the most common findings in SLE patients [32]. Furthermore, increased functional connectivity strength in the fronto-parietal cortex, in resting state, correlates to disease activity. A study, conducted by Shuang Liu et al., assessed fMRI data acquired from 118 non-NPSLE patients and 81 healthy controls [33]. Resting stated fMRI data was used to calculate regional homogeneity (ReHo) in all subjects. The results indicated decreased ReHo values in the fusiform gyrus and thalamus and increased ReHo values in the parahippocampal gyrus and uncus. Disease activity correlated positively with ReHo values of the cerebellum and negatively with values in the frontal gyrus. Therefore, the aforementioned study suggests that abnormal brain activities might occur before NPSLE and that they might be the underlying cause of depressive and anxiety conditions.

McKay et al. used fMRI to determine whether disease duration was associated with brain injury [34]. For this purpose, 13 SLE patients were stratified by disease duration of ≤2 years (short-term [ST]) or ≥10 years (long-term [LT]). Findings from this process include increased amygdale and superior parietal activation, as well as significantly increased cortical activation in the ST group in areas linked with cognition. These differences were attributable to SLE effects on the CNS and were related to disease activity.

The assessment of 9 NPSLE patients compared to 9 RA patients and 9 healthy controls demonstrated that NPSLE patients showed greater frontoparietal activation than other groups during the memory task [35]. This is possibly credited to the need for extra cortical pathways recruitment during such tasks, in order to supplement the impaired standard pathways. Another study of 14 subjects, using fMRI, dual-echo and DTMR images, aimed to investigate the degree of cortical reorganization in NPSLE patients and its association with the extent of brain pathology [36]. T2 sequences showed abnormalities in 11 NPSLE patients, while NPSLE subjects also demonstrated significant activation of the contralateral primary sensorimotor cortex, putamen and dentate nucleus. More specifically, sensorimotor activation was closely linked to the extent and severity of brain damage. Lastly, as demonstrated by DiFrancesco et al., fMRI irregularities can also be identified in childhood-onset SLE, as an imbalance between active and inhibitory responses to stimuli [37]. These altered activation patterns are likely the result of abnormalities in white matter connectivity and neuronal network dysfunction.

All in all, fMRI detects neuronal dysfunction in regional brain structures during cognitive tasks. The re-

cruitment of extra cortical pathways during such tasks leads to alterations in activation patterns, proportional to the extent and severity of brain degeneration.

Magnetization Transfer Imaging

Magnetization transfer imaging (MTI) is based on the principle that protons in macromolecules (e.g. myelin) which are not visible with conventional MRI, can be studied by measuring their effect on visible mobile protons. As a result, normal white matter that has a dense structure, has a high MT ratio. In general, MT provides valuable indications of demyelination and axonal loss in many chronic systemic diseases [38].

A study by Bosma et al., attempted to determine whether MTI histogram analysis can identify irregularities in patients with active NPSLE, and whether these findings can be compared with similar irregularities in MS patients [39]. Those results were encouraging, as it was observed that volumetric MTI analysis can indeed detect cerebral changes during the active phase of NPSLE. Furthermore, abnormalities in brain parenchyma of chronic NPSLE patients demonstrated similar MTI values to those of patients with inactive MS. On the contrary, MTI values in the active phase of NPSLE differed from those presented in the chronic state, most likely due to underlying inflammation.

A multimodal MRI study conducted on 9 active NPSLE patients, 9 SLE patients without NP symptoms and14 healthy controls showed that the co-analysis of MTI and DTI data contributes to the understanding of the microstructural damage in NPSLE and can improve diagnosis [40].

Steens et al. collected MTI data from 24 female SLE patients and 24 age- and sex-matched healthy controls. MTR maps were calculated for both grey matter and white matter separately and MTR histograms were produced [41]. The results indicated that SLE patients with a history of NP manifestations, with or without accompanying focal MRI abnormalities, had a significantly lower GM PH. This neuronal damage shows a susceptibility of the GM to small-vessel disease and the antineuronal action of auto-antibodies that managed to penetrate the compromised blood brain barrier. MTI was also recruited in a study aimed to assess its correspondence with clinical changes in NPSLE patients [42]. Twenty-four (24) pairs of scans corresponding to 19 patients were examined for significant differences. The peak height of whole-brain MTR histograms was found to match changes in the clinical status of NPSLE patients, suggesting that MTI could prove to be a useful tool for an effective clinical evaluation.

In conclusion, MTI detects cerebral changes, attributable to demyelization and axonal loss in patients with, either active NPSLE, or a history of NP symptoms. It is noteworthy that these changes may be undetectable using exclusively conventional MRI.

Diffusion Tensor Imaging

Diffusion tensor imaging (DTI) is an MRI technique that assesses the diffusion of water molecules. It is directly dependent on orientation, spacing and structural barriers in brain tissue, such as myelin and cellular membranes [43]. White matter (WM) restricts free water movement in the direction of diffusion that is perpendicular to the WM tracts with a mechanism called anisotropy. DTI employs anisotropic diffusion in order to estimate the axonal organization of the brain [44]. It holds great promise as a method of recognizing microstructural alterations and their progression with neuropathology and treatment. Thus, it can be used for the identification of white matter pathologies, such as ischemia, myelination, axonal damage, inflammation and edema [45].

Fifteen female SLE patients, with no history of major NP manifestations, underwent MRI with DTI at baseline and 1.5 years later. [46] The DTI abnormalities found included decreased fractional anisotropy and increased mean diffusivity in bilateral cerebral WM and GM. These abnormalities were not associated with emergent NP activity, medical decline or medication changes, nor were they developed on the grounds of an MRI visible macrostructural change. They are more likely to be considered the result of the ongoing inflammation.

The topological properties of brain WM structural networks in SLE patients were examined by Ling Zhao et al. [47]. Results from DTI datasets, acquired from 29 non-NPSLE patients and 24 healthy controls, were used to recreate their brain WM structural networks by using a deterministic fiber tracking approach. Abnormal diffusion parameters in the bilateral corticospinal tract and the right superior longitudinal fasciculus-temporal terminations were found in the non-NPSLE patients. These results suggest that brain WM connectivity appears to be damaged even in SLE patients who do not exhibit any NP symptoms.

A similar study applied DTI and tract based spatial statistics to examine 19 NPSLE patients, 19 non-NPSLE patients and 18 healthy controls [48]. All groups were age- and sex-matched. Data analysis of both SLE groups indicated several regions of compromised prefrontal

WM integrity. The alterations found in non-NPSLE patients were similar to those in the NPSLE group but less pronounced.

A systematic review of 37 articles with a total of 195 NPSLE patients, 299 no-NPSLE patients and 423 healthy controls, indicated that both SLE and NPSLE patients had reduced FA values, suggesting subclinical CNS involvement, and elevated MD values in most WM areas [49]. Nonetheless, follow-up studies are required in order to determine whether these microstructural alterations are transient or permanent.

All in all, DTI can be used to detect white matter pathologies indicative of ischemia, axonal damage or inflammation. Moreover, it may prove to be useful for monitoring disease progression and the differential diagnosis of transient and permanent microstructural damage.

Positron Emission Tomography

Positron emission tomography with fluorine-18 fluorodeoxyglucose [(18) F] FDG PET/CT assesses the increase in glucose uptake of infiltrating granulocytes and tissue macrophages. Moreover, due to the increased glucose metabolism of activated lymphocytes it can also be used to visualize large concentrations of these cells. One of the most common and remarkable PET/CT findings in NPSLE patients is parieto-occipital hypometabolism [50].

PET using F-18 –labelled fluorodeoxyglucose was performed in 28 SLE patients who were classified according to their clinical state, as having severe neuropsychiatric manifestations (n=12) or mild neuropsychiatric symptoms (n=11) or without any signs of CNS involvement (n=5). Subjects were also compared to 10 healthy controls [51]. PET scan results indicated hypometabolism in at least one brain region in all patients with severe or mild CNS symptoms, compared to non-symptomatic patients. Again, parieto-occipital regions were most commonly afflicted, followed by parietal regions. Comparatively, MRI images showed abnormalities in only 50% of NPSLE subjects and only in 25% of non-NPSLE patients.

Another study aimed to investigate the efficacy of (18) F-FDG-PET for the detection of CNS involvement in SLE patients with no MRI findings [52]. For this purpose, 20 NPSLE patients with headaches, seizures or mood disorders and a normal MRI underwent brain (18) F-FDG-PET. Significant abnormalities in glucose metabolism were observed in 15 out of 20 patients, mainly in the temporal, the occipital and the frontal lobe. Nonetheless, neuropsychiatric manifestations did not correlate geographically with specific imaging findings.

In summary, data indicates that (18) F-FDG-PET could be used as a diagnostic tool complementary to MRI, when the latter fails to provide confirmation of brain involvement in SLE patients.

Single-photon Emission Computed Tomography

Single-photon emission computed tomography is another method used to determine the connection between cerebral hypoperfusion, cumulative tissue damage and disease clinical activity. Two groups of patients underwent (99mTc-ECD) SPECT, while SLE disease activity index, SLICC/ACR damage index and native anti-DNA antibodies were also measured [53]. Group A was compiled of 10 women with SLE, but no history of major neuropsychiatric manifestations and no minor neuropsychiatric symptoms in the last six months, while group B included 57 unselected women with SLE. In group A, cerebral SPECT yielded abnormal findings (moderate or severe hypoperfusion) in five non-NPSLE patients. Moreover, patients with significant cerebral hypoperfusion had greater clinical disease activity and ESR. In group B, cerebral SPECT was normal in 30 patients and indicated moderate or severe hypoperfusion in 27. Thus, it may be assumed that cerebral hypoperfusion, identified using SPECT, is related to both clinical activity and cumulative tissue damage.

SPECT scans were also performed on 20 young patients with acute CNS manifestations, in order to determine whether this method can be used for monitoring CNS disease activity during childhood [54]. SPECT scan pattern was abnormal in 86% of patients, showing widespread small areas of decreased uptake, indicative of generalized hypoperfusion. However, it should be noted, that SPECT scans did not clearly indicate clinically visible CNS involvement in children.

A systematic review by Sahebari et al. assessed the diagnostic value of SPECT scan and fMRI as imaging tools for the detection of subtle brain abnormalities in SLE patients with cognitive impairment [55]. The analysis of 14 articles demonstrated that both SPECT and fMRI could be considerably beneficial for the diagnosis, as well as the initial management of SLE patients with CNS manifestations.

Overall, SPECT scans indicate areas of hypoperfusion caused by cumulative tissue damage that correlate positively to disease clinical activity. As a result, especially when combined with fMRI, SPECT may prove valuable for monitoring disease progression in non paediatric SLE patients with cognitive dysfunction.

Table 1. *Non-invasive techniques for the assessment of brain circulation and microstructure in systemic lupus erythematosus.*

Study	Sample	Assessment tool	Parameters assessed	Results	Associations
Shaharir et al.	83 SLE patients	MRI	WMHI lesions	Presence of WMHI lesions	- SLE activity - cerebral infarcts - aPLpositivity - inflammation - ischaemia
Nystedt et al.	85 subjects total: - 64 female SLE patients - 21 healthy controls	MRI DTI ADCI	WMHI lesions	↑ volumes of T1- /T2-weighted lesions ↑ cerebral atrophy	- interstitial edema - inflammatory microangiopathy - cytotoxic edema - microinfarction
Shuang Liu et al.	199 subjects total: - 118 non-NPSLE patients -81 healthy controls	fMRI	ReHo values	↑ ReHo ↑ fusiform gyrus and thalamus ↑ ReHo ↑ parahippocampal gyrus and uncus.	brain activity and disease severity
McKay et al.	13 SLE patients	fMRI	ReHo values	↑ amygdale and superior parietal activation ↑ ↑ cortical activation	correlation with disease duration and activity
Fitzgibbon et al.	27 subjects total: - 9 NPSLE patients - 9 RA patients - 9 healthy controls	fMRI	ReHo values	↑ frontoparietal activation during memory task	- extra cortical pathways - impaired standard pathways
Rocca et al.	14 subjects total: - 11 NPSLE - 3 nonNPSLE	fMRI, dual-echo and DTMR images	ReHo values	- T2 sequences↑ abnormalities - activation of contralateral primary sensorimotor cortex, putamen and dentate nucleus	- degree of cortical reorganization in NPSLE - association with the extent of brain pathology - sensorimotor activation ↑ extent and severity of brain damage
DiFrancesco et al.	10 patients with childhood-onset SLE	fMRI CPT N-Back task verb generation	ReHo values Composite Z maps	- alterations in regional PBF, MTT, PBV - alterations in quantitative pulmonary perfusion parameter maps	- imbalance between active and inhibitory responses - abnormalities in white matter connectivity - neuronal network dysfunction
Bosma et al	49 subjects total: - 8 female patients with active NPSLE 1 male patient with active NPSLE -10 female patients with chronic NPSLE - 10 female patients with SLE and no history of NPSLE - 10 female patients with inactive MS - 10 healthy controls	MTI	- MTI histogram analysis - volumetric MTI analysis	- irregularities in patients with active NPSLE	- abnormalities in the brain parenchyma of chronic NPSLE patients ↑ similar MTI values to inactive MS - MTI values in active NPSLE↑ differed from the chronic state

Table 1. *Non-invasive techniques for the assessment of brain circulation and microstructure in systemic lupus erythematosus (continued).*

Study	Sample	Assessment tool	Parameters assessed	Results	Associations
Ercan et al.	32 subjects total: - 9 active NPSLE patients - 9 non NPSLE patients - 14 healthy controls	-MTI -DTI	- FLAIR images - MTR - MD - FA - RD - AD maps - WM lesion maps	- NAWM changes: ↓ MTR ↓ FA ↑↑AD, RD and MD in NPSLE compared to HC	microstructural damage in NPSLE
Steens et al.	48 patients total: 24 female SLE patients - 24 age healthy controls	MTI	- MTR maps for GM and WM - MTR histograms	SLE patients with a history of NP manifestations: ↓↓ GM PH	neuronal damage → vulnerability of GM to small-vessel disease and antineuronal action of auto-antibodies
Emmer et al.	19 female patients	MTI	MTR histograms	4 patients clinically deteriorated→ ↓ peak height -14 patients with stable disease → peak height did not change significantly -6 patients clinically improved→ ↑ peak height	peak height of whole-brain MTR histograms matched changes in clinical status of NPSLE
Kozora et al.	15 female SLE patients, (no history of major NP symptoms)	MRI, DTI at baseline and 1.5 years later	-FA -MD	↓ fractional anisotropy ↑ mean diffusivity in bilateral cerebral WM and GM.	abnormalities associated with ongoing inflammation
Ling Zhao et al.	53 subjects total: - 29 non-NPSLE patients - 24 healthy controls	DTI	diffusion parameters	Abnormal diffusion parameters → bilateral corticospinal tract and right superior longitudinal fasciculus - temporal terminations → non-NPSLE patients	WM connectivity damage
Schmidt-Wilcke et al.	56 subjects total: - 19 NPSLE patients - 19 non-NPSLE patients - 18 healthy controls	DTI	FA	↓ FA prefrontal white matter in both SLE groups - changes in the non-NPSLE patients overlapped but not as pronounced with those in the NPSLE patients	changes in regional white matter integrity present in NPSLE patients and in non-NPSLE patients (lesser degree)
Costallat et al.	917 subjects total: 195 NPSLE patients - 299 no-NPSLE patients - 423 healthy controls	DTI	FA MD	SLE and NPSLE patients: - ↓ FA value - ↑ MD values in WM areas	transient and permanent microstructural alterations present

Table 1. *Non-invasive techniques for the assessment of brain circulation and microstructure in systemic lupus erythematosus (continued).*

Study	Sample	Assessment tool	Parameters assessed	Results	Associations
Weiner et al.	38 subjects total: - 28 SLE patients (12 with severe neuropsychiatric symptoms, 11 with mild neuropsychiatric symptoms, 5 without any signs of CNS involvement) - 10 healthy controls	(18) F-FDG PET/CT	Glucose uptake	- parieto-occipital hypometabolism - hypometabolism in at least one brain region in all patients with severe or mild CNS symptoms, compared to non symptomatic patients	hypometabolism in brain regions associated with clinical symptoms.
Lee et al.	20 NPSLE patients with headaches, seizures or mood disorders and a normal MRI	- (18) F-FDG PET/CT - MRI	- Glucose uptake - MRI findings	Significant abnormalities in glucose metabolism (15 out of 20 patients) in the temporal, the occipital and the frontal lobe	(18) F-FDG-PET as a diagnostic tool complementary to MRI in symptomatic SLE patients with normal MRI
López-Longo et al.	group A: 10 women with SLE, but no history of major neuropsychiatric manifestations and no minor neuropsychiatric symptoms in the last six months group B: 57 unselected women with SLE	(99mTc-ECD) SPECT	- disease activity index - SLICC/ACR - damage index - native anti-DNA antibodies	- cerebral hypoperfusion: ↑ clinical disease activity ↑ ESR Group B: cerebral SPECT normal in 30 patients → moderate or severe hypoperfusion in 27	cerebral hypoperfusion → clinical activity and cumulative tissue damage
Russo et al.	20 young patients with acute CNS manifestations	SPECT	- brain perfusion - disease activity	SPECT scan pattern abnormal → 86% of patients - widespread small areas of generalized hypoperfusion.	monitoring CNS disease activity during childhood
Sahebari et al.	Systematic review of 14 articles	SPECT fMRI	- brain perfusion - T2 weighted lesions	- damaged state of the native circuits - impairment in the visual and attention areas - ↑ activation Fusiform gyrus and visual associative cortex - ↑ number and volume of T2 lesion - enhanced lesion attenuation in the left superior and right posterior corona radiate - patients with cognitive problems had lower volume of the left hippocampus, amygdala, and right hippocampus	SPECT and fMRI sensitive for diagnosis of subtle brain damages in early stages of cognitive dysfunction

Table 1. *Non-invasive techniques for the assessment of brain circulation and microstructure in systemic lupus erythematosus (continued).*

Study	Sample	Assessment tool	Parameters assessed	Results	Associations
Zhuo et al	87 subjects total: - 31 NPSLE - 24 non-NPSLE patients - 32 healthy controls	3D ASL-MRI	- tissue perfusion rate - CBF	NPSLE patients: - ↑ CBF within WM - ↓ CBF within GM non-NPSLE patients: - ↑ CBF in both GM and WM. NPSLE group → ↓↓ CBF in the frontal gyrus, cerebellum and corpus callosum compared to non-NPSLE group	impaired cerebral perfusion
Jia et al	65 subjects total: - 16 NPSLE - 19 non-NPSLE - 30 healthy controls	3D ASL-MRI	- CBF	- Perfusion unevenly reduced → frontal, temporal, parietal and occipital lobes of all SLE patients. - all patients with impaired frontal lobe perfusion → acute CNS symptoms - 40% of the hypoperfusion in other regions was observed in non-NPSLE patients	CBF → biomarker for diagnosis and monitoring of disease progression in both NPSLE and non-NPSLE patients.
Papadaki et al. (2017)	76 patients total: - 37 primary NPSLE - 16 secondary NPSLE - 23 non-NPSLE - 31 healthy controls	MRI DSC-MRI	CBF	- primary NPSLE → lower CBF and volume in otherwise normal-appearing WM - primary NPSLE → lower CBF in the semioval centre bilaterally compared to non-NPSLE and secondary NPSLE	combination of conventional MRI and DSC-MRI → 94-100% specificity for discerning primary from secondary NPSLE
Papadaki et al. (2019)	73 subjects total: - 31 NPSLE - 19 non-NPLSE - 23 healthy controls	DSC-MRI	brain perfusion	Hypoperfusion → frontostriatal and limbic structures –positive correlation with severe anxiety symptoms - heamodynamic disturbances in NPSLE	assessment of brain regions linked with emotional response
Tinelli et al.	case report of 39 year-old female patient, diagnosed with arthritis, autoimmune thrombocytopenia, SLE and Sjogren's syndrome - control group of 6 SLE patients	H-MRS MRI	concentration of brain metabolites (NAA, Cho, Cr) and tissue lactate	- ↑↑ Cho/Cr peaks during headaches, compared to results during remission and data from CG - absence of fluctuation in NAA value	metabolic change → brain injury from microinfarction, cell infiltration, membrane activation or neuronal degradation

Table 1. *Non-invasive techniques for the assessment of brain circulation and microstructure in systemic lupus erythematosus (continued).*

Study	Sample	Assessment tool	Parameters assessed	Results	Associations
Simone Appenzeller et al.	113 subjects total: - 90 SLE patients (29 active NPSLE, 28 active non-NPSLE, 14 inactive NPSLE and 19 inactive non-NPSLE) - 23 healthy volunteers	single voxel proton MRS	concentration of brain metabolites (NAA, Cho, Cr) and tissue lactate	- ↓↓ NAA/Cr →patients with active SLE - ↑↑ NAA/Cr → 15 of patients with active SLE during initial MRS and inactive SLE at follow-up - ↓↓ NAA/Cr → 10 patients with active SLE in both initial MRS and follow-up - ↓↓↓ NAA/Cr 15 patients with inactive SLE at initial MRS, but active SLE at follow-up	axonal dysfunction in patients with active SLE (regardless of CNS involvement)
Axford et al.	17 subjects total: - 9 female NPSLE patients - 8 healthy controls	quantitative MRS	concentration of brain metabolites (NAA, Cho, Cr) and tissue lactate	mild SLE: - ↑↑ tCho - ↑mI severe SLE: - ↓↓ NAA - ↑↑ mI - tCho normal	neurometabolite changes indicate permanent neuronal loss
Zhang et al.	63 subjects total: - 22 NPSLE patients - 21 non-NPSLE patients - 20 healthy volunteers	- multivoxel MRS - VBM - DKI	concentration of brain metabolites (NAA, Cho, Cr) and tissue lactate -Diffusional kurtosis values	- metabolite concentrations ↓ in both patient groups → PCG and basal ganglia regions - more severely depleted in NPSLE patients - ↓ diffusional kurtosis values in the bilateral PCG compared to HC. - ↓ GM → PCG of NPSLE group	- neuronal degeneration and dysfunction - differentiation between NPSLE and nonNPSLE patients

Arterial Spin Labeling Magnetic Resonance Imaging

Arterial spin labeling (ASL) is a non-invasive MRI technique that measures cerebral blood flow, while also eliminating the risk of nephrogenic systemic fibrosis in patients with renal dysfunction, as is often the case with SLE patients [56]. Moreover, the absence of contrast agents and radiation exposure encourages the employment of this method for the assessment of paediatric patients [57]. ASL measures tissue perfusion rate and not macrovascular blood flow. Tissue perfusion, water and nutrient tissue exchange happens along the entire length of the capillaries and thus ASL uses blood water molecules as a free diffusible tracer from the arterial body to the tissue capillary bed [58].

A study by Zhuo et al. used 3D ASL-MRI to quantify cerebral perfusion of 31 NPSLE and 24 non-NPSLE patients compared to 32 healthy controls [59]. Results indicated that compared to the control group, NPSLE patients had increased blood flow (CBF) within WM, but decreased CBF within GM. On the other hand, non-NPSLE patients demonstrated increased CBF in both GM and WM. Additionally, compared to the non-NPSLE group, the NPSLE group showed considerably reduced CBF in the frontal gyrus, cerebellum and corpus callosum.

Jia et al. also used 3D ASL-MRI to estimate CBF in 16 NPSLE, 19 non-NPSLE and 30 healthy controls [60]. Perfusion was unevenly reduced in the frontal, temporal, parietal and occipital lobes of all SLE patients compared to controls. Whereas all patients with impaired frontal lobe perfusion had acute CNS symptoms, approximately 40% of the hypoperfusion in other regions was observed in non-NPSLE patients suggesting that a subclinical pathological process was underway. Consequently, CBF measured by non-invasive 3D ASL could potentially serve as a practical biomarker for the diagnosis and monitoring of disease progression in both NPSLE and non-NPSLE patients.

Dynamic Susceptibility Contrast-enhanced Perfusion Magnetic Resonance Imaging

Dynamic susceptibility contrast-enhanced perfusion MRI (DSC-MRI) is another non-invasive technique that measures cerebral perfusion and could contribute to distinguishing lupus from non-lupus neuropsychiatric events. Much like the aforementioned ASL-MRI, this method also limits patients' exposure to radiation, while at the same time providing higher spatial resolution and the ability to measure simultaneously cerebral blood volume and cerebral blood flow [61].

Papadaki et al. assessed a total of 76 patients (37 primary NPSLE, 16 secondary NPSLE, 23 non-NPSLE) and 31 healthy controls using conventional MRI and DSC-MRI [62]. Patients with primary NPSLE had a lower CBF and volume in otherwise normal-appearing WM areas compared to controls. Furthermore, they had a lower CBF in the semioval centre bilaterally, compared to both non-NPSLE and secondary NPSLE patients. In greater detail, this decrease in CBF was used to differentiate between primary NPSLE, secondary NPSLE and non-NPSLE, with an 80% sensitivity and 67-69% specificity. The combination of conventional MRI and DSC-MRI seems to grant 94-100% specificity for discerning primary from secondary NPSLE. Another study by the same team performed DSC-MRI on 31 NPSLE, 19 non-NPLSE and 23 healthy controls focusing on brain regions linked with emotional response [63]. Hypoperfusion in frontostriatal and limbic structures proved to correlate positively with more severe anxiety symptoms due to the heamodynamic disturbances in NPSLE.

According to the above data, alterations in cerebral tissue perfusion, detected by ASL-MRI and DSC-MRI, could aid to determine which NP signs may be attributed to SLE. Furthermore, it may be used to evaluate disease severity, as well as to differentiate between primary NPSLE, secondary NPSLE and non-NPSLE.

Magnetic Resonance Spectroscopy

As explained above, MRI may draw attention to focal ischemic lesions, white matter hyperintensity, ventricular dilation and cortical atrophy in SLE patients [64]. However, lack of MRI findings does not exclude neuronal dysfunction, as is the case with metabolic alterations [65,66]. Unlike MRI, MRS utilizes the signal from hydrogen protons to determine the concentration of brain metabolites, such as N-acetyl aspartate (NAA), choline (Cho), creatine (Cr) and tissue lactate [67].

Tinelli et al. reported the case of a 39-year-old female patient who underwent MRI and H-MRS scans when she presented with recurring catamenial migraines without aura on the grounds of pre-diagnosed arthritis, autoimmune, SLE and Sjogren's syndrome [68]. Multivoxel H-MRS was performed to assess posterior periventricular white matter, thalamus and basal ganglia that appeared normal in the cMRI. Results were compared to a control group of 6 other SLE patients without aura. Results from the H-MRS indicated a considerable increase in Cho/Cr peaks during headaches, compared to results during remission and to data acquired from the control group. This metabolic change may be attributed to brain injury, due to microinfarction, cell infiltration, membrane activation or neuronal degradation [69,70,71]. Moreover, while a decrease in NAA has been reported in SLE patients, possibly reflecting neuronal loss and dysfunctions, in the case of the aforementioned patient NAA values were normal [72]. The absence of fluctuation in NAA value can be attributed to the reversibility of symptoms and the absence of neuronal death.

Another study of 90 SLE patients and 23 healthy volunteers examined the axonal alterations in SLE using single voxel proton MRS [73]. Signals from NAA, Cho and Cr compounds were used to determine NAA/Cr ratios. Patients were then categorized into the following subgroups, according to disease activity; 29 patients with active NPSLE, 28 with active non-NPSLE, 14 patients with inactive NPSLE and 19 with inactive non-NPSLE. NAA/Cr ratios were considerably lower in patients with active SLE, regardless of whether they presented symptoms from the CNS, compared to the subgroup with inactive SLE and controls. There was a significant increase in NAA/Cr ratio in 15 of the patients who had active SLE during the initial MRS and inactive SLE at the follow-up. On the other hand, a noteworthy decrease in the NAA/Cr ratio was observed in 10 patients with active SLE in both the initial MRS and the follow-up, while an even greater reduction was noticed in 15 patients with inactive SLE at the initial

MRS, but active SLE at the follow-up. These findings confirm an axonal dysfunction in patients with active SLE regardless of CNS involvement. This dysfunction seems to be at least partly reversible during periods of disease regression.

Axford et al. used quantitative MRS to determine neurometabolite changes that herald permanent neuronal loss in 9 female NPSLE patients and 8 healthy sex- and age- matched volunteers [74]. Patients with mild SLE showed a considerable increase in tCho and a smaller reversible increase in myo-inositol levels (mI). Conversely, patients with severe SLE demonstrated a significantly and permanently reduced NAA and a greatly raised mI. In this group tCho levels were normal. The above findings were once again confirmed in a systemic review that examined 26 articles [75]. It concluded that NAA/Cr ratios were considerably lower and Cho/Cr ratios relatively more increased in several brain areas in patients with SLE, SS, RA and SSc.

Lastly, a multimodal imaging study was carried out in order to evaluate metabolic and microstructural changes in the brain of SLE subjects with cognitive impairment [76]. For this purpose, 22 NPSLE patients, 21 non-NPSLE patients and 20 healthy volunteers underwent multivoxel MRS, T1-weighted volumetric images for voxel-based morphometry (VBM) and diffusional kurtosis imaging (DKI) scans. The most prominent findings were located, but not limited to, the posterior cingulated gyrus (PCG), and they were also observed in other basal ganglia regions. Even though metabolite concentrations were reduced in both patient groups, they were more severely depleted in NPSLE patients. Moreover, both groups exhibited lower diffusional kurtosis values in the PCG bilaterally compared to healthy controls. VBM scans showed GM reduction in the PCG of the NPSLE group.

To recapitulate, MRS measures alterations in neurometabolite concentrations as a determinant of neuronal degeneration and dysfunction. More research is essential for asserting whether the aforementioned method can be used to differentiate between NPSLE and non-NPSLE patients.

CONCLUSION

SLE is an autoimmune disease that often targets the CNS, leading to the manifestation of neuropsychiatric symptoms of mild to severe intensity. Degeneration of the brain's perforating arterioles is the underlying vascular pathology that leads to cerebral small vessel disease. Disruption of the blood-brain barrier, increased cytokine production, as well as the neurotoxicity of circulating auto-antibodies are the key mechanisms leading to neuroinflammation, microangiopathy, chronic diffuse ischemia, thromboembolism and premature atherosclerosis. Timely diagnosis and careful monitoring of disease progression are vital for lowering mortality rates due to cerebrovascular events. Conventional MRI is considered the gold standard in diagnosing NPSLE as it is widely effective in identifying hyper intensive lesions in white matter on T2 and FLAIR weighted sequences, alterations attributable to inflammation and ischemia. On the other hand, fMRI uses differences in the ferromagnetic properties of oxygenated and deoxygenated blood to detect altered activation patterns in white matter connectivity and neuronal network dysfunction. MTI is suitable for locating signs of demyelination and axonal loss due to small-vessel disease in grey matter areas and the antineuronal action of auto-antibodies. DTI has proven to be of great assistance for identifying white matter pathologies, such as ischemia, myelination, axonal damage, inflammation, edema and impaired white matter connectivity. Additionally, FDG PET/CT demonstrates abnormalities in glucose metabolism, even in patients without any MRI findings indicative of CNS involvement. Especially when combined with fMRI, SPECT can recognize areas of cerebral hypoperfusion, due to cumulative tissue damage. Cerebral blood flow assessed using both ASL-MRI and DSC-MRI, indicates perfusion impairment located in the frontal, temporal, parietal and occipital lobes as well as the limbic structures. Lastly, MRS can detect neurometabolite changes in the posterior cingulated gyrus and other basal ganglia, which seem to signify neuronal damage or permanent neuronal loss. Future research will show whether the aforementioned non-invasive imaging techniques could be incorporated in a multimodal algorithm that would have a high sensitivity and specificity for effectively diagnosing and monitoring CNS involvement in SLE. Unfortunately, early diagnosis of NPSLE remains, to this day, a challenge for clinicians who are expected to rely mainly on observation and past patient experience.

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3. Systematic Reviews and Meta-analyses
4. Editorials
5. Letters to the Editor
6. Case Reports

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