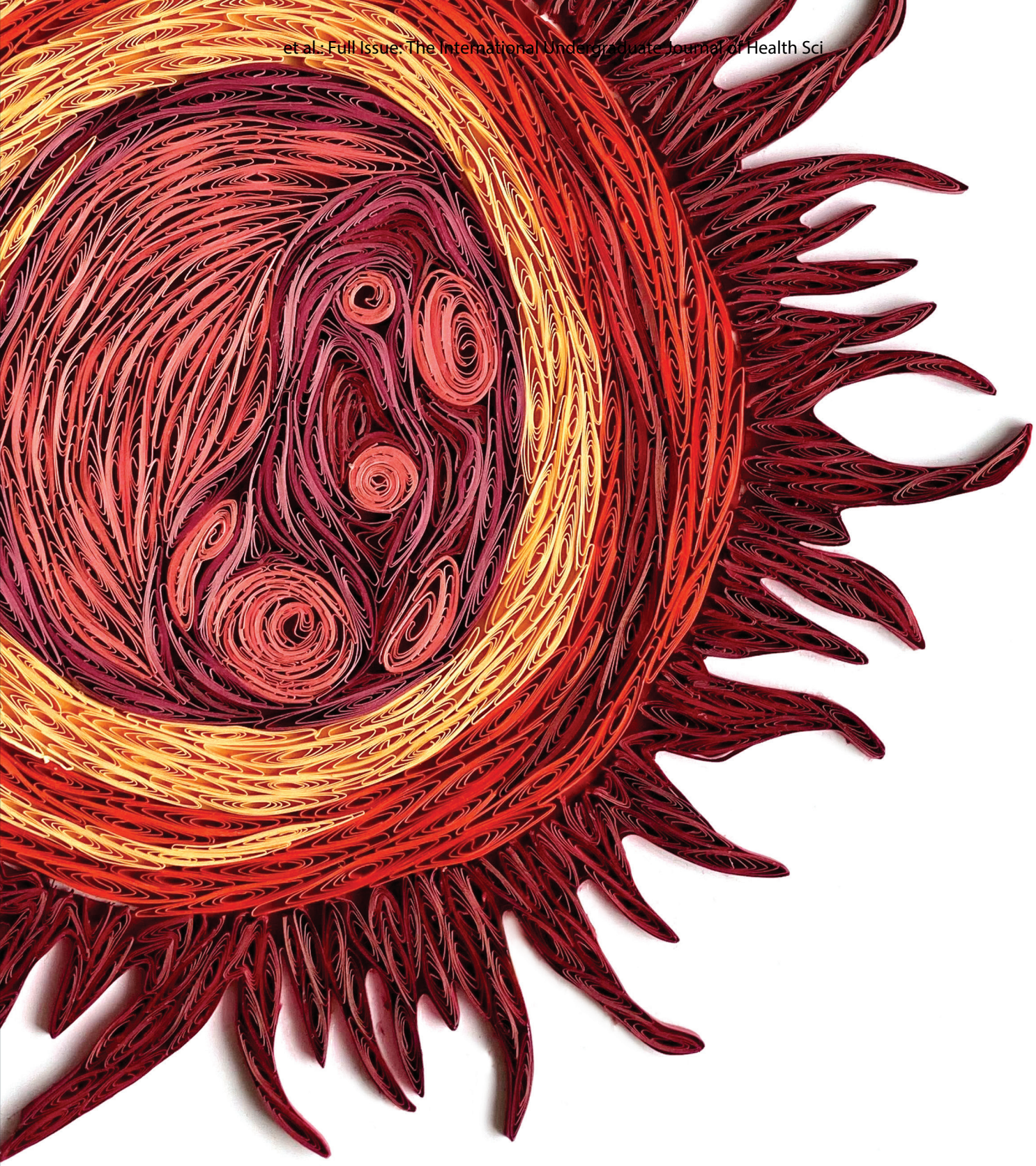

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International Undergraduate Journal of Health Sciences (IUJHS) is a student run, open access, peer- reviewed online journal that publishes original research papers, short communications, review papers, mini-review papers, letters to the editor and conference proceedings within the field of human health and medical science. The IUJHS is published bi-annually.

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June 2021

Editors' Foreword

This International Undergraduate Journal of Health Science (IUJHS) serves as a platform for undergraduate degree students to have their research, peer-reviewed, edited, and published. Getting published as an undergraduate student is a rare and perhaps intimidating prospect. The introduction of IUJHS will make publication more accessible to thousands of undergraduate students across all disciplines of health science. While this inaugural issue contains submissions from the MTU/UCC BSc (Hons) Biomedical Science students and the associated MTU Diploma in Clinical Laboratory Practice colleagues only, it is hoped and expected that the journal will expand and be accessible to a wider contribution from undergraduate students nationally and internationally. We hope that its content will further undergraduate health science education in addition to providing the usual benefits of peer-reviewed research reporting in any scientific journal.

IUJHS is a twice-yearly modern, student-run, and peer-reviewed journal. In keeping with the student-centered objective of this journal, the editorial board comprises of chief editors; Shane Cusack and Ruth Delahunty, assistant editors Katie O'Brien, Evelyn Hayes, and Clíodhna Ní Shúilleabháin and a panel of student reviewers (see a full list of editorial board members), all of whom are undergraduate students. The current editorial board looks forward to the prospects of the journal and the opportunities it will present to undergraduate students studying health science in Ireland and further afield.

After nearly 4 months since the first meeting of our editorial board, we are excited to present three reviews and four research articles in this inaugural issue. Each of the reviews provides a comprehensive analysis of a particular health science topic. Appropriately for the current global situation, Shannon Ginty presents an overview of coagulopathy in Covid-19. The role of immunomodulation in the treatment of Diabetes Mellitus is detailed by James Harte. The third and final review, by Adam Korneluk, presents Microangiopathic Haemolytic Anaemia, its diagnosis, and management in the haematological conditions TTP (Thrombotic Thrombocytopenic Purpura) and HUS (Haemolytic Uraemic Syndrome).

The research articles included in this issue are based on survey data and assess the areas of food allergies, organ donation, non-prescription medication, and cancer genetic screening. Bolger *et al.* highlight the need for improved healthcare supports for individuals suffering from food allergies. Hayes *et al.* present a qualitative study of the factors affecting third-level students when registering as organ donors. Cusack *et al.* critically evaluate knowledge of the risks and dangers associated with common over-the-counter painkillers, including aspirin, paracetamol, and ibuprofen. Finally, Cronin *et al.* assess knowledge of genetic screening, specifically testing for BRCA1 & BRCA2 mutations, and their results indicate that a positive test result for these mutations may influence lifestyle choices. These reviews and research articles make for some very interesting reading.

The editorial board would like to acknowledge our adjunct editors and IUJHS founders Dr. Lesley Cotter and Dr. Brigid Lucey from the Department of Biological Sciences MTU, and Ms. Sinead Hanrahan and Ms. Therese Ahern from the MTU library. This team has spent almost **two years** building the foundation on which this inaugural issue of IUJHS has now been launched following receipt of funding from the National Forum for the Enhancement of Teaching and Learning. This issue has seen many firsts for Munster Technological University

students, including the design of our cover by BA(Hons) student Ioana Cretu. We acknowledge other MTU departments including the Teaching and Learning Unit and the Technology Enhanced Learning Department for their assistance with getting this issue off the ground. Many other departments have helped get this issue. The editorial board is indebted to the IUJHS reviewer panel. We send our deepest thanks to our expert and student reviewers, who so willingly gave their time and expertise to see undergraduate work published. Their feedback was always constructive and in keeping with the aims of the journal, fostering a positive learning environment. This publication would not have been possible without many individuals, but especially our undergraduate peers. We thank them for their submissions to the journal and their engagement with the review process – long may this continue for our future issues.

Shane Cusack & Ruth Delahunty

Coagulopathy in COVID-19: An Overview

Shannon Ginty

Department of Biological Sciences, Munster Technological University.

ABSTRACT

Recent data has demonstrated that the pathophysiology of severe COVID-19 infection is associated with a significant pulmonary coagulopathy. Thrombotic complications have been reported in approximately 35-45% of patients with severe COVID-19. Entry of SARS-CoV-2 into the host cells leads to dysregulation in inflammatory signalling pathways, disrupting the normal coagulation mechanism. The hypercoagulability with abnormal clot formation is attributed to the inappropriately elevated immune response, culminating in a 'cytokine storm' with high levels of pro-inflammatory cytokines and subsequent thrombosis. The coagulopathy in COVID-19 affects many coagulation parameters such as D-dimer levels, fibrinogen levels, platelet count and prothrombin time. Coagulation parameters must be carefully monitored as they may indicate the requirement for clinical intervention. This suggests prophylactic anticoagulant treatment may be considered for COVID-19 patients with a predisposition to thrombosis. Current research indicates that immunomodulatory therapy may be beneficial to limit the propagation of the immune response. However, retrospective well-controlled studies are required to ensure that adverse effects do not outweigh therapeutic benefits. Despite the worldwide collaborative effort to elucidate COVID-19 pathophysiology, it is clear that further research and data analysis is required to uncover the immune mechanism causing the 'cytokine storm' in COVID-19 patients as well as therapeutic regimes to enable correct patient management and treatment of COVID-19 associated coagulopathies.

INTRODUCTION

As of February 2021, the World Health Organisation (WHO) has reported over 100 million COVID-19 cases globally, with over 2 million deaths. While the majority of COVID-19 cases are mild or asymptomatic, a small proportion of patients develop complications such as pneumonia, sepsis, respiratory failure and thromboembolisms (Miesbach and Makris, 2020). Recent data has shown that the pathophysiology of severe COVID-19 infection has an association with a significant pulmonary coagulopathy, with thrombotic complications reported in up to 35-45% of patients with severe COVID-19 (Levi et al., 2020).

Preliminary studies reported the occurrence of venous thromboembolism (VTE) in 25% of severe COVID-19 cases admitted to ICU, with a mortality rate of 40%. Despite prophylactic anticoagulation, a later study reported a 31% incidence of VTE in critically ill COVID-19 cases. Additionally, autopsy reports have linked pulmonary embolism (PE) to 33% of COVID-19 deaths (Görlinger et al., 2020). Post-mortem analyses of COVID-19 patients show the formation of microthrombi in the lungs (Martín-Rojas et al., 2020). Reports have shown that COVID-19 patients may present with a thromboembolic event in even asymptomatic cases (Zheng et al., 2020).

The coagulopathy associated with COVID-19 is comparable to that of severe acute respiratory syndrome (SARS). The pathophysiology involves damage to alveolar tissue by the virus, which can be exacerbated by mechanical ventilation, leading to the consumption of platelets due to platelet activation and aggregation (Jiang et al., 2020). Entry of SARS-CoV-2 into the host cells leads to dysregulation in inflammatory signalling pathways, disrupting the normal coagulation mechanism. The hypercoagulability with abnormal clot formation is attributed to the inappropriately elevated immune response, culminating in a 'cytokine storm' with high levels of pro-inflammatory cytokines and subsequent aberrant thrombotic events (Jose and Manuel, 2020).

The coagulopathy in COVID-19 affects many coagulation parameters such as D-dimer levels, fibrinogen levels, platelet count and prothrombin time. Critically ill COVID patients have an elevated D-dimer level which correlates to disease severity; underpinning the potential prognostic value of D-dimer levels in COVID-19 patients (Yao et al., 2020). Coagulation parameters must be carefully monitored as they may indicate the requirement for clinical intervention. This suggests prophylactic anticoagulant therapy should be considered for COVID-19 patients with a predisposition to thrombosis (Buijsers et al., 2020).

Despite the worldwide collaborative effort to elucidate COVID-19 pathophysiology, it is clear that further research and data analysis is required to uncover the immune mechanism causing the 'cytokine storm' in COVID-19 patients, as well as therapeutic regimes to enable correct patient management and treatment of COVID-19 associated coagulopathies. The aim of this review was to provide a comprehensive overview of the pathophysiology of the COVID-19 associated coagulopathy, affected laboratory parameters and potential therapeutic regimes.

PATHOPHYSIOLOGY OF SARS-CO-V-2 COAGULOPATHY

The pathophysiology of COVID-19 associated coagulopathy can be attributed to the hypercoagulable state manifested due to an imbalance between proinflammatory signalling cascades and its effect on coagulation when infected by COVID-19. It has been hypothesised that thrombosis is an immune mechanism to restrict dissemination of SARS-CoV-2 in vivo and lower the viral load (Colling and Kanthi, 2020).

THE ROLE OF ACE2 RECEPTORS IN COVID-19 INFECTION

The causative agent of COVID-19 is severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). SARS-CoV-2 enters the host cells via binding the angiotensin-converting enzyme 2 (ACE2) receptor. This receptor is highly expressed on ciliated airway epithelial cells and type-2 alveolar pneumocytes in the lungs. It has been reported that SARS-CoV-2 spreads systemically throughout the body via infected pulmonary epithelium and then via pulmonary endothelium. The cellular damage triggers a proinflammatory coagulation cascade as well as increasing microvascular permeability (Yi et al., 2020).

During SARS-CoV-19 infection, membrane-bound ACE2 (mACE2) is cleaved by the metalloprotease ADAM-17 leading to the shedding and release of soluble ACE2. This process is associated with pulmonary injury (Swärd et al., 2020). The decreased ACE2 activity leads to an increase in vascular permeability triggering the release of tissue factor in endothelial cells, further enriching the hypercoagulable milieu. ACE2 is thought to have antithrombotic properties, especially in control of the renin-angiotensin system (RAS) where ACE converts angiotensin I to angiotensin II which is then degraded by ACE2 (Ni et al., 2020). However, COVID-19 patients have increased levels of angiotensin II, which leads to the activation of plasminogen activator inhibitor 1 (PAI-1), inhibiting fibrinolysis. The increase in plasma angiotensin II correlates to increased viral load and subsequent pulmonary injury (Gue and Gorog, 2020). As seen in figure 1, the downregulation of ACE2 in COVID-19 infection promotes vasoconstriction, hypertrophy and enhances proinflammatory mechanisms (Ni et al., 2020).

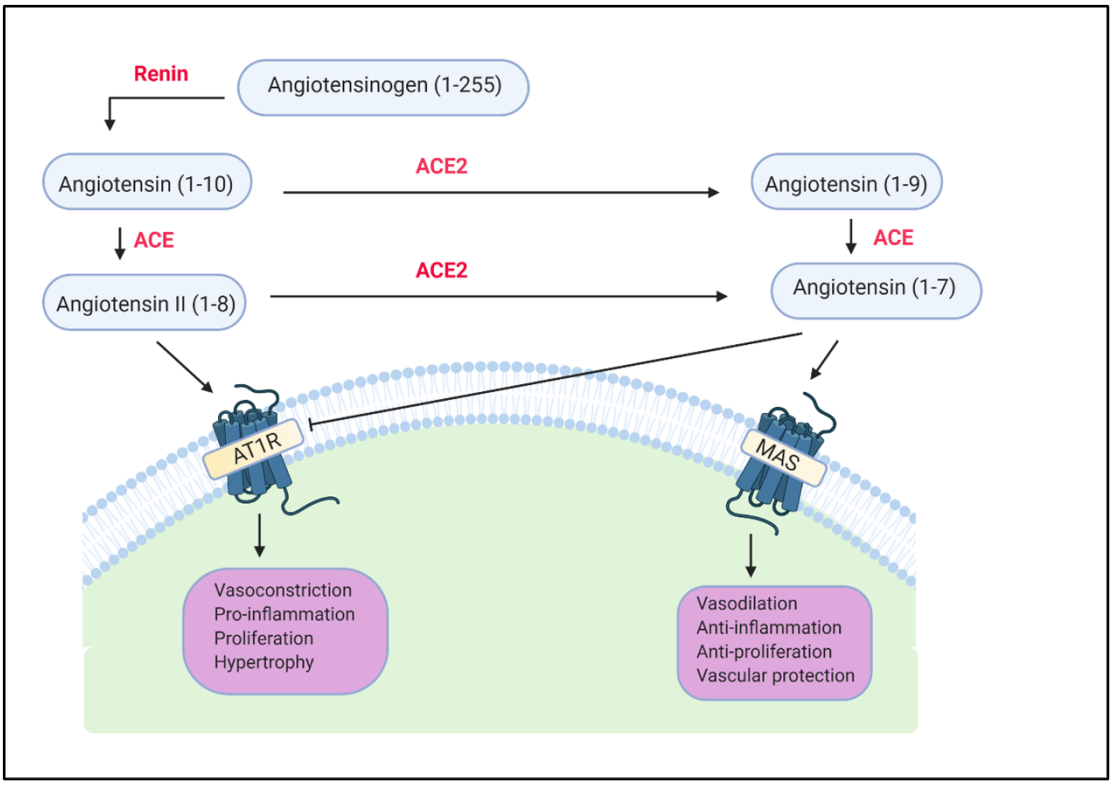

Figure 1: The Renin-Angiotensin system (RAS), ACE2 and the MAS receptor (adapted from Ni et al., 2020 and created using Biorender.com).

Renin converts angiotensinogen to angiotensin I (1-10), while angiotensin I is converted to angiotensin II by ACE. Angiotensin II then binds to the angiotensin type 1 receptor which promotes inflammation and vasoconstriction. ACE2 converts angiotensin II to angiotensin (1-7). Angiotensin (1-7) binds to the MAS receptor which is anti-inflammatory and promotes vasodilation (Ni et al., 2020).

Patient demographics may have a role in the risk of thrombotic complications in COVID-19 patients, with the Chinese population having up to a 4-fold increased risk when compared to the Caucasian populations. The risk is most elevated in the African American population. Polymorphisms in particular genes, such as the plasminogen activator inhibitor-1 (PAI-1) gene, the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) promoter and some proinflammatory cytokine genes, increase the risk of thrombotic complications (Görlinger et al., 2020).

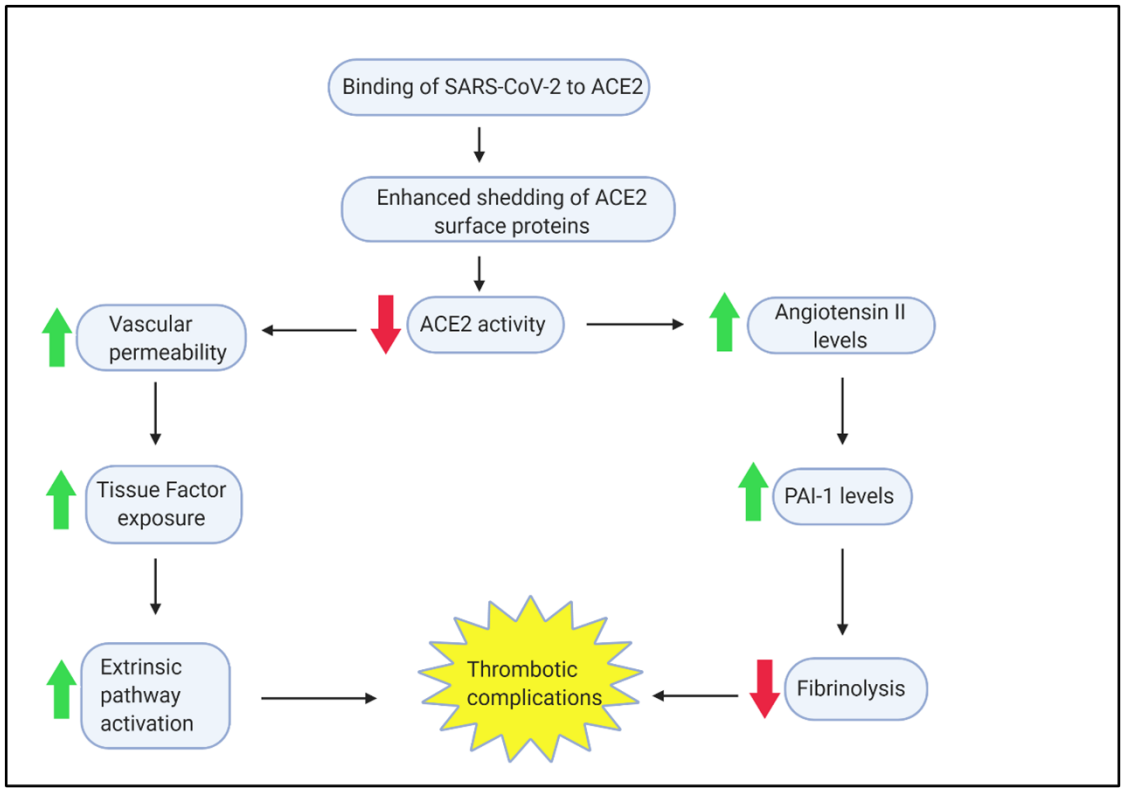

Figure 2: The role of ACE2 in thrombus formation in COVID-19 (adapted from Gue and Gorag., 2020 and created using BioRender.com).

Binding of SARS-CoV-2 to ACE2 receptors leads to increased ACE2 shedding, reducing ACE2 activity leading to increased tissue factor exposure and activation of the extrinsic coagulation pathway. Additionally, the decreased ACE2 activity leads to increased plasma angiotensin II levels with a subsequent increase in PAI-1 levels and decreased fibrinolysis (Gue and Gorag., 2020).

VIRCHOW'S TRIAD AND COVID-19 ASSOCIATED COAGULOPATHY

Many studies have implicated COVID-19 as a risk factor for arterial and venous thrombosis. Applying the basis of Virchow's triad, malformations in vascular endothelium, stasis in blood flow and blood hypercoagulability have been reported in COVID-19 patients, predisposing them to the development of thrombosis. Aberrant endothelial function, the release of PAI-1 and increased activation of platelets are implicated in this phenomenon. Due to these findings, research should focus on these novel targets to reduce mortality in COVID-19 (Ahmed et al., 2020).

Recurrent thrombosis occurs in COVID-19 patients despite prophylactic anticoagulation therapy, especially in ICU admitted COVID-19 patients (Zheng et al., 2020). All 3 elements of Virchow's triad are present in varying degrees in conditions such as atrial fibrillation, myocardial infarction and stroke, indicating these populations may benefit from prophylactic anticoagulation therapy on admission when diagnosed with COVID-19 to avoid thrombosis development (Ahmed et al., 2020).

As SARS-CoV-19 disseminates through the endothelium, the endothelial damage leads to the release of tissue factor, instigating the coagulation cascade (Yi et al., 2020). As seen in figure 2, binding of SARS-CoV-2 to the ACE2 receptor reduces ACE2 activity leading to increased tissue factor exposure as well as activation of the extrinsic clotting pathway leading to increased risk of thrombosis. Vasoconstriction and reduced blood flow occur as a result of COVID-19 induced hypoxia, stimulating the procoagulant properties of the endothelium. This further damages the endothelium and leads to the release of large von Willebrand factor (vWF) multimers (Joly et al., 2020). Abnormal blood flow is exacerbated by microthrombi development. Blood stasis is attributed to immobility in hospitalised

patients. The hypercoagulable state in COVID-19 stems from activation of the coagulation cascade, aberrant platelet function, complement activation and plasminogen inhibition (Ahmed et al., 2020).

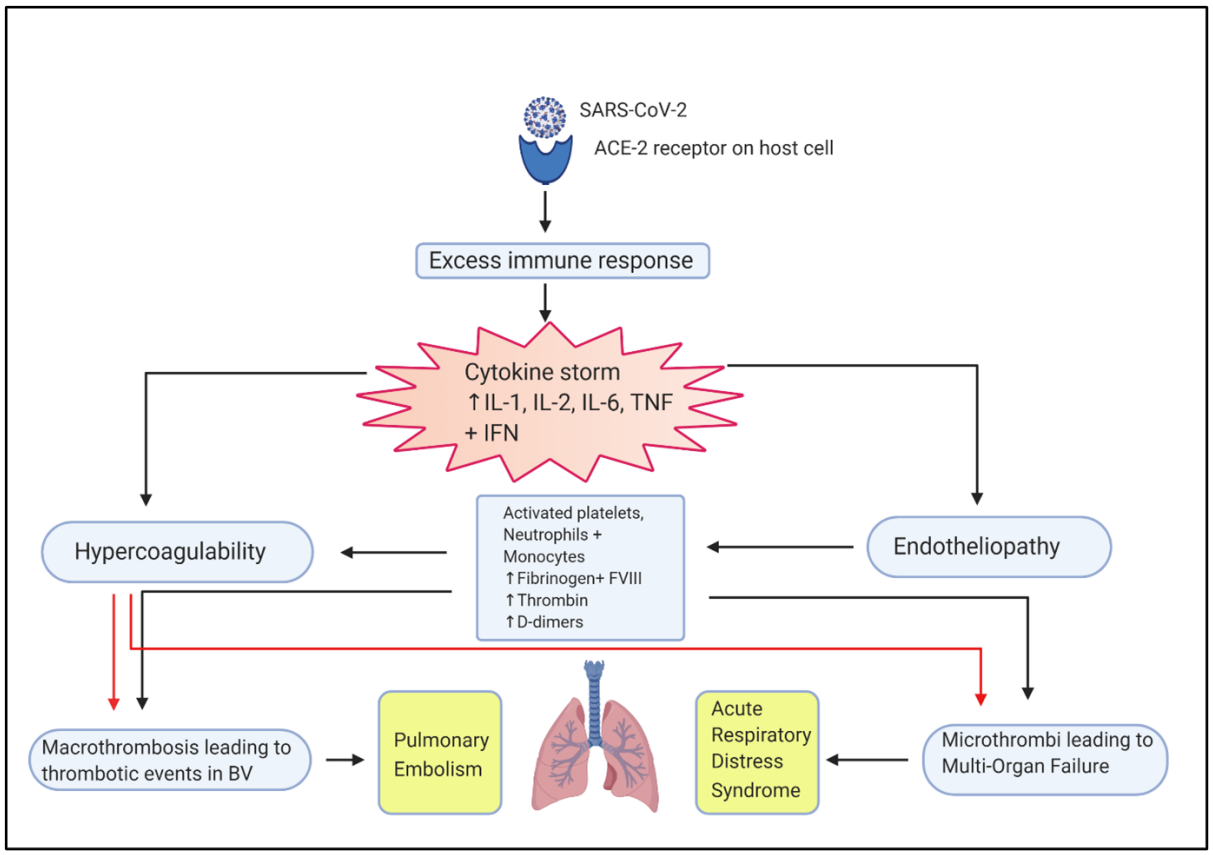

Figure 3: Pathophysiology of thrombosis in COVID-19 (adapted from Joly et al., 2020 and created using BioRender.com).

Binding of SARS-CoV-19 to the ACE2 receptor leads to an inappropriate release of proinflammatory cytokines culminating in a cytokine storm which triggers endothelitis and hypercoagulability, increasing the risk for thrombosis development. As seen in the Figure 3 above, the cytokine storm is thought to be the instigator of the hypercoagulable state and endothelial damage (Joly et al., 2020).

COVID-19 AND CYTOKINE STORM

The pathophysiology of COVID-19 is currently incompletely understood. However, the viral infection has been linked to an inappropriately amplified immune response leading to a 'cytokine storm' which has been implicated in abnormal coagulation and subsequently, acute respiratory distress syndrome (ARDS) and multiorgan failure (MOF). As a result, much interest has been generated in the identification of cytokines which may represent novel anti-inflammatory therapeutic targets (Iannaccone et al., 2020). Recent research demonstrates that the coagulopathy produced in COVID-19 is not due to specific properties of the virus, but due to the hyperactive immune response against the virus. However, unlike other RNA haemorrhagic fever viruses, significant bleeding does not accompany the coagulopathy in COVID-19 patients (Connors and Levy, 2020).

As seen in figure 3 above, binding of SARS-CoV-2 to respiratory epithelial cells via the ACE2 receptor elicits an aberrant inflammatory response of a cytokine storm with an influx of proinflammatory cytokines such as IL-1β, IL-2, IL-6 and TNF. Endothelial damage contributes to hypercoagulability by stimulating the production of tissue factor, P-selectin, Factor VIII, fibrinogen and vWF. Natural

anticoagulants such as thrombomodulin (TM) and endothelial protein C receptor (EPCR) are downregulated (Chan and Weitz, 2020).

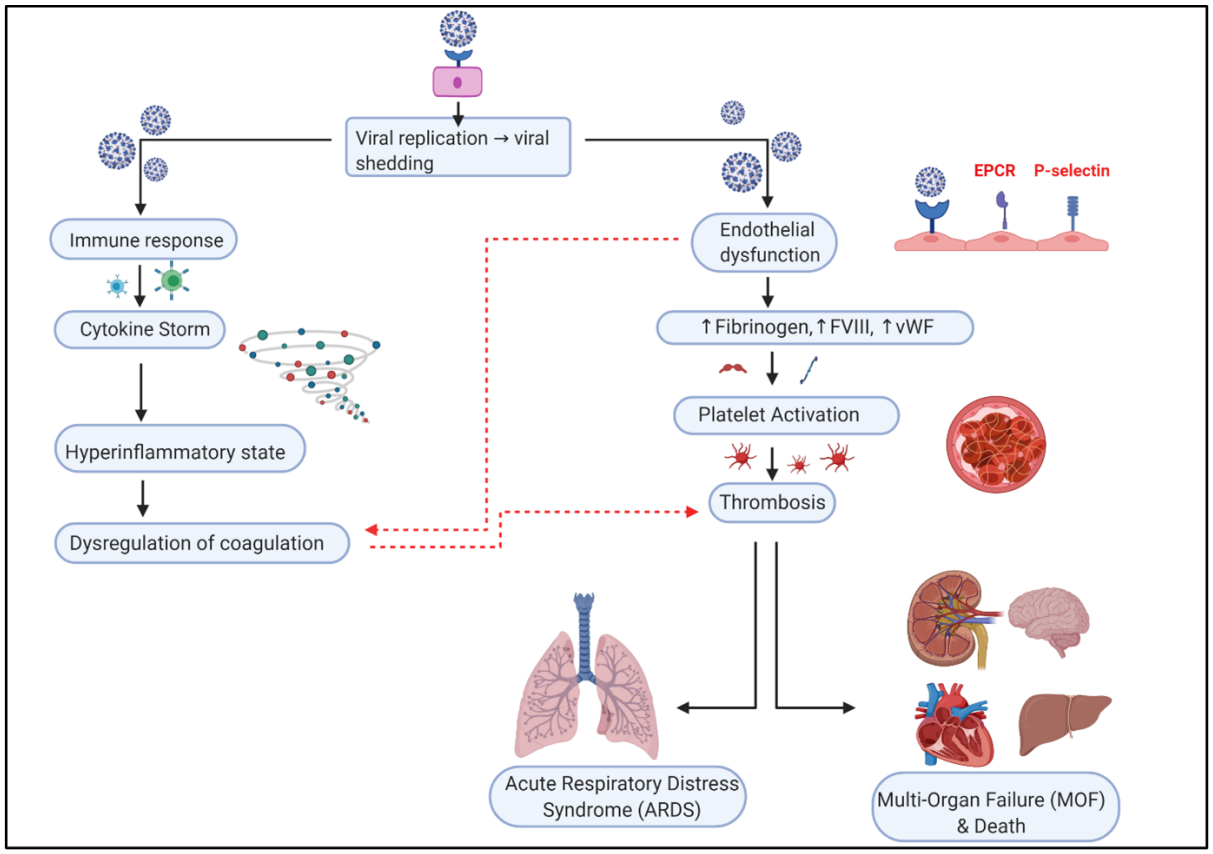

Figure 4: Coagulopathy and thrombosis associated with COVID-19 infection (adapted from Chan and Weitz, 2020 and created using BioRender.com).

As seen in Figure 4 the cytokine storm is fundamental to the progression to the hyperinflammatory state which leads to the dysregulation of coagulation with associated thrombosis. The coagulopathy can progress to complications such as ARDS and multiorgan failure and death (Chan and Weitz, 2020).

A hyperactive, overexaggerated immune response against the SARS-CoV-2 virus has been linked to the high mortality rates in COVID-19 patients. As demonstrated in figure 4 above, the inappropriately elevated levels of proinflammatory cytokines contributes to the development of ARDS and widespread tissue damage causing MOF. Reports of COVID-19 patient cytokine panels have demonstrated that the cytokine storm has a direct association with pulmonary damage and poor prognosis. Proinflammatory cytokines in this panel include interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-18 (IL-18), interferon-γ (IFN-γ) and tumour necrosis factor-α (TNF-α) (Ragab et al., 2020).

However, a preliminary study reported that COVID-19 is not characterised by a cytokine storm, with much lower levels of proinflammatory cytokines reported in COVID-19 patients vs other critically ill patients (e.g., bacterial septic shock). Studies have reported that IL-6 levels in severe COVID-19 cases are increased but are considerably lower than IL-6 level in non-COVID-19 related ARDS. For example, sepsis patients with ARDS have an IL-6 concentration of 376 pg/ml compared to only 48 pg/ml in COVID-19 patients with ARDS (Kox et al., 2020). Due to these findings, it is yet to be verified that cytokine directed therapies can be used to successfully treat the cytokine storm associated with poor outcome in COVID-19 patients.

ABNORMAL LABORATORY PARAMATERS IN COVID 19 INFECTION

Abnormal FBC results in COVID-19 and associated coagulopathy

A common finding in the full blood count of COVID-19 patients is lymphopenia with a mild thrombocytopenia. 5% of COVID-19 patients present with a platelet count less than 100×10^9 cells/L, while up to 95% of patients with severe disease have a platelet count of less than 150×10^9cells/L (Levi et al., 2020). COVID-19 induced thrombocytopenia occurs due to consumption of platelets in response to endothelial damage, as well as pulmonary microthrombi formation. Platelets in COVID-19 patients are reported to have an elevated mean platelet volume (MPV). The production of large, immature platelets may contribute to thrombosis due to their increased reactivity (Wool and Miller, 2020).

Neutrophilia has been reported in some COVID-19 patients (Pourbagheri-Sigaroodi et al., 2020). Increased neutrophil infiltration in the lungs and formation of neutrophil extracellular traps (NETs) exacerbate microthrombi formation by enhancing platelet aggregation (Tomar et al., 2020). The absence of eosinophils at presentation has been reported in 60% of COVID-19 patients compared to 16% of patients with influenza. This may be useful as a preliminary screen to isolate suspect COVID patients to prevent further spread of the virus while awaiting confirmatory testing (Tanni et al., 2020). Severe COVID-19 cases have recently been characterised by a higher neutrophil to lymphocyte ratio (NLR). An elevated NLR is a risk factor for mortality in infectious diseases (Ragab et al., 2020).

Abnormal coagulation parameters in COVID-19

Some of the abnormal coagulation parameters include a prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT). However, in a small subset of patients the aPTT has been shown to be shortened significantly, probably due to increases in the acute phase reactant FVIII (Wool and Miller, 2020). This phenomenon is encountered with patients on extracorporeal membrane oxygenation (ECMO). Although bleeding risk with COVID-19 patients is relatively low, it is important to consider such anomalies before proceeding with anticoagulant therapy (Mazzeffi et al., 2020).

COVID-19 coagulopathy is associated with an abnormal hypercoagulable state which progresses to a disseminated intravascular coagulopathy (DIC)-like state in the late stages of disease. There are marked differences between COVID-19 coagulopathy and DIC. In COVID-19 associated coagulopathy, there is mainly localisation of pulmonary microthrombi while in DIC this is more widespread. Bleeding is uncommon in COVID-19 coagulopathy while it is common in DIC. Fibrinogen levels are increased in COVID-19 coagulopathy but decreased in DIC. D-dimer levels are more markedly elevated in COVID-19 coagulopathy with a mild thrombocytopenia and mild clotting time prolongation when compared to DIC (Aggarwal et al., 2020).

COVID-19 associated coagulopathy can be divided into 3 stages. Stage 1 demonstrates an increase in D-dimer level, stage 2 demonstrates an elevated D-dimer level, mildly prolonged PT +/or aPTT with mild thrombocytopenia and finally, stage 3 shows a progression towards DIC-typical laboratory parameters. However, hypofibrinogenemia is rare (Wool and Miller, 2020). Tang et al noted that 71% of COVID-19 non-survivors developed DIC by day 4 of hospital admission compared to <1% of survivors, highlighting the importance of monitoring fibrinogen levels, PT and platelet count (Turshudzhyan, 2020). Due to the marked differences between COVID-19 induced coagulopathy and DIC, studies have shifted to identify a COVID-19 coagulopathy scoring system as DIC criteria are often not met (Hadid et al., 2020).

Recent research has demonstrated the presence of antiphospholipid antibodies associated with coagulopathy in COVID-19 patients which may account for the prolongation of the aPTT (Kipshidze et al., 2020). The presence of these antibodies may indicate requirements for early anticoagulation intervention. However, it is not uncommon to find these antibodies in patients with infection (Connell et al., 2020).

D-dimers have also been implicated as a prognostic marker (Pourbagheri-Sigaroodi et al., 2020). Research has shown that the increase in D-dimer levels is concurrent with the progressive activation of the coagulation cascade with simultaneous activation of pulmonary fibrinolysis (Fogarty et al., 2020).

Inflammatory markers in COVID-19

In severe disease, increased lactate dehydrogenase, serum ferritin and creatine kinase are found. Progression to multiple organ failure is marked by elevations in creatinine, ALT and AST (Ragab et al., 2020). The development of severe COVID-19 is denoted by increases in serum amyloid A (Zeng et al., 2020).

Recent research has identified the importance of lymphopenia as an indicator for development of the COVID-19 related cytokine storm which precedes the COVID-19 induced coagulopathy. The low number of T cell lymphocytes fails to control the viral infection and allows inappropriate propagation of the innate immune system. The ineffective elimination of inflammatory macrophages by CD8+ T cells may explain the surplus of proinflammatory cytokines (Caricchio et al., 2020). A retrospective study of 150 COVID-19 patients concluded that elevated ferritin and IL-6 correlates mortality with hyperinflammation. To identify COVID-19 patients who may benefit from immunosuppression therapy, increasing ferritin, thrombocytopenia and increased erythrocyte sedimentation rate (ESR) should be monitored (Mehta et al., 2020). One study correlated increased D-dimer levels with increased C-reactive protein (CRP), ferritin and procalcitonin (PCT) indicating that inflammatory markers may have the ability to predict onset of the cytokine storm preceding coagulopathy (Long et al., 2020).

Can biomarkers predict clinical severity and coagulopathy in COVID-19?

As the clinical presentation of COVID-19 ranges from asymptomatic to life-threatening, several studies have focused on identifying biomarkers to predict the disease course as well as predicting risk of complications such as thrombosis (Lippi et al., 2020). Biomarkers of coagulation, inflammatory response as well as clinical scoring systems can predict the clinical progression of COVID-19, patient outcome and requirement for increasingly scarce hospital resources such as ICU beds and ventilators (Görlinger et al., 2020).

Platelet counts can be used as an indicative marker of critical COVID-19 cases as thrombocytopenia is associated with a 3-fold increased risk of severe disease, with an increased mortality rate (Lippi et al., 2020). The importance of many lab parameters as potential prognostic indicators has been demonstrated, with findings having the ability to discriminate between mild and severe infection. Indicators of poor prognosis in COVID-19 patients include a low lymphocyte count and an increased D-dimer level, lactose dehydrogenase (LDH) and liver enzymes AST and ALT (Levi et al., 2020).

The most promising biomarker for COVID-19 disease severity and progression to aberrant coagulation is the D-dimer. D-dimer levels are highly elevated in COVID-19 non-survivors compared to survivors. Studies have shown that D-dimer levels commonly increase as the COVID-19 patient clinically deteriorates (Zhou et al., 2020). However, the use of D-dimer levels to guide anticoagulation therapy is not recommended in clinical practice and is reserved for clinical trial settings as further human trials are required (Flaczyk et al., 2020).

COVID-19 COAGULOPATHY TREATMENT

Therapeutic Anticoagulation

Research has demonstrated that although prophylactic anticoagulation reduces risk of thrombosis, the risk of major bleeding may increase to unacceptable levels in severely ill COVID-19 patients. Clinical trials conducting studies on anticoagulation dosing regimes are urgently needed, and fortunately, are in progress (Chan and Weitz, 2020). A retrospective study of over 4,000 participants demonstrated that anticoagulation therapy reduced incidence of mortality and intubation in COVID-19 patients (Nadkarni et al., 2020).

However, there are many clinical considerations before administering anticoagulants to all hospitalised COVID-19 patients. Some studies recommend administration in the following circumstances: patients on mechanical ventilation in ICU, non-ICU patients with pre-existing predisposition to thrombosis such

as diabetics, those with ischemic cardiomyopathy and patients with a history of PE or VTE (Zheng et al., 2020).

Heparin

Heparin exerts its anticoagulant effect by binding antithrombin III which amplifies its inhibitory effect on FXa and thrombin. A synthetic analogue, Fondaparinux, is indicated for prophylaxis of VTE (Costanzo et al., 2020). Heparin has been shown to decrease pulmonary emboli and inflammation of the lung while potentially acting as an antiviral agent against COVID-19. Other properties of heparin include the neutralisation of IL-6 and IL-8 that have a role in ARDS (Buijsers et al., 2020).

Low molecular weight heparin (LMWH) in particular plays a very important role in the treatment of COVID-19 infection through its ability to inhibit heparinase (HPSE). HPSE plays a role in the disruption of the endothelial glycocalyx barrier which is involved in the pathology of pulmonary oedema. LMWH inhibits synthesis of TNF-α and IFN-γ by blocking the NF-κB pathway. Heparin has demonstrated the ability to inhibit complement system activation which is thought to propagate the coagulopathy and thrombosis found in COVID-19 (Buijsers et al., 2020).

Research has demonstrated that prophylactic heparin administration leads to a 20% decrease in mortality in patients with an elevated D-dimer level and sepsis-induced coagulopathy score (Hippensteel et al., 2020). Studies have supported the use of nebulised unfractionated heparin (UFH) to treat COVID-19 associated ARDS and pneumonia. Also, UFH has a mucolytic effect which may relieve some of the pneumonic symptoms (Van Haren et al., 2020). Other effects include the release of tissue factor pathway inhibitor (TFPI), further enhancing its anticoagulative properties (Kipshidze et al., 2020).

Heparin dose adjustments should be made according to patient's weight, D-dimer levels, renal function and development of respiratory failure. UFH is recommended for patients with a reduced creatinine clearance (<30 ml/min). Increased anticoagulation is required in patients with highly elevated D-dimer levels. As previously discussed, heparin resistance may be attributed to decreased anti-thrombin levels and increased levels of fibrinogen. This may indicate the administration of anti-thrombin supplements which may enhance heparin's anticoagulative activity (Turshudzhyan, 2020; Zheng et al., 2020).

Although clinicians recommend the transition from vitamin K antagonist anticoagulants to direct oral anticoagulants (DOACs), further research is required to characterise the pharmacological interactions between anti-retroviral drugs and DOACs (Turshudzhyan, 2020). Although DOACs exhibit similar anti-inflammatory properties to heparin, clinical guidance recommends Rivaroxaban and Edoxaban are not administered with antiviral therapy, while Apixaban should be administered at a half dose. Dabigatran can be given at the normal dose (Kartsios et al., 2020).

Thrombolytic therapy

Alteplase is a thrombolytic agent that reportedly improves oxygenation in COVID-19 patients. This treatment could potentially alleviate some of the demand for mechanical ventilation by improving oxygenation in COVID-19 patients. However major bleeding has been reported in 10% of cases while intracranial bleeding has been reported in 1-2% of cases following systemic administration of this agent. As of late, this therapy is not indicated for treatment of COVID-19 related thrombosis (Barnes et al., 2020). It is recommended that thrombolytic therapy is only administered to those with haemodynamic instability who are progressively deteriorating (Rosovsky et al., 2020).

Anti-platelet drugs

A recent study has shown that in approximately 20% of COVID-19 patients, SARS-COV-2 RNA is detected in platelets (Zaid et al, 2020). The platelets also have hyperactive characteristics including increased degranulation and superior adhesion which contributes to inflammatory thrombus formation. For this reason, platelet activation pathways may represent a novel target for COVID-19 patients to prevent complications such as thrombi formation and consumptive coagulopathy (Zaid et al., 2020). Because thrombosis occurs in COVID-19 patients despite anticoagulation administration, research

should consider examining the value of antiplatelet drugs. Retrospective studies could be used to analyse if patients already taking antiplatelet drugs for an underlying disorder (such as cardiomyopathy) may be safeguarded from severe manifestations of COVID-19 (Thachil, 2020).

Dipyridamole (DIP) is an adenosinergic antiplatelet drug used globally to treat coagulopathy. Experimental analysis shows that DIP exerts anti-inflammatory and antiviral properties in COVID-19 cases (Liu et al., 2020). A limited clinical trial of COVID-19 patients demonstrated reduced D-dimer levels when treated with DIP. As DIP is universally available and moderately inexpensive with a high safety profile, further research is required to make use of this valuable antiplatelet agent (Colling and Kanthi, 2020).

Immune therapies

Due to the hyperactivation of the immune system, caused by SARS-CoV-2 infection, research has focused on targeting inflammatory signalling pathways to reduce the immune impact. Such therapies include disease-modifying anti-rheumatic drugs such as Tociluzimab, Baricitinib, Anakinra and hydroxychloroquine (Zhong et al., 2020). Once the cytokine storm has been activated, targeting upstream pathways may be more successful to limit the amplification of the hypercoagulable pathway (Colling and Kanthi, 2020).

Tocilizumab

IL-6 levels are elevated in COVID-19 patients, with its levels correlating to risk of mortality. Data suggests that IL-6 plays an important role in lung pathology in COVID-19 patients, indicating that it is an appropriate target for immunomodulatory therapy. Tocilizumab is a humanised IL-6 receptor antagonist which is clinically approved for treatment of rheumatoid arthritis and cytokine release syndrome. As COVID-19 associated pneumonia is thought to be attributed to the COVID-19 cytokine storm, research has shifted towards immune targeted therapies like Tocilizumab. Administration of Tocilizumab leads to a stable or decreased level of IL-6 as the drug competitively binds to IL-6 receptors (Song et al., 2020).

A preliminary trial in China used the monoclonal anti-IL-6 antibody Tocilizumab to target the cytokine storm associated with COVID-19 leading to a decrease in mortality and fewer mechanical ventilation requirements. The FDA has approved clinical trials to phase III for therapeutic utilisation of tocilizumab in 330 patients with severe COVID-19 related pneumonia (Ragab et al., 2020). Tocilizumab is still in its infancy for treatment of COVID-19 pneumonia. Randomised studies are required to confirm these findings, determine the optimal dose required, along with the critical timing of administration (Guaraldi et al., 2020)

Tocilizumab treatment has been associated with neutropenia and thrombocytopenia (McCreary and Pogue, 2020). Further studies are required to determine if Tociluzimab has a beneficial role in the prevention of COVID-19 associated thrombosis.

Baricitinib

Baricitinib is a Janus kinase inhibitor with anti-inflammatory properties currently used to treat rheumatoid arthritis. This therapy can reduce the influx of cytokines associated with the COVID-19 cytokine storm (Cantini et al., 2020). As the severe phase of COVID-19 is characterised by high pro-inflammatory cytokine levels and all of these implicated cytokines signal through the JAK-STAT transcription pathway, this immune therapy could potentially downregulate the COVID-19 associated cytokine storm. As illustrated in figure 5 below, Baricitinib selectively binds to JAK1/JAK2 and acts as a reversible ATP competitive kinase inhibitor. This suppresses the level of pro-inflammatory cytokines, primarily IL-6, IL-12 and IFN-γ. The reduction in the level of these cytokines can potentially reduce the inflammatory damage caused to the lungs and other organs in COVID-19 infection. SARS-CoV-2 viral endocytosis is reduced by Baricitinib due to its high affinity for AP2-associated protein kinase which acts as a regulator of clathrin-mediated viral endocytosis (X. Zhang et al., 2020).

However, there are reservations regarding JAK inhibitors in treatment of viral infection as the JAK-STAT signalling pathway leads to interferon production which kills virus infected cells (Favalli et al., 2020). However, the dose dependent association with thromboembolic events requires more research. As a result, this drug is only FDA approved at the 2 mg/day dose to reduce the risk of thrombotic events. Platelet count is exponentially increased in the first two weeks of Baricitinib therapy but there is no evidence to support that this is responsible for the hyperthrombotic state as another JAK inhibitor, tofacitinib, does not increase platelet count but also has an increased risk of thrombotic events. As a result, although Baricitinib may decrease the COVID-19 induced cytokine storm, therapeutic benefits may be outweighed by its adverse hypercoagulable risks (Jorgensen et al., 2020). There is currently an ongoing trial of Baricitinib therapy administered with the antiviral Remdesivir which may clarify the benefits of this therapy (Titanji et al., 2020).

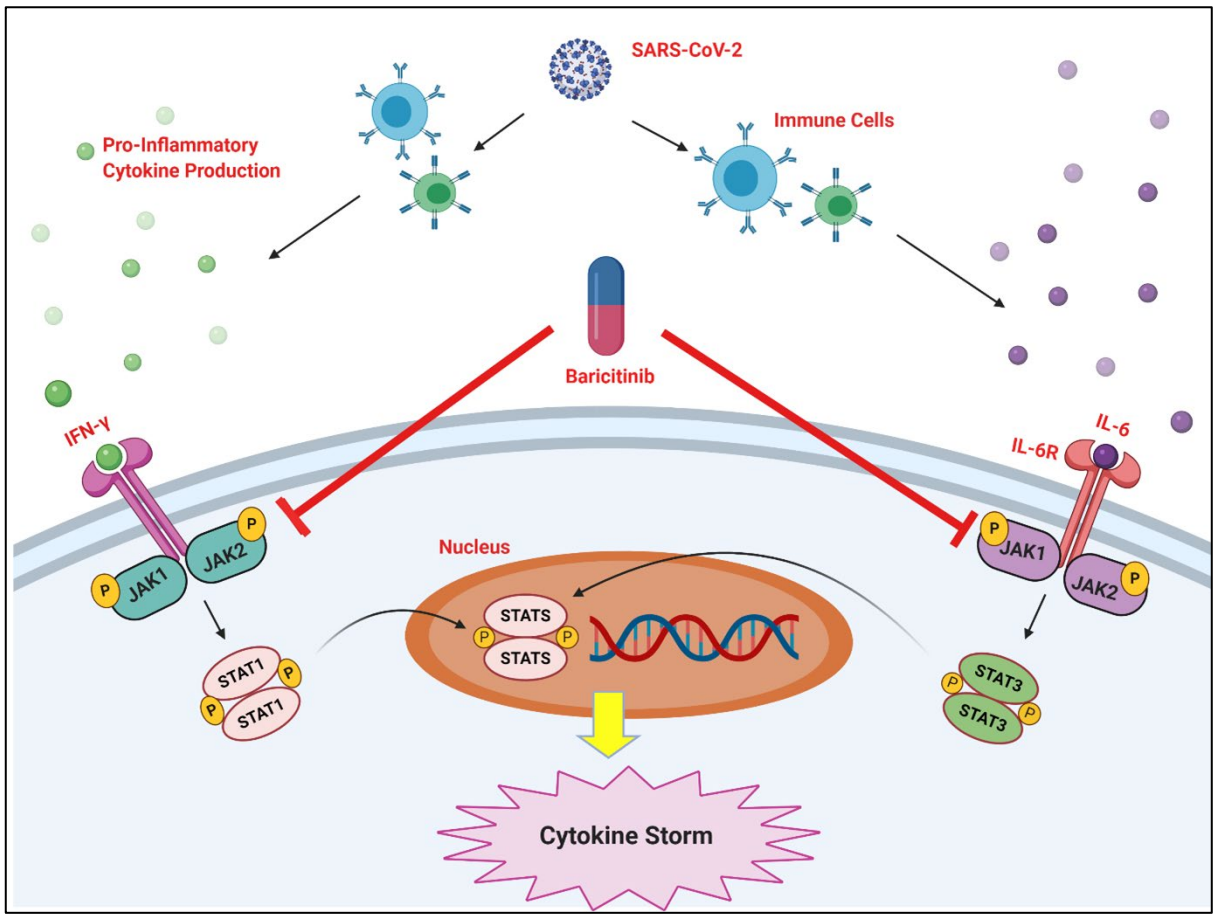

Figure 5: Baricitinib acts intracellularly to suppress the activation of JAKs, subsequently prohibiting the cytokine storm (adapted from X. Zhang et al., 2020 and created using BioRender.com).

When SARS-CoV-2 binds to ACE2, a large amount of cytokines such as IL-6 and IFN-γ are released. The pro-inflammatory cytokines bind to their receptors and activate JAKs which leads to the downstream activation and phosphorylation of STATs. The phosphorylated STATs enter the nucleus and lead to transcription of cytokine-responsive genes, culminating in a cytokine storm (X. Zhang et al., 2020).

Hydroxychloroquine

Hydroxychloroquine has demonstrated beneficial effects in vitro for control of COVID-19 associated coagulopathy. Studies have shown the anti-thrombotic properties of hydroxychloroquine in animal models by inhibition of platelet aggregation and reduced antiphospholipid antibody binding. The exact mechanism by which hydroxychloroquine exerts its anticoagulative effects is still largely unknown (Pal et al., 2020). Many studies demonstrated no in vivo clinical benefits of hydroxychloroquine in COVID-19 treatment or prevention (Li et al., 2020).

Intravenous immunoglobulin (IvIg)

Intravenous immunoglobulin (IVIg) is derived from human plasma and is used to treat autoinflammatory diseases due to its potent immunomodulatory abilities. Although, IVIg was used in the SARS 2003 epidemic to treat the associated cytopenias, there is no substantial clinical evidence for its use in the treatment of COVID-19 (Song et al., 2020). Also, there is apprehension that IVIg may trigger an inappropriate immune response termed antibody-dependent enhancement (ADE) (Nguyen et al., 2020). As complement is activated in COVID-19, monoclonal antibodies such as Eculizumab could be effective in dampening COVID-19 pathophysiology (Ahmed et al., 2020). It is clear that additional research is required to analyse the potential prothrombotic effect of IVIg in COVID-19 (Muccioli et al., 2020).

General COVID-19 Treatment

COVID-19 treatment is predominantly supportive. However, one must consider the effect of these therapies on the COVID-19 associated coagulopathy, especially in patients with one or more risk factors for thrombosis. Based on the results of preliminary studies, the FDA issued emergency authorisation of the use of the antiviral Remdesivir for treatment of hospitalised COVID-19 patients in the USA in May (Beigel et al., 2020). However, deep vein thrombosis (DVT) has been reported in COVID-19 recipients of Remdesivir. Further studies are required to determine if this antiviral agent increases the risk of coagulopathy in COVID-19 (Fan et al., 2020). Animal models have demonstrated that the corticosteroid, Dexamethasone, decreases the formation of neutrophil extracellular traps (NETs) which have been identified in COVID-19 coagulopathy (Fan et al., 2020, Arabi et al., 2020).

CONCLUSION

To conclude, despite worldwide collaboration, there is no clinically supported prophylactic anticoagulation regime to treat the coagulopathy associated with severe COVID-19 infection. This review has discussed the possible aetiology of the COVID-19 associated coagulopathy, affected lab parameters and prognostic indicators, as well as the requirement for anticoagulant therapy and potential novel therapeutic targets.

Contemporary data demonstrate that the COVID-19 coagulopathy is triggered by a ‘cytokine storm’ and due to this finding, research has queried that targeting this cytokine storm may represent a budding therapeutic entity to reduce the mortality associated with COVID-19 coagulopathy. However, some studies have shown that both incidence of coagulopathy and proinflammatory cytokine levels in general ICU patients and COVID-19 ICU patients are comparable which undermines the studies that suggest that the cytokine storm, and subsequent coagulopathy leading to a hyperthrombotic state, are unique to severe COVID-19 cases (Kox et al., 2020). Due to these findings, it is yet to be verified that cytokine directed therapies can be used to successfully treat the cytokine storm associated with poor outcome in COVID-19 patients.

The monitoring of FBC parameters is important in management of COVID-19 patients and progression to COVID-19 coagulopathy. Due to the abnormal coagulation parameters observed in COVID patients, the importance of multidisciplinary medical science in the management of COVID patients has been highlighted. Monitoring of coagulation parameters is paramount to reducing COVID-19 related mortality as certain thresholds may indicate the requirement for clinical intervention before the development of thrombosis.

Although guidelines have been published on indicative criteria for anticoagulation therapy in COVID-19, no guidelines have been adopted universally (Flaczyk et al., 2020). The two main approaches in the treatment and prevention of COVID-19 coagulopathy include both therapeutic and prophylactic anticoagulation as well as immunomodulatory therapies which target the inflammatory signalling cascade. Several plausible candidates have been applied to treatment of COVID-19 in attempt to reduce the occurrence of the potentially fatal coagulopathy. However, therapeutic benefits may not compensate for the adverse effects.

As a final remark, many studies support that COVID-19 associated coagulopathy is triggered by a cytokine storm. It is clear that retrospective randomised studies and clinical trials are required to validate anticoagulation regimes for COVID-19 coagulopathy, as well as identification of laboratory cut-off values to flag patients at risk of thrombosis.

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REFERENCES

Aggarwal, M., Dass, J. and Mahapatra, M., 2020. Hemostatic Abnormalities in COVID-19: An Update. *Indian Journal of Hematology and Blood Transfusion*, [online] 36(4), pp.616-626. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7418883/> [Accessed 10 November 2020].

Ahmed, S., Zimba, O. and Gasparyan, A., 2020. Thrombosis in Coronavirus disease 2019 (COVID-19) through the prism of Virchow's triad. *Clinical Rheumatology*, [online] 39(9), pp.2529-2543. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7353835/> [Accessed 24 October 2020].

Arabi, Y., Chrousos, G. and Meduri, G., 2020. The ten reasons why corticosteroid therapy reduces mortality in severe COVID-19. *Intensive Care Medicine*, [online] 46(11), pp.2067-2070. Available at: <https://link.springer.com/article/10.1007/s00134-020-06223-y#citeas> [Accessed 11 November 2020].

Barnes, G., Burnett, A., Allen, A., Blumenstein, M., Clark, N., Cuker, A., Dager, W., Deitelzweig, S., Ellsworth, S., Garcia, D., Kaatz, S. and Minichiello, T., 2020. Thromboembolism and anticoagulant therapy during the COVID-19 pandemic: interim clinical guidance from the anticoagulation forum. *Journal of Thrombosis and Thrombolysis*, [online] 50(1), pp.72-81. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7241581/> [Accessed 25 October 2020].

Beigel, J., Tomashek, K., Dodd, L., Mehta, A., Zingman, B., Kalil, A., Hohmann, E., Chu, H., Luetkemeyer, A., Kline, S., Lopez de Castilla, D., Finberg, R., Dierberg, K., Tapson, V., Hsieh, L., Patterson, T., Paredes, R., Sweeney, D., Short, W., Touloumi, G., Lye, D., Ohmagari, N., Oh, M., Ruiz-Palacios, G., Benfield, T., Fätkenheuer, G., Kortepeter, M., Atmar, R., Creech, C., Lundgren, J., Babiker, A., Pett, S., Neaton, J., Burgess, T., Bonnett, T., Green, M., Makowski, M., Osinusi, A., Nayak, S. and Lane, H., 2020. Remdesivir for the Treatment of Covid-19 — Final Report. *New England Journal of Medicine*, [online] Available at: <https://www.nejm.org/doi/full/10.1056/NEJMoa2007764> [Accessed 31 October 2020].

Buijsers, B., Yanginlar, C., Maciej-Hulme, M., de Mast, Q. and van der Vlag, J., 2020. Beneficial non-anticoagulant mechanisms underlying heparin treatment of COVID-19 patients. *EBioMedicine*, [online] 59, p.102969. Available at: <https://www.thelancet.com/journals/ebiom/article/PIIS2352-3964(20)30345-5/fulltext> [Accessed 4 October 2020].

Cantini, F., Niccoli, L., Matarrese, D., Nicastri, E., Stobbione, P. and Goletti, D., 2020. Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. *Journal of Infection*, [online] 81(2), pp.318-356. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7177073/> [Accessed 7 November 2020].

Caricchio, R., Gallucci, M., Dass, C., Zhang, X., Gallucci, S., Fleece, D., Bromberg, M. and Criner, G., 2020. Preliminary predictive criteria for COVID-19 cytokine storm. *Annals of the Rheumatic Diseases*, [online] pp.annrheumdis-2020-218323. Available at:

<https://ard.bmj.com/content/early/2020/09/25/annrheumdis-2020-218323.info> [Accessed 25 October 2020].

Chan, N. and Weitz, J., 2020. COVID-19 coagulopathy, thrombosis, and bleeding. *Blood*, [online] 136(4), pp.381-383. Available at: <https://ashpublications.org/blood/article/136/4/381/461407/COVID-19-coagulopathy-thrombosis-and-bleeding> [Accessed 24 October 2020].
Colling, M. and Kanthi, Y., 2020. COVID–19-associated coagulopathy: An exploration of mechanisms. *Vascular Medicine*, [online] 25(5), pp.471-478. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7306998/> [Accessed 1 November 2020].

Connell, N., Battinelli, E. and Connors, J., 2020. Coagulopathy of COVID-19 and antiphospholipid antibodies. *Journal of Thrombosis and Haemostasis*, [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267637/> [Accessed 6 November 2020].

Connors, J. and Levy, J., 2020. COVID-19 and its implications for thrombosis and anticoagulation. *Blood*, [online] 135(23), pp.2033-2040. Available at: <https://ashpublications.org/blood/article/135/23/2033/454646/COVID-19-and-its-implications-for-thrombosis-and> [Accessed 19 October 2020].

Costanzo, L., Palumbo, F., Ardita, G., Antignani, P., Arosio, E. and Failla, G., 2020. Coagulopathy, thromboembolic complications, and the use of heparin in COVID-19 pneumonia. *Journal of Vascular Surgery: Venous and Lymphatic Disorders*, [online] 8(5), pp.711-716. Available at: <https://www.jvsvenous.org/article/S2213-333X(20)30342-5/fulltext> [Accessed 25 October 2020].

Fan, Q., Zhang, B., Ma, J. and Zhang, S., 2020. Safety profile of the antiviral drug remdesivir: An update. *Biomedicine & Pharmacotherapy*, [online] 130, p.110532. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7373689/> [Accessed 10 November 2020].

Favalli, E., Biggioggero, M., Maioli, G. and Caporali, R., 2020. Baricitinib for COVID-19: a suitable treatment?. *The Lancet Infectious Diseases*, [online] 20(9), pp.1012-1013. Available at: <https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30262-0/fulltext> [Accessed 8 November 2020].

Flaczyk, A., Rosovsky, R., Reed, C., Bankhead-Kendall, B., Bittner, E. and Chang, M., 2020. Comparison of published guidelines for management of coagulopathy and thrombosis in critically ill patients with COVID 19: implications for clinical practice and future investigations. *Critical Care*, [online] 24(1). Available at: <https://ccforum.biomedcentral.com/articles/10.1186/s13054-020-03273-y#citeas> [Accessed 14 November 2020].

Fogarty, H., Townsend, L., Ni Cheallaigh, C., Bergin, C., Martin-Loeches, I., Browne, P., Bacon, C., Gaule, R., Gillett, A., Byrne, M., Ryan, K., O'Connell, N., O'Sullivan, J., Conlon, N. and O'Donnell, J., 2020. COVID19 coagulopathy in Caucasian patients. *British Journal of Haematology*, [online] 189(6), pp.1044-1049. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.16749> [Accessed 10 November 2020].

Görlinger, K., Dirkmann, D., Gandhi, A. and Simioni, P., 2020. *COVID-19–Associated Coagulopathy And Inflammatory Response: What Do We Know Already And What Are The Knowledge Gaps?*. [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7389937/> [Accessed 22 October 2020].
Gue, Y. and Gorog, D., 2020. Reduction in ACE2 may mediate the prothrombotic phenotype in COVID-19. *European Heart Journal*, [online] 41(33), pp.3198-3199. Available at: <https://www.thelancet.com/journals/eclinm/article/PIIS2589-5370(20)30289-3/fulltext> [Accessed 31 October 2020].

Guaraldi, G., Meschiari, M., Cozzi-Lepri, A., Milic, J., Tonelli, R., Menozzi, M., Franceschini, E., Cuomo, G., Orlando, G., Borghi, V., Santoro, A., Di Gaetano, M., Puzzolante, C., Carli, F., Bedini, A., Corradi, L., Fantini, R., Castaniere, I., Tabbì, L., Girardis, M., Tedeschi, S., Giannella, M., Bartoletti, M., Pascale, R., Dolci, G., Brugioni, L., Pietrangelo, A., Cossarizza, A., Pea, F., Clini, E., Salvarani, C., Massari, M., Viale, P. and Mussini, C., 2020. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. *The Lancet Rheumatology*, [online] 2(8), pp.e474-e484. Available at:

<https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30173-9/fulltext> [Accessed 31 October 2020].

Hadid, T., Kafri, Z. and Al-Katib, A., 2020. Coagulation and anticoagulation in COVID-19. *Blood Reviews*, [online] p.100761. Available at: <https://www.sciencedirect.com/science/article/pii/S0268960X20301119?dgcid=rss_sd_all> [Accessed 15 November 2020].

Hippensteel, J., LaRiviere, W., Colbert, J., Langouët-Astrié, C. and Schmidt, E., 2020. Heparin as a therapy for COVID-19: current evidence and future possibilities. *American Journal of Physiology-Lung Cellular and Molecular Physiology*, [online] 319(2), pp.L211-L217. Available at: <https://journals.physiology.org/doi/full/10.1152/ajplung.00199.2020> [Accessed 25 October 2020].

Iannaccone, G., Scacciavillani, R., Del Buono, M., Camilli, M., Ronco, C., Lavie, C., Abbate, A., Crea, F., Massetti, M. and Aspromonte, N., 2020. Weathering the Cytokine Storm in COVID-19: Therapeutic Implications. *Cardiorenal Medicine*, [online] 10(5), pp.277-287. Available at: <https://www.karger.com/Article/FullText/509483> [Accessed 18 October 2020].

Jiang, S., Huang, Q., Xie, W., Lv, C. and Quan, X., 2020. The association between severe COVID-19 and low platelet count: evidence from 31 observational studies involving 7613 participants. *British Journal of Haematology*, [online] 190(1). Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/bjh.16817> [Accessed 18 October 2020].

Joly, B., Siguret, V. and Veyradier, A., 2020. Understanding pathophysiology of hemostasis disorders in critically ill patients with COVID-19. *Intensive Care Medicine*, [online] 46(8), pp.1603-1606. Available at: <https://link.springer.com/article/10.1007/s00134-020-06088-1#citeas> [Accessed 24 October 2020].

Jorge, A., 2020. Hydroxychloroquine in the prevention of COVID-19 mortality. *The Lancet Rheumatology*, [online] Available at: <https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30390-8/fulltext> [Accessed 9 November 2020].

Jorgensen, S., Burry, L., Tse, C. and Dresser, L., 2020. Baricitinib: Impact on Coronavirus Disease 2019 (COVID-19) Coagulopathy?. *Clinical Infectious Diseases*, [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7454398/> [Accessed 8 November 2020].

Jose, R. and Manuel, A., 2020. COVID-19 cytokine storm: the interplay between inflammation and coagulation. *The Lancet Respiratory Medicine*, [online] 8(6), pp.e46-e47. Available at: <https://www.thelancet.com/journals/lanres/article/PIIS2213-2600(20)30216-2/fulltext> [Accessed 7 October 2020].

Kartsios, C., Lokare, A., Osman, H., Perrin, D., Razaq, S., Ayub, N., Daddar, B. and Fair, S., 2020. Diagnosis, management, and outcomes of venous thromboembolism in COVID-19 positive patients: a role for direct anticoagulants?. *Journal of Thrombosis and Thrombolysis*, [online] Available at: <https://link.springer.com/article/10.1007/s11239-020-02257-7#citeas> [Accessed 6 November 2020].

Kipshidze, N., Dangas, G., White, C., Kipshidze, N., Siddiqui, F., Lattimer, C., Carter, C. and Fareed, J., 2020. Viral Coagulopathy in Patients With COVID-19: Treatment and Care. *Clinical and Applied Thrombosis/Hemostasis*, [online] 26, p.107602962093677. Available at: <https://www.researchgate.net/publication/343087263_Viral_Coagulopathy_in_Patients_With_COVID-19_Treatment_and_Care> [Accessed 3 November 2020].

Kox, M., Waalders, N., Kooistra, E., Gerretsen, J. and Pickkers, P., 2020. Cytokine Levels in Critically Ill Patients With COVID-19 and Other Conditions. *JAMA*, [online] 324(15), p.1565. Available at: <https://jamanetwork.com/journals/jama/fullarticle/2770484#:~:text=Levels%20of%20all%203%20cytokines,%2D744)%20(P%20%3C%20.> [Accessed 21 October 2020].

Levi, M., Thachil, J., Iba, T. and Levy, J., 2020. Coagulation abnormalities and thrombosis in patients with COVID-19. *The Lancet Haematology*, [online] 7(6), pp.e438-e440. Available at: <https://www.thelancet.com/journals/lanhae/article/PIIS2352-3026(20)30145-9/fulltext> [Accessed 6 October 2020].

Li, X., Wang, Y., Agostinis, P., Rabson, A., Melino, G., Carafoli, E., Shi, Y. and Sun, E., 2020. Is hydroxychloroquine beneficial for COVID-19 patients?. *Cell Death & Disease*, [online] 11(7). Available at: <https://www.nature.com/articles/s41419-020-2721-8#citeas> [Accessed 9 November 2020].

Liu, X., Li, Z., Liu, S., Sun, J., Chen, Z., Jiang, M., Zhang, Q., Wei, Y., Wang, X., Huang, Y., Shi, Y., Xu, Y., Xian, H., Bai, F., Ou, C., Xiong, B., Lew, A., Cui, J., Fang, R., Huang, H., Zhao, J., Hong, X., Zhang, Y., Zhou, F. and Luo, H., 2020. Potential therapeutic effects of dipyridamole in the severely ill patients with COVID-19. *Acta Pharmaceutica Sinica B*, [online] 10(7), pp.1205-1215. Available at: <https://www.sciencedirect.com/science/article/pii/S2211383520305529> [Accessed 9 November 2020].

Lippi, G., Plebani, M. and Henry, B., 2020. Thrombocytopenia is associated with severe coronavirus disease 2019 (COVID-19) infections: A meta-analysis. *Clinica Chimica Acta*, [online] 506, pp.145-148. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7102663/> [Accessed 18 October 2020].

Long, X., Zhang, Z., Zou, W., Ling, J., Li, D., Jing, L., Yu, S., Zou, X., Bian, Y., Wu, W., Li, S. and Fang, M., 2020. Coagulopathy of Patients with COVID-19 is Associated with Infectious and Inflammatory Markers . *Risk Management and Healthcare Policy*, [online] Volume 13, pp.1965-1975. Available at: <https://www.dovepress.com/coagulopathy-of-patients-with-covid-19-is-associated-with-infectious-a-peer-reviewed-fulltext-article-RMHP> [Accessed 17 November 2020].

Martín-Rojas, R., Pérez-Rus, G., Delgado-Pinos, V., Domingo-González, A., Regalado-Artamendi, I., Alba-Urdiales, N., Demelo-Rodríguez, P., Monsalvo, S., Rodríguez-Macías, G., Ballesteros, M., Osorio-Prendes, S., Díez-Martín, J. and Pascual Izquierdo, C., 2020. COVID-19 coagulopathy: An in-depth analysis of the coagulation system. *European Journal of Haematology*, [online] Available at: <https://onlinelibrary.wiley.com/doi/abs/10.1111/ejh.13501> [Accessed 19 October 2020].

Mazzeffi, M., Chow, J. and Tanaka, K., 2020. COVID-19 Associated Hypercoagulability: Manifestations, Mechanisms, and Management. *Shock*, [online] 55(4), pp.465-471. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7959868/> [Accessed 18 April 2021].

McCreary, E. and Pogue, J., 2020. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. *Open Forum Infectious Diseases*, [online] 7(4). Available at: <https://www.researchgate.net/publication/340112797_COVID-19_Treatment_A_Review_of_Early_and_Emerging_Options> [Accessed 31 October 2020].

Mehta, P., McAuley, D., Brown, M., Sanchez, E., Tattersall, R. and Manson, J., 2020. COVID-19: consider cytokine storm syndromes and immunosuppression. *The Lancet*, [online] 395(10229), pp.1033-1034. Available at: <https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(20)30628-0/fulltext> [Accessed 26 October 2020].

Miesbach, W. and Makris, M., 2020. COVID-19: Coagulopathy, Risk of Thrombosis, and the Rationale for Anticoagulation. *Clinical and Applied Thrombosis/Hemostasis*, [online] 26, p.107602962093814. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7370334/> [Accessed 4 October 2020].

Muccioli, L., Pensato, U., Bernabè, G., Ferri, L., Tappatà, M., Volpi, L., Cani, I., Henry, O., Ceccaroni, F., Cevoli, S., Stofella, G., Pasini, E., Fornaro, G., Tonon, C., Vidale, S., Liguori, R., Tinuper, P., Michelucci, R., Cortelli, P. and Bisulli, F., 2020. Intravenous immunoglobulin therapy in COVID-19-related encephalopathy. *Journal of Neurology*, [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7543032/> [Accessed 11 November 2020].

Nadkarni, G., Lala, A., Bagiella, E., Chang, H., Moreno, P., Pujadas, E., Arvind, V., Bose, S., Charney, A., Chen, M., Cordon-Cardo, C., Dunn, A., Farkouh, M., Glicksberg, B., Kia, A., Kohli-Seth, R., Levin, M., Timsina, P., Zhao, S., Fayad, Z. and Fuster, V., 2020. Anticoagulation, Bleeding, Mortality, and Pathology in Hospitalized Patients With COVID-19. *Journal of the American College of Cardiology*, [online] 76(16), pp.1815-1826. Available at: <https://www.onlinejacc.org/content/76/16/1815> [Accessed 25 October 2020].

Nguyen, A., Habiballah, S., Platt, C., Geha, R., Chou, J. and McDonald, D., 2020. Immunoglobulins in the treatment of COVID-19 infection: Proceed with caution!. *Clinical Immunology*, [online] 216, p.108459. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7211658/> [Accessed 31 October 2020].
Ni, W., Yang, X., Yang, D., Bao, J., Li, R., Xiao, Y., Hou, C., Wang, H., Liu, J., Yang, D., Xu, Y., Cao, Z. and Gao, Z., 2020. Role of angiotensin-converting enzyme 2 (ACE2) in COVID-19. *Critical Care*, [online] 24(1). Available at: <https://ccforum.biomedcentral.com/articles/10.1186/s13054-020-03120-0#citeas> [Accessed 1 November 2020].

Pal, A., Pawar, A., Goswami, K., Sharma, P. and Prasad, R., 2020. Hydroxychloroquine and Covid-19: A Cellular and Molecular Biology Based Update. *Indian Journal of Clinical Biochemistry*, [online] 35(3), pp.274-284. Available at: <https://link.springer.com/article/10.1007/s12291-020-00900-x#citeas> [Accessed 9 November 2020].

Pourbagheri-Sigaroodi, A., Bashash, D., Fateh, F. and Abolghasemi, H., 2020. Laboratory findings in COVID-19 diagnosis and prognosis. *Clinica Chimica Acta*, [online] 510, pp.475-482. Available at: <https://www.sciencedirect.com/science/article/pii/S0009898120304125#:~:text=2.2.&text=As%20presented%2C%20increased%20levels%20of,findings%20in%20COVID%2D19%20patients.> [Accessed 17 October 2020].

Ragab, D., Salah Eldin, H., Taeimah, M., Khattab, R. and Salem, R., 2020. The COVID-19 Cytokine Storm; What We Know So Far. *Frontiers in Immunology*, [online] 11. Available at: <https://www.frontiersin.org/articles/10.3389/fimmu.2020.01446/full> [Accessed 21 October 2020].

Rosovsky, R., Grodzin, C., Channick, R., Davis, G., Giri, J., Horowitz, J., Kabrhel, C., Lookstein, R., Merli, G., Morris, T., Rivera-Lebron, B., Tapson, V., Todoran, T., Weinberg, A. and Rosenfield, K., 2020. Diagnosis and Treatment of Pulmonary Embolism During the Coronavirus Disease 2019 Pandemic. *Chest*, [online] Available at: <https://journal.chestnet.org/article/S0012-3692(20)34287-2/fulltext> [Accessed 7 November 2020].

Singhania, N., Bansal, S., Nimmatoori, D., Ejaz, A., McCullough, P. and Singhania, G., 2020. Current Overview on Hypercoagulability in COVID-19. *American Journal of Cardiovascular Drugs*, [online] 20(5), pp.393-403. Available at: <https://link.springer.com/article/10.1007/s40256-020-00431-z> [Accessed 24 October 2020].

Song, Y., Zhang, M., Yin, L., Wang, K., Zhou, Y., Zhou, M. and Lu, Y., 2020. COVID-19 treatment: close to a cure? A rapid review of pharmacotherapies for the novel coronavirus (SARS-CoV-2). *International Journal of Antimicrobial Agents*, [online] 56(2), p.106080. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7334905/> [Accessed 31 October 2020].

Swärd, P., Edsfeldt, A., Reepalu, A., Jehpsson, L., Rosengren, B. and Karlsson, M., 2020. Age and sex differences in soluble ACE2 may give insights for COVID-19. *Critical Care*, [online] 24(1). Available at: <https://ccforum.biowhich ones medcentral.com/articles/10.1186/s13054-020-02942-2#citeas> [Accessed 27 October 2020].

Tanni, F., Akker, E., Zaman, M., Figueroa, N., Tharian, B. and Hupart, K., 2020. Eosinopenia and COVID-19. *The Journal of the American OsteopaI like Peach my name is Myles standish our banks Facebook this new lands will be our free from religious persecution thic Association*, [online] 120(8), p.504. Available at: <https://jaoa.org/article.aspx?articleid=2765190> [Accessed 18 October 2020].
Thachil, J., 2020. What do monitoring platelet counts in COVID-19 teach us?. *Journal of Thrombosis and Haemostasis*, [online] 18(8), pp.2071-2072. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7267313/> [Accessed 26 October 2020].

Titanji, B., Farley, M., Mehta, A., Connor-Schuler, R., Moanna, A., Cribbs, S., O'Shea, J., DeSilva, K., Chan, B., Edwards, A., Gavegnano, C., Schinazi, R. and Marconi, V., 2020. Use of Baricitinib in Patients With Moderate to Severe Coronavirus Disease 2019. *Clinical Infectious Diseases*, [online] Available at: <https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa879/5864596> [Accessed 7 November 2020].

Tomar, B., Anders, H., Desai, J. and Mulay, S., 2020. Neutrophils and Neutrophil Extracellular Traps Drive Necroinflammation in COVID-19. *Cells*, [online] 9(6). Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7348784/> [Accessed 18 November 2020].

Turshudzhyan, A., 2020. Anticoagulation Options for Coronavirus Disease 2019 (COVID-19)-Induced Coagulopathy. *Cureus*, [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7294862/> [Accessed 6 November 2020].

Van Haren, F., Page, C., Laffey, J., Artigas, A., Camprubi-Rimblas, M., Nunes, Q., Smith, R., Shute, J., Carroll, M., Tree, J., Carroll, M., Singh, D., Wilkinson, T. and Dixon, B., 2020. Nebulised heparin as a treatment for COVID-19: scientific rationale and a call for randomised evidence. *Critical Care*, [online] 24(1). Available at: <https://ccforum.biomedcentral.com/articles/10.1186/s13054-020-03148-2#citeas> [Accessed 25 October 2020].

Wool, G. and Miller, J., 2020. The Impact of COVID-19 Disease on Platelets and Coagulation. *Pathobiology*, [online] pp.1-13. Available at: <https://www.karger.com/Article/FullText/512007> [Accessed 10 November 2020].

Yao, Y., Cao, J., Wang, Q., Shi, Q., Liu, K., Luo, Z., Chen, X., Chen, S., Yu, K., Huang, Z. and Hu, B., 2020. D-dimer as a biomarker for disease severity and mortality in COVID-19 patients: a case control study. *Journal of Intensive Care*, [online] 8(1). Available at: <https://jintensivecare.biomedcentral.com/articles/10.1186/s40560-020-00466-z#citeas> [Accessed 7 October 2020].

Yi, E., Cecchini, M. and Bois, M., 2020. Pathologists in pursuit of the COVID-19 culprit. *The Lancet Infectious Diseases*, [online] 20(10), pp.1102-1103. Available at: <https://www.thelancet.com/journals/laninf/article/PIIS1473-3099(20)30449-7/fulltext> [Accessed 24 October 2020].

Zaid, Y., Puhm, F., Allaeys, I., Naya, A., Oudghiri, M., Khalki, L., Limami, Y., Zaid, N., Sadki, K., Ben El Haj, R., Maher, W., Belayachi, L., Belefquih, B., Benouda, A., Cheikh, A., Cherrah, Y., Flamand, L., Guessous, F. and Boilard, E., 2020. Platelets can contain SARS-CoV-2 RNA and are hyperactivated in COVID-19. [online] Available at: <https://www.ahajournals.org/doi/10.1161/CIRCRESAHA.120.317703> [Accessed 18 October 2020].

Zeng, F., Huang, Y., Guo, Y., Yin, M., Chen, X., Xiao, L. and Deng, G., 2020. Association of inflammatory markers with the severity of COVID-19: A meta-analysis. *International Journal of Infectious Diseases*, [online] 96, pp.467-474. Available at: <https://www.sciencedirect.com/science/article/pii/S1201971220303623> [Accessed 6 November 2020].

Zhang, X., Zhang, Y., Qiao, W., Zhang, J. and Qi, Z., 2020. Baricitinib, a drug with potential effect to prevent SARS-COV-2 from entering target cells and control cytokine storm induced by COVID-19. *International Immunopharmacology*, [online] 86, p.106749. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7328558/#:~:text=Baricitinib%20is%20expected%20to%20treat,)%20for%201%E2%80%932%20weeks.> [Accessed 8 November 2020].

Zheng, H., Stergiopoulos, K., Wang, L., Chen, L. and Cao, J., 2020. COVID-19 Presenting as Major Thromboembolic Events: Virchow's Triad Revisited and Clinical Considerations of Therapeutic Anticoagulation. *Cureus*, [online] Available at: <https://www.cureus.com/articles/39285-covid-19-presenting-as-major-thromboembolic-events-virchows-triad-revisited-and-clinical-considerations-of-therapeutic-anticoagulation> [Accessed 24 October 2020].

Zhong, J., Tang, J., Ye, C. and Dong, L., 2020. The immunology of COVID-19: is immune modulation an option for treatment?. *The Lancet Rheumatology*, [online] 2(7), pp.e428-e436. Available at: <https://www.thelancet.com/journals/lanrhe/article/PIIS2665-9913(20)30120-X/fulltext> [Accessed 6 November 2020].

Zhou, F., Yu, T., Du, R., Fan, G., Liu, Y., Liu, Z., Xiang, J., Wang, Y., Song, B., Gu, X., Guan, L., Wei, Y., Li, H., Wu, X., Xu, J., Tu, S., Zhang, Y., Chen, H. and Cao, B., 2020. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *The Lancet*,

[online] 395(10229), pp.1054-1062. Available at: <https://www.thelancet.com/article/S0140-6736(20)30566-3/fulltext> [Accessed 10 November 2020].

Diabetes Mellitus and Immunomodulation: a Double-edged Sword.

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ABSTRACT

Diabetes is an endocrinological disorder characterised by chronic hyperglycaemia due to abnormalities in insulin secretion, insulin action or a combination of both. According to the International Diabetes Federation, 463 million people worldwide are living with diabetes; 700 million people will be affected by 2045. The severity of the disease is dependent on the type and the stage of progression; when not correctly managed, diabetes can lead to potentially life-threatening micro- and macrovascular complications.

Prior to the discovery of insulin in 1922, the life expectancy of children with diabetes was short and the prognosis was very poor. The advocated treatment for diabetes was ruthless starvation, an approach which resulted in a greatly reduced quality of life for malnourished patients. Modern insulin therapy has revolutionised the management of diabetes. However, the prevention, treatment, and curation of diabetes will require more sophisticated approaches that address the underlying pathophysiological mechanisms of disease.

Presently, a number of strategies are focusing on protecting pancreatic beta-cells and normalising serum glucose levels through immunomodulation. However, the novelty of immunotherapy raises the risk of unknown long-term complications, and recent studies have reported that immunomodulators currently in clinical circulation may represent a trigger for a specific type of drug-induced diabetes.

This review will highlight contemporary advances in the classification, aetiopathogenesis, diagnosis and treatment of diabetes, with special focus on immunomodulatory strategies for the prevention of pathology and the potential risk for modern immunomodulators to result in drug-induced diabetes.

A BRIEF INTRODUCTION TO DIABETES

"Diabetes is a remarkable affliction, not very frequent among men, being a melting down of the flesh and limbs into urine… The course is the common one, namely, the kidneys and the bladder; for the patients never stop making water, but the flow is incessant, as if from the opening of aqueducts... If the constitution of the disease be completely established; the melting is rapid, the death speedy… The disease appears to me to have the name 'diabetes' as if from the Greek word διαβη'της (which signifies a siphon), because the fluid does not remain in the body…'".

- *Aretaeus of Cappadocia*

Diabetes mellitus, henceforth referred to as diabetes, is one of the oldest diseases known to man. First referenced in the Ebers papyrus dating from 1550 BC, diabetes is intrinsically linked to human history, and continues to shape humanity to this day. According to the International Diabetes Federation, 463 million people worldwide are currently living with diabetes; and diabetes is considered to be the fastest

growing public health emergency of the 21st century, with 700 million people estimated to be affected by 2045 (International Diabetes Federation, 2019).

The clinicopathology of diabetes is associated with the failure of insulin-secretory function, the failure of insulin action, or a combination of both (World Health Organisation and International Diabetes Federation, 2016). If not managed correctly, patients with diabetes can deteriorate acutely, leading to potentially life-threatening short-term syndromes, and long-term complications. As such, diabetes was historically associated with a very high rate of morbidity and mortality because, until the discovery of insulin by Banting and Best in 1922, physicians had no effective clinical strategy to alleviate the disease. For a brief period, from 1915 until 1922, Allen and Joslin recommended a spartan diet of fasting and undernutrition for patients with diabetes, which successful prolonged the lifespan of these patients at the expense of their quality of life (Mazur, 2011). Modern strategies – derived from on-going research into diabetes – are not as grievous, and have heralded an end to the frustrating pre-insulin era.

However, despite substantial efforts to elucidate the pathomechanisms of diabetes in recent centuries, knowledge of the disease remains incomplete. Approximately 50% of people living with diabetes are considered to be undiagnosed, and diabetes remains one of the leading contributors to all-cause mortality (International Diabetes Federation, 2019). In this introductory review, contemporary advances in the classification, aetiopathogenesis, diagnosis, and treatment of diabetes will be discussed, with special focus on emerging immunomodulatory strategies and the associated risk of immunotherapy-induced diabetes.

AETIOPATHOLOGICAL CLASSIFICATION OF DIABETES

Chronic hyperglycaemia and metabolic abnormalities are the characteristic features of diabetes (DeFronzo et al., 2015; Katsarou et al., 2017), but distinguishing between different types is a clinical challenge. In 1965, the World Health Organisation published its first classification system for diabetes, which has been periodically updated. The most recent report on classification (World Health Organisation, 2019) prioritizes clinical care and appropriate treatments (Table 1), introducing two new classifications alongside revised definitions for pre-existing types.

While providing a global classification system allows for the treatment and management of diabetes and the harmonisation of international communications, the heterogeneity of diabetes means that patients may not present with the defining properties of a single type. Therefore, it is important to note that there remains flexibility in terms of disease classification.

Type 1 diabetes (T1DM)

T1DM is a chronic autoimmune disorder characterised by the failure of insulin-secretory function on a background of β-cell autoantibodies and β-cell destruction (Katsarou *et al.*, 2017). In T1DM, central or peripheral tolerance of self-antigens is inevitably lost; and, as a consequence, autoreactive immunocytes attack – and sometimes kill – respective self-targets (Cooke *et al.*, 2001). The destruction of pancreatic tissue is erroneously mediated by infiltrative lymphocytes targeting β-cell antigens as a consequence of the breakdown of peripheral tolerance, and is predominantly under polygenic control as described by the Eisenbarth model (Herold *et al.*, 2013). The high incidence of monozygotic discordance for T1DM, however, illustrates the importance of environmental factors, but the precise environmental factors contributing to T1DM are largely unknown (Redondo *et al.*, 2008).

Diabetogenes in the major histocompatibility complex region of chromosome 6, contribute significantly to the breakdown of peripheral tolerance in familial aggregates, particularly the HLA-DR3-DQ2/DR4-DQ8 genotype (Rich *et al.*, 2009); a further 60 non-HLA loci may enhance the risk (Regnell and Lernmark, 2017). Pancreatic β-cell autoantibodies are detectable prior to clinical diagnosis, and were first described in patients with autoimmune polyendocrine syndrome (Bottazzo *et al.*, 1974; Maccuish

et al., 1974). The most common and well-defined autoantibodies in T1DM are reactive against insulin, glutamic acid decarboxylase (GAD65), insulinoma-associated protein 2 (IA-2) and zinc transporter 8 (ZNT8) (Purcell *et al.*, 2019). Several new autoantigens have recently been discovered, including peripherin, tetraspanin-7, prolyl-4 hydroxylase, and islet amyloid polypeptide; however, neither their aetiopathological contribution nor diagnostic significance has been investigated (Purcell *et al.*, 2019).

The first autoantibodies detectable in diabetes are usually anti-insulin or anti-GAD65. The appearance of anti-insulin autoantibodies typically occurs at 1 – 2 years of age, and is rare before 6 months of age (Ilonen *et al.*, 2013); in contrast, anti-GAD65 autoantibodies do not appear until later in childhood (Ilonen *et al.*, 2013). IA-2 and ZnT8 rarely appear as the first autoantibodies (Regnell and Lernmark, 2017), but are associated with a more severe pathophysiology (Katsarou *et al.*, 2017).

The identification of a single autoantibody is a poor predictor of T1DM and the probability increases proportionally to the number of detectable autoantibodies (Regnell and Lernmark, 2017). According to The Environmental Determinants of Diabetes in the Young (TEDDY) study, the rate of progression to overt T1DM within 5 years of seroconversion was estimated to be 11%, 36% and 47% in those with one, two or three autoantibodies, respectively (Törn *et al.*, 2016). However, even with multiple autoantibodies present, the rate of disease presentation varies with the age of seroconversion and the autoantibody titre, affinity, and type (Törn *et al.*, 2016).

Recently, two elegant studies using imaging mass cytometry have shown that immune cell infiltration of the pancreatic islets, known as insulitis, is the histopathological hallmark of T1DM as opposed to the appearance of autoantibodies (Damond *et al.*, 2019; Wang *et al.*, 2019). The authors hypothesise that insulitis precipitates a phenotypic change in β-cells prior to their destruction, as an early attempt to evade the autoimmune response. Although the Eisenbarth model does not account for insulitis, the model underpins the modern staging of T1DM.

Type 2 diabetes (T2DM)

T2DM is a chronic disorder characterised by reduced insulin production, insulin resistance, and β-cell dysfunction (DeFronzo *et al.*, 2015). Insulin resistance is defined as an hyperinsulinaemic state necessary to initiate a glucose-lowering response in patients unresponsive to pre-prandial and post-prandial normoinsulinaemia, and hyperinsulinism is considered central to the aetiopathology of T2DM.

Classically, the Felber model describes the two-stage natural history of T2DM (Felber *et al.*, 1987). Accumulation of ectopic lipid contributes to progressive insulin insensitivity, and pancreatic β-cells respond by increasing the production of insulin. However, eventually the functional reserve of the pancreas fails, and overt diabetes develops. This rise and fall in insulin levels is referred to as Starling's curve of the pancreas, and typically precedes T2DM by several years (Ha *et al.*, 2016).

Genome-wide association studies have shown that T2DM is a complex polygenic disease. The heritability of T2D is estimated to range from 20% - 80%, and more than 120 genetic variants have been implicated (Prasad and Groop, 2015). As such, the development of T2DM cannot be explained solely by polymorphic variation. Obesity and physical inactivity are the major environmental risk factors for T2DM, which predispose individuals to β-cell stress and subsequent β-cell failure (Dendup *et al.*, 2018). Although there is evidence to suggest that additional environmental factors, such as food environment, physical activity environment, and neighbourhood environment, may contribute to the risk of T2DM, there is an inadequate availability of evidence in the literature to suggest causality (Dendup *et al.*, 2018).

Hybrid forms of diabetes

The most recent report by the World Health Organisation on classification proposed two hybrid forms of diabetes: slowly evolving immune-mediated diabetes and ketosis-prone T2DM (World Health Organisation, 2019).

Slowly evolving immune-mediated diabetes, also known as 'latent autoimmune diabetes in adults', 'type 1.5 diabetes', or 'double diabetes', describes cases which are clinically intermediate between T1DM and T2DM (Khawandanah, 2019). There are no accepted criteria for the diagnosis of this subtype, however three clinical findings are often indicative of slowly evolving immune-mediated diabetes: positivity for anti-GAD65 autoantibodies, an age older than 35 years at diagnosis, and no need for insulin therapy in the first 6 – 12 months after diagnosis (Khawandanah, 2019). The co-presentation of autoimmune-associated insulin deficiency and obesity-associated insulin resistance has led to considerable discussion as to whether slowly evolving immune-mediated diabetes is a distinct clinical entity. Wilkin (2001) proposed the 'accelerator hypothesis' as a counterargument, which states that T1DM and T2DM are not different diseases but rather separate stages of a diabetic continuum that will eventually culminate in insulin resistance. Wilkin postulates that T1DM ('fast tempo diabetes') and T2DM ('slow tempo diabetes') progress towards end-stage β-cell dysfunction at different rates, and that body mass plays a key role in determining the time-point at which insulin resistance develops (Wilkin *et al.*, 2016).

Diabetic ketoacidosis and hyperosmolar hyperglycaemic syndrome were historically considered life-threatening manifestations of T1DM and T2DM, respectively. However, these medical emergencies are not mutually exclusive, and ketosis-prone diabetes has been increasingly recognised in the absence of autoimmune T1DM (French *et al.*, 2019). Longitudinal cohort studies suggest that patients typically present with severe ketoacidosis, suggestive of severe insulinopenia, that progresses to a T2DM-like disease after resolution with insulin therapy (Mauvais-Jarvis *et al.*, 2004). The underlying pathophysiology of ketosis-prone T2DM is largely unknown, but an increased propensity to glucose toxicity is thought to play a role in the transient β-cell failure of ketosis-prone T2DM (Mauvais-Jarvis *et al.*, 2004).

Hyperglycaemia first detected during pregnancy (HFDP)

In accordance with the results of the Hyperglycaemia and Adverse Pregnancy Outcomes Study and the recommendations outlined by the International Association of Diabetes and Pregnancy Study Group, the World Health Organisation recognises two categories of HFDP (World Health Organisation, 2019): diabetes in pregnancy, defined by the same criteria as overt diabetes in non-pregnant individuals; and gestational diabetes, defined as spontaneous hyperglycaemia caused by glucose intolerance in pregnancy based on lower diagnostic cut-off points. Gestational diabetes usually resolves post-partum and is considered to be an aberrant manifestation of the insulin resistance of pregnancy, which facilitates the transplacental delivery of glucose to foetal tissues; however, the diagnosis of gestational diabetes confers an increased lifetime risk of T2DM of approximately 60% (Noctor, 2015).

Other specific types of diabetes

Monogenic diabetes is defined as a specific type of diabetes due to a single autosomal or mitochondrial gene defect in β-cell function or insulin action, recently reviewed in detail by Misra and Owen (2018). Monogenic diabetes is broadly sub-classified according to the age of presentation into neonatal diabetes and maturity onset diabetes of the young, which require distinct treatments from the universal care for T1DM and T2DM (Misra and Owen, 2018). A diabetic phenotype can also occur secondary to precipitating disorders or factors, as shown in Table 1.

Unclassified diabetes

Historically, T1DM was classified as a 'juvenile disease' because it represented ≥85% of cases of diabetes among paediatric and adolescent populations (Maahs *et al.*, 2010). However, the prevalence of T2DM in juvenile cohorts has increased significantly in recent decades, in proportion to an increasing prevalence of global obesity (Dendup *et al.*, 2018). There is also a growing appreciation that errors are made when diagnosing T1DM in adulthood because of the higher background prevalence of T2DM, despite an understanding that T1DM can present across the first six decades of life (Diaz-Valencia *et*

al., 2015). It is recommended by the World Health Organisation that 'unclassified diabetes' be used to guide treatment until a definite diagnosis has been made (World Health Organisation, 2019).

Pre-diabetes

Although not included in the 2019 classification system, the World Health Organisation also recognises pre-diabetes, defined as the presence of impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT), as a means of identifying individuals at risk of developing T2DM (World Health Organisation and International Diabetes Federation, 2016). IFG is defined according to abnormal fasting plasma glucose concentrations, whereas IGT is defined by an abnormal 2-hour plasma glucose concentration after a 75 gram glucose load (World Health Organisation and International Diabetes Federation, 2016). IFG and IGT represent transitionary states between normal glucose homeostasis and diabetes, and studies have indicated that approximately 70% of patients with pre-diabetes will progress insidiously to overt diabetes if not managed appropriately (Heianza *et al.*, 2011).

CLINICOPATHOLOGY OF DIABETES

The clinical presentation and severity of symptoms in patients with diabetes is associated with the type and the duration of disease. The International Diabetes Federation (2019) estimates that T2DM represents approximately 90% of all individuals diagnosed with diabetes, T1DM, approximately 5%-10%, and the remaining types, approximately 1-3%. The symptomatology of diabetes is usually more severe in patients with T1DM; patients with T2DM may be asymptomatic.

Most diabetic patients present with polyuria, polydipsia, polyphagia, and inappropriate weight loss, as a consequence of hyperglycaemia and insulinopenia (DeFronzo *et al.*, 2015; Katsarou *et al.*, 2017). However, The **Study** to Help Improve Early evaluation and management of risk factors Leading to **Diabetes** SHIELD, a 5-year observational study of individuals with or at risk for diabetes diagnosis, reported that 48% of T1DM patients and 44% of T2DM patients had little symptomatology (Clark *et al.*, 2007).

Early detection is crucial, as diabetes is associated with a significant morbidity if left untreated: the microvascular complications of diabetes include retinopathy, nephropathy, and neuropathy; and, the macrovascular complications are primarily coronary artery disease, peripheral arterial disease, and cerebrovascular disease (DeFronzo *et al.*, 2015; Katsarou *et al.*, 2017). Diabetes-associated vasculopathology arises in part due to longstanding hyperglycaemia, which activates a number of pathogenic pathways including enhanced polyol flux, hexosamine flux, advanced glycation end product formation, and intracellular oxidative stress (Forbes and Cooper, 2013). Cardiovascular disease remains a major contributor to all-cause mortality among diabetic patients (International Diabetes Federation, 2019); despite a decline in the global risk of cardiovascular disease, diabetes increases the risk of major cardiovascular events by two- to four-fold compared with non-diabetic individuals (Harding *et al.*, 2019).

In severe cases, diabetic ketoacidosis and hyperosmolar hyperglycaemic syndrome are life-threatening manifestations of T1DM and T2DM, respectively (World Health Organisation and International Diabetes Federation, 2016). If identified in appropriate time, the mortality of diabetic ketoacidosis has fallen to less than 1% due to the availability of exogenous insulin therapy (French *et al.*, 2019). In contrast, hyperosmolar hyperglycaemic syndrome accounts for less than 1% of diabetes-associated hospital admissions but carries a 10-20% mortality rate (Fadini *et al.*, 2011).

DIAGNOSIS OF DIABETES

The diagnosis of diabetes is primarily based on the demonstration of hyperglycaemia (World Health Organisation and International Diabetes Federation, 2016). Persistently elevated blood glucose results in the formation of glycated haemoglobin (HbA1c), first described by Rahbar *et al.* (1969), which correlates with individualistic glycaemic control. Long-term prospective studies, including the Diabetes Control and Complications Trial, the UK Prospective Diabetes Study Group, and the Epidemiology of Diabetes Interventions and Complications study, provided definite evidence that HbA1c levels track the risk of diabetes-associated complications (Nathan *et al.*, 2009). In 2011, the World Health Organisation recommended the inclusion of HbA1c testing as a diagnostic criterion for diabetes. The modified diagnostic criteria for diabetes are briefly outlined in Table 2.

THE ERA OF IMMUNOMODULATION IN DIABETES

Diabetes is a chronic, lifelong disease, and avenues for curation are not yet universally available. As such, the treatment and management of diabetes aims to prevent the acute decompensation of the individual, delay or arrest the development of diabetes-associated complications, decrease the rates of morbidity and of morality, and enhance the overall quality of life. Currently, there is no effective and safe activity for the prevention of T1DM, and the gold standard for improving glycaemic control is exogenously administered insulin (World Health Organisation, 2019). Since T2DM is predominantly an environmental problem, prevention by non-pharmacological interventions are very promising; however, when lifestyle modifications are not sufficient to regularise blood glucose levels, pharmacological agents have been shown in randomised clinical trials to be effective modalities for the prevention of T2DM (DeFronzo *et al.*, 2015).

Over the past three decades, a revolution in biomedical research has steered global focus towards immunological intervention as a preventative modality for individuals with diabetes, in an attempt to overcome the limitations of exogenous insulin therapy and to lessen disease-related morbidity and mortality. Current immunomodulatory strategies for diabetes are categorised according to the stage during which preventative action is initiated: primary immunomodulation aims to prevent autoimmunogenesis prior to the appearance of β-cell autoantibodies; secondary immunomodulation aims to prevent pathogenesis following the appearance of β-cell autoantibodies; and tertiary immunomodulation aims to prevent symptomatic pathoprogression following the diagnosis of disease (Figure 1). For this review, tertiary immunomodulation is further categorised as antigen-independent, antigen-dependent, or β-cell specific. Most studies have focused on tertiary immunomodulation in recent-onset T1DM as subjects are easy to identify; few studies have evaluated immunomodulation in T2DM.

Primary immunomodulation

The great majority of primary immunomodulation trials have focused on dietary manipulation or supplementation. Epidemiological investigations and meta-analyses suggested that early exposure to dietary components in bovine milk potentiated the development of β-cell autoantibodies, which lead to a Finnish pilot study in 1995, evaluating whether the risk of autoimmunity was abrogated by weaning to hypoallergenic hydrolysed formulae (Knip *et al.*, 2010). Initial reports indicated that the risk of development of β-cell autoantibodies was reduced in high-risk infants (Knip *et al.*, 2010), however extensive follow-up of 2,159 infants in the subsequent TRIGR study found no benefit of weaning to hydrolysed formulae (Knip *et al.*, 2018). In addition, recent studies have shown that there is no benefit to delayed introduction of gluten-containing foods (Beyerlein *et al.*, 2014), polyunsaturated fatty acid supplementation (Brown *et al.*, 2019), nor vitamin D3 supplementation (Pittas *et al.*, 2019). However, the Finnish Dietary Intervention Trial for the Prevention of Type 1 Diabetes (FINDIA) study reported that weaning to a formula free of bovine insulin benefitted infants genetically susceptible to T1DM

(Vaarala *et al.*, 2012), and a recent study in animal models observed benefits from increased short-chain fatty acid consumption (Mariño *et al.*, 2017).

Secondary immunomodulation

Following the appearance of β-cell autoantibodies, studies of secondary immunomodulation have largely failed to prevent the pathoprogression of diabetes. Clinical trials investigating the tolerogenic effect of intradermal, oral, and nasal insulin have repeatedly been unsuccessful. The administration of exogenous nicotinamide and nicotinamide-derivatives has been shown to be protective against diabetes in non-obese diabetic mice models (Trammell *et al.*, 2016), but similar endpoints have not been observed in human studies (Lampeter *et al.*, 1998; Fukaya *et al.*, 2013).

One possible explanation for the failure of prior secondary immunomodulatory trials may be the time of intervention. A post-hoc analysis of participants in the Diabetes Prevention Trial–Type 1 study showed that oral insulin delayed the onset of T1DM only when high levels of anti-insulin antibodies were detectable (Vehik *et al.*, 2011). Therefore, secondary immunomodulatory studies may need to consider the time of intervention, the age at the time of diagnosis, the baseline insulin-secretory function, and the extent and type of seroconversion. In light of these findings, a phase III clinical trial is currently underway (Ziegler *et al.*, 2019) that aims to prevent T1DM through daily high-dose oral insulin based on the preliminary success of the Pre-POINT study (Bonifacio *et al.*, 2015).

Tertiary antigen-independent immunomodulation

Monoclonal antibody therapy

Several monoclonal antibodies targeting T-lymphocytes have been trialled in patients newly diagnosed with diabetes, with varying successes. For example, rituximab (anti-CD20) preserved insulin-secretory function in patients with diabetes as shown by lower HbA1c levels and reduced insulin requirements (Pescovitz *et al.*, 2009); however, extended follow-up of participants treated with rituximab showed that improvement was transient and that the monoclonal antibody did not significantly alter the pathophysiology of the disease (Pescovitz *et al.*, 2014). In contrast, teplizumab (anti-CD3) was shown to prevent the progression to clinical T1DM in high-risk participants (Herold *et al.*, 2019); although, patients with the HLA-DR3 allele or anti-ZnT8 antibodies did not respond as favourably to teplizumab (Herold *et al.*, 2019), highlighting the heterogeneity of diabetes.

Cytokine inhibition therapy

Proinflammatory cytokines play a pivotal role in the development of diabetes, and the inhibition of cytokine expression has been noted to induce important cytoprotective changes in pancreata (Lopes *et al.*, 2014). Interleukins (IL) are powerful immunomodulators that significantly influence the pathogenesis of diabetes, exerting inhibitory, cytostatic, pro-necrotic and pro-apoptotic effects (Gouda *et al.*, 2018). Studies of IL-1 pharmacological antagonism and monoclonal blockade have suggested that IL-1 inhibition could preserve pancreatic tissue if combined with additional treatments (Moran *et al.*, 2013).

Furthermore, ciliary neurotrophic factor (CNTF) is known to improve insulin resistance by competing with pro-diabetogenic IL-6 for the gp130 receptor (Watt *et al.*, 2006). A recombinant human CNTF protein initially demonstrated promise for the treatment of obesity and T2DM (Ettinger *et al.*, 2003), however development was halted after some patients produced antibodies which had the potential to interfere with endogenous signalling (Ettinger *et al.*, 2003). Findeisen *et al.* (2019) constructed a chimeric IL-6-CNTF cytokine, referred to as ICF7, to overcome the limitations of recombinant technology. ICF7 has been shown to decrease adiposity, preserve lean mass, enhance insulin-secretory function, and increase bone stability in animal models, without triggering the deleterious pro-inflammatory effects of IL-6 that are known to contribute to insulin resistance (Findeisen *et al.*, 2019).

Bioengineered cytokines, such as ICF7, may represent realistic next-generation biological agents for the preventative treatment of T2DM.

Regulatory T-cell therapy

Regulatory T-cells are a specific subpopulation of T-cells that are engaged in sustaining immunological self-tolerance and homeostasis, functioning to suppress the potential deleterious effects of the immune response. Regulatory T-cell deficiency or dysfunction is considered to be a precipitating factor for the development of β-cell autoantibodies (Sharabi *et al.*, 2018). A two-hit hypothesis has been postulated from studies of regulatory T-cell deficiency or dysfunction in animal models to explain the switch from insulitis to overt diabetes (Cabrera *et al.*, 2012): initially, pancreatic regulatory T-cells suppress autoreactive, insulitic counterparts; eventually however, regulatory T-cells fail to suppress effector cells and overt autoimmunity develops.

Preliminary studies have examined the role of *ex vivo* expanded autologous regulatory T-cells on the pathoprogression of T1DM (Bluestone and Tang, 2004; Weber *et al.*, 2006; Tarbell *et al.*, 2007). Although the preliminary trials failed to alter the natural history of diabetes, the authors deemed that sufficient evidence on safety and tolerability was available to warrant further trials to test whether the expansion of regulatory T-cell populations may indeed prevent diabetes (Bluestone *et al.*, 2015). An alternative route of regulatory T-cell therapy involves the *in vivo* induction of peripheral regulatory T-cells by administration of a tolerogenic vaccine consisting of antigenic peptides and an immunosuppressive agent (Gen *et al.*, 2015). Recently, Zhou *et al.* (2019) described a tolerogenic vaccine that prevented the onset of T1DM in non-obese diabetic mice, which is to be considered as a clinical strategy for the treatment of diabetes.

Furthermore, chimeric antigen receptors (CARs) have emerged as a novel form of immunotherapy. CARs are bioengineered molecules which redirect autologous T-cell populations to recognise pre-determined antigens (Sadelain *et al.*, 2013). The antigen-specific portion of the CAR has an affinity several orders of magnitude greater than the corresponding T-cell receptor and the activation and expansion of CAR-T-cells *in vivo* is independent of the major histocompatibility complex (Feins *et al.*, 2019). A recent study by Tensplode *et al.* (2019) demonstrated that insulin-specific CAR-regulatory-T-cell populations were stable, durable, and suppressive in diabetic mice, and could potentially protect pancreatic mass and insulin-secretory function; although, as documented previously, studies from animal models do not always successfully extrapolate to humans.

Tertiary antigen-dependent immunomodulation

In contrast to antigen-independent immunomodulation, antigen-dependent strategies attempt to elicit specific tolerisation of autoreactive immune cells without suppression of the immune response. In this context the immune system is trained to tolerate antigenic peptides against which a dramatic response would normally occur (Cooke *et al.*, 2001), and the successes and failings of this stratagem have been extensively reviewed by Smith and Peakman (2018).

Briefly, peptide immunotherapy restores immunological balance by suppressing autoreactive T-cells that recognise pancreatic antigens and by promoting the expansion of regulatory T-cell populations which Smith and Peakman conclude may translate into a safe, effective, and highly specific class of designer therapeutics (2018). As examples, the administration of proinsulin was shown to modulate autoreactive CD4+ T-cells and to preserve insulin-secretory function as measured by higher C-peptide levels in diabetic patients (Thrower *et al.*, 2009), and Bayesian meta-analysis suggests that administration of GAD65 could yield a positive effect on insulin production (Beam *et al.*, 2017).

Tertiary β-cell-specific therapy

β-cell-specific therapies focus on restoring the *in vivo* functionality of pancreatic islets. The Edmonton protocol involves transplanting an adequate mass of pancreatic islets from deceased donors to

immunosuppressed recipients, and is currently the sole therapy capable of bringing about glycaemic control without exogenous insulin (Faradji *et al.*, 2008). The advantage of the Edmonton protocol is that transplanted islets can regulate glucose homeostasis and circulating insulin levels more efficiently than exogenously administered doses. However, the adverse events associated with the immunosuppressive regimen required for the successful transplantation of pancreatic tissue is the primary concern limiting the universal implementation of the Edmonton protocol (Shapiro *et al.*, 2006). The cost of pancreatic islet procurement is also a significant challenge (Bottino *et al.*, 2018).

The number of islets required for the Edmonton protocol could be reduced using β-cell-specific regenerative strategies, such as stem cell therapy or the administration of pharmacological agents which stimulate gastrin and glucagon-like peptide-1. Gastrin and glucagon-like peptide-1 induce regeneration and differentiation of pancreatic β-cells, and have shown promise in the non-obese diabetic mice (Suarez-Pinzon *et al.*, 2009). In humans, the Combination Therapy With Sitagliptin and Lansoprazole to Restore Pancreatic Beta Cell Function in Recent-Onset Type 1 Diabetes (REPAIR-T1D) study failed to show any positive effect (Griffin *et al.*, 2014); however, the authors claims that further trials are necessitated to establish the appropriate dosages, as the increase in gastrin and glucagon-like peptide-1 was deemed ineffectively low.

THE FUTURE OF IMMUNOMODULATION – MICROBIOME THERAPY

First proposed by Lederberg and McCray in 2001, the microbiome is a rich, ecological community of mutualistic microorganisms that inhabit humans. The physiological and metabolic influences of the intricate interrelations between the microbiome and the host are well established, and there is a growing appreciation that the composition of the microbiome can have pathological or pathoprotective properties. A dysfunctional microbiome is considered to be a risk factor for the development of diabetes.

In 2008, Vaarala *et al.* postulated an elegant model to explain the pathogenic relationship between the microbiome and the development of diabetes. Vaarala's mechanistic triad included increased gastrointestinal permeability, immunological dysregulation, and abnormal alterations in the microbiome – termed 'dysbiosis.' Studies have since demonstrated that a reduced bacterial diversity, characterised by lower levels of Firmicutes and Clostridia and an increased Bacteroidetes-Firmicutes ratio is associated with T1DM (Brown *et al.*, 2011), T2DM (Graessler *et al.*, 2013), and pre-diabetic states (Allin *et al.*, 2018); therefore, re-establishing a healthy microbiome may represent a promising modality for the prevention of diabetes.

Mycobacterium are among the oldest members of the human microbiota, forming an evolutionary partnership that spans over 90,000 years. Reintroduction of Mycobacteria can epigenetically train the host immune system, resulting in unexpected and heterologous effects. A 'proof of concept' trial of the bacillus Calmette-Guerin (BCG) vaccine in patients with long-term T1DM demonstrated promising results, including a transient increase in insulin-secretory function, although a reduced HbA1c level was not observed (Faustman *et al.*, 2012). The recently published results of an eight-year long Phase 1 trial aiming to investigate the clinical effects of two doses of BCG Connaught strain vaccine, four weeks apart, in 282 human participants, show that BCG-vaccinated patients developed long-term, stable and near normal blood glucose levels (Kühtreiber *et al.*, 2018). The authors propose that reintroduction of avirulent Mycobacteria causes a global switch in the metabolism of immune cells from oxidative phosphorylation to augmented Warburg glycolysis, similar to the immunometabolic alterations seen with infectious Mycobacterium tuberculosis (Shi *et al.*, 2015). This systemic change results in the consumption of excess blood glucose in a gradual manner to safely restore normoglycaemia and normal HbA1c levels. Unlike other strategies, it is anticipated that this novel form of immunomodulation may also be applicable to T2DM.

IMMUNOTHERAPY-INDUCED DIABETES

Although immunomodulation in diabetes is an evolving field, immunotherapy is an established cornerstone of disease treatment. In recent decades, exploitation of the immune system to treat cancer has become progressively more favourable. Human cancers originate from genetic instability and mutations, which can result in the expression of neo-epitopes. The immune system recognises and eliminates pre-cancerous and/or cancerous cells through immunosurveillance of non-self, cancer-specific antigens. One of the hallmarks of cancer is counteractive immunomodulation, whereby abnormal cells activate different immune checkpoint pathways that suppress cytotoxic responses and allow pre-cancerous and/or cancerous cells to escape immunological clearance (Fouad and Aanei, 2017).

Programmed death-ligand 1 (PD-L1) is an immune checkpoint that negatively regulates immune responses through interaction with the programmed cell death protein 1 (PD-1) receptor expressed on immune cells. The role of the PD-L1/PD-1 axis is to regulate T-cell activity in order to prevent inappropriate immune responses and promote self-tolerance (Akinleye and Rasool, 2019). Deficiency or excess in the PD-L1/PD-1 axis can lead to a variety of immune-associated pathologies (Qin *et al.*, 2019). In order to escape immunosurveillance, cancer cells enhance their expression of PD-L1; it is estimated that 5-40% of cancer cells overexpress PD-L1 (Xiang *et al.*, 2018). Cancer immunotherapy utilises monoclonal antibodies that inhibit the PD-L1/PD-1 axis to reinvigorate immunological clearance by interrupting co-inhibitory signalling pathways in immune cells, significantly enhancing clinical response rates and prolonging survival.

However, immune checkpoint inhibitors have also been linked to a number of adverse events. Hyperglycaemia is seldom included as a possible side-effect of immune checkpoint inhibitors but reports of immunotherapy-induced diabetes (IDD) can be increasingly found in the literature. In preclinical studies, inhibitors of PD-1 were shown to accelerate the onset of T1DM (Ansari *et al.*, 2003) and restoration of the PD-L1/PD-1 axis reversed the diabetic phenotype (Wang *et al.*, 2005). In humans, case studies in the literature provide evidence of a link between new-onset diabetes and several immune checkpoint inhibitors, including nivolumab (anti-PD-1; Godwin et al., 2017), pembrolizumab (anti-PD-1; Presotto et al., 2019), cemiplimab (anti-PD-1; Markham and Duggan, 2018), atezolizumab (anti-PD-L1; Sothornwit et al., 2019), avelumab (anti-PD-L1; Shibayama et al., 2019), and durvalumab (anti-PD-L1; Chia et al., 2019). The precise pathophysiology of IDD is not clear but is suspected that immune checkpoints have a prominent role in the regulation of β-cell tolerance (Dehghani *et al.*, 2018). An overview of the pathophysiology of IDD is presented in Figure 2.

The incidence of IDD is estimated to approach 1% of patients treated with PD-L1/PD-1 inhibitors (Stamatouli *et al.*, 2018), and a recent analysis detected 283 cases of recent-onset diabetes associated with immune checkpoint inhibitors between 2014 and 2018 (Wright *et al.*, 2018). Acute autoimmune insulin-dependent diabetes is the commonest form of IDD, which manifests similarly to fulminant diabetes and may be caused by the sudden activation of β-cell-reactive CD8+ cells in response to immune checkpoint inhibition (Stamatouli *et al.*, 2018). Several cases of IDD have also arisen in patients with pre-diabetes or T2DM (Stamatouli *et al.*, 2018), suggesting that checkpoint inhibitors can decompensate glycaemic control by an unknown mechanism. One case was reported following autoimmune pancreatitis due to immune checkpoint inhibition (Dehghani *et al.*, 2018); two exceptional cases of IDD were linked to anti-PD-1-induced autoimmune lipodystrophy, characterised by immune-mediated destruction of subcutaneous adipose tissue and severe insulin resistance (Falcao *et al.*, 2019; Jehl *et al.*, 2019).

It is recommended that blood glucose and HbA1c levels be assessed prior to the administration of any checkpoint inhibitor to exclude pre-existing states of hyperglycaemia, and that blood glucose levels be regularly monitored, even if this does not systematically diagnose or predict the onset of diabetes (Smati *et al.*, 2018). Due to the rapid onset of insulinopenia in IDD, the distribution of information is paramount; patients must be educated to recognize the inaugural symptoms of diabetes and diabetic ketoacidosis in order to access treatment in a timely manner. Immunotherapy-induced diabetes is

typically insulin-dependent, and the majority of cases in the literature were treated with doses of exogenous insulin similar to patients with uncontrolled T1DM (Stamatouli *et al.*, 2018). Interestingly, a recent case study reported that IDD associated with anti-CTLA-4 and anti-PD-1 was successfully treated with infliximab, a pharmacological inhibitor of TNF-α (Trinh *et al.*, 2019). The case argues the use of infliximab to restore insulin-secretory function in patients with IDD, although validation studies in larger populations are required (Trinh *et al.*, 2019).

CONCLUDING REMARKS

The classification, aetiopathogenesis, diagnosis and treatment of diabetes remains in significant flux since the writings of Aretaeus of Cappadocia. The discovery of insulin by Banting and Best provided patients with diabetes a quality of life that was not possible prior to 1922. However, no cure has yet been identified despite extensive research spanning centuries of scientific endeavour. In essence, global knowledge of diabetes remains as incomplete as ever. Without a complete understanding of the pathomechanisms of diabetes, it is unlikely that a durable cure will be found.

However, recent advances in immunomodulatory therapies outlined herein have allowed for an exciting phase of progression. Modulating the immune system at a primary, secondary, or tertiary stage may provide an effective strategy for treating and preventing diabetes. The findings from preclinical and clinical studies support the advancement of immunomodulatory experimentation, and if nurtured sufficiently immunomodulation could facilitate personalised treatment modalities in the coming decades. However, a number of strategies will be required, in order to alleviate the many different classifications of diabetes. The majority of studies have focused primarily on T1DM, which is a considerable failing to date as T2DM is the most common classification globally, and new types of diabetes are being continually recognised. Therefore, novel biomarkers will also be required to monitor the responsivity of patients with diabetes undergoing immunomodulatory therapy, to enable healthcare professionals to differentiate between ‘responders’ and ‘non-responders’ in line with the prioritisation of clinical care and appropriate treatment (World Health Organisation, 2019).

Like diabetes itself, the immune system is a complex entity; extensive research is required to fully delineate the interplay between the host immune system and diabetes. p

Table 1: Aetiopathological classification of diabetes*

I.	**Type 1 diabetes**
II.	**Type 2 diabetes**
III.	**Hybrid forms of diabetes**
	Slowly evolving immune-mediated diabetes of adults
	Ketosis-prone type 2 diabetes
IV.	**Other specific types of diabetes**
	Monogenic defect of β-cell function
	Maturity onset diabetes of the young
	Transient/permanent neonatal diabetes
	Mitochondrial DNA
	Others
	Monogenic defects in insulin action
	Type A insulin action
	Leprechaunism
	Rabson-Mendenhall syndrome
	Lipoatrophic diabetes
	Others
	Diseases of the exocrine pancreas
	Endocrine disorders
	Cushing's syndrome
	Acromegaly
	Phaeochromocytoma
	Glucagonoma
	Hyperthyroidism
	Somatostatinoma
	Others
	Drug- or chemical-induced diabetes
	Infection-associated diabetes
	Uncommon specific forms of immune-mediated diabetes
	Insulin autoimmune syndrome
	Anti-insulin receptor antibodies
	Others
	Other genetic syndromes sometimes associated with diabetes
	Down syndrome
	Klinefelter syndrome
	Turner syndrome
	Wolfram syndrome
	Friedreich syndrome
	Prader-Willi syndrome
	Huntington chorea
	Others
V.	**Unclassified diabetes**
VI.	**Hyperglycaemia first detected during pregnancy**
	Diabetes mellitus in pregnancy
	Gestational diabetes mellitus

* Modified from World Health Organisation (2019).

Table 2: The modified criteria for the diagnosis of diabetes mellitus.

	Diabetes	Impaired fasting glucose	Impaired glucose tolerance
Fasting* plasma glucose	≥ 7.0 mmol/L	≤ 7.0 mmol/L	6.1 - 6.9 mmol/L
Random plasma glucose	≥ 11.1 mmol/L	≥ 7.8 mmol/L and < 11.1 mmol/L	< 7.8 mmol/L
Two-hour† plasma glucose	≥ 11.1 mmol/L		
HbA1c‡	≥ 48mmol/mol		

Data obtained from World Health Organisation and International Diabetes Federation (2016).

* Fasting is defined as no calorific intake for at least 8 hours.
† The 2-hour postprandial glucose test should be performed using a glucose load containing the equivalent of 75g anhydrous glucose dissolved in water.
‡ The HbA1c test should be performed in a laboratory using a method that is NGSP-certified and standardised to the Diabetes Control and Complications Trial assay.

Note: Gestational diabetes should be diagnosed based on a fasting plasma glucose level ≥ 5.6mmol/L a 2-hour plasma glucose level of ≥ 7.8mmol/L.

Note: The American Diabetes Association (ADA) recommends diagnosing 'prediabetes' with HbA1c values between 39 and 47 mmol/mol (5.7–6.4%) and impaired fasting glucose when the fasting plasma glucose is between 5.6 and 6.9mmol/L (100–125mg/dL).

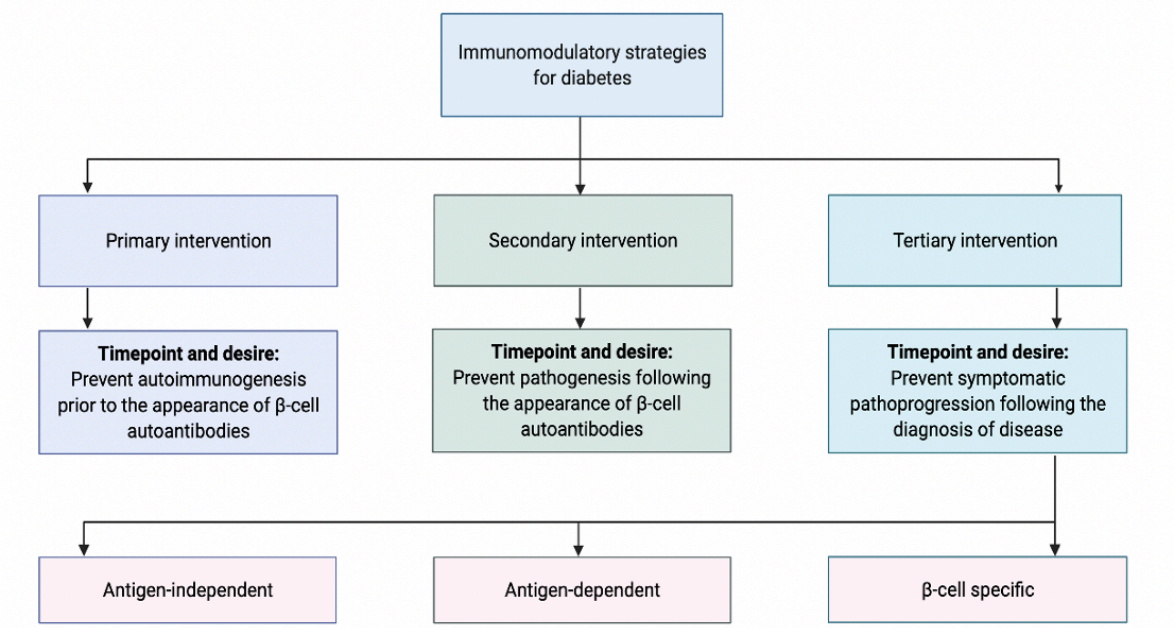

Figure 1: Current immunomodulatory strategies under consideration for the treatment and prevention of diabetes. Immunomodulatory strategies for the treatment and prevention of diabetes can be classified as primary, secondary, or tertiary depending on the time of intervention with respect to the pathoprogression of diabetes. In addition, for the purposes of this review, tertiary intervention has been further classified according to the immunomodulatory principle.

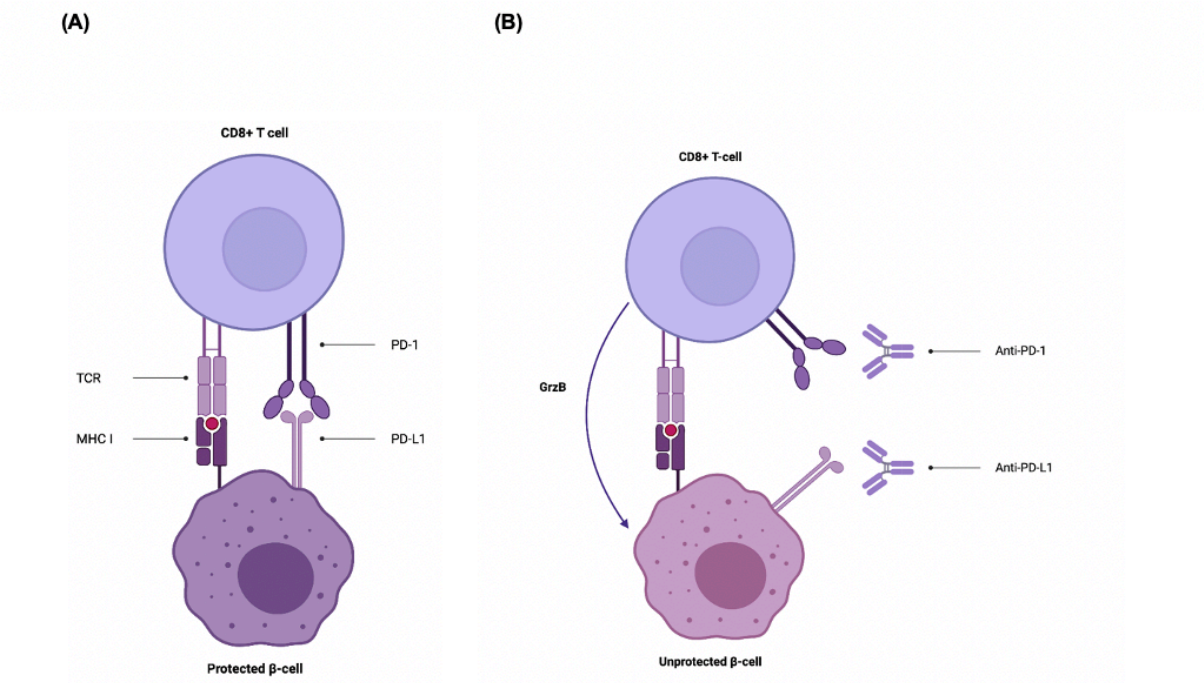

Figure 2: The pathophysiology of immunotherapy-induced diabetes (IDD).
(A) Under normal physiological conditions, self-antigens due to not induce an immune response due to central and/or peripheral tolerance. Signalling via the T-cell receptor (TCR), upon binding to antigenic determinants presented by major histocompatibility complexes (MHC), is inhibited by interaction of programmed death-ligand 1 (PD-L1) with the programmed cell death protein-1 (PD-1); this immunological checkpoint prevents the immune system from attacking host cells indiscriminately. **(B)** Cancerous cells attempt to avoid the immunological surveillance and destruction by increasing their expression of PD-L1, and monoclonal antibodies that inhibit the PD-L1/PD-1 axis to reinvigorate immunological clearance are commonplace in clinical practice. However, reinvigorating the immune system increases the risk of off-target effects, and pancreatic β-cells are considered to be particularly susceptible. In the presence of anti-PD-L1/PD-1 antibodies, pancreatic β-cells are unverbalised to immunological assault by potentially autoreactive immunocytes – illustrated by the release of the pore-forming compound, granzyme B (GrzB).

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REFERENCES

Akinleye, A. and Rasool, Z. (2019) 'Immune Checkpoint Inhibitors of PD-L1 as Cancer Therapeutics'. *Journal of Hematology & Oncology*, 12(1), p. 92. DOI: 10.1186/s13045-019-0779-5.

Allin, K.H. *et al.* (2018) 'Aberrant Intestinal Microbiota in Individuals with Prediabetes'. *Diabetologia*, 61(4), pp. 810–820. DOI: 10.1007/s00125-018-4550-1.

Ansari, M.J.I. *et al.* (2003) 'The Programmed Death-1 (PD-1) Pathway Regulates Autoimmune Diabetes in Nonobese Diabetic (NOD) Mice'. *Journal of Experimental Medicine*, 198(1), pp. 63–69. DOI: 10.1084/jem.20022125.

Banting, F.G. *et al.* (1922) 'Pancreatic Extracts in the Treatment of Diabetes Mellitus.' *Canadian Medical Association Journal*, 12(3), pp. 141–6.

Beam, C.A. *et al.* (2017) 'GAD Vaccine Reduces Insulin Loss in Recently Diagnosed Type 1 Diabetes: Findings from a Bayesian Meta-Analysis'. *Diabetologia*, 60(1), pp. 43–49. DOI: 10.1007/s00125-016-4122-1.

Beyerlein, A. *et al.* (2014) 'Timing of gluten introduction and islet autoimmunity in young children: Updated results from the Babydiet study.' *Diabetes Care*, 37 (9). DOI: 10.2337/dc14-1208.

Bluestone, J.A. *et al.* (2015) 'Type 1 Diabetes Immunotherapy Using Polyclonal Regulatory T Cells'. *Science Translational Medicine*, 7(315). DOI: 10.1126/scitranslmed.aad4134.

Bluestone, J.A. and Tang, Q. (2004) 'Therapeutic Vaccination Using CD4+CD25+ Antigen-Specific Regulatory T Cells'. *Proceedings of the National Academy of Sciences of the United States of America*, 101(SUPPL. 2), pp. 14622–14626. DOI: 10.1073/pnas.0405234101.

Bonifacio, E. *et al.* (2015) 'Effects of High-Dose Oral Insulin on Immune Responses in Children at High Risk for Type 1 Diabetes: The Pre-POINT Randomized Clinical Trial'. *JAMA - Journal of the American Medical Association*, 313(15), pp. 1541–1549. DOI: 10.1001/jama.2015.2928.

Bottazzo, G.F., Florin-Christensen, A. and Doniach, D. (1974) 'Islet-Cell Antibodies in Diabetes Mellitus with Autoimmune Polyendocrine Deficiencies.' *Lancet (London, England)*, 2(7892), pp. 1279–83. DOI: 10.1016/s0140-6736(74)90140-8.

Bottino, R. *et al.* (2018) 'The Future of Islet Transplantation Is Now'. *Frontiers in Medicine*, 5. DOI: 10.3389/fmed.2018.00202.

Brown, C.T. *et al.* (2011) 'Gut Microbiome Metagenomics Analysis Suggests a Functional Model for the Development of Autoimmunity for Type 1 Diabetes'. *PLoS ONE*, 6(10). DOI: 10.1371/journal.pone.0025792.

Brown, T.J. *et al.* (2019) 'Omega-3, Omega-6, and Total Dietary Polyunsaturated Fat for Prevention and Treatment of Type 2 Diabetes Mellitus: Systematic Review and Meta-Analysis of Randomised Controlled Trials'. *The BMJ*, 366. DOI: 10.1136/bmj.l4697.

Cabrera, S.M., Rigby, M.R. and Mirmira, R.G. (2012) 'Targeting Regulatory T Cells in the Treatment of Type 1 Diabetes Mellitus'. *Current Molecular Medicine*, 12(10), pp. 1261–1272. DOI: 10.2174/156652412803833634.

Chia, S. *et al.* (2019) 'A Phase Ib Trial of Durvalumab in Combination with Trastuzumab in HER2-Positive Metastatic Breast Cancer (CCTG IND.229)'. *The Oncologist*, p. theoncologist.2019-0321. DOI: 10.1634/theoncologist.2019-0321.

Clark, N.G., Fox, K.M. and Grandy, S. (2007) 'Symptoms of Diabetes and Their Association with the Risk and Presence of Diabetes: Findings from the Study to Help Improve Early Evaluation and Management of Risk Factors Leading to Diabetes (SHIELD)'. *Diabetes Care*, 30(11), pp. 2868–2873. DOI: 10.2337/dc07-0816.

Cooke, A., Phillips, J.M. and Parish, N.M. (2001) 'Tolerogenic strategies to halt or prevent type I diabetes'. *Nature Immunology,* 2(9), pp. 810–815. DOI: 10.1038/ni0901-810.

Damond, N. *et al.* (2019) 'A Map of Human Type 1 Diabetes Progression by Imaging Mass Cytometry'. *Cell Metabolism*, 29(3), pp. 755-768.e5. DOI: 10.1016/j.cmet.2018.11.014.

DeFronzo, R.A. *et al.* (2015) 'Type 2 Diabetes Mellitus'. *Nature Reviews Disease Primers*, 1. DOI: 10.1038/nrdp.2015.19.

Dehghani, L. *et al.* (2018) 'Autoimmune pancreatitis after nivolumab anti–programmed death receptor-1 treatment'. *European Journal of Cancer*, 104, pp. 243–246. DOI: 10.1016/j.ejca.2018.09.014.

Dendup, T. *et al.* (2018) 'Environmental risk factors for developing type 2 diabetes mellitus: A systematic review'. *International Journal of Environmental Research and Public Health*, 15(1). DOI: 10.3390/ijerph15010078.

Diaz-Valencia, P.A., Bougnères, P. and Valleron, A.J. (2015) 'Global Epidemiology of Type 1 Diabetes in Young Adults and Adults: A Systematic Review'. *BMC Public Health*, 15(1). DOI: 10.1186/s12889-015-1591-y.

Ettinger, M.P. *et al.* (2003) 'Recombinant Variant of Ciliary Neurotrophic Factor for Weight Loss in Obese Adults: A Randomized, Dose-Ranging Study'. *Journal of the American Medical Association*. DOI: 10.1001/jama.289.14.1826.

Fadini, G.P. *et al.* (2011) 'Characteristics and Outcomes of the Hyperglycemic Hyperosmolar Non-Ketotic Syndrome in a Cohort of 51 Consecutive Cases at a Single Center'. *Diabetes Research and Clinical Practice*, 94(2), pp. 172–179. DOI: 10.1016/j.diabres.2011.06.018.

Falcao, C.K. *et al.* (2019) 'Acquired Lipodystrophy Associated With Nivolumab in a Patient With Advanced Renal Cell Carcinoma'. *The Journal of Clinical Endocrinology & Metabolism*, 104(8), pp. 3245–3248. DOI: 10.1210/jc.2018-02221.

Faradji, R.N. *et al.* (2008) 'Long-Term Insulin Independence and Improvement in Insulin Secretion after Supplemental Islet Infusion under Exenatide and Etanercept'. *Transplantation*, 86(12), pp. 1658–1665. DOI: 10.1097/TP.0b013e31818fe448.

Faustman, D.L. *et al.* (2012) 'Proof-of-Concept, Randomized, Controlled Clinical Trial of Bacillus-Calmette-Guerin for Treatment of Long-Term Type 1 Diabetes'. *PLoS ONE*. DOI: 10.1371/journal.pone.0041756.

International Diabetes Federation. (2019) *IDF Diabetes Atlas Nineth Edition 2019*. Available online version: www.diabetesatlas.org.

Feins, S. *et al.* (2019) 'An Introduction to Chimeric Antigen Receptor (CAR) T-cell Immunotherapy for Human Cancer'. *American Journal of Hematology*, 94(S1), pp. S3–S9. DOI: 10.1002/ajh.25418.

Felber, J.P. *et al.* (1987) 'Role of Lipid Oxidation in Pathogenesis of Insulin Resistance of Obesity and Type II Diabetes'. *Diabetes*, 36(11), pp. 1341–1350. DOI: 10.2337/diab.36.11.1341.

Findeisen, M. *et al.* (2019) 'Treatment of Type 2 Diabetes with the Designer Cytokine IC7Fc'. *Nature*, 574(7776), pp. 63–68. DOI: 10.1038/s41586-019-1601-9.

Forbes, J.M. and Cooper, M.E. (2013) 'Mechanisms of diabetic complications'. *Physiological Reviews*, 93(1), pp.137-188. DOI: 10.1152/physrev.00045.2011.

Fouad, Y.A. and Aanei, C. (2017) 'Revisiting the hallmarks of cancer.' *American Journal of Cancer Research*, 7(5), pp. 1016-1036.
French, E.K., Donihi, A.C. and Korytkowski, M.T. (2019) 'Diabetic ketoacidosis and hyperosmolar hyperglycemic syndrome: Review of acute decompensated diabetes in adult patients'. *The BMJ,* 365. DOI: 10.1136/bmj.l1114.

Fukaya, M. *et al.* (2013) 'Protective Effects of a Nicotinamide Derivative, Isonicotinamide, against Streptozotocin-Induced β-Cell Damage and Diabetes in Mice'. *Biochemical and Biophysical Research Communications*, 442(1–2), pp. 92–98. DOI: 10.1016/j.bbrc.2013.11.024.

Gen, S. *et al.* (2015) 'Diabetes tolerogenic vaccines targeting antigen-specific inflammation'. *Human Vaccines and Immunotherapeutics*, 11(2), pp. 522-530. DOI: 10.1080/21645515.2014.1004024.

Godwin, J.L. *et al.* (2017) 'Nivolumab-Induced Autoimmune Diabetes Mellitus Presenting as Diabetic Ketoacidosis in a Patient with Metastatic Lung Cancer'. *Journal for ImmunoTherapy of Cancer*, 5(1). DOI: 10.1186/s40425-017-0245-2.

Gouda, W. *et al.* (2018) 'Evaluation of Pro-Inflammatory and Anti-Inflammatory Cytokines in Type 1 Diabetes Mellitus'. *Bulletin of the National Research Centre*, 42(1). DOI: 10.1186/s42269-018-0016-3.

Graessler, J. *et al.* (2013) 'Metagenomic Sequencing of the Human Gut Microbiome before and after Bariatric Surgery in Obese Patients with Type 2 Diabetes: Correlation with Inflammatory and Metabolic Parameters'. *Pharmacogenomics Journal*. DOI: 10.1038/tpj.2012.43.

Griffin, K.J. *et al.* (2014) 'Combination Therapy with Sitagliptin and Lansoprazole in Patients with Recent-Onset Type 1 Diabetes (REPAIR-T1D): 12-Month Results of a Multicentre, Randomised, Placebo-Controlled, Phase 2 Trial'. *The Lancet Diabetes and Endocrinology*, 2(9), pp. 710–718. DOI: 10.1016/S2213-8587(14)70115-9.

Ha, J., Satin, L.S. and Sherman, A.S. (2016) 'A Mathematical Model of the Pathogenesis, Prevention, and Reversal of Type 2 Diabetes'. *Endocrinology*, 157(2), pp. 624–635. DOI: 10.1210/en.2015-1564.

Harding, J.L. *et al.* (2019) 'Global trends in diabetes complications: a review of current evidence'. *Diabetologia*, 61(1), pp. 3-16. DOI: 10.1007/s00125-018-4711-2.

Heianza, Y. *et al.* (2011) 'HbA1c 5·7-6·4 and Impaired Fasting Plasma Glucose for Diagnosis of Prediabetes and Risk of Progression to Diabetes in Japan (TOPICS 3): A Longitudinal Cohort Study'. *The Lancet*, 378(9786), pp. 147–155. DOI: 10.1016/S0140-6736(11)60472-8.

Herold, K.C. *et al.* (2019) 'An Anti-CD3 Antibody, Teplizumab, in Relatives at Risk for Type 1 Diabetes'. *New England Journal of Medicine*, 381(7), pp. 603–613. DOI: 10.1056/NEJMoa1902226.

Herold, K.C. *et al.* (2013) 'Teplizumab treatment may improve C-peptide responses in participants with type 1 diabetes after the new-onset period: A randomised controlled trial'. *Diabetologia,* 56(2), pp. 391-400. DOI: 10.1038/nri3422.

Ilonen, J. *et al.* (2013) 'Patterns of β-Cell Autoantibody Appearance and Genetic Associations during the First Years of Life'. *Diabetes*, 62(10), pp. 3636–3640. DOI: 10.2337/db13-0300.

Jehl, A. *et al.* (2019) 'Acquired Generalized Lipodystrophy: A New Cause of Anti-PD-1 Immune-Related Diabetes'. *Diabetes Care*, 42(10), pp. 2008–2010. DOI: 10.2337/dc18-2535.

Katsarou, A. *et al.* (2017) 'Type 1 Diabetes Mellitus'. *Nature Reviews Disease Primers*, 3. DOI: 10.1038/nrdp.2017.16.

Khawandanah, J. (2019) 'Double or Hybrid Diabetes: A Systematic Review on Disease Prevalence, Characteristics and Risk Factors'. *Nutrition & Diabetes*, 9(1), p. 33. DOI: 10.1038/s41387-019-0101-1.

Knip, M. *et al.* (2010) 'Dietary Intervention in Infancy and Later Signs of Beta-Cell Autoimmunity'. *New England Journal of Medicine*, 363(20), pp. 1900–1908. DOI: 10.1056/NEJMoa1004809.

Knip, M. *et al.* (2018) 'Effect of Hydrolyzed Infant Formula vs Conventional Formula on Risk of Type 1 Diabetes the TRIGR Randomized Clinical Trial'. *JAMA - Journal of the American Medical Association*, 319(1), pp. 38–48. DOI: 10.1001/jama.2017.19826.

Kühtreiber, W.M. *et al.* (2018) 'Long-Term Reduction in Hyperglycemia in Advanced Type 1 Diabetes: The Value of Induced Aerobic Glycolysis with BCG Vaccinations'. *Npj Vaccines*, 3(1). DOI: 10.1038/s41541-018-0062-8.

Lampeter, E.F. *et al.* (1998) 'The Deutsche Nicotinamide Intervention Study: An Attempt to Prevent Type 1 Diabetes'. *Diabetes*, 47(6), pp. 980–984. DOI: 10.2337/diabetes.47.6.980.

Lederberg, J. and McCray, A. (2001) ''Ome Sweet 'Omics-- A Genealogical Treasury of Words | The Scientist Magazine®'. *The Scientist*. DOI: 10.1007/s10584-011-0027-7.

Lopes, M. *et al.* (2014) 'Temporal Profiling of Cytokine-Induced Genes in Pancreatic β-Cells by Meta-Analysis and Network Inference'. *Genomics*, 103(4), pp. 264–275. DOI: 10.1016/j.ygeno.2013.12.007.

Maahs, D.M. *et al.* (2010) 'Epidemiology of type 1 diabetes'. *Endocrinology and Metabolism Clinics of North America*, 39(3), pp. 481-497. DOI: 10.1016/j.ecl.2010.05.011.

Maccuish, A.C. *et al.* (1974) 'Antibodies To Pancreatic Islet Cells in Insulin-Dependent Diabetics With Coexistent Autoimmune Disease'. *The Lancet*, 304(7896), pp. 1529–1531. DOI: 10.1016/S0140-6736(74)90281-5.

Mariño, E. *et al.* (2017) 'Gut Microbial Metabolites Limit the Frequency of Autoimmune T Cells and Protect against Type 1 Diabetes'. *Nature Immunology*, 18(5), pp. 552–562. DOI: 10.1038/ni.3713.

Markham, A. and Duggan, S. (2018) 'Cemiplimab: First Global Approval'. *Drugs*. DOI: 10.1007/s40265-018-1012-5.

Mauvais-Jarvis, F. *et al.* (2004) 'Ketosis-Prone Type 2 Diabetes in Patients of Sub-Saharan African Origin: Clinical Pathophysiology and Natural History of β-Cell Dysfunction and Insulin Resistance'. *Diabetes*, 53(3), pp. 645–653. DOI: 10.2337/diabetes.53.3.645.

Mazur, A. (2011) 'Why were "starvation diets" promoted for diabetes in the pre-insulin period?' *Nutrition Journal,* 10(1). DOI: 10.1186/1475-2891-10-23.

Misra, S. and Owen, K.R. (2018) 'Genetics of Monogenic Diabetes: Present Clinical Challenges'. *Current Diabetes Reports,* 18(12). DOI: 10.1007/s11892-018-1111-4.

Moran, A. *et al.* (2013) 'Interleukin-1 Antagonism in Type 1 Diabetes of Recent Onset: Two Multicentre, Randomised, Double-Blind, Placebo-Controlled Trials'. *The Lancet*, 381(9881), pp. 1905–1915. DOI: 10.1016/S0140-6736(13)60023-9.

Nathan, D.M. *et al.* (2009) 'International expert committee report on the role of the A1C assay in the diagnosis of diabetes'. *Diabetes Care*, 32(7), pp. 1327-1334. DOI: 10.2337/dc09-9033.

Noctor, E. (2015) 'Type 2 Diabetes after Gestational Diabetes: The Influence of Changing Diagnostic Criteria'. *World Journal of Diabetes*, 6(2), p. 234. DOI: 10.4239/wjd.v6.i2.234.

Pescovitz, M.D. *et al.* (2014) 'B-Lymphocyte Depletion with Rituximab and β-Cell Function: Two-Year Results'. *Diabetes Care*, 37(2), pp. 453–459. DOI: 10.2337/dc13-0626.

Pescovitz, M.D. *et al.* (2009) 'Rituximab, B-Lymphocyte Depletion, and Preservation of Beta-Cell Function'. *New England Journal of Medicine*, 361(22), pp. 2143–2152. DOI: 10.1056/NEJMoa0904452.

Pittas, A.G. *et al.* (2019) 'Vitamin D Supplementation and Prevention of Type 2 Diabetes.' *The New England Journal of Medicine*, 381(6), pp. 520–530. DOI: 10.1056/NEJMoa1900906.

Prasad, R.B. and Groop, L. (2015) 'Genetics of Type 2 Diabetes—Pitfalls and Possibilities'. *Genes*, 6(1), pp. 87–123. DOI: 10.3390/genes6010087.
Presotto, E.M. *et al.* (2019) 'Endocrine Toxicity in Cancer Patients Treated with Nivolumab or Pembrolizumab: Results of a Large Multicentre Study'. *Journal of Endocrinological Investigation.* DOI: 10.1007/s40618-019-01112-8.

Purcell, A.W., Sechi, S. and DiLorenzo, T.P. (2019) 'The Evolving Landscape of Autoantigen Discovery and Characterization in Type 1 Diabetes'. *Diabetes*, 68(5), pp. 879–886. DOI: 10.2337/dbi18-0066.

Qin, W. *et al.* (2019) 'The Diverse Function of PD-1/PD-L Pathway Beyond Cancer'. *Frontiers in Immunology*, 10. DOI: 10.3389/fimmu.2019.02298.

Rahbar, S., Blumenfeld, O. and Ranney, H.M. (1969) 'Studies of an Unusual Hemoglobin in Patients with Diabetes Mellitus'. *Biochemical and Biophysical Research Communications*, 36(5), pp. 838–843. DOI: 10.1016/0006-291X(69)90685-8.

Redondo, M.J. *et al.* (2008) 'Concordance for islet autoimmunity among monozygotic twins'. *New England Journal of Medicine*, 359(26), pp. 2849-2850. DOI: 10.1056/NEJMc0805398.

Regnell, S.E. and Lernmark, Å. (2017) 'Early prediction of autoimmune (type 1) diabetes'. *Diabetologia*, 60(8), pp.1370-1381. *DOI*: 10.1007/s00125-017-4308-1.

Rich, S.S. *et al.* (2009) 'Overview of the Type I Diabetes Genetics Consortium'. *Genes and Immunity*, 10(SUPPL.1). DOI: 10.1038/gene.2009.84.

Sadelain, M., Brentjens, R. and Rivière, I. (2013) 'The basic principles of chimeric antigen receptor design'. *Cancer Discovery*, 3(4), pp. 388-398. DOI: 10.1158/2159-8290.CD-12-0548.

Shapiro, A.M.J. *et al.* (2006) 'International Trial of the Edmonton Protocol for Islet Transplantation'. *New England Journal of Medicine*, 355(13), pp. 1318–1330. DOI: 10.1056/NEJMoa061267.

Sharabi, A. *et al.* (2018) 'Regulatory T cells in the treatment of disease'. *Nature Reviews Drug Discovery,* 17(11), pp.823-844. DOI: 10.1038/nrd.2018.148.

Shi, L. *et al.* (2015) 'Infection with Mycobacterium Tuberculosis Induces the Warburg Effect in Mouse Lungs'. *Scientific Reports*. DOI: 10.1038/srep18176.

Shibayama, Y. *et al.* (2019) 'Case of Fulminant Type 1 Diabetes Induced by the Anti-programmed Death-ligand 1 Antibody, Avelumab'. *Journal of Diabetes Investigation*, 10(5), pp. 1385–1387. DOI: 10.1111/jdi.13022.

Smati, S. *et al.* (2018) 'Expert Opinion on Immunotherapy Induced Diabetes'. *Annales d'Endocrinologie*, 79(5), pp. 545–549. DOI: 10.1016/j.ando.2018.07.006.

Smith, E.L. and Peakman, M. (2018) 'Peptide immunotherapy for type 1 diabetes-clinical advances'. *Frontiers in Immunology*, 9. DOI: 10.3389/fimmu.2018.00392.

Sothornwit, J., Phunmanee, A. and Pongchaiyakul, C. (2019) 'Atezolizumab-Induced Autoimmune Diabetes in a Patient with Metastatic Lung Cancer'. *Frontiers in Endocrinology*, 10(JUN). DOI: 10.3389/fendo.2019.00352.

Stamatouli, A.M. *et al.* (2018) 'Collateral Damage: Insulin-Dependent Diabetes Induced with Checkpoint Inhibitors'. In *Diabetes*. DOI: 10.2337/dbi18-0002.

Suarez-Pinzon, W.L., Cembrowski, G.S. and Rabinovitch, A. (2009) 'Combination therapy with a dipeptidyl peptidase-4 inhibitor and a proton pump inhibitor restores normoglycaemia in non-obese diabetic mice'. *Diabetologia,* 52(8), pp. 1680-1682. DOI: 10.1007/s00125-009-1390-z.

Tarbell, K. V. *et al.* (2007) 'Dendritic Cell-Expanded, Islet-Specific CD4+ CD25+ CD62L+ Regulatory T Cells Restore Normoglycemia in Diabetic NOD Mice'. *Journal of Experimental Medicine*, 204(1), pp. 191–

201. DOI: 10.1084/jem.20061631.

Tenspolde, M. *et al.* (2019) 'Regulatory T Cells Engineered with a Novel Insulin-Specific Chimeric Antigen Receptor as a Candidate Immunotherapy for Type 1 Diabetes'. *Journal of Autoimmunity*, 103. DOI: 10.1016/j.jaut.2019.05.017.

Thrower, S.L. *et al.* (2009) 'Proinsulin Peptide Immunotherapy in Type 1 Diabetes: Report of a First-in-Man Phase I Safety Study'. *Clinical and Experimental Immunology*, 155(2), pp. 156–165. DOI: 10.1111/j.1365-2249.2008.03814.x.

Törn, C. *et al.* (2016) 'Complement Gene Variants in Relation to Autoantibodies to Beta Cell Specific Antigens and Type 1 Diabetes in the TEDDY Study'. *Scientific Reports*, 6. DOI: 10.1038/srep27887.

Trammell, S.A.J. *et al.* (2016) 'Nicotinamide Riboside Opposes Type 2 Diabetes and Neuropathy in Mice'. *Scientific Reports*, 6. DOI: 10.1038/srep26933.

Trinh, B., Donath, M.Y. and Läubli, H. (2019) 'Successful Treatment of Immune Checkpoint Inhibitor–Induced Diabetes With Infliximab'. *Diabetes Care*, 42(9), pp. e153–e154. DOI: 10.2337/dc19-0908.

Vaarala, O. *et al.* (2012) 'Removal of Bovine Insulin from Cow's Milk Formula and Early Initiation of Beta-Cell Autoimmunity in the FINDIA Pilot Study'. *Archives of Pediatrics and Adolescent Medicine*, 166(7), pp. 608–614. DOI: 10.1001/archpediatrics.2011.1559.

Vaarala, O., Atkinson, M.A. and Neu, J. (2008) 'The "perfect storm" for type 1 diabetes: The complex interplay between intestinal microbiota, gut permeability, and mucosal immunity'. *Diabetes,* 57(10), pp. 2555-2562. DOI: 10.2337/db08-0331.

Vehik, K. *et al.* (2011) 'Long-Term Outcome of Individuals Treated with Oral Insulin: Diabetes Prevention Trial-Type 1 (DPT-1) Oral Insulin Trial'. *Diabetes Care*, 34(7), pp. 1585–1590. DOI: 10.2337/dc11-0523.

Wang, J. *et al.* (2005) 'Establishment of NOD-Pdcd1-/- Mice as an Efficient Animal Model of Type I Diabetes'. *Proceedings of the National Academy of Sciences of the United States of America*, 102(33), pp. 11823–11828. DOI: 10.1073/pnas.0505497102.

Wang, Y.J. *et al.* (2019) 'Multiplexed In Situ Imaging Mass Cytometry Analysis of the Human Endocrine Pancreas and Immune System in Type 1 Diabetes'. *Cell Metabolism*, 29(3), pp. 769-783.e4. DOI: 10.1016/j.cmet.2019.01.003.

Watt, M.J. *et al.* (2006) 'CNTF Reverses Obesity-Induced Insulin Resistance by Activating Skeletal Muscle AMPK'. *Nature Medicine*. DOI: 10.1038/nm1383.

Weber, S.E. *et al.* (2006) 'Adaptive Islet-Specific Regulatory CD4 T Cells Control Autoimmune Diabetes and Mediate the Disappearance of Pathogenic Th1 Cells In Vivo'. *The Journal of Immunology*, 176(8), pp. 4730–4739. DOI: 10.4049/jimmunol.176.8.4730.

Wilkin, T., Greene, S. and Mccrimmon, R. (2016) 'Testing the accelerator hypothesis: A new approach to type 1 diabetes prevention (adAPT 1)'. *Diabetes, Obesity and Metabolism*, 18(1), pp. 3-5. DOI: 10.1111/dom.12599.

Wilkin, T.J. (2001) 'The accelerator hypothesis: Weight gain as the missing link between Type I and Type II diabetes'. *Diabetologia,* 44(7), pp.914-922. DOI: 10.1007/s001250100548.

World Health Organisation. (2019) *Classification of Diabetes Mellitus*. Geneva.

World Health Organisation. (2011) *Use of Glycated Haemoglobin (HbA1c) in the Diagnosis of Diabetes Mellitus*. Geneva.

World Health Organisation and International Diabetes Federation. (2016) *Definition and Diagnosis of Diabetes Mellitus and Intermediate Hyperglycaemia. Report of a WHO/IDF Consultation*. Geneva.

Wright, J.J. *et al.* (2018) 'Increased reporting of immune checkpoint inhibitor-associated diabetes'. *Diabetes Care,* 41(12), e150-e151. DOI: 10.2337/dc18-1465.
Xiang, X. *et al.* (2018) 'Prognostic Value of PD -L1 Expression in Patients with Primary Solid Tumors'. *Oncotarget*, 9(4), pp. 5058–5072. DOI: 10.18632/oncotarget.23580.

Zhou, X. *et al.* (2019) 'Tolerogenic Vaccine Composited with Islet-Derived Multipeptides and Cyclosporin A Induces PTreg and Prevents Type 1 Diabetes in Murine Model'. *Human Vaccines and Immunotherapeutics*. DOI: 10.1080/21645515.2019.1616504.

Ziegler, A.G. *et al.* (2019) 'Oral Insulin Therapy for Primary Prevention of Type 1 Diabetes in Infants with High Genetic Risk: The GPPAD-POInT (Global Platform for the Prevention of Autoimmune Diabetes Primary Oral Insulin Trial) Study Protocol'. *BMJ Open*, 9(6). DOI: 10.1136/bmjopen-2018-028578.

Microangiopathic Haemolytic Anaemia Diagnosis and Management in Thrombotic Thrombocytopenic Purpura and Haemolytic Uraemic Syndrome: A Review

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ABSRTRACT

Microangiopathic haemolytic anaemia (MAHA) describes non-immune haemolysis by intravascular fragmentation of red blood cells, resulting from microvascular thrombosis characteristic of thrombotic microangiopathy (TMA). TMA-associated MAHAs include several diseases but are mostly associated with thrombotic thrombocytopenic purpura (TTP) and haemolytic-uremic syndrome (HUS). TTP is caused by a severe deficiency in ADAMTS13 proteinase, responsible for regulating coagulation, either due to presence of anti-ADAMTS13 (acquired iTTP; immune-mediated) or mutations in ADAMTS13 itself (congenital cTTP). HUS is caused by abnormal and uncontrolled complement activation, either by bacterial toxin activity (typical dHUS) or lack of normal regulatory proteins (atypical aHUS). This review focuses on TTP and HUS in relation to MAHA aetiology, pathogenesis, diagnosis and treatment.

The overlap in clinical presentation between TTP and HUS emphasise the importance of specific diagnostic assays in differential diagnosis. Therapeutic plasma exchange (TPE) and renal replacement therapy (RRT) are reported as relatively effective standard treatment methods. However, novel therapies for TTP (Caplacizumab) and HUS (complement blockade therapy or Eculizumab) currently undergoing clinical trials should be reviewed for future use once approved and validated, to further improve patient prognosis, as both TTP and HUS mortality rates remain significantly high (5-16% and 15-33% respectively).

INTRODUCTION

Microangiopathic haemolytic anaemia (MAHA) describes non-immune haemolysis by intravascular fragmentation (mechanical injury) of red blood cells into fragments called schistocytes. As a microangiopathic subclass of anaemia it is characteristic of thrombotic microangiopathy (TMA), a specific pathologic lesion in vessel walls of arterioles and capillaries, resulting in microvascular thrombosis. Not all MAHA is caused by a TMA, but nearly all TMAs cause MAHA and thrombocytopenia (George and Nester, 2020). TMAs include several diseases but are most associated with thrombotic thrombocytopenic purpura (TTP) and haemolytic-uremic syndrome (HUS). TMA related MAHA is also associated with some infections (e.g. HIV), pregnancy (e.g. HELLP syndrome), bone marrow transplants, systemic vasculitis, and particular drugs (e.g. immunosuppressants). Severe thrombocytopenia and organ failure also accompany MAHA in TMAs (Shenkman and Einav, 2014). MAHA is also associated with disseminated intravascular coagulation (DIC), malignant hypertension and cancers (Vincent et al., 2018). MAHAs not related to TMAs are usually associated with intravascular mechanical devices (e.g. prosthetic heart valves) that cause mechanical injury to the red cells, resulting in non-immune haemolysis (Tsai, 2014). This review focuses on TTP and HUS in relation to MAHA aetiology, pathogenesis, diagnosis and treatment.

AETIOLOGY

TTP and HUS are the most common causes of TMA, where arterioles and capillaries become occluded by disseminated microthrombi formed from agglutinated platelets. Platelets are consumed which eventually results in severe thrombocytopenia. The occlusion causes increased shear force acting on red cells passing through the microvasculature resulting in haemolytic anaemia (MAHA) and organ ischaemia, which may progress to organ failure (Shenkman and Einav, 2014).

TTP is caused by a severe deficiency in ADAMTS13 protease enzyme responsible for cleaving von Willebrand factor (vWF) multimers and is divided into two types: acquired (immune-mediated; iTTP) and congenital (cTTP or Upshaw-Schulman syndrome). Acquired TTP is most frequent and is caused by the presence of autoantibodies against ADAMTS13, whereas congenital TTP is associated with mutations in gene coding for the enzyme (Joly et al., 2017). vWF is essential for platelet and sub-endothelial adhesion and plays a role in platelet-platelet cohesion and aggregation inside blood vessels. VWF is stored as high molecular weight multimers, which are more haemostatically competent than monomers and require homeostasis control by ADAMTS13. The absence of ADAMTS13 results in ultra-large vWF (ULVWF) multimers persisting in circulation after their release is stimulated, leading to spontaneous platelet aggregation and the formation of vWF-rich microthrombi. This process uses up platelets (thrombocytopenia) and occludes vessel flow, causing microangiopathic haemolytic anaemia (MAHA) and organ ischaemia (Kremer Hovinga et al., 2017).

HUS is divided into typical (Shiga toxin-associated) and atypical (aHUS) subtypes. Shiga toxin-associated HUS is caused by a Shiga toxin-producing bacterium but is mostly associated with *Escherichia coli* O157:H7 or Shigella infections. It is also referred to as diarrhoea-associated haemolytic–uraemic syndrome (dHUS), as it causes bloody diarrhoea. Atypical HUS does not present with bloody diarrhoea and is caused by activating mutations (inherited) or autoantibody-mediated (acquired) defects in the complement regulatory proteins. These proteins regulate deposition/activation of complement on cell surfaces, predominantly the endothelium. All HUS subtypes result in abnormal and uncontrolled complement activation, either by bacterial toxin activity (dHUS) or lack of normal regulatory proteins (aHUS). The resulting endothelial damage leads to platelet activation and thrombosis (Firth, 2019). dHUS is usually limited to the renal endothelium and hence associated with acute kidney injury (AKI), whereas aHUS is related to systemic multi-organ complications. Drug-induced HUS has also been reported and is commonly associated with the antimalarial drug Quinine. It is speculated to involve immune injury to endothelial cells, especially glomerular endothelium (Al-Nouri et al., 2015).

PATHOGENESIS

TTP is defined as a severe deficiency in ADAMTS13 (enzymatic activity <10%), which is the only biologic marker specific for this disorder (Joly et al., 2017). ADAMTS13 is primarily synthesized by hepatic stellate cells in the liver, as well as platelets, renal podocytes, renal tubular epithelial cells, and endothelial cells. It is released into circulation as an active enzyme, where it circulates free or bound to soluble von Willebrand Factor (3-5%). vWF is a multimeric plasma glycoprotein and the only known substrate for ADAMTS13. Storage of vWF is as ultra-large vWF (ULVWF) multimers in endothelial cells (Weibel–Palade bodies) or α-granules of megakaryocytes and platelets (Kremer Hovinga et al., 2017). vWF is essential for haemostasis, where its main function is supporting platelet adhesion and aggregation at sites of vessel injury, as well as storing and protecting FVIII from proteolytic degradation in the circulation. In its native conformation, it is inert for adhesive function and resists proteolysis by its regulator ADAMTS13. On exposure to repeated cycles of high shear stress levels, in capillaries and arterioles, it changes its conformation to an unfolded state in which it can support platelet adhesion and activation. The conformational responsiveness of vWF to shear stress is directly related to its size. ULVWF multimers are more responsive than smaller multimers, created by repetitive proteolysis by ADAMTS13, and therefore are more haemostatically competent (Tsai, 2014). In the absence of or at

substantially reduced activity of ADAMTS13, ULVWF multimers accumulate and become activated by shear stress in the circulation, resulting in platelet aggregation and microvascular thrombosis (Shenkman and Einav, 2014). Thrombocytopenia and MAHA ensue due to platelet consumption and mechanical destruction of circulating red cells as they pass through the occluded microvasculature see Figure 1 (Kalpatthi and Kiss, 2020).

Acquired TTP (immune-mediated; iTTP) involves the formation of polyclonal anti-ADAMTS13 (mostly IgG) autoantibodies against the cysteine-rich/spacer domain of ADAMTS13, which inhibit its proteolytic activity towards vWF. Significant amounts of ADAMTS13-specific immune complexes (ICs) have also been reported, which also contribute to severe deficiency of ADAMTS13 (Joly et al., 2017). Various causative factors of iTTP have been identified, including HIV infection, pregnancy and anti-platelet or immunosuppressive drugs. These trigger the formation of autoantibodies against ADAMTS13 or stimulate the secretion of large quantities of ULVWF multimers from endothelial cells (Shenkman and Einav, 2014).

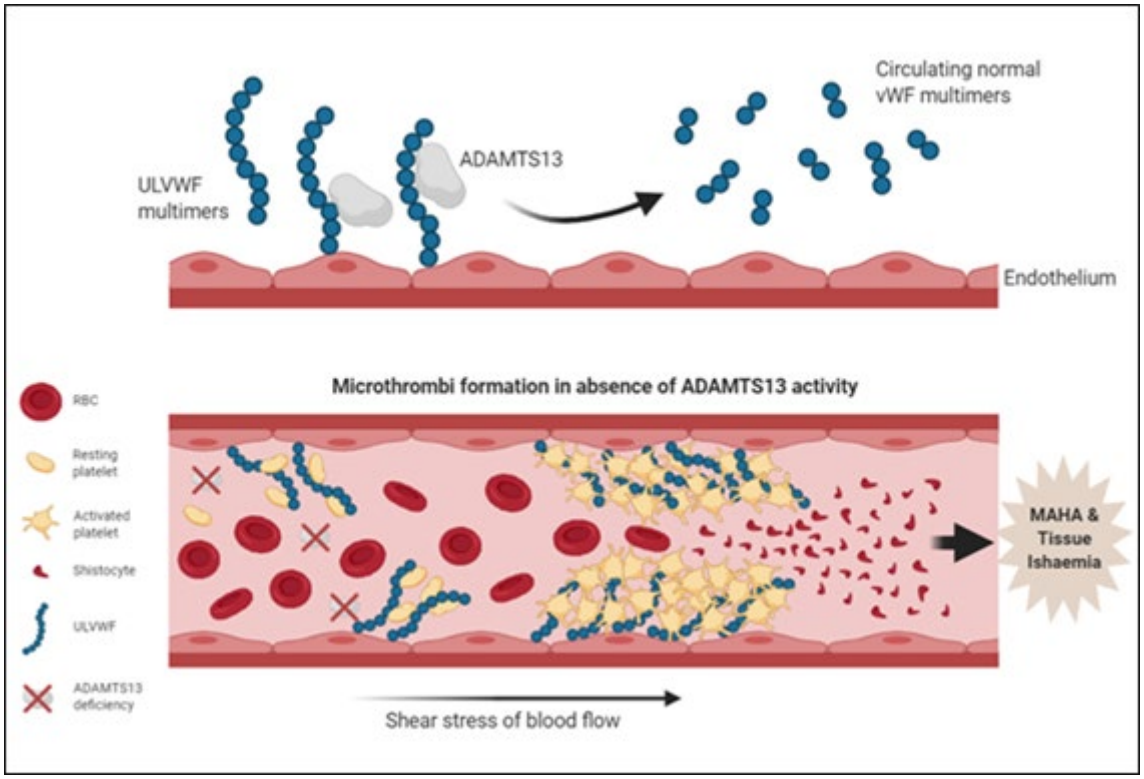

Figure 1: Pathophysiology of TTP. Under normal physiological conditions ULVWF multimers are cleaved into smaller multimers, which are less haemostatically competent (less adhesive to platelets). In the absence or reduced functional activity of ADAMTS13 (iTTP or cTTP), the accumulation of ULVWF multimers leads to widespread spontaneous platelet binding and thrombosis (microthrombi and thrombocytopenia). The resulting narrowing of the vessel lumen increases mechanical stress on the RBCs, leading to MAHA (schistocytes) and tissue ischaemia (figure adapted and redrawn from Joly et al., 2017).

Congenital TTP (cTTP or Upshaw-Schulman syndrome) is associated with rare recessive biallelic mutations in ADAMTS13, which are mostly responsible for quantitative ADAMTS13 defects. Approximately 150 distinct mutations have been identified spanning the entire ADAMTS13 gene, which mainly affect the N-terminal region (Joly et al., 2017). The most common mutations include the missense mutation c.3178C>T (p.R1060W) in exon 24, and the frameshift mutation c.4143_4144dupA in exon 29 (Kremer Hovinga et al., 2017).

In some acute TTP cases, severe ADAMTS13 deficiency may result from different and currently unclear mechanisms, as anti-ADAMTS13 IgG may not be detectable in 20-25% of these patients. The

following have been hypothesised: low sensitivity of the anti-ADAMTS13 IgG assays, unrecognised Ig isotypes, acute liver failure (low ADAMTS13 synthesis/secretion), degradation of ADAMTS13 by sepsis enzymes, and ADAMTS13 inhibition by free haemoglobin or interleukins (Joly et al., 2017). Although the deficiency in ADAMTS13 is the prime cause of microvascular thrombosis in TTP, the tendency to form vWF-platelet aggregation and thrombosis is also affected by responsiveness of vWF to shear stress (modified by plasma proteins such as FVIII, thrombospondin-1 and beta 2-glycoprotein 1) and the shear stress profile itself (Tsai, 2014).

HUS is a distinct type of TMA which has a pathology mainly associated with the kidneys (AKI). It is divided into two categories: typical/diarrhoea-associated HUS (dHUS) and atypical HUS (aHUS) (Afshar-Kharghan, 2016). Diarrhoea-associated HUS (dHUS) is most commonly a result of a Shiga toxin-producing E. coli (STEC) infection, O157:H7 being the most frequent serotype isolated. STEC are highly infectious organisms and the infection occurs through ingestion of contaminated food or water. In the stomach, intrinsic acid resistance allows them to survive the acidic environment and move on to colonise the intestinal mucosa. These bacteria use specific proteins to attach and efface the enterocytes, leading to loss of microvilli and formation of lesions (haemorrhagic colitis). Actin is accumulated within the host cells to further anchor the bacteria. The STEC then begin to produce the Shiga toxins (Stx), which are ribosome inactivating proteins. Two structurally similar types of Stx can be produced (Stx1 or Stx2), but Stx2 is more likely to cause HUS (Walsh and Johnson, 2018). These exotoxins are directly responsible for cell damage of the microvascular endothelium, due to expression of the toxin-specific receptors on these cells. Once endocytosed and transported inside the cell ribosomal peptide elongation is inhibited by the enzymatically active toxin subunit cleaving a single adenine base from the human rRNA. Protein synthesis inhibition then leads to cell death via an apoptotic pathway (Ibama et al., 2019).

Once the epithelium and endothelium are breached, the Stx toxins cross the intestinal wall into the bloodstream where they bind circulating platelets/leukocytes, activating them. These travel to distal sites such as the kidneys, where the microvascular endothelium expresses the primary cellular target: the globotriaosylceramide (Gb3) receptor. In the kidney, this receptor is also found on tubular cells, mesangial cells and podocytes. These cells are infiltrated by the same mechanism as above. Binding Gb3 and toxin endocytosis eventually leads to inhibition of protein transcription, apoptosis, induction of inflammatory cytokines (TNF-α, GM-CSF, IL-8) and cellular necrosis (Walsh and Johnson, 2018).

At higher Stx concentrations, endothelial apoptosis leads to cell detachment, exposing the subendothelial bed rich with prothrombogenic tissue factor and collagen (Joseph et al., 2020). Endothelial cell injury also inhibits prostacyclins and prostaglandins, activating thromboxanes, which induce platelet aggregation and microthrombi formation (Ibama et al., 2019). Stx toxin is also capable of inducing a prothrombogenic phenotype of the epithelial cells, which begin producing increased quantities of tissue factor and vWF (Joseph et al., 2020). Renal capillary lumen becomes partly occluded by the microthrombosis, increasing shear stress on the red cells and causing fragmentation into schistocytes (MAHA). Thrombocytopenia develops as platelets are used up in the formation of microthrombi (Ibama et al., 2019). Finally, recent studies have shown that Stx is also capable of activating the alternative complement pathway, by prompting the formation of platelet/red cell derived microvesicles coated with C3 and/or C9. Activated complement fraction C3a is then believed to trigger microvascular thrombosis by mobilizing P-selectin on the surface of endothelial cells, which plays a role in initial platelet adhesion (Joseph et al., 2020).

In atypical HUS (aHUS), the lack of normal regulatory proteins leads to abnormal activation of the complement cascade, which damages the renal endothelium and hence causes platelet activation and thrombosis. The defects in these complement regulatory proteins are either inherited or acquired (antibody-mediated) (Firth, 2019). Inherited/primary aHUS is associated with the dysregulation of the alternative complement pathway, mainly by mutations affecting proteins that regulate complement activation; FH (Factor H), FI (Factor I) and MCP (membrane cofactor protein). Some gain-of function mutations also occur in the alternative complement activators C3 and FB (Factor B) (Afshar-Kharghan, 2016). These mutations cause unregulated complement activation, and thus excessive generation of

activation proteins such as C3a, C5a and C5b-9 (membrane attack complex, MAC). C3a and C5a cause abnormal vascular permeability (oedema), while MAC causes endothelial cell lysis, resulting in endothelial swelling and injury. The resulting oedema and cellular proliferation cause subendothelial expansion. This, combined with endothelial cell swelling, may cause luminal stenosis and eventually lead to ischaemic organ injury, where initially MAHA occurs without thrombocytopenia (no thrombosis). Once the endothelium is damaged and prothrombotic components in the subendothelial matrix (collagen, vWF, fibrinogen, fibronectin and laminin) are exposed, the coagulation system is activated. Microvascular thrombosis ensues, leading to tissue ischaemia, thrombocytopenia and high shear stress, which fragments red cells into schistocytes (MAHA) (Tsai, 2014).

Acquired/secondary aHUS is associated with the presence of antibodies directed against complement Factor H (FH), and is typically detected in 5–10% of aHUS cases (Tsai, 2014). Anti-FH has been found in pregnancy, cancer, chemotherapy patients, post solid-organ/hematopoietic stem cell transplant and autoimmune disorders (Afshar-Kharghan, 2016). Factor H is the main regulator of the alterative complement pathway, functioning both as a cofactor for Factor I (FI; regulates complement activation) and in degradation of C3 convertase (contributes to formation of MAC). FH also has host recognition properties, allowing it to protect host cells by preventing complement activation on their surface. Anti-FH antibodies are mostly directed against the C-terminal responsible for host cell recognition, thus preventing the protective effect and causing complement activation with endothelial cell lysis (MAC) (Karpman et al., 2016). Endothelial injury leads to thrombosis, MAHA and thrombocytopenia.

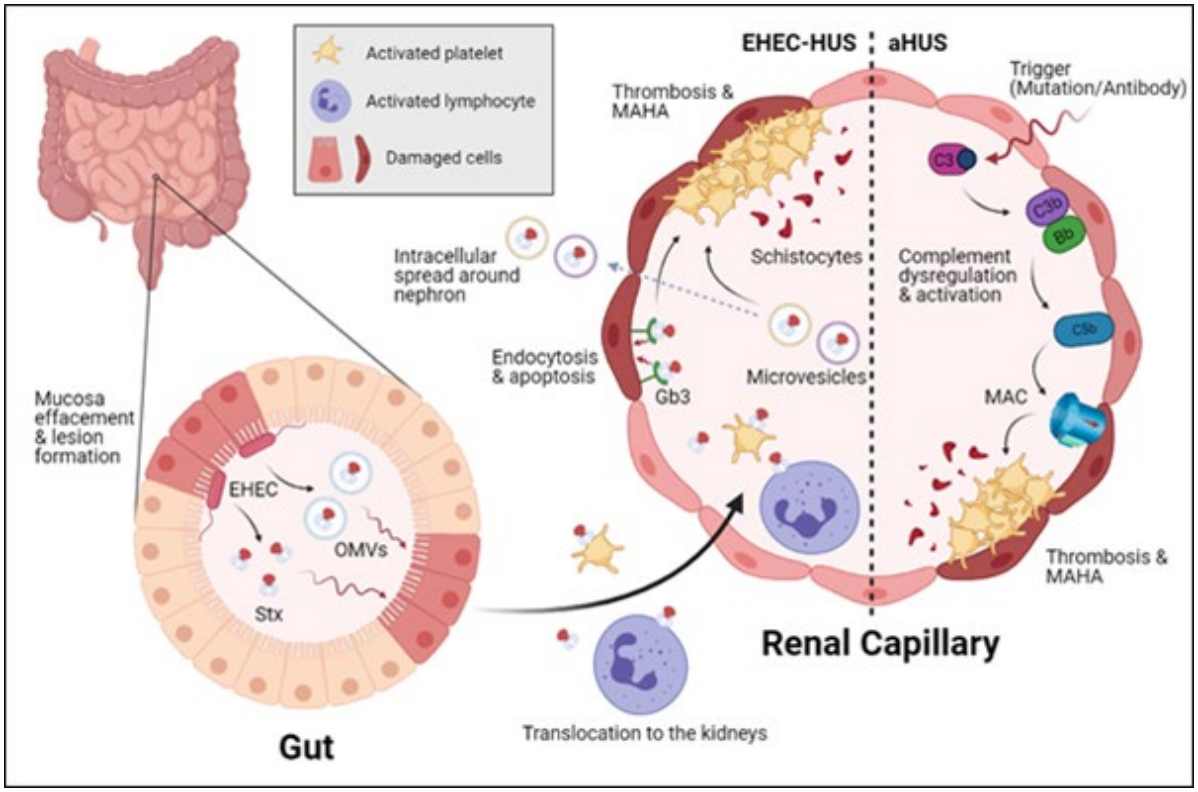

Figure 2: Pathophysiology of EHEC-HUS (STEC-HUS) and aHUS. Shiga toxin-producing Escherichia coli (STEC) ingestion results in gut colonisation and formation of lesions in the mucosa. Shiga toxins (Stx) are then produced and released, either as free particles or secreted through outer membrane vesicles (OMVs). Once the intestinal epithelium and endothelium is breached by the injury, the toxins enter the bloodstream and bind blood cells, which carry them to the kidneys. The toxins bind their target receptor globotriaosylceramide (Gb3) on the endothelial cells (glomerular and peritubular capillary) and undergo endocytosis. Infection leads to inhibition of protein synthesis and apoptotic cell death. The combination of damaged endothelium and activated platelets results in thrombosis, MAHA (schistocytes) and thrombocytopenia. Microvesicles also contribute to microvascular thrombosis, by triggering further platelet adhesion and allowing toxin transfer between cells and across basement membrane. This spreads the toxin around the nephron, eventually leading to acute kidney injury (AKI). In atypical HUS (aHUS), the uninhibited complement activation, either caused by autoantibodies or mutations in complement regulatory proteins, results in endothelial injury and thrombosis, leading to MAHA (figure adapted and redrawn from Karpman et al., 2016).

DIAGNOSIS

Clinical presentation of TTP

TTP is typically characterised by a severe disease course of acute onset. Although the disease onset is usually sudden, some flu-like early signs are frequently reported during the preceding days or at the time of diagnosis. These symptoms include fatigue and pains in the joints, muscles, abdomen or lumbar region (Kremer Hovinga et al., 2017). Clinical signs at onset include fever, signs of haemolytic anaemia (fatigue, dyspnoea, pallor and jaundice) and signs of thrombocytopenia (purpura, petechiae and skin/mucosal haemorrhaging). The renal and central nervous systems are most frequently affected. Neurological manifestations are often transient and range from a mild headache or mental changes/confusion to focal neurological deficits, seizures and coma. Variable degrees of renal dysfunction are observed but are generally mild (Kalpatthi and Kiss, 2020). The absence of bloody diarrhoea and severe renal dysfunction (oliguria or anuria) helps to differentiate TTP from HUS, where these symptoms are common (Chiasakul and Cuker, 2018). Other less common symptoms associated with TTP include cardiac arrhythmias (electrolyte imbalance), myocardial infarction (microthrombi) and gastrointestinal problems (nausea, vomiting, diarrhoea) (Kalpatthi and Kiss, 2020).

As the above clinical signs are not specific for TTP, a differential diagnosis should be carried out, where other TMAs with similar manifestations must be considered (HUS or other TMA syndromes associated with pregnancy, cancer, sepsis or organ transplantation). ADAMTS13 activity assays are pivotal in the differential diagnosis, but the results may not always be readily available. As TTP is classified a medical emergency requiring rapid diagnosis and treatment, a presumptive diagnosis must often be made and treatment initiated based on clinical presentation, history of conditions/comorbidities and routine laboratory assays (Kremer Hovinga et al., 2017). In the long term, the differential diagnosis is crucial, as patients with severe ADAMTS13 deficiency are more likely to respond to therapeutic plasma exchange (TPE) when compared with other TMAs (Joly et al., 2017).

Laboratory Investigation of TTP

Routine FBC (full blood count) detects evidence of anaemia and severe thrombocytopenia (typically <30 x10^9/L). Investigation of the blood smear reveals schistocytes (>1%). In some cases, delayed appearance of schistocytes may occur after onset of clinical signs and symptoms. In rare cases, especially relapse, they do not appear throughout the whole course of the disease (Chiasakul and Cuker, 2018). Other markers of MAHA include reticulocytosis (>120 x10^9/L), indirect hyperbilirubinemia, elevated LDH (lactate dehydrogenase) and decreased haptoglobin (Joly et al., 2017; Kalpatthi and Kiss, 2020). To aid differential diagnosis, other TMA-associated conditions should be ruled out. A basic metabolic panel (serum creatinine and blood urea nitrogen) can rule out severe renal impairment. A coagulation screen (PT, APTT), fibrinogen and D-dimers levels can rule out DIC. A pregnancy screen is performed on women of childbearing age. Cardiac involvement is associated with higher mortality rates and refractoriness to therapy; therefore, troponin-I, electrocardiogram and echocardiogram should be performed. HIV and antinuclear antibodies should also be investigated (Kalpatthi and Kiss, 2020).

As schistocytes are the morphological hallmark of TTP, they are instrumental in the diagnostic screen and require strict standardisation of microscopical identification criteria by the ICSH (International Council for Standardisation in Haematology). Schistocytes are always smaller than intact RBCs and the term encompasses all irregular triangular/crescent-shaped cells, including helmet cells, keratocytes (cells with pointed projections/horns), and microspherocytes (cells lacking central pallor; which are only included in count in the presence of other shapes mentioned) (Joly et al., 2017; Zini et al., 2011). A schistocyte count of >1% is a robust indicator of a TMA. If schistocytes are absent, but TMA is highly suspected, blood smear screening should be repeated daily, as in some cases their appearance can be delayed. Automated blood cell counters should only be used to complement microscopy or follow-up on true-positive samples (Zini et al., 2011).

Confirmatory assays for diagnosis of TTP include ADAMTS13 activity assay, ADAMTS13 functional inhibitor assay, and anti-ADAMTS13 antibody assay. These assays are used to differentiate between TTP and other TMAs, or between iTTP and cTTP. The ADAMTS13 activity assay uses fluorescence resonance energy transfer (FRET) methodology to assess the ability of the patient's enzyme to cleave vWF, where the resulting cleavage product is proportional to the level of ADAMTS13 activity (Chiasakul and Cuker, 2018). The antibody assays determine the presence of anti-ADAMTS13 autoantibodies and/or their inhibitory potential, while antigen assays measure the plasma concentration of ADAMTS13 (Kremer Hovinga et al., 2017). Undetectable ADAMTS13 activity (<10%), with a positive anti-ADAMTS13 antibody assay, confirms the diagnosis of iTTP (Kalpatthi and Kiss, 2020). ADAMTS13 activity should recover during remission. If severely reduced ADAMT13 activity persists during remission and the anti-ADAMTS13 antibody assay is negative, cTTP is suspected. Molecular analysis is then required to confirm that diagnosis (Kremer Hovinga et al., 2017). The diagnostic algorithm for TTP is presented in Figure 3.

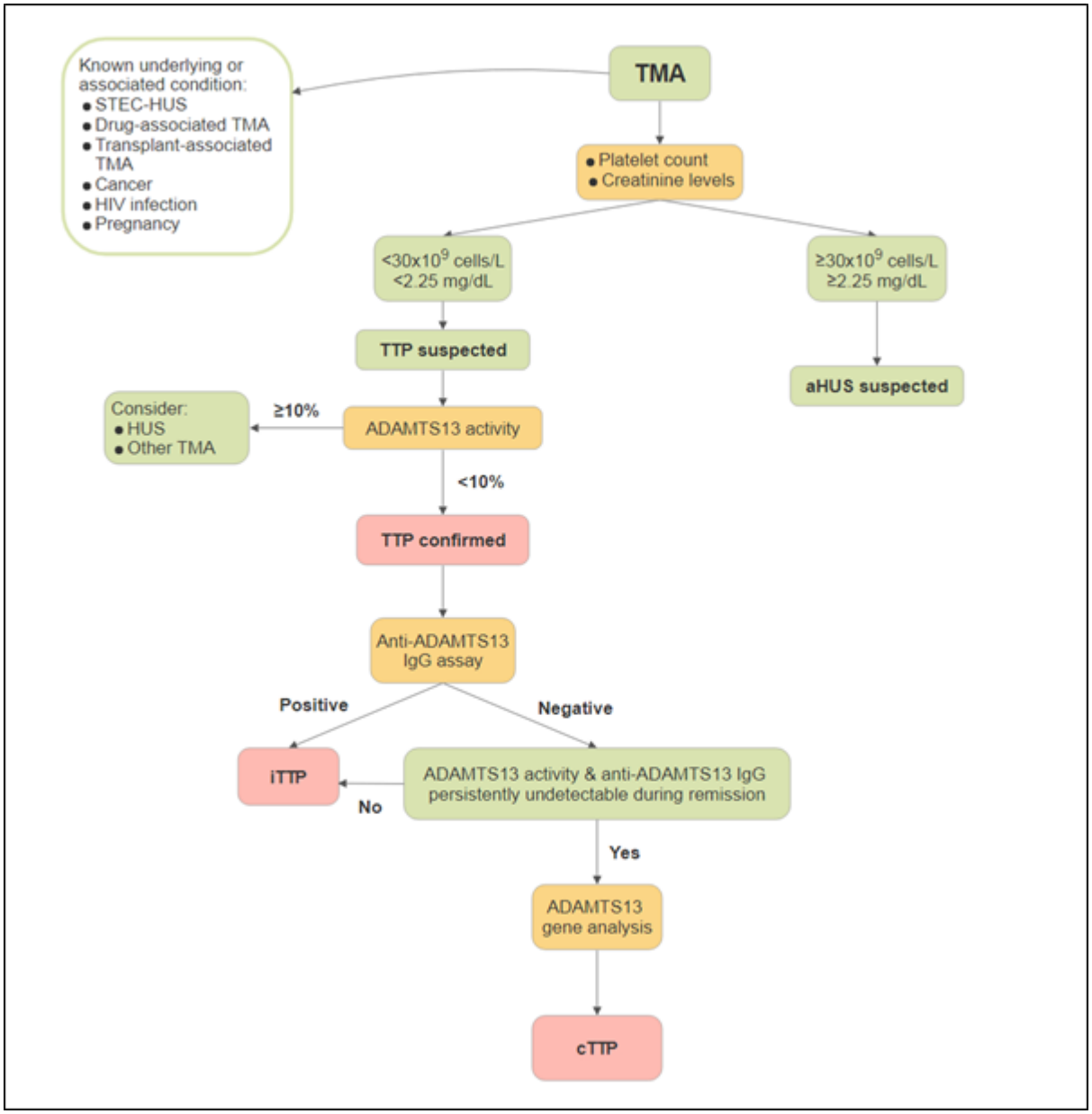

Figure 3: Diagnostic algorithm for diagnosis of TTP in a patient presenting with TMA, consisting of MAHA and thrombocytopenia, with or without organ failure (figure adapted and redrawn from Kremer Hovinga et al., 2017).

Clinical presentation of HUS

STEC-related disease exhibits a broad range of severity, from asymptomatic carriage to lethal dHUS. Rapid diagnosis is essential both for timely treatment and epidemic control. Symptoms at presentation can differ greatly with age (children <5 years old and elderly are more prone to developing HUS). However, patients generally present with severe abdominal cramps and painful diarrhoea. Nausea, vomiting and fever are less common, as the infection is usually limited to the colon (bacteraemia is rare) (Joseph et al., 2020). Severe gastroenteritis is associated with rectal prolapse, colonic gangrene or perforation. Haemorrhagic colitis manifests as bloody diarrhoea due to perforation of the intestinal wall. Haemolytic anaemia (MAHA), thrombocytopenia and AKI usually develop within 2-12 days after diarrhoea onset (Karpman et al., 2016). Bloody diarrhoea occurs 1-5 days after symptom onset but is not considered a defining diagnostic feature of dHUS, as it only occurs in 70-80% cases (Joseph et al., 2020). Signs of anaemia (acute pallor with or without jaundice) present within 3-14 days after the onset of bloody diarrhoea. Other manifestations include cardiac failure (fluid overload, oedema, and hypertension), CNS involvement (ranging from lethargy and irritability to seizures, coma or stroke), pancreatic inefficiency, or hepatomegaly (Ibama et al., 2019).

Patients with aHUS may present during any stage of life. Episodes may be triggered by pregnancy, infections or transplants. A preceding infection may manifest with diarrhoea, similarly to patients with STEC-associated HUS, although onset of aHUS is generally less sudden. This similarity presents a clinical challenge; therefore, patient history plays a key role in differential diagnosis, as the disease course is characterized by recurring episodes throughout the patient's life, especially post triggering events mentioned earlier. Acute episodes generally lead to end-stage renal failure, and in some cases already occur at presentation. Extra-renal manifestations include digital gangrene, cerebral/peripheral vessel stenosis, CNS involvement, and in some cases pancreatic and pulmonary complications (Karpman et al., 2016; Afshar-Kharghan, 2016).

Laboratory Investigation of HUS

The laboratory diagnosis of haemolytic uremic syndrome (HUS) involves haematological, biochemical and microbiological assays. These include FBC (thrombocytopenia, haemoglobin <8g/dL) and clotting assays (urgent blood film if TMA suspected – schistocyte count >1%, increased reticulocyte counts), haemolysis screen (high bilirubin, high LDH, low haptoglobins, negative Coombs test), renal function (elevated serum creatinine and urea, hyperkalaemia, hyponatraemia, metabolic acidosis) and urinalysis (microscopic haematuria, proteinuria and casts - glomerular injury), liver function (elevated liver enzymes), and ADAMTS13 activity (checked if TTP suspected) (Firth, 2019; Ibama et al., 2019).

Various microbiological assays are employed to differentiate dHUS, including faeces analysis (culture on sorbitol MacConkey Agar – where colourless non-haemolytic colonies are subsequently confirmed with E. coli O157:H7 antiserum, PCR for *stx*/*eae* genes, ELISA for free Stx), serology assay (ELISA for EHEC virulence factors, i.e., Stx, serotype-specific lipopolysaccharides, adhesins), blood culture (bacteraemia usually absent) and urine culture (urinary tract infections rare). Renal biopsies are rarely performed during the acute phase of disease, due to risk of bleeding associated with the thrombocytopenia (Ibama et al., 2019; Karpman et al., 2016). Therefore, these are reserved in the case of diagnostic uncertainty in context of STEC-HUS. Patients commonly display superimposed acute tubular damage and nonspecific features of TMA, including glomerular capillary thrombosis, endothelial swelling, congested glomeruli and capillary wall necrosis (luminal narrowing and thrombosis). Cortical infarcts are characteristic of severe and fatal disease (Joseph et al., 2020). Patients presenting with abnormal neurology should undergo CT or MRI scan of the head (Firth, 2019).

Patients with aHUS generally present with moderate to severe thrombocytopenia (<50 x10^9/L), however, around 15% of cases have normal platelet counts. Renal failure is severe and presents with elevated creatinine, haematuria, proteinuria, oedema, and hypertension. Some aHUS cases associated with MCP mutations have been reported to mimic TTP, with normal kidney function and mainly neurologic findings. Triggering events of aHUS are also frequently seen in other TMAs (e.g.,

pregnancy), which further complicates diagnosis (Afshar-Kharghan, 2016). Simple laboratory tests for the quick diagnosis of defective alternative complement regulation do not exist, hence diagnosis initially requires exclusion of TTP (ADAMTS13 analysis). Clinical history and presentation are also useful, such as significant renal failure, which favours a diagnosis of aHUS over TTP (Tsai, 2014). Several more indicative assay results (e.g., complement assays) are usually not available immediately, as most of these tests are performed in reference laboratories. ADAMTS13 activity, complement functional assays, and CH50, C3, C4, FI, FH and anti-FH antibody quantitation (ELISA) may take days to complete, while genetic studies on complement genes may take weeks to months.

As early treatment correlates with better prognosis, clinicians are forced to begin therapy based on general laboratory results and clinical indicators. The course of therapy can be modified based on response and the results of more specific investigations, when these results become available. Genetic studies are used to confirm aHUS diagnosis and guide long-term management, i.e., the length of anticomplement therapy or decisions regarding kidney transplantation (Afshar-Kharghan, 2016).

TREATMENT

TTP treatment should be started as soon as the provisional diagnosis has been made. Standard therapeutic plasma exchange (TPE) guidelines advise daily 1-1.5 times patient's plasma volume exchanges. These are continued until platelet count recovers (>150 x10^9/L) for a period of 2 consecutive days, haemolysis has ceased, and no additional organ dysfunction occurs. TPE is the treatment of choice for both iTTP and cTTP, and its use has drastically reduced mortality rates from >90% (untreated) to 5-16% (Kalpatthi and Kiss, 2020). Its use also correlates with higher survival rates compared with simple high-dose plasma infusion because it delivers greater quantities of functional ADAMTS13, without causing circulatory overload, and removes anti-ADAMTS13 antibodies. It may also remove ULVWF multimers and inflammatory cytokines, although this has yet to be proven (Kremer Hovinga et al., 2017). Regular plasma infusions are effective in preventing acute episodes in cTTP; however, no official guidelines indicate when they should be implemented.

Other available treatments include corticosteroids (immunosuppression in iTTP), Rituximab (anti-CD20 monoclonal antibody for patients with suboptimal response to TPE), N-Acetylcysteine (reduces the size of VWF multimers *in vitro* and inhibits platelet aggregation), splenectomy (last resort for patients who relapse after TPE/ Rituximab/ N-Acetylcysteine), and immunomodulatory drugs for suppression of anti-ADAMTS13 production (Cyclosporine A; last resort treatment in cases of suboptimal response to previous therapies mentioned).

Increased mortality and treatment refractoriness are generally associated with older age, increased plasma cardiac troponin (damaged myocardium), and very high LDH levels at diagnosis. The average survival rate from an initial episode is 80-90% (Kremer Hovinga et al., 2017; Rottenstreich et al., 2015). The definition of a full response to treatment includes platelets >150 x10^9/L for 2 consecutive days, with normal/normalising LDH, and clinical recovery. A full recovery is defined as a lasting response, for a minimum of 30 days, after TPE discontinuation (Joly et al., 2017).

Recent advances in TTP therapy include Caplacizumab (humanized anti-VWF antibody fragment), which inhibits the interaction between vWF multimers and platelets, reducing microvascular thrombosis. A recent study by Scully et al. (2019), has shown Caplacizumab can reduce mortality and refractoriness to 0% (compared with 4% in the placebo group), however, an 8% disease recurrence rate was observed once dosing had ceased. More bleeding was also observed in the test group (mainly epistaxis and from gums). These limitations, plus its high cost, may restrict its practical use. However, future studies on Caplacizumab combination with Rituximab and TPE may achieve lower mortality, faster recovery and reduced relapse rates, which could reduce overall health care costs (Kalpatthi and Kiss, 2020). Recombinant ADAMTS13 (BAX930) for cTTP has also shown promising results in

effectively restoring vWF-cleaving activity. Further results of ongoing trials are expected in 2023 (ClinicalTrials.gov, 2020; Kremer Hovinga et al., 2017).

HUS treatment should be started as soon as a diagnosis is suspected. Supportive care is the first line of action in any HUS subtype, but treatment is also directed towards the specific cause of the disease (dHUS or aHUS). Supportive care involves adequate nutrition and hydration, correction of acidosis and electrolyte imbalance, renal replacement therapy (RRT), as well as controlling hypertension and seizures (Karpman et al., 2016). Electrolyte and fluid imbalance (dehydration), caused by vomiting, diarrhoea and decreased fluid intake, is corrected by administering the appropriate electrolyte orally, intravenously or by tube. Hypertension (fluid overload) is treated with diuretics (oral or IV), to prevent hypertensive encephalopathy and congestive heart failure. If replacement of fluids is ineffective in correcting the imbalances, or if cardiac/pulmonary functions are already affected by severe fluid overload, the patient is switched to RRT (peritoneal dialysis or haemodialysis). Dialysis is also used to treat metabolic acidosis. Severe anaemia (Hb <7g/dL) is treated with red cell transfusion, while platelet transfusions are given only to patients with life-threatening bleeding (platelets <10 x10^9/L) or requiring surgery (Ibama et al., 2019; Karpman et al., 2016).

In STEC-HUS (dHUS), supportive therapy is the cornerstone of treatment. Early IV fluid administration has shown to reduce CNS-complications and mortality, however, it must be balanced against the risk of fluid overload (hypertension) once AKI is established. Fluid overload is generally managed with diuretics, calcium receptor blockers or angiotensin-converting enzyme inhibitors. Haemodialysis is preferred in adult patients, while children are frequently put on acute peritoneal dialysis. Packed red blood cell transfusions are preferred. Plasma exchange therapy has been theorized to remove the circulating toxins or complement factors, but there is little evidence of its effectiveness and randomised trials are currently pending (Joseph et al., 2020). Renal transplantation may be necessary if renal function cannot be restored after the acute phase of disease (Karpman et al., 2016). Other possible treatments currently undergoing trials include complement blockade therapy, Shiga toxin competitive inhibitors and anti-Stx monoclonal antibodies. STEC-HUS has relatively low lethality in paediatrics (<3%), but mortality can rise to 15-33% in adult and fragile populations, with long-term sequelae affecting a third of patients (Joseph et al., 2020).

In aHUS, plasma infusion (20-30 mL/kg body weight) or plasma exchange (1.5-2 times patient's plasma volume) are the standard treatments. They are effective at improving hematologic parameters and preventing relapse post kidney transplant. However, despite plasma therapy, many patients progress to ESRD (end-stage renal disease) or death because complement-mediated organ damage continues, as plasma exchange, in most cases, does not stop complement overactivation (Afshar-Kharghan, 2016). Even those who initially respond favourably can become dependent on, or resistant to, this treatment (Baines and Brodsky, 2017). Long-term follow-up is indicated, as 20-25% of patients develop some degree of CKD (Ylinen et al., 2020). Therapeutic response to plasma therapy mainly depends on complement mutations; therefore, genetic studies are useful in long-term management of the patient. However, specific mutations are identified in only about 50% of cases, hence prognosis is mainly dependent on presence of ESRD, extra-renal involvement, frequency of recurrence after kidney transplant, and the time delay between symptom onset and commencement of treatment. Therefore, anticomplement reagents (Eculizumab) should be considered as first-line therapy in relapsing patients, or in siblings of a patient with confirmed aHUS diagnosis (Afshar-Kharghan, 2016).

Eculizumab is a humanised monoclonal IgG antibody against complement C5. It effectively inhibits the terminal complement pathway and MAC formation, by preventing the conversion of C5 to C5a and C5b. Eculizumab is effective and safe for the treatment of aHUS in both adults and children (Firth, 2019; Walsh and Johnson, 2018). It significantly improves thrombocytopenia and renal function, leading to discontinuation of dialysis in some cases, especially if implemented early. The optimal treatment duration has not been defined; however, it may be safe to discontinue once the trigger of the acute episode has resolved. Some risk of relapse/recurrence still remains, with increased risk of Neisserial infections (require vaccination) (Baines and Brodsky, 2017). Treatment efficacy should be monitored by haematological (platelet counts) and biochemical (LDH, haptoglobin, creatinine) markers

of disease activity, levels of complement activation (CH50) and complement deposition on cells (Karpman et al., 2016). Immunosuppressive therapy might additionally be required if anti-FH antibody is detected. The length of therapy is determined based on anti-FH antibody titre monitoring. Kidney transplantation is considered a last resort for patients with ESRD. It requires rigorous risk assessment and preparation (vaccinations, prophylactic eculizumab). Patients with high risk of reoccurrence (FH, C3 and FB mutations) require life-long prophylactic Eculizumab therapy. Patients with low titres of anti-FH can discontinue the prophylaxis after a 12-month relapse-free period (Afshar-Kharghan, 2016).

CONCLUSION

Correct diagnosis and immediate treatment are crucial for positive patient prognosis. The similarities in clinical presentation of iTTP and STEC-HUS emphasise the importance of specific diagnostic assays (ADAMTS13 quantitation and PCR) in the differential diagnosis. Treatment of TTP and HUS should be reviewed as soon as more effective methods become validated and approved for use. Further studies are required on Caplacizumab, which has already shown promising results by potentially reducing mortality and refractoriness to 0%. Caplacizumab may also prove to be more effective if combined with Rituximab and TPE, potentially achieving faster recovery with reduced mortality and relapse rates. Novel STEC-HUS treatments currently undergoing trials (complement blockade therapy, Shiga toxin competitive inhibitors and anti-Stx monoclonal antibodies) may prove to be more effective and simple treatment options where available. Both TTP and HUS mortality rates remain significantly high (5-16% and 15-33% respectively) thus further innovation in therapeutics and development of staff educational programs, to further improve patient prognosis is required.

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REFERENCES

Afshar-Kharghan, V., 2016. Atypical hemolytic uremic syndrome. *Hematology Am Soc Hematol Educ Program 2016;* 2016(1), pp.217-225.

Al-Nouri, Z., Reese, J., Terrell, D., Vesely, S. and George, J., 2015. Drug-induced thrombotic microangiopathy: a systematic review of published reports. *Blood*, 125(4), pp.616-618.

Arepally, G., 2017. Heparin-induced thrombocytopenia. *Blood*, 129(21), pp.2864-2872.

Babu, K. and Bhat, G., 2016. Cancer-associated thrombotic microangiopathy. ecancermedicalscience, 10, p.649.

Baines, A. and Brodsky, R., 2017. Complementopathies. *Blood Reviews*, 31(4), pp.213-223.

Chiasakul, T. and Cuker, A., 2018. Clinical and laboratory diagnosis of TTP: an integrated approach. *Hematology. American Society of Hematology. Education Program*, 2018(1), pp.530-538.

Clinicaltrials.gov. 2020. *A Study Of Prophylactic And On-Demand Treatment Of Congenital Thrombotic Thrombocytopenic Purpura (Cttp) With BAX 930 (Radamts13) - Full Text View - Clinicaltrials.Gov*. [online] Available at: <https://clinicaltrials.gov/ct2/show/study/NCT03393975> [Accessed 15 November 2020].

Firth, J., 2019. Nephrology: thrombotic microangiopathy (haemolytic–uraemic syndrome). *Clinical Medicine*, 19(1), pp.72-74.

Hill, A., DeZern, A., Kinoshita, T. and Brodsky, R., 2017. Paroxysmal nocturnal haemoglobinuria. *Nature Reviews Disease Primers*, 3(1).

Ibama, O., Ibegbulem, E., Onwuli, D. and Ben-Chioma, A., 2019. The Health Implication of Enterohaemorrhagic Escherichia coli (EHEC) 0157:H7: A Review on Haemolytic Uraemic Syndrome. *Asian Journal of Research in Medical and Pharmaceutical Sciences*, pp.1-10.

Joly, B., Coppo, P. and Veyradier, A., 2017. Thrombotic thrombocytopenic purpura. Blood, 129(21), pp.2836-2846.

Joseph, A., Cointe, A., Mariani Kurkdjian, P., Rafat, C. and Hertig, A., 2020. Shiga Toxin-Associated Hemolytic Uremic Syndrome: A Narrative Review. *Toxins*, 12(2), p.67.

Kalpatthi, R. and Kiss, J., 2020. Thrombotic Thrombocytopenic Purpura, Heparin-Induced Thrombocytopenia, and Disseminated Intravascular Coagulation. *Critical Care Clinics*, 36(2), pp.357-377.

Karpman, D., Loos, S., Tati, R. and Arvidsson, I., 2016. Haemolytic uraemic syndrome. *Journal of Internal Medicine*, 281(2), pp.123-148.

Kitchens, C., Kessler, C., Konkle, B. and Garcia, D., 2019. *Consultative Hemostasis And Thrombosis*. 4th ed.

Kremer Hovinga, J., Coppo, P., Lämmle, B., Moake, J., Miyata, T. and Vanhoorelbeke, K., 2017. Thrombotic thrombocytopenic purpura. *Nature Reviews Disease Primers*, 3(1), p. 17020.

Lechner, K. and Obermeier, H., 2012. Cancer-Related Microangiopathic Hemolytic Anemia. *Medicine*, 91(4), pp.195-205.

Levi, M., 2018. Pathogenesis and diagnosis of disseminated intravascular coagulation. *International Journal of Laboratory Hematology*, 40 (Suppl 1), pp.15-20.

N George, J. and Nester, C., 2020. Approach To The Patient With Suspected TTP, HUS, Or Other Thrombotic Microangiopathy (TMA). [online] Uptodate.com. Available at: <https://www.uptodate.com/contents/approach-to-the-patient-with-suspected-ttp-hus-or-other-thrombotic-microangiopathy-tma> [Accessed 7 October 2020].

Rottenstreich, A., Hochberg-Klein, S., Rund, D. and Kalish, Y., 2015. The role of N-acetylcysteine in the treatment of thrombotic thrombocytopenic purpura. *Journal of Thrombosis and Thrombolysis*, 41(4), pp.678-683.

Schönermarck, U., Ries, W., Schröppel, B., Pape, L., Dunaj-Kazmierowska, M., Burst, V., Mitzner, S., Basara, N., Starck, M., Schmidbauer, D., Mellmann, A., Dittmer, R., Jeglitsch, M. and Haas, C., 2019. Relative incidence of thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome in clinically suspected cases of thrombotic microangiopathy. *Clinical Kidney Journal*, 13(2), pp.208-216.

Scully, M., Cataland, S., Peyvandi, F., Coppo, P., Knöbl, P., Kremer Hovinga, J., Metjian, A., de la Rubia, J., Pavenski, K., Callewaert, F., Biswas, D., De Winter, H. and Zeldin, R., 2019. Caplacizumab Treatment for Acquired Thrombotic Thrombocytopenic Purpura. New England Journal of Medicine, 380(4), pp.335-346.

Sheikh, B., Farooqi, S., Sheikh, Y., Bharat, A., Jain, N. and Copur, A., 2020. IMPORTANCE OF IDENTIFYING CATASTROPHIC ANTIPHOSPHOLIPID SYNDROME IN THE ICU. *Chest*, 158(4), p.A856.

Shenkman, B. and Einav, Y., 2014. Thrombotic thrombocytopenic purpura and other thrombotic microangiopathic hemolytic anemias: Diagnosis and classification. Autoimmunity Reviews, 13(4-5), pp.584-586.

Tsai, H., 2014. Reference Module In Biomedical Research - Microangiopathic Hemolytic Anemia. 3rd ed. E-Da Hospital, Kaohsiung, Taiwan: Elsevier Inc.

Vaught, A., 2016. Critical Care for the Obstetrician and Gynecologist. *Obstetrics and Gynecology Clinics of North America*, 43(4), pp.611-622.

Vincent, J., Castro, P., Hunt, B., Jörres, A., Praga, M., Rojas-Suarez, J. and Watanabe, E., 2018. Thrombocytopenia in the ICU: disseminated intravascular coagulation and thrombotic microangiopathies—what intensivists need to know. Critical Care, 22(1), p.158.

Walsh, P. and Johnson, S., 2018. Eculizumab in the treatment of Shiga toxin haemolytic uraemic syndrome. Pediatric Nephrology, 34(9), pp.1485-1492.

Ylinen, E., Salmenlinna, S., Halkilahti, J., Jahnukainen, T., Korhonen, L., Virkkala, T., Rimhanen-Finne, R., Nuutinen, M., Kataja, J., Arikoski, P., Linkosalo, L., Bai, X., Matussek, A., Jalanko, H. and Saxén, H., 2020. Hemolytic uremic syndrome caused by Shiga toxin–producing Escherichia coli in children: incidence, risk factors, and clinical outcome. *Pediatric Nephrology*, 35(9), pp.1749-1759.

ZINI, G., d'ONOFRIO, G., BRIGGS, C., ERBER, W., JOU, J., LEE, S., McFADDEN, S., VIVES-CORRONS, J., YUTAKA, N. and LESESVE, J., 2011. ICSH recommendations for identification, diagnostic value, and quantitation of schistocytes. *International Journal of Laboratory Hematology*, 34(2), pp.107-116.

An Investigation of Healthcare Supports for Those with Food Allergy in Ireland

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ABSTRACT

Introduction: In Ireland, around 5% of children and 3% adults have food allergy (134,000 people). This current paper describes a survey that was carried out on a subset of service-users with the aim of identifying whether there is a need for increased specialist medical services and/or for a funded charity such as Anaphylaxis Ireland, defunct since 2015.

Materials & Methods: These needs were assessed via an online survey using Google Forms. The survey was conducted from 17-27th February 2020. There were 31 questions in total, relating to topics such as symptoms, clinical wait times, satisfaction with care provided and demand for support services.

Results: There were 50 valid responses. Results showed that wait-times for referrals are shorter for privately referred patients (43% seen in 1 month) than public patients (20% seen in 1 month), most patients did not see a dietician (81.8%) and allergy management is generally effective (93% decrease in severe cases). Also, there is high demand for support services such as allergen-free food list (54.5% of respondents) and caterer's lists (54.5% of respondents).

Discussion: This is the first paper outlining food allergy care since 2015 in Ireland and the findings suggest the need for improved GP awareness of food allergy and filling consultant immunologist posts to reduce public wait times. Also, a funded support organisation should be reinstated to meet all the needs of food allergy patients.

KEYWORDS: food allergy, immunology, anaphylaxis, food allergen, dietetic service, Ireland

INTRODUCTION

Food allergy is an important public health issue that affects both children and adults and may be increasing in prevalence (Boyce *et al.*, 2010). In Ireland, statistics show that approximately 5% of children and 3% of adults suffer from food allergies (Irish Nutrition and Dietetic Institute (INDI), 2020). There has been a 615% increase in hospitalisations for anaphylaxis reported between 1992-2012 in the United Kingdom (Turner *et al.*, 2015). Food allergy has been defined as adverse reactions to food in which immunologic mechanisms have been demonstrated. This term therefore encompasses both immunoglobulin E (IgE)-mediated and non-IgE-mediated food allergies (Muraro *et al.*, 2014). This strict definition separates food allergy from food intolerance and hypersensitivity, metabolic conditions such as lactose intolerance, and coeliac disease (Hadley, 2006). Although any food may provoke a reaction, relatively few foods are responsible for most food allergic reactions, these include milk, egg, peanuts, tree nuts, fish, and shellfish (Sampson, 2003).

The clinical presentation of food allergy involves a large spectrum of symptoms including skin (urticaria, angioedema, atopic eczema), gastrointestinal (vomiting, abdominal pain, diarrhoea,

constipation), respiratory (rhinorrhoea, dyspnoea) and circulatory (cardiovascular collapse) (Muraro *et al.*, 2014). Both modifiable and non-modifiable early life risk-factors for food allergies have been identified, including male sex, ethnicity, genetics, allergen exposure (timing and route of exposure) and vitamin D insufficiency (Loh and Tang, 2018). The exact causative agent of food allergy development is unknown however, Platts-Mills (2015) argues changes in environment, hygiene and lifestyle have led to the increase in allergies in recent years. Improved hygiene, and the movement of children indoors means they are exposed to less antigens early in life, therefore they cannot be desensitised to those antigens. Consequently, this leads to exaggerated immune responses later in life in the form of allergies.

The current survey aimed to assess the provision of care for those with food allergies in Ireland and evaluate the strengths and weaknesses of the public healthcare system for the diagnosis and treatment of food allergy patients. The level of satisfaction with management and treatment options and any new approaches to treating food allergy patients were also examined. The respondents were asked to recommend any support services that may be helpful in managing their food allergy. The possible changes that may have occurred in the provision of care since the 2015 disbanding of Anaphylaxis Ireland were also interpreted, by comparing the satisfaction of food allergy patients who were diagnosed and treated prior to and after that year. The last paper on this topic in Ireland was published in 2014 (Conlon, *et al*, 2014).

MATERIALS AND METHODS

Survey Population

The target population for this study was individuals with food allergies in Ireland. To include the full range of those with food allergies, populations of both adult and paediatric patients were surveyed. Parents answered the survey on behalf of their children if aged under 18 years. Respondents were selected by asking individuals in the community either verbally, via social media or posters if they have a food allergy or if they know someone with a food allergy. If they knew someone, they were asked to pass the survey link to that individual. The population was sourced from locations across Ireland.

Method of Conducting the Survey

Responses were taken from the 17th of February 2020 to the 27th of February 2020**.** An online survey platform (Google Forms) was used to construct the survey. There were 31 questions in total, relating to topics including symptoms, clinical wait times, satisfaction with care provided and need for support services (shown in **Appendix 1**). The surveys were answered anonymously.

Data Analysis

Data analysis was conducted using Microsoft Excel and Google Sheets. The representation of the general population among the survey population was determined using the Census 2016 data (Central Statistics Office Census, 2016) and Prevalence Data (Irish Food Allergy Network Allergy, 2016). The Top 14 Allergens based on Regulation (EU) No 1169/2011 of the European Parliament (2011) on food labelling were used to identify “rare allergies” in this survey. The complete set of data was divided into four subgroups: the general population, paediatric patients, rare allergens and finally, severity sub-divisions (mild, moderate, and severe). Responses were considered valid if the subject consented to participation, is resident in Ireland and stated specifically they had a food allergy. Symptoms and patient experiences were used as validation of self-reported allergy severity.

RESULTS

There were 50 valid responses from 56 submissions. Six submissions were excluded as the respondents were either coeliac or not resident in the Republic of Ireland. Of the valid responses, 70% (n=35) of respondents were female and 30% (n=15) were male. Of the respondents, 76% (n= 38) were over 18 and 24% (n = 12) were parents of under 18 paediatric patients, answering on behalf of their child.

Regarding self-reported severity, 28% were mild (n=14), 42% were moderate (n=21) and 30% were severe (n=15). Survey respondents were asked to describe their medical experiences of food allergy and the prevalence of these experiences was examined across the range of food allergy severity presented in Table 1.

Table 1: Validation of Self-Reporting of Allergies by Comparison of Experiences of each Severity Group

Parameter of Severity	Mild Patients	Moderate Patients	Severe Patients
Anaphylactic shock	0%	5%	9%
Hospitalisation from allergy	0%	5%	50%
Adrenaline auto-injector (Epipen) carriage	0%	19%	64%

The number of respondents who reported allergy to each of the common allergens was noted and prevalence of that allergy among the respondents was calculated and can be seen in figure 1. This prevalence was compared with the prevalence of allergens among the severe cohort of patients. Some individuals had more than one allergy (n=23). Peanuts, other nuts, molluscs, and crustaceans represented some of the most common allergens.

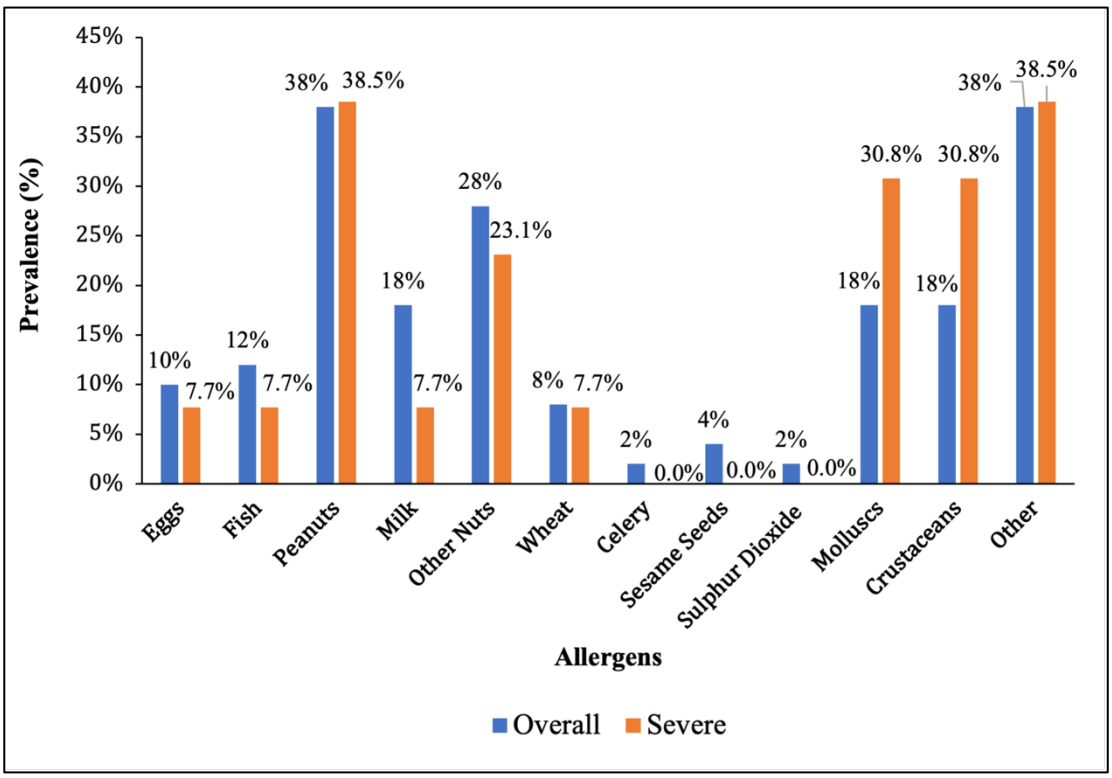

Figure 1: Frequency of Reported Food Allergens in Respondents

The respondents reported who they considered their primary care provider for their allergy and the frequency of these providers among this population was calculated. 12% (n=6) of respondents reported more than one individual as their predominate carer. Most respondents had care provided by General Practitioners. Of the total respondents 26% (n=13) had used complementary or alternative treatments for their food allergy outside of mainstream healthcare provider

The predominant healthcare provider for paediatric patients (n =12) were as follows: 58% an immunologist, 35% a GP and 7% did not state whether they had a healthcare provider.

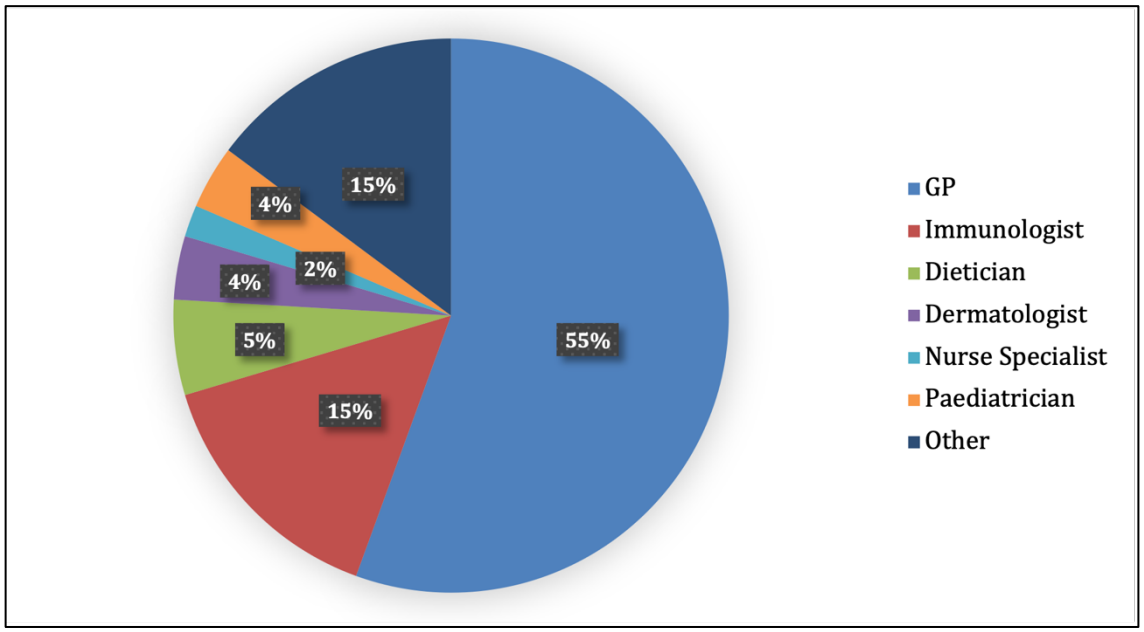

Figure 2: Predominant Health Care Provider for People with Food Allergies in Ireland.

Immunologist Referrals

Of the 50 responses, 60% (n=30) individuals were not referred to an immunologist. There were 13 respondents who had been referred in the past five years. The remainder (n=7) did not answer the question. Table 2 shows the wait time in months to see an immunologist for; patients referred through the private versus public system and paediatric versus adult populations. Half of paediatric patients were referred privately, 43% were referred publicly and 7% had no referrals

Wait times were also investigated dependent on allergy severity, and in those who have a close relative with food allergy versus those who do not. One patient referred privately who did not answer the wait-time question. One patient who answered that they were waiting 4-6 months did not answer whether they had a family history of food allergy.

Table 2: Wait Time (in months) to see an Immunologist

Wait Time (months)**	Private (n=7)*	Public (n=5)	Paediatric (n=7)	Adult (n=6)	Mild (n=1)	Moderate (n=4)	Severe (n=8)	Close Relative (n=2)	No Close Relative (n=10)
1	3	1	3	2	0	2	3	1	4
2-3	3	1	1	3	1	1	2	1	3
4-6	1	0	1	0	0	0	1	0	0
7-11	0	2	1	1	0	1	1	0	2
12+	0	1	1	0	0	0	1	0	1

* One patient referred privately did not answer the wait-time question.
** One patient who answered that they were waiting 4-6 months did not answer whether they had a family history of food allergy.

Dietician Referrals

A total of 84% (n=42) of respondents had not been referred to a dietician. Of those who had (n =8), three quarters were seen within a month (n=6) and the remainder were seen in two-three months (n=2). Of these individuals, 75% (n=6) reported having adequate information on their allergy and the supports available to them. In contrast, 52% (n=17) of those who had not seen a dietician reported having adequate information.

Efficacy of Food Allergy Treatment

As seen below, the number of respondents in each severity bracket was noted before treatment of their allergy and was compared with the proportion of severity after treatment. Note the decrease in severe cases and increase of mild cases after treatment.

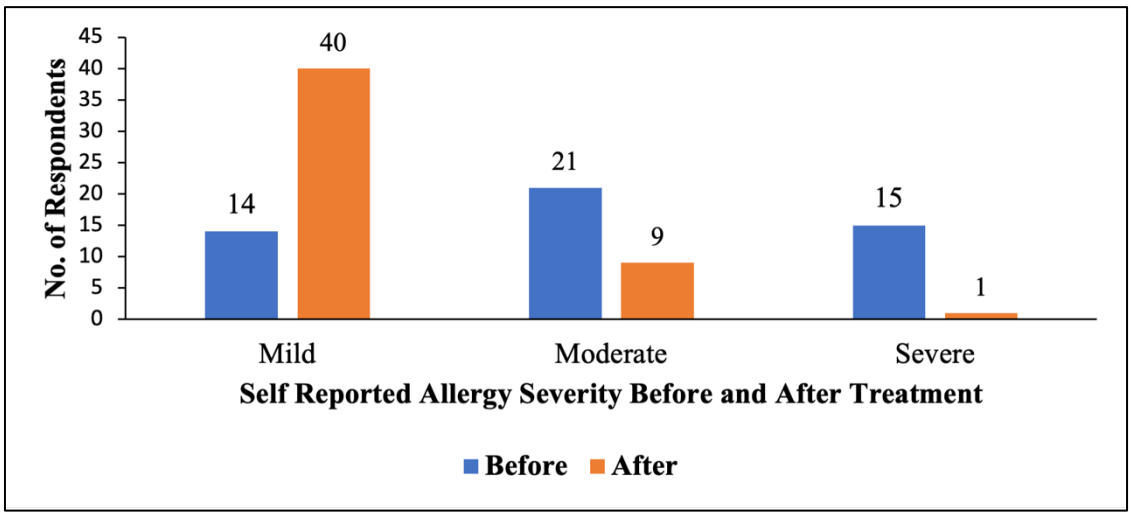

Figure 3: Change in Severity of Food Allergies Before and After Treatment

Table 3 compares the satisfaction with diagnosis and treatment among patient diagnosed prior to 2015 and subsequently.

Table 3: Level of Satisfaction with Allergy Diagnosis/Treatment in Long-Term and Short-Term Patients*

Time Since Diagnosis*	Satisfied	Dissatisfied
Short-term	57%	43%
Long-term	74%	26%

*Long term is defined as a diagnosis as prior to 2015 and short term since 2015.

Respondents (n=43) were asked to list the methods by which they had been tested for their food allergy. 20 respondents were tested by more than one method. Blood tests, followed by skin-prick tests and allergen exclusion were the most common testing methods see figure 4.

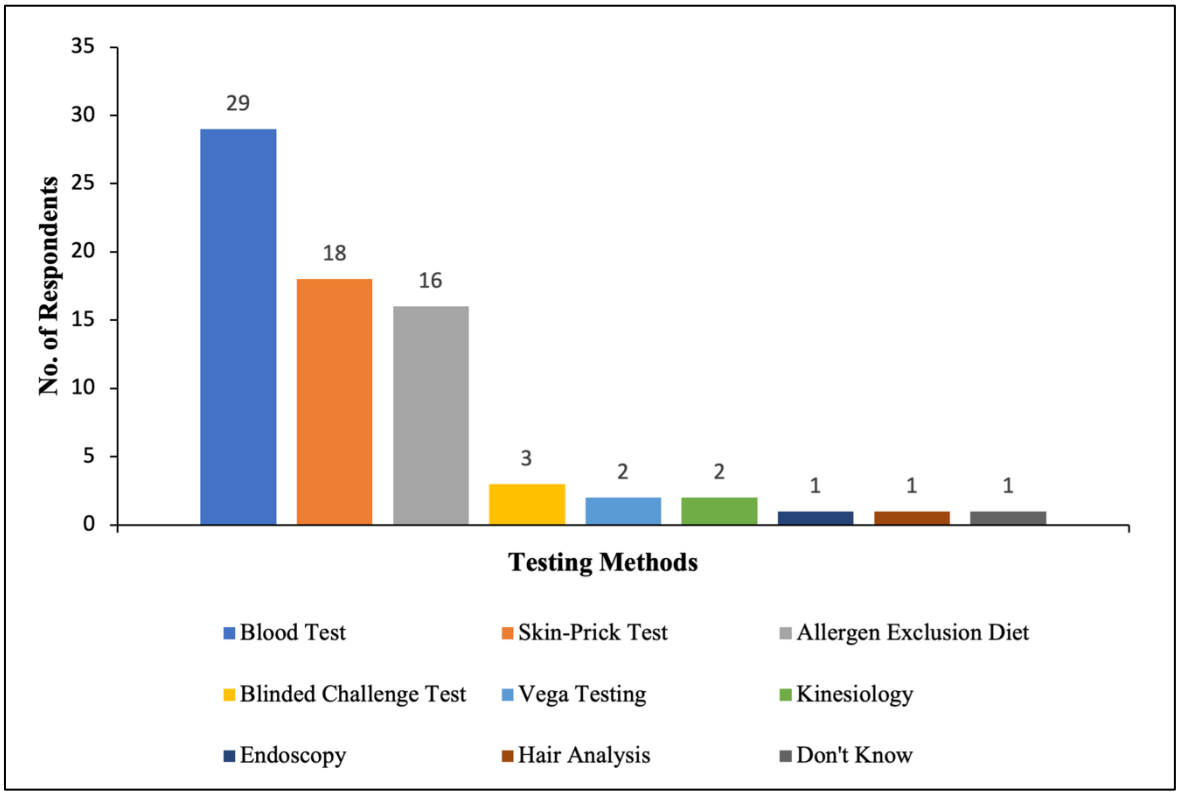

Figure 4: Number of Respondents Who Underwent Each Form of Food Allergy Testing

Respondents (n=48) were asked to list the methods they had used to manage their food allergy. The total number of people who used each method is seen in figure 5. Nineteen respondents had used more than one management method. Allergen exclusion was the most common management strategy.

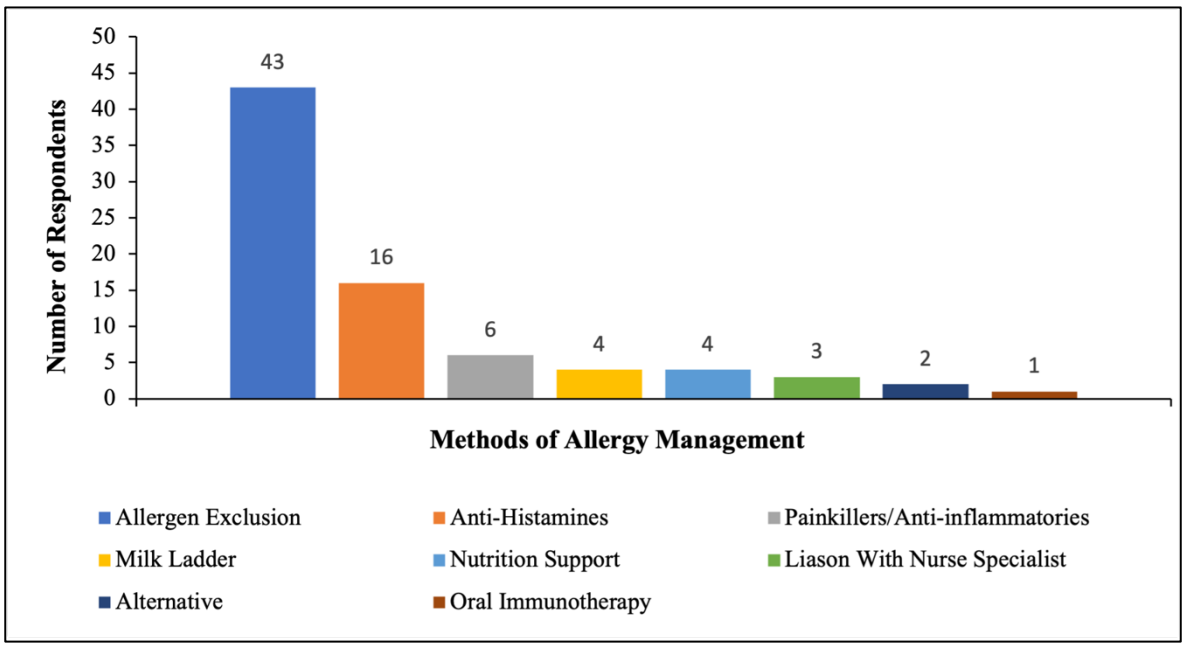

Figure 5: Number of Respondents Who Used Each Method of Food Allergy Management

The self-reporting efficacy of convention versus alternative treatments is seen in table 4 showing that the efficacy of convention methods is reported as better than alternative medicines.

Table 4: Self-Reported Efficacy of Conventional versus Alternative Treatment of Food Allergy*

Medical Pathway for Allergy Treatment	Average Self-Reported Efficacy (1-10 scale)
Conventional Medicine	8.1/10
Alternative Medicine	5.8/10

*Efficacy scale 1-10, where 1 is completely ineffective and 10 is extremely effective

Beneficial Changes Recommended by those with Food Allergies

Adult respondents were asked to list what services they felt would be most effective/necessary to support their food allergy care, the frequency of requests for each service was calculated. This was compared with the frequency of services requested by parents of children with food allergies to assess if their needs differ when compared to the adult population. Most parents wanted separate food preparation areas and issuing of a parental guide. Adult respondents wanted caterer's lists and updated food lists for their allergen.

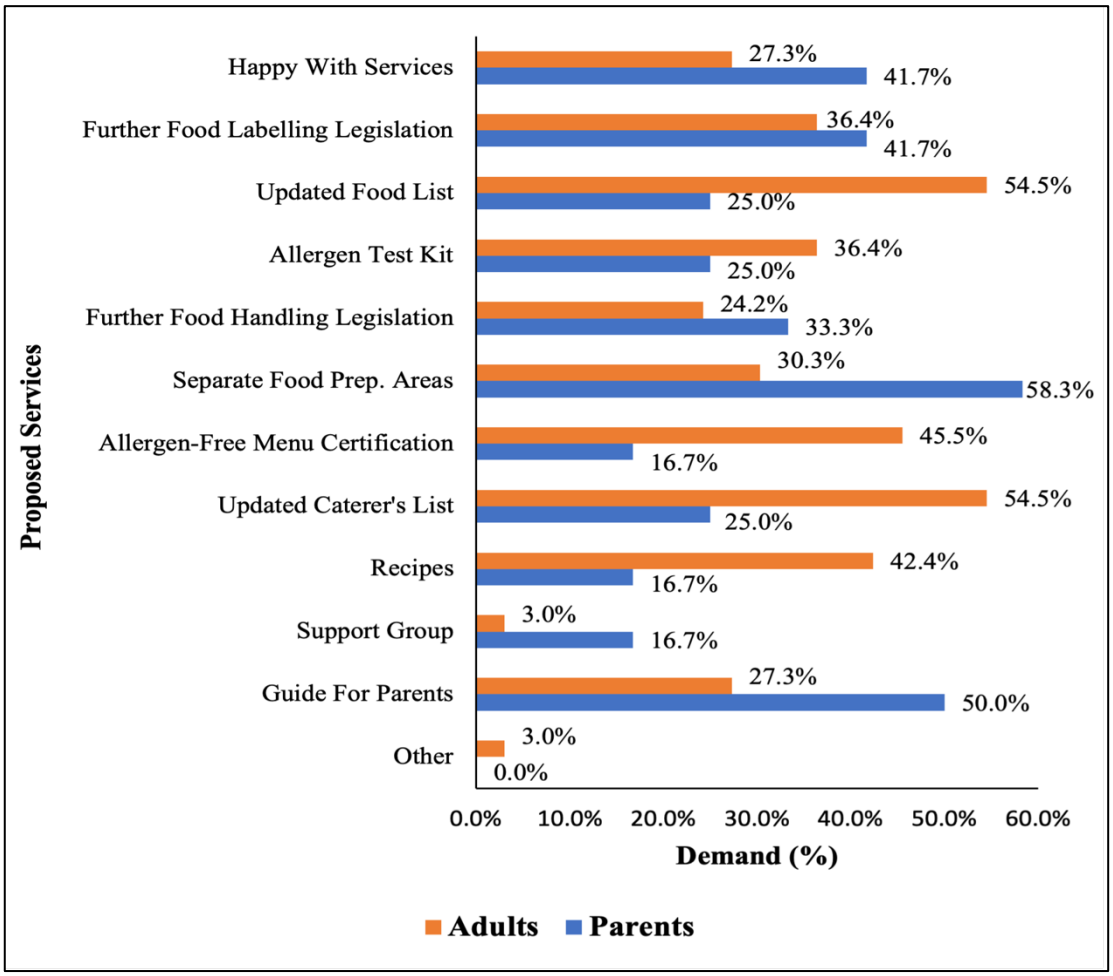

Figure 6: Suggested Needs for a Variety of Support Services Presented as a Percentage of Adult Respondents and Parent Respondents

DISCUSSION

The purpose of this study was to survey people with food allergies and assess the care they received in Ireland since the last published study by Conlon *et al*, (2014), which covered allergies in general, rather than food allergies specifically. With the closure of the only Irish food allergy support organisation Anaphylaxis Ireland in 2016 (Anaphylaxis Ireland, 2020), it may be beneficial to analyse the gaps in support post-diagnosis for food allergy patients. It is reported that approximately 3% of adults and 5% of children in Ireland have food allergies (IFAN, 2020). Of a given population 5% is taken as a representative sample, therefore the 50 subjects of this survey represent 1000 individuals with food allergies.

The most common foods that respondents were allergic to are shown in Figure 1. In Ireland these are peanut/other nuts, cow's milk, fish, and egg allergies, which together account for 90% of food allergies globally (Żukiewicz-Sobcza *et al*., 2013). In this survey, 80% of respondents reported having at least one of these allergies. Some people had an allergy to a foodstuff that fell outside the EU list of major food allergens (Annex II to Regulation 1169/2011 (FIC), 2011). These foods were collectively termed rare allergens and included strawberry, kiwi, yeast, and others. As the responses to this survey were gathered anonymously, allergy symptoms and experiences of the respondents were self-reported. One key area of self-reporting was the severity of an individual's allergy. As a measure of validation of this self-reporting Table 1 compares the frequency of symptoms and experiences of patients which is used as evidence to accept the three sub-divisions of mild, moderate, and severe in further analysis. As Table 1 shows, there was an increase in prevalence of all parameters measured in self-reported severe patients relative to moderate and mild patients. Anaphylaxis is used as a measure of severity as it represents a life-threatening, systemic hypersensitivity (Reber, *et al.* 2017). Hospitalisation for an allergic reaction often occurs due to anaphylactic shock (Banerji, *et al*. 2011). The most substantial difference between mild and severe patients was adrenaline auto-injector carriage (used in anaphylactic emergencies), this serves as a strong indicator of allergy severity.

An important aspect of allergy care in Ireland is the healthcare professional that manages diagnosis and treatment of that allergy. Of respondents, most were under a general practitioner's (GPs) care, with more complex cases referred to a specialist consultant immunologist as shown in Figure 2. Other practitioners consulted were dieticians and dermatologists. Testing for food allergy via blood test was reported by 67.4% of respondents (Figure 4). This is typically performed in GP practices. The GP will also note clinical and family history to diagnose the allergy (National Institute for Health Care and Excellence (NICE), 2011). Skin-prick tests were carried out in the diagnosis of 41.9% of those surveyed (Figure 4), which is usually conducted by dermatology outpatient departments (Purcell, 2019). Instances in which a GP may refer to an immunologist include where there is anaphylaxis or severe delayed reactions, faltering growth in young children or non-response to allergen exclusion (NICE, 2011).

When considering consultant immunologist referral wait-times, only those referred since 2015 were included, to allow appraisal since the publication of Conlon *et al.* in 2014 where they found there was an increasing demand for specialist public allergy services across Ireland. In this survey, 60% of individuals were not referred to an immunologist/allergist. As shown in Table 2, the wait times for private patients were shorter than for public referrals. National statistics on waiting times for public hospitals show that 742 people are currently (February 2020) on a waiting list to see an immunology consultant, a third of which have been waiting over 18 months (Outpatient by Specialty as at 27/02/2020, 2020). This represents a 7% increase since February 2015 (Outpatient by Specialty as at 26/02/2015, 2015). The key cause for the recent surge is both the lack of filled consultant vacancies and the rapid increase in referrals for allergic diseases, marking allergy as a "*major unmet need across the health service*" (Purcell, 2019). One consultant noted there are now only "*four and a half consultants in the country*" (Doyle, 2019). Previous studies detailing the effect of waiting times for outpatient services on the patient include a risk of physical deterioration, and higher levels of anxiety, uncertainty, and powerlessness whilst anticipating a disease outcome (Fogarty and Cronin, 2008). Paediatric patients were seen faster than their adult counterparts as shown on Table 2. Paediatric services may be better

resourced because there are more children with allergies (5%) in contrast with adults (1-2%) (Food Safety Authority of Ireland (FSAI), 2020).

Due to anaphylactic reaction risk, severe patients tend to be fast-tracked for immunology referrals (Purcell, 2019). Table 2 shows that a larger proportion of severe patients were seen within 1 month, however this trend was not observed in mild or moderate groups. In Table 2, those with a relative with food allergy were seen at an expedited rate, as family history is a significant clinical presentation of food allergy (The Royal College of Paediatrics and Child Health, 2011). It should be noted sample size was small for this parameter, so further study is needed. To help improve wait-times for allergy patients, the health service could encourage the recruitment of more consultant immunologists through in-house training initiatives and advertisement abroad to attract these specialists (Aronson, 2011). It was noted by Harding, *et al.* (2016) that a triage system reduced waiting lists by 40% compared to short-term investment (Kenis, 2006). Most patients reported their GP as their primary healthcare provider (Table 2). This may represent a lack of holistic care, with only 16% of respondents referred to a dietician in the past 5 years, and one respondent referred to a clinical nurse specialist.

It was found waiting times to see a dietician (from 2015 to 2020) were much shorter than that for the immunologist. Three-quarters of patients were seen within a month and the remainder in two-three months. As most (84%) respondents were not referred to a dietician, it is important to note the benefit reported by those who did. Of those who saw a dietician 75% felt they had adequate information on their allergen and support services available to them. In contrast, 52% of those not referred felt well-informed. An allergy patient is less likely to develop nutritional deficiencies due to allergen exclusion under the guidance of a registered dietician (Aronson, 2011). Under the guidance of dieticians, reintroduction of certain allergens (e.g. cow's milk, egg) has accelerated the rate at which infants grow out of their allergy (Brożek *et al.*, 2012; Dang *et al.,* 2016; Irish Food Allergy Network, 2020). Despite increased patient awareness and other benefits, dietetics is an underutilised service by healthcare-providers, and presents a valuable opportunity for more effective food allergy care

One successful aspect of food allergy care appears to lie in the management and treatment techniques used in the Irish health service. There is a distinct decline in those who self-report as severe and moderate after management (Figure 3). Mild cases greatly increased after treatment, encompassing 80% of respondents. The self-reported efficacy of conventional treatments in this survey was 8.1/10 (Table 4). In contrast, those patients using alternative/complementary treatments rated it 5.8/10. As no cure for food allergies exists, the standard and most basic management strategy is allergen avoidance (Lanser, *et al.* 2015). Of respondents, 87.5% practised allergen exclusion, while 33.3% used antihistamines (Figure 5). While theoretically allergen exclusion should result in lack of allergic reactions, this is often not the case due to contamination and mislabelling or risk-taking with allergens by patients (Sampson. *et al.* 2006). Allergy exclusion also carries psychological burdens and stress (Primeau, 2000). The patients who were diagnosed prior to 2015 were defined as long-term patients and those after 2015 were short-term patients. Most patients diagnosed prior to 2015 were satisfied with their diagnosis and current management strategy. However, those diagnosed in recent years are substantially less satisfied (Table 3). This may be attributed to the extended waiting times more recently or a lack of support services for those with allergies, for example the disbanding of Anaphylaxis Ireland (Figure 6). Other reasons for dissatisfaction reported were insufficient testing for those with multiple allergens and wrong tests being ordered (total IgE rather than specific-IgE), supporting the need for GP awareness of allergy. These, in combination with the challenges of allergen exclusion, suggests a need for newer, more effective forms of therapy for food allergies in Irish healthcare.

One promising new option is allergen immunotherapy, which one child from the current survey had undergone. This involved exposing the patient to increasing increments of their allergen with the aim of eventual desensitisation to the allergen (Licari, *et al.*, 2019). Some trials have shown up to 90% desensitisation, however there is a moderate risk of serious systemic allergic reaction, and thus require scrupulous adherence to maintenance doses (Nurmatov, *et al.* 2017; Nucera *et al.*, 2018; Licari, *et al.,* 2019; Chu *et al.*, 2019). Anti-cytokine therapies, gene therapy, probiotics and anti-IgE therapies may have applications in treating food allergies in the future (Kishida, *et al.* 2007; Licari, *et al.* 2019). These

treatments would not only act as effective management of food allergies but also remove the burden of allergen exclusion on Irish patients. Further research and development of these therapies could yield beneficial results for the care for allergies nationally and internationally.

With the rise of paediatric allergy cases worldwide, the care of children with food allergies presents unique challenges such as parental training and anxiety, nutritional insufficiencies and maintaining long-term management plans that change over a lifetime (The Royal College of Paediatrics and Child Health, 2011). According to the HSE (2013), paediatric allergy care was underdeveloped in Ireland, with most children not seeing an allergy specialist. Based on the current survey, most paediatric patients (58%) now see an immunologist which may represent an improvement in the provision of food allergy care for paediatrics since 2013. Half of paediatric patients were referred privately. Parents may have chosen the private referral route due to a faster diagnosis than the public route. Increased staff recruitment/specialist training in immunology and improved integration of the discipline into general practice will work to enhance the care provided to paediatric patients in Ireland.

The final component of food allergy care is post-diagnosis supports that improve the quality of life of a patient. The demand for a wide variety of services is shown in Figure 6. The adult cohort was less satisfied (27%) with current support services when compared with the parent population (42%). This coupled with the longer waiting times discussed above may indicate that adult food allergy care is not as effective or well-managed as paediatric care.

Of respondents, 36% recommended further legislation on food labelling and 42% of respondents recommended an updated list of products that are allergen-free. This suggests need for a funded entity in Ireland which maintains a food list in the same manner as the Coeliac Society of Ireland (2020), which is updated throughout the year. There is currently a high risk of allergen contamination in restaurants (36% had an allergic reaction while eating out). A combination of better food labelling and distribution of user-friendly, portable allergen test kits would reduce the incidence of contamination reactions (Ross, *et al.* 2018). An example is the smartphone-based microplate reader developed by Fu, *et al* (2016), which involves spectrophotometric analysis using the phone camera.

Besides labelling, some respondents (Figure 6) believed that food handling legislation should be changed. Under EU law, there are no specific regulations for handling of allergenic foodstuffs (Annex II to Regulation 1169/2011, 2011). A possible change in catering facilities includes separate preparation areas for allergen-free food (supported by 26% of respondents). A beneficial change recommended by 40% of respondents (Figure 6) was improved allergen-free menu certification, in a similar vein to the Coeliac Society of Ireland (2020)'s "*Gluten-Free Promise*" campaign. Similarly, an updated list of caterers reviewed for allergen-awareness was supported by 44% of respondents in the current survey. It was found that 17% of parents supported the establishment of a food allergy support group. Support groups can aid the child in developing self-sufficiency (e.g. adrenaline auto-injector tutorials) which can reduce parental burden (Sharma, *et al.* 2012). There currently exists a parental guide provided by IFAN (2018) to help parents manage their child's allergy as 50% (see Figure 6) of parents wanted a parental guide, this suggests a need for increased awareness of such guides among parents. Overall, the current demand for services such as these highlights the gap in food allergy care in Ireland left by Anaphylaxis Ireland.

One of the limitations of the study was the small sample size (n =50) which is not representative of the whole Irish population, also most respondents came from Cork (n =31). A more thorough distribution of the survey nationally with a greater sample size would address this limitation and provide more representative data. Due to the methods used to distribute the surveys, such as via college email, the older population were not represented as well as the younger population.

In conclusion, a notable strength in Irish allergy care is the effectiveness of the treatment strategies currently available. Faster wait times indicate that paediatric immunology appears better resourced than the adult counterpart. The 50% rise in Irish cases and 700% increase in hospitalisations in the past decade highlights food allergy as a serious and urgent public health issue (Cahill, 2020). Necessary

changes include a decrease in wait-times because they currently put public patients at risk and increasing the role of the dietician for a holistic approach. Better GP and public awareness of food allergies, as well as improved legislation for food labelling/handling are also needed. Reinstatement of Anaphylaxis Ireland is recommended to lobby for these services and fill the gaps seen in patient support in this country.

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REFERENCES

Anaphylaxis Ireland, 2011. Anaphylaxis Ireland | Supporting Those Living With Severe Allergies. [online] Available at: <http://www.irishanaphylaxis.org/> [Accessed 3 April 2020].

Anon, 2017. Census Of Population 2016 - Profile 3 An Age Profile Of Ireland. [online] Available at: < https://www.cso.ie/en/releasesandpublications/ep/p-cp3oy/cp3/> [Accessed 28 March 2020].

Anon, 2020. Allergy Help | Allergy Relief & Advice | Allergy UK. [online] Available at: <https://www.allergyuk.org/get-help> [Accessed 27 March 2020].

Anon, 2020. Eating Out | Allergy Friendly Restaurants | Allergy UK. [online] Available at: <https://www.allergyuk.org/get-help/eating-out> [Accessed 20 April 2020].

Aronson, J., 2011. How to attract, retain and nurture young academic clinicians. Journal of the Royal Society of Medicine, [online] 104(1), pp.6-14. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3014560/> [Accessed 30 March 2020].

Aronson, J., 2011. Nutrition and food allergies: the dietitian's role. Annals of Allergy, Asthma & Immunology, [online] 90(6), pp.115-116. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/12839125> [Accessed 30 March 2020].

Banerji, A., Rudders, S. A., Corel, B., Garth, A. P., Clark, S., & Camargo, C. A., Jr (2011). Predictors of hospital admission for food-related allergic reactions that present to the emergency department. *Annals of Allergy, Asthma & Immunology: official publication of the American College of Allergy, Asthma, & Immunology*, *106*(1), 42–48. https://doi.org/10.1016/j.anai.2010.10.011 [Accessed 30 March 2020].

Boyce, J., Assa'ad, A. and Burks, W., 2010. Guidelines for the Diagnosis and Management of Food Allergy in the United States. J Allergy Clin Immunol, [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4241964/> [Accessed 1 April 2020].

Brożek, J., Terracciano, L., Hsu, J., Kreis, J., Compalati, E., Santesso, N., Fiocchi, A. and Schünemann, H., 2012. Oral immunotherapy for IgE-mediated cow's milk allergy: a systematic review and meta-analysis. Clinical &

Experimental Allergy, 42(3), pp.363-374 “https://www.ncbi.nlm.nih.gov/pubmed/22356141” [Accessed 2 April 2020].

Cahill, O., 2020. Why has there been a global increase in food allergies?. *RTÉ Brainstorm*, [online] Available at: <https://www.rte.ie/brainstorm/2018/1112/1010346-why-has-there-been-a-global-increase-in-food-allergies/> [Accessed 18 May 2020].

Chu, D., Wood, R., French, S., Fiocchi, A., Jordana, M., Waserman, S., Brożek, J. and Schünemann, H., 2019. Oral immunotherapy for peanut allergy (PACE): a systematic review and meta-analysis of efficacy and safety. The Lancet, 393(10187), pp.2222-2232 “https://www.ncbi.nlm.nih.gov/pubmed/31030987”. [Accessed 29 March 2020]

Coeliac Society of Ireland, 2020. Benefits Of Membership - Coeliac Society Of Ireland. [online] Available at: <https://www.coeliac.ie/join-now/benefits-of-membership/> [Accessed 29 March 2020].

Conlon, N., *et al.* 2014. Allergy in Irish adults: a survey of referrals and outcomes at a major centre. Irish Journal of Medical Science (1971 -), [online] 184(2), pp.349-352. Available at: <https://link.springer.com/article/10.1007/s11845-014-1117-0#citeas>. [Accessed 29 March 2020].

Denman, A., 1981. What is clinical immunology?. Journal of Clinical Pathology, [online] 34(3), pp.277-286. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1146478/?page=3> [Accessed 30 March 2020].

Doyle, P., 2019. Non-Specialists Need To Improve Allergy Skills, Advises Consultant. [online] Irish Medical Times. Available at: <https://www.imt.ie/news/non-specialists-need-improve-allergy-skills-advises-consultant-30-09-2019/> [Accessed 29 March 2020].

Fogarty, C. and Cronin, P., 2008. Waiting for healthcare: a concept analysis. Journal of Advanced Nursing, 61(4), pp.463-471 “https://www.ncbi.nlm.nih.gov/pubmed/18234043”. [Accessed 1 April 2020].

FSAI, 2020. Food Hypersensitivity: Food Allergy and Intolerance Leaflet. Food Safety Authority Ireland, [online] pp.1-2. Available at: <https://www.fsai.ie/legislation/food_legislation/food_information_fic/allergens.html> [Accessed 30 March 2020].

Fu, Q, *et al.* 2016. Novel versatile smart phone based Microplate readers for on-site diagnoses. Biosensors and Bioelectronics, [online] 81, pp.524-531. Available at: <https://www.sciencedirect.com/science/article/pii/S0956566316302408?via%3Dihub> [Accessed 30 March 2020].

Hadley, C., 2006. Food allergies on the rise? Determining the prevalence of food allergies, and how quickly it is increasing, is the first step in tackling the problem. EMBO reports, [online] 7(11), pp.1080 -1083. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1679775/> [Accessed 1 April 2020].

Harding, K., Watts, J., Karimi, L., O’Reilly, M., Kent, B., Kotis, M., Leggat, S., Kearney, J. and Taylor, N., 2016. Improving access for community health and sub-acute outpatient services: protocol for a stepped wedge cluster randomised controlled trial. BMC Health Services Research, 16(1). “https://www.ncbi.nlm.nih.gov/pubmed/27506923”. [Accessed 30 March 2020].

Health Service Executive, 2013. A National Model of Care for Paediatric Healthcare Services in Ireland. [online] C.11, pp.2-8. Available at: <https://www.hse.ie/eng/services/publications/clinical-strategy-and-programmes/paediatric-allergy.pdf> [Accessed 30 March 2020].

IFAN, 2018. Parents And Carers. [online] Available at: <http://ifan.ie/parents-carers/> [Accessed 19 April 2020].

IFAN, 2020. Food Allergy In Summary. [online] Available at: <http://ifan.ie/food-allergy-in-summary/summary/> [Accessed 20 April 2020].

INDI, 2020. Food Allergies And Intolerances - Irish Nutrition & Dietetics Institute, INDI. [online] Available at: <https://www.indi.ie/diseases,-allergies-and-medical-conditions/food-allergy-and-intolerance/383-food-allergies-and-intolerances-factsheet.html> [Accessed 21 March 2020].

Kenis, P., 2006. Waiting lists in Dutch health care. Journal of Health Organization and Management, 20(4), pp.294-308 "https://www.ncbi.nlm.nih.gov/pubmed/16921814". [Accessed 31 March 2020].

Kishida, T., Hiromura, Y., Shin-Ya, M., Asada, H., Kuriyama, H., Sugai, M., Shimizu, A., Yokota, Y., Hama, T., Imanishi, J., Hisa, Y., & Mazda, O. (2007). IL-21 induces inhibitor of differentiation 2 and leads to complete abrogation of anaphylaxis in mice. *Journal of Immunology (Baltimore, Md. : 1950)*, *179*(12), 8554–8561. https://doi.org/10.4049/jimmunol.179.12.8554 [Accessed 30 March 2020].

Lanser, B. J., Wright, B. L., Orgel, K. A., Vickery, B. P., & Fleischer, D. M. (2015). Current Options for the Treatment of Food Allergy. Pediatric clinics of North America, 62(6), 1531–1549. https://doi.org/10.1016/j.pcl.2015.07.015 [Accessed 30 March 2020].

Licari, A., Manti, S., Marseglia, A., Brambilla, I., Votto, M., Castagnoli, R., Leonardi, S., & Marseglia, G. L. (2019). Food Allergies: Current and Future Treatments. Medicina (Kaunas, Lithuania), 55(5), 120. https://doi.org/10.3390/medicina55050120 [Accessed 30 March 2020].

Loh, W. and Tang, M., 2018. The Epidemiology of Food Allergy in the Global Context. International Journal of Environmental Research and Public Health, 15(9), p.2043. "https://www.ncbi.nlm.nih.gov/pubmed/30231558". [Accessed 31 March 2020].

Muraro, et al, 2014. EAACI Food Allergy and Anaphylaxis Guidelines: diagnosis and management of food allergy. Allergy, [online] 69(8), pp.1008-1025. Available at: <https://onlinelibrary.wiley.com/doi/full/10.1111/all.12429> [Accessed 1 April 2020].

NICE, 2016. Anaphylaxis Quality standards. National Institute for Health Care and Excellence, NICE [online] Available at: <https://www.nice.org.uk/guidance/qs119/chapter/Introduction> [Accessed 30 March 2020].

NICE, 2018. Food Allergy In Children And Young People, National Institute for Health Care and Excellence, NICE. [online] Available at: <https://www.nice.org.uk/guidance/cg116/evidence/full-guideline-136470061> [Accessed 27 March 2020].

NTPF, 2015. Outpatient National By Specialty As At 26/02/2015, the national treatment purchase fund, NTPF [online] Available at: <https://www.ntpf.ie/home/pdf//2015/02/nationalnumbers/out-patient/National02.pdf> [Accessed 28 March 2020].

NTPF, 2020. Outpatient By Specialty As At 27/02/2020, the national treatment purchase fund, NTPF [online] Available at: <https://www.ntpf.ie/home/pdf//2020/02/nationalnumbers/out-patient/National02.pdf> [Accessed 29 March 2020].

Nucera, E., Ricci, A., Rizzi, A., Mezzacappa, S., Rienzo, A., Pecora, V., Patriarca, G., Buonomo, A., Aruanno, A. and Schiavino, D., 2018. Specific oral immunotherapy in food allergic patients: transient or persistent tolerance?. Advances in Dermatology and Allergology, 35(4), pp.392-396. "https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6130139/". [Accessed 29 March 2020].

Nurmatov, U., Dhami, S., Arasi, S., Pajno, G. B., Fernandez-Rivas, M., Muraro, A., Roberts, G., Akdis, C., Alvaro-Lozano, M., Beyer, K., Bindslev-Jensen, C., Burks, W., du Toit, G., Ebisawa, M., Eigenmann, P., Knol, E., Makela, M., Nadeau, K. C., O'Mahony, L., Papadopoulos, N., Poulsen, LK., Sackesen, C., Sampson, H., Santos, AF., van Ree, R., Timmermans, F., Sheikh, A. (2017). Allergen immunotherapy for IgE-mediated food allergy: a systematic review and meta-analysis. *Allergy*, *72*(8), 1133–1147. https://doi.org/10.1111/all.13124 [Accessed 30 March 2020].

Peters, R., Dang, T. and Allen, K., 2016. Specific oral tolerance induction in childhood. Pediatric Allergy and Immunology, 27(8), pp.784-794. <http://europepmc.org/article/MED/27496561>. [Accessed 1 April 2020].

Platts-Mills, T., 2015. The allergy epidemics: 1870-2010. Journal of Allergy and Clinical Immunology, [online] 136(1), pp.3-13. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4617537/> [Accessed 3 April 2020].

Primeau, M., 2000. The psychological burden of peanut allergy as perceived by adults with peanut allergy and the parents of peanut-allergic children. Clinical & Experimental Allergy, [online] 30(8), pp.1135-43. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/10931121> [Accessed 30 March 2020].

Purcell, N., 2019. Frequently Asked Questions in the Clinical Immunology Department. St. James' Hospital Laboratory Directorate, [online] Available at: <http://www.stjames.ie/Departments/DepartmentsA-Z/I/Immunology/DepartmentOverview/Frequently%20Asked%20Questions%20in%20the%20Clinical%20Immunology%20Department.pdf> [Accessed 30 March 2020].

RCPH, 2011. Allergy Care Pathways for Children Guide, The Royal College of Paediatrics and Child Health, RCPH. [online] pp.1-10. Available at: <https://www.rcpch.ac.uk/sites/default/files/RCPCH_Care_Pathway_for_Children_with_Food_Allergy.pdf> [Accessed 30 March 2020].

Reber, L., Hernadez, JD., Galli, SJ.. 2017. The pathophysiology of anaphylaxis. Journal of Allergy and Clinical Immunology, [online] 140(2), pp.335-348. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5657389/> [Accessed 30 March 2020].

Regulation (EU) No 1169/2011 of The European Parliament and of The Council on the Provision of Food Information to Consumers, 2011, Annex II. Available at: <https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:02011R1169-20180101/>[Accessed 29 March 2020].

Ross, G., Bremer, M., & Nielen, M. (2018). Consumer-friendly food allergen detection: moving towards smartphone-based immunoassays. Analytical and Bioanalytical Chemistry, 410(22), 5353–5371. https://doi.org/10.1007/s00216-018-0989-7 [Accessed 30 March 2020].

Sampson. M, Munoz-Furlong, A., Sicherer, SH., 2006. Risk-taking and coping strategies of adolescents and young adults with food allergy. Journal of Allergy and Clinical Immunology, [online] 117(6), pp.1440-1445. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/16751011> [Accessed 30 March 2020].

Schäfer, T., 2004. Epidemiology of complementary alternative medicine for asthma and allergy in Europe and Germany. Annals of Allergy, Asthma & Immunology, [online] 93(2), pp.S5-S10. Available at: <https://www.ncbi.nlm.nih.gov/pubmed/15330007> [Accessed 30 March 2020]

Sharma, A., Prematta, T. and Fausnight, T., 2012. A Pediatric Food Allergy Support Group Can Improve Parent and Physician Communication: Results of a Parent Survey. Journal of Allergy (Cairo), [online] Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3191814/> [Accessed 30 March 2020].

Turner, P., Gowland, M., Sharma, V., Ierodiakonou, D., Harper, N., Garcez, T., Pumphrey, R. and Boyle, R., 2015. Increase in anaphylaxis-related hospitalizations but no increase in fatalities: An analysis of United Kingdom national anaphylaxis data, 1992-2012. Journal of Allergy and Clinical Immunology, 135(4), pp.956-963.e1. “https://www.ncbi.nlm.nih.gov/pubmed/25468198”. [Accessed 3 April 2020].

Żukiewicz-Sobczak, W., Wróblewska, P., Adamczuk, P. and Kopczyński, P., 2013. Causes, symptoms and prevention of food allergy. Advances in Dermatology and Allergology, 2, pp.113-116. Available at: <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3834685/>. [Accessed 4 April 2020].

A Qualitative Study on the Factors Affecting the Willingness of 3rd Level Students to Register as Organ Donors in Ireland

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ABSTRACT

A wide range of factors contribute to an individual's choice whether or not to register as an organ donor. The knowledge of the Irish population at large around the area of donation and transplantation is varied. A research survey was designed to be completed by third level students. The purpose of the research was to determine the most important factors that played a role in their decisions regarding opting-in to organ donation. A second aim of the survey was to determine the participants' levels of knowledge and understanding on the 'opt in' donation system in place here in Ireland.

The survey was designed on Google forms and distributed online. A total of 315 valid responses were received. The findings of the survey demonstrated that education, and influence from family and friends were the major contributing factors for Irish students when choosing whether or not to register as an organ donor. Other data indicated that over 90% of participants wished to see an 'opt- out' donation system in place in Ireland. The latter finding suggests a need for change to the current legislation. This legislation states that the donor register is compiled from the voluntary 'opting- in' of donors.

KEYWORDS: Organ donation, willingness to donate, 'opt-out' system, 'opt in' system, 3rd level students

INTRODUCTION

Organ donation involves the replacement of a recipient's failing organs with healthy donor organs. Organ transplantation is an essential area of medicine. For some, a transplant can be a matter of life and death. The first organ transplantation occurred in Ireland in 1964 and the rate of organ donation has increased steadily ever since (Umana *et al.*, 2018). WHO defines an organ donor as a 'Deceased or living person from whom at least one solid organ or part of it has been recovered for the purpose of transplantation' (World Health Organisation, 2009). Ireland has one of the highest rates of donation worldwide, with 20.3 donors per million population (Beaumont Hospital Kidney Centre, 2017). In 2019, a total of 274 transplants were performed in Ireland from 85 donors (Connor, 2019). This service could not be performed without a register of organ donors willing to support those in need. However, there is a shortage of organ donors in Ireland, particularly living donors. In 2018, 40 kidney transplants were performed, despite the fact that over 400 people were waiting for the lifesaving treatment at the end of the same year (McMahon, 2019).

Research suggests that a number of factors influence individuals' willingness to register to donate. People's knowledge regarding organ donation and their faith plays a role in their willingness to donate after death (Wakefield, Watts, Homewood, Meiser & Siminoff, 2010). In 2015 in Ireland, a survey was

carried out by Organ Donation and Transplant Ireland on Public Attitudes to Organ Donation. This research was carried out on Omnipoll, Ipsos MRBI's telephone omnibus service. The sample used was RDD (random digit dialling) to ensure that both listed and unlisted phone numbers have the same probability of being contacted. Interviews were conducted via landlines and mobile phones. Of the 1005 people surveyed, 51% (513) were female. This study included individuals from the age of 15 to 65+. It was found that while 81% of the individuals surveyed would be willing to donate organs, only 36% of these individuals carried an organ donor card. Other factors surveyed included the awareness of organ donation. Of the respondents, 26% of individuals stated they were not well informed about organ donation (HSE, 2015).

Furthermore, in Ireland there is currently an opt-in system in place whereby one must register in order to be an available organ donor. This study also investigated if participants would like to see an opt-out system in place, whereby each citizen must legally strike their names from the available donor list. The current study was conducted to determine the factors which affect third level student's willingness to donate organs in Ireland. Responses were sought from university or college students over the age of 18 who are resident in Ireland for at least 6 months of the calendar year. To determine the nature of these factors, the authors' constructed a survey which consisted of a series of questions designed to obtain information on people's attitudes towards organ donation. This paper investigates the attitudes that third level students, in Ireland, have towards organ donation and the various elements that affect their views and willingness to donate organs.

MATERIALS & METHODS

In order to compile information regarding factors affecting the willingness of 3rd level students to register as organ donors, a completely anonymised survey was conducted on 3rd level students over the age of 18 who are ordinarily a resident in Ireland for at least 6 months of the calendar year.

The survey tool used was Google forms. The questions asked included both open questions, where the respondent was able to compose a reply, and closed questions where multiple choices were included for the respondent to select from. All 17 questions were optional, so was possible for respondents to skip questions if desired. An ethics form was completed prior to the distribution of the survey and approved by the MTU Research Ethics Committee.

The survey was distributed using two methods:

1. Promotion using social media websites such as Facebook, Messenger, and WhatsApp.

2. Circulation by the service surveys@umail.ucc.ie which distributed the survey to University College Cork students.

Methods used to carry out hypothesis testing:

Hypothesis testing was used to determine if the differences between specific results were significantly different or just due to sampling. Z scores were calculated using the following formula:

$Z = ((\hat{p} - Po))/(\sigma\hat{p})$

Where $\hat{p}$ = the raw score

Po = the population mean

$\hat{p}$ = the standard deviation

The purpose of the z-scores was to eliminate bias from the results which may have arisen from the difference between population sizes between males and females, and school sizes. Consequently, the results of populations could be compared to one another despite population size differences.

RESULTS

A total of 323 responses was collected from the 19th of February - 2nd of March 2020. Eight responses were deemed invalid as the respondents were not ordinarily resident in Ireland for at least 6 months of the calendar year; 315 of the responses were used to compile the data presented in the results. Microsoft Excel was used to sort the data according to factors including gender, school/department of study and religious beliefs.

Table 2: Registered organ donors according to gender among surveyed college students in Ireland in 2020

	Total	Registered organ donors	Not registered organ donors
Respondents	315	208 (66%)	107 (34%)
Male respondents	77	43 (53.7%)	34 (42.7%)
Female respondents	239	165 (69.2%)	74 (30.8%)

Hypothesis testing to determine if the percentage of registered females could be compared with the percentage of registered males despite the difference in population size:

Null Hypothesis: 69.2% of Females Being Registered with a population of 239 Females can be *comparable to 53.7% of Males Being Registered with a population of 83 Males.*

Alternative Hypothesis: 69.2% of Females Being Registered with a population of 239 Females cannot be comparable to 53.7% of Males Being Registered with a population of 83 Males.

N = 1 $Z=(\hat{p}-po)/(\sigma\hat{p})=(0.692- 0.537)/0.407=0.381$

$\hat{p}$ = .692 $\sigma\hat{p}= \sqrt{(po(1-p)/N)}= \sqrt{(.537(1-.692)/1)}=$ **0.407**

Po = .537

As the Z value is greater than 0.05, the result is not significant, thus, the null hypothesis is accepted. Therefore, the results can be compared despite population size difference between male and female respondents.

The large majority of third-level student respondents were aware of the opt-in system for organ donation in place in Ireland (80.5% of females and 91% of males)

Furthermore, it was determined that 90.5% of respondents are in favour of changing current legislation to allow donation to an organ pool available to anybody in need. Figure 1 shows respondents' preference when it comes to being a living or post-mortem donor.

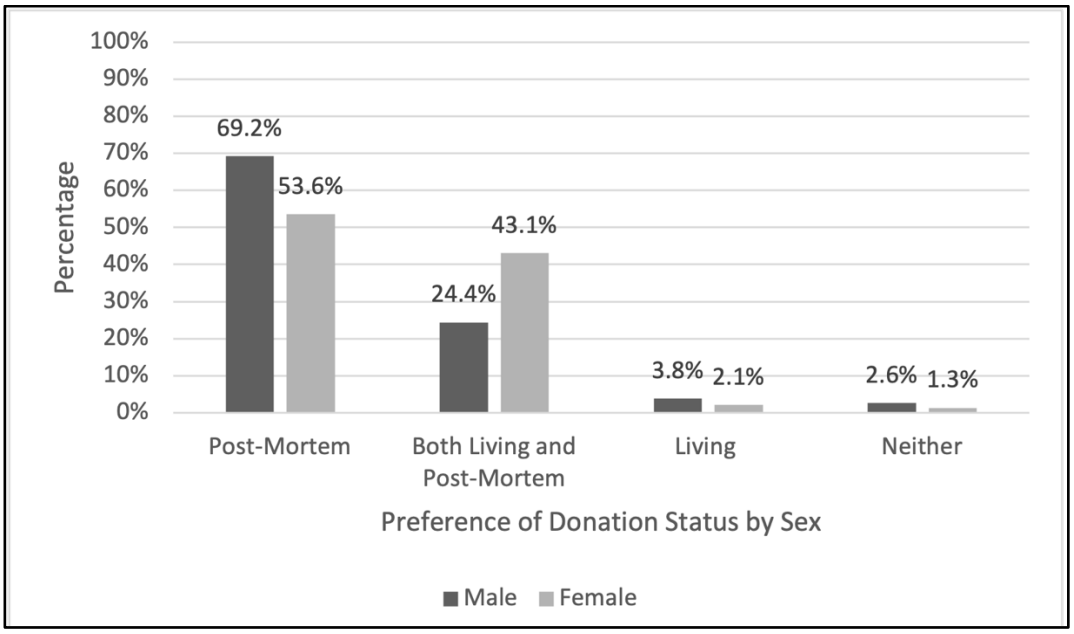

Figure 1: Respondent's preference of donation status (living, post-mortem or both) or non-donation status with results divided by sex (male/female).

Figure 2 examines the subset of the population willing to donate organs and indicates the percentage of the survey respondents willing to donate individual organ types, whereby trends were similar for males and females.

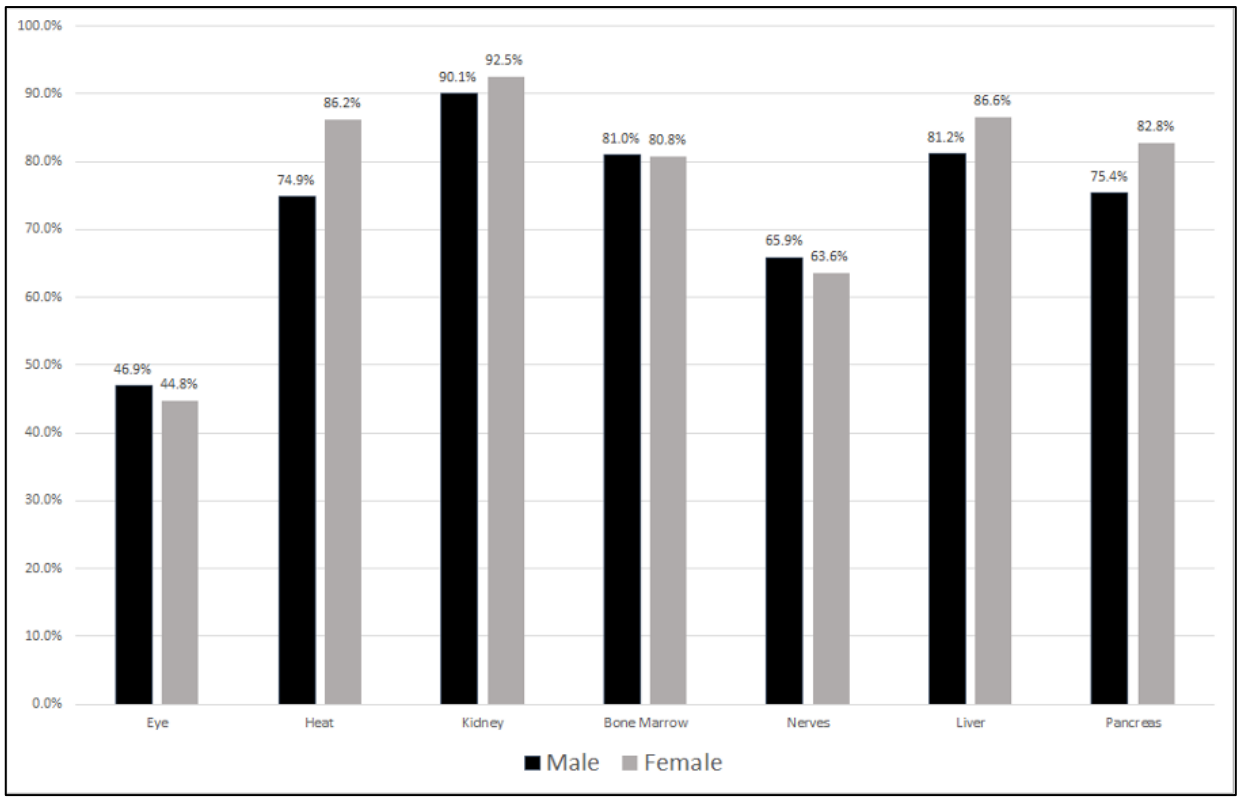

Figure 2: Willingness of participants (%) to donate individual organ types, responses differentiated by gender.

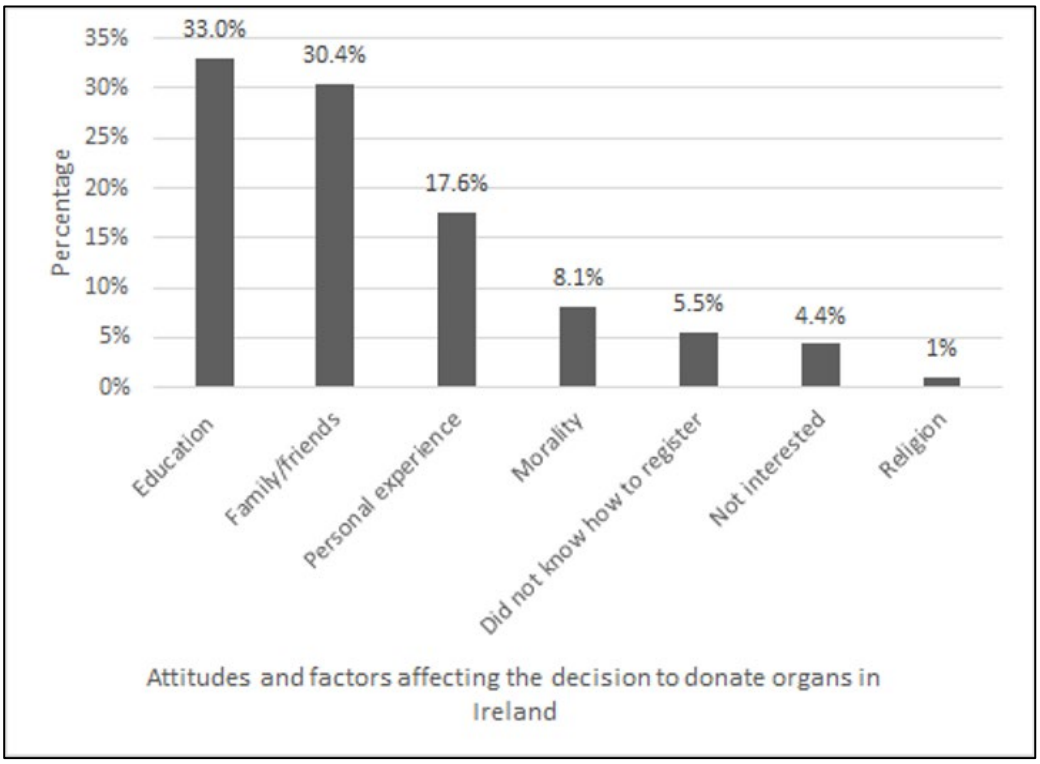

Figure 3: Factors that influence donating participants' decisions to donate organs

Figure 3 shows the results of asking respondents to indicate the factors influencing their decision to be an organ donor, whereby the influence of education and family and friends were the strongest factors cited.

Figure 4a gives an indication of the type of study being undertaken by the respondents and Figure 4b shows the proportion of each of these populations who are registered organ donors whereby the highest proportion of registered organ donors is among those studying medicine and health science (76%) and Science, Engineering and Food Science (66%) and despite population size differences, these comparisons are statistically valid, as indicated by the results of hypothesis testing shown in this section.

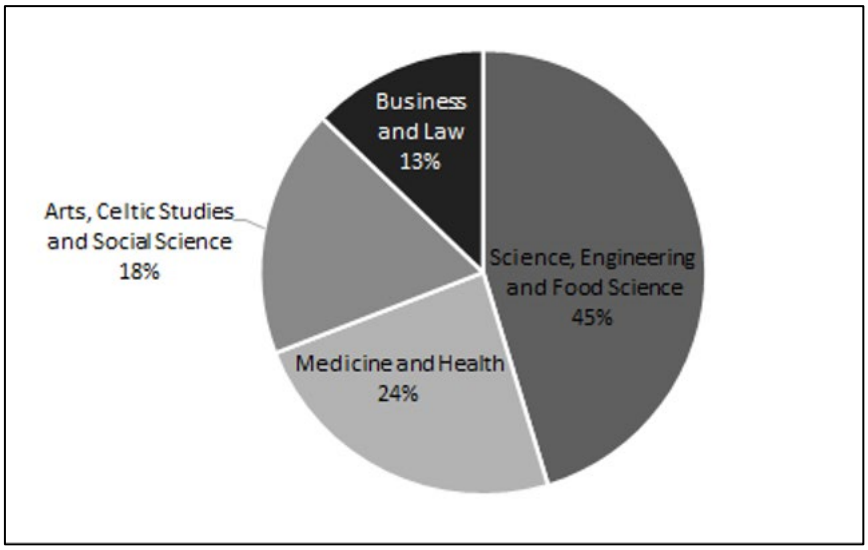

Figure 4 (a): Field of study of respondents

Hypothesis testing was carried out to determine if the results obtained from SEFS students can be compared to the results obtained from respondents from the other fields of study considered, despite differences in population size:

Null Hypothesis: 45.3% of SEFS Students can be comparable to the remainder schools, the lowest being 12.8% for Business & Law.

Alternative Hypothesis: 45.3% of SEFS Students cannot be comparable to the remainder schools, the lowest being 12.8% for Business & Law.

N = 1

p̂ = .453

Po = .128

$Z=(p\hat{}-po)/(\sigma p\hat{}\,)=(0.453-0.128)/0.265=1.23$

$\sigma p\hat{} = \sqrt{(po(1-p)/N)} = \sqrt{(.1.28(1-.453)/1)} = \mathbf{0.265}$

As the Z value is greater than 0.05, the result is not significant, thus, the null hypothesis is accepted, and the results can be compared despite population size differences.

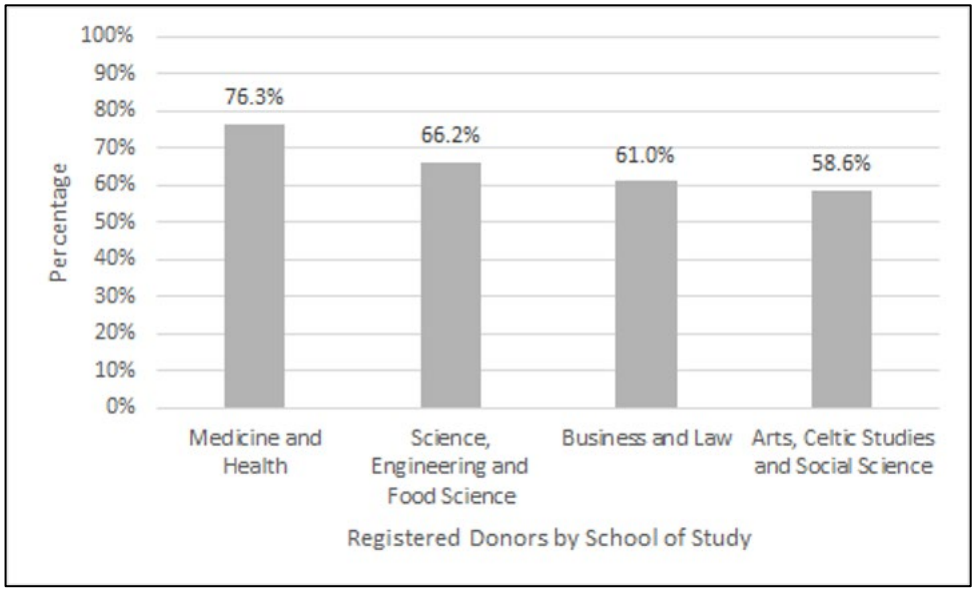

Figure 4 (b): Proportion of students that are registered organ donors, grouped by their field of study.

Figure 5 groups respondents according to whether they report themselves as religious or not and divides each of these two categories into organ donors and non-donors. It is shown that a higher proportion of non-religious respondents are organ donors (33%) versus those who classify themselves as religious (20%).

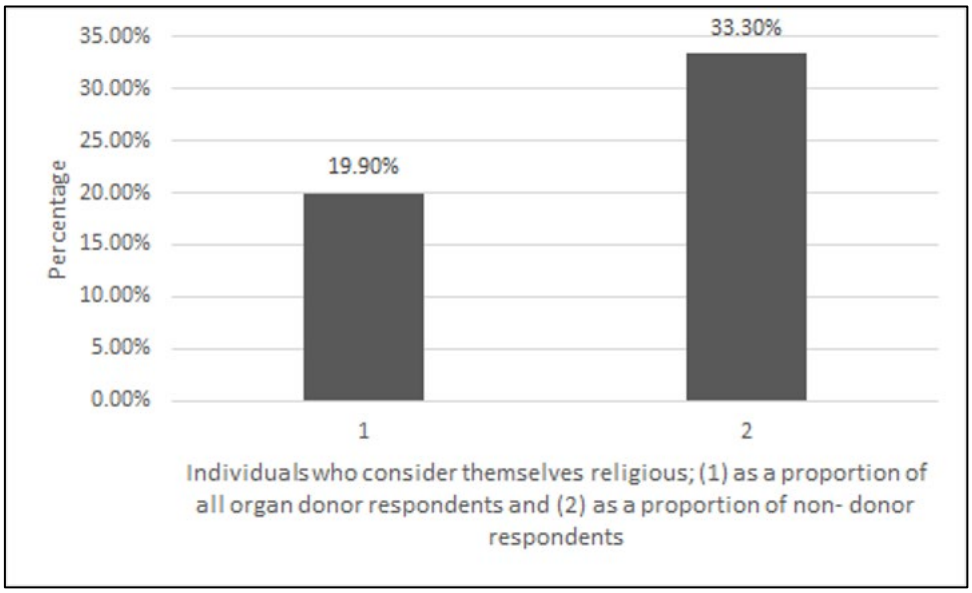

Figure 5: The proportions of all organ donor respondents who consider themselves religious vs. the proportion of all non- donor respondents who consider themselves religious

Hypothesis test to determine if the percentage of non-religious registered organ donors can be compared with the percentage of religious registered organ donors despite differences in population size:

$z > 0.05$

DISCUSSION

This study provided an insight into the various factors which influence Irish third-level students' willingness to register to donate organs in Ireland. According to the HSE 2019, an Irish individual is three times more likely to need a transplant than donate an organ (HSE, 2019). This statistic highlights the importance of encouraging young people to donate organs in Ireland. In Ireland in 2018, 81 donations were made by deceased individuals, resulting in 234 transplant surgeries. A total of 429 patients were awaiting renal transplantation (National Renal Transplant Centre Beaumont Hospital). It is therefore essential to identify the factors that influence willingness to become an organ donor. This survey will subsequently allow these factors to be examined and addressed to persuade more individuals to register to donate.

A total of 53.7% (43) of male respondents were registered organ donors, while 69.2% (165) of female respondents were registered organ donors. The higher percentage of organ donor registration in females may be attributed to several possible reasons. A study carried out by Wilczek-Ruzyczka et al. (2014) found that more females in a given study population tended towards becoming organ donors due to their higher levels of empathy and the influence of other psychological aspects such as beliefs and attitudes.

From the results illustrated in Figure 1, most male and female respondents indicated that they would rather be post-mortem donors than living donors, 69.2% and 53.6%, respectively. Comparably, only a small number of respondents, 3.84% of males and 2.1% of females preferred to be a living donor. This is reflected because only 40 of the total transplants carried out in Ireland in 2018 were living donations (ODTI, 2019). The preference towards post- humous donation may be due to the health repercussions associated with the invasive donation procedure and the potential impact on the quality of the donor's life. In 2009, a survey of living kidney donors was conducted in Germany by Wiedebusch et al. It was found that the donor's quality of life decreased post kidney resection. Pain and additional health

complications were common complaints. It was found that mental health in donors may also deteriorate post-donation, with some donors presenting with mental illnesses, including depression and anxiety.

It is essential to acknowledge that most respondents were interested in becoming organ donors, with only 1.3% and 2.7% of females and males indicating that they had no interest in becoming an organ donor. This percentage suggests that many of the unregistered organ donor participants, representing 34% of the total respondents, would be willing to donate if allowed to do so. This suggests that more practical support to facilitate easier registration for potential donors should be put in place. The current study's findings indicate that more campaigns should be carried out to promote the possibility of living organ donation and its benefits. Morgan et al. (2011) showed that increasing numbers of university staff and students signed organ donation cards and discussed donation with family members, following media and interpersonal campaigns through various university campuses.

From Figure 2, it is evident that respondents had differing preferences for which organs they would be willing to donate. Furthermore, the least popular option was cornea (eye) donation, the most popular being kidney donation. This could be due to several reasons, including concerns regarding disfigurement, the association of eyes with identity or the soul, and concerns about the need for eyes in an afterlife. These concerns are outlined in more detail in a paper titled Specific Unwillingness to Donate Eyes: The Impact of Disfigurement, Knowledge and Procurement on Corneal Donation (Lawlor et al., 2010). Kidneys were the most preferred organ to donate at 90.1%. This may be due to the fact kidney donations are more prominently featured in the media than others. For example, Selena Gomez and Sarah Hyland, two people presumed popular among young adults, have spoken publicly about their experience with kidney donation (National Kidney Foundation, 2017).

Figure 3 showed that there are different influencing factors on any individual's decision to become an organ donor, the strongest being education, family and friends and personal experience of the impact of organ donation. Education was the strongest influence on decisions whether to become an organ donor, suggesting the power of educational campaigns to increase the prevalence of organ donors among the population. Interestingly, in the current study, religious beliefs did not play a role in influencing respondents' decisions to register as an organ donor; shown in Figure 3, only 1% of students surveyed stated that religion influenced their decision to register as an organ donor.

As demonstrated in Figure 4(b), a total of 76.3% of the 76 students who responded studied in the school of Medicine and Health were registered organ donors. Medicine and Health students may be more likely to register as organ donors due to their experience in hospital-based placement. Science, Engineering and Food Science (SEFS) students had the second-highest percentage of registered organ donors with 66% of the 145 participants who study under this school registered. SEFS students may be more exposed to information regarding organ donation via their course content, such as through health and life science modules. These results may suggest a link between education and organ donation which concurs with the results derived that education was the single most significant factor influencing the cohort of students to become organ donors (see figure 4(b)). Business & Law and Arts, Celtic Studies & Social Science students were the least likely to be registered organ donors at 61% and 59%, respectively. It may be that students within these study fields are not likely to register as organ donors because they do not receive as much information on the topic as Medicine & Health and SEFS students.

While Figure 3 shows that only 1% of students surveyed stated that religion influenced their decision to register as an organ donor, there was a substantial difference between religious categories concerning registered organ donation. as illustrated in Figure 5, where 20% of the respondents who were registered organ donors considered themselves in some way religious. In contrast, 33% of the respondents who were not registered organ donors considered themselves religious. It can therefore be inferred that religion may play a subconscious role in the decisions of survey respondents to register as an organ donor or not. Although very few participants classed it as a significant influence, it is clear that those who consider themselves to be religious are less likely to become organ donors than those who are not inclined towards any religion.

The survey results indicated that many people are unaware that in Ireland, an 'opt in' system is in place to donate organs. Ireland is in the minority of European states using this 'opt in' system, with many other countries such as Spain and Croatia, have switched to an 'opt-out" system. 90% of survey respondents said they would like to see an 'opt out' system sanctioned by the Dáil. This 'opt-out' system could see increased organ donations as deceased individuals would be presumed to consent to organ donation before death. There may be ethical concerns about this such as sudden accidents to an individual before they can decide whether they want to be on or off the list. It would be important that next of kin or guardian can speak on behalf of the deceased to finalise whether the deceased's organs may be harvested.

In Ireland, living donors cannot donate organs to a stranger. The current legislation limits living donor programmes to a relative, spouse or close friend (Anatomy Act, 1832). Out of the total respondents, 90.5% of participants said that they would like to see this legislation changed to donate to an organ pool available to anybody in need. This could have an impact on the preference of individuals for living donation. If the current limiting Legislation is changed, it would be interesting to repeat this aspect of the survey to determine its impact on an individual's preference.

In conclusion, there are several factors that influence third level students participating in organ donation. The results of this study showed a substantial difference between the organ donation registration rate of males and females and differences in opinions between the two genders on organ donation. Furthermore, other factors that impacted the opinions of third level students on organ donation and willingness to register to donate included education, family and friends, personal experience, and religion. Many of these results can be compared to a study concerning the level of organ donation–related knowledge and attitude and willingness toward organ donation among a group of university students in Western China (Lei et al., 2018). It was also found in the current study that the majority of surveyed students were aware that there is an 'opt in' system in place for organ donation here in Ireland. This survey successfully illustrates the desire for change in Irish Legislation regarding organ donation. It is possible that if there is an opt-out system rather than an opt-in system in place, the willingness to register as an organ donor will increase. The findings of this study, while encouraging, suggest efforts are needed to heighten further students' awareness on the area of organ donation and transplantation, as it is clear that this plays a significant role in respondents' opinions.

One limitation to this study was that it was carried out online, so the respondents were not supervised by the survey creators and were therefore not able to ask questions about the survey. There may also be bias within the responses as people who have an interest in the topic of organ donation and transplantation are more likely to have completed it. Further research could be carried out with a larger population sample, i.e. either nationally or internationally. The increased sample size may help to obtain a more comprehensive understanding of the factors that affect people's willingness to donate organs in general. If an 'opt-out' system for organ donation is introduced in Ireland, it would be interesting to research the people who decided to deregister and their reasoning behind it. Furthermore, from this research, we see that education in respect of organ donation appears to have a bearing on an individual's decision-making and the fact that male respondents were less likely to be registered organ donors. It is possible that a more targeted education programme specifically for males would see a change in this finding.

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REFERENCES

Beaumont Hospital Kidney Centre (2017) *Facts about Organ Donation in Ireland - Beaumont Hospital*. Available at: http://www.beaumont.ie/kidneycentre-aboutus-nops-factsaboutdonation (Accessed: 29 April 2021).

Connor, Dyane. (2019). 'Record number of lung transplants in 2019'. RTE. 30 December 2019. Available at: https://www.rte.ie/news/ireland/2019/1230/1103603-organ-donation/ [Accessed: 1 April 2020].

HSE, 2019. Organ Donation Transplant Ireland (ODTI) Annual Report 2018. [online] Hse.ie. Available at: https://www.hse.ie/eng/services/news/media/pressrel/odti-report.pdf

Hse.ie. 2021. [online] Available at: <https://www.hse.ie/eng/about/who/acute-hospitals-division/organ-donation-transplant-ireland/publications/organ-donation-and-transplant-ireland-annual-report-2019.pdf>

Lawlor, M., Kerridge, I., Ankeny, R., Dobbins, T. and Billson, F., 2010. Specific Unwillingness to Donate Eyes: The Impact of Disfigurement, Knowledge and Procurement on Corneal Donation. [online] Wiley Online Library. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1600-6143.2009.02986.x

Lei, L., Deng, J., Zhang, H., Dong, H. and Luo, Y., 2018. Level of Organ Donation–Related Knowledge and Attitude and Willingness Toward Organ Donation Among A Group of University Students in Western China. [online] Science Direct. Available at: https://www.sciencedirect.com/science/article/pii/S0041134518302665?via%3Dihub

McMahon, Aisling. (2019). 'Will a new Policy Mean More Organ Donations in Ireland?' RTE. 25 October 2019. Available at: https://www.rte.ie/brainstorm/2019/1023/1085196-

Morgan, S. et al (2010). The University Worksite Organ Donation Project: a comparison of two types of worksite campaigns on the willingness to donate. [Viewed 21 April 2020]. Available at: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1399-0012.2010.01315.x

National Kidney Foundation, 2017. Selena Gomez Reveals She Had A Kidney Transplant. [online] National Kidney Foundation. Available at: https://www.kidney.org/newsletter/selena-gomez-announced-she-had-kidney-transplant

organ-donations-ireland/ [Accessed: 1 April 2020].

Organ Donation and Transplant Ireland. (2015) 'Annual Report 2015' ODTI. 2015. Available at: https://www.hse.ie/eng/about/who/organdonation/publications/odtiarjul15.pdf [Accessed 23 April 2020]

Organs. Transplantation Proceedings.46(8). 2505-2508. [Viewed 26 March 2020]. Available at: https://www.sciencedirect.com/science/article/abs/pii/S0041134514008100?via%3Dihub Rudge, C., Matesanz, R., Delmonico, F. and Chapman, J., 2012. International practices of organ donation. British Journal of Anaesthesia, 108, pp.i48-i55.

Shepherd, L., O'Carroll, R. and Ferguson, E., 2014. An international comparison of deceased and living organ donation/transplant rates in opt-in and opt-out systems: a panel study. BMC Medicine, 12(1).

Umana, E. *et al.* (2018) 'Attitudes and knowledge of healthcare professionals regarding organ donation. A survey of the saolta university health care group', *Irish Medical Journal*, 111(10), p. 838. Available at: https://imj.ie/attitudes-and-knowledge-of-healthcare-professionals-regarding-organ-donation-a-survey-of-the-saolta-university-health-care-group/

Wakefield, Claire E. et al. (2010) 'Attitudes toward organ donation and donor behavior: A review of the international literature' Progress in transplantation (Aliso Viejo, Calif.). December 2010. 20. 380-91. Available at:

https://www.researchgate.net/publication/49783866_Attitudes_toward_organ_donation_and_donor_behavior_A_review_of_the_international_literature [Accessed: 23 April 2020]

Wilczek- Ruzyczka E. et al., (2014). Demographic Variables on Willingness to Donate

Wiedebusch S. et al., (2009). Quality of Life, Coping, and Mental Health Status After Living Kidney Donation. Transplantation Proceedings. 41(5). 1483-1488. [Viewed 26 March 2020]. Available at: https://www.sciencedirect.com/science/article/pii/S0041134509004539 World Health Organisation, 2009.

Global Glossary of Terms and Definitions on Donation and Transplantation. [online] Geneva. Available at: <https://www.who.int/transplantation/activities/GlobalGlossaryonDonationTransplantation.pdf?ua=1> [Accessed 29 April 2021].

An Investigation into Over the Counter Painkiller Use

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ABSTRACT

This study comprises a survey to examine the use, risks, and awareness of over-the-counter (OTC) pain medication. The survey was a paper-based survey extended to the general public in Cork, Ireland from February 24th 2020 to March 14th 2020. A Microsoft Excel template (16.34 2020) was used to analyse the results of the 106 valid responses that were received. Responses showed that 105/106 individuals had taken an OTC painkiller in their lifetime. Paracetamol was the most used OTC painkiller with 98.1% of people having taken it in the past. The overall majority of individuals were aware of the risks associated with OTC painkiller use. However, there were a large number of people that were unaware of the serious risks and dangers. A higher proportion of individuals were willing to take a second dose sooner than recommended (41.9%), rather than a higher dose (36.2%), if they were in significant pain. In terms of taking a dose sooner than recommended; 43.7% of ibuprofen users and 35% of paracetamol users were unaware of the adverse health consequences. Regular users of OTC painkillers were generally more aware of the risks when compared to irregular users. This study supports the need for further education on the risks of OTC painkiller use as there was a large proportion of individuals willing to take higher doses than recommended, and many were unaware of the drug's associated risks.

INTRODUCTION

Analgesics, also known as painkillers, are drugs that are used to treat pain. Over-the-counter (OTC) painkillers can be bought without a prescription in stores or pharmacies. They are generally used to provide temporary relief from pain associated with inflammation. Painkillers have an estimated worldwide usage of more than 30 million per day (Singh, 1999). In Ireland, 23% of the Irish pharmaceutical market consist of analgesics (IPHA, 2009). OTC painkillers give consumers great freedom to self-medicate and have control over their health. However, there are risks associated with their use and thus users should adhere to the accompanying instructions. OTC painkillers should not be used for more than a few days in a row, and the specified maximum daily dose should not be exceeded (IQWIG, 2006).

Common OTC pain medications can be divided into nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen (Paracetamol, Panadol). NSAIDs include aspirin (Disprin, Excedrin), naproxen (Aleve), and ibuprofen (Nurofen, Buplex). According to the Health Products Regulatory Authority's (HPRA), many aspirin and paracetamol containing products are classified as a general sales medicine, which means they can be sold by both non-pharmacy and pharmacy retailers (HPRA, 2021). However, ibuprofen-containing products can only be sold by pharmacy retailers.

As NSAIDs, both aspirin and ibuprofen have an anti-inflammatory, analgesic, and antipyretic effect. They inhibit the production of prostaglandins (PGs), which are typically released in response to illness or injury, through the inhibition of two cyclooxygenase enzymes (COX-1 and COX-2) (Gunaydin and Bilge, 2018). Anti-platelet effect is also observed through the reduction of thromboxane A2 production

(Al-Saeed, 2011). Most common unwanted effects of NSAIDs involve the gastrointestinal tract (GI bleeding, symptomatic ulcer disease, perforation of the GI tract) due to the inhibition of gastric COX-1(Al-Saeed, 2011). COX-1 is responsible for the production of prostaglandins that inhibit acid secretion and protect the mucosa (Al-Saeed, 2011). While therapeutic doses are normally well tolerated in healthy individuals, they can cause renal insufficiency in susceptible individuals due to the inhibition of prostaglandin E2 and prostaglandin I2 which are involved in the maintenance of renal blood flow. Failure to excrete these drugs may exacerbate toxicity in other organs such as the liver (Hörl, 2010).

NSAIDs can also contribute to disturbances in platelet function. Inappropriate use of ibuprofen can result in serious cardiovascular events such as myocardial infarction, angina, stroke, and death (Al-Saeed, 2011). A British study involving two NHS hospitals in Merseyside in England showed that NSAIDs are responsible for 30% of hospital admissions for adverse drug reactions due to bleeding, heart attacks, strokes and renal damage (Davis & Robson, 2016).

Paracetamol (acetaminophen) is marketed as an analgesic-antipyretic agent that blocks the production of PG (Jozwiak-Bebenista *et al.*, 2014). They are weak anti-inflammatory agents (Jozwiak-Bebenista *et al.*, 2014). Increasing paracetamol dose above its therapeutic range mainly results in hepatotoxicity (Jozwiak-Bebenista *et al.*, 2014). As the drug is metabolised to a toxic metabolite (N-acetyl-p-benzoquinoneimine), long-term use may result in renal function disorder, higher blood pressure and increased prevalence of heart infarction (Jozwiak-Bebenista et al, 2014). Unfortunately, paracetamol overdoses can happen. In Ireland, there were 7933 recorded cases of drug overdose in 2004, of which 31% involved paracetamol (Mhaolain *et al*, 2007) It is estimated that in the United States nearly one in four adults consume a drug containing acetaminophen each week (Kaufman *et al*, 2002).

The study carried out specifically examined aspirin, ibuprofen, and paracetamol, as well as areas such as drug labelling, dose frequency and duration of use. The purpose of this survey was to note the general public's awareness of OTC painkillers in Ireland and to detect any patterns of misuse and negligence.

MATERIALS AND METHODS

The survey was a paper-based survey extended to third-level students and the authors' family, friends, and workplaces. The sample population studied were all over 18 years old in age. Upon analysing the survey, the individuals who have taken OTC painkillers were segregated into regular and irregular users for a particular drug to effectively analyse potential risks associated while taking that drug.

The survey was distributed from the 24th of February 2020 until the 14th of March 2020. Initially, the paper-based survey was tested on a control population to identify unnecessary question. These responses were not used in the data analysis. Paper surveys were and distributed over the course of two weeks (see appendix 1). A Microsoft Excel (Version 16.34, 2020) template was used to analyse the results.

RESULTS

The study consisted of 106 responses. All responses were deemed valid. Males represented 58% of respondents and females represented 42% of responses. The pharmacy was the most common place of obtaining an over-the-counter (OTC) painkiller, compared with retail shops, supermarkets or online.

Paracetamol was the most common OTC painkiller purchased among the Irish population (72.1%), with 23.1% and 4.8% most commonly purchasing Ibuprofen and aspirin respectively.. The data showed that 98.1% of the participants had previously taken paracetamol, 82.9% of participants had previously taken ibuprofen and 68.6% of participants had previously taken aspirin. The survey data also showed that

42.5% of the participants were determined to be regular OTC painkiller users and 57.5% were irregular users. A regular user was defined as someone who had consumed a painkiller more than once in the last 3 or 6 months. The data also shows that approximately half of respondents (51%) have purchased over-the-counter painkillers abroad for future use at home. Only 18.1% of respondents stated they did not think it was important to read the label on an over-the-counter medicine prior to use, while 81.9% of participants deemed it to be important. If using a drug for the very first time, 14.3% of participants were not likely to read the accompanying information compared to 85.7% of participants who would. Older survey participants were more likely to read the information when using an OTC for the first time. 52% of 26-39 year-olds, 59% of 40-59 year-olds and 56% of those 60+ years stated they would read the accompanying information when using the drug for the first time compared to 38% of those aged 18-25 years old. One participant out of the 106 had never taken an over-the-counter painkiller. Hence, figures 2-5 concern 105 of the 106 participants.

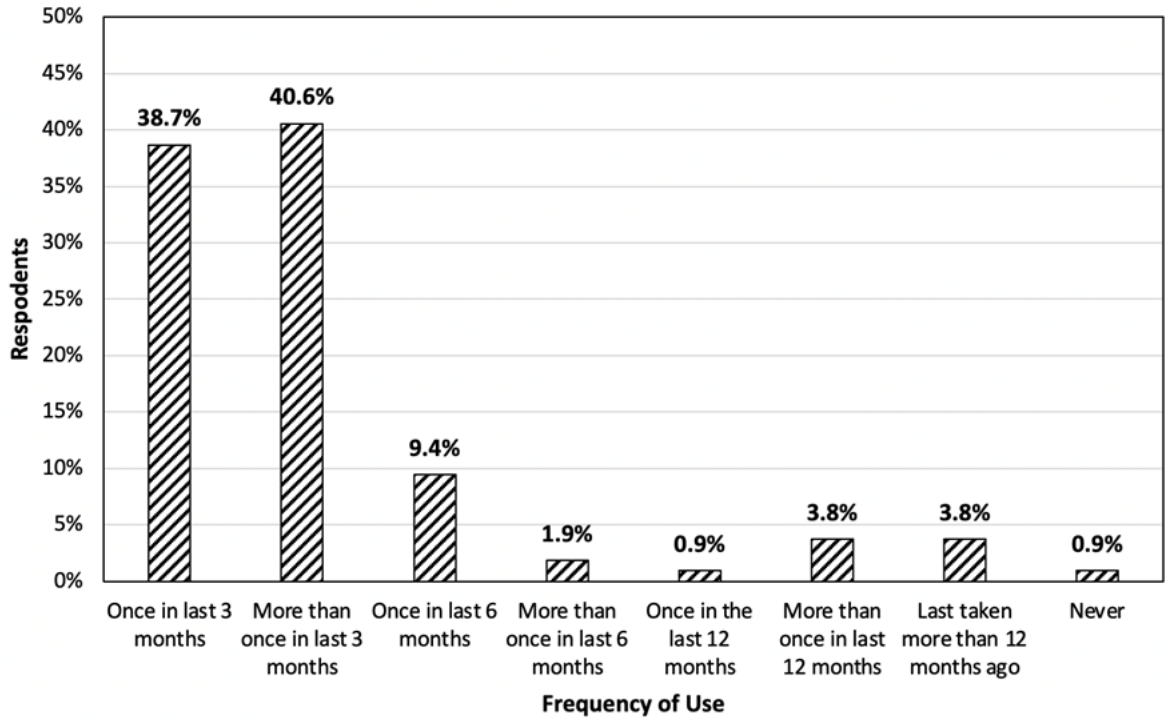

Figure 1: Frequency of over-the-counter painkiller intake among the Irish population in three-month period. The majority of the participants (79.3%) took an over-the-counter painkiller in the last three months as shown in figure 1.

A significant proportion of people were willing to take a higher dose than recommended for each drug and even more participants were willing to take a second dose sooner than recommended.

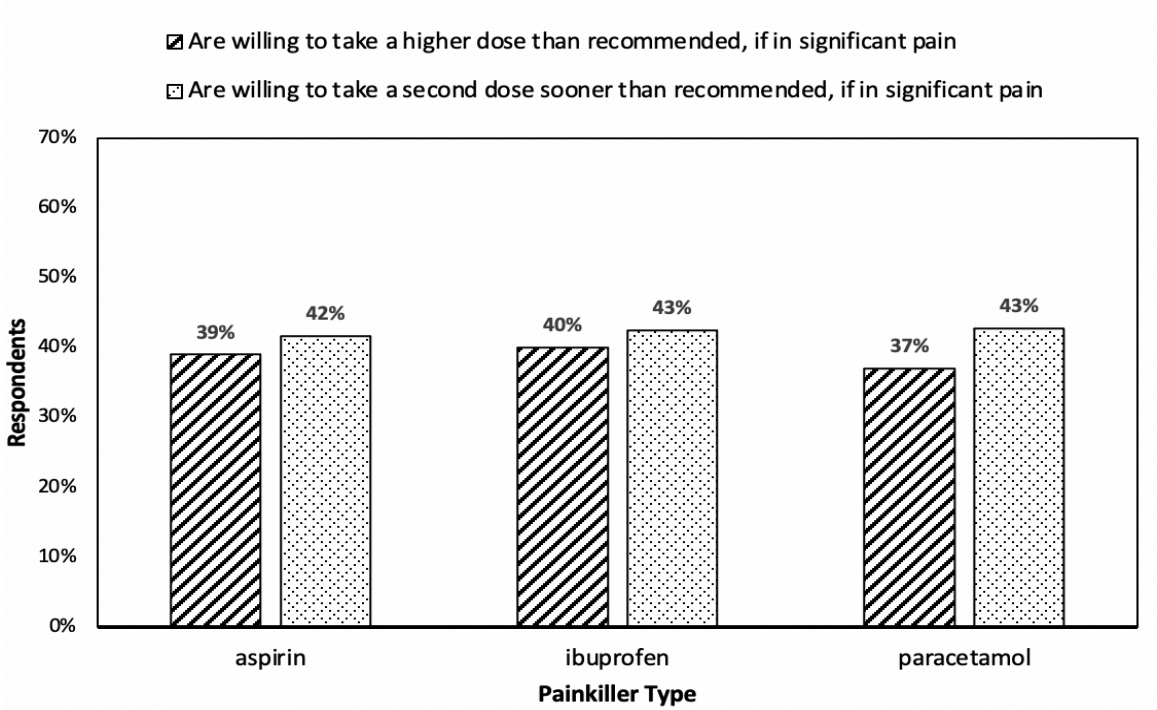

Figure 2: Percentage of participants who are willing to take a sooner subsequent dose or more than the recommended dose of aspirin, ibuprofen or paracetamol if they were experiencing significant pain

Overall, 36.2% of participants were willing to take higher than the recommended dose if they were in significant pain and 41.9% said they would be willing to take another dose sooner than recommended (not shown in figure).

Survey data also showed that 28.2% of respondents would not tell their dentist if they were taking aspirin prior to an appointment including 7% of regular aspirin users, 15.5% of respondents were unsure and 56.3% of respondents would tell their dentist prior to an appointment. Of respondents 12.5% believed high blood pressure could be treated with aspirin and 15.3% of respondents thought aspirin could be used to treat stomach pain both of which are not recommended.

A significant proportion of respondents were willing to take over-the-counter drugs, for multiple days. 25.35% of respondents were willing to take aspirin for more than 4 days, which is longer than recommended, 16.9% stated they would take it until the pain was gone. 21.84% of respondents stated they would take ibuprofen until the pain was gone as did 24.27% of respondents for paracetamol. Paracetamol should not be taken for over 10 days and 7.77% of participants stated they were willing to take the drug for more than 10 days.

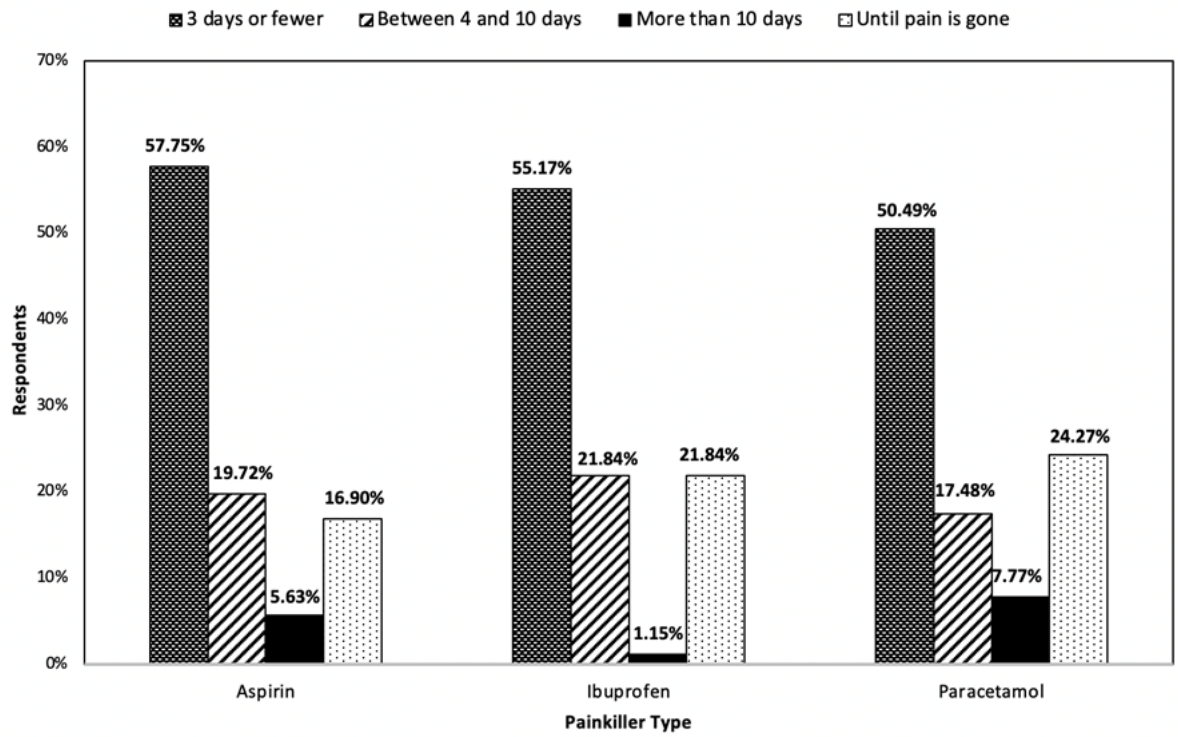

Figure 3: Percentage of individuals who were willing to take a specific drug for a certain period of time before seeking medical attention.

Of all Ibuprofen users, 49.4% were unaware of the health consequences or risks associated with the drug. This was reported as 50% for regular users and 48.8% for irregular users.

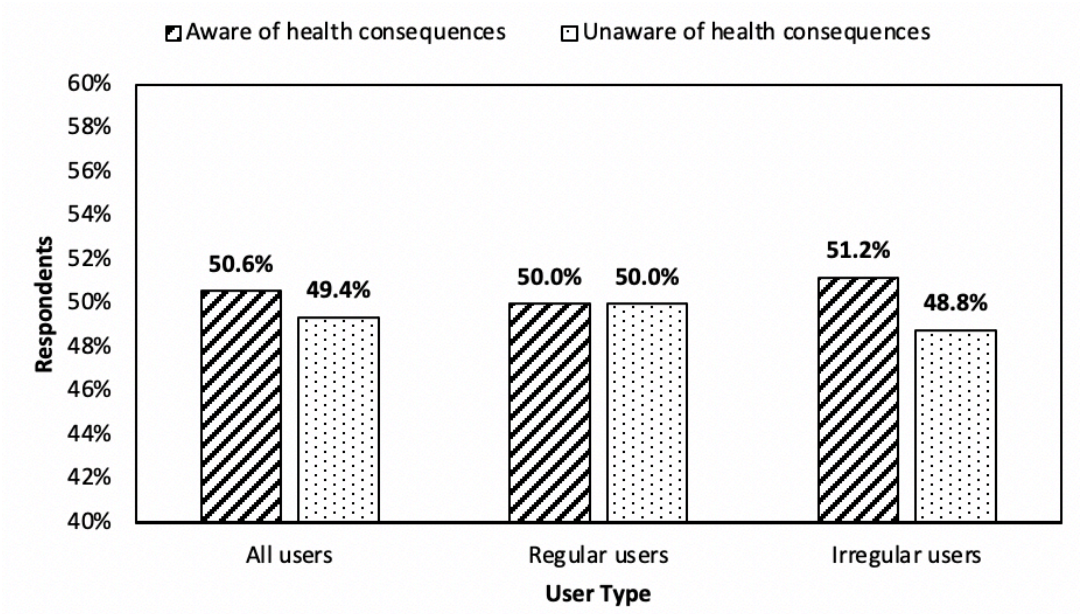

Figure 4: Analysis of the study population to determine their awareness of the health consequences associated with repeat doses of ibuprofen-containing product sooner than directed.

Survey data also shows that 34% of regular users of ibuprofen were unaware of their blood pressure status while 66% of users were aware (data not shown). The survey data also showed that 5.7% of ibuprofen users believed that it could treat hypertension.

Regular users of paracetamol were much more aware of the risks and health consequences than those who only used it occasionally, however 38% of regular users did not know the health consequences and risks associated with the drug. More awareness was seen in older age groups with 71% of those over 70 years old, 78% of those aged 60-70 and 66% of those 40-59 years old being aware of the health consequences compared to 33% of 18-25 year olds.

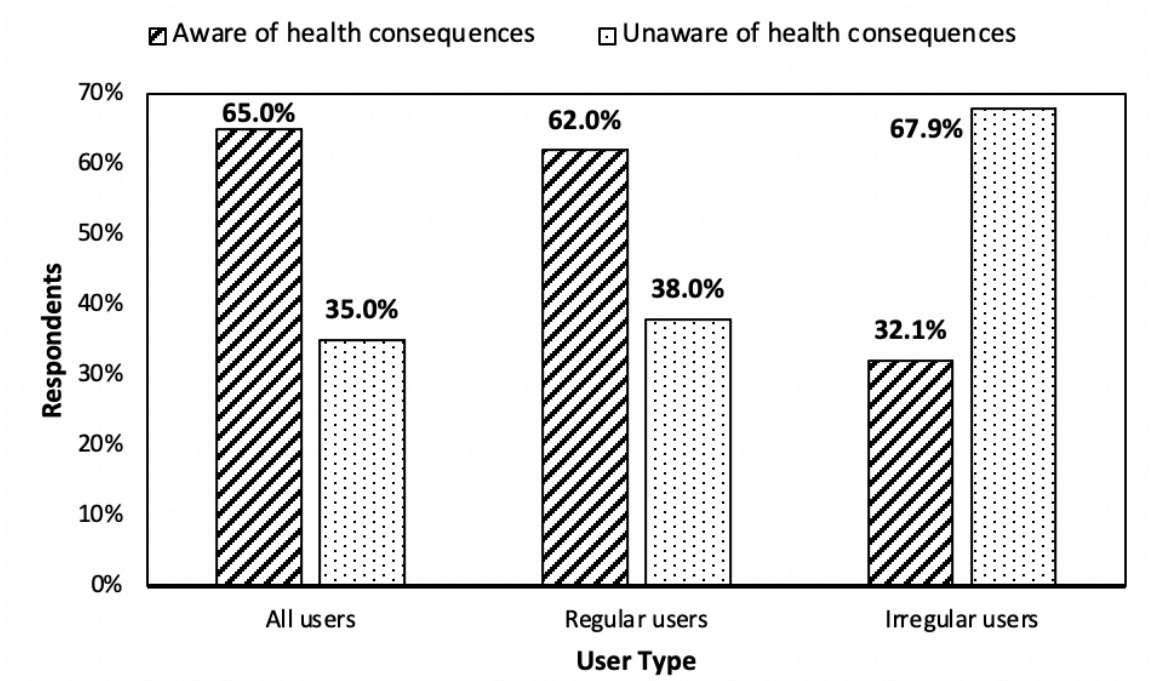

Figure 5: Analysis of the study population to determine their awareness of the health consequences associated with repeat doses of paracetamol taken sooner than directed.

DISCUSSION

As seen in Figure 1, survey data showed that 79.3 % of participants had taken non-prescription analgesics in the last three months prior to participation in the survey. Fifty-one percent of participants said they had purchased OTC painkillers, abroad for later use at home which are often of higher concentration than those available in Ireland. Up to recently, 600 mg ibuprofen tablets were available in Spain over-the-counter according to the Spanish Agency of Medicines and Medical Products (Agencia Española de Medicamentos y Productos Sanitarios, 2020). The ease of availability of these doses circumvents the strict protocols of pharmacists dispensing non-prescription products in Ireland. For instance, it is prohibited for pharmacies in Ireland to sell more than 24 paracetamol (500 mg) tablets in a single transaction (Mhaolain *et al*, 2007). Failing to adhere to regulations such as these may put consumers' health in danger.

The responses for the painkiller dosage questions in the survey revealed potentially dangerous practices within the Irish population. Almost a fifth of participants (19%) admitted to exceeding the dose of a painkiller in the past, but 36.2% of participants said they would be willing to take higher than the recommended dose if they were in significant pain. A higher percentage of people (41.9%) said they would be willing to take another dose sooner than recommended on the packaging (Figure 2). This shows that a number of participants would not be willing to take a higher dose when in significant pain and yet would take another dose sooner than recommended. The National Council on Patient Information and Education in 2002 (Harris Interactive, 2002) commissioned a survey to identify opinions that influenced self-medicating behaviours of Americans. The survey found that a third of Americans said that they take more than the recommended dose of a non-prescription medicine in order to increase the effectiveness of a product. Sixty-three percent reported taking the next dose sooner than

directed. The results from both of these surveys suggest that people are more likely to take a sooner subsequent dose rather than taking a higher dose at a given time. These results seem to suggest that there may be a belief that taking a sooner dose is not as dangerous as taking a higher dose despite the increased amount of drug in the body. Taking a dose sooner than directed can still increase the risk of overdosing (National Health Service, 2017).

The survey data showed that relatively few individuals (18.1%) believed reading the label including the dosage information to be important prior to taking the drug and 82.9% of people did not think doing this was important. It was also asked how likely participants were to read the labelling information when taking a specific brand/type of drug for the first time, to which 14.3% of participants stated they were not likely to read the information. This is concerning as these participants do not make themselves aware of the risks or dosage information prior to their first use and will likely not check the label and information routinely in the future. Overall individuals were more vigilant about checking the label when using the drug for the first time with increasing age. (38% of 18-25 years, 52% of 26-39 years, 59% of 40-59 years, 56% of 60+ years).

Most participants said they take OTC painkillers for 3 days or less before seeking medical attention (Figure 3). Most of the individuals were adhering to the recommended length of use for each drug e.g. 3 days or less for aspirin and 10 days or less for ibuprofen according to the National Health Service (2018) and 3 days or less for paracetamol (Pfizer Healthcare Ireland, 2018). However, there was still a large percent of individuals (17.48%) that would wait between 4 and 10 days for paracetamol and a smaller percentage of them that would wait for more than 10 days. The results showed that the percentage of individuals who would wait until the pain was gone, and not seek any medical advice were 16.9%, 21.84% and 24.27% for aspirin, ibuprofen and paracetamol respectively. This contradicts the information given with each of the over-the-counter painkillers and shows that a large percentage of individuals may not be aware of the effects of prolonged use of these OTC painkillers.

As can be seen in Figure 2, 39% of participants were willing to exceed the recommended dosage of aspirin if they were in significant pain. The main risks and complications of aspirin use include gastrointestinal bleeding and strokes (Ittaman *et al*. 2014). Aspirin is known to help prevent clot-related strokes (National Health Service, 2018); however, it may increase the risk of a bleeding stroke which is associated with taking high doses of aspirin or taking aspirin more frequently than recommended. The survey data obtained show that 57.7% of aspirin users would only take non-prescription aspirin for 3 days or less. This data is encouraging as it decreases their risk of bleeding and complications dramatically. In 2009, the FDA issued a warning about serious stomach bleeding risks with aspirin and other NSAIDS (FDA, 2009). The warning also stressed that those over 60 years of age are at a particularly high risk of bleeding. According to the NHS, aspirin should not be used for over 2-3 days without consulting a doctor (National Health Service, 2018). Almost a fifth of participants (19.7%) stated that they would use it between 4 and 10 days and 5.6% of people stated that they would take non-prescription aspirin for more than ten days putting them at an increased risk.

The dosage of over-the-counter aspirin is formulated to be taken only on a short-term basis. OTC aspirin intended for pain relief should not be used as a preventative measure by healthy individuals. Failing to do so may lead to haemorrhaging in the body (Ittaman *et al*, 2014). A Harvard study that consisted of over 14000 healthy adults, over 40 years old, and free of cardiovascular disease in the United States of America suggested that approximately ¼ of adults who do not have cardiovascular disease take OTC aspirin daily without a doctor's recommendation, (23% in the referenced study). The study concluded that the participants were putting themselves at risk without benefits (Harvard Medical School, 2019). A concerning trend identified in the survey data was that 12.5% of aspirin users said that OTC aspirin could be used to treat high blood pressure and 15.3% of users said aspirin could be used to treat stomach upset and pain. However, it is strongly advised in drug leaflets and by the FDA that any individuals with such conditions should consult a doctor before taking aspirin due to the increased risk of bleeding. (NHS as available 2020).

In the current study, 28.2% of aspirin users did not believe it was necessary to tell their doctor or dentist if they were taking aspirin and 15.5% were not sure if it was necessary to disclose this information. Aspirin inhibits platelet aggregation and causes increased bleeding. Bleeding complications related to aspirin after minor oral surgeries such as tooth extraction is of concern to dentists who are responsible for the dental care and management of these patients (Keun Lee, 2018). The study showed that 7% of regular users did not deem it necessary to tell their doctor or dentist if they were taking aspirin. These are the individuals most at risk of an adverse reaction to aspirin among the survey participants. It is evident that more education is needed on the associated risks as 39-42% of participants were willing to take an exceeded dose of aspirin (figure 2). These individuals are at a higher risk of bleeding, particularly patients who could have underlying conditions which could be exacerbated from excessive bleeding.

Figure 4 shows the percentage of ibuprofen users that were aware that taking another dose of ibuprofen sooner than recommended is associated with an increased risk of cardiac events. Even with regular users, only 50% were aware of this risk. Having a higher amount of ibuprofen in the body than is recommended significantly increases the risk of hypertension and other cardiac events as reiterated in 2015 by the FDA. The FDA strengthened warnings that non-aspirin NSAIDS, including ibuprofen, cause an increased risk of heart attack and stroke.

The data obtained from the survey conducted showed that 40% of ibuprofen users were willing to take a higher dose if in pain and 43% of ibuprofen users were willing to take another dose sooner than recommended on the packaging (Figure 2). In both of these scenarios the individual is exceeding the dose. Unknown underlying health conditions such as peptic ulcers may also exacerbate the effects and cause increased bleeding. (Drini, 2017). Respondents' knowledge of the conditions ibuprofen treats were relatively accurate except for a small proportion. However, 5.7% of participants believed ibuprofen could treat hypertension. This is both untrue and dangerous as ibuprofen can cause a significant increase in blood pressure. The survey data also showed that 34% of regular ibuprofen users were unaware of their blood pressure status. This puts individuals who may unknowingly have hypertension at a significant risk of cardiac events, especially if they take a higher dose of ibuprofen than recommended. All NSAIDs, including ibuprofen, may increase blood pressure and destabilise blood pressure controls in individuals (Grover *et al*, 2005). Researchers in Denmark studied 29,000 patients who suffered an out-of-hospital cardiac arrest and found a 31% increased risk of cardiac arrest when they were using ibuprofen (Sondergaard *et al*, 2016). This study suggested limiting ibuprofen to 1200 mg per day to reduce the associated risks. This is currently the limit in Ireland, however, the data shows that around 2/5 of participants are willing to exceed this (Figure 2).

Regular users of over-the-counter painkillers were more aware of the risks and dangers associated with paracetamol such as metabolic acidosis, depressed consciousness, renal toxicity, hepatotoxicity and liver failure (Tan & Sklar, 2017). As seen in Figure 5, 65% of paracetamol users, of which 62% were regular users, were aware of the risk of liver damage and hepatotoxic events when taking another dose of paracetamol sooner than is recommended. Overall, the respondents had a greater knowledge of the conditions that paracetamol can be used to treat compared to the other two drugs. While these figures were reassuring, there were still 38% of regular users who were not aware of its harmful effects on the liver. There was an interesting trend seen among the different age groups with only 33% of 18-25 year-olds were aware of paracetamol's associated risks to the liver compared to 53% of 26-39 year olds, 66% of 40-59 year olds, 78% of 60-70 year olds and 71% of those over 70 years of age. Older people seemed to have a better understanding of the risks of taking higher doses of paracetamol. Patients suffering from chronic alcohol problems are of particular concern as they are at a greater risk of hepatotoxicity at lower doses of paracetamol. (Chandok & Watt 2010).

Figure 2 shows that 37% of paracetamol users were willing to take a higher dose of the drug when in pain and 43% of users were willing to take another dose sooner than recommended. This is a significant amount of people at risk as the maximum dose within a 24 hour period of paracetamol should not be exceeded as it can lead to serious liver damage and can be fatal. Paracetamol overdose is one of the leading causes of liver failure. . In February 2017, The British Liver Trust stated in an article that

paracetamol overdoses represent approximately 20% of the required liver transplants across Europe, 52% of liver transplants in Ireland and 28% of liver transplants in the United Kingdom (British Liver Trust, 2017). Paracetamol may pose a higher risk to individuals due to the potential damage of one extra tablet over the recommended dose (British Liver Trust, 2017). For that reason, 37-43% of individuals being willing to exceed the dose is alarming (Figure 2).

It is important to emphasise that this research is a snapshot of both the use and awareness of the risks of OTC painkillers among the Irish population. Despite the study showing significant results, there are a number of factors that should be noted for further studies. Firstly, the access to, and scarcity of data on painkiller misuse and associated consequences in Ireland did pose a problem. There were also very little recent studies performed on this topic so some of the supporting studies were over 10 years old indicating a need for further research. Furthermore, a more comprehensive analysis of the awareness of the risks and dangers of OTC painkillers would have occurred if each of the age groups were represented equally in this study. The majority of the sample population was composed of 18-25 year old participants (37.7%), while the > 60 year old category consisted of 15% of the participants. Examining the understanding of package labelling could be an interesting area of future study. Factors including lifestyle habits, their effects on health, and relationship with OTC painkillers is another area that could lead to interesting results. This survey was paper based and hand-distributed, which may have resulted in less respondents when compared to digital methods. However, while the data is limited the results obtained are alarming and have not been previously reported in the Republic of Ireland.

In conclusion, a good understanding of OTC painkillers was seen among the Irish population. However, there remains a large percentage of individuals that take these drugs while being unaware of the risks and dangers associated with them. A concerning number of participants are willing to take more than the recommended dose, whilst being aware of the serious health consequences. Practices such as these show the need for further education, such as advertising campaigns, regarding dangers of OTC painkillers and warrant further studies.

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REFERENCES

Al-Saeed A. (2011) 'Gastrointestinal and Cardiovascular Risk of Nonsteroidal Anti-inflammatory Drugs', *Oman Medical Journal*, 26(6): *385-391.*

British Liver Trust. (2017) 'Researchers shed new light on how paracetamol causes liver damage'. [Online] Available at: https://britishlivertrust.org.uk/researchers-shed-new-light-paracetamol-causes-liver-damage/ [Accessed March 2020]

Davis A, Robson J. (2016) 'The dangers of NSAIDs: look both ways' – *British Journal of General Practice 66(645): 172–173.*

Drini M. (2017) 'Peptic ulcer disease and non-steroidal anti-inflammatory drugs' –*Australian Prescriber 40(3): 91–93.*

Food and Drug Administration. (2015) 'FDA Drug Safety Communication: FDA strengthens warning that non-aspirin non-steroidal anti-inflammatory drugs (NSAIDs) can cause heart attacks or strokes' [Online] Available: https://www.fda.gov/drugs/drug-safety-and-availability/fda-drug-safety-communication-fda-strengthens-warning-non-aspirin-nonsteroidal-anti-inflammatory [Accessed April 2020].

Food and Drug Administration. (2009) 'Warning: Aspirin-Containing Antacid Medicines Can Cause Bleeding' [Online] Available at: https://www.fda.gov/consumers/consumer-updates/warning-aspirin-containing-antacid-medicines-can-cause-bleeding [Accessed March 2020]

Grover SA, Coupal L, Zowall H. (2005) 'Treating osteoarthritis with cycloxygenase-2-specific inhibitors: What are the benefits of avoiding blood pressure destabilization?' - *Hypertension 45:92*

Gunaydin C and Bilge S. (2018) 'Effects of Nonsteroidal Anti-Inflammatory Drugs at the Molecular Level', *The Eurasian Journal of Medicine*, 50(2*): 116–121.*

Harris Interactive. (2002) 'Attitudes and Beliefs about the Use of Over-The-Counter Medicines: A Dose of Reality. A National Survey of Consumers and Health Professionals' [Online] Available at: https://www.bemedwise.org/wp-content/uploads/2019/12/final_survey.pdf [Accessed March 2020]

Harvard Medical School. (2003) Updated December 2019. 'Drug Expiration Dates — Do They Mean Anything? – FDA gets to the heart of expired medicine and safety' [Online] Available at: https://www.health.harvard.edu/staying-healthy/drug-expiration-dates-do-they-mean-anything [Accessed March 2020]

Harvard Medical School. (2019) 'A major change for daily aspirin therapy' [Online] Available at: https://www.health.harvard.edu/staying-healthy/a-major-change-for-daily-aspirin-therapy [Accessed March 2020]

Health Products Regulatory Authority (2021). 'Latest General Sales Product Information'. [Online] The Health Products Regulatory Authority. Available at: https://www.hpra.ie/img/uploaded/swedocuments/latest_general_sales_list.pdf [Accessed April 2020].

Hörl W. (2010) 'Nonsteroidal Anti-Inflammatory Drugs and the Kidney', *Pharmaceuticals*, 3(7): *2291-2321.*

Institute for Quality and Efficiency in Healthcare. (2006) 'Using medication: The safe use of over-the-counter painkillers'. Published online 2016 Available at: https://www.ncbi.nlm.nih.gov/books/ NBK361006/ [Accessed April 2020].

Irish Pharmaceutical Healthcare Association. (2009) '*IPHA* Healthcare Facts and Figures 2009' [Online] Available at: https://www.slideshare.net/iphadotie/ipha-healthcare-facts-and-figures-2009-finalsales -nx-power-lite [Accessed April 2020].

Ittaman SV, VanWormer JJ, Rezcalla SH. (2014) 'The Role of Aspirin in the Prevention of Cardiovascular Disease - *Clinical Medicine & amp; Research*, *12(3-4): 147-154*.

Jozwiak-Bebenista M, and Nowak JZ. (2014) 'Paracetamol: Mechanism of Action, Applications and Safety Concern', *Polish Pharmaceutical Society, 71(1): 11-23.*

Kaufman DW, Kelly JP, Rosenberg L, Anderson TE, Mitchell AA. (2002) 'Recent Patterns of Medication Use in the Ambulatory Adult Population of the United States'- *Journal of the American Medical Association*, 287(3), p.337.

Keun Lee J. (2018) 'Dental management of patients on anti-thrombotic agents'– *Journey of the Korean Association of Oral and Maxillofacial surgery 44(4): 143–150.*

Mhaolain AN, Kelly BD, Breen EG, Casey P. (2007) 'Legal limits for paracetamol sales'- *The Lancet,* 369 (9570): *1346.*

National Health Service. (2017) 'What Should Patients Do If They Miss A Dose Of Their Medicine?' [Online] Available at: <https://www.sps.nhs.uk/wp-content/uploads/2013/12/UKMi_QA_Missed-doses-of-medicines_update_Feb-2017_FINAL.pdf> [Accessed March 2020].

National Health Service. (2018) 'Ibuprofen for Adults: Painkiller' [Online] Available at: <https://www.nhs.uk/medicines/ibuprofen-for-adults/> [Accessed March 2020].

National Health Service. (2018) 'Aspirin for Pain Relief" [Online] Available at: <https://www.nhs.uk/medicines/aspirin-for-pain-relief/> [Accessed March 2020].

National Health Service. (2020) 'Low Dose Aspirin' [Online] Available at: https://www.nhs.uk/medicines/low-dose-aspirin/ [Accessed March 2020]

Pfizer Healthcare Ireland. (2018) 'Pfizer Paracetamol 500mg Film-coated tablets Package Leaflet' [Online] Available at: https://www.hpra.ie/img/uploaded/swedocuments/2205799.PA0822_167_001.f23db9e a-fd91-40a1-9b49-ba0e04159f2e.000001Product%20Leaflet%20 Approved.180509.pdf [Accessed: 25 Mar 2020].

Singh G. (1999) 'Triadafilopoulos G. Epidemiology of NSAID induced gastrointestinal complications' - *Journal of Rheumatology*, *56: 18–24.*

Sondergaard KB, Weeke P, Wissenberg M, Schjerning Olsen AM, Fosbol EL, Lippert FK, Torp-Pedersen C, Gislason GH, Folke F. (2016) 'Non-steroidal anti-inflammatory drug use is associated with increased risk of out-of-hospital cardiac arrest: a nationwide case–time–control study' - European Heart Journal -Cardiovascular Pharmacotherapy, 3(2):100-107.

Spanish Agency of Medicines and Medical Products. Agencia Española de Medicamentos y Productos Sanitarios. Agencia Española de Medicamentos y Productos Sanitarios. (2020) [Online]. Available at: https://cima.aemps.es/cima/publico/lista.html [Accessed: 24 Mar 2020].

Tan CJ and Sklar GE. (2017) 'Characterisation and outcomes of adult patients with paracetamol overdose presenting to a tertiary hospital in Singapore'- *Singapore Medical Journal, 58(12): 695-702.*

An Investigation on the Irish Population's Attitudes and Knowledge Towards Genetic Screening for Cancer

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ABSTRACT

Genetic mutations are alterations in DNA that may result in the development of a disease later in life. A BRCA gene is a tumour suppressor gene that helps to prevent the development of some cancers, particularly breast cancer. If a mutation occurs, this gene no longer functions at preventing these cancers. Genetic screening is when a population is tested for a mutation in an attempt to identify a group of people that are positive for the mutation. This can help identify cancer in different populations as well as track their inheritance. This study was conducted online, questioning the Irish populations opinions on how a genetic mutation would alter their life. Topics covered included having children, illness prevention therapies if a mutation were discovered, and what impact would a mutation have on their life. Comparisons were made between genders, and age groups to demonstrate if differences of opinions exist between each group selected was compared with the overall attitude of the population. It was discovered that there was an overall difference of opinion between the different age groups, but in some questions like the ones regarding children, the opinions were similar. In this study, an investigation was conducted regarding the Irish population's attitudes and existing knowledge towards genetic screening and how testing positive for a genetic mutation, specifically in either the BRCA1 or BRCA2 gene, would influence lifestyle choices.

KEYWORDS: BRCA, gene, mutation, cancer, Ireland, genetic screening

INTRODUCTION

BRCA1 and BRCA2 are tumour suppressor genes whose normal function is to prevent the over-proliferation of cells resulting in tumour growth. The BRCA gene's normal functions are to encode the proteins that are responsible for repairing DNA double strands if they become separated. A mutation causes these repairs to occur incorrectly which can cause disease-causing errors in the DNA sequence resulting in the over proliferation of cells and tumour growth (Stoppa-Lyonnet, 2016). BRCA1 and BRCA2 mutations are hereditary which means they are passed from generation to generation in a bloodline and studies have shown that there is a 50% chance the mutation will be passed down to children if the parent has the BRCA mutation. Both men and women can have the BRCA gene mutation and those carrying a mutation in either gene have a significantly higher risk of developing a number of different cancers.

The most commonly associated cancer with BRCA gene mutation is breast cancer, but there is also associations of ovarian cancer and prostate cancer with mutated BRCA genes. According to extensive studies, 72% of women who inherit a mutated BRCA1 gene and 69% of women who inherit a mutated

BRCA2 gene will develop breast cancer by the age of 80 (Kuchenbaecker, et al., 2017) and approximately 30% of all breast and ovarian cancers are due to mutations in BRCA1 and BRCA2 genes (Mehrgou & M, 2016). Men with a BRCA mutation are at 20% risk of developing prostate cancer and 7-8% chance of developing breast cancer (Ibrahim, et al., 2018).

It is known that there are certain risk factors that may make a person more susceptible to acquiring cancer. These include gender, weight, alcohol consumption and smoking habits (Feng, et al., 2018). By reducing exposure to carcinogens the risk of developing cancer can be reduced. Nicotine, an active ingredient in cigarettes, is carcinogenic and can result in the production of DNA adducts which can evade cellular repair mechanism allowing for permanent mutations, increased cell proliferation in breast tissue, and induction of migration of cancer cells which enhances tumour progression (Kispert & McHowart, 2017). Simple lifestyle choices such as regular exercise and a healthy, balanced diet can improve overall health and reduce risk of developing disease (Harvie, et al., 2015). Positive lifestyle changes, such as by incorporating physical activity and healthy dietary options into everyday routines, could prevent 25% to 30% of cases of breast cancer.

Preventative therapies are available that can reduce the chances of breast cancer, especially among people who are most at risk. These actions of prevention are only an option once genetic screening is performed for the mutation and following extensive counselling, if required. These therapies include selective oestrogen receptor modulators and neoadjuvant therapies, as well as mastectomy (Slepicka, et al., 2019). Therefore, genetic testing for pathogenic (disease-causing) mutations in these genes is very important. Importantly, knowing which gene is mutated will help determine the correct targeted gene therapy to be used. One such strategy is genetic correction, in which the therapy is molecularly targeted to cancer cells and should leave normal cells unharmed (Obermiller, et al., 2000)

MATERIALS AND METHODS

Survey Design and Distribution

A survey was constructed in Google Forms to determine how the presence of BRCA genes affects lifestyle choices. Questions were included that would gather information about topics including family planning, genetic testing of children, lifestyle, and cancer prevention treatments. The survey was trialled on 20 people so that weaknesses in the survey could be identified before the composition of the finalised survey was established (see Appendix 1).

RESULTS

In total, there were 300 participant responses. A total of 300 responses were gathered, 252 of these were from female participants (84%) and 48 responses were from male participants (16%). Regarding age of participants 60% of the responses were between the ages of 18 and 25, and the remaining 40% were aged 26 or older.

Figure 1 shows the male and female responses received to the question posed regarding children – whether to tell them that one had tested positive for the BRCA gene, whether to test them for the gene having tested positive, or indeed, whether to have children at all.

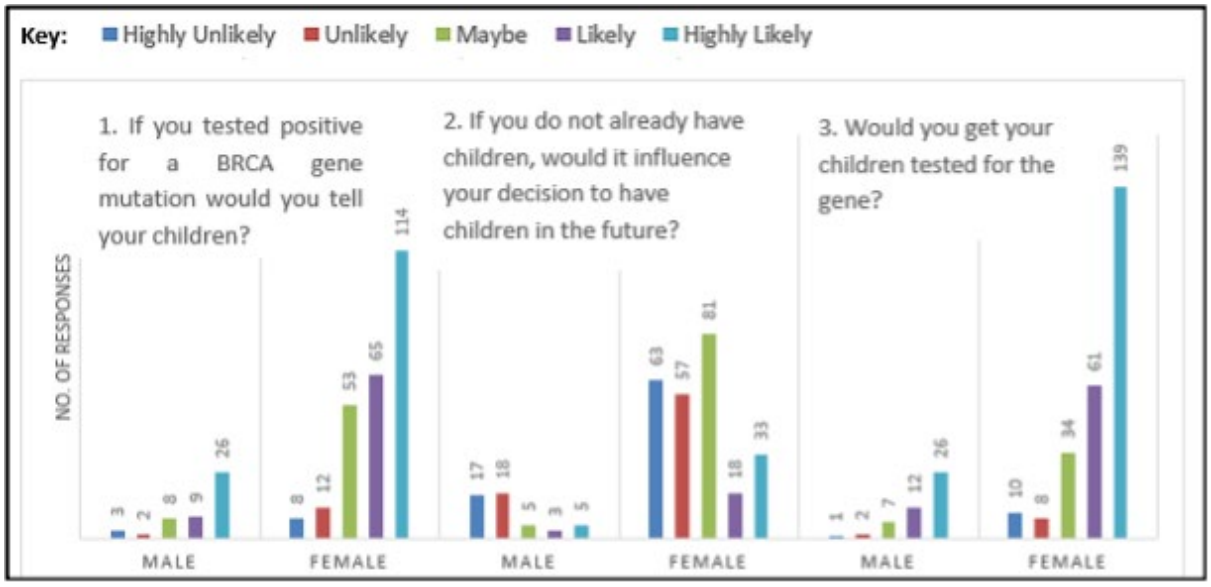

Figure 1. Survey participants' views of the anticipated effect of testing positive for the BRCA gene on their decisions of whether to have children, or, if having children, whether to inform them about the gene

Results of investigating for differences in attitudes by age of the views of the anticipated effect of testing positive on choices regarding children are shown in figure 2. Results show major respondent age-related differences to the question about whether or not to have children.

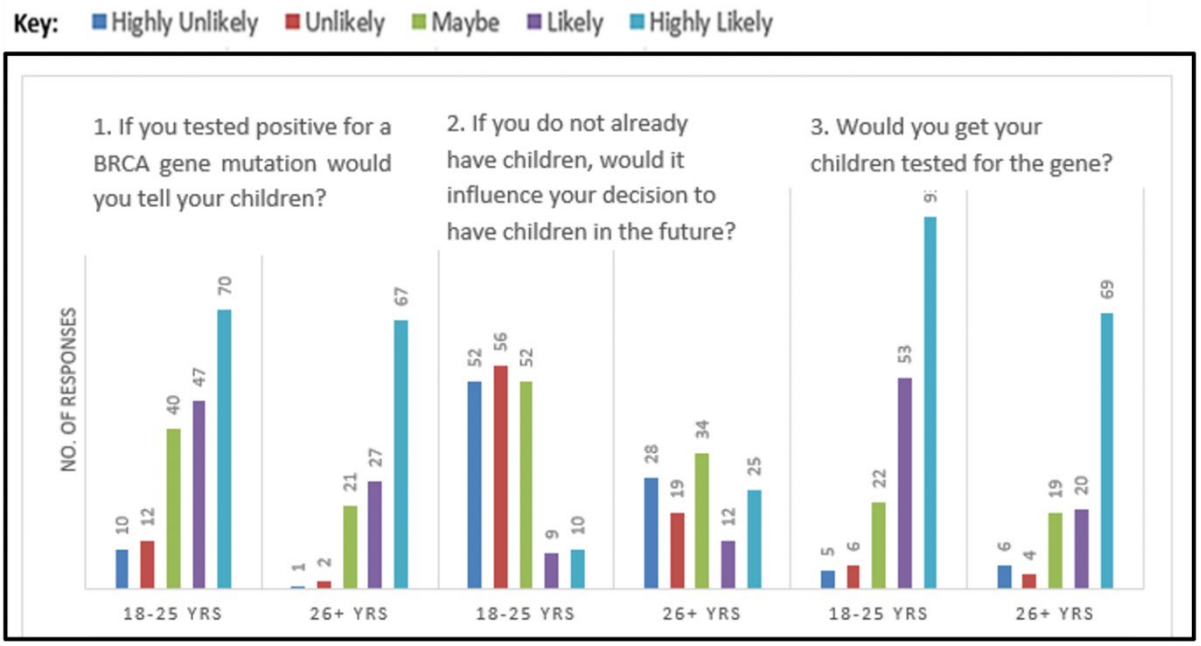

Figure 2. Investigation for age-related differences in response to considerations relating to children after respondents putatively test positive for the BRCA gene

Respondents were asked to consider whether they would opt for a mastectomy after testing positive for the BRCA gene. Figure 3 shows that there is a greater difference between age-groups (18-25y vs >25y) than between male and female respondents.

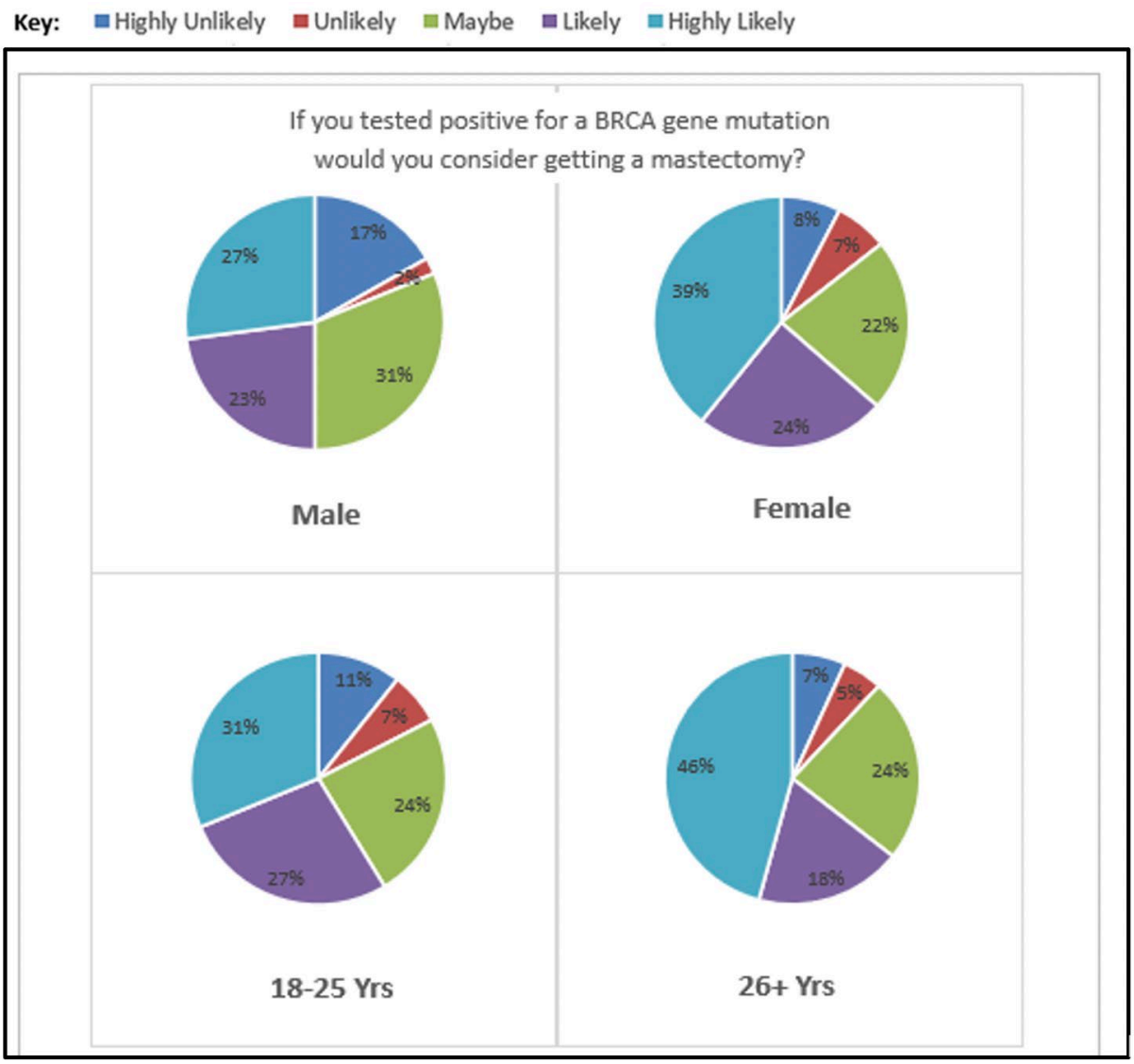

Figure 3. Opinion of respondents when asked whether they would opt for a mastectomy after testing positive for the BRCA gene.

The survey results indicated differences in opinion between different groups when asked about prevention therapies as shown in figure 4. There are differences in opinion between the two age-groups and between male and female respondents.

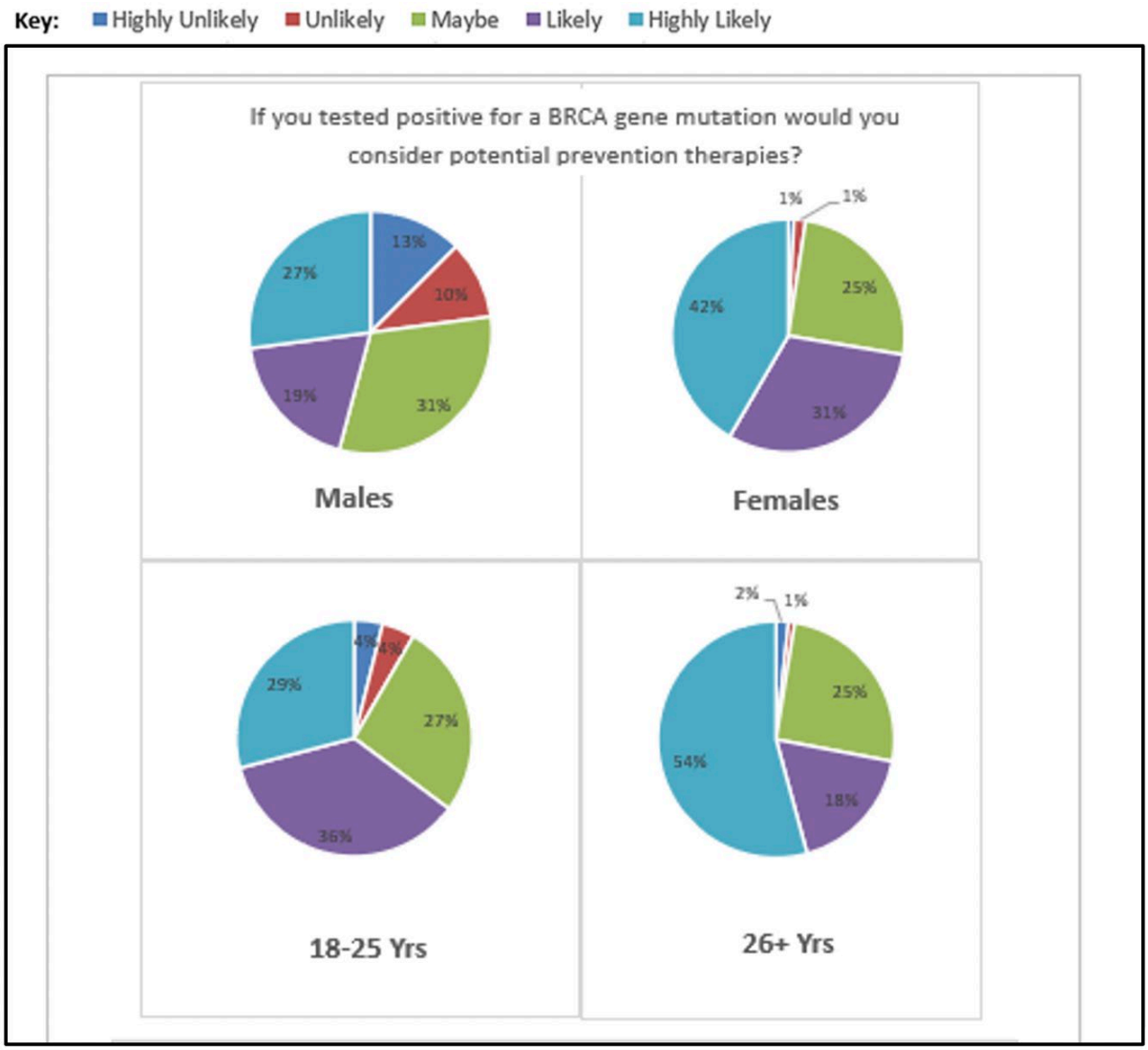

Figure 4. Opinion of survey participants when asked whether they would consider availing of prevention therapies after testing positive for the BRCA gene

In response to the question posed as to whether having tested positive for the BRCA gene would affect their life positively or negatively, in each category of respondent (male, female, those 18-25 and those over 25) the majority of the respondents were accounted for by the combined total of undecided/slightly negative impact categories see Figure 5.

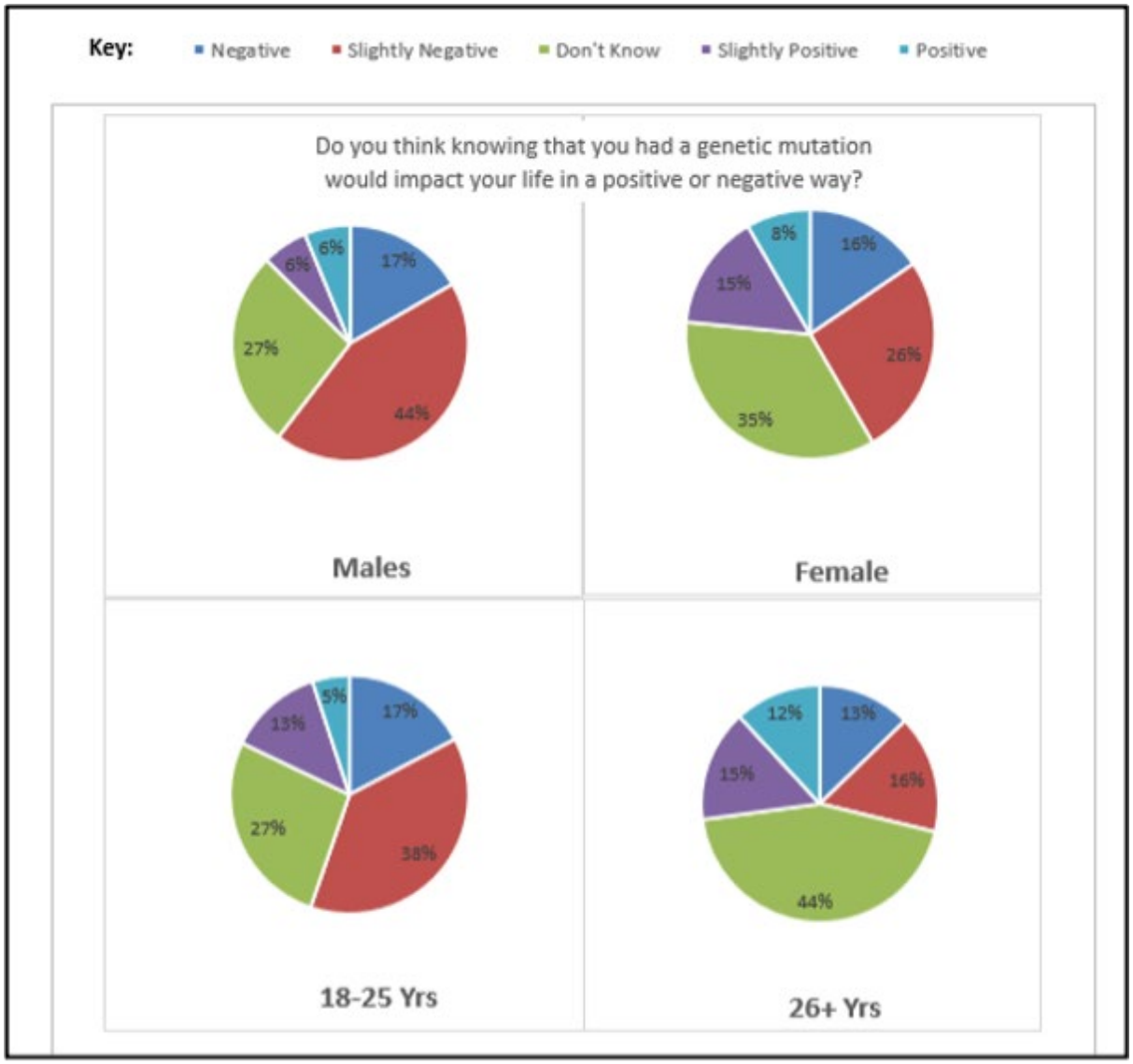

Figure 5. Survey respondents' opinion on whether they considered that having tested positive for the BRCA gene would impact their lives in a positive or a negative way

DISCUSSION

BRCA1 and BRCA2 are two genes that normally play a role in preventing tumour growth. However, in some people these genes are mutated. When mutated, they can increase chances of developing breast cancer. In females, it is estimated that the presence of a BRCA gene mutation increases their chances of developing breast cancer by 70% (Kotsopoulos, 2018). When males carry a BRCA gene mutation, it is estimated that they are up to 10% more likely to get breast cancer (breatcancer.org, 2019). However, those carrying the mutation may never develop breast cancer. The carrier of the mutated gene has a 50% chance of passing the gene mutation to one of their children (Rauscher, et al., 2019). There are also a number of risk factors that may increase the likelihood of developing illnesses such as cancer, including smoking or drinking alcohol.

Breast cancer can occur in both males and females. Breast cancer in females is quite common, with 1 in 9 women in Ireland affected by breast cancer at some stage in their lives (Breast Cancer Ireland, 2020). In comparison, a significantly lower number of males are affected by breast cancer in their lives, estimated at 1 in 1000 (Salman, 2017). Approximately 3,000 women are diagnosed with breast cancer

in Ireland annually while approximately only 25 men are diagnosed with breast cancer every year in Ireland (Irish Cancer Society, 2020).

In this study, the aim was to analyse how the diagnosis of a mutated BRCA gene mutation could affect aspects of life, and their opinions regarding certain topics. The aspects investigated in this study included family planning, treatment options to prevent the development of breast cancer and the impact of diagnosis of mutated gene. There have been no Irish studies conducted on the subject, but some similar studies have been carried out in the United States. The profile of the survey responses chosen were Irish nationals over the age of 18. Three hundred people participated in this survey, of which 252 (84%) were female and 48 (16%) were male.

When participants were asked about the topic of family planning and genetic screening there were similarities seen in the responses to certain topics while other areas highlighted differences of opinion. When participants were asked would get their children tested upon finding out they themselves had the mutated BRCA gene, both age groups concurred, answering they would get their children tested. Similarly, as seen in figure 1.1, males and females both answered they would likely get their children tested. The participants were then asked if they tested positive for a BRCA gene mutation would they inform their children. As seen in figure 1.2, the response from both age groups was similar with the majority of participants in the two age categories responding highly likely. Similarly, both males and females responded that they were likely to tell their children if they have a BRCA gene mutation (figure 1.1). This data highlights that regardless of age or gender, people have great interest in the area of genetic screening and are willing to get tested for the benefit of their health and their children's health.

However, there was contrasting opinions recorded in response to other questions regarding family. As seen from figure 1.2, the 26+ year olds are much more likely than the 18-25-year-olds to let the presence of the gene mutation affect their decision to have children in the future. Figure 1.1 showed most males felt the diagnosis would unlikely affect their decision to have children in the future. In comparison, females seemed indecisive about their decision with 32% responding maybe, while only 23% deemed the diagnosis unlikely to affect their decision to have children. This response supports the theory that females may feel more at risk at developing breast cancer than males and this may account for this increased level of indecision in the female cohort.

The differences in opinion between the age groups could be due to the fact that the two groups are at different stages of their lives when it comes to having children. The survey results indicate that 99% of the 18-25-year-old participants did not have children, whereas 88% of the 26+ year olds did have children. This difference of opinion might be accounted by the majority of the 26+ group answered the questions on children from a position of having children, whereas the 18-25-year-olds were answering the questions in a hypothetical sense.

Treatment options are available which can help decrease risk of developing breast cancer. Two different preventative measures were proposed to the participants. The first option was the removal of breast tissue in a mastectomy procedure with the second being the use of prevention therapies, such as selective oestrogen receptor modulators and neoadjuvant therapies

The preferred preventative treatment chosen by all participants was the use of prevention therapies. As seen in figure 3, 65% of 18-25-year-olds and 72% of 26+ year olds were 'likely/highly likely' to utilise this therapy measure. In contrast, only 58% of 18-25-year-olds and 64% of 26+ year olds were 'likely/highly likely' to consider getting a mastectomy. With regards to male and females, figures 2 and 3 again show that preventative therapies are a more popular option than a mastectomy. It was noted that females were more likely than males to consider preventative measures. Due to the low incidence of breast cancer among males (Irish Cancer Society, 2020), they potentially do not feel the need to undergo preventative measures as much as females do.

Prevention therapies are more appealing to participants as it is less invasive and does not require an operative procedure and can in most cases reduce the chances of getting breast cancer by between 44%

and 69%. However, these therapies may sometimes carry side effects (Slepicka, et al., 2019). Undeniably, the removal of breast tissue is a procedure associated with much emotional and physical trauma, so this is a decision not taken lightly. Post-mastectomy can induce many issues including, but not limited to, an effect on self-esteem, distorted self-image, reduced sex drive as well as physical problems such as pain and lymphoedema (Rodriguez, 2009). Therefore, it is unsurprising that participants were less likely to consider such an invasive procedure especially since a diagnosed mutated BRCA gene does not always lead to breast cancer but is only an increased risk factor. However, it must be noted that in BRCA gene mutation carriers, mastectomy is an effective procedure reducing the chances of getting breast cancer by 90% (Thorat & Balasubramanian, 2019). Additionally, advances in technology in the medical field has made many post-surgery options available to improve life post-mastectomy including breast reconstruction, breast prosthesis and mental health support groups and foundations (Rodriguez, 2009).

Participants were asked if they would inform their health insurance provider about the discovery of a mutated BRCA gene, considering that their premium may increase. Most males 31% responded that it was highly unlikely they would inform the insurance company while mast females (29%) were indecisive. However, 28% of females responded that they were highly likely to inform their insurance provider of the diagnosis. Comparing the responses from the different age groups, the older age category was more likely to inform the provider with most of the younger participants indecisive. An article published in the Irish Times suggested that if people felt obliged to inform their insurance provider it may discourage people from genetic testing performed as it may ultimately increase their insurance premium (McConnell, et al., 2000). These findings highlight the need for raised awareness of the benefits of genetic screening in Ireland as the public appear to be more concerned about their insurance rates increasing than recognising the true benefit of genetic screening. Genetic screening can aid the early detection of illnesses and in some cases preventative measures can be used to result in improved health outcomes (FORCE - Facing Our Risk of Cancer Empowered, 2020). Interestingly, many Irish health insurance providers promote genetic testing by assisting customers in financing the tests (VHI Healthcare, 2020).

In a study published in the International Journal of Biological Sciences, it was stated that the modern western diet, particularly the excess consumption of saturated fats, is associated with mortality and poor prognosis in breast cancer patients. This study also reported that increased breast cancer risk is associated with high alcohol intake and smoking at an early age (Sun, et al., 2017). Participants were asked if they would consider lifestyle changes to improve their overall health and help reduce risk of disease development. Most participants indicated they would take steps to improve their lifestyle such as exercise, diet, smoking and alcohol consumption. However, there was as a small number of participants answered that they were unwilling to change their current lifestyle and would not improve their smoking/drinking habits. Overall, the investigation of lifestyle factors was positive as it highlights that the vast majority of the public would be more conscientious of their life choices upon a gene mutation diagnosis.

Finally, participants were asked the impact the diagnosis of a mutated BRCA gene would have on a persons' outlook on life. The 18-25 year-olds consider the presence of a BRCA gene mutation would have a negative impact on their life. While the majority of the 26+ age group answered that they did not know what effect it would have on their life. This may indicate that the older age group may be able to see both the positives and negatives of having a BRCA gene mutation.

Interestingly, a greater number of males would consider the presence of a BRCA gene mutation to have a negative impact on their lives in comparison to females, as seen in figure 4. Only a small number of participants would consider the diagnosis to have a positive effect, 12% of males and 23% of females. There was a significant negative response from the male participants about the diagnosis and although the opinions of participants cannot be presumed, it could be suggested that this response is to male stigmatization surrounding breast cancer. A research investigation conducted by the National Centre for Biotechnology Information (NCBI) in 2018 titled 'Men With a "Woman's Disease": Stigmatization of Male Breast Cancer Patients—A Mixed Methods Analysis (Dorak & Karpuzoglu, 2012) analysed

how male patients felt following a diagnosed with breast cancer and their journey during cancer treatment. In the study, the patients were asked a series of questions regarding their diagnosis and cancer treatment. A significant 26% of the participants responded that they had experienced sexual stigmatization during their treatment because of their gender. Some doctors refused to treat the patients because they were male and other participants even recalled being called by a female pronoun in a waiting room due to gender presumption that 'only' females can develop breast cancer.

It was important to investigate how the participants felt about the potential diagnosis of a mutated BRCA gene in this survey. Firstly, the findings highlighted that the participants had a more pessimistic view on the diagnosis and failed to recognise the positive aspect of the situation. This further reiterates that the public is not sufficiently informed about genetic screening and that people fail to recognise that the diagnosis of a mutated gene although upsetting can save lives. It is important to stress that having the mutated gene does not guarantee someone will ever develop breast cancer in their lifetime. Confirmation of the mutation allows preventative steps to be taken to stop disease development as seen in mastectomy where breast cancer development risk is reduced by 90% (Thorat & Balasubramanian, 2019). Identifying the mutation gives the person the choice as to whether they wish to act upon the diagnosis or not. For those who do not avail of a genetic test, they remain unaware of their options.

With regards family planning, the diagnosis of a mutated BRCA gene allows people to discuss with their partner the risk of gene inheritance and make informed decisions about starting a family. Someone who has not had a genetic screening test is unaware of any genetic mutations they may carry and the potential risk posed if they decide to have children, especially if this mutation is potentially life threatening.

Lastly, it could be said that having the knowledge of a mutated BRCA gene may reap benefits for the person as it can make them more conscientious of their health by encouraging the person to improve their diet and participate in active exercise. Not availing of genetic screening may lead to a lack of utilisation of preventative measures and lack of positive steps to improve their health.

One major limitation in this study is the lack of diversity with the participants. The survey was circulated through social media platforms and because of this, the population was quite limited. The survey should be circulated more widely using multiple different platforms to gather data from as many different subgroups of people.

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REFERENCES

Breast Cancer Ireland, 2020. *Breast Cancer Ireland.* [Online]
Available at: https://www.breastcancerireland.com/education-awareness/facts-and-figures/
[Accessed 25 March 2020].

breatcancer.org, 2019. *What to Do if Your Genetic Test Results Are Positive.* [Online]
Available at: https://www.breastcancer.org/symptoms/testing/genetic/pos_results
[Accessed 25 March 2020].

Dorak, M. T. & Karpuzoglu, E., 2012. Gender Differences in Cancer Susceptibility: An Inadequately Addressed Issue. *Frontiers in Genetics,* 3(268).

Feng, Y., Spezia, M., Huang, S. & Yuan, C., 2018. Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & Diseases,* 5(2), pp. 77-106.

FORCE - Facing Our Risk of Cancer Empowered, 2020. *If Cancer Runs in Your Family… Information about BRCA Testing for Men.* [Online]
Available at: https://www.facingourrisk.org/understanding-brca-and-hboc/publications/documents/Info%20for%20Men%20Flyer%207.16.14.pdf
[Accessed 25 March 2020].

Harvie, M., Howell, A. & Evans, D., 2015. Can diet and lifestyle prevent breast caner: What is the evidence?. *American Society of Clinical Oncology Educational Book,* Volume 35.

Ibrahim, M., Yadav, S., Ogunleye, F. & & Zakalik, D., 2018. Male BRCA mutation carriers: Clinical characteristics and cancer spectrum. *BMC Cancer,* 18(1), p. 179.

Irish Cancer Society, 2020. *Cancer Information and Suport.* [Online]
Available at: https://www.cancer.ie/cancer-information-and-support/cancer-types/breast-cancer/
[Accessed 25 March 2020].

Kispert, S. & McHowart, J., 2017. Recent insights into cigarette smoking as a lifestyle risk factor for breast cancer. *Breast Cancer (Dove Med Press),* Volume 9, pp. 127-132.

Kotsopoulos, J., 2018. BRCA Mutations and Breast Cancer Prevention. *Cancers (Basel),* 10(12), p. 524.

Kuchenbaecker, K. et al., 2017. Risks of breast, ovarian and contralateral breast cancer for BRCA1 and BRCA2 mutation carriers. *JAMA,* 20 June, 317(23), pp. 2402-2416.

Liede, A., Metcalfe, K. & D, H., 2000. Evaluation of the Needs of Male Carriers of Mutations in Brca1 or BRCA2 Who have undegone Genetic Counselling. *American Journal of Human Genetics,* 67(6), pp. 1494-1504.

McConnell, D. et al., 2000. Genetic Testing and Insurance. *The Irish Times*, 31 October.

Mehrgou, A. & M, A., 2016. The importance of BRCA1 and BRCA2 gene mutations in breast cancer development. *Medical Journal of the Islamic Republic of Iran,* Volume 30, p. 369.

Mogilner, A., Otten, M. & Cunningham, J. D., 1998. Awareness and Attitudes concerning BRCA Gene Testing. *Annals of Surgical Oncology,* Volume 5, pp. 607-612.

National Cancer Registry Ireland, 2016. *Cancer Trends No 29. Breast Cancer,* s.l.: National Cancer Registry .

Obermiller, P., Tait, D. & Hotl, J., 2000. Gene therapy for carcinoma of the breast: Therapeutic genetic correction strategies. *Breast Cancer Research,* 2(1), pp. 28-31.

Rauscher, E. A., Dean, M., Campbell-Salome, G. & Barbour, J. B., 2019. "How do we rally around the one who was positive?" Familial uncertainty management in the context of men managing BRCA-related cancer risks. *Social Science & Medicine,* Volume 242.

Rodriguez, D., 2009. *Everyday Health.* [Online]
Available at: https://www.everydayhealth.com/breast-cancer/mastectomy-emotions.aspx
[Accessed 26 March 2020].

Salman, D. R., 2017. *Independent.ie.* [Online]
Available at: https://www.independent.ie/life/health-wellbeing/12-facts-about-breast-cancer-36350114.html
[Accessed 25 March 2020].

Slepicka, P. F., Cyrill, S. L. & dos Santos, C. O., 2019. Pregnancy and Breast Cancer: Pathways to Understand Risk and Prevention. *Trends in Molecular Medicine,* 25(10), pp. 866-881.

Stoppa-Lyonnet, D., 2016. The Biological Effects and Clinical Implications of BRCA Mutations: Where do we go from here?. *European Journal of Human Genetics,* 24(Suppl 1), pp. S3 - S9.

Sun, Y.-S.et al., 2017. Risk Factors and Preventions of Breast Cancer. *International Journal of Biological Sciences,* 13(11), pp. 1387-1397.

Thorat, M. & Balasubramanian, R., 2019. Breast cancer prevention in high-risk women. *Best Practice & Research Clinical Obstetrics & Gynaecology,* Volume 63.

VHI Healthcare, 2020. *VHI Healthcare.* [Online]
Available at: https://www.vhi.ie/members/genetic-testing
[Accessed 25 March 2020].

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