



**Trinity College Dublin**  
Coláiste na Tríonóide, Baile Átha Cliath  
The University of Dublin

**The Pathological Associations of Hyperuricaemia in Sub-Clinical Gout,  
Cardiovascular and Cardiopulmonary Diseases.**

A thesis presented to the School of Postgraduate Studies, Trinity College Dublin

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Medical Doctorate

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Rachael Flood

March 2023.

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## **List of Abbreviations**

% Percentage

°C Degrees Celsius

< Less than

≤ Less than or equal to

Ab Antibody

ACR American College of Rheumatology

AH Asymptomatic Hyperuricaemia

AMPK AMP-associated protein kinase

ASC apoptosis associated speck-like protein

cAMP 3'-5'-cyclic adenosine monophosphate

CAPS Cryopyrin-associated periodic syndromes

CARD Caspase activation and recruitment domain

CKD Chronic Kidney Disease

cm<sup>2</sup> Centimetre squared

COVID-19 Coronavirus Disease 2019

CRP C-reactive protein

CVD Cardiovascular Disease

DAMPs Damage associated molecular patterns

DC Dendritic cell

DCA Deoxycholic acid

DECT Dual Energy Computed Tomography

DL Decilitre

DMARD Disease Modifying Anti-Rheumatic Drug

DMSO Dimethyl sulfoxide

DNA Deoxyribonucleic acid

DNase Deoxyribonuclease

dNTP Deoxyribonucleotide triphosphate

ECM Extracellular matrix

ED Endothelial Dysfunction

EGF Epidermal growth factor

eGFR estimated glomerular filtration rate

ELISA Enzyme-linked immunosorbent assay

eNOS Endothelial Nitric Oxidase

EULAR European Alliance of Associations for Rheumatology

FBC Full blood count

FCU Familial Cold Urticaria

FMD Flow Mediated Dilatation

g Gram

g/dl Grams per decilitre

g/l Grams per litre

GAPDH Glyceraldehyde-3-phosphate dehydrogenase

G-CAN Gout, Hyperuricaemia and Crystal Associated Disease Network.

GOF Gain of Function

GGT Gamma-glutamyl transferase

GMP Guanine Monophosphate

GRA Global Rheumatology Alliance

Hb Haemoglobin

HDL high-density lipoprotein

Hr Hour

Hrs Hours

ICAM-1 Intracellular adhesion molecule 1

IgG Immunoglobulin G

IL Interleukin

IL-1 $\beta$  IL- 1 Beta.

IMRD Immune Mediated Rheumatic Disease

IUGR intrauterine growth retardation

l Litre

LDH Lactate dehydrogenase

LFTs Liver function tests

m Metre

M Molar

MDDC Monocyte-derived dendritic cell

mg/kg Milligram per kilogram

mg/l Milligrams per litre

mg/ml Milligrams per millilitre

MHz megahertz

min Minute

mins Minutes

ml Millilitres

mM Millimolar

MMP Matrix metalloproteinases

MSU Monosodium Urate

MTP Metatarsophalangeal

MWS Muckle-Wells Syndrome

NaCl Sodium chloride

ng Nanogram

NLR Nod Like Receptor

nm Nanometres

NMD Nitroglycerin Mediated Dilatation

NO Nitric Oxide

NF- $\kappa$ B Nuclear Factor Kappa B Subunit 1

NSAIDs Non-steroidal Anti-Inflammatory Drugs

OA Osteoarthritis

PAP Pulmonary Artery Pressure

PAGE Polyacrylamide gel electrophoresis

PBMC Peripheral Blood Mononuclear Cell

PBS Phosphate buffered saline

PCR Polymerase Chain Reaction

PDGF Platelet-derived growth factor

PH Pulmonary Hypertension

PPWT Pulmonary Pulse Wave Transit Time

PNP purine nucleoside phosphorylase

q-PCR Real-time quantitative polymerase chain reaction

RAGE receptor for advanced glycation end products

RCT Randomised Controlled Trial

RHC Right Heart Catheter

ROS Reactive Oxygen Species.

RNA Ribonucleic Acid

rpm Rotations per minute

RT Reverse transcriptase

RT-PCR Reverse transcriptase polymerase chain reaction

s Second

SARS-CoV-2 severe acute respiratory syndrome coronavirus 2

SD Standard deviation

SEM Standard error of the mean  
S1PR2 Sphingosine-1-phosphate receptor 2  
sUA Serum Uric Acid  
TBS Tris-buffered saline  
TIMP Tissue inhibitors of matrix metalloproteinases  
TNF $\alpha$  Tissue necrosis factor-alpha  
TLR Toll Like Receptor  
VDR Vitamin D Receptor  
UA Uric Acid  
 $\mu$ g Micrograms  
 $\mu$ g/ml Micrograms per millilitre  
 $\mu$ g/ $\mu$ l Micrograms per microlitre  
 $\mu$ l Microlitres  
ULT Urate Lowering Therapy  
 $\mu$ M Micromolar  
US Ultrasound  
XO Xanthine Oxidase  
XOI Xanthine Oxidase Inhibitor

## **Abstract - Introduction**

Gout is a common and treatable disease caused by the deposition of MSU crystals in articular and non-articular structures. Hyperuricaemia is the most important risk factor for the development of gout. Gout management is suboptimal worldwide resulting in significant socio-economic impact due to permanent disability. Gout is independently associated with increased morbidity and mortality due to CVD. Although the associations between hyperuricaemia and CVD are well described, it has not been definitively established whether uric acid is merely a marker for risk or a causative agent. At present ACR/EULAR 2015 gout classification criteria state that a patient must have at least one acute gout flare prior to a diagnosis being confirmed. This thesis firstly examines early clinical presentations of gout which do not fulfil the current diagnostic criteria, secondly, the effect of hyperuricaemia on surrogate markers of adverse CVD outcomes and thirdly, MCC950 as a selective inhibitor of the NLRP3 inflammasome and a potential new therapeutic agent for the treatment of gout. The impact of the COVID-19 pandemic on our research is also discussed.

## **Methods.**

Patients with hyperuricaemia and healthy controls who met the study criteria were recruited. Baseline clinical and demographic information was recorded. Hyperuricaemic cases with foot pain underwent assessment with validated pain scores and US evaluation of the first MTP joint to examine for evidence of urate crystal deposition (DC sign, tophus and erosions). Cases were treated with ULT and assessments repeated after a period of six months. To investigate associations with CVD, patients underwent flow and nitroglycerin mediated dilatation studies of the brachial artery and assessment of pulmonary haemodynamics via pulmonary pulse wave transit. These examinations were repeated after 3 months of ULT. Synovial biopsies and PBMCs were isolated from patients during an acute gout flare. Levels of IL-6 and IL-1 $\beta$  were examined by ELISA and qPCR and compared to osteoarthritis (OA) control samples. Subsequently, the effects of MCC950 on the secretion of IL-6 and IL-1 $\beta$  was assessed.

## **Results.**

Results from this study indicate that hyperuricaemic cases with non-specific foot pain commonly have US features of uric acid crystal deposition. The presence of DC sign or tophus on MTP US predicted a significant improvement in pain score following ULT. Hyperuricaemic cases had impaired FMD, NMD and pPTT compared to normal healthy controls, these indices improved following 3 months of treatment with ULT. Knee samples taken from patients with acute gout had elevated IL-6 and IL-1 $\beta$  compared to OA patients and treatment with MCC950 reduced levels of IL-6 and IL-1 $\beta$  in a dose dependant manner.

## **Conclusion.**

We describe an early clinical presentation of gout previously unrecognised which, through the use of US, can be diagnosed prior to the first acute flare. We propose that inclusion of highly sensitive and specific US indicators of early gout be included in diagnostic criteria so that diagnosis and

earlier, effective treatment may commence prior to disease progression. Hyperuricaemia is associated with surrogate markers that predict future adverse CVD outcomes. MCC950 reduced pro-inflammatory mediators associated with gout and has potential to be further investigated as a novel therapeutic agent.

### **Lay Abstract:**

**Introduction.** Gout is now the most common form of inflammatory arthritis. Gout is caused by high uric acid levels in the blood. Uric acid crystals can deposit in joints and cause an acutely swollen and tender joint, known as a gout flare. Although gout flares typically last 3-5 days and resolve, if repeated gout flares are not treated appropriately, and ULT not prescribed, MSU crystals in the joint can cause long-term damage. If gout is diagnosed and managed appropriately with ULT, permanent damage to the joint may be avoided, improving outcomes for sufferers. At present guidelines state that a patient must have at least one acute gout flare prior to a diagnosis being confirmed. It is becoming increasingly apparent that there are alternative presentations of pain in patients with high serum uric acid levels which do not necessarily meet the criteria for diagnosis. Gout is commonly associated with heart disease but the mechanisms by which this occurs are not completely understood. This thesis examines alternative presentations of early gout, some of the underlying mechanisms which cause an acute gout flare at a cellular level, how hyperuricaemia is associated with medical conditions such as heart disease and potential new treatment options. . The impact of the COVID-19 pandemic on our research is also discussed.

**Methods.** Patients with hyperuricaemia and healthy controls who met the study criteria were recruited and underwent informed consent. Baseline clinical and demographic information was recorded. Cases with foot pain underwent assessment of their pain with focused pain scores. Cases underwent US evaluation of their large toe joint to examine for evidence of urate crystal deposition and damage. Cases were treated with urate lowering medication and the assessments repeated after a period of six months. To investigate associations with heart disease, patients underwent specific ultrasound examinations of an artery in their arm and a focused assessment of their heart. These examinations were repeated after 3 months treatment with urate lowering medications. Samples were taken from patients with an acute gout flare, they were assessed for specific markers of gouty inflammation. Following this, certain samples were treated with a new potential therapeutic molecule called MCC950, the response of these markers of inflammation to this molecule was recorded.

**Results.** Results from this study indicate that people with high uric acid levels and non-specific foot pain commonly have US features of uric acid crystal deposition in the joints of their feet. When treated with uric acid lowering medication, over 6 months, their pain reduced significantly. People with high uric acid levels had impaired markers of heart disease compared to normal healthy controls. These indices demonstrated a trend towards improvement following 3 months of treatment with urate lowering medications. Samples taken from acute gout patients had elevated markers of inflammatory proteins compared to controls with osteoarthritis. Treatment with a new molecule named MCC950 reduced the levels of these inflammatory proteins.

**Conclusion.** Gout is a common condition caused by high levels of uric acid in the blood. Different presentations of pain occur outside the typical acute gout flare in the setting of hyperuricaemia. These presentations are associated with evidence of urate crystal deposition in the joints and respond to treatment with urate lowering medications. High uric acid levels may also be associated

with heart disease. MCC950 reduced inflammatory proteins associated with gout and has the potential to be a new therapeutic agent. Gout is a common and disabling disease, however earlier clinical diagnosis and potential new targeted therapies may lead to a brighter future, with an improved quality of life for sufferers.

### **Aims and Hypothesis of the Project.**

**Hypothesis:** I hypothesize that hyperuricaemia identifies an at-risk population, and that early intervention with urate lowering therapy (ULT) ameliorates future disease with improved long-term outcomes for clinical gout and its associated cardiovascular diseases.

### **Aims:**

- To investigate whether ULT reduces non-episodic foot pain in patients who fail to meet ACR/EULAR 2015 gout classification criteria.
- To investigate the effect of hyperuricaemia on endothelial dysfunction.
- To further investigate the association of pulmonary hypertension and hyperuricaemia.
- To determine the effects of NLRP3 overexpression in samples from patients with an acute flare of gout.
- To report on outcomes of COVID-19 infection in patients with gout and immune mediated rheumatic diseases in Ireland.



### **Value of Research**

Gout is a common form of inflammatory arthritis, caused by elevated serum uric acid levels, which typically presents with an acutely swollen and tender joint. Although there are low cost, effective, urate-lowering therapies available the management of patients with gout is suboptimal due to delayed diagnosis, slow initiation of treatment and poor adherence to therapy (1). In the last decade, the focus of rheumatology research has been in inflammatory rheumatic diseases such as rheumatoid arthritis and significant therapeutic advances have occurred in this area, whereas improvement in gout outcomes have plateaued. In correlation with an ageing population with multiple co-morbidities, there has been an increase in the number of patients with gout and therefore the burden of gout as a proportion of rheumatology 'ill-health' is on the rise. Our research responds to the clinical and research need to focus on this common, debilitating disease.

At present, guidelines state that a patient must have at least one acute gout flare, defined as an episode of swelling, pain or tenderness of a peripheral joint/bursa before a gout diagnosis can be confirmed. It is becoming increasingly apparent that there are alternative presentations of pain in patients with high uric acid levels, which do not meet the criteria for diagnosis and therefore treatment. Our research identifies presentations of pain in

patients with high uric acid levels, who are likely to have 'early clinical gout' before they have a 'classical gout attack.' By identifying this group of patients at an early stage, tight urate control will be easier to achieve, resulting in less joint damage, improved outcomes and reduced socio-economic burden of this debilitating disease. As part of this research, we used musculoskeletal ultrasound to examine if there are any specific ultrasound features of urate deposition in the joints of patients with early clinical gout, our findings provide clinicians with further validation of MSK ultrasound for use in the diagnosis of rheumatic diseases.

Patients who suffer from gout are at increased risk of heart disease. At present, there are a lack of clinical studies examining whether the high uric acid levels in gout directly cause vascular changes, leading to heart disease, or if the association is because patients with gout often have other contributory metabolic conditions, such as obesity and type 2 diabetes. Our work investigates the effect of hyperuricaemia on surrogate markers of adverse cardiovascular outcomes, contributing to the scientific understanding of this complex relationship. A better understanding of the association between high uric acid levels and heart disease will help physicians to make more informed decisions surrounding management of high urate levels. This research on vascular changes in hyperuricaemia is of great clinical relevance given the high incidence and considerable morbidity of heart disease in patients with gout.

At present the long-term treatment of gout involves medications that lower uric acid levels in the blood including allopurinol and febuxostat. Understanding how urate crystals cause an acute episode of inflammation at a cellular level is important for the identification and development of future therapies. This research identifies inflammatory proteins which are

elevated in an acute gout flare and may contribute to the cascade of events, which occur in cells, to cause the pain and swelling witnessed by patients. Our research examines the effect, at a cellular level, of a new molecule known as MCC950, which has been found to specifically inhibit part of the inflammatory response to urate crystals. This preliminary work is important as it may identify an area for future development of a potential drug therapy.

This MD research project began just before the emergence of the COVID-19 health emergency. The resulting pandemic created a serious threat to population health, which occurred in the face of a pathogenic threat about which almost nothing was known. The management of hospitals changed dramatically, with access restricted to those who required face-to-face urgent care only. The recruitment and follow-up of patients who took part in the study was paused for a prolonged period given the circumstances. Faced with an urgent and pressing need to respond to our changed health environment, and to use our research resources in the best way possible, we rapidly designed and executed a research project to quantify the extent of COVID-19 related hospitalisation among patients with immune mediated rheumatic diseases which commonly require treatment with immune modulating therapies. The onset of the COVID-19 pandemic raised concerns of severe disease outcomes in patients with immune mediated rheumatic diseases (IMRDs) such as rheumatoid arthritis. Patients with IMRDs are considered to be at an increased risk of opportunistic infections due to their immunocompromised state, resulting both from their underlying immune disease and due to the use of targeted immune-modulating therapies. Our data reported a lower hospitalisation rate for patients with IMRDs and COVID-19 infection than was reported by other rheumatology registries at the time,

including the Global Rheumatology Alliance (GRA) online COVID-19 Registry. This finding suggested a reporting bias towards the registering of already hospitalized patients to online registries and helped guide how we interpreted data early in the pandemic. Our findings provided reassurance to clinicians and patients given the prior lack of evidence regarding management of immunocompromised patients with IMRDs and COVID-19. The identification of factors associated with hospitalisation in this group of patients allowed for risk stratification and informed clinical decision making where evidence-based medicine was not yet available, due to the novel nature of the SARS-CoV-2 virus. Although not initially an aim or objective for this thesis, reporting on outcomes of COVID-19 in patients with IMRDs became a valuable and essential research agenda in the changing medical landscape of 2020.

In summary, the research conducted as part of this thesis contributes to the scientific, clinical and real world understanding of hyperuricaemia, gout and their pathological associations.

## **Outputs**

- **Prizes:**

### **Irish Society of Rheumatology Young Investigator of the Year Award 2021**

- Top abstract prize at ISR Autumn Meeting.
- Urate-lowering Therapy Reduces Non-Episodic Foot Pain in Patients Who Fail to Meet ACR/EULAR 2015 Gout Classification Criteria: An Effect Predicted by Ultrasound. Flood RM, Kirby C, Alammari Y, Kane D, Mullan RH.

### **Irish Society of Rheumatology Young Investigator of the Year Award 2020**

- Top abstract prize at ISR Autumn Meeting.
- Anticytokine Therapies for Inflammatory Rheumatic Disease (IRD) Are Associated with Reduced Hospitalisation Following Community COVID-19 Infection; Results of the Trinity Rheumatology and Covid-19 Registry – TRACR. Flood R, Conway R, Kane D, Mullan R.

### **G-CAN Early Career-Investigator Award**

- Presentation at Gout, Hyperuricaemia and Crystal-Associated Disease Network international conference October 2020 and October 2022.

- **Publications:**

**Correspondence to: 'Characteristics associated with hospitalisation for COVID-19 in people with rheumatic disease: data from the COVID-19 Global Rheumatology Alliance physician-reported registry by Gianfrancesco et al. Results of the Trinity Rheumatology**

**& COVID-19 Registry – TRACR.** Flood R, Conway R, Gheta D, Kirby D, Kane D, Mullan RH. Annals of the Rheumatic Diseases 2020.

<http://dx.doi.org/10.1136/annrheumdis-2020-218733>

**Temporal Trends in COVID-19 outcomes in People with Rheumatic Diseases in Ireland: data from the COVID-19 Global Rheumatology Alliance registry.** Richard Conway, Elena Nikiphorou, Rachael Flood, Geraldine McCarthy et al. Rheumatology (Oxford) 2022 Mar 8;keac142.

DOI: 10.1093/rheumatology/keac142.

**COVID-19 in Pregnant Women With Rheumatic Disease: Data From the COVID-19 Global Rheumatology Alliance.** Bonnie L Bermas, Milena Gianfrancesco, Helen L Tanner, Rachael Flood, Philip C Robinson et al. Journal of Rheumatology 2022.

DOI: 10.3899/jrheum.210480.

**Predictors of hospitalization in patients with rheumatic disease and COVID-19 in Ireland: data from the COVID-19 global rheumatology alliance registry.** Richard Conway, Elena Nikiphorou, Christiana A. Demetriou, Rachael M Flood, Geraldine M. McCarthy et al Rheumatology Advances in Practice 2021;0:1–7

DOI:10.1093/rap/rkab031

**Urate Lowering Therapy (ULT) reduces non-episodic foot pain in patients who fail to meet ACR/EULAR 2015 Gout Classification Criteria; an effect predicted by Ultrasound and potential rationale for re-classification.** Alammari Y, Gheta D, Flood R, Boran G, Kane D, Mullan RH. Annals of the Rheumatic Diseases 2018.

DOI: 10.1136/annrheumdis-2018-214305

- **Conference Presentations – International**

**Urate-lowering Therapy Reduces Non-Episodic Foot Pain in Patients Who Fail to Meet ACR/EULAR 2015 Gout Classification Criteria: An Effect Predicted by US** (updated results) Poster Presentation, ACR Convergence virtual 2022. Flood RM, Kirby C, Alammari Y, Kane D, Mullan RH.

**Pulmonary Vascular Dysfunction Occurs in Association with Hyperuricaemia: Assessment by a Novel Non-Invasive Measurement of Pulmonary Pulse Wave Transit Time** Poster Presentation, ACR Convergence virtual 2022. Rachael Flood, Colm Kirby, David Kane, Ronan H Mullan, Tallaght University Hospital, Dublin, Ireland.

**Hyperuricemia Is Associated with Vascular Endothelial Dysfunction – The Impact of Hyperuricemia on Flow Mediated and Nitroglycerin Mediated Dilatation of the Brachial**

**Artery.** Poster Presentation, ACR Convergence virtual 2021. Rachael Flood, Colm Kirby, David Kane, Ronan H Mullan, Tallaght University Hospital, Dublin, Ireland.

**The Pathological Associations of Hyperuricaemia in Sub-clinical Gout, Cardiovascular and Cardiopulmonary Diseases.** Flood R, Fearon U, Kane D, Mullan RM, Oral presentation of preliminary findings at New Horizons in Rheumatology Conference. December 2020.

**Anticytokine Therapies for Inflammatory Rheumatic Disease (IRD) Are Associated with Reduced Hospitalisation Following Community COVID-19 Infection; Results of the Trinity Rheumatology and Covid-19 Registry – TRACR.** Flood R, Conway R, Kane D, Mullan R. Oral Presentation American College of Rheumatology Convergence Meeting 2020.

**Metatarsophalangeal Joint Medial Collateral Ligament Measurement, A Novel Ultrasound Feature of Monosodium Urate Deposition in the Joint** Flood R, Kane D, Mullan R. Rheumatology Department, Tallaght University Hospital. Poster presentation at EULAR scientific meeting, Frankfurt, June 2020.

<http://dx.doi.org/10.1136/annrheumdis-2020-eular.2182>

- **Conference Presentations National.**

**Pulmonary Vascular Dysfunction Occurs in Association with Hyperuricaemia: Assessment by a Novel Non-Invasive Measurement of Pulmonary Pulse Wave Transit Time** Oral Presentation, ISR Belfast, 2022. Rachael Flood, Colm Kirby, David Kane, Ronan H Mullan, Tallaght University Hospital, Dublin, Ireland.

**Urate-lowering Therapy Reduces Non-Episodic Foot Pain in Patients Who Fail to Meet ACR/EULAR 2015 Gout Classification Criteria: An Effect Predicted by Ultrasound.** Flood RM, Kirby C, Alammari Y, Kane D, Mullan RH.

Oral presentation & winner of the young investigator award, Irish Society of Rheumatology Meeting 2021.

**Anticytokine Therapies for Inflammatory Rheumatic Disease (IRD) Are Associated with Reduced Hospitalisation Following Community COVID-19 Infection; Results of the Trinity Rheumatology and Covid-19 Registry – TRACR.** Flood R, Conway R, Kane D, Mullan R. Oral Presentation & winner of the young investigator award, Irish Society of Rheumatology Meeting 2020.

## **1.1 Introduction**

Gout, which is caused by the deposition of monosodium urate (MSU) crystals in the setting of hyperuricaemia, is now the most common form of inflammatory arthritis (2). In tandem with sedentary lifestyles and rising obesity rates, hyperuricaemia is increasingly prevalent in Ireland, with a reported adult prevalence of 25% (3). In addition to gout, hyperuricaemia has been implicated both in the development and potentiation of a wide range of diseases including type 2 diabetes, cardiovascular disease (CVD), stroke, systemic hypertension, pulmonary hypertension and chronic kidney disease (CKD). Alongside their significant socioeconomic costs, these diseases are associated with increased morbidity and mortality on a global scale. All are associated with a decades-long pre-clinical prodrome, which if effectively identified, could be amenable to primary prevention.



I postulate that hyperuricaemia identifies an at-risk population, and that early intervention with urate lowering therapy (ULT) ameliorates future disease with improved long-term outcomes for clinical gout and its associated cardiovascular and pulmonary vascular diseases.

This introduction will provide an overview of hyperuricaemia, gout and the burdensome impact this common condition has on today's society. The current American College of Rheumatology (ACR) 2015 classification criteria for gout will be discussed, along with its diagnostic limitations. The extra-articular pathological associations of hyperuricaemia and their proposed mechanisms will be reviewed alongside the management challenges surrounding 'asymptomatic hyperuricaemia'. Finally, the aims and objectives of this thesis will be outlined.

## **1.2 Uric Acid.**

### **1.2.1 The Proposed Natural History of Uric Acid.**

Uric acid is a product of purine metabolism that is generated during the enzymatic degradation of xanthine (4). Relative to humans, most other mammals have low uric acid levels due to the presence of the enzyme uricase, which further degrades urate to allantoin. Phylogenetic analysis indicates that around 20 million years ago during the Miocene epoch, a mutation occurred in humans which resulted in complete loss of the uricase enzyme. As a result of the loss of uricase activity, humans have higher levels of uric acid than most other mammals. One hypothesis as to why this deletion may have occurred is that it allowed for an increase in serum uric acid to provide greater antioxidant activity,

and that this may account for the greater longevity of humans compared with most other mammals (4). Free radicals are a group of small reactive molecules that play critical roles in the regulation of various cell functions and biological processes. Although essential for homeostasis, the uncontrolled production of free radicals has been implicated in many pathological processes including vascular injury, cardiovascular disease and malignancy. Endogenous anti-oxidants function as checkpoints to avoid the consequences of uncontrolled free radicals (5). Uric acid is a strong antioxidant, it accounts for over half of the free radical scavenging activity in human blood (6).

### **1.2.2. Urate Metabolism**

Uric acid is a  $C_5H_4N_4O_3$  (7,9-dihydro-1H-purine-2,6,8(3H)-trione) heterocyclic organic compound with a molecular weight of 168 Da. Many enzymes are involved in the conversion of the two purine nucleic acids, adenine and guanine, to uric acid (Figure 1.1). Initially, adenosine monophosphate (AMP) is converted to inosine via two different mechanisms; either first removing an amino group by deaminase to form inosine monophosphate (IMP) followed by dephosphorylation with nucleotidase to form inosine, or by first removing a phosphate group by nucleotidase to form adenosine followed by deamination to form inosine. Guanine monophosphate (GMP) is converted to guanosine by nucleotidase. The nucleosides, inosine and guanosine, are further converted to purine base hypoxanthine and guanine, respectively, by purine nucleoside phosphorylase (PNP). Hypoxanthine is then oxidized to form xanthine by xanthine-

oxidase (XO), and guanine is deaminated to form xanthine by guanine deaminase. Xanthine is again oxidized by xanthine oxidase to form the final product, uric acid (7).

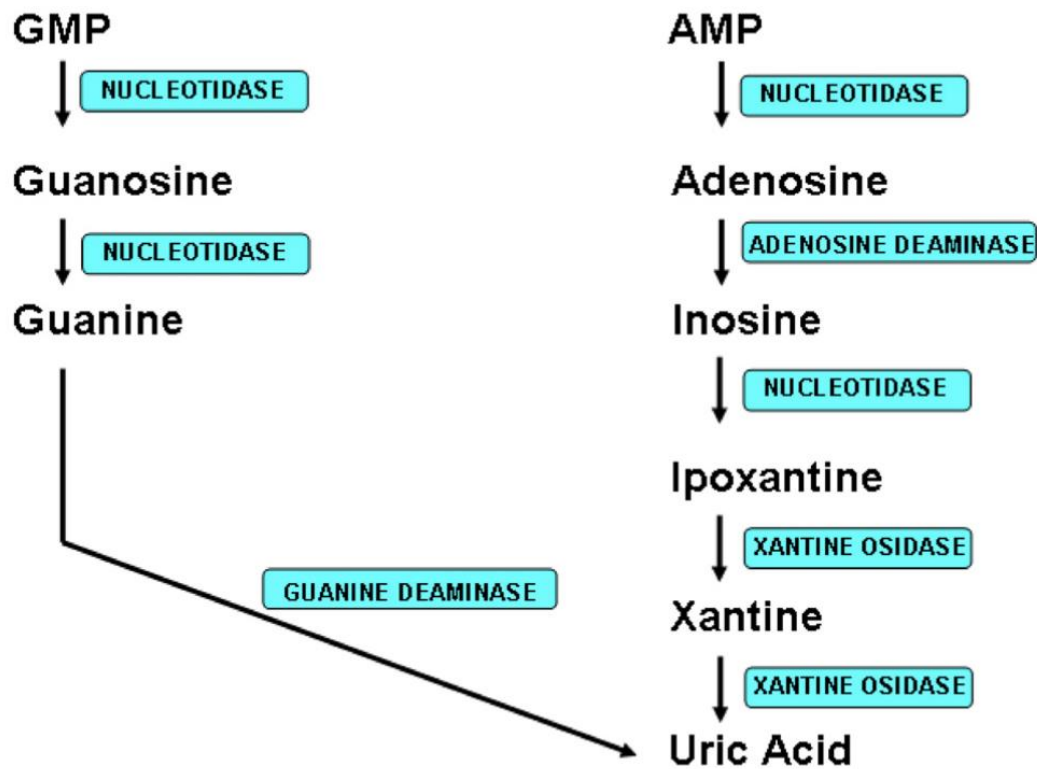


Figure 1.1 Enzymatic Degradation of Purines in humans. (7)

### 1.2.3 The Protective to Pathological Change of Serum Uric Acid.

Paradoxically, the expected protective antioxidant effects of serum uric acid on human longevity remain largely unseen in modern observational cohort studies, where higher uric acid levels tend to associate with increased risk for cardiovascular disease (CVD) and increased rather than decreased mortality (8). To explain this paradoxical association, an alternative hypothesis has been proposed in which the uricase mutation may have resulted in increased salt sensitivity and higher arterial blood pressures. It is accepted that paleolithic hunter gatherer societies survived on a very low dietary sodium. Therefore any evolutionary adaptation favouring salt and water retention may have conferred an

evolutionary advantage by maintaining osmotic turgor leading to increases in systolic blood pressure. Studies inhibiting the uricase enzyme in rats have resulted in increased blood pressure. Higher uric acid levels induce endothelial dysfunction, followed by uric acid-induced arteriolosclerosis of the renal vasculature, in turn leading to persistent salt sensitive hypertension (4). There is evidence that a similar process of uric acid induced endothelial dysfunction may also occur in humans (9). Experimental studies have indicated the possibility that uric acid absorbed into endothelial cells via activation of uric acid transporters causes endothelial dysfunction (10)(11). While the uricase mutation may have conferred a blood pressure advantage to our early hominoid ancestors as they transitioned to a bipedal gait, this mutation is now no longer beneficial due to the fact that human diets have changed substantially though large increases in the availability of dietary salt. Climate change driving CKD induced hypertension and CVD is also now an evolving issue for global health (12)(13). In the 21<sup>ST</sup> century hyperuricaemia in mammals is synonymous with gout, CVD and the metabolic syndrome. It is reasonable therefore to postulate that efforts to reduce hyperuricaemia may therefore ameliorate the incidence and prevalence of a range of modern diseases associated with Western dietary habits lifestyles.

### **1.3 Gout.**

#### **1.3.1 The Gout, Hyperuricaemia and Crystal- Associated Disease Network (G-CAN) common language definition of gout**

“Gout is a common condition caused by high levels of urate (also known as uric acid) in the body. Having too much urate causes crystals to form in the joints that can result in inflammation and damage. People are usually diagnosed with gout when they have an

attack of a very painful arthritis, which can cause difficulty sleeping, walking and working. This is called a gout flare. For most people, gout happens when the kidneys do not remove enough urate from the body, with genetic make- up playing a critical role for some groups. Gout often occurs alongside other health problems such as high blood pressure, heart disease, diabetes and kidney disease, with shared risk factors including being overweight, diet and alcohol consumption. Gout flares are treated in the short term with anti-inflammatory medicines. Long- term daily medicines such as allopurinol decrease the amount of urate in the body and dissolve the urate crystals, resulting ultimately in prevention of gout flares and joint damage. Healthcare workers such as doctors, nurses and pharmacists can help people with gout understand the condition, reduce stigma and work with them to make management choices”(14).

### **1.3.2 The History of Gout.**

Gout has been hailed “A disease of kings, the king of diseases.” It was first identified by the Egyptians in approximately 2640 B.C. The oldest recorded example of uric acid deposition is the demonstration of a uric acid containing renal calculus found in an Egyptian mummy dating back 7000 years (15). Hippocrates of Kos (c. 460–c. 370 B.C.) was a Greek physician often referred to as the Father of Western Medicine, described gout in the fifth century B.C. as the “unwalkable disease,” a condition of older men and a product of high living (16). The term gout originated from the Latin word “gutta” meaning a drop, as the ancient belief has it that the devil causes the disease by instilling a poisonous humour into the joint of the victim drop by drop.



**Figure 1.2** A self-indulgent man afflicted with 'the gout': the pain is represented by a demon burning his foot. Coloured lithograph by G. Cruikshank, 1818. (Licence: Public Domain Mark)

In 1969, McCarty and Hollander (17) proved the association between acute arthritis in gout and deposition of uric acid crystals in the joints. In 1988, Sir James Black, Gertrude Elion and George Hitchings shared the Nobel Prize in Physiology or Medicine for their discoveries of important principles for drug treatment of this condition.

### **1.3.3 Epidemiology of Gout.**

Gout is a common disease with increasing incidence and prevalence, particularly in Western or westernising countries, paralleling the obesity epidemic over recent decades (18)(2). With an estimated 41 million people worldwide, gout is now the most common form of inflammatory arthritis (19). Gout carries a significant global socio-economic burden, available data suggests that gout patients incur substantially greater direct and indirect costs as compared with gout-free individuals (20). It is more common in men, with a prevalence of 5.2% in men and 2.7% in women (21). Gout is rare in women before the

menopause and does not usually affect children. It is more common in some ethnicities such as Māori, Pacific Peoples and Taiwanese (22).

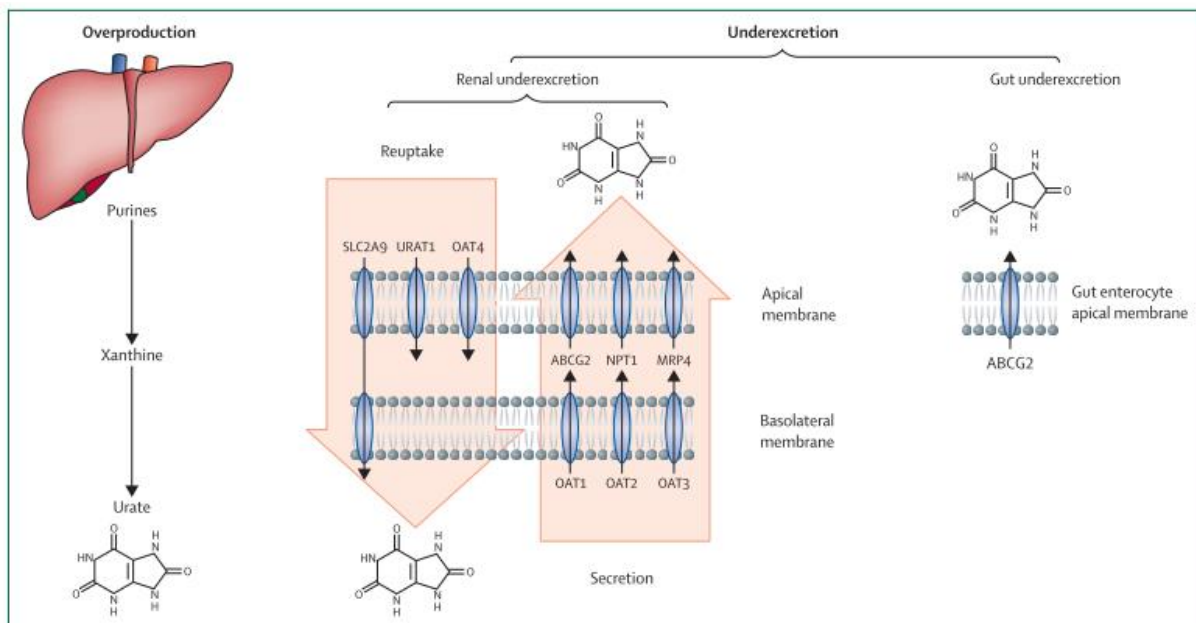
#### **1.3.4 Pathogenesis of Gout.**

The progression of hyperuricaemia and gout can be considered to take place over four pathophysiological stages: development of hyperuricaemia, deposition of monosodium urate crystals, clinical presentation of gout flares due to an acute inflammatory response to deposited crystals, and clinical presentation of advanced disease characterised by tophi (23).

Pathological hyperuricaemia has been defined as the serum urate concentration above which monosodium urate crystals form in vitro at physiological pH and temperature (24). While hyperuricaemia can occur due to increases in dietary purine ingestion or overproduction from hepatic metabolism and cell turnover, progressive renal under-excretion is the typical mechanism directly leading to the development of clinical gout. The majority of uric acid is excreted through the kidneys with the remaining one third excreted via the gut (25). This process is controlled by a suite of secretory and reabsorptive molecules (URAT1/SLC22A12, OAT4/SLC22A11, OAT10/SLC22A3), the reabsorptive GLUT9/SLC2A9 urate transporter, secretory anion-exchange transporters (OAT1, OAT2, OAT3) sodium- phosphate transporter proteins (NPT1/SLC17A1 and NPT4/SLC17A3), and the ATP-driven secretory efflux pump MRP4/ABCC4. In the gut, the secretory transporter ABCG2 is important for excretion of uric acid (25) (Figure 1.3). A reduction in function of these transporters results in elevated serum uric acid levels and hyperuricaemia. High-purine diets or other factors (such as alcohol and fructose intake, diuretics and  $\beta$ -blockers,

high intake of meat, seafood, fructose, alcohol, and sodium) increase serum urate and the risk of incident gout (26)(27).

Medical conditions causing high cell turnover, such as psoriasis and myeloproliferative disorders, also lead to increased serum urate concentrations due to overproduction of urate.



**Figure 1.3: Mechanisms of Hyperuricaemia (25).**

### 1.3.5 The acute inflammatory response to monosodium urate crystals.

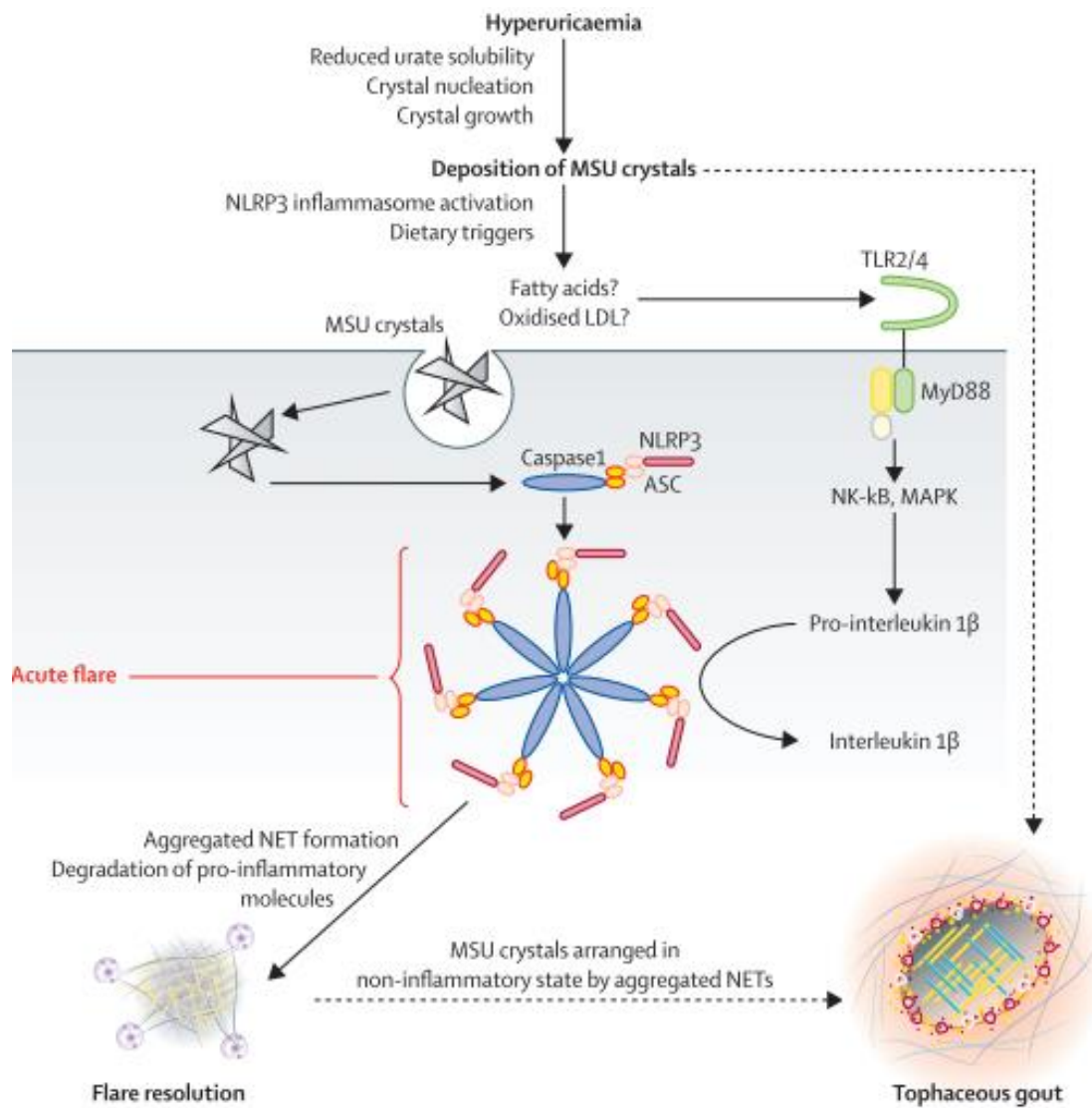
The acute inflammatory response, manifesting as acute gout flares is thought to be initiated when MSU crystals interact with resident macrophages to form and activate the NLRP3 inflammasome (figure 4) (28). NLRP3 activation leads to the release of interleukin 1 beta (IL-1 $\beta$ ) and a subsequent cascade of pro-inflammatory cytokines including IL-18 and IL-6.



The NLRP3 inflammasome is dependent on a two-signal initiation system, which avoids unregulated activation that would damage the host. The first signal results in stimulation of nuclear factor kappa B subunit 1 (NF- $\kappa$ B) via toll like receptor (TLR) 4 and TLR2, and synthesis of pro interleukin 1 beta (pro-IL-1 $\beta$ ) and inflammasome components (29). The second signal is activated by monosodium urate crystals which cause the assembly of the inflammasome and activation of caspase-1, which proteolyzes pro-IL-1 $\beta$  to mature IL-1 $\beta$  (30). IL-1 $\beta$  then triggers a downstream signalling cascade involving proinflammatory cytokines and chemokines, resulting in the recruitment of neutrophils and other cells to the site of crystal deposition.

The initiation of the NLRP3 inflammasome requires a two-step initiation process, therefore deposition of monosodium urate crystals alone does not necessarily result in inflammation, explaining how monosodium urate crystals can be present in the joint without clinically apparent inflammation (31)(32).

Factors that stimulate the first signal, such as free fatty acids (induced by intake of a large meal or alcohol), gut microbiota, and other microbiological components, can induce the acute inflammatory response in the presence of deposited monosodium urate crystals (33).



**Figure 1.4: Checkpoints in the progression from hyperuricaemia to the clinical manifestations of gout.**

Factors controlling deposition of MSU crystals are not well understood. The acute flare results from production of mature interleukin 1 $\beta$ , after activation of the NLRP3 inflammasome, that occurs after ingestion of crystals by monocytes which in humans requires a second signal through TLRs. Flare resolution involves NETs which bind MSU crystals (depicted in yellow). The NETs probably contribute to the formation of tophi.

Images from Dalbeth et al, Lancet 2016. (205) MSU – Monosodium urate. LDL – Low density Lipoprotein. ASC – apoptosis associated speck like protein containing a caspase recruitment domain. MAPK – mitogen-activated protein kinase. NET -Neutrophil extracellular trap. TLR - toll like receptor.

### **1.3.6 Clinical Presentation of Gout.**

In certain patients with elevated serum uric acid (sUA) levels, deposition of MSU crystals occur predominantly in peripheral joints and surrounding tissues. The characteristic clinical presentation is of rapidly developing monoarticular synovitis in peripheral joints; an acute 'attack' that is extremely painful but typically self-limiting. However, long-term deposition of MSU crystals can result in joint damage and disfiguring subcutaneous tophi. This may cause significant disability and loss of function to the patient in the longer term.

### **1.3.7 ACR/EULAR 2015 Gout Classification Criteria.**

Optimal management of gout requires prompt diagnosis before long term damage to the joint occurs, followed by aggressive reduction of sUA with urate lowering therapy (ULT) in a treat to target sUA approach which aims to bring the serum concentration below the saturation threshold through which crystal formation would otherwise occur. Despite recent observations that ULT should be considered early to reduce disease chronicity, diagnosis is frequently delayed, leading to suboptimal clinical outcomes (34). The current ACR/European League Against Rheumatism (EULAR) 2015 gout classification entry criterion requires the history of a prior episode of swelling, pain or tenderness of a peripheral joint/bursa before confirmation either through MSU crystal identification in synovial fluid or through achieving a score of >8 using a predefined scoring system of radiological, laboratory and clinical features. One such feature, a gout 'episode', is clearly defined both in terms of its intensity (joint erythema, tenderness, reduced/inhibited walking ability) and duration (time to maximal pain from onset <24 hour; resolution to baseline <14 days) (35).

	Categories	Score
<b>Step 1:</b> Entry criterion (only apply criteria below to those meeting this entry criterion)	At least 1 episode of swelling, pain, or tenderness in a peripheral joint or bursa	
<b>Step 2:</b> Sufficient criterion (if met, can classify as gout without applying criteria below)	Presence of MSU crystals in a symptomatic joint or bursa (i.e., in synovial fluid) or tophus	
<b>Step 3:</b> Criteria (to be used if sufficient criterion not met)		
<b>Clinical</b>		
Pattern of joint/bursa involvement during symptomatic episode(s) ever†	Ankle or midfoot (as part of monoarticular or oligoarticular episode without involvement of the first metatarsophalangeal joint)	1
	Involvement of the first metatarsophalangeal joint (as part of monoarticular or oligoarticular episode)	2
Characteristics of symptomatic episode(s) ever		
• Erythema overlying affected joint (patient-reported or physician-observed)	One characteristic	1
• Can't bear touch or pressure to affected joint	Two characteristics	2
• Great difficulty with walking or inability to use affected joint	Three characteristics	3
Time course of episode(s) ever		
Presence (ever) of ≥2, irrespective of antiinflammatory treatment:		
• Time to maximal pain <24 hours	One typical episode	1
• Resolution of symptoms in ≤14 days	Recurrent typical episodes	2
• Complete resolution (to baseline level) between symptomatic episodes		
Clinical evidence of tophus (Figure 2)		
Draining or chalk-like subcutaneous nodule under transparent skin, often with overlying vascularity, located in typical locations: joints, ears, olecranon bursae, finger pads, tendons (e.g., Achilles)	Present	4
<b>Laboratory</b>		
Serum urate: Measured by uricase method.		
Ideally should be scored at a time when the patient was not receiving urate-lowering treatment and it was >4 weeks from the start of an episode (i.e., during intercritical period); if practicable, retest under those conditions. The highest value irrespective of timing should be scored.		
Synovial fluid analysis of a symptomatic (ever) joint or bursa (should be assessed by a trained observer)§	MSU negative	-2
<b>Imaging (Figure 3)¶</b>		
Imaging evidence of urate deposition in symptomatic (ever) joint or bursa: ultrasound evidence of double-contour sign# or DECT demonstrating urate deposition**	Present (either modality)	4
Imaging evidence of gout-related joint damage: conventional radiography of the hands and/or feet demonstrates at least 1 erosion††	Present	4

\*

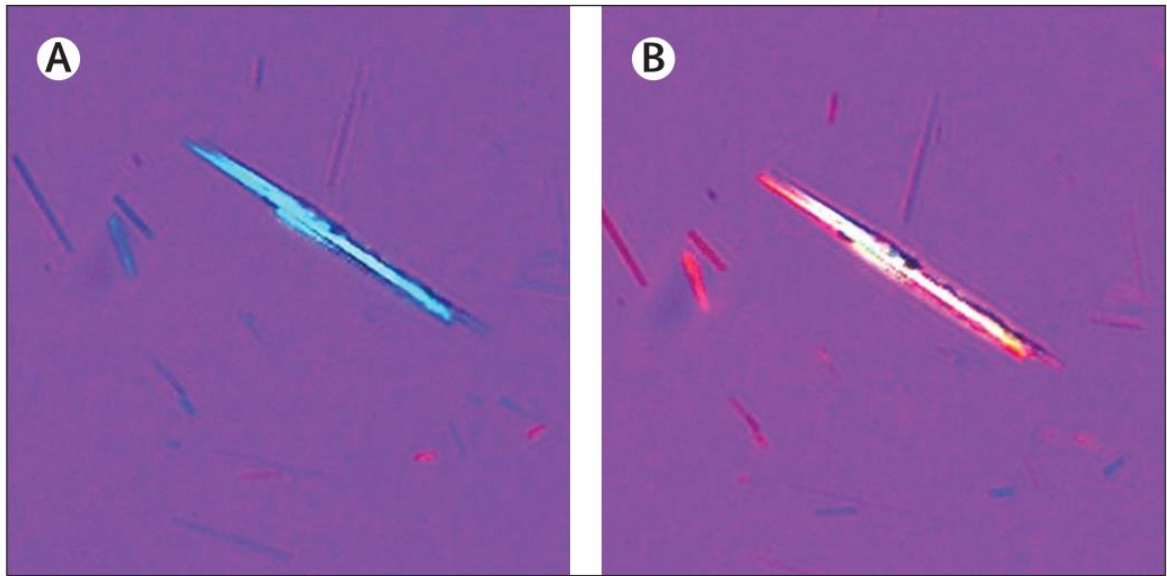
**Table 1.1: The ACR/EULAR 2015 Gout Classification Criteria.** (36) \* For the purpose of the thesis urate was measured in umol/L. <4mg/dl = 240umol/L (-4). 6-8mg/dl = 360 – 480 umol/L (2). 8-10mg/dl = 480-600 umol/L (3). >10mg/dl = >600umol/L (4).

### **1.3.8 The Role of Imaging in the Diagnosis of Gout.**

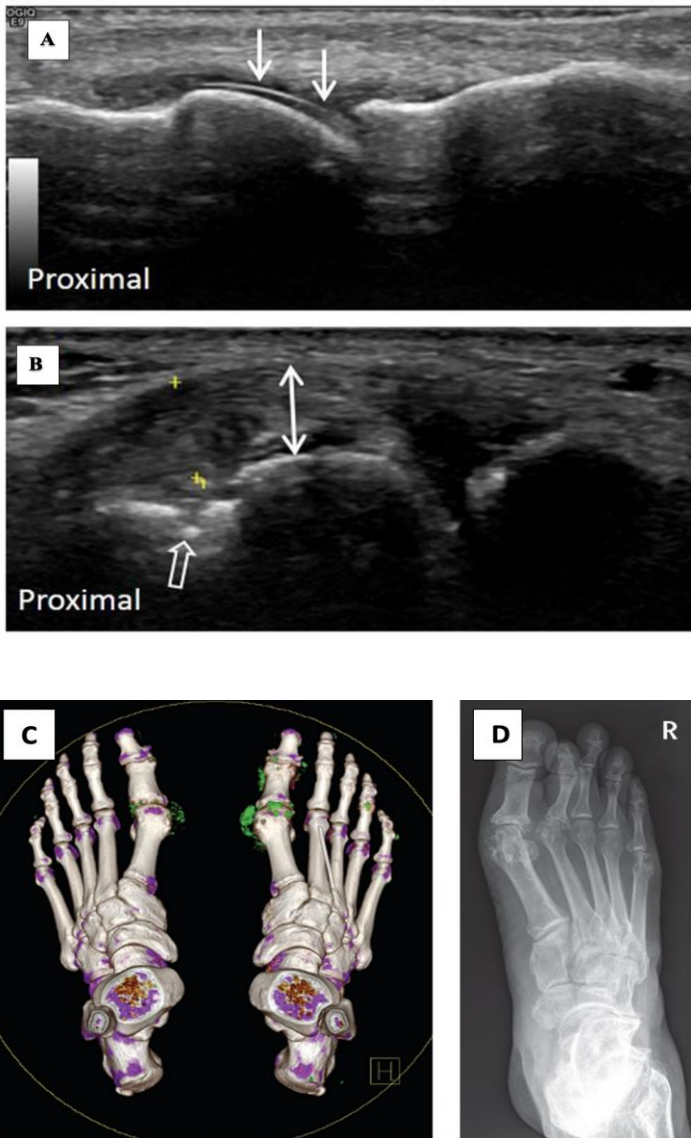
The diagnostic gold standard for gout remains aspiration and identification of monosodium urate (MSU) crystals under polarised light microscopy (25). Joint aspiration is invasive and in certain patients with suspected gout may not be possible. The 2015 gout classification criteria include imaging evidence of urate deposition in the diagnostic criteria. The imaging modalities with sufficient published data and investigator experience to support their utility in identifying urate deposition accurately are conventional X-ray, ultrasound (US), and dual energy computed tomography (DECT) (35). At the time of first presentation, radiographs are usually normal, except for non-specific soft tissue swelling of the affected joint making x-ray less useful for diagnosis. Bone erosion on radiography is a feature of advanced gout and is typically characterised by juxta-articular positioning, a sclerotic rim

and overhanging edge (25). For US evidence of urate deposition, the common finding is the double-contour sign, defined as hyperechoic irregular enhancement over the surface of the hyaline cartilage that is independent of the insonation angle of the US beam. The OMERACT US working group have published consensus based definitions for elementary lesions (tophus, double contour sign (DC) aggregates and erosions) used as outcome measures in the diagnosis of gout by US (37). For DECT, urate deposition is defined as the presence of colour-coded urate at articular or periarticular sites (35).

While DECT allows for automated acquisition, it is expensive to perform and equipment may not be readily available. Conversely, US is now more largely available but can be operator dependant and reliability depends on the availability of a trained musculoskeletal sonographer. US and DECT are more sensitive for detection of urate deposition earlier in the disease course than plain x-ray. While there is limited data available on the comparison of US and DECT imaging modalities, it is suggested both exhibit comparable sensitivity for the detection of urate deposition (38). Although US and DECT are both candidates to quantify urate deposition and monitor urate depletion (39), it is still unknown whether these techniques provide the same quantification of the extent of urate deposition in a given patient.



**Figure 1.5. Polarising light microscopy of synovial fluid demonstrating negatively birefringent monosodium urate crystals left panel perpendicular to lambda axis, and right panel parallel to lambda axis, both at 40× magnification (32).**



**Figure 1.6. Radiology of Gout. (A) Ultrasound features of gout. Dorsal longitudinal ultrasound of first metatarsophalangeal joints (MTP1). The presence of double contour sign due to articular cartilage monosodium urate deposition (solid arrows). (B) Medial longitudinal ultrasound of MTP1. The presence of tophus (double-headed arrow) and juxta-articular erosion (open arrow) over the medial surface of the first metatarsal is shown. Greyscale images obtained using LogiqE9 at 15 MHz. (C) Dual-energy CT of a patient with gout, showing deposition of monosodium urate crystals (shown in green), particularly at the first metatarsophalangeal joints. (D) Plain radiograph of the right foot demonstrating erosive gout in a patient with extensive subcutaneous tophi. Note the involvement of the first, second, and fifth metatarsophalangeal joints, and toes (32).**



### **1.3.9 Current Gout Therapies – Management of the Gout Flare**

The acute gout flare is exceptionally painful and the focus of management is the control of pain and suppression of joint inflammation. Early administration of anti-inflammatory medications is recommended, first line options include oral corticosteroids, non-steroidal anti-inflammatory drugs (NSAIDs) or colchicine. The choice of medication depends on the patients' co-morbidities, renal function, previous experience of therapies and concomitant medications. IL-1 inhibitors are reserved for patients who are unable to tolerate or have contraindications to the first-line anti-inflammatory drugs (40). Intraarticular corticosteroid injection may have a role for patients with an isolated joint involvement. Patient education on the pathophysiology of gout, importance of lifestyle modification, healthy eating and avoidance of food/alcohol which may trigger an attack is also essential.

### **1.3.10 Current Gout Therapies – Principles of Long-term Management.**

In the first instance education around the disease, advice with regard to lifestyle and assessment of co-morbidities should occur. Prophylactic ULT can be commenced during a flare with cover from an anti-inflammatory medication such as colchicine for the first 3-6 months. Allopurinol, an xanthine oxidase inhibitor (XOI) is the current first line ULT. A typical initial dose of 100mg, with up-titration by 100mg every second week to 300mg, based on renal function and serum urate in a treat to target programme. Although allopurinol is usually well tolerated, a potentially severe hypersensitivity cutaneous drug eruption may rarely occur as a known side effect. If allopurinol is not tolerated, febuxostat, a potent non-purine xanthine oxidase inhibitor is a second line treatment option. At present, following the controversial CARES trial, treatment with febuxostat in patients with pre-existing major cardiovascular diseases should be avoided, unless no other therapy

options are appropriate (41). Uricosuric medications such as probenecid, which increase the urinary excretion of urate are an alternative but now rarely used second line therapy, due to the potential risk of nephrotoxicity. If target sUA is not achieved with an XOI or in combination with a uricosuric the agent pegloticase may be considered(40). Pegloticase is a pegylated uricase, produced by a genetically modified strain of Escherichia Coli that catalyses the oxidation of uric acid into allantoin, a more soluble end product. Clinical trials of pegloticase demonstrated improvement in gout flares, tophus size, activity limitation, and health- related quality of life over 6 months (42). Although efficacious, infusion reactions are common in patients receiving pegloticase, reactions are associated with anti-polyethylene glycol antibody formation and the resultant loss of serum urate-lowering efficacy.

#### **1.4 Asymptomatic Hyperuricaemia and Early Gout.**

The updated ACR 2020 guidelines for the management of gout recommends against initiation of ULT for the first gout attack and for those with asymptomatic hyperuricaemia (AH) (43). The prognostic importance of asymptomatic monosodium urate crystal deposition is unclear; specifically, it is unknown what proportion of these individuals will develop symptomatic gout, and without this information, it is difficult to accurately assess the risk–benefit ratio of therapy. Treatment with ULT is associated with medication-related adverse events. Serious adverse events, including fatal hypersensitivity reactions, have been reported in people receiving allopurinol for asymptomatic hyperuricaemia (44). The newer xanthine oxidase inhibitor febuxostat can be associated with hepatotoxicity and hypersensitivity reactions, albeit rarely.

Uric acid is biologically active and may have a role in activating intracellular inflammation through its role in multiple pathways, including inhibiting AMP-associated protein kinase (AMPK), a central regulator of gluconeogenesis, inflammation, and functions associated with metabolic syndrome (45). Efforts to study the impact of AH are stymied by several issues including that animal models of hyperuricemia are limited, as most mammals possess uricase, and therefore have low serum urate levels, rendering them unsuitable as models for hyperuricaemia. While observational cohort studies have indicated associations between AH and multiple comorbidities, including hypertension and CVD, the question as to whether AH plays a causative role in these diseases remains an area of investigation, with divided expert opinion on the need for AH intervention.

Whether or not AH is truly 'asymptomatic' remains a clinical uncertainty, given that imaging techniques demonstrate urate deposits in around 30% of individuals who have never experienced a classical gout attack (46). It is reasonable to propose the alternative hypothesis therefore that US evidence of urate deposition prior to a first acute attack may constitute an early presentation of gout, with MSU deposition on cartilage leading to future joint destruction over time. Whether or not a threshold of urate crystal volume is needed before clinically apparent disease occurs, patients with so-called AH have already been shown to report greater overall non-specific foot pain and disability resulting in increased activity limitation when compared to normouricaemic controls. This emerging evidence that joints of AH individuals contain MSU deposits and that alternative presentations of foot pain occur in hyperuricaemia suggests that both preclinical and early clinical phases may occur prior to a first episodic gout attack (47). Therefore, although the current guidelines recommend against initiation of ULT for AH, there remains clinical uncertainty

as to whether more atypical presentations of pain also constitute a manifestation of gout, and whether earlier treatment of hyperuricaemia in such circumstances would lead to improved long-term outcomes.

### **1.5 Extra-articular manifestations of Hyperuricaemia & Gout.**

The role of ULT in the management of co-morbidities associated with gout is complex and not yet fully elucidated. The association of gout with hypertension, diabetes, kidney disease, and CVD has been observed since the 19th century (48). Investigators from this era hypothesized uric acid to be a cause of hypertension or renal disease. In the 21<sup>st</sup> century there is a wealth of research documenting the association between uric acid and these conditions however it has not been definitively established whether uric acid is merely a marker for risk or a causative agent (49). Studies have found sUA to be independently associated with CVD risk and events (50) (51), but there is currently a lack of evidence regarding treatment targeting sUA levels and improved outcomes for diseases other than gout (52).

#### **1.5.1. Hyperuricaemia and Cardiovascular Disease**

A large number of epidemiologic studies have reported a relationship between serum uric acid levels and a wide variety of cardiovascular conditions, including hypertension, metabolic syndrome, CVD and cerebrovascular disease (27)(51). Tuttle et al, found serum uric acid level was linearly related to coronary artery disease severity in women undergoing coronary angiograms, which likely explains the stronger association with clinical events and mortality. A meta-analysis by Grayson et al, found hyperuricemia is associated with an increased risk for incident hypertension, independent of traditional

hypertension risk factors (53). Further research is needed to fully elucidate the mechanisms linking hyperuricaemia and CVD.

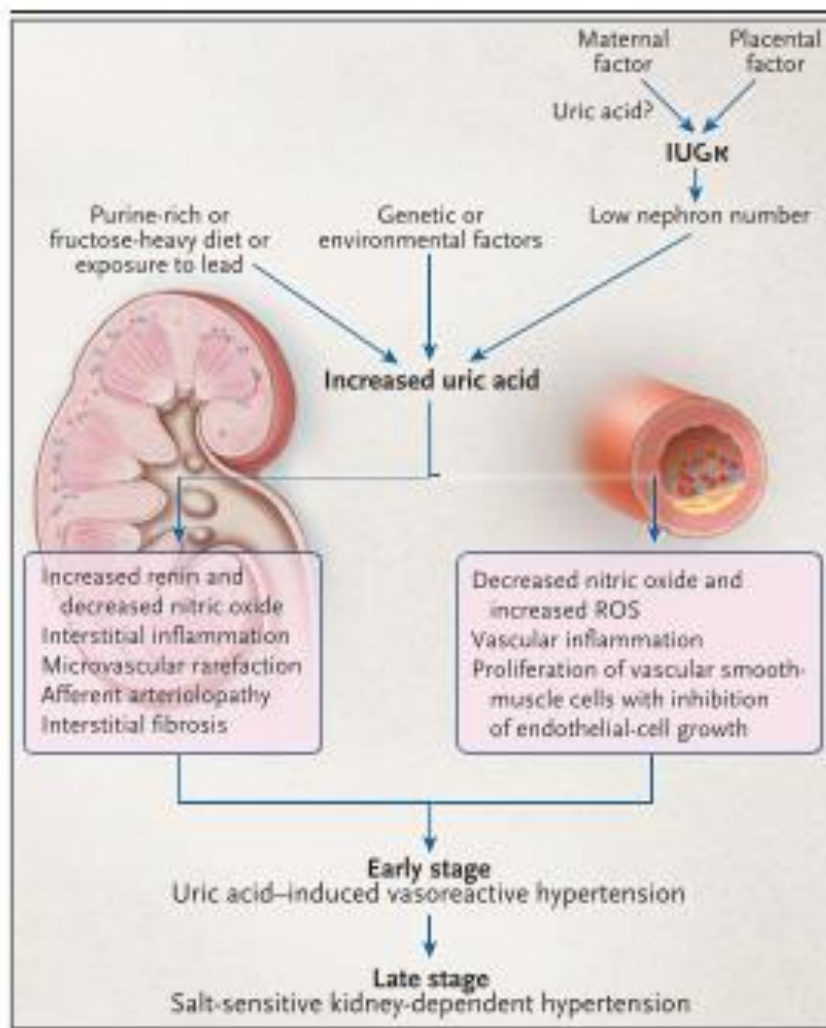
### **1.5.2. Hyperuricaemia and Hypertension.**

The association of hyperuricaemia with hypertension is independent of the traditional cardiovascular risk factors, including age, obesity, hypercholesterolaemia, hypertriglyceridaemia, low high-density lipoprotein (HDL) cholesterol, diabetes, family history of hypertension, smoking and alcohol consumption, suggesting causality (54).

An elevated uric acid consistently predicts the development of hypertension (55)(56)(57). Elevated uric acid is observed in 25-60% of patients with untreated essential hypertension and in nearly 90% of adolescents with essential hypertension of recent onset (58). Reducing the uric acid level with XO1 lowers blood pressure in adolescents with hypertension of recent onset (59).

The proposed mechanism linking hyperuricaemia with hypertension (figure 1.7) involves a combination of environmental factors including diet (such as a fructose or purine rich diet), medications (diuretics), exposure to lead and genetic factors resulting in chronic hyperuricaemia (48)(60). Maternal factors can also contribute, such as hyperuricaemic mothers who transfer uric acid into the fetal circulation through the placenta (61). This transfer may ultimately contribute to intrauterine growth retardation (IUGR) and a reduction in nephron number increasing sUA levels in later life. Chronic hyperuricemia is

postulated to stimulate the renin–angiotensin system and inhibit release of endothelial nitric oxide, contributing to renal vasoconstriction and increasing blood pressure. Persistent renal vasoconstriction may contribute to arteriolosclerosis and the development of salt-sensitive hypertension, which persists even if the hyperuricemia is later corrected (48). This mechanism has been demonstrated in rat models but yet to be fully elucidated in humans (62).



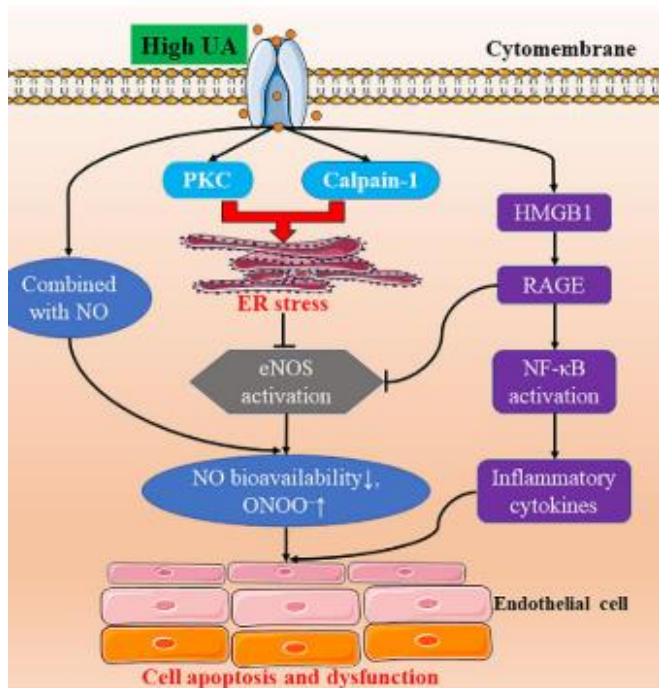
**Figure 1.7: Proposed mechanism for Uric-Acid mediated Hypertension (48). ROS denotes reactive oxygen species.**

Few studies have examined the effects on blood pressure of ULT specifically. A meta-analysis in 2013 reported on 10 clinical studies with a total of 738 individuals (63). All studies used allopurinol as the urate-lowering therapy. In subjects who received allopurinol, the mean decrease in systolic blood pressure was 3.3 mmHg (95% CI: 1.4–5.3 mmHg) and mean decrease in diastolic blood pressure was 1.3 mmHg (95% CI: 0.1–2.5 mmHg) compared to placebo (63).

### **1.5.3 Hyperuricaemia and Endothelial Dysfunction**

A proposed mechanism of hyperuricaemia induced CVD is through impairment of endothelial cell function. Vascular endothelial cells line the entire circulatory system, from the heart to the smallest capillaries. These cells have very distinct and unique functions that are paramount to vascular biology (64). Endothelial cells secrete a variety of vasoactive substances, including vasodilators (nitric oxide, prostaglandin I<sub>2</sub>, etc.) and vasoconstrictors (endothelin-1, thrombin A<sub>2</sub>, Ang II, etc.) (65). Uric acid inhibits the protective vasodilator effect of nitric oxide (NO), NO inhibition plays a key role in the development of early atherosclerosis. Increased uric acid levels in cells combine with NO, resulting in decrease of NO bioavailability and the increased production of the unstable isomer of NO, peroxynitrite (ONOO<sup>-</sup>) a highly potent oxidant, which can cause DNA damage, cell death, and lipid peroxidation (Figure 6) (65)(66). High uric acid also induces endothelial cell apoptosis and endothelial dysfunction through endoplasmic reticulum stress via the HMGB1/RAGE pathway. UA increases high mobility group box chromosomal protein 1 (HMGB1) expression and extracellular release in endothelial cells. HMGB1 is an inflammatory cytokine that interacts with the receptor for advanced glycation end

products (RAGE), inducing an oxidative stress and inflammatory response, which leads to endothelial dysfunction (67). Endothelial dysfunction plays a critical role in the development and progression of atherosclerosis, leading to serious cardiovascular events.



**Figure 1.8: The molecular mechanisms of high UA-inducing oxidative stress, endoplasmic reticulum stress, and endothelial dysfunction. High UA induces endothelial cell apoptosis and endothelial dysfunction through endoplasmic reticulum stress and the HMGB1/RAGE pathway.**

eNOS, endothelial nitric oxide synthase; ER, endoplasmic reticulum; ERK, extracellular signal- regulated kinase; HMGB1, high mobility group box chromosomal protein 1; NF-κB, nuclear factor κB; NO, nitric oxide; Nrf2, NF-E2-related factor 2; p-AKT, phospho-Akt; PI3K, phosphatidylinositol 3-kinase; p-IRS1, phospho-insulin receptor substrate 1; PKC, protein



kinase C; RAGE, receptor for advanced glycation end products; ROS, reactive oxygen species; UA, uric acid (68).

#### **1.5.4 Hyperuricaemia and Pulmonary Vascular Disease**

Elevated serum uric acid levels are common in subjects with pulmonary hypertension (PH). Some findings suggest up to 80% of adult patients with PH have hyperuricaemia (69). The pathogenesis and aetiologies of PH are complex, but in many cases (e.g. lung disease) it appears to be due to hypoxia-dependent pulmonary vasoconstriction with secondary vascular remodelling (70). Based on pathophysiological, clinical, and therapeutic considerations, PH is divided into five groups: pulmonary arterial hypertension; pulmonary hypertension due to left-sided heart disease; pulmonary hypertension due to lung disease or hypoxia; chronic thromboembolic pulmonary hypertension; and pulmonary hypertension with unclear or multifactorial mechanisms (71). Pulmonary arterial hypertension, especially the idiopathic form, although still a rare disease with an incidence of 2–5 per million adults, is increasingly being diagnosed in elderly people (71).

A high serum uric acid is common in subjects with pulmonary hypertension (PH). In a retrospective analysis of 39 patients with PH 79% of cases were found to have elevated serum uric acid levels (72). This study also found a significant correlation between urate levels and the cardiac index ( $r=0.48$ ;  $p=0.0021$ ) and an even stronger correlation between serum urate levels and mean right atrial pressures ( $r=0.83$ ;  $p<0.0001$ ). Data suggests that

sUA levels are correlated with poor outcomes in PH and may be used as a prognostic marker (69). In severe pulmonary hypertension tissue hypoxia may provoke urate overproduction, tissue ischemia, depletion of ATP and increased expression of the xanthine oxidase enzyme that elevates xanthine, hypoxanthine and uric acid levels (73). There is also evidence to suggest that uric acid through its role in nitric oxide inhibition and endothelial dysfunction may be a potential mediator of PH (74). When vascular endothelial cells are incubated with uric acid, a dose-dependent decrease in nitric oxide (NO) production has been shown (75). Reduced bioavailability of NO impairs vascular dilatation and may contribute to altered pulmonary haemodynamics. The associations between hyperuricaemia and PH have been described in the literature, however causality which is amenable to ULT has not yet been fully investigated.

Pulmonary hypertension (PH) is defined as elevated mean pulmonary arterial pressure (PAP, typically >25 mm Hg at rest) and is associated with the development of right heart failure and increased mortality. Right heart catheterization (RHC) is currently the gold-standard method for evaluating pulmonary hypertension, however, by virtue of its invasive nature, other non-invasive methods are needed for refining diagnosis, predicting prognosis and to assess response to therapy. Advances in transthoracic Doppler echocardiography over recent years have led to novel echocardiographic methods that provide the opportunity to assess pulmonary hemodynamic parameters. Pulmonary Pulse Wave Transit Time (PPWT) is a novel echocardiographic measurement being evaluated as a potential alternative to RHC in the diagnosis of PH (76).

### **1.5.5 Hyperuricaemia and Chronic Kidney Disease (CKD)**

A large body of evidence links hyperuricaemia with the development of CKD. Studies in the general population have demonstrated that hyperuricaemia is an independent risk factor for the development of CKD (77)(78). In addition, there is increasing evidence that even mild hyperuricaemia correlates with early kidney damage, as shown by albuminuria and kidney ultrasound abnormalities (79). Serum urate level has been shown to negatively correlate with decreasing estimated glomerular filtration rate (eGFR) as a result of reduced excretion (80). It is unclear whether elevated serum urate levels play a causative role in the progression of kidney disease, or is an indirect marker of decreased kidney function, or both. As discussed previously, MSU crystals, which form in the presence of hyperuricaemia, cause gout flares by activating monocytes and macrophages, with resultant NLRP3 inflammasome-mediated IL-1 $\beta$  release. In this context, evidence supports a low-grade inflammatory phenotype in CKD, which is linked with increased serum concentrations of C-reactive protein, pro-inflammatory cytokines, prostaglandins and leukotrienes (81). MSU crystal-driven inflammation may therefore directly affect renal structure and function in patients with gout. Despite these associations there is a lack of randomised controlled trial (RCT) evidence to support use of ULT to improve eGFR in the context of CKD. The PERL and CKD-FIX trials found no evidence of clinically meaningful benefits of serum urate reduction with allopurinol on kidney outcomes among patients (82)(83). Results from one systematic review and metanalysis did find ULT use for one year was associated with significant improvement in serum creatinine and proteinuria/albuminuria, based on low certainty evidence only (84). A G-CAN consensus statement has highlighted this as an important research priority, commenting that quality evidence to guide management is lacking (85).

### **1.5.6 Hyperuricaemia and Insulin Resistance.**

A number of studies have reported an increased risk of type 2 diabetes in individuals with elevated uric acid levels (86)(87). A meta-analysis, which included 11 observational cohort studies and 42,834 participants, concluded that the risk of developing type 2 diabetes is increased by 17% for each 1 mg/dL (60umol/L) increment in serum uric acid concentration (87). Furthermore, an analysis of data from the European Prospective Investigation into Cancer and Nutrition (EPIC)-InterAct study comprising 24,265 individuals aged 35–70 years, concluded that the risk of developing type 2 diabetes increases by 20% for every 1 mg/dL (60umol/L) increase in uric acid concentration (88). Although the significant positive correlation between hyperuricemia and insulin resistance has been documented, it is largely unknown which is the precursor. The molecular mechanism of hyperuricaemia induced insulin resistance is still unclear. The NLRP3 inflammasome plays a central role in obesity and insulin resistance (89) and as discussed, uric acid crystals are known to activate the inflammasome (28). Uric acid may be a causative factor in the development of insulin resistance but novel analyses and rigorous clinical trials are needed to provide further clarity.

### **1.6 COVID-19 Infection and Outcomes in patients with Gout and Immune Mediated Rheumatic Diseases.**

In March 2020 severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and the resulting Coronavirus disease 2019 (COVID-19) presented a global health crisis which would impact on the provision of medical care and research throughout the world. Due to increased clinical risks associated with SARS-CoV-2 infection, patient recruitment into non-essential clinical studies, including my own research on the pathological associations of

hyperuricaemia was abruptly paused. Necessary redeployment of researchers to the medical 'front line' occurred throughout departments thus altering research agendas and timelines significantly.

The emergence of the COVID-19 pandemic raised concerns amongst clinicians of increased mortality in infected immunocompromised patients including those with immune mediated rheumatic diseases (IMRDs). Many of these patients are considered at-risk for opportunistic infections due to an immunocompromised state resulting both from their underlying immune disease and also due to use of targeted immune-modulating therapies such as conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), Anti-TNF inhibitors (TNFi), and targeted synthetic DMARDS (90). The risk for serious infection is not equal across all patients however and other factors ( e.g. age, glucocorticoids, co-morbidity) are important in assessing infectious risk for any given patient.

With the worldwide spread of SARS- CoV-2, obesity and impaired metabolic health emerged as important determinants of COVID-19 outcome. According to two US studies, patients with SARS- CoV-2 infection were at increased risk of hospital admission if they had obesity (91)(92).

Petrilli et al, examined factors associated with hospital admission and critical illness among 5279 people with COVID-19 in New York City: They found the strongest risks for critical illness aside from age were heart failure (1.9, 1.4 to 2.5), BMI >40 (1.5, 1.0 to 2.2), and male sex (1.5, 1.3 to 1.8). Increased body mass index (BMI) (BMI >40: 2.5, 1.8 to 3.4) was also a risk factor for hospital admission with COVID-19 infection. Price-Haywood et al, examined odds ratios for hospitalization among 3481 Covid-19 Positive Patients and found patients with obesity were at increased odds for hospitalisation (1.43, 1.20 to 1.71) (92). Bode et al

demonstrated that amongst 1122 patients in 88 US hospitals, COVID-19 patients with diabetes and/or uncontrolled hyperglycemia had a longer length of stay in hospital and markedly higher mortality than patients without diabetes or uncontrolled hyperglycemia (93). There is a large volume of data inferring poorer COVID-19 outcomes in patients with features of metabolic syndrome. Due to the well described complex interconnections between gout and metabolic syndrome there was an urgent need to investigate patients with COVID-19 infection and gout. Although the pandemic provided an unexpected turn for my research objectives it provided an opportunity to complete some important work on outcomes of COVID-19 infection in patients with IMRDs and gout.

## **1.7 Summary**

The pathological associations of hyperuricaemia remain a diagnostic and management challenge for Rheumatologists and general physicians around the globe. This thesis strives to provide further clarification on the definitions of sub-clinical gout and the potential benefits of early intervention with ULT, alongside examining the role of hyperuricaemia in cardiovascular, cardiopulmonary and infective diseases.

## **Chapter 2.**

### **Urate-lowering Therapy Reduces Non-Episodic Foot Pain in Patients Who Fail to Meet ACR/EULAR 2015 Gout Classification Criteria: An Effect Predicted by Ultrasound.**

#### **2.1 Introduction**

This case–control study evaluated urate deposition in hyperuricaemic individuals not fulfilling the current gout classification criteria and the potential therapeutic role for urate lowering therapy (ULT) in this cohort.

##### **2.1.1 Current recommendations for diagnosis and initiation of urate lowering therapy in hyperuricaemia.**

Gout, which is caused by monosodium urate (MSU) deposition within joints in the presence of hyperuricaemia, is now the leading cause of inflammatory arthritis within developed countries (94)(95) Despite recent observations that ULT should be considered early to reduce disease chronicity, diagnosis is frequently delayed, leading to suboptimal clinical

outcomes (95)(40). The current American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) 2015 gout classification entry criterion requires the history of a prior episode of swelling, pain or tenderness of a peripheral joint/bursa before confirmation, either through MSU crystal identification in synovial fluid or through achieving a score of >8 using a predefined scoring system of radiological, laboratory and clinical features. (Figure 2.1) One such feature, a gout 'episode', is clearly defined both in terms of its intensity (joint erythema, tenderness, reduced/inhibited walking ability) and duration (time to maximal pain from onset <24 hour; resolution to baseline <14 days).

Two previous randomised controlled trials (RCTS), designed to study the effect of urate lowering therapy on chronic kidney disease (CKD) and cardiovascular disease (CVD) outcomes, demonstrated that commencing ULT in those with asymptomatic hyperuricaemia (AH) prevented subsequent gout flares when compared to control groups not receiving ULT (96)(97). In one study, 24 patients were required to receive treatment with ULT for 3 years to prevent a single (incident) gout flare, indicative of an unacceptably low rate of return for the treatment of asymptomatic disease. Current guidelines therefore do not recommend the institution of treatment for asymptomatic hyperuricaemia. The updated 2020 ACR management guidelines for gout conditionally recommend against initiating ULT for AH, including in patients with radiographic evidence of crystal deposition (43). In this study we aimed to identify an even earlier stage of clinical gout for intervention with ULT, where the opportunity/cost of therapy would still favour a clinical benefit.



	Categories	Score
<b>Step 1:</b> Entry criterion (only apply criteria below to those meeting this entry criterion)	At least 1 episode of swelling, pain, or tenderness in a peripheral joint or bursa	
<b>Step 2:</b> Sufficient criterion (if met, can classify as gout without applying criteria below)	Presence of MSU crystals in a symptomatic joint or bursa (i.e., in synovial fluid) or tophus	
<b>Step 3:</b> Criteria (to be used if sufficient criterion not met)		
Clinical		
Pattern of joint/bursa involvement during symptomatic episode(s) ever†	Ankle <i>or</i> midfoot (as part of monoarticular or oligoarticular episode without involvement of the first metatarsophalangeal joint)	1
	Involvement of the first metatarsophalangeal joint (as part of monoarticular or oligoarticular episode)	2
Characteristics of symptomatic episode(s) ever		
• Erythema overlying affected joint (patient-reported or physician-observed)	One characteristic	1
• Can't bear touch or pressure to affected joint	Two characteristics	2
• Great difficulty with walking or inability to use affected joint	Three characteristics	3
Time course of episode(s) ever		
Presence (ever) of $\geq 2$ , irrespective of antiinflammatory treatment:		
• Time to maximal pain $< 24$ hours	One typical episode	1
• Resolution of symptoms in $\leq 14$ days	Recurrent typical episodes	2
• Complete resolution (to baseline level) between symptomatic episodes		
Clinical evidence of tophus (Figure 2)		
Draining or chalk-like subcutaneous nodule under transparent skin, often with overlying vascularity, located in typical locations: joints, ears, olecranon bursae, finger pads, tendons (e.g., Achilles)	Present	4
Laboratory		
Serum urate: Measured by uricase method. Ideally should be scored at a time when the patient was not receiving urate-lowering treatment and it was $> 4$ weeks from the start of an episode (i.e., during intercritical period); <i>if</i> practicable, retest under those conditions. The highest value irrespective of timing should be scored.		
Synovial fluid analysis of a symptomatic (ever) joint or bursa (should be assessed by a trained observer)§	MSU negative	-2
Imaging (Figure 3)¶		
Imaging evidence of urate deposition in symptomatic (ever) joint or bursa: ultrasound evidence of double-contour sign# <i>or</i> DECT demonstrating urate deposition**	Present (either modality)	4
Imaging evidence of gout-related joint damage: conventional radiography of the hands and/or feet demonstrates at least 1 erosion††	Present	4

**Figure 2.1 The ACR/EULAR 2015 Gout Classification Criterion (35).** \* For the purpose

of the thesis urate was measured in  $\mu\text{mol/L}$ .  $< 4\text{mg/dl} = 240\mu\text{mol/L}$  (-4).  $6\text{-}8\text{mg/dl} = 360 - 480 \mu\text{mol/L}$  (2).  $8\text{-}10\text{mg/dl} = 480\text{-}600 \mu\text{mol/L}$  (3).  $> 10\text{mg/dl} = > 600\mu\text{mol/L}$  (4).

The 2020 ACR guideline for the management of gout recommends commencing ULT for gout patients with the following: 1 or more subcutaneous tophi; radiographic damage (any modality) attributable to gout; frequent gout flares ( $> 2/\text{year}$ ); those who have previously experienced  $> 1$  flare but have infrequent flares ( $< 2/\text{year}$ ); and for patients experiencing

their first flare with concomitant CKD stage >3, serum uric acid >9 mg/dl (535 umol/L) , or urolithiasis (43). The guidelines recommend against initiation of ULT in patients with asymptomatic hyperuricemia and MSU crystal deposition as noted on imaging tests such as ultrasound or dual- energy computed tomography.

Our hypothesis is that foot pain in association with hyperuricaemia exists on a continuum and that the current gout classification criteria are not sensitive enough to specifically capture pain from urate deposition at an early stage. Since any temporal delay in the treatment of pain due to urate deposition will increase the burden of disease, the development of a new classification criteria which identifies early gout will open a window of opportunity to improve outcomes. Our concern is that the current classification criteria may contribute to delayed diagnosis, suboptimal management and increased risk of long-term joint damage and disability due to refractory disease.

### **2.1.2. Early gout - Presentation & Symptoms.**

Emerging evidence that the joints of AH individuals contain MSU deposits and that alternative presentations of foot pain occur in hyperuricaemia suggests that preclinical and clinical phases may occur prior to a first episodic gout attack (98)(47). Failure of current gout classification criteria and management guidelines in identifying these patients with early gout delays treatment and may contribute to long term joint damage. Patients with AH when compared to normal controls have increased odds of disabling foot pain (OR 4.2,  $p = 0.013$ ), increased activity limitation, decreased lower limb function for daily living and recreational activities (98). According to the normative aging study, while risk of gout is based on serum urate levels, not all patients with hyperuricaemia will go on to develop

incident gout (99). Prior serum urate levels of 9 mg/dl (535umol/L) or more were associated with an annual incidence rate of gouty arthritis of 4.9%, compared with 0.5% for urate levels of 7.0 to 8.9 mg/dl. (416-530 umol/L) Where urate levels were 9mg/dl (535umol/L) or higher, the cumulative incidence of gouty arthritis reached 22% after five years (99). The lack of accurate predictors for gout means that many patients who could benefit from early ULT intervention are overlooked. Rheumatology physicians should therefore be aware that alternative presentations of foot pain in the setting of hyperuricaemia may be a harbinger of early gout. We hypothesise that identifying patients with non-episodic foot pain in the setting of hyperuricaemia and initiating ULT prior to the 2015 ACR/EULAR gout diagnostic criteria being met may significantly improve outcomes for joint pain and disability long term.

### **2.1.1 Role of Ultrasound in Early Gout Diagnosis**

Ultrasound (US) is a readily available, inexpensive, non-invasive imaging modality that is becoming more established in the diagnosis of multiple rheumatic diseases. With regard to US evidence of urate deposition, a strongly suggestive finding is the double-contour (DC) sign, defined as a hyperechoic irregular enhancement over the surface of the hyaline cartilage that is independent of the insonation angle of the US beam. Furthermore, the OMERACT US working group have published consensus based definitions for the elementary lesions (tophus, DC sign, aggregates and erosions) used as outcome measures in the diagnosis of gout by US (37). Recent advanced imaging studies have reported that urate crystal deposition is present in some asymptomatic individuals with hyperuricaemia, suggesting that subclinical urate deposition occurs prior to presentation with symptomatic

disease (100)(31). Ultrasound features of gout, including DC sign, accurately predict the presence of MSU crystals in the joint of AH patients indicating that US may be used as a modality for diagnosis of early gout (31). The results of the 'Sons of Gout' study indicate that ultrasonographic evaluation of both first MTP joints is sufficient to identify all individuals with MSU crystal deposition. This research suggests that men with a high risk of gout (e.g. those with a positive family history) could undergo serum urate measurement and ultrasonography of both first MTP joints to screen for MSU crystal deposition (47). While ultrasonographic examination of multiple peripheral joints may be time consuming, assessment of both first MTP joints takes only 5 to 10 minutes, making it possible for asymptomatic MSU crystal deposits to be easily identified. Timely detection would allow consideration of primary preventative measures to halt development of symptomatic gout and permit initiation of therapy at an earlier symptomatic stage.

### **2.1.2 Assessing pain and disability in early gout.**

In order to assess and monitor foot pain and functional disability, an objective and reproducible tool is required. Patient-reported outcome measures (PROMs) are clinical outcome assessment tools used to measure subjective symptoms and the effect of treatment to patients' health. The ideal assessment instrument is responsive to clinical change, reliable, validated, and its scores are repeatable (101). The Manchester Foot Pain and Disability Index (MFPDI) is a PROM developed and validated to measure pain specifically related to a foot disability. The MFPDI is a suitable instrument for assessing the impact of painful foot conditions in community and clinical populations (102). It is a self-administered or interview scoring system. Items are assigned severity level values of 1–3,

corresponding to increasing severity, studies have indicated scores are reliable and consistent (103)(104).

The visual analogue scale (VAS) is a validated, subjective PROM for acute and chronic pain (105). Scores are recorded by making a handwritten mark on a 10-cm line that represents a continuum between “no pain” and “worst pain.” Evidence supports the reliability and validity of the VAS pain score across many populations (106). These validated pain scores are used to assess foot pain and monitor treatment response with ULT in patients with hyperuricaemia in this study.

### **2.1.3 The Role of ULT in AH.**

Allopurinol, a xanthine oxidase inhibitor, is the first line agent for long term management of gout (43). Although allopurinol is a commonly prescribed, usually well tolerated medication, it has been associated with serious and potentially fatal hypersensitivity syndromes in patients with AH (44). Febuxostat, a non-purine inhibitor of xanthine oxidase and a commonly used second line agent, has liver abnormalities listed among its common side effects and the FDA has issued a black box warning for patients with a history of cardiovascular disease (41). The updated 2020 ACR management guidelines for gout conditionally recommend against ULT in AH, commenting the benefits of ULT would not outweigh potential treatment costs or risks for the large number of patients unlikely to progress to gout (43).

Given the association of gout with hypertension, CVD and kidney disease, the use of ULT has been suggested to have benefit in the management of these conditions (59)(48). This has led to discussion surrounding the use of ULT in patients with AH and these co-morbidities. Although the association between AH and CVD has been well documented, it

remains to be mechanistically proven as to whether elevated levels of serum urate are an independent contributor to the development of CVD. At present, given the lack of data on the benefit of ULT in CVD, and given the potential side effect profile of ULT medications, their use is currently not routinely indicated for these conditions (52).

In this chapter we suggest that ULT may have an additional role in the management of hyperuricaemic patients with non-episodic foot pain who fail to meet ACR/EULAR 2015 gout classification criteria and that this effect is predicted by US. Earlier intervention in this specific cohort of patients may improve pain and may also impact on the long-term clinical outcomes for this group.

#### **2.1.4 Specific Objectives of this Study.**

- To investigate whether ULT reduces non-episodic foot pain in patients who fail to meet ACR/EULAR 2015 gout classification criteria.
- To evaluate US evidence of urate crystal deposition in the 1<sup>st</sup> MTP joint in patients with hyperuricaemia and non-episodic foot pain.
- To compare ultrasound features of urate crystal deposition in patients with hyperuricaemia and non-specific foot pain versus asymptomatic hyperuricaemic controls.
- To compare ultrasound features of urate crystal deposition in patients pre and post treatment with ULT.

## **2.2 Methods**

### **2.2.1 Patient recruitment and inclusion criteria**

A specialist 'early gout' outpatient clinic was established in Tallaght University Hospital and patients were referred from general practitioners, allied health practitioners (physiotherapy, occupational health), rheumatology services and other specialities throughout the hospital and community. This clinic was run by a specialist registrar (Rachael Flood) with a special interest in gout and with oversight from a consultant rheumatologist.

Following receipt of informed consent patients with hyperuricaemia and non-specific foot pain who met the following inclusion criteria were recruited to the study;

- Age > 18
- Elevated serum urate (> 420umol Men. > 360umol Women.)
- No previous diagnosis of Inflammatory Arthritis
- No previous treatment for gout.
- Estimated glomerular filtration rate (eGFR) > 30.
- Happy to take part.

Patients included in the non-specific foot pain arm of this study included anyone who answered "yes" to the question: 'Do you have any regular pain or discomfort in your feet?'

Exclusion Criteria

- Age <18
- Normal Serum Urate (<420umol Men. <360 Women.)
- History of Inflammatory Arthritis
- eGFR <30
- Currently on treatment for gout.
- Unable to complete informed consent.

Age and sex matched hyperuricaemic patients with no history of foot pain who met the inclusion criteria were also recruited.

### **2.2.2. Baseline Anthropomorphic assessment**

Baseline demographics including age, sex, co-morbidities (diabetes, hypertension, cardiovascular disease, pulmonary disease, chronic kidney disease and history of malignancy) were recorded. Baseline anthropomorphic assessment of height, weight, body mass index and blood pressure were completed. Baseline serum was taken and the following markers recorded uric acid (umol/L) egfr ml/min/1.73<sup>2</sup>, c-reactive protein mg/dl (crp) erythrocyte sedimentation rate (mm/hr) (ESR) cholesterol (mmol/L) fasting glucose (mmol/L) Homocysteine (umol/L).

### **2.2.3 Assessment of Foot Pain.**

Foot pain was assessed using two validated PROMs, the Manchester Foot Pain and Disability Index (MFDPI) and the 24hour and 7-day visual analogue scale (VAS) (Figure 2.2) The MFDPI is a self-administered or interview scoring system. Items have assigned severity level values of 1–3, corresponding to increasing severity. The VAS scores are recorded by making a handwritten mark on a 10-cm line that represents a continuum between “no pain” and “worst pain” which was then equated to a score of between 0 –



100. The PROMs were self-administered at the beginning of each visit. Results were subsequently calculated and recorded in a secure database in TUH. Pain scores were assessed and recorded at each visit.

**A. Below are some statements about problems people have because of pain in their feet.**

For each statement indicate if this has applied to you **during the past month**. If so, was this

**PLEASE TICK A BOX FOR EACH STATEMENT.**

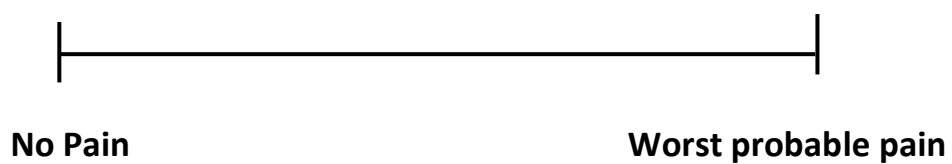
		<b>During the past month this has applied to me</b>		
		<b>None of the time</b>	<b>On some days</b>	<b>On most/ every day(s)</b>
<b>Because of pain in my feet:</b>				
	I avoid walking outside at all	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I avoid walking long distances	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I don't walk in a normal way	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I walk slowly	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I have to stop and rest my feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I avoid hard or rough surfaces when possible	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I avoid standing for a long time	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I catch the bus or use the car more often	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I need help with housework/shopping	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I get irritable when my feet hurt	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Because of pain in my feet:</b>				
	I feel self-conscious about my feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I feel self-conscious about the shoes I have to wear	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I still do everything but with more pain and discomfort	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I have constant pain in my feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	My feet are worse in the morning	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	My feet are more painful in the evening	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
	I get shooting pains in my feet	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
<b>Because of pain in my feet:</b>				<b>Not applicable</b>

**B**

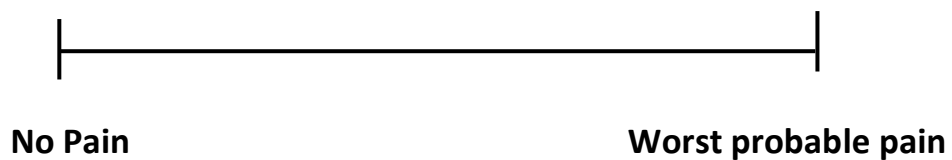
**Generalised Joint Pain Scale**

Please draw a vertical line through the horizontal line below to answer the following questions which relate to the amount of joint pain you have experienced throughout your pain in the last 24 hours and last 7 days.

1- How much pain have you experienced over the last 24 hours.



2- How much pain have you experienced over the last 7 days.



**Figure 2.2. PROMS used to assess foot pain in patients with hyperuricaemia. A: The Manchester Foot Pain and Disability Index. B: 24 Hour and 7 Day Visual Analogue Scale Score.**

#### **2.2.4 Ultrasound Assessment of First MTP Joint.**

Patients underwent musculoskeletal ultrasound (US) assessment of the first MTP joints bilaterally by two trained ultrasonographers. Greyscale images were obtained using LogiqE9 or E10 at 15 MHz. Features of urate deposition according to the OMERACT working group consensus guidelines, including DC sign, tophus and juxta-articular erosions were recorded (37). Both experienced ultrasonographers conducted the ultrasound together and had to agree on the presence of each finding for it to be recorded as positive. The MTP ultrasound assessment was completed pre and post treatment with ULT.

#### **2.2.5 Commencement of ULT**

Cases were treated with ULT daily for 6 months. Allopurinol was the first line agent used, if there was a contraindication or intolerance febuxostat 80mg was used as second line. Allopurinol was commenced at 100mg and uptitrated by 100mg every second week until established on 300mg. Afterwards, serum uric acid levels were checked and dosing was increased as required in a treat to target programme. Patients' renal function and serum uric acid were monitored throughout their time on treatment. Patients were prescribed Colchicine 500mcg twice daily (BD) prophylaxis for the first 8 weeks on ULT. Patients who did not adhere to treatment or failed to reach target serum uric acid levels for a minimum of three months were excluded.

### **2.2.6. Follow up.**

Patients were assessed in the clinic at three and six month time points. Serum uric acid levels were checked six weeks post commencement of ULT and then repeated regularly until target serum uric acid levels were reached. At each study visit, serum urate levels, as well as data on demographic characteristics, lifestyle factors, comorbidities, and drug prescriptions were collected. Targeted musculoskeletal assessment was performed, height, weight, and blood pressure were measured. MFDPI and VAS PROMS were self-administered and targeted MTP US was completed. Bloods as mentioned previously were collected and processed at the chemical pathology laboratory in TUH.

### **2.2.7 Statistical Analysis.**

Statistical analysis was performed using SPSS V.26 software. Continuous variables were reported as the mean  $\pm$  standard error (SE). Categorical variables were reported as the number and percentage. In univariable analyses, differences between cases and controls were compared using chi-squared tests or Fisher's exact tests, as appropriate, for categorical variables and Mann-Whitney U-tests for continuous variables. For the comparison of pain scores the difference in score compared with baseline was assessed

using a paired t test and an independent samples t test was used to compare the difference in score between groups. P value of < 0.05 was deemed significant.

## **2.3 Results.**

### **2.3.1 Baseline Demographics.**

Recruitment was completed between August 2018 and August 2021. 68 Hyperuricaemic patients with non-specific foot pain met the inclusion criteria and were recruited following informed consent. 25 Hyperuricaemic patients with no pain met the inclusion criteria and were recruited, following informed consent, as controls. Table one outlines the baseline demographics between cases and controls. There was no significant difference in age between the two groups, Mean age  $\pm$  SE for cases was  $52.93 \pm 1.9$  versus  $50.28 \pm 3.2$  for controls. There was a significantly higher percentage of males in the case v control groups (60.3% v 36%,  $P < 0.05$ ). There was significantly more hypertension present in the case versus control group (36.% v 12%,  $P < 0.05$ ) other co-morbidities were evenly matched in the two groups. Serum uric acid was significantly higher in cases versus controls (  $474 \pm 12.7$  versus  $402 \pm 17.4$ ,  $P < 0.05$ ) as was egrfr (  $79.94 \pm 3.2$  versus  $68.55 \pm 2.8$ ,  $P < 0.05$  )

Parameter (Mean ± SE)	Case (N = 68)	Control (N= 28)
Age (years)	52.93 ± 1.9	50.82 ± 2.9
Male (N, %)	41 (60.3)	12 (42.9)
uric acid umol/L	474 ± 12.7*	413 ± 16.74
eGFR mL/min/1.73 m <sup>2</sup>	79.94 ± 3.2*	66.87 ± 2.8
CRP (mg/dl)	5.49 ± 1.1	3.12 ± 0.7
ESR (mm/hr)	18.27 ± 2.1	14.08 ± 1.9
Cholesterol (mmol/L)	4.79 ± 0.1	4.94 ± 0.14
Fasting Glucose (mmol/L)	5.76 ± 0.1	6.01 ± 0.35
Homocysteine (umol/L)	18.17 ± 0.1	15.15 ± 1.2
Systolic BP. (mm/hg)	143.73 ± 2.8	139.33 ± 3.8
Diabetes (N,%)	8 (11.8)	2 (7.1)
Hypertension (N, %)	25 (36.8)	5 (17.9)
CKD (N, %)	10 (14.7)	4 (14.3)
IHD (N, %)	7 (10.3)	2 (7.1)
Malignancy (N, %)	2 (2.9)	1 (3.6)
BMI (kg/m <sup>2</sup> )	33.34 ± 0.8	30.44 ± 1.6

**Table 2.1: Baseline Demographics of Cases and Controls. \*P=<0.05**

eGFR - estimated glomerular filtration rate. CRP – C Reactive Protein. ESR – Erythrocyte Sedimentation Rate. BP – Blood Pressure. CKD – Chronic Kidney Disease. IHD - Ischaemic Heart Disease. BMI – Body Mass Index.

### 2.3.2 Excluded Cases.

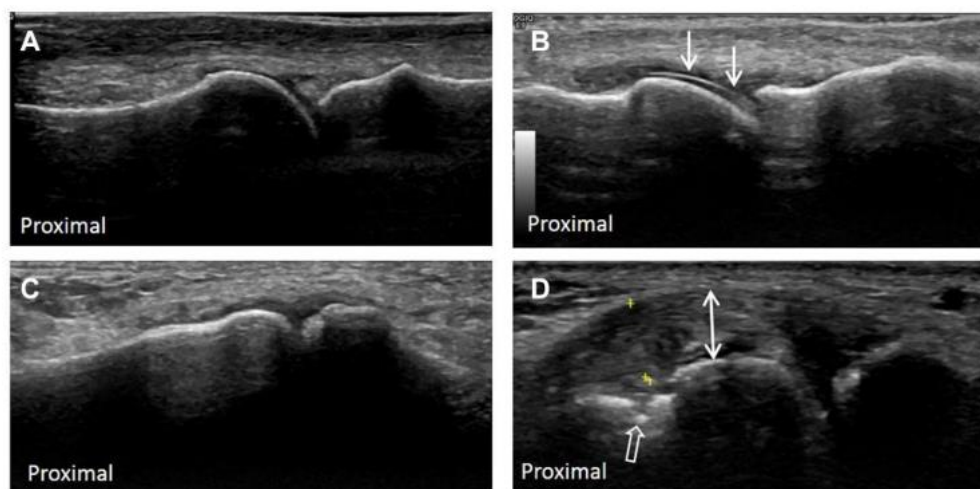
Of the 68 cases recruited for the study 52 completed 6 months of ULT and follow up MTP ultrasound imaging. 16 cases did not complete the study protocol and were excluded. Non-compliance with ULT was the main indication for exclusion. Compliance with ULT was assessed at each study visit by asking the patient directly and regular monitoring of serum urate levels. 10 of the 16 patients did not attend follow up study visits and no longer wished to take part or were lost to follow up. 6 patients no longer wished to take ULT. Table 2.2 demonstrates the baseline demographics between cases and those who were excluded from the study. There was no significant difference in baseline demographics or baseline pain scores between the two groups.

<b>Parameter (Mean ± SE )</b>	<b>Case (N=52)</b>	<b>Excluded Case (N=16)</b>
<b>Age (Years)</b>	53.04 ± 2.13	52.56 ± 4.34
<b>Male (N,%)</b>	35 (67.3%)	6 (37.5%)
<b>Uric Acid (umol/L)</b>	479.15 ± 16.06	456.19 ± 13.64
<b>egfr (ml/min/1.73<sup>2</sup>)</b>	135.3 ± 3.52	74.60 ± 7.00
<b>CRP (mg/dl)</b>	5.77 ± 1.42	4.55 ± 0.75
<b>ESR (mm/hr)</b>	17.98 ± 2.56	19.21 ± 3.25
<b>BMI (kg/m<sup>2</sup>)</b>	33.28 ± 0.95	33.62 ± 1.20
<b>MFDPI</b>	16.06 ± 1.35	17.94 ± 2.47
<b>24 Hour VAS (0-100)</b>	43.12 ± 3.89	50.35 ± 6.93
<b>7 Day VAS (0-100)</b>	55.22 ± 3.97	68.25 ± 5.87

**Table 2.2 Baseline Demographics and PROMS for Cases and Excluded Cases.**

### 2.3.3 Ultrasound Findings Pre and Post Treatment with ULT.

The presence of Tophus, double contour sign and erosions were assessed via a Logiq E9 or E10 ultrasound using a 15mhz linear probe at baseline and following a minimum of six months on ULT.



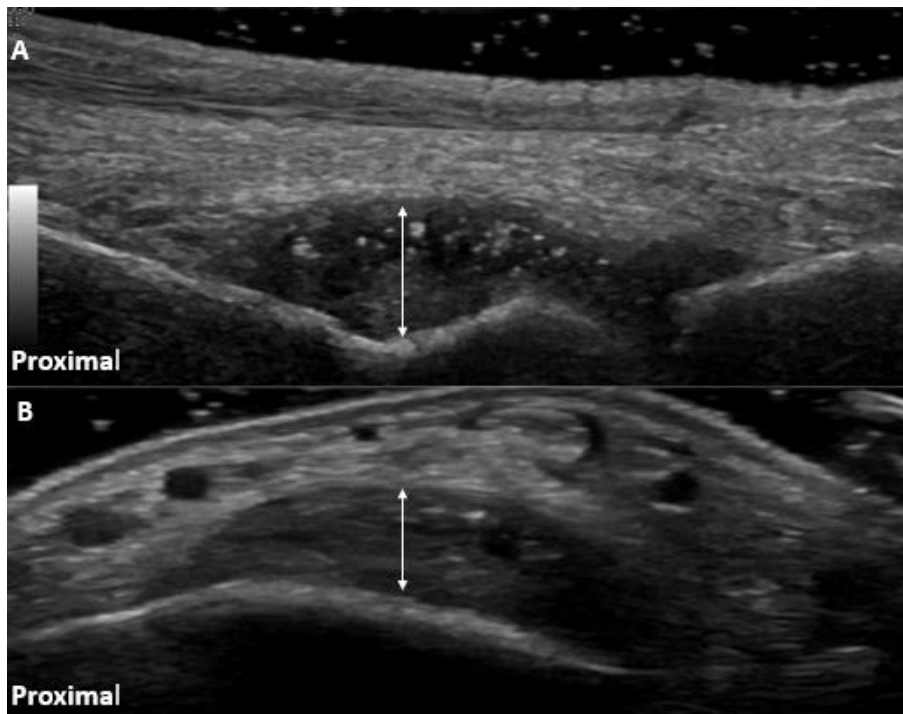
**Figure 2.3: Ultrasound features in patients with hyperuricaemia.** Dorsal longitudinal ultrasound of first metatarsophalangeal joints (MTP1) in (A) isolated hyperuricaemia and (B) hyperuricaemia with non-specific foot pain. The presence of double contour sign due to articular cartilage monosodium urate deposition (solid arrows) is shown in (C). Medial longitudinal ultrasound of MTP1 in (C) isolated hyperuricaemia and (D) hyperuricaemia with non-specific foot pain. The presence of tophus (double-headed arrow) and juxta-articular erosion (open arrow) over the medial surface of the first metatarsal is shown in (D). Greyscale images obtained using LogiqE9 at 15 MHz.



Of the 52 cases that completed the study protocol tophus was present in N= 32, 61.5% of cases at baseline and N= 35, 67.3% of cases following six months of therapy. Double Contour sign was present in N= 35, 67.3% of cases at baseline and N=31, 59.6% of cases at 6 months. Erosions were present in N = 16, 30.7% of cases at baseline and N= 15, 28.8% following six months of therapy. There was no significant resolution in ultrasound features (Tophus, DC, Erosions) of urate deposition following six months of therapy. MSK US assessment of the MTPs was completed on asymptomatic hyperuricaemic controls, 1 patient had evidence of tophus and double contour sign on examination. 27 asymptomatic controls did not have features of urate deposition on US.

<b>Ultrasound Feature</b>	<b>Baseline (N, %)</b>	<b>6 Months. (N, %)</b>
<b>Tophus</b>	32, 61.5%	35 67.3%
<b>Double Contour</b>	35, 67.3%	31 59.6%
<b>Erosions</b>	16, 30.7%	15, 28.8%

**Table 2.3: Ultrasound Features of Urate Deposition at Baseline and 6 Months.**



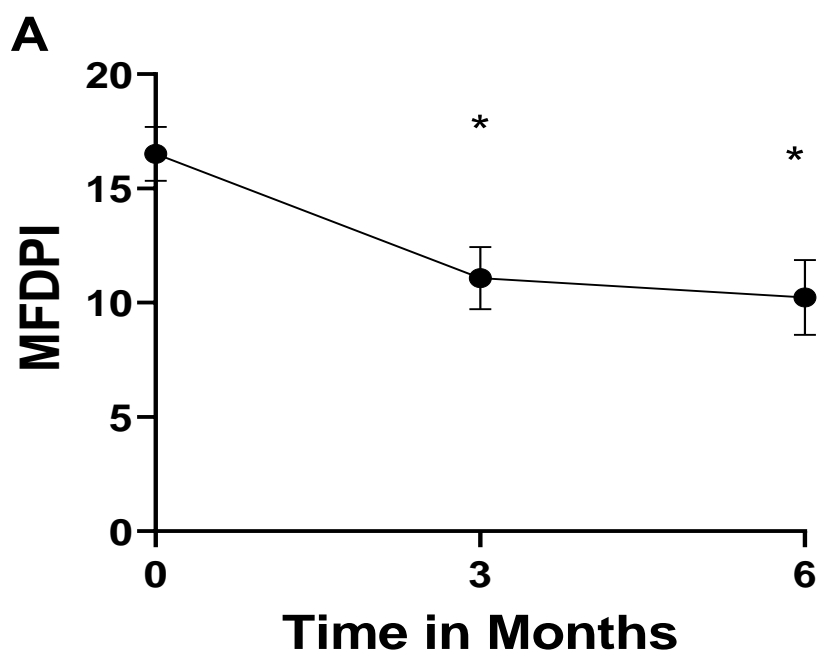
**Figure 2.4 Ultrasound features in control case with asymptomatic hyperuricaemia.** Dorsal ultrasound of first metatarsophalangeal joints (MTP1) (A) Longitudinal view and (B) Transverse view in a patient with hyperuricaemia and no pain. The presence of a tophus due to articular cartilage monosodium urate deposition (double-headed arrows) is shown. Greyscale images obtained using LogiqE10 at 15 MHz

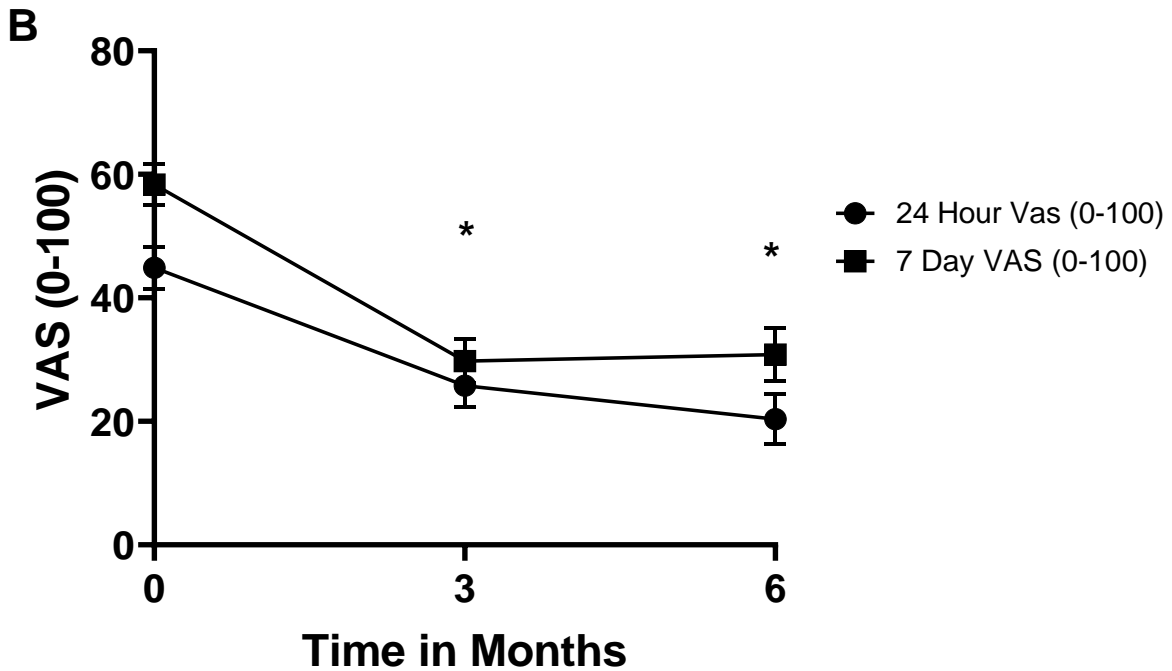
### 2.3.4 MFDPI, 24hr VAS and 7-day VAS Scores.

Baseline pain scores for MFDPI, 24hr VAS and 7-day VAS significantly improved in cases following 3 and 6 months' treatment with ULT (Table 2.4). Serum urate in cases fell at 3 months ( $327 \pm 20$ ;  $p < 0.01$ ) and 6 months ( $298 \pm 18$ ;  $p < 0.01$ ). MFDPI significantly reduced from  $16.51 \pm 1.18$  at baseline to  $11.08 \pm 1.36$  at three months and  $10.23 \pm 1.64$  at six months,  $P < 0.05$ . 24-hour VAS significantly reduced from  $44.85 \pm 3.40$  at baseline to  $25.77 \pm 3.49$  at three months and  $20.38 \pm 4.05$  at six months,  $P < 0.05$ . 7 Day VAS significantly reduced from  $58.33 \pm 3.38$  at baseline to  $29.78 \pm 3.61$  at three months and  $30.81 \pm 4.25$  at six months,  $P < 0.05$ .

Pain Score	Baseline	3 Months	6 Months
<b>MFDPI</b>	16.51 ± 1.18	11.08 ± 1.36*	10.23 ± 1.64*
<b>24 Hour VAS (0-100)</b>	44.85 ± 3.40	25.77 ± 3.49*	20.38 ± 4.05*
<b>7 Day VAS (0-100)</b>	58.33 ± 3.38	29.78 ± 3.61*	30.81 ± 4.25*

Table 2.4: PROMs Scores in Cases at Baseline and 3 & 6 months post ULT. N = 52; \*= P <0.05 significant difference from baseline.



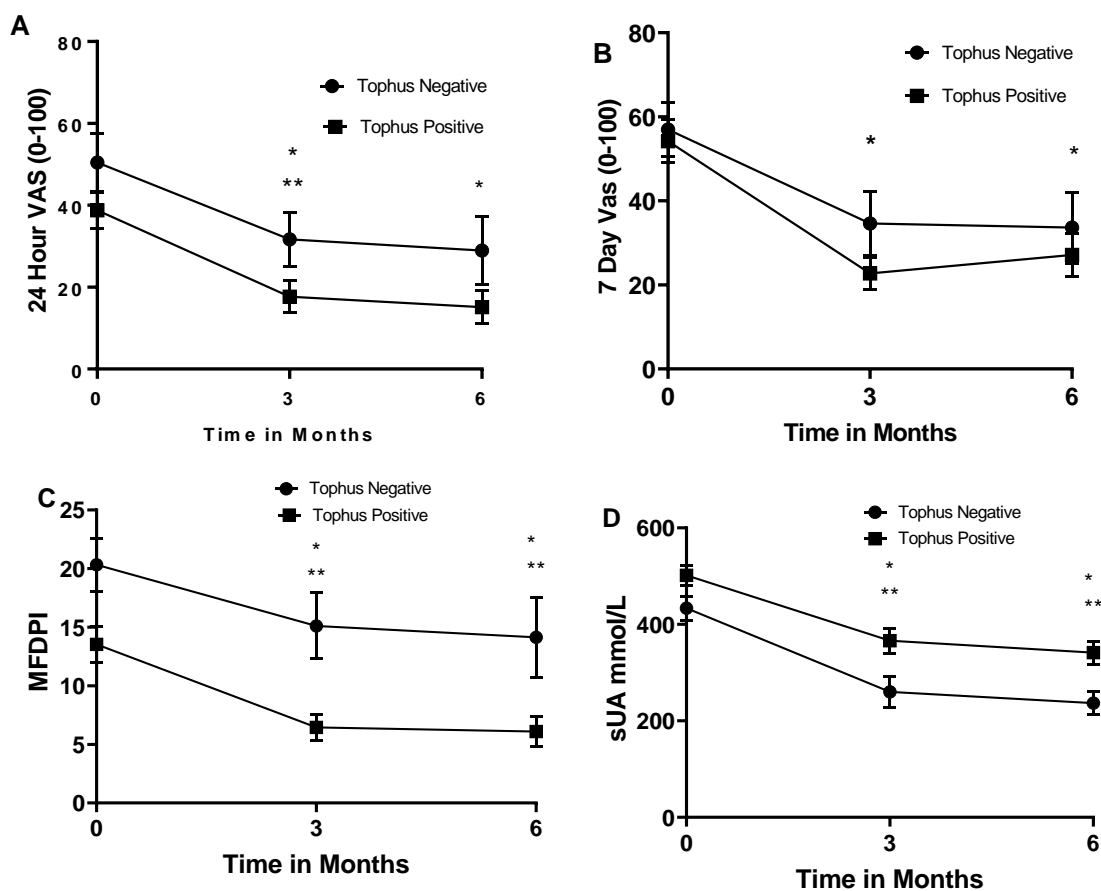


**Figure 2.5: A. MFPDI Pain Scores significantly reduce from baseline following ULT. B. 24 Hour and 7 Day VAS Pain Scores significantly reduce from baseline following ULT. (n=52; \*= P < 0.05 significant difference from baseline).**

**2.3.5. The Presence of Baseline Tophus on US is associated with a greater improvement in pain scores following treatment with ULT**

When cases were grouped according to the presence (n=32) or absence (n=20) of tophus on baseline US, no differences were observed for baseline 24 hour and 7day VAS Scores. MFPDI however was significantly lower in tophus positive cases at baseline (Figure 2.5). Baseline 24-hour VAS was 38.78±4.4 in tophus positive cases versus 50.42±7.1; P=NS in tophus negative cases. Baseline 7-day VAS was 54.19±5.09 in tophus positive cases versus 56.95.5±6; P=NS in tophus negative cases. Baseline MFPDI was 13.53±1.5 in tophus positive cases versus 20.32±12.3; P<0.05 in tophus negative cases. Following ULT however, 24-hour pain VAS were significantly lower in tophus positive patients at 3 months (17.65±3.9 tophus positive vs 31.68±6.5 tophus negative; p<0.05) and lower at 6 months

( $15.10 \pm 4.1$  vs  $28.93 \pm 8.2$ ;  $p=NS$ ). The 7-day pain VAS were lower in tophus positive patients at 3 months ( $22.71 \pm 3.8$  vs  $34.63 \pm 6.5$ ;  $P=NS$ ) and 6 months ( $27.14 \pm 5.07$  vs  $33.62 \pm 8.4$ ;  $p=NS$ ). The MFDPI was significantly lower in tophus positive patients at 3 months ( $6.45 \pm 1.1$  vs  $15.11 \pm 2.8$ ;  $p < 0.05$ ) and 6 months ( $6.09 \pm 1.3$  vs  $14.13 \pm 3.3$ ;  $p < 0.05$ ). The mean serum urate (umol/L) was significantly higher in tophus positive cases at baseline ( $501 \pm 19.9$  vs  $433 \pm 24.8$ ;  $p < 0.05$ ) three months ( $366 \pm 25.8$  vs  $259 \pm 31.5$ ;  $p < 0.05$ ) and six months ( $341 \pm 24.0$  vs  $236 \pm 23.6$ ;  $p < 0.05$ ). In all cases, there were significant reductions from baseline in pain score and serum urate at three and six months.

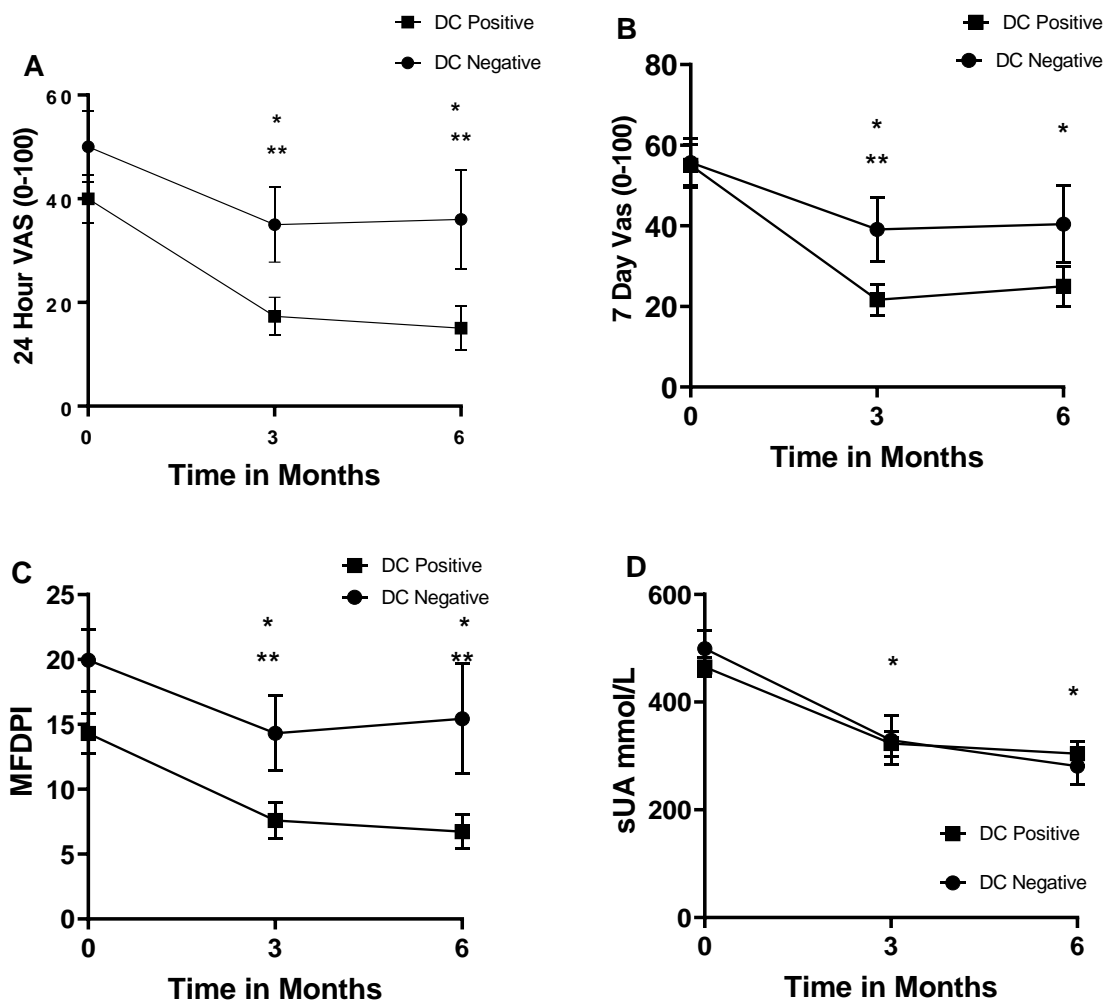


**Figure 2.6 Baseline US presence of tophus is associated with a greater reduction in non-specific foot pain at 3 and 6 months. Patient reported (A) 24-hour pain VAS (0–100 mm), (B) 7-day pain VAS (0–100 mm), (C) MFPDI and (D) serum urate concentration (umol/L) at 0–6months compared between patients with baseline positive tophus (Square, N=32) or negative tophus (Circle, N=20). Mean and SE values shown. \*P<0.05, significant difference compared with baseline, paired t-test. \*\*P<0.05 significant differences between groups, independent samples t-test.**

### **2.3.5 The Presence of Baseline Double Contour Sign on US is associated with a greater improvement in pain scores following treatment with ULT.**

When cases were grouped according to the presence (n=35) or absence (n=17) of DC on baseline US, no significant differences were observed for baseline VAS or MFDPI scores (figure 2.6). Baseline 24-hour VAS was  $39.97 \pm 4.6$  in DC positive cases versus  $50.00 \pm 6.8$ ; P=NS in DC negative cases. Baseline 7-day VAS was  $55.00 \pm 5.1$  in DC positive cases versus  $55.69 \pm 6.1$ ; P=NS in DC negative cases. MFDPI scores were  $14.29 \pm 1.56$  in DC positive patients versus  $19.94 \pm 2.3$ ; p=NS in DC negative cases at baseline. Following ULT however, 24-hour pain VAS were significantly lower in DC positive patients at 3 month ( $17.32 \pm 3.6$  DC positive vs  $35.00 \pm 7.2$  DC negative;  $p < 0.05$ ) and significantly lower at 6 months ( $15.04 \pm 4.2$  vs  $36.00 \pm 9.5$  P= $< 0.05$ ). The 7-day pain VAS were significantly lower in DC positive patients at 3 months ( $21.65 \pm 3.9$  vs  $39.12 \pm 7.8$ ;  $p < 0.05$ ) and lower at 6 months ( $25.0 \pm 4.9$  vs  $40.42 \pm 9.5$ ; P=NS). The MFDPI was significantly lower in DC positive patients at 3 months

( $7.59 \pm 1.4$  vs  $14.31 \pm 2.9$ ;  $p < 0.05$ ) and 6 months ( $6.73 \pm 1.3$  vs  $15.4 \pm 4.2$ ;  $p < 0.05$ ). There was no significant difference in the mean serum urate ( $\mu\text{mol/L}$ ) at baseline in DC positive cases versus negative cases ( $465 \pm 17.6$  vs  $499 \pm 34.1$ ,  $P = \text{NS}$ ) or at three months ( $323 \pm 23.0$  vs  $329 \pm 45.6$ ;  $P = \text{NS}$ ) and six months ( $304 \pm 22.8$  vs  $281 \pm 34.8$ ;  $P = \text{NS}$ ). In all cases, there were significant reductions from baseline in pain score and serum urate at three and six months.



**Figure 2.7** Baseline DC sign is associated with a greater reduction in non-specific foot pain at 3 and 6 months. Patient reported (A) 24-hour pain VAS (0–100 mm), (B) 7-day pain VAS (0–100 mm), (C) MFPDI and (D) serum urate concentration ( $\mu\text{mol/L}$ ) at 0–6months compared between patients with baseline positive DC sign (square,  $N=35$ ) or negative DC

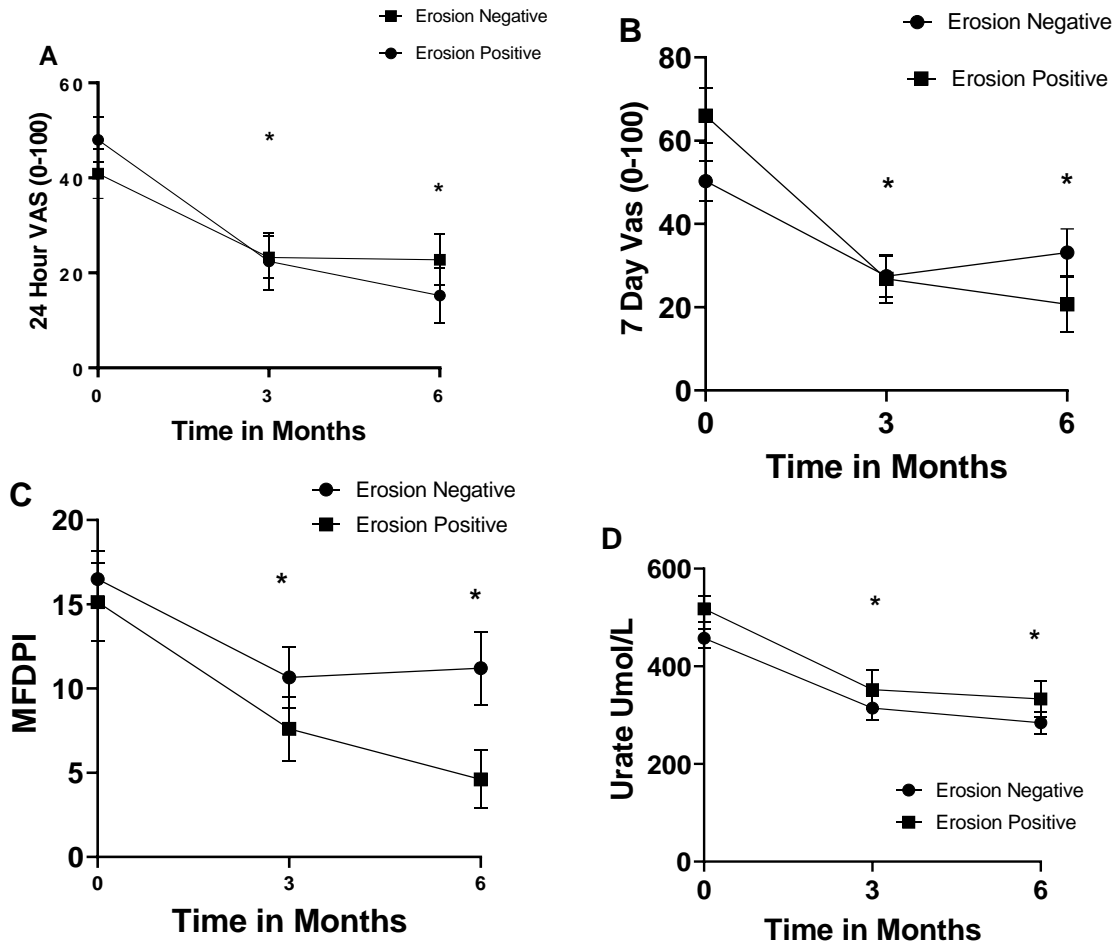
sign (circle, N=17). Mean and SE values shown. \*P<0.05, significant difference compared with baseline, paired t-test. \*\*P<0.05 significant differences between groups, independent samples t-test.

### **2.3.6. No Significant Improvement in Pain Scores Seen in Cases with Erosions Present versus Absent on US Following Treatment with ULT.**

When cases were grouped according to the presence (n=16) or absence (n=36) of erosions on baseline US, no significant differences were observed for baseline pain scores (figure 6). Baseline 24-hour VAS was  $48.00 \pm 4.8$  in erosion positive cases versus  $40.89 \pm 5.2$ ; P=NS in erosion negative cases. Baseline 7-day VAS was  $66.00 \pm 6.6$  in erosion positive cases versus  $50.29 \pm 4.7$ ; P=NS in erosion negative cases. Baseline MFPDI was  $15.13 \pm 2.3$  in erosion positive cases versus  $16.49 \pm 1.6$ ; P=NS in erosion negative cases. Baseline US presence of erosions is not associated with a greater reduction in non-specific foot pain at 3 and 6 months. Following 3 months of ULT 24-hour pain VAS were  $22.40 \pm 6.0$  in erosion positive vs  $23.23 \pm 4.4$ ; P=NS in erosion negative patients and at 6 months  $15.2 \pm 5.8$  vs  $22.74 \pm 5.5$ ; P=NS. Following 3 months of ULT 7-day pain VAS were  $26.8 \pm 5.7$  in erosion positive vs  $27.43.71 \pm 4.8$ ; P=NS in erosion negative patients and at 6 months  $20.07 \pm 6.5$  vs  $33.14 \pm 5.6$ ; P=NS. Following 3 months of ULT MFDPI were  $7.6 \pm 1.9$  in erosion positive vs  $10.66 \pm 1.8$ ; P=NS in erosion negative patients and at 6 months  $4.60 \pm 1.7$  vs  $11.21 \pm 2.1$ , P=NS. There was no significant difference in the mean serum urate (umol/L) at baseline in erosion positive cases versus negative cases ( $517.8 \pm 26.5$



vs  $457.3 \pm 19.5$ ,  $P=NS$ ) or at three months ( $352.2 \pm 39.5$  vs  $314.3 \pm 25.1$ ,  $P=NS$ ) and six months ( $333.1 \pm 36.3$  vs  $284.5 \pm 22.0$   $P=NS$ ). In all cases, there were significant reductions from baseline in pain score and serum urate at three and six months.



**Figure 2.8.** Baseline US presence of erosions is not associated with a greater reduction in non-specific foot pain at 3 and 6 months. Patient reported (A) 24-hour pain VAS (0–100 mm), (B) 7-day pain VAS (0–100 mm), (C) MFDPI and (D) serum urate concentration (umol/L) at 0–6months compared between patients with baseline positive erosion (square, N=16) or negative erosion (circle, N=36). Mean and SE values shown. \* $P < 0.05$ , significant difference compared with baseline, paired t-test. \*\* $P < 0.05$  significant differences between groups, independent samples t-test.

## 2.4 Discussion

This is a large study examining the relationship between atypical foot pain in hyperuricaemic patients and features of uric acid deposition on musculoskeletal ultrasound. In this chapter, we aimed to demonstrate that persistent, non-episodic foot pain in hyperuricaemia is associated with US features of MSU deposition and is responsive to ULT. We have showed that ultrasound features of MSU deposition, including tophus and double contour sign, were frequently found on baseline examination of the MTP joints in patients with non-specific foot pain, who did not meet the ACR/ EULAR 2015 gout classification criteria. Our work adds to the growing body of evidence that urate deposits are frequently found in the joints of hyperuricaemic patients and such deposits may cause inflammation(31)(46)(107). It is difficult to predict which cases of hyperuricaemic deposits will go on to develop classic gout and require treatment. Given the controversy surrounding empiric treatment with ULT, our study identifies a group with 'early clinical gout' who may benefit from treatment with ULT.

Patients with hyperuricaemia and atypical foot pain had a significant improvement from baseline of both MFDPI and VAS pain scores, following a period of ULT. The presence of either double contour sign or tophus on MSK ultrasound predicted a significantly greater improvement in pain compared to cases without these features on ultrasound. The presence of erosions on MTP US was not associated with a significantly greater reduction in pain over the treatment period. In contrast to DC and tophus, which are considered to be highly specific for gout, we hypothesize that the lack of an association between the

presence of joint erosions and subsequent reduction of foot pain following ULT is because an erosive appearance at the MTP joint is not specific to a diagnosis of gout and may occur in numerous other inflammatory and non-inflammatory conditions (osteoarthritis) which do not respond to ULT (108) (109). The current ACR 2020 gout management guidelines cautiously recommend against therapy for patients with hyperuricaemia who do not meet the diagnostic criteria for gout. Our results indicate an early disease presentation of 'patients with atypical foot pain and US features of urate deposition' - which if correctly identified would benefit from earlier intervention with ULT.

In the 52 cases that completed the study protocol we did not find there to be a significant reduction in features of urate deposition (DC Sign, Tophus, Erosions) detected on MSK US following six months of ULT. There was a small, non-significant reduction in the presence of double contour sign over the six month study period, this was despite a significant reduction in serum uric acid over the treatment period. The disappearance of US signs of urate deposition after sustained sUA normalization has been previously shown in smaller studies of patients with gout (110)(111). One larger study of 209 patients followed over a 12 month period demonstrated that DC sign was shown to be the feature most sensitive to change (112). Although there are ultrasound definitions by OMERACT for the elementary lesions (DC, tophi and aggregates) there are currently no validated scoring systems for the different types of ultrasound depositions. The studies mentioned were mainly focused on patients with an established diagnosis of gout and our work is the first of its kind, examining change in patients with early gout. The reduced urate burden and duration of disease in our cohort may make the initial findings more subtle and therefore the detection of change more difficult. Features of urate deposition were recorded as present or absent, tophus

burden and DC thickness was not specifically reviewed. 6 months is a relatively short timeframe, and it would be interesting to examine if features resolved after a longer period of targeted therapy.

The strengths of the present study include the high number of participants followed for 6 months of effective ULT. The participants were followed in a specific early gout clinic with short waiting lists and regular monitoring. In addition, only high-end ultrasound machines were used, and two skilled ultrasonographers had to agree on each elementary lesion recorded.

68 patients were initially recruited to the study, alongside 28 age and sex matched controls. 52 cases completed the study protocol, with the main reason for exclusion being non-compliance with ULT. This finding is in keeping with the consensus that the management of gout is suboptimal worldwide (1)(21). There were no significant differences in baseline demographics between excluded cases and those who completed the study therefore reducing the impact on the overall results. Seemingly universal low adherence rates to ULT globally is likely to be due to many factors, including systems barriers (e.g. time constraints, cost, and scarcity of incentives), the intermittent flaring nature of the illness which can have long asymptomatic inter-critical periods, stigma and incomplete knowledge about the illness and its management (113). Poorly controlled gout causes significant disability and incurs significant societal costs (20). In addition, gout is associated with an increased risk of death (from cardiovascular disease), even after adjusting for age, sex, and other comorbidities (114). Early intervention with ULT and education surrounding the disease and the importance of compliance with treatment is essential to improve outcomes in this cohort of patients.

Other limitations of this study include the short duration of follow up, due in part to the impact of the COVID-19 pandemic. A larger sample size would increase the power of the study. The number and sex matching of hyperuricaemic controls could be optimised. It may also be of benefit to follow controls to assess if pain or gout features develop in the future. Although ultrasound is a useful tool for diagnosing and monitoring urate deposition, its reliability depends on the user and there will be inter-user variations in reporting. While VAS and MFDPI PROMs are validated tools for accessing pain, the scoring is subjective and difficult to standardise between cases. Due to the impact of the COVID-19 pandemic, recruitment for this study was paused for a period and for some cases follow-up timepoints were completed by phone call with MSK US completed retrospectively.

In conclusion, symptomatic hyperuricaemia occurring prior to episodic gout represents an earlier or alternative disease presentation. Changes to the ACR/ EULAR classification criteria to include non-episodic foot pain in the presence of US features of gout may increase the sensitivity of disease classification at an early stage, leading to improved future treatment strategies and long-term outcomes.

## **Chapter 3**

### **The Pathological Association of Hyperuricaemia and Cardiovascular and Cardiopulmonary Diseases.**

#### **3.1 Introduction.**

##### **3.1.1. General Introduction.**

In addition to the pathogenesis of gout, hyperuricaemia has been associated with cardiovascular (CVD) and cardiopulmonary diseases (48)(69). Although the associations between hyperuricaemia and vascular disease are well described, it has not been definitively established whether uric acid is a marker for risk or a causative agent (49). In this chapter we investigate the associations between hyperuricaemia and surrogate markers of adverse cardiovascular outcomes, firstly, of the systemic arterial vasculature via flow mediated and nitroglycerin mediated dilatation studies of the brachial artery and secondly of the pulmonary vasculature by a novel echocardiogram index: pulmonary pulse wave transit time (pPTT). Furthermore, using serial measurement of dilatation and pPTT before and after treatment with urate lowering therapy (ULT) we investigated whether reducing serum uric acid levels has an impact on these indices of vascular dysfunction.

##### **3.1.2. Hyperuricaemia and Cardiovascular Disease**

A large number of epidemiologic studies have reported a relationship between serum uric acid (sUA) levels and cardiovascular conditions, including hypertension, metabolic syndrome, CVD and cerebrovascular disease (27)(51). The prevalence of metabolic syndrome, based on the National Health and Nutrition Examination Survey (NHANES) data,

showed a stepwise increase from 18.9% in individuals with serum uric acid concentrations <6 mg/dL (357umol/L) to 70.7% in those with serum uric acid concentration  $\geq$ 10 mg/dL (595umol/L) (115). The Brisighella heart study also reported a significant correlation between elevated serum uric acid levels and hypertension and atherosclerosis (including both increased carotid intima-media thickness and pulse wave velocity) (116). Despite the documented association, the mechanism by which hyperuricaemia infers causality of CVD is not yet fully understood. Additional data is required to better understand if treating sUA levels will affect CVD outcomes. One randomised controlled trial (RCT) demonstrated that long-term effective control of serum uric acid by allopurinol may improve blood pressure and carotid intima-media thickness (IMT) in patients with type 2 diabetes (T2DM) and asymptomatic hyperuricaemia (AH) (97). In this study, 176 patients with T2DM and AH were randomly allocated to the conventional or allopurinol treatment groups. Changes in the carotid IMT, biochemical indexes, CRP and the incidence of hypertension in patients before and after three years of treatment were examined and compared between the groups. Systolic blood pressure, diastolic blood pressure and the carotid IMT in the allopurinol group were significantly lower than those in the conventional group after three years of treatment ( $p < 0.01$ ). sUA has been shown to be independently associated with CVD risk and events (117)(118). A population-based, prospective cohort study of 1423 middle-aged Finnish men found, in age-adjusted analyses, high serum uric acid levels to be associated with a greater than 2.5 fold higher risk of death from CVD (118). However, there are also compelling arguments that the apparent association is primarily a function of sUA being strongly collinear with established CV risk factors (119). Outcomes from the Framingham heart study indicated that uric acid does not have a causal role in the development of coronary heart disease or death from CVD and any apparent association

with these outcomes is probably due to the association of uric acid level with other risk factors.

### **3.1.3. Hyperuricaemia and Endothelial Dysfunction**

A proposed mechanism of hyperuricaemia induced CVD is through impairment of endothelial cell (EC) function. Vascular EC line the entire circulatory system, from the heart to the smallest capillaries. These cells have very distinct and unique functions that are vital for vascular biology (64). ECs form an inner monolayer along the blood vessel wall, as well as forming a barrier between blood and the walls of vessels. ECs perform endocrine functions through the paracrine and endocrine release of mediators of vascular relaxation. ECs secrete a variety of vasoactive substances, including vasodilators (nitric oxide, prostaglandin I<sub>2</sub>, etc.) and vasoconstrictors (endothelin-1, thrombin A<sub>2</sub>, Ang II, etc.) (65). They also perform as an immune organ and express cell surface receptors and adhesion molecules such as ICAM-1 and VCAM-1 at the sites of inflammation which enable the attachment and ingress of monocytes, macrophages and lymphocytes.

Uric acid inhibits the protective vasodilator effect of nitric oxide (NO). NO inhibition plays a key role in the development of early atherosclerosis. sUA has been shown to down regulate NO production through at least three mechanisms, including modulation of the phosphorylation status of endothelial nitric oxide synthase (eNOS), potentiation of arginase activity and increasing intracellular superoxide (66). Decreased NO bioavailability results in increased production of the unstable isomer of NO, peroxynitrite (ONOO<sup>-</sup>) which is a highly potent oxidant, causing DNA damage, cell death and lipid peroxidation (65)(66). High uric acid also induces endothelial cell apoptosis and endothelial dysfunction through endoplasmic reticulum stress and the HMGB1/RAGE pathway. Uric acid increases high mobility group box chromosomal protein 1 (HMGB1) expression and extracellular release

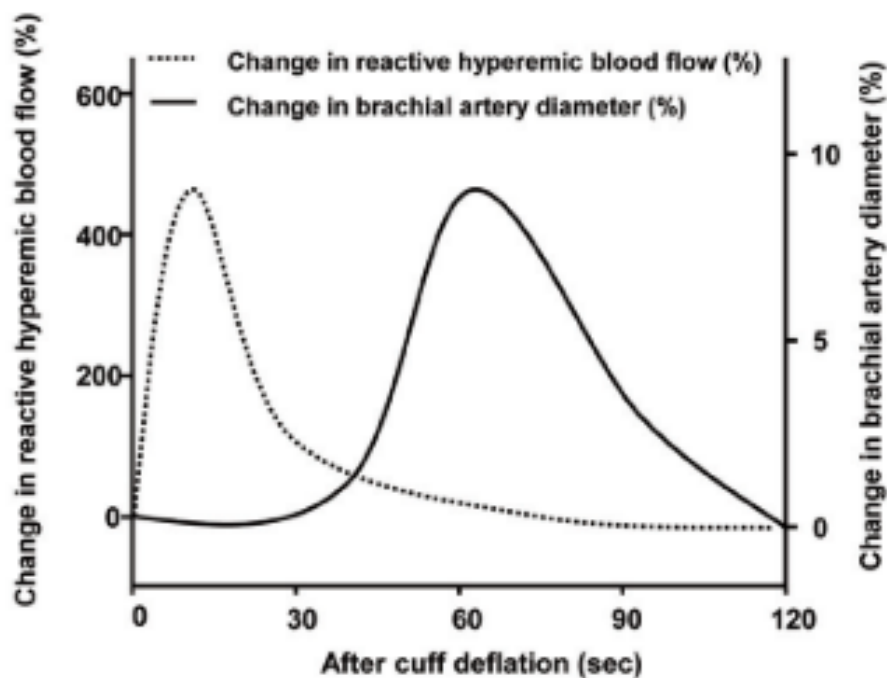


in endothelial cells. HMGB1 is an inflammatory cytokine that interacts with the receptor for advanced glycation end products (RAGE), inducing an oxidative stress and inflammatory response, which leads to endothelial dysfunction (67).

In summary, damage to the endothelium upsets the balance between vasoconstriction and vasodilation. Endothelial dysfunction (ED) and resultant increased endothelial permeability, platelet aggregation, leukocyte adhesion and generation of inflammatory cytokines plays a critical role in the development and progression of atherosclerosis, leading to serious cardiovascular events (120).

#### **3.1.4 Evaluation of Endothelial Dysfunction.**

Direct measurement of impaired endothelial-dependent dilation during coronary angiography is the historical “gold-standard” for evaluation of ED (121). A less invasive approach, measurement of brachial artery diameter via high resolution ultrasound imaging (flow mediated dilatation (FMD)) was first used as a method for evaluating ED in 1992 (122). FMD has been validated both as a sensitive predictor and surrogate endpoint in cardiovascular risk reduction interventional studies. Close concordance between this measurement with coronary artery endothelial-dependent vasodilation has been established (123)(124). During FMD studies, the vascular response to reactive hyperaemia (induced by inflation and then release of a blood pressure cuff) in the brachial artery is used for assessment of endothelium-dependent function. Although the precise mechanism by which vasodilation occurs during reactive hyperemia in FMD measurement has not been fully elucidated, shear stress-induced NO has been proposed as a principal mediator of FMD (Figure 3.1)(125) (126).



**Figure 3.1** Representative data on change in reactive hyperemic blood flow and change in brachial artery diameter after cuff deflation. Modified from Soga J, et al. *Arterioscler Thromb Vasc Biol* 2011; 31: 2353-9.

Following FMD studies, nitroglycerin-induced vasodilation, an index of endothelium-independent vasodilation, is assessed as a control test to ensure that impaired FMD is not due to underlying vascular smooth muscle dysfunction or alterations in vascular structure but truly a consequence of endothelial dysfunction (127). Nitroglycerin-induced vasodilation has also been found to be impaired in subjects with cardiovascular risk factors or with coronary atherosclerosis (128).

An objective of this chapter was to compare vascular endothelial cell function in hyperuricaemic patients with normouricaemic controls via flow mediated dilatation (FMD) and nitroglycerin mediated dilatation (NMD) of the brachial artery and the subsequent effect of treatment with urate lowering therapy (ULT). We hypothesise that individuals with hyperuricaemia will have impaired vascular ED and that treatment with ULT will result in enhanced vascular function as evidenced by improved FMD and NMD of the brachial artery.

### **3.1.5. Hyperuricaemia and Pulmonary Hypertension**

Pulmonary hypertension (PH) is defined as elevated mean pulmonary arterial pressure (PAP, typically >25 mm Hg at rest) and is a progressive disorder associated with the development of right heart failure, leading to significant morbidity and mortality (129). Based on pathophysiological, clinical, and therapeutic considerations, PH is divided into five groups: pulmonary arterial hypertension; pulmonary hypertension due to left-sided heart disease; pulmonary hypertension due to lung disease or hypoxia; chronic thromboembolic pulmonary hypertension; and pulmonary hypertension with unclear or multifactorial mechanisms (71). Pulmonary arterial hypertension, especially the idiopathic form, although still a rare disease with an incidence of 2–5 per million adults, is increasingly being diagnosed in elderly people (71). A high serum uric acid is common in subjects with pulmonary hypertension (PH). In a retrospective analysis of 39 patients with PH 79% of cases were found to have elevated serum uric acid levels (72). This study also found a significant correlation between urate levels and the cardiac index ( $r=0.48$ ;  $p=0.0021$ ) and an even stronger correlation between serum urate levels and mean right atrial pressures

( $r=0.83$ ;  $p<0.0001$ ). Data suggests that sUA levels are correlated with poor outcomes in PH and may be used as a prognostic marker (69). In severe pulmonary hypertension, tissue hypoxia may provoke urate overproduction, tissue ischemia, deplete ATP and stimulate the expression of the xanthine oxidase enzyme that elevates xanthine, hypoxanthine and uric acid levels (73). There is also evidence to suggest that uric acid, through its role in nitric oxide inhibition and endothelial dysfunction, may be a potential mediator of PH (74). When vascular endothelial cells are incubated with uric acid a dose-dependent decrease in nitric oxide (NO) production has been shown (75). Reduced bioavailability of NO impairs vascular dilatation and may contribute to altered pulmonary haemodynamics. The associations between hyperuricaemia and PH have been described in the literature however causality, which is amenable to ULT, has not yet been fully investigated.

### **3.1.6. Evaluation of Pulmonary Hypertension.**

Right heart catheterization (RHC) is currently the gold-standard method for evaluating pulmonary hypertension, however, by virtue of its associated procedure morbidity other non-invasive methods are needed to confirm PH diagnosis, predict prognosis and assess response to therapy. Advances in transthoracic Doppler echocardiography over recent years have led to the development of non-invasive measures that exploit the haemodynamic effects of blood flow through the pulmonary circulation due to changes in pulmonary vascular tone.

### **3.1.7 Pulmonary Pulse Wave Transit Time.**

Pulmonary Pulse Wave Transit Time (pPTT) is an echocardiographic measurement being evaluated as a potential alternative to RHC in the diagnosis of PH (76). pPTT is defined as the

delay between onset of ventricular electrical activity (on electrocardiogram) and the arrival of the pulse wave in the pulmonary vein (as determined by Doppler echocardiography of the pulmonary vein). Wibmer et al showed that pPTT was reduced in 6 patients with World Health Organization (WHO) group 1 pulmonary hypertension (129). Pulse wave velocity (PWV) and the inversely related pulse transit time (pPTT) are physiologic measures that have been identified as clinically important surrogates in the assessment of increased arterial stiffness and blood pressure seen in pulmonary hypertension (130). While PWV is the speed at which the pulse pressure wave travels along an arterial segment, pPTT refers to the time it takes the pulse pressure wave to travel from one arterial site to another. In healthy tone, the pressure wave caused by the ejection of blood from the right ventricle is ameliorated to some extent by the natural compliance (distensibility) of the arterial circulation. This distensibility enables a temporary increase in the pulmonary arterial volume, allowing the increased blood volume to be accommodated whilst smoothing out the peak of the pressure wave, slowing down and lengthening the time for blood to flow from one side to the other, increasing transit time. In PH, reduced distensibility forces the blood through at higher pressure, with increasing arterial stiffness and reduced distensibility PWV increases and thus pPTT shortens (131).

Our preliminary objective was a proof of concept study evaluating pPTT in patients with known PH compared to normal controls. Subsequently we sought to evaluate pPTT in patients with hyperuricaemia compared to normal healthy controls. We hypothesise that

due to ED secondary to elevated sUA, pPTT would be shorter in patients with hyperuricaemia. Furthermore, to investigate for a causal role between the development of hyperuricaemia leading to PH, we examined whether treatment of hyperuricaemia may improve pulmonary haemodynamic parameters.

### **3.2 Specific Aims and Objectives of this Chapter**

- To compare vascular endothelial cell function in hyperuricaemic patients versus normouricaemic controls via FMD and NMD of the brachial artery.
- To evaluate FMD and NMD in hyperuricaemic patients post treatment with urate lowering therapy (ULT).
- To evaluate pPTT in patients with known PH versus normal healthy controls and examine its reliability as a novel non-invasive index to assess pulmonary haemodynamics.
- To evaluate pPTT in patients with hyperuricaemia compared to normal healthy controls.
- To evaluate pPTT in patients with hyperuricaemia post treatment with ULT.

### **3.3 Methods.**

#### **3.3.1. Methods - Flowed Mediated Dilatation & Nitroglycerin Mediated Dilatation of the Brachial Artery Study.**

##### **3.3.1.1 Recruitment**

Following informed consent patients with hyperuricaemia who met the following inclusion criteria were recruited to the study;

- Age > 18.
  - Elevated serum urate (> 420umol Men. > 360umol Women.)
  - Diagnosis of gout.
  - No previous diagnosis of Inflammatory Arthritis.
  - Not currently taking urate lowering therapy.
  - estimated glomerular filtration rate (eGFR) > 30.
- Happy to take part.

##### Exclusion Criteria

- Age <18.
- Normal Serum Urate (<420umol Men. <360 Women.)
- History of Inflammatory Arthritis.

- eGFR <30.
- Currently on treatment for gout.
- Unable to complete informed consent.

Patients were assessed and recruited from a specialist 'early gout' outpatient clinic established in Tallaght University Hospital (TUH). Patients were referred to this clinic from general practitioners, allied health practitioners (physiotherapy, occupational health), rheumatology services and other specialities throughout the hospital and community. This clinic was run by a specialist registrar (Rachael Flood) with a special interest in gout and with oversight from a consultant rheumatologist.

Age and sex matched patients with normal serum uric acid levels who met the inclusion criteria were also recruited.

### **3.3.1.2 Baseline Anthropomorphic assessment**

Baseline demographics including age, sex, co-morbidities (diabetes, hypertension, cardiovascular disease, pulmonary disease, chronic kidney disease and history of malignancy) and regular medications were recorded. Baseline anthropomorphic assessment of height, weight, body mass index and blood pressure were completed. Baseline serum was taken and the following markers recorded; uric acid (umol/L) eGFR ml/min/1.73<sup>2</sup>, c reactive protein mg/dl (crp) erythrocyte sedimentation rate (mm/hr) (ESR), cholesterol (mmol/L) fasting glucose (mmol/L) Homocysteine (umol/L).

### **3.3.1.3 Flow Mediated Dilatation of The Brachial Artery.**

All studies were performed in a similar environment with the patient in the resting, supine state between the hours of 08.00 and 10.00am. All antihypertensive and diuretic



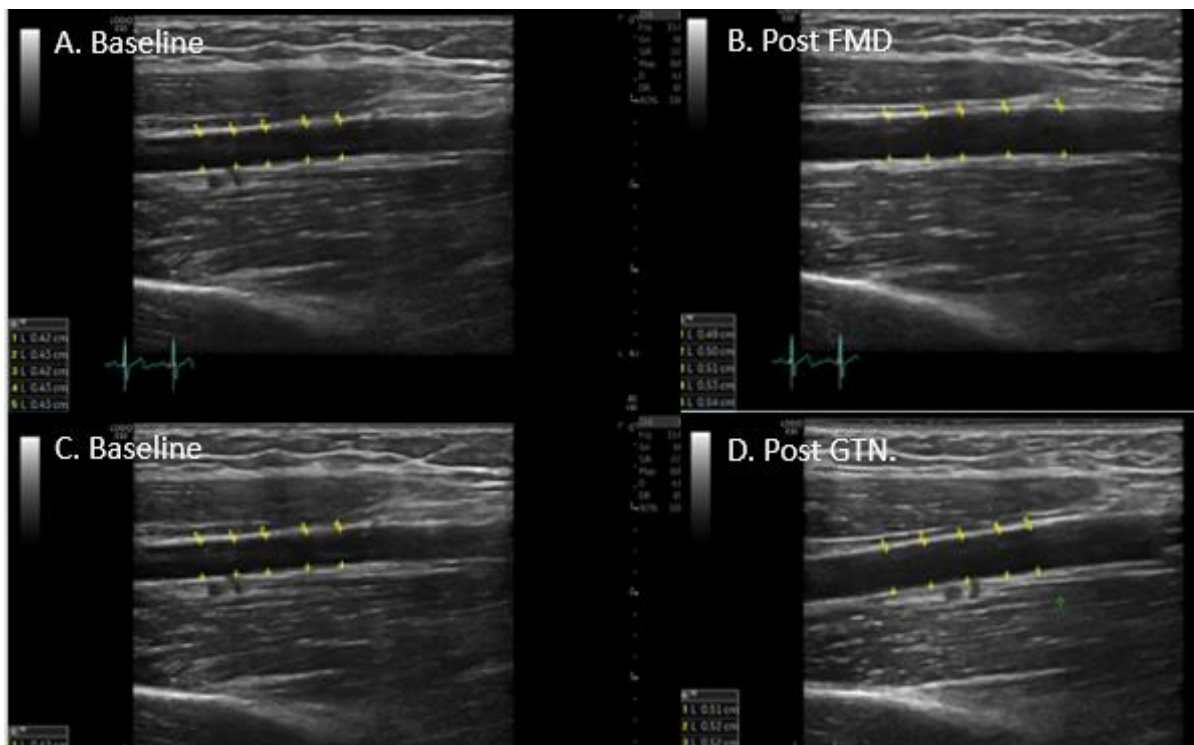
medications were held on the morning of the examination. An electrocardiogram (ECG) was monitored continuously and blood pressure and heart rate were recorded from the right arm. The subjects' left arm was comfortably immobilised in the extended position to allow consistent access to the brachial artery for imaging. All imaging was performed by a single, highly skilled sonographer (RF). Brachial artery diameter was imaged using a 16MHz linear array transducer from a Logiq E10 ultrasound machine. A 5-cm segment of artery was measured at 3-5cm above the anterior cubital fossae. Images were obtained in a longitudinal view with great care taken to maximize vessel diameter and provide optimal blood-vessel wall definition. (Figure 3.2) The image depth was set at 4 cm, and gain settings were adjusted to optimally delineate the linear-arterial wall interface. Images were saved for later analysis.

Arterial diameter was calculated within the 5cm segment as the mean of five evenly spaced measurements of the distance between the arterial walls along a line perpendicular to the artery's long axis. All vasodilatation measurements were made at end diastole (concurrent with onset of QRS complex on the ECG). Changes in brachial artery diameter were expressed as percentage change relative to the baseline vessel diameter.

Following baseline measurements an adult sized blood pressure cuff was placed over the ipsilateral upper left arm just above the transducer and inflated for 5 minutes. Inflation pressure was maintained at 200 mmHg for the duration of the occlusion and then the cuff was suddenly deflated. Brachial artery diameter was measured at 1, 3 and 5 minute intervals post cuff deflation and results recorded. The maximum vessel dilation reading was noted.

### 3.3.1.4 Nitroglycerin Mediated Dilatation.

Following a rest period baseline recordings of arterial diameter were repeated, after which 0.4 mg sublingual nitroglycerin was administered. Arterial diameter was recorded at 1,3 and 5 minute intervals. The maximum vessel dilation reading was noted.



**Figure 3.2. Ultrasound Images taken using GE E10 with 16mHZ probe. A: Baseline Measurement of Brachial Artery (BA). B: Enlargement of the BA diameter is clearly seen at 3 minutes post FMD. C: Baseline Measurement of BA. D: Enlargement of the BA diameter is clearly seen at 3 minutes post 0.4mg sublingual GTN.**

### 3.3.1.4. Commencement of Urate Lowering Therapy

Cases were treated with ULT daily. Allopurinol was the first line agent used, if there was a contraindication or intolerance febuxostat 80mg was used as second line. Allopurinol

was commenced at 100mg and uptitrated by 100mg every second week until established on 300mg, serum uric acid levels were then checked and dosing increased as required in a treat to target programme. Patients renal function and serum uric acid was monitored throughout their time on treatment. Colchicine 500mcg twice daily (BD) prophylaxis was prescribed for the first 8 weeks on ULT. Patients who did not adhere to treatment or failed to reach target serum uric acid levels for a minimum of three months were excluded.

#### **3.3.1.5 Follow up.**

After a minimum period of three months of serum uric acid being within target range (< 420umol Men. < 360umol Women), cases were reviewed. At each study visit data on demographic characteristics, lifestyle factors, comorbidities, and drug prescriptions were collected. Height, weight, and blood pressure were measured. Bloods as mentioned previously were collected and processed at the chemical pathology laboratory in TUH. FMD and NMD studies were repeated using the same protocol as the initial visit.

### **3.3.2 Pulmonary Pulse Wave Transit Time Study.**

#### **3.3.2.1 Recruitment**

(i) For the proof of concept study 'pulmonary hypertension is associated with a reduced pPTT', 10 patients with established pulmonary hypertension, confirmed at prior right heart catheterisation and/or echocardiogram, were recruited from the rheumatology outpatient department at Tallaght University Hospital.

(ii) Following informed consent patients with hyperuricaemia who met the following inclusion criteria were recruited to the study;

- Age > 18.
  - Elevated serum urate (> 420umol Men. > 360umol Women.)
  - Diagnosis of gout.
  - No previous diagnosis of Inflammatory Arthritis.
  - Not currently taking urate lowering therapy.
  - estimated glomerular filtration rate (eGFR) > 30.
- Happy to take part.

#### Exclusion Criteria.

- Age <18.
- Normal Serum Urate (<420umol Men. <360 Women.)
- History of Inflammatory Arthritis.

- eGFR <30.
- Currently on treatment for gout.
- Unable to complete informed consent.

(iii) Age and sex matched normal controls for both the (i) PH patient cohort and (ii) hyperuricaemia cohort were recruited from the outpatient department at Tallaght University Hospital.

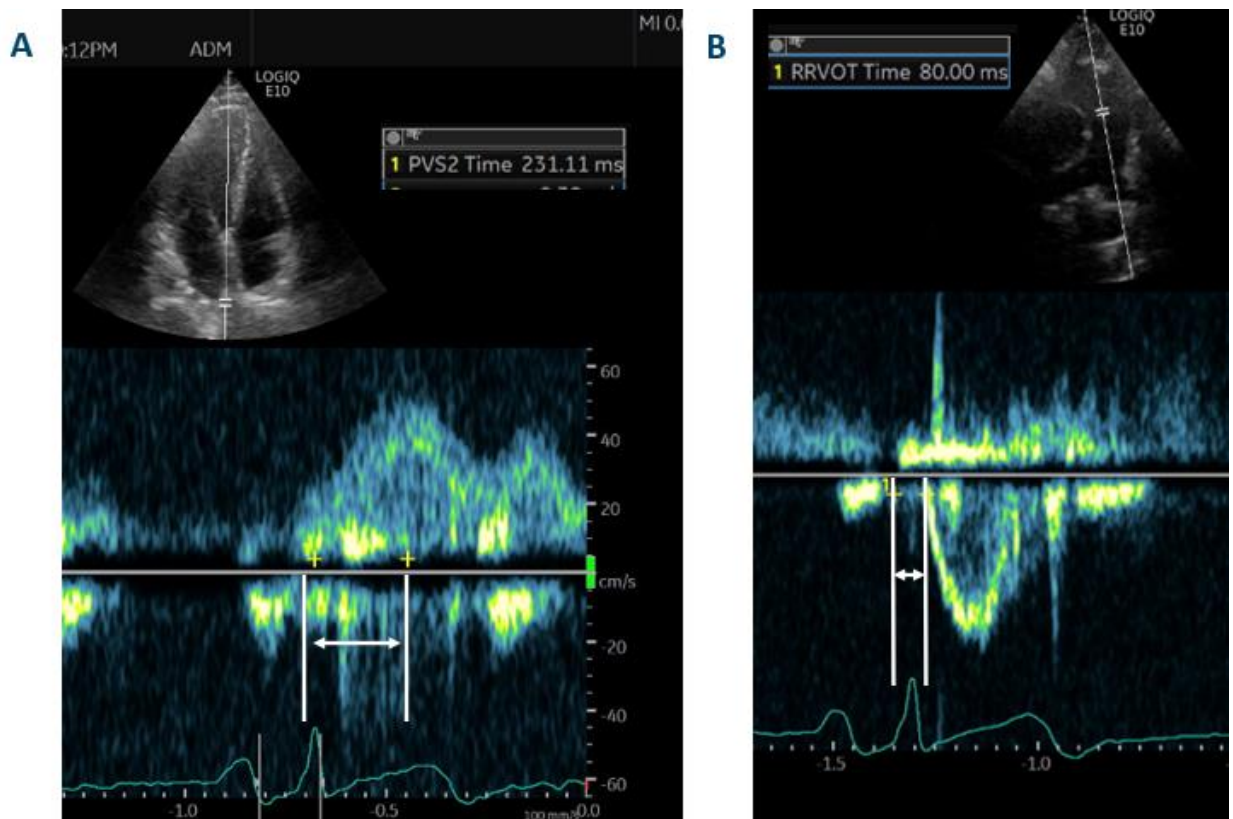
### **3.3.2.2 Baseline Anthropomorphic assessment**

Baseline demographics including age, sex, co-morbidities (diabetes, hypertension, CVD, pulmonary disease, chronic kidney disease and history of malignancy) regular medications, PH mediations and supplemental oxygen requirements were recorded. Baseline anthropomorphic assessment of height, weight, body mass index and blood pressure were completed. Baseline serum was taken and the following markers recorded; uric acid (umol/L) egfr ml/min/1.73<sup>2</sup>, c reactive protein mg/dl (CRP) erythrocyte sedimentation rate (mm/hr) (ESR), cholesterol (mmol/L) fasting glucose (mmol/L) Homocysteine (umol/L). N-terminal proB- type Natriuretic Peptide (NT- Pro BNP) (pg/dl)

### **3.3.2.3 Echocardiographic Assessment of pPTT**

Echocardiograms were performed under the supervision of a trained technician with over 15 years of experience. Over one month, two rheumatologists with significant ultrasound experience developed the skill to perform focused echocardiograms on patients and calculate pPTT. Independent scans were only completed once the clinicians were confident in the technique and results were reproducible.

Echocardiography was performed with patients in the standard left lateral decubitus position using a Logiq E10 with a 3.5-MHz multiphase array probe. All antihypertensive and diuretic medications were held on the morning of the examination. pPTT was defined as the time taken by the pressure wave to travel from the pulmonic valve/right ventricular outflow tract (RVOT) to the pulmonary vein. (RVOT) flow was studied using the pulsed-wave Doppler with the Doppler sample volume placed in the main pulmonary artery just distal to the pulmonic valve in the left parasternal, short-axis view. Pulmonary vein flow was studied by the pulsed-wave Doppler with the Doppler sample volume placed in the right inferior pulmonary vein in the apical 4-chamber view, according to guidelines of the American Society of Echocardiography (132). All the Doppler recordings were made at a sweep speed of 75 mm/s with a simultaneous superimposed electrocardiogram. We measured the time interval between the R-wave in the electrocardiogram and the corresponding onset of RVOT pulse Doppler flow velocity for 2 to 3 consecutive cardiac cycles and calculated the mean (R-RVOT) (Figure 3.3). We then measured the time interval between the R-wave in the electrocardiogram and the corresponding peak late-systolic pulmonary vein flow velocity for 2 to 3 consecutive cardiac cycles and calculated the mean (R-PVs2) (Figure 3.3). This late-systolic pulmonary vein flow pulse wave has been shown to be closely related to the forward-traveling pressure wave originating from the right ventricle (133). We then calculated pPTT as the difference between R-PVs2 and R-RVOT intervals, normalized to cardiac cycle length. We normalized pPTT to cardiac cycle length, because shorter cardiac cycles permit less time between pulse waves. Thus, although pPTT is a measure of time, it is unitless when normalized to cardiac cycle length.



**Figure 3.3.** Images taken using Logiq E10 with a 3.5-MHz multiphase array probe of Doppler interrogation of A.) pulmonary veins. R-PVS2. Arrows show interval between the R-wave in the electrocardiogram and the corresponding peak late-systolic pulmonary vein flow velocity B.) Right ventricular outflow track (RVOT). Arrows show interval between the R-wave in the electrocardiogram and the corresponding onset of RVOT pulse Doppler flow velocity.

#### 3.3.2.4. Commencement of Urate Lowering Therapy

Hyperuricaemic cases were treated with ULT daily. Allopurinol was the first line agent used, if there was a contraindication or intolerance febuxostat 80mg was used as second line. Allopurinol was commenced at 100mg and uptitrated by 100mg every second week until established on 300mg, serum uric acid levels were then checked and dosing increased as required in a treat to target programme. Patients renal function and serum

uric acid was monitored throughout their time on treatment. Colchicine 500mcg twice daily (BD) prophylaxis was prescribed for the first 8 weeks on ULT. Patients who did not adhere to treatment or failed to reach target serum uric acid levels for a minimum of three months were excluded.

### **3.3.2.5 Follow up.**

After a minimum period of three months of sUA within target range (< 420umol Men. < 360umol Women.) cases were reviewed. At each study visit data on demographic characteristics, lifestyle factors, comorbidities, and drug prescriptions were collected. Height, weight, and blood pressure were measured. Bloods as mentioned previously were collected and processed at the chemical pathology laboratory in TUH. Echocardiogram was repeated as per the protocol above.

### **3.3.2.6 Statistical Analysis**

Statistical analysis was performed using SPSS V.26 software. Continuous variables were reported as the mean  $\pm$  standard error (SE). Categorical variables were reported as the number and percentage. In univariable analyses, differences between cases and controls were compared using chi-squared tests or Fisher's exact tests, as appropriate, for categorical variables and Mann-Whitney U-tests for continuous variables. P value of < 0.05 was deemed significant. Spearman's bivariate correlation analysis was used to test the relationships between the numerical variables when appropriate. A P value of < 0.05 was deemed significant.



### **3.4 Results.**

#### **3.4.1 Flow Mediated Dilatation & Nitroglycerin Mediated Dilatation Studies.**

##### **3.4.1.1. Baseline Demographics.**

Recruitment was completed between August 2018 and August 2021. 34 hyperuricaemic patients met the inclusion criteria and were recruited following informed consent. 16 age and sex matched controls were recruited following informed consent. Table 3.1 outlines the baseline demographics between cases and controls. There were no significant differences in age between the two groups, mean age  $\pm$  SE for cases was  $50.56 \pm 2.7$  versus  $42.6 \pm 3.3$  for controls. Systolic blood pressure was significantly higher in the case versus control group ( $141.56 \pm 4.1$  versus  $122.75 \pm 3.4$ ,  $P < 0.05$ ) Body Mass Index (BMI) was significantly higher in the case versus control group ( $33.2 \pm 1.2$  versus  $25.94 \pm 1.0$ ,  $P < 0.05$ ) other co-morbidities were evenly matched in the two groups. Serum uric acid was significantly higher in cases versus controls ( $474 \pm 14.1$  versus  $283 \pm 13.8$ ,  $P < 0.05$ ).

Parameter (Mean ± SE)	Case (N = 34)	Control (N= 16)
Age (years)	50.56 ± 2.7	42.6 ± 3.3
Male (N, %)	24 (70.6)	8 (50)
uric acid umol/L	474 ± 14.1*	283 ± 13.8
eGFR mL/min/1.73 m <sup>2</sup>	92.93 ± 2.9	94.03 ± 4.7
CRP (mg/dl)	8.94 ± 2.8	4.87 ± 2.5
ESR (mm/hr)	16.43 ± 3.3	10.2 ± 2.4
Cholesterol (mmol/L)	4.95 ± 0.2	4.77 ± 0.2
Fasting Glucose (mmol/L)	5.6 ± 0.2	5.1 ± 0.2
Homocysteine (umol/L)	15.11 ± 1.9	13 ± 1.4
Systolic BP. (mm/hg)	141.56 ± 4.1*	122.75 ± 3.4
Diabetes (N,%)	1 (2.9)	0 (0)
Hypertension (N, %)	10 (29.4)	6 (37.5)
CKD (N, %)	0 (0)	0 (0)
IHD (N, %)	1 (2.9)	1 (6.3)
Malignancy (N, %)	1 (2.9)	0 (0)
BMI (kg/m <sup>2</sup> )	33.2 ± 1.2*	25.94 ± 1.0

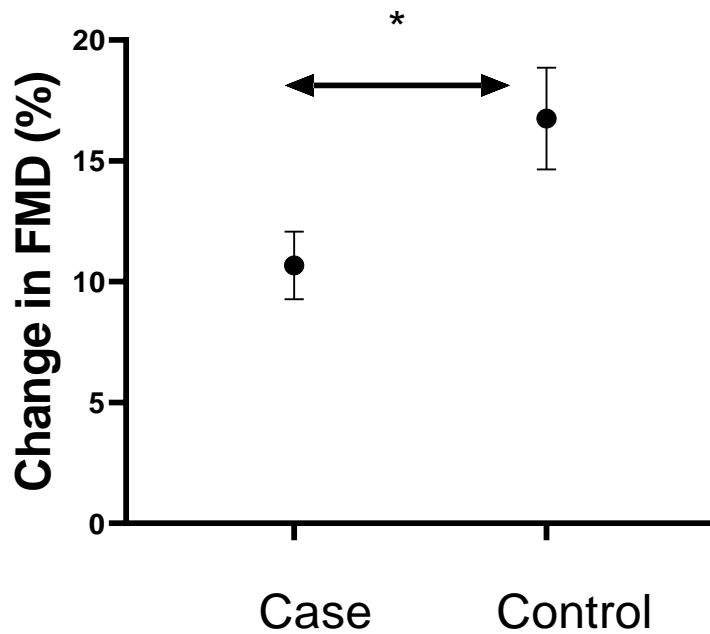
**Table 3.1. Baseline Demographics of Cases and Controls.** \*P = <0.05

eGFR - Estimated glomerular filtration rate, CRP – C – Reactive Protein, ESR – Erythrocyte Sedimentation Rate, BP – Blood pressure, CKD – Chronic Kidney Disease, IHD – Ischaemic Heart Disease, BMI – Body Mass Index.

#### **3.4.1.2. FMD & NMD of the Brachial Artery is Reduced in Hyperuricaemic Patients in Comparison to Normal Controls.**

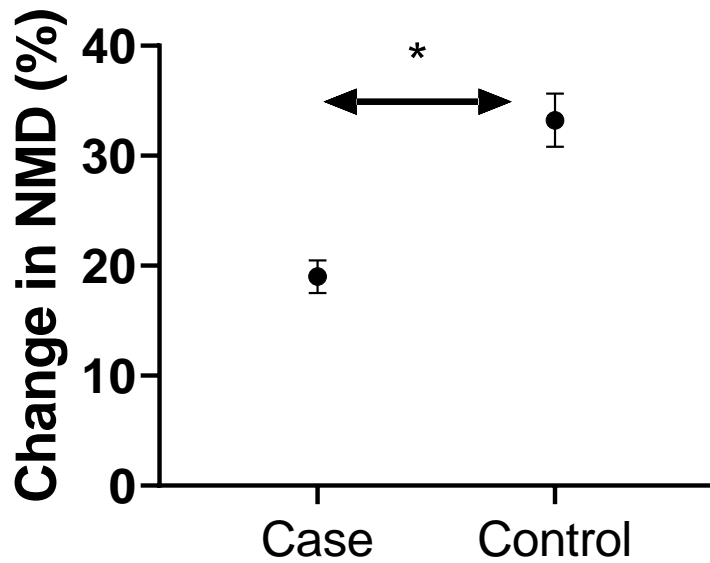
In one subject the ultrasound scans were of insufficient quality for assessment of vessel diameter. One subject declined sublingual GTN. All subjects tolerated the studies well. No case or control had clinical evidence of vascular disease, nor any ultrasound evidence of arterial narrowing in the vessels studied. Flow mediated dilatation of the brachial artery (maximum mean percentage (%) change from baseline ± SE) was significantly reduced in

hyperuricaemic cases in comparison to normouricaemic matched controls. (N=33, 10.67% ± 1.40 versus N=16, 16.75% ± 2.10. P=<0.05) (Figure 3.4)



**Figure 3.4: Mean % Change From Baseline FMD is Reduced in Hyperuricaemic Cases Versus Normal Controls. (N=33, 10.67% ± 1.40 versus N=16, 16.75% ± 2.10. \* P= <0.05 )**

Nitroglycerin mediated dilatation of the brachial artery (maximum mean % change from baseline ± SE) was significantly reduced in hyperuricaemic cases in comparison to normouricaemic matched controls. (N=32 19.01% ± 1.48 versus N=16 33.23% ± 2.42. P=<0.01) (Figure 3.5)



**Figure 3.5: Mean % Change From Baseline NMD is Reduced in Hyperuricaemic Cases Versus Normal Controls.** (N=32 19.01% ± 1.48 versus N=16 33.23% ± 2.42. \* = P<0.01)

### **3.4.1.3. Improvement in FMD & NMD Studies Following 3 months of Urate Lowering Therapy.**

Cases underwent treatment with ULT daily for three months. There was a significant reduction in sUA levels at visit 2 following three months of ULT (  $369.58 \pm 17.96$  P = <0.05). Two cases were excluded for failing to reach target serum uric acid levels due to non-compliance with ULT. ULT was well tolerated among cases. There was a non-significant improvement in maximum mean % change from baseline FMD (N=33, 10.67% ± 1.40) in treated cases at Visit 2 (N = 31, 11.46% ± 1.16 ) Figure 3.6. There was a significant improvement in mean % change from baseline NMD (N=32 19.01% ± 1.48) in treated cases at Visit 2 (N = 31, 23.66% ± 1.32 P=<0.05) Figure 3.7.

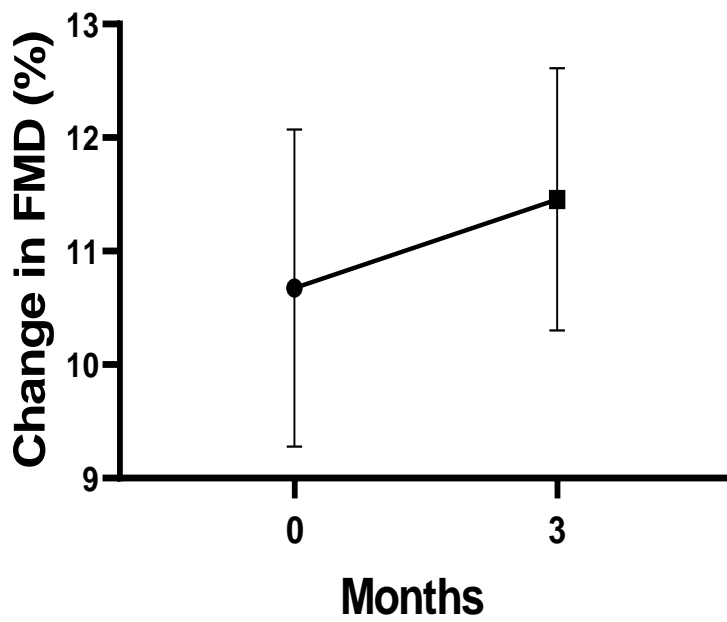


Figure 3.6: Improvement in Mean % change from baseline FMD of the brachial artery following 3 months of ULT. (N=33, 10.67% ± 1.40 v N = 31, 11.46% ± 1.16, P=NS)

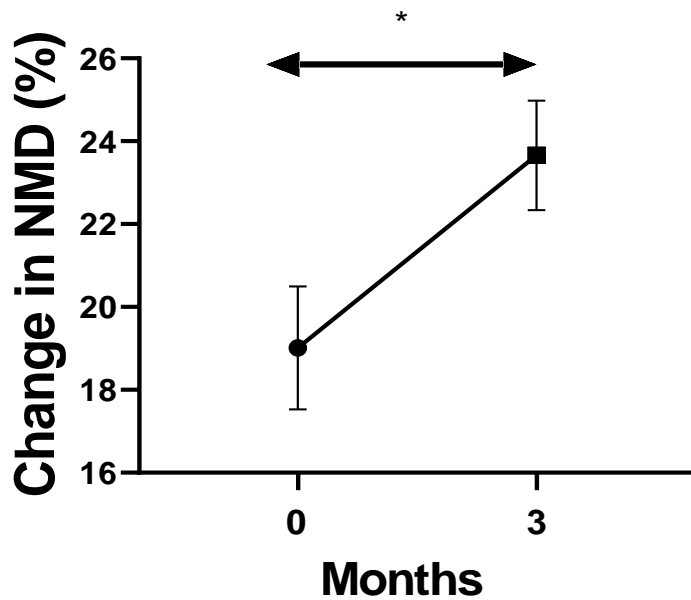


Figure 3.7: Improvement in Mean % change from baseline NMD of the brachial artery following 3 months of ULT. (N=32 19.01% ± 1.48 v N = 31, 23.66% ± 1.32 \*P<0.05)

#### 3.4.1.4. Relationship between serum uric acid levels and % Change in FMD & NMD.

Percentage change in NMD in hyperuricaemic cases was inversely correlated with serum uric acid levels (  $R = -0.44$ ,  $P = <0.01$ ) Figure 3.8. A significant correlation in percentage change in FMD and serum uric acid in hyperuricaemic cases was not seen. Figure 3.9.

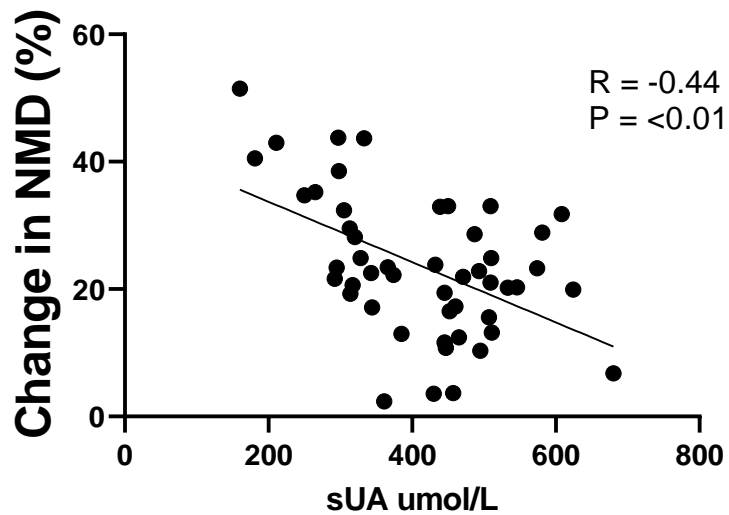


Figure 3.8: Spearman Rho Correlation between % Change From Baseline NMD and serum uric acid levels.

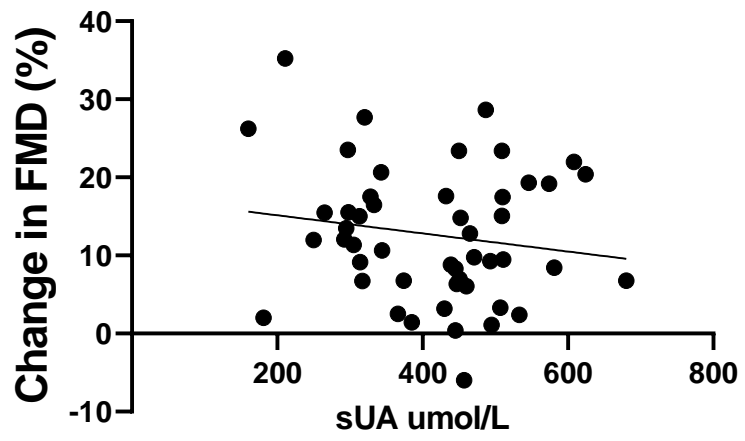


Figure 3.9: Spearman Rho Correlation between % Change From Baseline FMD and Serum Uric Acid Levels.

### 3.4.2 Results - Pulmonary Pulse Wave Transit Time Study.

#### 3.4.2.1. Baseline Demographics – Established Pulmonary Hypertension Cohort.

Initially we measured pPTT in 13 healthy controls and 10 patients with established pulmonary hypertension, who were recruited from the rheumatology outpatient department at Tallaght University Hospital (Table 3.2). There were no significant differences in age in cases ( $59.1 \pm 4.1$ ) versus controls ( $48.46 \pm 4.7$ ). Uric acid levels and NTproBNP were significantly higher and HB significantly lower in cases versus controls. (Table 3.2)

Parameter (Mean $\pm$ SE)	Case (N = 10)	Control (N= 13)
Age (years)	$59.1 \pm 4.1$	$48.46 \pm 4.7$
Male (N, %)	3, 30%	7, 53.8%
uric acid umol/L	$466 \pm 61.1^*$	$296.77 \pm 14.7$
eGFR mL/min/1.73 m <sup>2</sup>	$66.47 \pm 9.8$	$82.22 \pm 8.1$
HB (g/dl)	$12.41 \pm 0.5^*$	$14.14 \pm 0.4$
Cholesterol (mmol/L)	$4.3 \pm 0.4$	$4.6 \pm 0.3$
NT proBNP (pg/ml)	$2856.6 \pm 1270.2^*$	$98.0 \pm 31.0$
Systolic BP. (mm/hg)	$140.67 \pm 8.1$	$136.67 \pm 4.6$
Diabetes (N,%)	1, 10%	1, 7.7%
Hypertension (N, %)	5, 50%	4, 30.8%
IHD (N, %)	1, 10%	0, 0%
BMI (kg/m <sup>2</sup> )	$27.6 \pm 2.6$	$29.17 \pm 1.7$
Statin (N, %)	3, 30%	4, 30.8%
Beta Blocker (N, %)	2, 20%	1, 7.7%
Ace Inhibitor (N, %)	2, 20%	2, 15.4%
Diuretic (N, %)	7, 70%	1, 7.7%
PDE – 5 Inhibitor (N, %)	7, 70%	1, 7.7%
LTOT	2, 20%	0, 0%

**Table 3.2: Baseline Clinical Characteristics of Patients with Pulmonary Hypertension and Healthy Controls.**

eGFR – estimated glomerular filtration rate, HB- Haemoglobin, NT proBNP – N- Terminal proB Type Naturitic Peptide, BP – blood pressure, IHD – Ischaemic Heart Disease, BMI –



Body Mass Index, PDE- 5- Phosphodiesterase – 5. LTOT – Long Term Oxygen Therapy. \* = P <0.05

### 3.4.2.2. pPTT Results – Established Pulmonary Hypertension Cohort.

There was a significant reduction in R-PVs2 in patients with PH (167.40msec ± 10.5) when compared with control patients (208.95msec ± 17.01, P=< 0.05.) The distribution of R-PVs2 in controls and patients with PH is depicted in Figure 3.10.

There was a significant reduction in pPTT in patients with PH (0.104 ± 0.01) when compared with control patients (0.168 ± 0.02, P=< 0.01.) The distribution of pPTT in controls and patients with PH is depicted in Figure 3.11

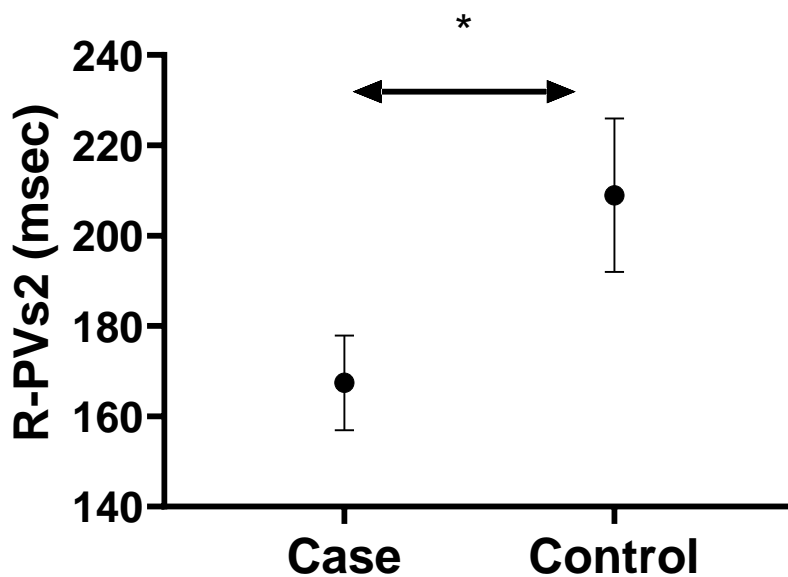


Figure 3.10 R-PVs2 is Significantly Reduced in Patients with Established Pulmonary Hypertension. N=10, 167.40msec ± 10.5 versus N=13, 208.95msec ± 17.01,\* P = <0.05

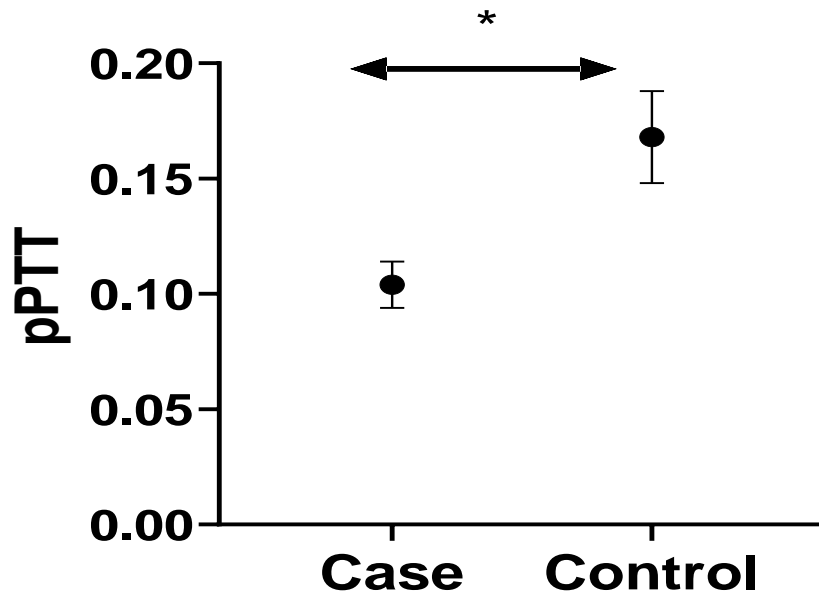


Figure 3.11. pPTT is Significantly Reduced in Patients with Established Pulmonary Hypertension. N=10,  $0.104 \pm 0.01$  v N=13,  $0.168 \pm 0.02$ ,  $P < 0.01$ . \*  $P = < 0.01$

#### 3.4.2.3 Baseline Demographics – Hyperuricaemia Cohort.

Subsequently we assessed pPTT in 15 healthy controls and 22 hyperuricaemic cases who were recruited from the rheumatology outpatient department at Tallaght University Hospital (Table 3). There was no significant difference in age between cases ( $51.33 \pm 4.6$ ) and controls ( $49.0 \pm 2.8$ ;  $P = \text{NS}$ ). There were significantly more males in the case (21, 95.5%) versus control group (8, 53.3%  $P = < 0.01$ ). Uric acid levels were also significantly higher in cases ( $486.36 \pm 16.3$ ) versus controls ( $291.07 \pm 16.1$   $P = < 0.01$ ). Other clinical characteristics were evenly matched. (Table 3.3)

Parameter (Mean ± SE)	Case (N = 22)	Control (N= 15)
Age (years)	49.0 ± 2.8	51.33 ± 4.6
Male (N, %)	21, 95.5% *	8 53.3%
uric acid umol/L	486.36 ± 16.3*	291.07 ± 16.1
eGFR mL/min/1.73 m <sup>2</sup>	84.6 ± 4.1	92.8 ± 9.9
HB (g/dl)	14.5 ± 0.3	14.1 ± 0.4
Cholesterol (mmol/L)	5.1 ± 0.3	4.6 ± 0.3
NT proBNP (pg/ml)	108.0 ± 58	104.78 ± 28.2
Systolic BP. (mm/hg)	130.72 ± 4.2	136.0 ± 4.0
Diabetes (N,%)	3, 13.6%	2, 13.3%
Hypertension (N, %)	6, 27.3%	4, 26.7%
IHD (N, %)	1, 4.5%	0, 0%
BMI (kg/m <sup>2</sup> )	29.9 ± 1.2	28.67 ± 1.7
Statin (N, %)	3, 13.6%	4, 26.7%
Beta Blocker (N, %)	2, 9.1%	1, 6.7%
Ace Inhibitor (N, %)	5, 22.7%	2, 13.3%
Diuretic (N, %)	1, 4.5%	1, 6.7%
PDE – 5 Inhibitor (N, %)	0, 0%	1, 6.7%
LTOT	0, 0%	1, 6.7%

**Table 3.3: Baseline Clinical Characteristics of Hyperuricaemic Cases and Healthy Controls.**

eGFR – estimated glomerular filtration rate, HB- Haemoglobin, NT proBNP – N- Terminal proB Type Natriotic Peptide, BP – blood pressure, IHD – Ischaemic Heart Disease, BMI – Body Mass Index, PDE- 5- Phosphodiesterase – 5. LTOT – Long Term Oxygen Therapy. \* = P <0.05

#### **3.4.2.4 pPTT Results – Hyperuricaemic Cohort.**

There was a non-significant reduction in mean R-PVs2 in cases with hyperuricaemia (176.63msec ± 11.6) when compared with control patients (200.75msec ± 15.73; P= 0.20 .)

The distribution of R-PVs2 in controls and hyperuricaemic cases is depicted in Figure 3.12.

There was a significant reduction in pPTT in cases ( $0.12 \pm 0.02$ ) when compared with control patients ( $0.16 \pm 0.01$ ;  $P < 0.05$ .) The distribution of pPTT in controls and cases with hyperuricaemia is depicted in Figure 3.13.

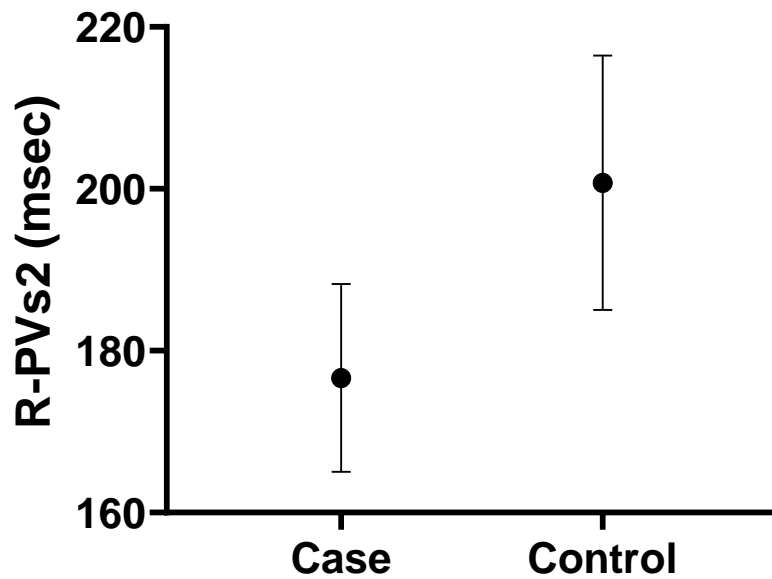


Figure 3.12. Mean R-PVs2 is Reduced in Hyperuricaemic Cases. (N=22, 176.63msec  $\pm$  11.6 v N=15, 200.75msec  $\pm$  15.73; P= 0.20 )

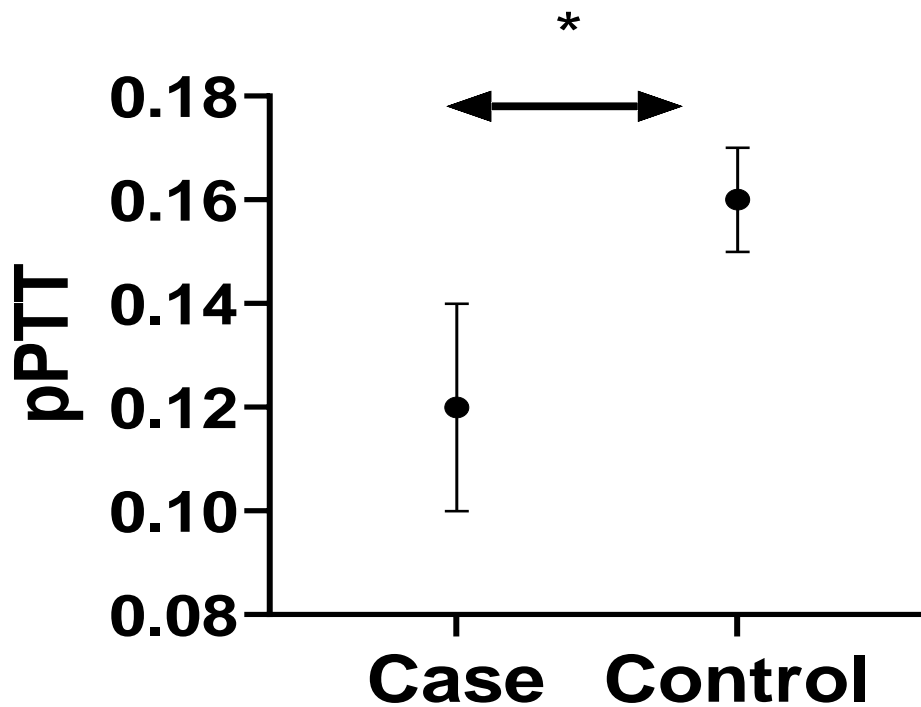


Figure 3.13. pPTT is Significantly Reduced in Hyperuricaemic Cases. (N=22,  $0.12 \pm 0.02$  v N=15,  $0.16 \pm 0.01$ ; \* =P<0.05.)

#### 3.4.2.5 pPTT Results Following 3 Months of ULT.

pPTT was reassessed in 13 cases following a sustained period of 3 months of normal sUA levels. There was a significant decrease in mean sUA levels between the baseline visit ( $487.15 \pm 24.15$ ) and three months ( $309.38 \pm 20.78$  P=<0.01). There was a non-significant increase in mean R-PVs2 from baseline (  $159.42\text{ms} \pm 14.37$  ) following three months of ULT (  $168.84\text{ms} \pm 10.94$ ; P=NS) There was a non- significant increase in mean pPTT from baseline ( $0.09 \pm 0.02$ ) following three months of ULT ( $0.12 \pm 0.02$ ; P=NS)

### 3.5 Discussion

This chapter examines the impact of hyperuricaemia on markers of vascular dysfunction; flow and nitroglycerin mediated dilatation studies and pPTT. FMD & NMD of the brachial artery have been developed as a non-invasive means of identifying patients with ED and a surrogate marker for predicting CVD. Our study found impaired FMD and NMD in patients with hyperuricaemia compared to normouricaemic matched controls. Subsequently, following a period of treatment with ULT, we found a trend towards improved indexes of FMD and significantly improved NMD. These findings support the growing body of evidence that hyperuricaemia contributes to the development of CVDs.

FMD of the brachial artery after occlusion is an endothelium- dependent function of the vascular tree. Vasodilatation in FMD measurement is caused by the generation of nitric oxide (NO) in response to sheer stress. A mechanism proposed for uric acid-induced ED and subsequent FMD impairment is the reaction between uric acid and NO in a rapidly irreversible manner, resulting in depletion of endogenous NO and subsequent reduced bioavailability (134), in addition to the inhibition of endothelial NO synthase activity (135) and potentiation of arginase activity, increasing intracellular superoxide. Through creation of reactive oxidant species and inhibition of NO, hyperuricaemia is postulated to cause vascular ED resulting in arterial stiffness and subsequent atherosclerosis.

By contrast, in NMD, GTN causes vasodilatation by direct action on the smooth muscle; its effect is therefore independent of the endothelium and is a measure of endothelial independent vasodilation (122). NMD is also known to be impaired in those with CVD (128). This suggests that CVD is not strictly secondary to ED and functional changes in the vessel wall in CVDs may not be limited to the endothelium. The reduction in vasodilatation and

arterial stiffness seen in CVDs may be mediated, in part, by changes in vascular smooth muscle. Uric acid has been shown to activate NOD like receptor protein 3 (NLRP3)-inflammasome and alter vascular smooth muscle cells (VSMC). Hui et al demonstrated that uric acid increases the proliferation of VSMC (136). Since gout is independently associated with increased mortality and death due to CVD, hyperuricaemia may contribute to this risk through secondary reduced NO bioavailability, subsequent ED and NLRP3 mediated proliferation of VSMCs causing endothelium independent vascular remodelling.

BMI and systolic blood pressure were significantly higher in cases compared to controls which is to be expected given the strong association between gout and metabolic syndrome (27). A cross sectional study of 9408 Chinese participants found those who were overweight or obese had higher risk for hyperuricemia (OR=1.90, 95% CI=1.46–2.47) and a dose-response relationship was identified between BMI and the risk of hyperuricemia (137). Evidence has also supported the possible role of uric acid as a mediator of high blood pressure. Both animal model data and tissue culture experiments suggest that uric acid may increase blood pressure via uric acid mediated vasoconstriction or induction of renal afferent arteriosclerosis and altered pressure natriuresis leading to a sodium dependent hypertension (138). Feig et al assessed children with newly diagnosed essential hypertension through cross-sectional studies and clinical trials. Elevated uric acid was closely associated with new onset, essential hypertension in children and preliminary data suggests that lowering uric acid can lower blood pressure in some patients (139). Alongside education and lifestyle changes we hypothesize that earlier intervention with ULT may improve outcomes in both gout and its pathological associations therefore reducing the socioeconomic burden of these metabolic conditions.

Dilatation studies were repeated after a period of three months of sustained target uric acid levels following treatment with ULT. There was a trend towards improved FMD & a significant improvement in NMD of the brachial artery after treatment. This further implies that uric acid may have an effect on the impairment of dilatation and suggests that treatment with ULT improves dilatation parameters. The proposed mechanism is due to a combination of endothelial dependant impairment (seen in FMD) via reduced NO bioavailability and, secondly, endothelial independent (NMD) impairment via VSMC proliferation, mediated by uric acid induced NLRP3 activation. Improvement in endothelial function post treatment with allopurinol lies in its ability to reduce vascular oxidative stress through inhibition of xanthine oxidase (140).

Our findings support the work of two other studies, Ho et al examined FMD in 46 hyperuricaemic patients compared to age and sex matched controls. The FMD values were significantly lower in the hyperuricaemic patients than in the controls [4.45% (3.13%) vs 7.10% (2.48%);  $P < 0.001$ ] (141). Wong et al examined the clinical significance of hyperuricaemia in relation to vasomotor response of the brachial artery in 304 subjects with coronary artery disease and/or diabetes. They found high cardiovascular risk patients with hyperuricaemia had impaired NMD compared to those with normouricaemia. In addition, hyperuricaemia was independently associated with NMD after multivariable adjustments (142). To the best of our knowledge this is one of the only studies examining FMD & NMD in treatment naïve patients with early gout.

Our study found subjects with hyperuricaemia were also more likely to have deficits in endothelial independent smooth muscle responsiveness, as measured by NMD. NMD of the brachial artery was inversely correlated with sUA ( $R = -0.44$ ,  $P < 0.01$ ). This supports a



similar correlation between serum uric acid level and NMD demonstrated by Wong et al (R = -0.250, P < 0.001) (142). An inverse correlation of sUA with FMD has previously been demonstrated in a study by Krasnokutsky et al which compared 34 gout cases with 64 healthy controls (R = - 0.5, p = 0.003) (143). Our observation adds to the current literature and suggests hyperuricaemia impacts CVD risk via direct or indirect effects on arterial function.

pPTT is a novel echocardiogram index for detecting pulmonary hemodynamic and vascular alterations associated with PH. Our objective was to validate pPTT as a non-invasive, cost effective, bedside technique for the rheumatologist to develop for diagnosis and monitoring of patients with PH. PH is a significant contributor to mortality in many rheumatic conditions, diagnosis is often delayed due to PH being clinically silent until late in the disease process and difficulty accessing RHC required for diagnosis. RHC is an invasive and expensive method of diagnosing and monitoring treatment in PH requiring speciality cardiac input outside the clinical scope of the rheumatologist. The accuracy and standardization of Doppler-derived measurement techniques are limited and currently no single echocardiographic parameter is able to diagnose pulmonary hypertension specifically (144). Ultrasound is a more readily available resource compared to RHC in Irish hospitals. The development of pPTT as a non-invasive and bedside tool could be a useful, cost saving measure in the assessment of patients at risk of PH and may ultimately lead to earlier diagnosis and improved long-term outcomes.

Patients with established PH and matched controls were recruited for the initial proof of concept study. sUA levels were significantly higher in patients with PH when compared to normal controls, supporting our hypothesis that sUA levels are a potential mediator of PH

through ED. NT-proBNP levels were significantly higher in cases versus controls, given the association of PH with right heart failure we would expect BNP to be elevated in PH cases. Cases and controls were otherwise evenly matched for this aspect of the study. Echocardiograms were performed under the supervision of a trained technician with over 15 years of experience. Over one month, two rheumatologists with significant ultrasound experience developed the skill to perform focused echocardiograms on patients and calculate pPTT. Independent scans were only completed once the clinicians were confident in the technique and results were reproducible. As predicted, mean R-PVs2 was shorter and subsequently calculated mean pPTT was significantly lower in PH cases versus controls. Our preliminary findings suggest reliability of this echocardiogram technique and confirm the potential for pPTT to be used as a non-invasive index in the assessment of PH. We demonstrated through our findings that with appropriate supervision and training calculation of pPTT via focused echocardiogram is a skill which can be easily developed for use by the rheumatologist.

A further objective of this study was to assess pPTT in hyperuricaemic cases compared to normal healthy controls. Hyperuricaemia has been associated with development, severity and poor prognosis in PH (145). We hypothesized that pPTT would be shorter in patients with hyperuricaemia compared to normal healthy controls. Hyperuricaemic cases were recruited for this study and compared with matched normouricaemic controls. While there was a trend towards shorter mean R-PVs2 in hyperuricaemic cases when compared to controls, pPTT was significantly shorter in hyperuricaemic cases when compared with controls. Previously pPTT has been suggested as a surrogate marker of hemodynamic and vascular alterations in PH by Wibmer et al (129). In this regard, shorter pPTT may suggest

underlying changes in pulmonary haemodynamics in this cohort of patients with hyperuricaemia. We propose potential mechanisms driving pulmonary haemodynamic change secondary to hyperuricaemia are similar to those causing systemic arterial change evident in dilatation studies; a combination of endothelial dependant impairment via impaired NOS activity and reduced NO bioavailability and secondly endothelial independent impairment via VSMC proliferation mediated by uric acid induced NLRP3 activation. These vascular changes may contribute to the documented association of hyperuricaemia with poor outcomes in PH and indeed overall CV morbidity in this cohort.

There was a trend towards improvement in both R-PVs2 and pPTT in cases who underwent treatment with ULT for a period of three months. Although a non-significant trend, these preliminary findings further demonstrate that hyperuricaemia may contribute to pulmonary haemodynamic change therefore also increasing the risk of CVD developing over time. This contributes to our hypothesis that maintaining sUA levels within normal parameters may improve outcomes for both gout and its pathological associations including CVD. Further research needs to be completed in this area to confirm these results and better understand the physiological mechanisms between hyperuricaemia and PH.

Our study has a number of strengths, the sample is a typical representation of patients with hyperuricaemia and gout. The potential causal role of hyperuricaemia in altered arterial and pulmonary haemodynamics is supported by improvement in these surrogate markers of vascular function following a period of treatment with ULT. High end ultrasound imaging was used to complete the study and assess vessel diameter. Patients sUA levels were closely followed and studies repeated following a period of sUA within target range.

The study does have some limitations, some of which were inherent and some of which occurred due to external factors including the outbreak of the COVID-19 pandemic. It is an observational and a single-centre design which can be susceptible to unidentified confounders. Due, in part, to the impact of the COVID-19 pandemic there were a smaller number of patients recruited into all samples than initially planned and a shorter follow up time, reducing the power of the studies. It was not possible to blind the ultrasonographer to the patients' condition which could introduce some unintentional bias in results. Studies require a skilled ultrasonographer and there can be inter-user variability in US assessments. Although performed under the supervision of a trained echocardiogram technician, pPTT studies were carried out by two rheumatologists without formal qualifications in echocardiography. While not trained to perform full echocardiography examinations the rheumatologists became highly proficient when limited to focused pPTT measurement. The study conditions were kept as consistent as possible, however there are many external variables which can affect artery vasoconstriction and therefore impact results.

In conclusion, we have demonstrated through impaired dilatation studies and shortened pPTT on echocardiogram that hyperuricaemia may have a casual effect on the development and potentiation of vascular haemodynamic changes. This may contribute to the independent association of gout and CVDs which is the source of significant morbidity and mortality among sufferers. Earlier intervention with ULT may have implications for improving outcomes both in gout and its associated pathological associations.

## **Chapter Four**

### **NLRP3 Inflammasome Activity In The Acute Gout Joint.**

#### **4.1 Introduction**

##### **4.1.1. The Inflammasome**

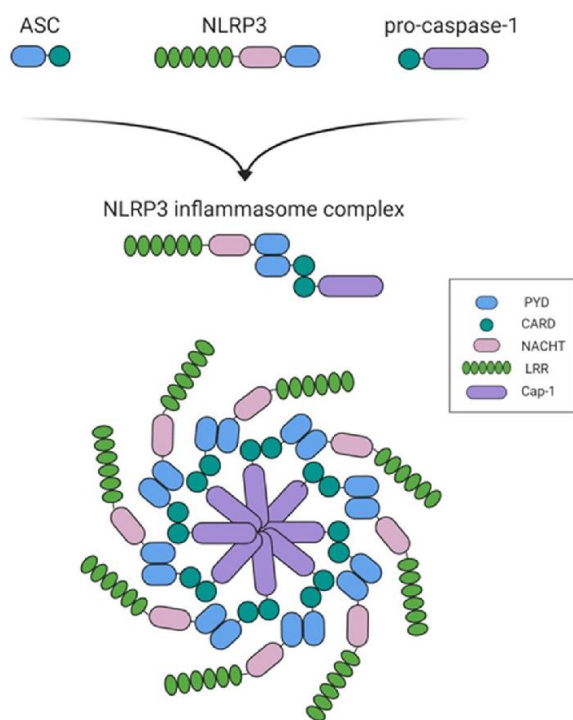
Inflammasomes are multimeric protein complexes present within the cytosol of cells, which when activated trigger a downstream inflammatory response as part of the innate immune defences (146). Inflammasome activation occurs through activation of Pattern Recognition Receptors (PRR) which may recognise Pathogen Associated Molecular Patterns (PAMPS) on microbial components in infection or Damage Associated Molecular Patterns (DAMPS) present on endogenous host cellular components (147). Dysregulated inflammasome activity has been associated with human heritable and acquired inflammatory diseases.

##### **4.1.2. The NOD-like receptor family proteins**

The NOD-like receptor (NLR) family members, NLRP1, NLRP3, NLRC4, and AIM2 (a member of the PYHIN protein family), have been identified as being capable of forming multi-protein inflammasome complexes that activate caspase-1, which leads to the processing and secretion of pro-inflammatory cytokines IL-1 $\beta$  and IL-18. Among NLRs, NLRP3 is

currently the most fully described inflammasome. It consists of the NLRP3 scaffold, adaptor apoptosis-associated speck-like protein (ASC) containing a caspase activation and recruitment domain (CARD) and pro-caspase-1 (Figure 4.1). NLRP3 is an intracellular signalling molecule that senses many pathogen, environmental and host-derived factors (148) and it is an important innate immune sensor that is activated in response to structurally diverse DAMPs such as toxins(149), ATP(149), excess glucose(150), urate(28), and cholesterol crystals(151).

Upon activation, NLRP3 interacts with ASC via its caspase activation and recruitment domain (CARD). ASC in turn interacts with the cysteine protease caspase-1 to form the active inflammasome. This results in the activation of caspase-1, which cleaves the pro-inflammatory cytokines IL-1 $\beta$  and IL-18 to their active forms and mediates a type of inflammatory cell death known as pyroptosis (152). A major function of a subfamily of NLR proteins is inflammasome activation, which has been implicated in a multitude of disease models and human diseases including gout (146).



**Figure 4.1 NLRP3 inflammasome structure.** The NLRP3 inflammasome is a complex consisting of NLRP3, ASC, and procaspase-1. NLRP3 consists of three regions: the pyrin domain (PYD) in the amino terminus, the NACHT domain, and the leucine-rich repeat domain (LRR) in the carboxy terminus. NLRP3 recruits ASCs through PYD-PYD interactions. In turn, procaspase-1 is recruited by ASC through CARD-CARD interactions to form the NLRP3-ASC-procaspase-1 inflammasome.

#### **4.1.3. NLRP3 and Autoinflammatory Diseases.**

A gain of function (GOF) mutation is a type of mutation in which the altered gene product possesses a new molecular function or a new pattern of gene expression. The discovery of a GOF mutation in a NLRP3 activating inflammasome as a cause of systemic autoinflammatory diseases was a significant step in understanding these conditions (153). This GOF mutation was initially identified in genetic screening of patients with familial cold urticaria (FCU) and Muckle–Wells syndrome (MWS) (154). FCU and MWS belong to a group of autoinflammatory diseases known as cryopyrin-associated periodic syndromes (CAPS), which arise from GOF point mutations in the NACHT domain of NLRP3, leading to a constitutively activated inflammasome (155). They are characterised clinically by recurrent fevers, joint and ocular symptoms, skin rashes, amyloidosis and neurological complications (156,157). In accordance, monocytes and macrophages isolated from CAPS patients display a basal spontaneous secretion of mature IL-1 $\beta$  in the absence of an external stimuli (158). In addition to its central role in the pathogenesis of auto-inflammatory disorders, the NLRP3 inflammasome has emerged as an unexpected sensor for metabolic danger and stress. It has since been implicated in the development of major diseases including gout, type 2 diabetes and obesity-induced insulin resistance (159). Haneklaus et al suggest that

it is possible that under normal physiology NLRP3 is homeostatic and maintains the metabolic balance. However, upon chronic activation (e.g. in obesity or hypercholesterolemia), NLRP3 becomes pathologic and promotes disease (159).

#### **4.1.4. NLRP3 & Gout**

The activation of the NLRP3 inflammasome by monosodium urate crystals is a central factor to the initiation and potentiation of an acute flare of gout (32). Martinon et al demonstrated that MSU crystals engage the caspase-1-activating NLRP3 inflammasome, resulting in the production of active interleukin (IL)-1 $\beta$  and IL-18 through the analysis of peritoneal macrophages (PMFs) derived from mice deficient in various key proteins of the inflammasome complex. They found that murine PMFs, stimulated with MSU, activated caspase-1 and secreted mature IL-1 $\beta$  (28). It is postulated that during a destabilising event, MSU crystals ingested by macrophages trigger a cytosolic DAMP response, leading to the assembly and activation of the NLRP3 inflammasome. NLRP3 protein activation leads to the recruitment and activation of the adaptor ASC and caspase-1 via PYD-PYD and CARD-CARD homotypic interactions, resulting in the processing and maturation of pro-IL-1 $\beta$  into its biologically active form, IL-1 $\beta$  (160).

As the NLRP3 inflammasome is considered central to the pro-inflammatory response to MSU crystals in gout, strategies that may impede its activation or affect its activity could reduce gouty inflammation. Martinon et al also demonstrated that in NLRP3-deficient mice, IL-1 $\beta$  release was impaired after MSU exposure suggesting that attenuation of NLRP3



may have an effect on disease progression (28). Colchicine, which is often indicated for treatment of an acute gout flare, acts through inhibition of microtubule driven rearrangement of mitochondria, which blocks MSU crystal mediated NLRP3 activation (161). The biologic agent Anakinra is a recombinant IL-1 $\beta$  receptor antagonist that targets activated IL-1 $\beta$  following its cleavage by NLRP3. Anakinra is often used in the management of gout, it is also indicated for treatment of other NLRP3 mediated diseases including RA and CAPS. The beneficial effects of compounds, such as xanthine oxidase inhibitors, that inhibit reactive oxygen species (ROS) production and decrease oxidative stress are regularly considered in gout. Xanthine oxidase inhibitors such as allopurinol, which reduce serum urate levels directly, also affect mitochondrial ROS production by inhibiting MSU crystal mediated inflammasome activation (162).

Nonspecific blockade of NLRP3, through mechanisms such as ROS inhibition, may have undesirable side effects including immunosuppression caused by complete blockade of IL-1 $\beta$  production during infection and inhibition of the antimicrobial response (152). The development of a more specific NLRP3 inhibitor has been an area of focus for researchers. MCC950, a novel specific NLRP3 inhibitor, has emerged as a potential therapeutic of interest (152).

#### **4.1.5. IL-1 $\beta$ & Gout.**

It is now widely accepted that IL-1 $\beta$  is a pivotal cytokine in acute gout (28). IL-1 $\beta$  is a highly inflammatory cytokine which is also known as the endogenous pyrogen. Production of IL-1 $\beta$  is tightly controlled by at least three distinct steps; Firstly, the production of the pro-IL-

1 $\beta$  protein (p35); secondly cleavage of the precursor pro-IL-1 $\beta$  to produce the active IL-1 $\beta$  protein (p17), and thirdly IL-1 $\beta$  is released into the extracellular environment. The middle step, processing of pro-IL-1 $\beta$ , involves the activation of a caspase-1-activating complex, the best characterized being the inflammasome (30). Activated IL-1 $\beta$ , in turn, triggers a cascade of proinflammatory mediators (including IL-8, IL-6 and TNF $\alpha$ ) leading to endothelial activation and leukocyte recruitment contributing to the inflammatory response (163).

#### **4.1.6. IL-6 & Gout**

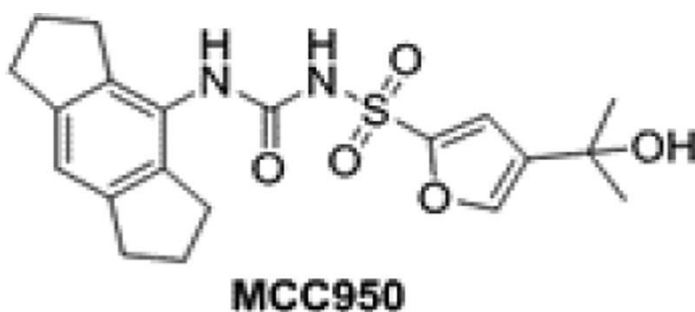
The presence of uric acid crystals causes oligomerization and dysfunctional activity of NLRP3 inflammasome macromolecules, in turn causing secretion of IL-1 $\beta$  and IL-18. These cytokines trigger a further downstream cascade of pro-inflammatory mediators including IL-6 and IL-8. IL-8 plays a key role in the recruitment and activation of neutrophils to the site of infection (164). IL-6 is a pleiotropic cytokine that when combined with sIL-6R, has been demonstrated to lead to activation of synovial cells, secretion of matrix metalloproteinases and formation of osteoclasts. This may contribute to bone damage via mediation of osteoclastogenesis and the increased bone-absorbing activity of osteoclasts (165)(163)(166).

Serum IL-6 levels have been shown to be associated with increased ESR and CRP levels and also associated with the presence of tophi and deformities in patients with gout (163). In the general population increased serum IL-6 has been correlated with increased risk for CVD (167). This is particularly relevant given that patients with gout and high serum uric acid levels are already known to be predisposed to greater cardiovascular risk than the general population (168). IL-6 may have a direct role in heightened risk among these patients.

#### 4.1.8. MCC950

MCC950 is a diarylsulfonylurea containing compound that was initially identified as an inhibitor of extracellular ATP-mediated maturation of IL-1 $\beta$  (152). MCC950 specifically inhibits the NLRP3 inflammasome therefore presenting certain advantages over biologic inhibitors of IL-1 $\beta$ . This molecule blocks NLRP3-induced ASC oligomerization in mouse and human macrophages without affecting the activation of NLRP1, AIM2 or NLRC4 inflammasomes. MCC950 may therefore have less immunosuppressive effects when compared to biologics (152). The effects of several NLRP3 activators, including MSU crystals, were inhibited by MCC950 indicating that the drug might directly act on a conserved NLRP3 activating mechanism; however, exactly how this drug affects NLRP3 activation is not yet clear (152)(169).

Although MCC950 has been shown to be a potent selective NLRP3 inhibitor that is active both in mice *in vivo* and in human cells *ex vivo*, it has not been specifically investigated in samples from patients with acute gout. In this chapter we aim to assess the effects of MCC950 on inflammasome components and pro-inflammatory mediator IL-6 in gout *ex vivo* synovial tissue and PBMCs.



**Figure 4.2:** Structure of MCC950.

*Reproduced from J. Med. Chem.* 2022, 65, 8, 6250-6260 (170).

## 4.2 Specific Aims of this Chapter

- To investigate if the NLRP3 inflammasome is active in the gout joint by assessing if inflammasome components are expressed in gout *ex vivo* synovial tissue compared to OA *ex vivo* tissue
- To assess the effects of novel inhibitor MCC950 on inflammasome components and pro-inflammatory mediator IL-6 in the gout *ex vivo* synovial tissue model
- To determine the effects of MCC950 on the secretion of IL-1 $\beta$  and pro-inflammatory mediator IL-6 in PBMCs from patients with gout.

## **4.3 Materials and Methods**

### **4.3.1 Patient Recruitment**

Patients with gout were recruited during an acute gout attack from the outpatient clinics at the Department of Rheumatology, Tallaght University Hospital (TUH). Patients with an actively inflamed knee joint and fulfilling the ACR/EULAR 2015 gout classification criteria were recruited for synovial biopsy (35). Serum samples for isolation of PBMCs were taken from patients fulfilling the ACR/EULAR 2015 gout classification criteria, currently having an acute gout flare but without a significantly inflamed joint. Ultrasound guided biopsies were performed under local anaesthetic using a 2.7mm grasping forceps inserted into the target joint. Biopsies were then obtained from the site of inflammation under direct ultrasound visualisation. For non-inflammatory disease controls, synovial biopsies from osteoarthritis (OA) patients were harvested from knee joints at the time of knee joint replacement surgery. All research was performed in accordance with the Declaration of Helsinki and approved by the TUH/SJH ethics committee. Fully informed written consent was obtained from each patient prior to inclusion.

#### **4.3.2 Ex vivo Synovial Explants**

Synovial biopsies were sectioned into three pieces and cultured in 96-well plates in full RPMI. This culture model maintains the synovial architecture and cell-cell contact, therefore closely reflecting the *in vivo* environment. For inhibition experiments, biopsies were cultured in the presence of novel small molecule inhibitor MCC950 (Sigma-Aldrich) (500nM-1 $\mu$ M) for 24 hrs. For each experiment, three technical replicates are combined (three individual biopsies from one patient) to give one biological sample and this was performed on a number of different patients (n=2 gout, n=2 OA). This is important to allow for heterogeneity within the patient joint and between patients. Following incubation, culture supernatants were harvested and assayed for cytokine expression by specific ELISA as outlined below. Biopsies were weighed and stored in RNA $later^{\circledR}$  (Invitrogen) at -20°C for future RNA extraction as outlined below.

#### **4.3.3 RNA extraction and Reverse Transcription from ex vivo Tissue**

*Ex vivo* biopsies were homogenised for 3 x 10 sec in lysis buffer (600 $\mu$ l Buffer RLT (Qiagen) supplemented with 10%  $\beta$ -Mercaptoethanol) using a 0.45cm diameter Tissue Tearor (BioSpec, Oklahoma, USA). Total RNA was isolated using the RNeasy Mini Kit (Qiagen) according to the manufacturer's protocol. The integrity of RNA samples was assessed using a bioanalyzer (Agilent). Samples with a 260:280 nm ratio of 1.8 and above and an RNA integrity number between 7 and 10 were used in subsequent experiments. Isolated RNA was stored at -80°C. Total RNA (300ng) was reverse transcribed to cDNA using a high-

capacity cDNA reverse transcription kit (Applied Biosystems) and stored at -20°C until further use.

#### **4.3.4 qPCR Analysis**

Relative quantification of gene expression was analysed with pre-optimised conditions on the QuantStudio 5 Dx Real-Time PCR System (Applied Biosystems). Specific SYBR primers for NLRP3, IL-1 $\beta$  and IL-6 were used (Table 4.1) (Microsynth) and primers for GAPDH and RPLPO were used as endogenous controls. Amplification reactions contained 1  $\mu$ l of cDNA, 6.25 $\mu$ l of Power SYBR<sup>®</sup> Green PCR Master Mix (Biosciences), 1 $\mu$ l FWD primer, 1 $\mu$ l REV primer and 3.75 $\mu$ l RNase free water. All reactions/negative controls were performed in triplicate using 96-well plates on the QuantStudio 5 Dx Real-Time PCR System (Applied Biosystems). Thermal cycling conditions were as recommended by the manufacturer (Microsynth). Relative changes in gene expression were determined using the comparative Ct method.

#### **4.3.5 Isolation of Peripheral Blood Mononuclear Cells (PBMC)**

Serum samples for isolation of PBMCs were taken from patients fulfilling the ACR/EULAR 2015 gout classification criteria, currently having an acute gout flare. Blood was drawn into heparin containing tubes. PBMC were isolated by Ficoll-Metrizoate density gradient centrifugation (Lymphoprep; Nycomed), from fresh blood samples. Cells were seeded into 12-well plates at a density of  $1 \times 10^6$  cells/1ml full RPMI 1640 medium and allowed to adhere

overnight. The Inflammasome Activation assay was then performed on these cells as detailed below.

#### **4.3.6 Inflammasome Activation Assay**

To measure the effects of MCC950 on TLR4-induced (LPS) inflammasome activation, PBMC were isolated and seeded into 12-well plates at a density of  $1 \times 10^6$  cells/ml in full RPMI 1640 medium as explained above. Cells were first primed with LPS (Enzo Life Sciences) (10ng/ml) for 3 hrs. Following this, medium was removed and discarded, and cells were incubated in fresh medium containing MCC950 (500nM-1 $\mu$ M) or basal control medium for 45 minutes. Finally, cells were then stimulated with Monosodium Urate (MSU) Crystals (InvivoGen) (200 $\mu$ g/ml) and incubated overnight. Supernatants were then harvested and stored at -20°C until future use. Cells were lysed with 600 $\mu$ l Buffer RLT (Qiagen) supplemented with 10%  $\beta$ -Mercaptoethanol and stored at -80°C. Due to time restraints, qPCR was not performed on these samples.

IL-1 $\beta$  measurement by specific ELISA was performed as a marker of NLRP3 activation (outlined below in section 4.3.7). Pro-inflammatory mediator IL-6 was also measured by specific ELISA.

#### **4.3.7 Cytokine Measurement of IL-1 $\beta$ and IL-6**

To assess the effects of LPS, MCC950 and MSU crystals on NLRP3 inflammasome, cytokines were quantified by ELISA. Protein concentration of secreted IL-1 $\beta$  and IL-6 from harvested supernatants were measured by IL-1 $\beta$  and IL-6 DuoSet ELISAs (R&D Systems) according to



the manufacturer's protocol. The ELISA standards ranged from 250 pg/ml to 3.91 pg/ml for IL-1 $\beta$  and from 600 pg/ml to 9.38 pg/ml for IL-6.

#### 4.3.8 Statistical Analysis

SPSS26 system (SPSS Inc, Chicago, Illinois, USA) for windows was used for statistical analysis. Wilcoxon Signed Rank test was used for analysis of non-parametric data. p-values of less than 0.05 (\* p<0.05) were determined as statistically significant. All data is represented as mean  $\pm$  SEM.

#### 4.3.9 Impact of COVID-19 on Experiments.

Due to the COVID-19 pandemic and time constraints the number of biopsy specimens available were curtailed and it was not possible to complete certain elements of the experimental design, namely qPCR on remaining PBMC samples. These samples have been stored for future analysis.

Targeted cDNA	Forward Primer Sequence	Reverse Primer Sequence
GAPDH	5'-GGG AAG CTT GTC ATC AAT GGA- 3'	5'-TCT CGC TCC TGG AAG ATG GT- 3'
RPLPO	5'-GCG TCC TCG TGG AAG TGA CAT CG-3'	5'-TCA GGG ATT GCC ACG CAG GG- 3'
NLRP3	5'-TCT GGA TGA GGA AAC TGA AGT TGA-3'	5'-TGA TTA CGG GGC TAT GAC ATT GG-3'

IL-1 $\beta$	5'-CTC AAG TGT CTG AAG CAG CCA T- 3'	5'-CAT CAT TTC ACT GGC GAG CTC A-3'
IL-6	5'-CCC TGA GAA AGG AGA CAT GTA AC-3'	5'-CCT CTT TGC TGC TTT CAC ACA TG-3'

**Table 4.1 SYBR Primers**

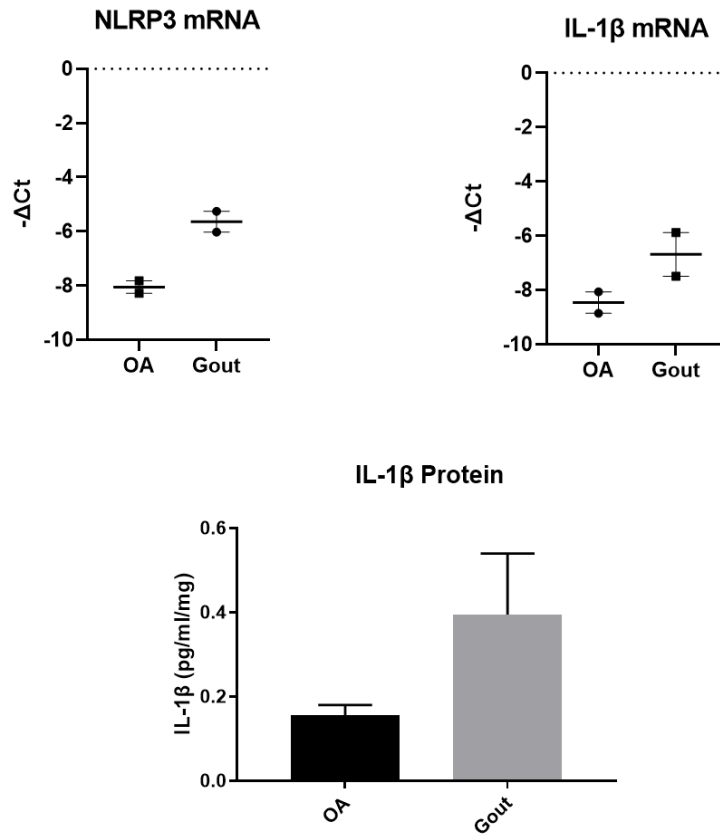
## 4.4 Results

### 4.4.1 NLRP3 inflammasome components and IL-1 $\beta$ are highly expressed in Gout compared to OA *ex vivo* synovial explants

To investigate if NLRP3 is active in the gout joint, primary experiments examined the effects of NLRP3 and IL-1 $\beta$  mRNA in gout compared to OA synovial tissue. NLRP3 and IL-1 $\beta$  mRNA levels in whole tissue biopsies were measured by qPCR and transcripts were found to be

more highly expressed in gout compared to OA (Figure 4.3). This was mirrored by an increase in protein levels of IL-1 $\beta$  (Figure 4.3). To further examine the expression of inflammasome components we utilised a well-established *ex vivo* synovial tissue explant model which maintains the synovial architecture and cell-cell contact and closely reflects the *in vivo* microenvironment. As these explants are in culture within 15 mins of biopsy, they continue to spontaneously release inflammatory mediators into the culture medium (171) . Figure 4.3 (lower panel) shows culture medium harvested from *ex vivo* explants from gout patients had higher protein levels of active IL-1 $\beta$  (pg/ml/mg) (OA  $0.155 \pm 0.025$  V gout  $0.395 \pm 0.145$ ). Although the numbers of samples precludes the evaluation of this result for statistical proof, this preliminary data taken together suggests that the NLRP3 inflammasome is active in the gout joint, and its components are more highly expressed in gout synovial tissue than OA synovial tissue.

**Figure 4.3:**



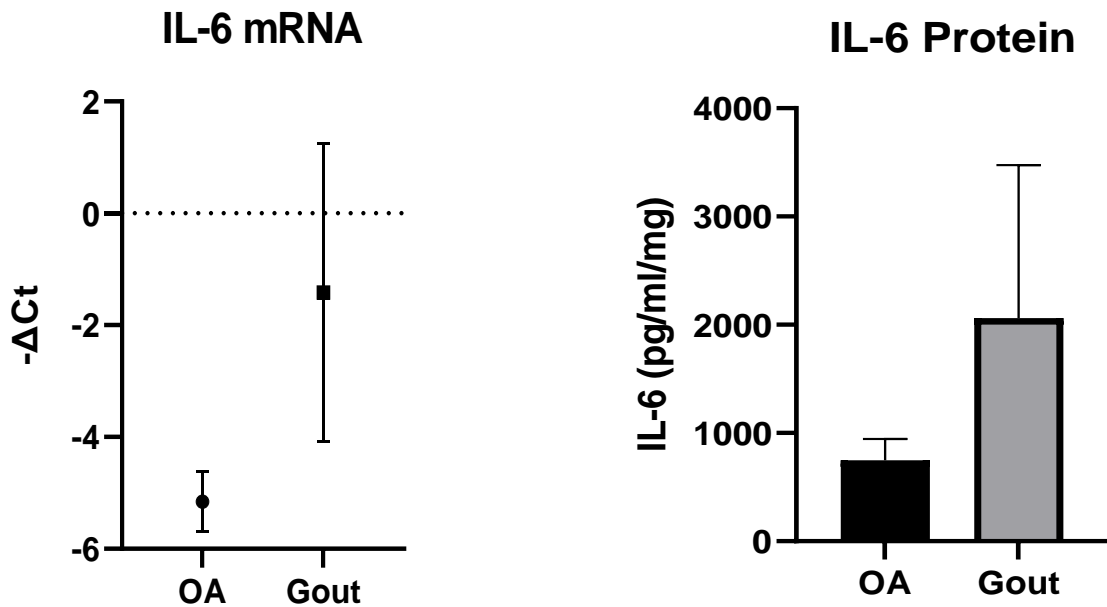
**Figure 4.3: NLRP3 and IL-1 $\beta$  are higher in Gout compared to OA synovial tissue.**

Representative line graphs (top panels) demonstrating increased expression of NLRP3 and IL-1 $\beta$  mRNA levels in gout (n=2) versus OA (n=2) synovial tissue. Bar graph (lower panel) represents spontaneous release of active IL-1 $\beta$  from gout (n=2) and OA (n=2) *ex vivo* explants. mRNA data is expressed as  $-\Delta\text{Ct}$  Mean  $\pm$  SEM compared to endogenous controls (RPLPO and GAPDH). Cytokine data is expressed as Mean  $\pm$  SEM.

**Results 5.4.2. IL-6 is more highly expressed in Gout compared to OA *ex vivo* synovial explants**

IL-6 mRNA levels in whole tissue biopsies were measured by qPCR and transcripts were found to be more highly expressed in gout compared to OA  $\Delta Ct$  ( $-5.159 \pm 0.53$  v  $-1.415 \pm 2.662$ ) (Figure 4.4). This was mirrored by an increase in protein levels of IL-6 pg/ml/mg ( $749.2 \pm 195.4$  v  $2061 \pm 1414$ ) (Figure 4.4).

Figure 4.4



**Figure 4.4 IL-6 levels are higher in Gout compared to OA synovial tissue.** Representative line graphs demonstrating increased expression of IL-6 mRNA levels in gout (n=2) versus OA (n=2) synovial tissue. Bar graph represents spontaneous release of active IL-6 from gout (n=2) and OA (n=2) *ex vivo* explants. mRNA data is expressed as  $-\Delta Ct$  Mean  $\pm$  SEM compared to endogenous controls (RPLPO and GAPDH). Cytokine data is expressed as Mean  $\pm$  SEM.

#### 4.4.3. MCC950 significantly decreases IL-6 Protein levels in Gout PBMC.

To measure the effects of MCC950 on TLR4-induced (LPS) inflammasome activation, PBMC from patients in acute gout flare were isolated and firstly primed with LPS (10ng/ml) for 3 hrs. Following this, medium was removed and discarded, and cells were incubated in fresh medium containing MCC950 (500nm-1 $\mu$ M) or basal control medium for 45 minutes. Finally, cells were then stimulated with Monosodium Urate (MSU) Crystals (200 $\mu$ g/ml) and incubated overnight. Results found that MCC950 inhibits IL-6 and IL-1 $\beta$  protein levels in gout PBMCs in a dose dependant manner. Figure 4.5 shows the effect of MCC950 on IL-6 protein levels (pg/ml), following stimulation with LPS, protein levels significantly increased when compared to basal control ( $81.51 \pm 40.60$  v  $22.59 \pm 15.13$  p= 0.03). Treatment with 500nM of MCC950 significantly decreased IL-6 protein levels when compared with LPS control ( $19.02 \pm 8.81$  v  $81.51 \pm 40.60$ , p=0.03). Treatment with LPS and 1 $\mu$ M MCC950 resulted in a significant decrease in IL-6 protein levels compared with LPS control ( $19.50 \pm 9.83$  v  $81.51 \pm 40.60$  p=0.03). Incubation with LPS and MSU crystals resulted in a significant increase in IL-6 protein levels compared to LPS stimulation alone ( $160.8 \pm 82.35$  v  $81.51 \pm 40.60$  p=0.03). Cells treated with LPS, 1 $\mu$ M MCC950 and MSU demonstrated lower levels of IL-6 protein secretion when compared with LPS and MSU alone ( $105.3 \pm 49.83$  v  $160.8 \pm 82.35$ ), however this change was not significant. IL-1 $\beta$  protein levels followed a similar trend to IL-6 but MCC950 did not significantly reduce the levels of IL-1 $\beta$  protein in gout PBMC.

Figure 4.5

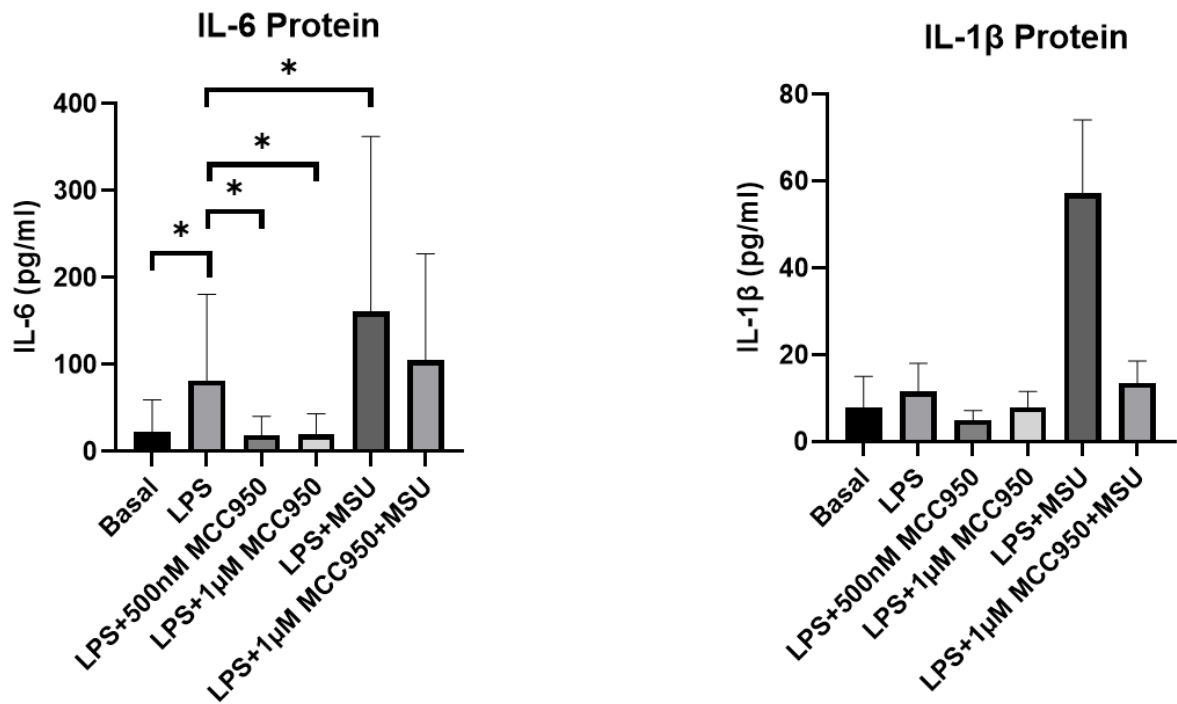


Figure 4.5: MCC950 significantly decreases IL-6 Protein levels in Gout PBMC.

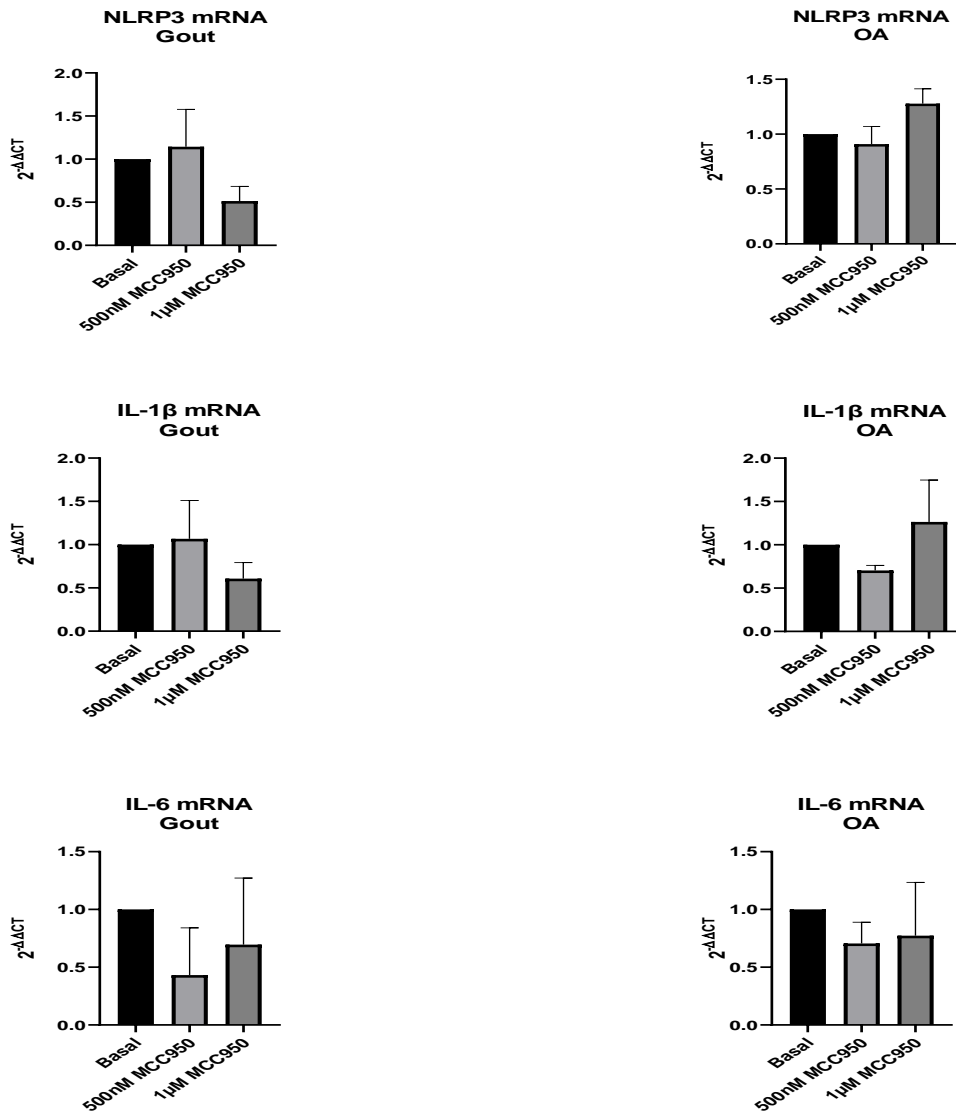
Representative line graphs demonstrating decreased levels of IL-6 protein following treatment with MCC950 in gout PBMC (n=6). MCC950 did not significantly reduce the levels of IL-1β protein in gout PBMC (n=5). Cytokine data is expressed as Mean ± SEM, \* $p < 0.05$  significantly different to control.

**Results 4.4.4 : MCC950 stimulation is associated with a dose dependant decrease in NLRP3, IL-1 $\beta$  and IL-6 mRNA in Gout but not in OA synovial tissue.**

To investigate whether MCC950 downregulates the mRNA expression of NLRP3, IL-1 $\beta$  and IL-6 mRNA in synovial explant cultures, mRNA was extracted at the end of the MCC950 inhibition cultures experiments described above and probed for NLRP3, IL-1 $\beta$  and IL-6 by qPCR, Results were compared to non-inflammatory OA tissue. Figure 4.6 shows the effect of MCC950 on NLRP3 mRNA, in which representative line graphs demonstrate a dose dependant decrease in NLRP3, IL-1 $\beta$  and IL-6 mRNA levels in gout synovial tissue (n=2). This trend was not observed in OA synovial tissue (n=2).



Figure 4.6



**Figure 4.6: mRNA Expression of NLRP3, IL1 and IL6 in Gout and OA Synovial tissue Following Exposure to MCC950**

Representative line graphs demonstrating a dose dependant decrease in NLRP3, IL-1β and IL-6 mRNA levels in gout synovial tissue (n=2). This trend was not observed in OA synovial tissue (n=2). mRNA data is expressed as fold change ( $2^{-\Delta\Delta CT}$ ) mean  $\pm$  SEM compared to endogenous controls (RPLPO and GAPDH).

#### **4.4.5. Protein levels of IL-6 and IL-1 $\beta$ following treatment with MCC950 in Gout and OA synovial tissue.**

To assess the effects of novel inhibitor MCC950 on the NLRP3 inflammasome, cytokines were quantified by ELISA. Protein concentration of secreted IL-1 $\beta$  and IL-6 from gout *ex vivo* synovial tissue, were measured by IL-1 $\beta$  and IL-6 DuoSet ELISA following incubation with MCC950 for 24 hrs (500nM-1 $\mu$ M). No significant change in the protein levels of IL-6 or IL-1 $\beta$  in gout (n=2) or OA (n=2) synovial tissue was observed following treatment with MCC950 (Figure 4.7). Although a reduction in protein levels of IL-6 and IL-1 $\beta$  was not seen post incubation with MCC950 basal levels of IL-1 $\beta$  and IL-6 were numerically higher with gout than with OA. (2061  $\pm$  1414 v 749.2  $\pm$  195.4)

Figure 4.7:

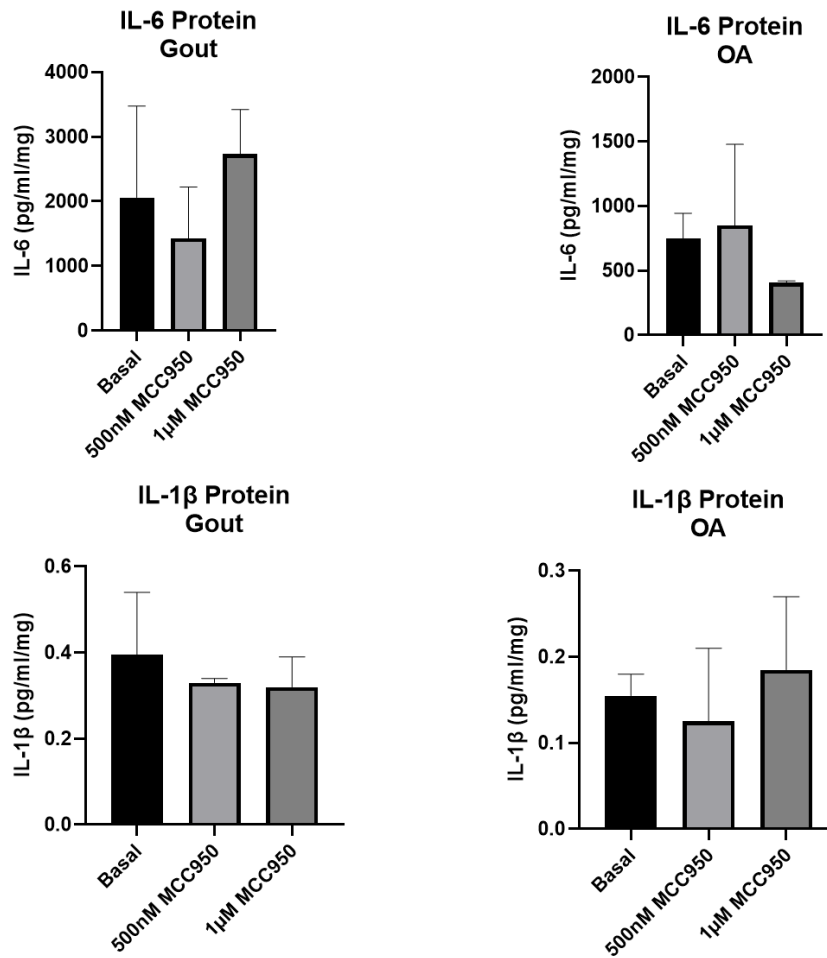


Figure 4.7 Protein levels of IL-6 and IL-1β following treatment with MCC950 in Gout and OA synovial tissue. Representative line graphs demonstrating no significant change in the protein levels of IL-6 or IL-1β in gout (n=2) or OA (n=2) synovial tissue following treatment with MCC950.

#### 4.5 Discussion.

Gout is a chronic inflammatory disorder in which the NLRP3 inflammasome and potent inflammatory cytokine IL-1 $\beta$  are implicated in disease pathogenesis (172). This study examines proinflammatory components related to NLRP3 in acute gout and a potential therapeutic inhibitor of the NLRP3 inflammasome, MCC950. In this study, we demonstrate that the NLRP3 inflammasome is highly active in 'ex-vivo' synovial tissue explants taken from the inflamed knee joints of patients during an acute gout attack. We have shown increased expression and activity of the NLRP3 inflammasome and related proinflammatory cytokines IL-1 $\beta$  and IL-6 in gout compared to OA synovial tissue. Our findings reveal that TLR2-induced inflammasome activation was inhibited in MCC950 treated PBMCs in a dose dependant manner. Finally, we demonstrate for the first time the inhibitory effects of specific NLRP3 inhibitor MCC950 on the inflammasome and pro-inflammatory mediators in a gout *ex vivo* explant model.

NLRP3 is known to potently potentiate IL-1 $\beta$  activation therefore the use of NLRP3 blockade as a molecular target to treat inflammatory diseases where IL-1 $\beta$  plays a central role holds promise. NLRP3 inhibition has been demonstrated to attenuate type 2 diabetes(173), atherosclerosis(151), multiple sclerosis(174), Alzheimer disease(173), and gout (159)(28). Heneka et al demonstrated that NLRP3 negative mice carrying mutations associated with familiar Alzheimer's disease (AD) were largely protected from loss of spatial memory and other AD-associated sequelae and demonstrated reduced brain caspase-1 and IL-1 $\beta$  activation (173). Duewell et al showed that cholesterol crystals activate the NLRP3 inflammasome in phagocytes and that NLRP3 is required for atherogenesis (151). Furthermore, Stienstra et al demonstrated that mice deficient in NLRP3 were

resistant to the development of high fat diet induced obesity and protected from obesity induced insulin resistance (89). Thus, identification of endogenous mechanisms that control NLRP3 inflammasome deactivation may provide insights into the control of several chronic diseases.

In 2001, a number of diarylsulfonylurea-containing compounds were identified as novel IL-1 $\beta$  processing inhibitors, however their mechanism of action and relation to NLRP3 was unknown (175). Subsequently, our colleagues in Trinity College Dublin identified MCC950 as a selective inhibitor of the NLRP3 inflammasome. Coll et al initially described the molecule MCC950 as reducing IL-1 $\beta$  production in vivo and attenuating the severity of experimental autoimmune encephalomyelitis (EAE), a disease model of multiple sclerosis (152). Furthermore, MCC950 treatment rescued neonatal lethality in a mouse model of CAPS and was active in ex vivo samples from individuals with Muckle-Wells syndrome (152). The selective inhibitor has been trialled in several NLRP3 mediated diseases including diabetes and shown promising results. Sharma et al demonstrated in diabetic apolipoprotein E knockout mice models that MCC950 reduces plaque development, promotes plaque stability, and improves vascular function. This suggests that targeting NLRP3-mediated inflammation is a novel therapeutic strategy to improve diabetes-associated vascular disease (176). Ward et al showed that NLRP3 inflammasome inhibition with MCC950 improved diabetes-mediated cognitive impairment and vasoneuronal remodelling after ischemia in high-fat diet, diabetic male Wistar rats (177). MCC950 has been found to be a potential therapeutic target for NLRP3 associated syndromes, including autoinflammatory and autoimmune diseases.

In this study, we demonstrate increased expression of IL-1 $\beta$  and IL-6 in gout compared to OA synovial tissue, at both the mRNA level and protein level. Our work supports the findings by Martinon et al which demonstrated that MSU crystals engage the caspase-1-activating NLRP3 inflammasome, resulting in the production of active IL-1 $\beta$ . Until now, strategies for targeting the NLRP3 inflammasome pathway have been focused on IL-1 $\beta$  blockade, this approach has been successful for the treatment of CAPS. Improved mechanistic understanding of NLRP3 activation of the inflammasome by MSU crystals during a gout attack provides researchers with potential future therapeutic targets to treat this debilitating disease.

We sought to identify if MCC950 could inhibit the NLRP3 inflammasome in *ex-vivo*' synovial tissue explants taken from the inflamed knee joints of patients during an acute gout attack. To measure the effects of MCC950 on TLR4-induced (LPS) inflammasome activation, PBMC from active gout patients were isolated, primed and treated with MCC950 at varying doses. Initial experiments demonstrated that MCC950 could significantly inhibit NLRP3-induced IL-6 and revealed a trend for inhibition of IL-1 $\beta$  secretion in a dose dependent manner in gout PBMC. Bryan et al demonstrated that murine hosts require TLR-2 and TLR-4 for macrophage activation and development of inflammation in response to MSU crystals. TLR-2 and TLR-4 negative bone marrow derived macrophages (BMDMs) demonstrated impaired uptake of MSU crystals in vitro and impaired MSU crystal-induced production of IL-1 $\beta$ . These findings indicate that innate immune recognition of naked MSU crystals by specific TLRs is involved in determining the inflammatory response of MSU crystal deposits and the course of clinical gout in vivo (29). In our experiment PBMCs were incubated with MSU crystals to replicate more closely what occurs during a gout flare in vivo. MSU crystals

are deposited at the site of inflammation and form an antigenic trigger leading to the activation of the inflammatory response in gout. Following incubation with MSU, cells treated with MCC950 expressed numerically reduced levels of pro-inflammatory cytokines IL-6 and IL-1 $\beta$  supporting its inhibitory role of NLRP3. To further investigate the effects of novel inhibitor MCC950 on the NLRP3 inflammasome, primary experiments examined the effect of various doses of MCC950 on NLRP3 mRNA in gout compared to OA synovial tissue by qPCR. In ex-vivo synovial tissue MCC950 demonstrated a trend towards inhibition of the expression of NLRP3.

Finally, to assess the effects of novel inhibitor MCC950 on the NLRP3 inflammasome at the protein level, cytokines were quantified by ELISA. Protein concentration of secreted IL-1 $\beta$  and IL-6 from gout *ex vivo* synovial tissue, were measured by IL-1 $\beta$  and IL-6 ELISA following incubation with MCC950. No significant change in the protein levels of IL-6 or IL-1 $\beta$  in gout or OA synovial tissue was observed following treatment with MCC950. We postulate the lack of change seen in this experiment may be secondary to the low numbers used in this experiment. Another consideration may be that change at the protein level may require a longer time to occur compared to mRNA.

The findings from this pre-liminary work taken together support the need for further investigation into MCC950 as a potential therapeutic target for patients with gout. Narros-Fernández et al recently reported the synthesis and biological evaluation of new selective NLRP3 inflammasome inhibitors obtained by the replacement of the hexahydroindacene moiety of MCC950 with various differently substituted benzenes. They refer to this molecule as compound 4b and have found it to have a good NLRP3 inhibition profile both in vitro and in vivo in a mouse model of gout (170). To our knowledge, this is the only other

work specifically examining the interaction of MCC950 with NLRP3 activation in gout models.

Further development of a selective inhibitor of the inflammasome may have several benefits. Coll et al demonstrated that MCC950 does not block the major anti-microbial inflammasomes NLRC4 and NLRP1. Specific targeting of NLRP3 will therefore not cause complete blockade of IL-1 $\beta$  production during infection and antimicrobial responses may remain intact (152). MCC950 may therefore have less immunosuppressive effects when compared to biologics which are known to increase the risk of serious infections (178). MCC950 has a shorter half-life compared to bDMARDs and can be withdrawn more rapidly should unwanted effects occur. A small molecule such as MCC950 may also be more cost effective than biologic agents (179). The future clinical development of MCC950 or derivatives may result in the development of new anti-inflammatory therapies for NLRP3 related diseases such as type 2 diabetes, MS, Alzheimer's disease and gout.

Our study has a number of limitations; the ability to recruit patients for synovial biopsy was reduced as a result of the COVID-19 pandemic. As such, the numbers for this preliminary work were reduced, thereby greatly limiting the power of the study. Unfortunately, we did not complete the inflammation assay on non-inflammatory control PBMCs and therefore lack appropriate control data for comparison in this experiment, however we aim to recruit and process these for during future experiments. In addition, future work currently underway in our department includes on-going recruitment and further analysis of 'ex-vivo' synovial tissue explants taken from the inflamed knee joints of patients during an acute gout attack.



In conclusion, this study shows that the NLRP3 inflammasome is highly active in gout and MSU crystals may induce NLRP3 in cells *in vitro/ex vivo*. MCC950, a novel compound, can inhibit the inflammasome in gout *ex vivo* tissue and further inhibit pro-inflammatory cytokines. Targeting NLRP3 may present certain advantages over the use of biological inhibitors, which have previously been examined for the treatment of gout. This study highlights the importance of the inflammasome in the pathogenesis of gout and the potential therapeutic benefit of specifically targeting NLRP3.

**Anticytokine Therapies for Immune Mediated Rheumatic Diseases are associated with Reduced Hospitalisation following community COVID-19 infection; Results of the Trinity Rheumatology and Covid-19 Registry - TRACR**

**5.1 Introduction.**

**5.1.1. A Changing Research Agenda.**

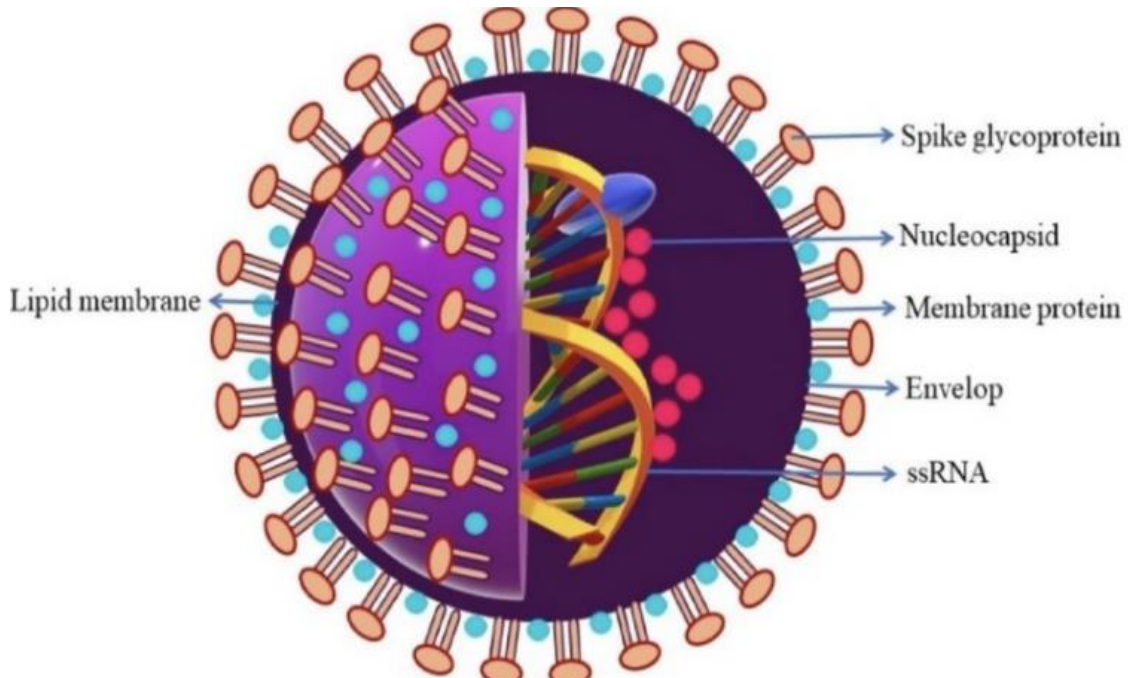
In March 2020 the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and resulting Coronavirus Disease 2019 (COVID-19) presented a global health crisis which dramatically altered the provision of medical care and research throughout the world. Due to the increased clinical risk of COVID-19 transmission between patients in the hospitalised setting, patient recruitment into non-essential clinical studies, including my own research on the pathological associations of hyperuricaemia was abruptly paused. Necessary redeployment of researchers to the medical 'front line' occurred throughout departments thus altering research agendas and timelines significantly.

In advance of the first pandemic wave reaching Ireland, other national healthcare systems were quickly overwhelmed by exponential increases in hospitalisations for severe respiratory disease, many of whom were refractory to standard supportive care, leading to high mortality rates. Early reports indicated that elderly patients with pre-existing respiratory diseases and patients with severe obesity suffered higher COVID-19 mortality rates. There was an acute lack of information on the risk profiles for patients with autoimmune conditions including immune mediated rheumatic diseases receiving immunosuppressive disease modifying therapies. To both address the lack of data and redirect our research resource to where it was most urgently needed, we rapidly designed and executed a research project to quantify the extent COVID-19 related morbidity among patients with immune mediated rheumatic diseases occurring within the catchments of hospitals affiliated with Trinity College Dublin during the first wave of the COVID-19 pandemic. The results of this work are detailed in this chapter.

### **5.1.2 COVID-19 – Background.**

On the 30<sup>th</sup> of January 2020, the World Health Organization (WHO) initially declared a global emergency following the novel coronavirus outbreak in Wuhan, a city located in China's Hubei province. This outbreak of coronavirus would subsequently become a major global pandemic. Belonging to the Coronaviridae family, coronaviruses cause respiratory infection in mammals, including bats, rodents, and camels. It may also occur in avian species (180). In humans, coronavirus infections may be asymptomatic or accompanied by fever, cough, shortness of breath and gastrointestinal irritation (181). Coronavirus infections may lead to severe pneumonia and subsequently death, more commonly in vulnerable cohorts such as the elderly or immunocompromised (182). Coronaviruses were

named after the Latin word corona, meaning crown or halo, owing to their crown-like spikes on the surface as seen when viewed under an electron microscope (183). The SARS-CoV-2 virus is genetically closely related to severe acute respiratory syndrome coronavirus (SARS-CoV) the first pandemic threat of a deadly coronavirus that emerged in late 2002 and caused an outbreak of severe acute respiratory syndrome (SARS). SARS-CoV had a high mortality but cases diminished quickly after intense public health mitigation measures including contact tracing, isolation, use of face masks and hand hygiene (184). Similar intense public health measures were introduced globally following the emergence of SARS-COV-2 including social distancing, isolation, hand hygiene and 'lockdown' of entire countries which included 'stay at home' policies and severe restriction of movement significantly impacting on the day to day lives of most in society in 2020.



**Figure 5.1: Schematic diagram of SARS-CoV-2. Credit: *Sci. Rep.* 11, 23122 (2021)**

Usually residing in an animal reservoir for example bats, mice, rats, camels or chickens, Coronaviruses have developed the ability to initiate an epidemic by adapting to humans via zoonotic transmission. There are a number of human factors influencing emerging zoonotic diseases including; factory farming practices, the destruction of forests and other habitats leading to increased contact between humans and previously unknown viruses and evolving human behaviour, cultural practices and food preferences (185)(186). Bats have been reported to be the primary carrier and reservoir for a vast range of viruses, including Coronavirus (187). SARS-CoV-2 is postulated to have jumped the species barrier from bats sold at the Huanan South China Seafood Market in Wuhan, Hubei Province, China, as described in a phylogenetic study by Lu et al (188). In this study next-generation sequencing of samples from bronchoalveolar lavage fluid was completed and cultured isolates from nine inpatients, eight of whom had visited the Huanan seafood market in Wuhan. Complete and partial SARS-CoV-2 genome sequences were obtained from these individuals. The ten genome sequences of SARS-CoV-2 obtained were extremely similar, exhibiting more than 99.98% sequence identity. Notably, SARS-CoV-2 was closely related (with 88% identity) to two bat-derived severe acute respiratory syndrome (SARS)-like coronaviruses, bat-SL-CoVZC45 and bat-SL-CoVZXC21, collected in 2018 in Zhoushan, eastern China. As bat derived coronavirus rarely directly transmit to humans, researchers suggest it is likely that a second intermediate animal host, such as a Malayan pangolin may be involved in the zoonotic transmission. (189) These animals are of growing importance and interest because they are the most illegally trafficked of any group of mammal, they are used as both a food source and their scales are utilized in traditional Chinese medicine. Once established in humans, it is now recognized that the main form of human-to-human transmission of SARS-CoV-2 occurs through respiratory droplets expelled by an infected

individual, transmission can also occur as a result of direct contact with contaminated inanimate objects, known as fomite transmission (190). Once inhaled, viral particles are primarily deposited on the nasal mucosa where SARS-CoV-2 infects ACE2 expressing ciliated cells in the nasal passage, it then replicates and is released apically infecting neighbouring cells. In response to infection, the epithelial cells initiate an innate immune response by secreting type I and type III interferons and downstream pro-inflammatory cytokines which activate humoral and cell specific responses to COVID-19.

SARS-CoV-2 when confined to upper airway and nasal passages causes mild or asymptomatic disease. However, the extension of infection into the gas exchange portions of the lung is the primary cause of severe morbidity and mortality in patients with COVID-19. Infection in this region produces progressive hypoxia associated with pulmonary infiltrates. Early pathologic changes include alveolar flooding and inflammatory cell infiltrates. The disease can rapidly progress to acute respiratory distress syndrome (ARDS) with diffuse alveolar damage, hyaline membrane, epithelial and microvascular injury and, in some cases, thrombi of small and large vessels (191). The majority of patients with severe COVID-19 are elderly males (M:F ratio 2:1–3:1), with a mean age of 73–81.5 years (191). Those with severe infection usually have one or more co-morbidities such as, hypertension, atherosclerosis, ischemic cardiomyopathy and/or coronary heart disease, chronic obstructive pulmonary disease, diabetes, ATTR amyloidosis, and obesity (192).

### **5.1.3. Initial Response to COVID-19.**

When COVID-19 was declared as a pandemic by the World Health Organisation on March 11<sup>th</sup> 2020, there were no known effective or specific treatment options such as antiviral drugs or vaccines available against SARS-CoV-2 and no evidence base for certain pre-

existing medications such as Tocilizumab which now have additional indications in COVID-19 infection. With the emergent fatalities evidenced across the globe and the need to urgently address the evidence base deficit in defining effective treatment strategies, SARS-CoV-2 rapidly became a major focus for global researchers. Initial treatment administered for COVID-19 patients was based on the clinical presentation of the patient and largely focused on supportive care, there was a lack of evidence and experience with specific therapeutics. High infection rates, severe infection, increasing mortality and overwhelmed healthcare systems highlighted the need for strategies to develop novel therapeutics, vaccines, and other anti-viral drugs to manage this global pandemic. Establishing evidence-based management guidelines was essential but challenging given the unknown and unpredictable nature of virus. Due to the fact that this virus had not been encountered previously, and therefore humans had no effective immunity (cell-specific responses to infection /immunological memory) there was a lot of uncertainty and fear at the outset of the pandemic. In particular there was concern for vulnerable and at risk populations such as the elderly and immunocompromised, the role of public health measures including shielding and social isolation was yet to be clarified. There was an urgent need to identify factors which put patients at risk of severe COVID-19 infection and implement appropriate risk mitigation strategies.

#### **5.1.4. COVID-19 and Immune Mediated Rheumatic Diseases.**

The emergence of the COVID-19 pandemic raised concerns amongst clinicians of increased mortality in infected immunocompromised patients including those with immune mediated rheumatic diseases (IMRD). Many of these patients are considered at-risk for opportunistic infections due to an immunocompromised state resulting both from their

underlying immune disease and also due to use of targeted immune-modulating therapies such as conventional synthetic disease modifying anti-rheumatic drugs (csDMARDs), Anti-TNF inhibitors (TNFi), and targeted synthetic DMARDs (tsDMARDs) (90). The risk for serious infection is not equal across all patients however and other factors ( e.g., age, glucocorticoids, co-morbidity) are important in assessing infectious risk for any given patient.

#### **5.1.5. Metabolic Syndrome and COVID-19**

With the worldwide spread of SARS- CoV-2, obesity and impaired metabolic health emerged as important determinants of COVID-19. According to two US studies, patients with SARS- CoV-2 infection were at increased risk of hospital admission if they had obesity (91)(92).

Petrilli et al examined factors associated with hospital admission and critical illness among 5279 people with coronavirus disease 2019 in New York City: They found the strongest risks for critical illness aside from age were heart failure (1.9, 1.4 to 2.5), BMI >40 (1.5, 1.0 to 2.2), and male sex (1.5, 1.3 to 1.8). Increased body mass index (BMI) (e.g., for BMI >40: 2.5, 1.8 to 3.4) was also a risk factor for hospital admission with COVID-19 infection. Price-Haywood et al examined odds ratios for hospitalization among 3481 Covid-19–Positive Patients and found patients with obesity were at increased odds for hospitalisation (1.43, 1.20 to 1.71). (92). Bode et al demonstrated that amongst 1122 patients in 88 U.S. hospitals COVID-19 patients with diabetes and/or uncontrolled hyperglycaemia had a longer length of stay in hospital and markedly higher mortality than patients without diabetes or uncontrolled hyperglycaemia (93). There is a large volume of data inferring poorer COVID-19 outcomes in patients with features of metabolic syndrome. Due to the well described

complex interconnections between gout and metabolic syndrome there was an urgent need to investigate patients with COVID-19 infection and gout. Although the pandemic provided an unexpected turn for my research objectives it provided an opportunity to complete some important work on outcomes of COVID-19 infection in patients with IMRDs and gout.

#### **5.1.6. bDMARDs and COVID-19**

bDMARDs used in the treatment of IMRDs are known to increase the risk of serious and non-serious infections, the most common sites of infection being the respiratory tract, skin, soft tissue, and the urinary tract (90). The proinflammatory role of cytokines including TNF, IL-1, and IL-6, which mitigate inflammatory disease pathogenesis, are also important for the host immune response to a number of microbial pathogens. Large meta-analyses have demonstrated increased risk of serious infections in patients with IMRDs treated with bDMARDs such as TNF Inhibitor (TNFi) therapy (193). There was strong clinical reasoning for concern with regard to severe COVID-19 infection in patients with IMRDs on bDMARD therapy.

However, there is also evidence TNFi may be associated with less risk of sepsis among patients hospitalised with infection, due to mitigation of the cytokine storm, and some therapies used to treat rheumatic diseases are under investigation as potential therapies for COVID-19 (194)(195). Richter et al investigated the impact of bDMARDs on the outcomes of serious infections (SIs) in patients with rheumatoid arthritis and found compared with csDMARDs, the risk was significantly lower when patients were exposed to bDMARDs at the time of SI (OR: 0.56, 0.38 to 0.81) (194). The severe forms of COVID-19 resemble other hyperinflammatory conditions such as macrophage activation syndrome



(MAS) with elevated inflammatory markers such as CRP, IL-6, TNF and IL-8 (196). Therefore, several immunosuppressive therapies were the subject of investigation as potential therapeutic targets for the treatment of severe COVID-19 infection.

#### **5.1.8 Establishment of a COVID-19 Physician registry.**

An urgent need for clinical outcome data on COVID-19 infection in patients with IMRDs led to the rapid establishment of The Trinity Rheumatology and COVID-19 Registry- (TRACR). TRACR was established to record COVID-19 infection outcomes on all rheumatology patients attending two large academic teaching centres, Tallaght University Hospital and St James's Hospital during the first wave of the pandemic, March to June 2020. Alongside clinical duties during a period of redeployment from March to July 2020, this work became my primary research focus. At this time, due to clinical risk of infection with COVID-19, patient recruitment for other non-essential research objectives was not possible. Although not initially an aim or objective for this thesis, reporting on outcomes of COVID-19 in patients with IMRDs became a valuable and essential research agenda in the changing medical landscape of 2020. In this chapter, we report on factors associated with hospitalisation and COVID-19 infection outcomes in patients with IMRDs.

#### **5.2 Specific Aims of this Chapter.**

- Our primary aim was to collect demographic, disease status, immunotherapy and hospitalisation information on all rheumatology patients diagnosed with COVID-19 in the Tallaght University Hospital (TUH)/St James's Hospital (SJH) catchment area.
- Our secondary aim was to complete a sub-analysis on risk of hospitalisation in patients specifically with a diagnosis of gout.

- Our third aim was to add this data to the GRA COVID-19 registry and promote reporting amongst our rheumatology colleagues. Through presentation of registry data at national and international meetings we aimed to increase the input of physician reported COVID-19 outcome data to the GRA registry nationally.

### **5.3. Methods.**

#### **5.3.1. Patient Recruitment**

Contact tracing of all COVID-19 cases in patients attending the rheumatology services at TUH and SJH during the first wave of the pandemic from March 1<sup>st</sup> to June 3<sup>RD</sup> 2020 was completed. All patients attending the rheumatology departments at each hospital were contacted by an automated text messaging service and requested to contact the department by phone or email if they had been given a diagnosis of COVID-19. Patients who responded to the text message were then contacted by a physician via phone and data regarding individuals with rheumatic disease who developed COVID-19 infection were entered into a secure data portal hosted by the hospital.

### 5.3.2. Data Collection

Data was collected on baseline rheumatic disease diagnosis and classified as either an Immune Mediated Rheumatic Disease (IMRD); Rheumatoid Arthritis, Psoriatic Arthritis, Systemic Lupus Erythematosus, Sjogren's, Juvenile Idiopathic Arthritis, Un-differentiated connective tissue disease, Polymyalgia Rheumatica, Hypocomplementemic Urticarial Vasculitis Syndrome and Ankylosing Spondylitis or Non-Immune Mediated Rheumatic Disease (nIMRD) including; Ehlers Danlos Syndrome, OA – Osteoarthritis, Costochondritis, Fracture, Fibromyalgia or Gout. Demographic and clinical variables were recorded as follows: age, sex, smoking status, rheumatic disease diagnosis, disease activity, and co-morbidities including Cardiovascular Disease (CVD), Pulmonary Disease, Type 2 Diabetes Mellitus (T2DM), Gout and Malignancy. Data on prescribed medications at the time of COVID-19 infection were recorded and were categorised as follows; conventional synthetic disease-modifying antirheumatic drugs (csDMARDs); hydroxychloroquine, azathioprine, cyclophosphamide, cyclosporine, leflunomide, methotrexate, mycophenolate mofetil/mycophenolic acid, sulfasalazine, tacrolimus. Biologic DMARDs (bDMARDs); abatacept, CD-20 inhibitors, IL-1 inhibitors, IL-6 inhibitors, IL-12/IL-23 inhibitors, IL-17 inhibitors, tumour necrosis factor inhibitors (anti-TNF) and targeted synthetic DMARDs (tsDMARDs) namely Janus Kinase (JAK) inhibitors. Data collected on COVID-19 infection included the method of diagnosis of COVID-19, place of diagnosis of COVID-19 (Community testing centre, Hospital, Nursing Home or Clinical Diagnosis by G.P.) COVID-19 symptoms and the outcomes of COVID-19 disease (recovered, hospitalised or deceased). Cross-referencing with test-centre positive polymerase chain reaction (PCR) results and mortality data was performed to ensure complete collation of cases. Figures for disease incidence

per 100,000 and hospitalisation were calculated based on a catchment area of 720,000 people and compared to the incidence in the population in the greater Dublin area the at time as taken from the Central Statistics Office.

### **5.3.3 Entry to the GRA Registry**

Data was also shared with the GRA registry, via the European data entry portal ([eular.org/eular\\_covid19\\_database.cfm](http://eular.org/eular_covid19_database.cfm); hosted by The University of Manchester, UK). This study was approved by the Irish National Research Ethics Committee for COVID-19 (20-NREC-COV-010). The committee waived the need for written informed consent as the data was fully anonymised.

### **5.3.4 Statistical Analysis.**

Results analysed with SPSS v.26 software. In order to avoid overestimates of overall morbidity a sub-analysis was performed on those with community acquired disease. Continuous variables were reported as the mean [standard deviation (SD)]. Categorical variables were reported as number and percentage. In univariable analyses, differences according to hospitalization status and mortality were compared using chi-squared tests or Fisher's exact tests as appropriate for categorical variables and Mann-Whitney U-tests for continuous variables.

## **5.4 Results**

### **5.4.1 Baseline Characteristics.**

A total of 7,500 patients comprising 4,524 (60.32%) with IMRDs and 2,976 (39.68%) with nIMRDs were contacted via automated text message. 210 patients responded to the text message by either email or phone. 78 cases met the criteria for PCR or physician diagnosed COVID-19. The mean age (SD) at time of diagnosis was 56.40 (17.0), with 57/78 (73.1%) female. The most common IMRD diagnosis was rheumatoid arthritis at 24.4% (N=19) and the most common nIMRD diagnosis was a fracture at 11.5% (N=9), TUH runs a large fracture prevention service. The breakdown of primary disease diagnosis is represented in

Table 5.1. Of the identified 78 cases of PCR or physician diagnosed COVID-19, 68 acquired the infection in the community with 10 cases incidentally acquired while already hospitalised for another reason.

44 (56%) of patients recruited had an underlying co-morbidity, the most common of which was a history of cardiovascular disease, N=17 (21.8%). Table 5.2 outlines the breakdown of concomitant comorbidities among patients recruited to TRACR. For two cases gout was the 'primary' disease for which the patient was attending rheumatology, gout also occurred in 4 others as a 'co-morbidity' where the patient also had a different primary rheumatoid disease.

	<b>Primary Diagnosis</b>	<b>N</b>	<b>Percentage %</b>
<b>IMRD N= 49</b>	<b>RA</b>	<b>19</b>	<b>24.4</b>
	<b>PsA</b>	<b>7</b>	<b>9</b>
	<b>SLE</b>	<b>5</b>	<b>6.4</b>
	<b>UCTD</b>	<b>5</b>	<b>6.4</b>
	<b>AS</b>	<b>5</b>	<b>6.4</b>
	<b>Sjogren's</b>	<b>2</b>	<b>2.6</b>
	<b>JIA</b>	<b>2</b>	<b>2.6</b>
	<b>PMR</b>	<b>2</b>	<b>2.6</b>
	<b>Vasculitis</b>	<b>1</b>	<b>1.3</b>
	<b>HUVS</b>	<b>1</b>	<b>1.3</b>
	<b>nIMRD N=29</b>	<b>Fracture</b>	<b>9</b>
<b>OA</b>		<b>8</b>	<b>10.2</b>
<b>Fibromyalgia</b>		<b>8</b>	<b>10.2</b>
<b>Gout</b>		<b>2</b>	<b>2.6</b>
<b>Costochondritis</b>		<b>1</b>	<b>1.3</b>
<b>EDS</b>		<b>1</b>	<b>1.3</b>

**Table 5.1. Primary Rheumatic Disease Diagnosis in all COVID-19 Positive Patients**

Legend: RA – Rheumatoid Arthritis. PsA – Psoriatic Arthritis. SLE – Systemic Lupus Erythematosus. JIA – Juvenile Idiopathic Arthritis. uCTD – Undifferentiated Connective Tissue Disease. PMR – Polymyalgia Rheumatica. AS- Ankylosing Spondylitis, EDS -Ehlers Danlos Syndrome. OA – Osteoarthritis. HUVS – Hypocomplementemic Urticarial Vasculitis Syndrome.

<b>Co-morbidity</b>	<b>N</b>	<b>%</b>
<b>CVD</b>	17	21.8
<b>Pulmonary Disease</b>	10	12.8
<b>T2DM</b>	6	7.7
<b>Gout</b>	6	7.7
<b>Malignancy</b>	5	6.4

**Table 5.2 Co-Morbidities in all COVID-19 Positive Patients (N=78)**

**5.4.2 Baseline Data on Community Acquired COVID-19 Infected Patients.**

Of the 68 patients with community acquired COVID-19, 40 (58.9%) had an underlying immune mediated rheumatic disease (IMRD) and 28 (41.2%) had a non-immune mediated rheumatic disease (nIMRD). The mean age (SD) in the IMRD group was 51.8 (16.5) versus 56.46 (13.6) in nIMRD group. 31 (77.5%) of IMRDs were female versus 20 (71.4%) of nIMRDS. (Table 5.3)

There was significantly more CVD in the nIMRD group (N=10, 35.7%) compared to the IMRD group (N=4, 10%, P<0.05). There was no significant difference in body mass index (BMI), Type 2 Diabetes (T2DM) or glucocorticosteroid use between the two groups (Table 5.3)

There was no significant difference in cumulative COVID-19 disease incidence per 100,000 between the two groups (884 IMRD v 940 nIMRD) (Table 5.3)

In the IMRD group 18 (45%) of patients were on a conventional synthetic DMARD (9 on methotrexate and 9 on Hydroxychloroquine) at the time of diagnosis with COVID-19. 16 (40%) were on a bDMARD and 2 (5%) on glucocorticosteroids. (Table 5.4)

<b>Parameter</b>	<b>IMRD</b>	<b>nIMRD</b>
<b>COVID-19 Positive (n, %)</b>	40 (58.9)	28 (41.2)
<b>Age in Years (Mean, SD)</b>	51.8 (16.5)	56.46 (13.6)
<b>Female (n, %)</b>	31 (77.5)	20 (71.4)
<b>CVD (n,%)</b>	4 (10)	10* (35.7)
<b>BMI &gt;30 (n, %)</b>	11 (27.5)	8 (28.6)
<b>T2DM (n, %)</b>	3 (7.5)	1 (3.6)
<b>GC (n, %)</b>	2 (5)	1 (3.6)



<b>Incidence (per 100,000)</b>	884	940
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**Table 5.3. Baseline Data between IMRDs and nIMRDs in Community Acquired COVID-19**

**Patients**

CVD – Cardiovascular Disease. BMI – Body Mass Index. T2DM – Type 2 Diabetes Mellitus. GC – Glucocorticoids. RA – Rheumatoid Arthritis. PsA – Psoriatic Arthritis. SLE – Systemic Lupus Erythematosus. JIA – Juvenile Idiopathic Arthritis. uCTD – Undifferentiated Connective Tissue Disease. PMR – Polymyalgia Rheumatica. AS- Ankylosing Spondylitis, EDS -Ehlers Danlos Syndrome. OA – Osteoarthritis. HUVS – Hypocomplementemic Urticarial Vasculitis Syndrome. \* P = <0.05

<b>Drug</b>	<b>N</b>	<b>%</b>
<b>csDMARD</b>	18	45
<b>bDMARD</b>	16	40
<b>GC</b>	2	5
<b>tsDMARD</b>	0	0

## **Table 5.4. Breakdown of DMARDS prescribed in Community Acquired Covid-19 Infected**

### **Patients (IMRD, N = 40)**

CS DMARD – Conventional Synthetic Disease Modifying Antirheumatic Drug. HCQ - Hydroxychloroquine. bDMARD - biologic DMARD. tsDMARD -Targeted Synthetic DMARD. GC – Glucocorticosteroids.

### **5.4.3 Comparison of Hospitalised and Non-Hospitalised Community Acquired COVID-19 infected patients.**

No significant differences were seen in cumulative incidence/100,000 of COVID-19 between IMRD (1083), nIMRD (940), when compared to the COVID-19 incidence rate for metropolitan Dublin (887). Hospitalisation rates following community acquired COVID-19 infection in IMRD (15%), equivalent to national figures (13%) were observed. There was no significant difference in hospitalisation rates between nIMRDs and IMRDs (N=9, 32% v N=6, 15%, P=0.093) There were 8 deaths in total with no significant difference between groups (N =5, 12.5% IMRD v N=3, 10.7% nIMRD).

In all community acquired cases (Table 5.5) hospitalisation was associated with increased age (68.1 (12.2) v 49.6 (13.8), P<0.01), CVD (N=8, 53.3% V N=6, 11.3% P<0.01) those diagnosed with type 2 diabetes (N=3, 20% V N=1, 1.9% p<0.05) and those on glucocorticosteroids (N=3, 20% v N=0 P=<0.01). In subgroup analysis of patients with IMRDs with community acquired infection (n=40) hospitalisation was associated with increased age (68.9 (15.1) v 48.7 (15.0), P<0.01), those receiving glucocorticosteroids (N=2, 33.3% V N=0, p<0.05), those with CVD (N=5, 33.3% V N=2, 5.9% P<0.01) and those

diagnosed with type 2 diabetes (N=2, 33.3% V N=1, 2.9% p<0.05). Hospitalisation was statistically less likely in patients receiving long-term bDMARD therapies (p<0.05,). (Table 5.5) This significance was lost when hospital acquired cases were included in the analysis. In total, six patients had a diagnosis of gout. In a subgroup analysis of those with community acquired Covid-19 infection (N=68) 5 patients had a gout diagnosis, 4 of these were subsequently hospitalised, indicating a significant association between a diagnosis of gout and hospitalisation for patients diagnosed with community acquired COVID-19 outside the hospital setting ( P <0.05, chi square).

Parameter	All Patients (n=68)		IMRD Only (n=40)	
	Non-Hospitalisation	Hospitalisation	Non-Hospitalisation	Hospitalisation
<b>COVID-19 Positive (n, %)</b>	53 (77.9)	15 (22.1)	34 (85)	6(15)
<b>Age in Years (Mean, SD)</b>	49.6 (13.8)	68.1*(12.2)	48.7 (15)	68.9* (15.1)
<b>Female (n, %)</b>	41 (77.4)	10 (66.7)	26 (76.5)	5 (83.3)
<b>CVS Disease (n, %)</b>	6 (11.3%)	8*(53.3)	2 (5.9%)	2 (33.3)*
<b>BMI &gt;30 (n, %)</b>	13 (24.5)	6 (40)	8 (23.5)	3 (50)
<b>T2DM (n, %)</b>	1(1.9)	3 <sup>†</sup> (20)	1 (2.9)	2 <sup>†</sup> (33.3)

csDMARD (n, %)	N/A		15 (44.1)	5 (83.3)
HCQ (n,%)	N/A		7 (20.6)	2 (33.3)
bDMARD (n,%)	N/A		16 (47.1)	0 <sup>†</sup> (0)
tsDMARD (n,%)	N/A		0 (0)	0 (0)
GC (n, %)	0(0)	3* (20)	0 (0)	2 <sup>†</sup> (33.3)

**Table 5.5. Stratifying Hospitalised and Non-Hospitalised Data in Community Acquired COVID-19 Infection; all data as n(%).**

CVS – Cardiovascular Disease. BMI – Body Mass Index. T2DM – Type 2 Diabetes Mellitus. csDMARD- conventional synthetic disease modifying antirheumatic drug. HCQ – hydroxychloroquine. bDMARD- biologic disease modifying antirheumatic drug. tsDMARD- targeted synthetic disease modifying antirheumatic drug. GC – Glucocorticoids.

\* P=<0.01 † P=<0.05

## 5.5 Discussion.

In this study we aimed to identify and record outcomes of all cases of COVID-19 infection amongst patients attending two large academic teaching hospitals associated with Trinity College Dublin. In patients with rheumatic diseases who contracted COVID-19 in the community we found similar disease incidence rates compared the greater Dublin area at the time. Similar disease incidences rates were found between those with an immune and non-immune mediated rheumatic disease. We did not find hospitalisation rates amongst

patients with an IMRD to be significantly higher than the general population at the time. Hospitalisation in COVID-19 infection was found to be associated with increased age, CVD, T2DM and glucocorticosteroid use, hospitalisation was significantly less likely for patients with an IMRD on a bDMARD.

In tandem with the establishment of TRACR, the international need for clinical data on COVID-19 infection outcomes in patients with rheumatic diseases led to the development of the Global Rheumatology Alliance (GRA) COVID-19 registry. The COVID-19 GRA registries are the collaborative work from an international group of rheumatologists, researchers, and patient partners. These registries are a global effort to collect information pertinent to COVID-19 infection in patients with rheumatologic disease. Although the GRA data provides useful information on disease morbidity patterns its design, which fails to account for the denominator population at risk, has been unable to provide disease incidence data for comparison. A New York case series published in 2020, which reported an incidence rate no different from the general population, was reassuring for patients with IMRDs, but required further validation (197). The suggestion of a potential reporting bias towards hospitalised cases in GRA registry data was raised following an initial publication reporting that nearly half of all patients with COVID-19 and IMRDs were hospitalised (198).

To test our hypothesis that the initial GRA physician-reports of 49% (2) hospitalisation was due to strong reporting bias towards patients with more severe infection, TRACR aimed to record outcomes on all rheumatology patients attending two large academic teaching centres, Tallaght University Hospital and St James's Hospital, during the first wave of the pandemic March to June 2020. By recording outcomes of all patients with IMRDs who contracted COVID-19 infection in these two centres we aimed to negate the potential

recording bias towards hospitalisation noted in the GRA registry and gather information on disease incidence in this cohort compared to the general population. Unintentional bias in reporting hospitalised COVID-19 cases to IMRD registries and the inclusion of hospital acquired cases during data analysis may lead to spurious overestimates of overall morbidity. Hospitalisation rates for community acquired COVID-19 infection in patients with IMRDs equivalent to national figures were observed from TRACR data. As far as we could control for there was no significant difference in disease incidence per 100,000 between our rheumatology population and the disease incidence in the greater Dublin area at the time of the study.

Our specific study design also provided a comparison group of those with an underlying immune mediated (for example, RA) versus non-immune mediated rheumatic disease (fracture, fibromyalgia.) There was no significant difference in the incidence of COVID-19 infection per 100,000 between the IMRD and nIMRD groups, the larger GRA registry outputs focused on patients with IMRDs and therefore did not have this comparison group. This finding suggests that those with an underlying IMRD were not at a greater risk of becoming infected with the COVID-19 virus. These results should be interpreted with caution given the advice around isolation and shielding at the time, patients with IMRDs on immunosuppression were advised to shield from the general public which may have reduced COVID-19 incidence in this cohort.

The most common co-morbidity in our cohort was CVD which is representative of the strong association between rheumatic disease and cardiovascular disease (199). TRACR found CVD to be associated with increased risk of hospitalisation in COVID-19 infection. In a study examining 105 patients entered in the COVID-19 GRA registry from Ireland and their disease-related characteristics, CVD was not shown to be an independent predictor

of hospitalisation but was identified as a specific co-morbidity associated with mortality (23.1%, P= 0.014) (200). This finding is supported by Strangfeld et al, in a study of 3729 patients with COVID-19, cardiovascular disease was a key factor associated with COVID-19 related death in patients with rheumatic diseases (OR: 1.89, 1.31 to 2.73) (201). Other factors identified from TRACR as associated with hospitalisation in COVID-19 include age, T2DM and glucocorticosteroid use. These findings are supported by outcomes from larger registries which identified similar factors (198)(200)(91).

Gout was identified as a risk factor for hospitalisation from TRACR data. This finding was supported by national GRA COVID-19 data which also found a gout diagnosis to be associated with increased odds of hospitalisation in patients with COVID-19 infection and Rheumatic Disease (200). In this study of 105 patients, 21 had a diagnosis of gout, 100% of which were admitted to hospital with complications related to COVID-19 infection. Conway et al commented that this result may be affected by selection bias as gout is usually managed in primary care in Ireland and given that the GRA COVID-19 registry is a rheumatologist-entered registry these cases could be less likely to be recorded. In addition to gout, hyperuricaemia has been implicated both in the development and potentiation of a wide range of diseases including type 2 diabetes, cardiovascular disease (CVD), systemic hypertension and the metabolic syndrome. These diseases are independently associated with increased morbidity and mortality in COVID-19 infection, so therefore it is perhaps expected that a diagnosis of gout predicts a poorer outcome with COVID-19 infection.

Interestingly, in subgroup analysis of community acquired infection, hospitalisation was statistically less likely in patients receiving long-term anticytokine biological therapies. This significance was lost when hospital acquired cases were included, supporting our hypothesis that inclusion of hospitalised cases may mask critically important information

on the role of anticytokine therapies. This finding was supported by data from the GRA COVID-19 registry which found TNFi to have a decreased odds of hospitalisation in patients with rheumatic disease and COVID-19 (198). There are a number of trials on going at time of writing investigating potential therapeutic roles for TNFi in treating COVID-19 infection, infliximab (SOLIDARITY, CATALYST, ACTIV-1 IM and RECOVERY trials) and adalimumab (COMBAAT Trial,) the outcomes of which are eagerly awaited. Aside from a role for IL-6 inhibition in severe COVID-19 infection there has been limited robust evidence for a role of other DMARDs in the treatment of COVID-19 to date (202).

The TRACR dataset was not large enough for in depth analysis examining the effect of specific classes of bDMARDs. Recent data from the GRA registry found among patients with RA, at the onset of COVID-19, rituximab and JAKi users were at increased odds for worse COVID-19 outcomes compared with TNFi. In contrast, they did not find an association between abatacept or IL-6 inhibitor use with worse COVID-19 outcomes when compared with TNFi users (203).

The patients recruited for this study were entered into the GRA registry via the European data entry portal. Presentation of TRACR findings at national meetings was used as a means to raise awareness and therefore improve reporting of outcomes by our rheumatology colleagues nationally. National data extracted and analyzed from the GRA registry has given rheumatologists in Ireland accurate and important information on COVID-19 infection outcomes in patients with IMRDs (200). These observations can inform decision making for rheumatologists, specifically on who should be prioritized for risk mitigation strategies.

Our study has a number of inherent strengths. The design aimed to remove the selection bias towards severe cases seen in other similar registries that relied on physician reporting



alone. Similar disease incidence rates and characteristics between immune mediated and non-immune mediated groups is reassuring for patients with IMRDS, larger registries including the GRA registry do not have this data for comparison. Analysis of community acquired COVID-19 infection with exclusion of hospital acquired cases demonstrated the potential protective role of bDAMRDs in COVID-19 disease outcome. Inferences can be drawn on disease incidence in this cohort as the rate of COVID-19 infection in the denominator population for the data is known, this is not the case in many of the larger national registries. This is the only registry which attempts to record outcomes of all patients with rheumatic diseases and COVID-19 infection in a defined catchment area. In order to capture all cases of COVID-19 in our catchment area results were cross referenced with test-centre positive polymerase chain reaction (PCR) results and mortality data to ensure complete collation, however it is difficult to confirm we recorded all cases.

Our study has limitations; the small number of cases recorded reduces the statistical power of the findings. This data was collected during the first wave of the pandemic therefore it relates to a time before more effective evidence-based treatment strategies, vaccines and the introduction of specific anti-viral therapies such as nirmatrelvir-ritonavir. Therefore, the information on morbidity/hospitalisation may no longer be clinically relevant where the vast majority of the population now have a degree of immunity against infection. However, this information is still highly relevant in that it provides us with valuable information on the initial impact and outcome of infection with COVID-19. Patients with rheumatic diseases might have behaved differently with regard to risk behaviour during the COVID-19 pandemic. In Ireland, a strict national lockdown was in place for a large part of the study period. Individuals judged to be at high risk at the start of the pandemic, including many with rheumatic diseases, were specifically advised to stay at home as much

as possible and to avoid social interactions.

<https://www2.hse.ie/conditions/covid19/people-at-higher-risk/overview/> this policy may have reduced disease incidence in this cohort.

In conclusion, TRACR's findings support larger registries in confirming that increasing age, co-morbidity burden and glucocorticoid use are associated with hospitalisation in COVID-19 infected patients with rheumatic diseases. Although our dataset is small, these results suggest that patients on bDMARDs have a reduced association with hospitalisation in COVID-19 infection, the results of ongoing randomized controlled trials of bDMARDs in COVID-19 will provide clarity. Further analysis using existing updated datasets will continue to identify disease characteristics and factors associated with hospitalisation of COVID-19 infected patients with IMRDs.

## **6. Overall Discussion.**

Gout is now the most common form of inflammatory arthritis (2). Although there are low cost, effective urate-lowering therapies available, the management of patients with gout is suboptimal due to delayed diagnosis, slow initiation of treatment and poor adherence to therapy (1). In the last decade, the focus of rheumatology research has been in immune

mediated rheumatic diseases such as rheumatoid arthritis and significant therapeutic advances have occurred in this area, whereas improvement in gout outcomes have plateaued. In contrast to RA where disease prevalence remains static or is falling, there has been an increase in the incidence and prevalence of gout. This increased burden of gout has occurred in concert with our ageing population, therefore the burden of gout as a proportion of rheumatology 'ill-health' is on the rise. The understanding of gout pathogenesis is incomplete, and many uncertainties remain., Therefore there is a renewed need from a clinical and research perspective to focus on this common debilitating disease to improve health outcomes.

The current gout classification criteria, while specific for making a diagnosis, may not be sensitive enough to pick up early clinical presentations prior to an initial 'classical' gout attack. The gout classification criteria require the occurrence of at least one episode of swelling, pain, or tenderness in a peripheral joint or bursa prior to diagnosis (35). Alternative, non-classical, presentations of foot pain in the setting of hyperuricaemia are often described and provide a diagnostic challenge for physicians. This thesis examined presentations of early clinical gout which do not meet the current classification criteria. Clarification of the diagnostic criteria is important as current guidelines recommend against initiation of ULT prior to a diagnosis of gout (43). This may prevent timely initiation of ULT in early clinical gout and contribute to increased disability for patients in the long term (43).

In chapter two, we demonstrated that ultrasound features of monosodium urate crystal deposition (DC sign, tophus and erosions) are frequently found in the MTP joint of patients who present with persistent non-specific foot pain and hyperuricaemia. MSU crystals

precipitate on the articular surface of hyaline cartilage forming a hyperechoic, bright band, visible on US, that parallels the hyperechoic bony cortex known as “double contour.” Double contour sign is considered a finding strongly suggestive of gout and is already included in the ACR 2015 gout classification criteria (35) (204). Utilising MSK ultrasound of the MTP joint, we found that hyperuricaemic patients with atypical foot pain who have either DC sign or tophus evident on examination will have a greater improvement in pain score following treatment with ULT than those who do not. These findings highlight a group of patients with early gout before they have a ‘classical gout attack.’ By identifying this group of patients at an early stage, tight urate control will be easier to achieve which will result in less joint damage, improved outcomes and a reduction of the socio-economic burden caused by this debilitating disease. MSK ultrasound is a widely available, non-invasive and cost-effective modality which can be utilised by the rheumatologist to aid with diagnosis of rheumatic conditions such as gout. We would make a recommendation that persistent, non-specific foot pain, in the setting of hyperuricaemia with features of DC and/or tophus on US examination of the MTP joint be included in an updated diagnostic criteria for gout. Our results indicate that this inclusion would not reduce the sensitivity or specificity of the gout diagnostic criteria. DC sign is considered strongly suggestive of gout. Subject to further confirmatory studies, the demonstration of DC sign in the setting of hyperuricaemia and foot pain could be included as a new ‘gold standard test’ for early diagnosis, providing a basis for ULT to be introduced prior to disease progression and disability.

In addition to gout, hyperuricaemia has been implicated in the development and potentiation of a wide range of diseases including type 2 diabetes, cardiovascular disease

(CVD) and cardiopulmonary diseases. Although the associations between hyperuricaemia and cardiovascular and cardiopulmonary diseases are well described, it has not been definitively established whether uric acid is merely a marker for risk or a causative agent, Chapter three of this thesis further examined this. The association between hyperuricaemia and cardiovascular and cardiopulmonary diseases was examined through experiments involving the systemic arterial vasculature, via flow and nitroglycerin mediated dilatation studies of the brachial artery and secondly of the pulmonary vasculature by a novel echocardiogram index: pulmonary pulse wave transit time (pPTT). Flow and nitroglycerin mediated dilatation studies are validated surrogate markers of adverse cardiovascular outcomes. Proof of concept experiments comparing normal controls with known pulmonary hypertension cases, further validated the use of pPTT as a marker for non-invasive PH management that can be used in a variety of healthcare settings. Research has found sUA to be independently associated with CVD risk and events (50) (51), but there are currently a lack of studies investigating whether hyperuricaemia contributes to the development of CVDs, or indeed whether the lowering of serum urate levels pharmacologically is associated with improvement of vascular tone (52). Our study found that hyperuricaemic patients have evidence of altered vascular haemodynamics, as borne out by reduced brachial artery dilatation compared to normal controls and shortened pPTT compared to normal controls. The potential causal role for hyperuricemia in altered arterial and pulmonary haemodynamics is supported by improvement in these indices following a period of treatment with ULT. These results infer that hyperuricaemia potentially contributes to early vascular dysfunction which over time, through development of arterial stiffness and atherosclerosis, may contribute to the development of CVDs. Gout is known to be independently associated with CVD, in depth analysis of the

mechanisms underlying this relationship is important to guide treatment, manage risk and improve outcomes in this common disease (48). We suggest that earlier intervention with ULT in the setting of hyperuricaemia may have a role in improving markers of vascular dysfunction which in turn would reduce CVD risk. However, more research is needed in this area to further define our findings.

The activation of the NLRP3 inflammasome by monosodium urate crystals has been proposed as a central factor to the initiation and potentiation of an acute flare of gout (28). In chapter four of this thesis, we further investigated if NLRP3 and related pro-inflammatory cytokines (IL-1 $\beta$  and IL-6) are active in the inflamed joint of those in an acute gout attack, by examining levels of NLRP3, IL-1 $\beta$ , IL-6 mRNA and proteins in gout compared to OA synovial tissue. NLRP3, IL-1 $\beta$  and IL-6 mRNA levels in whole tissue biopsies, measured by qPCR, were found to be more highly expressed in an acute flare of gout compared to OA. This was mirrored by an increase in protein levels of IL-1 $\beta$  and IL-6 in gout *ex vivo* synovial tissue compared to OA synovial tissue. This preliminary data taken together suggests that the NLRP3 inflammasome is active in the gout joint, and its components are more highly expressed in gout synovial tissue than OA synovial tissue. Our findings contribute to the growing body of evidence that NLRP3 inflammasome activation has a key role in the pathogenesis of gouty inflammation. Improved mechanistic understanding of NLRP3 activation of the inflammasome by MSU crystals during a gout attack, indicates a potential future therapeutic target to treat this debilitating disease.

While current treatment strategies for gout treatment, such as xanthine oxidase inhibitors, involve long term suppression of serum urate, there are no targeted or specific therapies for the management or prevention of an acute attack caused by the presence of existing

urate crystals in vivo. Until now, strategies for targeting the NLRP3 inflammasome pathway have been focused on IL-1 $\beta$  blockade. IL-1 $\beta$  is the major downstream pro-inflammatory cytokine produced by NLRP3, non-specific blockade may inhibit the host immune response and increase the risk for serious infections. In 2001, a number of diarylsulfonylurea-containing compounds were identified as novel IL-1 $\beta$  processing inhibitors, however their mechanism of action and relation to NLRP3 was at the time unknown (175). Subsequently, our colleagues in Trinity College Dublin identified MCC950 as a selective inhibitor of the NLRP3 inflammasome. A benefit of the selective inhibitory effect of MCC950 is it may have a reduced risk of serious infections compared to current therapeutics, such as anakinra, which non-specifically block IL-1 $\beta$  (152).

We sought to further identify if MCC950 could inhibit the NLRP3 inflammasome and related proinflammatory cytokines, in *ex-vivo* synovial tissue explants taken from the inflamed knee joints of patients during an acute gout attack. Initial experiments demonstrated that MCC950 could significantly inhibit NLRP3-induced IL-6 and a trend for inhibition of IL-1 $\beta$  secretion in PBMCs isolated from patients during an acute flare of gout. The effects of novel inhibitor MCC950 on the NLRP3 inflammasome were further examined in *ex-vivo* synovial tissue biopsies to precisely mimic the in-vivo environment it will target. The effect of various doses of MCC950 on NLRP3 mRNA in gout compared to OA synovial tissue was examined by qPCR. In *ex-vivo* synovial tissue, MCC950 demonstrated a trend towards inhibition of the expression of NLRP3 in a dose dependant manner. These preliminary findings suggests that MCC950 has potential to be a specific therapeutic target for use in the management of gout. This is the first study investigating the effect of MCC950 on *ex-vivo* synovial tissue explants taken from the inflamed knee joints of patients during an acute gout attack.

This MD research project began just before the emergence of the COVID-19 health emergency. The resulting pandemic created an existential threat to the health of the population, caused by a pathogenic threat about which almost nothing was known. Hospital access was placed on a war-footing with access restricted only to those most in need of face-to-face urgent care. Both the recruitment of and subsequent follow-ups with patients as part of this research work was unavoidably paused for a prolonged period. Faced with an urgent and pressing need to respond to our dramatically altered health environment and to use our research resources in the best way possible, we rapidly designed and executed a research project to quantify the extent of COVID-19 related morbidity among patients with immune mediated rheumatic diseases (“IMRDs”) occurring within the catchments of hospitals affiliated with Trinity College Dublin during the first wave of the COVID-19 pandemic. The COVID-19 pandemic raised concerns of increased mortality in patients with immune mediated rheumatic diseases. Many of these patients are considered at-risk for opportunistic infections due to an immunocompromised state resulting both from their underlying immune disease and also due to the use of targeted immune-modulating therapies. We established the TRACR registry to record COVID-19 outcomes from all patients with rheumatic diseases attending TUH and SJH. Our data collection, which was designed to collect across the whole outpatient rheumatic disease population in our catchment area, reported a much lower rate of hospitalization than the 49% which was reported contemporaneously by the GRA-COVID registry (198). This reflects the highly skewed reporting bias towards the registering of already hospitalized patients to the GRA-COVID online registry by treating rheumatologists. Our own research design sought to negate such reporting bias and therefore obtain a truer reflection of COVID-19 related morbidity in this vulnerable group of patients. We identified factors associated with



hospitalisation in patients with rheumatic diseases and COVID-19 infection including age, CVD, T2DM, gout and glucocorticosteroid use. Outputs from TRACR revealed there was not an increased risk of hospitalisation for community acquired infection in patients prescribed bDMARDs. These findings were subsequently supported by further data from larger registries, including the GRA- COVID-19 registry, which similarly found reassuring data for patients on bDMARDs (not including rituximab) and COVID-19 infection (198) (200). Insights gained from research conducted during the COVID-19 outbreak indicate that different study designs are required when managing and collecting data during an evolving pandemic. A totally different approach is required to measure disease over time when the vast bulk of illness happens on a global population scale and does not present to hospitals in the same manner as non-communicable diseases do. Although not initially an aim or objective for this thesis, reporting on outcomes of COVID-19 in patients with IMRDs became a valuable and essential research agenda in the changing medical landscape of 2020.

The design and execution of our gout studies have several inherent strengths. The sample is a typical representation of patients with hyperuricaemia and gout which means our results accurately describe this cohort. The cases and controls recruited for each study were age and sex matched where possible, reducing confounding. Improvement of surrogate indices of arterial and pulmonary haemodynamics in hyperuricaemic cases post treatment with ULT, suggest hyperuricaemia may have a casual role in CVD pathogenesis. High end ultrasound machines with high-definition imaging were used throughout the study, indicating a role for the use of high-end ultrasound as the new 'gold standard' in the diagnosis and management of both gout and haemodynamic complications of

hyperuricaemia. Patients sUA levels were closely followed and studies repeated following a period of sUA within target range contributing to reliability of results.

This study does have some limitations, some of which were inherent and some of which occurred due to external factors stemming from the outbreak of the COVID-19 pandemic. It is an observational and single-centre study, this design can be susceptible to unidentified confounders. Due in part to the impact of the COVID-19 pandemic there were a smaller number of patients recruited into all samples than initially planned and a shorter follow up time, reducing the power of the studies. It was not possible to blind the ultrasonographer to the patients' condition which could introduce some unintentional bias in results.

Additional work currently underway in our department includes compounding our results by following a larger number of patients over a more prolonged period to confirm any significant findings. By analysing a larger cohort of patients, we aim to control and diminish the statistical error caused by small sample sizes. Larger population sample sizes will also allow for the use of compound statistics, such as regression analysis, to indicate whether associations of hyperuricaemia with our clinical measures, such as pPTT time and FMD, are truly independent of other cardiovascular risks including BMI, age, and smoking. To fully understand if there is a potential therapeutic role for ULT in the setting of cardiovascular and cardiopulmonary diseases then large scale RCTs with intention to treat analysis are required. PBMCs taken from patients with an acute gout flare were prepped and stored at -80°C. Due to time restraints qPCR was not performed on these samples at the time and a short-term future aim is to complete qPCR on these samples along with matched healthy controls to further understand the effect of MCC950. MCC950 is a potential new targeted

therapy for the management of gout however, more synovial biopsy and ex-vivo studies are required to prove a therapy is effective before moving into human in-vivo clinical trials.

In summary, this thesis describes an early clinical presentation of gout previously unrecognized but which, using ultrasound, can be diagnosed more effectively and at an earlier stage. We propose that inclusion of highly sensitive and specific US indicators of early gout are included in gout classification criteria so that diagnosis and effective treatment may commence prior to disease progression. Hyperuricaemia is associated with ultrasound surrogate markers that predict future adverse cardiovascular outcomes. Improvement in these ultrasound indices is seen post-treatment, suggesting that urate lowering strategies may improve cardiovascular outcomes. We have discussed some of the molecular mechanisms behind the inflammatory process in gout and provided preliminary evidence to support the use of MCC950, a selective inhibitor of the NLRP3 inflammasome, as a future therapy. Gout is a common and disabling disease, however earlier clinical diagnosis and potential new targeted therapies may lead to a brighter future, with an improved quality of life for sufferers.

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