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THE EFFECT OF ACUTE GOUT ON INFLAMMATORY MARKERS IN HYPERURICEMIC PATIENTS

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Summary

Introduction: Gout is a painful form of acute inflammatory arthritis associated with elevated uric acid crystal deposition especially in the joints, but also in tendons and the kidney. Between 1 and 2% of Western populations are affected and in severe cases, gout sufferers can be completely incapacitated. Despite the number of gout sufferers, the high number of risk factors and high incidence of adverse drug reactions using the standard treatment regimens, little research involving gout has been done within the highly diverse multiracial and multicultural population of South Africa.

Hypothesis: This study was a hypothesis generating observational study to assess whether serum levels of pro-inflammatory cytokines and acute phase protein levels could be used as markers of the gout status of a patient.

Method: Thirty gout patients were enrolled onto the study and attended two visits. At the screening visit; medical history, vital signs and demographic details were collected from intercritical gout patients. At both visits, patients completed visual analogue scales; namely: subject's assessment of pain and subject's assessment of disease activity. A doctor completed the physician's assessment of disease activity at both of the visits. At the end visit, patients experiencing an acute gout attack were asked to list various foods and beverages that triggered said attacks. Patients were requested to return for their second visit as soon as they experienced a gout attack, however, those patients that did not experience a gout attack were asked to return to the clinic to complete the follow up visit four months after their baseline visit. Uric acid, IL-1β, TNF-α and CRP were measured for each patient at both visits.

Results: Many of the patients displayed risk factors for metabolic syndrome. The mean subject's assessment of pain score increased from 31mm at the screening visit to 40mm at the end visit ($p=0.1947$; $n=26$), while the mean subject's assessment of disease activity score and the mean physician's assessment of disease activity increased from 30mm to 37mm ($p=0.3196$; $n=26$) and 23mm to 35 mm ($p=0.0937$; $n=26$) respectively.

Uric acid levels decreased from 1.053mmol/L to 0.871mmol/L between visits (p=0.0926; n=25) while CRP concentrations increased significantly from 10.2mg/L to 26.6mg/L (p=0.0278, n=24). IL-1β concentrations remained similar (12.17pg/ml to 12.54pg/ml) while TNF-α concentrations decreased from 12.63pg/ml to 3.54pg/ml, however neither of these were statistically significant differences.

Upon stratifying results into active and non-active patients, both IL-1β and TNF-α concentrations decreased between non-active and active patients, while CRP and urate concentrations increased. However, none of these differences were statistically significant.

Conclusion: The visual analogue scales all showed an increase between the screening and final visits, although this was not statistically significant. Uric acid concentrations decreased between visits, however this increase was once again not statistically significant. There appears to be no association between inflammatory markers and the level of gout activity, although this needs to be tested in a larger sample population. Results in South African patients have confirmed results from previous studies where gout patients are at a higher risk of metabolic syndrome than the normal population.

Keywords: gout, uric acid, CRP, TNF-α, IL-1β, visual analogue scales

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List of abbreviations

A

ACTH	Adrenocorticotropic hormone
ACR	American College of Rheumatology
ARA	American Rheumatism Association

B

BD	Becton & Dickinson

C

CAPS	Cryoprin-associated periodic syndromes
CBA	Cytometric bead array
CEO	Chief executive officer
CRP	C-reactive protein

D

ºC	Degrees Celsius

E

ELISA	Enzyme-linked immunosorbent assay
ESR	Erythrocyte sedimentation rate
EULAR	European League Against Rheumatism

F

Fc	Fragment crystallisable region

H

HbA_{1c}	Glycosylated haemoglobin
HBsAg	Hepatitis B surface antigen
HCV	Hepatitis C antibody
HDL	High density lipoprotein
HIV	Human immunodeficiency virus

I

IA	Intra-articular
IgG_1	Immunoglobulin G_1
IL-1β	Interleukin 1 beta
IM	Intramuscular
IV	Intravenous

L

L	Litre
LDL	Low density lipoprotein
LIFE	Losartan intervention for endpoint reduction

M

MRFIT	Multiple risk factor intervention trial
mg/dL	Milligrams per decilitre
ml	Millilitre
mmol/L	Millimole per litre

N

ng/ml	Nanogram per millilitre
NHLS	National Health Laboratory Service
NLRP3	Nucleotide-binding domain leucine-rich repeat and pyrin domain containing receptor
nm	Nanometre
NSAIDs	Non-steroidal anti-inflammatory drugs

P

PAD1	Physician's assessment of disease activity for visit one

PAD2	Physician's assessment of disease activity for visit two
pg	Picogram
pg/ml	Picogram per millilitre
pH	Percentage/parts hydrogen

Q

QOL	Quality of life

S

SAD1	Subject's assessment of disease activity for visit one
SAD2	Subject's assessment of disease activity for visit two
SAP1	Subject's assessment of pain for visit one
SAP2	Subject's assessment of pain for visit two
SF-36	Short form (36) health survey

T

TB	Tuberculosis
TNF-α	Tumor necrosis factor alpha
TRIS	Tris(hydroxylmethyl)aminomethane

U

UK	United Kingdom
µl	Microlitre
µmol/l	Micromole per litre

V

VAS	Visual analogue scale

Chapter one: Introduction

1.1 Literature review

1.1.1 Introduction

Gout was described by Hippocrates as “the disease of kings” due to its association with a rich diet.[1] Known risk factors for gout include high dietary purine consumption, e.g. various types of meat, seafood and certain vegetables, as well as alcohol intake, obesity and the use of particular drugs such as diuretics and low-dose aspirin.[2,3] Gender is also considered a risk factor, due to the higher prevalence of gout in men than in women.[1] The incidence of gout increases rapidly between the ages of 30 and 50 years, and the prevalence then continues to increase with age.[4] However, in patients over the age of 60, gout affects both men and women equally.[5] Furthermore, postmenopausal women are more prone to develop gout, possibly due to oestrogen increasing the renal clearance of uric acid.[1] Above the age of 80, more women than men are afflicted by gout.[5]

The Western civilisation has had two major gout epidemics, one during the reign of the Roman Empire and the other during the height of the British Empire. It is speculated that we are currently in the third epidemic due to the present high incidence of this condition.[2] The search for new treatments for gout has increased in the last few years as a result of this increase in the incidence of gout and the associated adverse renal and cardiovascular effects of this disease.[6] However no new drug therapies have been approved by the regulatory authorities for more than 30 years.

1.1.2 Epidemiology

The epidemiology of gout is well documented and a number of risk factors that influence the development of gout have been identified, including; age, hereditary, dietary, environmental and ethnic factors.[1] The Normative Aging Cohort Study provided useful information with regard to the incidence of gout in male patients. In hyperuricemic patients with baseline serum uric acid levels of greater than 9.0 mg/dL, the annual incidence rate of gout was 4.9%. In patients with baseline serum uric acid levels of between 7.0 mg/dL and 8.9 mg/dL, the annual incidence rate decreased to 0.5%, and this further decreased to 0.1% in patients with baseline serum uric acid levels below 7.0 mg/dL.[7] As this concentration is still regarded as hyperuricemic, this clearly implicates hyperuricemia as a risk factor for the development of gout.[7]

1.1.3 Prevalence

A 4.1% prevalence of hyperuricemia was found in a study of people older than 75 years.[2] Another study, the Rochester Epidemiology Project, revealed that the incidence of gout in a random population increased from 45/100 000 in 1977/8 to 63.2/100 000 in 1995/6.[7]

However, care should be taken when comparing prevalence rates of gout in different studies, as there are many variables that must be taken into consideration.[8] Examples of these variables include population status, age and gender, co-morbidities, diagnostic criteria, sample size and even seasonal changes in serum uric acid levels.[8]

1.1.4 Pathophysiology

Uric acid is formed from the metabolic breakdown of purines.[1] Increased uric acid production may be caused by various factors, including nutritional, haematological

and genetic factors. Other miscellaneous factors, such as obesity and excessive alcohol consumption, also increase urate production.[1] While certain medications, such as cytotoxic drugs may increase uric acid production, other medications, such as cyclosporine, may decrease the renal excretion of uric acid. Various renal, metabolic and genetic factors decrease the excretion of uric acid.[1]

Hyperuricemia is caused by an imbalance in the rates of production and excretion of uric acid. This causes the body fluids to become supersaturated with uric acid, which occurs at a level of 6.8 mg/dL.[7] In men and postmenopausal women, hyperuricemia is defined as a serum uric acid level of greater than 7.0 mg/dL. In premenopausal women, hyperuricemia is characterised by a serum uric acid level that is greater than 6.0 mg/dL.[1]

Hyperuricemia results in formation of monosodium urate crystals, which are deposited in various tissues and around joints during the asymptomatic hyperuricemic gout stage.[1,2,6] The deposited monosodium urate crystals form tophi[1,2] which cause inflammation leading to the symptoms of an acute gout attack.[2,6] The presence of tophi can also lead to soft tissue damage and destruction of afflicted joints, which causes the deformities associated with gout.[1] However, it is important to note that, while hyperuricemia is a dominant risk factor for gout, not all patients with hyperuricemia develop gout.[1]

1.1.5 Clinical phases of gout

There are four recognised sequential clinical stages in gout development, namely asymptomatic hyperuricemia, followed by the acute gouty attack or recurrent gout, then the intercritical period and finally chronic tophaceous gout.[1]

The first stage of gout, asymptomatic hyperuricemia, may continue for many years without detection until the first gouty attack. In approximately half of gout patients, the first metatarso-phalangeal joint is the first joint affected.[2] Gouty attacks are initially infrequent, but, as the disease develops, the gouty attacks increase in frequency, duration and in the number of joints involved.[2]

The intercritical period of gout occurs after the symptoms of the first gouty attack subside. This is the stage when the affected joints appear to return to normal, although it is usually followed by the chronic gouty arthritis stage, during which the accumulation of monosodium urate crystals in the joints is evident even between attacks.[2]

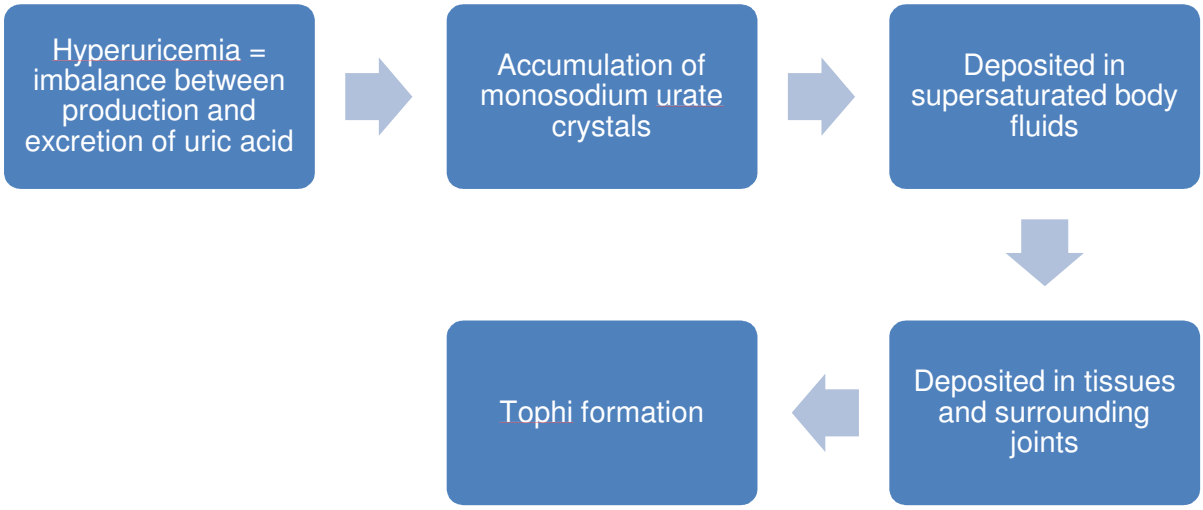

Figure 1: Diagram of the progression of symptoms of gout from hyperuricemia to formation of tophi

1.1.5.1 Asymptomatic hyperuricemia

Asymptomatic hyperuricemia appears to have adverse cardiovascular and renal effects and it is possible that serum urate-lowering therapy could decrease these adverse effects. The LIFE study, in which Losartan, an angiotensin receptor blocker, decreased adverse cardiovascular outcomes, is perhaps an example of this. The study investigators attributed the decrease in adverse cardiovascular effects to Losartan's urate-lowering properties. Numerous risks are associated with the prophylactic treatment of asymptomatic hyperuricemia, including the induction of gout flares, but there is also the possibility that prophylactic treatment at this stage would prevent progression to acute/recurrent gout.[9] However, the use of prophylactic treatment for asymptomatic hyperuricemia has been discouraged by numerous authors. Lifestyle modification is recommended.[10,11]

1.1.5.2 Acute gouty attack or recurrent gout

An acute gout flare is the result of a large inflammatory response to the deposition of the monosodium urate crystals.[1] An acute attack of gout is characterised by intense pain, swelling and erythema in the affected joint. Non-steroidal anti-inflammatory drugs (NSAIDs) are considered to be the first-line treatment for acute or recurrent gout.[12] The symptoms of an acute gout attack tend to be most pronounced eight to twelve hours after the start of the attack.[7]

The monosodium urate crystals that accumulate in hyperuricemic patients activate both the humoral and cellular components of the immune system. These crystals stimulate the recruitment and consequent adhesion of neutrophils, leading to inflammation. The crystals activate various intracellular receptors, leading to the activation of various inflammatory mediators and bringing about increased production of IL-1β, as demonstrated in Figure 1.[7]

1.1.5.3 Intercritical gout

Intercritical gout is characterised by hyperuricemia without any painful attacks. This period can be of short duration or can last up to many years. During this phase uric acid crystals are deposited into tissue and joints but do not result in the characteristic painful inflammatory reaction. The lower the uric acid levels in plasma the lower the risk of the following acute attack. In a study of the uricosuric drug, probenecid, it was found that 70% of intercritical gout patients with a serum urate level of above 9 mg/dL had one or more gouty attacks in one year. Studies performed with allopurinol, benzbromarone and febuxostat have shown that lowering serum urate levels to below 6 mg/dL decreases the incidence of gouty attacks, depletes monosodium urate crystals in the joints, inhibits the formation of new tophi and decreases the size of existing tophi.[9] It is apparent that urate-lowering therapy is necessary in order to prevent long-term joint destruction in gout patients.

1.1.5.4 Chronic tophaceous gout

In chronic tophaceous gout, the painful symptoms continue between attacks, leading to significant discomfort.[7] Urate-lowering therapy, either a xanthine oxidase inhibitor or a uricosuric, is thus indicated. This therapy decreases the excess uric acid levels in the patient, consequently reducing the size of the tophi. In serious cases, surgical excision of the tophi may be performed. A serum urate level of 6.0 mg/dL or less has proved to be an important target level with regard to the treatment of chronic tophaceous gout.[9]

1.1.6 Complications

Complications may arise in gout patients due to the tophi that form from the accumulation of monosodium urate crystals in the affected joints. These tophi can cause damage to the adjoining joints and tissues, leading to chronic pain, and may even lead to deformities.[1] Various renal complications may also occur. These complications include, but are not limited to, kidney stones, uric acid calculi and acute and chronic uric acid nephropathy.[1] These complications are not only caused

by the gout itself, but also by diseases that often accompany gout, such as hypertension, hyperlipidemia, heart disease and type 2 diabetes.[8] Furthermore, drugs that are prescribed to treat gout may bring about further complications, as discussed in the treatment section.

1.1.7 Diagnosis

An accurate diagnosis of gout forms an important part in treatment of gout as opposed to other inflammatory conditions.[9]

Diagnosis of gout may be confirmed by demonstrating monosodium urate crystals in the affected tissue or joints usually from biopsy. If this procedure is not possible to perform, gout may also be diagnosed by means of a review of medical history or the observation of any two of the following: podagra, tophus, an abrupt attack of monoarthritis and/or a response to colchicine within 48 hours.[8]

The diagnosis of gout has also been made by ultrasound-based methods that have the ability to diagnose gout between acute attacks and even in patients with asymptomatic hyperuricemia.[2] Furthermore, ultrasound allows for the inspection of tophaceous deposits without the need for painful needle aspirations. As such, this technique is gaining importance in the diagnosis of gout.[2] Physicians also use the criteria developed by the American College of Rheumatology to confirm or dismiss a diagnosis of gout.[3]

1.1.8 Treatment

Treatment is based on the particular clinical phase of gout that the patient is experiencing. Treatment for acute gout is aimed at reducing the pain and inflammation that accompany acute gout attacks, whereas treatment for the

intercritical period of gout aims to maintain low levels of serum uric acid in order to prevent the formation of tophi. Chronic tophaceous gout is treated by initiating long-term hypouricemic therapy. There is currently no evidence to suggest that the treatment of asymptomatic hyperuricemia prevents further development of gout.[7]

Treatment can also be divided into three groups, based on the purpose of the treatment. One group of drugs is intended to treat acute gout and the attacks that occur during this phase. The second group of drugs is used for the prevention of recurrences of gout, while the third group of drugs is aimed at lowering serum uric acid.[2]

1.1.8.1 NSAIDs

NSAIDs are considered to be the drugs of choice to treat acute gout.[12] NSAIDs are also used prophylactically.[2] In the treatment of an acute attack, high doses of NSAIDs are prescribed during the first three to four days of an attack. Thereafter standard doses are prescribed for maintenance.[7]

In a study comparing the use of rofecoxib, diclofenac sodium and meloxicam in the treatment of acute gout, rofecoxib was found to be the most effective of these three NSAIDs, displaying a rapid onset of pain relief after dosing, but all three drugs gave similar side effect profiles.[12]

Etoricoxib and lumiracoxib are two cyclo-oxygenase 2-selective inhibitors that have the same efficacy as NSAIDs in the treatment of acute gout. Two individual studies have suggested that the above two drugs may be of use in the treatment of acute gout in patients in whom standard NSAIDs are contraindicated.[7]

Although rofecoxib and lumiracoxib have since been removed from the market due to unwanted drug effects, there are still other cyclo-oxygenase inhibitors available, including the previously mentioned etoricoxib and meloxicam.[13,7,12]

Etoricoxib, in particular, has shown comparable efficacy to indomethacin in an acute gout clinical trial.[9] Indomethacin is a NSAID used to give relief during acute gout attacks as well as in gout prophylaxis.[1]

One of the major adverse effects associated with NSAIDs in general is gastrointestinal damage.[14] A proton pump inhibitor can be prescribed for gout patients administered NSAIDs to avoid potential gastrointestinal risks.[9] NSAIDs can also cause hypertension, fluid retention, congestive heart failure and central nervous system effects, in addition to the common gastrointestinal side effects.[1,2] All of these side effects are important to note due to the many co-morbidities present in the typical gout patient.[2]

As such, NSAIDs are often completely contraindicated in the elderly, where many co-morbidities exist in patients using warfarin, as well as in patients with cardiac, renal and hepatic diseases.[7] Other treatments for acute gout include corticotropin, corticosteroids and colchicine.[1] The above-mentioned drugs are generally effective in the treatment of acute gout, although they are associated with numerous side effects.[1]

The choice of therapy is determined according to the stage, the severity of the acute gout attack and any existing patient co-morbidities that may interfere with the therapy. There are a number of different drugs that can be used for treatment of an acute gout attack.[9]

1.1.8.2 Colchicine

Colchicine, a plant alkaloid known to reduce uric acid levels and suppress inflammation, has been used for centuries in the treatment of gout and is effective in reducing the symptoms of an acute gout attack.[1,2] However, colchicine has a toxic effect and a very small therapeutic index. Gastrointestinal side effects associated with colchicine are nausea, cramping and diarrhoea. Colchicine accumulates in patients with renal insufficiency, and is only recommended in patients without renal failure.[2] Acute and long-term side effects of colchicine include neuropathy, muscle damage, neutropenia, nephrotoxicity, cardiac effects and vacuolar myopathy.[7] The long-term side effects are often irreversible and require monitoring. Both NSAIDs and colchicine can be used for the prophylaxis of gout.[2]

1.1.8.3 Corticosteroids

Corticosteroids have been found to be effective in the treatment of acute gout, whether they are administered intra-articularly, systemically or orally.[2] They are indicated for use in gout patients who cannot tolerate, or have contraindications for the use of, NSAIDs and colchicine.[1] The concern with the use of corticosteroids is the many long-term side effects of the steroidal drugs.[1,15] Some of these side effects include Cushing's syndrome, diabetes mellitus, hypertension and osteoporosis.[15]

However, as demonstrated in a clinical trial comparing the use of oral indomethacin and paracetamol to the use of oral prednisolone and paracetamol, prednisolone (corticosteroid) proved to be just as effective as indomethacin (NSAID) with regard to the treatment of acute gout, and was associated with fewer adverse effects than indomethacin. It was concluded that the short-term use of a corticosteroid, such as prednisolone, can be effective in the treatment of acute gout without the risks of the long-term side effects of corticosteroids.[15]

1.1.8.4 Corticotropin

Corticotropin, or ACTH as it is commonly known, has been used in the treatment of acute gout for many years. In one clinical trial in which indomethacin was compared to the use of ACTH in acute gout, ACTH brought about faster pain relief than indomethacin, and the ACTH treatment group had far fewer side effects than the indomethacin group.[1]

Corticotropin is used as a treatment for polyarticular flares in acute gout when the first-line therapies have either failed or are contraindicated.[1,7] When ACTH is used in conjunction with colchicine, it is effective in the treatment of acute gout and this combination causes less side effects than indomethacin.[1] The side effects of colchicine and ACTH combination therapy include fluid retention, poor control of glucose levels in diabetes, hypokalemia and the relapse of gout or rebound arthritis.[1,7]

1.1.8.5 Allopurinol

Allopurinol is not used in asymptomatic hyperuricemia, but is indicated in long-term hypouricemic therapy. Allopurinol is a xanthine oxidase inhibitor that decreases acute gout attack incidence by decreasing the amount of serum uric acid through the inhibition of the xanthine oxidase enzyme, which is involved in uric acid synthesis.[2]

Allopurinol is considered to be very effective in terms of long-term hypouricemic therapy, and is recommended for use in patients with high serum uric acid levels, nephrolithiasis and renal impairment.[2] Serum urate levels need to be monitored continuously in order to maintain the appropriate dosage of allopurinol. Elderly patients, as well as patients with impaired kidney function, are generally prescribed lower doses of allopurinol.[7]

Although allopurinol is effective, various side effects have been noted that vary in degree of severity. Some patients complain of only mild gastrointestinal side effects. On the other end of the scale is the life-threatening "allopurinol hypersensitivity syndrome", which is characterised by fever, rash, eosinophilia, hepatitis and renal failure.[2,7] Risk factors for the development of allopurinol hypersensitivity syndrome include allopurinol therapy initiation, renal insufficiency and the use of diuretics.[7]

Allopurinol exhibits serious drug interactions, for example with azathioprine, antibiotics, cytotoxic drugs, immune suppressants and diuretics. Allergic reactions to allopurinol have also been reported often, thus patients on allopurinol require monitoring for possible adverse events .[2]

Probenecid is also used for long-term hypouricemic therapy. It is a uricosuric drug that lowers serum uric acid levels by increasing the amount of urinary excretion of uric acid.[1,2] Probenecid requires two daily doses, which may affect compliance. While probenecid does have interactions with certain drugs and is considered to be less effective than allopurinol, it is sometimes the drug of choice for long-term hypouricemic therapy due to far fewer side effects than allopurinol.[2]

Other uricosuric drugs that may be used to reduce hyperuricemia include benzbromarone, sulfinpyrazone, losartan and fenofibrate.[7] These uricosuric drugs are not widely used in practice because of their many drug interactions, their loss of effectiveness in patients with low glomerular filtration rates, and their possible role in promoting nephrolithiasis.[7]

New drugs for the treatment of gout include febuxostat and uricase. Febuxostat proved to be an effective hypouricemic therapy in a clinical trial comparing the use of febuxostat and allopurinol, but febuxostat, upon initiation of therapy, induced acute

gout flares in up to 70% of patients. Furthermore, there are concerns about the long-term safety of febuxostat with regard to hepatic and cardiovascular adverse events.[7]

A stabilised uricase enzyme, namely polyethylene glycol-linked uricase, is being investigated in Phase 3 clinical trials in order to determine whether it is effective in controlling hyperuricemic patients with chronic gout, and in patients who have not responded to conventional therapy.[6,7] Long-term hypouricemic therapy aims to decrease levels of serum uric acid in order to decrease acute gout attacks, while at the same time preventing damage over time to the patient's joints by decreasing the size and number of tophi.[2]

1.1.8.6 Diet and lifestyle

In addition to the medical interventions necessary in controlling gout, patients are encouraged to make certain diet and lifestyle changes. The aim is to reduce obesity, decrease the intake of purine-rich foods, such as various types of meat, seafood, certain vegetables, and to reduce alcohol consumption.[1,3] Examples of purine-rich foods include animal liver, beef, tuna, peas and lentils.[3] Lifestyle modification would involve a drastic change in the patient's diet, cutting out many of the purine-rich foods.[1] It has also been suggested that an increased intake of dairy products may decrease the incidence of gout.[3] While the various drugs available for the treatment of gout clearly play a vital role, it is important to note that diet and lifestyle modifications also form an essential part of the treatment plan. Furthermore, diet and lifestyle modification have the dual benefit of not only improving gout symptoms, but also many of the diseases associated with gout, such as hypertension, hyperlipidemia and diabetes mellitus.[8]

1.1.9 New research

Symptoms of an acute gout attack include pain, inflammation and erythema of the afflicted joint.[12] The inflammation is caused by the release of various cytokines, including interleukin-1β (IL-1β). IL-1β is an inflammatory cytokine that is associated with the leukocytosis and fever that often accompany acute gouty attacks.[16]

The monosodium urate crystals that are present in gout stimulate leukocytes to increase the production of IL-1β. Once the cryoprin (NLRP3) inflammasome is activated, pro-inflammatory caspase 1 cleaves and activates pro-IL-1β to form the active mature IL-1β.[16] Both intracellular and extracellular signals activate the inflammasome.[16,17]

Tumor necrosis factor (TNFα) triggers the production of IL-1β. TNFα appears to be directly associated with numerous inflammatory diseases. Antagonists of TNFα have various applications in inflammatory diseases, such as rheumatoid arthritis.[18]

Rilonacept, which is also referred to as IL-1 trap is an engineered protein, consisting of fused parts, namely:

- Ligand binding domains of the extracellular portions of the IL-1 receptor found in humans and
- IL-1 accessory protein (IL-1RAcP) which is linked to the
- Fc portion part of human IgG_1 (immunoglobulin G_1)[17]

The mechanism of action of the drug is that it acts as a soluble decoy receptor. This decoy receptor binds to IL-1β which blocks IL-1β signaling. Rilonacept is thus a targeted IL-1β inhibitor. In 2008 Rilonacept was approved by the FDA for use in

cryoprin-associated periodic syndromes (CAPS), a group of rare auto-inflammatory conditions. Regeneron is exploring the potential for Rilonacept as a gout treatment, due to the inflammatory nature of gout and its association with the NLRP3 inflammasome.[17]

A pilot study using another IL-1 blocker, Anakinra, relieved the inflammatory symptoms of gout in ten patients who did not tolerate or had failed to respond to the standard anti-inflammatory therapies used to control acute gout attacks.[19]

Current long term treatments for gout are aimed at decreasing serum levels of uric acid and treating the causes of gout. New research is currently under way to explore the potential use of an IL-1β blocker to treat gout symptoms.[17] TNF and IL-1 thus have high potential as targets for acute gout attack treatment.

The purpose of this study is to determine the effect of acute gout on these two inflammatory markers in the South African population in order to further explore the potential of these inflammatory markers as specific targets for gout treatment.

1.1.10 Summary

Hyperuricemia, one of the major risk factors for the development of gout, is caused by an imbalance between the rates of production and excretion of uric acid. An excess of uric acid thus supersaturates the body fluids, leading to the deposition of monosodium urate crystals in tissues and joints, which in turn leads to the initiation of an acute gout attack.

Gout is a painful, occasionally debilitating disease and is divided into four clinical phases, namely asymptomatic hyperuricemia, acute gouty attack or recurrent gout,

intercritical gout and chronic tophaceous gout. Each of these clinical phases have various possible treatments. NSAIDs, colchicine, corticosteroids and corticotrophin are used for the treatment of acute gout attacks in order to relieve pain. NSAIDs and colchicine are prescribed for the prophylaxis of gout. Allopurinol, probenecid and other uricosurics are used in long-term hypouricemic therapy in order to decrease serum uric acid levels and to prevent long-term damage to joints.

Due to the untoward and, in some cases, potentially life-threatening side effects of the various gout therapies, there are opportunities for novel therapies to treat gout.

Detailed patient evaluation is the basis of a clinician's decision on the choice of therapy for gout patients. More attention needs to be given to patient evaluation in order to personalise gout therapy for every patient while avoiding the high risk of adverse drug reactions. Emphasis should also be placed on the importance of diet and lifestyle modifications in addition to gout therapy so as to prevent gout and acute gout attacks.

Aim and objectives

Aim

The aim of this study is to assess whether there is an association between inflammatory markers (IL-1β, TNFα and CRP) and the level of disease activity in gout.

Objectives

To determine the following in patients:

1. Determine the serum uric acid levels in patients during the inter-critical stage and again during a gout attack.

2. Patient assessment of disease activity (Visual Analogue Scale) at each visit.

3. Physician's assessment of disease activity at each visit.

4. Patient assessment of pain (Visual Analogue Scale) at each visit.

5. Determine serum levels during the inter-critical stage and during a gout attack of:

    a. IL-1β.

    b. TNF-α.

    c. CRP

6. Investigate the association between the inflammatory markers and the disease activity and pain levels

Hypothesis

This study is a hypothesis generating observational study to assess whether the serum levels of pro-inflammatory cytokines and acute phase protein levels can be used as markers of the gout status of a patient.

Chapter two: Materials and methods

2.1 Clinical trial

2.1.1 Defining the research problem

Gout is a painful form of acute inflammatory arthritis associated with elevated uric acid crystal deposition especially in the joints, but also in tendons and the kidney. Between 1 and 2% of Western populations are affected and in severe cases, gout sufferers can be completely incapacitated. Despite the number of gout sufferers, the high number of risk factors and high incidence of adverse drug reactions using the standard treatment regimens, little research involving gout has been done within the highly diverse multiracial and multicultural population of South Africa.

Research into many aspects of gout may provide a better understanding of the disease and information that could lead to improved gout treatment outcomes by altering prescribing practices of general practitioners.

2.1.2 Study design

This was an observational study that might be used to generate a hypothesis driven study in the future. Thirty gout patients meeting the inclusion and exclusion criteria were assessed in this study. The sample size was approved by a statistician.

Numerous patients were pre-screened according to their hospital files. Thirty one patients were screened, of which thirty were enrolled onto the study. The patients were requested to come into the clinic for two separate visits. At the first visit, patients were “gout-attack free” for four weeks. They were requested to come back to the clinic for their final visit during their next gout attack. However, if a gout attack

did not occur within a four month period, patients were asked to return for their final visit to end their participation.

At their first visits, patients signed an informed consent form and were screened according to the inclusion and exclusion criteria of the study. Demographic information and medical and surgical histories were collected from the patients. Patients were asked to complete a subject's assessment of pain visual analogue scale and a subject's assessment of disease activity visual analogue scale. A physician performed a complete physical examination on each patient and completed a physician's assessment of disease activity visual analogue scale.

At their final visits, patients were requested to complete a gout related questionnaire, containing information on their diets. Once again both the patient and the physician completed the above-mentioned visual analogue scales.

Approximately 5ml of blood was drawn from the patient at both visits. Gel serum separator gold top BD (Becton Dickinson and Co.) vacutainers were used to collect the blood, which were then left to clot for half an hour. Thereafter the tubes were centrifuged at 1500g for 15 minutes and the serum was transferred into 200µl aliquots. These aliquots were frozen at -20℃ and used when needed for the various measurements.

2.1.3 Ethical considerations

This study received approval from the Faculty of Health Sciences Research Ethics Committee (Protocol S41/2009). Informed consent was performed in accordance with Good Clinical Practice guidelines and the Declaration of Helsinki as amended.

2.1.4 Patient selection

Patients with serum uric acid levels of ≥7.5 mg/dL were contacted and invited to volunteer for the study. The CEO of Steve Biko Academic Hospital, Dr AP Van Der Walt, gave permission to access hospital patient records to identify possible candidates.

A set of inclusion and exclusion criteria were assessed and the most influential parameters included in order to avoid invalid results.

2.1.4.1 Inclusion criteria

1. Male or female patients that are 18-80 years of age
2. Subjects who have previously met the preliminary criteria of the American Rheumatism Association (ARA) for the classification of the acute arthritis of primary gout (if any 6 or more of the 13 criteria are present, serially or simultaneously, during any interval of observation) or if monosodium urate microcrystals have been identified in joint fluid.
3. Serum uric acid ≥7.5 mg/dL at screening and, according to the treating physician or investigator, the subject is a possible candidate for treatment with allopurinol.
4. A self-reported history of ≥2 gout flares in the year prior to the initial Screening Visit
5. Subjects who are willing, committed, and able to return for all clinic visits and complete all study-related procedures
6. Subjects who are able to read, understand and willing to sign the informed consent form
7. Subjects who are able to read, understand, and complete study-related questionnaires
8. Patients are allowed to be on anti-gout treatment, but this is not necessary to be included in the trial

2.1.4.2 Exclusion criteria

1. Subjects with an acute gout flare in the 4 weeks prior to or during the Screening Visit
2. Subjects who are pregnant, nursing, or planning a pregnancy or fathering a child during the course of the trial
3. Subjects with chronic active gouty arthritis
4. Subjects who have a known or suspected current active infection or a history of chronic or recurrent infectious disease including but not limited to chronic renal infection, chronic chest infection, sinusitis, recurrent urinary tract infection, and open, draining, infected skin wounds
5. History of an allergic reaction to allopurinol or inadequate urate-lowering response to allopurinol
6. Uncontrolled diabetes, defined as HbA1c ≥ 9.0% at the Screening Visit
7. Subjects requiring dialysis
8. Subjects who have had an organ transplant
9. Treatment with any systemic immunosuppressants (e.g. methotrexate, azathioprine, cyclosporine, mercaptopurine, mycophenolate mofetil, tacrolimus, sirolimus, leflunomide, etanercept, adalimumab, infliximab, abatacept, natalizumab, rituximab) within 6 months prior to Screening Visit, or anakinra within 30 days of Screening Visit
10. Treatment with pegloticase within 6 months of Screening Visit
11. History of a demyelinating disease or symptoms suggestive of multiple sclerosis
12. Use of oral, intra-articular (IA), intramuscular (IM) or, intravenous (IV) glucocorticoids in the 4 weeks prior to the Screening Visit
13. Treatment with a live (attenuated) virus vaccine during the 3 months prior to the Screening Visit
14. History of HIV by clinical or serological history
15. History of Hepatitis B surface antigen (HBsAg) and/or Hepatitis C antibody (HCV) by serologic testing

16. Chest radiograph (or historic results within 3 months of Screening Visit) that shows evidence of malignancy or any abnormalities suggestive of prior tuberculosis infection, including, but not limited to, apical scarring, apical fibrosis, or multiple calcified granulomata. This does not include non-caseating granulomata
17. History of active TB prior to screening
    - Signs or symptoms suggestive of active TB (e.g. new cough of > 14 days in duration or a change in chronic cough, persistent fever, unintentional weight loss, night sweats) upon review of medical history and/or physical examination
    - Have recent close contact with a person with active TB
    - History of latent untreated TB
18. Any other arthritic or medical condition that in the opinion of the investigator could adversely affect the subject's participation or interfere with evaluations. This includes significant concomitant illness such as, but not limited to, cardiac, renal, neurological, endocrinological, metabolic, pulmonary, gastrointestinal, or psychiatric diseases.
19. History or presence of malignancy within 5 years of the Screening Visit (other than a successfully treated non-metastatic cutaneous squamous cell or basal cell carcinoma and/or localized carcinoma in situ of the cervix)
20. History of a myeloproliferative disorder
21. Any investigational drug within 30 days or within 5 half lives, whichever, is longer, prior to the Screening Visit

2.2 Visual analogue scales

Visual analogue scales will be used to determine:

- Patient assessment of pain
- Patient assessment of disease activity
- Physician's assessment of disease activity

The Visual Analogue Scale or VAS has been compared to multi-item questionnaires in patients and has been found to be valid and reliable. It has been recommended for use in clinical trials to assess various parameters that have an effect on the patient's quality of life globally.[20] The strength of the VAS is its comparative use for people either over a specific time period or over different populations. Although the patients assessment of both pain and disease activity is subjective, the doctor's assessment is objective. Also, another benefit of using VAS is that it provides statistical information or quantitative values for subject self-reporting, which is normally data collected qualitatively.[21] The visual analogue scale has been referred to as the 'gold standard' in research and clinical practice.[22]

The patients were asked to indicate their level of pain by making a single vertical line on the bar, as seen below. A mark made close to the 0mm side of the bar indicated that the patient felt very little pain at that particular visit, whereas a mark made close to the 100mm side of the bar indicated that the patient was experiencing a lot of pain at that particular visit, as seen in the example below.

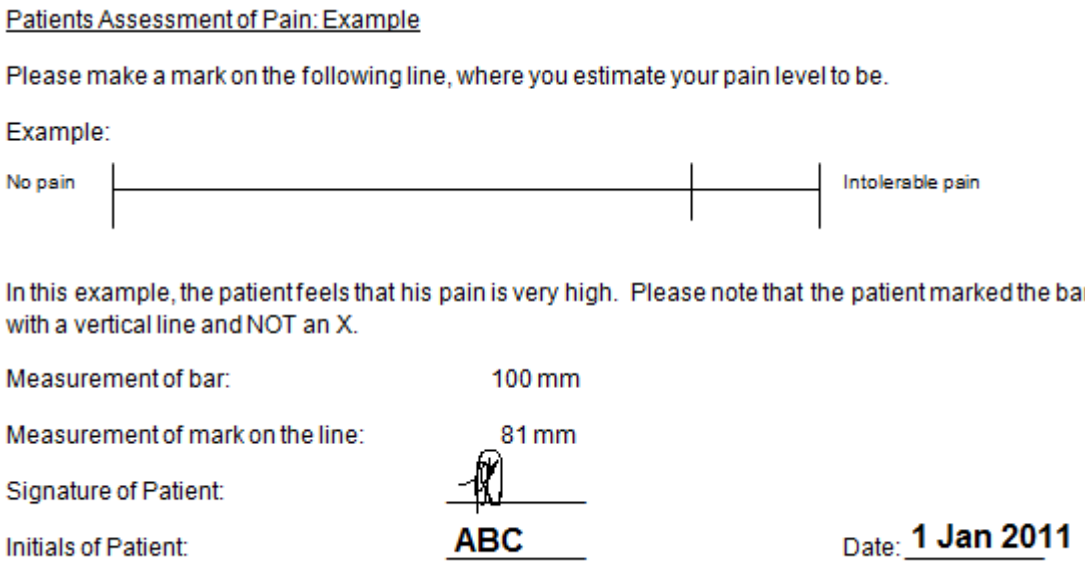

Figure 2: Visual analogue scale example (not to scale)

This procedure was then repeated for the patients assessment of disease activity, except that “no pain” was changed to “no disease activity” and “intolerable pain” was changed to “very high disease activity”. Physicians also assessed the patients level of disease activity, using the same procedure.

2.3 Cytokine measurement

The serum levels of the two pro-inflammatory cytokines, IL-1β and TNFα were determined at both patient visits.

Flow cytometry was used to determine the concentrations of the two cytokines. A cytometric bead array (CBA) kit was used to determine the concentrations of IL-1β and TNF-α. There are various benefits in using this method in flow cytometry. Firstly, both of the cytokines may be determined simultaneously in the same sample. Secondly, due to the increased sensitivity of this method, minimal sample volumes are required. Finally, the use of a CBA kit provides high levels of reproducibility.[23] In flow cytometry, the analytes are captured on specific beads of known size and fluorescent intensity and are then exposed to a second quantitating secondary fluorescent antibody. The beads are then characterised by size and fluorescent intensity at multiple wavelengths to determine the quantitative and qualitative data simultaneously.[24]

2.3 FlowCytomix human 2plex IL-1β and TNFα kit

2.3.1 Intended use

The FlowCytomix basic kit can be used in combination with up to twenty different simplex kits. For the purpose of this experiment, two simplex kits; namely IL-1β and TNFα, were combined with the basic kit. This enabled quantitative detection of the

above-mentioned analytes in serum. This kit is intended for research use only and is not intended for use in diagnostic or therapeutic procedures.

2.3.2 Summary

IL-1β and TNFα are cytokines that are both involved in a variety of immunomodulatory actions.

Low levels of IL-1β are found in normal serum, however IL-1 genes are said to be activated by tissue damage and infection. Much higher levels of IL-1β have been found in a variety of inflammatory diseases, including Crohn's disease.[25]

It has been reported that TNF-α plays a significant role in the pathophysiology of numerous inflammatory diseases. Elevated levels of this cytokine have been found in myocarditis, septicemia, parasitic infections and meningococcal disease.[26]

2.3.3 Principles of the test

The principles of the fluorescent bead immunoassay are similar to those of ELISA.

Two differently sized bead sets were used in this assay, one bead set coated with monoclonal antibody to IL-1β and the other bead set coated with monoclonal antibody to TNFα. The different sizes of the beads makes it possible to distinguish between different beads in the same fluorescent channel.

These two bead sets are coated with antibodies that bind specific analytes (either IL-1β or TNFα). A secondary biotinylated antibody binds to the specific analyte, streptavidin-phycoerythrin binds to the biotin and emits fluorescent signals.

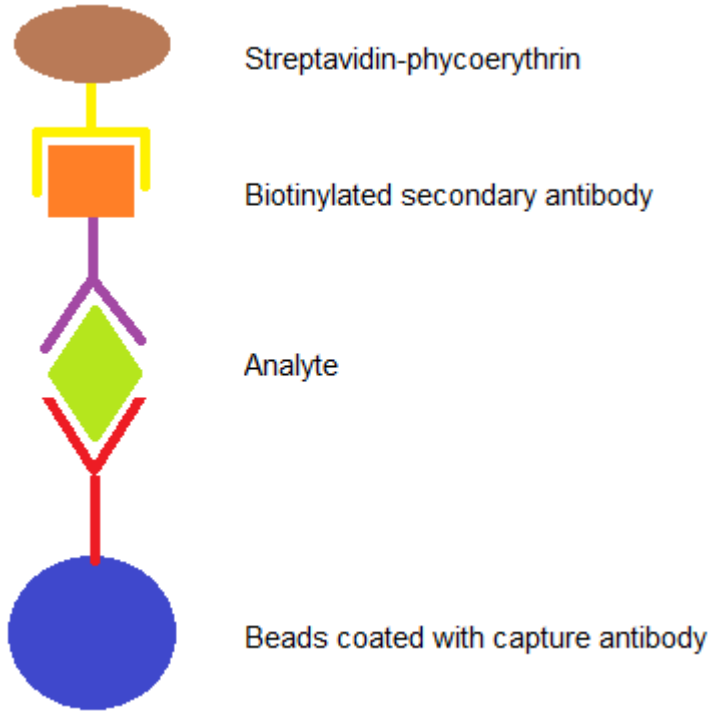

Figure 3: Principles of the CBA test

2.3.4 Materials

The FlowCytomix simplex kits provided the following materials:

175µl fluorescent beads coated with monoclonal antibody to IL-1β

175µl fluorescent beads coated with monoclonal antibody to TNFα

Lypophilized IL-1β standard

Lypophilized TNFα standard

350µl biotin-conjugate anti-human IL-1β monoclonal antibody

350µl biotin-conjugate anti-human TNFα monoclonal antibody

The FlowCytomix basic kit provided the following materials:

Vial setup beads

50ml assay buffer

13ml reagent dilution buffer

200µl Streptavidin-Phycoerythrin

The following materials were required but not provided:

Beckman Coulter FC500 flow cytometer

Centrifuge

Flow cytometer sample acquisition tubes

Aluminium foil

5ml and 10ml graduated pipettes

10µl to 1000µl adjustable single channel micropipettes with disposable tips

Beakers, flasks, cylinders necessary for the preparation of reagents

Glass-distilled or deionised water

Vortex mixer

2.3.5 Method

Blood samples were drawn from patients into Becton & Dickinson gold top gel separator tubes and allowed to clot for 30 minutes. The samples were then centrifuged at 1500g's for 15 minutes. Thereafter plasma was divided into 200 µl

aliquots and stored and frozen at -20℃. All samples were brought to room temperature and vortexed before use.

Preparation of the assay buffer

The assay buffer concentrate was mixed well and 50ml of this concentrate was added to 450ml distilled water. This was gently mixed and stored at 2℃ to 8℃.

Preparation of IL-1β and TNFα standards

The lypophilised standards were centrifuged for 15 seconds to collect the lypophilised standards at the bottom of the vials. Thereafter standards were reconstituted with distilled water according to instructions on the particular vials. Vials were gently swirled to allow quantitative solubilisation of contents and were allowed to reconstitute for 10 minutes before pipetting. The final concentrations of both the IL-1β and TNFα standards were 400ng/ml.

10µl of both the IL-1β and TNFα reconstituted standards were added to a flow cytometer acquisition tube labelled Standard 1. 180µl of assay buffer was added to Standard 1 and was mixed well to bring the total volume to 200µl.

Serial dilution of standard mixture

100µl of assay buffer was added to flow cytometer acquisition tubes labelled Standard 2 to 7. 50µl of Standard 1 was added to Standard 2 and the contents were mixed well. 50µl of Standard 2 was then added to Standard 3 and this procedure was repeated for all 7 standard dilutions. This procedure was repeated in order to create a second round of 7 standard dilutions.

Preparation of bead mixture

For each test 25µl of bead mixture was needed. 96 tests were performed including plasma samples, blanks and standards

The final volume of bead mixture needed was calculated in the following manner:

96 tests x 25µl = 2400µl

This value was rounded up to 3000µl in order to provide a pipetting reservoir and to account for operator error.

Individual bead vials were vortexed for a few seconds each.

A mixture of beads was created by adding 150µl (1/20 of the final volume) of the IL-1β bead set and 150µl (1/20 of the final volume) of the TNF-α bead set to 2700µl of reagent dilution buffer.

This bead mixture was washed once by centrifugation at 3000g's for 5 minutes with reagent dilution buffer and vortexed.

Preparation of the biotin-conjugate mixture

For each test 50µl of biotin-conjugate was needed. 96 tests were performed including plasma samples, blanks and standards

The final volume of biotin-conjugate needed was calculated in the following manner:

96 tests x 50µl = 4800µl

This value was rounded up to 6000µl in order to provide a pipetting reservoir and to account for operator error.

A mixture of biotin-conjugate was created by adding 300µl (1/20 of the final volume) of the IL-1β biotin-conjugate and 300µl (1/20 of the final volume) of the TNF-α biotin-conjugate to 5400µl of reagent dilution buffer.

Preparation of the streptavidin-phycoerythrin mixture

For each test 50µl of streptavidin-phycoerythrin was needed. 96 tests were performed including plasma samples, blanks and standards

The final volume of streptavidin-phycoerythrin needed was calculated in the following manner:

96 tests x 50µl = 4800µl

This value was rounded up to 5500µl in order to provide a pipetting reservoir and to account for operator error.

176µl of concentrated streptavidin-phycoerythrin was added to 5324µl of assay buffer.

Test procedure using flow cytometer acquisition tubes

25µl of Standards 1-7 were added to flow cytometer acquisition tubes. 25µl of assay buffer was added to two "blank" tubes. 25µl of patient plasma was then added to the remaining tubes. The following was added to all of the tubes: 25µl bead mixture and 50µl biotin-conjugate. The tubes were covered with aluminium foil and incubated for 2 hours at 18℃ to 25℃.

Thereafter, 1ml of assay buffer was added to each tube and each tube was washed twice by centrifuging for 5 minutes at 200g's. After each washing step, the supernatant was carefully discarded, leaving 100µl of pellet in the tubes. Care was taken not to disturb the pellet when removing the supernatant after each wash step.

50µl of streptavidin-phycoerythrin solution was added to each tube and the tubes were then covered with aluminium foil. Tubes were incubated for 1 hour at 18℃ to 25℃.

Thereafter, 1ml of assay buffer was added to each tube and tubes were each washed twice by centrifuging for 5 minutes at 200g's. After each washing step, the supernatant was carefully discarded, leaving 100µl of pellet in the tubes. Care was taken not to disturb the pellet when removing the supernatant after each wash step.

500µl of assay buffer was added to each tube and samples were analysed on the flow cytometer.

2.3.6 Analysis of results

Analysis of results was based on the FlowCytomix Pro 2.4 software.

2.4 Uric acid measurement

2.4.1 Intended use

This method is intended for research use only and is not intended for use in diagnostic or therapeutic procedures

2.4.2 Summary

Uric acid concentration is a blood parameter that is involved in numerous physiological disorders and biochemical changes, of which gout is one example. Serum uric acid was measured at both the screening and acute gout attack visits.[27]

There are various laboratory methods that can be used to determine uric acid concentration. One of the widely used methods is a reduction reaction method whereby phosphotungstic acid is reduced to a blue tungsten salt as a result of the oxidation of uric acid to allantoine. However, various interferences are seen in this method as numerous other antioxidants in serum can produce this same reaction.[20, 27]

2.4.3 Principles of the test

The method that was used in this project, involved the use of the specific enzyme uricase. Uricase catalyzes the oxidation of uric acid to allantoine through the action of oxygen in the air. The uric acid method was based on the ACA method referred to by Elin and colleagues.[28]

The principle of the test is as follows:

A standard curve was created with known concentrations of uric acid. The absorption maximum of 283nm was determined by performing a spectrophotometric scan. Uricase enzyme was added to patient serum samples and absorbance values were then obtained for these samples on the Perkin-Elmer Lambda UV/VIS spectrophotometer. Samples were then incubated for three hours at 37ºC and thereafter absorbance values were once again obtained for these samples.

During the incubation period, the uricase enzyme broke down the uric acid into its various constituents. The difference between the first and second sets of absorbance values for the samples may be extrapolated into the standard curve equation in order to obtain the concentration of uric acid in the sample.

2.4.4 Materials

350ml Tris(hydroxymethyl)aminomethane (TRIS) buffer; pH=8.5

50ml uricase

50ml uric acid stock solution

Quartz cuvette

Pasteur pipettes

15ml test tubes

Test tube racks

10µl to 1000µl adjustable single channel micropipettes with disposable tips

Beakers, flasks, cylinders necessary for the preparation of reagents

Distilled water

Vortex mixer

2.4.5 Method

Blood samples were drawn from patients into Becton & Dickinson gold top gel separator tubes and allowed to clot for 30 minutes. The samples were then centrifuged at 1500g's for 15 minutes. Thereafter plasma was then divided into 200 µl aliquots and stored and frozen at -20℃. All samples were brought to room temperature and vortexed before use.

Tris(hydroxymethyl)aminomethane (TRIS) buffer

For each test 1000µl of 0.1mol/L TRIS buffer was needed. 56 tests were performed. An additional 150ml of 0.1mol/L TRIS buffer was required for the preparation of the uricase solution, as well as for the standard curve samples.

This was rounded to 350ml in order to provide a pipetting reservoir. 4.2396g of TRIS was dissolved in 350ml of distilled water. Thereafter the pH was adjusted to 8.5.

Uricase

For each test 400µl of 3mg/ml uricase was needed. 56 tests were performed.

This was rounded to 50ml in order to create a pipetting reservoir. 150mg of uricase was dissolved in 50ml TRIS buffer.

Uric acid stock solution

A stock solution of 4000µM was made up by dissolving 33.65mg of uric acid in 50ml of TRIS buffer.

Standard curve samples

1000µl of uric acid stock solution was added to a 15ml tube and was labelled standard 1. 1000µl of TRIS buffer was added to15ml tubes labelled standard 2 to 9. 1000µl of uric acid stock solution was added to Standard 2 and the contents were mixed well. 1000µl of Standard 2 was then added to Standard 3 and this procedure was repeated for all 9 standard dilutions.

600µl of standard 1 was added to a 5ml tube and was labelled 4000µM. 4200µl of TRIS buffer was also added to this tube and the contents were mixed well. This procedure was repeated with standards 2-9.

Sample preparation

For each test 200µl of patient plasma was needed. 56 tests were performed. Samples were prepared by adding 1000µl of TRIS buffer and 200µl of patient sample to 56 individually-labelled 15ml tubes. The contents were mixed well. Thereafter 400µl of uricase was added to each tube. Grossly lipaemic samples were diluted before analysis on the spectrophotometer.

Test procedure

The standard curve samples were analysed on the spectrophotometer, using an absorbance of 283nm in order to generate a calibration curve. The samples were prepared and then immediately analysed on the spectrophotometer, using an absorbance of 283nm. The quartz cuvette was rinsed with distilled water between samples. The samples were then incubated for three hours in a 37ºC incubator and

analysed once again on the spectrophotometer, using the same wavelength. Results were recorded and analysed.

2.4.6 Analysis of results

GraphPad Prism version 5.0 was used to analyse the results

2.5 CRP measurement

CRP, an acute phase protein that increases rapidly in serum during inflammation and infection,[29] was also quantified at both of the patient's visits. This assay was performed by the National Health Laboratory Service (NHLS) by means of ELISA kits specific for human CRP which covered the full range of possible CRP levels. These levels were expected to be high during the acute gout attack and low during the inter-critical phase.

ELISA relies on the capture of the analyte by an antibody specific to the analyte. After washing away the unbound sample, the analyte will be quantified using a second antibody that is conjugated with biotin, which is then targeted with a horse radish peroxidase bound streptavidin. A colour reaction is used to quantify the analyte by comparison to a similarly treated set of calibration standards of the analyte.

Chapter three: Results

3.1 Clinical trial

3.1.1 Baseline characteristics

The following baseline characteristics and demographic information were obtained from patients at their baseline visit and analysed as follows:

Table 1: Patient demographics and baseline characteristics (n=30)

Baseline characteristics	
Age: years	
Mean	55
Range	33 – 75
Race: n (%)	
Caucasian	20 (67)
Black	8 (27)
Indian	1 (3)
Coloured	1 (3)
Sex: n (%)	
Male	28 (93)
Female	2 (7)
Comorbidities: n (%)	
Diabetes	2 (7)
Hypertension	24 (80)
Hyperlipidaemia	12 (40)
Obesity	12 (40)
Body Mass Index (BMI): kg/m^2	
Mean	29.8
Range	20.9 – 46.5
BMI categories: n (%)	
20.0 - 25.0	4 (13)
25.1 – 30.0	14 (47)
30.1 – 35.0	9 (30)
>35.1	3 (10)

3.1.2 Dietary questionnaire

Analysis of the dietary questionnaires completed by patients at their gout attack visits revealed various potential food and beverage triggers for acute gout attacks.

Table 2: Potential dietary triggers for acute gout attacks as listed by patients in their dietary questionnaires.

FOOD TRIGGER	NUMBER OF VOTES	%
Red meat	23	25
Alcohol	22	24
Tomato	14	15
Soft drinks	12	13
Other	8	9
Fruit	7	8
Green vegetables	4	4
White bread	3	3

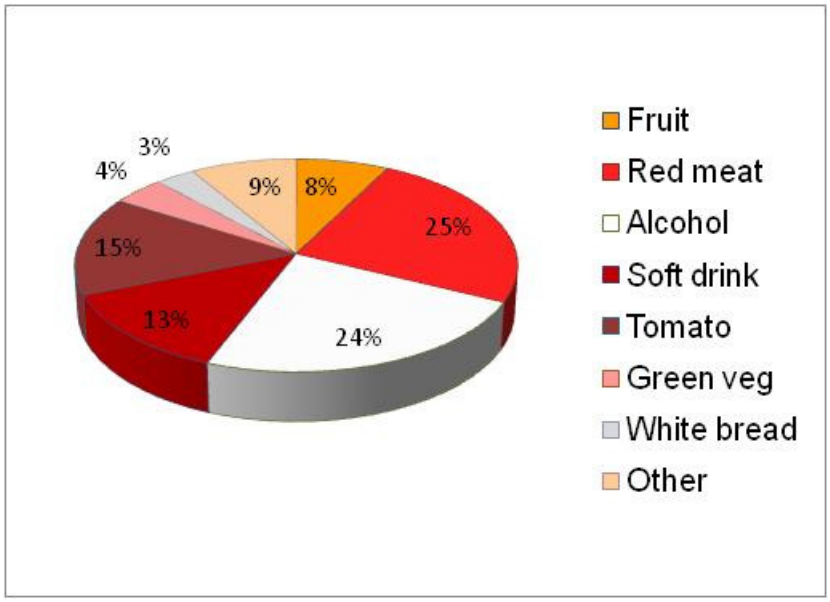

Figure 4: Potential food and beverage triggers for acute gout attacks, as listed by patients in their dietary questionnaires.

3.2 Visual analogue scales

Patients were asked to complete visual analogue scales at both visits; namely subject's assessment of pain (SAP) and subject's assessment of disease activity (SAD), as seen below. Physicians were asked to complete a physician's assessment of disease activity (PAD) for each patient at both visits.

Results for patients 2, 3, 24 and 27 were not analysed with the rest of the data as these patients only attended one clinic visit and, for various reasons, declined to return for their second visit.

Results were analysed by means of a T-test. Upon analysis of results, an increase was found in all three visual analogue scales between baseline and second visits, however none of these increases were statistically significant.

The mean subject's assessment of pain score showed an increase from 31mm at the screening visit (SAP1) to 40mm at the end visit (SAP2) ($p=0.1947$; $n=26$)

The mean subject's assessment of disease activity score increased from 30mm at the screening visit (SAD1) to 37mm at the end visit (SAD2) ($p=0.3196$; $n=26$). However, this was not a statistically significant increase.

The mean physician's assessment of disease activity score once again showed an increase from 23mm at the screening visit (PAD1) to 35 mm at the end visit (PAD2) ($p=0.0937$; $n=26$), however this was once again not a statistically significant increase.

Individual patient's scores for each visual analogue scale for both the baseline and the second visits are listed in table 3 and graphically represented in figures 5-8. A general trend was observed in these figures, all three mean visual analogue scale scores increased between baseline and second visits, however none of these increases were statistically significant.

The twenty-six patients' two visits were then further subdivided, based on gout activity. An overall visual analogue scale score was determined for each patient for each visit by determining the average of SAP, SAD and PAD. Patients with an overall score of greater than or equal to 50mm were classified as having active gout (acute gout), whereas patients with a score less than 50mm were classified as having non-active gout (intercritical gout). This overall visual analogue scale score was used to determine level of disease activity, as it includes both the subjective SAP and SAD scores, as well as the more objective PAD score.

SAP and SAD were also averaged for each patient for each visit in order to determine the subject's overall visual analogue scale score. Active gout was defined as a subject's score of greater than or equal to 50mm.

The overall visual analogue scale score was compared with both the subject's overall visual analogue scale score and the physician's assessement of disease activity score in order to analyse objectivity of the scales, as seen in table 4 and 5. Table 4 compared these three scores at the patients' baseline visits, while table 5 compared these three scores at the patients' second visits.

Table 3: Individual patients' scores for the visual analogue scales performed at both baseline and second visits (n=26).

Patient Number	SAP1 (mm)	SAP2 (mm)	SAD1 (mm)	SAD2 (mm)	PAD1 (mm)	PAD2 (mm)
1	54	55	54	33	72	29
4	3	60	3	52	3	55
5	54	51	55	25	23	4
6	72	18	97	19	76	19
7	47	19	46	21	42	16
8	27	23	25	7	1	9
9	79	52	70	77	20	50
10	4	18	16	16	2	20
11	58	12	51	21	71	17
12	3	12	6	11	23	56
13	7	0	5	3	34	0
14	29	20	11	12	14	8
15	85	38	14	41	28	30
16	0	1	14	1	1	4
17	3	50	2	56	2	41
18	23	44	23	41	29	37
19	16	23	20	8	27	51
20	13	64	17	78	32	59
21	27	41	77	74	3	91
22	28	23	13	20	4	5
23	11	40	19	33	1	5
26	27	78	21	63	1	75
28	27	94	30	88	42	91
29	1	63	1	8	2	11
30	48	50	63	66	11	59
31	52	78	29	76	23	65
Average	**31**	**40**	**30**	**37**	**23**	**35**

SAP1 & SAP2: Individual scores for subjects assessment of pain for visit 1(baseline visit) and visit 2 (gout attack visit) respectively.

SAD1 & SAD2: Individual scores for subjects assessment of disease activity for visit 1(baseline visit) and visit 2 (gout attack visit) respectively.

PAD1 & PAD2: Individual scores for the physician's assessment of disease activity for visit 1(baseline visit) and visit 2 (gout attack visit) respectively

The results of the following patients: 2, 3, 24, 25 and 27 were excluded from the table. Patient 25 was a screen failure and never took part in the trial, while the results of the other patients were excluded as they dropped out of the clinical trial without returning for their second visits.

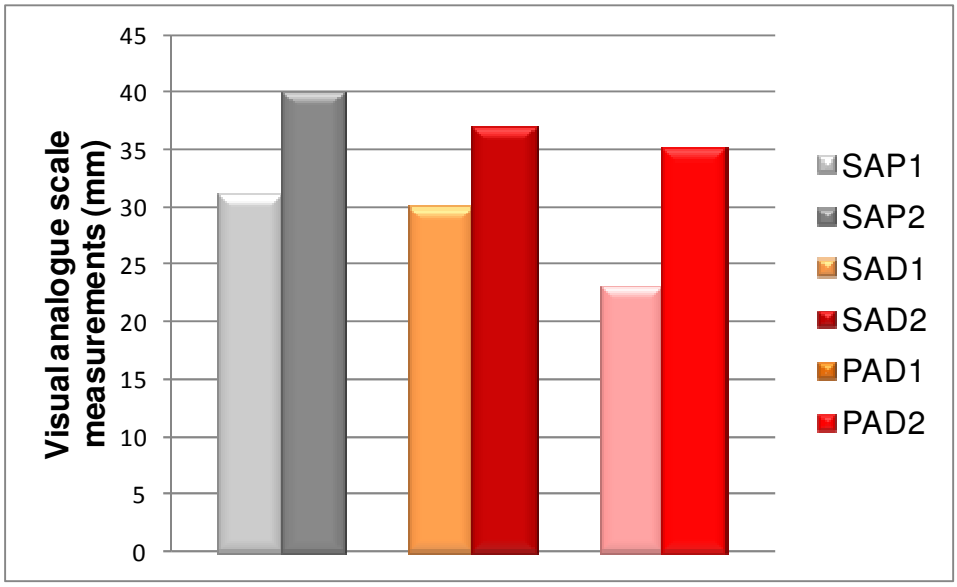

Figure 5: Comparison of mean visual analogue scale scores between baseline and second visits (n=26).

SAP1 & SAP2: Mean scores for subjects assessment of pain for visit 1(baseline visit) and visit 2 (second visit) respectively.

SAD1 & SAD2: Mean scores for subjects assessment of disease activity for visit 1(baseline visit) and visit 2 (second visit) respectively.

PAD1 & PAD2: Mean scores for the physician's assessment of disease activity for visit 1(baseline visit) and visit 2 (second visit) respectively.

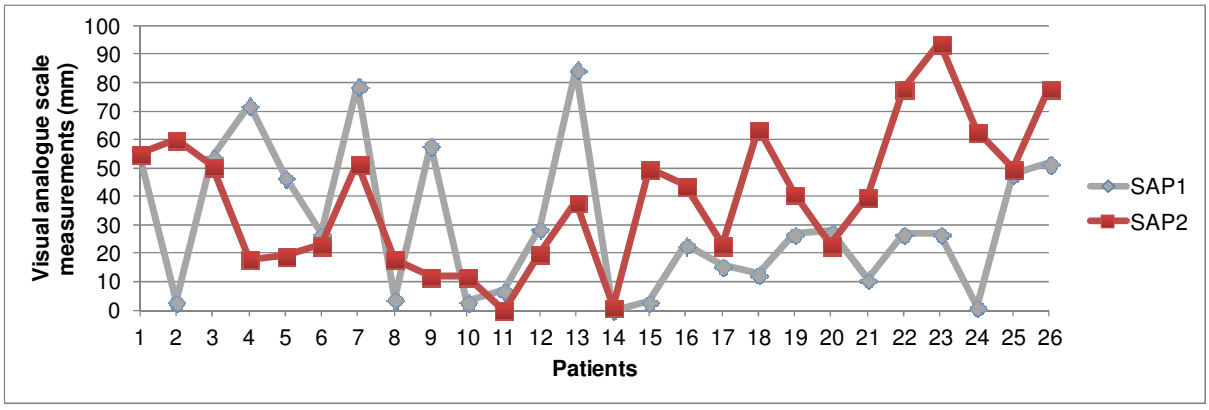

Figure 6: Comparison of individual patients' scores for the subjects assessment of pain between baseline and second visits.

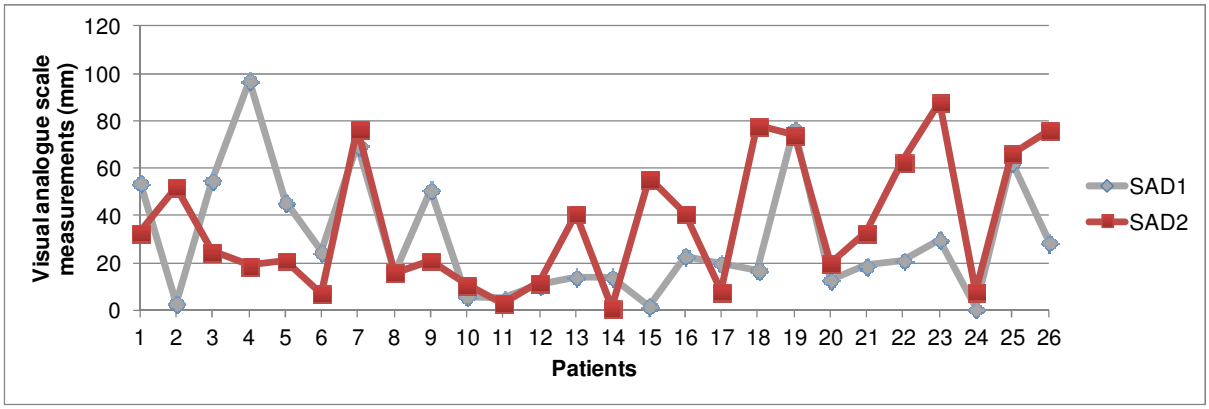

Figure 7: Comparison of individual patients' scores the for subjects assessment of disease activity between baseline and second visits.

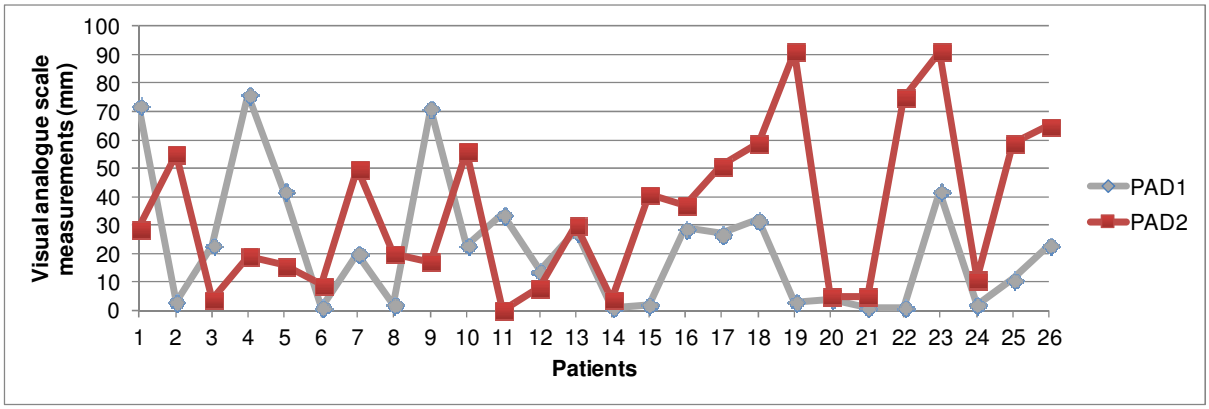

Figure 8: Comparison of individual patients' scores for the physician's assessment of disease activity between baseline and second visits.

Table 4: Comparison of gout activity by assessment of overall, subject's and physician's scores for individual patients at the baseline visit.

Patient Number	Overall score (mm)	Active (Y/N)	Subjects score (mm)	Active (Y/N)	Physicians score (mm)	Active (Y/N)
1	60	Y	54	Y	72	Y
4	3	N	3	N	3	N
5	44	N	55	Y	23	N
6	82	Y	85	Y	76	Y
7	45	N	47	N	42	N
8	18	N	26	N	1	N
9	56	Y	75	Y	20	N
10	7	N	10	N	2	N
11	60	Y	55	Y	71	Y
12	11	N	5	N	23	N
13	15	N	6	N	34	N
14	18	N	20	N	14	N
15	42	N	50	Y	28	N
16	5	N	7	N	1	N
17	2	N	3	N	2	N
18	25	N	23	N	29	N
19	21	N	18	N	27	N
20	21	N	15	N	32	N
21	36	N	52	Y	3	N
22	15	N	21	N	4	N
23	10	N	15	N	1	N
26	16	N	24	N	1	N
28	33	N	29	N	42	N
29	1	N	1	N	2	N
30	41	N	56	Y	11	N
31	35	N	41	N	23	N
Average	**28**	**N**	**30**	**N**	**23**	**N**

Based on their overall visual analogue scale scores, patients 1, 6, 9 and 11 were classified as having active gout at their first visits, while the rest of the patients were classified as having intercritical gout. The overall score was used to assess activity because it combines both the subject's and physician's scores. The overall score, subject's score and physician's score tended to reach the same conclusion about activity in the majority of patients.

Table 5: Comparison of gout activity by assessment of overall, subject's and physician's scores for individual patients at the second visit.

Patient Number	Overall score (mm)	Active (Y/N)	Subjects score (mm)	Active (Y/N)	Physicians score (mm)	Active (Y/N)
1	39	N	44	N	29	N
4	56	Y	56	Y	55	Y
5	27	N	38	N	4	N
6	19	N	19	N	19	N
7	19	N	20	N	16	N
8	13	N	15	N	9	N
9	60	Y	65	Y	50	Y
10	18	N	17	N	20	N
11	17	N	17	N	17	N
12	26	N	12	N	56	Y
13	1	N	2	N	0	N
14	13	N	16	N	8	N
15	36	N	40	N	30	N
16	2	N	1	N	4	N
17	49	N	53	Y	41	N
18	41	N	43	N	37	N
19	27	N	16	N	51	Y
20	67	Y	71	Y	59	Y
21	69	Y	58	Y	91	Y
22	16	N	22	N	5	N
23	26	N	37	N	5	N
26	72	Y	71	Y	75	Y
28	91	Y	91	Y	91	Y
29	27	N	36	N	11	N
30	58	Y	58	Y	59	Y
31	73	Y	77	Y	65	Y
Average	**37**	**N**	**38**	**N**	**35**	**N**

Once again based on their overall visual analogue scale scores, patients 4, 9, 20, 21, 26, 28, 30 and 31 were classified as having active gout at their second visits, while the rest of the patients were classified as having intercritical gout. It was expected that a much higher percentage of patients would have active gout at the second visit, however for numerous reasons, patients failed to return to the clinic during their gout attacks.

Similarly to what was found in table 4, the overall score, subject's score and physician's score tended to reach the same conclusion about activity in the majority of patients.

3.3 Cytokine measurement

Concentrations of IL-1β and TNF-α cytokines were determined by means of a fluorescent bead immunoassay. The majority of the patients had undetectable cytokine levels, consequently these patients' results were not displayed in table 6 and 7.

Results were analysed using the Wilcoxon matched-pairs signed rank test, as the results were not normally distributed. The following tables indicate whether patients experienced an increase or a decrease in cytokine concentrations between baseline and second visits.

Table 6: Concentrations of IL-1β cytokine for individual patients at both the baseline and second visits.

Patient	Baseline visit (pg/ml)	Second visit (pg/ml)	Change (pg/ml)
IL-1β			
1	0	231	+231
5	47	0	-47
16	0	70	+70
20	198	0	-198
22	47	0	-47
Average	**12.17**	**12.54**	**+0.37**

Table 7: Concentrations of TNF-α cytokine for individual patients at both the baseline and second visits.

Patient	Baseline visit (pg/ml)	Second visit (pg/ml)	Change (pg/ml)
TNF-α			
1	0	58	+58
5	139	0	-139
7	54	0	-54
9	22	0	-22
20	32	0	-32
29	1	27	+26
31	55	0	-55
Average	**12.63**	**3.54**	**-9.09**

A slight increase of 0.37pg/ml was observed in IL-1β concentrations between baseline and second visits, whereas TNF-α concentrations decreased by 9.09pg/ml between visits.

3.4 Uric acid

Serum uric acid levels were determined using a uricase spectrophotometric method. Absorbance values were determined for each patient sample before and after the addition of uricase. The change in the absorbance values was then compared to calibration curves of uric acid standards in order to determine uric acid concentrations. Experiments were performed in triplicate. This procedure was repeated for each patient for both the baseline and the second visits.

The following table indicates whether patients experienced an increase or a decrease in serum uric acid concentrations between baseline and second visits. Results for patients 2, 3, 15, 24, 25 and 27 have been excluded from the table. (n=25) Patients 2, 3, 24 and 27 did not return for their second clinic visit, patient 25 was considered to be a screen failure and patient 15 had a negative uric acid concentration value due to experimental error.

Results were analysed using the Wilcoxon matched-pairs signed rank test, as the results were not normally distributed.

Table 8: Change in individual patients' uric acid concentrations between the baseline and second visits (n=25).

Patient	Baseline visit (mmol/L)	Second visit (mmol/L)	Change (mmol/L)
1	1.935	1.282	-0.653
4	0.750	0.990	+0.240
5	0.808	0.912	+0.104
6	1.415	0.647	-0.768
7	1.012	0.958	-0.054
8	1.372	0.741	-0.631
9	0.873	1.082	+0.209
10	0.778	1.119	+0.341
11	1.452	0.677	-0.775
12	0.603	1.236	+0.633
13	1.044	0.464	-0.580
14	1.029	0.465	-0.564
16	1.086	0.496	-0.590
17	1.119	0.428	-0.691
18	1.063	0.522	-0.541
19	0.695	0.658	-0.037
20	0.892	0.845	-0.047
21	0.836	1.303	+0.467
22	0..793	1.281	+0.488
23	0.800	1.162	+0.362
26	1.000	0.791	-0.209
28	0.937	0.827	-0.110
29	0.969	1.063	+0.093
30	1.114	1.069	-0.045
31	1.943	0.767	-1.176
Average	**1.053**	**0.871**	**-0.182**

A decrease was observed in the mean patient serum uric acid concentration between baseline and second visits. This trend was followed in 64% of patients.

3.5 C-Reactive Protein (CRP)

Serum samples were sent to the National Health Laboratory Service (NHLS) for analysis of CRP levels at both patient visits. CRP concentrations were determined by means of an ELISA kit. The following table indicates whether patients experienced an increase or a decrease in CRP concentrations between baseline and second visits. Results were analysed using the Wilcoxon matched-pairs signed rank test, as the results were not normally distributed.

Results for patients 2, 3, 12, 24, 25, 27 and 28 have been excluded from the table. (n=24) Patients 2, 3, 24 and 27 did not return for their second clinic visit, patient 25 was considered to be a screen failure and CRP results were unavailable for both patient 12 and 28.

A statistically significant increase in mean patient CRP concentrations was seen in table 7. CRP concentrations increased from 10.2mg/L at the baseline visit to 26.6mg/L at the second visit. An increase in CRP concentrations was noted in 63% of patients.

Table 9: Change in individual patients' CRP concentrations between the baseline and second visits (n=24).

Patient	Baseline visit (mg/L)	Second visit (mg/L)	Change (mg/L)
1	6.5	8.3	+1.8
4	1.1	2.0	+0.9
5	3.7	6.0	+2.3
6	22.4	33.4	+11.0
7	4.9	11.4	+6.5
8	3.3	3.9	+0.6
9	6.0	4.5	-1.5
10	2.2	1.0	-1.2
11	8.3	6.2	-2.1
13	5.6	4.8	-0.8
14	31.5	60.9	+29.4
15	3.8	2.0	-1.8
16	1.7	1.5	-0.2
17	16.4	17.6	+1.2
18	17.6	35.6	+18.0
19	13.0	5.4	-7.6
20	4.9	16.5	+11.6
21	60.0	362.7	+302.7
22	14.3	21.4	+7.1
23	1.4	1.3	-0.1
26	4.5	6.2	+1.7
29	6.9	5.9	-1.0
30	3.7	5.5	+1.8
31	1.3	14.5	+13.2
Average	**10.2**	**26.6**	**16.4**

3.6 Summary

Blood parameter results for patients were separated according to visit number, as seen in Figure 9, and according to level of gout activity (by using the overall visual analogue scale scores) in order to ascertain whether an association exists between level of gout activity and inflammatory markers, as seen in Figure 10.

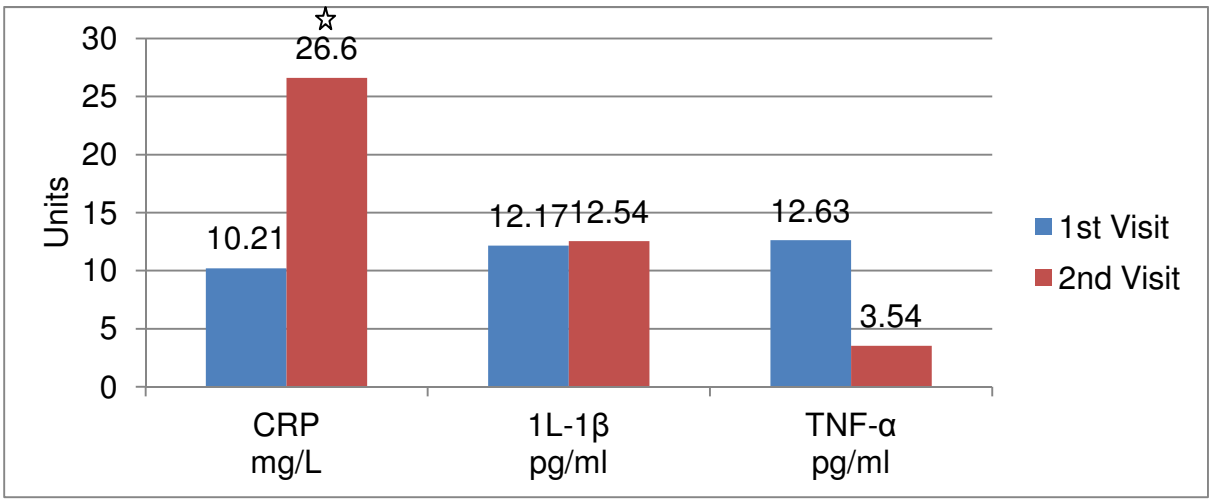

Figure 9: Mean serum inflammatory marker results separated according to baseline and second visits. [☆ Indicates statistical significance (p<0.05)]

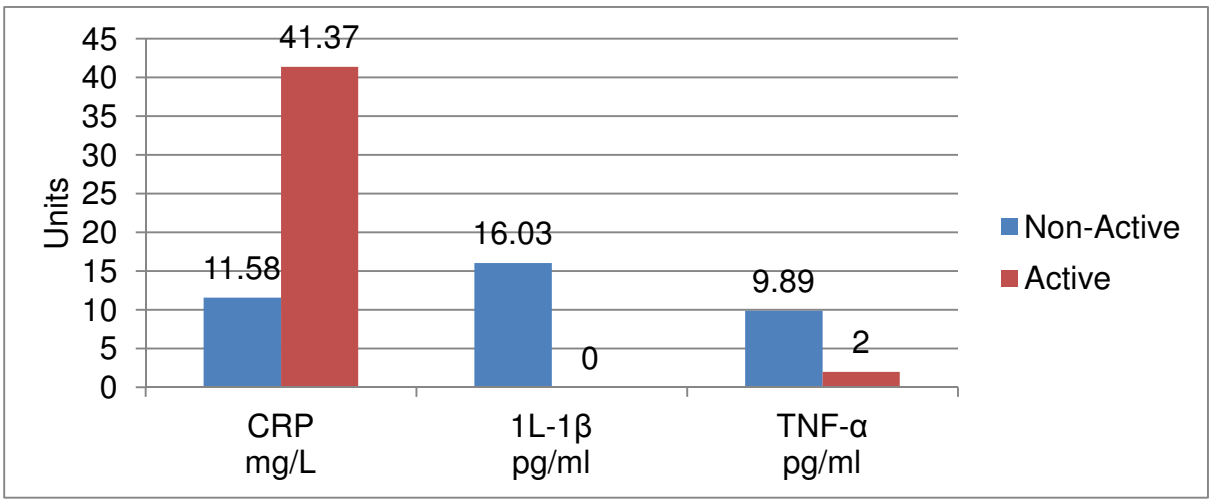

Figure 10: Mean serum inflammatory marker results separated according to level of gout activity based on the overall visual analogue scale scores.

Chapter 4: Discussion

4.1 Clinical trial

4.1.1 Demographics

Patients completed a demographic questionnaire, the results of which showed that 20 of the patients (67%) were Caucasian, 8 (27%) were Black, 1 (3%) was Indian and 1 (3%) was Coloured.

Although previous studies have shown the prevalence of gout is higher in black men than in white men, the majority of patients in this study were Caucasian and Black patients only made up a small percentage of the total sample. However, while this might not be representative of the South African population, this patient sample was randomly selected. The first thirty responders who met the study requirements were enrolled and this selection process did not take race into consideration.[30]

The mean age of the patients was 55 years, with a range of 33 to 75 years. The incidence of gout increases rapidly between the ages of 30 and 50 years, and the prevalence then continues to increase with age.[4]

The vast majority of the patients screened; 28 (93%) were male, while only 2 (7%) were female. Literature states that the prevalence of gout is generally higher in men than in women, although this is reversed after women reach menopause, whereupon women are more afflicted by gout than men.[1] Both of the women enrolled in the trial were postmenopausal.

4.1.2 Diet

It is well known that various foodstuffs are associated with acute gout attacks.[2,3] The following triggers of acute gout attacks were listed by patients in their food questionnaires: red meat, alcoholic beverages, tomatoes, carbonated beverages, fruit, green vegetables, white bread, sweets, nuts, porridge and fried eggs.

Of the ninety-three food and beverage triggers listed by patients, 25% were classified as red meat, 24% as alcoholic beverages, 15% as tomatoes, 13% as carbonated beverages, 8% as fruit, 4% as green vegetables, 3% as white bread and 9% as other foodstuff.

This clearly indicates the importance of red meat and alcoholic beverages as potential triggers for acute gout flares. Many of the patients stated that they tried to decrease their intake of the above-mentioned foodstuffs in order to avoid acute gout attacks.

The Third National Health and Nutrition Examination survey performed in the US assessed the relationship between serum uric acid levels and various foodstuffs by means of a food frequency questionnaire. The study showed that serum uric acid levels increased with increased total meat or seafood intake, while an increased dairy intake decreased serum uric acid levels. However, although this was a nationally representative sample of men and women, gout patients, as well as patients receiving hypouricemic therapy, were excluded.[31]

Increased seafood intake has long been referred to as a risk factor for gouty flares.[2,3] Contrary to this, not a single patient listed any form of seafood as a potential trigger during completion of their food questionnaires. This could be due to the fact

that many of the patients informed the investigator that seafood was not part of their regular diet, due to the high cost of seafood.

While patients in this study listed fruit as a potential trigger for acute gout attacks, another study has stated that the risk of gout actually declines with increasing fruit intake, although this could be due to the fact that those patients who ate large amounts of fruit also maintained healthier lifestyles in general.[32]

4.1.3 Body mass index (BMI)

An increased body mass index (BMI) and waist circumference has been said to increase the risk of gout.[32] In fact, in one particular study, when comparing patients with a BMI of 21-22.9 to patients with a BMI of larger than or equal to 35, multivariate relative risk of gout increased from 1.95 to 2.97 respectively.[33]

The mean BMI of the patients in this study was 29.8, which is classified as overweight. Of the thirty patients screened; 4 (13%) fell within the normal healthy range with a BMI of 20-25, 14 (47%) were classified as overweight with a BMI of 25-30, 9 (30%) were classified as obese with a BMI of 30-35 and 3 (10%) were classified as morbidly obese with a BMI greater than 35.

A calorie restriction pilot study was performed in 13 non-diabetic gout patients with an average BMI of 30.5. Investigators restricted patients' calorie intake to 1600kcal per day for 16 weeks, which resulted in significantly decreased serum uric acid levels and improved dyslipidemia. Patients, on average, lost 7.7 kg and decreased their serum cholesterol, triacylglycerol and LDL-C levels while increasing their HDL-C levels.[34]

86% of the patients in this gout trial were classified as either; overweight, obese or morbidly obese based on their body mass indexes (BMI's) and as such, could also possibly benefit from a similar calorie reduction, particularly as the calorie reduction in the above-mentioned study resulted in a significant decrease in the amount of gout flares experienced by the patients.

4.1.4 Hypertension

Upon examination of all of the patients' medical histories, 24 patients (80%) confirmed that they had been diagnosed with hypertension and were being treated for this condition. Hypertension has been linked with gout on numerous occasions, in one study the presence of hypertension in patients increased the multivariate relative risk of gout.[33]

Hypertension can cause hyperuricemia by decreasing renal blood flow which can cause increased reabsorption of urate.[35] As such, hypertension remains an important parameter to monitor in the management of chronic gout.

4.1.5 Hyperlipidemia

Twelve (40%) of the patients had previously been diagnosed with hyperlipidemia, however this was not confirmed with laboratory testing. Patients 1, 2, 3, 6 and 15 had grossly lipemic serum samples that needed to be diluted before analysis on the spectrophotometer. Of these patients, only patient 1 and 3 actually confirmed a history of hyperlipidemia, leading us to speculate that the percentage of patients with hyperlipidemia may have been even higher than stated.

4.1.6 Diabetes

Only two of the patients (7%) were confirmed diabetics, however many of the patients displayed risk factors for the development of type II diabetes mellitus. This, however, was once again not confirmed with laboratory testing.

Insulin influences the excretion of uric acid from the kidneys by increasing urate reabsorption. This makes insulin resistance an important parameter to monitor in gout patients.[32]

A 6-year follow-up of the Multiple Risk Factor Intervention Trial (MRFIT) established that men with gout have a higher risk of developing type II diabetes. These results were consistent with those obtained from the Finnish diabetes prevention study.[36]

Given the severity of the long term effects of type II diabetes and the association of gout with this disease, gout patients should be frequently tested for diabetes.

4.1.7 Risk for metabolic syndrome

High serum uric acid levels and gout have been associated with metabolic syndrome, a condition consisting of all of the above-mentioned comorbidities; namely: hypertension, hyperinsulinemia, dyslipidemia and obesity.[37, 38]

Studies have shown that up to 76% of gout patients also have metabolic syndrome.[35] Many of the patients involved in this study display the risk factors for this disease.

4.2 Visual analogue scales

Patients were asked to complete visual analogue scales at both visits; namely subject's assessment of pain and subject's assessment of disease activity. Physicians were asked to complete a physician's assessment of disease activity for each patient at both visits.

Patients 2, 3, 24 and 27 dropped out of the clinical trial for various reasons and as such only completed one set of visual analogue scales. Although the clinic tried to contact both patient 2 and patient 24 on numerous occasions, they did not answer any of their contact numbers. Patient 3 chose not to come back for his follow-up visit, while patient 27 moved further away from the clinic and informed the clinic that he could no longer attend a follow-up visit.

All of the visual analogue scales showed an increase between the two visits, however these increases were not statistically significant. Of particular interest is the physician's assessment of disease activity. The major criticism of the use of the visual analogue scales is that they are subjective, as pain in particular is inherently subjective. The physician's assessment of disease activity is, however, objective as the same independent physician assesses the same patient's disease activity at both visits.

When comparing the baseline mean subjects assessment of disease activity score to that of the baseline mean physician's assessment of disease activity score, it can be seen that the patients mean score of 30mm is much higher than that of the physician's mean score of 23mm.

This could possibly indicate that patients tend to overestimate their disease activity in periods where they do not experience gout attacks. This leads us to question

whether the subjects assessment of pain was also overestimated? This question can unfortunately not be answered, as only the patients, and not the physician, completed an assessment of pain.

Upon comparison of the follow-up visit's mean assessments of disease activity, it was found that both the mean subjects assessment of 37mm and the mean physician's assessment of 35mm were similar to each other, indicating that while each individual patient's assessment of disease activity may not have agreed with that of the physician, the general trend in mean scores shows similarity.

The visual analogue scale scores were then stratified according to overall, subject and physician's scores and these were then used to define active or non-active gout. Upon comparison of these three scores for the patients' baseline visits, all three of these reached the same conclusion in 21 of the 26 patients (81%). Similarly, upon comparison of these scores for the patients' second visits, all three scores reached the same conclusion in 23 of the 26 patients (88%).

So, while scores show that patients tend to overestimate the severity of their gout attacks, their scores generally remain in the same range as the physician's scores, which shows that although the patients' assessment of pain and disease activity is subjective, it is still a reliable indicator of gout activity.

A study recently completed in the UK assessed the quality of life of gout patients compared to that of control patients. This study required the patients of two general practices to complete quality of life (QOL) questionnaires. Analysis of the returned questionnaires displayed impaired satisfaction with health, impaired physical-related health quality of life (QOL) as well as impaired overall quality of life (QOL) for the

gout patients as compared to the control patients. These decreases were all statistically significant.[39]

Quality of life can be assessed in numerous ways, but visual analogue scales are frequently used to assess gout in clinical trials. As such the validity of these scales needs to be established. One research team assessed the validity of pain and patient global scales in chronic gout patients involved in the pegloticase clinical trials. They discovered that visual analogue scales correlated with results obtained from tender and swollen joint counts as well as the SF-36, a body pain subscale. The authors concluded that the visual analogue scales are valid outcome measures for the evaluation of gout patients.[40]

These results show that visual analogue scales are a reliable indicator of gout activity.

4.3 Cytokine measurement

The proposed mechanism for the inflammatory reaction experienced in an acute gout attack is that phagocytosed monosodium urate crystals activate the NLRP3 inflammasome which in turn activates caspase 1. Caspase 1 is responsible for the cleavage of pro IL-1β and the formation of mature IL-1β. IL-1β then initiates the production of numerous other inflammatory cytokines, TNF-α is included amongst these. As such, high concentrations of both of these pro-inflammatory cytokines were expected during acute gout flares.[41]

At the baseline visit, patients had not had a gout attack for four weeks. As such it was expected that the pro-inflammatory cytokine concentrations would be negligible. However, it was expected that the pro-inflammatory cytokine concentrations would

show a statistically significant increase from the patients' baseline visits to the second visits.

This was, however, not the case. The mean IL-1β concentrations were similar for both baseline and second visits, 12.17pg/ml and 12.54pg/ml respectively. Conversely, mean TNF-α concentrations actually displayed a decrease between baseline (12.63 pg/ml) and second visits (3.54 pg/ml), although this difference was not statistically significant. This could be due to the fact that very few patients experienced acute gout attacks at their second visits.

Very low cytokine concentrations were determined for all the patients for both IL-1β and TNFα. In fact, the majority of patients displayed concentration values that were below the limit of detection for this experiment.

The standards included in the kit which were diluted and used to create a standard curve, produced expected results. This indicates that the fault did not lie with the kit. A possible reason for these unexpected results could be that IL-1β and TNF-α were present for a short time period only after production during inflammation and that patients reported their gout attacks too late, thereby missing this period.

Patients for whom results were available were classified as having either active or non-active gout according to their overall visual analogue scale scores. Upon analysis of these results, only 11 cases of active gout were found, while 37 cases of non-active gout were found.

Many of the patients, upon returning for their second clinic visit, claimed that they had experienced numerous gout attacks during the interval between clinic visits,

however, they chose not to come into the clinic during these flare-ups. Numerous reasons were provided for this, two of the most frequently experienced issues were transport problems and the disabling nature of the gout attacks which prevented normal every-day functioning.

Upon stratifying mean values of patients' inflammatory cytokines, namely; IL-1β and TNF-α, according to gout activity from the patients' overall visual analogue scale scores, once again no statistically significant differences were found. IL-1β concentrations decreased ($p=0.2116$) from 16.03pg/ml to 0pg/ml between non-active and active gout patient groups respectively. TNF-α concentrations also decreased ($p=0.4058$) from 9.89pg/ml to 2.0pg/ml between non-active and active gout patient groups respectively.

This study has shown that there appears to be no definite association between the level of gout activity and these two pro-inflammatory cytokines, this could once again be due to the timing of the blood withdrawals and patient clinic visits, as discussed above.

Although high concentrations of both IL-1β and TNF-α have been found in the synovial fluid of gout patients, little research has been done on the serum concentration of these two pro-inflammatory cytokines in acute gout.

One particular study demonstrated that no significant differences were found in either IL-1β or TNF-α concentrations between intercritical gout and acute gout. This supports the conclusion in this study, but leads us to question how the synovial fluid concentrations and serum concentrations of these cytokines can differ so greatly.

The first hypothesis is that the cytokines were rapidly removed from the blood and broken down, before the blood was drawn from the patient. The second hypothesis is that IL-1β and TNF-α could remain localised within the affected joints. Either one of these hypotheses could explain the low concentrations of cytokines found in this study.[42]

4.4 Uric acid

High serum uric acid levels have been associated with numerous diseases including; chronic renal disease, metabolic syndrome, gout, hypertension and cardiovascular disease. Furthermore, over the past few years, low serum uric acid levels have been associated with increased prevalence and progression of certain neurological disorders.[43]

Normal serum uric acid levels range between 240µmol/L and 480µmol/L or 0.24mmol/L and 0.48mmol/L. However these normal values do tend to vary between laboratories.[44]

Patients with hyperuricemia may be treated with a variety of urate-lowering drugs, including but not limited to; allopurinol, probenecid and febuxostat.[1,2,9] The aim of chronic urate-lowering therapy is to reduce patient serum uric acid levels to below 360µmol/L as this is below the saturation point of monosodium urate. This therapy thus aims to prevent the formation of new monosodium urate crystals, as well as to dissolve any crystals that are already present.[45]

Four patients; namely patients 2, 3, 24 and 27 dropped out of the clinical trial after the first visit and did not return for their gout attack visit. These patients values were thus not included in the statistical analysis of the mean serum uric acid concentrations between visits 1 and 2.

Patients 1, 2, 3 and 6 from the first visit and patient 15 from the second visit had grossly lipemic samples that needed to be diluted before analysis on the spectrophotometer. Despite these dilutions, these patient samples had very high absorbance values and consequently much higher serum uric acid concentrations than the rest of the patient population. However, it is not known whether these high absorbance values were due to high serum uric acid levels or if they were simply the result of high serum lipid content.

Studies have shown there is an association between an atherogenic serum lipid profile and more active rheumatoid arthritis. An atherogenic serum lipid profile is said to have pro-inflammatory properties, this hypothesis was tested in gout patients in order to determine whether atherogenic serum lipid profiles predict gout attacks.[46]

Although numerous other studies have confirmed that an atherogenic serum lipid profile is associated with higher incidence of gout, [46, 47, 48] one particular study indicated that low serum levels of HDL-C was an important predictor of more frequently occurring gout attacks. However, a major limitation of this study was that the 96% of these patients were either of ethnic Chinese or Malay origin, which makes comparison difficult with this study, as the majority of the patients enrolled were of Caucasian origin. Despite this limitation, this study still leads us to question whether optimisation of serum HDL-C levels in gout patients might reduce the occurrence of gouty flares.[46]

Patient 3 and patient 15 displayed “negative” serum uric acid values, which is clearly impossible. This is due to the fact that the initial absorbance values before incubation of these two samples were lower than the absorbance values after incubation, which is clearly incorrect and is due to operator error. A possible reason

for this error could be that less than the required 1.5ml of sample was added to the cuvette for analysis before incubation. These patients' values were thus not included in the statistical analysis of the mean serum uric acid concentrations between visits 1 and 2.

A limit of detection was determined for the standard curve, although this limit was considerably lower than the range of serum uric acid concentrations obtained and the limits of detection did as such not affect any of the patient values.

The mean serum uric acid concentration for the first visit was 1.053mmol/L, this mean decreased to 0.871mmol/L for the second visit. Although there was an decrease in the mean serum uric acid values between the patient visits, this decrease was not statistically significant ($p=0.0926$; $n=25$)

A possible reason for this decrease could be that 80% of the patients were using allopurinol which decreased the serum uric acid concentrations. Another possible explanation could be that the serum uric acid concentrations dropped due to crystallisation of the uric acid in the patients' tophi.

The mean serum uric acid concentration obtained for the first visit was more than double that of the upper limit of the normal range of serum uric acid concentrations stated above, whereas the mean value obtained for the second visit was also much higher than the upper limit of the normal range. These results demonstrate patients with exceedingly high serum uric acid concentrations. This could be due to uncontrolled gout, however as the majority of these patients (80%) were using the urate-lowering drug allopurinol, it is more likely a direct result of atherogenic serum samples that increased absorbance values and consequently, concentration values, as found with the grossly lipemic samples for patients 1, 2, 3, 6 and 15.

Upon stratifying mean serum uric acid concentrations according to gout activity by using the patients' overall visual analogue scale scores, once again no statistically significant differences were found. Serum uric acid concentrations increased from 0.912mmol/L for patients with inactive gout to 1.138mmol/L for patients with active gout (p=0.0678).

Research on the impact of acute gout on serum uric acid levels demonstrates varying results. A Japanese retrospective cohort study found that almost half of the patients with acute gout had normal serum uric acid levels, whilst the Framingham Heart study found normal serum uric acid levels in one third of gout patients.[49]

Other studies have also found that acute gout attacks are accompanied by an increase in serum uric acid concentrations.[50] However, other studies have disputed this by demonstrating by means of a clinical trial that almost half of the patients with acute gout attacks (49%) have decreased or normal serum uric acid levels.[51]

Despite the fact that the American College of Rheumatism (ACR) criteria include hyperuricemia as one of the possible criteria for the diagnosis of gout, the European League Against Rheumatism (EULAR) has stated that serum uric acid levels have "limited diagnostic value" during acute gout. This is due to the fact that normal serum uric acid levels have been found in gout patients with confirmed urate crystals. The EULAR hypothesise that this could be due to increased excretion of uric acid by the kidneys during acute flares or that uric acid could possibly act as a negative acute-phase reactant.[49]

4.5 C-Reactive Protein (CRP)

In certain instances, gout attacks may cause a systemic inflammatory reaction, whereby fevers and leukocytosis may be experienced in conjunction with increased C-Reactive Protein (CRP) levels and increased erythrocyte sedimentation rates (ESR). Due to this, high levels of CRP were expected in patients experiencing acute gout attacks.[52]

A negative correlation has been found in previous studies between serum uric acid levels and CRP levels during acute attacks, CRP levels increase while serum uric acid levels decrease.[51]

While the mean serum uric acid concentrations in this study did show a decrease between baseline and second visits, when stratifying uric acid concentrations based on gout attack visits, mean serum uric acid concentrations increased slightly, although neither of these increases were deemed statistically significant. It should still be noted that serum uric acid levels were perhaps falsely elevated in this study due to interferences of lipids in the absorbance values that were used to calculate serum urate concentrations.

The mean CRP concentrations demonstrated a statistically significant increase ($p=0.0278$; $n=24$) between patients' first and second visits from 10.21mg/L to 26.60mg/L.

When comparing the patients' CRP results to those of the normal range published by the NHLS on their laboratory report forms, 0.1-7.5mg/L, the majority of the patients' baseline results fell within normal limits (67%). This decreased to 58% with the patients' second visit CRP results.

One particularly noteworthy patient, patient 21, displayed a decidedly large increase in CRP concentration from 60mg/L at his baseline visit to 362.7mg/L at his gout attack visit. This particular patient was hospitalised during his gout attack visit with an enlarged and inflamed tophus on his knee. If this particular patient's result is excluded from the statistical analysis, the mean CRP levels still show a statistically significant increase between the two visits from 8.043mg/L to 11.00mg/L($p=0.0480$, $n=23$).

Upon stratifying mean values of patients' inflammatory CRP levels according to gout activity from the patients overall visual analogue scale scores, no statistically significant difference was found. CRP concentrations increased from 11.58mg/L to 41.37mg/L ($p=0.1812$) between non-active and active gout patient groups respectively.

However, the major limitation in stratifying the uric acid, inflammatory cytokines and CRP concentrations according to gout activity, was the small number of patients that were classified as having acute gout ($n=11$). Due to the fact that this small sample did not display a Gaussian distribution, it was necessary to use non-parametric tests which limited the power of the tests.

Thus there appears to be no association between level of gout activity, as measured by overall visual analogue scales (Table 4 and table 5) and CRP levels. While this appears to contraindicate information given above, where CRP levels significantly increased ($p=0.0278$; $n=24$) between chronological visits from 10.21mg/L to 26.60mg/L, this is not the case.

It must be taken into consideration that numerous patients did not come to the clinic while experiencing a gout attack, but instead only came back after the four month

period of the trial elapsed. Only 11 patients had active or acute gout, as determined by the overall visual analogue scale scores (Table 4 and table 5). Thus, in the majority of patient cases the second visit is not actually a gout attack visit.

Therefore the results where patients were stratified according to chronological visit numbers, are different from those where patients were stratified by level of gout activity, as determined by the overall visual analogue scale scores. The results obtained for the latter group were used to reject the hypothesis that there is an association between level of gout activity and inflammatory markers.

Conclusion

Visual analogue scales proved to be a valid measure of gout activity. It was found that patients tend to slightly overestimate their level of disease activity when comparing patient responses to those of the physician. The results of these scores were used to stratify gout patients according to whether they were experiencing active gout or not at the time of their clinic visits.

Serum uric acid concentrations decreased between patient visits, although this was not a statistically significant decrease. Serum uric acid concentrations were elevated in this study, which is probably due to the interference of high cholesterol levels with the absorbance values that were used to determine urate concentrations.

Although there appeared to be no significant association between levels of gout activity and inflammatory markers; CRP, IL-1β and TNF-α, certain trends were noted. CRP levels increased between non-active and active gout patient groups, while IL-1β and TNF-α levels decreased.

However, only 11 patients had active gout, as determined by their overall visual analogue scales. This small population did not display a Gaussian distribution and, as such, it was necessary to use non-parametric tests to analyse the results of the non-active (intercritical gout) and active (acute gout) patients. This limited the power of the statistical analyses of non-active and active gout patients.

Antagonists of TNF-α and IL-1β are effective in numerous inflammatory disorders, including rheumatoid arthritis and cryoprin-associated periodic syndromes. Antagonists of IL-1β are currently under investigation for use in chronic gout patients.

In order to establish whether these antagonists will be effective in the treatment of South African gout patients, it is necessary to demonstrate increased levels of the above-mentioned pro-inflammatory cytokines in patients experiencing acute gout attacks.

In order to adequately demonstrate this association between level of gout activity and inflammatory markers, a larger sample size will be required in order to establish a population with a normal distribution. Furthermore, due to the problems experienced with patients not coming to the clinic during their gout attacks, it might be helpful to monitor these patients in a phase I clinic setting. Patients' serum uric acid, 24-hour urinary uric acid, CRP and pro-inflammatory cytokine levels could be measured continuously, while patient diets are closely monitored.

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