Low-dose methotrexate: not the hepatotoxic medication we once thought

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CHAPTER 1:   ABSTRACT

Methotrexate is a highly efficacious and frequently utilised disease-modifying medication. Concern regarding methotrexate-related hepatotoxicity has impeded the widespread application of the drug, despite a lack of high-quality evidence demonstrating a causal relationship.

Methotrexate monitoring guidelines differ across various specialities. A single centre audit (n=150) demonstrated monitoring guidelines are not adhered to in over 2/3rds of patients evaluated, and hepatological concern was a significant cause of methotrexate cessation. Risk factors for alternative causes of liver disease such as Non-alcoholic fatty liver disease were commonplace, and alcohol intake was poorly documented.

A large cross-sectional study of 600 individuals attending outpatient rheumatology and dermatology secondary care demonstrated a prevalence of liver fibrosis of 17.5%. There was no significant difference in prevalence between those taking methotrexate, and those who had never been exposed to it. Markers of adiposity; body mass index, waist circumference and fat mass were associated with an elevated FibroScan® score. Multiple linear regression demonstrated neither methotrexate prescription nor cumulative dose of methotrexate were significant predictors of liver fibrosis.

To our knowledge, this is the largest cohort study evaluating methotrexate use with liver fibrosis. There was no demonstrable relationship between the two. Although at odds with historically published reports, our findings are in keeping with the contemporaneous evidence. It seems likely that hepatotoxicity related to non-alcoholic fatty liver disease was incorrectly attributed to methotrexate.

A survey of 300 patients taking methotrexate reinforced the positive effect it had had on individuals' lives; 41% of respondents citing it's advantageous consequences. Four in ten participants reported concerns regarding potential side-effects of methotrexate, demonstrating an apprehension about potential consequences, including hepatotoxicity. This survey suggests that the out-dated concerns relating to methotrexate-related hepatotoxicity are still negatively impacting patients to this day.

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Co-Authored Publications:

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CHAPTER 6:   INTRODUCTION

6.1   Methotrexate

6.1.1   *The history of a steroid sparing agent*

Low-dose Methotrexate (MTX) is an effective treatment for a variety of immune mediated diseases; the clinical significance of which is reflected by its inclusion within WHO's list of essential medications(1). Low-dose MTX is defined as a dose ranging between 5 to 30mg of MTX given weekly(2, 3).

MTXs use dates back to 1948, when it was first reported by Farber and colleagues, to have induced remission in five children with acute leukaemia. Sidney Farber was a pathologist working in Boston's Children's Hospital. His breakthrough was a result of a disastrous clinical trial in New York, where children with advanced cancer and leukaemia were unknowingly given a folic acid agonist, rather than antagonist. Rather than curing the children, it stimulated rapid tumour growth, an 'acceleration phenomenon', with devastating consequences(4). Recognising folic acid is key to tumour replication Subbarow, a biochemist, formulated some of the early versions of MTX, aiming to competitively inhibit folic acid synthesis (5, 6). Initial formulations of the drug included 4-amino-10-methylfolic acid, 4-aminopteroylglutamic acid, aminopterin and finally amethopterin(7). The molecular structure of amethopterin, later termed MTX, is demonstrated in Figure-6-1.

Whilst Aminopterin was initially used within oncology(7, 8), Gubner (a cardiologist based in New York) and colleagues, were first to publish its steroid-sparing effects in patients with rheumatoid arthritis (RA), psoriasis, lupus and dermatitis(9). These early studies described a striking improvement in psoriatic lesions in four subjects with "psoriasis and rheumatoid arthritis", (most likely psoriatic arthritis), and amelioration of arthritic symptoms in three out of the four participants(10). However, the group cautioned against the side effects of the agent: "The toxic effects of sodium aminopterin place practical limitation on its use as a therapeutic agent"(9).
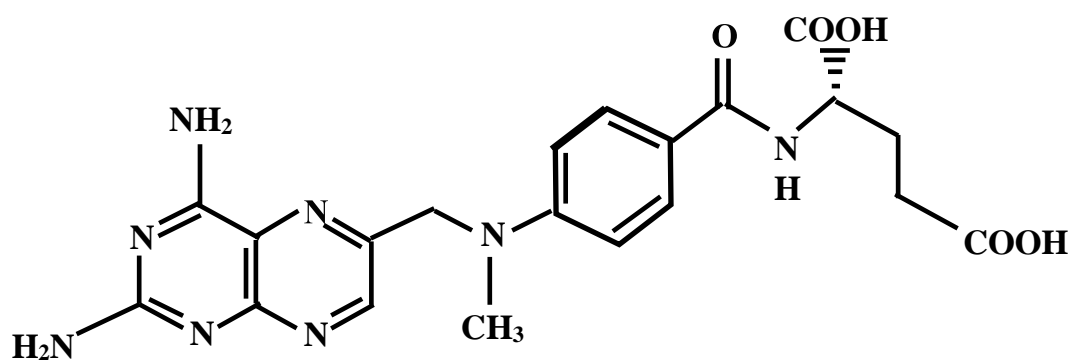
Figure-6-1 Molecular structure of MTX. Adapted from Bleyer 1978 (11)

Progress within the dermatology community outstripped that of rheumatology. In 1958, Edmundson and Guy demonstrated improved symptoms following the use of folic acid antagonists in 62 participants with psoriasis(12). O'Brien et al. replicated those findings in participants with psoriatic arthropathy (PsA), but similar improvement was not seen in their cohort of five patients with RA(13). This small, preliminary report had a big impact – shifting the focus away from RA, and firmly onto psoriasis and PsA. This study was expanded and modified, with the inclusion of MTX, rather than aminopterin, given its reported side-effects, and difficulty manufacturing the drug. In 1964 the group published the results of a double-blind cross over study in which 21 participants with PsA received MTX and placebo. Both cohorts demonstrated biochemical and clinical improvement following MTX exposure(14).

Animal studies demonstrating the immunomodulating properties of MTX (15, 16) were followed by comparator studies. Rees and Bennett's was first in patients with psoriasis (n = 37)(17), followed by Skrakosch *et al.* (n = 127) reviewing efficacy and toxicity in PsA. Both found aminopterin was clinically superior to MTX, but was associated with poorly tolerated side-effects – largely gastrointestinal in nature. This settled the debate; aminopterin's adverse side-effects rendered it a clear second place to MTX, and research focussed on the latter from here onwards(18).

The 1960s saw dermatologists gradually adopt MTX predominantly for use in psoriasis. Treatment regimens varied; orally – up to 5 times per week (2.5mg to 5mg each day) – or weekly either orally or intramuscularly at 25 to 50mg were commonplace(19, 20). Now referred to as 'low-dose', these were significantly smaller doses than oncological regimens, where higher doses were typically given intravenous or intrathecally(21). Weinstein and Frost published a new dosing regime in 1971 which became standard practice – 3 doses within 24 hours over a one week period(22). The following ten years saw this gradually replaced with the once weekly prescribing schedule still used to this day.

MTXs investigation and implementation within the rheumatology community was significantly more cautious than their dermatology colleagues. It's use as a chemotherapy agent afforded it an apparent 'drug of last resort' reputation. Furthermore, the discovery

and utilisation of 'compound E', later known as corticosteroids, for which Philip Hence and colleagues were awarded the Nobel Prize in 1950 (23), dominated. MTX was thought to be inferior to corticosteroids, and toxic in comparison (24).

It took until the mid 1960s for MTX's potential as a steroid-sparing agent to be revisited by rheumatologists, even then progress was slow. Miescher and Riethmüller considered MTX in the treatment of systemic lupus erythematosus (SLE) after exhaustion of azathioprine, prednisolone and 6 mercaptopurine. Weekly doses of 50 to 100mg intravenous were used with good effect(25).

The late 1960s and 1970s witnessed numerous studies considering and comparing the newly-termed disease modifying anti-rheumatic drugs (DMARDs) in the treatment of RA including d-penicillamine (26, 27), azathioprine (28, 29), cyclophosphamide (30), and chlorambucil (27). There was a resurgence of interest in MTX in the 1980s; case reports were published (31-33), leading to the first randomised controlled trials of MTX compared to placebo for RA; Thompson *et al.* (n = 48) 1984(34), Anderson *et al.* (n = 12) 1985(35), Weinblatt *et al.* (n = 28) 1985(36) and Williams *et al.* (n = 189) 1985(37). Three years later the United States Food and Drug Administration (FDA) approved the use of MTX in RA, 40 years following its discovery.

Head-to-head comparator studies, published in the late 1980s and early 1990s, followed. These initially compared MTX with the then standard of care for RA, gold, demonstrating equal efficacy and reduced adverse effects (38, 39). Studies comparing MTX with other DMARDs were also published, again demonstrating the superiority of MTX (40). By the early 1990's, MTX was established as standard of care for RA(24).

The rheumatology communities use of low-dose MTX (10 to 25mg/week) became increasingly prevalent, as evidence for its ability to modify disease-related damage in RA accumulated and the anticipated MTX-toxicity failed to materialise. In RA, MTX has since been shown to reduce disease-related damage, morbidity and mortality (41), and is now considered the first-line DMARD for RA treatment across continents (42) (43) (44).

Absorption of MTX, when taken orally, is via the protein coupled folate transporter(45) within the small intestine and varies amongst individuals(46). Low-dose MTX reaches peak plasma concentrations 1-2 hours following ingestion, and is largely undetectable at 24 hours(47). Intracellular concentrations of MTX builds up over a period of weeks, explaining the delay in efficacy seen in clinical practice(48). Excretion is predominantly renal – via glomerular filtration and active secretion from the tubule. A smaller proportion of MTX, around 10% is also metabolised within the liver and excreted within the bile(49).

The method of action of low-dose MTX is incompletely understood. MTX has a similar structure to folic acid, essential for cell proliferation, and acts as a folic acid antagonist. Specifically, the production of thymidine monophosphate, an essential element of deoxyribonucleic acid (DNA), requires folate cofactors. Thus, MTX, when given in high doses, competitively inhibits dihydrofolate reductase, reducing the availability of folate co-factors, and thereby decreasing both DNA synthesis and cell division(50). This, in turn, has been demonstrated to reduce de novo production of nucleotides and total purine pools within human T lymphocytes(51).

Oncological regimes of MTX require significantly higher doses than those commonly used for immunomodulation, when folic acid is routinely co-prescribed. Indeed, MTX activity is most visible in actively dividing cells, which explains why highly proliferating cancer cells are so susceptible to the cytotoxic effect of MTX and emphasises the role of folate antagonism as critical to its anti-tumour action. However, folate antagonism only applies with high doses of MTX and does not explain the immunomodulating properties seen with low-dose MTX, where an alternative mode of action is required.

One possible hypothesis suggests that low-dose MTX exhibits anti-inflammatory properties by altering adenosine pathways, thus preventing inflammation and oedema. Adenosine is an important signalling molecule and endogenous anti-inflammatory agent. MTX has been shown to increase local adenosine release by both fibroblasts and endothelial cells(52). Adenosine regulates inflammation by promoting anti-inflammatory macrophages(53) and inhibiting cytokine and osteoclast formation(54, 55). MTX has also been shown to reduce T lymphocyte levels of adenosine triphosphate (ATP) and

Guanosine-5-triphosphate (GTP) and increase levels of uridine triphosphate (UTP), in turn, decreasing cell proliferation(56).

Furthermore, MTX has been shown to suppress human Janus Kinases/Signal Transducers and Activators of Transcription (JAK/STAT) signalling, which also play an important role in inflammatory and immune pathways(57).

Despite the above hypotheses, the method of action of low-dose MTX is not completely understood.

6.1.3   The divergence of methotrexate related guidelines

Low-dose MTX is prescribed by a range of specialities for a large number of indications, as per Table 6-1. A lack of consensus regarding MTX monitoring has been present since it's advent, and divergent guidelines persist to this day (58-60). Much of this variation is across specialities, however it has also been demonstrated within specialities (61-64).

A multitude of guidelines has been published over the past 50 years, as show in Table 6-2. Dermatologists were first to print formal guidelines in 1972. As reports of liver cirrhosis secondary to MTX were published and medico-legal suits ensued, it was hoped that the writing of an unambiguous guideline would put a stop to this: "Medicolegal situations soon developed with the potential that large sums might be awarded. It became clear that the situation was getting out of hand at a rapidly accelerating pace." (65)

Dermatological guidelines (1972 – 88) allowed the use of MTX only in those with "severe psoriasis" defined as "life-ruining physically, emotionally or economically" (65-69). A liver biopsy was mandatory pre-MTX initiation (1972 and 73); and thereafter every 1.5grams of cumulative MTX given, or every 1gram if there were co-existent risk factors (67). This equated to a liver biopsy every 12.5 or 10 months for individuals taking 20mg or 25mg once weekly respectively; publications detail examples of patients having over 10 precautionary biopsies to ensure fibrosis had not occurred.

Clinical uses of MTX		
Rheumatology	Rheumatoid arthritis	Juvenile idiopathic arthritis
	Psoriatic arthritis	Systemic lupus erythematosus
	Connective tissue disease	Polymyalgia rheumatica
	Felty's syndrome	Vasculitis
	Early undifferentiated arthritis	Osteosarcoma
	Spondyloarthropathies	Myositis
	Mixed connective tissue disease	Scleroderma
Oncology	Gestational choriocarcinoma	Gestational trophoblastic neoplasia
	Bladder cancer	Chorioadenoma destruens
	Hydatidiform mole	Epidermoid cancers of head and neck
	Breast cancer	Lung cancer
Haematology	Cutaneous T cell lymphoma	Non-Hodgkin's lymphoma
	Acute lymphoblastic leukaemia	Acute promyelocytic leukaemia
Dermatology	Psoriasis	Atopic eczema
	Mycoses fungoides	Lymphomatoid papulosis
	Pityriasis lichenoides	Cutaneous polyarteritis nodosa
	Behçet's disease	Erythema elevatum diutinum
	Systemic sclerosis	Lupus erythematosus
	Pyoderma gangrenosum	Cutaneous Langerhans cell histiocytosis
	Dermatomyositis	Alopecia areata
	Bullous Pemphigus	Bullous Pemphigoid
	Necrobiosis lipoidica	Granuloma annulare
	Linear IgA disease	Epidermolysis bullosa acquisita
	Chronic idiopathic urticaria	Hailey-Hailey disease
	Lichen planus	Extragenital lichen sclerosis
	Morphea	Palmoplantar pompholyx
Gastroenterology	Crohns	Ulcerative colitis
Neurology	Inflammatory myopathies and neuropathies	Immune-mediated central and peripheral nervous system diseases
	Myasthenia gravis	Multiple sclerosis
Gynaecology	Ectopic pregnancy	
Respiratory	Sarcoidosis	Asthma
	Interstitial lung disease	Pulmonary vasculitis
Ophthalmology	Scleritis	Vasculitis
	Mixed connective tissue disease	

Table 6-1 Clinical indications for MTX(58-60, 70)

Speciality	Year published	Title
Dermatology	1972	Use of MTX in psoriasis (65)
Dermatology	1973	Methotrexate guidelines - revised (66)
Dermatology	1982	Methotrexate guidelines - revised (71)
Rheumatology	1987	Methotrexate in rheumatoid arthritis. Health and Public Policy Committee, American College of Physicians (72)
Rheumatology	1988	Methotrexate in rheumatoid arthritis (73)
Gastroenterology	1988	Methotrexate-induced chronic liver injury: guidelines for detection and prevention(74)
Dermatology	1988	Methotrexate in psoriasis – revised guidelines (68)
Rheumatology	1994	Methotrexate for rheumatoid arthritis (75)
Dermatology	1998	Methotrexate in psoriasis: consensus conference (69)
Gastroenterology	2004	Guidelines for the management of inflammatory bowel disease in adults(76)
Rheumatology	2006	Methotrexate Therapy for Rheumatoid Arthritis: Clinical Practice Guidelines Based on Published Evidence and Expert Opinion (77)
Gastroenterology	2006	European evidence-based consensus on the diagnosis and management of Crohn's disease: current management(78)
Rheumatology	2008	American College of Rheumatology 2008 Recommendations for the use of Non biologic and biologic disease-modifying antirheumatic drugs in Rheumatoid Arthritis (79)
Rheumatology	2009	Multinational evidence-based recommendations for the use of methotrexate for rheumatic disorders with a focus on rheumatoid arthritis: integrating systematic literature research and expert opinion of a broad international panel of rheumatologists in the 3E Initiative (80)
Dermatology	2009	Methotrexate and Psoriasis: 2009 National Psoriasis Foundation Consensus Conference (81)
Dermatology	2010	Guidelines on the use of methotrexate in psoriasis (82)
Gastroenterology	2011	Guidelines for the management of inflammatory bowel disease in adults(83)
Dermatology	2016	British Association of Dermatologists' guidelines for the safe and effective prescribing of methotrexate for skin disease (58)
Rheumatology	2017	BSR and BHPR guideline for the prescription and monitoring of non-biologic disease-modifying anti-rheumatic drugs (84)
Dermatology	2017	Methotrexate use and monitoring in patients with psoriasis: a consensus report based on a Danish expert meeting (85)
Gastroenterology	2017	Safety of treatments for inflammatory bowel disease: Clinical practice guidelines of the Italian Group for the Study of Inflammatory Bowel Disease(86)
Rheumatology	2019	Japan College of Rheumatology Guideline for the use of Methotrexate in patients with Rheumatoid Arthritis (42)
Gastroenterology	2019	British Society of Gastroenterologists consensus guidelines on the management of Inflammatory Bowel Disease in adults(60)
Dermatology	2020	Joint American Academy of Dermatology – National Psoriasis Foundation guidelines of care for the management of psoriasis with systemic non biologic therapies(87).

Table 6-2 Published guidelines regarding the use and monitoring of MTX

Divergence began in 1987 when the American College of Physicians published what were the first of the rheumatology-centred guidelines (72). Studies demonstrating an increased prevalence of liver disease in those with psoriasis, had not been replicated in individuals with RA (32, 88). A pre-treatment liver biopsy was not mandatory. Furthermore, ongoing monitoring differed too, in the frequency and range of blood tests recommended, once MTX was established.

The following year, Furst and Kremer published their approach when treating RA with MTX. Contrary to the dermatological guidelines, they proposed that obesity and diabetes mellitus should *not* be contraindications for MTX treatment, rather advising use with appropriate caution. They did not recommend supplementary folate in those taking MTX, supporting consideration of leucovorin rescue therapy in cases of life-threatening toxicity (73). In addition, these authors recognised the significant morbidity and mortality associated with liver biopsies, questioning the risk benefit ratio. In 1994 the same authors went further, revising the previous guidance including a recommendation limiting the requirement for a pre-treatment liver biopsy to high-risk patients only, rather than a biopsy being mandatory for all potential recipients (75). By contrast, the dermatological guidelines continued to mandate pre-treatment liver biopsy, at or near the beginning of MTX therapy, until as recently as 2009 (81).

Guidelines were updated relatively frequently within rheumatology and dermatology specialities, however the same experts featured recurrently as authors. Roenigk, Maibach and Weinstein authored five sets of dermatological guidance from 1972 to 1998, likely explaining the lack of significant variation. Conversely, no specific monitoring guidelines have been published within other specialities, such as respiratory or ophthalmology.

The divergence between the two main MTX-using specialities continued for decades. Rheumatologists became increasingly relaxed regarding apparent MTX-related hepatotoxicity; successive guidelines were each less prescriptive than the last. Increasing evidence of MTXs safety in long-term cohort studies were published(89) and the predicted tide of MTX-related liver injury never arrived. There are several likely reasons for this. Firstly, the appropriate screening of patients prior to MTX initiation allowed for identification of co-existing liver disease such as hepatitis B, hepatitis C and alcohol

excess. Secondly, the discovery of non-alcoholic fatty liver disease (NAFLD) of as a potential cause of liver disease, and thirdly, a change in prescribing habits, as recommended doses and frequency of MTX, reduced.

Consensus for the relaxation of guidelines appeared to be led by clinicians themselves(90, 91). In 2003, Yazici *et al*. surveyed American rheumatologists regarding adherence to, and opinion on, the American College of Rheumatologists 1994 monitoring guidelines for MTX in RA (75). 123 rheumatologists responded, with 41% of rheumatologists agreeing that liver monitoring guidance should be changed. 59% advocated to reduce liver blood test (LBT) monitoring to 3-4 monthly, rather than every 4-8 weeks (92). This paper prompted much discussion. Whilst the authors defended the robust nature of their initial 1994 guideline(93, 94), others felt sufficient evidence had come to light in the intervening nine years to warrant updating and easing the guidance(95).

Similarly, dermatologists, whose guidelines continued to advocate for liver biopsy in certain circumstances until 2016(81), were beginning to move towards the use of procollagen type III N-terminal peptide (PIIINP), a serological marker, as an alternative to repeated liver biopsies(96, 97). A questionnaire surveying 376 British dermatologists' prescribing practices with MTX in 2008, revealed significant variation regarding the use of liver biopsy and the criteria justifying it. This survey also reported four deaths as a direct result of liver biopsy, in comparison with two deaths from liver failure (without specified alcohol excess) and two from hepatorenal failure(98).

Consensus statements were increasingly incorporated in guidelines from the late 1990's (69, 77, 80, 81, 85, 99) in an effort to reduce discordance in the face of disagreement (88, 100-102). Current guidance, as detailed in Table 6-3, are the most concordant to date. Gastroenterological guidance(86) has incorporated a recognition that MTX causes a seemingly harmless elevation of transaminases, which doesn't require action. Recommendations for liver biopsies have been almost entirely replaced with referral to a specialist, particularly important following the advent of many non-invasive methods for assessing liver fibrosis and cirrhosis. All guidelines advise folic acid supplementation and repeated measurements of FBC, renal function and liver transaminases, although frequencies do still vary.

	British Society of Rheumatologists 2017 (84)	British Association of Dermatologists 2016 (58)	British Society of Gastroenterologists 2019(60)
Baseline investigations	FBC GFR ALT and/or AST and albumin Height and weight Blood pressure Comorbidity assessment	FBC U&Es LFTs HBV, HCV and HIV VZV PIIINP (psoriasis) +/- CXR & exam +/- TB	FBC U&Es LFTs HBV, HCV and HIV VZV CXR TB screening Immunisation status
Folic acid	Minimum 5mg once weekly	Between 5mg OD to once weekly	Advise 1mg OD or 5mg once weekly
Immediate monitoring	**Every 2 weeks until stable dose for 6 weeks:** FBC Creatinine / GFR ALT and/or AST and albumin	**Every 1-2 weeks until stable dose:** FBC U&Es LFTs	**At 2, 4, 8 and 12 weeks:** FBC U&Es LFTs
3/12 after dose stabilised	**Every 3 months:** FBC Creatinine / GFR ALT / AST	**Every 2-3 months:** FBC U&Es LFTs PIIINP (psoriasis only)	**Every 3 months:** FBC U&Es LFTs
Dose increases	**Every 2 weeks until stable dose for 6 weeks:** FBC ALT / AST Creatinine / GFR	Not stated	Not stated
Serious infection	Temporarily discontinue until the patient has recovered	Not stated	Not stated
Alcohol	Not stated	"Well below national guidelines"	Not stated
Action if abnormal LBTs	ALT / AST > 100 U/I Unexplained albumin < 30g/L Platelets < 140 x 10^9/L WCC < 3.5 x 10^9/L Neuts < 1.6 x10^9/L MCV > 105 fL Creat increase > 30% over 12 months and/or GFR < 60ml/min Unexplained eosinophilia >0.5 x 10^9/L	ALT/AST < 2 fold rise – repeat in 2-4 weeks ALT/AST > 2-3 times normal - withhold/decrease MTX, consider other risk factors and d/w gastroenterologist Platelets < 100 x 10^9/L WCC < 3 x 10^9/L Neuts < 1 x 10^9/L MCV > 105 fL	ALT/AST > 2 times normal: withhold MTX

FBC: Full blood count, GFR: Glomerular filtration rate, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, WCC: White cell count, Neuts: Neutrophils, MCV: Mean cell volume, Creat: Creatinine, U&Es: Urea and electrolytes, LFTs: Liver function tests, HBV: Hepatitis B virus, HCV: Hepatitis C virus, HIV: Human immunodeficiency virus, VZV: Varicella zoster virus, PIIINP: Procollagen III n-terminal peptide, CXR: Chest Xray, TB: Tuberculosis, OD: once daily, MTX: Methotrexate

Table 6-3 Summary of MTX monitoring guidelines

In summary, decades of conflicting guidelines, a reflection of the sparsity of high-quality evidence, has resulted in varied practices both within and between specialities(103). Clarity regarding presumed MTX related hepatotoxicity is required.

6.2 Hepatotoxicity

6.1.2   *The pathogenesis of liver injury*

The pathology of most liver disease is a spectrum spanning from inflammation, through various staging of fibrosis, to cirrhosis, and sometimes progressing further, to malignancy. This liver inflammation can be initiated by a number of potential insults, including infections (HBV, HCV), the immune system itself (AIH, PBC, PSC) and fat deposition (NAFLD, alcoholic hepatitis). The cycle of cellular inflammation causing injury, with consequential fibrosis causes progressive liver disease. Cirrhosis is the end-product of all chronic liver disease, occurring when repair and regeneration, are overwhelmed by the ongoing inflammatory insult.

Macrovesicular hepatic steatosis – where fat vacuoles can be observed within hepatocytes, is the first stage of liver injury observed in fatty liver disease. Fat vacuoles are made up of triglycerides, phospholipids and cholesterol esters (104). Fat accumulation is reversible should the causal insult be removed, e.g., alcohol or excess weight. Fat accumulation is mediated by fatty acid synthesis induction and fatty acid ß-oxidation inhibition. Increased sterol regulatory element-binding protein c (SREBP-c) expression and reduced peroxisome proliferator-activated receptor alpha (PPARα) expression both play a role in altering the genetic transcription of free fatty acid transportation and oxidation(104). Certain genes have been cited as potentially responsible for increased fat deposition within the liver; PNPLA3(105), MTP G allele (106), and microsomal triglyceride transfer protein 493 GT polymorphism (107).

The molecular mechanism transforming simple steatosis to steatohepatitis is not entirely understood, but is optimally explained by a 'multi-hit hypothesis' involving lipotoxicity, mitochondrial dysfunction and ATP synthesis. Fat accumulation itself is thought to be noxious to hepatocytes; lipotoxicity - causing altered lysosomal metabolism (108) and endoplasmic reticular stress (109). As a result of this lipotoxicity, the likelihood of cell

apoptosis is significantly increased. In addition, mitochondrial dysfunction has been shown to play a critical role, culminating in the inability to maintain sufficient ATP levels (110). The metabolite of alcohol, acetaldehyde, damages hepatic mitochondria and the microtubules of hepatocytes (111). Increased hydrogen ion transfer across mitochondrial membranes, reduces the membrane potential, and therefore the amount of ATP synthesised(112). All these mechanisms together play a role in promoting liver injury, hence termed the 'multi-hit hypothesis' (113).

Cellular inflammation is regulated by cytokines – molecular mediators which play a key role directing the inflammatory cascade and ultimately fibrogenesis. Platelet derived growth factor (PDGF) (114), transforming growth factor (TGF)-ß (115), tumour necrosis factor (TNF)-α(116), and interleukin (IL) (117) all demonstrate increased expression in liver fibrosis. Inflammatory cytokines lead to polymorph infiltration, reactive oxygen species and consequential hepatocyte damage. The damaged proteins are degraded, but promote hepatocyte injury and cytokeratin aggregates lead to the development of intracytoplasmic hyaline bodies and Mallory-Denk bodies (118). Cytokines are ubiquitous regardless of hepatic disease aetiology. The following pro-inflammatory cytokines have been demonstrated to be upregulated; IL-2, IL-8 and TNFα in PBC (119), IL-2, IL-6 and IL-8 in autoimmune hepatitis (120), IL-2, and T helper-1 in HCV (121) TNFα, and IL-6 in NAFLD (122, 123), and TNFα in alcoholic hepatitis (124).

Oxidative stress is another crucial step in hepatotoxicity and subsequent fibrogenesis. Increasing levels of oxidative stress correlate with non-alcoholic steatohepatitis (NASH) severity in animal (125) and human (126) studies. Oxidative stress arises from the breakdown of free fatty acids within hepatocytes, a process which relies upon PPARα, and has been shown to be present at higher levels in those with NAFLD (127). An increase in reactive oxygen species impacts upon protein and nucleotide synthesis, which in turn leads to inflammation, and the development of liver fibrosis (128).

What is still not entirely clear, is which of the above mechanisms are a consequence of liver dysfunction, or mediators playing a causal role in inducing it. Regardless, they effect changes to the normal hepatic infrastructure.

Hepatic stellate cells (HSC) are the main source of excess collagen synthesis and other extracellular matrix proteins resulting in hepatic fibrosis and cirrhosis (129). They are found in the gap between the sinusoids, where blood flows through the liver, and the hepatocytes themselves. In their quiescent state, HSCs act as a storage facility for vitamin A amongst other things, but under certain conditions, including oxidative stress described above, as well as numerous other novel pathways and mediators, they transition from a quiescent to activated state. Activation transforms the essentially innocuous HSC into a proliferative, fibrogenic myofibroblast (130). Such activated HSCs secrete an arsenal of extracellular matrix proteins including collagens, glycoproteins and proteoglycans. If the insult is chronic and the HSC remains activated, the normal extracellular matrix of the liver is damaged, followed by deposition of collagen types I, III and IV with consequential liver fibrosis (131).

Liver sinusoidal endothelial cells, normally acting as a sieve between the components within sinusoidal blood and parenchymal hepatocytes, become defenestrated in liver cirrhosis, and develop a basement membrane (132). This membrane prevents adequate substrate exchange and further interferes with hepatocyte dysfunction.

Kupffer cells are the resident macrophage within the liver, where they reside within the sinusoidal lining picking up pathogens entering from the portal or arterial circulation. Kupffer cells, once activated, have been shown to produce harmful mediators to destroy hepatocytes as well as act as antigen-presenting cells (133). They also play a key role in cascading the inflammatory response by recruiting and activating other immune cells such as T lymphocytes, natural killer cells, neutrophils (134, 135) and hepatic stellate cells (136, 137) by way of pro-inflammatory cytokines (138).

Hepatocytes, the primary liver parenchymal cell, are a target for most liver pathogens. In addition, hepatocyte apoptosis can be initiated by a wide range of factors, including oxidative stress, TNF FAS ligand, as well as several other stressors. Promoted hepatocyte apoptosis itself, releases reactive oxygen species and fibrogenic mediators (139, 140). Hepatocytes have been demonstrated to be a major source of matrix metalloproteinases and tissue inhibitors of matrix metalloproteinases (141), resulting in extracellular matrix modulation, fibrosis and ultimately cirrhosis.

The culmination of these steps – basement membrane development, collagen synthesis, and myofibroblast transformation, result in distortion of the architecture of the liver. This distortion of the liver architecture, combined with regenerative nodules and endogenous vasoconstrictors, results in increased intrahepatic portal pressure and explains many of the clinical features associated with chronic liver disease such as ascites or varices.

In summary, the spectrum of liver pathology is broad and is likely to be heavily influenced by genotype. Steatohepatitis is mediated by numerous cytokines causing an inflammatory cascade resulting in an altered extracellular matrix. Mitochondrial injury, oxidative stress, increased collagen deposition and cellular apoptosis are all key features in the development of fibrosis and cirrhosis.

6.1.3   *The clinical consequences of progressive liver fibrosis*

As fibrosis in the liver advances to cirrhosis, a series of serious clinical consequences are unleashed, impacting upon morbidity and mortality(142). Deaths from liver disease are increasing; premature mortality (death under the age of 75) due to liver disease was 18.5 per 100,000 population between 2015 and 2017, compared with 15.8 per 100,000 in 2001 to 2003(143). The following section details the clinical consequences and sequelae of liver fibrosis.

Portal hypertension is the clinical syndrome that develops as a result of an increase in vascular resistance at any level within the portal venous vasculature. It is measured as the pressure gradient between the portal vein and hepatic veins and under physiological conditions, should be under 5mmHg. Portal hypertension, a pressure gradient above 5mm.Hg, may be due to pre-hepatic, intrahepatic or post-hepatic causes, but the most common is the intrahepatic condition of liver cirrhosis (approximately 90%)(144). Increased resistance seen in liver cirrhosis is secondary to the sinusoidal architectural variations of fibrosis, regenerative nodules, vasoconstriction within the distorted liver structure coupled with increased portal inflow from splenic vasodilatation(145). Mounting resistance within the portal system promotes the development of portosystemic collaterals and the resulting varices can rupture (see below). Portal hypertension has been demonstrated to predict clinical consequences, such as hepatocellular cancer (HCC)(146)

and decompensation of patients with liver cirrhosis(147), underlining it's critical significance in the development of clinical consequences.

Ascites is the most common complication of liver cirrhosis and frequently results in hospitalisation. In the context of portal hypertension, ascites describes the accumulation of low-protein fluid within the peritoneal cavity(148). The key event of portal hypertension, is the development of splanchnic vasodilatation (149) secondary to local vasodilators such as nitric oxide, increasing capillary permeability, resulting in lymph formation accumulating within the splanchnic organs(150). Splanchnic vasodilatation also leads to a decrease in central arterial pressure; baroreceptors prompt activation of the renin-angiotensin system, causing anti-diuretic hormone (ADH) and sodium and water retention as the kidneys attempt to compensate(151). Fluid retention, coupled with relative hypoalbuminaemia, and splanchnic changes within the microcirculation, results in fluid leaking into the peritoneal cavity(152). Ascites has a significant impact upon quality of life (153), it increases risk of infection, hepatorenal syndrome (HRS) (154) and is associated with a poor prognosis; mortality is approximately 40% at 1 year and 50% at 2(155).

Spontaneous bacterial peritonitis (SBP), defined by an ascitic fluid neutrophil count > 250 cells/mm^3, was first described in 1963(156). Studies to demonstrate the prevalence of SBP in a population with cirrhotic ascites have varied between 3.5 - 12%(157-159), higher in decompensated hospital inpatients as compared with asymptomatic outpatients. Risk factors for developing SBP include a lower ascites protein concentration(148) and abnormalities within the innate immune system(160, 161) which has been demonstrated in patients with liver cirrhosis. Genetic predisposition may also play a role in susceptibility of developing SBP and influence mortality, once acquired(162, 163). SBP is generally thought due to bacterial translocation from the bowel lumen, facilitated by the loss of gut mucosal integrity in those with cirrhosis whose liver disease has advanced(164). *Escherichia coli* is the commonest causal bacteria, however non-enteric bacteria have also been found to cause SBP suggesting alternative mechanisms of pathological contamination are yet to be identified(165).

Hepatorenal syndrome (HRS), is characterised by a reduced glomerular filtration rate (GFR) secondary to renal vasoconstriction with sodium and water retention, in the setting of liver disease with portal hypertension(166). Unlike acute kidney injury of other causes, the condition is not responsive to cessation of diuretic therapy or rehydration, and does not feature typical markers of parenchymal renal disease, such as proteinuria or haematuria(167). Depending on rapidity of onset, HRS can be subdivided into types 1 and 2. HRS can be precipitated by further flares of hepatitis – prompted by toxins such as alcohol or drugs, or bacterial infections. Clinically, it poses a challenge to treat and is associated with a very high mortality, approximately 50% at 30 days(168, 169).

Hepatopulmonary syndrome (HPS) is seen in individuals with portal hypertension, hypoxia, and intra-pulmonary vascular dilation in the absence of significant other pulmonary disease(170). The dilatation of pulmonary vessels results in impaired oxygen transfer and shunting away from ventilated areas of the lungs, leading to a ventilation-perfusion mismatch. The main symptom of dyspnoea, typically worsens in an upright position. HPS prevalence ranges between 4 and 47% (171, 172) in those with liver disease, but up to 80% in those undergoing liver transplantation assessment(173). Morbidity and mortality is significantly increased in those with HPS (23% 5 year survival) compared to individuals without it (63% 5 year survival), as demonstrated by Swanson *et al*.(174). Liver transplantation is the only cure for HPS.

Portal vein thrombosis (PVT) was first described in a 20 year-old tailor from Glasgow in 1868 (175), who presented with abdominal pain, ascites, splenomegaly and oesophageal varices. PVT prevalence is significantly higher in individuals with liver cirrhosis, particularly when cirrhosis is severe and associated with liver failure (176). Rudolf Virchow was first to explain this phenomena as a result of endothelial damage, stasis and hypercoagulability (177), (178). The relative stiffening of the liver architecture which occurs in cirrhosis, slows portal flow velocity and thus increases the likelihood of thrombosis. PVT, in addition to being more likely in the setting of portal hypertension, also causes a consequential increase in portal hypertension itself, increasing mortality and morbidity in patients with liver cirrhosis (179).

Gastroesophageal varices develop as a result of increased portal pressure, causing collateral vessels to dilate and enlarge over time. Varices have been demonstrated to be present in approximately 52% of patients with liver cirrhosis(180) with increasing prevalence as severity of liver disease escalates. Variceal haemorrhage occurs more frequently in larger varices, in those with more advanced liver disease (Childs C) and where stigmata are evident, such as red wale marks(181). The development, monitoring and treatment of oesophageal varices is imperative, as the 30-day mortality of variceal haemorrhage is 15%(182, 183).

Hepatic encephalopathy (HE) refers to the neurological consequences seen secondary to liver impairment; manifesting as spectrum from mildly impaired concentration, disrupted sleep-wake cycle, altered mental status through to coma(184). HE results from changes in the metabolism of urea secondary to hepatocyte dysfunction; an increase in ammonia and pro-inflammatory cytokines are seen. Astrocytes swell due to increased oncotic pressure, resulting in cerebral oedema and microglia further activate immune pathways causing oxidative stress and neuroinflammation(185). HE has been shown to have a significant impact upon quality of life for patients and their relatives(186), whilst 12-month survival rates as low as 42% are reported(187).

Hepatocellular carcinoma (HCC) is a highly prevalent cancer world-wide; significantly more prevalent, but not exclusive to, those with liver cirrhosis(188). HCC develops as a result of sustained inflammation; necrosis, increased hepatocyte cell turnover, local toxins and regenerative proliferation all increase the risk of DNA mutation (189). Cancer is the leading cause of death, and liver cancer is the fourth most common cause of cancer death worldwide(190, 191).

In summary, liver disease is highly prevalent and it's sequalae are clinically significant, being a leading cause of morbidity and mortality world-wide.

6.2   Diagnosing liver fibrosis

The accurate and timely assessment of liver fibrosis is critical to establishing the diagnosis, the prognosis and the likelihood of developing complications, in patients with chronic liver disease. Liver biopsy, for many years considered the gold standard test for

establishing presence and magnitude of fibrosis, has some limitations, hence the emergence of non-invasive tests including serum markers and imaging techniques. The ideal test would have the following characteristics: inexpensive, reliable, easy to perform, accurate with a high specificity and sensitivity, liver specific, and consistently reproducible. This section explores the range of available tests, identifying their strengths and weaknesses.

6.2.1 Liver biopsy

The accurate evaluation of the extent and degree of liver fibrosis is integral to clinical decision-making, prognostication and appropriate treatment. Liver biopsy is widely considered to be the gold standard investigation for detection of liver fibrosis, however its invasive nature is associated with both morbidity and mortality(192, 193). However, a liver biopsy can only be considered truly representative of the entire liver, and thus earn it's moniker of gold standard, if it is of sufficient size. Guidelines specifying what constitutes an appropriate sample size vary between 10mm (194) and 30mm (195) length with agreement that a minimum of 11 complete portal tracts are required for accurate staging and grading of disease. In practice, these criteria are rarely met; with a meta-analysis demonstrating mean length and number of portal tracts as 1.8cm and 7.5 tracts respectively(196). Furthermore, samples can be confounded by biopsy location, as an adult biopsy sample corresponds to around 1/50,000[th] of the total liver volume(197, 198). Despite the widespread use of staging scores, inter-observer variation continues to reduce the reliability of biopsy interpretation(199), with pathologist experience also having an impact (200).

6.2.2 Serological markers of liver fibrosis

A blood test to gauge the presence and/or severity of liver disease has long been the holy grail of physicians interested in liver disease. Standard liver blood tests (alanine transferase, bilirubin, alkaline phosphatase and albumin), are poor at detecting liver fibrosis, and cannot be relied upon as a marker of liver function, fibrosis or cirrhosis(201). Indeed, the National Institute for Health and Care Excellence (NICE) guidelines reflect this, suggesting liver blood tests should not be used to rule out liver disease(202, 203).

Whilst standard liver blood tests can demonstrate advanced liver disease once established, a more useful blood test would be one that gave warning at a much earlier stage, when intervention may be effective at altering the outcome.

Serological tests of fibrosis are less-invasive, less expensive, easily reproducible and do not require specialist interpretive skills, as compared to other test modalities, such as a liver biopsy. However, they are rarely organ specific, relying upon clearance and excretion rates, and they lack a high degree of accuracy(204). Serological tests can be divided into *direct* and *indirect* markers, where direct markers generally correlate with the deposition of excess extracellular matrix (including collagen subtypes especially types I and III, glycoproteins & elastin) and indirect markers are biomarkers that are altered as a consequence of liver parenchymal distortion and associated fibrosis (e.g. AST to platelet count ratio (APRI), see below).

One such direct serological marker is Procollagen III amino peptide (PIIINP), the amino terminal peptide of type III procollagen, released from the precursor peptide during the synthesis and deposition of type III collagen. Widely utilised by dermatologists in the setting of PsA and MTX, it has been shown to increase in the presence of hepatic fibrosis(205). However, it's sensitivity and specificity for detecting liver fibrosis are reported as 74% and 77%, respectively(206), making it sufficiently unreliable for current authors to conclude that it is unhelpful in PsA and frequently elevated, despite normal liver histology(207). Recent evidence demonstrating alternative serological markers may be more sensitive, may bring about a change in guidelines over the coming years(208).

Compilation markers, frequently patented, use a combination of tests to increase their diagnostic accuracy. Table 2 details the diagnostic accuracy of the most commonly utilised compilation markers and algorithms in detecting significant liver fibrosis (stage 3-4). Aspartate aminotransferase (AST) to platelet index ratio (APRI), initially appeared promising in detecting fibrosis in those with chronic HCV; this was not replicated in other disease aetiologies including alcohol-related fibrosis(209) and autoimmune hepatitis(210). The Enhanced Liver Fibrosis (ELF) test is a direct marker of fibrosis measuring Hyaluronic acid, PIIINP and Tissue inhibitor of metalloproteinase 1 (TIMP-1). ELF has one the highest sensitivities in detecting liver fibrosis (83%), and hence is

recommended as the biochemical marker of choice to assess NAFLD in the NICE guidelines(203), and has also been shown to be useful in screening for fibrosis in alcohol-related liver disease (ArLD)(211).

Composite markers, like all tests, have to balance sensitivity and specificity when defining a cut-off value(212); the NAFLD fibrosis score is an exception, reporting an 'indeterminate' result (between < -1.455 and >0.676), in addition to negative (>0.676) and positive (< -1.455)(213) values. This 'grey area' acknowledges uncertainty and allows the clinician a degree of flexibility in interpretation, depending on the clinical circumstances. Perhaps this explains why the NAFLD fibrosis score is one of the most widely adopted scores in clinical practice.

6.2.3   Radiological techniques for detecting liver fibrosis

Ultrasonography is low cost, painless, radiation-free and historically a commonly utilised modality, however, its diagnostic accuracy for detection of liver fibrosis is poor – sensitivity and specificity 57% and 88% respectively(214). A higher body mass index has been demonstrated to reduce sensitivity further(215), particularly relevant given that obesity and associated NAFLD is a leading cause of liver disease within the United Kingdom (UK).

		Serological composite		Diagnostic accuracy	
				Sensitivity	Specificity
AST to platelet ratio index (APRI)(210)	$APRI = \dfrac{AST}{Platelet\ count} \times 100$	AST	Platelet count	75%	68%
European Liver Fibrosis (ELF®)(216)	$ELF = 2.278 + 0.851\ 1n(HA) + 0.751\ 1n(PIIINP) + 0.394\ 1n(TIMP - 1)$	HA TIMP-I	PIIINP	83%	73%
Fibrometer®(217)	$\begin{aligned} Fibrometer = &\ 0.418xglucose + 0.070xAST + 0.0008xferritin \\ &+ 0.010xplatelets - 0.026xALT + 0.0459xweight \\ &+ 0.084xage + 11.623 \end{aligned}$	Platelet count Prothrombin index AST	HA Urea α2macroglobulin	80%	84%
Fibrospect®(218)		HA α-2-macroglobulin	TIMP-II	72%	74%
Fibrotest® (Fibrosure® in USA)(219)	$\begin{aligned} FibroTest = &\ 4.467xlog10[alph2macroglobulin] \\ &- 1.357xlog10[haptoglobin] + 1.017xlog10[\gamma GT] \\ &+ 0.0281x[age] + 1.737xlog10[bilirubin] \\ &- 1.184x[apolipoproteinA1] \\ &+ 0.301xsex[female = 0, male = 1] - 5.54 \end{aligned}$	Age Gender γGT α-2-macroglobulin	Total bilirubin Haptoglobin Apolipoprotein-A	61%	80%
Forns Index(220)	$\begin{aligned} Forns = &\ 7.811 - 3.131.In[platelets] + 0.781In[\gamma GT] + 3.467In[age] \\ &- 0.014.[cholesterol] \end{aligned}$	Platelet count ∝GT	Age Cholesterol	30%	95%
Fibrosis-4 (Fib-4)(221)	$Fib4 = \dfrac{Age \times AST}{Platelets} \times \sqrt{ALT}$	Age AST	Platelet count ALT	54%	88%
HepaScore®(222)	$\begin{aligned} HepaScore = \exp\ [&-4.186 - (0.025xage) + (0.746xsex) \\ &+ (1.004x\alpha2macroglobulin) + (0.030xhyaluronic\ acid) \\ &+ (0.069xbilirubin) - (0.001x\gamma GT)] \end{aligned}$	∝GT Age Gender	HA Total bilirubin α2- macroglobulin	70%	79%
For NAFLD only					
BARD score(223)	$BARD = (BMI > 28 = 1) + \left(\dfrac{AST}{ALT} > 0.8 = 2\right) + (DM\ yes = 1)$ If total score 2 – 4 then BARD score positive	BMI AST / ALT ratio	T2DM	74%	66%
NAFLD Fibrosis Score (NFS)(213)	$\begin{aligned} NFS = &-1.675 + 0.037\ x\ age + 0.094\ x\ BMI + 1.13\ x\ IGF\ (yes = 1, no = 0) \\ &+ 0.99\ x\dfrac{AST}{ALT} - 0.013xplatelet - 0.66\ x\ albumin) \end{aligned}$	Age BMI IFG / Diabetes	AST / ALT ratio Platelet count Albumin	77%	71%

AST: Aspartate aminotransferase, HA: Hyaluronic Acid, TIMP-I: Tissue inhibitor of metalloproteinase I, PIIINP: Procollagen III aminopeptide, TIMP-II: Tissue inhibitor of metalloproteinase II, ∝GT: gamma glutamyl transferase, BMI: Body mass index, ALT: Alanine aminotransferase, T2DM: Type 2 Diabetes Mellitus, IFG: impaired fasting glucose

Table 6-4 Sensitivity and specificity of non-invasive diagnostic markers in detecting liver fibrosis (223, 224)

Transient elastography (TE) – FibroScan® – was introduced to the European market in 2003 by the French company, EchoSens®. This ultrasound-based technology utilises sound waves to calculate liver fibrosis, specifically, the velocity of the reflected shear wave correlates with liver stiffness(225). TE is quick (less than five minutes), painless and reproducible(226); although the requirement of expensive equipment (£30,000 to £70,000 per unit(227)) is a significant drawback. Undertaking the examination requires an individual to lay supine with their right arm elevated, as demonstrated in Figure 6-2. The probe is then placed overlying the intercostal space (9th to 11th) and 10 measurements are taken.

Following its introduction, TE has rapidly gained popularity as a reliable non-invasive method of assessing liver fibrosis across a broad variety of disease modalities. This is demonstrated by the incorporation of TE into national(228) (229, 230) and international(231) guidelines. It was approved for use by the US FDA in April 2013 and has been extensively adopted both in UK-based hospitals and developed countries across the world.

The sensitivity and specificity of TE, for detecting significant fibrosis, is 70-79% and 78-84% respectively and for cirrhosis 81-89% and 88-95% respectively (232) (233-237). Obesity, ascites, high ALT, alcoholic hepatitis and food consumption within the preceding 4 hours have all been shown to reduce the sensitivity of the test(238). TE is highly reproducible, and has been demonstrated to be more accurate in diagnosing liver fibrosis in a general population than ALT, NAFLD Fibrosis score, or Fib-4(239). Initial concerns centred on meeting manufacturers requirements (requiring ≥10 valid readings, interquartile range ≤30% of the median liver stiffness measurements) and one study suggested results may be invalid in up to 20% of cases(240). However, as users have become increasingly familiar with the technology this concern has largely been allayed.
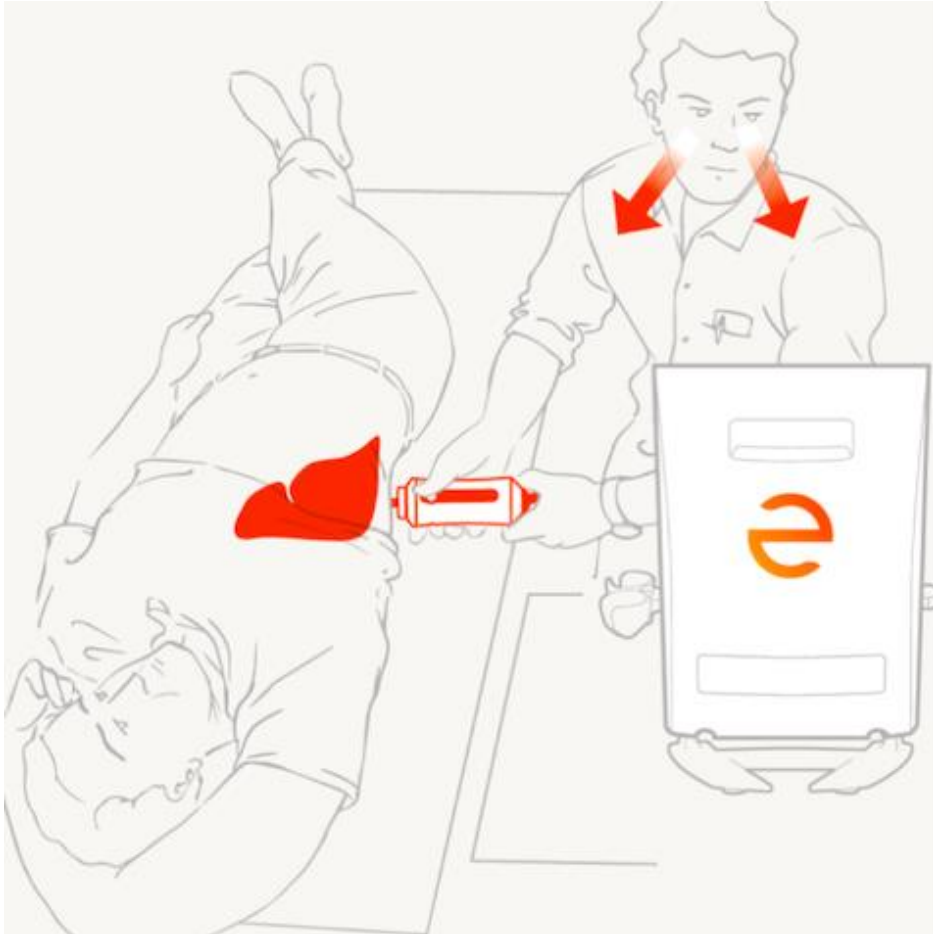
Figure 6-2 Position required to undertake Transient Elastography(241)

TE has also been evaluated in a population of patients taking MTX both in those with RA(242, 243) and psoriasis(244, 245) showing similar efficacy, and superior performance to APRI, HepaScore, Fib-4, ELF and PIIINP. Initial studies were small (n<100 participants) and didn't compare TE to the gold standard of liver histology. In time, larger review articles were published directly comparing TE to liver biopsy in a population of individuals taking MTX for a variety of benign inflammatory conditions(246-248). Marsh *et al.* reported on 1536 participants across 15 studies, concluding TE was 50-100% sensitive and 50-88% specific in detecting liver fibrosis as compared with liver biopsy(249), similar figures were reported by Rongngern *et al.*(250).

TE has undoubtedly transformed hepatological practice over the past 15 years. It has been demonstrated to be a reliable non-invasive tool for assessing liver fibrosis regardless of aetiology. One example being the inclusion of TE in the most recent American Dermatology guidelines for use of MTX in psoriasis, instead of liver biopsy, demonstrating how integral it has become(87).

Acoustic radiation force imaging (ARFI) utilises conventional ultrasound imaging and then additionally generates a shear wave within a region of interest in the liver tissue, calculating liver stiffness too. The region of interest being directly within the liver, reduces interference from ascites, but is over a smaller area than with TE (1-2cm versus 5cm) and is therefore vulnerable to variation within the liver(251-253). Meta-analyses reviewing detection of significant fibrosis in a range of aetiologies demonstrated a sensitivity of between 74-92% and a specificity of 83-85% (254, 255). ARFI has been most extensively evaluated in those with viral hepatitis, particularly HCV (256, 257). However, studies have explored its reproducibility in NAFLD with similar, if not better, results(258, 259).

Computed tomography (CT) is considered to be inferior in detection of liver fibrosis (sensitivity and specificity 83-84% and 76-81% respectively(260, 261)) when compared to the alternatives. Furthermore, the associated radiation exposure to the patient has meant it has not been widely adopted as a first-line method of diagnosis.

Magnetic resonance elastography (MRE) combines MR and mechanical waves to give a highly accurate assessment of fibrosis across a range of aetiologies, boasting 100%

sensitivity and specificity of 91%(262). There is no consensus, as yet, over variables such as scanner technique or wave software and this is likely to cause a degree of disparity. Time to acquire the images is significantly greater than all of the alternative technologies. In addition to this, computational time prior to required analysis of the images is typically measured in hours, ruling out the option of an immediate result(263). Finally, the cost of MRE is significantly greater than that of the alternatives, and it is not yet widely available in the UK. These limitations mean the widespread adoption of MRE has not yet taken place, further research is required and standardisation of techniques will be necessary, before this method can be embraced.

6.3 Clinical evidence of liver fibrosis with methotrexate

The discovery of MTX and its subsequent utilisation across a variety of clinical conditions has been outlined in section 7.1. Once efficacy was broadly accepted, the emergence of side-effects, particularly affecting the liver, inevitably dampened enthusiasm for its use. This section explores the origins of MTX-related hepatotoxicity and evaluates the supporting evidence within the context of specific diseases where pre-existing liver disease and/or disease-related hepatotoxicity have emerged.

6.3.1 Historical case reports

O'Rourke and Eckerk's landmark publication in 1964 was the first to postulate a relationship between MTX, as an immunomodulator, and hepatotoxicity. The authors detailed the case of a 62-year-old lady found to have liver fibrosis on biopsy following three years of MTX treatment(264). A steady stream of case reports and later, case-control series, were published during the 1970's and 1980's describing MTX as causal for liver fibrosis, some suggesting a prevalence as high as 27%(265-268). Table 6-5 details studies published with histological liver assessment following treatment with MTX prior to 2000.

Authors	Type of study	Published	n =	Disease	Pre-MTX biopsy?	Post-MTX biopsy Fibrosis (%)	Post-MTX biopsy Cirrhosis (%)	BMI	EToH	Other liver disease	Meds	Female	Cumulative dose relationship?
Colsky *et al.*(269)	Case Series	1955	5	Leukaemia	No	100%	0%	n/a	n/a	N	N	20%	N/a
Hutter *et al.*(270)	Case Series	1960	72	Leukaemia	No	35%	--	n/a	n/a	N	Y	--	N/a
O'Rourke and Eckert(264)	Case Report	1964	1	PsA	No	n/a	n/a	N	Y	Y	N	100%	N/a
Taft(271)	Case Series	1965	7	Leukaemia	No	n/a	n/a	N	N	N	Y	--	N/a
Hersh *et al.*(272)	Case Series	1966	10	Leukaemia	No	10%	0%	N	Y	Y	N	--	N/a
Coe and Bull(265)	Case Series	1968	3	Psoriasis	No	n/a	n/a	N	N	N	N	33%	N/a
Epstein and Croft(273)	Case Report	1969	1	Psoriasis	No	n/a	n/a	N	Y	N	Y	0%	N/a
Muller *et al.*(266)	Case Series	1969	7	Psoriasis	No	n/a	n/a	N	N	N	N	33%	N/a
Sharp *et al.*(274)	Cross Sectional	1969	10	Leukaemia	No	unclear	unclear	n/a	n/a	N	N	--	N/a
Dubin and Harrell(275)	Case Series	1970	3	Psoriasis	No	n/a	n/a	N	Y	Y	Y	0%	N/a
Weinstein *et al.*(276)	Case-Control	1970	21	Psoriasis	No	10%	5%	N	Y	N	N	43%	N/a
Berge *et al.*(277)	Cross Sectional	1970	3	Psoriasis	No	0%	0%	Y	Y	N	Y	0%	N/a
Dahl *et al.*(267)	Case-Control	1971	37	Psoriasis	No	27%	19%	N	Y	Y	N	--	Yes
Roenigk *et al.*(278)	Case-Control	1971	37	Psoriasis	No	5%	16%	Y	Y	N	N	44%	No
Almeyda *et al.*(279)	Cross Sectional	1971	39	Psoriasis	No	31%	8%	N	Y	N	N	--	Yes
Filip *et al.*(280)	Case Report	1971	1	Psoriasis	No	n/a	n/a	N	Y	N	N	0%	N/a
Zachariae and Schiodt(281)	Case-Control	1971	36	Psoriasis	Some	Not stated	0%	Y	Y	Y	N	49%	No
Almeyda *et al.*(282)	Cohort	1972	42	Psoriasis	Some	29%	7%	N	Y	N	N	--	Yes
Dahl *et al.*(268)	Cohort	1972	44	Psoriasis/PsA	No	25%	14%	N	Y	N	N	45.5%	--
Hoffmeister *et al.*(283)	Cross-sectional	1972	29	RA	No	22%	0%	N	N	N	N	--	N/a
Ryan *et al.* (284)	Case Series	1972	4	Psoriasis	No	0%	50%	N	Y	N	N	--	--
Weinstein *et al.*(20)	Cross Sectional	1973	550	Psoriasis	No	13%	3%	Y	Y	N	N	**42%**	Yes
Podurgiel et al.(285)	Cross Sectional	1973	35	Psoriasis	No	11%	14%	N	Y	Y	Y	51%	No
Palmer(286)	Cross Sectional	1973	23	Psoriasis	No	17%	13%	N	Y	N	N	--	No

Study	Type	Year	N	Condition	Col6	Col7	Col8	Col9	Col10	Col11	Col12	Col13	Col14
Tobias and Auerbach(287)	Cross Sectional	1973	69	Psoriasis	No	6%	7%	N	Y	N	N	**55%**	Yes
Pai et al.(288)	Case Report	1973	1	Psoriasis	No	100%	0%	Y	Y	N	Y	**0%**	N/a
Coughlin et al.(289)	Case Report	1973	1	Psoriasis	No	0%	100%	N	Y	N	N	100%	N/a
Millward-Sadler and Ryan(290)	Cross Sectional	1974	19	Psoriasis	No	11%	16%	N	N	N	N	**58%**	Not stated
Reese et al.(91)	Case-Control	1974	35	Psoriasis	No	6%	3%	N	Y	N	N	Not stated	No
Warin et al.(291)	Cross Sectional	1975	25	Psoriasis	Yes	4%	0%	Y	Y	N	Y	Not stated	No
Nyfors and Poulsen(292)	Cohort	1976	88	Psoriasis	Yes	6%	7%	Y	Y	N	Y	52%	No
Nyfors and Hopwood(293)	Cross Sectional	1977	24	Psoriasis	Yes	--	--	N	N	N	N	**67%**	No
McIntosh et al.(21)	Cohort	1977	8	Leukaemia	No	63%	0%	N	N	N	N	Not stated	No
Nyfors(294)	Cohort	1977	160	Psoriasis	Some	7%	1%	Y	Y	N	N	50%	No
Horvath et al.(295)	Cross Sectional	1978	52	Psoriasis	No	10%	2%	N	Y	Y	Y	**40%**	No
Zachariae et al.(296)	Cross Sectional	1980	183	Psoriasis	Some	Not stated	10%	Y	Y	Y	Y	Not stated	Yes
Parker et al.(297)	Case Series	1980	8	Leukaemia / Lymphoma	No	13%	13%	n/a	n/a	N	N	Not stated	n/a
Robinson et al.(298)	Cross Sectional	1980	43	Psoriasis	Yes	26%	0%	Y	Y	Y	Y	Not stated	No
Willkens et al.(32)	Cohort	1980	5	RA	No	0%	0%	N	N	N	N	Not stated	No
Asthon et al.(299)	Cross Sectional	1982	38	Psoriasis	Yes	24%	5%	N	Y	Y	N	55%	No
Groff et al.(300)	Case Series	1983	5	RA	No	0%	0%	N	N	N	Y	Not stated	No
Hoffmeister (33)	Cohort	1983	34	RA	Some	21%	0%	N	N	N	N	Not stated	Not stated
Lance et al.(301)	Cross Sectional	1985	30	Numerous	Yes	13%	0%	N	Y	N	N	57%	Not stated
Mackenzie(302)	Cohort	1985	60	RA	No	0%	0%	N	Y	N	N	64%	No
Weinstein et al.(303)	Cross Sectional	1985	17	RA	No	35%	0%	N	Y	N	N	67%	No
Tolman et al.(304)	Corss Sectional	1985	29	RA	No	3%	0%	N	Y	N	N	41%	No
Van de Kerkhof(305)	Cohort	1985	44	Psoriasis	No	16%	5%	N	Y	N	Y	41%	No
Boh et al.(306)	Cohort	1986	21	Inflammatory arthritis	Some	0%	0%	N	N	N	N	54%	No
Reynolds and Lee(307)	Cross Sectional	1986	14	Variety	No	27%	7%	Y	Y	N	N	Not stated	No
Kremer and Lee(308)	Cohort	1986	29	RA	Yes	0%	0%	N	N	N	Y	76%	No
Szanto et al. (309)	Cross Sectional	1987	17	RA	No	6%	0%	N	N	N	N	53%	No
Weinblatt et al.(310)	Cohort	1988	17	RA	No	0%	0%	N	Y	N	Y	62%	No

Shergy et al.(311)	Cohort	1988	399	Variety	No	2%	0.4%	Y	Y	Y	Y	Not stated	No
Bjorkman et al.(312)	Cohort	1988	26	RA/psoriasis	No	15%	0%	N	Y	N	Y	81%	No
Aponte and Petrelli(313)	Cross Sectional	1988	23	RA	No	24%	0%	Y	Y	N	Y	57%	Not stated
Rau et al. (314)	Cross Sectional	1989	30	RA	Yes	3%	0%	Y	Y	N	N	80%	No
Brick et al. (315)	Case Series	1989	88	RA	Yes	11%	2%	Y	Y	Y	Y	68%	No
O'connor et al.(316)	Cohort	1989	78	Psoriasis	Some	24%	0%	Y	Y	N	N	Not stated	No
Kremer et al.(89)	Cohort	1989	27	RA	Yes	52%	0%	Y	Y	N	Y	74%	Yes
Mitchell *et al.*(317)	Cohort	1990	51	Psoriasis	No	20%	6%	N	N	N	N	Not stated	Not stated
Keim et al.(318)	Case Report	1990	1	RA	Yes	n/a	n/a	N	N	N	Y	100%	N/a
Drosos et al.(319)	Cohort	1990	41	RA	No	15%	0%	N	N	N	Y	Not stated	N/a
Willkens *et al.*(320)	Cross Sectional	1990	52	RA	No	29%	0%	N	Y	N	N	55.7%	No
Scully et al.(321)	Cohort	1991	40	RA	No	30%	0%	Y	Y	N	N	Not stated	No
Weinblatt et al.(322)	Cohort	1992	10	RA	No	10%	0%	N	Y	N	N	Not stated	No
Tishler et al.(323)	Cohort	1992	10	RA	Yes	10%	0%	N	N	N	N	100%	No
Graham et al.(324)	Cross Sectional	1992	12	RA	No	0%	0%	n/a	n/a	Y	Y	Not stated	No
Phillips et al.(325)	Case Series	1992	43	RA	No	1%	1%	Y	Y	N	Y	74%	No
Themido et al.(326)	Cross Sectional	1992	30	Psoriasis	Yes	23%	10%	Y	Y	Y	Y	Not stated	No
Minocha et al.(327)	Cross Sectional	1993	24	RA	No	4%	8%	Y	Y	Y	Y	67%	Not stated
Arias et al.(328)	Cohort	1993	16	RA	No	6%	0%	N	N	N	N	Not stated	No
Bjorkman et al.(329)	Cohort	1993	15	RA	No	unclear	0%	Y	Y	N	N	20%	No
Chandran *et al.*(330)	Case Series	1994	3	RA	Some	N/a	N/a	Y	Y	Y	Y	0%	N/a
Van Dooren-Greebe et al.(331)	Cross Sectional	1994	55	Psoriasis	Yes	13%	4%	N	Y	Y	Y	Not stated	No
Boffa et al.(332)	Cross Sectional	1995	49	Psoriasis	No	22%	0%	N	Y	N	N	39%	No
Lower and Baughman(333)	Cross Sectional	1995	33	Sarcoidosis	No	15%	--	N	N	N	N	86%	Not stated
Malatjalian et al.(334)	Cross Sectional	1996	104	Psoriasis	Yes	20%	3%	N	Y	Y	Y	43%	Not stated
Boffa et al.(335)	Cohort	1996	87	Psoriasis	Some	21%	3%	N	N	N	N	Not stated	Not stated
Kremer et al.(336)	Cross Sectional	1996	94	RA	No	0%	0%	N	N	N	N	64%	Not stated
ter Borj(337)	Case Report	1996	1	RA	Yes	n/a	n/a	Y	Y	Y	Y	100%	N/a

Kugathasan et al.(338)	Cohort	1996	9	Juvenile RA	No	0%	0%	N	Y	Y	Y	100%	No
Jaskiewicz et al.(339)	Cohort	1996	20	Psoriasis	Some	0%	0%	N	Y	Y	N	53%	No
Beyeler et al.(340)	Cohort	1997	16	RA	No	13%	0%	N	N	N	N	81%	Not stated
Hashkes *et al*.(341)	Cross Sectional	1997	14	Juvenile RA	No	0%	0%	Y	Y	Y	Y	Not stated	No
Richard et al. (342)	Cohort	2000	57	RA	Yes	0%	0%	N	N	N	N	81%	No
Lemann et al. (343)	Case Series	2000	11	Crohns	No	9%	0%	N	N	N	N	Not stated	Not stated
Te et al. (344)	Cross Sectional	2000	20	IBD	No	5%	0%	Y	Y	Y	Y	60%	Yes
PsA: Psoriatic arthritis, RA: Rheumatoid arthritis, IBD: Inflammatory Bowel Disease, RCT: Randomised control trial													

Table 6-5 A summary of historical studies published investigating MTX hepatotoxicity evaluated by liver biopsy until 2000

A handful of further publications, prior to O'Rourke(269, 345), reported the presence of liver fibrosis, predominantly detected at autopsy, in children treated with higher doses of MTX for malignancy. These findings were given relatively little attention, largely because the interpretation of histopathological changes in the context of multiple cytotoxic agents, varying degrees of leukaemic infiltration and terminal infection, was near impossible(271).

Over-estimation of the prevalence of liver fibrosis in these early publications is likely to be multifactorial. Studies were small, rarely including more than 40 participants(281, 291) and took place over relatively short periods of time. Selection bias is evident, particularly within earlier publications, where patients who demonstrated hepatotoxic phenomena, such as persistently abnormal liver blood tests or clinical signs of liver disease, were far more likely to undergo biopsy(299). As clinicians were aware of the risks associated with liver biopsy, there had to be significant concern to justify this invasive test. Dahl *et al.*'s study in 1971, published in the British Medical Journal provides an example. Of the 37 participants, the authors state 9 were selected for further investigations due to clinical concern of liver damage, a further 5 patients had abnormal liver blood tests prior to initiation of MTX. Their final, selected cohort demonstrated a prevalence of liver fibrosis or cirrhosis in 46% of MTX-treated patients(267), a startling figure when not interpreted in context. In fact, as they only biopsied 14 of the total 37 patients they have found fibrosis or cirrhosis in 16% of the total cohort, rather than 46%. This degree of selection bias is widespread throughout the published literature, and explains some of flawed conclusions, particularly in studies published prior to the publication of guidelines recommending routine biopsy.

Historically, strict, inflexible dose regimens of MTX were prescribed. Daily dosing(346), intravenous routes(347), higher doses(348) and variable co-prescription of folic acid(349) are in contrast to 21st century dosing regimens of weekly doses administered either orally or subcutaneously(350). There is some evidence to suggest these former dosing regimens did adversely influence histological findings(20).

Ashton *et al.*'s case series of 38 patients in 1982 is an example of one of the more detailed retrospective series published in the period. Participants underwent liver biopsy pre- and

post- MTX treatment for psoriatic arthritis. The study details 9 of the 38 patients (24%) who developed fibrosis following 28 months of MTX prescription. However, the authors concede one (of the nine) patients had a pre-existing diagnosis of alcoholic liver disease, 4 (of the nine) patients admitted to heavy alcohol consumption, a further one to 'moderate' intake. In keeping with this, 3 of the 9 biopsies demonstrated "classical features of alcoholic hepatitis", and a further patient was found to have alpha 1 antitrypsin deficiency(299). Other risk factors for liver disease, such as weight or BMI are not reported. Furthermore, the cohort initially included 56 patients, however only 38 had repeat liver biopsies. The reason for this variance is not detailed – presumably these individuals had no evidence of liver dysfunction and were therefore spared a second biopsy. Accounting for these two confounding diagnoses reduces the proportion of patients with fibrosis of an unclear cause from 24% to 9%. It is not clear whether other causes of liver disease were ruled out. The authors, as with many others in the period, assumed MTX to be causal for all changes within the liver, despite no relationship between cumulative dose of MTX and the incidence of liver fibrosis(291, 298, 299).

Ashton et al.'s study demonstrates the most significant confounding factor of the period, specifically, the failure to rule out other causes of liver disease (265, 266). The presence or absence of viral hepatitis, serological markers for genetic and immune-related conditions (e.g. AIH, PBC and haemochromatosis) and particularly alcohol intake were not documented in many of the studies, making retrospective interpretation of the data challenging. This is particularly relevant for NAFLD, which prior to 1980 was an unknown phenomena(351). Gastroenterologists previously referred to the condition – with the same histopathological changes as 'MTX-related' hepatotoxicity, as cryptogenic. With hindsight, it is easy to see how MTX may have been blamed for this common, unknown but significant cause of liver disease.

NAFLD is particularly relevant in a cohort of rheumatological and dermatological patients. Reduced mobility secondary to joint pathology and frequent co-prescription of corticosteroids increases the likelihood of an elevated BMI and the presence of metabolic syndrome within this cohort. Furthermore, patients with psoriatic arthritis are known to have an increased prevalence of metabolic syndrome, significantly increasing the likelihood of having co-existing NAFLD (352-354).

Numerous studies caused alarm by reporting cohorts of patients whose liver biopsy after only a few months of MTX treatment, demonstrated fibrosis or cirrhosis. Minocha *et al.* is an example of such a study, reporting liver biopsies from 25 patients with RA taking MTX, that identified 2 (8%) with cirrhosis. However, despite both individuals in question being reported as obese and diabetic, the authors ascribed their cirrhosis to MTX-induced hepatotoxicity(327). This study was published in 1993, 13 years after the discovery of NAFLD. Even so, both dermatologists and rheumatologists appeared to be relatively dogmatic in attributing all abnormal liver biopsy findings to MTX whilst remaining poorly informed on the emerging concerns of metabolic syndrome, and the associated, very identical, histological anomalies.

The dissemination of increasingly stringent guidelines throughout the 1980's necessitated a liver biopsy prior to initiation of MTX, as well as during treatment. This heralded a landmark in clinicians' perspectives, as those with RA(89, 315, 355) and PsA(291, 356) were found to have significant liver disease on pre-MTX liver biopsies, which could not, therefore, be ascribed to the drug. Clearly there are numerous reasons as to why patients may have liver disease; patients deemed appropriate for treatment with MTX were those with severe debilitating disease – having exhausted trials of multiple other potentially hepatotoxic medications including gold, non-steroidal anti-inflammatory drugs (NSAIDs), prednisolone, arsenic and vitamin A(357). However, this did appear to prompt a shift in opinion, and heralded an era of improved appreciation that alternative causes for histological findings should be considered(314, 327).

Studies began to emerge detailing alcohol misuse in patients with psoriasis(358-360), with some authors suggesting alcohol excess may explain, or at the very least contribute to, the hepatotoxicity seen with therapies such as MTX(361, 362). By contrast, there is little published regarding alcohol misuse in the rheumatological population.

A small number of historical studies do demonstrate the acquisition or progression of liver fibrosis whilst on MTX treatment(89, 296). Putting aside the limitations of liver biopsy, and that not all studies have replicated these findings(363), there may be alternative explanations for this apparent correlation. MTX was often co-prescribed with other hepatotoxic medications, such as NSAIDs, prednisolone and other DMARDs(309).

Reactivation of viral hepatitis, particularly HBV, may also account for new fibrosis in the context of immunosuppression. Historically screening for HBV and HCV was not mandatory prior to initiation of MTX, and HBV reactivation has been demonstrated in cases of acquired fibrosis (305, 364). Finally, although cumulative dose of MTX was not commonly associated with liver damage, age(294, 298) and duration of disease(365) was. It is therefore possible other confounding factors may have contributed to the observed changes.

A causal relationship between MTX and liver fibrosis is impossible to determine from the current evidence. Histopathological changes noted secondary to apparent MTX-induced hepatotoxicity are indistinguishable from those seen with alternative liver aetiologies(293), and similar to other liver disease aetiologies including NASH(366) and alcohol excess(367). The lack of pathognomonic histopathological changes, including those features typically seen with drug induced liver injury, casts yet further doubt on this hypothesised relationship. Quintin *et al.* analysed liver histology on 41 individuals with abnormal liver blood tests taking MTX, these revealing histology in keeping with AIH 41%, NAFLD 32%, and PBC 5%(368). Thus demonstrating the pitfalls of attributing all liver disease in this population to MTX.

Meta-analyses have helped to shed light on this complex, multifactorial topic. MTX is undoubtedly associated with an increased risk of transaminase elevation, however, as with almost every other liver condition, the clinical significance of this change is unclear(41). Visser and van der Heijde reviewed 34 studies containing data on liver biopsies performed in 2179 patients with RA. Pre-treatment biopsies on 372 patients demonstrated mild fibrosis in 9.1%, severe fibrosis 0% and cirrhosis in 0.3% of patients. Of those participants who had serial liver biopsies (689), only 43 patients (6.2%) demonstrated progression from a normal liver architecture to mild fibrosis following MTX. Indeed, risk factors for hepatotoxicity were documented in only 4 of the 34 studies, seriously undermining any meaningful conclusions with respect to causation. However, multivariate analysis on those 4 studies did suggest that alcohol, duration of RA, age and albumin were associated with Roenigk score, a histological measure of liver damage(365).

Meta-analyses are clearly only as dependable as the evidence upon which they are based. The absence of a control cohort, selection bias, and failure to attain pre-treatment liver biopsies for comparison, are examples of the multiple design flaws within the existing, historic, literature. Failure to demonstrate a cumulative dose relationship (as detailed in Table 6-5), means evidence demonstrating a causal link between MTX and liver fibrosis is weak and circumstantial at best(41). Conway *et al.*'s meta-analysis was confined to double-blind randomised controlled trial data, overcoming some of the above limitations. Only studies with greater than 100 participants, studies with a control cohort and those featuring a minimum of 24 weeks exposure to MTX were included. The authors analysed 32 studies, including 13,177 participants, and demonstrated no increased risk of liver fibrosis compared to controls. Control cohorts were a combination of placebo and active comparators (other DMARDs or biological therapies) to MTX for RA, psoriasis, PsA, and IBD(369). A key limitation of the meta-analysis, however, is that it relies upon liver blood tests to evaluate liver fibrosis rather than liver biopsy, which was not performed routinely.

In summary, despite significant historical concerns it remains unclear and unproven, as to whether patients receiving MTX are at an increased risk of developing liver fibrosis. Meaningful stratified analysis of the existing evidence-base is thwarted by pervasive, flawed study-design that generally exclude established liver risk factors and liver histology. With hindsight, attributing all liver disease in those taking MTX to the drug itself appears naïve, at best. A cohort of patients, such as those requiring MTX, have increased risk factors for liver disease, increased risk of metabolic syndrome (in those with psoriasis) and NAFLD was ignored. In an era where NAFLD was poorly defined, it seems that MTX was 'blamed' for liver disease more likely attributable to NAFLD. Correlation does not imply causation.

6.3.2   *Risk factors for fibrosis progression in those taking Methotrexate*

Over the past twenty years the obesity epidemic has become increasingly relevant to clinical practice. In 2018, 60% of women and 67% of men in England were overweight or obese(370). Described for the first time in the 1970s, NAFLD is highly prevalent; the global prevalence now thought to be around 24%(371).

Psoriasis, whilst best-known for its skin and joint manifestations, is now widely acknowledged as causing systemic inflammation. Hence patients with psoriasis are more likely to develop liver fibrosis when compared to many other inflammatory conditions requiring MTX treatment(365). A population-based study in Rotterdam (n = 1535) demonstrated the prevalence of liver fibrosis in patients with psoriasis as 8.1%, compared with 3.6% in those without psoriasis(354). Such systemic inflammation in individuals with psoriasis is thought to lead to insulin resistance, endothelial cell dysfunction and consequent metabolic syndrome and cardiovascular disease(372). Thus, patients with psoriasis have a 40% increased risk of metabolic syndrome as compared with the general population(372). Risk factors for NAFLD, such as diabetes and being overweight (BMI > 25kg/m^2), are the same risk factors that promote liver fibrosis in psoriatic patients; suggesting that NAFLD may be the underlying aetiology, rather than the MTX(373).

Unsurprisingly, other recognised causes of liver disease, such as high alcohol consumption(363, 373), chronic viral hepatitis(69), diabetes(374, 375), elevated BMI(41, 327) and hyperlipidaemia(374) have all been shown to be risk factors for liver fibrosis development in the MTX cohort, as well as the non-MTX cohort. What remains unresolved is whether the acquired liver fibrosis is related solely to the risk factor or whether MTX plays a causal role, a synergistic role or is just an innocent bystander.

No review of the relationship between MTX and liver fibrosis is complete without exploring the potential role of cumulative dose. Indeed, some smaller studies (89, 376) do suggest a relationship, but the majority(207, 373, 377), along with several systematic reviews(375, 378), have failed to demonstrate any relationship between cumulative dose of MTX and liver fibrosis. This discrepancy within the literature is most likely attributable to the relative poor quality of the existing publications in this field, where risk factors and other confounding factors are often under-reported and there is a paucity of biopsy-based studies. The absence of a correlation between liver fibrosis and cumulative dose in the majority of studies suggests MTX could be an innocent bystander, rather than a risk factor for liver fibrosis itself.

6.4   Body Mass Composite

6.4.1   The Body Composition Analyser

Overweight or obesity is defined as abnormal or excessive fat accumulation that may impair health; the World Health Organisation (WHO) describes this as one of today's most neglected health problems(379). Health Survey for England data suggest that 64% of adults in England are overweight or obese(380). Precise quantification is important given the associated health risks (diabetes(381), cardiovascular disease(382) and NAFLD(383)) and increased mortality associated with excess adipose tissue(384). BMI (weight in kilograms divided by height in metres, squared) is most commonly used as a practical assessment of adiposity. It is simple to measure, low cost and has been shown to correlate with mortality(385). However, BMI fails to differentiate between tissue type (muscle and adipose) or distribution (visceral and subcutaneous) which can lead to incorrect classification(386, 387). Ethnicity causes disparity in height, fat distribution, and risk of metabolic syndrome, which leads to varying reliability of BMI to correlate with significant morbidity(388).

Alternative anthropometric parameters that correlate with morbidity and mortality include waist circumference(384), waist to hip ratio(389), waist to height ratio(390), and composite scores such as A Body Shape Index (ABSI)(391, 392). NICE guidance recommends considering the use of waist circumference in conjunction with BMI(393). However, all anthropometric measures are vulnerable to the same inconsistencies as BMI(389).

Bioelectrical impedance analysis (BIA) to assess body composition was described by Thomasset(394) and then Hoffer(395) throughout the 1960's and became commercially available to assess human body composition in the 1990's(396). The technique measures the impedance of an electrical current through body fluid. Demographic and anthropometric information including gender, ethnicity, age, level of physical activity, height, weight and waist circumference are then utilised to calculate free fat mass (FFM), total body water (TBW), skeletal muscle mass (SMM) and visceral adipose tissue (VAT) amongst others parameters(397). BIA is non-invasive(398) and safe(399, 400); it provides more information than anthropometric tests in isolation(401, 402). The

equipment required is costly at the outset and reproducibility across different companies' products is inconsistent(403).

6.5 Summary

MTX is an highly effective therapy, widely utilised across a broad range of specialities since the 1960's. However, concern regarding potential MTX-related hepatoxicity has damaged its reputation caused much consternation, even though high-quality evidence demonstrating a causal relationship is lacking. The absence of a robust, consistent, validated clinical pathway to assess the risk of liver fibrosis development in patients receiving MTX, has led to erratic clinical monitoring and the inappropriate continuation and discontinuation of MTX in many recipients.

Fatty liver disease is increasingly prevalent and relevant given the burgeoning obesity epidemic. Liver fibrosis cannot be detected by standard liver blood tests alone, but novel, non-invasive measures, such as transient elastography, provide an exciting opportunity to detect and monitor liver disease.

CHAPTER 7: AIMS AND HYPOTHESIS

The aims of this study were to:

1) To assess compliance with MTX monitoring guidelines locally
2) To ascertain consideration given to alcohol intake when prescribing MTX
3) To assess response in the face of abnormal LBTs in patients prescribed MTX
4) To establish the prevalence of liver fibrosis and cirrhosis (by way of FibroScan®) in a population of patients who take low-dose MTX, compared with those who have never taken this medication
5) To establish risk factors for liver disease in this population, including age, alcohol, BMI and physical activity levels.
6) To establish patients awareness of serological monitoring when taking MTX
7) To quantify how much alcohol patients were drinking whilst taking MTX
8) To establish patient's opinions regarding MTX

The hypothesis of the study were:

Low-dose MTX does not cause liver fibrosis

CHAPTER 8:   METHODS

8.1   Electronic record-based audit

8.1.1   Audit design

An IT search identified patients prescribed MTX at YTHT over a 4 year period (n=3409). Retrospective electronic note review was performed on a random sample (n=150) establishing demographic details, blood tests, interventions following MTX prescription and consequential actions.

8.1.2   Regulatory considerations and approvals

The audit design, objectives and proposed outcomes were approved by YTHT clinical effectiveness team following appropriate committee review.

8.2   Assessing liver fibrosis in an outpatient population

8.2.1   Study design

A cross-sectional study was adopted; evaluating a group of participants who had received MTX, for at least 6 months, and a cohort who had never taken MTX. The study design is illustrated in Figure 8-1. Rheumatology and dermatology outpatient clinics were identified in advance, based on equipment availability, appropriate workspace, and personnel. An Invite Letter (Appendix E) and Participant Information Sheet (Appendix F) were sent to all patients over 18 years of age, prior to attendance. The study was advertised in the outpatient department by way of display posters (detailed in Appendix K) and clinicians were encouraged to offer participation to patients.

Disseminating information
- Patients attending specified rheumatology and dermatology outpatient clinics in York Hospital recieve an Invite letter (Appendix E) and Participant Information Sheet (Appendix F) regarding the study prior to their appointment.

Consent obtained
- Participants who wish to partake are identified at their clinic appointment and discuss the risks and benefits of the study with a member of the research team.
- Appropriate consent taken, after eligibility is confirmed.

Data anonymisation
- Participants are allocated a study ID number, which is used on study documentation.

Gathering data
- Participants complete a MTX questionnaire (Appendix J) (only if in the MTX cohort) and the IPAQ questionnaire (Appendix I) with appropriate support as required.
- A clinical proforma (Appendix H) is completed by a member of the research group.
- Measurements including height, weight, a liver FibroScan® and body mass composite score are performed.

Data transfer
- Manual transfer of the written information gathered is recorded on an EXCEL spreasheet on a trust encrypted, secure, information technology system.
- Data is sense checked at this time to ensure no obvious errors or missing data.

Data analysis
- Data analysis is performed to establish risk factors of liver disease - by way of multiple regression analysis - and to evaluate whether patients taking MTX have a higher prevalence of liver disease. Findings will then be presented regionally, nationally and internationaly with results published in peer reviewed journals.

ID: Identification, MTX: Methotrexate, IPAQ: International physical activity questionnaire

Figure 8-1 Flowchart of study design

Recruitment was similar in both arms of the study. Recruiting both cohorts from the same outpatient setting increased the probability that the cohorts would share similar attributes such as prevalence of immune-related illnesses, exposure to similar medications (prednisolone and other DMARDs), and limitations on physical activity. Modelling was performed to ensure sufficient eligible participants within the targeted population, see Recruitment. A research team member (with the appropriate space and equipment) was also in attendance at the identified clinics, allowing study enrolment and participation at a single consultation. Some participants chose to contact the research team in advance to make a mutually agreeable time to meet and participate in the study outside of their scheduled outpatient attendance. A member of the research team consented participants into the study, gathered the appropriate information from their medical history, participant questionnaires, and specialist tests.

8.2.2   Rationale for adopting current study design

The central question running through this thesis is whether MTX causes liver fibrosis. The optimal study design to address this central question, was carefully considered. Alternative study designs were contemplated; RCTs are gold standard in demonstrating causal relationships(404). Indeed the efficacy of MTX in treating many inflammatory conditions is well-established using just such a trial design, however, an RCT to establish efficacy is conceptually rather different to an RCT to evaluate a potential side-effect. The breadth of the underlying diseases present within our target cohort makes randomisation to suitable alternatives problematic. Equally, MTX is established as superior compared with other treatment options in certain diseases(43); depriving participants of one of the most effective treatments with either a comparison drug or a placebo would be unethical. The interventional nature of an RCT makes the study design inappropriate and alternative approach needed to be considered.

A prospective cohort study has the benefit of assessing liver health specifically, in a stipulated group before and after they have received MTX. However, the lengthy study design is significantly more resource-heavy, costly and leads to issues with loss of follow-up. Optimal methods of assessment of diagnosing liver fibrosis and cirrhosis would likely change over the decades required for this study design, and this would cause difficulties

in comparing methods of detection of disease. The combination of these factors would have made a prospective cohort study impractical in our setting.

A cross-sectional study would aim to examine liver health in a population either receiving or not receiving MTX, at a specific point in time. Such an approach has several limitations, for example it ignores liver status prior to exposure to MTX. However, a clear strength of the study design is the ability to incorporate multiple underlying diagnoses within the study population, as the same study population incorporates both the MTX-recipient arm and the non-MTX or control arm. A cross sectional study is relatively quick to undertake, does not suffer from a loss of follow-up and is economical(405). The key deficiencies of cross-sectional methodology centre around potential for recall bias and unreliable timing of events; however, MTX use is well-documented meaning these weaknesses could be overcome in our cohort.

8.2.3 Study setting

The study was conducted at York Teaching Hospitals Trust (YTHT) and coordinated from an office within the Hepatology Department, at York Hospital. Adopting a multi-centre approach was considered, however due to the specialist equipment required (body mass composite), this was not feasible. A contingency plan of widening participation to other hospitals should recruitment be insufficient was considered, including Scarborough Hospital and Harrogate and District NHS Foundation Trust; this was not required.

Participants of the study were met and assessed in the Outpatient Department of York Hospital. This area facilitated safe and confidential working spaces and allowed access to the specialist equipment required including the FibroScan® and body mass composite machine.

8.2.4 Data collection – participant history

A history was taken from the participant acquiring details of past medical history, drug history, current and historical alcohol history as per the proforma (Appendix H). This information was corroborated by checking the patient's electronic record. Rarely, where

information could not be recalled and was not available electronically, a patient's historical medical notes were reviewed.

8.2.5 Data collection – electronic record

Data from the patient's electronic record; including blood test results, duration and cumulative dose of MTX prescribed, historical weights and postcode were collected. The electronic record was also used to corroborate information as stated in the above section, 'Data collection – participant history'.

8.2.6 Data collection – questionnaires

Participants within the MTX arm of the study were asked to complete an anonymous questionnaire focusing on their thoughts and opinions regarding MTX (Appendix J). Participants were given adequate time and assisted with any special needs as appropriate. The questionnaire included six questions based around a participants' experience, concerns and monitoring whilst taking MTX.

All participants were also asked to complete the International Physical Activity Questionnaire (IPAQ) - short version (Appendix I). The IPAQ is short and easy to execute for participants. It's a validated tool available in multiple different languages and is easily scored. Appropriate time and assistance were provided for participants to complete the seven questions.

8.2.7 Data collection – special tests

A liver FibroScan® and body composition analysis were performed on all subjects. If a participant's FibroScan® was greater than 7kPa they were informed of the result, the implications and the planned next steps including a further appointment for a repeat scan and an hepatologist's review. FibroScan® acquisition required participants to lie down flat. Body mass composite analysis required participants to stand, bare-footed and maintain a grip onto handles. In some instances, participants could not adopt or maintain these positions e.g., due to limb loss or reluctance to remove footwear, these participants were excluded. No undue pressure was applied on potential participants to take part.

8.2.8   Eligibility criteria

Inclusion criteria for the study were as follows:

- Participants must be greater than 18 years-old
- MTX cohort: must have been prescribed and currently taking either oral or subcutaneous preparations of MTX for a minimum of 6 months prior to inclusion
- Control cohort: participants have never been prescribed or taken MTX
- Capable of giving informed consent

Exclusion criteria were as follows:

- Participants less than 18 years
- Pregnant females
- Individuals who had a history of MTX exposure but who were no longer taking the medication

8.2.9   Sample size

Evidence regarding the prevalence of liver fibrosis in patients receiving MTX is variable, and reported in between 5-10% of patients who are prescribed MTX(244, 406), in contrast to 1.3% in a UK population not taking MTX(371, 376).

Dr Mona Kanaan, an Associate Professor, based at the Hull and York Medical School (HYMS), helped to advise regarding an appropriate sample size given the above presumed prevalence. Using a case control ratio of 1:1 and significance level of 0.05, the total number of participants within the study would need to be at least 698.

Preliminary analysis of the first 219 participants was performed to assess accuracy of sample size, by Professor Martin Bland, a professor of statistics at HYMS. Using FibroScan® as a quantitative outcome, a case-control ratio of 1:1 and a power of 90%, a sample size of 600 participants was deemed statistically sufficient. If FibroScan® is utilised as a dichotomous outcome - fibrosis (>7kPa) or no fibrosis (≤7Pa), with a detectable odds ratio of 1.55 a sample size of 2000 participants would be required. Based

on this further calculation the study recruitment target was 600 participants – with FibroScan® used as a quantative outcome, rather than qualitative.

8.2.10   Recruitment

Patients attending specific rheumatology and dermatology clinics received an Invitation Letter (Appendix E) and Participant Information Sheet (Appendix F) prior to their outpatient appointment. This allowed participants to read and consider the information in their own time, prior to their appointments, and discuss partaking in the study, with others, should they so wish. Contact details of the research team were included within the literature, enabling individuals to seek further information or an alternative time to meet the research team in advance of their appointment.

Appropriate clinics were identified following discussion with relevant specialities including drug monitoring clinics in dermatology and connective tissue disease clinics in rheumatology. Modelling was performed to ensure the designated clinics would include a sufficient number of eligible patients each week (Figure 8-2) to ensure recruiting the total number of participants required was feasible. These clinics were appropriately manned with a member of the research team, an allocated room and access to the equipment required.

Healthcare professions running the relevant clinics were encouraged to mention the study to eligible candidates. When potential participants expressed interest in the study, they were directed to a member of the research team who could provide them with further information, and recruit them into the study if eligible, should an individual wish.

Prior to recruitment, the participant would be taken through a series of short questions to ensure they were eligible to take part in the study (Figure 8-2). The process in which participants were recruited was similar for both arms of the study. Both cohorts were identified from rheumatology and dermatology clinics.
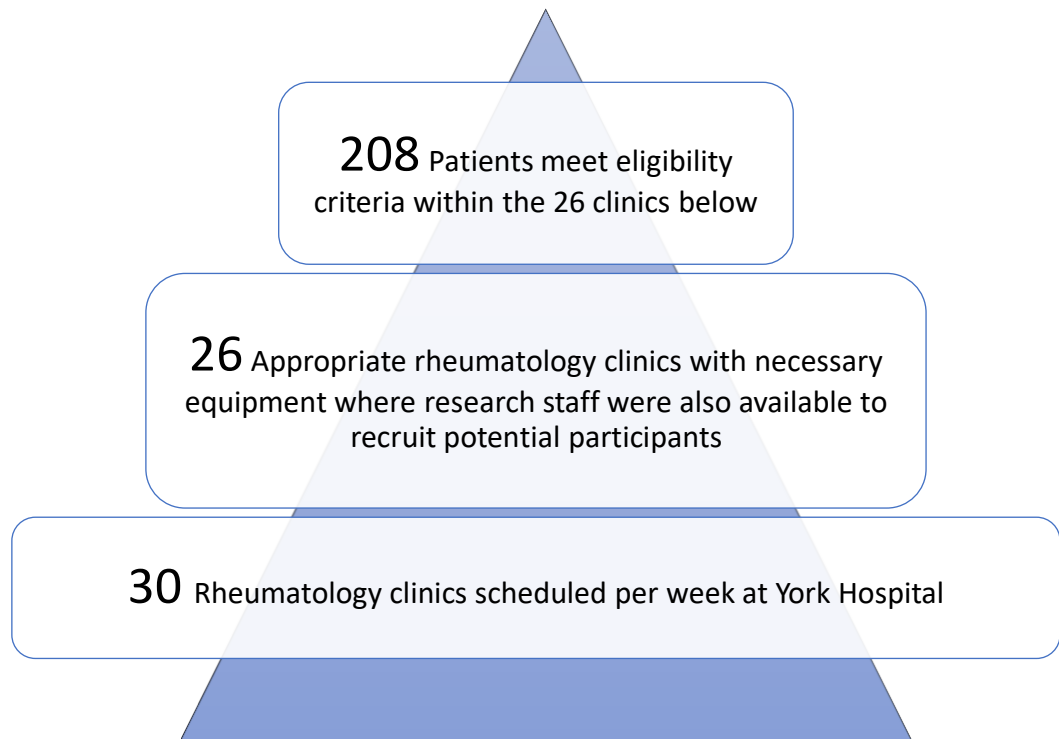
Figure 8-2 Modelling potential recruitment

Calculations were performed based on the total number of clinics (30) within the relevant speciality in a typical week at York Hospital. Clinics where appropriate equipment or research staff were not available were excluded (4), leaving 26 clinics from which to potentially recruit participants. Patient records were then scrutinised to assess whether they would be eligible to take part in the study, should they so wish. This resulted in 208 potential participants each week being eligible for recruitment to the study, should the patients be willing.

The Principal Investigator (PI) monitored recruitment throughout the study period, to ensure it was sufficient and approximately equal numbers of each cohort were enrolled. Alternative processes were considered should recruitment be poor; a pharmacy-based search detailing those who have been prescribed MTX, speciality-specific databases of patients prescribed MTX and widening recruitment to involve other hospitals. However, none of these additional measures proved necessary, as although initial recruitment was slow, once the study was established, recruitment proved to be better than forecast.

Websites were not used to recruit participants, and there was no payment or reimbursement provided to participants. Every effort was made to conduct the sole meeting required for the study at the same time as an existing hospital appointment, so as to minimise the inconvenience to the study participant.

8.2.11 Consent

All members of the research team are Good Clinical Practice (GCP) trained and aware of the principles of informed consent. Receiving study information prior to their clinic attendance meant individuals had time to consider participation and discuss this with others, including the research team, prior to attendance. If participants expressed interest in the study, they were shown into an appropriate quiet area within the department, with a member of the research team. The participant information leaflet (Appendix E) and consent form (Appendix G) were both outlined verbally, including the procedure and time requirement for participation. Confirmation of eligibility was undertaken with a series of short questions prior to consent being taken, as demonstrated in Figure 8-3.

Informed consent was only taken by appropriately trained personnel, only adults who had capacity were recruited into the study. Those with additional requirements were facilitated in any way appropriate; largely this was making accommodation for mobility issues such as wheelchair access and increased time in those with mobility disorders. All participants had the opportunity to ask questions at all stages of the process. Participants were never coerced into enrolling into the study.
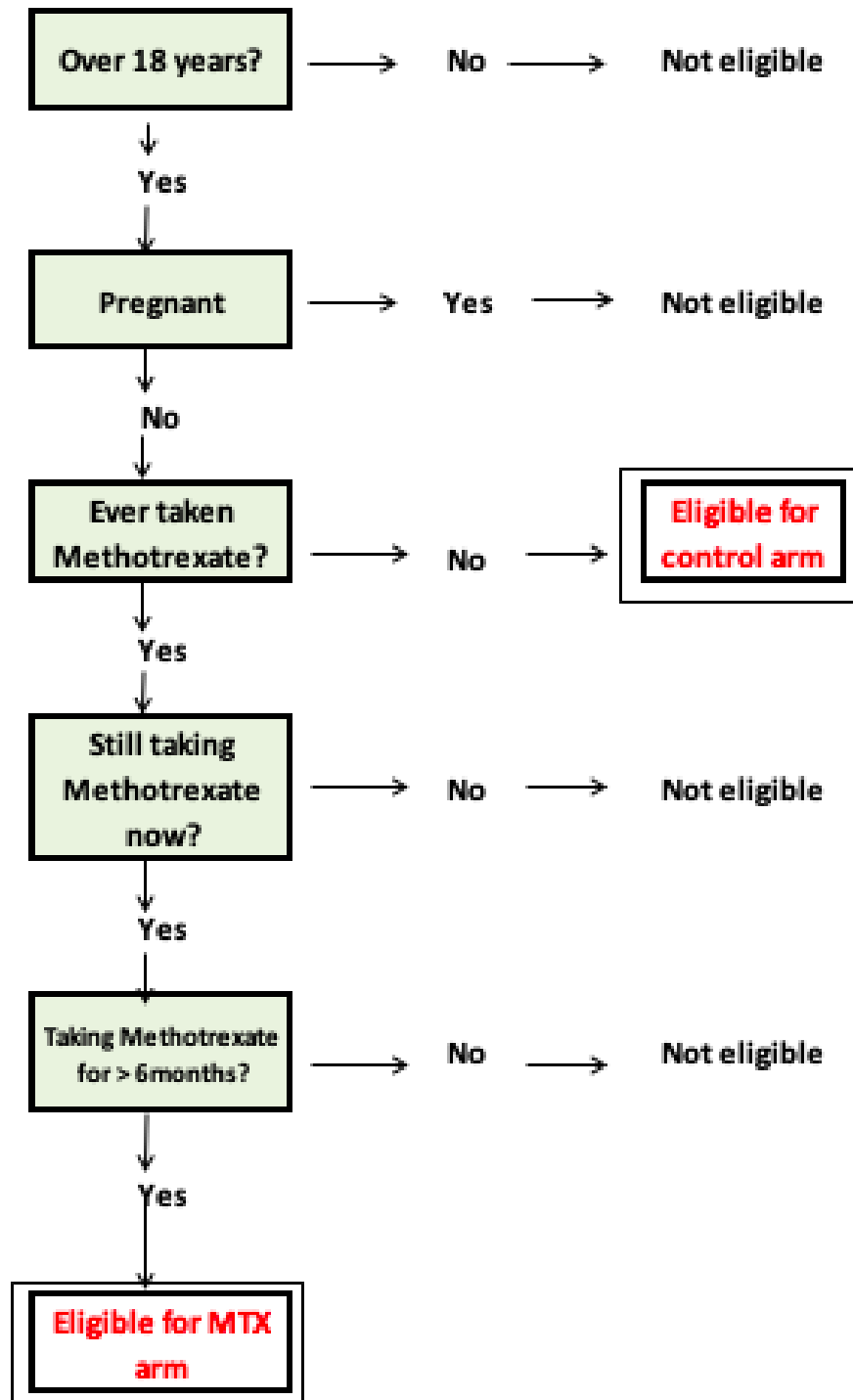
Figure 8-3 Eligibility flowchart

8.3 Patient and public involvement

8.3.1 The process

A service user team reviewed the patient-facing documents adopted within the study; the Participant Invite Letter (Appendix E), Participant Information Sheet (Appendix F), Participant Consent form (Appendix G), and non-validated MTX Questionnaire (Appendix J).

Feedback from members was written and anonymised. This feedback was largely positive, such as the example below:

 "It's a polite, comprehensive sheet which covers all aspects which could be asked or worried about from a patient's point of view. It reads well and the methodology is explained well."

The complete feedback is detailed in Appendix D.

8.3.2 Syntax and grammar

Much of the feedback centred around grammatical amendments and sentence structure. The alterations predominantly reduced sentence size to make them easier to comprehend.

8.3.3 Negative connotations

Feedback emphasised the importance of removing negative words or phrases; 'problem' was replaced with 'issue' and other more benign synonyms. The advice suggested the content left the reader with a negative view of both MTX and taking part in the study. For example, in explaining the possible benefits of taking part, the Participant Information Sheet stated the following:

"It is possible that the health checks carried out during the study could show up a problem that you didn't know about. If this happens, you will be referred for suitable assessment and investigations and your General Practitioner (GP) will also be informed".

Advice following review suggested the following addition to the sentence:

"This referral may be to your advantage".

Well-meaning efforts to not coerce individuals to take part in the study or falsely overstate the benefit of partaking, had resulted in the risks being over-stated. Careful review was undertaken to remove bias that may have led to this.

8.3.4 Impact upon the participant

Feedback encouraged the research team to consider the study from a patient perspective. Recruitment of the control cohort was an example of this; the proposed Invite Letter discussed MTX in some detail with only brief mention about a control cohort being required. It was observed that this could be confusing and daunting to half our intended cohort, who were not taking MTX. The invite letter was altered accordingly.

Overall, the acceptability of the research from the service user team, was reported as good.

8.4 Assessment and management of risk

8.4.1 Informed consent

The consent process was only undertaken by appropriately trained research practitioners, all of whom are GCP accredited. The provision of written information being disseminated prior to a participant's clinic attendance afforded individuals time to consider the study and discuss it with others, should they wish. By way of reinforcement, a member of the research team outlined the study verbally with participants, reviewed the Participant Information Sheet and detailed the practicalities of study involvement prior to commencing consent. Opportunity for questions was provided and time taken to reiterate that a decision to decline to take part, would not have any adverse consequences for the patient.

Individuals who lacked capacity were not included in the study, given its voluntary nature and being additional to standard care.

8.4.2   Request to withdraw

A participants' right to withdraw from the study was highlighted to them during the consent process, prior to enrolment. Participation in the study involved one meeting, with no planned follow-up, therefore requests for withdrawal from the study were thought to be relatively unlikely but planned for, regardless. Where participants chose to withdraw prematurely, a reason (if volunteered) was documented, and the PI was notified.

8.4.3   Risk to participants

This study was observational in nature – acquiring information, rather than intervening, trialling or altering treatments. The main risk would be unmasking previously undiagnosed liver fibrosis or cirrhosis following the FibroScan®. Should a FibroScan® score be abnormal (> 7kPa) the participant was informed that their result was outside of the 'low risk' category, by appropriately trained personnel (Figure 8-4). Participants were informed of the ongoing plan - referral to the hepatology team, and were reminded of the research teams' contact details, prior to departure, should they have concerns. All datasets were reviewed by the PI and GPs were informed as a courtesy.

8.4.4   Risk to researchers

The research study was compiled with the guidance and support of individuals who are highly experienced in both hepatology and research methodology. Contact with abusive individuals was deemed unlikely to occur, given the voluntary nature, but if this were to happen, YTHT Trust Policy would have been followed at all times. The working environment was familiar to the research personnel, helping to reduce risk and ensure team cohesiveness.

The supervision of the Research and Development team at YTHT was a necessary safeguarding mechanism to ensure ongoing adherence to regulations throughout the timespan of the project.

```
                        ┌─────────────────────────┐
                        │   FibroScan® performed  │
                        └─────────────────────────┘
                            ↙               ↘
        ┌─────────────────────┐       ┌─────────────────────┐
        │  FibroScan® ≤ 7 kPa │       │  FibroScan® > 7 kPa │
        └─────────────────────┘       └─────────────────────┘
                    ↓                           ↓
    ┌──────────────────────┐   ┌──────────────────────────────┐
    │ No additional action │   │ Inform participant FibroScan® │
    └──────────────────────┘   │ result suggests above 'low    │
                               │ risk' category for liver      │
                               │ fibrosis                      │
                               └──────────────────────────────┘
                                   ↙               ↘
```

Participant already aware of potential liver problem and known to liver team

Participant not already aware of a potential liver problem

Ensure existing liver outpatient review, if not in place, email PI to arrange

Explain next steps to participant including offering a hepatology appointment where the FibroScan® will be repeated following fasting
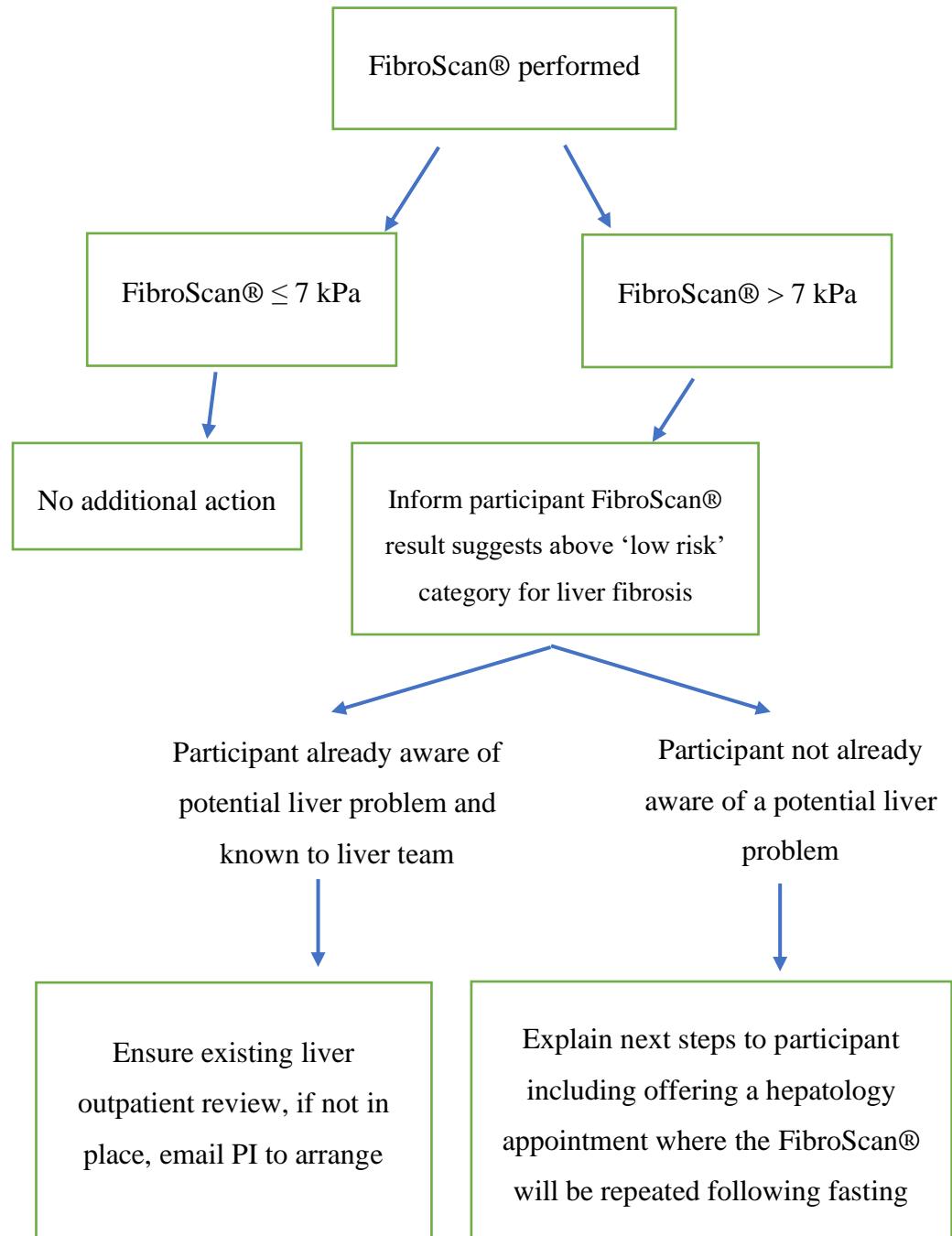
Figure 8-4 Action required dependent upon FibroScan® result

8.4.5 Risk to reliability of results

Appropriate powering of the study helps to ensure study results are sufficient to draw meaningful conclusions, without unnecessarily involving too many participants. Statisticians were involved during the powering of this research study and interim analysis was planned to ensure this could be revised if necessary, as detailed in Section 8.2.9.

Given the study was sponsored by YTHT, the research and development team performed serial exercises, as per Trust guidelines. The team were vigilant for deviations from the protocol or fraud. All research members, being GCP trained, are aware of the absolute requirement of adherence to the protocol, at all times.

Information acquired throughout the study was verified using an alternative source. Hence, participant history was confirmed with electronic patient records, and when this was not possible, historical notes were used. This improved the accuracy of data collected. All datasets were then reviewed by the PI, where incongruous results were checked again.

8.4.6 Risk to organisation

Funding of the research teams' time was comprehensively assessed prior to management approval within YTHT. The equipment used (FibroScan® and body composite analyser) were already owned by the Trust and thus incurred no additional monetary cost.

Indemnity arrangements have been considered in section Indemnity below.

In assessing 600 individuals' liver health, the study anticipated finding new cases of liver fibrosis or cirrhosis, prompting referral into local hepatology services. Capacity was considered; in the unlikely event of the service being overwhelmed a reserve plan was put in place to increase capacity in the short-term until resolution.

Although unlikely, a research member may be made aware of a potential risk to a participant, or another individual e.g., a safeguarding concern. Team members would act in accordance with YTHT guidance at all times, and therefore inform the safe-guarding team, as per mandatory training within the Trust.

8.5 Ethical and regulatory considerations

8.5.1 Sponsorship

Sponsorship was awarded from YTHT following application, and committee scrutiny, as detailed in Appendix A. Prior to approval, the research group were required to demonstrate the following steps had been undertaken:

- A clear protocol with scientific peer review and statistical review as necessary
- Appropriate funding be sourced as necessary
- Drafts of all patient-facing documentation including advertisements, invite letter, patient information sheet and consent form
- Drafts of all communication with patients, GPs, or recruitment advertisements
- A study risk assessment
- Curriculum Vitaes for all investigators within the team (407)

Necessary applications were made for full sponsorship review at YTHT in early November 2018 and granted in April 2019.

8.5.2 Research Ethics Committee

The study team applied for the relevant ethical approval via the national Integrated Research Application System (IRAS) to achieve Health Regulation Approval (HRA), prior to commencing recruitment. This was awarded on the 17th June and 18th June 2019 respectively, as detailed in Appendices B and C.

All correspondence with the Research Ethics Committee (REC) has been retained. The PI will notify the REC at the end of the study, or sooner if the study is ended prematurely, including reasons for termination. Currently the study is still ongoing. An annual progress report is submitted to the REC within 30 days of the anniversary date on which the favourable opinion was given, and annually until the study is declared ended. Within one year from the end of the study, the PI will submit a final report with the results, including any publications and abstracts, to the REC.

Minor amendments to the study were made throughout the recruitment process, to alter the wording of the Patient Information Sheet and later to amend the total recruitment target; approvals were issued in both cases. The YTHT research and development department worked in conjunction with the team to coordinate amendments and to confirm their ongoing support for the study. No substantial amendments were undertaken. Protocol and associated document amendments were tracked using different versions of the documents.

8.5.3 Data management plan

Microsoft Excel was used to construct and hold all study results. This database was password-protected on an National Health Service (NHS) Trust encrypted IT system. Only members of the research team had access to the database. Saved data is backed up every 24 hours, as part of the York NHS Trust standard IT operating procedures.

Data transfer, from paper datasets onto the electronic database, was undertaken on NHS property; all paper datasets are stored in a locked cupboard within the R&D department. Data held on the database are anonymised, using an allocated participant study identification (ID) number. Statistical analysis has been performed intermittently throughout data collection (interim analyses) and will be repeated once data collection is complete. All data are archived as per the YTHT Trust policy and supervised by the York NHS Trust R&D team.

8.5.4 Data protection and patient confidentiality

All participants were issued with a study ID number at recruitment, to ensure data were anonymised. The study number was used on all documents, rather than identifiable information, meaning if and when data are shared with co-investigators or sponsors, it will already be anonymised. Any identifiable data were stored in a site file held in a locked room on NHS premises. Non identifiable data were held on YTHT trust, password-protected computers. Full medical confidentiality is upheld; the PI acted as data custodian for the study. All research members complied with the Data Protection Act 2018 with regards to data collection, storage, processing and disclosure of personal information. The Act's core principles were upheld at all times.

Data are to be stored for the minimum time necessary so that adequate work may be performed enabling meaningful conclusions to be reached. This will be for the two-year timespan of the Medical Doctorate (M.D.) and should continue until at least one year following completion of recruitment, allowing for analysis and publishing.

8.5.5 *Indemnity*

Insurance and indemnity are in keeping with YTHT protocol, and the NHS indemnity scheme. Investigators and collaborators will ensure their activity is covered on their own professional indemnity; however, they will also be covered by YTHT overarching insurance and indemnity.

Equipment used throughout including computers, networks and specialist machines (FibroScan® and body composition analyser) will be appropriately maintained and calibrated. This equipment is covered as the property of York Teaching Hospitals NHS Foundation Trust.

8.5.6 *Protocol compliance*

Rigorous training of all team members was undertaken prior to joining the delegation log in an effort to reduce protocol deviations or breaches. If protocol deviations did occur they were documented and reported to the sponsor, PI immediately. This prompted reiteration of training if required and review to ensure repeated deviations were not likely.

8.6 Statistical analysis

8.6.1 *Summary statistics*

SPSS 17.0 was used for statistical analyses. Descriptive statistics for both the MTX and control cohorts were calculated, including gender, age, BMI, waist circumference, proportion of cohort overweight (BMI > $25kg.m^2$), self-reported alcohol intake, weekly physical activity levels, prescribing speciality and presence or absence of diabetes mellitus, hypercholesterolaemia, hypertension and psoriasis. Mean was calculated for continuous variables with independent two tailed t-test for statistical significance. Categorical or nominal data were represented using frequencies and percentages. Chi-

squared was used for statistical significance, or Fisher's Exact test when assumptions were not met (in the case of gender, ethnicity and prescribing speciality). A p-value of < 0.05 was deemed as significant.

Odds ratios were used to calculate likelihood of liver fibrosis within the cohorts. Pearson's correlation coefficient was used to assess correlation between continuous variables. Where data was not normally distributed, appropriate adjustments were made such as the reciprocal used.

8.6.2 *Multiple regression analysis*

Multiple regression analysis was used to adjust for confounding variables, to assess the relationship between cumulative dose of MTX and liver fibrosis. Independent variables included gender, age, BMI, waist circumference, fat mass (%), self-reported Alcohol Use Disorder Identification Test (AUDIT- C) score, average alcohol intake, ALT, cumulative MTX dose and physical activity levels.

CHAPTER 9:   PRESCRIBING METHOTREXATE – REAL WORLD EXPERIENCE

9.1   Introduction

Low-dose MTX's versatility as an immune modulator explains why it is widely used as an effective treatment for a variety of conditions across several specialities, including rheumatology, dermatology, and gastroenterology, amongst others. Monitoring guidelines differ across these diverse specialities, as discussed in Chapter 8. Many of these guidelines incorrectly infer, overtly or inadvertently, that a normal LBT excludes significant liver disease, that changes in LBT's correlate with liver damage and that medications for which liver monitoring are not required, are *not* hepatotoxic.

In practice, adherence to guidelines is suboptimal(408-411). Escalas *et al*. demonstrated a 52% adherence to DMARD monitoring guidelines in a French cohort of 782 patients with RA. A lack of robust evidence behind guidelines has been postulated as a cause for deviation, implying clinician scepticism(412). However, regardless of high levels of agreement with guidelines, clinician adherence has still been demonstrated to lag behind self-reported rates of compliance(413). This phenomenon is multifactorial. Logistics and impracticalities of 'real-life' patient care play a role, for instance, patient non-attendance. Personal preference from both prescribers and patients, with hesitation regarding perceived side-effects and adverse reactions, is also likely to impact practice(98, 99). Economic considerations are less relevant within the UK, but provider hesitation regarding intensification of therapy are pertinent(414) (415).

A scoping audit was undertaken to evaluate local practice regarding MTX prescriptions. A retrospective electronic note review of 150 patients was performed consisting of a random selection of those prescribed MTX at YTHT identified in an IT search, as per Figure 9-1. Demographic details, medication history, blood tests, interventions and actions following MTX prescription were detailed. Relevant guidelines were cross referenced dependent upon prescribing speciality and prescription date (58, 59, 84, 98).
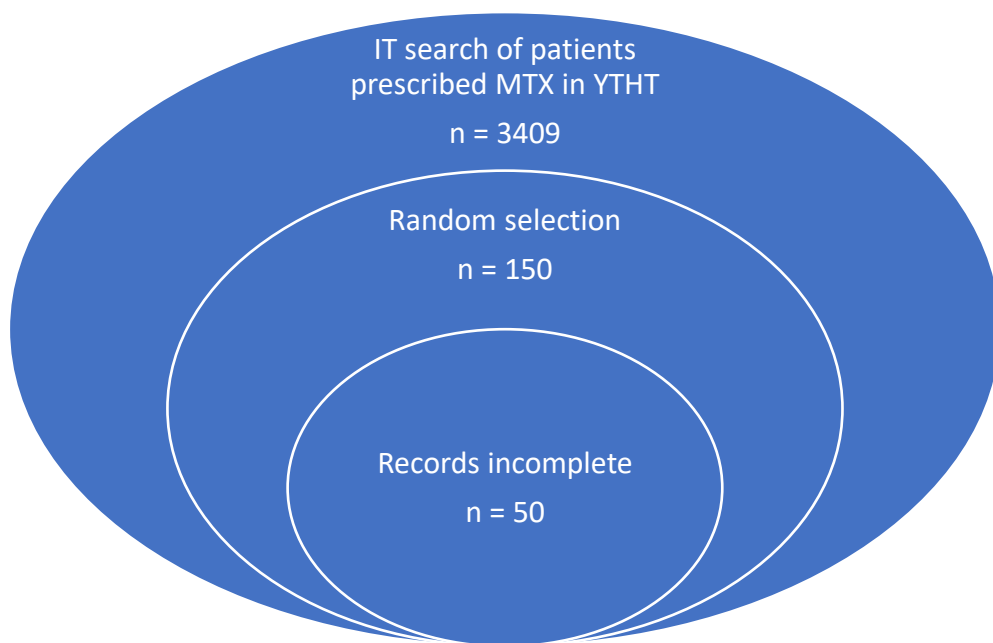
IT: Information technology, MTX: Methotrexate, YTHT: York Teaching Hospitals Trust

Figure 9-1 Patient selection for methotrexate prescribing audit

Aims included:

- To assess compliance with MTX monitoring guidelines
- To review risk factors for NAFLD in a cohort of individuals prescribed MTX
- To ascertain consideration given to alcohol intake when prescribing MTX
- To assess response in the face of abnormal LBTs in patients prescribed MTX

9.2 Results

9.2.1 Risk factors for liver disease are commonplace

Rheumatologists instigated the majority of prescriptions (88%) with RA (44%) the most prevalent indication, followed by PsA (27%), as demonstrated in Figure 9-2. The average age of patients being commenced on MTX was 51 years (range 4 - 81), 69% of whom were female, as per Table 9-1. Risk factors for NAFLD were present in the cohort as follows: hypertension (31%), co-prescription of statin (20%) and diabetes (8%), as illustrated in Table 9-2. Mean BMI was 28.6 kg/m^2 (range 17.8 – 44.1), and 64% of participants were overweight (BMI > 25kg.m^2) prior to initiation of MTX.

A lack of documentation of alcohol intake in the cohort made interpretation of drinking habits problematic, as per Table 9-3. Where appropriate documentation was recorded, mean intake was 7.4 and 11.3 units/week (p=0.197) in the rheumatology and dermatology population respectively (Table 9-1).
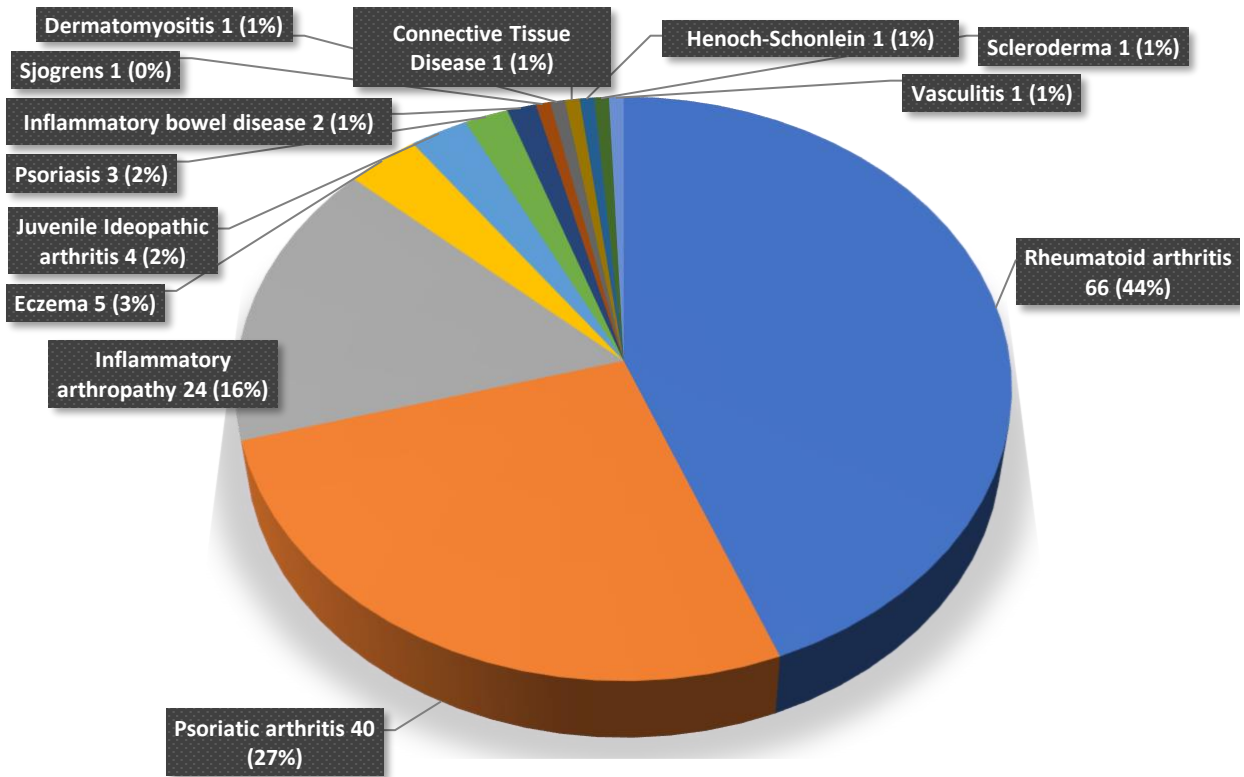
Figure 9-2 Proportion of indications for MTX prescription (%)

	Rheumatologists n (%)	Dermatologists n (%)	Paediatrics n (%)	Gastroenterology n (%)	Total cohort n (%)
Total cohort (n, %)	132 (88.0%)	13 (8.7%)	3 (2.0%)	2 (1.3%)	150 (100%)
Mean age (years)	54.4	41.6	11.7	57	51
Mean BMI (kg/m^2)	28.7	27.7	n/a	Not stated	28.6
Female (n, %)	95 (72.0%)	5 (38.5%)	2 (66.7%)	1 (50%)	103 (68.7%)
Alcohol (units/week)	7.4	11.3	0	Not stated	7.8

Table 9-1 Summary statistics of the included population

	Rheumatology	Dermatology	Paediatrics	Gastroenterology	Total cohort
Diabetes	11 (8.3%)	1 (7.7%)	0 (0%)	0 (0%)	12/150 (8.0%)
Hypertension	43 (32.3%)	3 (23.1%)	0 (0%)	1 (50%)	47/150 (31.3%)
Co-prescription of a statin	28 (21.2%)	2 (15.4%)	0 (0%)	0 (0%)	30/150 (20.0%)
Overweight (BMI > 25 kg/m^2)	36/56 (64.3%)	2/4 (50.0%)	n/a	Not stated	38/59 (64.4%)
Co-prescription of prednisolone	41/132 (31.1%)	0/13 (0%)	2/3 (66.7%)	1/2 (50%)	44/150 (29.3%)

Table 9-2 Risk factors for NAFLD within the cohort

	Rheumatology	Dermatology	Paediatrics	Gastroenterology	Total cohort
Alcohol intake not documented	41/104 (39.4%)	7/12 (58.3%)	n/a	1/2 (50%)	49/118 (41.5%)
Units/week not documented	64/104 (66.3%)	7/12 (58.3%)	n/a	2/2 (100%)	73/118 (61.9%)

Table 9-3 Documentation of alcohol history prior to prescription of MTX

9.2.2 Adherence to rheumatological guidelines

Of those patients prescribed MTX by rheumatology; 23% did not have a baseline CXR and 17% did not have baseline blood tests prior to MTX initiation. 69% failed to have ongoing blood tests as per monitoring guidelines, as demonstrated by Figure 9-3. A significantly smaller proportion of patients (n = 12) were prescribed MTX by dermatology team members. Within the cohort 33% did not have a baseline CXR, 46% did not have adequate baseline bloods and 80% did not have PIIINP testing, as represented in Figure 9-4.

Adherance to rheumatology guidelines for MTX monitoring
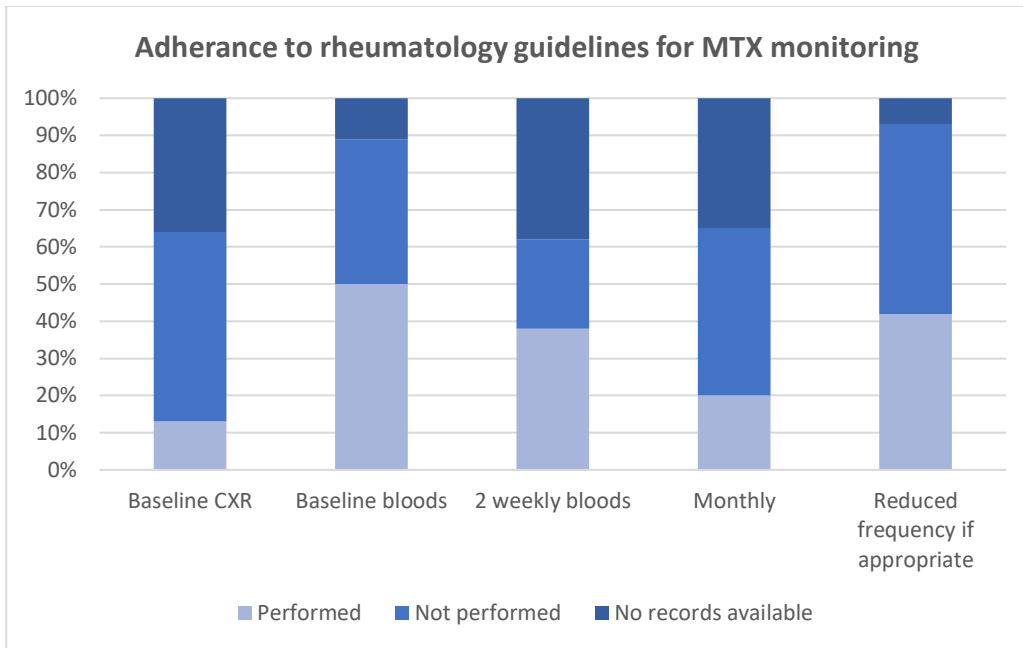
MTX: Methotrexate, CXR: Chest Xray

Figure 9-3 Adherence to rheumatological guidelines for MTX monitoring

Adherence to dermatology guidelines for MTX monitoring
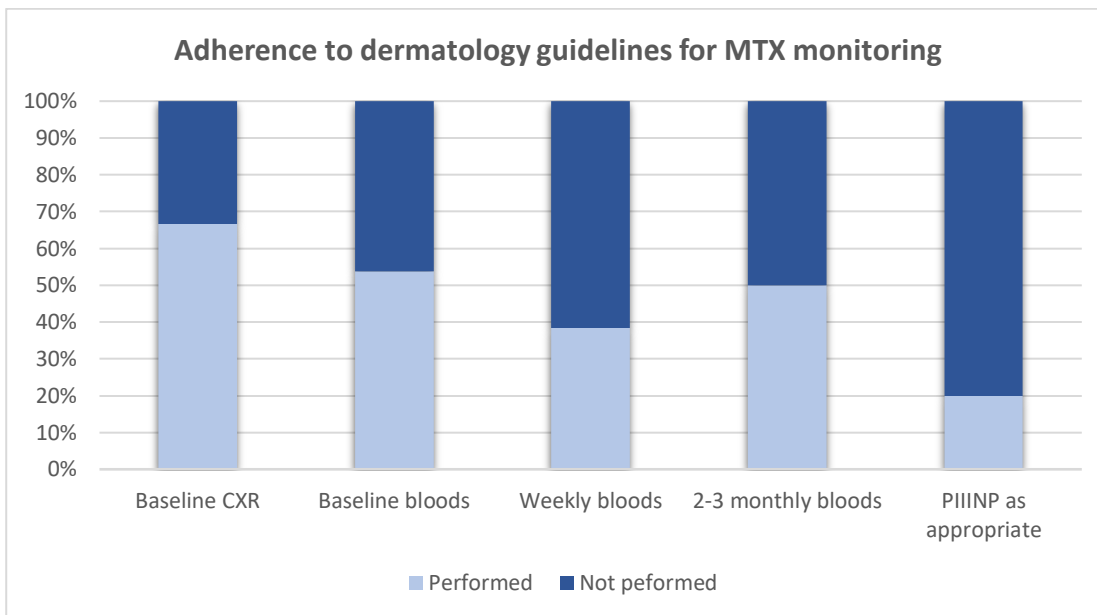
MTX: Methotrexate, CXR: Chest Xray, PIIINP: Procollagen type III N-terminal peptide

Figure 9-4 Adherence to dermatology guidelines for MTX monitoring

9.2.3   Abnormal LBTs – worth worrying about?

In our cohort 41% of patients had abnormal LBTs prior to MTX initiation and 61% had abnormal LBTs during treatment. Action taken in response to abnormal LBTs differed, as demonstrated in Figure 9-5. Of those with abnormal LBTs; a non-invasive liver screen was sent in 1%, imaging of the liver was obtained in 21% and an hepatology referral requested in 4%, as per Figure 9-6. Figure 9-7 demonstrates causes for MTX cessation; hepatological concern was responsible for 13%. Of the entire cohort 27% went onto start biological therapy (n=40), 45% of whom had had to stop MTX.

Abnormal LBTs throughout treatment

n = 91

No action

n = 56

Stop MTX until improved

n = 15

Continue MTX
repeat bloods early

n = 9

Document and monitor

n = 6

Stop MTX

n = 4

MTX later stopped and restarted

n = 3

MTX never re-started

n = 2
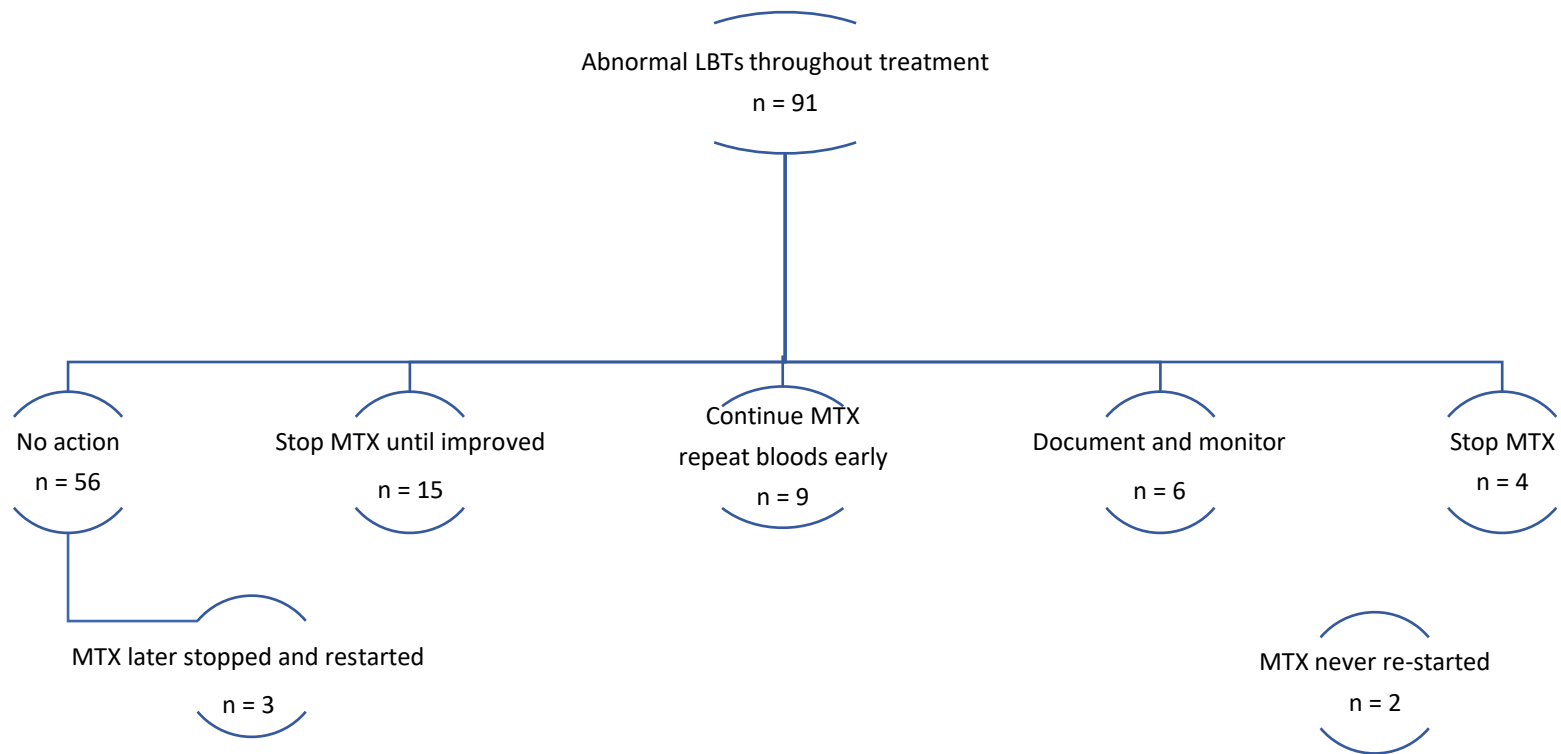
LBTs: Liver blood tests, MTX: Methotrexate

Figure 9-5 Action when LBTs abnormal during MTX treatment
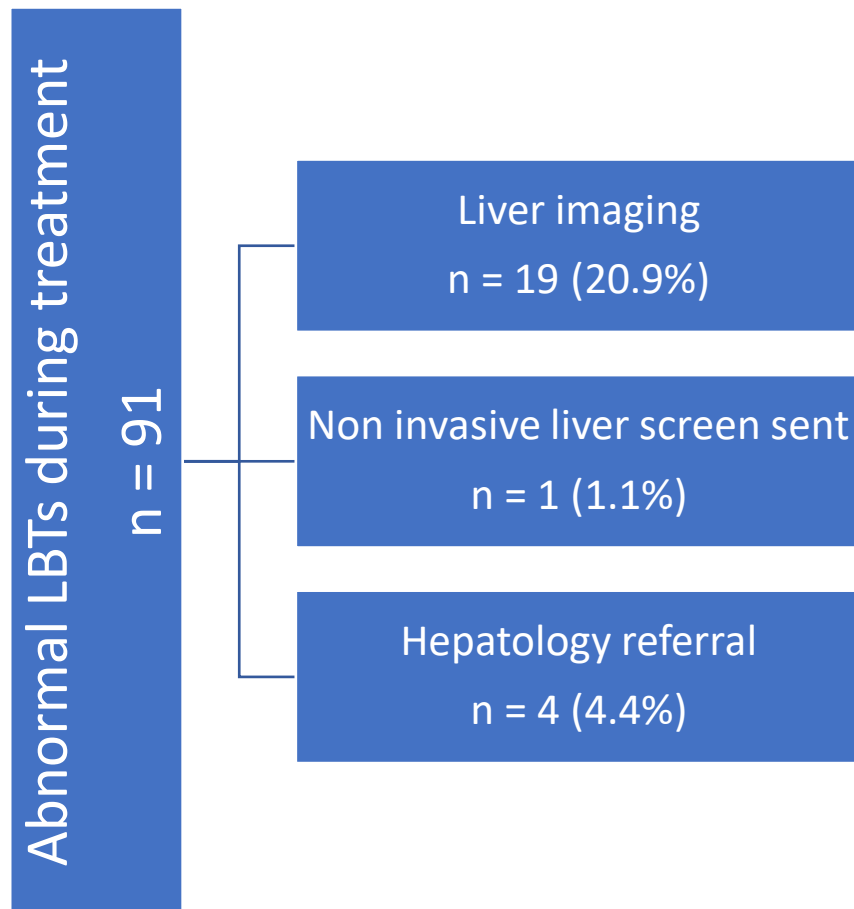
LBTs: Liver blood tests

Figure 9-6 Actions following abnormal LBTs whilst prescribed MTX

*Intolerance equated to hair loss, low mood and patient concern causing Peyronies disease

GI: Gastrointestinal

Figure 9-7 Causes for MTX cessation

9.3   Discussion

This audit aimed to compare national recommendations with a real-life cohort to assess adherence and risk factors for liver disease within the local population. The demograph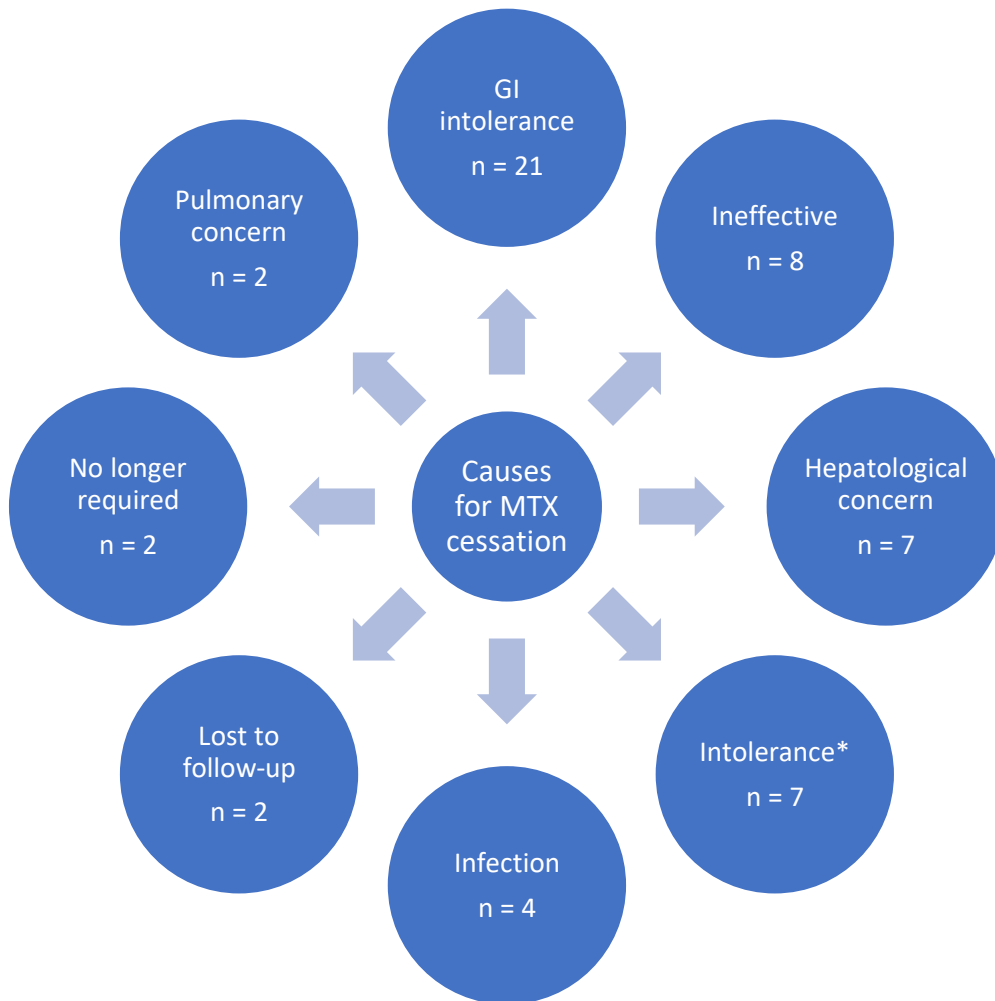ic for our cohort, with a female predominance, is not unlike the published literature(410, 416). A lower mean age is a reflection of the incorporation of the paediatric population.

Risk factors for NAFLD, such as metabolic syndrome and a high BMI, were commonplace in patients being commenced on MTX. Rheumatology, the most frequent prescribers, have a patient cohort likely to have mobility issues (hindered physical activity) and exposure to corticosteroids (associated weight gain). PsA, the second most common indication for commencing MTX, is associated with a higher prevalence of metabolic syndrome(417) (418). The prevalence of NAFLD within the UK has been postulated to be around 24%(371); it is likely this cohort will be at higher risk of NAFLD, given these factors.

Published audits have demonstrated that adherence to national MTX monitoring guidelines is initially variable (419-421), our study has demonstrated that ongoing monitoring was also poor. Compliance with necessary blood monitoring fell from 83% to 31%, as treatment duration increased. Studies have demonstrated that the incidence of side-effects with DMARDs are relatively low, with speciality interventions and admissions due to complications, rare(422). These results may reflect a reduction in vigilance from both clinician and patient, behaviour potentially based on previous reassuring experience(92, 95). Our results are in keeping with the published literature(410, 423). Lack of confidence regarding safe DMARD prescribing in general practitioners has also been demonstrated and may play a role in reduced adherence to serial monitoring, once responsibility falls to the community-based team(424, 425).

Alcohol intake was poorly documented, suggesting thorough alcohol history was not recorded and appropriate advice regarding alcohol intake, therefore not given to patients.

MTX cessation due to concerns about liver damage was surprisingly common, this rarely involved hepatology input or further investigation. This project provides evidence that clinicians are stopping MTX and switching to biological therapies because of concerns

around hepatotoxicity, which has significant cost implications, as demonstrated elsewhere in the literature(426).

Audit, as a research tool, relies upon effective documentation of events from which results and conclusions may be drawn, retrospectively(427). The quality of the documentation is a potential weakness which should be taken into account when evaluating these results. An example of this is patient's weight, often not recorded on clinic letters, but is documented at *every* clinic attendance. A further example is units of alcohol ingested. The authors have made the assumption that if alcohol units were not documented in the clinical notes then it was not discussed. It is possible that alcohol units may have been discussed and simply not recorded.

It was not possible to retrieve the 150 written medical notes for this scoping audit. As a consequence, there was a reliance on the electronic records for information gathered. Although note-keeping within the hospital is almost entirely electronic now, this was not the case for historical records and some historical blood tests were not available to review electronically and could not be captured in the data collection.

Finally, audit is a blunt instrument, which may be insufficient to capture what are often complex, multifactorial decision-making processes. Recording data on why patients may have commenced biological therapies following MTX is an example of this. It may be that there were minor adverse effects brought on by MTX which weren't documented or perhaps a sub-optimal therapeutic response, however the documented trigger which prompted stopping MTX was an elevated ALT. Decisions within clinical medicine are often complicated, may have multiple influences and rely upon both clinician and patient agreement. They are rarely one dimensional or binary, as can be captured and recorded in an audit.

In summary, this single-centre audit demonstrated that MTX-monitoring guidelines were not adhered to in over 2/3rds of recipients, suggesting a degree of apathy or perhaps disregard towards the requirement of repeated, long-term blood monitoring in this cohort. Although this is not out of keeping with the published data, it suggests the problem is more significant than previously documented. Furthermore, risk factors for NAFLD were

commonplace in the population, and alcohol intake was poorly documented, demonstrating ample risk factors for liver disease related to other causes.

CHAPTER 10:   RISK FACTORS FOR LIVER FIBROSIS IN AN OUTPATIENT POPULATION

10.1 Introduction

Low-dose MTX, a highly effective treatment for many immune-related diseases, has been over-shadowed by concerns regarding hepatotoxicity for many decades. Evidence demonstrating MTX-related hepatotoxicity is weak, circumstantial and does not demonstrate causality, as discussed in Section 6.3.1. Despite significant historical concerns it remains unclear, and unproven, as to whether patients taking MTX are at an increased risk of developing liver fibrosis. Meaningful stratified analysis of the existing evidence-base is thwarted by a lack of documented risk factors. With hindsight, it appears rather coincidental that both NAFLD and MTX-related liver injury share very similar histological findings and both are significantly more common in individuals with psoriatic arthritis. In an era when NAFLD was an unknown disease, it seems likely that liver disease secondary to NAFLD was incorrectly attributed to MTX?

The advent of novel non-invasive measures to assess liver fibrosis allows large scale assessment of liver disease using techniques that are risk-free in comparison to liver biopsy. Transient elastography is a reliable measure of liver fibrosis and has been widely adopted within the UK.

We undertook a large cross-sectional study to assess the prevalence of liver fibrosis in an outpatient population of patients who may be prescribed low-dose MTX.

Aims were as follows:

- To establish the prevalence of liver fibrosis, including cirrhosis, using FibroScan® in a population of patients who take low-dose MTX, compared with those with similar diseases but who have never taken this medication
- To establish risk factors for liver disease in this population, including age, alcohol, BMI and physical activity levels.

10.2 Results

10.2.1 Summary statistics

Descriptive statistics to compare the MTX and control cohorts are demonstrated in Table 10-1. The two groups were similar in characteristics. The cohort was predominantly Caucasian, as per Table 10-2. Age and BMI distribution were similar in both populations, as demonstrated in Figure 10-1. The only statistically significant differences between the MTX and control cohorts were mean age (62.2 and 59.6 years), co-prescription of biological therapy (26% and 4%) and average physical activity undertaken per week (4034 and 5202 Multiple Energy Expenditure (MET)/week) respectively. The underlying medical condition being treated spanned a wide range, most common of which was RA (52%), psoriatic arthropathy (22%) and undifferentiated inflammatory arthropathy (20%), as demonstrated in Figure 10-2 and Figure 10-3. Risk factors for metabolic syndrome were commonplace in both cohorts; 71% had a BMI greater than $25kg/m^2$, 25% had hypertension, 21% were prescribed a statin, and 12% were taking prednisolone. They were not statistically different between the two cohorts.

The cumulative dose of those individuals who had taken MTX (n = 300) ranged from 200mg to 120.5g. The mean cumulative dose was 6949.25mg and the median dose was 20mg per week, as detailed in Table 10-3. The distribution of doses is demonstrated in Figure 10-4.

10.2.2 Prevalence of liver fibrosis

Of the total cohort, 17.5% had a FibroScan® result above 7kPa. This equates to a prevalence of liver fibrosis of 17,500 per 100,000 in our population.

	MTX	Control	P value
Female	204 (68%)	211 (70%)	0.32
Mean age (years)	62.2	59.6	**0.02**
Mean BMI (kg/m^2)	28.3	28.4	0.77
Caucasian	295 (98%)	296 (99%)	1.00
Co-existing HTN	83 (28%)	69 (23%)	0.19
Co-existing DM	24 (8%)	20 (7%)	0.53
Co-prescription of a statin	63 (21%)	60 (20%)	0.76
Co-prescription of prednisolone	33 (11%)	39 (13%)	0.45
Co-prescription of biological therapy	77 (26%)	12 (4%)	**<0.01**
Waist circumference (m)	0.92	0.91	0.37
Overweight (BMI > 25 kg.m^2)	213 (71%)	212 (71%)	0.93
Prevalence of psoriasis	68 (23%)	33 (11%)	**<0.01**
Prescribed by rheumatology	292 (97%)	283 (94%)	0.11
Self-reported mean AUDIT-C score	3.9	3.6	0.28
Mean physical activity (MET/week)	4034	5202	**0.03**

BMI: Body mass index, HTN: Hypertension, DM: Diabetes mellitus, AUDIT-C: Alcohol Use Disorder Identification Test, MET: Multiple energy expenditure

Table 10-1 Summary statistics of the MTX and control cohorts, statistical significance measured by the independent 2-tailed t test, Chi squared or Fisher's Exact test as appropriate.

Ethnicity	MTX	Control
Caucasian	295 (98.3%)	296 (98.7%)
Asian	2 (0.7%)	2 (0.7%)
Black African	3 (1%)	2 (0.7%)

Table 10-2 A table to show the ethnicity of the population
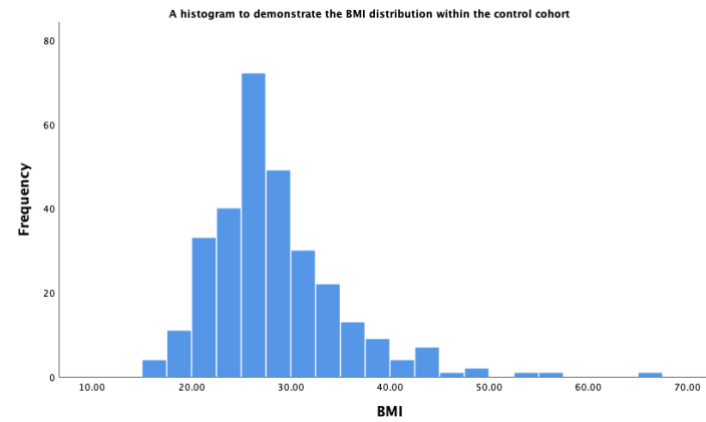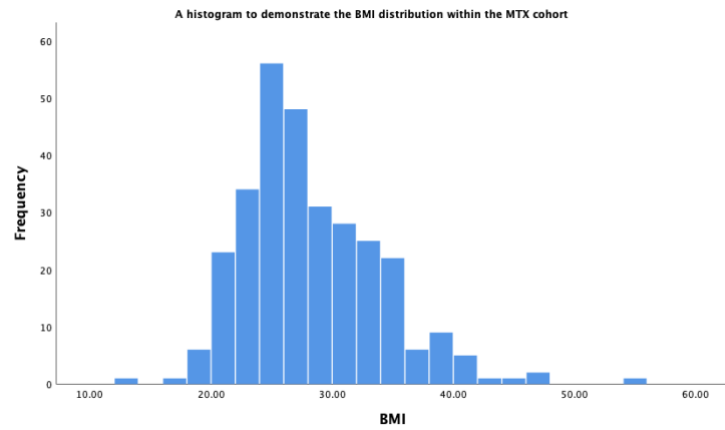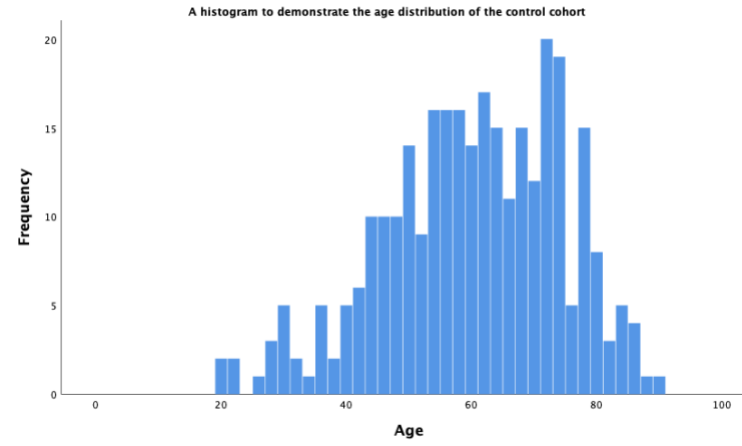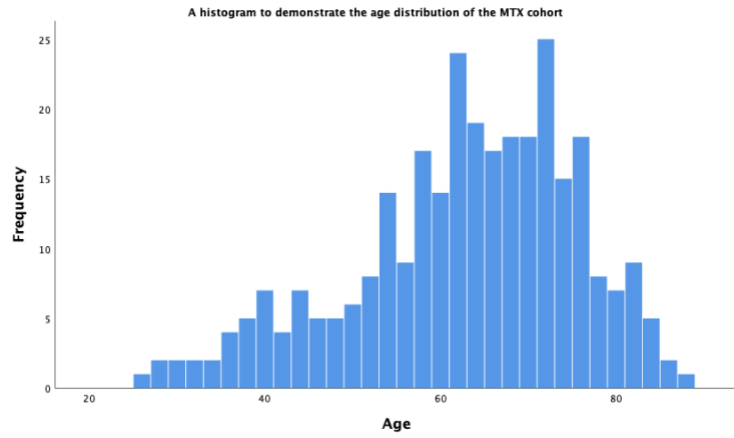
Figure 10-1 Histograms to show the distribution of age and BMI within the MTX and control cohort

A Graph to Demonstrate the underlying diagnosis within the control cohort

Legend:
- Inflammatory arthropathy
- Rheumatoid arthritis
- Osteoarthritis
- Psoriatic arthritis
- Ankylosing Spondylitis
- Sjogrens
- Osteoporosis
- Giant Cell Arteritis
- Psoriasis
- Spondyloarthropathy
- Connective Tissue Disease
- Polymyalgia Rheumatica
- Lupus
- Eczema
- Fibromyalgia
- Tenosynovitis
- Scleroderma
- Vasculitis
- Systemic Lupus Erythematosus
- Sarcoidosis
- Sicca
- Acne
- Ehlers-Danlos Syndrome
- IBD Arthropathy
- Pseudogout
- Sciatica
- Stills disease
- Sclerosis
- Systemic Sclerosis
- Mechanical Joint Pain
- Reactive Arthritis
- Oligoarthritis
- Dermatomyositis
- Folliculitis
- IgG4 Disease
- Fibromyaglia
- Bullous Pemphigoid
- Polychondritis

Figure 10-2 A Graph showing the range of underlying diagnoses for which the patients were attending the outpatient clinics for

A graph to demonstrate the indications for low-dose-MTX

Legend:
- Rheumatoid Arthritis
- Inflammatory Arthropathy
- Psoriatic Arthritis
- Eczema
- Ankylosing Spondylitis
- Osteoarthritis
- Spondyloarthropathy
- Vasculitis
- Connective Tissue Disease
- Psoriasis
- Dermatomyositis
- Giant Cell Arteritis
- Lupus
- Myositis
- Sjogrens
- Systemic Lupus Erythematosus
- Polymyalgic Illness
- Sclerouveitis

Values shown: 154 (52%), 55 (18%), 67 (22%), 4 (1%), 3 (1%), 2 (1%), 2 (1%), 2 (1%), 1 (0%), 1 (0%), 1 (0%), 1 (0%), 1 0%, 1 (0%), 1 (0%), 1 (0%), 1 (0%), 1 (0%)

Figure 10-3 A graph showing the range of underlying diagnoses for which patients were receiving low dose MTX

Methotrexate dose range (mg/week)	5 - 30
Median methotrexate dose (mg/week)	20
Oral preparations	63%
Methotrexate cumulative dose (mg)	200 – 120,500
Mean cumulative dose (mg)	6949

Table 10-3 The range of doses of MTX in those prescribed it

Figure 10-4 Histogram to show the distribution of doses of methotrexate

10.2.3 Methotrexate as a cause of liver fibrosis

The range of FibroScan® results in both the MTX cohorts, and the control cohorts are demonstrated in Table 10-4 and Table 10-5 respectively. The prevalence of liver fibrosis in our population did not differ significantly, regardless of whet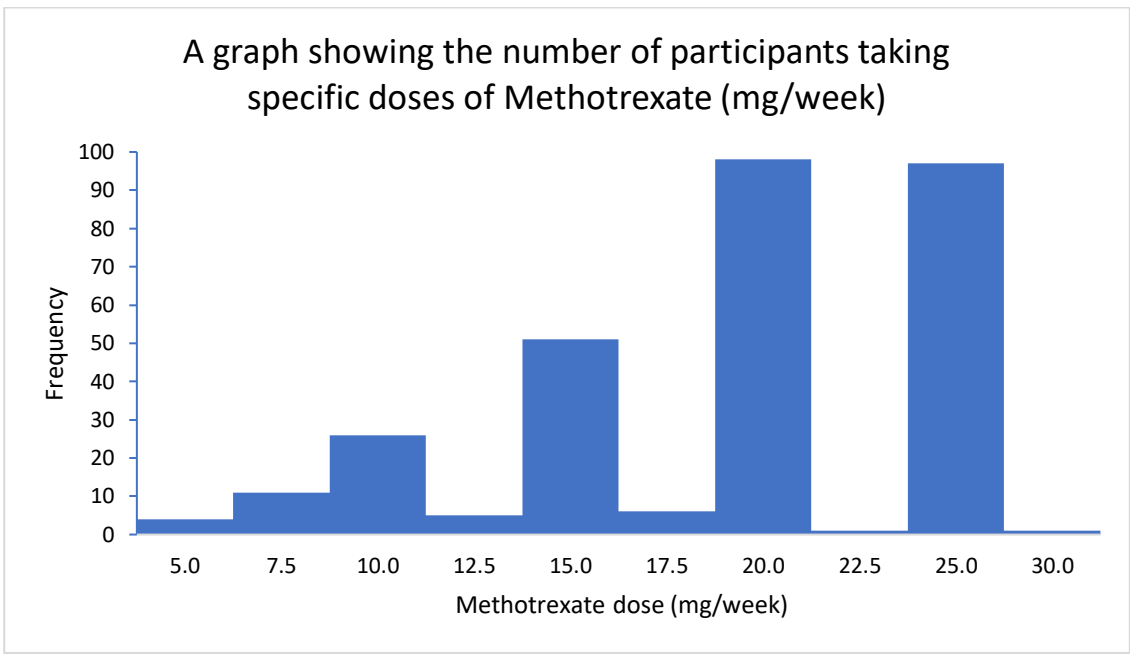her participants had taken MTX or not (X2(df=1)=0.31, p=0.578). Similarly, the odds ratio of having an abnormal FibroScan® following MTX, compared with the control cohort, was 1.127 (95% CI 0.75 – 1.72). Given the confidence interval crosses 0, this is not a meaningful relationship. Considering FibroScan® score as a quantative, rather than as a dichotomous outcome (fibrosis versus no fibrosis) there was still no statistical difference between the MTX group (5.9kPa, 95% CI 5.3 - 6.5) and controls (6.5kPa, 95% CI 5.6 - 7.2) ($p = 0.28$).

10.2.4 Risk factors for liver fibrosis

Correlation between known risk factors for liver disease and FibroScan® result was calculated, as per Table 10-6. A positive correlation was demonstrated with measurements related to weight; BMI (0.48, p<0.01), waist circumference (0.41, p<0.001) and fat mass (0.42, p<0.01). There was no significant correlation between cumulative dose of MTX and FibroScan® score (-0.042, p=0.31).

Multiple regression analysis was undertaken to predict whether MTX affects liver fibrosis, whilst adjusting for known risk factors of liver disease. Quantitative FibroScan® score was the outcome variable (logged to improve the model fit), with explanatory variables including age, ALT, AUDIT-C score, average alcohol intake, BMI, co-prescription of statin, diabetes, gender, hypertension, MTX use and physical activity. Results are demonstrated in Table 10-7. MTX use was not a significant predictor of liver fibrosis (p=0.798). Positive predictors for liver fibrosis were ALT (p=0.001), BMI (p=<0.001) and diabetes (p=0.029).
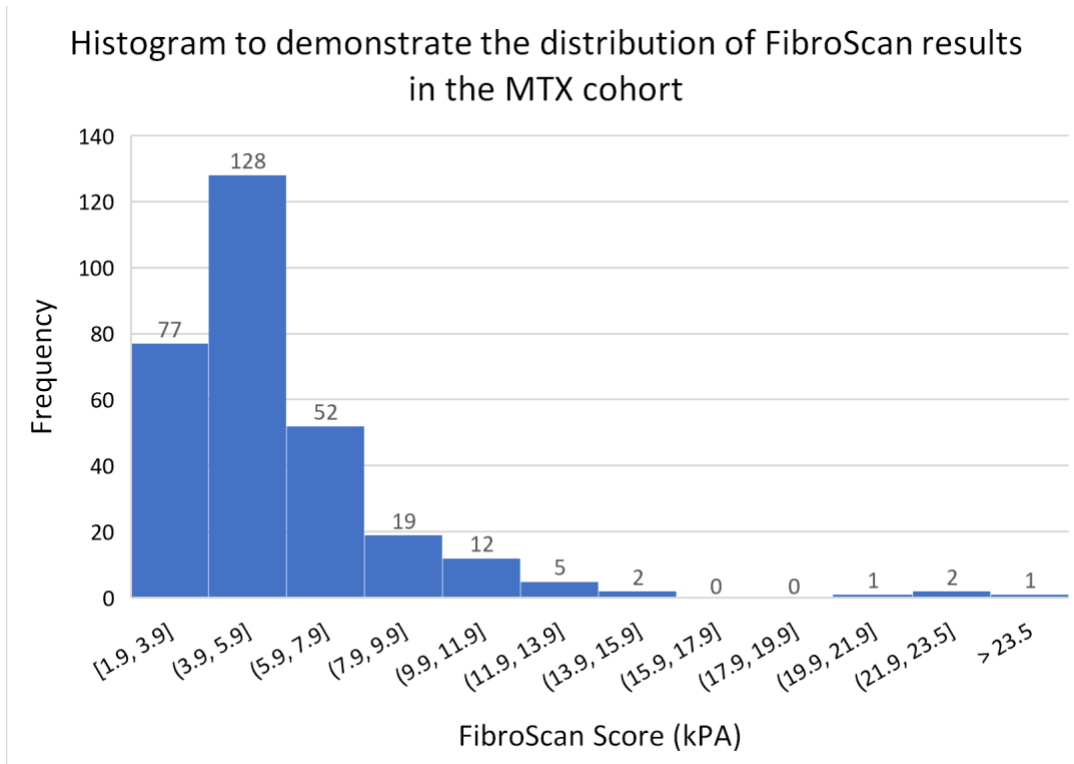
Table 10-4 Histogram demonstrating FibroScan result distribution in the MTX cohort
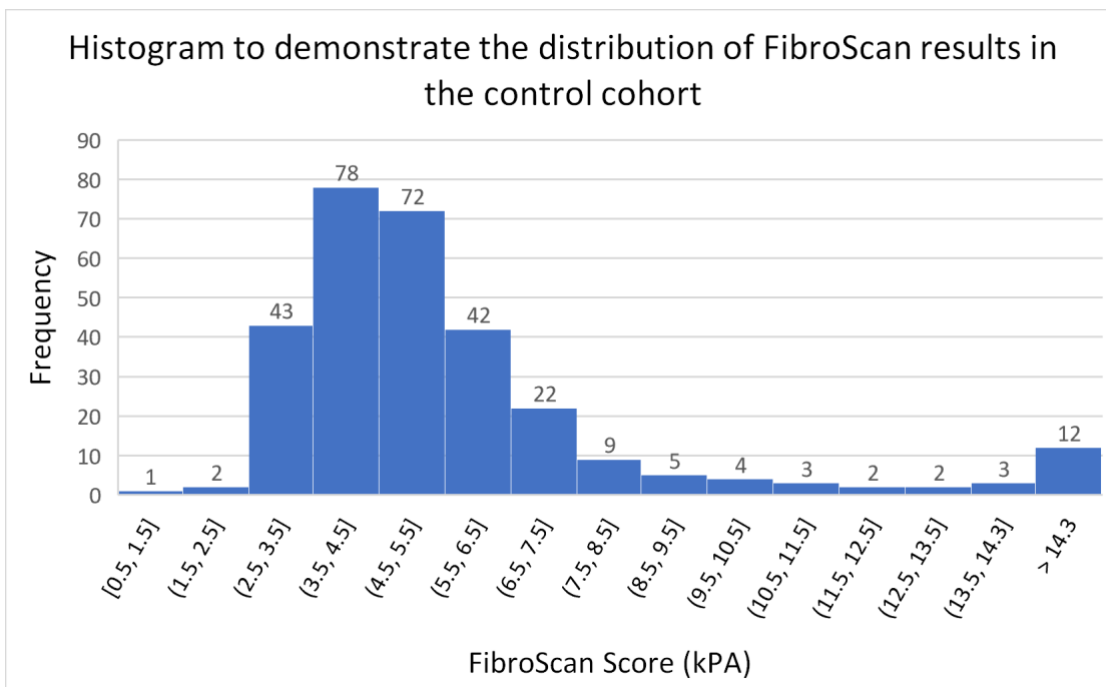

Table 10-5 Histogram demonstrating FibroScan result distribution in the control cohort

Correlation with FibroScan	Correlation coefficient	P value
Age	-0.020	0.632
AUDIT-C	0.03	0.941
Average alcohol intake	-0.008	0.846
Bilirubin	0.036	0.399
BMI	0.482	<0.001
Cumulative MTX dose	-0.042	0.311
Fat Mass (kg)	0.423	<0.001
Physical activity levels (MET/week)	-0.049	0.239
Waist circumference	0.406	<0.001

AUDIT-C: Alcohol Use Disorder Identification Test, BMI: Body mass index, MTX: Methotrexate, MET: Multiple energy expenditure

Table 10-6 Correlation of risk factors for liver disease measured against FibroScan® result. Pearson's correlation

	Co-efficient (B)	Standard error	P value
Constant	0.496	0.149	0.001
Age	0.002	0.002	0.179
ALT	0.005	0.002	0.001
AUDIT-C score	-0.008	0.006	0.207
Average alcohol intake	-3.771E-5	0.000	0.054
BMI	0.033	0.003	<0.001
Co-prescription of statin	0.044	0.048	0.366
Diabetes	0.156	0.071	0.029
Gender	0.042	0.040	0.301
Hypertension	-0.014	0.046	0.766
Methotrexate use	-0.009	0.36	0.798
Physical activity (MET/week)	-3.493E-6	0.000	0.205

ALT: Alanine aminotransferase, AUDIT-C: Alcohol Use Disorder Identification Test, BMI: Body mass index, MET: Multiple energy expenditure

Table 10-7 Multiple regression analysis demonstrated predictors for liver fibrosis, of which MTX was not.

10.3 Discussion

Despite its use as a highly efficacious immunomodulator for many decades, concerns regarding MTX-related hepatotoxicity has hindered it's use since the 1960s. However, on closer scrutiny, it's tarnished reputation is predicated almost entirely on data that ignored other hepatotoxins such as alcohol or obesity, and did not include liver biopsy. Whilst the rather basic error of ignoring co-morbidity is perplexing to modern observers, the option of employing relatively novel, non-invasive methods of assessing liver fibrosis (such as TE) allow new insight into this much debated topic, where the risks of liver biopsy can no longer be justified(376).

This large cross-sectional study of 600 participants has demonstrated no increased prevalence of liver fibrosis (by way of FibroScan®) in patients taking MTX, compared with those who have not. Multiple regression analysis, performed to adjust for recognised risk factors for liver disease, demonstrated neither MTX prescription nor cumulative dose of MTX were significant predictors of liver fibrosis. However, risk factors for NAFLD were significant predictors of liver pathology in this cohort.

These results, although in stark contrast to the historical claims regarding the hepatotoxicity of MTX(279, 282, 288), are not out of keeping with more recent publications(377),(428, 429),(430). Berends *et al*. is one such example. They performed a retrospective review of 278 liver biopsies, from 125 patients. They found no association between cumulative dose of MTX, weekly dose, age or duration of treatment with MTX on liver injury. Similar to our cohort, risk factors for NAFLD, such as obesity and diabetes, were significant risk factors for liver injury(377).

They are a minority of studies, published recently, which have demonstrated a relationship between cumulative dose of MTX and liver fibrosis(431, 432) . These data are based on much smaller cohorts, and of note both studies fail to include a control cohort. Previous data have demonstrated that patients with psoriasis(433) and rheumatological conditions such as RA(428, 434) have an increased risk of liver fibrosis, even if MTX-naïve, compared with matched healthy controls. As previously discussed, this is likely to be attributable to co-prescription of other medication (such as

corticosteroids), reduced ability to undertake physical activity with potential weight gain and increased risk of co-existent autoimmune pathology. Therefore, a higher FibroScan® score may well be seen in a cohort who have taken MTX for a longer period of time - but this may be attributable to other factors; presumably those individuals are older, and have had an underlying immune-mediated disease for longer too? Without a control cohort it is not clear what has caused the increased liver fibrosis and confounding factors risk thwarting any meaningful interpretation of the data.

Risk factors for metabolic syndrome; an elevated BMI, hypertension, hypercholesterolaemia, use of corticosteroids and diabetes were commonplace within our population. This finding is mirrored in other studies (433, 435). These factors, in conjunction with mobility issues potentially reducing physical activity levels, conspire to increase the risk and prevalence of NAFLD in a population of patients with rheumatological conditions(434). The increased risk of those with psoriasis to develop metabolic syndrome has also been well documented. Numerus published data have demonstrated histological features of NASH in liver biopsies of this patient cohort, regardless of whether they are taking MTX or not (366) (436, 437). This is in keeping with the author's theory that perhaps a significant proportion of historic, so-called, MTX-related hepatotoxicity was mis-labelled NAFLD.

The prevalence of liver fibrosis (taken as a FibroScan® > 7kPa) within our study population was 17.5%. This is similar to other studies where prevalence of liver fibrosis is reported between 3-23% in those with RA and between 14-17% in those with psoriasis or PsA(438, 439). This high figure demonstrates the importance of diagnosing and actively treating the commonest risk factors for liver disease in the cohort, which in the UK remain metabolic syndrome and alcohol.

This study was a single-institution, cross-sectional, cohort study. In order to draw meaningful conclusions from cross-sectional studies we must assume that the sample population is representative and can be generalised to the population of interest. Numerous measures were taken to reduce selection bias within our cohort, including the provision of mobility aids and additional time as required, adaptations to study material (e.g. large-format text for those with visual impairment), and the provision of research

clinics every weekday from 0700 to 1900 as required for those who were in full-time employment. These efforts were made to ensure that the study was as inclusive as possible and didn't inadvertently exclude any part of the population.

Cross-sectional studies are vulnerable to recall bias and factors changing over time(440). Information acquired from participants was cross-checked with secondary sources where possible, usually their electronic patient records (e.g. medication history and past medical history) was sufficient, but also historical case notes, as required. Variables most vulnerable to bias include historical alcohol intake and the duration or frequency of breaks off MTX, due to social desirability bias and the recall period respectively. These were also the most challenging variables to cross-reference, as were not always documented in electronic or paper records.

In our study, self-reported alcohol consumption, recorded using the AUDIT-C questionnaire, was not a positive predictor of liver fibrosis. This is not what you may expect from alcohol, given it's well known relationship with liver fibrosis(441) despite the known inconsistency in whom and to what degree, it causes liver injury(442). This is noted in other studies within the field(433, 439). Possible reasons for this discrepancy may include participant reluctance to reveal accurate historic amounts of alcohol. Those taking MTX are likely to be aware of the risks of liver injury and presumably warned regarding alcohol consumption by healthcare professionals previously, making social desirability bias even more marked in this cohort. Members of the research team do not have the foundation of relationship, trust, and loyalty which patients afford to their clinicians, as part of the doctor-patient relationship(443) which may exacerbate reporting bias. Finally, it could be a reflection of the bluntness of the data collection tool. AUDIT-C, consisting of only 3 questions, is brief and it will fail to identify some hazardous and harmful alcohol consumption(444). Despite these limitations it is surprising that alcohol was not identified to correlate with liver fibrosis in our cohort and this is a limitation of the study.

This study was reliant upon Transient Elastography (FibroScan®) to accurately measure liver fibrosis, which was not confirmed histologically. As previously discussed, in Section 6.2.1, the gold standard assessment of liver fibrosis is liver biopsy. The authors

did not feel the risks of acquiring liver histology was appropriate or ethical given the reliability and availability of non-invasive methods of assessment of liver fibrosis(236). FibroScan® is one such way of undertaking this(445), with reproducible accuracy, simplicity and ease of incorporation into the outpatient setting(446). FibroScan® does have its limitations; it cannot be used in ascites(447), lean patients with narrower intercostal spaces increase the risk of failure as does obesity(446) and liver stiffness values are likely to be higher in the setting of acute inflammation or transaminitis(448). On balance it was felt that FibroScan® was still the better modality in this setting, as patients in our cohort were highly unlikely to have ascites, failure rate proved not to be an issue and a known acute inflammation of the liver or transaminitis would hopefully be revealed when evaluating past medical history with the participant. Overwhelmingly though, FibroScan® is safe, a characteristic that fundamentally cannot be stated of the acquisition of liver histology. Reassuringly, our results have been replicated in other studies when alternative methods to assess liver fibrosis are utilized such as ARFI(449) and MRI(450).

The manufacturers of FibroScan®, Echosens®, recommend only undertaking a FibroScan® reading if fasted by at least 2 hours, on the grounds that this improves the reliability of the test(447). Limiting the recruitment of potential participants to those who had fasted prior to attendance was liable to significantly impact on recruitment figures within the time available, and therefore was not considered to be feasible within this study. Unfasted FibroScan® results have still been demonstrated to be of good reliability, particularly in those without underlying liver disease(451).

Summary statistics reveal differences in the baseline characteristics of the two cohorts – those who had never been exposed to MTX, and those who had taken it for more than 6 months. The MTX group were older, more likely to be taking biological therapies, had a higher prevalence of psoriasis and lower levels of physical activity as compared with the control cohort. These factors are all likely to increase the risk of liver fibrosis in the MTX group, rather than reduce it, and therefore we do not feel this has significantly impacted upon our study results.

Our cohort included 300 participants who had taken MTX for at least six months. Of those who had been exposed to MTX the mean cumulative dose was 6949mg and the median was 20mg per week, closely followed by 25mg per week. The mean cumulative dose is equivalent to an uninterrupted prescription of MTX at 20mg/week for over 6.6 years, or 25mg/week for 5.3 years. Given patients in real-world cohorts are prescribed MTX for decades, an ideal study may include participants who had received MTX for a minimum of 10-20 years. However, limiting the pool of patients from which to recruit participants to 1 or 2 decades would have extended the period of recruitment to many years. The MTX cumulative doses within our data are larger than other published studies (449) (428), and notably more than that of the studies reporting a relationship between liver fibrosis and cumulative dose(431, 432), refuting the notion that an insufficient cumulative dose in our population was responsible for a conflicting finding.

In conclusion we report a large cohort study evaluating MTX use and liver fibrosis and have been unable to identify any association. We conclude the phenomenon of MTX-related hepatotoxicity is likely to have been historically over-estimated, if it exists at all. Markers of adiposity (BMI, fat mass and waste circumference) correlated with FibroScan® score. Cumulative MTX dose was not correlated with FibroScan score. These results suggest that this cohort had NAFLD as the underlying cause of liver fibrosis.

CHAPTER 11: METHOTREXATE – PATIENTS' PERCEPTIONS

11.1 Introduction

Patient acceptability is a major aspect of any medication's efficacy and uptake(452, 453). Patient's perceptions of both their disease, and medications prescribed, have an important role in how prescribers manage disease(454), and patient adherence to recommended treatment(455).

A large survey including 1313 patients with rheumatoid arthritis in Australia suggested most patients had a positive perception of MTX. 82% considered it important, and 60% preferred to continue taking MTX. However, reported adverse effects were relatively commonplace – occurring in 38% of the population(456). A scoping audit performed locally (as detailed in CHAPTER 9:) demonstrated that adverse events were the commonest cause for MTX cessation, occurring in 64%. The most frequent of these were GI intolerance (40%) and hepatological concerns (13%). Furthermore, it demonstrated long-term compliance to monitoring guidelines was relatively low, with 69% and 80% of rheumatology and dermatology patients evaluated not having appropriate blood monitoring, respectively. Patient cooperation is an essential element of adequate blood monitoring.

Hazardous alcohol intake is commonplace within the UK, with 21% of adults drinking more than 14 units of alcohol per week in England(457). Alcohol is also the leading cause of advanced liver disease within Europe(458). Given the long-standing concern regarding potential hepatotoxicity with MTX, it is important to understand alcohol intake in those being prescribed MTX, as a potential confounding risk factor.

A questionnaire was designed and given to the 300 participants within our cohort of patients who were prescribed MTX (see Appendix J).

Aims were as follows:

- To establish patients' awareness of serological monitoring when taking MTX
- To quantify how much alcohol patients were drinking, whilst taking MTX

- To establish patient's opinions regarding MTX

11.2 Results

11.2.1 Alcohol – how much is too much?

Of our cohort of patients (n=300), 51% felt it was safe to drink alcohol whilst taking MTX, compared with 24% who felt it wasn't, as in Figure 11-1. Of those who felt it was safe to drink alcohol whilst taking MTX; we asked them to quantify how many units per week was acceptable. The range of responses was between 0 and 76 units/week, as demonstrated in Figure 11-2. The mean response was 7.6units/week and the median 5units/week. Of those that responded (n=205) only 6% felt it was safe to drink more than 14 units per week, whilst taking MTX. Men reported it was safer to drink higher amounts of alcohol compared with women, reporting a mean 9.8units/week and median 10units/week in comparison to women mean 6.4units/week and median 4units/week.

11.2.2 Prescription and monitoring

The majority of participants, 90%, recalled being given an information leaflet prior to being prescribed MTX, as opposed to 5% who reported not being given one, demonstrated in Figure 11-3.

With regard to the frequency of required blood tests whilst taking MTX, 73% reported having blood tests every 3 months. Only 5% of participants reported having blood tests either less frequently than this, not at all (neither in line with national guidelines), or that they weren't sure, as in Figure 11-4.

When questioned about who was responsible for acting on the blood tests, the majority of the cohort were aware it was partially the responsibility of the GP (77%) and 76 people gave multiple answers to this question. Just under half (48%) felt it was the responsibility of either the hospital doctor or nurse to act on these results. The remaining results are demonstrated in Figure 11-5.
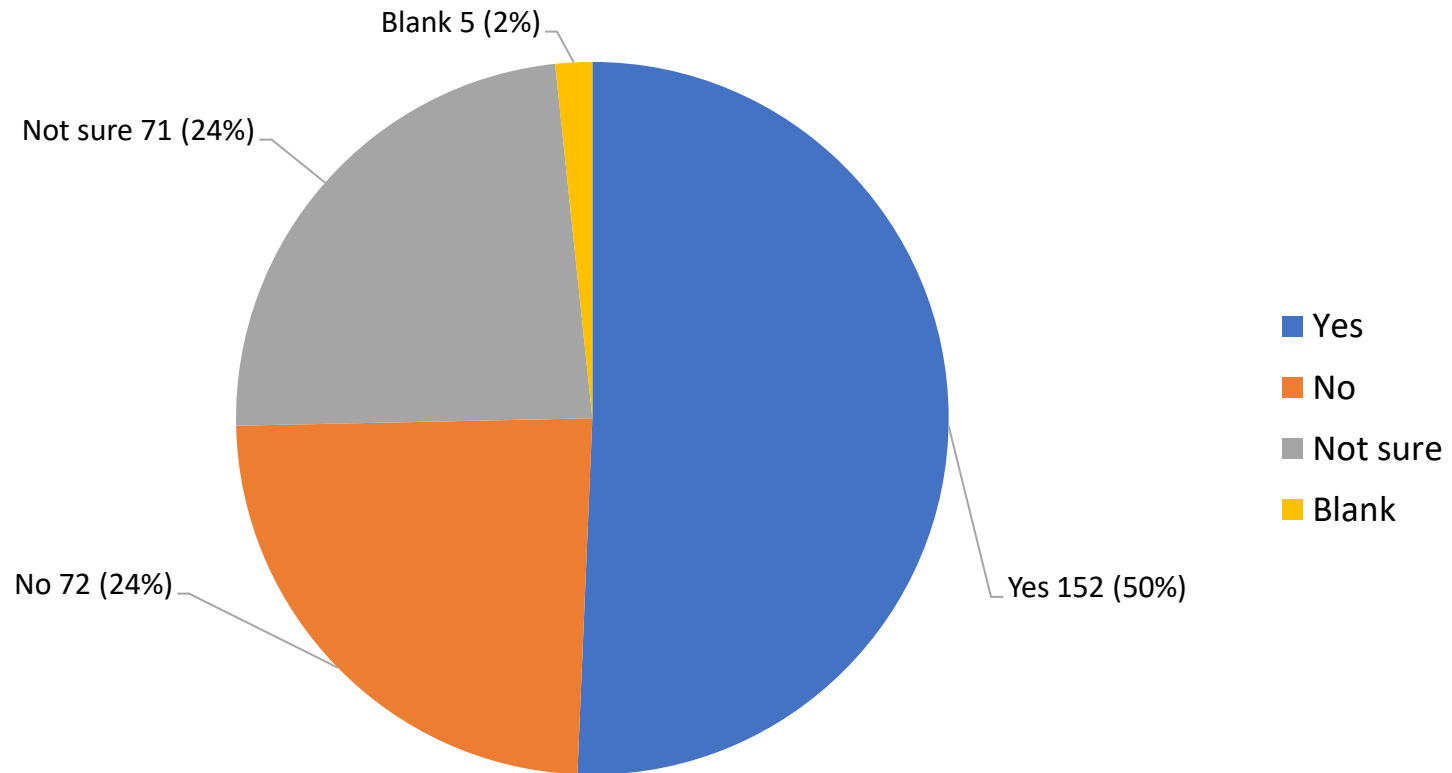
Figure 11-1 The proportions of participants who felt it was safe to drink alcohol whilst taking MTX

A histogram showing responses when asked how much alcohol is safe with MTX
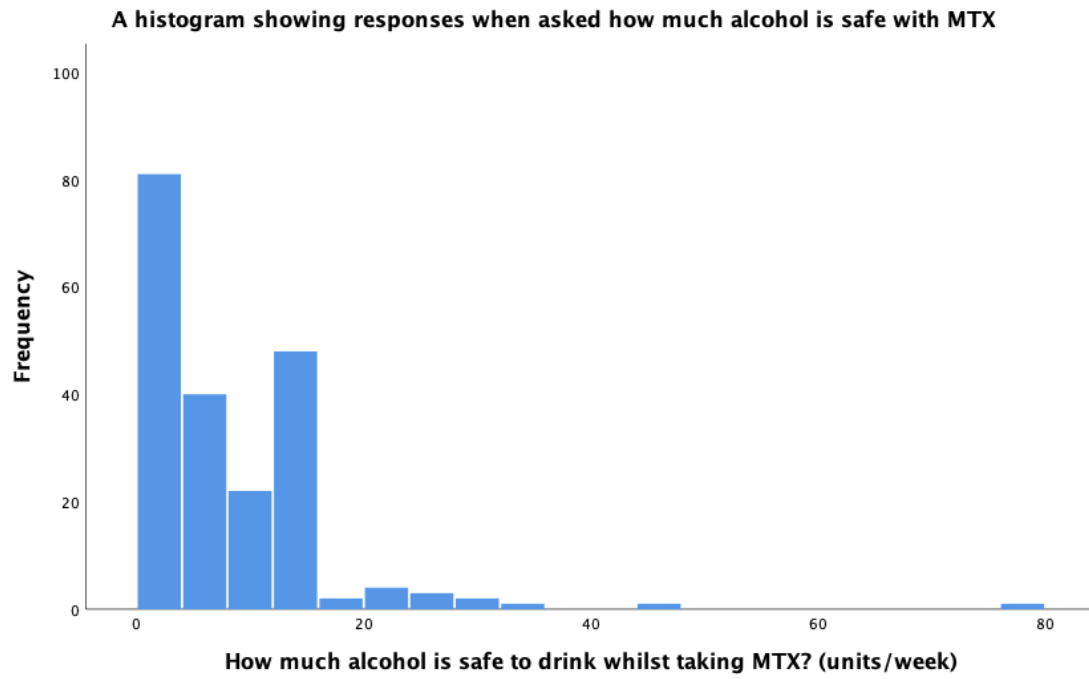
Figure 11-2 A graph showing the quantity of alcohol (units/week) that participants felt was safe to drink whilst taking MTX

A Graph to demonstrate the proportion of participants given an information leaflet prior to starting MTX
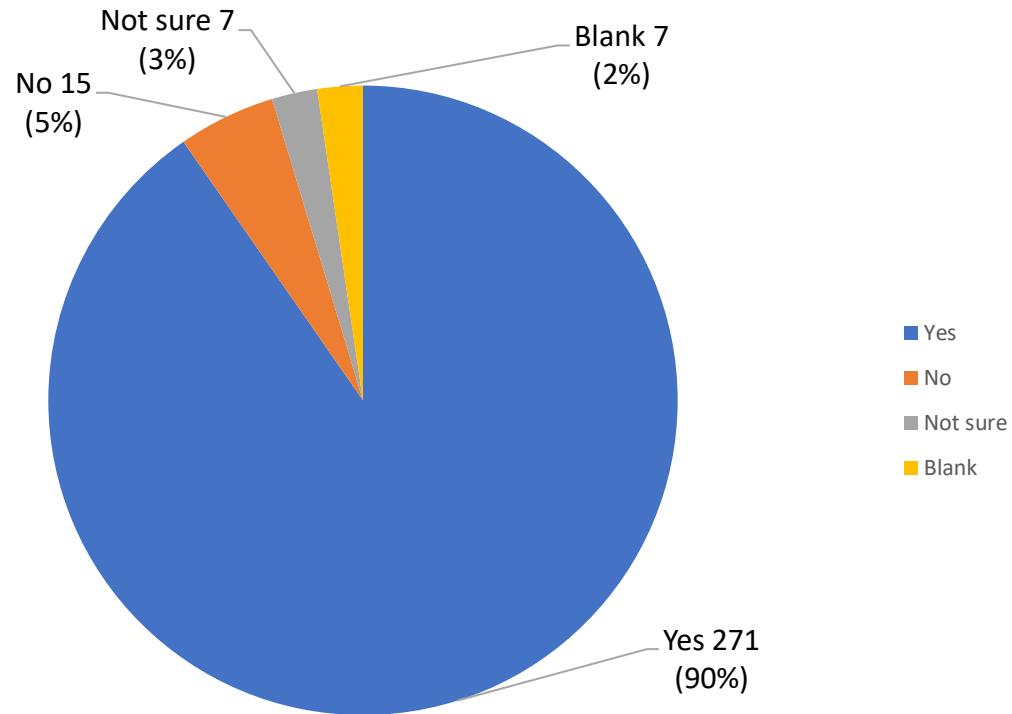
Figure 11-3 A pie chart demonstrating the proportion of participants who were given an information leaflet prior to starting MTX.

Figure 11-4 Survey responses when asking about the frequency of blood tests required whilst taking MTX

Figure 11-5 Responses to survey question 'Who is responsible for acting on monitoring blood tests taken?' Some respondents choose multiple responses.

11.2.3 Methotrexate – a toxic medication?

Participants were asked about their experience of taking MTX. When analysing the responses, three themes were apparent. The first was that MTX had had a positive impact on individual's lives. 41% of the comments referenced MTX's advantageous effect, examples of which are below:

"Methotrexate has changed my life for the better, minimal side effects"

"It has been beneficial, despite the the days of tiredness"

"It has helped with my RA. I do not have the pain I used to have"

"Excellent, no problems, a life changer"

"Very good, I couldn't do basic activities before starting, it had a massive

improvement"

Positive comments (41%) were significantly more common than negative comments (2%), which were relatively rare in this cohort.

Adverse effects (AE) were the second common theme which was mentioned within the participant's responses. Nausea was the most commonly reported AE (15%), followed by increased frequency of infections (2%), headaches (2%), fatigue (2%) and other digestive complaints (2%). Some individuals remarked on how these AEs settled with time:

"Nausea initially, then fine"

"At first felt very poorly, sickly and lightheaded. This settled after a few

months."

"Slow start feeling sick, now happy with the medication"

"Ok, seemed to cause fatigue. Initally it caused nausea, but that wore off after a few months"

The third theme related to switching from an oral preparation of MTX to subcutaneous injection, with participants largely reporting feeling better as a consequence.

"The drug has been effective. Tablets caused nausea, injection much better"

"Wasn't good in the beginning, but much improved now as on the injection"

"Tablets caused vomiting and nausea. Quickly adapted to injection. More satisfactory"

"I didn't feel well on oral methotrexate, but I'm fine now I inject"

In a further free-text question, participants were asked whether they had any concerns about taking MTX. The majority, 59%, reported no concerns, however, 41% did report concerns. Of those with concerns, the vast majority referenced potential side effects which MTX may induce (95%). Some detailed the potential side effects that could be experienced including concern regarding liver damage (25%), immunosuppression causing infections (9%) and hair loss (7%). The majority of concerns focused on the potential for MTX cause harm, even if the absence of current side-effects (37%). Examples of this include:

"Just reading all the side effects"

"It has potentially very high risk of side effects impacting / effecting your other organs"

"What long term taking of methotrexate can have"

"Knowing that it can cause organ damage to internal organs"

11.3 Discussion

Our results suggest the majority of patients prescribed MTX in our cohort were offered an information leaflet prior to prescription and had a good understanding of the frequency of blood tests required, as per the national guidelines. There was some confusion as to who was responsible for acting on those blood tests, with many participants believing this was shared across the hospital and GPs simultaneously. In fact, shared care guidelines suggest that this is the responsibility of the GPs once patients are on a stable dose (detailed in Appendix L). This finding is not out of keeping with the existing literature, demonstrating a deficit in patient(459) and nurse specialist(460) education regarding the correct monitoring guidelines. This misunderstanding may come about as a consequence of the inevitable increase in Clinical Nurse Specialist supervision that occurs around the time of starting MTX, contrasting with the relative paucity of input from their primary care team.

Opinions regarding the ingestion of alcohol varied considerably within our cohort and this is likely reflects the historically ambiguous guidance from national bodies regarding safe alcohol intake during MTX therapy, as detailed in Section 6.2.1. One quarter of patients felt no amount of alcohol was safe whilst taking MTX. Of those who thought alcohol was safe, most stated less than 14 units was appropriate.

These results are in keeping with the existing literature. Humphrey's *et al.* reviewed 11,839 patients with RA on MTX reporting 33% didn't drink any alcohol(461). Eight percent of their cohort drank over 14 units/week, corresponding closely to our results reporting only 6% of the group felt it was safe to drink more than 14 units/week. However, it is well documented that individuals with psoriasis consume more alcohol than the

general population, with some data suggesting between 13% and 30% of patients with psoriasis found drink harmfully(359, 362, 462). Drinking alcohol in moderation (less than 14 units/week) is in keeping with advice from wider rheumatology and dermatology communities within the UK(463-466). The relaxation of guidelines regarding alcohol intake in those taking MTX has occurred over decades and has followed the publication of large, long-term, cross-sectional studies demonstrating the apparent safety of smaller quantities of alcohol with low-dose MTX(467).

Participants reported predominantly positive opinions of MTX. It is worth considering that the inclusion criteria for this study (participants who had taken MTX for at least 6 months) may have introduced selection bias into this element of the study. Patients who did not find MTX beneficial, or those who found the AEs too arduous, would likely have stopped treatment prior to the 6-month milestone upon which inclusion into our study was dependent. Participants did report frustration with common AEs –nausea, fatigue, hair loss and infection. These same AEs are reported in other studies(468-470). It is note-worthy though, that despite the frequently mentioned AEs, participants were still overwhelming positive towards the medication, suggesting it's efficacy significantly outweighs the detrimental consequences.

Perhaps most significantly, our survey has demonstrated an apprehension and pre-occupation with potential unknown but deleterious consequences of long-term MTX in our cohort, particularly in relation to hepatotoxicity and nephropathy. This was commonly cited by MTX-recipients, when asked about concerns over their treatment and is likely to be related to MTXs much publicised and now questioned association with lung(471) and liver disease, (detailed in 6.3.2). This study provides evidence that the much hypothesised and discussed 'toxicity' of MTX continues to tarnish its reputation amongst patients today, despite decades of safe and effective use(41, 369). This is in keeping with other, similar studies(472).

These data described in this study were acquired via a participant questionnaire. Unlike other elements of data collection in this thesis, it is not possible to cross-check patient opinion and unlike medical diagnoses or weight, it is much less likely to be previously referenced in the medical notes. Care was taken during study design to ensure that

questions were not leading and were clearly written, so as to reduce the potential risk of bias or the acquisition of flawed or incorrect data. Participants were asked to complete this survey following recruitment into the study. Part of the recruitment process explained the rationale for the study, mentioning the historic concerns regarding MTX and liver disease. It is, of course, possible that this may have reminded participants, or prompted them when then asking them whether they had any concerns regarding taking MTX, leading to an over-reporting of concern regarding hepatotoxicity.

To conclude, patient-related factors (expectations, perception and motivation) all influence adherence to prescribed treatments. This study has demonstrated four out of ten of our participants reported concerns about MTX due to potential adverse effects, including hepatotoxicity and nephrotoxicity. Despite this, participants were overwhelmingly positive about MTX, which many described as a "life-changer".

CHAPTER 12:   DISCUSSION

12.1 Introduction

Despite its use over many years, low-dose MTX gained a reputation as a toxic and potentially dangerous drug, which for decades was only advocated in 'life-ruining circumstances'(65-69). More recently, evidence emerged supporting its safety, (369, 406) contradicting previous suggestions that its use lead, inevitably, to liver disease.

Historically, routine monitoring of MTX-recipients depended on regular blood test monitoring, ignoring the fact that LBTs are poor markers of liver function and PIIINP has a low sensitivity and specificity, if used to identify liver damage(206). Whilst MTX is known to commonly cause a harmless transaminitis, these very same blood tests only very rarely correlate with histological damage and are too insensitive to differentiate between aetiology. Hence, reliance upon LBTs, regardless of their ease of monitoring, is illogical and potentially misleading. In the absence of highly sensitive and specific serological tests, an alternative method of assessment prior to and during MTX therapy should be employed. Liver biopsy, at first sight, fulfils these requirements, but is invasive, expensive and not without risk, especially in the context of the demonstrated low risk of hepatotoxicity associated with MTX. A non-invasive alternative, which accurately identifies liver damage in an at risk population, such as overweight individuals or those imbibing a harmful amount of alcohol, has proved elusive until recently. There are now several such tests widely used in general hepatology, which could be used routinely to monitor patients before and during MTX therapy.

12.2 Methotrexate and liver fibrosis – real world experience

The audit of patients taking MTX in YTHT demonstrated that risk factors for NAFLD were commonplace, close to one third of patients had co-existing hypertension or were prescribed corticosteroids, and over two thirds were overweight. This is significant, as NAFLD is likely one of the historically largest confounding elements of the published data regarding hepatotoxicity which was assumed to be related to MTX. The prevalence of NAFLD is thought to be 24% worldwide(371). A population attending rheumatology

and dermatology outpatient clinics are likely to have an even higher prevalence given the increased risk in a psoriatic population(354), concomitant medication, such as corticosteroids and potential mobility problems impacting upon BMI.

Furthermore, our study has demonstrated that alcohol intake was not documented in close to two thirds of those starting MTX treatment. Over 1 in 5 people in the UK drink over 14 units/week(457). This project provides evidence that alcohol-related liver damage is likely to also be a confounding diagnosis within our cohort. Thorough historical evaluation of previously published trials, demonstrated in Table 6-5 reinforces that alcohol was often not considered as an alternative cause for liver damage in this cohort.

As previously discussed in Section 9.3; audit, as a research tool, does have limitations. A weakness of this scoping audit was a reliance on data from clinical records, with a paucity of historical electronic records in some cases and the nuances of multifactorial, complex decision-making can be lost. Despite these limitations, as an initial method of data capture, the audit provided an interesting insight into current practice within one teaching hospital. The audit certainly portrays an inappropriate reliance upon LBTs as a marker for liver disease within both the rheumatological and dermatological communities.

12.3   Risk factors for liver fibrosis

The large cross-sectional study, set within a population of patients attending outpatient rheumatology and dermatology clinics, demonstrated no difference in prevalence of liver fibrosis and no increased risk of developing liver fibrosis, regardless of whether an individual has taken MTX or not. The presence of liver fibrosis did, however, correlate with BMI, waist circumference and fat mass percentage, all known risk factors for the development of NAFLD.

Our results build on similar findings in the existing literature, which challenge the prevailing dogma that low-dose MTX is hepatotoxic. A multitude of published studies have failed to demonstrate a relationship between MTX and liver disease when examined using liver biopsy, including cohort(339, 342) and cross-sectional(334-336) studies, literature reviews(365, 473) and meta-analyses(369, 474). However, perhaps because of MTXs common adverse effect of what is considered 'harmless' elevation of LBTs(369),

there appears to have been an understandable reluctance to declare it safe, and particularly "not hepatotoxic". This has resulted in uncertainty and limitations in the use of what is undoubtedly a highly efficacious and remarkably safe medication for a wide range of immune-related pathologies.

Since 2000 there have been very few studies which evaluate liver disease in those prescribed MTX by histology, as it became evident that the risks of biopsy frequently outweighed the benefit of the test(475, 476). The minority of studies which did include histological analysis, reinforced that MTX was not causing hepatotoxicity(246, 368, 477). National guidelines for monitoring MTX changed to reflect this across specialities, as detailed in section 6.1.3. More recently, studies have used alternative methods of non-invasive assessment. In 2007 Berends *et al.* used both histological and transient elastography assessment to demonstrate that FibroScan® was a viable and reliable alternative to detecting fibrosis in a population of patients taking MTX(246). Further reports have gone onto demonstrate the use of transient elastography in this setting (248, 478, 479) all of which demonstrate MTX is not associated with liver fibrosis. Lahari *et al.* is an example of one of these – including 518 participants with a variety of benign inflammatory conditions. Both MTX and controls groups were evaluated. Liver fibrosis, as measured by FibroScan®, was associated with BMI and alcohol use but not MTX(479). This pattern is replicated in studies based on patients with RA(478), psoriasis(480, 481) and IBD(248, 482) Attalah *et al.*'s recently published study included participants with both RA and psoriasis. FibroScan and ELF were used to evaluate liver fibrosis in this large prospective study (n=999), which also demonstrated no relationship between MTX cumulative dose nor duration and liver fibrosis (483).

Our study revealed liver fibrosis in 1 in 6 patients of those attending rheumatological and dermatology clinics. Importantly there was no increase in liver fibrosis in those taking MTX. The use of multivariate analysis allows us to account for confounding factors including weight, alcohol intake and medical comorbidities, a weakness of many other published studies in the field.

12.4 Patient's Perceptions of Methotrexate

The results of the questionnaire of 300 patients who take MTX suggest the majority received appropriate information regarding the medication prior to its prescription, and our cohort largely understood the frequency of blood tests required, as per national guidelines. However, similar to other studies(459, 460) there was confusion as to who was responsible for acting on abnormal blood results, with a misconception this was the hospital team, rather than GP. These findings are likely a result of increased Clinical Nurse Specialist supervision, who are responsible for educating patients prior to MTX commencement and overseeing the initial serological monitoring.

Opinions regarding the ingestion of alcohol varied within our cohort. Only 6% felt it was safe to drink more than 14 units/week, in contrast, one quarter felt no amount of alcohol was safe. These findings mirror the existing literature when evaluating alcohol intake in MTX-recipients(461), long-term ambiguity from national bodies regarding safe alcohol intake has most likely played a role in this significant variation(463-466).

Although MTX-recipients reported predominantly positive opinions of MTX, we have also highlighted significant patient concern regarding both known, but largely unknown, AEs of MTX. Just under half of participants cited potential organ damage (liver and kidney), and other serious, unknown, AEs were a source of concern to them even after years of taking the treatment. This is important as it suggests that out-dated literature implying MTX causes significant organ damage (such as liver disease) is still widespread today, and causing apprehension within the patient cohort; the apparent 'toxicity' of MTX continues to tarnish its reputation amongst patients today, despite decades of safe and effective use(41, 369). Patient-related factors influence adherence to prescribed treatments, and it is highly likely that this has an impact upon MTX cessation rates, despite being a highly efficacious, in-expensive, disease-modifying medication.

12.5 Limitations

No study design is perfect and we recognise that both participants and doctors enrolled into research studies can behave differently in comparison with real world cohorts(484). Different study designs were utilised to acquire data gathered, the limitations of all are described in more detail above. Audits risk over-simplifying complex clinical decisions

and rely upon adequate documentation. Cross sectional studies are subject to recall bias. Questionnaires are similarly vulnerable to self-reporting bias, although they are suitable for attaining participant's opinions and perspectives which was one of the key aims of the study(485). Participants were asked to complete our survey following recruitment into the study. This process cited the historical concerns regarding MTX causing liver disease as rationale for the study – important context to obtain valid consent. However, this may have led to an over-reporting of potential MTX-related hepatotoxicity when later questioned.

This study assessed liver fibrosis by way of FibroScan®. FibroScan® provides a validated, non-invasive method of assessing liver damage and as such has been commonly adopted across the UK. It could be argued that histological examination would have provided more robust results, however the risks associated with liver biopsy would have made the study unethical. FibroScan® measurements within our study were not undertaken in fasted subjects, as they would have ideally been. This was due to the practicalities of running the study.

MTX prescription within the UK is subject to national guidance; the British Association for Dermatologists(58), British Society of Rheumatologists(84) and British Society for Gastroenterologists(60), as detailed in section 6.1.3. Our results demonstrating no increased risk of liver fibrosis despite exposure to MTX, could be seen as proof of success of these guidelines - reinforcing that the requirement for 3 monthly monitoring of LBTs and/or PIIINP. The authors hope to have demonstrated the poor sensitivity and specificity of LBTs alone as markers of liver fibrosis, not just unnecessary monitoring and cost for patients, but also falsely reassuring. Furthermore, the relative ease by which other non-invasive methods are now available, means there are suitable alternatives to LBTs. International studies, where guidelines differ, have also demonstrated no relationship between MTX use, or cumulative dose, and liver fibrosis, suggesting this is not just related to the success of the UK guidelines(479, 486).
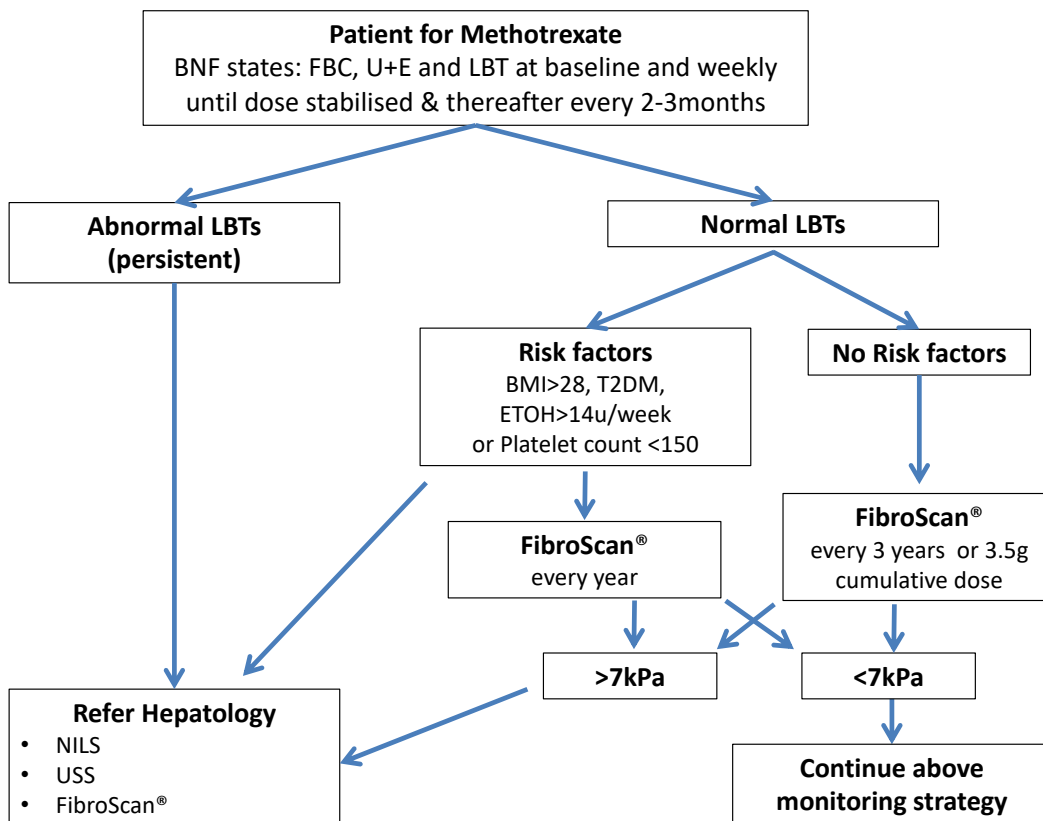
Self-reported alcohol, measured by way of the CAGE questionnaire, was not associated with liver fibrosis, which is contrary to what we may expect given the relationship between alcohol and liver disease. Section 10.3 reviews why this discrepancy may have

arisen, including participants potential failure to disclose an entirely accurate alcohol history, particularly historical, to research workers and weaknesses of the AUDIT-C tool to capture historical excess alcohol.

This study had low levels of missing data, reducing the risk of potential bias and invalidating the powering of the study.

12.6 Recommendations

This review has highlighted the weakness of existing methods to monitor liver damage with LBTs, given their low sensitivity and specificity for liver fibrosis. Guidelines differ across various countries and specialities, as discussed in Section 6.1.3. The most recent American guidelines for MTX use in psoriasis have incorporated composite hepatological scores and non-invasive assessment including transient elastography to reduce reliance on LBTs alone(87). Evidence has demonstrated that reduced frequency of serological monitoring for MTX does not increase serious adverse events, arguing against the use of more frequent and multiple liver enzyme measurement (487). Given the compilation of evidence the authors believe it is no longer appropriate to rely solely upon LBTs as a marker for liver fibrosis and would advocate for a monitoring pathway to include assessment of risk factors and alternative blood tests such as platelet count. A proposed pathway is demonstrated in Figure 12-1. This proposed flowchart is in keeping with current UK guidelines from the British Association of Dermatologists(58), British Society of Rheumatologists(84) and British Society of Gastroenterologists(60) to have at least quarterly blood tests once maintained on a stable dose of MTX. The addition of FibroScan® ensures thorough assessment of liver fibrosis, and allows ongoing monitoring including highlighting significant change and involving hepatology teams appropriately.

Patient for Methotrexate
BNF states: FBC, U+E and LBT at baseline and weekly until dose stabilised & thereafter every 2-3months

Abnormal LBTs (persistent)

Normal LBTs

Risk factors
BMI>28, T2DM, ETOH>14u/week or Platelet count <150

No Risk factors

FibroScan®
every year

FibroScan®
every 3 years or 3.5g cumulative dose

>7kPa

<7kPa

Refer Hepatology
- NILS
- USS
- FibroScan®

Continue above monitoring strategy

BNF: British National Formulary, FBC: Full blood count, U&E: Urea and electrolytes, LBT: Liver blood tests, BMI: Body mass index, T2DM:Ttype 2 diabetes mellitus, EToH: Alcohol, NILS: Non-invasive liver screen, USS: Ultrasound

Figure 12-1 A proposed pathway for commencing and monitoring MTX. Amended following personal communication from Dr Charles Millson

12.7 Closing Remarks

MTX is an effective, inexpensive, treatment with over 65 clinical indications and consequently utilised worldwide. For many decades concern regarding MTX-related hepatotoxicity have curtailed it's use. This study aimed to evaluate real-world adherence to the existing guidelines within the UK, establish the prevalence of liver fibrosis within the population of patients who are prescribed the medication, and finally to establish MTX-recipients opinion of this medication.

Our study has concluded that adherence to current MTX-monitoring guidelines, although fair initially, declines over time. Rates of liver fibrosis are not higher in those prescribed the medication in contrast to controls from the same population, rebuking the long-suggested relationship, and finally that the ongoing suggestion of potential organ damage secondary to MTX weighs heavily on those prescribed the medication.

Our proposed MTX monitoring pathway incorporates consideration of risk factors for liver disease and FibroScan assessment, given its significantly higher sensitivity and specificity for detecting liver fibrosis than LBTs alone. The proposed pathway also provides more robust guidance to MTX prescribers as to when to involve hepatologists who may help to facilitate the ongoing use of MTX with appropriate monitoring.

This work adds to the growing body of evidence demonstrating the safety of MTX with no discernible evidence found that it attributes or causes liver disease.

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CHAPTER 14:   APPENDICES

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Appendix A

York Teaching Hospital NHS Foundation Trust Sponsorship Documents

Methotrexate Sponsorship application - resubmission following January 2019 meeting

Phillips, Deborah <Deborah.Phillips@York.nhs.uk>

To: Turner, Lucy - Hepatology Research Fellow <Lucy.Turner2@YORK.NH   Fri 05/04/2019 12:26
Cc: Hutchinson John (YORK TEACHING HOSPITAL ... **+1 other**

Dear Lucy,

I have now discussed your resubmission for the above study with the Chairman of the R&D Group and I am pleased to be able to confirm that the Trust will act as Sponsor for your study.

The Chairman has asked for some further consideration about the statistical aspects but we can perhaps work on this together after Easter – it does not stop you from progressing in the meantime.
I do have some further additional comments from the Group members that I will compile into a separate email but they are advisory only.

Congratulations!

Kind regards
Deborah

Kind regards

Deborah

Dr Deborah Phillips
Research Adviser
York Teaching Hospital NHS Foundation Trust
Usual working days are Tuesday - Thursday

Appendix B

Integrated Research Application System Approval

![NHS Health Research Authority letter]

NHS
Health Research Authority

North East - York Research Ethics Committee
NHSBT Newcastle Blood Donor Centre
Holland Drive
Newcastle upon Tyne
NE2 4NQ

Telephone: 0207 1048091

> **Please note:** This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

17 June 2019

Dr Lucy Turner
Hepatology Research Fellow
York Teaching Hospitals NHS Trust
Wigginton Road
York
YO31 8HE

Dear Dr Turner

Study title:	A Study to Investigate the Liver Function of Patients taking Methotrexate, and risk stratify them accordingly
REC reference:	19/NE/0176
Protocol number:	n/a
IRAS project ID:	264991

Thank you for your letter of 11 June 2019, responding to the Committee's request for further information on the above research [and submitting revised documentation].

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date

A Research Ethics Committee established by the Health Research Authority

Appendix C

Health Research Authority Approval

Ymchwil Iechyd
a Gofal Cymru
Health and Care
Research Wales

NHS
Health Research
Authority

Dr Lucy Turner
Hepatology Research Fellow
York Teaching Hospitals NHS Trust
Wigginton Road
York
YO31 8HE

Email: hra.approval@nhs.net
Research-permissions@wales.nhs.uk

18 June 2019

Dear Dr Turner

**HRA and Health and Care
Research Wales (HCRW)
Approval Letter**

Study title:	A Study to Investigate the Liver Function of Patients taking Methotrexate, and risk stratify them accordingly
IRAS project ID:	264991
Protocol number:	n/a
REC reference:	19/NE/0176
Sponsor	Organization not set

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, **in line with the instructions provided in the "Information to support study set up" section towards the end of this letter**.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Appendix D

Patient and Public Involvement– Individual 1

CONSENT FORM
Alter AND RISK STRATIFY THEM ACCORDINGLY in title to e.g. Categorise them into low/medium/high risk

Is number 4 usual? Consent to use information gathered in any and all future research seems like a big ask!

PIS
YOU WILL VISIT CLINIC
This MAY..... Surely research should be arranged so that it WILL otherwise extra visits to clinic will be time consuming and costly for participants.
WHAT ARE POSSIBLE DISADVANTAGES.....
Alter PARTICIPANT to YOU as rest of the form is in the second person

QUESTIONNAIRE
I assume this is not for the people who are not taking the methotrexate.
Maybe define a unit of alcohol in terms of volume of wine or beer.

INVITATION LETTER
How are the people not taking the methotrexate to be selected?
This will need explaining in the letter otherwise the invitation will be a surprise and potential worry, or a separate letter written for these controls?

ILESHA STUDY LAY PERSPECTIVE

<u>Consent Form.</u>

Para 3. This would read better with no commas.
Para 6. Delete (*if appropriate*). It either is or isn't!

<u>Participant Information Sheet.</u>

What is the purpose of this study? Immediately you say that …*assess the health of their liver and check for liver damage…* potential participants will think that methotrexate causes liver damage. It would read better with a full stop after ..*their liver* and delete the rest.
Why have I been invited? Suggest insert at the end …as a control group.
You will visit clinic. Suggest "You will visit a clinic" In the last line, delete "problems" and insert "interventions" or some other less negative term.
What are the possible benefits of taking part? Delete "problem" and insert a less negative word, such as issue.
Reword the last sentence to read "the culmination of this research will hope to better inform clinicians about the side effects on the liver for in those individuals taking methotrexate, helping to shape future guidelines." The original is very negative and only serves to reinforce the view that methotrexate causes liver damage.

What are the possible disadvantages and risks of taking part? You say that there is a small risk that the health checks performed show up a problem, but you cannot assess the level of risk of a health check to each individual. For some it might turn out to be a major risk. Better to delete "small risk" and insert "possibility".

<u>Participant Questionnaire.</u>

I had never heard of methotrexate before seeing this questionnaire. However, my answer to question 10 would be a resounding **NO**, even with no knowledge of the drug. If methotrexate can cause more than one of the symptoms listed in question 9, then there must be a large query as to why it is, or has been, used. The questionnaire appears to be heavily weighted against methotrexate.

<u>Invite Letter.</u>

The reason for the study is delightfully vague. What does "better monitor" mean and imply?

<u>ILESHA Summary.</u>

In the title, it would look better to use capital first letters throughout.

Feedback / Comments on the ILESHA study

Consent form

Consider removing first comma in statement 3

Consider adding a witness to the signature section

Participant Information Sheet

We 'would like to' invite you to take part in the ILESHA research study

When reviewing the possible benefits of taking part in the study include a paragraph such as 'this referral may be to your advantage'

Invite Letter

No changes advised

Methotrexate Questionnaire

Question 5 – use 'taking' rather than 'prescribed' MTX

Question 5 – make it clearer that you are referring to alcohol here

My lay comments as agreed for the ILESHA study

Consent Form
The form is fine but could the end of the first line "and risk Stratify them accordingly" be simplified. Is it necessary?
The other seven statements of agreement are fine and the consent form is very readable.

Participant Information Sheet
It's a polite, comprehensive sheet which covers all aspects which could be asked or worried about from a patient's point of view.
It reads well and the methodology is explained well.

Participant Questionnaire
Comprehensive in its lay out and not too many questions.
The first line emphasises "not to return and amend answers" ....possibly a patient would want to add more or change something if they rushed the questions.
Patient Invitation
Good but hospitals could be named instead of "regional"
Summary
I only changed six words so it reads well overall.

Possibly the questions although in bold type, which is good but could the font be larger ; say 14 I'm thinking of older patients' eyesight.

Invitation Letter To Patient
A polite invitation which would be acceptable to all patients.
The only addition would be to add the named hospitals in the second paragraph rather than "Yorkshire" region to show how varied the study will be.
ILESHA Study
Summary
This reads well and I only changed five words in the six paragraphs so the whole summary is readable and comprehensive.

Comments on ILESHA Study

Ilesha Summary document

I am not sure at whom the summary is aimed but it is not clear to me that it explains why fibrosis is being used as a predictor in preference to other indicators. It refers to "presumed adverse effects, such as abnormal liver blood tests, liver fibrosis and cirrhosis". This suggests that liver fibrosis is just one of several possible adverse effects and the Summary does not explain whether fibrosis can be expected to be present in all forms of liver damage that might be related to use of methotrexate or whether there might be other forms of damage which are not accompanied by fibrosis. Further along the Summary, we see "(FibroScan) uses pulse-echo ultrasonography to calculate liver stiffness as a surrogate marker of fibrosis. It is a novel, quick, non-invasive method of liver assessment". Here then, stiffness is a "surrogate marker of fibrosis" but it is not clarified whether it is a surrogate marker of other liver damage; in particular, whether lack of fibrosis is an indicator of good liver health. Unless the Summary is aimed at liver specialists, I think this reliance on fibrosis as a surrogate needs to be further explained and justified. Otherwise, there is an obvious question as to why the study is using FibroScan rather than, for example, a set of blood tests.

Invite Letter

The Invite Letter seems fine to me.

Consent Form

Item 6.

(i)   I do not understand what "If appropriate" means.
(ii)   "may be used to help contact me or provide information about my health status."
 May be used to help who or what contact me? My GP, other parts of the NHS, third parties? ...provide information about my health status to whom? To me, to my GP, to other parts of the NHS, to third parties? Elsewhere in the Consent Form, care has been taken to make very specific reference to GP, NHS Trust etc. But here, the vagueness is unsettling.

Participant Information Sheet

Purpose of Study

"so that we can assess the health of their liver and check for liver damage"

Here, I think it would be better to omit "and check for liver damage". It is fine to refer to liver damage further down the document but putting it right within the "purpose" makes it seem that the researcher expects methotrexate to be causing liver damage. Checking for liver damage is surely part of assessing liver health and it seems unnecessary to emphasise it in the Purpose heading.

What would taking part involve

"You will be given a questionnaire to complete focussing on your views of methotrexate and also your previous alcohol intake."

Needs a comma after "complete". (As it stands, the sentence indicates that the questionnaire brings about the completion of focussing).

I do have some serious issues with the questionnaire itself and I explain those under its separate heading, below. Those concerns make consideration of commas somewhat redundant.

Possible benefits, Possible disadvantages and risks

I feel that these two sections are rather confusing. Largely, they confuse benefits with advantages and confuse risks with disadvantages, and also to some extent confuse risk with probability. Further, there is overlap between the advantages and disadvantages.

"The culmination of this research will hope to better inform clinicians about risk factors for liver injury in those individuals taking methotrexate, helping to shape future guidelines."

That is definitely a potential benefit of the study and I agree with the term "risk" in that sentence. Depending on the timing and nature of the future guidelines, this benefit might or might not actually apply to participants in the study. It might apply to future patients some years hence. This distinction should be made clear.

"It is possible that the health checks carried out during the study could show up a problem that you didn't know about." Yes, this is a possible advantage, though it only becomes a benefit if the "problem" turns out to be both significant and treatable in an acceptable manner.

"There is a small risk that the health checks performed show up a problem."
Given that this has just been highlighted as a "benefit" (I prefer "advantage"), it is confusing to see it also as a "risk". In any case, it is not a risk though it may be a disadvantage. The statement "there is a small risk that the health checks performed show up a problem" is, I suggest, a confusion between risk and probability. "Risk" is an adverse event or adverse effect – examples of "risk" might be death, or cancer, or liver damage. Rather, here, what is being highlighted is that there is a small probability or small likelihood or small chance that the tests show up a problem. That is distinct from a risk unless the researcher is suggesting that the checks might actually cause the problem, in which case that would be a risk. For example, in a study involving invasive tests (colonoscopy, cystoscopy etc), there is a risk of causing damage though that risk might have a small probability.

In any case, showing up a problem is not a "risk" (because it is not in itself harmful). It might be a disadvantage if it showed up a situation which caused merely worry or unnecessary treatment which would not have caused a problem if it had been left undiagnosed. There might be risks (in the proper sense) associated with the treatments for such problems.

I think that the whole area of showing up problems should be rewritten without classifying it as either an advantage or a disadvantage, and still less as benefit or risk. For example, it could be given a separate heading, such as "What the tests might show". Then it could be worded along the following:

What the tests might show

The culmination of this research will hope to better inform clinicians about risk factors for liver injury in those individuals taking methotrexate, helping to shape future guidelines.

It is possible that the health checks you receive during the study could show up a condition that you didn't know about. In this circumstance you and your GP would be informed immediately and you would be advised on whether any further investigations, tests, treatments or changes to medication would be appropriate. We appreciate that discovery of health related findings can cause worry and the staff are on hand to discuss any concerns you might have.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time. If you choose to withdraw we would like to use the data collected up to the time of your withdrawal.

Does the patient have the option of also stopping the use of data collected up to the time of withdrawal? This should be clarified either way. In particular, if data cannot be withdrawn, patients might be reassured if they know that all data is anonymised.

What happens when the research study stops?

After the research study is completed, the results will be analysed and we hope to publish the findings in the medical literature/journals.

Again here, it needs to make clear that the published findings would be anonymised and not identify individuals.

Participant Questionnaire

I have huge problems with this Questionnaire. I find it very hard to see the point of phrasing the questions in this way and even harder to see what you might deduce from the answers. It comes over as really quite aggressive and even as an attempt to show the participant up for their lack of correct knowledge (which I am sure is not actually the researcher's intention). Many of those questions have a right or wrong answer. The expert knows and the participant (possibly) doesn't but is being asked to guess or show their ignorance.

The only acceptably worded questions on the Questionnaire are 8 and 10. It is acceptable to ask who is doing blood tests and whether the patient feels safe on methotrexate.

But all the other questions are frankly unacceptable to me. If I were presented with this questionnaire face to face, together with the instruction that my first (implied uninformed) impressions were sought, I would walk off the study rather than complete that questionnaire. I am sorry that this comment itself seems aggressive but I cannot disguise my strong adverse reaction to the Questionnaire.

If I am offered a medication that I have not investigated, I first at least ask the specialist what are the possible adverse effects and possible benefits, together with their probabilities. For "serious" medications, such as methotrexate (or 6-mercaptopurine or humira, both of which I take), I would certainly first go away and look them up on the EMC and the BNF. I would probably also look at the NICE guidelines on the condition being treated although I place more weight on EMC and BNF. Then I would discuss my concerns about precautions, contraindications and adverse effects with the specialist before making a decision.

What I would definitely not do is base my decision or concerns on my initial prejudices in the absence of information from either the consultant or the published reference sources.

Once on a medication, I might well forget the details of many of the adverse effects and then be unable to recall those details if asked. For example, I would defy anybody to be able to recite all the possible adverse effects, together with their probabilities, of humira or other monoclonal antibodies, for which the list is extremely long and complex (as indeed it is for methotrexate), without concurrent reference to one of the published sources. Indeed, it is common for doctors to refer to this material whilst discussing it with me, an action which I respect and which gives me confidence.

To return then to the suggested Questionnaire, a patient might already be on methotrexate but have forgotten the side effects because, having agreed to take it, they no longer need to remember them all. Or they might not be taking it and would not be likely either to know them all or to base a future decision on their guesses.

It would be more relevant and much less provocative, in the Questionnaire, to provide information on some specific side effects or cautions which you believe to be pertinent to the study, together with probabilities, and ask whether the participant feels:
(a) Not worried; (b) Concerned but not deterred from taking methotrexate; (c) Deterred from taking methotrexate and would prefer another option.

For example: you could state the position on alcohol consumption (the Sandoz SmPC for 10mg Methotrexate tablets states that alcohol should be avoided or greatly reduced) and ask the participant's reaction to that statement (I don't drink alcohol anyway so compatibility with alcohol consumption would be irrelevant to me). Or you could state the position regarding pregnancy and ask for the participant's reaction (appropriate for male participants, post-menopausal women?). Or you could state the recommended frequency of blood tests (the Sandoz SmPC says "every 2-3 months") and ask the (a) (b) (c) choice as above.

But to ask for initial impressions in the absence of quality information is rather demeaning and I cannot see the point of doing so.

In summary, I would urge a complete rethink and redesign of the questionnaire, preceded by a re-evaluation of what purpose is intended for it. I can understand why you would want to collect a history and factual information about how and when the patient is being monitored. But the list of precautions, contraindications and adverse effects of methotrexate is very long and complex. It is hard (for me) to understand why the singling out of a few points on a questionnaire is appropriate or helpful especially when based on other than information.

As a layman, these five documents impress me and I've actually little to say about them! I think they're clear and well-written and each document seems to me to do the job it sets out to.

Here are the few comments I would make.

THE CONSENT FORM. -Fine.

THE PARTICIPENT INFORMATION SHEET.   -In the section "What are the possible benefits of taking part?", it refers at one point to "risk factors for liver INJURY "(my capitals). I wonder whether it would be better to say "liver DAMAGE"? ("damage" rather than "injury" is in fact used earlier, in the second paragraph of this sheet.)

THE PATIENT QUESTIONNAIRE. -Presumably Lucy wants to know what patients taking methotrexate know about it. If I were filling this in, I think I'd go to the patient information leaflet supplied with the drug, to find the answers to her questions. If she's expecting patients to do this, it's unclear why she's asking these questions. If not, and she's trying to discover what patients remember about the drug, I'm not sure of the value of this. I've been comparing this with my own experience with a drug I take as a migraine preventive-propanolol. If I want to check something about it, for instance if I can drink alcohol while using it, I'll go straight to the patient information leaflet. I don't memorise all the important facts about a drug I'm prescribed, but instead rely on being able to check them when I need to. (I know Lucy has included this : "We would like your first answer to each question, please don't return and amend your answers.", but I don't think this really invalidates the point I'm making here.)

Perhaps I'm barking up the wrong tree here and there's something I'm missing?

THE INVITE LETTER. -Fine.

THE SUMMARY. - In the first line, the apostrophe in "people's" is in the wrong place. It ought to be where I've just put it! (in the summary it says : "peoples' ").

I'm wondering who this summary is aimed at? If it's for medics, I expect it's fine. If it's intended to be accessible to lay people, I'm not sure "immune-mediated" in the first paragraph means a lot. But then , nor does "transient elastography" or a number of other medical terms in the summary. So although Lucy has included this overview of the project which you suggested we lay people might like to read before reviewing the whole thing, in fact I think it's probably intended for medics.

In the fifth paragraph "liver injury" is used again, so the comment I made about this earlier applies again. (Under "The patient information sheet" above).

In the second line of the sixth paragraph, the "that" is redundant!

I hope this is useful. As I said at the top, I think Lucy's documents are good; I certainly understand in general terms what it's all about!

Hi Lydia,

My lay comments as agreed for the ILESHA study

Consent Form

The form is fine but could the end of the first line "and risk Stratify them accordingly" be simplified. Is it necessary?

The other seven statements of agreement are fine and the consent form is very readable.

Participant Information Sheet

It's a polite, comprehensive sheet which covers all aspects which could be asked or worried about from a patient's point of view.

It reads well and the methodology is explained well.

Participant Questionnaire

Comprehensive in its lay out and not too many questions.

The first line emphasises "not to return and amend answers" ....possibly a patient would want to add more or change something if they rushed the questions.

Patient Invtation

Good but hospitals could be named instead of "regional"

Summary

I only changed six words so it reads well overall.

Best wishes

Appendix E

Participant Invite Letter

York Teaching Hospital **NHS**
NHS Foundation Trust

STrATIFy Methotrexate

<Patient Title> <Patient forename> <Patient surname>

<Patient Address 1>

<Patient Address 2>

<Patient City>

<Patient Postcode>

<Date>

Dear [*Title*] [*Patient*]

You are being invited to participate in a research study.

The purpose of the study is to establish whether we could better monitor individuals who are taking the medication methotrexate.

You are receiving this letter as we believe you would be suitable to participate in this study if you would like to. The study is being held at hospitals within the Yorkshire region.

Enclosed is a participant information leaflet, so that you may find out more information about the study.

If you wish to be involved please inform your health care professional at your next outpatient clinic appointment. If you have any questions or concerns prior to this please contact Dr Lucy Turner, Research Coordinator, on 01904 725467, or email 'lucy.turner@york.nhs.uk'.

Yours Sincerely,

Doctor Lucy Turner

MBChB BSc (Hons) MRCP Honorary Lecturer at HYMS

Hepatology Research Fellow

York Teaching Hospital NHS Foundation Trust

Invitation Letter
Version 1.0

IRAS: 264991
14/03/2019

Appendix F

Participant Information Sheet (page 1)

York Teaching Hospital **NHS**

NHS Foundation Trust

A study investigating liver fibrosis – STrATIFy Methotrexate

PARTICIPANT INFORMATION SHEET

An invitation

We invite you to take part in the STrATIFy METHOTREXATE research study. Before you decide we would like you to understand why the research is being done and what it would involve for you. One of our team will go through the information sheet with you and answer any questions you have. This should take about 10 minutes. Talk to others, family or friends, about the study if you wish.

What is the purpose of the study?

The aim of this study is to assess the liver health of individuals taking methotrexate, compared with those who are not. We would like to collect information on both patients who are taking methotrexate, and those who have never taken it. This is so we may assess the health of their liver. Longer term, this will help us to establish whether we could better monitor the liver function of people who are taking methotrexate, compared to those people who are not taking this drug.

Why have I been invited?

The study is assessing changes in liver health. We are hoping to study individuals who are taking methotrexate, and those who have never taken methotrexate as a control group.

Do I have to take part?

No, it is up to you to decide to join the study. We will describe the study here and go through this information sheet with you in clinic. If you agree to take part, we will then ask you to sign a consent form. You are free to withdraw at any time, without giving a reason. This would not affect the standard of care you receive.

What would taking part involve?

You will complete a questionnaire:
You will be given a questionnaire to complete, focussing on your views of methotrexate and also lifestyle questions, such as your alcohol intake.

You will visit clinic:
To gather information required we will review you in a clinic setting, this may be at the same time as an existing clinic appointment. The consultation will involve a study member asking questions about your medical history, including other medication you are taking and previous medical issues you've had.

You will have special tests done:
As part of the study we will perform various tests. These include measuring your height, weight and body mass composite. It will also include a measure of the stiffness of your liver, by way of a transient elastography (FibroScan). This involves putting a small probe on the right side of your abdomen whilst a soundwave is sent into your liver, similar to an ultrasound scan. It is painless and the procedure takes about 5 minutes.

Participant Information Sheet (page 2)

STrATIFy Methotrexate

What are the possible advantages of taking part?

It is possible that the health checks carried out during the study could show up an issue that you didn't know about. If this happens, you will be referred for suitable assessment and investigations and your GP will also be informed; this referral may be to your advantage.

The culmination of this research should better inform clinicians about risk factors for potential liver injury in those individuals taking methotrexate, and help to shape future guidelines.

What are the possible disadvantages and risks of taking part?

There is a possibility that the health checks performed show up a problem. In this circumstance you would be informed immediately and the next appropriate steps will be organised and made clear to you. We appreciate the discovery of health related findings can cause worry and the staff are on hand to discuss any concerns you might have.

Who will be able to see my confidential information?

We will follow ethical and legal practice and all information about you will be handled in confidence.

York Teaching Hospital is the sponsor for this study based in the United Kingdom. We will be using information from you and your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. York Teaching Hospital will keep identifiable information about you for 12 months after the study has finished.

Your rights to access change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

You can find out more about how we use your information by contacting the R&D Department on 01904 7255123..

York Hospital will collect information from you and your medical records for this research study in accordance with our instructions.

York Teaching Hospital will use your name, hospital number, and contact details to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Individuals from York Teaching Hospitals and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The only people in York Teaching Hospital who will have access to information that identifies you will be people who need to contact you to about the study or audit the data collection process. The people who analyse the information will not be able to identify you and will not be able to find out your name, hospital number or contact details.

York Teaching Hospital will keep identifiable information about you from this study for 12 months after the study has finished.

Participant Information Sheet (page 3)

Your data will be collected:
If you join the study, some parts of your medical records and the data collected for the study will be looked at by authorised persons from the York Teaching Hospitals NHS Trust. All will have a duty of confidentiality to you as a research participant.

Your medical records will be checked:
To make sure the information collected in the study is accurate, it will be need to be checked by study personnel working in York Teaching Hospitals NHS Foundation Trust. We ask you give permission for these authorised people to see your medical records. They will keep the information confidential and your rights to privacy will be protected.

What will happen if I don't want to carry on with the study?

You can withdraw from the study at any time. If you choose to withdraw we will ask your permission to use the data collected up to the time of your withdrawal. This would be anonymised, and we would only do this with your permission.

What happens when the research study stops?

After the research study is completed, the results will be analysed and we hope to publish the findings in the medical literature/journals. All data collected from the study is anonymised, so it would not be possible to identify any individuals.

Further information and contact details

Any queries please contact:

Dr Lucy Turner, Chief Investigator
Hepatology Research Fellow
Telephone: 01904 725467

York Teaching Hospital NHS Foundation Trust
Email: lucy.turner@york.nhs.uk

Appendix G

Consent Form

STrATIFy Methotrexate

York Teaching Hospital **NHS**
NHS Foundation Trust

| IRAS ID: 264991 | Centre number: .... | Study Number: .... |

Participant Identification Number for this trial:

CONSENT FORM:

A Study to Investigate the Liver Function of Patients Taking Methotrexate, and risk Stratify them Accordingly

Name of Researcher: Lucy Turner

Please initial box

1. I confirm that I have read the information sheet dated 14/03/19 (version 1.0) for the above study. I have had the opportunity to consider the information, ask questions and have had these answered satisfactorily. ☐

2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐

3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by individuals from York Teaching Hospital NHS Foundation Trust, by regulatory authorities or from the NHS Trust where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

4. I understand that the information collected about me may be used to support other research in the future, it may be shared anonymously with other researchers. ☐

5. I agree to my General Practitioner being involved in the study, including any necessary exchange of information about me between my GP and the research team. ☐

6. I understand that the information held and maintained by York Teaching Hospital NHS Foundation Trust may be used to help contact me or provide information about my health status. ☐

7. I agree to take part in the above study. ☐

_____   _____   _____
Name of Participant   Date   Signature

_____   _____   _____
Name of Person   Date   Signature
taking consent

Consent Form
Version 1.0   IRAS: 264991
                                                                    14/03/2019
When completed: 1 for participant; 1 for researcher site file; 1 to be kept in medical notes.

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Appendix H

Data Collection Proforma (page 1)

York Teaching Hospital NHS
NHS Foundation Trust

STrATIFy Methotrexate

Demographics	
Height (m)	....
Weight (kg)	....
BMI (kg/m²)	....
Ethnicity	....
Gender	M / F

Study ID number	
Date today	........./........./...........

Body Mass Composite Score	

Comorbidities (circle as relevant)							
T1DM	T2DM	Chol	HTN	Psoriatic A			
All others:							

Medications (pls detail all)			

Methotrexate	
Dose (mg)	....
Frequency of administration	Weekly / ....
Route	PO / SC / ....
Speciality overseeing	Rheum / Derm / Gastro / ....
Prescribed for...	RA / Psoriatic arthritis / Inflammatory arthropathy / JIA / ....
When first prescribed?	_____ / _____ (month/year)
Any breaks?	

FibroScan Results	
Date	........./........./...........
Result (kPA)	....
IQR	....
CAP	....

If first prescribed > 1 yr ago.....	
Weight when first prescribed:	...............(kg)
BMI when first prescribed:	.... (kg/m2)

Proforma
Version 1.0

IRAS: 264991
14/03/19

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Data Collection Proforma (page 2)

STrATIFy Methotrexate

York Teaching Hospital **NHS**
NHS Foundation Trust

What does 1 unit of alcohol look like?

| 218ml | 76ml | 25ml | 250ml | 250ml |

Standard 4.5% cider | Standard 13% wine | Standard 40% whiskey | Standard 4% beer | Standard 4% alcopop (275ml)

You shouldn't regularly exceed **14** units

drinkaware

Age	Average unit intake / week
16 – 30 years	
30 – 40 years	
40 – 50 years	
50 – 60 years	
60 - 70 years	
70 – 80 years	
80 +	

Audit C completed?	Yes – Complete
If Audit C score > 4 perform AUDIT	Yes – Complete / Not applicable

Proforma
Version 1.0

IRAS: 264991
14/03/19

Appendix I

International Physical Activity Questionnaire (page 1)

York Teaching Hospital **NHS**

NHS Foundation Trust

Physical Activity Related Questions:

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the last 7 days. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the vigorous activities that you did in the last 7 days. Vigorous physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

1. During the last 7 days, on how many days did you do vigorous physical activities like heavy lifting, digging, aerobics, or fast bicycling?

_____ days per week

☐　　　No vigorous physical activities　→　**Skip to question 3**

2. How much time did you usually spend doing vigorous physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐　　　Don't know/Not sure

Think about all the moderate activities that you did in the last 7 days. Moderate activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the last 7 days, on how many days did you do moderate physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? (Do not include walking).

_____ days per week

☐　　　No moderate physical activities　→　**Skip to question 5**

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International Physical Activity Questionnaire (page 2)

York Teaching Hospital **NHS**

NHS Foundation Trust

4. How much time did you usually spend doing moderate physical activities on one of those days?

_____ hours per day

_____ minutes per day

☐   Don't know/Not sure

Think about the time you spent walking in the last 7 days. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the last 7 days, on how many days did you walk for at least 10 minutes at a time?

_____ days per week

☐   No walking   ➔   Skip to question 7

6. How much time did you usually spend walking on one of those days?

_____ hours per day

_____ minutes per day

☐   Don't know/Not sure

The last question is about the time you spent sitting on weekdays during the last 7 days. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the last 7 days, how much time did you spend sitting on a week day?

_____ hours per day

_____ minutes per day

☐   Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

Appendix J

Methotrexate Questionnaire

Appendix K

Advertisement of study

Appendix L

Yorkshire Rheumatological Shared Care Guidelines, revised 2019

Methotrexate

Dose:	Treatment may begin at a dose of 10-20mg WEEKLY using 2.5mg tablets and increased to 20mg after 2-4 weeks. Folic acid should be co-prescribed, but patients should be advised not to take it on the day they take their methotrexate. The day of administration plus strength of tablet should be specified. Consider changing to the subcutaneous route if there is gastric intolerance or a lack of efficacy at the higher end of the dose range. Maximum recommended dose oral or SC = 30mg weekly.
Baseline Tests:	FBC/U&E/LFT Consider pregnancy test All patients should have a pre-treatment CXR and consider PFT (in RA). Where TLCO less than 70% or clinical concern a baseline HRCT chest may be advisable (lung toxicity is increased when fibrosis is present)
Routine Monitoring:	FBC and LFTs every 2 weeks until on stable dose for 6 weeks Once on stable dose, monthly for 3 months and then every 3 months Patients at risk of renal impairment may need U&Es checked regularly or if not annually More frequent monitoring is appropriate in patients at higher risk of toxicity, or when clinically indicated. NPSA still recommend MTX monitoring books for patients Dose increases should be monitored by FBC and LFTs at 2 and 6 weeks and then every 3 months
Indications for Stopping Therapy:	**Stop medication and contact local rheumatology service if:** WCC <3.5 x 10⁹L or below local normal range Neutrophils < 1.6 x 10⁹L or below local normal range Platelets <140 x10⁹L or below local normal range AST or ALT > 3 times normal range (iu/L) Oral ulceration/Unusual bruising/Rash/Nausea/Alopecia Any new respiratory symptoms including cough Fever **Consider the need for folinic acid rescue - refer to BNF for dosage recommendations and discuss with Rheumatology Service**
Assessment of Response:	Clinical effect usually within 2 to 4 months.
Additional information:	**Warnings/Caution:** Avoid in significant hepatic impairment Not recommended in severe renal impairment (creatinine clearance <10ml/min) the dose should be reduced by 50% if the CrCl is between 10-20ml/min. Also consider dose reduction if CrCl 20-50ml/min. Pre-existing haematological condition Underlying chest disease Where history of excessive alcohol intake **Drug interactions:** Concomitant administration of folate antagonists such as trimethoprim and nitrous oxide should be avoided. Use of co-trimoxazole may occur in patients with GPA, under specialist supervision Penicillins may potentiate levels of methotrexate (Patients should stop taking methotrexate if they have any infection/require antibiotics and restart once the antibiotic course is completed and the infection has resolved) Acitretin - severe hepatitis reported when combined with MTX Vitamin preparations containing folic acid
Pregnancy & Breastfeeding:	Refer to Section 9 (Guidance on use of DMARDS in pregnancy)
Please refer to licensed datasheet for more comprehensive prescribing information: http://www.medicines.org.uk/EMC/medicine/6005/SPC/Maxtrex+Tablets+10+mg/	

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CHAPTER 15:   DEFINITIONS

ABSI	A Body Shape Index
ADH	Anti-diuretic hormone
AE	Adverse event
AIH	Autoimmune Hepatitis
ALT	Alanine aminotransferase
AS	Ankylosing Spondylitis
APRI	Aspartate aminotransferase to platelet count ratio
ARFI	Acoustic Radiation Force Imaging
ArLD	Alcohol-related liver disease
AST	Aspartate aminotransferase
ATP	Adenosine triphosphate
AUDIT-C	Alcohol use Disorders Identification Test
CT	Computerised Tomography
FDA	Food and Drug Administration
BAD	British Association of Dermatologists
BIA	Bioelectrical Impedance Analysis
BMI	Body mass index
CTD	Connective tissue disease
CXR	Chest Xray
df	Degrees of freedom

DM	Diabetes Mellitus
DMARDs	Disease modifying anti-rheumatic drugs
DNA	Deoxyribonucleic acid
ELF	Enhanced Liver Fibrosis
FBC	Full blood count
FFM	Free Fat Mass
FIB-4	Fibrosis-4
GCA	Giant Cell Arteritis
GCP	Good Clinical Practice
GFR	Glomerular Filtration Rate
GGT	Gamma-glutamyl transpeptidase
GI	Gastrointestinal
GP	General Practitioner
GTP	Guanosine 5-triphosphate
HA	Hyaluronic acid
HCC	Hepatocellular carcinoma
HE	Hepatic encephalopathy
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
HIV	Human immunodeficiency virus
HSC	Hepatic stellate cells
HPS	Hepatopulmonary syndrome

HRA	Health Regulation Approval
HRS	Hepatorenal syndrome
HTN	Hypertension
HYMS	Hull and York Medical School
IBD	Inflammatory Bowel Disease
IFG	Impaired Fasting Glucose
IL	Interleukin
IPAQ	International Physical Activity Questionnaire
IRAS	Integrated Research Application System
IT	Information Technology
JAK/STAT	Janus Kinases/Signal Transducers and Activators of Transcription
Kg	Kilograms
kPa	KiloPascals
LBT	Liver blood test
LFT	Liver function test
M	Meter
MCV	Mean cell volume
MET	Multiple energy expenditure
Mg	Milligrams
MRE	Magnetic resonance elastography
MTX	Methotrexate
NASH	Non-alcoholic steatohepatitis

NAFLD	Non-alcoholic fatty liver disease
NICE	National Institute for Health and Care Excellence
NIHR	National Institute for Health and Care Research
NSAIDs	Non-steroidal anti-inflammatory drugs
OD	Once daily
PIII3NP	Procollagen type III N-terminal peptide
PBC	Primary Biliary Cholangitis
PDGF	Platelet derived growth factor
PI	Principal Investigator
PMR	Polymyalgia Rheumatica
PPARα	Peroxisome proliferator-activated receptor alpha
PsA	Psoriatic arthritis
PSC	Primary Sclerosing Cholangitis
PVT	Portal vein thrombosis
R&D	Research and Development
RA	Rheumatoid arthritis
REC	Research Ethics Committee
SBP	Spontaneous bacterial peritonitis
SLE	Systemic lupus erythematosus
SMM	Skeletal Muscle Mass
SREBP-c	Sterol regulatory element-binding protein C
T2DM	Type 2 diabetes mellitus

TBW	Total Body Water
TE	Transcient elastography
TGF	Transforming growth factor
TIMP-1	Tissue inhibitor of metalloproteinase 1
TNF	Tumour necrosis factor
U&Es	Urea and electrolytes
UK	United Kingdom
UTP	Uridine triphosphate
VAT	Visceral Adipose Tissue
VZV	Varicella Zoser virus
WCC	White cell count
WHO	World Health Organisation
YTHT	York Teaching Hospitals NHS Foundation Trust