THE ROLE OF "NON-ALCOHOLIC STEATOHEPATITIS (NASH)" IN METHOTREXATE INDUCED LIVER INJURY

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DECLARATION

I, Gerald Langman, hereby declare that the work on which this thesis is based is original and that neither the whole work nor any part of it has been nor will it be submitted for a degree in this or any other University.

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..... Gerald Langman

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7 – 12 – 2001

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ABBREVIATIONS

ASH	alcoholic steatohepatitis
BMI	body mass index
BX	biopsy
CYP 2E1	cytochrome 2E1
DM	diabetes mellitus
FFA	free fatty acid
GL	Gerald Langman
GSH	glutathione
HCV	hepatitis C virus
MTX	methotrexate
NASH	nonalcoholic steatohepatitis
NEC	necrosis
ROS	reactive oxygen species
PH	Pauline Hall
PROG	progression
P3NP	type III procollagen
SH	steatohepatitis
TNFα	tumour necrosis factor-alpha
VLDL	very low density lipoprotein

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ABSTRACT

Hepatotoxicity, especially liver fibrosis, is the major concern with long-term, low-dose oral methotrexate (MTX) therapy for psoriasis. The histological features are nonspecific and may resemble those of nonalcoholic steatohepatitis (NASH). Moreover most of the risk factors of MTX induced liver injury are also associated with NASH. In this study serial liver biopsies of 24 psoriatic patients on long term MTX were investigated to determine whether they had risk factors for NASH and the histological features of a NASH-like pattern of liver injury that could have been contributing to the prevalence and progression of liver injury that occurred while on MTX. There was a positive correlation between presence of risk factors for NASH, and also cumulative dose of MTX, with progression of liver injury while on MTX when the biopsies were scored by a grading and staging classification for NASH-like features, but not when scored with the Roenigk system. Thirteen of the 17 patients who had a NASH-like pattern of liver injury also had the risk factors for NASH-obesity and/or diabetes, and all had progressive liver injury. The other four patients had no risk factors but a mean cumulative dose of 6.5g. Seven patients, who did not have a NASH-like pattern of injury, had a mean cumulative dose of 3.8g. Five had no other risk factors and the remaining two, both of whom abused alcohol, had significant fibrosis but no steatosis. In conclusion, 13 of the 24 patients on long-term MTX had risk factors for NASH and the histological features of a NASH-like pattern of injury and all showed progressive liver injury. Four patients had a NASH-like pattern of injury attributable, at least partly, to a large cumulative dose of MTX. The conditions that predispose to NASH appear to contribute to the liver injury in

psoriatic patients. The possibility that psoriasis itself may be influencing the liver pathology is also discussed.

CHAPTER 1

INTRODUCTION

1.1 METHOTREXATE (MTX) IN THE TREATMENT OF PSORIASIS

Methotrexate (amethopterine), a folic acid antagonist initially developed for the treatment of leukaemia (1), is an established and highly effective alternative in the control of severe psoriasis. Favourable results but unacceptable side effects accompanied the aggressive daily oral and even intravenous schedules used in the early studies on psoriatic patients (2). In 1969 weekly "low-dose" oral MTX therapy was found to be equally effective but with reduced toxicity (3). Today therapy is commenced with a low, once-weekly, dose of MTX, which is slowly increased until the desired clinical effect is obtained. Dosages range from 2.5mg to 25mg weekly, depending on age, size, renal function and response to therapy (4). This regimen is associated with the least side-effects and optimal therapeutic effect (2).

1.1.1. Mechanism of Action

MTX exerts its antiproliferative action by competing with folic acid for the enzyme dihydrofolate reductase, thereby inhibiting the formation of tetrahydrofolate (5). This decreases reduced folate necessary for supplying methyl donor groups for nucleic acid and protein synthesis and thereby directly interferes with epidermal cell division (6,7). Proliferating keratinocytes and activated lymphoid cells, prominent in psoriatic skin lesions, would appear to be the main targets of the antiproliferative effects of MTX (2), however the mechanism(s) of action of once-weekly, "low-dose" MTX therapy is poorly understood. Rats fed simultaneously with MTX and choline, which has a lipotropic effect and the synthesis of which is dependent on methylation, do not show the pathological changes in the liver characteristic of MTX treatment alone (7). This strongly suggests that, in the rat model, MTX causes liver toxicity by virtue of an

effect other than the inhibition of dihydrofolate reductase. The failure of folic acid to reduce the antipsoriatic efficiency (8) and the efficacy of "low-dose" MTX in severe non-proliferative inflammatory disorders including rheumatoid arthritis, inflammatory bowel disease and asthma (9-11) further supports the experimental evidence.

1.1.2. Side-effects

The use of MTX for psoriasis is associated with a well-defined toxicity profile. This ranges from common and mild side-effects like nausea and abdominal discomfort to life threatening agranulocytosis (Table 1).

Table 1. Side-effects of methotrexate in psoriasis (12)

COMMON AND MILD	UNCOMMON	RARE
Nausea Abdominal pain Leukopenia Hepatotoxicity	Fatigue Headache Vomiting Thrombocytopenia	Stomatitis Burning sensation Chills Fever Dizziness Depression Hair loss Anorexia Gastrointestinal ulceration Agranulocytosis Pneumonitis Toxic epidermal necrolysis Diffuse fibrosis

The fact that MTX has remained the standard systemic therapy for psoriasis is due largely to its high benefit-toxicity ratio (4). Meticulous pre-MTX clinical evaluation with close attention to indications and contraindications, ongoing monitoring of therapy and the performing of serial liver biopsies is mandatory if this ratio is to remain favourable.

Bone marrow suppression is infrequent with the MTX dosages used in psoriasis (12). The main long-term concern with "low-dose" MTX therapy is hepatotoxicity.

1.2 METHOTREXATE AND THE LIVER

1.2.1. Pathology

The histological changes described in the liver with "low-dose" MTX therapy are non-specific. Macrovesicular steatosis, focal liver cell necrosis, nuclear variability, chronic portal tract inflammatory cell infiltrate, fibrosis in the perivenular, pericellular and portal tract regions (Fig 1a-d) and cirrhosis have all been observed (13). No single lesion is pathognomonic and similar histological features may be encountered with alcohol abuse, nonalcoholic steatohepatitis (NASH), hepatitis C virus (HCV) infection and drugs (Table 2). Indeed, many of these factors may contribute to the liver pathology in psoriatics on MTX and in such circumstances it is often difficult or impossible to ascribe the abnormal histological findings to MTX alone. This is particularly the case if the first liver biopsy is performed months or years after commencing MTX.

Table 2. Histological and biochemical similarities between MTX and other lesions associated with steatosis and inflammation. Modified from Zimmerman and Ishak (14)

	FAT	NEUTROPHILIC INFLAMMATION	ZONE 3 PERI-CELLULAR FIBROSIS	MALLORY BODIES	INTERFACE HEPATITIS	AST/ ALT
NASH	3-4+	+/-	+	+	-	~1
MTX	2-4+	-	+	?	-	~1
ASH	1-3+	+	+	2+	-	>2
Amiodarone	+/-	+	+	2+	-	~1
HCV	1-3+	-	-	-	+	<1

1-4+, increasing degrees of severity; -, absent; +/-, present or absent

ASH, alcoholic steatohepatitis

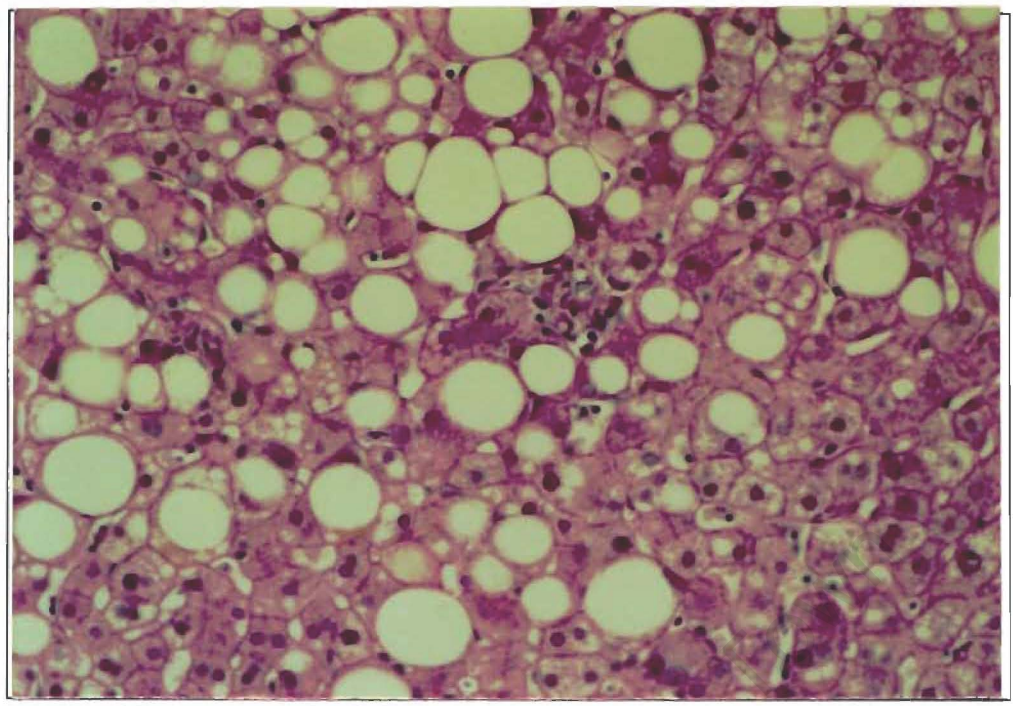


Fig 1(a). MTX-induced hepatotoxicity. Macrovesicular steatosis with focal lobular inflammation and liver cell necrosis. (Original magnification X 200; H&E.)

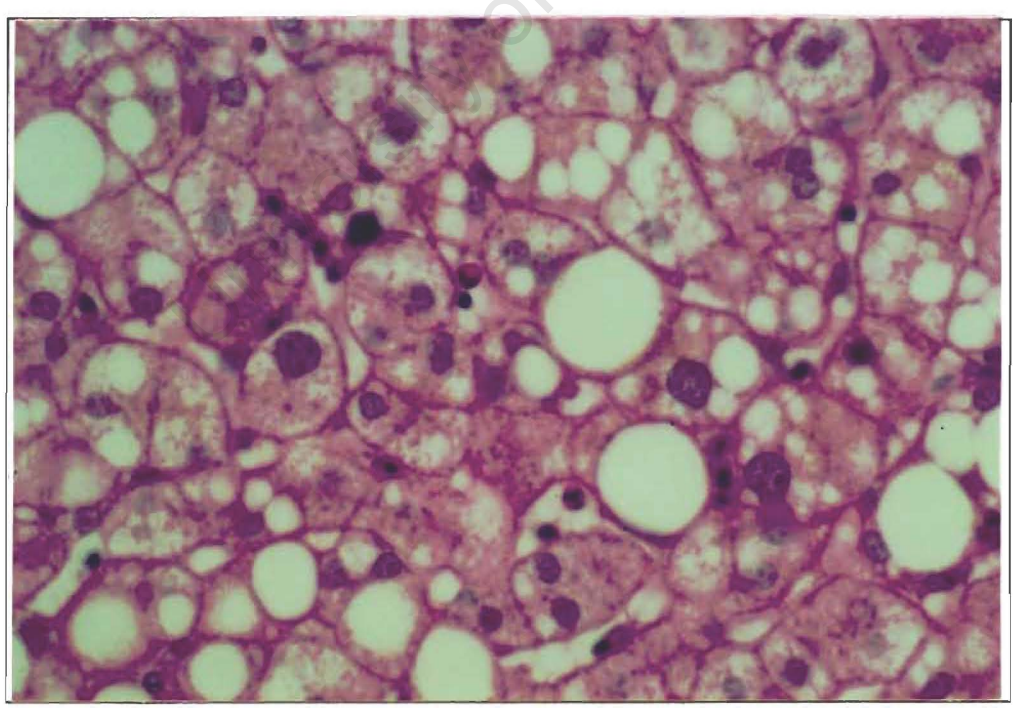


Fig 1(b). MTX-induced hepatotoxicity. Nuclear variability and macrovesicular steatosis. (Original magnification X 400; H & E.)

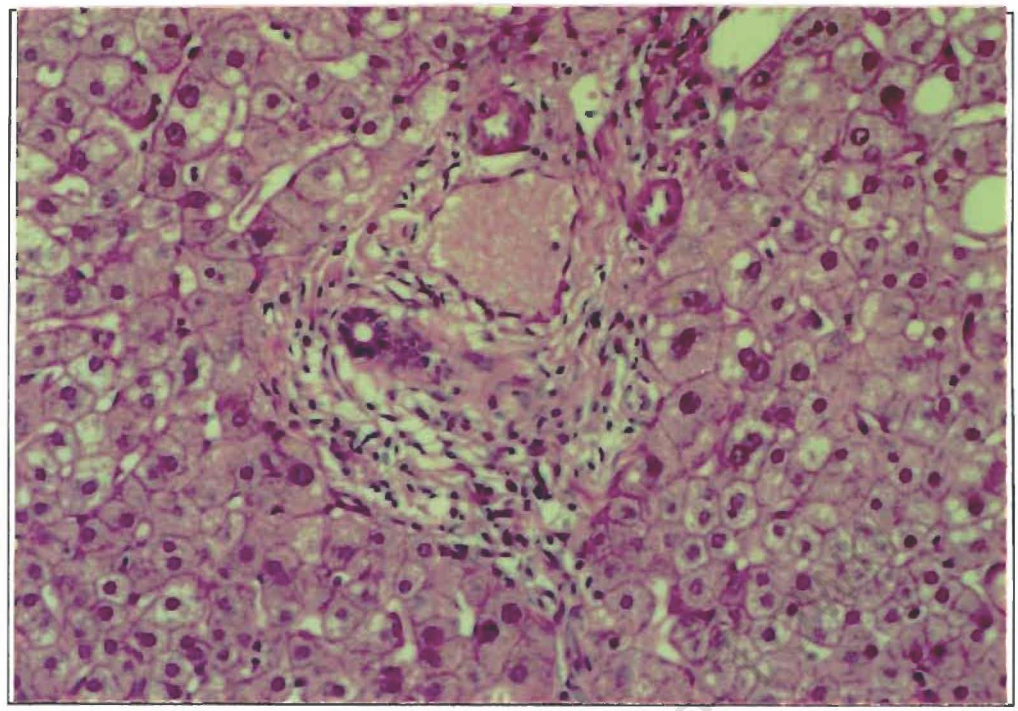


Fig 1(c). MTX-induced hepatotoxicity. A mild chronic inflammatory cell infiltrate involves this portal tract. (Original magnification X 200; H & E.)

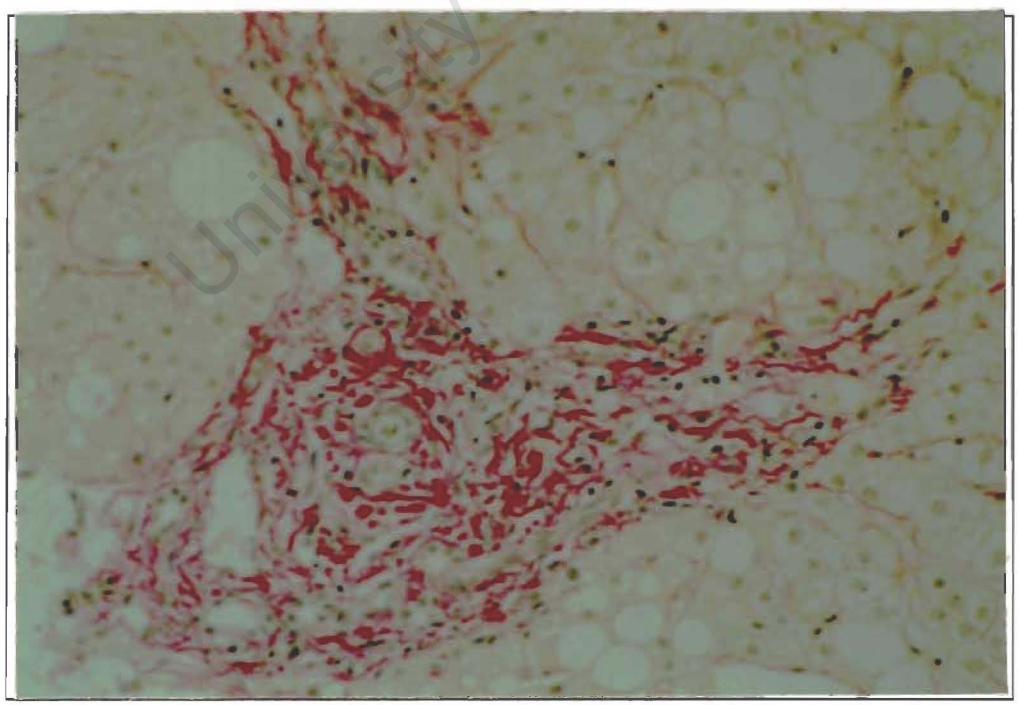


Fig 1(d). MTX-induced hepatotoxicity. Fibrous septa originating from the portal tract extend into the lobule. (Original magnification X 200; Sirius red.)

1.2.2. Risk factors

In order to avoid the occurrence of serious hepatic injury it is important in the selection of patients for MTX therapy to identify risk factors that might aggravate the injury. Indeed, an important aim of many of the studies investigating the relationship between MTX and histologic hepatic abnormalities is to identify the contribution of these risk factors to the liver injury. A large meta-analysis has associated alcohol and total cumulative dose with a significant risk for liver toxicity (15). Pre-existing liver disease, renal insufficiency, obesity and diabetes are other factors associated with an increased risk of MTX hepatotoxicity (16).

1.2.3. Grading

A uniformly accepted grading system is essential for clinical interpretation and comparison of different studies. In 1972 Roenigk and co-workers, as part of the Psoriasis Task Force of the National Program of Dermatology USA, developed a histological classification based on liver biopsy findings (17). A connective tissue stain to assess the degree of fibrosis is an essential requirement. Despite subsequent modifications (Table 3) (18, 19), this grading scheme remains subjective and insensitive to small histological changes.

Table 3. Roenigk grading system (19)

Grade I	Normal; mild fatty infiltration, mild nuclear variability, mild portal inflammation
Grade II	Moderate to severe fatty infiltration, moderate to severe nuclear variability, moderate to severe portal tract expansion, portal tract inflammation and necrosis
Grade IIIA	Fibrosis, mild (portal fibrosis here denotes formation of fibrous septa extending into the lobules; slight enlargement of the portal tracts without disruption of limiting plates or septum formation does not classify the biopsy specimen as grade III.)
Grade IIIB	Fibrosis, moderate to severe
Grade IV	Cirrhosis (regenerating nodules as well as bridging of portal tracts must be demonstrated.)

Other classifications have been used in an attempt to improve the sensitivity (20,21) while video image analysis has been performed to improve subjectivity (21, 22).

1.2.4. Pathogenesis

Whereas the effect of large dose daily MTX and subsequent side-effects in the therapy of malignancies is related to the drugs' antiproliferative action, the pathogenesis of chronic MTX hepatotoxicity in psoriasis is poorly understood. A genetic predisposition, related to the frequency of HLA A1 and B8 antigens in patients who developed MTX induced cirrhosis (23) and the accumulation of MTX and its polyglutamate metabolites in hepatocytes and other tissues (24), have been suggested by some as possible mechanisms. These hypotheses have not been supported in other studies (16). Hepatic stellate cells, vitamin A containing lipocytes, are the major source of excess collagen in cirrhosis (25). When stimulated, they acquire a myofibroblastic phenotype capable of secreting collagen and extracellular matrix (26). An increase in the number and size of hepatic stellate cells has been found in liver biopsies after MTX therapy, supporting a role for these cells in MTX induced fibrosis (27).

Many argue whether low dose MTX is a hepatotoxin at all. Several publications have shown pre-existing liver disease in patients with both psoriasis and rheumatoid arthritis. Rau et al. (28) described pre-treatment Roenigk grade IIIA changes in liver biopsy specimens in 9 of 60 (15%) patients with rheumatoid arthritis while Themido et al. (29) showed 20 of 63 (32%) patients with psoriasis with grade III pre-treatment biopsies. Furthermore studies comparing pre- and post-MTX liver biopsies found 6% to 24% of biopsies showed histological improvement during MTX therapy (16). Kaplan, commenting on the role of MTX in the treatment of primary sclerosing cholangitis and primary biliary cirrhosis, believes that the hepatotoxicity of MTX is greatly exaggerated (30, 31). That a putative hepatotoxic drug such as MTX should be effective in treating patients with serious liver diseases was an unexpected finding in the study group he examined (30). Much of this controversy would be settled if there were a suitable animal model. A rat model exists for MTX hepatotoxicity, however the liver injury was only produced on daily oral therapy and the histology lacked some of the classically described features (32).

1.2.5. Role of the liver biopsy

The liver biopsy remains the gold standard for the detection of MTX induced hepatotoxicity (16). A non-invasive test would greatly simplify the management of patients on long term MTX therapy, but no investigation has the sensitivity and specificity of the liver biopsy in detecting incipient fibrosis. The lack of correlation between abnormalities in hepatic enzyme levels and liver fibrosis is well recognised (16) while radiological and imaging techniques are similarly unhelpful (16). Recently measurement of serum levels of type III procollagen (P3NP) has attracted much interest (33). Levels have been shown to correlate with the presence and degree of hepatic fibrosis, however it is not liver specific and raised levels have been reported in other conditions where there is accumulation and degradation of collagen (33). Although there is unlikely to be significant fibrosis with consistently normal P3NP levels, insufficient evidence has been provided to substantiate obtaining liver biopsies at less frequent intervals than those recommended by the Psoriasis Task Force (19).

Controversies regard the risk of development of liver fibrosis (34) and the recommendations regarding the performing of liver biopsies as a means to monitor therapy (12). The Psoriasis Task Force advises a baseline liver biopsy within 2 to 4 months of commencement of therapy in those with risk factors. In patients with no risk factors for liver disease, the first biopsy is done at approximately 1,0 to 1,5 gm of cumulative MTX (Table 4).

Studies have shown that the risk of development or progression of liver disease in patients receiving "low-dose", once-weekly oral MTX for psoriasis is small (20, 35). Even though the risk of complications (0.06-0.32%) and mortality (0.009%) is extremely low (36), it may be difficult to justify repeated biopsies. This view is certainly echoed by many physicians who do not follow the guidelines set forth regarding liver biopsy recommendations in patients on MTX therapy. Petrazzuoli et al. found that 66% of gastroenterologists in Connecticut and Massachusetts, USA, who responded to a questionnaire did not perform routine liver biopsies (37) while Peckham et al. showed in a national survey from the USA that 50% of MTX-treated patients had never undergone a liver biopsy (38).

Table 4. Recommendations for liver biopsy in patients with or without risk factors for liver disease (19).

LIVER BIOPSY	APPROXIMATE CUMULATIVE MTX DOSES (GM)
In patients without risk factors	
First	1.0 – 1.5
Repeat	3.0
Repeat	4.0
In patients with risk factors	
First	2 – 4 months of therapy
Repeat	1.0 – 1.5
Next repeat	3.0
Following repeat	4.0

Studies have shown that the risk of development or progression of liver disease in patients receiving "low-dose", once-weekly oral MTX for psoriasis is small (20, 35). Even though the risk of complications (0.06-0.32%) and mortality (0.009%) is extremely low (36), it may be difficult to justify repeated biopsies. This view is certainly echoed by many physicians who do not follow the guidelines set forth regarding liver biopsy recommendations in patients on MTX therapy. Petrazzuoli et al. found that 66% of gastroenterologists in Connecticut and Massachusetts, USA, who responded to a questionnaire did not perform routine liver biopsies (37) while Peckham et al. showed in a national survey from the USA that 50% of MTX-treated patients had never undergone a liver biopsy (38).

1.2.6. Rheumatoid arthritis and methotrexate

When rheumatologists from the USA, followed by other parts of the world, started to use MTX for rheumatoid arthritis in the early 1980's, they followed the recommendations set forth by the American Association of Dermatology (39). It soon became apparent that patients with rheumatoid arthritis were less susceptible to MTX-induced heptotoxicity than those with psoriasis (39). The reasons for this are unclear but it may be associated with a lower dose schedule (16) or fewer risk factors in those with rheumatoid arthritis selected for therapy (37). Indeed, there is a well established

link between psoriasis and high alcohol intake (40). Conflicting results exist with respect to the inherent susceptibility of psoriatic patients to MTX hepatotoxicity compared to patients with rheumatoid arthritis. Lanse et al. showed that when risk factors for hepatotoxicity were avoided, there was no difference in the liver injury in patients with rheumatoid arthritis or psoriasis (41). Whiting-O'Keefe et al. reported in a meta-analysis involving 636 patients that those with rheumatoid arthritis were less likely than those with psoriasis to develop hepatic fibrosis, even when one controls for total cumulative dose (15). Based on these observations, the American College of Rheumatology excluded either baseline or surveillance liver biopsies in their 1994 guidelines (42). The exceptions were prior excessive alcohol consumption, persistent abnormal baseline serum aminotransferase values or evidence of chronic hepatitis B or C infection (42).

1.3. NONALCOHOLIC STEATOHEPATITIS

Ludwig first introduced nonalcoholic steatohepatitis (NASH) in 1980 to describe liver disease in obese diabetic women (43). More recently it has been accepted that this entity occurs in other clinical settings that are all characterised by a morphological pattern of liver injury resembling alcoholic hepatitis (Table 5).

Table 5. Factors associated with nonalcoholic steatohepatitis (44)

Metabolic	Obesity
	Diabetes mellitus and hyperglycaemia
	Hyperlipidaemia
	Rapid weight loss
	Acute starvation
	Total parental nutrition
Surgical Procedures	Jejunal bypass
	Intestinal resection
Drug Therapy	Amiodarone
	Perhexilene maleate
	Glucocorticoids
	Synthetic oestrogens
	Tamoxifen
Miscellaneous	Small bowel diverticulosis with bacterial overgrowth
	Partial lipodystrophy

1.3.1. Histology

The diagnosis of NASH is based on histological features similar to those seen in alcoholic hepatitis but in the absence of a history of "significant" alcohol consumption (43) (Fig 2 a - b). Recent studies have accepted macrovesicular steatosis with parenchymal inflammation only as sufficient criteria for NASH (45). As emphasised by Lee stricter criteria are required for the diagnosis of NASH (46) and in addition to steatosis and a mixed inflammatory cell infiltrate there should be hepatocyte ballooning degeneration, with or without Mallory hyaline bodies, or fibrosis (44). The hepatic fibrosis is typically pericellular in a zone 3 (perivenular) distribution in the early lesion with subsequent development of fibrous septa (44).

Recently it has been accepted that a number of other patterns of liver injury are seen in alcoholic liver disease, including non-specific steatohepatitis without a neutrophil polymorph infiltrate and balloning degeneration of hepatocytes, and that this nonspecific pattern of liver injury can precede the development of alcoholic cirrhosis in both animal models and humans (46a). Much of the confusion regarding the criteria for the diagnosis of NASH might be explained by the existence of a similar spectrum of injury in 'non-drinkers' ranging from steatosis, through steatosis with lobular inflammation (non-specific steatohepatitis) to the classic histological features of NASH as described above.

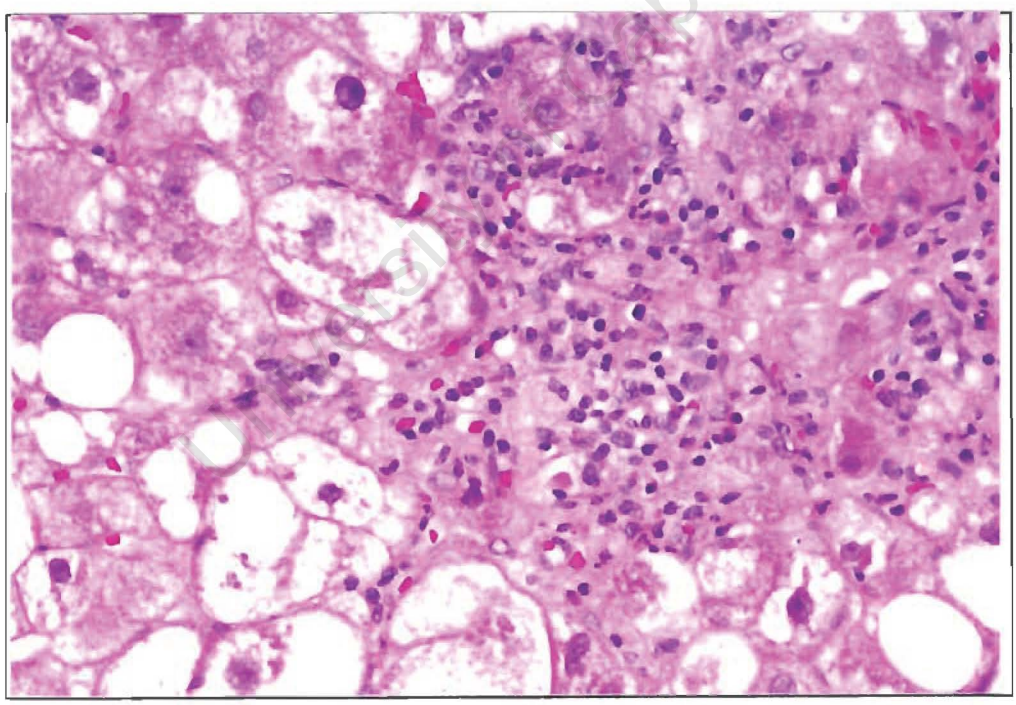


Fig 2(a). NASH. Steatosis with a mixed inflammatory cell infiltrate and ballooning degeneration. (Original magnification X 400; H & E.)

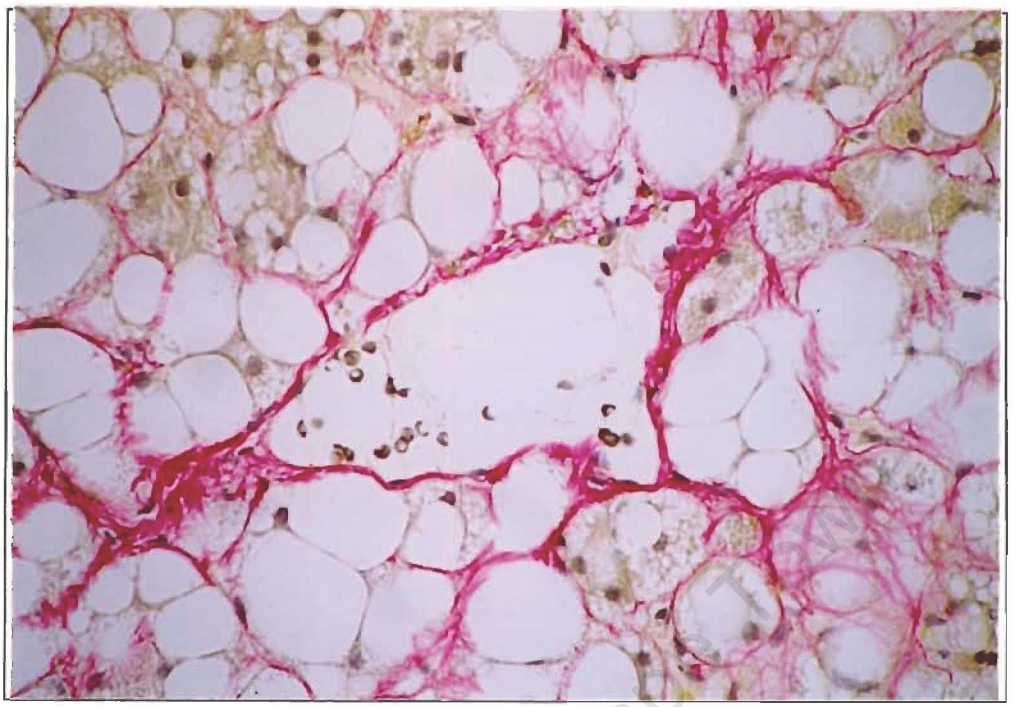


Fig 2(b). NASH. Steatosis with prominent pericellular fibrosis. (Original magnification X 200; Sirius red.)

1.3.2. Pathogenesis

Although the course of events in the development of NASH is unclear, the final pathway, like alcohol, is thought to be oxidative stress. The subsequent generation of oxygen free radicals is capable of causing damage to mitochondria, protein and DNA as well as resulting in peroxidation of the polyunsaturated fatty acid side-chains of membrane phospholipids (47).

Steatosis occurs more frequently than steatohepatitis. It is present in up to 80% of patients with diabetes mellitus (48) and 76% with obesity (49). The prevalence of NASH in the general population has not been defined in the USA (44) nor has it been studied in South Africa. Wanless and Lentz, however, found 18,5% of markedly obese patients in a large autopsy study from Toronto Canada had steatohepatitis (50). Although this indicates that fat is not causal in the development of steatohepatitis, it is

not entirely innocent and it is probably the first insult in a progressive disease culminating in inflammation and eventually scarring (51).

The increase in oxidative stress, which occurs when reactive oxygen species (ROS) are produced faster than they can be removed by the cellular defence mechanisms, is derived from induction of the P450 cytochrome 2E1 (CYP2E1) system, a switch towards peroxisomal beta-oxidation and endotoxin mediated cytokine release (47). Weltman et al. showed that, as in alcoholic steatohepatitis (ASH), there is induction of CYP2E1 in livers with NASH compared to normal controls (52). In addition the distribution of CYP2E1 was similar to ASH with increased zone 3 (perivenular) intensity and more extensive acinar distribution. Cytokines, especially tumour necrosis factor-alpha (TNFα), are important mediators of multiple types of liver injury, including alcoholic hepatitis and NASH (53, 54). In steatohepatitis the release of cytokines is mediated through increased serum levels of endotoxins caused by increased gut permeability (53) with hepatocyte injury triggered by the production of free radicals (55). Finally Burt et al. (47) suggested that, in NASH, excess fatty acids might saturate mitochondrial beta-oxidation with a switch to peroxisomal beta-oxidation and the subsequent generation of hydroxyl radicals. A defect in the function of the peroxisomes has also been entertained (56).

CHAPTER 2

HYPOTHESIS AND AIMS

2.1. HYPOTHESIS: METHOTREXATE INDUCED HEPATOTOXICITY IS ASSOCIATED WITH NONALCOHOLIC STEATOHEPATITIS

Not only are the important clinical entities associated with NASH also the risk factors for MTX hepatotoxicity, but the histological features of these two forms of liver injury are similar. This suggests that similar pathogenetic mechanisms may be contributing to the liver injury in psoriatics on long-term MTX therapy and/or that pre-existing NASH/NASH-like liver injury, possible aggravated by MTX, may be the main cause of the liver damage. No study to date has investigated the incidence of NASH in cohorts of patients on long term MTX for psoriasis. An association between MTX-induced liver damage and NASH/NASH-like liver injury may not only help redefine the criteria for liver biopsy but could also open new avenues of investigation into the pathogenesis of MTX hepatotoxicity and ultimately new treatment modalities.

2.2. AIMS

The aims of this study are:

1. To correlate clinical parameters with the histological assessment of serial liver biopsies in a cohort of patients on long term MTX therapy for psoriasis.
2. To investigate the association between MTX-induced hepatotoxicity and a NASH-like pattern of liver injury.
3. To identify factors involved in the pathogenesis of MTX hepatotoxicity.

CHAPTER 3

PATIENTS AND METHODS

3.1. CLINICAL FEATURES AND MTX SCHEDULE

The surgical pathology files as well as the dermatology records at Groote Schuur Hospital, Cape Town were searched for psoriatic patients receiving "low-dose", once-weekly oral MTX therapy. Only patients who had undergone two or more liver biopsies were selected and 24 such cases, 12 male and 12 female, were identified. When more than two biopsies were available only the first and last were reviewed. The clinical records were scrutinised for details regarding the age, sex, MTX schedule as well as risk factors for liver injury that included alcohol, diabetes mellitus and obesity (Table 6). The cumulative dose was determined by the summation of the weekly dose from the commencement of therapy to the last biopsy. In addition, the cumulative dose before the first biopsy was also calculated. In two cases (5 and 19) the first biopsy was too small for assessment while in another two cases (14 and 21) there was no connective tissue stain or available tissue in the wax block to perform extra stains. In these cases, all of which had more than 2 biopsies, the second in the series was examined and the cumulative dose before the first biopsy was adjusted accordingly. The duration of therapy represented the time, in months, during which the patient received MTX and not simply the interval from the start of treatment to the end of the study. The lowest MTX dose, excluding that used for the commencement of therapy, and the highest dose received were documented as was the dose that was most frequently used to achieve the desired clinical response. The body mass index (BMI) was calculated by dividing the weight in kilograms by the square of the height in meters and obesity was defined when the value was equal to or greater than 30.

Table 6. Clinical features and MTX schedule

NO	AGE	SEX	TOTAL MTX DOSE (MG)	CUMULATIVE DOSE MTX-1ST BIOPSY (MG)	DURATION (MONTHS)	WEEKLY DOSE *	RISK FACTORS	BMI
1	65	M	7405	4145	72	15-30(22.5)	DM	28
2	46	F	4925	1475	51	25-30(25)	DM, obese	42
3	54	M	378	128	6	20-25(20)	DM, obese	31
4	58	M	4135	1345	44	20-25(22.5)		24
5	55	M	22750	15600	210	25	obese	36
6	61	M	8870	3780	108	10-25(20)		26
7	51	F	5945	0	66	10-25(20)	obese	31
8	47	M	2315	0	29	12.5-25(20)		19
9	50	M	12765	6750	177	15-25(20)		20
10	51	F	6020	1480	70	20	DM, obese	29
11	51	F	2032	280	41	7.5-15(10)	obese	30
12	48	M	3535	1705	46	17.5-20(20)	alcohol	22
13	50	F	3650	1300	39	25		27
14	49	F	3270	1500	60	10-15(10)		23
15	65	F	6620	1620	94	20-25(22.5)	alcohol	28
16	61	M	4025	1245	32	25-40(30)	DM, obese	42
17	56	F	1685	0	41	5-15(10)		24
18	38	M	2245	180	27	10-20(15)	obese	30
19	28	F	3052	1280	38	15-25(20)	obese	35
20	49	F	4910	130	53	15-25(20)		28
21	49	M	8330	3150	69	15-30(25)		25
22	61	M	5000	1435	121	7.5-20(12.5)	diabetes	27
23	52	F	4260	0	53	15-20 (17.5)	obese	36
24	52	F	3945	450	62	15	obese	35

BMI, body mass index; DM, diabetes
* most commonly used dose in parenthesis

3.2. LIVER PATHOLOGY

Haematoxylin and eosin stained sections of liver tissue were examined for steatosis, lobular and portal tract inflammation, hepatocyte necrosis and nuclear variability. A sirius red stain for collagen was assessed for the presence of pericellular and perivenular fibrosis as well as expansion of the portal tracts by fibrous tissue. Two pathologists (GL, PH), blinded to the patient's name, clinical history and prior biopsy results, examined each biopsy. In addition, one pathologist (PH) was uninformed of the sequence of the biopsies. The findings were graded according to the Roenigk classification as previously described (page 6) with progression being defined as an increase of one grade between the "initial" and last liver biopsy. In addition, a semiquantitative assessment based on the recently proposed grading and staging system for NASH was applied (57). The histological features analysed and the numerical score attributed to each are as follows (Table 7).

1. Macrovesicular steatosis: The percentage of hepatocytes containing fat was graded from 0 to 4 (0 is no steatosis; 1 is less than 25%; 2 is between 25 and 50%; 3 is between 50 and 75%; and 4 is greater than 75%).

2. Lobular inflammation: Characterised by the presence of single small clusters of mononuclear cells, often accompanied by necrotic hepatocytes, lobular inflammation was graded from 0 to 3 based on the number of such foci and the size of the inflammatory infiltrate (0 is no inflammation; 1 is occasional small foci; 2 is mild to moderate numbers of foci, the majority of which are small; and 3 is frequent inflammatory foci, many of which are large).

3. Fibrosis: A sirius red stain was evaluated for the pattern and severity of collagen, taking into account the typical distribution encountered in NASH (0 is no fibrosis; 1 is zone 3 pericellular and perivenular fibrosis present focally or extensively; 2 is zone 3 pericellular and perivenular fibrosis together with focal or extensive periportal fibrosis; 3 is as described for 2 but with any form of bridging fibrosis; and 4 is cirrhosis).

As a result of the omission of polymorphs as a requirement of the lobular inflammatory infiltrate, the histological features selected for this study do not satisfy the current strict criteria for NASH. Instead a non-specific steatohepatitis or a NASH-like pattern of injury has been chosen for the purpose of this study. Hepatocellular ballooning was not encountered with any degree of frequency to warrant the inclusion of this feature in the scoring system. Furthermore, when enlarged hepatocytes were encountered it frequently proved difficult to separate the hydropic change from microvesicular steatosis in haematoxylin and eosin stained sections. Only those biopsies for which a score of 1 or more was attributed for each of the three variables examined were diagnosed as having a NASH-like pattern of injury. Progression was deemed to have occurred when there was an increase in fibrosis, steatosis or necroinflammation by a score of 1.

Table 7. Histological criteria and corresponding numerical value used.

GRADE/ STAGE	MACROVESICULAR STEATOSIS	LOBULAR INFLAMMATION	FIBROSIS
	% hepatocytes containing fat	Clusters of mononuclear cells, often accompanied by necrosis	Pattern and severity of collagen deposition
0	No steatosis	No lobular inflammation	No fibrosis
1	< 25%	Occasional small foci	Zone 3 pericellular and perivenular fibrosis, focal or extensive
2	25 – 50%	Mild to moderate numbers of foci, the majority small	Grade 1 and focal or extensive periportal fibrosis
3	50 – 75%	Frequent inflammatory foci, the majority large	Grade 2 with bridging fibrosis
4	> 75%		Cirrhosis

CHAPTER 4

RESULTS

Table 8 summarises the results of the Roenigk and semiquantitative grading and staging score of the 24 patients who met the criteria for inclusion in this study. The age of the patients ranged from 28 to 65 years with a mean of 52 years. The total cumulative dose of MTX administered ranged from 378mg to 22750mg with a mean of 5502mg. The mean duration of therapy was five years and seven months with a range of six months to 17 years and six months. MTX was stopped in one patient (case 3), an obese diabetic, after six months and a cumulative dose of 378mg. The risk of further liver injury in this case was thought to outweigh the benefits of the therapy. Nine patients had no risk factors for liver injury, eleven had one and four had two risk factors.

Twenty-one of the 24 patients showed progressive liver injury when the biopsies were scored using the grading and staging method. The mean cumulative dose of MTX/patient in this group was 5813mg compared to 3330mg for the three patients that showed no progression (Fig 3).

Seventeen of the 24 patients had a NASH-like pattern of liver injury, 15 of who showed a progression in the semiquantitative grading and staging score. One of the two patients (case 13) who showed no progression had no risk factors. The other patient (case 16) was an obese 61-year old male diabetic who showed bridging fibrosis in both biopsies. Although the fibrosis was more pronounced in the second biopsy, it did not fulfil the criteria for cirrhosis and therefore could not be reflected in either scoring system (Fig 4 a - d). Thirteen of the 17 patients who had a NASH-like pattern of injury also satisfied the clinical criteria. Seven were obese, two were diabetic and four were obese and diabetic. Four patients, three males and one female, had a NASH-like pattern of injury without any risk factors for liver injury (Fig 5).

Table 8. Results of the Roenigk and grading and staging score.

NO	TOTAL MTX DOSE (MG)	RISK FACTORS	BX. NO	FAT	NEC	FIBR	NASH	PROG	ROENIGK GRADE
1	7405	DM	a b	1 2	1 1	2 2	Y Y	Y	IIIA IIIA
2	4925	Diabetes Obese	a b	3 4	1 2	2 3	Y Y	Y	IIIA IIIB
3	378	Diabetes Obese	a b	1 1	0 1	1 2	N Y	Y	I I
4	4135	-	a b	0 0	0 0	0 1	N N	Y	I I
5	22750	Obese	a b	1 1	2 1	1 2	Y Y	Y	I I
6	8870	-	a b	1 2	2 2	1 1	Y Y	Y	II II
7	5945	Obese	a b	0 1	0 1	1 2	N Y	Y	I IIIA
8	2315	-	a b	0 0	0 0	0 0	N N	N	I I
9	12765	-	a b	1 1	0 1	0 1	N Y	Y	II II
10	6020	Diabetes Obese	a b	0 1	0 1	1 2	N Y	Y	I IIIB
11	2032	Obese	a b	1 1	1 2	0 1	N Y	Y	II II
12	3535	Alcohol	a b	0 0	0 0	1 2	N N	Y	II II
13	3650	-	a b	2 2	2 1	2 2	Y Y	N	IIIA IIIA
14	3270	-	a b	0 0	0 0	1 2	N N	Y	I II
15	6620	Alcohol	a b	0 0	1 0	1 2	N N	Y	II IIIA
16	4025	Diabetes Obese	a b	4 4	2 2	3 3	Y Y	N	IIIB IIIB
17	1685	-	a b	0 1	0 0	0 1	N N	Y	I I
18	2245	Obese	a b	4 4	1 1	1 2	Y Y	Y	II IIIA
19	3052	Obese	a b	1 1	1 1	1 2	Y Y	Y	II IIIA
20	4910	-	a b	0 1	0 0	2 2	N N	Y	IIIA IIIA
21	8330	-	a b	0 1	1 1	1 2	N Y	Y	II IIIA
22	5000	Diabetes	a b	0 1	0 1	0 2	N Y	Y	I II
23	4260	Obese	a b	2 2	1 2	1 1	Y Y	Y	II II
24	3945	Obese	a b	3 4	2 2	2 2	Y Y	Y	IIIA IIIA

BX, biopsy; NEC, necrosis; PROG, progression.

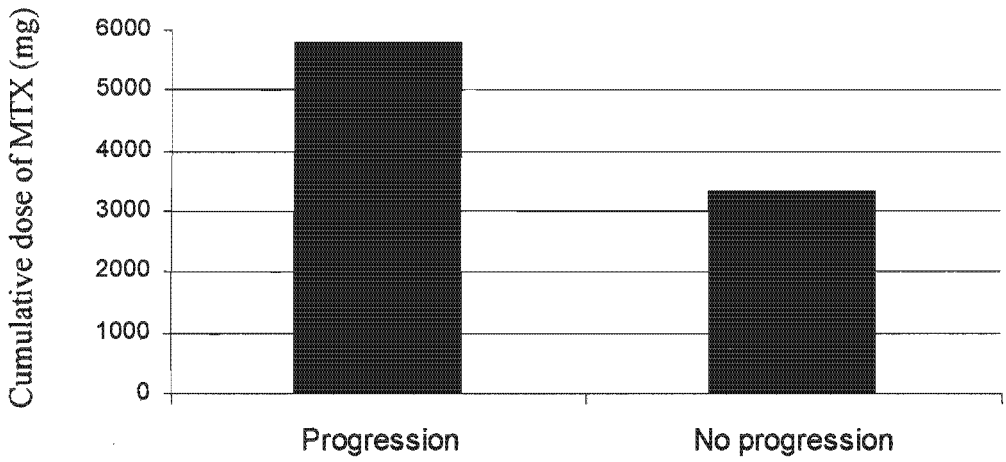


Fig 3. Mean cumulative dose of MTX for patients with and without progression as scored using the grading and staging method.

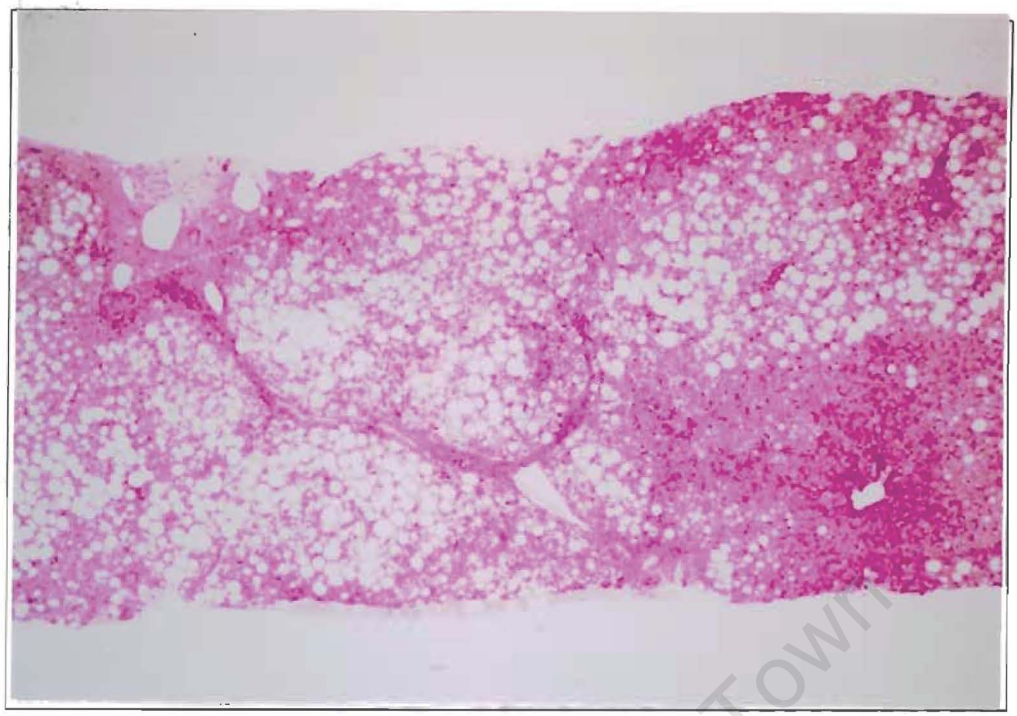


Fig 4(a). Case 16. Photomicrograph from the first liver biopsy shows extensive steatosis and fibrosis. (Original magnification X 40; H & E.)

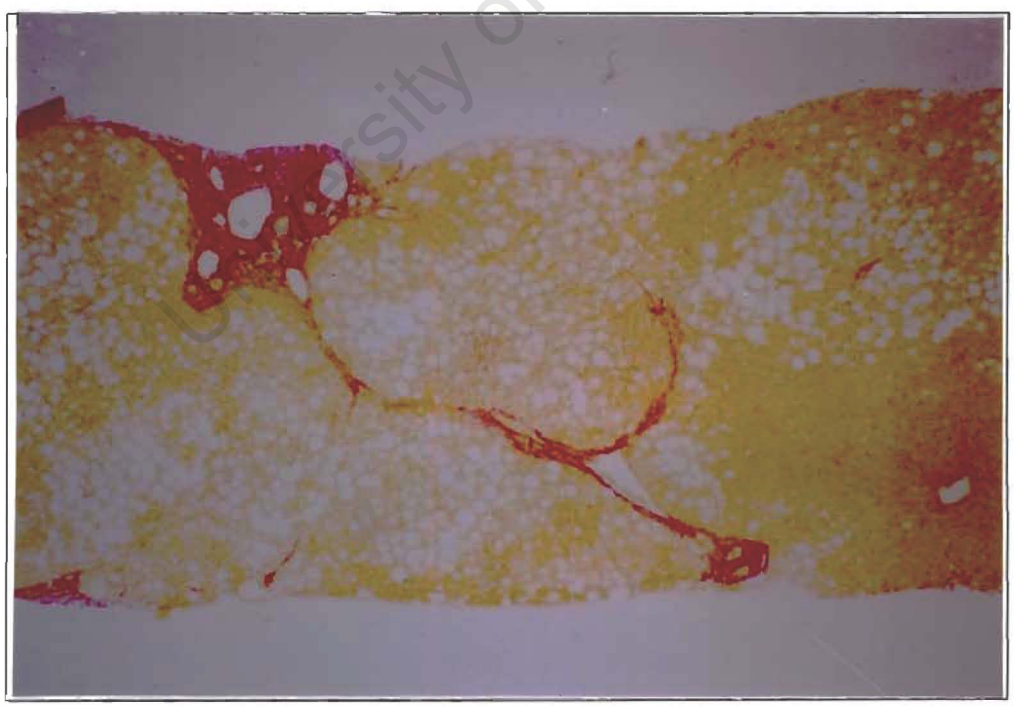


Fig 4(b). Case 16. Connective tissue stain of the same biopsy above showing bridging fibrosis. (Original magnification X 40; sirius red.)

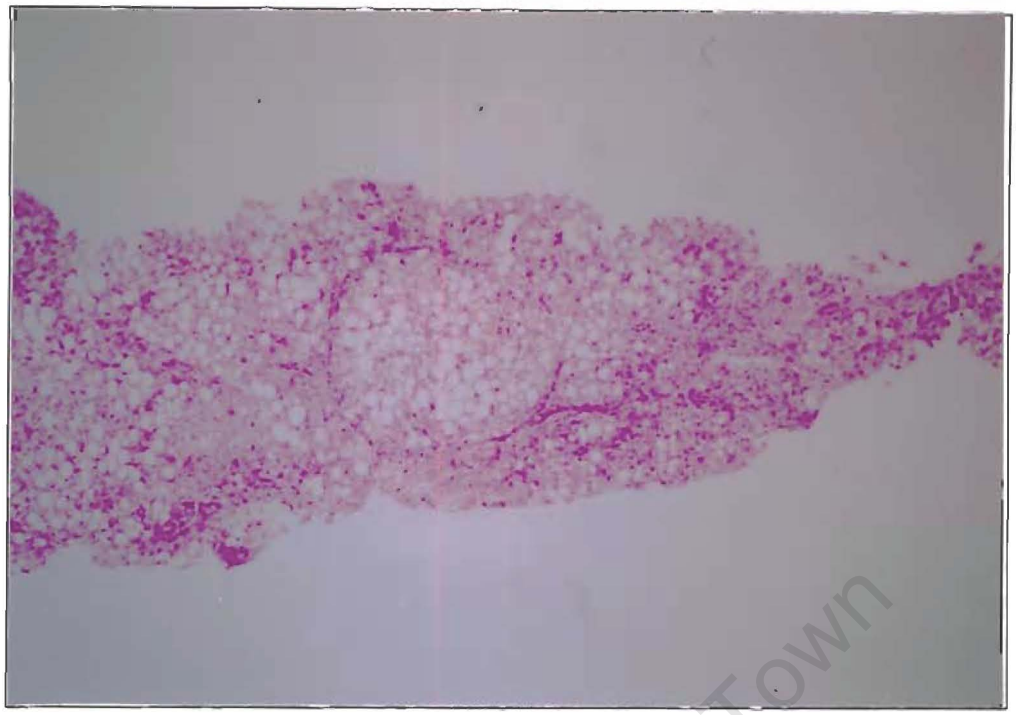


Fig 4 (c). Case 16. The second biopsy also shows extensive steatosis and fibrosis. (Original magnification X 40; H&E.)

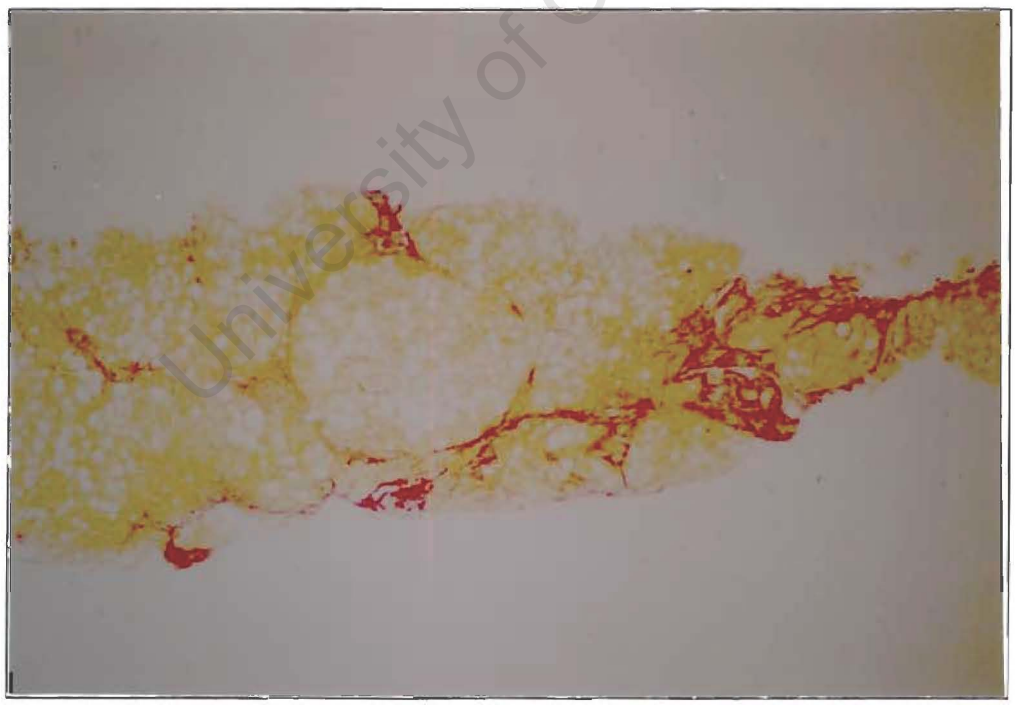
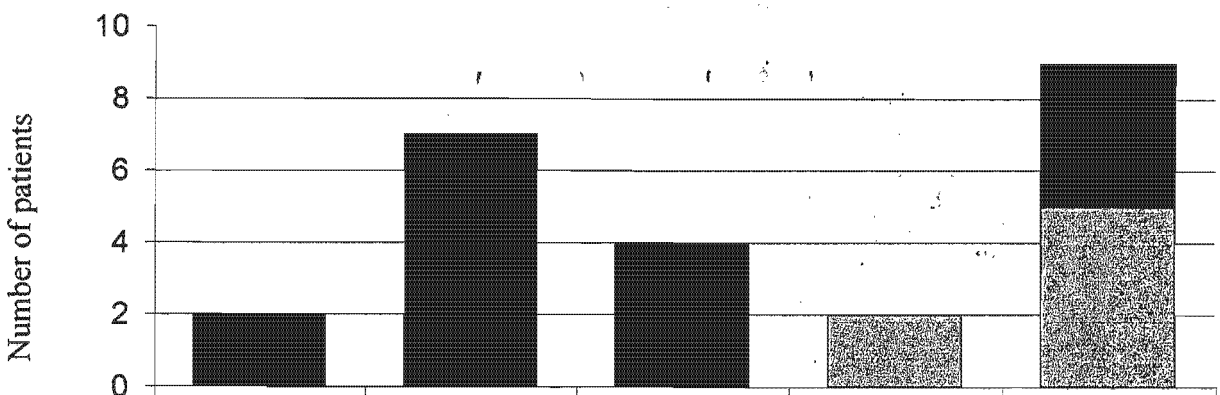


Fig 4 (d). Case 16. The connective tissue stain of the second biopsy shows progressive fibrosis. A small regenerative nodule is apparent, but there were insufficient features in the rest of the biopsy to justify a diagnosis of cirrhosis. (Original magnification X 40; Sirius red.)



	Diabetes mellitus	Obese	DM & obese	Alcohol *	No risk Factors
SH	2	7	4	0	4
No SH	0	0	0	2	5

Risk factors for liver injury

Fig 5. Distribution of risk factors between patients with and without a NASH-like pattern of liver injury (* past history of alcohol abuse; SH, steatohepatitis; DM, diabetes mellitus).

Of the seven patients who did not have a NASH-like pattern of liver injury, five had no risk factors while the other two had abused alcohol in the past. Both of the latter had significant fibrosis but no steatosis or necrosis suggesting that they had not been drinking at the time of the biopsy. Case 20 had stage 2 fibrosis after a cumulative dose of 130mg of MTX that did not progress despite a further 4780mg. Three patients had stage 1 or 2 fibrosis (cases 4, 14, 17) and, except for mild steatosis in case 17, there was no evidence of necroinflammatory activity.

Two of the four patients without risk factors had a NASH-like pattern of injury on the initial biopsy taken after 3780mg (case 6) and 1300mg (case 13). Neither, however, showed an increase in fibrosis despite a further 5090mg and 2350mg of MTX respectively. The mean total cumulative dose of MTX in these four patients, as assessed from the first biopsy showing a NASH-like picture, was 6544mg. This was

higher than the 3800mg that represented the mean cumulative dose of MTX of the seven patients who did not have a NASH-like pattern of liver injury (Fig 6).

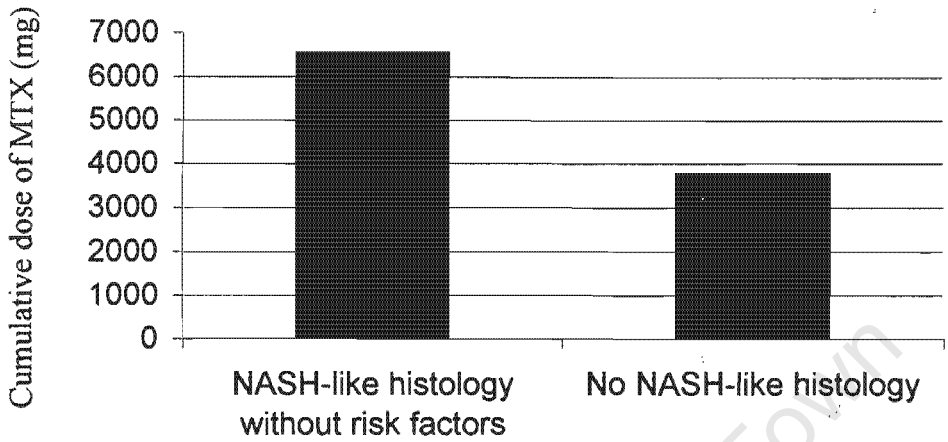


Fig 6. Mean cumulative dose of MTX for patients with a NASH-like pattern of injury and no risk factors for liver injury and patients without a NASH-like picture.

Ten patients showed progressive liver injury when the Roenigk grade of the two biopsies were compared while in 14 patients there was no change. No patient showed a reduction in the histological score. The mean cumulative MTX dose for those that progressed was 4894mg. This was lower than the 5938mg that represented the mean for the 14 patients who did not deteriorate (Fig 7). Although eight of the 10 patients who progressed had risk factors for liver injury, so did seven of the 14 in whom there was no change in the Roenigk grade.

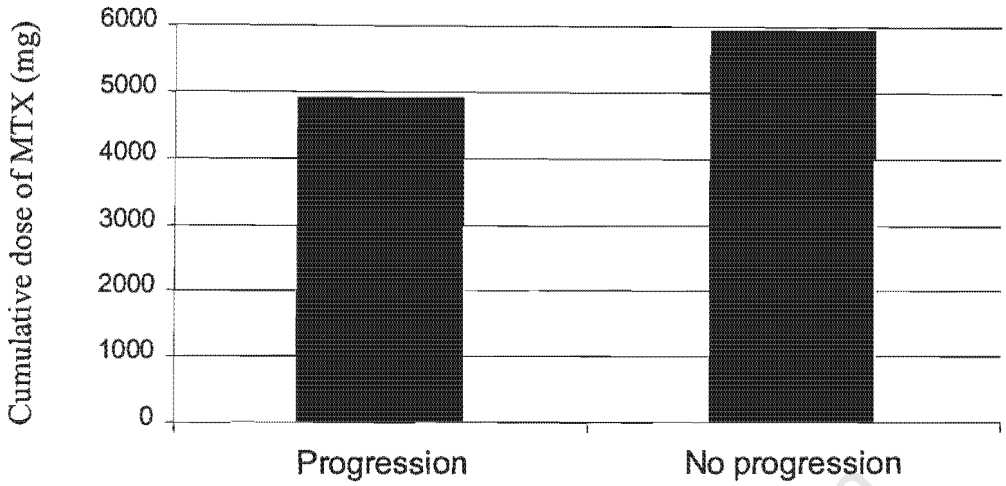


Fig 7. Mean cumulative dose of MTX for patients with and without progression as scored by the Roenigk classification.

CHAPTER 5

DISCUSSION

5.1. THE HISTOLOGICAL CLASSIFICATION SYSTEM

A uniformly accepted grading system is essential for clinical interpretation and comparison of different studies of MTX related liver injury. In 1972 Roenigk and co-workers developed a histological classification based on liver biopsy findings (17). Despite subsequent modification (18,19), this grading scheme remains subjective and insensitive to small histological changes, especially in the interpretation of the severity of fibrosis, and other classifications have been used in an attempt to improve the sensitivity (20,21,22). The liver fibrosis in this study was quantified using a microcomputer image analysis system (results not shown). The inability to distinguish portal tract fibrosis from the normal variation in portal tract size and the variation in the intensity of the sirius red stain, despite staining all the slides in one batch, compromised the measurement of the collagen. For these reasons this method was not used further.

We were unable to show an association between liver injury and a higher cumulative MTX dose when the biopsies were graded by the Roenigk scoring system, an observation that has been made by others (20, 35, 58). The 10 patients who showed a progressive liver injury when scored by the Roenigk method had a mean cumulative MTX dose of 4894mg. This was lower than the mean of the 14 patients who showed no change in the histological score (5938mg). In addition, seven of the 15 patients with risk factors were in the latter group. Perivenular and pericellular fibrosis are not accounted for in the Roenigk classification and in the present study it was the major reason why so many patients with risk factors did not appear to show progression. Nevertheless many of the studies investigating the relationship between hepatotoxicity, risk factors and long term MTX therapy for psoriasis have used the Roenigk classification to assess liver injury.

When a needle biopsy is used to evaluate an organ as large as the liver, the possibility of sampling error needs to be considered. It is generally accepted that most hepatic diseases affect the organ homogeneously and this has been shown to be the case for steatosis and alcoholic hepatitis (59). Nevertheless, when assessing and comparing early or mild cases of steatohepatitis and MTX-induced liver injury, it is reasonable to assume that there may be some sampling variability. This, together with the intra- and interobserver variability that is inherent to any semiquantitative scoring system, may result in certain irregularities. That this is occurring in this study cannot be refuted, however it is unlikely to affect the global outcome and general conclusions.

No non-invasive test can be used reliably to monitor progressive liver fibrosis and the liver biopsy remains the "gold standard" for monitoring patients on long term MTX therapy (16). An objective and sensitive system is therefore essential to detect subtle changes in the liver injury. Such information is useful not only in monitoring the patient, but also for assessing the role of potential risk factors or biochemical markers of hepatotoxicity in cohorts of patients. In common with chronic hepatitis (viral, drug or autoimmune) and NASH, MTX-induced liver injury can be evaluated by grade of necroinflammatory activity and the stage of fibrosis. The grade and stage of disease reflects different aspects of the liver injury and a final score derived from adding these categories together, as previously described for MTX-induced hepatotoxicity (21), may not be an accurate reflection of the severity and progression of the disease. While staging, which reflects irreversible structural damage, may be regarded as a more serious marker of liver injury, histological grade or activity provides a measure of severity at the time of the biopsy (60). The two patients in the study who abused alcohol both had grade IIIA lesions on the Roenigk classification and while this gives some indication of the stage of the injury, it does not reflect the activity. The lack of steatosis and necroinflammation in these patients indicates abstinence from alcohol at the time of the biopsy. A system that incorporates both the grade and stage is a more accurate assessment of the injury and is in keeping with current trends in scoring necroinflammatory lesions of the liver (61). Twenty-one of the 24 patients showed progressive liver injury when scored by the method incorporating grade and stage. The mean cumulative MTX dose of this group was higher than that of the 3 patients who showed no worsening of their liver injury (5813mg and 3330mg respectively). In 16 of the 21 patients the progression was due to an increase in fibrosis while the

remaining five had an increase in either steatosis or necroinflammation; there was no difference in the mean cumulative MTX dose of these two groups (5793mg and 5878mg respectively). Two of the three patients who did not progress had no risk factors for liver injury. The third patient (case 16) had two risk factors and is discussed in more detail below. That a strong correlation between cumulative MTX dose and progression was obtained in this study when the biopsies were interpreted for grade and stage compared to the Roenigk system, supports the use of such a classification in assessing MTX-induced liver injury.

5.2. THE INTERACTION BETWEEN NASH AND MTX

Not only does long-term MTX therapy cause a liver injury that resembles NASH, but the risk factors are similar. To test the hypothesis that NASH contributes to liver injury in psoriatic patients on MTX, the liver biopsies were scored with a grading and staging system for a NASH- like pattern of liver injury. Seventeen of the 24 patients in this study satisfied these histological NASH-like criteria. In 13 the clinical criteria were also met, and all except one showed progressive liver injury. The patient who failed to show objective progression (case 16) had bridging fibrosis in both biopsies. Although there was definite deterioration with respect to the amount of fibrosis, the liver was not cirrhotic and as such this subjective impression could not be reflected in either scoring system. Therefore MTX appears to contribute to the progressive liver injury in those patients with the risk factors for NASH and the histological features of a NASH-like pattern of liver injury. This interaction has also been demonstrated experimentally. Neuman et al. exposed cells to MTX in the presence of either ethanol or acetaminophen, known inducers of cytochrome P450 2E1 (62). Increased cytotoxicity was observed which was secondary to an increase in oxidative stress in MTX-exposed cells by raised levels of TNFα and depletion of both cytosolic and mitochondrial fractions of GSH (glutathione) (62). The other 4 patients had a NASH-like pattern of injury but no other known risk factors for liver injury suggesting that MTX alone causes or is associated with NASH-like histology.

Seven patients, two of whom had risk factors for NASH, did not satisfy the NASH-like histological criteria used in this study. This group had a lower mean cumulative dose of MTX (3780mg) when compared to the four patients who had histological features of a NASH-like pattern of injury but without any known risk factors for liver injury (6544mg). Although this suggests that, with a large enough cumulative dose, MTX contributes to a NASH-like picture, other factors may be contributing. Case 13, for example, had a NASH-like pattern of injury after 1300mg of MTX and did not show deterioration despite a further 2350mg. There were no risk factors for liver injury nor was there any history of weight fluctuations between the biopsies. That other unknown or undisclosed risk factors are playing a role in this case, to explain the development of steatohepatitis after a cumulative dose of MTX lower than that of the group that did not develop a NASH-like pattern of liver injury, cannot be

excluded. Six of the seven patients without steatohepatitis showed fibrosis, the pattern of which was still similar to those that had steatohepatitis. The two patients who abused alcohol both had a significant increase in fibrosis when the serial biopsies were compared, but neither had inflammation or fatty change. As such they do not qualify for the diagnosis of steatohepatitis although it is likely that episodes of steatohepatitis occurred prior to the first biopsy and/or in the interval between the biopsies to account for the typical pattern of scarring. Three patients showed progressive fibrosis but no evidence of steatohepatitis. Whether this is via a direct effect, possibly through the activation of hepatic stellate cells as previously suggested (63), or secondary to transient steatohepatitis due to an alcohol binge, fluctuation in weight between biopsies or additional hepatotoxic medication, is not known.

5.3. PATHOGENETIC LINK BETWEEN NASH AND MTX-INDUCED HEPATOTOXICITY

The histological similarities between ASH and NASH have encouraged researchers to explore a common pathogenetic link. Indeed, Weltman et al. has shown that hepatic CYP 2E1 is induced in both conditions (52). Several lines of evidence suggests a role for endotoxin-mediated cytokine release in alcoholic hepatitis and NASH (53,54). Furthermore, the final pathway resulting in the generation of reactive oxygen species and lipid peroxidation is also similar (47). Currently the pathogenesis of NASH is still unclear but a clearer picture is emerging involving an interaction between steatosis, oxidative stress and endotoxin mediated cytokine release (Fig 8). Is there experimental evidence of a similar link between NASH and MTX-induced hepatotoxicity?

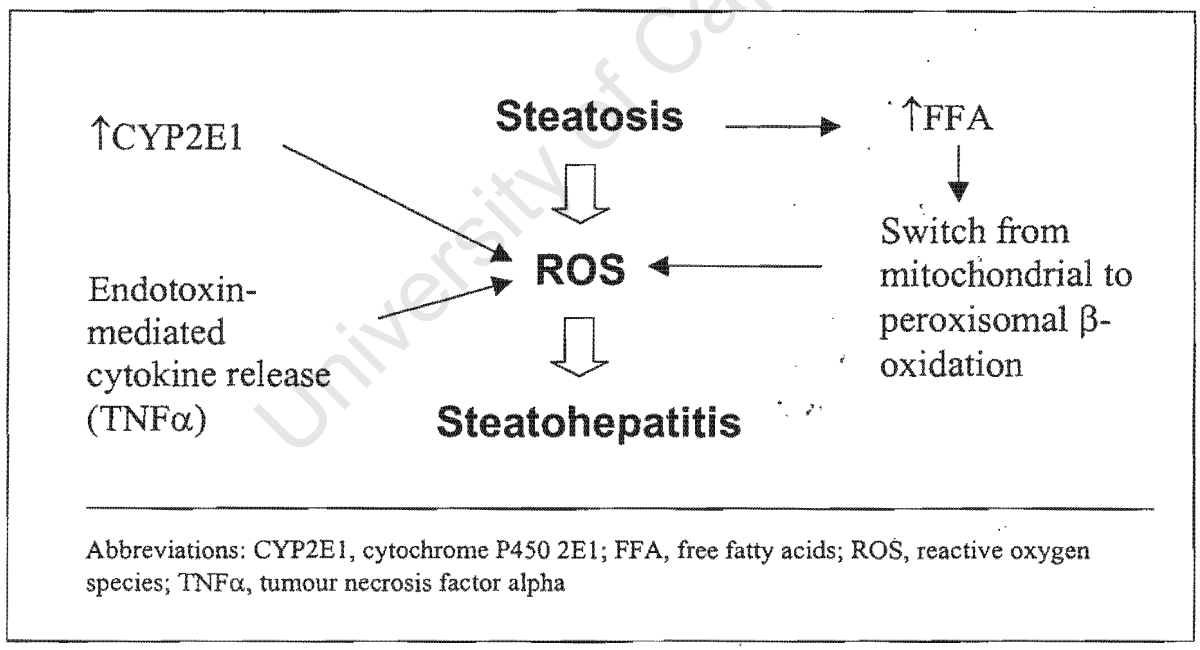


Fig 8. The interaction between steatosis and oxidative stress in the pathogenesis of NASH. The saturation of mitochondrial β-oxidation by excess fatty acids induces a switch towards peroxisomal β-oxidation with the subsequent generation of hydroxyl radicals.

Early experiments performed on perfused isolated rat livers have shown that MTX exerts a marked effect on the metabolism of lipids (64). Alterations at many points in the complex lipid pathway could lead to the accumulation of triacylglycerol within hepatocytes and thus steatosis. Aarsaether et al. found a slight rise in the hepatic lipids and a moderate reduction in the serum level when MTX was administered to rats (65). The associated increase in palmitoyl-CoA synthetase activity and microsomal glycerophosphate acyltransferase activity in the liver was consistent with failure to transfer the newly formed triacylglycerols into the plasma. They also demonstrated that the fatty liver of MTX-exposed rats could not be attributed simply to a reduction of fatty acid oxidation as the carnitine palmitoyltransferase activity, an enzyme involved in the transport of long chain fatty acids into the mitochondria, was increased. Perhexiline maleate and amiodarone, two therapeutic agents that have been associated with NASH, are known to inhibit mitochondrial beta-oxidation (66). Unlike these drugs, it would appear that MTX results in steatosis by a mechanism other than the inhibition of mitochondrial beta-oxidation. In summary, the development of fatty change in the liver of patients treated with "low dose", long term MTX is uncertain, but experimental evidence suggests that there may be impairment in the secretion of triacylglycerol. Whether this is due to a deficiency of apoproteins or other factors affecting the synthesis of very-low-density lipoprotein (VLDL) or the result of cytoskeleton abnormalities affecting the exocytosis of VLDL is unknown. Changes in the cytoskeleton morphology have been observed in MTX-treated cells, but the concentration is higher than that used for psoriasis (67).

Cheung et al. found no effect of MTX on the total catalytic activity of cytochrome P450 isozymes or the mRNA content of the CYP3A2 and 2C11 isozyme in the liver of male rats (68). Although the effect on hepatic CYP 2E1, a key cytochrome increased in patients with both ASH (69) and NASH (52) was not examined, a significant role of this or any other isozyme is unlikely in the face of an unchanged total cytochrome P450 catalytic activity. No effect on the peroxisomal beta-oxidation activity however has been found when MTX is administered to normal fed rats (70). Glutathione peroxidase is one of the body's principal means of protection against oxidative damage (71). It catalyzes the reduction of hydrogen peroxide and lipid peroxides by GSH, the sulfhydryl groups of which serve as electron donors. MTX has

been shown to decrease the cellular levels of GSH which would lead to a reduction in the effectiveness of the antioxidant enzyme defence system (72).

As alluded to earlier, the release of cytokines, important mediators of liver injury in NASH, is mediated through increased gut permeability and subsequent endotoxaemia (53). Stomatitis and gastrointestinal ulceration, which may lead to portal endotoxemia, are uncommon side effects when MTX is used for the treatment of psoriasis (12). Yadav et al. has however shown that the exposure of peripheral blood mononuclear cells of normal individuals to MTX augmented spontaneous TNFα production (73). A summary of the proposed pathogenetic mechanisms, based on the above experimental evidence, whereby MTX causes a NASH-like pattern of injury, is illustrated in figure 9.

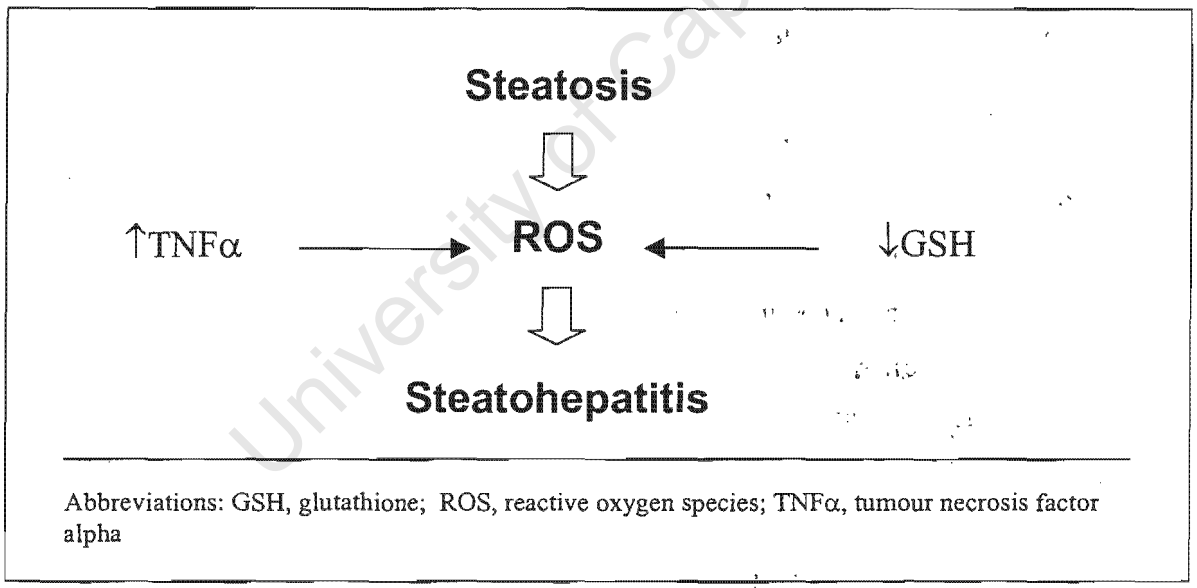


Fig 9. Proposed pathogenesis of MTX-induced liver injury.

5.4. THE MISSING LINK

Even though there are pathogenetic mechanisms to support the histological similarities between NASH and MTX-induced hepatotoxicity, many questions remain unanswered. Why is MTX, a hepatotoxic drug, effective in treating patients with potentially fatal liver diseases like primary sclerosing cholangitis and primary biliary cirrhosis (30, 31)? Why is there a difference in the frequency of hepatotoxicity in MTX-treated psoriasis patients compared to patients with rheumatoid arthritis (74)? Whiting-O'Keefe et al. raised the possibility that some other unknown factor could account for the difference (15). Studies have shown that over 50% of patients with psoriasis have abnormal baseline liver biopsies, mainly steatosis and nonspecific inflammation, prior to MTX therapy and fibrosis was also documented, even in patients without additional risk factors (16). Case 20 in our study had pericellular and perivenular fibrosis together with expansion of the portal tracts in the absence of any known risk factors for liver injury. That this was present in the first biopsy taken after only 130mg of MTX is in keeping with these studies and suggests that an additional factor may be contributing to the liver injury in patients treated with MTX. No one has yet investigated the possibility that psoriasis per se could be dictating the pattern of liver injury. Is psoriasis the missing link?

The role of TNFα in psoriasis is well known (75). Its importance has been emphasised by a recent report showing a dramatic decrease in the clinical activity of psoriatic lesions when treated with monoclonal antibodies against TNFα (76). Endotoxaemia has been demonstrated in 6 of 11 psoriatic patients tested by Belew et al. (77). The presence of oedema, granularity and rarely friability in 60%, and microscopic changes in 100% of colonic biopsies from psoriatics without bowel symptoms, (78) suggests that, as in ASH and NASH, endotoxaemia may be due to an increase in gut permeability. In addition, increased lipid peroxidation and decreased antioxidants have been found in the blood of psoriatics compared to normal controls (79). Rather than playing a primary role in psoriasis, it is probably secondary to the generation of reactive oxygen species by the chronic inflammatory process.

By the generation of oxidative stress, either through endotoxin mediated cytokine release or a reduction in antioxidants, psoriasis and presumably the activity thereof

may contribute to the liver injury associated with MTX (Fig 10). This could partly explain the development of steatohepatitis in those patients without known risk factors for liver injury and the lower risk of significant liver disease when "low dose" MTX treatment is used for rheumatoid arthritis.

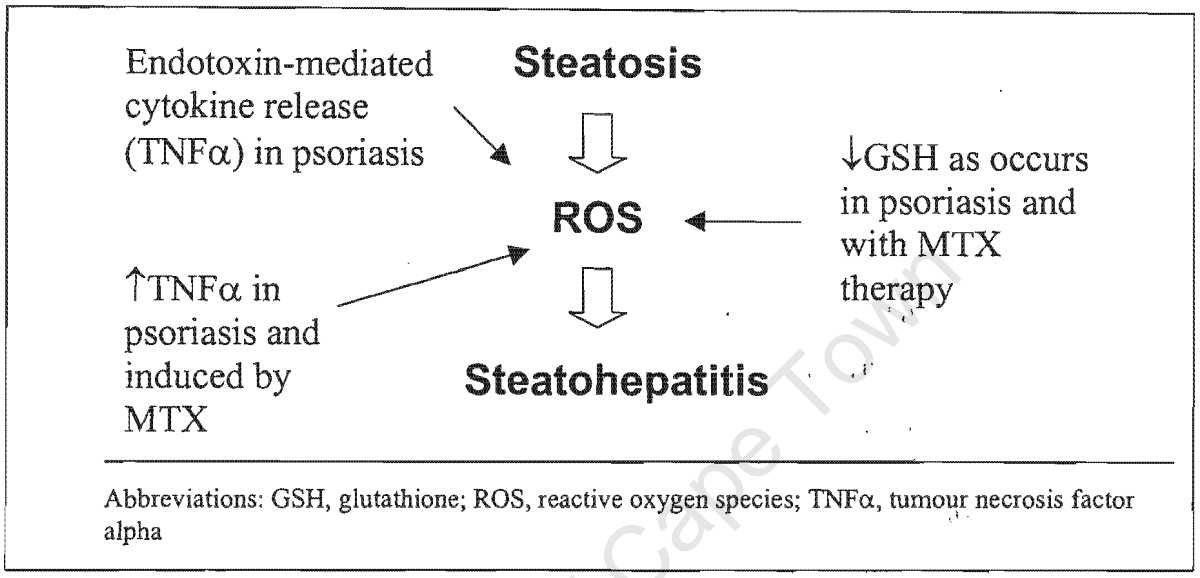


Fig 10. Proposed interplay between MTX and psoriasis in the pathogenesis of the NASH-like pattern of injury. The reduction in GSH and increase in TNFα has been demonstrated both during MTX therapy as well as in psoriasis.

5.5. NEW GUIDELINES FOR LIVER BIOPSIES

The interaction between pre-existing NASH and MTX therapy suggests the need for pre-MTX liver biopsy in patients with risk factors for NASH as well as frequent assessment of liver histology if MTX is commenced.

In this study MTX, without risk factors for liver injury, was associated a NASH-like pattern of liver injury only after a mean cumulative dose of 6544mg. This does not necessarily mean that the high dose of MTX was the only factor contributing to liver injury, but rather suggests that when there are no apparent risk factors for liver injury, that the initial liver biopsy can be delayed until after a large cumulative dose of MTX.

Proposed indications for liver biopsies in patients on MTX therapy for psoriasis are outlined in table 9.

Table 9. Proposed recommendations for liver biopsies in patients on MTX therapy for psoriasis.

LIVER BIOPSY	INDICATION
Pre-treatment	Abnormal liver function tests Alcohol Risk factors for NASH – obesity, diabetes mellitus
Follow-up	Persistent abnormal liver enzymes Alcohol Risk factors for NASH A previous biopsy showing mild liver injury – MTX continued
After treatment started	No risk factors – biopsy after a total of 6gm

5.6. PROPOSALS FOR FUTURE STUDIES

1. A similar study investigating the relationship between NASH and MTX therapy for rheumatoid arthritis would be interesting. A lower rate of progression in patients with pre-existing NASH and the absence of a NASH-like pattern of injury in patients without associated risk factors for liver injury would strengthen the role of psoriasis in the pathogenesis of MTX-induced hepatotoxicity.

2. The overlapping pathogenetic mechanism between MTX-hepatotoxicity and a NASH-like pattern of liver injury paves the way for studies investigating these pathways as well as exploring treatment modalities currently used for NASH. Strategies for patients 'at-risk" for NASH are directed at reducing the severity of steatosis and avoiding or removing the triggers of necroinflammation (51). Ursodeoxycholic acid, the seven-beta epimer of chenodeoxycholic acid (80), which stabilises cells membranes and protects against cytotoxic insults (81), has been shown to significantly reduce liver enzymes and hepatic steatosis in the treatment of patients with NASH (82). It has been suggested that antibiotic therapy may be of value in NASH by reducing portal endotoxaemia (51). Although this was the case in patients treated with antibiotics following jejuno-ileal bypass surgery for obesity (83), a recent study has shown that antibiotic therapy exacerbated fibrosis in the alcohol/carbon tetrachloride rat model (84). Apart from weight reduction and glucose control, specific treatment modalities applicable to MTX therapy for psoriasis include ursodeoxycholic acid, antioxidants, inhibitors of TNFα and possibly antibiotics.

CHAPTER 6

CONCLUSION

In this study we reviewed the clinical details and liver biopsies of 24 patients on long term, "low dose" MTX therapy for psoriasis to determine whether NASH contributes to the prevalence, progression and pathogenesis of MTX-induced liver injury. In summary, the following conclusions can be drawn.

1. *Improved scoring system.*

Scoring the liver biopsies for NASH-like criteria, rather than the Roenigk method, shows improved correlation between cumulative dose, risk factors and severity of injury. In addition incorporating the grade and stage in the scoring system is a more accurate reflection of the liver injury.

2. *Interaction between MTX-induced liver toxicity and NASH.*

MTX contributes to progressive liver injury in those patients with the risk factors for NASH and the histological features of a NASH-like pattern of liver injury. Furthermore MTX alone can cause a NASH-like pattern of injury which is at least partly due to a higher cumulative dose.

3. *Psoriasis and NASH.*

Psoriasis, via endotoxin-cytokine mediated cell damage and a reduction in antioxidants, may be a risk factor for NASH.

4. *"New" guidelines for liver biopsies.*

New guidelines are proposed which reflect the contribution of NASH to the liver injury associated with MTX.

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