H.13 Methotrexate and monitoring for hepatotoxicity

H.13.1 Liver enzyme tests

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>M. Newman, R. Auerbach, H. Feiner, R. S. Holzman, J. Shupack, P. Migdal, M. Culubret, P. Camuto, and H. Tobias. The role of liver biopsies in psoriatic patients receiving long-term methotrexate treatment. Improvement in liver abnormalities after cessation of treatment. Arch.Dermatol. 125 (9):1218-</td>
<td>Observational: Case series and within-group comparison

Retrospective

• Patient selection: New York University Hospital and office records; attempted to identify all those undergoing biopsy 1968-1986
• Index tests: LFTs – within 3 days of reference standard; unclear method of selection of threshold (may have been post-hoc)
• Reference standard: biopsy classification according Roenigk grading (grade IIIA-IV =</td>
<td>N: 168

Total of 364 biopsies (85 before treatment)

49% had biopsies before starting methotrexate (279/346 biopsies were from those with at least 1 months</td>
<td>Inclusion criteria: Patients who have diagnosed psoriasis unresponsive to previous treatment; liver biopsy before and/or during therapy with MTX

Exclusion criteria: none stated

<table>
<tr><th>Parameter</th><th>All (n=168)</th></tr>
<tr><td>Mean age (at biopsy) – years</td><td>47.7</td></tr>
<tr><td>Gender M/F (%)</td><td>52/48</td></tr>
<tr><td>Pre-MTX biopsy (%)</td><td>49%</td></tr>
<tr><td>Median monthly MTX dose before biopsy (range)</td><td>67.3 (7.5-205.6) mg</td></tr>
<tr><td>Duration of treatment (median)</td><td>48 months</td></tr>
</table>
</td>
<td>Liver function tests:
Alanine aminotransferase
Aspartate aminotransferase
Bilirubin
Alkaline phosphatase
Prothrombin time
Albumin</td>
<td>Histological techniques:

Biopsy by Menghini technique; fixed and embedded in paraffin; staining with H&E, reticulin and trichrome stains

Diagnosis made by blinded assessor according to Roenigk grading:

Grade 1: normal tissue, no/mild fatty change, no/mild nuclear pleomorphism, no fibrosis, mild portal inflammation

Grade 2: moderate/severe fatty</td>
<td>Honors Research Program of New York State University School of Medicine; partial funding from Lederle Laboratories</td>
</tr>
</table>

1224, 1989. Ref ID: NEWMAN1989	abnormal); blinded to clinical data Reviewed by 3 pathologists (experience unclear); inter-reader agreement periodically checked (questionable specimens reviewed by all and consensus made) • **Flow and timing:** Max 3 days between tests (index test second); unclear if all patients included in the analysis/if all received LFTs because raw data/2x2 table not available	MTX)	<table><tr><td>IBD or gallbladder disease</td><td>0</td></tr><tr><td>History of high alcohol intake</td><td>14 (8 of whom received MTX)</td></tr><tr><td>Diabetes</td><td>16</td></tr><tr><td>Obese</td><td>67 (40%)</td></tr></table> **Methotrexate schedule:** Most received oral administration in either a single weekly or a divided weekly dose MTX treatment stopped when biopsy specimen was grade IIIB or greater		changes, moderate/severe nuclear pleomorphism, no fibrosis, moderate/severe portal inflammation Grade 3a: mild fibrosis, portal fibrotic, septa, extending in the lobuli, portal tract enlargement Grade 3b: moderate/severe fibrosis Grade 4: cirrhosis, regenerating noduli and bridging of the portal tracts	

Effect Size

Outcomes

Pre-test probability/prevalence

17/83 patients had abnormal biopsy before taking MTX

2/31 patients with biopsy before and after MTX had abnormal biopsy at both time points

Pre-test probability not available for group with biopsy after MTX and not before

Note: raw data not available (based on both those taking methotrexate and those who had biopsy before methotrexate, but unclear if analysed on a per patient basis – taking the most severe biopsy sample for each patient – or if multiple biopsies per patient were included)

Test	Abnormal range	Sensitivity	Specificity	Predictive value	
				Positive test	Negative test
Alanine aminotransferase	≥40 U/L	0.05 (0.006-0.17)	0.85 (0.72-0.94)	0.22 (0.03-0.48)	0.52 (0.40-0.63)
Aspartate aminotransferase	≥40 U/L	0.20 (0.13-0.30)	0.90 (0.84-0.93)	0.49 (0.33-0.65)	0.70 (0.62-0.76)
Bilirubin	≥2 µmol/l	0.19 (0.12-0.29)	0.86 (0.80-0.90)	0.41 (0.26-0.57)	0.60 (0.63-0.75)
Alkaline phosphatase	≥100 U/L	0.38 (0.28-0.49)	0.71 (0.63-0.77)	0.39 (0.28-0.49)	0.70 (0.63-0.77)
Prothrombin time	≥14.5 s	0.01 (0.00-0.05)	0.99 (0.94-0.99)	0.25 (0.06-0.80)	0.66 (0.61-0.72)
Albumin	≥35 g/l	0.19 (0.11-0.29)	0.76 (0.68-0.83)	0.33 (0.19-0.48)	0.61 (0.52-0.68)

Author's conclusion

- No single test was a good predictor of liver damage; although the specificity for prothrombin time was high, the prevalence of abnormal values was such that the predictive values were low for both a positive and negative test

Reference	Study type	Number of patients	Patient characteristics	Index test	Reference standard	Outcome measures	Source of funding
G. T. O'Connor, E. M. Olmstead, K. Zug, R. D. Baughman, J. R. Beck, J. L. Dunn, P. Seal, and J. F. Lewandows	**Observational:** Retrospective case series and within-group comparison • **Patient selection:** Patients who had undergone MTX treatment for psoriasis at Dartmouth-Hitchcock Medical Centre, USA between 1972-1986 (unclear if included all/consecutive sample)	N: 78 (147 biopsies; 52 before and 95 after treatment)	**Inclusion criteria:** Psoriasis patients who had undergone biopsy associated with MTX therapy **Exclusion criteria:** Not stated	**Liver function tests:** total bilirubin, aminotransferase, alkaline phosphatase Abnormal levels in effect	**Liver biopsy** Graded by the Roenigk classification by blinded assessor **Histological techniques:**	Hepatotoxicity by biopsy and LFTs -sensitivity, specificity, PPV and NPV	None stated

ki. Detection of hepatotoxicity associated with methotrexate therapy for psoriasis. Arch.Dermatol. 125 (9):1209-1217, 1989. Ref ID: OCONNOR 1989	• **Index tests: LFTs –** obtained during week prior to reference standard; obtained using standard methods during regular clinical care threshold selection based on normal ranges that were in effect at the time the test was performed (note that this changes during the study period for AST and ALP) • **Reference standard:** biopsy classification according Roenigk grading (grade IIIA-IV = abnormal); single pathologist blinded to clinical data **Note:** the specimens were obtained during regular care but re-graded for this analysis (biopsy sample method unclear) • **Flow and timing:** Max 1 week between tests (index test first); not all biopsies were included in the analysis (2 of the pre-Tx and 9 of the post-Tx biopsies were excluded because complete laboratory and demographic data were not available Excluded from analysis if from patient with abnormal pre-Tx biopsy or if incomplete data set	Analysis restricted to 50 before and 86 after Tx with full lab and Tx data	No baseline data presented **Methotrexate**: Dosing schedules not stated	at the time of assessment	Biopsy by Menghini technique; staining with H&E, and Masson trichrome		

Effect Size

Outcomes

Pre-test probability: 28/147 (19%)

Biopsy grade	**Overall**	**Pre-treatment**	**Post-treatment**
I	96	41	55
II	23	6	17
IIIa	20	3	17
IIIb	7	1	6
IV	1	1	0
Total 'abnormal'	**28**	**5**	**23**

Note: 2 and 9 from the pre- and post-treatment groups respectively were not included in the analysis due to incomplete data sets (unclear what biopsy grade they had). For the 2 pre-treatment biopsies, laboratory and/or demographic data were not available. For the 9 post-treatment biopsies complete laboratory and treatment data were not available and/or there were abnormal findings before treatment

Pre-treatment test accuracy

Test	**Biopsy specimens *before* treatment (n=50)**					
	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR+**	**LR-**

AST	0.40 (0.05-0.85)	0.89 (0.76-0.96)	0.29 (0.04-0.71)	0.93 (0.81-0.99)	3.76 [0.97,15]	0.67 [0.33,1.38]
ALP	0.40 (0.05-0.85)	0.77 (0.60-0.87)	0.15 (0.02-0.45)	0.92 (0.83-0.97)	1.71 [0.52,5.63]	0.78 [0.38,1.63]
TB	0.20 (0.07-0.72)	0.96 (0.85-0.99)	0.33 (0.01-0.91)	0.91 (0.80-0.98)	4.7 [0.51,43]	0.84 [0.54,1.30]
AST or ALP	0.60 (0.15-0.95)	0.71 (0.56-0.84)	0.19 (0.04-0.46)	0.94 (0.80-0.99)		
AST, ALP or total bilirubin	0.60 (0.15-0.95)	0.71 (0.56-0.84)	0.19 (0.04-0.46)	0.94 (0.80-0.99)		

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	**TP:** AST = 2 ALP = 2 TB = 1	**FP:** AST = 5 ALP = 11 TB = 2
Index test -ve	**FN:** AST = 3 ALP = 3 TB = 4	**TN:** AST = 42 ALP = 36 TB = 45

Post-treatment test accuracy

Test	Biopsy specimens *after* treatment (n=86)					
	Sensitivity	**Specificity**	**PPV**	**NPV**	**LR+**	**LR-**
AST	0.43 (0.22-0.66)	0.86 (0.75-0.93)	0.50 (0.26-0.74)	0.82 (0.71-0.91)	3.13 [1.49,6.56]	0.66 [0.45,0.95]
ALP	0.57 (0.34-0.78)	0.72 (0.60-0.83)	0.40 (0.23-0.59)	0.84 (0.72-0.92)	2.03 [1.21,3.41]	0.6 [0.37,0.98]
TB	0.10 (0.02-0.30)	0.95 (0.87-0.99)	0.40 (0.05-0.85)	0.76 (0.65-0.85)	1.57 [0.31,8.00]	0.97 [0.84,1.11]
AST or ALP	0.81 (0.58-0.95)	0.60 (0.47-0.72)	0.40 (0.25-0.56)	0.91 (0.78-0.95)		
AST, ALP or total bilirubin	0.86 (0.64-0.97)	0.58 (0.46-0.71)	0.40 (0.26-0.56)	0.93 (0.80-0.98)		

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	**TP:** AST = 10 ALP = 13 TB = 2	**FP:** AST = 10 ALP = 20 TB = 4
Index test -ve	**FN:** AST = 13 ALP = 10	**TN:** AST = 62 ALP = 52

	TB = 21	TB = 68

Test	Association of abnormal LFT and biopsy specimen grade III or IV					
	Crude analysis			Adjusted analysis (age and history of cholecystitis)		
	OR	X^2	p-value	OR	X^2	p-value
AST	4.7	7.98	0.005	14.7	12.83	<0.001
ALP	3.5	5.99	0.014	2.1	1.58	0.209
TB	2.2	0.69	0.406	5.1	2.38	0.123
AST or ALP	6.4	10.50	0.001	5.5	6.78	0.009
AST, ALP or total bilirubin	8.4	12.27	<0.001	14.7	8.00	0.005

Author's conclusion

- The benefit of performing liver biopsies serially is markedly reduced in the absence of clinical suspicion of hepatotoxicity and/or abnormal liver function test results

Reference	Study type	Number of	Patient characteristics	Index test	Reference standard	Source

<table>
<tr><td></td><td></td><td>patients</td><td></td><td></td><td></td><td>of

funding</td></tr>
<tr><td>M. S. Ho, E. Cheah, S. N. Tham, L. B. Teh, H. S. Ng, and S. Thirumoorthy. Liver biopsies from psoriatics treated with methotrexate. Ann.Acad. Med.Singapore 15 (2):210-214, 1986.

Ref ID: HO1986</td><td>Observational: Case series

Prospective

• Patient selection: Study conducted in Singapore Limited to those indicated for biopsy investigation (indication of potential liver damage according to cumulative dose or SGPT/ALT) Consecutive patients meeting the inclusion criteria
• Index tests: LFTs – time between tests unclear; unclear method of selection of threshold (may have been post-hoc)
• Reference standard: biopsy classification according Robinson grading; all specimens assessed by 2 independent pathologists with no prior knowledge of clinical or biochemical details of patients (experience unclear); biopsy sample size unclear
• Flow and timing:</td><td>N: 18</td><td>Inclusion criteria: Patients who have diagnosed psoriasis unresponsive to topical treatment and requiring systemic MTX; either minimum total dose of 1500 mg or SGPT/ALT more than twice normal values

Exclusion criteria: none stated

<table><tr><th>Parameter</th><th>All (n=18)</th></tr><tr><td>Mean age (years)</td><td>55 (39 – 77)</td></tr><tr><td>Gender M/F (%)</td><td>66.7/33.3</td></tr><tr><td>Cumulative MTX dose (mean, range)</td><td>1808 (20-3500) mg</td></tr><tr><td>Duration of treatment (mean, range)</td><td>88 months (2 weeks to 208 months)</td></tr><tr><td>History of high alcohol intake</td><td>0</td></tr></table></td><td>Liver function tests:
Alanine aminotransferase (ALT/SGPT)</td><td>Liver biopsy

Histological techniques:

Biopsy by Menghini technique; fixed and embedded in paraffin; staining with H&E, reticulin and trichrome stains, Perl’s stain and periodic acid Schiff

Diagnosis made by independently by 2 blinded assessors according to the following grading based on degree of fatty change, parenchymal cell necrosis, portal inflammation, nuclear vacuolation, periportal and septal fibrosis and cirrhosis):
1. Minimal change
2. None-specific hepatitis</td><td>None stated</td></tr>
</table>

	Unclear time between tests; all patients included in the analysis and all received LFTs		PsA: 1 (5.6%) Diabetes: 0 Obese: 0 **Methotrexate schedule:** Not stated		3. Fibrosis (septum formation) 4. Cirrhosis	

Effect Size

Outcomes

Pre-test probability/prevalence

5/18 had fibrosis or cirrhosis (27.8%)

Summary of findings

Biopsy grade	**Number of biopsies**	**Number of ALT tests (using threshold of approx 32 U/l)**		**Number of ALT tests (using threshold of approx 40 U/l)**	
		Normal	**Abnormal**	**Normal**	**Abnormal**
Normal	2	2	0	2	0

Minimal change	3	3	0	3	0
Non-specific hepatitis	8	6	2	5	1
Fibrosis	4	2	2	2	2
Cirrhosis	1	1	0	1	0

Note: failed to detect the one case of cirrhosis

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined and ALT threshold of >32

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 2	FP: 2
Index test -ve	FN: 3	TN: 11

Summary statistic	
Pre-test probability/prevalence	27.8%
Sensitivity	40 (79-71.3)%
Specificity	84.6 (72.3-96.7)%
PPV	50 (9.8-89.2)%
NPV	78.6 (67.1-89.8)%
LR +	2.60 [0.49,14]

LR-	0.71 [0.33,1.50]

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined and ALT threshold of >40

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 2	FP: 1
Index test -ve	FN: 3	TN: 12

Summary statistic	
Pre-test probability/prevalence	27.8%
Sensitivity	40 (8.0-58.9)%
Specificity	92.3 (80.0-99.6)%
PPV	66.7 (13.4-98.2)%
NPV	80.0 (69.3-86.3)%
LR+	5.20 [0.60,45]
LR-	0.65 [0.31,1.35]

Author's conclusion

- SGPT was not a good predictor of liver damage

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>P. Lenler-Petersen, H. Sogaard, K. Thestrup-Pedersen, and H. Zachariae. Galactose tolerance test and methotrexate-induced liver fibrosis and cirrhosis in patients with psoriasis. Acta Derm.Venereol. 62 (5):448-449, 1982.

Ref ID: LENLERPETERSEN 1982</td>
<td>Observational: Case series

Retrospective

• Patient selection
Setting = out-patient clinic
Limited to those known to have developed fibrosis or cirrhosis Consecutive patients meeting the inclusion criteria during 1972-1981 (investigated previous paired test results)
• Index tests: LFTs – time between tests unclear; pre-defined selection of threshold
• Reference standard: biopsy grading unclear; all specimens assessed by one of 2 pathologists (experience and blinding unclear); biopsy sample size unclear
• Flow and timing:
Unclear time between tests; all patients included in the analysis and all received LFTs</td>
<td>N: 45

151 concurrent biopsy and tests</td>
<td>Inclusion criteria: Patients who have diagnosed psoriasis and MTX-induced liver fibrosis

Exclusion criteria: none stated

<table><tr><th>Parameter</th><th>All (n=45)</th></tr><tr><td>Fibrosis</td><td>22</td></tr><tr><td>Cirrhosis</td><td>23</td></tr></table>
Methotrexate schedule:

Not stated</td>
<td>Liver function tests:
Galactose tolerance test (abnormal >3 g galactose excretion per litre of urine)

40 g galactose given orally to fasting patient and urine collecting during ensuing 8-h period</td>
<td>Liver biopsy

Histological techniques:

Biopsy by Menghini technique

Assessed by one of 2 pathologists

Tested at approximately 1-year intervals for biopsy and GTT

Unclear how biopsies were classified/categorised</td>
<td>None stated</td>
</tr>
</table>

Effect Size

Outcomes

Pre-test probability/prevalence
All had fibrosis or cirrhosis by last follow-up (100%)
At first comparative investigation 10 had fibrosis and 6 had cirrhosis (35.5%)

Summary of findings

Biopsy grade	**Number of biopsies**	**Galactose tolerance test**	
		Normal	**Abnormal**
Normal	46	43	3
Fibrosis	64	57	7
Cirrhosis	41	33	8

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 15	FP: 3
Index test -ve	FN: 90	TN: 43

Summary statistic	
Pre-test probability/prevalence	69.5%
Sensitivity	14.3 (10.2-16.4)%
Specificity	93.5 (84.1-98.3)%
PPV	83.3 (59.5-95.5)%
NPV	32.3 (29.1-34.0)%
LR +	2.19 [0.67,7.20]
LR-	0.92 [0.82,1.02]

Author's conclusion

- Oral GTT is not sensitive enough to reveal methotrexate-induced liver fibrosis or cirrhosis

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>J. Paramsothy, R. Strange, H. Sharif, M. Collins, P. Shaw, and C. M. Lawrence. The use of antipyrine clearance to measure liver damage in psoriatic patients receiving methotrexate. Br.J.Dermatol. 119 (6):761-765, 1988.

Ref ID: PARAMSOTHY1988</td>
<td>Observational: Case control study (included group not receiving MTX, but they did not receive a biopsy)

Prospective

• Patient selection
Unclear
• Index tests: LFTs – time between tests unclear; pre-defined selection of threshold based on normal ranges
• Reference standard: biopsy grading according to extent of fat, inflammatory cells per portal tract, fibrosis and liver cell necrosis; all specimens assessed blind by one pathologists (experience unclear); biopsy sample size unclear
• Flow and timing:
Unclear time between tests; all patients included in the analysis and all received LFTs</td>
<td>N: 15</td>
<td>Inclusion criteria: Patients who have diagnosed psoriasis receiving MTX

Exclusion criteria: none stated

<table>
<tr><th>Parameter</th><th>All (n=15)</th></tr>
<tr><td>% male</td><td>60%</td></tr>
<tr><td>Age, mean years</td><td>48.3 (31-64)</td></tr>
<tr><td>Cumulative MTX dose (mean)</td><td>2194 (992-4450) mg</td></tr>
<tr><td>Alcohol consumption (mean, range)</td><td>3.5 (0-16) units/week</td></tr>
<tr><td>On other medicines</td><td>53.3%</td></tr>
</table>

Methotrexate schedule:</td>
<td>Liver function tests:
Plasma concentration of albumin and bilirubin and the activities of alkaline phosphatase (AP), aspartate aminotransferase (AST) and γ-glutamyl transferase (GGT) measured with a Sequential Multiple Analyser with Computer System</td>
<td>Liver biopsy

Histological techniques:

Biopsy technique unclear

Examined and scored blind by a pathologist

Assessment of fat, inflammatory cells per portal tract, fibrosis and liver cell necrosis (each graded 0-3 depending on severity)</td>
<td>None stated</td>
</tr>
</table>

	except one for whom no GGT value was available		Not stated			

Effect Size

Outcomes

Pre-test probability/prevalence
Cases of fibrosis = 7/15 (46.7%)
Summary of findings

Patient number	**Liver biopsy score**					**Liver function tests**				
	Fatty change	**Infiltrate**	**Fibrosis**	**Necrosis**	**Total**	**Albumin (g/l)** *Ref range: 39-150*	**AP (u/l)** *Ref range: 45-120*	**AST (u/l)** *Ref range: 0-40*	**GGT (u/l)** *Ref range: 0-35*	**Bilirubin (µmol/l)** *Ref range: 0-17*
13	0	0	0	0	0	44	54	29	**77**	10
15	0	0	0	0	0	45	87	25	29	7
5	0.5	0	0	0.5	0.5	46	**151**	26	**142**	7
6	0.5	0	0	0.5	0.5	47	66	18	15	8
14	0.5	0	0	0	0.5	45	78	19	23	3
7	1	0	0	0	1	47	68	26	28	5
9	2	0	0	2	4	47	87	26	27	**19**

1	2	0.5	1	1	4.5	45	113	**44**	-	9
10	2	0	0.5	2	4.5	44	**168**	24	23	12
8	2	1	0	2	5	42	**208**	28	**162**	6
11	0	1	2	2	5	43	78	23	33	8
4	2	0.5	1	2	5.5	51	59	**57**	21	15
3	3	0	1	2	6	42	95	20	10	5
12	2	0.5	2	3	7.5	**30**	**225**	25	**85**	4
2	2	1	3	2	8	**36**	**186**	32	**38**	6

Note: no cases of cirrhosis were observed

ALB

2 x 2 table – based on definition of abnormal biopsy as any fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 2	FP: 0
Index test -ve	FN: 5	TN: 8

Summary statistic	
Pre-test probability/prevalence	46.7%
Sensitivity	29%
Specificity	100%
PPV	100 (21-100)%
NPV	62%
LR +	Infinity [0.31,101]
LR-	0.71 [0.44,1.19]

AP

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	Reference test -ve
Index test +ve	TP: 3	FP: 2
Index test -ve	FN: 4	TN: 6

Summary statistic	
Pre-test probability/prevalence	46.7%
Sensitivity	42.9 (14.1-65.6)%

Specificity	75.0 (49.9-94.9)%
PPV	60.0 (19.8-91.9)%
NPV	60.0 (39.9-75.9)%
LR +	1.71 [0.39,7.48]
LR-	0.76 [0.36,1.62]

AST

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 2	FP: 0
Index test -ve	FN: 5	TN: 8

Summary statistic	
Pre-test probability/prevalence	46.7%
Sensitivity	29%
Specificity	100%
PPV	100 (21-100)%

NPV	62%
LR +	Infinity [0.31,101]
LR-	0.71 : [0.44,1.19]

GGT

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 2	FP: 3
Index test -ve	FN: 4	TN: 5

Summary statistic	
Pre-test probability/prevalence	42.9%
Sensitivity	33.3 (6.7-65.8)%
Specificity	62.5 (42.5-86.8)%
PPV	40 (8.0-79.0)%
NPV	55.6 (37.8-77.2)%
LR +	0.89 [0.21,3.76]
LR-	1.07 [0.49,2.33]

Bilirubin

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 0	FP: 1
Index test -ve	FN: 7	TN: 7

Summary statistic	
Pre-test probability/prevalence	46.7%
Sensitivity	0%
Specificity	88%
PPV	0 (0-87)%
NPV	50 [41%,58%]
LR +	0
LR-	1.14 [0.80,1.58]

Author's conclusion

- Standard liver function tests are a poor marker for histological liver damage

H.13.2 Liver scintigraphy and ultrasound scans

Reference	Study type	Number of patients	Patient characteristics	Index test	Reference standard	Source of funding
R. G. Geronemus, R. Auerbach, and H. Tobias. Liver biopsies upsilon liver scans in methotrexate-treated patients with psoriasis. Arch.Dermatol. 118 (9):649-651, 1982. Ref ID: GERONEMUS1982	**Observational:** Retrospective case series • **Patient selection:** Psoriasis patients receiving long-term MTX therapy (unclear if included all relevant individuals/consecutive sample) • **Index tests: Liver scintigraphy: Tc 99m sulphur colloid liver scan –** Evaluated for commonly accepted pre-specified abnormalities • **Reference standard:** biopsy classification according Roenigk grading (grade IIIA-IV = abnormal); unclear if blinded, who made the classification and size of biopsy sample • **Flow and timing:** Time between tests: 23/24 pairs performed within 2	N: 24	**Inclusion criteria:** Psoriasis patients receiving long-term MTX therapy (at least 1 year) **Exclusion criteria:** concomitant diseases that might affect liver or reticuloendothelial system Baseline data: **% male**: 54% **Mean age**: 53 years (range 27-70) **Methotrexate**: Administered in a 1-5 year period either once	**Liver scintigraphy:** Tc 99m sulphur colloid liver scan Evaluated for commonly accepted abnormalities each of which have been shown to correlate with histologically proved liver diseases: **heterogeneous uptake** (irregular distribution of Tc), **hepatomegaly** (width >18 cm and height >17cm), **extra hepatic uptake** (increased Tc distribution in spleen, bone marrow or lungs relative to liver), **focal defects** (areas of absent or minimal radioactive colloid uptake with surrounding tissue concentration)	**Liver biopsy** Graded by the Roenigk classification **Histological techniques:** Biopsy by Menghini technique Biopsy graded (according to Roenigk) 1. Normal or mild fatty infiltration, nuclear variability and portal inflammation 2. Moderate to severe fatty infiltration, nuclear variability and portal	None stated

	weeks and one within a 2-month period while MTX therapy was continued (test order unclear); data available for all		weekly intramuscular or orally (either once weekly or in divided doses during a 24-h period). Total MTX dose per patient: 800mg-4g	Positive = presence of any of the above abnormalities	inflammation and focal necrosis 3. Fibrosis (septum formation) 4.Cirrhosis	

Effect Size

Summary of findings

Biopsy grade	**Number of biopsies**	**Number of liver scans**		**Specific abnormalities**
		Normal	**Abnormal**	
1	13	8	5	1 hepatomegaly 3 extra-hepatic uptake 1 extra-hepatic uptake, hepatomegaly and heterogeneous uptake
2	4	3	1	1 heterogeneous uptake
3	5	3	2	1 heterogeneous uptake 1 extra-hepatic uptake
4	2	0	2	2 extra-hepatic uptake and heterogeneous uptake

Note: both cases of cirrhosis were identified correctly

2 x 2 table – based on definition of abnormal biopsy as grade 3 or 4 (fibrosis or cirrhosis) combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 4	FP: 6
Index test -ve	FN: 3	TN: 11

Summary statistic	
Pre-test probability/prevalence	29.2%
Sensitivity	57.1 (22.7-86.7)%
Specificity	64.7 (50.5-76.9)%
PPV	40.0 (15.9-60.7)%
NPV	78.6 (61.3-93.3)%
LR +	1.62 [0.65,4.02]
LR-	0.66 [0.26,1.67]

Authors' conclusion:

Hepatotoxic reactions from long-term methotrexate use in psoriasis cannot be reliably evaluated by the Tc 99m sulphur colloid liver scan

Reference	Study type	Number of patients	Patient characteristics	Index test	Reference standard	Source of funding
P. M. McHenry, E. A. Bingham, M. E. Callender, P. B. Delvin, M. D. O'Hara, W. R. Ferguson, J. D. Laird, and D. Burrows. Dynamic hepatic scintigraphy in the screening of psoriatic patients for methotrexate-induced hepatotoxicity. Br.J.Dermatol. 127 (2):122-125, 1992. Ref ID: MCHENRY1992	**Observational:** Retrospective case series • **Patient selection:** All psoriasis patients about to receive or receiving MTX therapy since 1981 (consecutive sample) • **Index tests:** Liver scintigraphy: Tc 99m sulphur colloid liver scan – Evaluated for portal contribution (threshold <50%) • **Reference standard:** biopsy classification according Warin grading by a single independent pathologist, unclear size of biopsy sample • **Flow and timing:** Time between tests: max 4 weeks (test order unclear); data	N: 63 (23 had data while on MTX) 87 paired biopsy and DHS scans (49 prior to MTX and 38 during therapy)	**Inclusion criteria:** psoriasis patients about to receive or receiving MTX therapy with both biopsy and DHS since 1981 (max interval 4 weeks) **Exclusion criteria:** not stated Baseline data: **% male**: 65% **Mean age**: 52 years (range 21-84) **Methotrexate**: Schedule unclear Mean cumulative MTX dose in 23 patients: 1.5 g (60mg-7g) and mean duration of MTX	**Liver scintigraphy:** Tc 99m sulphur colloid liver scan Sequence of images taken (using gamma camera operated in conjunction with nuclear medicine computer) over an 80-s interval following Tc99 injection showing uptake of colloid in liver and spleen Analysis of time activity curves allows estimation of the proportion of the total colloid uptake represented by the portal venous contribution Positive = portal contribution of <50% of total hepatic uptake of colloid at 30s	**Liver biopsy** **Histological techniques:** Biopsy by Tru-Cut needle stained with H7E, reticulin, Masson trichrome, Perl's and orcein Fibrosis graded as none, very mild, mild (sparse interlobular), moderate (just bridging portal tracts) or severe (bridging portal tracts Abnormal = at least moderate Abnormal cut-off chosen because MTX may be continued in the presence of sparse intra-lobular fibrosis	None stated

	available for all		32 months (1 month-9 years)	Threshold chosen based on reference range in 50 patients with no liver disease (mean portal contribution 64.4±11.7%)	Biopsy performed prior to MTX and after every 1-1.5 g MTX (or at approximately yearly intervals)	

Effect Size

Summary of findings

- Sensitivity: 83.3%
- Specificity: 81.5%
- NPV: 98.5%
- PPV: 25%

2 x 2 table – based on definition of abnormal biopsy as grade 2 or 3 (portal fibrosis) and DHS threshold of <50% portal contribution

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 5	FP: 15
Index test -ve	FN: 1	TN: 66

Note: the one false negative result had a portal contribution of 51% so a 2% increase in the threshold would have allowed all patients with portal fibrosis to have been detected by DHS

Summary statistic	
Pre-test probability/prevalence	6.9%
Sensitivity	83.3 (38.0-99.1)%
Specificity	81.5 (78.1-82.6)%
PPV	25.0 (11.4-29.7)%
NPV	98.5 (94.4-99.9)%
LR +	4.50 [2.52,8.04]
LR-	0.20 [0.03,1.23]

Authors' conclusion:

Dynamic hepatic scintigraphy may therefore offer a means to reduce the number of liver biopsies necessary in patients receiving methotrexate for psoriasis

Reference	Study type	Number of patients	Patient characteristics	Index test	Reference standard	Source of funding
D. Mitchell, R. J. Johnson, H. J. Testa, N. Y. Haboubi, and R. Chalmers. Ultrasound and radionuclide scans - Poor indicators of liver damage in patients treated with methotrexate. Clin.Exp.Dermatol. 12 (4):243-245, 1987. Ref ID: MITCHELL1987	**Observational:** Prospective case series • **Patient selection:** Psoriasis patients receiving long-term MTX therapy (unclear if included all relevant individuals/consecutive sample) • **Index tests:** assessed without prior knowledge of liver histology or duration of MTX treatment **Liver scintigraphy: Tc 99m sulphur colloid liver scan –** Evaluated for pre-specified abnormalities by consultant in nuclear medicine **Ultrasound:** assessed by either a radiologist or a qualified ultrasound radiographer for signs of abnormality according to standard proforma • **Reference standard:** biopsy classification by one observer (experience unclear); unclear if blinding and size of biopsy sample • **Flow and timing:** Time between tests: scans performed 1 day prior to biopsy;	N: 49	**Inclusion criteria:** Psoriasis patients receiving long-term, low dose, once weekly oral MTX therapy requiring biopsy **Exclusion criteria:** not stated **Baseline data** **% male**: 53.1% **Median age**: 46 years (range 22-69) **Cumulative dose** MTX: median 2.8 g (0.5-10 g) **Median duration of MTX therapy**: 5.3 years (range: 1-13)	**A. Liver scintigraphy:** Tc 99m sulphur colloid liver scan (Tc99 given intravenously 10 min before scan) Consultant in nuclear medicine evaluated anterior, posterior and lateral views for: size of liver and spleen, pattern of uptake in these organs and degree of extrahepatic uptake Positive = presence of any of the above abnormalities **B. Ultrasound** Examination carried out by one of two operators (radiologist or a qualified ultrasound radiographer); scan images obtained at 1-cm intervals through the liver in 2 planes	**Liver biopsy** **Histological techniques:** Biopsy by Menghini technique; staining with H&E, periodic acid Schiff, Perl's stain for iron, reticulin fibre stain and haematoxylin picrosirus red Biopsy graded as: 1.Normal 2.Fatty change (steatosis) alone 3.Inflammation 4. Fibrosis	None stated

	data available for all		**Methotrexate**: long-term, low dose, once weekly oral MTX therapy	Assessed for liver size, shape, echo pattern and information about the biliary and vascular system according to a standard proforma	(graded mild, moderate or severe) 5. Cirrhosis	

Effect Size

Summary of findings: ultrasound

Biopsy grade	**Number of biopsies**	**Number of liver scans**	
		Normal	**Abnormal**
Normal	13	12	1
Steatosis alone	7	6	1
Inflammation	17	14	3
Fibrosis	9	9	0
Cirrhosis	3	3	0

Note: ultrasound failed to detect any of those with fibrosis or cirrhosis

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 0	FP: 5
Index test -ve	FN: 12	TN: 32

Summary statistic	
Pre-test probability/prevalence	24.5%
Sensitivity	0%
Specificity	86%
PPV	0%
NPV	73%
LR +	0
LR-	1.16 [0.95,1.33]

Summary of findings: scintigraphy

Biopsy grade	**Number of biopsies**	**Number of liver scans**		**Specific abnormalities detected on scan**
		Normal	**Abnormal**	

Normal	13	9	4	Mostly patchy tracer uptake No splenomegaly or extra-hepatic uptake
Steatosis alone	7	6	1	
Inflammation	17	12	5	-
Fibrosis	9	5 – mild fibrosis	4 – moderate fibrosis	5 splenomealy 3 patchy tracer uptake 2 increased extra-hepatic uptake
Cirrhosis	3	1	2	

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 6	FP: 10
Index test -ve	FN: 6	TN: 27

Summary statistic	
Pre-test probability/prevalence	24.5%
Sensitivity	50.0 (24.2-74.9)%
Specificity	73.0 (64.6-81.1)%

PPV	37.5 (18.2-56.2)%
NPV	81.8 (72.4-90.9)%
LR +	1.85 [0.85,4.02]
LR-	0.69 [0.38,1.25]

Authors' conclusion:

Hepatotoxic reactions from long-term methotrexate use in psoriasis cannot be reliably evaluated by the Tc 99m sulphur colloid liver scan or by ultrasound scan, so these cannot replace biopsy

Reference	Study type	Number of patients	Patient characteristics	Index test	Reference standard	Source of funding
I. H. Coulson, J. Mckenzie, V. S. Neild, A. E. Joseph, and R. A. Marsden. A comparison of liver ultrasound with liver biopsy histology in psoriatics receiving long-term methotrexate therapy. Br.J.Dermatol. 116 (4):491-495, 1987. Ref ID: COULSON1987	**Observational:** Prospective case series • **Patient selection:** Psoriasis patients receiving or about to receive MTX (unclear if included all relevant individuals/consecutive sample) • **Index test** **Ultrasound:** assessed by experienced radiologist for signs of fatty change and fibrosis (according to pre-defined echo pattern) Assessed blind to clinical details • **Reference standard:** biopsy classification by one observer (experience unclear);performed before scans so no need for blinding; 5µm sections sampled • **Flow and timing:**	N: 28 54 paired observations (7/54 were pre-MTX)	**Inclusion criteria:** Severe psoriasis patients receiving or about to receive MTX **Exclusion criteria:** not stated **Baseline data** **% male**: 71.4% **Age**: range 30-74 **Methotrexate schedule:** once-weekly oral dose	**Ultrasound** Examination carried out by an experienced radiologist; scan images obtained using a 3.5 or 5 mHz transducer by a single experience operator unaware of clinical details Graded as normal or abnormal (fatty change and/or fibrosis) **Fatty change** identified by hyper-echoic liver tissue with fine packed echoes **Fibrosis without fatty change** = coarse echo pattern **Fibrosis with fatty**	**Liver biopsy** at least 5 days after last MTX dose **Histological techniques:** Biopsy by Tru-Cut needle; 5µm sections cut and stained with haematoxylin and van Gieson stain **Grading** Subjective microscopic assessment based on method of Warin et al of fat, inflammation, fibrosis (each graded 0, 0.5, 1, 2, or 3) and cirrhosis (not graded)	None stated

	Time between tests: scans performed within 1 month of biopsy; data available for all			**change** (difficult to identify) = coarse echoes (pin-head echoes) within the fine echo pattern of fatty change		

Effect Size

Summary of findings: ultrasound

Biopsy grade	**Number of biopsies**	**Number of liver scans**		
		Normal	**Steatosis**	**Fibrosis**
Normal	11	9	2	0
Steatosis ± inflammation	17	5	12	0
Mild fibrosis (intralobular)	6	4	2	0
Marked fibrosis (portal)	20	0	15	5
Cirrhosis	0	0	0	0

Note: no cases of cirrhosis

2 x 2 table – based on definition of abnormal biopsy as any degree of fibrosis or cirrhosis combined and abnormal ultrasound as showing fibrosis (not fatty change)

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 5	FP: 0
Index test -ve	FN: 21	TN: 28

Summary statistic	
Pre-test probability/prevalence	48.2%
Sensitivity	19.0%
Specificity	100%
PPV	100 (39-100)%
NPV	57%
LR +	Infinity [0.69,204]
LR-	0.81 [0.67,0.99]

2 x 2 table – based on definition of abnormal biopsy as portal fibrosis (in accordance with Roenigk criteria) or cirrhosis combined and abnormal ultrasound as showing fibrosis (not fatty change)

	Reference	**Reference**

	test +ve	**test -ve**
Index test +ve	TP: 5	FP: 0
Index test -ve	FN: 15	TN: 34

Summary statistic	
Pre-test probability/prevalence	37.0%
Sensitivity	25.0%
Specificity	100.0%
PPV	100% (39-100)%
NPV	69%
LR +	Infinity [1.07,315]
LR-	0.75 [0.58,0.97]

Authors' conclusion:

- No patient with a normal ultrasound scan showed significant fibrosis and thus such patients may be spared liver biopsy and safely continue with methotrexate therapy.
- Ultrasound cannot reliably distinguish between fatty change and fibrosis, so all patients with abnormal scans require liver biopsy

H.13.3 PIIINP

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>M. J. Boffa, A. Smith, R. Chalmers, D. Mitchell, B. Rowan, T. W. Warnes, M. Shomaf, and N. Y. Haboubi. Serum type III procollagen aminopeptide for assessing liver damage in methotrexate-treated psoriatic patients. Br.J.Dermatol. 135 (4):538-544, 1996.

Ref ID: BOFFA1996</td>
<td>Observational:
Prospective case series and case control study

• Patient selection: Unclear if a random or consecutive sample was taken
• Index tests: PIIINP – serum sample immediately prior reference standard (unclear when analysed); unclear method of selection of threshold)
• Reference standard: biopsy classification; 2 observers blinded to MTX dose (experience unclear); biopsy sampling unclear
• Flow and timing:</td>
<td>N: 87

147 paired liver biopsies and serum samples</td>
<td>Inclusion criteria: Long-term low-dose once weekly oral MTX for severe psoriasis; 1 or more biopsies with simultaneous serum sampling

Exclusion criteria: Not stated

<table>
<tr><th>Parameter</th><th>Treated (n=87)</th></tr>
<tr><td>Median age – years at first biopsy</td><td>50 (22-75)</td></tr>
<tr><td>Cumulative MTX dose at first biopsy, median (range)</td><td>2.2 (0.3-10.0) g</td></tr>
<tr><td>Duration of treatment at first biopsy, median weeks</td><td>206 (26-738)</td></tr>
</table>
</td>
<td>PIIINP (^{125}I) – Orion Diagnostica

Serum samples collected at least 5 days after last dose of MTX and immediately prior to liver biopsy

Samples stored at -20°C until analysis

Abnormal values of PIIINP >4.2 ng/ml

BUT in 3 control groups (normal enzyme levels and no history/clinical features of liver disease the reference ranges were:</td>
<td>Hepatotoxicity by histology score – assessed blind to MTX dose by 2 assessors

Histological techniques:
Biopsy by Menghini technique; staining with H&E, periodic acid Schiff, Perl's stain for iron, orcein, Hep B surface antigen and metallothionein, untoned reticulin and haematoxylin/picrosirius red for collagen

Grading:
1. Normal histology
2. Steatosis alone
3. Inflammation (±steatosis) without fibrosis
4. Fibrosis (±steatosis ± inflammation)

5. Cirrhosis</td>
<td>Skin disease research fund</td>
</tr>
</table>

	Tests taken immediately after each other (index test first); all received both index and reference tests		(range) **Methotrexate**: Long-term low-dose once weekly oral MTX	1. 17 healthy patients with non-inflammatory skin disorders: 1.6-4.9 ng/ml 2. 18 people with moderate-severe psoriasis with no history of systemic treatment: 2.1-4.7 ng/ml 3. 11 PsA with no MTX use: 2.2-4.6 ng/ml		

Effect Size

Outcomes

Summary of findings

Pre-test probability/prevalence: 21/87 (24.1%)

Biopsy grade	**Number of biopsies**	**Number with raised PIIINP**

Normal	28	5 (18%)
Steatosis	12	5 (42%)
Inflammation	26	14 (54%)
Fibrosis	18	14 (78%)
Cirrhosis	3	3 (100%)

Note: all cases of cirrhosis were identified correctly; proportion with raised PIIINP increased with increasing severity of histological damage

Based on single biopsy specimen – **fibrosis**: sensitivity = 81%; specificity = 62%

Based on all 147 paired observations – **fibrosis**: sensitivity = 77%; specificity = 66%

2 x 2 table – based on definition of abnormal biopsy as fibrosis or cirrhosis combined (data from time of first biopsy only)

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 17	FP: 24
Index test -ve	FN: 4	TN: 42

Summary statistic	
Pre-test probability/prevalence	24.1%

Sensitivity	81.0 (60.3-93.5)%
Specificity	63.6 (57.1-67.6)%
PPV	41.5 (30.9-47.9)%
NPV	91.3 (81.9-97.0)%
LR +	2.23 [1.52,3.26]
LR-	0.30 [0.12,0.74]

Authors' conclusion:

PIIINP-O is of value in detecting liver damage and, particularly if measured serially, may reduce the need for liver biopsy in MTX-treated patients. Although the test does not detect all patients with fibrosis, it would appear that the risk of missing significant liver damage in patients with persistently normal PIIINP-O is low

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>H. Zachariae, L. Heickendorff, and H. Sogaard. The value of amino-terminal propeptide of type III procollagen in routine screening for methotrexate-induced liver fibrosis: a 10-year follow-up. Br.J.Dermatol. 144 (1):100-103, 2001.

Ref ID: ZACHARIAE2001</td>
<td>Observational: Retrospective case series (follow-up 1-11 years)

• Patient selection: Unclear if a random or consecutive sample was taken; psoriasis patients on MTX in 1989-90
• Index tests: PIIINP – all had at least 2 assays prior to or at time of biopsy and all but one had at least 3 analyses within a year around the time of the biopsy (unclear when analysed); unclear method of selection of threshold)
• Reference standard: biopsy classification; unclear who assessed and unclear if blind to ref standard (experience unclear); biopsy sampling unclear
• Flow and timing:
all received both index and reference tests, but order unclear and all received more index test analyses than</td>
<td>N: 70

189 biopsies and 329 PIIINP analyses</td>
<td>Inclusion criteria: psoriasis patients on MTX in 1989-90; studied with both biopsy and PIIINP; initial biopsy and PIIINP normal and continued to take MTX

Exclusion criteria: Not stated

<table>
<tr><th>Parameter</th><th>All (n=70)</th></tr>
<tr><td>% male</td><td>55.7%</td></tr>
<tr><td>Cumulative MTX dose at latest biopsy, mean (range)</td><td>3.5 (0.6-16.8) g</td></tr>
<tr><td>Duration of treatment at first biopsy, mean years (range)</td><td>4 (1-20)</td></tr>
<tr><td>PsA</td><td>27 (38.6%)</td></tr>
</table>
Methotrexate:</td>
<td>PIIINP (^{125}I) – Orion Diagnostica

Abnormal values of PIIINP >4.2 ng/ml

Note: serial analyses of PIIINP were performed; therefore not a 1:1 relationship with biopsies. Those who tested positive on both tests could also have had several negative PIIINP tests within a year of a positive biopsy</td>
<td>Fibrosis on biopsy

Histological techniques: Biopsy by Menghini technique; staining with H&E, van Gieson and Masson's trichrome stain

Grading:

Unclear classification system, only fibrosis reported</td>
<td>None stated</td>
</tr>
</table>

	reference standards 1 patient excluded after finding a positive biopsy result had been found prior to 1989		Dosing regimen not stated			

Effect Size

Outcomes

Only 2/6 showed increased PIIINP without either biopsy verified liver fibrosis or verified PsA (one of the false positives had PsA)

2 x 2 table –fibrosis (note: a positive result on the index test was based on at least 1 elevated PIIINP reading, but among the serial analyses at least one could also have been normal, but data on this are not reported, although the 4 true positives all had 2 elevated PIIINP tests)

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 4	FP: 2
Index test -ve	FN: 0	TN: 63

Summary statistic	
Pre-test probability/prevalence	5.8%

Sensitivity	100%
Specificity	97%
PPV	66% [30%,84%]
NPV	100%
LR +	32 (6.80,83)
LR-	0 (0.01,1.44)

Authors' conclusion:

As long as PIIINP is consistently normal in serial investigations there is minimal risk of development of substantial liver fibrosis

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>P. D. Maurice, A. J. Maddox, C. A. Green, F. Tatnall, J. K. Schofield, and D. J. Stott. Monitoring patients on methotrexate: hepatic fibrosis not seen in patients with normal serum assays of aminoterminal peptide of type III procollagen. Br.J.Dermatol. 152 (3):451-458,</td>
<td>Observational:
Retrospective case series

• Patient selection: All dermatology patients in 3 adjacent dermatology departments undergoing MTX treatment entered into a database established in 1999 (consecutive sample)
• Index tests: PIIINP – serial analyses before and after biopsy; unclear if blinded to biopsy grade but should be objective; prior selection of threshold according to laboratory normal range)
• Reference standard: biopsy classification according to Roenigk grade; single blinded pathologist</td>
<td>N: 34

70 biopsies and 306 PIIINP analyses</td>
<td>Inclusion criteria: dermatology patients on MTX before or after the database was established who had both serial PIIINP assays and at least one liver biopsy

Exclusion criteria: Not stated

<table>
<tr><th>Parameter</th><th>All (n=34)</th></tr>
<tr><td>% male</td><td>68%</td></tr>
<tr><td>Median age at latest biopsy</td><td>50.5 (21-81) years</td></tr>
<tr><td>Weekly MTX dose at latest biopsy, median (range)</td><td>2740 (150-23,955) mg</td></tr>
<tr><td>Cumulative MTX dose at latest biopsy, median (range)</td><td>15 (0-30) mg/wk</td></tr>
<tr><td>Duration of treatment, mean years (range)</td><td>0.5-20 y</td></tr>
</table>
</td>
<td>PIIINP (^{125}I) – Orion Diagnostica

Abnormal values of PIIINP >4.2 ng/ml

Serum samples sent to biochemistry laboratory where PIIINP assays were performed using the radioimmunoassay

Laboratory normal range is 1.2-4.2 ug/l

Note: serial analyses of PIIINP were performed; therefore not a 1:1 relationship with biopsies. PIIINP assays performed at 3-mothnly intervals

Selected data on all biopsies where PIIINP</td>
<td>Fibrosis on biopsy

Histological techniques:
Biopsy by percutaneous route using ultrasound control with an 18 gauge Biopince needle; staining with H&E and van Gieson for fibrosis; reticulin for liver architecture; Perl's stain, periodic acid –Schiff and orcein stain used on most biopsies

Grading:

According to Roenigk classification (abnormal = Grade IIIA-IV)

Assessed by one pathologist blind to MTX dose and PIIINP values</td>
<td>R&D group of West Hertfordshire Hospitals NHS trust</td>
</tr>
</table>

2005. Ref ID: MAURICE 2005	(experience unclear); biopsy sampling by 18 gauge needle • **Flow and timing:** all received both index and reference tests, and all received more index test analyses than reference standards (some before and some after ref test); some biopsies excluded if not within right time frame relative to assays		<table><tr><td>Psoriasis</td><td>33 (97%)</td></tr><tr><td>Pemphigus foliaceus</td><td>1 (3%)</td></tr><tr><td>Mean (SD) body weight</td><td>Male: 87.6 (18.2) kg Female: 70.3 (14.5) kg</td></tr><tr><td>No alcohol intake</td><td>71% (the median intake for 29% was 5 units/week (range: 1-21)</td></tr><tr><td>Inflammatory arthritis</td><td>22%</td></tr></table> **Methotrexate**: Oral once weekly dosing	assays had been performed within 12 months (6 months preceding and 6 months following) of biopsy to compare assay levels with biopsy grade Note: not all 70 biopsies included because some were not at the mid-point of the series of assays		

Effect Size

Outcomes

Summary of findings

Pre-test probability/prevalence: 8/70 biopsies (11.4%); 5/34 patients (14.7%)

Biopsy grade	Number of biopsies	Number with raised PIIINP
I	91	16 (18%)
II	60	33 (55%)
Fibrosis	15	24 (62%)
Cirrhosis	0	0

Note: Some biopsies counted more than once as paired with more than one PIIINP assay

Numbers of biopsies and contemporaneous PIIINP assays

Proportion PIIINP abnormal	Roenigk grade		
	I	II	IIIA/B
None	15	5	20
<50%	4	2	8

≥50%	5	2	8
All	0	7	10
Total	24	16	46

Note: 3 liver biopsies in 2 morbidly obese patients who also had maturity-onset diabetes were graded II on Roenigk classification but showed signs of NASH (rather than portal fibrosis which is more often associated with MTX)

Note: 6 grade II biopsies were from 4 patients with inflammatory arthritis; 4 of these biopsies had elevated PIIINP in **all** associated readings; the other two biopsies had **some** abnormal PIIINP readings

2 x 2 table –fibrosis (note: PIIINP and biopsy not matched 1:1 and more than one result per patient; based on 46 biopsies with contemporaneous PIIINP assays [number of PIIINP assays per biopsy ranged from 2 to 6])

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 15	FP: 49
Index test -ve	FN: 9	TN: 102

Summary statistic	
Pre-test probability/prevalence	13.7%
Sensitivity	62.5 (42.1-79.8)%
Specificity	67.5 (64.3-70.3)%
PPV	23.4 (15.8, 29.9)%

NPV	91.9 (87.5, 95.6)%
LR +	1.93 (1.31,2.83)
LR-	0.56 (0.33,0.94)

Authors' conclusion:

- Follow-up liver biopsies for patients on long-term low-dose methotrexate can be avoided if PIIINP levels are consistently normal.
- The PIIINP assay will also be helpful in the management of patients on methotrexate in whom liver biopsy is contraindicated, and in patients with nonalcoholic steatohepatitis

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>J. Risteli, H. Sogaard, A. Oikarinen, L. Risteli, J. Karvonen, and H. Zachariae. Aminoterminal propeptide of type III procollagen in methotrexate-induced liver fibrosis and cirrhosis. Br.J.Dermatol. 119 (3):321-325, 1988.

Ref ID: RISTELI1988

H. Zachariae, H. Sogaard, and L. Heickendorff. Serum aminoterminal propeptide of type III procollagen. A non-invasive test for liver fibrogenesis in methotrexate-treated psoriatics. Acta Derm.Venereol. 69 (3):241-244, 1989.

Ref ID: ZACHARIAE1989</td>
<td>Observational: Prospective case control study (included untreated group with no biopsy measurement)

• Patient selection: Unclear if a random or consecutive sample was taken (state it represents their total experience with PIIINP)
• Index tests: PIIINP – unclear if performed blind but should be objective; prior selection of threshold
• Reference standard: biopsy classification; assessed by single pathologist blind to clinical and laboratory data (experience unclear); biopsy sampling unclear
• Flow and timing:
all received both index and reference tests</td>
<td>N: 24 for pilot and 73 for full study (including the original 24)</td>
<td>Inclusion criteria: psoriasis patients on MTX for at least 6 months, no more than slight fibrosis before MTX

Exclusion criteria: Not stated

<table>
<tr><th>Parameter</th><th>Pilot group (n=24)</th></tr>
<tr><td>Median age (range)</td><td>50 (32-75)</td></tr>
<tr><td>% male</td><td>56.3%</td></tr>
<tr><td>Duration of treatment, mean years (range)</td><td>7.6 (0.5-20)</td></tr>
<tr><td>PsA</td><td>11 (45.8%)</td></tr>
<tr><td>PP</td><td>3 (12.5%)</td></tr>
</table>

Note: baseline data not available for full group</td>
<td>PIIINP

Stored at -20°C and measured by radioimmunoassay based on human propeptide

Abnormal values of PIIINP >4.2 ng/ml (based on 88 Finnish blood donors)

Serial PIIINP performed in 11 patients (one in pilot study)</td>
<td>Hepatotoxicity on biopsy

Biopsies taken at 1-2 year intervals while on MTX (used biopsy closest to time of PIIINP measurement)

Histological techniques:
Biopsy by Menghini technique; staining with H&E, periodic acid Schiff, van Gieson and Masson's trichrome stain, Perl's stain for iron, reticulin fibre stain

Grading:

Unclear classification system, included normal, pronounced steatosis, slight fibrosis, fibrosis, cirrhosis</td>
<td>None stated</td>
</tr>
</table>

	(except one in full study who refused biopsy), but order unclear and time between tests unclear (used biopsy closest to time of PIIINP measurement)		**Methotrexate**: Weekly divided oral dose of 5 mg with 12-h intervals once weekly			

Effect Size

Outcomes

Summary of findings in pilot study (N=24)

Diagnosis	**PIIINP (ug/l)**	**Liver histology**
PsA	**7.6**	Steatosis
Pustular psoriasis	**7.4**	Fibrosis
Psoriasis	**7.2**	Fibrosis
PsA	**5.9**	Fibrosis
PsA	**?**	Cirrhosis

	PsA	**4.8**	Fibrosis
	PsA	**4.6**	Fibrosis
	Psoriasis	**4.5**	Fibrosis
	PsA	**4.3**	Cirrhosis
	Psoriasis	4.1	Fibrosis
	Pustular psoriasis	4.1	Fibrosis
	PsA	3.9	Normal
	Psoriasis	3.7	Slight fibrosis
	Pustular psoriasis	3.5	Slight fibrosis
	Psoriasis	3.5	Normal
	Psoriasis	3.4	Cirrhosis
	PsA	3.4	Normal
	Psoriasis	3.4	Normal
	Psoriasis	3.1	Fibrosis
	PsA	3.1	Normal
	PsA	3.0	Normal
	Psoriasis	3.0	Normal
	Psoriasis	2.8	Normal
	PsA	2.7	Normal

2 x 2 table –fibrosis and cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 9	FP: 1
Index test -ve	FN: 6	TN: 9

Pre-test probability/prevalence	60%
Sensitivity	60.0 (41.0-66.3)%
Specificity	90.0 (61.6-99.5)%
PPV	90.0 (61.6, 99.5)%
NPV	60.0 (41.0, 66.3)%
LR +	6.0 (0.89, 40)
LR-	0.44 (0.23, 0.85)

Subgroup analysis for PsA and non-PsA populations

2 x 2 table –fibrosis and cirrhosis combined - **no PsA**

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 3	FP: 0
Index test -ve	FN: 6	TN: 4

Pre-test probability/prevalence	69.2%
Sensitivity	33.0%
Specificity	100%
PPV	100 (33-100)%
NPV	40%
LR +	Infinity
LR-	0.67

2 x 2 table –fibrosis and cirrhosis combined - PsA

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 4	FP: 1
Index test -ve	FN: 0	TN: 5

Pre-test probability/prevalence	40%
Sensitivity	100%
Specificity	83%
PPV	80 (40-92)%
NPV	100%
LR +	6.00 [0.99,18]
LR-	0.00 [0.01,1.82]

Summary of findings in full study (N=72 including original 24)

2 x 2 table –fibrosis and cirrhosis combined

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 19	FP: 1
Index test -ve	FN: 6	TN: 46

Summary statistic	
Pre-test probability/prevalence	34.7%
Sensitivity	76.0 (61.8-79.8)%
Specificity	97.9 (90.3-99.9)%
PPV	95.0 (77.2, 99.7)%
NPV	88.5 (81.6, 90.3)%
LR +	36 (5.07,251)
LR-	0.25 (0.12,0.49)

Authors' conclusion:

The study indicates that PIIINP can be utilized as a valuable non-invasive marker of fibrogenesis in the liver. This analysis is not specific for the liver, but it seems that the number of liver biopsies probably can be reduced in people with psoriasis on methotrexate who have normal levels of PIIINP

H.13.5 Fibrotest and Fibroscan

<table>
<tr><th>Reference</th><th>Study type</th><th>Number of patients</th><th>Patient characteristics</th><th>Index test</th><th>Reference standard</th><th>Source of funding</th></tr>
<tr>
<td>M. A. Berends, J. Snoek, E. M. de Jong, J. H. Van Krieken, R. J. de Knegt, M. G. van Oijen, P. C. van de Kerkhof, and J. P. Drenth. Biochemical and biophysical assessment of MTX-induced liver fibrosis in psoriasis patients: Fibrotest predicts the presence and Fibroscan predicts the absence of significant liver fibrosis. Liver International 27 (5):639-</td>
<td>Observational:
Retrospective case series

• Patient selection: unclear, only 34 contacted and 24 agreed
• Index tests:
Fibrotest– Measurements were performed immediately on fresh obtained samples using validated methods; pre-defined selection of threshold)

Fibroscan – performed on the same day as the biological parameters input to Fibrotest; used the median</td>
<td>N: 24</td>
<td>Inclusion criteria: of all 60 psoriasis patients on MTX treatment at the end of 2005 those who had biopsy and fibrotest and fibroscan within 18 months as part of their regular MTX monitoring

Exclusion criteria: Not stated

<table>
<tr><th>Parameter</th><th>All (n=24)</th></tr>
<tr><td>% male</td><td>45.8%</td></tr>
<tr><td>Mean age</td><td>55 (34-73)</td></tr>
<tr><td>Cumulative MTX dose (range)</td><td>3352 (314-20235) mg</td></tr>
<tr><td>Follow-up,</td><td>346</td></tr>
</table>
</td>
<td>Fibrotest and Fibroscan

Fibrotest is an artificial intelligence alogorithm consisting of a biochemical fibrosis index using inputs from 5 serum markers (g-GT, bilirubin, a2-macroglobulin apolipoprotien A1 and heptoglobin) and is corrected for age and sex leading to a composite value between 0 and 1 to determine the presence of significant liver fibrosis

Measurements performed immediately on fresh obtained samples using validated methods</td>
<td>Hepatotoxicity by histology score – all biopsies assessed blind by 2 investigators and verified by an experienced pathologist (disagreements resolved by consensus)

Histological techniques:
Biopsy by Menghini technique (1.6 mm needle); staining with H&E, von Gieson

Grading: Metavir system
F0 = no fibrosis
F1 = portal fibrosis without septa
F2 = portal fibrosis with few septa
F3 = numerous septa without cirrhosis

F4 = cirrhosis</td>
<td>None stated</td>
</tr>
</table>

645, 2007. Ref ID: BERENDS2007B	value of successful measurements • **Reference standard:** biopsy classification; 2 independent observers and verified by an experienced pathologist (disagreements resolved by consensus = difficult to diagnose cases included); biopsy sampling by 1.6 mm needle • **Flow and timing:** Time between tests up to 18 months; all received Fibrotests and Fibroscan but only 20 had evaluable scans; it failed in four people with obesity		median weeks (range) (111-2162) Median BMI (range) 26 (20-38) kg/m^2 N overweight (BMI>25) 14 N alcohol consumption 10 (1 excessive >14U/wk) Diabetes 4 Note: all patients underwent a liver biopsy after a median dosage of 1635 mg MTX (range 162–2354 mg). Only one patient had elevated liver enzymes more than twice the ULN. Sixteen patients were biopsied before treatment. **Methotrexate**: Dosing unclear	The quantitative output estimate of liver fibrosis stage corresponds to stages F0-F4 of metavir scale Based on the literature a cut-off of 0.31 was chosen to identify Metavir ≥F2 **Fibroscan** measures liver elasticity using 1 dimensional transient elastography, as the liver stiffness roughly correlates with the degree of hepatic fibrosis (a fine correlation has been established between significant fibrosis (Metavir F2) and Fibroscan outcome It uses an ultrasound transducer that generates vibrations that cause a slow elastic shear wave. The propagation and velocity of the wave in the liver are tracked by pulse-echo ultrasound and		

				correlate to tissue stiffness. Measurements were performed on the right lobe of the liver, at the same target area for liver biopsy. The procedure was performed through the intercostals space while the patients were lying on their backs with their arms in maximal abduction behind their heads. Conducted by an experienced physician blinded to histological outcome Each patient underwent a series of 10 validated electrographic measures. A cut-off value of 7.1 kPa was chosen to identify significant fibrosis (≥F2) Only procedures with 10 validated measurements and a success rate of at least 60% were considered to be reliable.		

Effect Size

Outcomes

Summary of findings

Pre-test probability/prevalence (at least F2): 6/24 (25%)

Biopsy grade	**Number of people**
F0	5
F1	13
F2	4
F3	1
F4	1

Test accuracy as reported in study (not possible to derive 2x2 table for Fibroscan from the text)

	Fibrotest **≥F2**	**Fibroscan** **≥F2**
Optimal cut-off	0.31	7.1 kPa

Sensitivity (%)	83	50	
Specificity (%)	61	88	
Negative predictive value (%)	92	86	
Positive predictive value (%)	42	33	

Note: 16 people had more than one biopsy and unclear if analysed on a per-patient basis

Fibrotest 2 x 2 table – (at least F2)

	Reference test +ve	**Reference test -ve**
Index test +ve	TP: 5	FP: 7
Index test -ve	FN: 1	TN: 11

Summary statistic	
Pre-test probability/prevalence	25%
Sensitivity	83.3 (40.8-99.1)%
Specificity	61.1 (46.9-66.4)%
PPV	41.7 (20.4-49.6)%
NPV	91.7 (70.4-99.6)%

LR +	2.14 [1.08,4.23]
LR-	0.27 [0.04,1.69]

Discordance between fibrotest and fibroscans

- In nine patients, Fibroscans and Fibrotest resulted in different Metavir scores with a discordance of two stages. In four of them, the total Fibroscans procedure failed because of the presence of obesity. In the remaining five, biopsy length was significantly shorter compared with the biopsy length of the remaining patients. There was no significant difference in BMI between those patients. Other potential confounders for failure of Fibrotest such as Gilbert, haemolysis and acute inflammation were ruled out.

Authors' conclusion:

Fibrotest seems to be good in detecting and the Fibroscans seems to be good in excluding significant MTX-induced liver fibrosis (FZ2) in patients with psoriasis treated with MTX. This suggests that the combined use of Fibrotest and Fibroscans may be beneficial in establishing the grade of liver fibrosis in MTX-induced liver fibrosis in psoriasis patients