PRIMARY BILIARY CIRRHOSIS:
NEW THERAPEUTIC OPTIONS?

Medical Grand Rounds

October 5, 1989

Burton Combes, M.D.

Introduction to the Disease

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Mitochondrial Antigens and Antibodies

Antimitochondrial antibodies (AMA) are detected in the sera of the vast majority of patients with PBC. Antibody is usually detected by an immunofluorescent test with frozen sections of tissue serving as antigen. The test is positive in 85 to 95 percent of patients with PBC. In PBC, AMA antibodies react with non-organ or species specific antigens associated with either inner (M_2) or outer mitochondrial membranes (M_4, M_8, M_9).

Anti-M_2 antibodies appear to be specific for the diagnosis of PBC. The presence of anti-M_4 and anti-M_8 tend to correlate with progression of disease. Anti-M_9 may be especially helpful for early diagnosis of PBC patients who are still AMA negative. In addition, anti-M_9 appears to correlate with a benign course of the disease.

Molecular techniques have led to the elucidation of the M_2 autoantigen. In fact, at least four proteins comprise the M_2 autoantigen system. Utilizing the DNA map identified by Gershwin et al, that coded for a polypeptide reactive with PBC sera, Yeaman and associates identified the protein as the E_2 component of the pyruvate dehydrogenase enzyme complex (PDC). Subsequently, the M_2 autoantigens have been shown to consist of the E_2 components of all three 2-oxo acid dehydrogenase complexes, i.e., the branched-chain 2-oxo acid dehydrogenase complex and the 2-oxoglutarate dehydrogenase complex. In addition, the X component of the pyruvate dehydrogenase complex contains an epitope that reacts with all positive anti-PDC E_2 sera.

Current nomenclature of mitochondrial autoantigens[a]

Antigen	Clinical correlation	Biochemical definition	Molecular weight	Organ specificity	Trypsin sensitivity	Location[b]
M1	Syphilis	Cardiolipin	Unknown	No[c]	No	Inner
M2	Very specific for PBC	See Ref. (1) for sequence	70, 45, 39 kd complex	No	Yes	Inner
M3	Pseudolupus	Unknown	Unknown	No	No	Outer
M4	"Overlap" PBC and CAH	Unknown	Unknown	No	No	Outer
M5	Connective tissue disease	Unknown	Unknown	No	No	Outer
M6	Drug-induced hepatitis	Unknown	Unknown	Liver	No	Outer
M7	Cardiomyopathies	Unknown	Unknown	Myocardium	Yes	Inner
M8	PBC (may correlate with severity)	Unknown	Unknown	No	Yes	Outer
M9	PBC (but not specific)	Unknown	Unknown	No	No	Outer

[a] As emphasized in the text, this nomenclature must eventually be superceded by molecular definitions. Antigens located in the outer membrane mitochondria may turn out to be microsomal in origin.

[b] Inner vs. outer mitochondrial membranes.

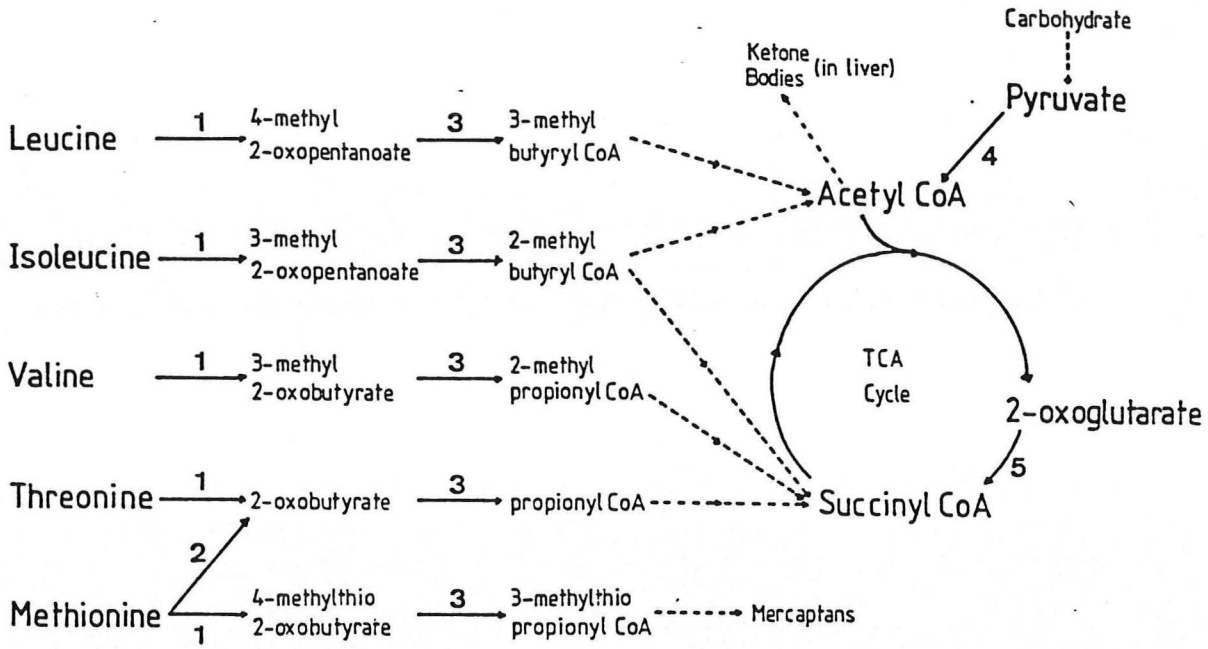
[c] Reacts equally well with mitochondrial preparation from a variety of organs.

From Gershwin et al, Hepatology 8:147, 1988

M_2 autoantigens are contained in the 2-oxo acid dehydrogenase complexes

(2-Oxo acid dehydrogenase complexes) (α-Keto acid dehydrogenase complexes)[a]
Pyruvate dehydrogenase complex Abbreviations: PDC, PDH, PDHC
Branched-chain 2-oxo acid dehydrogenase complex Abbreviations: BCOADC, BCKD
2-Oxoglutarate dehydrogenase complex Abbreviations: OGDC, KGDH
Component enzymes E1: 2-oxo acid dehydrogenase E2: dihydrolipoamide acyltransferase (E2 of PDC is an acetyltransferase) (E2 of OGDC is a succinyltransferase) (E2 of BCOADC is a branched-chain acyltransferase)

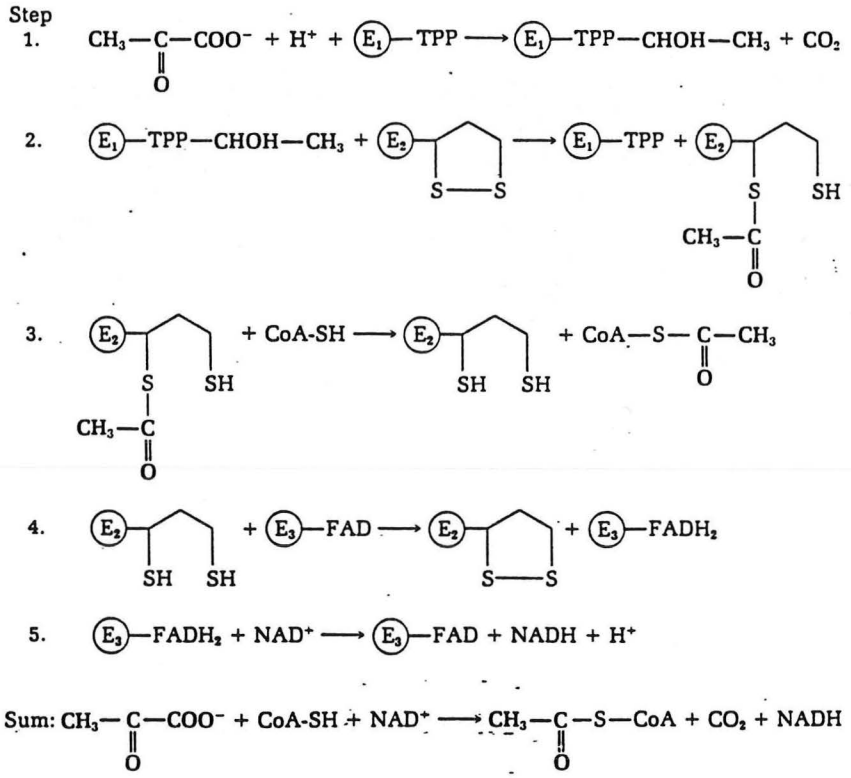
From James et al, Hepatology 10:247, 1989



Outline of the metabolic role of the 2-oxoacid dehydrogenase complexes. 1, transamination; 2, trans-sulphuration; 3, branched-chain 2-oxoacid dehydrogenase; 4, pyruvate dehydrogenase; 5, 2-oxoglutarate dehydrogenase. The broken lines indicate that several reaction steps are involved.

From Yeaman, TIBS 11:293, 1986

Steps in the oxidative decarboxylation of pyruvate to acetyl-CoA by the pyruvate dehydrogenase complex



Key: E_1 = pyruvate dehydrogenase; TPP = thiamine pyrophosphate; TPP-CHOH-CH_3 = α-hydroxyethylthiamine pyrophosphate; E_2 = dehydrolipoyl transacetylase; E_3 = dihydrolipoyl dehydrogenase.

Adapted from Lehninger, Principles of Biochemistry, Worth Publishers, Inc., 1982

Reactivity of sera from 40 PBC patients with the E2 components of mammalian 2-oxo acid dehydrogenase complexes

PDC E2 (and X)	BCOADC E2	OGDC E2	No. of PBC patients (%)
+	+	+	21 (52.5)
+	+	−	2 (5)
+	−	+	6 (15)
+	−	−	9 (22.5)
−	+	+	2 (5)

From Fussey et al, Proc. Natl. Acad. Sci. USA 85:8657, 1988

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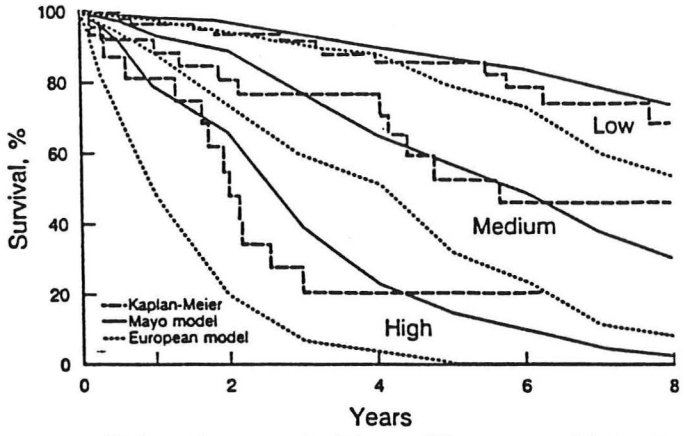
Prognosis

A number of clinical observations has been found to provide prognostic information about patients with primary biliary cirrhosis. Serum bilirubin alone is useful. The development of predictive survival models based on many more variables appears to provide substantially more power.

The Mayo Clinic has developed a Cox survival model based on 312 patients seen between 1974 - 1984 by using stepwise modeling on a subset of 12 noninvasive, easily collected variables that required only clinical evaluation and a blood sample. These were age, albumin, bilirubin, alkaline phosphatase, cholesterol, AST, prothrombin time, hepatomegaly, ascites, and edema. Statistical selection procedures chose five of these variables: $\log_e$(bilirubin), $\log_e$(albumin), $\log_e$(prothrombin time), age, presence or absence of edema.

Predicted survivals based on these variables compared well with actual survival (Kaplan-Meier) curves for three risk groups contained in a series of 106 cross-validation patients.



Risk tertiles comparing Mayo and European models to actual (Kaplan-Meier) survival curves for the 106 Mayo cross-validation patients. Time zero is time of entry into trial.

From Dickson et al, Hepatology 10:1-7, 1989

To use the model, a risk score is calculated for each patient:

$R = 0.871 \log_e$ (bilirubin in mg/dl) + -2.53 $\log_e$ (albumin in gram/dl) + 0.039 age in years + 2.38 $\log_e$ (prothrombin time in sec) + 0.859 edema.

where edema score is:
0: No edema and no diuretic therapy for edema
0.5: Edema present - not on diuretics; or
Edema resolved with diuretic therapy
1: Edema despite diuretic therapy

The probability of survival for at least t more years is read from a table based on the mean risk score (R = 5.07) calculated for a group of 418 patients (initial 312 patients, plus the validation group of 106 patients).

Underlying survival function for the final Mayo model

t (years)	1	2	3	4	5	6	7
$S_0(t)$	0.970	0.941	0.883	0.833	0.774	0.721	0.651

$S_0(t)$ gives the survival probabilities for a patient with risk score 5.07, the mean of the combined Mayo data set.

One then calculates the survival probability of a given patient for any period up to 7 years from the risk score calculated for that person:

$$S(t) = [So(t)]^{\exp.\ (R\ -\ 5.07)}$$

Sample calculations are provided in the publication of Dickson et al, Hepatology 10:1-7, 1989.

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Therapy

General References

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34. Kaplan MM. Medical treatment of primary biliary cirrhosis. Sem. Liver Dis. 9:138-143, 1989.

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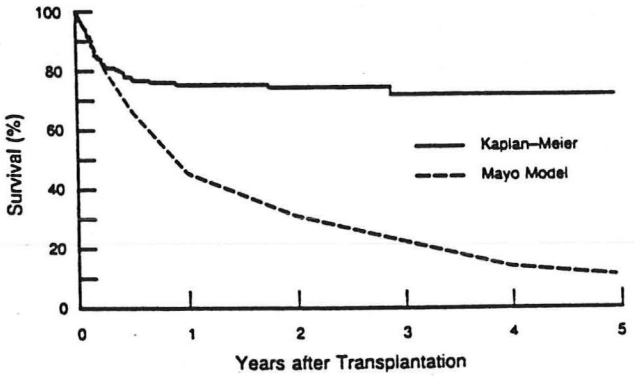
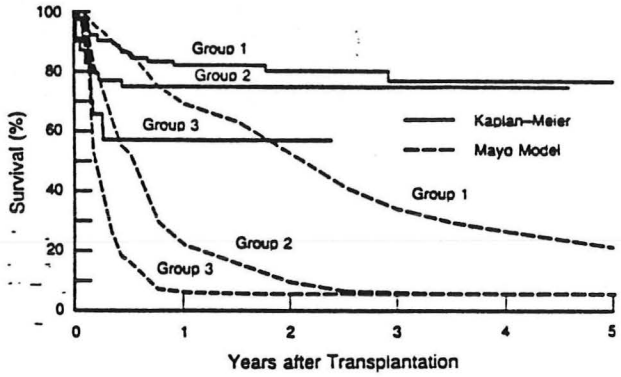
Liver Transplantation

The Mayo Clinic Cox regression model for predicting the probability of survival in patients with primary biliary cirrhosis and treated conservatively has been particularly valuable in assessing the contribution of liver transplantation in the management of patients with primary biliary cirrhosis.

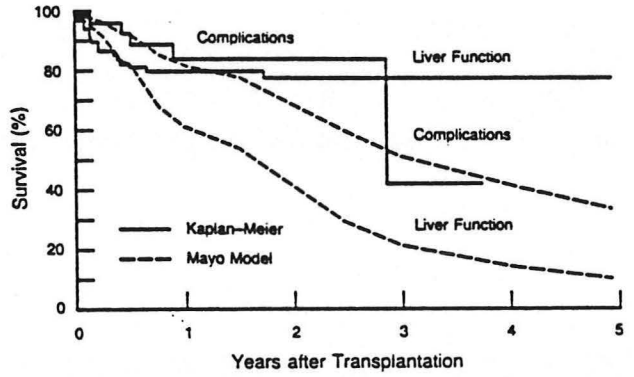


Actual (Kaplan–Meier) Survival after Transplantation in 161 Patients with Primary Biliary Cirrhosis and Estimated Survival without Transplantation as Predicted by the Mayo Model (Simulated Control).



Actual (Kaplan–Meier) Survival after Transplantation in Three Risk Groups of Patients with Primary Biliary Cirrhosis and Estimated Survival without Transplantation as Predicted by the Mayo Model.

The risk groups were formed on the basis of pretransplantation Mayo-model risk scores. Group 1 (low risk) comprised 98 patients with risk scores below 8.67; Group 2 (medium risk), 41 patients with scores between 8.67 and 9.93; and Group 3 (high risk), 22 patients with scores above 9.93.



Actual (Kaplan–Meier) Survival after Transplantation in Two Subgroups of Low-Risk Patients, and Predicted (Mayo Model) Survival without Transplantation.

The subgroups were 70 patients with primary biliary cirrhosis who required transplantation because of poor liver function and 28 patients with primary biliary cirrhosis who required liver transplantation because of other severe complications.

Variables in 98 Patients in Group 1, According to Indication for Transplantation.

Variable	Poor Liver Function (N = 70)	Gastrointestinal Bleeding or Osteodystrophy (N = 28)
	mean ±SD	
Age (yr)	45.7±8.0	48.4±9.3
Bilirubin (mg/dl)*	15.2±7.5	4.4±3.6
Albumin (g/dl)	3.1±0.5	3.2±0.6
Prothrombin time (sec)	13.4±1.3	13.2±1.6
Edema score†	0.35±0.44	0.39±0.39
Risk score†	7.68±0.84	6.55±1.08

*To convert to micromoles per liter, multiply by 17.1.

†According to the Mayo model.

From Markus et al, N. Engl. J. Med. 320:1709-1713, 1989

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Azathioprine

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Prednisone

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D-Penicillamine

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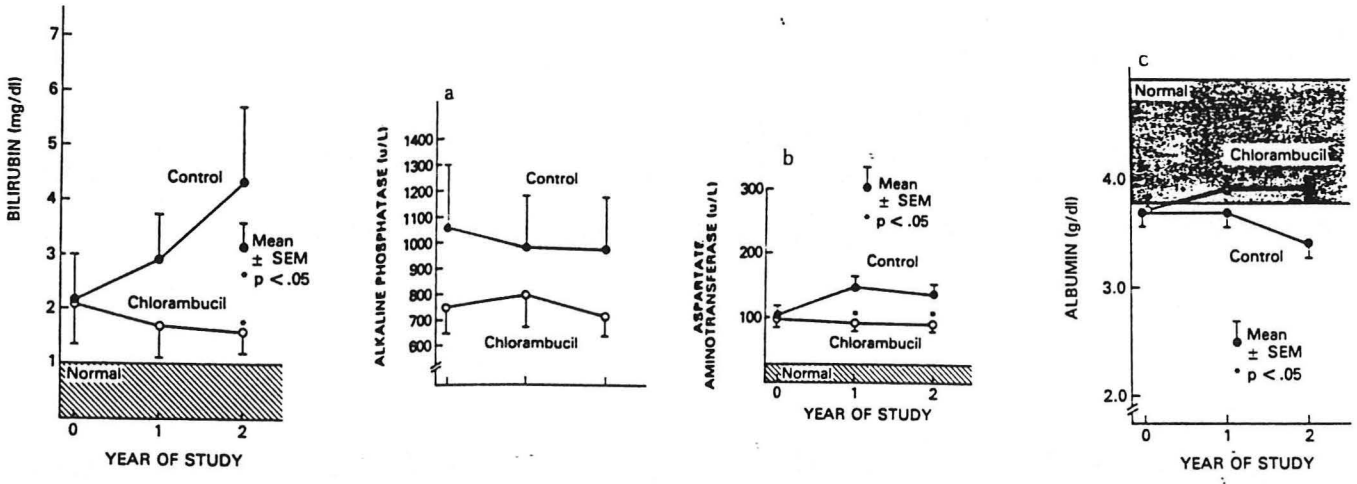
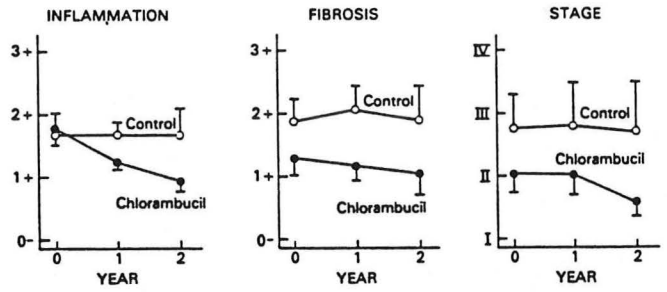
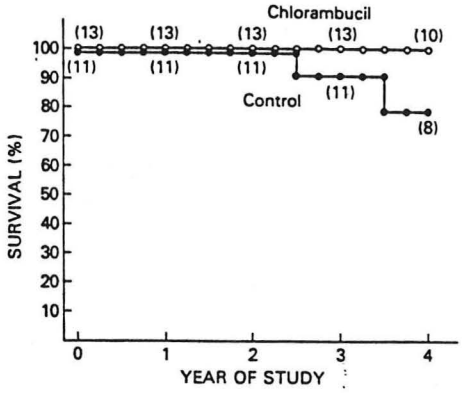
Chlorambucil



From Hoofnagle et al, Gastroenterology 91:1327-1334, 1986



Average degree of hepatic inflammation, fibrosis, and stage of PBC as assessed by three independent observers who read the liver biopsy histology under code—without knowledge of the patient, timing of the biopsy, or treatment.



Survival in the 24 patients with PBC analyzed by the Kaplan–Meier method. *Numbers in parentheses* indicate the number of patients followed at that time period.

From Hoofnagle et al, Gastroenterology 91:1327-1334, 1986

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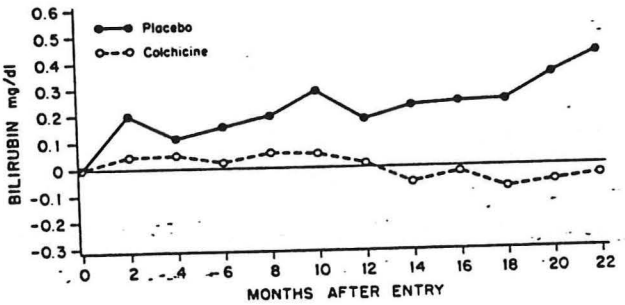
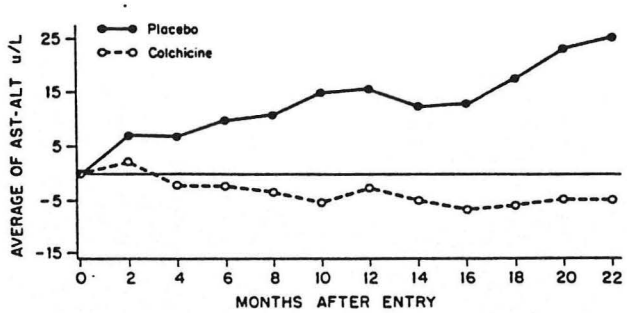
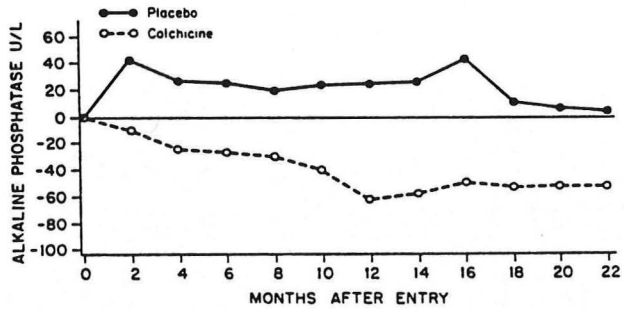
Colchicine

Bilirubin, Aminotransferase (Average of AST–ALT), and Alkaline Phosphatase Levels in the Colchicine and Placebo Groups.

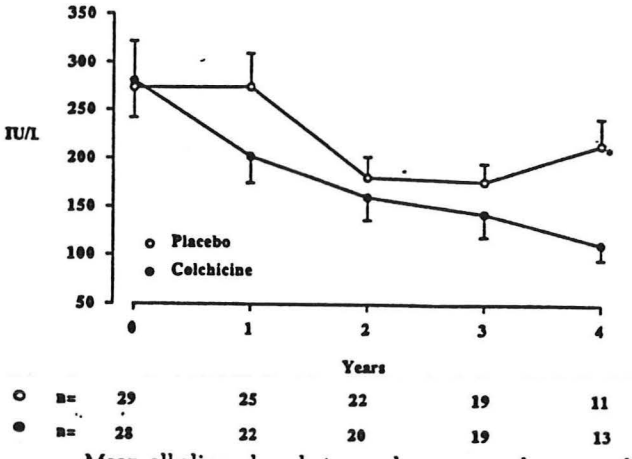
Values are deviations from base line of geometric mean values. AST–ALT denotes an average of levels of aspartate and alanine aminotransferases.







From Kaplan et al, N. Engl. J. Med. 315:1448-1454, 1986



Mean alkaline phosphatase values assessed at annual intervals during the trial. The difference between the curves is significant at 4 yr (*p < 0.01). *Error bars* indicate SEM.

Comparison of Biochemical Values Between Treatment Groups During the Trial

Test	Colchicine	Placebo	p[a]
Alkaline phosphatase	0.61	0.89	<0.05
ALT	0.68	0.94	<0.05
Bilirubin	1.36	1.99	0.12

ALT, alanine aminotransferase. [a] p value indicates the level of significance of the comparison of the last/first ratios between colchicine and placebo groups.

Changes in Biochemical Values During the Trial

	Last/first ratios					
Group	AP	p[a]	ALT	p[a]	Bilirubin	p[a]
Placebo	0.89	>0.3	0.94	0.55	1.99	<0.01
Colchicine	0.61	<0.0001	0.68	<0.0001	1.36	0.10

ALT, alanine aminotransferase; AP, alkaline phosphatase. [a] p values indicate the level of significance of the difference of the last/first ratios from unity.

From Bodenheimer et al, Gastroenterology 95:124-129, 1988

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Cyclosporin

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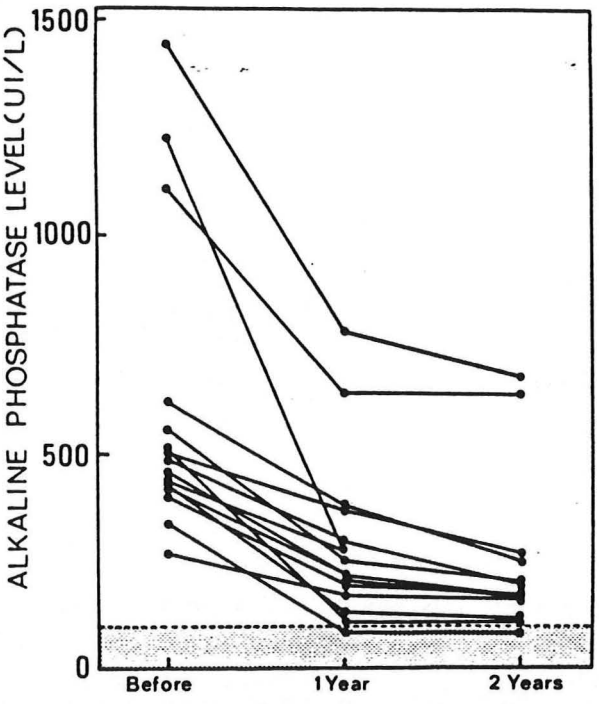
Ursodeoxycholic acid



Changes in serum alkaline phosphatase levels over 2 years of UDCA treatment.

Dotted line = upper limit of normal.

CLINICAL AND BIOCHEMICAL DATA

	Mean (SEM) or %		
—	At entry (n = 15)	At 1 year (n = 15)	At 2 years (n = 12)
Total serum bile acid (μmol/l)	33 (6)	39 (8)	32 (12)
UDCA (% of total bile acids)	0	62 (7)‡	58 (9)‡
% with pruritus	53%	27%	8%†
% with hyperbilirubinaemia (> 34 μmol/l)	30%	7%	0%*
Alkaline phosphatase (N < 100 IU/l)	612 (90)	287 (50)‡	213 (47)‡
Gamma-glutamyltranspeptidase (N < 40/l)	554 (116)	316 (66)‡	122 (37)‡
ALAT (N < 30 IU/l)	104 (13)	45 (7)‡	33 (6)‡
ASAT (N < 30 IU/l)	62 (7)	34 (5)‡	28 (5)‡
Bilirubin (μmol/l)	36 (10)	23 (6)‡	22 (6)‡
BSP fractional clearance (%/min)	7·5 (1·0)	8·2 (0·7)	9·7 (0·9)*
Globulin (g/l)	15·0 (3·2)	13·5 (2·6)	15·0 (2·1)
IgM (g/l)	4·9 (0·29)	3·7 (0·21)	4·7 (0·29)

Statistics: *p < 0·05, †p < 0·01, ‡p < 0·001 for comparison between pretreatment and 1 yr or 2 yr values.
N = upper limit of normal, defined as 95th percentile of normal population of chemical laboratory. ALAT = alanine aminotransferase; ASAT = aspartate aminotransferase.

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Rifampin

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Methotrexate

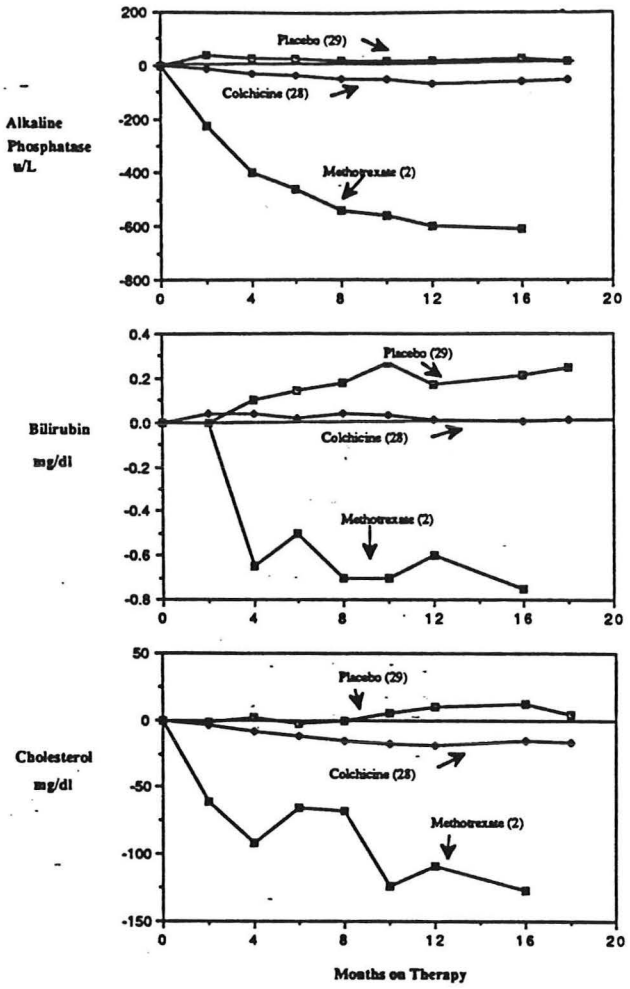

Figure 1. Effects of methotrexate, colchicine, and placebo treatment on the serum alkaline phosphatase (*top*), bilirubin (*middle*), and cholesterol (*bottom*) levels in patients with primary biliary cirrhosis. The numbers in parentheses refer to the number of patients who received the specific therapy. Values are mean deviation from initial mean values. Results for patients receiving methotrexate are shown for the first 16 months of treatment.

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