Clinical rheumatology, 1990, **9**, N° 3 333-341

Methotrexate therapy in rheumatoid arthritis. A two year prospective follow-up

A.A. DROSOS, D. PSYCHOS, A.P. ANDONOPOULOS, S. STEFANAKI-NIKOU*, E.B. TSIANOS, H.M. MOUTSOPOULOS.

Departments of Internal Medicine and *Pathology, Medical School, University of Ioannina, Ioannina, Greece.

SUMMARY *One hundred and thirty seven rheumatoid arthritis (RA) patients refractory to D-penicillamine and some of them (15%) refractory to other slow active drugs were treated with oral methotrexate (MTX) (10-15mg weekly). After 12-24 months of treatment, 94 and 74 patients respectively showed a significant improvement as judged by duration of morning stiffness ($p<0.0001$), grip strength ($p<0.0001$), degree of joint swelling ($p<0.01$) and tenderness ($p<0.0001$) compared to pre-treatment values. This clinical improvement was also associated with a decrease of erythrocyte sedimentation rate ($p<0.0001$), decrease of C-reactive protein ($p<0.0001$) and with improvement of anaemia ($p<0.05$). No changes were seen in rheumatoid factor titres. Seventy-four of the patients were followed for up to 24 months. Thirty-one of them (23%) had complete remission and 43 (31%) had an excellent response. Adverse drug reaction during MTX therapy included: elevated liver enzymes in 34 patients, mucosal ulcers in 21, nausea and vomiting in 8, diarrhoea in 4, leukopenia in 2, interstitial pneumonitis in one, intestinal bleeding in one and finally septic arthritis in another patient. The majority of these side effects were resolved without sequelae. However, 15 patients (11%) with adverse drug reactions had to discontinue the treatment. Forty-one of our patients who received a cumulative mean dose of MTX of 1550.5 ± 235.5 mg underwent a percutaneous liver biopsy. Ten patients had normal tissue, 12 had minimal changes, 13 nonspecific changes and 6 patients had mild fibrosis. We conclude that MTX therapy in refractory RA patients appears to be effective, but requires close monitoring for toxicity. Hepatotoxicity with fibrosis and cirrhosis due to long term MTX therapy may be relatively uncommon in RA patients.*

Key words: Methotrexate, Rheumatoid Arthritis, Toxicity, Hepatotoxicity, Liver Biopsy.

INTRODUCTION

Methotrexate (MTX), a folic acid antagonist, has been used for the treatment of non-neoplastic diseases such as: psoriasis, psoriatic arthritis, polymyositis and Reiter's disease for many years (1-5).

In 1951, Gubner reported that MTX can suppress rheumatoid synovitis (6). Many studies in recent years have reported its efficacy in the treatment of refractory rheumatoid arthritis (RA) patients (7-21). Although the effectiveness of MTX has been observed in all short-term studies (7-15) its therapeutic benefit in long-term studies, however, has been less well appreciated (16-21). In addition, the long-term hepatic effects of MTX in RA patients are controversial (16, 22-23).

In this study, we evaluated prospectively the efficacy and the safety of MTX in 137 RA

Received: 30 November 1989
Revision-accepted: 21 January 1990
Correspondence to: Dr. Alexandros A. DROSOS, Department of Internal Medicine, Medical School, University of Ioannina, 451 10 Ioannina, Greece.

patients, the majority of whom were treated for more than 24 months.

MATERIAL AND METHODS

This study included 137 patients who were seen at the Department of Internal Medicine of the Ioannina Medical School and prospectively followed in order to determine the efficacy and safety of MTX in RA. All our patients included in this study fulfilled the American Rheumatism Association (ARA) criteria for RA (24). Before entering this study, all patients had received D-penicillamine. Twenty-one had received hydroxychloroquine, 24 had received gold salts and 2 had also received azathioprine. Methotrexate therapy was instituted at a dose of 10 to 15 mg once weekly per os, usually in two doses (0.2 mg/kg/body weight). In addition, all our patients were also taking nonsteroid antiinflammatory drugs (usually diclophenac or naproxen), while none had received corticosteroid therapy.

Clinical and laboratory disease variables were determined by the same investigator (AAD or DP) before treatment, then monthly for 6 months and every 2 months thereafter. All patients were assessed for disease activity by determinating the number of tender and swollen joints, by the score of tender and swollen joints (mild = 1+, moderate = 2+, severe = 3+), duration of morning stiffness and mean grip strength. The following laboratory values were obtained in each patient: complete blood count, differential and platelet count. Serum levels of glutamyc oxalacetic, glutamyc pyruvic transaminase, alkaline phosphatase and gamma glutamyl transferase were also performed. Albumin, total bilirubin, total protein, creatinine, glucose and urinalysis were also obtained at each visit. In addition, C-reactive protein (CRP) (single radial immunodiffusion), titres of rheumatoid factor (RF) (latex fixation test) and erythrocyte sedimentation rate (ESR) (Westergren method) were also obtained at each clinical assessment. A decrease of leukocytes (WBC $<4.000/mm^3$) and an increase of transaminase levels up to three times normal values were considered abnormal. For the definition of clinical remission we used the preliminary criteria of Pinals et al (25).

Patients with a history of alcohol consumption, with liver or kidney abnormalities, blood dyscrasias, diabetes mellitus and obesity were excluded from the study. None of the above patients was taking salicilates, colchicine or other drugs known to interfere with MTX (26). Finally, for monitoring liver toxicity in patients who received more than 1gr of MTX (27) all gave their informed consent for liver biopsy.

For statistical analysis, we used chi-square analysis and Student-t-test when indicated.

RESULTS

Of the 137 patients, 34 were male and 103 female, with mean age 56.05 ± 12.67 years and disease duration 8.76 ± 5.5 years. One hundred and three patients had positive RF test ($>1/40$) at the time of entry. All patients were at the time of entry in functional class II-III according to Steinbrocker's classification (28). Extra-articular manifestations were present in 14 patients, i.e. subcutaneous nodules in 5, pleurisy in one, pericarditis in one; 7 had secondary Sjögren's syndrome (29).

After 12 months of treatment, 94 patients (69%) were on MTX and after 24 months 74 patients (54%) were still on MTX. All these patients showed a significant clinical improvement as judged by duration of morning stiffness ($p<0.0001$), grip strength ($p<0.0001$), degree of joint swelling ($p<0.01$) and tenderness ($p<0.0001$), compared to pretreatment values (Table I). This improvement was also associated with a decrease of ESR ($p<0.0001$), decrease of CRP ($p<0.0001$) and with improvement of anaemia ($p<0.05$) (Table II). No changes were observed in RF titres and also no changes were noted regarding the extraarticular manifestations during the follow-up. Patients with extraarticular manifestations stopped

Table II: *Changes of laboratory parameters in RA treated patients with MTX*

Parameters	Duration of treatment								
	Before treatment n:137	3 months n:127	p	6 months n:114	p	12 months n:94	p	14 months n:74	p
Ht (%)	38.5±5.0	38.8±4.4	N.S.	39.7±4.0	<0.05	39.7±4.1	<0.05	40.0±3.9	<0.05
ESR (mm/h)	57.9±30.7	42.5±29.2	<0.001	37.8±23.4	<0.0001	36.2±23.2	<0.0001	32.7±27.0	<0.0001
CPR (%)	35.1±33.1	33.5±35.2	N.S.	27.7±26.7	<0.05	15.1±21.8	<0.0001	10.2±15.9	<0.0001

n = number of patients, N.S. = not statistical

Table I: *Changes of clinical parameters in RA patients treated with MTX*

Parameters	Duration of treatment								
	Before treatment n:137	3 months n:127	p	6 months n:114	p	12 months n:94	p	24 months n:74	p
Morning stiffness (min)	103.5±128.1	29.7±61.4	<0.0001	22.3±57.9	<0.0001	20.0±57.8	<0.0001	21.0±38.5	<0.0001
Grip strength(mmHg)									
right	89.7±58.2	124.6±76.9	<0.001	137.5±80.7	<0.0001	160.3±83.1	<0.0001	198.2±86.1	<0.0001
left	87.7±57.7	121.3±76.1	<0.001	135.7±81.5	<0.0001	161.3±82.4	<0.0001	196.4±87.0	<0.0001
Joint count									
swelling	16.6±15.2	13.3±13.7	N.S.	12.6±13.2	<0.05	12.0±10.2	<0.01	11.1±9.0	<0.01
tenderness	18.0±10.2	9.0±7.5	<0.0001	8.2±7.2	<0.0001	6.3±6.9	<0.0001	3.9±5.6	<0.0001
Joint score									
swelling	17.3±16.2	13.7±14.5	N.S.	13.0±13.0	<0.05	12.3±14.2	<0.05	11.1±11.0	<0.01
tenderness	20.8±12.2	9.6±8.3	<0.0001	9.0±8.3	<0.0001	6.9±9.4	<0.0001	4.1±6.0	<0.0001

n = numbers of patients, NS = not statistical.

taking MTX because of exacerbation after a follow-up period of 12 months (see below). Seventy-four of our patients were followed up to 24 months. Thirty-one of them (23%) had complete clinical remission.

Adverse drug reactions during MTX therapy included: increased liver enzymes in 34 (25%), mucosal ulcers in 21 (15%), nausea and vomiting in 8 (6%). Four patients developed diarrhoea, two maculopapular rash and two others leukopenia. Finally, one patient developed interstitial pneumonitis, one intestinal bleeding and another septic arthritis (Table III). In patients with increased liver enzymes the dose of MTX decreased from 15mg to 7.5 mg for two weeks. After that, in 28 patients the liver enzymes were normalized and they continued to take 10 mg of MTX weekly without any side effect. The majority of side effects in our patients were resolved without sequelae. However, 15 patients (11%) with adverse drug reactions had to stop the treatment (Table IV). Six patients continued to have elevated liver enzymes after a decrease of the MTX dose. After that, MTX was discontinued for two weeks with normalization of liver enzymes. These six patients, however, refused to undergo a percutaneous liver biopsy and they were withdrawn from the study. One of these six patients also had oral ulcers. Two patients with nausea and vomiting and two with mucosal ulcers, as well as two with leukopenia, one with interstitial pneumonitis, one with intestinal bleeding and finally, one with septic arthritis were also withdrawn from the study because of side-effects (Table IV).

Table III: *Side effects of RA patients treated with MTX*

Adverse drug reactions	Number of patients	Percentage
Increased liver enzymes	34	25
Mucosal ulcers	21	15
Nausea vomiting	8	6
Diarrhoea	4	3
Rash	2	1.5
Leukopenia	2	1.5
Interstitial pneumonitis	1	0.75
Intestinal bleeding	1	0.75
Septic arthritis	1	0.75

Table IV: *Patients withdrawn from the study because of side effects*

Adverse drug reaction	Number of patients	Occurrence (months)
Elevated liver enzymes	6	1-18
Nausea, vomiting	3	1-8
Mucosal ulcers	2	1-6
Leukopenia	2	2-12
Interstitial pneumonitis	1	1
Intestinal bleeding	1	6
Septic arthritis	1	6

In Table V the number of patients and the reason for which they were withdrawn from the study are shown. Fifteen patients were

Table V: *Patients withdrawn from the study*

Reasons for withdrawal	Duration of treatment (months) 0	3	6	12	24	TN	%
Side effects	4	5	4	2		15	11
Lack of response	2	3				5	3.6
Exacerbation	2	2	13	6		23	16.8
Lost to follow-up	2	3	3	2		10	7.3
Personal reasons*				6		6	4.4
Other reasons**				4		4	3
Total number	10	13	20	20		63	46

TN: Total number of patients; *Patients in complete remission who refused to undergo liver biopsy; **Patients in complete remission. One developed diabetes mellitus, two started drinking and another began to gain weight.

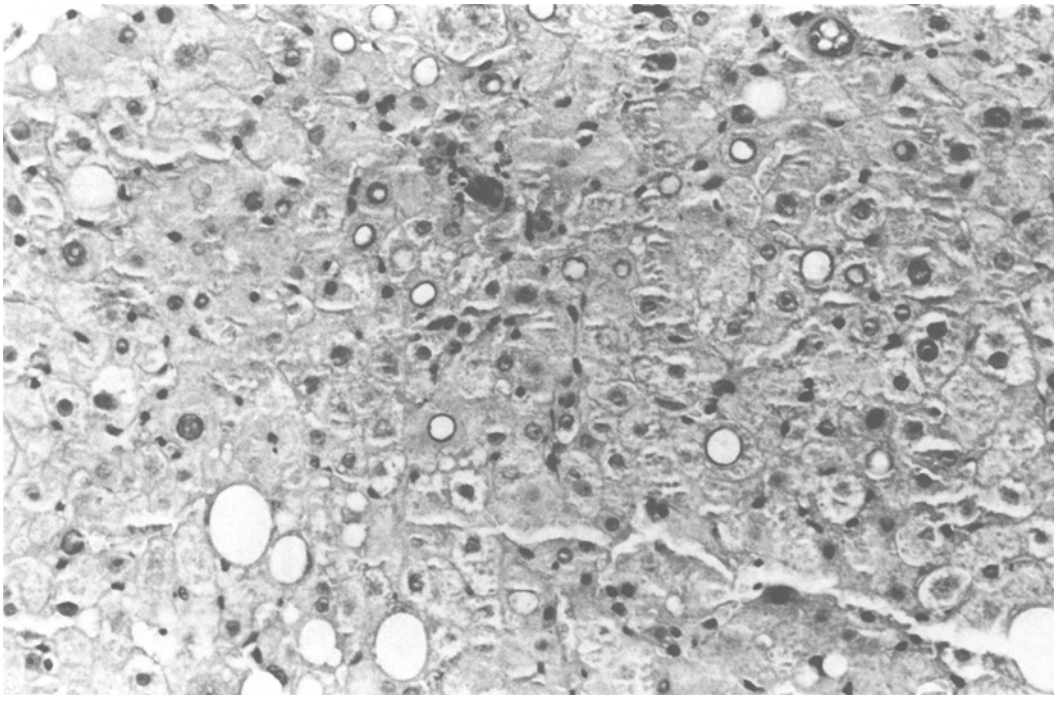

Fig. 1: Liver histology. Minimal changes: anisonucleosis, nuclear vacuolation and fatty changes (hematoxylin and eosin X 40).

dropped out because of side effects (11%), 5 because of lack of response to treatment (3.4%); 23 (16.8%) had synovitis flare-up during follow-up. Most of these patients had extraarticular manifestations. Ten patients were lost during follow-up (7.3%). In addition, six patients in complete clinical remission refused to undergo a percutaneous liver biopsy after a total cumulative dose of 1440 ± 50 mg of MTX and were withdrawn from the study. Finally, 4 more patients in complete clinical remission were also withdrawn from the study because two started drinking, one developed diabetes mellitus and another began to gain weight. The total number of patients who were withdrawn from the study was 63 (46%) and the remaining (54%) are still on MTX.

To monitor liver toxicity, 41 patients underwent a percutaneous liver biopsy after a total cummulative dose of 1550.5 ± 235.5 mg of MTX. We found normal tissue in 24%, minimal changes in 29% (Fig. 1), no specific changes in 32% (Fig. 2) and mild fibrosis in 15% (Fig. 3) (Table VI). In the patients who underwent liver biopsy, no correlation was found between histologic findings and increased liver enzymes during MTX therapy.

DISCUSSION

Many patients with RA require additional therapy after lack of response or toxic reac-

Table VI: *Histological findings of the liver in 41 RA patients treated with MTX*

Liver findings	Number of patients	Percentage
Normal tissue	10	24%
Minimal changes*	12	29%
Non-specific changes**	13	32%
Mild fibrosis***	6	15%

*Anisonucleosis, nuclear vacuolation and fatty changes; **Portal round cell infiltrates, focal hepatocellular necrosis and fatty changes; ***Portal round cell infiltrates, mild portal fibrosis with or without short fibrotic septa extending into the lobule

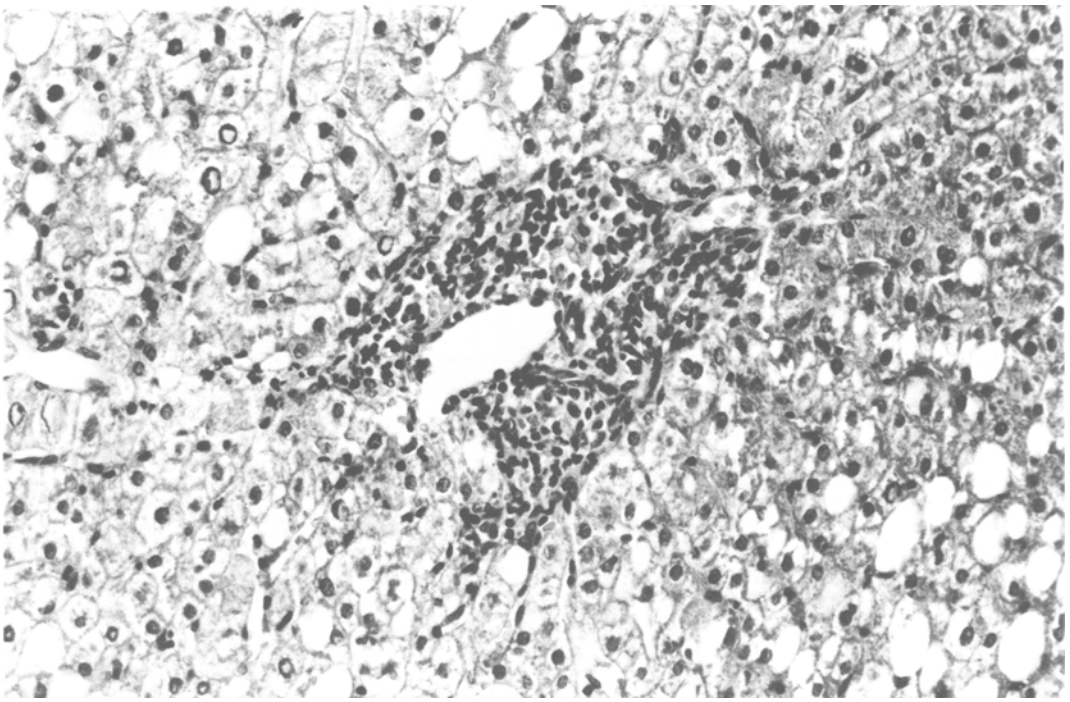

Fig. 2: Liver histology. Non-specific changes: portal round cell infiltrates, focal hepatocellular necrosis and fatty changes (hematoxylin and eosin X 40).

tions to slow acting anti-rheumatic drugs, such as gold salts, D-penicillamine or hydroxychloroquine. A variety of cytotoxic drugs has been extensively used including azathioprine, cyclophosphamide and methotrexate. Azathioprine has been compared favourably to placebo in controlled trials of RA-treated patients (30-32). The data suggest that it is as effective as gold or D-penicillamine (30,31). However, the use of azathioprine for long-term studies has been associated with serious side effects including malignancy (32-34). Cyclophosphamide has been used to treat RA for over a decade and has been found to have a significant effect in RA patients (35-37). Toxic effects of cyclophosphamide is the major problem. The most prominent side effects are sterility, hemorrhagic cystitis, bladder squamous cell carcinoma and other lymphoreticular malignancies (38-40).

In an effort to exploit a less toxic but still effective agent, many investigators have suggested the potential value of MTX in the treatment of active RA refractory to slow-acting drugs. Many published studies indicate that MTX is effective in the short-term therapy of patients with refractory RA (7-15). Its therapeutic effect in long-term studies, however, has been less well appreciated (16-21). In addition, the long-term toxic effect of MTX in RA patients is controversial (16, 22-23).

The results of this study demonstrated that MTX is effective in the management of RA that is unresponsive to traditional therapy. We demonstrated that 54% of our patients continued receiving clinical benefit from MTX for at least two years after the beginning of therapy. Furthermore, as we have shown, 23% of our patients had true clinical remission. All these patients showed a significant clinical improvement as judged by duration of morning stiffness, grip strength, degree and score of swollen joints and tender-

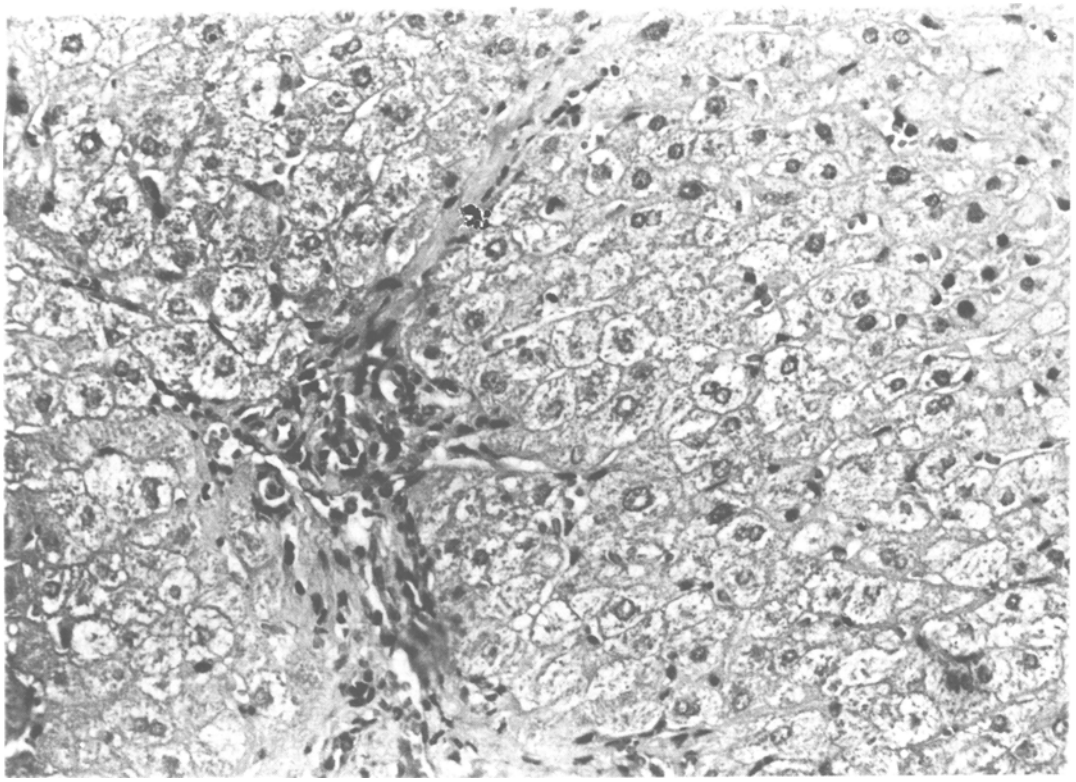

Fig. 3: Liver histology. Portal round cell infiltrates, mild portal fibrosis with short fibrotic septa extending into the lobule (hematoxylin and eosin X 40).

ness, compared to pre-treatment values. In addition, this clinical improvement was associated with a decrease of ESR and CRP and improvement of anaemia. Results in this study are in accordance with other controlled and uncontrolled studies (16-21).

Hepatotoxicity is considered to be the most serious effect limiting the long-term use of low dose MTX (41). Hepatic fibrosis or cirrhosis have been reported in psoriatic patients receiving prolonged MTX therapy (42,43). Serious hepatotoxicity and other severe toxic effects using MTX have been also described in RA treated patients (23, 44,45). Clinical side effects in this group of patients were common but usually mild. No serious toxicity was seen and all side effects were resolved without sequelae after discontinuation of therapy. However, 15 of our patients (11%) had to stop the treatment because of various side effects (Table IV). In our study, nonserious hepatotoxicity was found in the 41 patients who underwent a percutaneous liver biopsy after a cumulative dose of 1550.5 ± 235.5 mg of MTX. The lack of serious hepatotoxicity in our patients is in agreement with other previous studies (16,22,46) and in contrast with the experience in psoriasis (42,43) and RA patients (23). These discrepancies may be due to the use of high doses of MTX in psoriatic patients which is considered more toxic (47) and the allowance of alcohol consumption in some patients of Kremer's study (23), which is another risk factor for fibrosis and cirrhosis (42-43).

Our findings suggest that MTX therapy in refractory RA patients appears to be effective; however, it requires close monitoring for toxicity. Hepatotoxicity with fibrosis and cirrhosis due to long term MTX therapy may

be relatively uncommon in RA patients. We are continuing to follow up our patients and will repeat liver biopsies every two years to see if these patients develop severe hepatotoxicity.

Acknowledgements: We wish to thank Mr. George E. Papanikolaou for excellent secretarial assistance.

REFERENCES

1. Bleyer, W.A. The clinical pharmacology of methotrexate: new applications of an old drug. Cancer 1978, 41, 36-51.
2. Nyfors, A. Methotrexate therapy of psoriasis. Dan Med Bull 1980, 27, 74-96.
3. Black, R.L., O'Brien, W.M., Van Scott, E.J., Averbach, R., Eisen, A.Z., Bunim, J.J. Methotrexate therapy in psoriatic arthritis. JAMA 1964, 189, 743-747.
4. Owen, E.T., Cohen, M.L. Methotrexate in Reiter's disease. Ann Rheum Dis 1979, 38, 48-50.
5. Bookbinder, S.A., Espinosa, L.R., Fenske, N.A., Germain, B.F., Vasey, F.B. Methotrexate: its use in the rheumatic diseases. Clin Exp Rheumatol 1984, 2, 185-193.
6. Gubner, R., August, S., Ginsburg, V. Therapeutic suppression of tissue reactivity. II. Effect of aminopterin in rheumatoid arthritis and psoriasis. Am J Med Sci 1951, 221, 176-182.
7. Willkens, R.F., Watson, M.A., Paxson, C.S. Low dose pulse methotrexate therapy in rheumatoid arthritis. J Rheumatol 1980, 7, 501-505.
8. Steinsson, K., Weinstein, A., Korn, J., Abeles, M. Low dose methotrexate in rheumatoid arthritis. J Rheumatol 1982, 9, 860-866.
9. Thompson, R.N., Watts, C., Edelman, J., Esdaile, J., Russell, A.S. A controlled two centre trial of parenteral methotrexate therapy for refractory rheumatoid arthritis. J Rheumatol 1984, 11, 760-763.
10. Willkens, R.F. Short-term efficacy of methotrexate in the treatment of rheumatoid arthritis. J Rheumatol 1985, Suppl. 12, 21-24.
11. Weinblatt, M.E., Coblyn, J.S., Fox, D.A., Holdsworth, D.E., Glass, D.N., Tzentham, D.E. Efficacy of low dose methotrexate in rheumatoid arthritis. N Engl J Med 1985, 302, 818-822.
12. Williams, H.J., Willkens, R.F., Samuelson, C.O.Jr., et al. Comparison of low dose oral pulse methotrexate and placebo in the treatment of rheumatoid arthritis: a controlled clinical trial. Arthritis Rheum 1985, 28, 721-730.
13. Hamdy, H., McKendry, R.J.R., Mierins, E., Liver, J.A. Low dose methotrexate compared with azathioprine in the treatment of rheumatoid arthritis. Arthritis Rheum 1987, 30, 361-368.
14. Andersen, P.A., West, S.G., O'Dell, J.R., Via, C.S., Claypool, R.G., Kotzin, B.L. Weekly pulse methotrexate in rheumatoid arthritis. Ann Intern Med 1985, 103, 489-496.
15. Boerbooms, A.M.Th., Jeurissen, M.E.C., Westgeest, A.A.A., Theunisse, H., Van de Putte, L.B.A. Methotrexate in refractory rheumatoid arthritis. Clin Rheumatol 1988, 7, 249-256.
16. Weinstein, A., Marlowe, S., Korn, J., Farouhan, F. Low dose methotrexate treatment of rheumatoid arthritis. Long term observations. Am J Med 1985, 79, 331-337.
17. Kremer, J.M. Long term methotrexate therapy in rheumatoid arthritis: A review. J Rheumatol 1985, Suppl. 12, 25-28.
18. Kremer, J.M., Lee, J.K. The safety and efficacy of the use of methotrexate in long-term therapy for rheumatoid arthritis. Arthritis Rheum 1986, 29, 822-831.
19. Nordstrom, D.M., West, S.G., Andersen, P.A., Sharp, J.T. Pulse methotrexate therapy in rheumatoid arthritis. Ann Intern Med 1987, 107, 797-801.
20. Tishler, M., Caspi, D., Rosenbach, T.O., et al. Methotrexate in rheumatoid arthritis: a prospective study in Israeli patients with immunogenetic correlations. Ann Rheum Dis 1988, 47, 654-659.
21. Fehlauer, C.S., Carson, C.W., Cannon, G.W., et al. Methotrexate therapy in rheumatoid arthritis: 2-year retrospective follow-up study. J Rheumatol 1989, 16, 307-312.
22. Aponte, J., Pertrelly, M. Histophathologic findings in the liver of rheumatoid arthritis patients treated with long-term bolus methotrexate. Arthritis Rheum 1988, 31, 1457-1464.
23. Kremer, J.M., Lee, R.G., Tolman, K.G. Liver histology in rheumatoid arthritis patients receiving long-term methotrexate therapy. Arthritis Rheum 1989, 32, 121-127.
24. Arnett, F.C., Edworthy, S.M., Bloch, D.A., McShane, D.J., Fries, J.F., Cooper, N.S., Healey, L.A., Kaplan, S.R., Liang, M.H., Luthra, H.S., Medsger, T.A.Jr., Mitchell, D.M., Neustadt, D.H., Pinals, R.S., Schaller, J.G., Sharp, J.T., Wilder, R.L., Hunder, G.G. The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. Arthritis Rheum 1988, 31, 315-324.
25. Pinals, R.S., Masi, A.T., Larsen, R.A. Preliminary criteria for clinical remission in rheumatoid arthri-

tis. Arthritis Rheum 1981, 24, 1308-1315.
26. Evans, W.E., Cristensen, M.L. Drug interactions with methotrexate. J Rheumatol 1985. Suppl. 12, 15-20.
27. Roenigk, H.H.Jr., Aurbach, R., Maibach, H.J., Weinstein, G.D. Methotrexate guidelines revised. J Am Acad Dermatol 1982, 6, 145-155.
28. Steinbrocker, O., Traeger, C.H., Batterman, R.C. Therapeutic criteria in rheumatoid arthritis. JAMA 1949, 140, 651-662.
29. Andonopoulos, A.P., Drosos, A.A., Skopouli, F.N., Acritidis, N.C., Moutsopoulos, H.M. Secondary Sjögren's syndrome in rheumatoid arthritis. J Rheumatol 1987, 14, 1098-1103.
30. Urowitz, M.B., Gordon, D.A., Smythe, H.A., Pruzanski, W., Ogryzlo, M.A. Azathioprine in rheumatoid arthritis: a double-blind cross-over study. Arthritis Rheum 1973, 16, 411-419.
31. Berry, H., Liyanage, S.P., Durance, R.A., Barnes, C.G., Berger, L.A., Evans, S. Azathioprine and penicillamine in treatment of rheumatoid arthritis: a controlled trial. Br Med J 1976, 1, 1052-1054.
32. Woodland, J., Chaput de Saintonge, D.M., Evans, S.J.W., Sharman, V.L., Currey, H.F. Azathioprine in rheumatoid arthritis: double-blind study of full versus half doses versus placebo. Ann Rheum Dis 1981, 40, 359-368.
33. Penn, I. Occurrence of cancer in immune deficiencies. Cancer 1974, 34, 858-866.
34. Coban, C.D., Sheon, R.P., Kirsner, A.B. Immunosuppressive drugs and acute leukemia. Ann Intern Med 1973, 79, 131-132.
35. Cooperative Clinics Committee of the American Rheumatism Association: A controlled trial of cyclophosphamide in rheumatoid arthritis. N Engl J Med 1970, 283, 883-889.
36. Townes, A.S., Sowa, J.M., Shulman, L.E. Controlled trial of cyclophosphamide in rheumatoid arthritis. Arthritis Rheum 1976, 19, 563-573.
37. Lidsky, M.D., Sharp, J.T., Billings, S. Double-blind study of cyclophosphamide in rheumatoid arthritis. Arthritis Rheum 1973, 16, 148-153.
38. Wall, R.L., Clansen, K.P. Carcinoma of the urinary bladder in patients receiving cyclophosphamide. N Engl J Med 1975, 293, 271-273.
39. Tannenbaum, H., Schur, P.H. Development of reticulum cell sarcoma during cyclophosphamide therapy. Arthritis Rheum 1974, 17, 15-18.
40. Love, R.R., Sowa, J.M. Myelomonocytic leukemia following cyclophosphamide therapy of rheumatoid disease. Ann Intern Med 1977, 86, 199-204.
42. Zachariae, H., Kragballe, K., Sogaard, H. Methotrexate-induced liver cirrhosis: studies including serial liver biopsies during continued treatment. Br J Dermatol 1980, 102, 407-412.
43. Robinson, J.K., Baughman, R.D., Auerbach, R., Cimis, R.J. Methotrexate hepatotoxicity in psoriasis; consideration of liver biopsies at regular intervals. Arch Dermatol 1980, 116, 413-415.
44. Weinblatt, M.E. Toxicity of low dose methotrexate in rheumatoid arthritis. J Rheumatol 1985, Suppl. 12, 35-39.
45. Gispen, J.S., Alarcon, G.S., Johnson, J.J., Acton, R.T., Barger, B.O., Koopman, W.J. Toxicity to methotrexate in rheumatoid arthritis. J Rheumatol 1987, 14, 74-79.
46. Tolman, K.G., Clegg, D.O., Lee, R.G., Ward, J.R. Methotrexate and the liver. J Rheumatol 1985, Suppl. 12: 29-34.
47. Furst, D.E., Koehnke, R., Burmeister, L.F., Kohler, J., Cargill, I. Increasing methotrexate effect with increasing dose in the treatment of resistant rheumatoid arthritis. J Rheumatol 1989, 16, 313-320.