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REFERENCES

1. Beral V, Booth M. Predictions of cervical cancer incidence and mortality in England and Wales. Lancet 1986; i: 495.
2. Gissman L, Wolnik L, Ikenberg H, Koldovsky U, Schnurch HG, ZurHausen H. Human papillomavirus type 6 and 11 DNA sequences in genital and laryngeal papillomas and in some cervical cancers. Proc Natl Acad Sci USA 1983; 80: 560–563.
3. McCance DJ, Campion MJ, Clarkson PK, Chesters PM, Jenkins D, Singer A. The presence of human papillomavirus type 16 DNA sequences in cervical intraepithelial neoplasia and invasive carcinoma. Br J Obstet Gynaecol 1985; 92: 1101–1105.
4. McCance DJ. Human papillomavirus and cancer. Biochim Biophys Acta 1986; ii: 237–240.
5. Campion MJ, McCance DJ, Cuzick J, Singer A. The progressive potential of mild cervical atypia: a prospective cytological, colposcopic and virological study. Lancet 1986; ii: 237–240.
6. Scheurlen W, Gissman L, Gross G, ZurHausen H. Molecular cloning of two new HPV types (HPV 37 and HPV 38) from a kerato-acanthoma and a malignant melanoma. Int J Cancer 1986; 37: 505–510.
7. Lewis FA, Griffiths S, Dunnicliff R, Wells M, Dudding N, Bird CC. Sensitive in situ hybridisation technique using biotin–streptavidin–polyalkaline phosphatase complex. J Clin Path 1987; 40: 163–166.
8. Lee JAH, Peterson GR, Stevens RG, Vesanen, K. The influence of age, year of birth and date on mortality from malignant melanoma in the populations of England and Wales, Canada and the white populations of the United States. Am J Epidemiol 1979; 110: 734–739.
9. McKie RM, Soutar DS, Watson ACH et al. Malignant melanoma in Scotland 1979–83. Lancet 1985; ii: 859–862.
10. Morson BC, Volkstadt H. Malignant melanoma of the anal canal. J Clin Pathol 1963; 16: 126–131.
11. Hartveit F, Maehle BO, Skaarland E, Sandstad E, Lisaeth T. Acta Derm Venereol (Stockh) 1988; 68: 144–148.
12. Milton GW, Shaw HM, McCarthy WH. Resection margins for melanoma. Aust NZ J Surg 1985; 55: 225–226.

Serum Aminoterminal Propeptide of Type III Procollagen

A Non-invasive Test for Liver Fibrogenesis in Methotrexate-treated Psoriatics

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Serum aminoterminal propeptide of type III procollagen (PIIINP) was studied in 73 psoriatics receiving methotrexate and in 11 selected for trial with methotrexate or etretinate. 72 of the patients on methotrexate were also investigated with liver biopsies. The highest PIIINP value was found in a patient with ascites and her PIIINP decreased after medication was discontinued. Psoriatics with fibrosis or cirrhosis in their liver biopsies had a significantly higher mean PIIINP than patients without fibrosis, who had the same mean value as psoriatics prior to treatment. Based upon the individual data together with data from serial PIIINP investigations of 11 patients studied during treatment, it is concluded that PIIINP can be utilized as a valuable non-invasive test for liver fibrogenesis in methotrexate-treated psoriatics. PIIINP is not specific for the liver, but the study indicates that the number of liver biopsies can be reduced in psoriatics on methotrexate who have normal levels of PIIINP.

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The recent accumulation of new knowledge concerning the structure and immunochemistry of connective tissue constituents has led to the development of non-invasive assays of connective tissue metabolism. Radio-immunoassays of the aminoterminal propeptide of type III procollagen (PIIINP) have been proposed as means of clinical chemical diagnosis and follow-up of fibrotic liver disease (1, 2). In collaboration with the researchers who developed a new rapid equilibrium type of radio-immunoassay for this purpose (3), we have recently published preliminary results of investigations on methotrexate-induced liver fibrosis and cirrhosis (4). This study indicated that analyses of PIIINP in serum could be utilized as a valuable non-

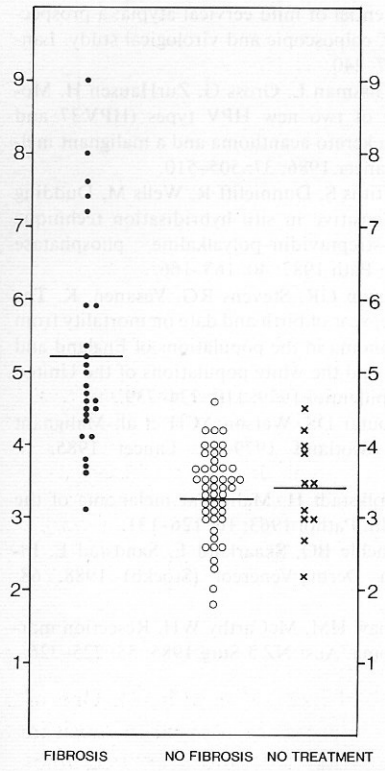

Fig. 1. Serum PIIINP levels in MTX-treated psoriatics with fibrosis and/or cirrhosis in their liver biopsies, MTX-treated psoriatics without liver fibrosis, and psoriatics investigated prior to MTX. All values are in μg/l.

invasive marker of fibrogenesis in liver in these patients.

The present report is a follow-up of that study, including data performed by one of us (L. H.) using the now commercially available kit from FARMOS Diagnostica, Oulunsalo, Finland. The study was performed on serum from psoriatics receiving methotrexate (MTX) or selected for trial with MTX or etretinate.

PATIENTS AND METHODS

Seventy-three adult psoriatics receiving MTX and 11 patients selected for trial with MTX or etretinate were studied. 72 of the 73 patients had been investigated with liver biopsies, while the 11 patients studied prior to treatment, according to the procedures of our Department, have no biopsies taken until the final decision for continuing long-term treatment is taken.

The liver biopsies were performed at intervals of 1–2 years. The histological specimens were obtained by the Menghini technique. All biopsies were stained with hematoxylin-eosin, periodic acid-Schiff reagent, van Gieson's stain, Masson's trichromic stain, with Perl's method, and reticular fibre stain *a.m.* Foot. The diagnosis of fibrosis or cirrhosis was made by the pathologist (H. S.) without knowledge of the laboratory data. In none of the patients had initial liver biopsies, taken prior to or earlier in treatment, shown any cirrhosis or more than slight fibrosis. The biopsy taken closest to the time of the first measurement of PIIINP was used in the present study.

Serum PIIINP levels were measured by the radio-immunoassay based on the human propeptide (5). The reference range based on healthy Finnish blood donors ($n=88$) is 1.7–4.2 μg/l; similar results on healthy Danish controls were 2.3–4.6 μg/l ($n=39$). The 95% specific cut-off levels were 2.1–4.3 μ/l. Serial measurements were performed on 9 of the psoriatics receiving MTX. One of the patients with psoriasis, who developed ascites and highly elevated liver transaminases and alkaline phosphatases during MTX medication, was studied, although no liver biopsy was available due to the patient's refusal to have this investigative procedure performed. In this case PIIINP was measured immediately before and 3 weeks after discontinuation of the drug.

RESULTS

The psoriatics with fibrosis or cirrhosis in their liver biopsies had a mean PIIINP value of 5.2 ± 1.5 (S.D.) μg/l. This differed significantly ($p<0.001$) from PIIINP in psoriatics with liver biopsies without fibrosis, who had an average PIIINP of 3.3 ± 0.6 μg/l and from psoriatics investigated prior to MTX with an average PIIINP of 3.4 ± 0.7 μg/l. The individual data appear from Fig. 1. These data include the results of the already published preliminary study (4), and together with the results of the serial investigations represent our present total experience on PIIINP in psoriasis.

The data from the serial investigations appear from Fig. 2. The highest value of the study (13.5 μg/l) was found in the patient who developed ascites during MTX, but refused to have a liver biopsy performed. Her values were reduced to 8.6 μg/l 3 weeks after discontinuing the drug. One patient with fibrosis who showed two PIIINP values which were normal had developed a MTX-induced liver cirrhosis in 1977, but since 1983 his biopsies have been without manifest cirrhosis (5) following reduction in MTX and probably reduced alcohol intake. The remaining 7 patients had all liver biopsies without fibrosis or cirrhosis prior to or at the time of their first PIIINP investigation. Two of these patients had determinations above the upper reference level. One of them received a combination of MTX and etretinate, which seems more hepatotoxic than when each drug is taken alone (6).

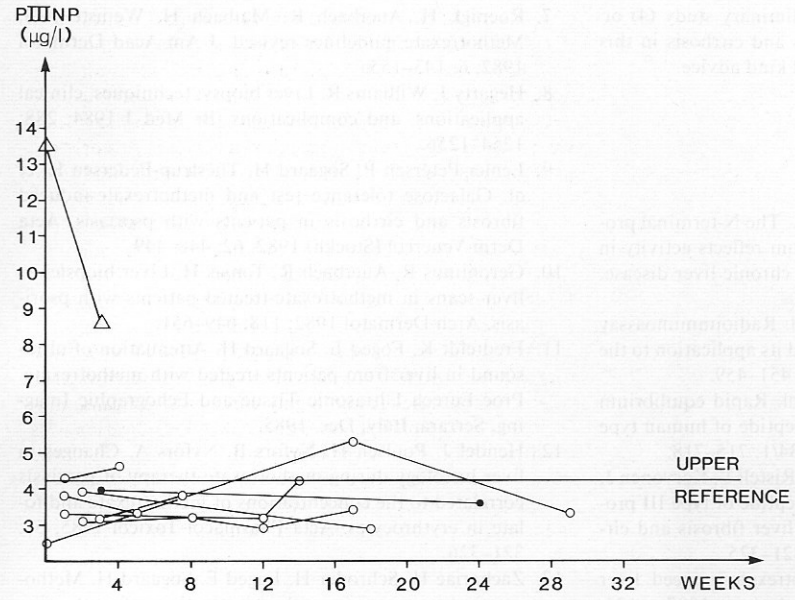

Fig. 2. Serial serum PIIINP levels from 11 psoriatics receiving MTX. The highest values were found in a patient with ascites. Her second investigation was performed 3 weeks after discontinuing the drug.

DISCUSSION

Liver biopsy is the most reliable method of assessing long-term MTX-induced liver damage, and liver biopsies are recommended in the control of MTX-treated psoriatics (7). Obtaining percutaneous liver specimens is, however, an uncomfortable procedure, associated with a certain morbidity (8). This has led to several attempts to test non-invasive procedures (9–13), but none of the tests has been sufficiently successful as to replace histology.

The present investigation indicates that PIIINP can be utilized to obtain a more dynamic picture of the processes, which may lead to hepatic fibrosis in patients on MTX. Our data also suggest that the number of liver biopsies can be reduced significantly in psoriatics—as long as they have normal PIIINP values.

The present data are consistent with our preliminary investigation as well as with previous studies on other precirrhotic liver diseases (1, 2). It should be stated that there are no organ-specific steps in the collagen metabolic pathway, and since PIIINP cannot be specific for liver fibrosis, one should be cautious when drawing conclusions in individual cases.

PIIINP cannot directly reflect the histology of the liver. But, as was demonstrated in our earlier studies (4), the more severe the liver damage is, the higher were the propeptide concentrations found in serum. It should be noted, however, that we have found a small number of liver biopsies with fibrosis in patients with normal PIIINP values (Fig. 1). One of these patients had, as already mentioned, cirrhosis in 1977, but no cirrhosis and less fibrosis in serial biopsies since 1983 after reducing his MTX intake and probably also his alcohol intake (5). This may reflect the reduced fibrogenesis.

The highest PIIINP value was found in a patient with ascites, and her PIIINP decreased (Fig. 2) together with her laboratory data on alkaline phosphatases and serum transaminases after discontinuing MTX. Also these results may illustrate the dynamic picture obtained by PIIINP.

On the basis of the present study and in accordance with our already published data (4) it is our conclusion that psoriatics on long-term MTX medication should still have a liver biopsy performed—at least when their total cumulative dose of MTX exceeds 1.5 g (5) but that thereafter subsequently, if no significant fibrosis has appeared, and as long as PIIINP is normal, liver biopsies may be reduced to a minimum. As long as PIIINP is normal there seems to be no great risk of developing fibrosis or cirrhosis. We propose that PIIINP should be investigated 3 to 4 times a year.

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REFERENCES

1. Frei A, Zimmerman A, Weigand K. The N-terminal propeptide of collagen type III in serum reflects activity in degree of fibrosis in patients with chronic liver disease. Hepatology 1984; 4: 830–834.
2. Rohde H, Vargas L, Hahn E, et al. Radioimmunoassay for type III procollagen peptide and its application to the disease. Eur J Clin Invest 1979; 9: 451–459.
3. Risteli J, Niemi S, Trivedi P, et al. Rapid equilibrium assay for the amino-terminal propeptide of human type III procollagen. Clin Chem 1988; 34/1: 715–718.
4. Risteli J, Søgaard H, Oikarinen A, Risteli L, Karvonen J, Zachariae H. Aminoterminal propeptide of type III procollagen in methotrexate-induced liver fibrosis and cirrhosis. Br J Dermatol 1988; 119: 321–325.
5. Zachariae H, Søgaard H. Methotrexate-induced liver cirrhosis—a follow-up. Dermatologica 1987; 175: 161–165.
6. Zachariae H. Dangers of methotrexate/etretinate combination therapy. Lancet 1988; i: 422.
7. Roenigk H, Auerbach R, Maibach H, Weinstein G. Methotrexate guidelines revised. J Am Acad Dermatol 1982; 6: 145–155.
8. Hegarty J, Williams R. Liver biopsy: techniques, clinical applications, and complications. Br Med J 1984; 288: 1254–1256.
9. Lenler-Petersen P, Søgaard H, Thestrup-Pedersen K, et al. Galactose tolerance test and methotrexate-induced fibrosis and cirrhosis in patients with psoriasis. Acta Derm Venereol (Stockh) 1982; 62: 448–449.
10. Geronimus R, Auerbach R, Tobias H. Liver biopsies v. liver scans in methotrexate-treated patients with psoriasis. Arch Dermatol 1982; 118: 649–651.
11. Fredfeldt K, Foged E, Søgaard H. Attenuation of ultrasound in liver from patients treated with methotrexate. Proc Eurech Ultrasonic Tissue and Echographic Imaging, Serrara, Italy, Dec. 1985.
12. Hendel J, Poulsen H, Nyfors B, Nyfors A. Changes in liver histology during methotrexate therapy of psoriasis correlated to the concentrations of methotrexate and folate in erythrocytes. Acta Pharmacol Toxicol 1985; 56: 321–326.
13. Zachariae H, Schrøder H, Foged E, Søgaard H. Methotrexate hepatotoxicity and concentrations of methotrexate and folate in erythrocytes—relation to liver fibrosis and cirrhosis. Acta Derm Venereol (Stockh) 1987; 67: 336–340.

Trichophyton Rubrum Abscesses in Immunocompromized Patients

A Case Report

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A 72-year-old immunocompromised man with myelodysplastic syndrome who developed multiple erythematous, scaly abscesses like lesions on his left foot and lower leg is described. He also had dry scaly lesions on his soles and lesions on several toe nails. A punch biopsy showed abscesses with fungal elements and *Trichophyton rubrum* was cultured from skin scales and the biopsy. A diagnosis of *T. rubrum* abscesses should be suspected in all immunocompromised patients with signs of superficial dermatophyte infection.

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Infections with *Trichophyton rubrum* are usually localized to the stratum corneum, hairs and nails. Deep infection with *T. rubrum* has been described in immunocompromised patients. Meinhoff describes multiple *T. rubrum* abscesses as a type of dermatophytosis distinguishable clinically and histopathologically from Kerion celsi and Majocchi's granuloma (1). Subsequently other cases have been described in the literature (2, 3).

CASE REPORT

A 72-year-old man, in whom pancytopenia was diagnosed in December 1984, is presented. The bone marrow was hypocellular in repeated biopsies, and he was initially considered as a case of aplastic anaemia. Blood transfusions had to be given at 4–8 week intervals; otherwise he remained general good health for nearly 3 years. In May 1985, non-A, non-B hepatitis was diagnosed.

When the patient was followed with repeated bone marrow examinations, his cellularity eventually increased and he became hypercellular. He developed morphologic abnormali-