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Comparison of serum and cerebrospinal fluid levels of methotrexate in man during high-dose chemotherapy for aggressive non-Hodgkin's lymphoma*

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Summary. The relationship between plasma and cerebrospinal fluid levels of methotrexate was studied in five patients, four with aggressive non-Hodgkin's lymphoma and one with mixed epithelial mesothelial tumour, who were treated with high-dose methotrexate (1.5 g/m^2) as part of combination chemotherapy. Cerebrospinal fluid was sampled for 24 h via a permanent indwelling lumbar catheter. No complications were observed with this technique.

In two patients with central nervous system involvement adequate "cytotoxic" levels ($>10^{-6}$ *M*) were obtained for greater than 12 h. The remaining three patients, with no direct evidence of central nervous system involvement, never attained adequate cytotoxic methotrexate levels in the cerebrospinal fluid. Serum levels were therapeutic in all patients.

These results suggest that patients with central nervous system tumour involvement may receive adequate doses of methotrexate in the cerebrospinal fluid. Patients with occult central nervous system tumour involvement may not attain adequate cerebrospinal fluid levels. A 24-h serum methotrexate level of $>10^{-5}$ *M* may indicate that patients have achieved therapeutic cerebrospinal fluid levels of methotrexate. Cranial irradiation following chemotherapy is still recommended in this tumour group until adequate cytotoxic levels of methotrexate can be obtained in all patients for prolonged periods.

Introduction

The treatment of advanced high-grade non-Hodgkin's lymphoma has undergone many changes in the last decade [9, 22]. The recent intensification of combined-modality therapy in high-grade non-Hodgkin's lymphoma was initiated to lengthen the duration of remission and to prevent central nervous system (CNS) relapse. There is considerable evidence [12, 24] that such relapse is one of the causes of recurrent disease in this patient group. The addition of high-dose methotrexate (MTX) to recent regimens [9] has been suggested, since it has been shown to pass into the CNS, which appears to act as a sanctuary site for malignant lymphoma.

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Methotrexate enters cells by passive diffusion and the rate is therefore dependent on the concentration of the MTX in the plasma. Tissue concentrations may also be affected by other drugs, e.g., vincristine, steroids, cephalosporins [12]. Several workers have studied the concentration of MTX in the cerebrospinal fluid (CSF) after bolus and infusions of MTX [3, 7]. Their studies yielded varied results, but indicated that the greater the infusion dose of MTX the higher the MTX concentration in the CSF. The achievement of high levels of MTX for longer periods of time in the CNS has direct therapeutic implications and could negate the need for prophylactic cranial irradiation [9].

This paper compares serum and CSF levels of MTX in five patients receiving combination chemotherapy, in each of whom an *indwelling CSF catheter* was used to measure the extent of MTX penetration into the CSF.

Patients and methods

Patients. Four patients with-high grade non-Hodgkin's lymphoma and one with a rare epithelioid tumour met the selection criteria for the study (see Table 1). The cytotoxic regimen was the same for all patients. The study was performed 10 days after receiving IV doxorubicin (40 mg/m^2), etoposide (200 mg/m^2), and cyclophosphamide (750 mg/m^2); IM bleomycin (15 mg); and 5 days of oral prednisolone (100 mg/day). On the study day patients received IV vincristine 2 mg, MTX (bolus) 300 mg/m^2, and MTX (12 h IV infusion) 1200 mg/m^2 and IM bleomycin 15 mg at time zero. At 1 h prior to cytotoxic therapy, IV cannulae were inserted into both arms. One cannula was used for the administration of 3 l N saline per day and the other for blood sampling. Intravenous folinic acid (50 mg/m^2) rescue was commenced 24 h after the MTX bolus. This together with IV hydration was continued until the serum MTX level had fallen below 10^{-7} *M* (0.1 μ*M*).

Sample technique. Blood samples (10 ml) were obtained at 0, 5, 15, 30, 45, 60, 90, and 120 min and at 3, 4, 5, 6, 8, 12, 15, and 24 h. Serum was separated and stored at 4 °C until analysis. Matched CSF samples (0.7 ml) were withdrawn through a clear nylon catheter situated in the dorsolumbar subarachnoid space and were stored at 4 °C. The catheter was inserted under aseptic conditions with the patient in the left lateral position [14]. An 18-g Tochy needle (Epidural Minipack, Portex) was inserted in the L2-3 or L3-4 in-

Table 1. Patients' clinical, histological and biochemical data

Patient	Histology	Malignant Cells in DSF	Age (years)	Sex	Weight (kg)	Creatinine clearance ml/min	MTX dose (mg)	
							Bolus	Infusion
1. EC	Histiocytic lymphoma	−ve	49	F	36	108	390	1560
2. FW	Diffuse centroblastic lymphoma	−ve (cranial nerve involvement)	48	M	87	109	450	1800
3. MK	Diffuse centroblastic lymphoma	+ve	57	F	70	91	510	2000
4. LMcG	Epithelial/mesothelial mediastinal tumour	−ve	28	F	46	70	420	1680
5. ET	Diffuse centroblastic lymphoma	−ve	54	F	70	87	525	2100

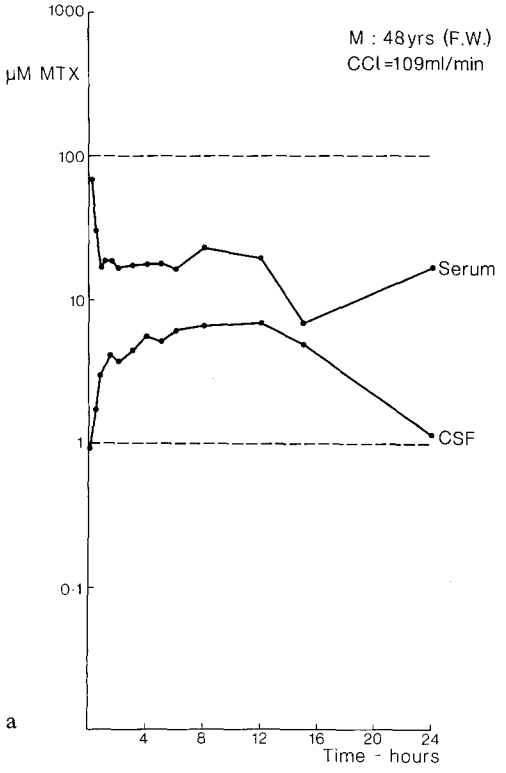
terspace and advanced until the sensation of dural puncture was felt. When CSF flow was confirmed the catheter was passed through the needle for 10 cm in a cephalad direction. A three-way tap was connected and the catheter was taped securely to the skin. The CSF catheter was left in situ for 24 h. Samples of CSF were also taken, on insertion of catheter and prior to its removal, for microscopy, culture, and cytology.

Assay procedure. Serum and CSF MTX was determined by the enzyme-multiplied immunoassay technique (EMIT) (Syva, Palo Alto, Calif, USA). The coefficient of variation of the assay was ±6% for both serum and CSF samples at a level of 10^{-6} *M*.

Results

Clinical and biochemical data on the patients are noted in Table 1. The serum and CSF data for 24 h are shown in Figs. 1 and 2. All patients attained initial serum levels of approximately 10^{-4} *M* (100 μ*M*). In only two patients did levels remain above 10^{-5} *M* for 24 h. In the remaining three patients levels of less than 10^{-5} *M* were achieved for a period of 12 h.

In only two patients (FW, MK) did CSF MTX levels reach adequate levels i.e. $> 10^{-6}$ (Fig. 1). These patients also demonstrated sustained serum levels of $> 10^{-5}$ *M* for 24 h. This effect appeared to be independent of age, sex, weight, creatinine clearance, and MTX dose. Both these



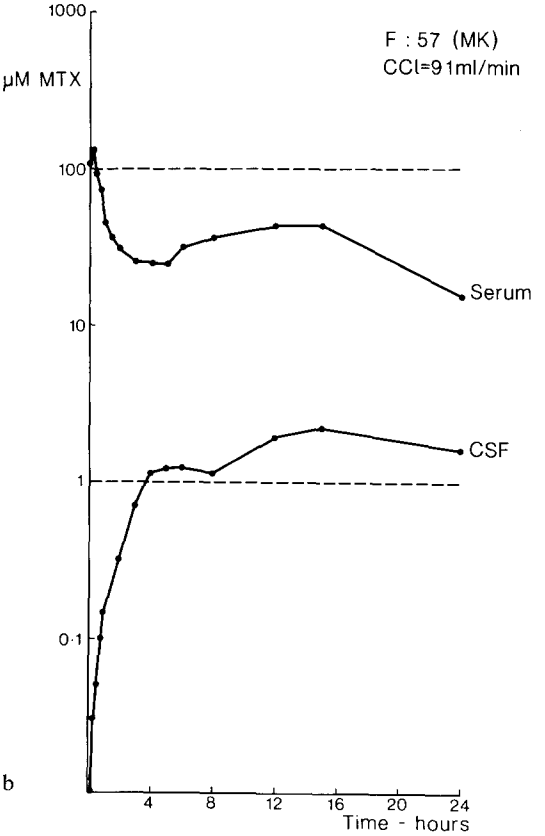
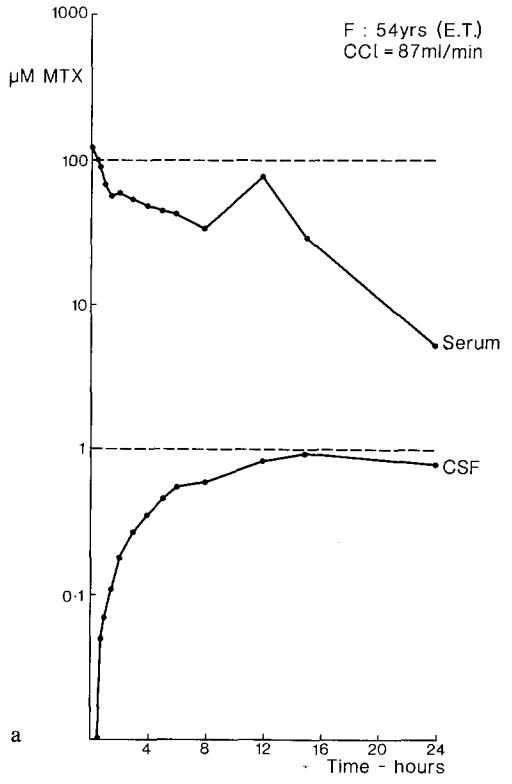
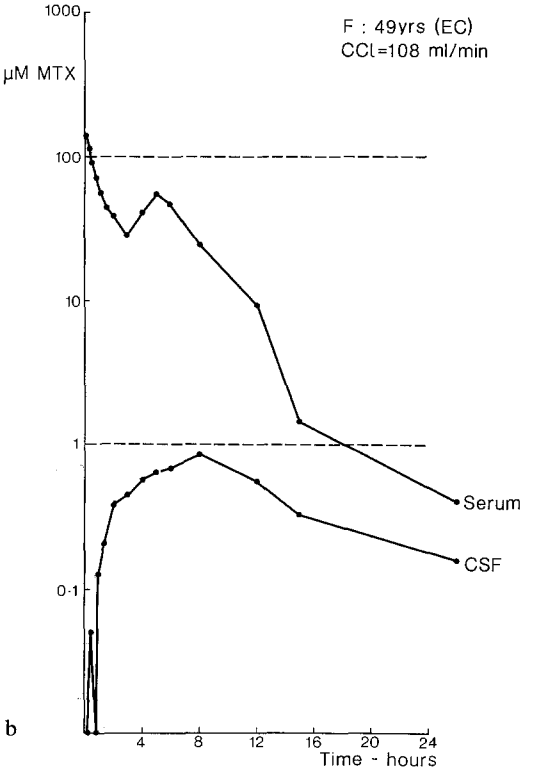


Fig. 1a, b, Serum and CSF levels of MTX in patients FW **(a)** and MK **(b)** in whom CSF levels exceeded 1 μ*M* (10^{-6} *M*) and were maintained above 1 μ*M* for up to 24 h and serum levels were above 10 μ *M* at 24 h





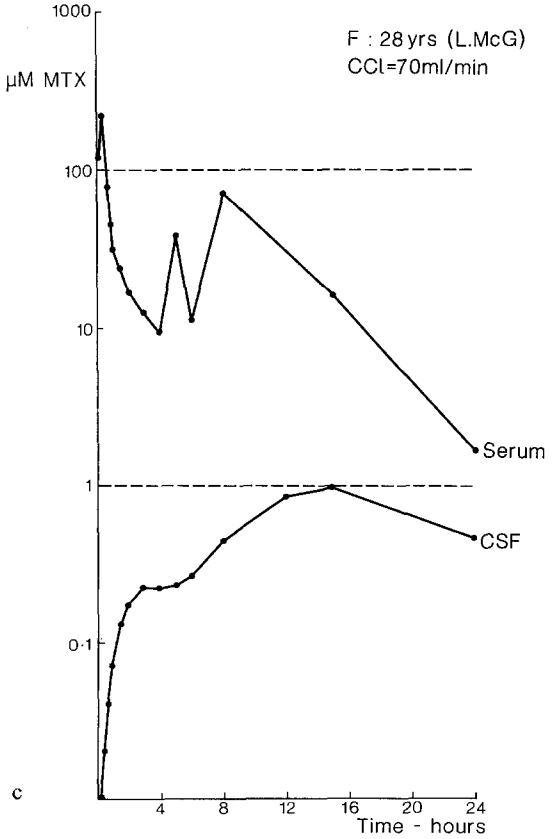


Fig. 2a–c. Serum and CSF levels of MTX in patients ET **(a)**, EC **(b)**, and LMcG **(c)**, in whom CSF levels failed to exceed $>1\ \mu M$ at any time

patients had evidence of CNS involvement. The remaining patients with no evidence of CNS involvement failed to obtain satisfactory CSF levels, although their plasma levels during the infusion were satisfactory (Fig. 2).

No adverse side effects from the CSF catheter were noted. One patient developed headache 4 days after removal of the catheter, which resolved after 24 h of bed rest. Microbiology and culture of CSF were negative at the time of insertion and immediately before catheter removal. No subsequent episodes of meningitis have been observed over an 8-month period.

Discussion

This study of MTX CSF levels in patients with aggressive non-Hodgkin's lymphoma demonstrated the uncertainty of CNS prophylaxis with this high-dose MTX regimen. Involvement of the CNS with lymphoma or CNS relapse is not uncommon. In high-grade aggressive lymphoma up to 20% of patients can have CNS involvement at different stages of the disease [12, 24]. Bone marrow involvement by tumour is reported to indicate a higher risk of CNS involvement [24]. In our patients none had bone marrow involvement according to single marrow aspirate and trephine biopsy, but two had direct evidence of CNS involvement (FW, MK). This highlights the inaccuracy of bone marrow investigations, and the present need for CNS prophylaxis in all patients.

The minimum therapeutic concentration of methotrexate in body fluids of $10^{-6}\ M$ ($1\ \mu M$) was determined in human tumour in vitro [11, 13]. Other workers, using a murine tumour model [1] arrived at a minimum effective con-

centration of 10^{-8} *M*. However, in our study, as in others, we have accepted a minimum effective concentration of 10^{-6} *M* for inhibition of dihydrofolate reductase [11, 13]. Other factors, such as cell resistance [4] and duration of exposure, are important [13]. Brief periods of less than 6 h are reported to be less effective than those over 6 h, which places more emphasis on attaining a satisfactory cytotxic level for periods of greater than 6 h [6, 18].

The relationship between plasma and CSF MTX levels has been studied before, but not in aggressive non-Hodgkin's lymphoma. Shapiro et al. studied patients with meningeal leukaemia and carcinomatosis using a 24 h infusion of 500 mg/m^2 of MTX [20]. In spite of obvious CNS involvement, levels of just over 1×10^{-6} *M* were obtained in only a few patients. This work was confirmed by Freeman et al. [11] in patients with acute lymphocytic leukaemia (ALL), all of whom had levels lower than 10^{-6} *M*. This problem has been overcome by either lumbar or Ommaya reservoir injection of MTX either at the same time as or independently of MTX infusion. The lumbar method of injection has many drawbacks, including toxicity [15, 21] and poor cephalad distribution of MTX giving rise to low ventricular levels ($< 10^{-6}$ *M* [21], and Ommaya reservoirs are not practical for prophylaxis in patients with lymphomas. Higher dose infusions of 1000 mg/m^2 (MTX) have been used in children with ALL, but again poor CSF levels ($< 10^{-6}$ *M*) were obtained, which could be marginally improved with intrathecal MTX [7].

The MTX regimen reported here satisfies the high dose criterion [5] and compares with other studies in which similar or higher doses [23] were used and MTX levels of 1×10^{-6} *M* were obtained. The adequate CSF levels of MTX found in our two patients were also accompanied by high serum levels ($> 10^{-5}$ *M*) at 24 h. Evans et al. have recently reported that a delay in systemic clearance of MTX reduces the probability of relapse in children with ALL [8], and it is possible that high (10^{-5} *M*) serum levels of MTX at 24 h may act as a marker of adequate CNS prophylaxis in this group of lymphomas. Alteration of the blood-brain barrier is known to occur with CNS tumour [20] involvement, and drugs such as vincristine are also known to influence membrane permeability. This study differs from other reports [7, 11, 21] in the use of other drugs (vincristine, bleomycin) and a higher dose of MTX. In spite of this, no real differences in serum or CSF MTX levels were observed, suggesting that vincristine did not enhance CNS uptake and only a large increase in the MTX dosage could lead to adequate CSF levels.

This study highlights a number of points in the use of MTX for CNS prophylaxis. First, CNS involvement can be difficult to document by means of bone sampling techniques and CSF cytology. If there is significant involvement the CNS levels of MTX may be satisfactory. Secondly, there is some doubt about the therapeutic level of MTX [11] needed in the CSF. However, it is currently accepted that levels of 10^{-6} *M* are required for at least 6 h [6, 11, 13, 18]. It has been shown that nervous tissue cell levels are approximately the same as those in the CSF [17]. Despite this Fisher et al. describing their ProMACE and MOPP chemotherapy [9] with a similar MTX regimen to ours (1.5 mg/m^2) and accepting MTX's CNS penetration, still felt that the addition of prophylactic cranial irradiation was necessary to ensure a low rate of CNS relapse. Thirdly, since our results and those of others [11, 21] suggest that the passage of MTX is the CNS is dependent upon overt CNS involvement [20], those patients with minimal involvement are unlikely to achieve prophylactic levels. Fourthly, we have to ask whether the CSF levels can be increased either by increasing MTX dosage or adding such drugs as mannitol to increase CNS penetration. Finally, it appears that serum levels at 24 h after MTX delivery could be used as a guide to CNS levels [7, 8].

It is clear from our study that a proportion of our patients are not receiving adequate CNS MTX exposure and that MTX is thus not providing sufficient CNS prophylaxis. Recent reports have demonstrated pharmacological methods of altering the blood-brain barrier [16], and this may provide an alternative method of obtaining higher CSF drug levels. Until better CNS penetration is achieved all patients with aggressive non-Hodgkin's lymphoma treated with our regimen will need cranial irradiation to ensure adequate CNS prophylaxis.

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