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Area under the curve of methotrexate and creatinine clearance are outcome-determining factors in primary CNS lymphomas

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Although high-dose methotrexate (HD-MTX) is the most effective drug against primary CNS lymphomas (PCNSL), outcome-determining variables related to its administration schedule have not been defined. The impact on toxicity and outcome of the area under the curve (AUC_{MTX}), dose intensity (DI_{MTX}) and infusion rate (IR_{MTX}) of MTX and plasmatic creatinine clearance (CL_{crea}) was investigated in a retrospective series of 45 PCNSL patients treated with three different HD-MTX-based combinations. Anticonvulsants were administered in 31 pts (69%). Age >60 years, anticonvulsant therapy, slow IR_{MTX} ($\leqslant 800\,mg\,m^{-2}\,h^{-1}$), and reduced DI_{MTX} ($\leqslant 1000\,mg\,m^{-2}\,wk^{-1}$) were significantly correlated with low AUC_{MTX} values. Seven patients (16%) experienced severe toxicity, which was independently associated with slow CL_{crea}. A total of 18 (40%) patients achieved complete remission after chemotherapy, which was independently associated with slow CL_{crea}. In all, 22 patients were alive at a median follow-up of 31 months, with a 3-year OS of $40 \pm 9\%$; slow CL_{crea} and $AUC_{MTX} > 1100\,\mu mol\,h\,l^{-1}$ were independently associated with a better survival. Slow CL_{crea} and high AUC_{MTX} are favourable outcome-determining factors in PCNSL, while slow CL_{crea} is significantly related to higher toxicity. AUC_{MTX} significantly correlates with age, anticonvulsant therapy, IR_{MTX}, and DI_{MTX}. These findings, which seem to support the choice of an MTX dose $\geqslant 3\,g\,m^{-2}$ in a 4–6-h infusion, every 3–4 weeks, deserve to be assessed prospectively in future trials. MTX dose adjustments in patients with fast CL_{crea} should be investigated.
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High-dose methotrexate (HD-MTX) is the most effective drug against primary central nervous system lymphomas (PCNSL) (Reni *et al*, 1997; Ferreri *et al*, 2002b). Any chemotherapy regimen without HD-MTX is associated with outcomes no better than those obtained with radiotherapy (RT) alone in these malignancies (Schultz *et al*, 1996; O'Neill *et al*, 1999; Mead *et al*, 2000), while the survival benefit of the addition of other drugs to HD-MTX remains a matter of debate (Reni *et al*, 2001; Ferreri *et al*, 2002b). In spite of its central role in PCNSL treatment, the optimal dose, administration schedule, and dose timing of MTX have not been clearly defined. Moreover, contrary to what is observed in other malignancies in which MTX plays a crucial role, such as acute leukaemia (Evans *et al*, 1986) and osteosarcoma (Graf *et al*, 1994; Delepine *et al*, 1995; Bacci *et al*, 1998), where a significant association between MTX parameters and outcome has been reported, the impact on toxicity and outcome of MTX administration schedule has not been investigated in PCNSL.

The analysis of some MTX parameters, such as the area under the curve (AUC_{MTX}), dose, dose intensity (DI_{MTX}), and infusion rate (IR_{MTX}), as well as the plasmatic creatinine clearance (CL_{crea}) could allow us to identify subgroups of patients with increased risk of severe toxicity or disappointing outcome, as well as to define the optimal MTX administration schedule against PCNSL. This paper reports the analysis of the impact on toxicity and outcome of the above-mentioned variables in a retrospective multicentre series of 45 immunocompetent patients with PCNSL treated with HD-MTX-based primary chemotherapy.

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PATIENTS AND METHODS

Study population

A questionnaire requesting epidemiological, clinical, histopathological, therapeutic, and survival data of immunocompetent patients with PCNSL treated with primary chemotherapy containing HD-MTX ($\geqslant 1\,g\,m^{-2}$), followed or not followed by RT, was sent to the seven participating centres. In particular, the questionnaire included body weight and body surface area, creatinine clearance, theoretical and really administrated dose of MTX, duration of infusion, timing dose, and MTX serum levels at 0, 24, 48, and 72 h. The use of anticonvulsants, type and dose, was also analysed

considering the capacity of some of these drugs to interfere with MTX metabolism (Jacobs *et al*, 1976). This study conformed to the tenets of the Declaration of Helsinki and all the patients accessioned provided signed informed consent to the treatment. This consent extended to the use of biological, histopathological, radiological, biochemical, and clinical data for scientific purposes.

MTX variables

CL_{crea} value, determined before the start of chemotherapy, was obtained by the formula of Cockcroft and Gault (1976):

$$CL_{crea}(ml\,min^{-1}) = \frac{(140 - age) \times body\ weight}{creatinine\ serum\ level \times 72}$$

CL_{crea} value in females was considered as 85% of the value for males.

The MTX variables investigated were AUC_{MTX}, dose, DI_{MTX}, and IR_{MTX}. The individual AUC_{MTX} ($\mu mol\,h\,l^{-1}$) related to the first course of chemotherapy was determined according to a one-compartment model by using the statistical population pharmacokinetic program P-PHARM-Version 3 (InnaPhase, 77420 Champs-sur-Marne, France), considering MTX dosage and serum levels at 0, 24, 48, and 72 h after drug infusion for calculation. DI_{MTX}, expressed as $mg\,m^{-2}\,wk^{-1}$, was calculated by the Hryniuk method (Hryniuk and Goodyear, 1990). This was a ratio between the total dose of MTX administered ($mg\,m^{-2}$) and the treatment duration expressed in days divided by 7. Treatment duration was calculated from the first day of the first course to the 22nd or 29th day of the last course (respectively for regimens administered every 3 or 4 weeks) (Hryniuk and Goodyear, 1990). The IR_{MTX}, expressed as $mg\,m^{-2}\,h^{-1}$, was defined as the MTX dose ($mg\,m^{-2}$) administered per hour during the first chemotherapy course.

Statistical considerations

Correlations between AUC_{MTX} and the other variables were analysed by the Spearman test. The impact of studied variables on severe toxicity and complete response rate was analysed by logistic regression. Severe toxicity was defined by the onset of one of two major events: toxic death or interruption of chemotherapy due to toxicity. Complete response was defined as the disappearance of all evidence of lymphoma.

CL_{crea}, AUC_{MTX}, DI_{MTX}, and IR_{MTX} were firstly analysed as continuous variables; then, quartiles values were applied as cutoff to differentiate the risk groups (categorical variables): lower quartile for CL_{crea} ($85\,ml\,min^{-1}$) and upper quartile for DI_{MTX} ($1000\,mg\,m^{-2}\,wk^{-1}$), for AUC_{MTX} ($1100\,\mu mol\,h\,l^{-1}$), and for IR_{MTX} ($800\,mg\,m^{-2}\,h^{-1}$).

Survival curves were generated by the Kaplan–Meier method. The overall survival (OS) was calculated from diagnosis to the date of death or the last date of follow-up. Impact on survival of clinical and therapeutic variables was evaluated through the log-rank test. The independent prognostic value of variables was analysed using Cox proportional hazard model. All the probability values were two-sided. All the analyses were carried out using the Statistica 4.0 statistical package for Windows (Statsoft Inc, 1993, Tulsa, OK 74104, USA).

RESULTS

Study group

The study group consisted of 45 patients treated between 1995 and 2001 (Calderoni and Aebi 2002; Pasini *et al*. 2002; Ferreri *et al*. 2002a). Patients' characteristics and extent of disease at diagnosis are summarised in Table 1. Chemotherapy regimens and MTX administration schedules are reported in Table 2. All patients were treated with adequate pre-MTX hydration, urinary alkalinisation, and escalated leucovorin dosages according to MTX serum levels. Dehydration, aciduria, renal or cardiac dysfunction, pleural effusion, or gastrointestinal tract obstruction were excluded before commencing treatment in all cases. Post-chemotherapy RT, which

Table 1 Patients' characteristics and extension of disease at diagnosis

	Entire series
No.	45
Median age (range)	54 (25–76)
>70 years	2 (4%)
Males	29 (64%)
Performance status (ECOG score)	
0–1	19 (42%)
2	13 (29%)
3	11 (24%)
4	2 (4%)
Prior cancer[a]	2 (4%)
Histotype (REAL/WHO Classification)	
Diffuse large B-cell lymphoma	42 (93%)
Anaplastic large-cell Ki1 lymphoma	1 (2%)
Unclassified	2 (4%)
High LDH serum level[b]	13/38 (34%)
Intraocular disease[b]	1/40 (3%)
Positive CSF cytology examination[b]	2/27 (7%)
Elevated CSF protein levels[b,c]	13/19 (68%)
Multiple lesions[b]	25/45 (56%)
Involvement of deep structures[b,d]	25/44 (57%)

CSF = cerebrospinal fluid. [a]Prior cancers: renal cell carcinoma and Waldenstrom's macroglobulinaemia. [b]Ratio between the number of positive cases and the number of assessed patients. [c]The cutoff to define normal CSF protein levels was $45\,mg\,dl^{-1}$ in patients ⩽60 years and $60\,mg\,dl^{-1}$ in patients >60 years. [d]Deep structures of the brain: basal ganglia, corpus callosum, brain stem, and cerebellum. ECOG = Eastern Cooperative Oncology Group; LDH = Lactic Dehydrogenase; REAL = Reversed European American lymphoma.

Table 2 Chemotherapy regimens

Regimen	No. of patients	MTX dose ($mg\,m^{-2}$)	MTX infusion (h)	MTX dose day	Other drugs	i.t. CHT	Planned no. of courses	Courses every weeks
MTX alone (Calderoni and Aebi, 2002)	10	1000–3000 8000	4 24	1 and 7	—	Yes/no	2–3	4
MATILDE (Ferreri *et al*, 2002a)	11	3500	3[a]	1	AraC $2\,g\,m^{-2} \times 2\,d$ 2 IDA $15\,mg\,m^{-2} \times d$ 1 TTP $25\,mg\,m^{-2} \times d$ 3	No	3	4
MTX+AraC (Pasini *et al*, 2002)	24	1000–2000[b]	24	1	AraC $2\,g\,m^{-2} \times 2\,d$ 2–3	no	3	3

MTX = methotrexate; i.t. CHT = intrathecal chemotherapy; AraC = cytarabine; IDA = idarubicin; TTP = thiotepa. [a]Infusion preceded by an initial MTX bolus. [b]Four patients received $3500–8000\,mg\,m^{-2}$ in 24-h infusion. DI_{MTX} (mean ± s.d.) of the used chemotherapy regimens were 1638 ± 1642, 844 ± 180, and 780 ± 1080 $mg\,m^{-2}\,week^{-1}$ ($P = 0.01$), respectively, for HD-MTX alone, MATILDE, and MTX+Ara-C combination.

consisted of whole-brain irradiation, followed or not followed by a tumour-bed boost, was planned in all cases, but it was in fact performed as part of the first-line therapy in 31 patients, with median brain and tumour-bed doses of 36 ± 5 and 42 ± 9 Gy, and as part of salvage therapy in six cases. Anticonvulsants were administered in 31 patients (69%), and consisted of phenobarbital ($100-150\,mg\,d^{-1}$) in 27 cases, hydantoin ($100-300\,mg\,d^{-1}$) in two cases and carbamazepine ($400-800\,mg\,d^{-1}$) in two cases.

MTX parameters

The mean value $\pm$ s.d. of CL_{crea} was $119 \pm 57\,ml\,min^{-1}$. The mean value $\pm$ s.d. of AUC_{MTX} was $731 \pm 525\,\mu mol\,h\,l^{-1}$. Patients $\leqslant 60$ years old displayed a faster CL_{crea} (mean $\pm$ s.d.: 118 ± 38 *vs* $94 \pm 28\,ml\,min^{-1}$; $P = 0.01$), and a higher AUC_{MTX} (846 ± 562 *vs* $502 \pm 359\,\mu mol\,h\,l^{-1}$; $P = 0.02$) with respect to patients >60. According to the PS, patients with an ECOG score of 3–4 displayed a similar CL_{crea} (100 ± 28 *vs* $112 \pm 35\,ml\,min^{-1}$; $P = 0.17$) and a similar AUC_{MTX} (1397 ± 1208 *vs* $1238 \pm 836\,\mu mol\,h\,l^{-1}$; $P = 0.53$) with respect to patients with a PS of 0–2. The mean value $\pm$ s.d. of DI_{MTX} was $992 \pm 1140\,mg\,m^{-2}\,week^{-1}$. Seven patients (16%) received only one course of chemotherapy (severe toxicity in five, no response in two), 16 (36%) received two, 14 (31%) received three, and eight (18%) received more than three courses. No difference in the used MTX dose according to age or PS was observed; the proportion of patients $\leqslant 60$ years old and >60 treated with a dose $>3\,g\,m^{-2}$ was similar (47 *vs* 47%, $P = 0.99$); 53% of patients with PS 0–2 and 31% of patients with PS 3–4 received an MTX dose $>3\,g\,m^{-2}$ ($P = 0.19$). No cases of reduction of more than 25% of the MTX dose in further courses with respect to the planned dose were observed. The MTX dose of the further courses was increased by more than 25% with respect to the MTX dose of the first course in three (7%) cases. The mean value $\pm$ s.d. of IR_{MTX} during the first chemotherapy course was $475 \pm 423\,mg\,m^{-2}\,h$. This parameter remained unmodified during further courses in all cases.

AUC_{MTX}-determining variables

Variables significantly correlated with the AUC_{MTX} are reported in Table 3. Anticonvulsant use and age correlated inversely with AUC_{MTX}, while a direct correlation between AUC_{MTX} and IR_{MTX} and DI_{MTX} was observed. No correlation with sex, performance status (PS), and CL_{crea} was observed. Patients treated with MATILde chemotherapy regimen achieved significantly higher AUC_{MTX} values.

Severe toxicity

The predictive value of MTX variables on severe toxicity was analysed considering toxic death ($n = 2$) and interruption of chemotherapy due to toxicity ($n = 5$) as events. Severe toxicity consisted of pulmonary thromboembolism in two cases (lethal in both), sepsis in two, acute renal failure in one, and persistent grade IV thrombocytopenia in two. These events were observed during the first two courses of chemotherapy. As reported in Table 4, CL_{crea} was independently associated with severe toxicity; a significantly higher toxicity rate was observed in patients with a CL_{crea} $\leqslant 85\,ml\,min^{-1}$. Importantly, a DI_{MTX} $>1000\,mg\,m^{-2}$/week, and an AUC_{MTX} $>1100\,\mu mol\,h\,l^{-1}$ were not related to a higher toxicity.

Objective response

After primary chemotherapy, 18 patients (40%) achieved a complete remission and 16 (36%) a partial response (overall response rate = 76%); four patients (9%) had stable disease, five (11%) experienced progressive disease, and two (4%) died of toxicity. As reported in Table 4, a slow CL_{crea} ($\leqslant 85\,ml\,min^{-1}$) was significantly and independently associated with a higher complete remission rate.

Table 3 Correlations between AUC_{MTX} and the other analysed variables

Variables	Subgroups	No.	AUC_{MTX} (mean $\pm$ s.d. $\mu mol\,h\,l^{-1}$)	*P*
Age	$\leqslant 60$ years	30	846 ± 562	
	>60 years	15	502 ± 359	0.02
Sex	Females	16	540 ± 398	
	Males	29	837 ± 562	0.08
PS	0–1	19	927 ± 614	
	2–4	26	589 ± 405	0.08
Anticonvulsants	No	14	1028 ± 662	
	Yes	31	598 ± 394	0.04
IR_{MTX} ($mg\,m^{-2}\,h^{-1}$)	$\leqslant 800$	33	635 ± 564	
	>800	12	996 ± 274	0.003
DI_{MTX} ($mg\,m^{-2}\,week^{-1}$)	$\leqslant 1000$	33	559 ± 392	
	>1000	12	1206 ± 567	0.0003
CL_{crea} ($ml\,min^{-1}$)	$\leqslant 85$	12	604 ± 434	
	>85	33	778 ± 553	0.35
Chemotherapy regimen	HD-MTX	10	857 ± 658	
	MATILde	11	1043 ± 240	
	MTX+AraC	24	536 ± 491	0.008

PS = performance status according to the ECOG score.

Overall survival

A total of 26 patients experienced failure: early progression of the disease in nine cases, relapse after initial response (complete and partial) in 15 and toxic death in two cases, with a 2-year failure-free survival of $50 \pm 8\%$. In all, 22 patients are alive (19 NED) at a median follow-up of 31 months (range 4–72 months), with a 3-year OS of $40 \pm 9\%$. The cause of death was lymphoma in 20 cases, acute toxicity in two, and unrelated disorder in one.

Univariate analyses showed that patients with a slow CL_{crea} ($\leqslant 85\,ml\,min^{-1}$) survived longer than patients with a fast CL_{crea} ($>85\,ml\,min^{-1}$), with 3-year OS values of 88 ± 13 and $25 \pm 9\%$ ($P = 0.0005$), respectively (Figure 1). Patients treated with an AUC_{MTX} $>1100\,\mu mol\,h\,l^{-1}$ survived significantly longer than patients treated with lower levels (3-year OS: 78 ± 12 *vs* $32 \pm 9\%$; $P = 0.05$) (Figure 2). The use of anticonvulsants and IR_{MTX} was not associated with survival, and no significant difference in the efficacy of the used chemotherapy regimens was observed. Multivariate analysis (Table 5) confirmed the independent prognostic value of CL_{crea} and AUC_{MTX}.

DISCUSSION

The present study focused on the impact on toxicity and outcomes of CL_{crea}, AUC_{MTX}, DI_{MTX}, and IR_{MTX} in a multicentre retrospective series of 45 immunocompetent patients with PCNSL. This series is representative of PCNSL patients currently treated with HD-MTX-based chemotherapy, since it displays similar median age, PS distribution, histotypes, and ocular and meningeal infiltration rates with respect to more comprehensive unselected

Table 4 Logistic regression: variables correlated to severe toxicity ($n=7$) and complete remission rate ($n=18$) after primary chemotherapy

Variables	Subgroups	No.	Severe toxicity	*P*	Complete response	*P*
Age	⩽60 years	30	3 (10%)		11 (37%)	
	> 60 years	15	4 (27%)	0.28	7 (46%)	0.26
PS	0–1	16	2 (13%)		10 (62%)	
	2–4	29	5 (17%)	0.14[a]	8 (28%)	0.19
Anticonvulsants	No	14	1 (7%)		6 (43%)	
	Yes	31	6 (19%)	0.97	12 (39%)	0.51
IR_{MTX} (mg m^{-2} h^{-1})	⩽800	33	4 (12%)		15 (45%)	
	>800	12	3 (33%)	0.11	3 (25%)	0.49
DI_{MTX} (mg m^{-2} week^{-1})	⩽1000	33	6 (18%)		14 (42%)	
	>1000	12	1 (8%)	0.48	4 (33%)	0.52
CL_{crea} (ml min^{-1})	⩽85	12	4 (33%)		8 (67%)	
	>85	33	3 (9%)	0.05	10 (30%)	0.02
AUC_{MTX} (μmol h l^{-1})	⩽1100	34	6 (17%)		14 (41%)	
	>1100	11	1 (9%)	0.45	4 (36%)	0.95
Chemotherapy regimen	HD-MTX	10	0 (0%)		2 (20%)	
	MATILde	11	3 (27%)		3 (27%)	
	MTX+AraC	24	4 (17%)	0.25	13 (54%)	0.08

HD-MTX = high-dose methotrexate. [a]The incidence of severe complications was significantly higher in patients with a PS > 2 with respect to the others (41 *vs* 9%, $P=0.002$). An additional logit analysis with patients grouped according to PS ⩽2 *vs* >2 confirmed the independent association between toxicity and CL_{crea}.

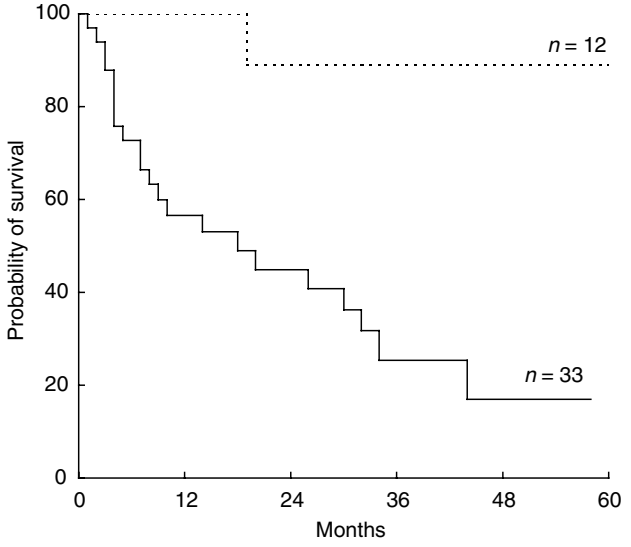

Figure 1 OS curves for patients grouped according to the CL_{crea}. Patients with a slow CL_{crea} (⩽85 ml min^{-1}; dotted line) showed a better OS with respect to patients with a fast CL_{crea} (>85 ml min^{-1}; continued line).

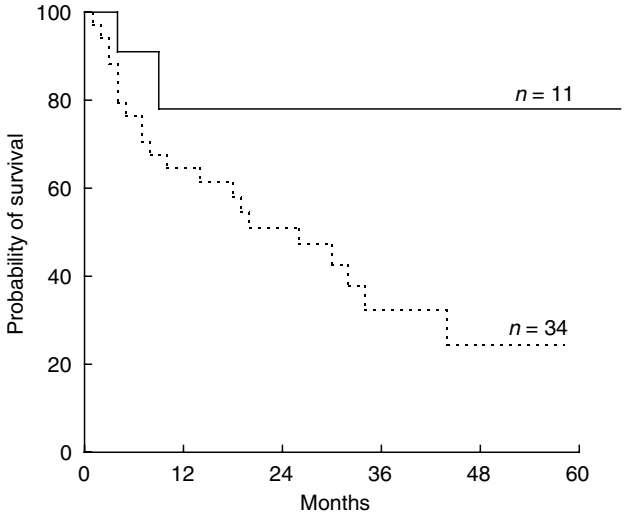

Figure 2 OS curves for patients grouped according to the AUC_{MTX}. Patients treated with an AUC_{MTX} >1100 μmol h l^{-1} (continued line) showed a significantly better survival with respect to those treated with an AUC_{MTX} ⩽1100 μmol h l^{-1} (dotted line).

retrospective series (Ferreri *et al*, 2002b), and to the largest published prospective trials (O'Brien *et al*, 2000; Deangelis *et al*, 2002). A clear relationship between the studied variables and therapeutic outcome is difficult to establish, considering the multitude of other factors, such as protein binding, membrane transport, dihydrofolate reductase levels, tissue distribution, or concurrent drugs, which may also influence the efficacy of MTX. Nevertheless, as has been reported for other malignancies (Evans *et al*, 1986; Graf *et al*, 1994; Delepine *et al*, 1995; Bacci *et al*, 1998), the characterisation of the MTX variables investigated could be useful to identify different risk groups and to define the optimal administration schedule of this drug in PCNSL patients.

HD-MTX is the most effective drug against PCNSL; any regimen without this drug is associated with outcomes which are no better than with RT alone (Schultz *et al*, 1996; O'Neill *et al*, 1999; Mead *et al*, 2000). When used as primary treatment, alone or combined

Table 5 Multivariate analysis: impact on overall survival of studied variables

Variables	Subgroups		Odds ratio (CI 95%)	P
Age	Continuous variable		1.06 (1.01–1.11)	0.04
PS	0–2	3–4	2.55 (1.92–7.02)	0.05
Anticonvulsant	No	Yes	1.46 (0.28–7.53)	0.65
IR_{MTX} ($mg\,m^{-2}\,h^{-1}$)	$\leqslant 800$	>800	0.51 (0.11–2.49)	0.41
DI_{MTX} ($mg\,m^{-2}\,week^{-1}$)	$\leqslant 1000$	>1000	3.38 (0.53–4.98)	0.21
CL_{crea} ($ml\,min^{-1}$)	$\leqslant 85$	>85	6.01 (3.04–9.77)	0.005
AUC_{MTX} ($\mu mol\,h\,l^{-1}$)	$\leqslant 1100$	>1100	0.11 (0.01–0.77)	0.03
Cytarabine[a]	No	Yes	1.19 (0.34–4.11)	0.78
Alkylating agents[a]	No	yes	4.41 (0.75–5.61)	0.16

[a]Similar results were obtained when analysis was performed according to the chemotherapy regimen.

with other drugs, followed or not followed by RT, HD-MTX produces a response rate of 70–80%, with a 2-year OS of 60–70% (Ferreri *et al*, 2000). The survival benefit of the addition of other drugs to HD-MTX is matter of debate, considering that not only do randomised trials comparing mono-chemotherapy with HD-MTX and poly-chemotherapy not exist, but also that the activity of these drugs has not been assessed as a single drug in prospective trials. Thus, HD-MTX remains a crucial drug against PCNSL, being an irreplaceable component of primary chemotherapy. Nevertheless, the prognostic role of the AUC_{MTX} and DI_{MTX} as well as the optimal MTX dose, IR and dose timing of this drug have not been clearly defined in PCNSL. A single study comparing some MTX parameters in PCNSL patients treated with blood–brain barrier disruption or with systemic chemotherapy has been reported (Zylber-Katz *et al*, 2000), but their impact on outcome has not been analysed. Conversely, the prognostic role of MTX pharmacokinetics has been reported in other malignancies in which this drug plays a critical role, such as acute leukaemia (Evans *et al*, 1986) and osteosarcoma (Graf *et al*, 1994; Delepine *et al*, 1995; Bacci *et al*, 1998). Patients with acute leukaemia have been grouped according to a slow, medium or fast CL_{MTX}, obtaining an inverse association with outcome (Evans *et al*, 1986). A significant correlation between a faster CL_{MTX} and lower serum and cerebrospinal fluid (CSF) MTX concentrations has also been documented, suggesting an insufficient treatment both of the brain and meninges, and a greater risk of CNS relapse in this subgroup of leukaemia patients (Evans *et al*, 1983). Likewise, a significant survival effect of serum peak concentration of MTX has been reported in osteosarcoma (Graf *et al*, 1994).

Our study suggests that CL_{crea} and AUC_{MTX} are independent predictors of MTX efficacy in PCNSL patients also. A $CL_{crea} \leqslant 85\,ml\,min^{-1}$ was associated with a higher complete remission rate and better survival, which was independent of age, PS, DI_{MTX}, IR_{MTX}, and other therapeutic variables. Patients treated with an $AUC_{MTX} > 1100\,\mu mol\,h\,l^{-1}$ showed a significantly better survival with respect to those treated with lower AUC_{MTX} levels. CL_{MTX} is defined based upon CL_{crea}; decreased CL_{crea} represents decreased CL_{MTX}, which for a given dose would produce an increased AUC_{MTX}. However, in the present series, CL_{crea} and AUC_{MTX} are two independent variables, which is explained by the heterogeneity in MTX dose, DI_{MTX}, and IR_{MTX}, as well as by differences in the MTX metabolism, according to the drug infusion duration (see below). A strongly inverse correlation between CL_{crea} and AUC_{MTX} could be observed, for example, in a prospective trial, where the used MTX dose and schedule is the same for the entire series. Considering that AUC_{MTX} is significantly correlated, among others, with DI_{MTX}, IR_{MTX}, and anticonvulsant therapy, changes in these parameters could lead to significant changes in AUC_{MTX} and efficacy. Importantly, as reported in Table 4, a higher AUC_{MTX} was not associated with a higher incidence of severe toxicity (9 *vs* 17%, $P = 0.45$). The single case of severe toxicity in the group of patients treated with an $AUC_{MTX} > 1100\,\mu mol\,h\,l^{-1}$ consisted of nonlethal persistent thrombocytopenia, without bleeding complications in a patient with a CL_{crea} of $186\,ml\,min^{-1}$. On the other hand, four of the five cases of severe renal and haematological toxicity were observed in patients with slow CL_{crea}. A close follow-up with haematologic profile and renal function assessment appears advisable in this subgroup of patients.

Pretreatment CL_{crea} assessment could also be useful to identify groups of PCNSL patients with different MTX efficacy, and the use of higher doses in patients with a fast CL_{crea} should be critically considered. The choice of the MTX dose is a relevant issue in PCNSL, especially because of the high interpatient and intrapatient variability of MTX pharmacokinetics. For example, in a recently published trial (Batchelor *et al*, 2003), the MTX dose ($8\,g\,m^{-2}$) was adjusted based on the pre-chemotherapy CL_{crea}. The dose was reduced by the percentage decrease of this variable below $100\,ml\,min^{-1}$. The schedule employed was well tolerated; however, a detailed analysis of the impact on activity and toxicity of this strategy was not provided. Our data suggest that changes in MTX dose according to the pretreatment CL_{crea} could lead to more suitable AUC_{MTX} levels, with a consequent efficacy improvement. This interesting hypothesis will be studied in a prospective series treated with a homogeneous MTX schedule.

DI is a debated outcome-conditioning factor in aggressive systemic lymphomas, while its role has not been investigated in PCNSL patients treated with conventional strategies. In the present series, DI_{MTX} was not associated with survival or toxicity. However, the significant association between a $DI_{MTX} > 1000\,mg\,m^{-2}\,week^{-1}$ and higher AUC_{MTX} levels seems to suggest that, when administered every 3–4 weeks, a MTX dose $\geqslant 3000\,mg\,m^{-2}$ could produce better results than lower doses. This also seems to be supported by the MSKCC and RTOG experience (Deangelis *et al*, 1992, 2002). In a previous trial (Deangelis *et al*, 1992), MTX administered at a dose of $1\,g\,m^{-2}$ produced an overall response rate of 64% and a 5-year OS of 28%, while, in a recently reported trial (Deangelis *et al*, 2002), the use of a $3.5\text{-}g\,m^{-2}$ MTX dose produced an overall response rate of 90% and a 5-year OS of 50%. Analysed together, these data suggest that a higher amount of MTX administered in a single dose increases drug exposure and activity. Moreover, as previously reported (Pitman and Frei, 1977; Evans *et al*, 1983), this strategy seems to lead to a higher diffusion of the drug across the blood–brain barrier and increased drug concentrations in the CSF, with a potential positive impact on efficacy against PCNSL.

From the present analysis, no significant association between IR_{MTX} and survival was observed. This could be due to the strong correlation observed between this parameter and AUC_{MTX}. The identification of the best IR_{MTX} in PCNSL needs further studies. In the meantime, an IR_{MTX} of $>800\,mg\,m^{-2}\,h^{-1}$ appears advisable, since it is associated with higher AUC_{MTX} and does not display significantly higher toxicity in comparison to slower rates.

Anticonvulsants are commonly used in PCNSL patients presenting seizures. These drugs interact with hepatic aldehyde oxidase, which constitutes a major mechanism for MTX degradation (Jacobs *et al*, 1976). Anticonvulsant therapy increases the systemic CL_{MTX}, as well as the level of other cytostatics, and is associated with lower efficacy of chemotherapy in children treated for acute lymphoblastic leukaemia (Relling *et al*, 2000). This effect seems to be more intense in patients treated with HD-MTX by a 24-h infusion, in whom about 40% of the drug is metabolised in the liver, whereas, when HD-MTX is administered as a short intravenous infusion (4–6 h), most of the drug is cleared by the kidneys (Evans *et al*, 1983). In the present analysis, no association between anticonvulsant use and toxicity and outcome was observed, and the impact of these drugs in patients treated with a 24-h infusion cannot be analysed, considering that all these patients received anticonvulsant therapy. The hypothesis that MTX

dose adjustments are needed when anticonvulsants are contemporarily used should be better explored.

To identify new active drugs and combinations remains the most important strategy to improve therapeutic results in PCNSL patients, and any effort to define the best administration schedule for HD-MTX should be encouraged. With certain limitations due to their retrospective nature, our data seem to suggest that slow CL_{crea} and high AUC_{MTX} are independently associated with better outcome in PCNSL patients. Comprehensively, these interesting findings deserve to be assessed in prospective trials. In the meantime, a MTX dose $\geqslant 3000\,mg\,m^{-2}$ administered in a 4- or 6-h infusion, every 3–4 weeks, appears an advisable schedule to adopt in clinical practice. The need to increase the MTX dose to ensure adequate exposure, such as higher AUC_{MTX} values, in patients with a fast CL_{crea}, should be critically considered.

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