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Population pharmacokinetics of adjuvant cyclophosphamide, methotrexate and 5-fluorouracil (CMF)

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Abstract

Despite the success of adjuvant cyclophosphamide, methotrexate (MTX), 5-fluouracil (5-FU) (CMF) treatment for early stage breast cancer, more than 35% of patients die within 5 years of diagnosis. Optimisation of the dose of each component drug may improve survival and reduce toxicity. In this study, the pharmacokinetics of intravenous (i.v.) cyclophosphamide (600 mg/m^2), MTX (40 mg/m^2) and 5-FU (600 mg/m^2) were determined in 46 women, with data on two consecutive courses available for 41 patients. A population analysis using NONMEM was performed to investigate the effect of patient covariates on pharmacokinetics (PK), and to estimate the relative magnitude of interindividual and interoccasion variability. Patient weight had a significant influence on the clearance of cyclophosphamide and on the volume of central compartment for MTX, whose clearance was dependent on renal function. For all three drugs, interoccasion variability was of the same order (20–40%) as that between individuals, suggesting a limited potential for dose-optimisation of this regimen.

Keywords: Breast cancer; Adjuvant; Pharmacokinetics; Cyclophosphamide, Methotrexate; 5-Fluorouracil; Population analysis

1. Introduction

The application of population pharmacokinetics (PK) in oncology is based on the assumption that clear pharmacodynamic relationships exist between plasma drug concentrations and clinical effect. The latter should include antitumour effects but, more commonly, toxicity has been used as a pharmacodynamic endpoint. A further assumption is that accounting for PK variability will result in optimal dose individualisation. Such an approach has been applied for carboplatin [1], docetaxel [2] and epirubicin [3]. Regimens for the treatment of cancer usually involve multiple agents administered on a number of occasions, such that population PK models must also consider the effect of co-administered drugs and the degree of interoccasion variability in PK. Few studies have addressed the former issue [4], and fewer still the latter. The subject of the current study, the adjuvant chemotherapy of breast cancer, offers an opportunity to explore these issues in a clinically-relevant setting.

A meta-analysis of 6700 patients with breast cancer has shown that adjuvant chemotherapy reduces the annual risk of recurrence by up to 27% and of death by 18% [5]. The combination of cyclophosphamide, methotrexate (MTX) and 5-fluorouracil (5-FU) (CMF) has been commonly used in the adjuvant treatment of breast cancer and is usually given for a total of six cycles. Very mature data indicate that the survival benefit from CMF chemotherapy is maintained for up to at least 20 years after its initiation [6].

Despite the benefits of adjuvant therapy, 35% of patients treated will still die of metastatic breast cancer within 5 years and only 44% will be alive at 10 years [5]. In order to improve these survival data, the adjuvant use of anthracyclines and/or taxanes, and high-dose chemotherapy has been evaluated [7–10]. Given the well established safety profile of this regimen, as opposed to the currently unknown long-term effects of other treatments, it is important that attempts are also made to maximise the efficacy of CMF therapy by individualising the doses of the three drugs administered to each patient.

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The impact of dose on the efficacy of the CMF regimen has been extensively studied in both retrospective analyses and prospective trials. These studies have shown that outcome is dose dependent and, in one randomised study, a 2-fold difference in dose resulted in a significant difference in both relapse-free and overall survival [11]. Furthermore, the magnitude of the impact of dose in these studies may have been confounded by pharmacological variability and hence a very significant therapeutic gain may be achievable by individualised dosing to produce a uniformly high, effective yet safe drug exposure in each patient. Plasma concentrations generated by a given dose have been shown to be important in terms of both toxicity and tumour response for the individual components of the CMF regimen [12–14].

In order to design a study to elucidate the potential gain from the pharmacological optimisation of CMF treatment, it is essential to determine the extent of both the inter subject and inter occasion variability in the PK of each component drug. The oxazaphosphorine cyclophosphamide requires metabolic activation to exert its cytotoxic effect [15]. Previous studies suggest that the antitumour and cardiotoxic effects of high-dose cyclophosphamide may be inversely related to the plasma concentrations of the parent drug, presumably because this represents unmetabolised, non-activated compound [12]. In the latter study, of high-dose cyclophosphamide following conditioning with busulphan or radiotherapy, there was a high degree of intersubject variability (coefficient of variation (CV) up to 61%) and also a strong inverse relationship between the area under the plasma concentration–time curve (AUC) value for the parent drug and that for the active 4-hydroxy metabolite [16]. Studies with both intravenous (i.v.) and oral preparations of cyclophosphamide given at conventional doses indicate that interoccasion variability is relatively small (less than 20%) [17,18].

The clinical importance of monitoring MTX levels in plasma is well established and PK data are routinely used to predict which patients will require folinic acid rescue [19]. In children with leukaemia, independent studies have shown that patients who eliminate the drug rapidly are at a higher risk of relapse [13,20]. Furthermore, dose intensification to a target plasma concentration has been shown to improve treatment outcome [21]. Interoccasion CV for MTX pharmacokinetics has been reported to be less than 20% [22,23] and prospective studies have shown that dose adjustment on the basis of plasma MTX concentrations can compensate for PK variability [24].

For 5-FU, relationships between the PK of the drug and both toxic and antitumour effects of 5-FU have been demonstrated [14,25,26]. Whilst only limited interoccasion variability data have been reported, this appears to be less than the interpatient variability [27]. For example, in a population study of 5-FU, epirubicin and cyclophosphamide, CVs for intersubject and interoccasion variability for 5-FU clearance were 30 and 11%, respectively [4].

Given the potential benefit of dose optimisation for the adjuvant treatment of breast cancer, a strategy for individualising doses of chemotherapy is desirable. In this study the relative degrees of intersubject and interoccasion variation in the PK of CMF were determined from the plasma concentrations of each drug measured in 46 patients during two courses of chemotherapy. Population PK analysis was employed using NONMEM to assess interoccasion variability and the influence of patient covariates.

2. Patients and methods

A total of 46 patients were recruited to the study, conducted at the Northern Centre for Cancer Treatment (NCCT), Newcastle General Hospital. All patients gave written informed consent and the study was approved by the Ethics Committee of the combined Newcastle Hospitals and University. Eligible patients were either axilliary lymph-node negative, but with other features of a poor prognosis (such as grade 3 histology, lymphatic, vascular or perineural invasion) or patients with 1–3 positive nodes. Each patient was scheduled to be treated with six cycles of adjuvant cyclophosphamide (600 mg/m^2), MTX (40 mg/m^2) and 5-FU (600 mg/m^2).

Patients were studied on their first two courses of treatment, with a 3-week interval. Prior to the day of administration, a full record of patient characteristics, including renal function, blood biochemistry and liver function were obtained. Repeat observations of patient characteristics, except clearance of ethylene diamine tetra-acetic acid (EDTA), were obtained prior to the second course of chemotherapy. Haematological and other toxicities were recorded after each course and any treatment delay or dose reduction noted.

Drugs for administration were prepared according to the manufacturer's instructions and administered as short i.v. infusions (1–5 min). The order of administration was cyclophosphamide, MTX then 5-FU. The exact start and stop times of each drug administration were carefully recorded. Blood samples (10 ml) were obtained pretreatment, at the end of the 5-FU administration and at 5, 15, 30, 60, 90, 120, 180, 240, 360 and 480 min after the end of administration. Plasma was prepared immediately by centrifugation at 2000*g* and stored at −20 °C prior to analysis.

High performance liquid chromatography (HPLC) assays for each of the component drugs were based on published methods. Briefly, for cyclophosphamide [18], 50 μl of internal standard (250 μg/ml ifosfamide) was added to 1 ml of each plasma sample or standard

followed by 100 μl 1M NaOH and 10 ml ethyl acetate. After vortex mixing for 30 s, the organic phase was separated by centrifugation for 10 min at 3500*g* and the solvent evaporated under nitrogen at 37 °C. Samples were reconstituted in 200 μl mobile phase and 100 μl injected into the HPLC. To each MTX standard or sample [28], 50 μl of internal standard (8-chlorotheophylline, 1 mg/ml) was added, followed by vortex mixing for 30 s. Four ml of acetonitrile was added and mixing repeated for 1 min. Samples were centrifuged at 3000 rpm for 10 min at 4 °C. Four ml of supernatant were removed and evaporated under nitrogen at 40 °C. Reconstitution and HPLC injection were as for cyclophosphamide. For 5-FU [29], 50 μl internal standard (0.25 μg/ml 5-fluorocytosine) was added to 0.4 ml of each standard or sample and vortex mixed for 30 s. Ethyl acetate (2.5 ml) was added and after vortex mixing for 2 min and centrifuge tubes at 3000*g* for 10 min at 4 °C, 2 ml of supernatant was removed and the solvent evaporated under nitrogen at 40 °C. Reconstitution and HPLC injection were as for cyclophosphamide. Each assay was validated for linearity, intra- and interday reproducibility and quality assurance standards were included in every analytical run.

The HPLC instruments were the same for analysis of all three compounds, with a Waters Alliance Separations module and either a Waters 2487 dual wavelength or Waters 996 diode array detector. Wavelengths for detection for cyclophosphamide, MTX and 5-FU, were 197, 290 and 290 nm, respectively. Columns and mobile phases were: cyclophosphamide—Waters NovaPak C_{13} 3.9×300 mm 5 μ, H_2O/acetonitrile 75:25% v/v; MTX—Waters Spherisorb® $S3C_6$ 4.6×150 mm, acetate buffer pH 4/acetonitrile 95:5% w/w; 5-FU—4.6×250 mm Waters Spherisorb® S5 ODS1, sodium phosphate buffer pH 4.5/acetonitrile 99.8/0.2% w/w.

Data from each course of chemotherapy for each of the drugs were subjected to population PK analysis using the NONMEM software (version V). The first-order conditional estimation method was used, retaining interaction between interindividual random effects and the residual error term. Selection of structural models was based on the fit of the model to the data, consistent with reliable parameter estimation and significant change in objective function. Models incorporated interoccasion variability in all parameters, as well as interindividual variability between patients. The influence of patient covariates on clearance and volume parameters were investigated. Decisions on the fit of models to the data and the inclusion of covariates were based on objective function values (greater than 10-point change in objective function), plots of parameters versus covariates and significance of coefficients.

Given the small number of patients in the study, no attempt was made to correlate PK with efficacy, but relationships with toxicity were explored.

3. Results

Of the 46 patients recruited to the study, data for all three drugs on two consecutive courses was available in 41. The patient characteristics are given in Table 1. Plasma concentration time profiles for each of the drugs in the CMF regimen in a representative patient are shown in Fig. 1. Five patients withdrew from the PK study after the first course, but in every case this was due to patient preference and was not a reflection of toxicity or other adverse outcome. Thirty nine patients received a total of six cycles of CMF, 4 patients received only two cycles, 1 each three, four or eight cycles. Reasons for cessation of treatment included progressive disease and complications of mastectomy. Toxicities were uncommon, with only 12 patients experiencing any degree of haematological toxicity. There were five patients who experienced Grade 3 or higher neutropenia or leucopenia. Grade 1 or 2 neutropenia or leucopenia occurred in a further 7 patients, and was accompanied by Grade 2 anaemia in 1 patient. None of these toxicities could be related to individual PK parameters of the three drugs. Haematological toxicity resulted in treatment delay, dose reduction or cessation of treatment in only 4 cases. Non-haematological toxicity included stomatitis, diarrhoea, skin rashes and nausea, but 36 patients did not experience any non-haematological toxicity.

No patients were receiving concomitant medication with any drugs reported to interact pharmacokinetically with the component drugs of CMF, although 1 patient was being treated with verapamil and 1 with nifedipine, both of which are substrates and potential competitive inhibitors of CYP450 metabolism of cyclophosphamide. These patients did not exhibit any distinct PK for any of the compounds and were included in the overall analysis. Of the 44 patients for whom full clinical data were available, 28 were on tamoxifen or anastrozole (Arimidex®) treatment and disease-free at the time of follow-up. Thirteen patients were disease-free with no further

Table 1
Patient characteristics. Median and (range) ($n=46$)

Patient characteristics	Course 1	Course 2
Age (years)	51 (34–79)	
Menopausal status	Pre $n=18$	
	Post $n=21$	
	Hysterectomy $n=7$	
Weight (kg)	70 (52–102)	
Surface area (m^2)	1.71 (1.48–2.06)	
Creatinine (μmol/l)	76 (64–159)	81 (65–181)
Bilirubin (μmol/l)	7 (1–26)	6 (2–28)
AST (IU/l)	20 (10–36)	38 (13–55)
ALT (IU/l)	17 (9–61)	21 (12–174)
ALP (IU/l)	67 (26–178)	76 (9–166)

AST, asparate aminotransferase; ALT, alanine aminotransferase; ALP, alkaline phosphatase.

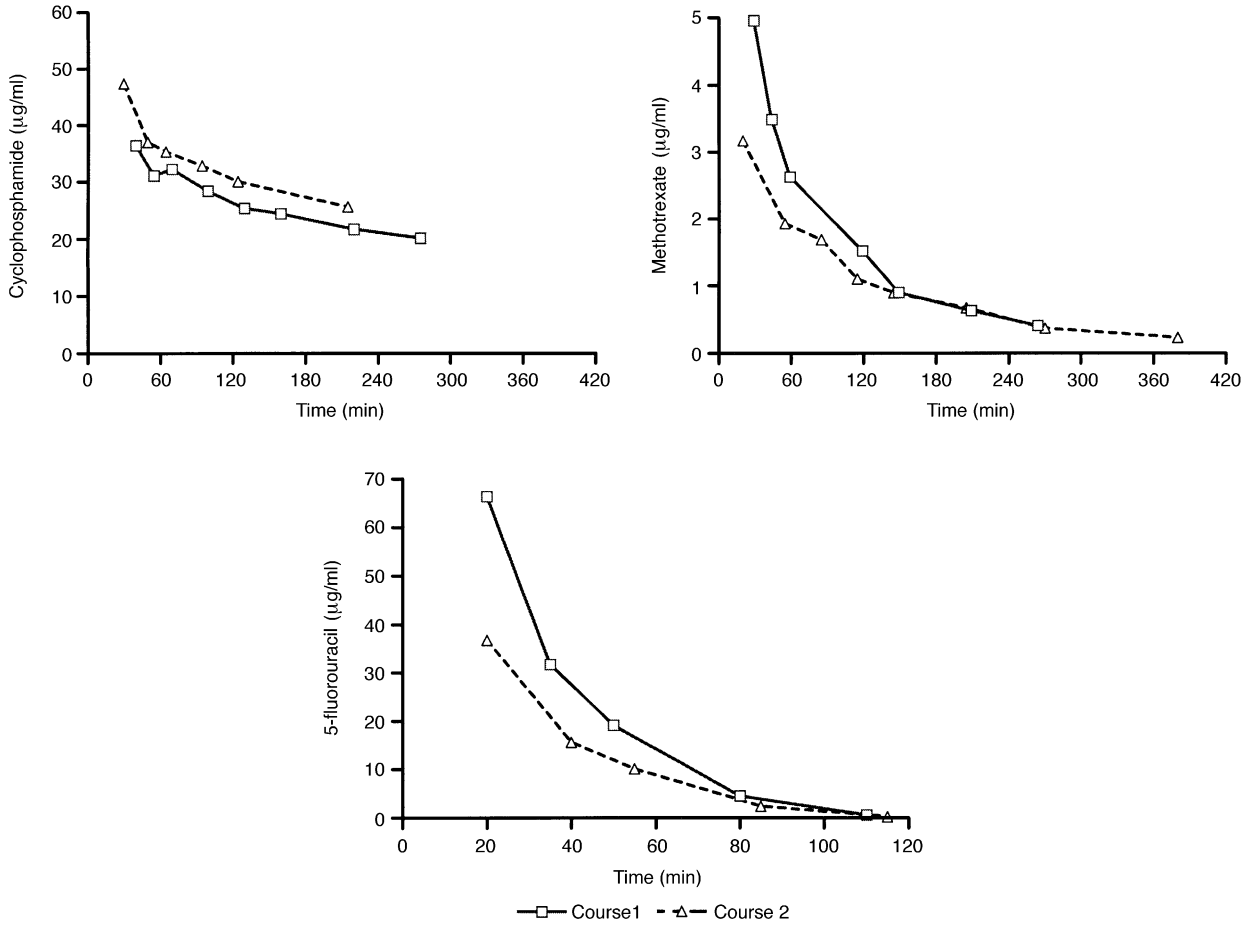

Fig. 1. Plasma concentration time profiles for each of the drugs in the cyclophosphamide, methotrexate (MTX) 5-fluouracil (5-FU) (CMF) regimen in a representative patient.

treatment and 3 patients had shown signs of relapse or progression.

Cyclophosphamide PK were best described by a one-compartment model, with interindividual and inter-occasional random effects on clearance (CL) and volume (Table 2). Residual error was described by an additive error model on the log-scale. The raw data were log-transformed in order to minimise the dependence of estimation of mean parameters on the variance function. A lower CL was seen in the second course compared with first course in 28 patients, (median decrease Cl = 23% (range 4–62%), with 12 patients having an increase in Cl on course 2 relative to course 1 (median change 11%, range 1–42%) (Fig. 2a). A marginal positive correlation between clearance and weight was found (Fig. 2b). Interindividual variability in clearance was 35% (37% without the effect of weight), compared with 21% for interoccasion variability.

There were some initial problems in the analysis of the MTX data. A subset of the population appeared to have enormously divergent volumes of distribution (5- to 10-fold higher than the majority of patients). This discrepancy may have been the result of analytical error or mis-dosing. Dosing and analytical methods were

Table 2
Pharmacokinetic models with covariates for each component drug of CMF regimen

Drug	Cl	V	Q	V2
Cyclophosphamide (C)	70.1 (4.4) + 0.907 (0.151)*(WT-70) + 13.6 (3.11)*OCC1	30.1 (0.8)		
Methotrexate (M)	1 28 (5)-(GFR-80)×1.05 (0.2)	15.5 (1.5–3.33 (0.7)*OCC1 + O.229 (0.09)×(WT-75)	206 (23)	10.9 (0.8)
5-Fluorouracil (F)	0.907 (0.042) + 7.94 (2.22)*(Age 55)	1 5.2 (0.7)		

Cl, clearance; V, volume; GFR, glomerular filtration rate. Estimates of parameters and coefficients given as population mean (standard error). Cl and Q in ml/min, V1 and V2 in l. OCC1 0 (course 1) or 1 (course 2). WT in kg; GFR in ml/min; Age in years.

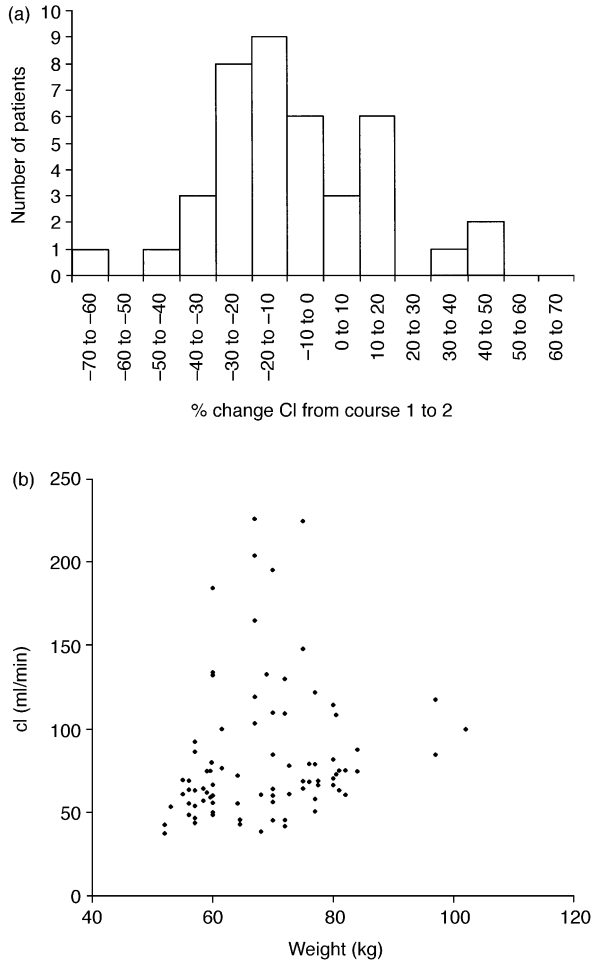

Fig. 2. (a) Frequency plot of change in clearance (CL) of cyclophosphamide comparing course 1 to course 2; (b) plot of cyclophosphamide clearance (CL) against weight for all courses studied.

checked thoroughly, and the latter repeated where possible. Neither of these potential causes could account for the variability observed and this degree of variability has not been reported previously [30]. Analysis of the full dataset resulted in violations of the distributional assumptions of NONMEM. Population analysis was therefore restricted to a subset of 34 patients in whom PK parameters appeared to be homogeneous.

A two-compartment model was fitted to the data, with interindividual and interoccasional random effects on all four parameters (Cl, Vl, Q and V2, Table 2). A proportional error model best described the pattern of residual error and the raw data were log-transformed to minimise the dependence of mean parameters on estimation of the variance function.

Even on the subjectively edited dataset, there was a clear increase in the volume of distribution from course 1 to course 2 (Fig. 3a). 22 patients had an increase in central volume of between 4 and 109%. Weight had a modest influence on MTX V1 (decrease in interindividual variability from 42 to 37%). A small effect of glomerular filtration rate (GFR) (estimated from clearance of EDTA) on MTX clearance (decrease variability 24–20%) was also identified (Figs. 3b and c).

The pharmacokinetics of 5-FU could parsimoniously be described by a one-compartment model with interindividual and interoccasional random effects on clearance only. Log transformation did not improve the fit and a combined additive and proportional model best described the pattern of residual error (Table 3). No evidence of a second compartment or of nonlinear pharmacokinetics could be discerned in these data from a limited dose range. Allowing clearance to increase as a function of age reduced interindividual variability (from 14 to 10%), but this effect was modest (Table 2 and Fig. 4). Interoccasion variability was of the same order as interindividual variability (10–14%).

There were no correlations among the absolute values of clearance for the three compounds. Nor were there any correlations among the three agents in the clearance changes observed between courses 1 and 2 (Fig. 5).

Due to the low incidence of toxicity, both haematological and non-haematological, no relationship could be discerned between the pharmacokinetics of any of the component drugs and toxicity. Similarly, dose reduction or treatment delay could not be accounted for by variation in PK. Given that these patients were at a low risk for recurrence, the expected relapse rate during the available follow-up period is unlikely to reveal any relationship with the pharmacology of the drugs used in the adjuvant treatment and was not evaluated.

Table 3
Details of population models used for each of component drug of the CMF regimen

Drug	IIV (%)				IOV (%)				Residual model	Slope
	Cl	Vl	Q	V2	Cl	V1	Q	V2		
Cyclophosphamide (C)	35	14			21	13			Log	13%
Methotrexate (M)	20	37	28	22	15	31	20	35	Log	17%
5-fluorouracil (F)	10				13				Slope 31%	Intercept 0.00945

Percentages indicate degree of variation accounted for by interindividual (IIV) or interoccasion (IOV) variability in each model paramater. The residual error for cyclophosphamide is modelled as additive on the log-scale. Hence, residual error is reported as the geometric coefficient of variation. Characteristics of the residual error model for each component drug are given in the last two columns.

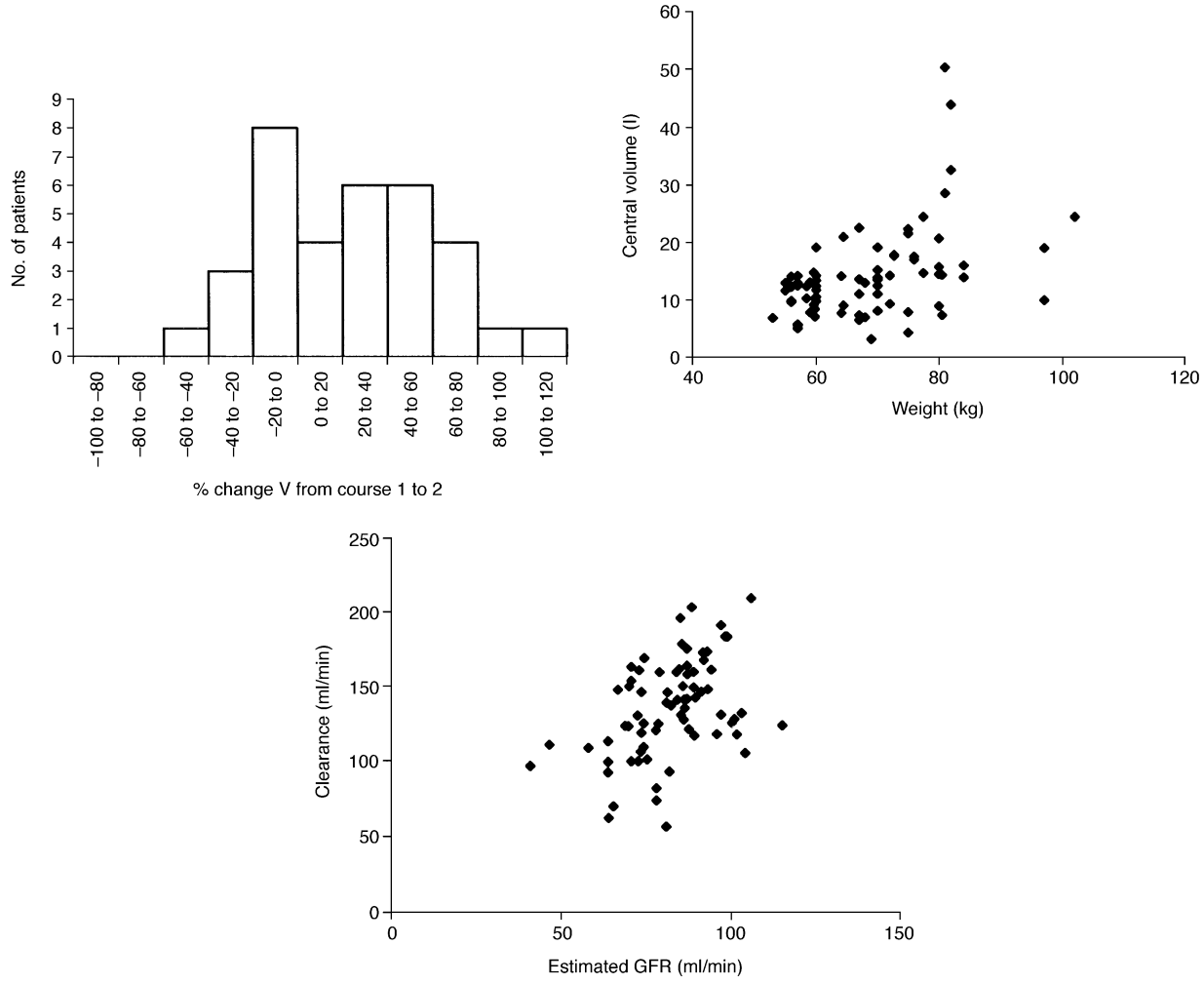

Fig. 3. (a) Frequency plot of change in volume (V) of central compartment for methotrexate (MTX) comparing courses 1 and 2; (b) Effect of weight on volume of central compartment (V) for MTX; (c) effect of glomerular filtration rate (GFR) (estimated from clearance of ethylene diamine tetra acetic acid (EDTA) on clearance for MTX.

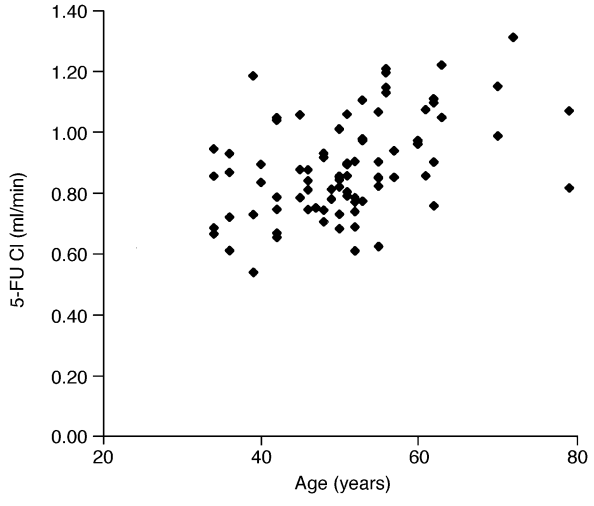

Fig. 4. Effect of age on clearance of 5-fluorouracil (5-FU).

4. Discussion

The concept of individualisation of drug doses in cancer chemotherapy has been applied for a number of agents. The most common application is the dosing of carboplatin to a target AUC, using a measure of GFR to calculate clearance [31]. The utility of therapeutic monitoring of etoposide during continuous infusion has also been demonstrated [32], and modulation of the MTX dose to achieve a target plasma concentration improves outcome in patients with leukaemia [21]. Adjuvant treatment of early stage breast cancer with CMF could offer a further opportunity to apply such an approach. Patients receive repeated courses of chemotherapy, thus allowing for adaptive dosing following course 1, and a dose–response relationship in terms of survival and time to relapse has been established [6].

Previously, only one study of the pharmacokinetics of CMF, specifically as used in the adjuvant treatment of breast cancer, has been reported. In a cohort of 23 women undergoing adjuvant chemotherapy with the CMF regimen, the interpatient variability in the AUC for each of the component drugs of the regimen was found to be 3- to 4-fold [30]. Interoccasion variation in the pharmacology of CMF was less than 50% in the majority of patients, i.e. substantially less than the intersubject variability (Prof. M.J. Moore, Ontario Cancer Institute, Canada).

The most commonly used pharmacodynamic correlate in oncology is the AUC, although more plausible mechanistic models have been suggested [33]. For the component drugs of the CMF regimen, an AUC-based model is probably not optimal. All three agents are prodrugs, although MTX can act directly, and the pharmacology of MTX and 5-FU may be governed by time above a threshold plasma concentration. Therefore, dose-adaptation based solely on the plasma clearance of the parent drugs may not be appropriate. Further characterisation of tumour activation of these prodrugs relative to plasma concentrations would be required before dose optimisation could be implemented based on measurement of the latter. However, the utility of dose individualisation based on plasma concentration (inversely related to clearance) for CMF treatment will be explored in this discussion.

For cyclophosphamide, a systematic decrease in clearance was detected between course 1 (mean 84 ml/mm) and course 2 (70 ml/mm), although this effect was not well-characterised. A marginal correlation between weight and clearance was also demonstrated. However, the degree of interoccasional variability (21%) was of the same order as that of interindividual variability for clearance, suggesting that pharmacologically-guided dosing could control systemic exposure of this drug only to a modest extent.

For MTX, clearance has been shown to be related to the GFR only at low concentrations (less than 0.4 μM). Tubular secretion and reabsorption are also important, and the latter is saturable at the concentrations attained after a short infusion. Using the estimate of GFR obtained from the population PK of EDTA [34], a

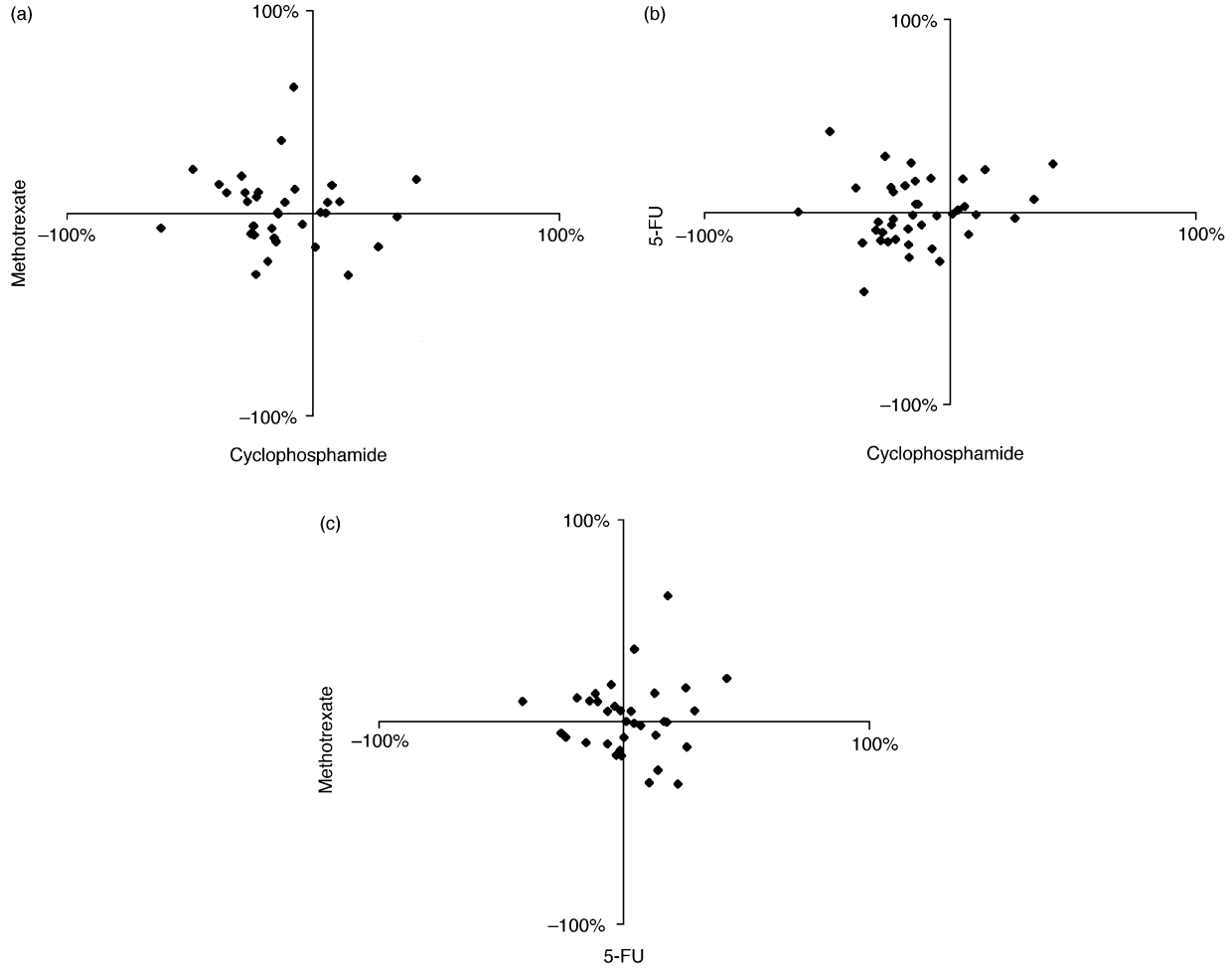

Fig. 5. Correlation between percentage changes in clearance from course 1 to course 2 for the three component drugs of the cyclophosphamide methotrexate (MTX), 5-fluorouracil (5-FU) (CMF) regimen.

correlation with observed clearance was demonstrated. The estimation of clearance is necessarily time-averaged, given the likely non-linearity of excretion processes over the concentration range in plasma.

The substantial increase in the volume of the central compartment between course 1 and course 2 for MTX is difficult to explain. It is possible that exposure to MTX in course 1 causes upregulation of either cellular transport proteins or of folylpolyglutamate synthethase, both of which would act to increase uptake and retention of MTX in cells. Such a change was not noted in the previous investigation of CMF PK [30].

The estimates of interoccasional and interindividual variability for 5-FU were small (14%) and approximately equal. The validity of these estimates may be limited due to the assumption of a linear single-compartment model and the high residual error (31%). When data from studies using a longer duration of infusion have been analysed, several important covariates were identified [35]. These included time of day, peripheral mononuclear cell dihydropyrimidine dehydrogenase and elapsed infusion time, none of which were available or applicable to the current study. In addition, clearance appeared to decrease when a raised alkaline phosphatase was reported and with increasing age [35]. The effect of alkaline phosphatase was not observed in the current study and age appeared to influence clearance in the opposite direction, i.e. older patients had a higher clearance. This unexpected finding may be spurious, or may reflect some other unrecorded covariate. In a previous population analysis of 5-FU PK, a saturable clearance model was used [4], although the estimated Km was high (27 mg/ml) relative to the concentrations observed in our data set.

Adaptive dosing of 5-FU has been attempted, but only when administered as a longer infusion. For example, a study of the relationship between AUC and toxicity in 89 patients with head and neck cancer [25], led to the development of a strategy for dose reduction in patients whose AUC following initial therapy exceeded a certain threshold. A reduction in the incidence of toxicity was associated with fewer dose delays and a higher response rate. The improvement in therapeutic index may be related to the proportion of early-stage patients in the two arms of the study. Applying such an approach based on the analysis performed in the current study would require a larger study with more pharmacodynamic information in order to identify target plasma concentrations.

The 2–3-fold interindividual variation in pharmacokinetics for the three drugs suggests that further refinement of dosing in individual patients would be beneficial, based on analysis of patient covariates. In this relatively small initial patient population, no covariate was found to significantly influence the pharmacokinetics of 5-FU. Clearance of cyclophosphamide was positively correlated with body size, while that of MTX was related to renal function. The degree of interoccasion variation and the impact of these covariate effects require further validation in a separate group of patients. These data indicate that optimisation of CMF dosing may provide further improvement in adjuvant chemotherapy, although the potential benefit, in terms of uniformity of plasma concentration–time profiles, may be limited by interoccasion variability.

Acknowledgements

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