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Malnourished Malawian patients presenting with large Wilms tumours have a decreased vincristine clearance rate

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ABSTRACT

Introduction: In developing countries, patients with a Wilms' tumour often present late with a high degree of malnutrition and large tumours. We investigated whether this affects vincristine pharmacokinetics.

Methods: Patients newly diagnosed with Wilms' tumour in Malawi and the UK were included. We documented anthropometric parameters, nutritional status and tumour size. Vincristine (1.50 mg/m^2) was administered as part of the standard chemotherapy regimen. Vincristine plasma concentrations were measured at several time points by liquid chromatography–mass spectrometry. Vincristine pharmacokinetic parameters (clearance and area under the curve) were calculated by non-compartmental analysis.

Results: Eleven Malawian and 8 UK patients were included. Mean Z-score of (corrected) weight for height was significantly lower in the Malawian patients than in the UK patients (−2.3 versus 0.42, $p < 0.0001$). Mean tumour weight at diagnosis was significantly larger in Malawian patients (2.8 kg versus 0.7 kg, $p = 0.007$). Mean vincristine log Clearance was lower in Malawian as compared to UK patients (2.2 versus 2.6 ml/min, $p = 0.001$). Mean log AUC values were higher in Malawian than in UK patients (3.8 versus 3.5 μg/ml min, $p = 0.003$). This difference is reflected in the, on average, 1.98-fold larger vincristine AUC values for Malawian patients. The difference in AUC values was statistically significantly explained by nutritional status ($p = 0.043$).

Conclusion: Malnourished patients in Malawi exhibited lower vincristine clearance rates and thus higher AUC values than a comparable patient population with a better nutritional status in the UK. In malnourished patients, dose reductions may need to be considered to prevent an increased incidence and severity of toxicity.

1. Introduction

In resource limited countries, patients with a Wilms' tumour often present late with large tumours and a marked degree of malnutrition.[1–4] This study aimed to evaluate the pharmacokinetics of vincristine in these patients, as compared to control patients treated with a comparable vincristine dosing regimen in the United Kingdom.

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Dosing regimens implemented for the treatment of Wilms' tumour patients may be particularly important as vincristine, in common with many other anticancer drugs, has a relatively narrow therapeutic index and is associated with potentially life-threatening toxicity in the form of neuropathy. There are a limited number of reports concerning the pharmacokinetics of vincristine in children. Considerable intra- and interpatient variation in pharmacokinetic parameters have previously been reported.[5–7] Further clinical pharmacology studies in defined patient populations are required in order to obtain information which may be used to improve its future therapeutic potential.[8–13]

Differences in vincristine pharmacokinetics between children with normal nutritional status and those with malnutrition may be expected, relating to altered liver and renal function and differences in body composition, which may impact on drug distribution.[14] Additionally, as vincristine exhibits a relatively high level of plasma protein binding, decreased concentrations of plasma proteins in malnourished children may affect plasma protein binding and the proportion of free drug.[15]

2. Patients and methods

Patients younger than 18 years with a localised Wilms tumour were included after informed consent was obtained. We documented anthropometric data. Laboratory evaluation included electrolytes, liver enzymes, creatinine and protein status. Tumour size was determined by ultrasonography (Malawian patients), CT scan or MRI analysis. The tumour was measured in three dimensions and the tumour volume calculated using a standard ellipsoid formula.[16] Corrected weight (body weight – estimated tumour weight) was calculated and Z-scores for (corrected) weight for height (WHZ) were derived in reference to the 1978 NCHS growth curve (HANES data) to express the degree of acute malnutrition.[17]

Vincristine 1.5 mg/m^2 was given by IV bolus. Doses were reduced by 1/3 if weight was below 12 kg. Blood samples for the measurement of vincristine concentration were obtained at 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 24 h. In Malawian patients, additional blood samples were taken at 15 min and 6 h to determine unbound vincristine concentrations. Following withdrawal, blood samples were centrifuged at 1200*g* for 10 min at 4 °C and the plasma was stored at −20 °C prior to transport to the laboratory.

For the quantification of vincristine in samples analysed in Amsterdam, a previously described high-performance liquid chromatography coupled to tandem mass spectrometry (LC–MS/MS) method was used.[18] Analysis of samples in Newcastle was carried out using a validated liquid chromatography–mass spectrometry (LC–MS) assay. These methods have a lower limit of quantification (LLOQ) of 0.25 and 0.50 ng/ml, respectively. Cross-validation of the assays was carried out. For the quantification of the protein-unbound vincristine fraction, ultrafiltrate was prepared from plasma.

Vincristine pharmacokinetic parameters were calculated for all patients by non-compartmental analysis using the Stata (Release 10) software package for intravenous bolus injection. The area under the plasma concentration-time curve (AUC) was calculated from 0 to 24 h using the trapezoidal rule.

Statistical differences in numerical patient characteristics between the patient populations were investigated using the independent sample *t*-test. Because of small sample size *t*-test results were verified with a non-parametric Mann–Whitney *U*-test. Since the tests coincided, only *t*-test results are denoted. Difference in categorical characteristics was tested with a Fisher Exact test. Pharmacokinetic parameters (vincristine clearance and AUC) were 10log-transformed before analysis, because of their skewed distribution. Again population differences were tested with an independent *t*-test. Linear regression was used to assess the correlation between log AUC and patient characteristics that might influence pharmacokinetics.

3. Results

Patient characteristics, nutritional status, tumour weight and details of vincristine treatment are detailed in Tables 1, 2 and Fig. 1. Analysis of electrolytes, creatinine and liver enzymes showed no significant abnormalities in Malawian patients.

For Malawian patients, the estimated tumour weight at diagnosis also represented the tumour weight when the pharmacokinetic study was carried out. For UK patients, the pharmacokinetic study was carried out at a later date, with a mean time interval of 1.1 months (range 0.7–24 months).

3.1. *Vincristine pharmacokinetics*

A total of 6 samples were obtained from each of the 11 Malawian study patients, with 5–7 samples obtained from 8 patients studied in the UK. Plasma concentration–time curves for both groups are shown in Fig. 2.

Table 1 – Patient characteristics, tumour size and nutritional status.

	Malawi $N = 11$	United Kingdom $N = 8$	*p*-Value
Male	9 (82%)	4 (50%)	0.319
Age (yr)	4.5 ± 2.6	4.6 ± 2.1	0.912
Body weight (kg)	15 ± 3.9	20 ± 8.4	0.091
Surface area (m^2)	0.65 ± 0.13	0.79 ± 0.22	0.113
Tumour weight (kg)	2.8 ± 2.1	0.7 ± 0.4	0.007
Z-score corrected weight for height	−2.3 ± 1.3	0.4 ± 0.8	<0.0001

Values are *n* (%) or mean (±SD)

Table 2 – Vincristine dosing and pharmacokinetic parameters in patients with a Wilms tumour treated in the UK and Malawi.

Patient	Country	Vincristine dose (mg)	Vincristine dose (mg/m^2)	Vincristine AUC_{0-24h} (ng/ml min)	Vincristine Cl (ml/min)	Vincristine Cl (ml/min/m^2)
1	Malawi	1.0	1.56	4941	202.4	316.2
2	Malawi	1.1	1.45	5207	211.3	278.0
3	Malawi	1.1	1.53	3753	293.1	407.1
4	Malawi	1.4	1.57	5883	238.0	267.4
5	Malawi	0.9	1.41	7467	120.5	188.3
6	Malawi	0.4	0.91	4278	93.5	212.5
7	Malawi	1.1	1.43	11385	96.6	125.5
8	Malawi	0.5	1.11	4142	120.7	268.3
9	Malawi	0.95	1.51	6124	155.1	246.2
10	Malawi	1.0	1.52	8244	121.3	183.8
11	Malawi	0.9	1.55	7745	116.2	200.4
12	UK	2.0	1.54	2231	896.5	689.6
13	UK	1.15	1.53	1858	618.9	825.3
14	UK	0.55	1.0	1591	345.7	628.5
15	UK	1.2	1.56	2925	410.3	532.8
16	UK	1.0	1.41	8310	120.3	169.5
17	UK	1.3	1.53	3455	376.3	442.7
18	UK	0.98	1.51	3153	310.8	478.2
19	UK	1.1	1.49	3855	285.3	385.6

Abbreviations: AUC, area under the plasma concentration–time curve and Cl, clearance.

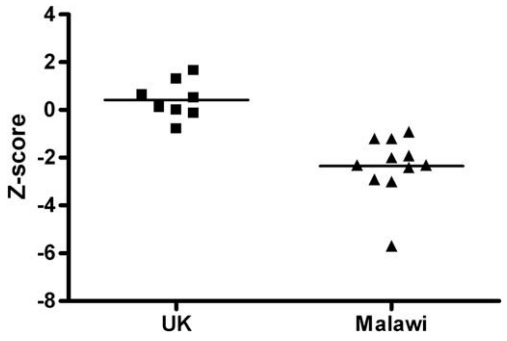

Fig. 1 – Z-scores (corrected weight for height) observed in United Kingdom and Malawian Wilms tumour patient populations.

3.2. *Clearance*

Median vincristine clearance (Cl) was lower in Malawian patients (121.3 ml/min, range 93.5–293.1) than in UK patients (361.0 ml/min, range 120.3–896.5). Correspondingly, mean log Clearance was lower in Malawian as compared to UK patients, with mean log Cl values of 2.2 ml/min (SD 0.17, range 2.0–2.5) and 2.6 ml/min (SD 0.26, range 2.1–3.0), respectively ($p = 0.001$). Expressed in terms of body surface area, mean log Cl in Malawian patients was 2.4 ml/min/m^2 (SD 0.14, range 2.1–2.6) as compared to 2.7 ml/min/m^2 (SD 0.21, range 2.2–2.9) in UK patients ($p = 0.001$). Fig. 3A shows a comparison of vincristine log Clearance values in Wilms tumour patients treated in the UK and Malawi.

3.3. *Area under the curve (AUC)*

AUC values of 1.6 to 11.4 μg/ml min were observed across Wilms' tumour population studied, with log AUC values of 3.2–4.1 μg/ml min. Mean log AUC values were higher in Malawian than in UK patients, with mean values of 3.8 μg/ml min (SD 0.15, range 3.6–4.1) and 3.5 μg/ml min (SD 0.22, range 3.2–3.9), respectively ($p = 0.003$). This difference is reflected in, on average, 1.98-fold larger vincristine AUC values for Malawian patients than for UK patients (95% confidence interval (CI) 1.39, 2.99). A comparison of vincristine log AUC values in these two patient populations is shown in Fig. 3B.

3.4. *Analysis of variables possibly influencing AUC*

We calculated the log(10)AUC to transform the AUC into a less skewed distributed variable which is a (pre)condition to perform a linear regression analysis as presented below. Across the whole patient population, age did not explain the difference in AUC ($p = 0.82$) and neither did vincristine dose as expressed per m^2 ($p = 0.42$). Body weight ($p = 0.3$) and vincristine dose ($p = 0.6$) did not significantly contribute to the difference in log AUC.

3.5. *Nutritional status*

Nutritional status, expressed as Z-score for corrected weight for height, was shown to significantly contribute to the difference in log AUC. Linear regression analysis indicated that a decrease of −1 in Z-score was associated with a change in log10 AUC of +0.061 ($p = 0.043$). This translates into a 13% increase in AUC on the non-transformed scale, i.e. higher AUC values were exhibited by the more malnourished patients. This association is shown in Fig. 4.

3.6. *Tumour weight*

Tumour weight did not significantly contribute to the differences found in log AUC across the population studied ($p = 0.20$). In UK patients, tumour weight was estimated at

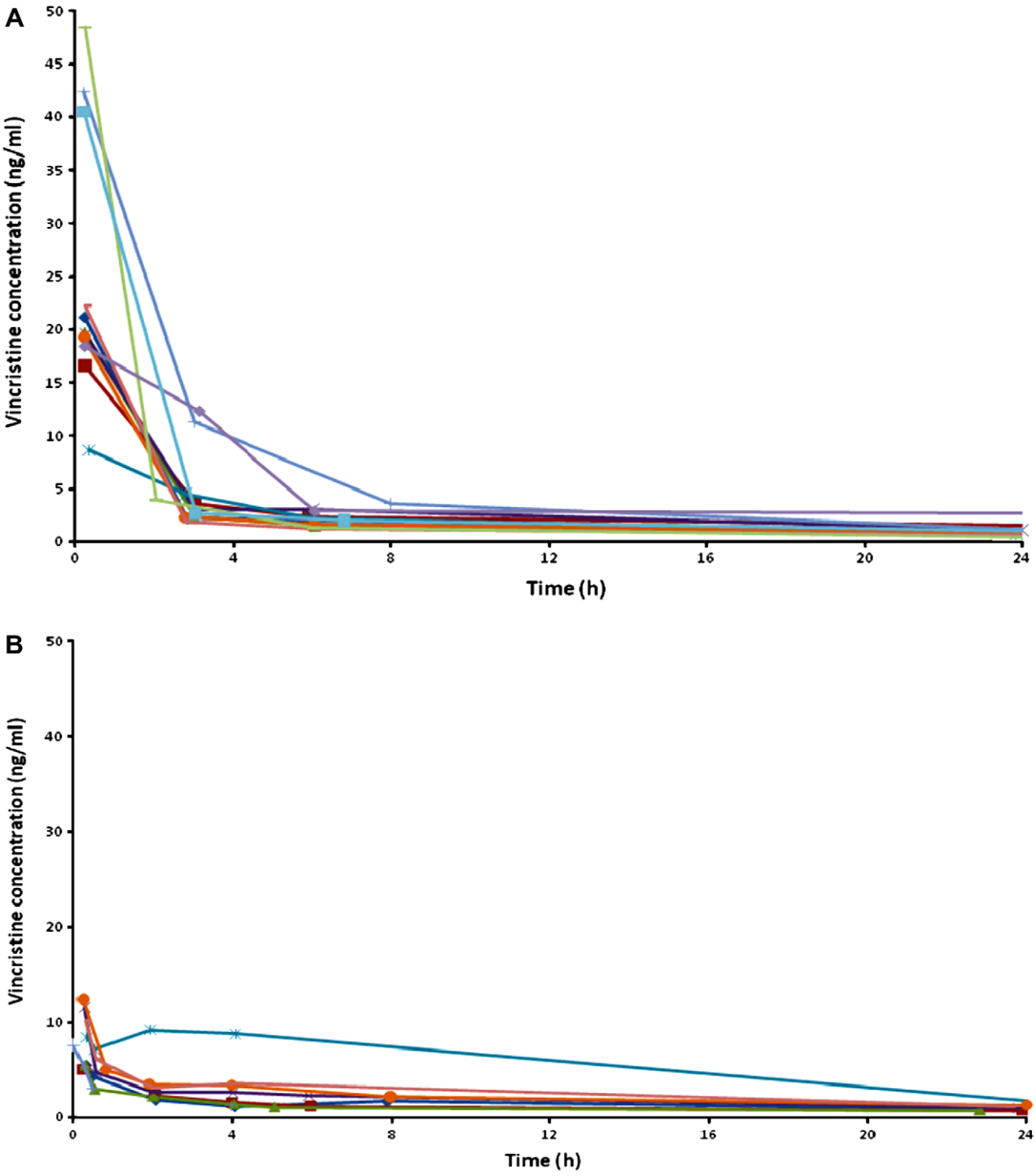

Fig. 2 – Vincristine plasma concentration–time curves in (A) Malawian and (B) UK Wilms tumour patient populations.

diagnosis and pharmacokinetics sampling carried out later. For this reason, we also analysed the relationship between tumour size and log AUC separately for the Malawian patients only. Again, no significant relationship between tumour weight and log AUC was observed ($p = 0.338$).

3.7. *Correlation nutritional status and tumour weight*

Nutritional status, as determined by Z-score for corrected weight for height, and tumour weight were closely correlated ($r = -0.848$, $p < 0.0001$).

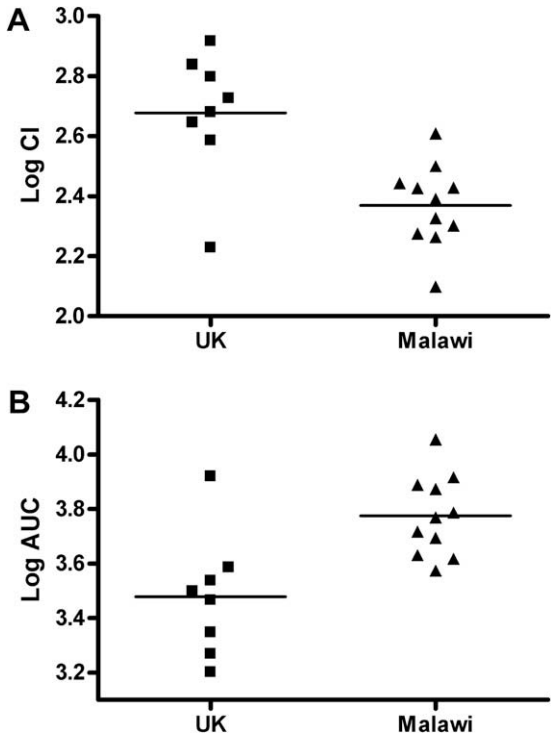

Fig. 3 – Vincristine log Clearance (A) and log AUC (B) values in UK and Malawian Wilms' tumour patient populations.

3.8. *Protein binding of vincristine*

Protein status was evaluated in the malnourished Malawian patient population to investigate whether this affected protein binding of the drug. Nine of 11 patients had an albumen level below the normal values of 37–55 g/l, with a mean value of 30 g/l (range 22–38 g/l). Four of 11 patients had a total protein below the normal values of 60–80 g/l, with a mean value of 65 g/l (range 51–82 g/l). The fraction of vincristine bound to protein in the Malawian patient population was found to be 69.4% (SD 4.6, range 62.4–74.9%).

4. Discussion

In developing countries, patients with Wilms' tumour often present late with large tumours and a marked degree of malnutrition.[1–4] Preoperative chemotherapy for localised disease consists of a combination of intravenous vincristine and actinomycin D for four weeks.[19] We studied the pharmacokinetics of vincristine in these patients, as compared to control patients treated with a comparable vincristine dosing regimen in the UK.

A clear difference in nutritional status between the two patient populations was found, highlighted by the fact that the patient with the poorest nutritional status in the UK group had a higher Z-score than the least malnourished Malawian patient. A limitation in this assessment is that the actual pharmacokinetic study was carried out in the UK patients at

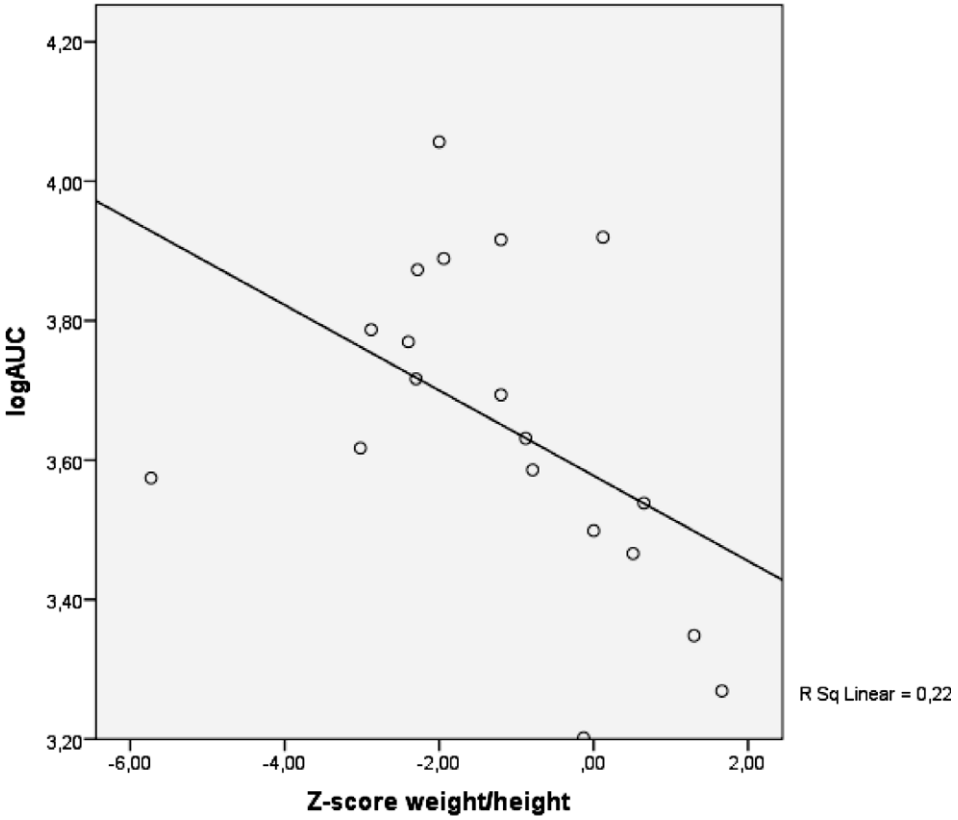

Fig. 4 – Relationship between patient nutritional status as determined by Z-score for corrected weight for height and log AUC of vincristine in patients with Wilms' tumour.

variable times after the tumour weight was estimated. Assuming though that most tumours will shrink during preoperative chemotherapy, had tumour weight been estimated at the time of the pharmacokinetic study, the actual tumour weight would have been lower, the corrected weight higher and thus the difference between the two groups even larger. Similarly with respect to tumour weights, which were higher in Malawian patients, it is likely that this difference would only have been larger if tumour weight in the UK patients had been determined at the time of the pharmacokinetic study.

A significantly lower vincristine clearance and correspondingly higher AUC was found in the Malawian children studied. This could be clinically relevant as the Malawian patients exhibited vincristine AUC values approximately 2-fold larger than those in UK patients.

Following linear regression analysis, nutritional status (as evaluated with Z-score for corrected weight for height) was shown to be a significant contributor to the differences in AUC found. A decrease of −1 in Z-score was associated with a 13% increase in AUC ($p = 0.043$). In this study, we did not find statistically significant evidence for a contribution of tumour weight to the differences in AUC. However, we cannot exclude a contribution of tumour weight *per se* in this study, bearing in mind that malnutrition and tumour size are highly correlated, the imperfect documentation of tumour size in the control patients in this study and the small sample size.

It would be interesting to know whether the increased vincristine AUC observed in Malawian patients is associated with an increased tumour response. This study does not provide data to support or reject that hypothesis. In a previously published study, involving many patients included in the current study, we found that tumour responses in Malawian patients with localised disease were comparable to those documented in European patients in the SIOP 9 study.[4,19]

An additional question would be whether an increased vincristine AUC is associated with an increased toxicity in Malawian patients. In this respect positive correlations between vincristine exposure and toxicity have previously been described. A study involving 27 patients receiving vincristine, including both adults and children, reported a significant correlation between drug exposure, in terms of vincristine AUC, and degree of neurotoxicity.[20] This finding is supported by other studies investigating the potential clinical impact of vincristine pharmacokinetic variation between patients.[21,22] However, it should be noted that several studies have also been published which do not support relationships between vincristine exposure and either clinical response or toxicity in patients.[5,6] Further studies are required to provide more definitive data concerning vincristine pharmacokinetic and pharmacodynamic relationships. While it is difficult to draw comparisons between studies, it is interesting to note that in our previous study we found that toxicity was increased in Malawian as compared to European patients.[4,19] It is unknown whether this increased toxicity is caused by a reduced patient tolerance, related to nutritional status, or due to an increased exposure to the drug in terms of AUC.

In our study, 69.4% of the vincristine was found to be bound to proteins in Malawian patients. Despite their very low albumen and relatively low total protein levels, this percentage of binding is comparable to the percentage of 58% protein binding found by Donigian.[15]

The laboratory values in Malawian patients do not indicate any liver or kidney dysfunction associated with malnutrition which would explain the decreased clearance and correspondingly higher AUC.

A limitation of this study is that pharmacogenetics in these, racially different, groups were not studied. Variation in drug metabolising enzymes such as CYP3A4 and CYP3A5, or drug transporters, many of which exhibit racial differences in prevalence of genetic variants, cannot be ruled out. Expression of CYP3A5, which plays a key role in vincristine metabolism, varies significantly according to race, with a greater proportion of African-Americans and Asians being high expressors as compared to Caucasians.[23] However, such an impact of CYP3A5 expression would cause an increased clearance in association with an increase in vincristine metabolism, i.e. the reverse of the trend observed in our study.

Conflict of interest statement

None declared.

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