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ORIGINAL ARTICLE

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Peak plasma concentrations of doxorubicin in children with acute lymphoblastic leukemia or non-Hodgkin lymphoma

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Abstract *Purpose:* The peak plasma concentrations seem to play an important role in the toxicity of the anthracyclines. As there are only limited data in the literature about the distribution of doxorubicin in children, we assessed the peak plasma concentrations of doxorubicin in pediatric patients. *Patients and methods:* We collected 87 plasma samples at the end of infusion from 27 children with acute lymphoblastic leukemia (ALL) or non-Hodgkin lymphoma (NHL) treated with 30 mg/m^2 doxorubicin as a 1- or 2-h infusion once weekly for four weeks in the ALL-BFM 95 or NHL-BFM 95 protocol. Plasma concentrations of doxorubicin were quantified by capillary electrophoresis, and the peak plasma levels for a uniform 2-h infusion were calculated. *Results:* The geometric mean of all samples was 273 μg/l with a geometric coefficient of variation of 46.0%. This is in accordance with the peak plasma concentrations expected from simulations based on literature data from adults. High inter-individual as well as substantial intra-individual variability was observed. Girls had slightly higher peak plasma levels than boys. Age, weight, and body mass index as well as laboratory parameters had no influence on the peak plasma concentrations. No cumulation of doxorubicin during therapy was observed. *Conclusion:* The peak plasma concentrations are similar in adults and children for both the absolute values as well as the variability; this indicates that there are no major differences in the distribution of doxorubicin in children and adults.

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Introduction

Doxorubicin is a very important cytostatic drug in the treatment of acute lymphoblastic leukemia (ALL) and non-Hodgkin lymphoma (NHL). However, the cardiotoxicity of doxorubicin is a serious problem, especially for long-term survivors, as congestive heart failure might occur years after treatment. There is an ongoing discussion about the best schedule for anthracyclines [1, 13, 23], as reduced acute cardiotoxicity was found with prolonged infusions compared to bolus injections [8, 11]. In an investigation with a mean follow-up of 8.1 years, a significant association with a higher rate of administration of doxorubicin and increased afterload was found [12]. While bolus injections result in high peak plasma concentrations (C_{max}) prolonged infusions substantially reduce C_{max} due to the short initial half-life of doxorubicin. However, a study published recently on a smaller number of patients with gastric carcinoma suggested a slightly reduced efficacy with 8-h infusions compared to bolus injections [15]. Bielack et al. [2] reviewed 14 studies where doxorubicin bolus injections were compared to schedules with prolonged infusions or fractionated administration. They concluded that there is substantial evidence that schedules leading to lower peak plasma levels are much less cardiotoxic. The data further suggest that with prolonged infusions there is no decline of efficacy together with cardiotoxicity.

Thus, the role of the C_{max} for the efficacy and safety of the doxorubicin therapy is unclear. Our hypothesis is that lower peak plasma concentrations are associated with a lower risk of developing congestive heart failure (CHF). There are very little pharmacokinetic data on doxorubicin in children in the literature. To our knowledge, there is one study indicating a reduced clearance of doxorubicin in infants

compared to older children [13]. Another group compared the C_{max} of doxorubicin and epirubicin after concomitant administration of both drugs [4]. However, this study focused on differences in the kinetics between doxorubicin and epirubicin. In adults, substantial inter-individual variability was observed; this suggests dose adjustment based on plasma concentration measurements might be useful to optimize anthracycline chemotherapy [16]. The aim of our investigation was to quantify the C_{max} and its intra- and inter-individual variability of doxorubicin in children with ALL or NHL and to get information about the pharmacokinetics of doxorubicin in children from the C_{max}. It has been shown that the C_{max} of doxorubicin correlates with the area under the curve (AUC) [6]. Further, we investigated if covariates such as age, weight, gender, and liver and renal function might influence the kinetics of doxorubicin. We decided not to conduct a classical pharmacokinetic study with a rich sampling protocol, to reduce the burden on the individual patients.

Patients and methods

This investigation was approved by the local ethics committee. A total of 27 patients were included and the patients or their parents gave informed consent to the blood sampling. The patients were treated according to the ALL-BFM 95 or NHL-BFM 95 protocol. Doxorubicin was administered during protocol II (22 weeks after the start of therapy) as a 1- or 2-h infusion every 7 days over 4 weeks at a dose of 30 mg/m^2 from a perfusor syringe, which ensured a constant flow rate during the entire infusion. Patients had already received a cumulative dose of 60 to 180 mg/m^2 daunorubicin in the induction regimen depending on the risk status. Co-medication included dexamethasone 10 mg/m^2 per day, asparaginase medac 10,000 U/m^2, and vincristine 1.5 mg/m^2, on the same day.

About 50 µl blood was collected with heparinized capillaries from the fingertip 3 to 5 min before the end of infusion (i.e., after the perfusor alarm sounds) and was immediately centrifuged at 4 °C. Experiments done previously showed that this procedure is suitable to ensure reproducible results [10]. Plasma was stored at –20 °C until analysis. The samples were analyzed by a validated method using capillary electrophoresis with laser-induced fluorescence detection [7].

During this investigation, the infusion time for doxorubicin was increased from 1 to 2 h. Due to the very short half-life of the α-phase of doxorubicin, the peak plasma level drastically decreases with increasing infusion duration [17]. In clinical practice, the duration of infusion was not exactly 1 or 2 h. Therefore, the exact time of infusion for every application was documented. For further comparison, a pharmacokinetic model based on literature data [5] was used to transform the observed C_{max} to C_{max} for a theoretical 2-h infusion: based on the reported pharmacokinetic parameters for a three-compartment model, the C_{max} as a function of time of infusion for a uniform dose was calculated for all 21 patients reported in the literature [5]. The mean ratio $C_{max\ actual}/C_{max\ 2\text{-}h\ infusion}$ was used for the correction of peak plasma levels for the infusion time of our own data set.

For the statistical analysis, the C_{max} values were log-transformed, as pharmacokinetic data such as C_{max} and AUC are known to be log-normally distributed [9]. Inter- and intra-individual variability was quantified by the geometric mean $mean_{geo}$, the geometric standard deviation SD_{geo} and the geometric coefficient of variation CV_{geo} [20]:

$$mean_{geo} = e^{\left(\frac{1}{n}\sum_{i=1}^{n}\ln x_i\right)}$$

$$SD_{geo} = \sqrt{\frac{1}{n-1}\left(\sum_{i=1}^{n}\ln(x_i)^2 - \frac{1}{n}\left(\sum_{i=1}^{n}\ln(x_i)^2\right)\right.}$$

$$CV_{geo} = 100\sqrt{e^{SD_{geo}^2} - 1[\%]}$$

For graphical presentation, the geometric mean is shown with the 16th and 84th percentiles. The percentiles were calculated using the following formulae:

$$16^{\text{th}}\ \text{percentile} = \frac{mean_{geo}}{e^{SD_{geo}}}$$

$$84^{\text{th}}\ \text{percentile} = mean_{geo} * e^{SD_{geo}}$$

The log-transformed C_{max} values of two different groups were compared by the t test. To test for possible differences between the first, second, third, and fourth administration of doxorubicin to the same patient, the log-transformed C_{max} values were compared by the one-way repeated-measures analysis of variance. Correlations between peak levels as dependent variables and different independent factors were investigated by linear regression analysis. The software SigmaStat 2.03 was used.

Results

The patients' characteristics are summarized in Tables 1 and 2. The age distribution is typical for this tumor entity. Laboratory parameters were in the usual range, except for two patients with elevated liver enzyme parameters.

Table 1 Data on patients and available blood samples

	No. of patients	No. of blood samples
Overall	27	87
Patients with ALL	24	78
Patients with NHL	3	11
Boys	12	39
Girls	15	48

Table 2 Patients' characteristics and laboratory parameters

	No. of samples	Median	Range
Age (years)	27	4.13	1.56–19.99
Height (cm)	27	106	79–181
Weight (kg)	27	22.5	10.4–73.0
Body surface area (m^2)	27	0.78	0.48–1.94
Body-mass index (kg/m^2)	27	16.7	13.7–22.2
Hematocrit (%)	83	37.2	23.8–48.8
Serum protein (g/l)	55	57	48–70
Serum creatinine (mg/dl)	55	0.5	0.4–0.7
Total bilirubin (mg/dl)	54	0.3	0.0–1.0
AST (U/l)	55	10.0	6–81
ALT (U/l)	55	20.0	3–263
γ-GT (U/l)	55	13.0	7–107

While this investigation was running, the infusion time for doxorubicin was increased from 1 h to 2 h. As expected, the peak plasma concentrations (without transformation) decreased linearly from a geometric mean of 505 μg/l (CV_{geo} 44.9%, $n = 15$ from 6 patients) with a 1-h infusion to 256 μg/l (CV_{geo} 63.3%, $n = 81$ from 21 patients) with a 2-h infusion. This is in accordance with the simulations based on pharmacokinetic parameters reported in the literature. After transformation of the 1-h data by the procedure described above, the geometric mean was 271 μg/l with a CV_{geo} of 44.9%.

Figure 1 shows simulations for 21 individuals for a 2-h infusion of doxorubicin (30 mg/m^2) as box plots, on the basis of data published by Eksborg et al. [5] in adults and the data collected in this study. A three-compartment model was used. The simulated geometric mean C_{max} of 278 μg/l is in good agreement with our data (273 μg/l). In addition, the variability found in literature is similar to what we found in children (CV_{geo} 46.0%, simulated 36.9%).

The deviations between patients were substantial over the four administrations given. The C_{max} values from 13 patients of all four repeated administrations could be collected, whereas for the others only incomplete data sets were available. In Fig. 2, the data from the four consecutive administrations are compared. The geo-

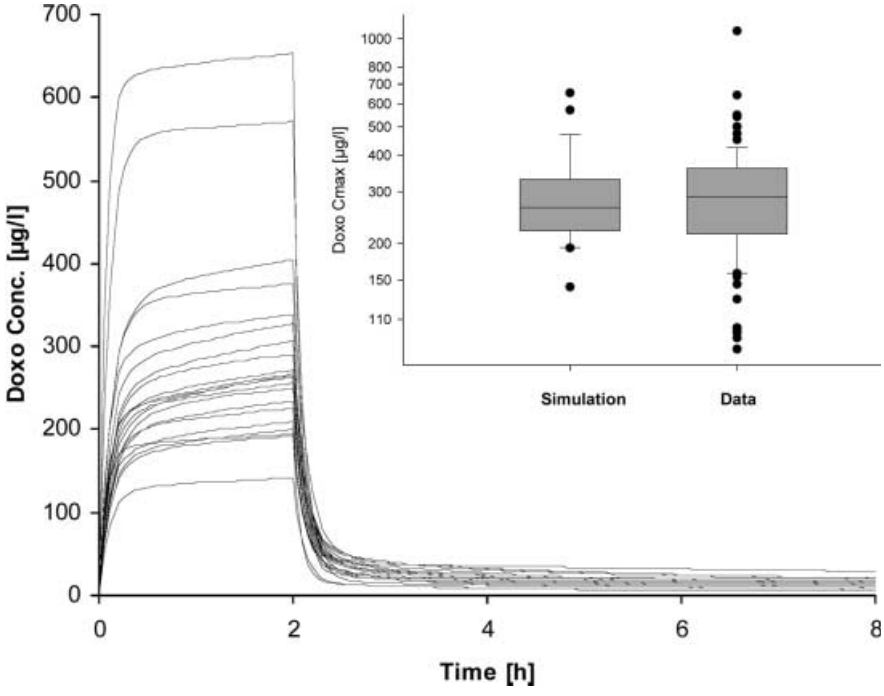


Fig. 1 Simulations of 2-h infusions of 30 mg/m^2 doxorubicin (Doxo) for 21 adults based on data from the literature. The *box plots* represent the data collected in this study vs the C_{max} of the simulations

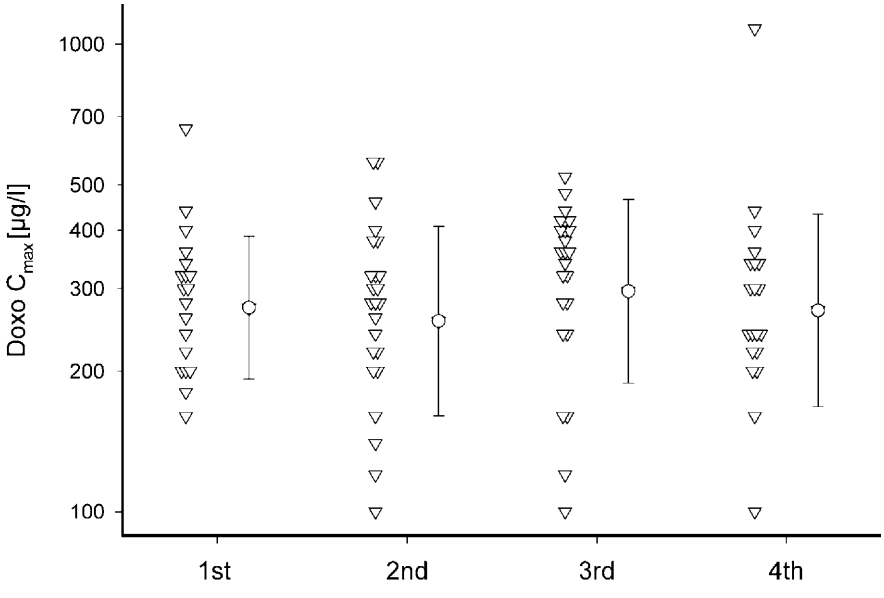


Fig. 2 Doxorubicin (Doxo) C_{max} after consecutive administrations. The *triangles* represent single data points, the *circles* are the mean$_{geo}$ values, and the *bars* represent the 16th and 86th percentiles

metric means for all patients were 273, 256, 296, and 270 μg/l for the first, second, third, and fourth infusions, respectively. This indicates that, as expected from data in adults, there is no cumulation with weekly infusions of doxorubicin. The variability was also similar in the consecutive infusions, with CV_{geo} values of 36.2, 49.3, 47.6, and 50.2%, respectively. Statistical analysis of the 13 complete data sets, by the one-way repeated measures of variance test, also showed that there were no differences between the C_{max} of the consecutive administrations ($P = 0.904$).

Figure 3 illustrates the variability of the C_{max} parameter. Variability between patients was substantial with a CV_{geo} of 46.0% when only the first C_{max} per patient is taken into account. However, the intra-individual deviations ranged from 3.5 to198.1%, with a median of 26.2% for all patients. Some patients, such as patients 10 or 16, have a very low intra-individual variability, whereas others, such as patient 5, display an extremely high variability with unpredictable C_{max} values.

The C_{max} was 255 μg/l ± 53.9% in boys and 289 μg/l ± 38.3% in girls ($mean_{geo} \pm CV_{geo}$, Fig. 4). If only the first data point per patient is taken into account, the difference is more pronounced, with 201 μg/l ± 44.0% for boys and 291 μg/l ± 41.6% for girls (*t* test, $P = 0.027$). However, the low number of patients does not allow one to draw the general conclusion that the C_{max} of doxorubicin is higher in girls than in boys; this is also indicated by the power of 0.525 of the statistical test.

In Fig. 5, C_{max} is plotted against the patients' characteristics. Age, weight, and body-mass index were

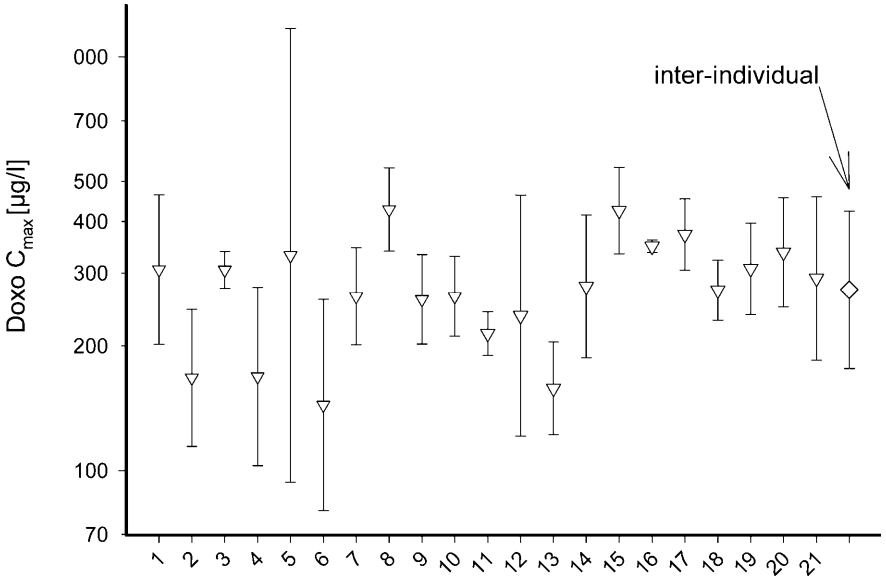


Fig. 3 Intra-individual and inter-individual variability of the doxorubicin (Doxo) C_{max} values in 21 patients with more than two data points available ($mean_{geo}$ and 16th and 84th percentiles)

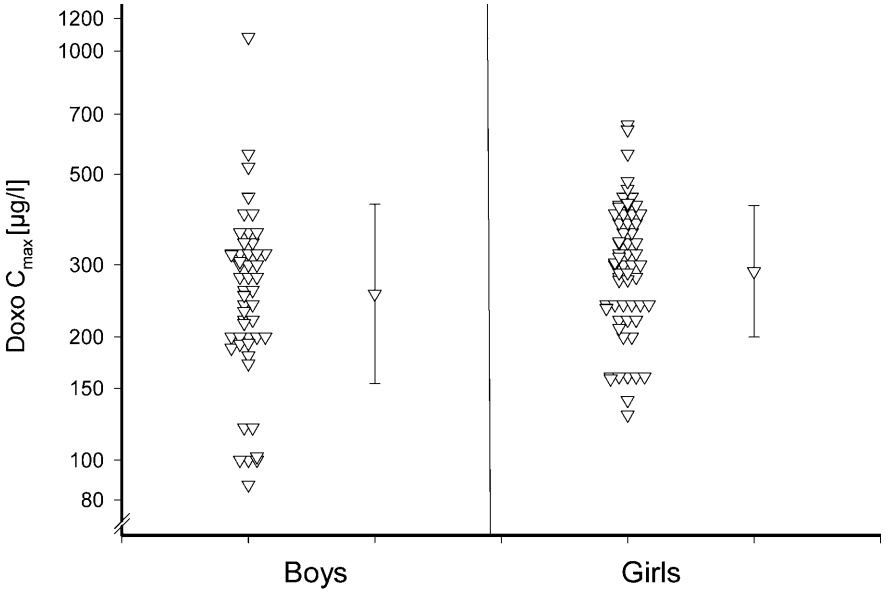


Fig. 4 Comparison of the doxorubicin (Doxo) C_{max} values of boys and girls. (All data present; $mean_{geo}$ and CV_{geo} values as *bars*)

investigated for correlation to the C_{max} of doxorubicin. No correlation or visible graphical trend was observed.

In addition, laboratory parameters were tested for their influence on the C_{max} of doxorubicin. In Fig. 6, the data are plotted against the laboratory parameters.

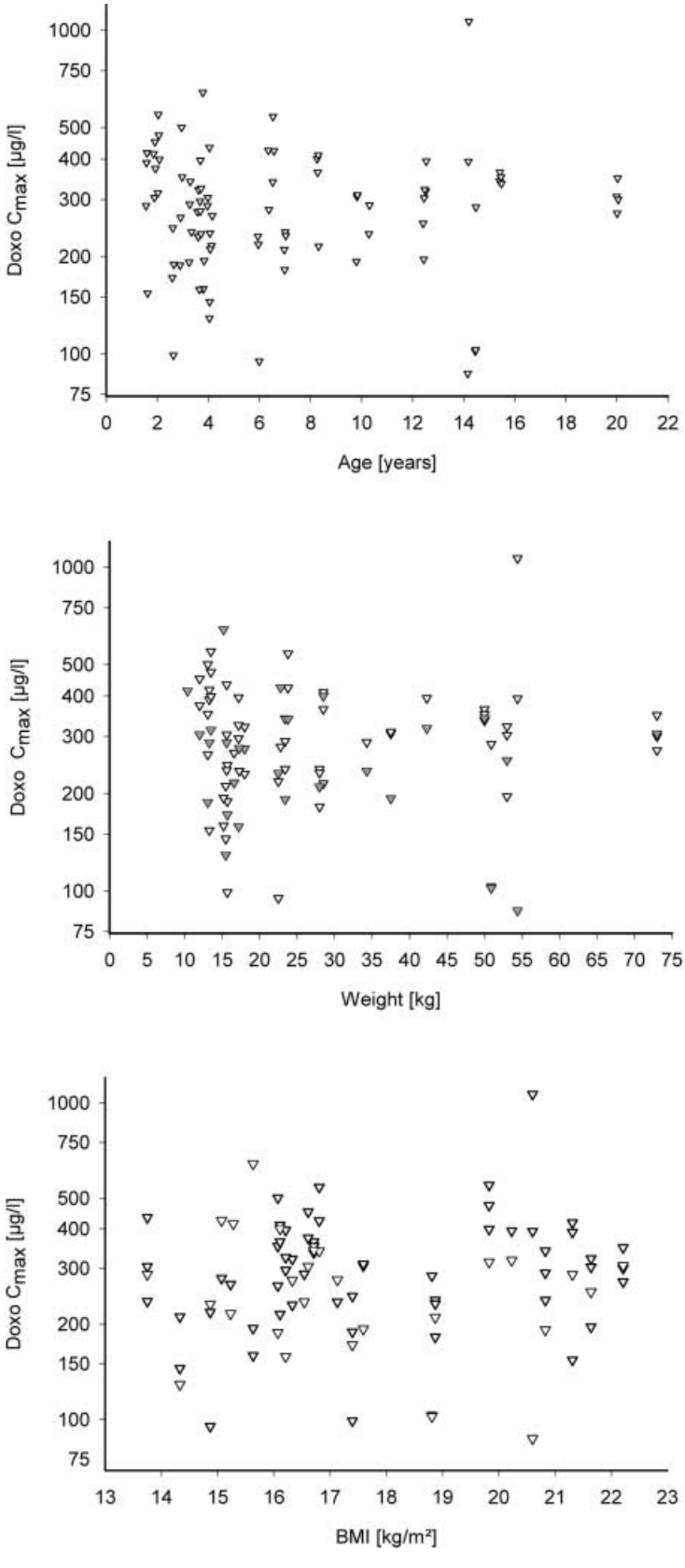


Fig. 5 Plots of various patient characteristics versus doxorubicin C_{max}

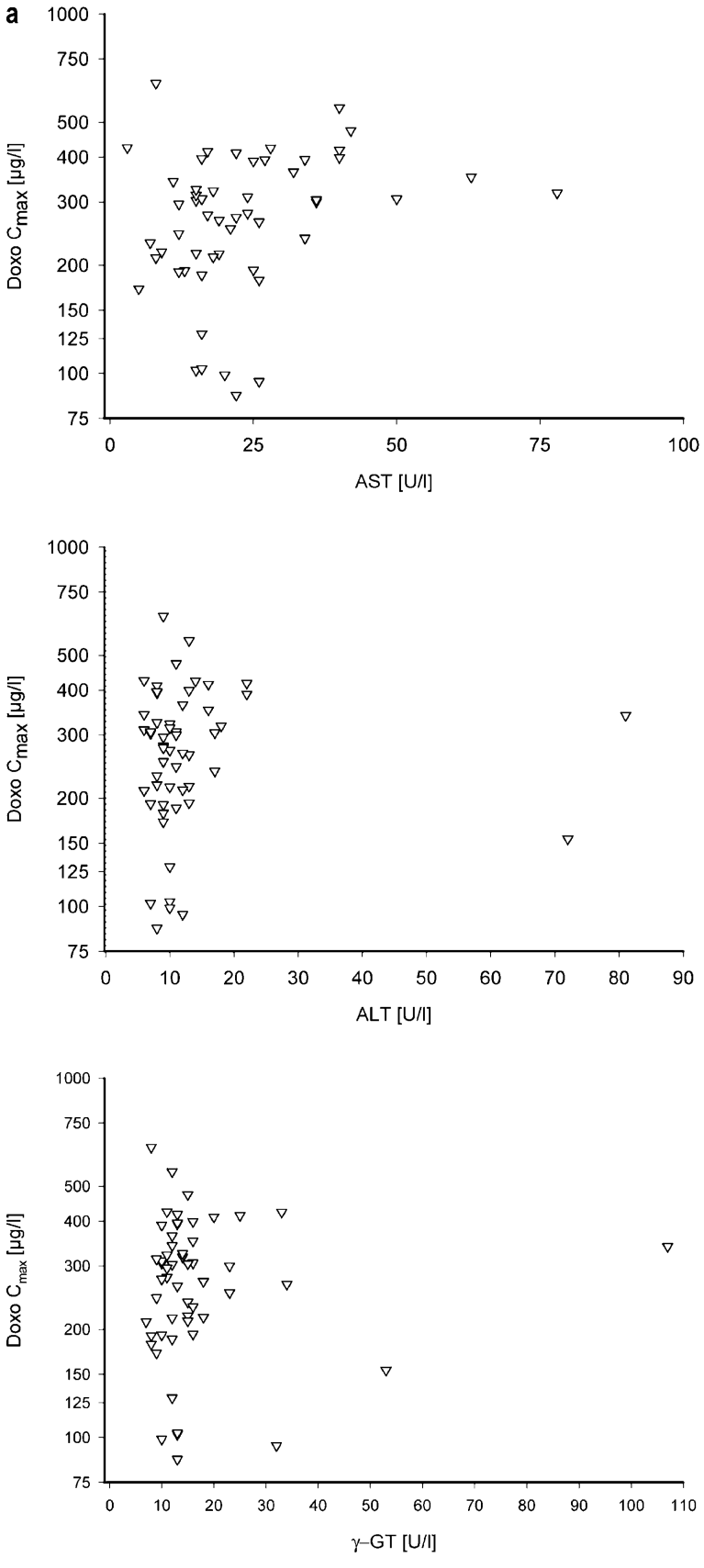
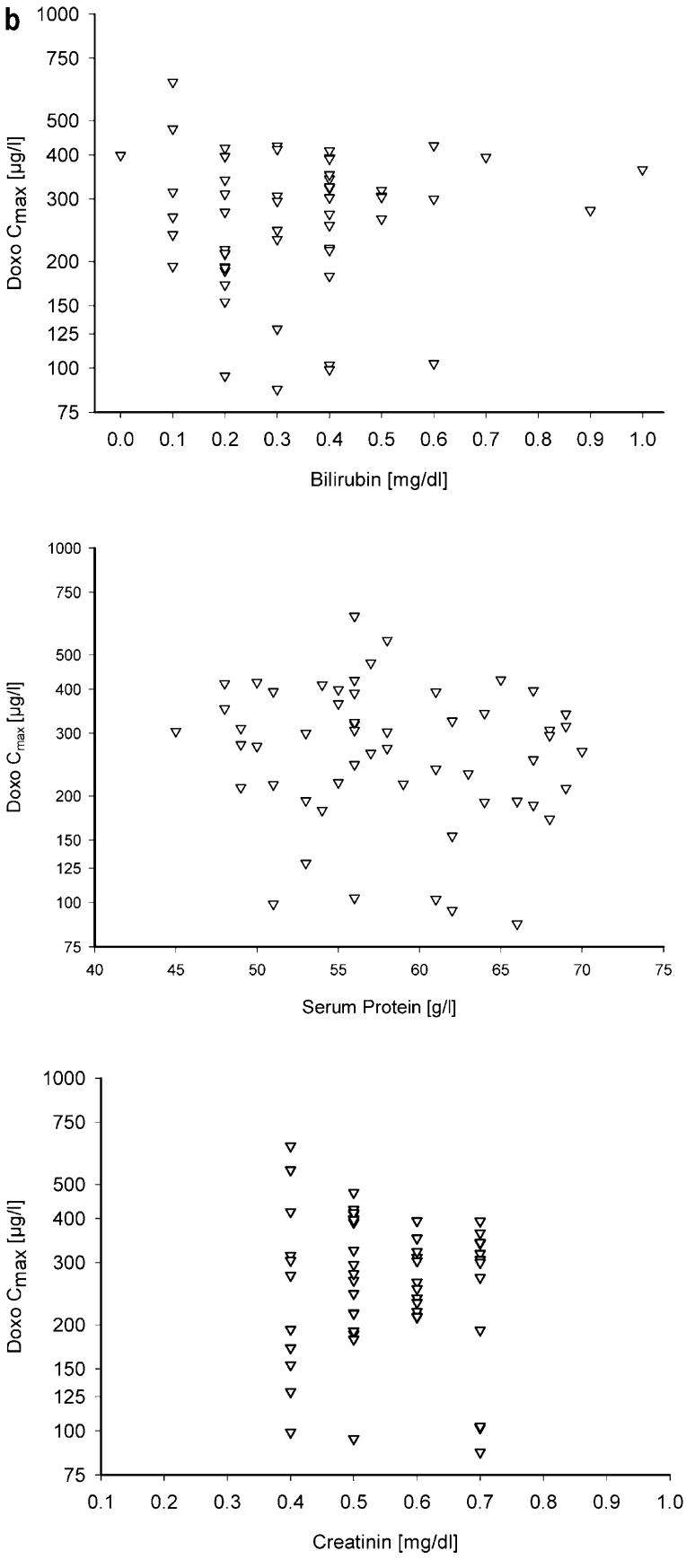


Fig. 6a–b Plots of various laboratory parameters versus doxorubicin C_{max}. There were no statistically significant correlations between C_{max} and any of the parameters

Fig. 6a–b (Contd.)



Although there may be some trend towards a lower doxorubicin C_{max} with increasing serum protein ($P = 0.283$) or with lower serum creatinine concentrations ($P = 0.559$), no statistically significant correlation was found between any of the laboratory parameters listed in Table 2 and the C_{max} of doxorubicin.

Discussion

There is an ongoing discussion about the best schedule of administration for anthracyclines. Both toxicity and efficacy can be influenced by the duration of infusion: because of the short initial half-life of about 4 min, the C_{max} for 30 mg/m^2 doxorubicin varies between approximately 1500 μg/l for a 3-min infusion down to 100 μg/l for an 8-h infusion [5]. However, the AUC is not influenced by the infusion time, as shown by the majority of studies [18], although one study found a greater overall exposure with a 4-day infusion compared with the same dose given as a bolus injection [21]. As the C_{max} seems to play a key role in the toxicity of doxorubicin, we decided to collect the C_{max} from children in different treatment schedules, and to identify possible cofactors which might influence the kinetics of doxorubicin.

To reduce the patients' burden during this investigation, we sampled only the peak plasma concentrations instead of conducting a classical pharmacokinetic study with many blood samples per patient and administration. Further, we used capillary electrophoresis (CE) instead of high-performance liquid chromatography (HPLC) to quantify doxorubicin in plasma, as CE requires only very small sample volumes. A drawback of this design is that we have only limited information available about the elimination of doxorubicin in the body. In a three-compartment model, the central volume of distribution and the initial half-life has the greatest influence on C_{max}. In addition, it was demonstrated by the use of limited sampling models that other parameters such as the area under the curve (AUC) can also be calculated from C_{max} with acceptable precision [6]. Therefore, C_{max} can be used to get some information about the pharmacokinetics of anthracyclines, mainly the distribution phase.

To our knowledge, only very little data appear in the literature about the kinetics of doxorubicin in children [4, 14]. Therefore, we compared our C_{max} data with the pharmacokinetic parameters for doxorubicin described in the literature for adults. No differences between the mean C_{max} as well as its variability were found. This indicates that the distribution of doxorubicin in children and adults is similar. However, assumptions about the elimination of the drug can not be drawn from our data. McLeod et al. found the pharmacokinetics in children aged 3–18 years to be similar to that of adults, and a lower clearance in infants than in older children [13].

Because literature data indicate that toxicity is reduced with prolonged infusions [1, 8, 11], the protocol was modified to lengthen the duration of infusion from 1 h to 2 h. A subgroup of six patients received the 1-h infusion. The 15 peak plasma concentrations of this subset were analyzed after transformation together with the other data. Analysis of the data showed no differences between the 1-h infusions and the 2-h infusions. Analysis of the 2-h data alone led to the same results as analysis of the whole data set.

In a retrospective study, Lipshultz et al. [12] identified the cumulative dose, age at time of treatment, and gender as risk factors for children with ALL to develop congestive heart failure later in life. We investigated whether or not patients with these risk factors have higher C_{max} values for doxorubicin. In the data presented here, no correlation was found between age and C_{max} (Fig. 5). However, data from only two children younger than 2 years were available. Eksborg et al. [4] found that the C_{max} values of doxorubicin were higher in a group of 15 children below 4.9 years than in 16 older children receiving 24-h infusions. However, the cut-off point of 4.9 years is rather arbitrary, and does not allow a conclusion about a general age dependency to be drawn. In the patient population described here, no such differences between these age groups were seen. In a group of 37 patients aged between 17 and 74 years, Robert et al. found a decreasing clearance with increasing age [17]. This may be due to a decreased early clearance in patients older than 60 years. Our data indicate that these findings in adults cannot be interpolated to the kinetics in children.

Girls had slightly higher doxorubicin C_{max} values than boys. However, this difference was only significant when the first measurement of each patient was analyzed. In adults, the doxorubicin clearance was found to be higher in men than in women in a group of 27 patients [3]. We are currently addressing this question in an analysis conducted in our laboratory, involving a larger number of patients.

Reduced clearance has been observed in obese patients [19]. From the data shown here, no clear dependency of the C_{max} on the body-mass index could be found.

Twelves et al. [22] found reduced clearance in 24 adults with abnormal liver biochemistry test results. In the patient population analyzed here, only two patients with transient increases in AST, ALT, and γ-GT were present. These patients had no elevated C_{max} levels. However, it cannot be excluded that the elimination in these patients is slower than in patients with normal liver function.

Dose individualization based on plasma concentration measurements has been suggested to reduce the variability in the drug exposure of individuals [16, 21]. From the data shown here it is obvious that dose individualization cannot be based on C_{max}, owing to the high intra-individual variability. However, it remains to be tested if a dose adaptation based on plasma concentrations collected later after administration, that is, 24-h levels, is possible.

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