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Vincristine pharmacokinetics is related to clinical outcome in children with standard risk acute lymphoblastic leukemia

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Summary

Vincristine is a key drug in the treatment of childhood and adult acute lymphoblastic leukemia (ALL), and many other childhood malignancies. Despite decades of wide clinical use, no data on the correlation between vincristine pharmacokinetics and long-term clinical outcome have been published. We here report clinical data (median follow-up time 10·5 years, range 7·3–12 years) for 86 children with B-cell precursor ALL, in whom vincristine kinetics were studied on treatment day 1. The median total plasma clearance was 429 and 331 ml/min per m^2 and the area under the plasma concentration-time curve (AUC) was 4·49 and 5·40 mg/l × min in relapse and non-relapse patients, respectively (not significant). In standard risk patients, where treatment depends more heavily on vincristine than in other subgroups, the relative risk (RR) of relapse was significantly increased for patients with clearance values above median (RR 5·2; $P = 0·036$), or AUC values below median (RR 5·8; $P = 0·025$). Our data suggest a relationship between the antileukemic effect and the systemic exposure of the drug, which warrants further studies.

Keywords: acute lymphoblastic leukemia, childhood, vincristine, pharmacokinetics, drug effect.

Vincristine has been used in clinical practice since the early 1960's (Costa *et al*, 1962; Johnson *et al*, 1963). Today, it is a key drug in the combination chemotherapy for childhood and adult acute lymphoblastic leukemia (ALL). Vincristine is also part of the front-line treatment for lymphomas, Wilms tumor, rhabdomyosarcoma, Ewing sarcoma, neuroblastoma and many brain tumors in children. Until 1991, pharmacokinetic studies were hampered by the lack of specific and sensitive analytical methods for measuring vincristine concentrations in biological fluids. The introduction of a specific high performance liquid chromatographic (HPLC) assay, sufficiently sensitive for clinical use in children, made the appropriate studies of vincristine possible (Bloemhof *et al*, 1991). A number of studies in children with ALL (Crom *et al*, 1994; de Graaf *et al*, 1995; Groninger *et al*, 2002; Frost *et al*, 2003), Wilms tumor (Gidding *et al*, 1999) and brain tumors (Kellie *et al*, 2004) have described the pharmacokinetics of vincristine. After an i.v. push, there is a short distribution phase (t½ about 6 min) and a slow elimination phase, with a terminal half-life of around 15 h. The volume of distribution is high (>200 l/m^2), indicating extensive tissue binding. Inter-patient variability was large in pediatric cancer patients (>10-fold for all kinetic parameters studied). Correlation to sex or age has not been found for clearance or other pharmacokinetic parameters in children, but tumor type, concomitant medication and liver function were found to be of importance (Gidding *et al*, 1999; Groninger *et al*, 2002; Frost *et al*, 2003).

In spite of the fact that vincristine has played a key role in treatment of childhood ALL for several decades (Chessells, 2000), little is known about the relationship between the pharmacokinetics and the clinical effect of the drug. In a unique study, Groninger *et al* (2005) investigated vincristine pharmacokinetics and the response to monotherapy in an up-front study including 54 children with newly diagnosed ALL.

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Response to a single dose of vincristine was determined on bone marrow and peripheral blood smears after 3 d. However, the variability of pharmacokinetics did not explain the variability in early response to vincristine monotherapy.

Between 1995 and 1999 we studied vincristine pharmacokinetics in 98 children with newly diagnosed ALL (Frost *et al*, 2003). In the present study, we have collected clinical follow-up data for this cohort of patients, to see if there was any correlation between vincristine pharmacokinetics and clinical outcome. To our knowledge, no such data have been presented for any age group, neither for acute leukemia nor solid tumors. Since the treatment of ALL is adapted to risk group, and the relative importance of vincristine varied considerably in the treatment given to the three major risk groups here, our strategy was to analyse both the whole patient material and the three risk groups separately.

Patients and methods

Patients

We previously reported vincristine pharmacokinetics as studied on treatment day 1 in 98 patients with ALL (Frost *et al*, 2003). Two patients were reclassified as having acute myeloid leukemia and treatment was changed accordingly. One patient with ALL died during induction therapy on treatment day 9. One patient was resistant to the initial treatment as shown by >25% blast cells in a bone marrow aspirate at day 29 and was then treated by another protocol. Eight children had T-ALL, a disease that in many respects differ from B-cell precursor (BCP) ALL. The present study focussed on the 86 patient with BCP ALL who, at the end of induction therapy, were in complete remission and received further treatment according to the Nordic Society of Paediatric Haematology and Oncology (NOPHO) ALL-92 protocol (Gustafsson *et al*, 2000).

Three major risk groups were recognised in NOPHO ALL-92: standard risk (SR), intermediate risk (IR), and high risk (HR). The criteria for SR were age 2–<10 years, white blood cell count (WBC) $\leq 10 \times 10^9$/l, no extramedullary leukemia (EML), and no cytogenetic high risk features [*MLL* rearrangements or t(9;22)(q34;q11)], whereas those for IR were age 1–<2 or >10 years, WBC count 10–$\leq 50 \times 10^9$/l, no EML, and absence of cytogenetic high risk features. The criteria for HR were WBC $>50 \times 10^9$/l, EML, lymphomatous leukemia and/or cytogenetic high risk features. HR also included slow responders with day 15 M3 bone marrow (≥25% blasts) or day 29 M2 (5–24% blasts)/M3 bone marrow. Induction treatment was identical for all of the risk groups, except that HR patients received an extra dose of doxorubicin on treatment day 8. After induction treatment (50 d), further chemotherapy was adapted according to risk group.

Vincristine, 2 mg/m^2 body surface area (BSA), maximum of 2 mg, was given as an i.v. bolus injection over 1 min. The first dose was administered on treatment day 1, and the total number of doses was 11 in the SR group, 14 in the IR group, and 15 in the HR group. Details on drugs and dosages have been reported previously (Gustafsson *et al*, 2000).

Assessment of the treatment results was based on bone marrow morphological analyses, peripheral blood tests and cerebrospinal fluid examinations. Complete remission (CR) was defined as less than 5% leukemic blasts in a representative bone marrow sample and no EML, and relapse as more than 5% blast cells in bone marrow and/or manifestation of EML. The results were evaluated from annual reports submitted from the treating clinicians to the Nordic registry at the Childhood Cancer Research Unit in Stockholm.

Patients and/or parents gave informed consent. The local ethics committees approved the study.

Plasma samples and pharmacokinetic methodology

Blood was collected before and 10, 30, 360, and 1380 min after vincristine injection. Vincristine plasma concentrations were measured by HPLC with electrochemical detection (Koopmans *et al*, 2001). A limited sampling strategy and Bayesian analysis was used to fit a two-compartment model to the concentration-time data, using the parameter estimation module of the ADAPT II pharmacokinetic software package (Biomedical Simulations Resource, University of Southern California, Los Angeles, CA, USA (D'Argenio, 1981; D'Argenio & Schumitzky, 1979). Priors for the Bayesian analysis were obtained from previous studies (Crom *et al*, 1994; de Graaf *et al*, 1995). Estimated primary pharmacokinetic parameters included volume of central compartment, first-order rate constant for overall elimination of drug from central compartment, and first-order rate constants for drug transport between central and peripheral compartment. Primary parameters were used to derive the following secondary parameters: distribution and elimination half-lives ($t_{½}\,\alpha$ and $t_{½}\,\beta$), total body clearance (Cl), and volume of distribution at steady-state (Vdss). Area under the concentration-time curve (AUC) was calculated using the formula Cl = Dose/AUC.

Statistical analysis

Non-parametric methods were used throughout. Differences in distribution of variables were tested with the Mann-Whitney U test or the chi-square test. The Spearman correlation coefficient was used to examine relationships between continuous variables. Curves illustrating the probability of disease-free survival (p-DFS) were calculated by the Kaplan-Meier method, where patients in continuous complete remission (CCR) were censored at the time of the latest follow-up. The log rank test was used to compare survival curves. Statistical comparisons of outcome were conducted by simple (univariate) and multivariate Cox proportional hazard regression analyses, with stratification for risk group when the whole patient material was analysed. The Statistical Package for the Social Sciences (SPSS) v. 14.0 software was used for the calculations. All analyses were two-tailed and the level of statistical significance was set at $P < 0{\cdot}05$.

Results

The median follow-up time of the 86 patients studied was 10·5 years (range 7·3–12 years). During follow-up, 25 children relapsed: 22 patients relapsed in the bone marrow (in five cases combined with extramedullary manifestations), and three patients experienced extramedullary relapses. The median time to relapse was 3·3 years (range 1·7–11 years), and the p-DFS 0·69, standard error (SE) ± 0·06 (0·76 ± 0·05 at 5 years).

There were no statistically significant differences in baseline parameters between patients in CCR and relapse patients, but a trend to a male preponderance in the relapse group (Table I). Cytogenetic high risk features, i.e. *MLL* rearrangements and t(9;22), were not found in any of the patients. Data on t(1;19) and t(12;21) were only available for a minority of the patients, but did not show differences in distribution between the two groups (not shown).

Table I. Patient characteristics and pharmacokinetic parameters for vincristine.

	CCR	Relapse	*P* value
No.	61	25	
Age, years			
Median	4·1	5·2	0·45
Range	1·2–17·4	1·9–16·1	
Sex (*n*)			
Male	30	18	0·10
Female	31	7	
WBC, 10^9/l			
Median	6·4	6·0	0·98
Range	0·8–148	1·3–274	
Risk group (*n*)			
Standard	29	11	0·64
Intermediate	21	11	
High	11	3	
Clearance, ml/min per m^2			
Median	331	429	0·24
Range	134–1703	171–1194	
25th–75th	250–450	258–502	
AUC, mg/l × min			
Median	5·40	4·49	0·25
Range	0·93–14·9	1·08–11·7	
25th–75th	3·84–7·46	3·74–7·14	
T½ α, min			
Median	6·1	6·9	0·35
Range	0·8–11·8	3·0–10·4	
25th–75th	5·6–7·5	5·7–7·9	
T½ β, min			
Median	1033	1031	0·76
Range	258–2323	496–1966	
25th–75th	886–1261	665–1314	
Vdss, l/m^2			
Median	442	441	0·82
Range	174–873	139–1113	
25th–75th	340–543	337–621	

CCR, continuous complete remission; WBC, white blood cell count at diagnosis; AUC, area under the concentration-time curve; T½ α, distribution half-life; T½ β, elimination half-life; Vdss, volume of distribution at steady-state; 25th–75th, 25th to 75th percentile.

The median total plasma clearance of vincristine was 429 and 331 ml/min per m^2, and the AUC 4·49 and 5·40 mg/l × min, in relapse and CCR patients, respectively (not significant). Distribution and elimination half-lives, and volumes of distribution were similar in the two groups (Table I).

To analyse the correlation between exposure to the drug and clinical outcome, we dichotomised the clearance and AUC values. The p-DFS for patients with clearance values below median tended to be higher than for those with clearance values above median, p-DFS 0·75 (SE ± 0·09) and 0·63 (±0·07), respectively ($P = 0·065$) (Fig 1). Similar curves were obtained when the Kaplan-Meier analysis was based on dichotomised AUC values; p-DFS 0·74 (±0·09) and 0·63 (±0·07), respectively ($P = 0·081$). When risk groups were studied separately, significant differences were found in the SR group (Fig 2), with p-DFS of 0·90 (±0·07) and 0·55 (±0·11) for patients with clearance below and above median, respectively ($P = 0·019$). p-DFS for patients with AUC above and below median was 0·90 (±0·06) and 0·53 (±0·12), respectively ($P = 0·011$). No differences or any trend to differences were found in the IR and HR groups (not shown). When AUC values in the SR group were divided into tertiles, six out of 14 patients in the group with the lowest AUC values relapsed, compared to 4/13 in the middle group, and 1/13 in the group with the highest AUC values. The median AUC values in the three groups were 3·7, 5·8, and 9·6 mg/l × min, respectively.

Cox regression analysis was performed to study the prognostic impact of pharmacokinetic parameters and baseline

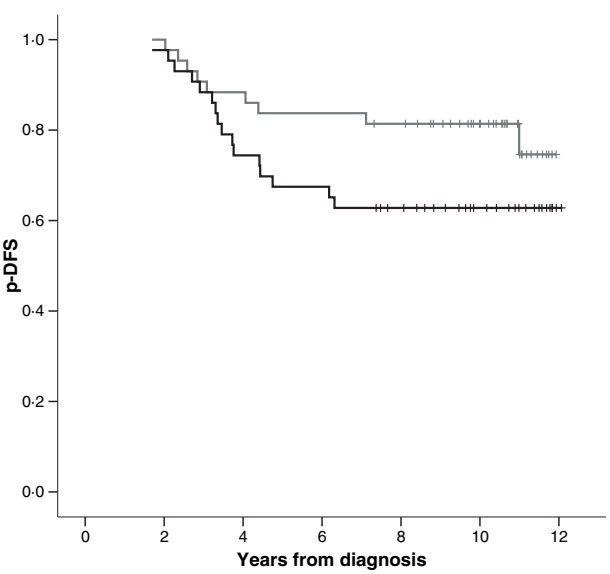

Fig 1. The relationship between vincristine plasma clearance and probability of disease-free survival (p-DFS) in 86 children with B-cell precursor ALL. The upper curve represents children with clearance values below the median and the lower curve represents those with values above the median ($P = 0·065$).

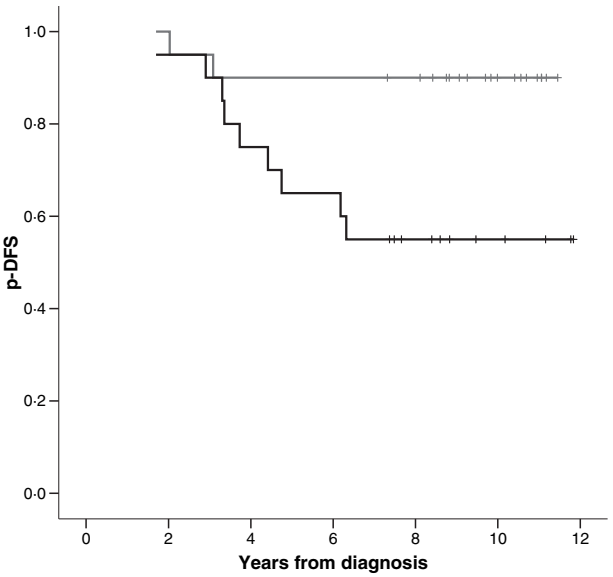

Fig 2. The relationship between vincristine plasma clearance and p-DFS in 40 children with standard risk B-cell precursor ALL. The upper curve represents children with clearance values below the median and the lower curve represents those with values above the median ($P = 0{\cdot}019$).

patient characteristics. For the whole study population, a trend to increased relative risk for relapse was found for clearance above median and AUC below median, as well as for sex (higher risk for males compared to females; see Table II). In the SR group, the risk of relapse was significantly higher for patients with clearance values above median (RR 5·2, $P = 0{\cdot}036$) or AUC values below median (RR 5·8, $P = 0{\cdot}025$). The results of multivariate analyses including clearance and sex, or AUC and sex, are displayed in Table III.

Table II. Relative risk (RR) of relapse in 86 children with B-cell precursor acute lymphoblastic leukemia.

Factor	RR	95% CI	*P* value
Clearance			
Score 2 vs. score 1	2·1	0·94–4·8	0·071
AUC			
Score 1 vs. score 2	2·0	0·90–4·7	0·087
Sex			
Male vs. female	2·3	0·97–5·6	0·06
Age			
≥10 vs. 1–9·99 years	1·2	0·41–3·7	0·50
WBC			
10–50 vs. $<10 \times 10^9$/l	1·2	0·39–3·7	0·67
>50 vs. $<10 \times 10^9$/l	0·8	0·22–2·6	0·67

Univariate Cox regression analysis with stratification for risk group. Clearance and AUC values below median were given a score of 1 and those above median a score of 2.
CI, confidence interval; AUC, area under the concentration-time curve; WBC, white blood cell count at diagnosis.

Table III. Relative risk (RR) of relapse in 40 children with standard risk B-cell precursor acute lymphoblastic leukemia.

Factor	RR	95% CI	*P* value
Univariate analysis			
Clearance			
Score 2 vs. score 1	5·2	1·1–24	0·036
AUC			
Score 1 vs. score 2	5·8	1·3–27	0·025
Sex			
Male vs. female	2·8	0·73–10	0·14
Multivariate analysis including clearance and sex			
Clearance			
Score 2 vs. score 1	4·5	0·96–21	0·057
Sex			
Male vs. female	2·1	0·56–8·2	0·26
Multivariate analysis including AUC and sex			
AUC			
Score 1 vs. score 2	5·0	1·1–24	0·045
Sex			
Male vs. female	1·9	0·50–7·4	0·34

Cox regression analysis. Clearance and AUC values below median were given a score of 1 and those above median a score of 2. Age and WBC count were not included in the analysis, as standard risk patients by definition were below 10 years of age and had a WBC count $<10 \times 10^9$/l.
CI, confidence interval; AUC, area under the concentration-time curve.

As the clearance and AUC values were closely correlated (rho = −0·94; $P < 0{\cdot}001$), a multivariate analysis including both parameters was not possible.

Discussion

In spite of its extensive use in pediatric and adult hematology and oncology for more than three decades, there appears to be a complete lack of published studies on the correlation between the pharmacokinetics and the long-term anticancer effects of vincristine. Possible explanations include the lack of specific and sensitive methods for analysis of vincristine until 1991, practical and ethical problems in multiple blood sampling in children, and the need for extended follow-up time, especially in ALL, where many relapses occur 3–5 years after diagnosis.

We here present data on 86 children with BCP ALL, in whom vincristine pharmacokinetics were investigated on treatment day 1, with a subsequent median follow-up time of 10·5 years (range 7·3–12 years). No patient was lost to follow-up. A very late relapse occurred in one patient 11 years after diagnosis. Not unexpectedly, this patient was found to have t(12;21) at diagnosis, a cytogenetic aberration related to the occurrence of very late relapses. Even if single relapse(s) might still occur, we considered our patient material mature enough to evaluate the clinical outcome of treatment. Twenty-five out of 86 patients relapsed during the observation period, resulting in a p-DFS of 0·69 (0·76 at 5 years). Corresponding

figures for the whole cohort of Nordic children treated according to the NOPHO ALL-92 protocol are 0·75 (0·79 at 5 years) (unpublished observations).

Our main finding was that total body clearance and AUC, parameters reflecting drug exposure, were related to clinical outcome in children with standard risk BCP ALL. A similar trend was seen in the whole patient group, but significant differences were found in the SR group only. This might be explained by the fact that vincristine has a relatively more important role in the treatment of SR patients, than in the other risk groups. SR, IR and HR patients received a total of 11, 14, and 15 doses of vincristine, respectively, but the IR and HR protocols were much more intense and included more anthracyclines, cyclophosphamide, and cytarabine, to mention some of the major differences (Gustafsson *et al*, 2000). Furthermore, the SR patients represented a very homogeneous group, where all children were below 10 years of age and had a WBC count below 10×10^9/l at diagnosis. As previously reported, there were no differences in pharmacokinetics by age or gender, nor was there any evidence of dose-dependent pharmacokinetics (Frost *et al*, 2003).

Ideally, our data should be confirmed in a larger study. If a relationship between the vincristine antileukemic effect and systemic exposure to vincristine is confirmed, this might provide a rationale for pharmacokinetically guided, individualised dosing of vincristine, with the aim to achieve a target systemic exposure in each patient (Evans *et al*, 1991). This strategy of individualising chemotherapy has been successfully applied for methotrexate during postremission treatment of childhood ALL (Evans *et al*, 1998). Unfortunately, pharmacokinetic studies after an i.v. injection necessitates multiple blood samples, which makes a large scale study very labour intensive, and the method less well suited for tailoring therapy in clinical routine. Another approach would be to try to find single nucleotide polymorphisms (SNPs) that are associated with differences in vincristine pharmacokinetics. Plasschaert *et al* (2004) studied the influence of functional polymorphisms of *ABCB1* on vincristine pharmacokinetics in childhood ALL (Plasschaert *et al*, 2004). *ABCB1* encodes for P-glycoprotein (P-gp), and the authors hypothesized that P-gp could influence the biliary excretion of vincristine. They found, however, that the genetic variants in *ABCB1* did not explain the large variability in vincristine pharmacokinetics observed.

In vitro and *in vivo* data support a dominant role for CYP3A enzymes in the elimination of vincristine. Dennison *et al* (2006, 2007) studied vincristine metabolism with human liver microsomes, and found that polymorphic expression of CYP3A5 may be a major determinant in the biotransformation of vincristine. We suggest further efforts in this field, and plan to retrieve biobanked material from as many as possible of our patients, to study SNPs on a genome-wide scale. Modern array-based techniques make this feasible and, if a ''pharmaco-genomic profile'' can be found that correlates to vincristine pharmacokinetics, this might become clinically useful. The final goal would be an individualised dosing, which ensures sufficient antileukemic effect in children who clear vincristine rapidly and avoids overdosing in patients who eliminate the drug slowly.

In conclusion, this study found that the clinical outcome in children with standard risk B-cell precursor ALL was related to vincristine pharmacokinetics as studied on treatment day 1. These data suggest a relationship between the antileukemic effect and the systemic exposure to the drug, which warrants further exploration. Future studies will hopefully help to explain inter-individual differences in response to therapy, and might form the basis for a more individualised treatment than is available today.

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