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Paediatric Update

Treatment of acute lymphoblastic leukaemia in countries with limited resources; lessons from use of a single protocol in India over a twenty year peroid

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Abstract

In the 1970s, survival rates after treatment for acute lymphoblastic leukaemia (ALL) in children and young adults (less than 25 years) in India were poor, even in specialised cancer centres. The introduction of a standard treatment protocol (MCP841) and improvements in supportive care in three major cancer centres in India led to an increase in the event-free survival rate (EFS) from less than 20% to 45–60% at 4 years. Results of treatment with protocol MCP841 between 1984 and 1990 have been published and are briefly reviewed here. In addition, previously unpublished data from 1048 patients treated between 1990 and 1997 are reported. Significant differences in both patient populations and treatment outcome were noted among the centres. In one centre, a sufficiently large number of patients were treated each year to perform an analysis of patient characteristics and outcome over time. Although steady improvement in outcome was observed, differences in the patient populations in the time periods examined were also noted. Remarkably, prognostic factors common to all three centres could not be defined. Total white blood cell count (WBC) was the only statistically significant risk factor identified in multivariate analyses in two of the centres. Age is strongly associated with outcome in Western series, but was not a risk factor for EFS in any of the centres. Comparison of patient characteristics with published series from Western nations indicated that patients from all three Indian centres had more extensive disease at presentation, as measured by WBC, lymphadenopathy and organomegaly. The proportions of ALLs with precursor T-cell immunophenotypes, particularly in Chennai, were also increased, even when differences in the age distribution were taken into consideration (in <18-year olds, the range was 21.1–42.7%), and in molecular analyses performed on leukaemic cells from over 250 patients less than 21-years-old with precursor B-cell ALL, a lower frequency of *TEL-AML1*-positive ALL cases than reported in Western series was observed. The worse outcome of treatment in Indian patients compared with recent Western series was probably due to the higher rate of toxic deaths in the Indian patients, and possibly also due to their more extensive disease – which is, at least partly, a consequence of delay in diagnosis. Differences in the spectrum of molecular subtypes may also have played a role. The higher toxic death rates observed are likely to have arisen from a combination of more extensive disease at diagnosis, co-morbidities (e.g., intercurrent infections), differences in the level of hygiene achievable in the average home, poor access to acute care, and more limited supportive care facilities in Indian hospitals. Toxic death was not associated with WBC at presentation, and hence would tend to obscure the importance of this, and, potentially, other risk factors, as prognostic indicators. Since the prevalence of individual risk factors varies in different populations

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and over time, their relative importance would also be expected to vary in different centres and in different time periods. This was, in fact, observed. These findings have important implications for the treatment of ALL in countries of low socioeconomic status; it cannot be assumed that risk factors defined in Western populations are equally appropriate for patient assignment to risk-adapted therapy groups in less affluent countries. They also demonstrate that heterogeneity in patient populations and resources can result in significant differences in outcome, even when the same treatment protocol is used. This is often overlooked when comparing published patient series.
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1. Introduction

The population of India recently exceeded a billion people, of which almost 50% are less than 25 years of age. Although data for all of India are not available, population-based data from various Indian cancer registries suggest that approximately 10 000 new cases of acute lymphoblastic leukaemia (ALL) occur each year in this age group. Very few of these patients are adequately treated, and the majority die from their disease. This contrasts with present cure rates of over 70% in Western countries. Reasons for the poor results in India as a whole are multiple, and include paucity of cancer treatment facilities, lack of human and physical resources required for effective management (both of which lead to lack of access to effective medical care) and the poverty and low level of education of the population. All of these factors contribute to delay in diagnosis and consequent presentation with more advanced disease as well as to a high rate of abandonment of therapy before completion and subsequent loss to follow-up. The generally poorer quality of data management and analysis also throws doubt upon the accuracy of some of the earlier published results.

A major factor in improving survival rates in ALL in children and adolescents in Western nations has been the formation of cooperative groups and the conduct of sequential clinical trials leading to stepwise increments in knowledge and resultant stepwise increments in survival rates [1–5]. Because of the lack of resources, few high quality clinical trials are conducted in developing countries, such that the only available data on which to base treatment decisions are those obtained from clinical trials conducted in Western countries. It is generally assumed that risk factors are equally applicable to developing countries, but this is not necessarily so. Patients in low resource settings tend to have more extensive disease at presentation and an increased risk of toxic death. There are also large differences in environments and in population genetics between developing countries and affluent nations. A likely consequence is that there will be corresponding differences both in the biology of ALL, e.g., in the proportions of immunophenotypically and molecularly defined subtypes, and in the patients themselves, e.g., with respect to drug metabolism, nutritional status and the presence of significant co-morbidities such as hepatitis. All of these factors, which may be variably applicable to different populations, could potentially influence survival rates, and therefore the relative importance of various prognostic factors.

This article describes two decades of experience in characterising and treating ALL in three Indian centres. The data presented demonstrate not only differences in patient characteristics from Western series, but also the more limited value of the most widely used risk factors, particularly, in the case of precursor B-cell ALL (pre B-ALL), age, which was not associated with treatment outcome in any of the centres, and total white blood count at presentation (WBC). These findings strongly suggest that the evidence-base for cancer treatment in developing countries must, to a large extent, be derived from clinical trials carried out in those same countries. The conduct of well designed clinical studies in developing countries has an additional advantage. When such studies include the provision of care to newly diagnosed patients, such patients are likely to receive better therapy than they would in a non-research setting.

2. Establishment of a common protocol

Because of the very poor results (less then 20% long-term survival) being achieved in children and adolescents with ALL seen at the Cancer Institute (WIA), Madras (now Chennai), a collaboration was established between this institute and the National Cancer Institute, Bethesda, United States of America (USA), in the early 1980s. A more intensive treatment protocol (MCP841) than that in use at the time was designed. Although this carried a risk of increased toxicity, in view of the poor results being obtained and the extensive disease in most patients, the added risks appeared worth taking. The protocol (Fig. 1) was based on standard treatment principles with a four drug induction regimen, I_1 (vincristine, prednisone, daunorubicin and asparaginase), followed by a second induction cycle, I_2 (cyclophosphamide, cytarabine and mercaptopurine), which included cranial radiation. Intrathecal therapy was given in both induction cycles. I_2 was followed by a reinduction cycle identical to I_1, consolidation with cyclophosphamide, vincristine, cytarabine and mercaptopurine, then continuation therapy until a total of 2 years of therapy had been completed. Treatment elements felt to be difficult to administer or particularly costly in the Indian setting

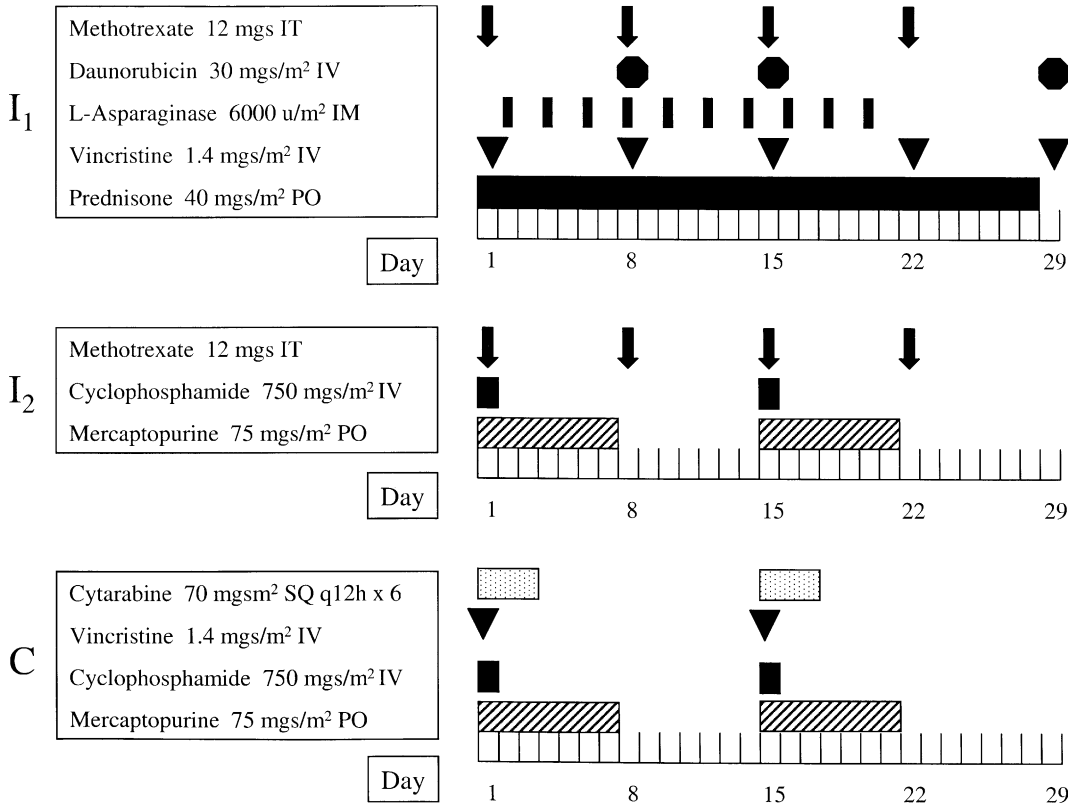


Fig. 1. Treatment schema of protocol MCP841. Three treatment modules are shown: I_1 (first induction cycle), I_2 (second induction cycle) and C (consolidation). Patients were treated initially with I_1, then I_2, followed by a repeat of I_1 then C. After C, patients began maintenance therapy consisting of vincristine,1.4 mg/m^2 IV on day 1, daunorubicin, 30 mg/m^2 IV on day 1, and L-asparaginase 6000 u/m^2, IM on days 1, 3, 5 and 7. Methotrexate, 15 mg/m^2 PO, was given once a week, missing every fourth week for a total of 12 weeks, and beginning on day 15, mercaptopurine, 75 mg/m^2 PO daily, was commenced, also being given for 3 weeks in every 4 weeks for a total of 12 weeks. A total of 6 cycles of maintenance therapy were given. Each therapy cycle was initiated as soon as the neutrophil count was $\geqslant 1 \times 10^9$/l and the platelet count was $\geqslant 100 \times 10^9$/l. Intrathecal therapy (IT) was given as shown for patients $\geqslant 3$ years; patients aged two years received 10 mg; patients aged one year received 8 mg; and patients less than one year received 6 mg. Cranial irradiation, 200 CGy daily $\times$ 9 days was given to a total of 1800 cGy in cycle I_2 from 1990. Prior to this, a total dose of 2000 cGy had been given. IV, intravenously; IM, intramuscularly; PO, orally.

(such as high-dose methotrexate) were avoided. Preliminary data from the phase II study of MCP841 initiated in the early 1980s at the Cancer Institute (WIA) indicated that the treatment plan was feasible with respect to toxicity, at least in the context of a major cancer centre in India, and that the survival rate would likely be significantly better. Although the toxic death rate was high, it was felt that if this would probably decrease over time as supportive care improved, with a corresponding further improvement in survival. The protocol was subsequently initiated at the Tata Memorial Hospital in Bombay (now Mumbai) in 1986 and at the All Indian Institute of Medical Sciences in New Delhi in 1992. The expanded objectives were to determine the toxicity pattern and survival rates of treatment with MCP841 in several Indian centres, and to attempt to identify prognostic factors in these patient populations. The study was open to patients aged 1–24-years-old. Detailed case report forms were developed, and training in data management also provided. Meetings were held at intervals, usually in India, to address these problems.

There was strong evidence from Western studies, that patients between 3 and 9-years-old, with a WBC less than 10×10^9/l and no organomegaly were likely to fall into a "good-risk" category. At first, these patients did not receive the reinduction cycle. However, very few patients were classified as "good-risk" and several relapses occurred even in these few patients, leading to a decision to treat all future patients with the same therapy regardless of age and WBC.

3. Early results

In 1996, Shanta and colleagues [6] published the results of a series of 97 patients treated according to protocol MCP841 between 1984 and 1988 at the Cancer Institute (WIA), Madras. Of these patients, 72% were either younger than 3 years or older than 6 years, 60% had a WBC at diagnosis greater than 10×10^9/l, 92% had hepatosplenomegaly and 66% had FAB L2 morphology. Of those patients who achieved remission,

51% remained in remission, and event-free survival (EFS) at 4 years was 38%. This result, in spite of a high toxic death rate, was at least twice as good as had been previously achieved at the WIA, and was comparable to results being achieved at that time in high-risk patients, variously defined, in Western series – such as the particularly high-risk "lymphoma syndrome", which included patients with bulky disease and T lineage leukaemia [7–9]. The majority of patients treated at the WIA would have fallen into this category.

In the same year (1996), Vaidya and colleagues [10], from the Tata Memorial Hospital published a comparison between 260 patients treated according to protocol MCP841 and 162 patients treated previously at the same hospital using various treatment approaches. The rate of relapse *after the completion of therapy* appeared to be much lower in patients treated according to MCP841 (15% compared with 27% with "less intensive" protocols) and almost 90% of all relapses occurred within 4 years from the start of therapy (2 years after completion of therapy). In a subsequent publication (1997), Sagar and colleagues [11] from Madras updated their results with MCP841, describing 163 patients, aged 14 years or younger, treated between 1984 and 1992. Only 81% of patients achieved complete remission, due primarily to early toxic deaths. Of the 132 complete responders, 38% relapsed while receiving therapy. The majority had recurrent bone marrow involvement, but 18% and 8% developed isolated testicular or isolated central nervous system (CNS) relapse, respectively.

Unexpectedly, a study of 285 previously untreated patients seen at the WIA revealed that 126 (44%) expressed T cell markers [12,13]. This fraction is higher than that observed at the All India Institute of Medical Sciences in New Delhi a few years earlier [14], and much higher than that reported from the Tata Memorial Hospital in Mumbai (21%) a few years later [15]. The reason for this very high proportion of T cell cases in Madras, which has persisted over time, remains unknown, although it is interesting that a similarly high percentage of T cell lineage ALLs (50%), in both children and adults, has also been reported from Egypt [16]. While further investigations will be required to elucidate the reason for the differences among the Indian centres, it is of note that the referral patterns of the three Indian centres are different – Cancer Institute (WIA) being primarily a regional centre, whilst the others receive patients from wider regions of India.

The Tata Memorial Hospital series reported in 1999 included 530 previously untreated patients less than 25 years of age who received therapy with protocol MCP841 between 1986 and 1993 [15]. Most of these patients had hepatosplenomegaly (80%) or lymphadenopathy (79%) and 21% had a precursor T-cell ALL (pre T-ALL). Complete remission was achieved in 91%, the majority of remission failures (36, or 7%) being due to toxic deaths. There were also 49 remission deaths (9%). After 1990, there were significantly fewer toxic deaths, deaths during induction dropping from 10% to 4.6% and remission deaths from 19% to 6%. A high WBC (above 60×10^9/l) was a poor risk factor for EFS, but in this group a low haemoglobin conferred additional risk, whilst amongst patients with a lower WBC (less than 60×10^9 per mm^3), the presence of lymphadenopathy was associated with a worse EFS. In a multivariate analysis, only WBC, haemoglobin and lymphadenopathy were associated with EFS overall, but when immunophenotype was taken into consideration, these risk factors were shown to apply to common ALL (C-ALL, defined as pre B-ALL expressing the common ALL antigen, CD10) but not to (pre T-ALL). In pre T-ALL, older age and high haemoglobin were moderately significant poor risk factors. Among all patients, low haemoglobin, lymphadenopathy at presentation as well as low height and weight for age, using Indian standard data, were statistically significant risk factors for toxic death. Notably, application of the National Cancer Institute (NCI) risk criteria for pre B-ALL (essentially age and WBC) did not efficiently separate patients with different treatment outcomes.

4. Comparison of patient populations treated at the three centres between 1990 and 1997

In order to examine the apparent differences in patient populations and treatment results in more detail, an analysis was subsequently conducted on data derived from prospective series consisting of all patients entered on study between 1st January, 1990 and December 31st, 1997 (the latter date chosen to give adequate follow-up) from the three centres. For the purposes of comparing patients in different eras, an additional 205 patients treated with MCP841 at the Tata Memorial Hospital between 1986 and 1989 were included. Immunophenotyping was carried out in the majority of patients; both to confirm the diagnosis of ALL and to provide information regarding the proportions of pre T-ALL and pre B-ALL cases at each centre. Patients were characterised at presentation with respect to clinical features, including the presence and degree of lymphadenopathy and hepatosplenomegaly, as well as haematological characteristics, including the WBC prior to the start of treatment, haemoglobin level and platelet and blast cell counts (some of these measurements were not available in a small number of patients). Only patients able to remain close to the treatment centre during the period of induction and consolidation were considered eligible for entry on protocol, in order to ensure effective follow-up. Although

potentially introducing some bias, this criterion was effective – less than 4% of patients were lost to follow-up in the period 1990–1997.

EFS was chosen as the most satisfactory primary end-point to use as an overall indicator of efficacy and toxicity of treatment since events included all deaths as well as disease progression. The number of patients treated at each centre, the numbers of toxic deaths and relapses in the observation period, and EFS at 4 years are shown in Table 1. Differences in EFS for the period 1990–97 among the centres, which ranged between 41% and 60%, were statistically significant ($P < 0.0001$). Differences in toxic deaths and relapse rates were also statistically significant ($P < 0.001$ and 0.002, respectively). Survival curves of EFS and overall survival in each centre are shown in Figs. 2 and 3.

The highest relapse rate (41%) was observed at the WIA. The other two centres had similar relapse rates (29–30%). Toxic deaths were higher at the All India Institute of Medical Sciences and lowest at the Tata Memorial Hospital. These differences could relate either to differences in patient management, or to differences in the patient populations seen at each centre, or to a combination of both. Table 2 provides a comparison of patients seen at each of the centres with respect to age and presenting features. There were a number of statistically significant differences in the patients entered on study at the three centres, including age, immunophenotype, and measures of disease burden at presentation, such as WBC, hepatosplenomegaly, lymphadenopathy and mediastinal mass. A broad range of possible risk factors for survival were examined. Univariate and multivariate analyses of risk factors for EFS revealed WBC at presentation to be significant at the Tata Memorial Hospital and the All India Institute of Medical Sciences ($P = 0.002$ and 0.0005, respectively, in the multivariate analysis). When pre T and pre B patients were analysed separately, WBC was a significant risk factor only in pre B-ALL patients. No other factors associated with EFS were found (Tables 3–5). When all patients were included in the analysis, it was not possible to identify risk factors common to all three centres. The rate of response to therapy, measured at various points during or after the first induction cycle, was not determined in this series of patients, but will be examined in future studies.

Because the upper age limit in this series is higher than in many Western series of childhood ALL, additional analyses were performed with patients less than 18-years-old, who comprised 90% of the patients at the Tata Memorial Hospital and the All India Institute of Medical Sciences, and 86% at the Cancer Institute (WIA). In this age group, the fractions of patients with pre T-ALL were 21.1%, 31% and 42.7% for the Tata Memorial Hospital, All India Institute of Medical Sciences and Cancer Institute (WIA), respectively. Univariate analyses for the impact of initial WBC on EFS in this same age group were <0.0001, 0.014 and 0.11, respectively (compared with 0.002, 0.0011 and 0.066 for patients less than 25 years – see Tables 3–5). Only 3%, 6% and 7% of patients, respectively, were 21 years or older in these three centres. Thus, the different age range does not account for the findings.

Table 1
Outcome of treatment in three Indian centres, 1990–1997

Centre	No. patients	CR (%)	Toxic deaths (%)	Relapses (%)	EFS at 4 years (%)
TMH	652	94.8	10.6	28.8	60
AIIMS	228	83.3	22.8	30.5	41
CI	168	86.9	16.7	41.1	43

TMH, Tata Memorial Hospital; AIIMS, All India Institute of Medical Sciences; CI, Cancer Institute (WIA); CR, complete response; EFS, event-free survival.

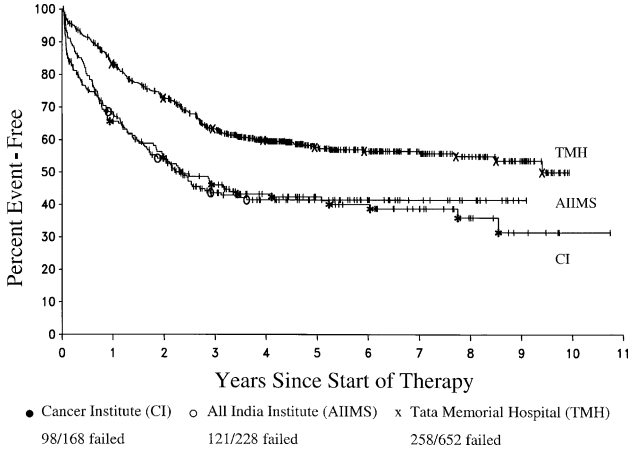


Fig. 2. Event-free survival (EFS) at the three centres.

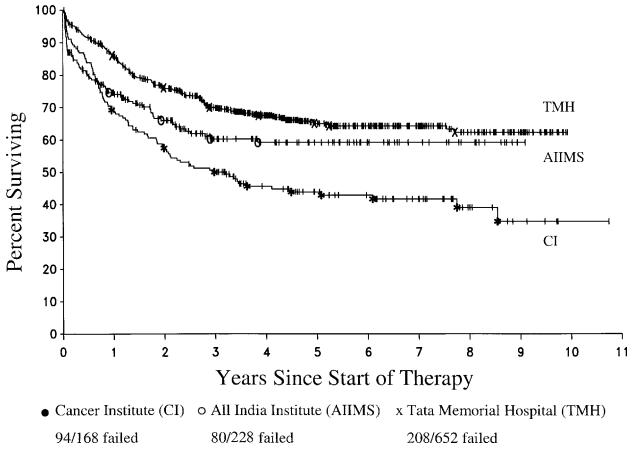


Fig. 3. Overall survival at the three centres.

Table 2
Patient's characteristics at presentation in the three Indian centres, 1990–1997

Characteristic	TMH	AIIMS	CI	*P* value
Number of patients	652	228	168	–
Median age	7.2	7.6	10	0.007[a]
Pre B-ALL	75.2%	59.5%	45.4%	<0.0001[b]
Pre T-ALL	20.7%	31.8%	43.1%	<0.0001[c,d]
Other phenotype	4.1%	8.7%	11.5%	n/a
WBC[e] $< 10 \times 10^9$/l	44.0%	37.3%	39.9%	0.18[f]
WBC 10–50×10^9/l[g]	31.4%	31.6%	25.6%	0.035[h]
WBC 50–100×10^9/l	10.0%	13.2%	11.3%	
WBC $> 100 \times 10^9$/l	14.6%	18.0%	23.2%	0.023[i]
Lymphadenopathy	74.2%	83.8%	72.0%	0.006
Hepatomegaly/splenomegaly	76.0%	89.5%	85.1%	<0.0001
Mediastinal mass, all patients	1.8%	7.8%	2.4%	0.0041[j]
Mediastinal mass, pre T-ALL patients	33.6%	46.8%	35.7%	0.21[k]

TMH = Tata Memorial Hospital; AIIMS = All India Institute of Medical Sciences; CI = Cancer Institute (WIA); n/a, not available; WBC, white blood cell count.

[a] For age treated as a continuous variable (Kruskal–Wallis test).

[b] Pre B-ALL *vs.* pre T-ALL/other compared across centres, χ^2 test, $n = 913$. % pre B-ALL and pre T-ALL revised and based upon number of patients immunophenotyped.

[c] Pre T-ALL *vs.* pre B-ALL/other compared across centres, χ^2 test, $n = 913$.

[d] Pre B-ALL *vs.* pre T-ALL compared across centres centre, χ^2 $P < 0.0001$, $n = 857$ (no "other cells").

[e] Median WBC: Chennai, 18.1×10^9/l; Delhi, 17.0×10^9/l; and Mumbai, 12.8×10^9/l.

[f] WBC $< 10 \times 10^9$/l *vs.* WBC $\geqslant 10 \times 10^9$/l compared across centres, χ^2 test.

[g] WBC $< 50 \times 10^9$/l *vs.* WBC $\geqslant 50 \times 10^9$/l compared across centres, $P = 0.014$ (χ^2 test).

[h] Each of the four WBC groups compared across centres, Kruskal–Wallis test. (Testing WBC as a continuous variable, $P = 0.045$.)

[i] WBC $> 100 \times 10^9$/l *vs.* WBC $> 100 \times 10^9$/l compared across centres, χ^2 test.

[j] Fisher–Freeman–Halton test, $n = 617$.

[k] Fisher–Freeman–Halton test, $n = 240$.

5. Potential explanations for inability to identify common risk factors

There are several possible explanations that could account for the inability to identify common risk factors among the three centres. Since Indian patients presented with more extensive disease than those in Europe and the USA, the range of variation in prognosis was narrower than that observed in Western series, making differences potentially more difficult to detect. In addition, since toxic death rates were much higher than observed in Western series (at the All India Institute of Medical Sciences, for example, the toxic death rate was more than 50% of the relapse rate), they had a correspondingly greater influence on survival, and could have masked risk factors which would otherwise have been detected. A number of analyses were conducted to explore these issues. Multivariate analysis was used to determine whether there were separate risk factors for toxic death *vs.* relapse, and whether separate prognostic factors could be identified for pre B-ALL *vs.* pre T-ALL (Table 6). It was determined that WBC at presentation was not significantly associated with toxic death in any of the three centres. Thus, toxic deaths would tend to dilute the influence of WBC on survival. Conversely, WBC might be expected to have a greater prognostic significance in populations (or in time periods) with lower toxic death rates. The data were consistent with this interpretation. The influence of WBC also varied in pre B-ALL *vs.* pre T-ALL and among the three centres. WBC was a powerful prognostic indicator for relapse in pre B-ALL in Mumbai, somewhat less so in Delhi, and not at all in Chennai. The association with relapse in pre T-ALL was non-significant for all of the centres.

6. Comparison of treatment outcome in three different time periods

If presenting features and risk factors differed in the ALL patient series treated in three major Indian centres, it was possible that they may differ in a single institution over time. The Tata Memorial Hospital series was large enough to examine this question. Presenting features of patients are shown in three consecutive time periods (Table 7). There were significant differences in the proportions of patients with pre B-ALL in each of the time periods, as well as in presenting features (WBC, lymphadenopathy and organomegaly). Over time, the proportion of patients with lower WBCs rose, while the proportion with lymphadenopathy and/or organomegaly fell. Interestingly, the proportion of patients with pre T-ALL did not change over time, although the proportion of patients with pre B-ALL rose and that of "other" (null) phenotypes fell. This could be a consequence of improvements in immunophenotyping

Table 3
Univariate and multivariate analysis of risk factors at presentation in the Tata Memorial Hospital, 1990–97

Characteristic, phenotype, or risk factor	Univariate *P*-value[a]			Multivariate *P*-values[d,g]
	All patients	Pre B-ALL[b]	Pre T-ALL[c]	All patients
Age	0.86	0.33	0.1	0.74
Gender	0.17	0.24	0.95	0.44
WBC[e]	0.0002	<0.0001	0.63	0.002[h]
Blast count[e]	0.0001	<0.0001	0.36	0.53
	$n = 616$	$n = 417$	$n = 117$	$n = 611$
Platelet count[e]	0.0009	0.052	0.061	0.011[i]
	$n = 647$	$n = 439$	$n = 121$	
LDH[e]	0.01	0.001	0.84	0.39
	$n = 596$	$n = 402$	$n = 116$	$n = 591$
LDH > 500 i.u. (*vs.* LDH < 500 i.u)	0.002	0.042	0.076	0.1
	$n = 596$	$n = 402$	$n = 116$	$n = 591$
Phenotype[j]	0.71	n/a	n/a	0.17
	$n = 588$			$n = 584$
Haemoglobin	0.51	0.35	0.12	0.79
Lymphadenopathy	0.097	0.42	0.49	0.49
Hepatosplenomegaly	0.22	0.37	0.97	0.92
	$n = 642$	$n = 434$		$n = 637$
Mediastinal mass	0.63	0.15	0.97	0.92
	$n = 642$			
Year of accrual (1990–1997)	0.96	0.58	0.2	0.47
	$n = 642$			
Median height for age[f]	0.84	0.84	0.7	0.75
	$n = 644$	$n = 436$	$n = 120$	$n = 639$
Median weight for age[f]	0.32	0.4	0.59	0.39
	$n = 647$	$n = 437$		$n = 642$
Median height and weight for age[f]	0.4	0.29	0.45	0.5
	$n = 644$	$n = 436$	$n = 120$	$n = 639$

LDH, lactate dehydrogenase.
[a] $n = 652$, unless indicated otherwise.
[b] $n = 442$, unless indicated otherwise.
[c] $n = 122$, unless indicated otherwise.
[d] $n = 647$, unless indicated otherwise.
[e] A $\log_{10}$ transformation was performed on the variable prior to analysis.
[f] Median (50th percentile) for age.
[g] Likelihood ratio P-values, adjusted for WBC count and platelet count except where noted.
[h] Adjusted for platelet count.
[i] Adjusted for WBC count.
[j] Pre B-ALL *vs.* pre T-ALL and "other" (pre T-ALL and "other" groups were non-significantly different, $P = 0.19$).

techniques. EFS also improved (Fig. 4 and Table 8). This was predominantly due to the marked reduction in toxic deaths in successive time periods (25%, 13%, and 8%, respectively), and a smaller, although still statistically significant, reduction in the fraction of patients with recurrent disease was observed (35%, 28% and 27%). Disease-free survival (DFS) for the entire group, which reflects the relapse rate (toxic deaths were censored in this analysis), was significantly improved between the first and second, and first and third periods, but not between the second and third periods. The reduction in toxic deaths could have been due either to improved supportive care or to changes in the patient population in each time period, but the former is the most likely explanation [17], particularly since the toxic death rate was not associated with the initial WBC. While the duration of follow-up in each time period differed, most relapses (over 90%) occur in the first 4 years after the initiation of treatment [18] such that follow-up duration is very unlikely to have influenced the fractions of patients who relapsed. Indeed, the proportion relapsing remained the same in the last two time periods.

A comparison, by multivariate analysis, of risk factors for the two treatment periods – 1990–1993 and 1994–1997 – revealed some interesting findings. In the first, WBC was a major risk factor, which was modified, as described above, by lymphadenopathy in those with a WBC below 60×10^9/l and by haemoglobin level in the group with a WBC above 60×109/l (lower levels being associated with a worse prognosis). In the second period, blast count (cut-off 3.1×10^9/l) was a better predictor of prognosis, but blast count also proved to be a highly significant risk factor in the first period. Initial haemoglobin level (although not to the same degree) remained a significant risk factor,

Table 4
Univariate and multivariate analysis of risk factors at presentation in the All India Institute for Medical Sciences, 1990–1997

Characteristic, phenotype, or risk factor	Univariate *P*-value[a]			Multivariate *P*-values[d,g]
	All patients	Pre B-ALL[b]	Pre T-ALL[c]	All patients
Age	0.1	0.21	0.28	0.2
Gender	0.22	0.64	0.32	0.45
WBC[e]	0.0011	0.0025	0.95	0.0005[h]
	0.073	0.052	0.45	0.39
Blast count[e]	$n = 218$	$n = 113$	$n = 59$	$n = 208$
	0.041	0.0047	0.88	0.025[i]
Platelet count[e]	$n = 218$	$n = 113$	$n = 59$	
	0.83	n/a	n/a	0.99
Phenotype	$n = 195$			$n = 198$
Haemoglobin	0.64	0.69	0.28	0.94
Lymphadenopathy	0.62	0.34	0.94	0.66
Hepatosplenomegaly	0.8	0.98	0.42	0.58
Mediastinal mass	0.93	0.84	0.53	0.32
Year of accrual	0.62	0.93	0.9	0.077
Median height for age[f]	0.9	0.49	0.061	0.65
Median weight for age[f]	0.99	0.92	0.22	0.7
Median height and weight for age[f]	0.89	0.83	0.35	0.79

[a] $n = 228$, unless indicated otherwise.
[b] $n = 116$, unless indicated otherwise.
[c] $n = 62$, unless indicated otherwise.
[d] $n = 218$, unless indicated otherwise.
[e] A $\log_{10}$ transformation was performed on the variable prior to analysis.
[f] Median (50th percentile) for age.
[g] Likelihood ratio *P*-values, adjusted for both WBC and platelet counts except where noted.
[h] Adjusted for platelet counts.
[i] Adjusted for WBC counts.

Table 5
Univariate and multivariate analysis of risk factors at presentation in the Cancer Institute (WIA), 1997–1990

Characteristic, phenotype, or risk factor	Univariate *P*-value[a]			Multivariate *P*-values
	All patients	Pre B-ALL[b]	Pre T-ALL[c]	All patients
Age	0.16	0.95	0.087	NS[f]
Gender	0.37	0.34	0.11	NS
WBC[d]	0.066	0.68	0.039	NS
Blast count[d]	0.029 ($n = 118$)	0.51 ($n = 46$)	0.082 ($n = 42$)	NS
Platelet count[d]	0.19	0.11	0.47	NS
LDH[d]	0.58 ($n = 101$)	0.97 ($n = 39$)	0.1 ($n = 35$)	NS
LDH > 500 i.u. (*vs.* LDH $\leqslant$ 500 i.u.)[d]	0.45 ($n = 101$)	0.53 ($n = 39$)	0.31 ($n = 35$)	NS
Phenotype	0.19 ($n = 130$)	n/a	n/a	
Haemoglobin	0.65	0.67 ($n = 59$)	0.52 ($n = 56$)	NS
Lymphadenopathy	0.84	0.99	0.73	NS
Hepatosplenomegaly	0.25	0.77	0.43	NS
Mediastinal mass	0.19	0.28	0.092	NS
Year of accrual	0.38	0.59	0.017	NS
Median height for age[e]	0.82	0.44	0.48	NS
Median weight for age[e]	0.78	0.49	0.47	NS
Median height and weight for age[e]	0.64	0.2	0.67	NS

[a] $n = 168$, unless indicated otherwise.
[b] $n = 59$, unless indicated otherwise.
[c] $n = 56$, unless indicated otherwise.
[d] A $\log_{10}$ transformation was performed on the variable prior to analysis.
[e] Median (50th percentile) for age .
[f] Non-significant.

but lymphadenopathy did not. These differences in the relative importance of risk factors were associated with differences in the EFS and toxic death rates in these two time periods and emphasise that risk factors should not be assumed to remain constant over time, even in the same institution and when the same treatment protocol is used.

Table 6
Comparison of WBC as a prognostic factor for toxic death and relapse in patients with Pre B-ALL and Pre T-ALL in each of the three centres

Sub-type	No. patients	Outcome	No. events	Log OR[a]	*P* value
Tata Memorial Hospital					
All	652	EFS	258	0.34	0.0002
		Relapse	192	0.42	<0.0001
		Toxic death	66	0.12	0.51
Pre B-ALL	442	EFS	181	0.59	<0.0001
		Relapse	140	0.76	<0.0001
		Toxic death	41	0.02	0.93
Pre T-ALL	122	EFS	42	0.1	0.63
		Relapse	27	0.17	0.53
		Toxic death	15	−0.01	0.98
All India Institute of Medical Sciences					
All	228	EFS	118	0.45	0.0011
		Relapse	65	0.52	0.006
		Toxic death	53	0.37	0.071
Pre B-ALL	116	EFS	63	0.65	0.0023
		Relapse	35	0.76	0.012
		Toxic death	28	0.54	0.076
Pre T-ALL	62	EFS	33	−0.02	0.95
		Relapse	20	0.01	0.98
		Toxic death	13	−0.06	0.89
Cancer Institute (WIA)					
All	168	EFS	98	0.28	0.066
		Relapse	65	0.19	0.32
		Toxic death	33	0.43	0.089
Pre B-ALL	59	EFS	40	0.12	0.68
		Relapse	26	0.04	0.91
		Toxic death	14	0.24	0.6
Pre T-ALL	56	EFS	28	0.65	0.035
		Relapse	18	0.67	0.082
		Toxic death	10	0.62	0.23

EFS, event-free survival.
[a] Relative risk of the outcome for an increase in WBC by a factor of 10 (OR = Odds Ratio).

7. Molecular studies

The four major chromosomal translocations observed in pre B-ALL in children include the t(12;21), that results in the fusion of the *TEL* (ETV6) and *AML1* genes, t(1;19), resulting in a chimeric protein of E2A and PBX1, t(9;22), yielding a BCR-ABL fusion, and t(4;11), which juxtaposes *MLL* to *AF4*. These translocations define clinicopathological entities that have also been used in risk-stratification of ALL, at least, in the USA and Europe [19–22]. Data pertaining to the relative proportions of each subgroup in other geographical regions are sparse, but it would be surprising, given the very large variations in the environment and in population genetics, if there were not differences in the distribution of molecular subtypes of ALL in various world regions. The relative proportions of these molecularly defined entities are not only relevant to understanding pathogenetic mechanisms, but also to treatment strategies. This could be of particular value in patient populations in which standard risk factors, such as age and WBC, appear to be less predictive of outcome than in Western populations, although risk groups defined in this way would probably be rather small. An analysis of the presence or absence of several of the more common chromosomal translocations was therefore undertaken in patients from two of the participating institutions. This revealed significant differences from data published by centres in the West.

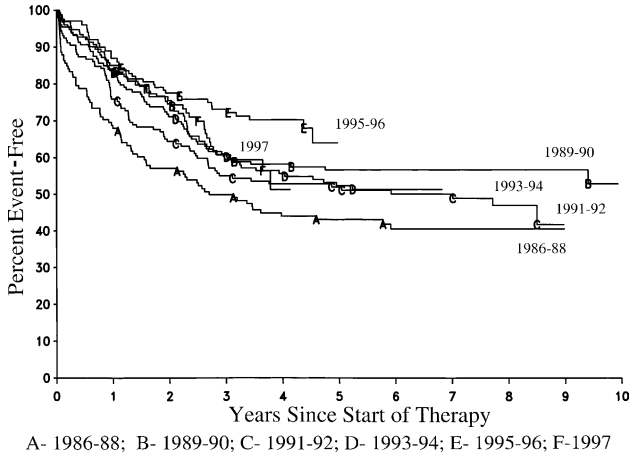
More than 250 newly diagnosed cases of pre B-ALL in which adequate material was available from two of the centres, Tata Memorial Hospital and All India Institute of Medical Sciences, were analysed by real-time multiplex reverse transcriptase–polymerase chain reaction (RT–PCR) for the four leukaemia-specific translocations mentioned above [23]. Only samples in which patients were less than 21 years of age were studied. At least one of these leukaemia-specific translocations was detected in 19% of cases. This is fewer than is regularly reported in Western series, where as many as one third of ALLs belong to one of these subgroups. Less common subgroups in Western series, which were not tested for here, could prove to be more frequently encountered in Indian patients.

The translocation 4;11 (*MLL;AF4*), which is present in over 80% of infant leukaemias, was not encountered

Table 7
Data for the Tata Memorial Hospital for three different time periods

Characteristic	1986–1989	1990–1993	1994–1997	*P* value
No. patients	205	317	335	–
Median Age	6.4	8	6.6	0.084[a]
Pre B-ALL	64.1%	72.4%	78.0%	0.001[b]
Pre T-ALL	20.1%	20.5%	21.0%	0.82[c,d]
Other phenotype	15.8%	7.1%	1.0%	n/a
WBC $< 10 \times 10^9$/l[e]	33.2%	40.1%	47.8%	0.0007[f]
WBC $10–50 \times 10^9$/l	42.4%	33.1%	29.9%	0.008[g]
WBC $50–100 \times 10^9$/l	11.7%	10.1%	9.9%	
WBC $> 100 \times 10^9$/l	12.7%	16.7%	12.5%	0.80[h]
Lymphadenopathy	80.5%	79.2%	69.5%	0.002
Hepato/splenomegaly	81.0%	80.8%	71.6%	0.005
Mediastinal mass pre T-ALL	27%	45.9%	21.3%	0.30[i]

[a] For age treated as a continuous variable (Kruskal–Wallis test).
[b] Pre B-ALL *vs.* pre T-ALL/other compared across the time periods, Cochran–Armitage trend test, $n = 772$. % Pre B-ALL and pre T-ALL revised and based upon number of patients immunophenotyped.
[c] Pre T-ALL *vs.* pre B-ALL/other compared across the time periods, Cochran–Armitage trend test, $n = 772$.
[d] Pre B-ALL *vs.* pre T-ALL compared across the time periods, Cochran–Armitage trend test $P = 0.52$, $n = 719$ (other phenotypes not included).
[e] Median WBC: 1986–1989, 18.5×10^9/l; 1990–1993, 16×10^9/l; and 1994–1997, 10.9×10^9/l.
[f] WBC $< 10 \times 10^9$/l *vs.* WBC $\geqslant 10 \times 10^9$/l compared across the time periods, Cochran–Armitage trend test.
[g] Each of the four WBC groups compared across the time periods, Jonckheere-Terpstra trend test. (Testing WBC as a continuous variable compared across the time periods, Spearman rank correlation, coefficient = −0.08, $P = 0.015$, $n = 857$.)
[h] WBC $> 100 \times 10^9$/l *vs.* WBC $< 100 \times 10^9$/l compared across the time periods, Cochran–Armitage trend test.
[i] $n = 159$.



A- 1986-88; B- 1989-90; C- 1991-92; D- 1993-94; E- 1995-96; F-1997

Fig. 4. EFS in patients treated at the Tata Memorial Hospital during sequential time periods.

in this series, one reason being because infants less than 1 year of age were not included. Even so, only two cases with *MLL* rearrangements were identified. In an earlier series from the All India Institute of Medical Sciences, which did include infants, 17 rearrangements of *MLL* were identified among 130 pre B-ALLs (70% in infants) [24]. It is important to note that at least 40 different translocations involving *MLL* have been described [25] and it remains possible that differences amongst Indian populations will be identified, both with respect to the frequency and type of translocations involving MLL.

The relative distribution of other molecular subgroups also differed in the 259 Indian cases studied compared with results reported from Western series [25] and collected references in *Leukemia* 2000, **14**, 2196–320]. The frequency of t(12;21), which is associated with a good prognosis in protocols which include high cumulative exposure to asparaginase [21], was significantly lower in the Indian series (7%) than in the USA (22%) or Europe (23%) ($P < 0.005$). In contrast, t(1;19) and t(9;22), the latter being associated with a poor prognosis, were more commonly seen in the Indian series (7% and 5%, respectively) than in Western series (<3.8% and <2.2%). This finding was even more pronounced when the specific type of t(9;22) associated with chronic myeloid leukaemia (p210) was studied. A recent analysis of 248 cases from the Tata Memorial Hospital revealed a p210 type of t(9;22) in 10 (9 B and 1 T lineage) of 248 ALLs (4%) [26].

Preliminary studies at the All India Institute of Medical Sciences of rearrangements of TAL-1, a gene which is activated in a third of pre T-ALL leukaemias, suggest that the frequency of these rearrangements in Indian pre T-ALL is similar to that in the USA, although the proportions of different types of rearrangement may differ [27].

It will be important to determine whether the molecular abnormalities in Indian patients are associated with differences in prognosis similar to those reported in studies from other countries and important, too, in analysing such results, to take age and treatment into consideration, since both have been associated with outcome in

Table 8
Treatment outcome at the Tata Memorial Hospital over three different time periods

	Survival at 4 years for the given time period			*P* values[a]			
	1. 1986–1989	2. 1990–1993	3. 1994–1997	Overall	1 *vs.* 2	1 *vs.* 3	2 *vs.* 3
Survival (all patients)							
EFS	45%	58%	61%	<0.0001	0.001	0.0001	0.43
DFS	51%	62%	64%	0.007	0.010	0.006	0.71
CR rates and events (all patients)							
CR[b]	88.3%	94.0%	95.8%				
Toxic Death	25%	13%	8%				
Relapse	35%	28%	27%				
Survival (precursor B-cell patients)							
EFS	44%	59%	58%	0.003	0.002	0.011	0.68
DFS	52%	63%	60%	0.87	0.32	0.29	0.33

CR, complete response; DFS, disease-free survival.
[a] *P* values are unadjusted for multiple comparisons.
[b] Nearly all patients who failed to achieve CR did so because of death prior to evaluation (at the end of induction cycle, 1_1).

these various translocation subgroups. Unfortunately, at the present time, too few patients in each group have been identified for a meaningful analysis to be conducted. Ultimately, more detailed molecular studies, including gene expression profiling, if feasible, are likely to be of great interest.

8. Implications of these findings

These results demonstrate a number of important principles that require emphasis in the context of developing countries, particularly in low income countries with ethnically varied populations and contrasting environments, such as India. Firstly, because patients in low income developing countries generally present with more advanced disease, the majority of patients require more intensive treatment. More intensive treatment regimens inevitably result in higher toxic death rates, particularly in the early phases of using such protocols, but in the final analysis, more intensive therapy is likely to give higher survival rates than less intensive therapy in patients with extensive disease. As demonstrated by the reduction in toxic deaths in sequential periods in the data presented here, supportive care improves over time, resulting in a continued improvement in survival rates. Improvement is likely because toxic deaths are not confined to patients with the highest leukaemia burdens. Secondly, because patient populations may be very different in different world regions, or in different socioeconomic strata within the same country, it is essential to identify locally relevant risk factors and not to assume that those identified in more affluent countries will automatically apply. Differences in the genetics of the population (e.g. in the frequency of polymorphisms in enzymes involved in drug metabolism), in the molecular abnormalities present in leukaemias, and in co-morbidities (e.g, hepatitis and other chronic infections or infestations, and malnutrition), coupled to the generally more advanced disease of patients in poorer countries, are likely to influence both treatment tolerance and outcome. In these circumstances, it is essential to develop a solid foundation on which to build better treatment through a series of clinical trials. A reasonable strategy, as adopted in India in the mid-1980s, is to first conduct a single arm study using the same treatment protocol (judged feasible in the context of available resources and the patient population) for all patients, and to collect information on response, toxicity and survival rates, as well as patient and leukaemia characteristics potentially associated with survival. This approach is designed to provide a foundation of locally relevant data on which to build whilst simultaneously providing better patient care. Moreover, since data is being collected and scrutinised, continuous improvements in the discipline with which the treatment protocol is administered, and patients are monitored and supported, are likely to occur, particularly when ''hands-on'' training for staff, both with respect to patient care and data management, is simultaneously provided. Collaboration with more experienced centres is important in this regard. Finally, communication between participating centres and centralized study coordination result in improved access of patients to experts in their disease.

The findings in the present series, comprised of young patients with ALL treated with the same treatment protocol (MCP841) at three major cancer centres in India, suggest that not only are there significant differences between Indian populations and patients in the USA or Europe, but that differences are also present among patients seen at different centres in India. This is not surprising, given the heterogeneity of Indian populations (e.g., multiple ethnic, language and religious groups and a broad range of socioeconomic levels), as well as the presence of institutional biases with respect to the populations they draw from. Population differences

and the quality of care may influence patient compliance and the frequency of toxic deaths, and these two factors probably account for our inability to identify risk factors common to patients treated at all three centres. Indeed, even risk factors identified in patients treated with MCP841 in an earlier era at the Tata Memorial Hospital hospital [15], which were reproduced, albeit with a lower level of significance at the All India Institute of Medical Sciences (data not shown), were not as apparent in later years, although at the Tata Memorial Hospital, blast count rather than WBC was a more reproducible risk factor across the three time periods.

A higher upper age limit (24 years) was used in this Indian series than is usual in Western series. It is particularly surprising, therefore, that age, an important prognostic factor in Western series, was not associated with outcome. However, age is merely a surrogate marker and probably reflects differences in a number of other factors, such as the specific molecular subtype of ALL (which tends to be age-associated), and perhaps immunological and behavioural factors. It is possible that the lack of an association of age with outcome in India is indicative, at least in part, of differences in the pattern of molecular subtypes. Molecular characterisation performed to date supports this interpretation since it has revealed that the predominant subtype in pre B-ALL in Western series, the *TEL-AML1* translocation, which accounts for 22% of patients and also tends to be associated with younger age, is present in a much smaller fraction of the Indian patients. This finding is also consistent with observations relating to the emergence of the early age peak in ALL, which was coupled to an increased incidence of ALL in various populations throughout the last century [28]. The early age peak is now known to be associated with the predominant, common ALL subtype (c-ALL) of pre B-cell ALL. Even when such an age peak is absent, or ill-defined, in a population of patients with ALL, it is readily detectable in the common ALL subgroup – in this respect, it provides a crude measure of the relative proportions of pre B-ALL and pre T-ALL. The findings in various Indian series of a lower, but increasing proportion of c-ALL cases over time, coupled to the lower incidence of leukaemia recorded in population-based registries in India, are consistent with the evidence suggesting that common ALL is in some way associated with socioeconomic or technological development. Interestingly, these considerations also imply that the higher the proportion of common ALL, the lower the median age of the leukaemic population is likely to be.

Table 9
Comparison of WBCs in various series in India and the United States of America (USA) or Europe

Institution	$<50\times10^9$/l	$>100\times10^9$/l	EFS (4–5 years)
Cancer Institute (WIA)	65.5%	23.2%	43%
All India Institute of Medical Science	68.9%	18.0%	41%
Tata Memorial Hospital	75.4%	14.6%	60%
UK ALL XI (1990–1997)[a]	77.9%	12.0%	63%
POG ALinC14 and 15 studies[b]	85%	6.6%	66.6%
ALL-BMF 83[c]	80.2%	11.3%	64.3%
ALL-BMF 90[c]	77.7%	12.4%	78.0%
St. Jude 1988–1991[d]	77.7%	13.8%	67.6%
St. Jude 1991–1994[d]	73.3%	14.5%	76.9%
Dana Farber Consortium (USA)[e]	81.7%	10.9%	83%

UK, United Kingdom; POG, Pediatric Oncology Group.
[a] Reference [1].
[b] Reference [4].
[c] Reference [2].
[d] Reference [35].
[e] Reference [36].

As shown in Table 9, the extent of disease at presentation, using WBC as a marker of leukaemia cell burden, differed between the three Indian series and various series from the USA and Europe. Delayed diagnosis and a higher WBC at presentation probably accounts for at least some of the generally (but not universally) worse outcome in the Indian series compared with Western series although biological and molecular genetic factors in the leukaemia cells and the host are also likely to contribute. In this respect, the apparent discrepancy between the frequency of lymphadenopathy and hepatosplenomegaly at the Cancer Institute (WIA), but not at the other centres, as well as the apparent lack of an association of WBC with outcome in pre B-ALL (compare Table 5 with Tables 3 and 4), could reflect differences either in the biology of the leukaemic cells, or in the patient populations, or both. It is possible that the presence of a significant leukaemia burden outside the bone marrow and peripheral blood compartments in Indian patients, coupled to the paucity of patients with a low leukaemia burden, partly accounts for the lesser impact of WBC as a risk factor (particularly at the Cancer Institute (WIA)) – with the added inference that WBC alone may not be a sufficient indicator of the differences in leukaemia burden between the Indian and Western series. The suggestion from the Tata Memorial Hospital series that blast count may be a better prognostic indicator then WBC, was not borne out in the All India Institute of Medical Sciences series and the relative value of WBC and blast count may well be dependent upon other as yet undefined variables.

The higher toxic death rate in Indian patients is also partly, if not largely, responsible for the poorer treatment outcome, although the relapse rate at the Cancer Institute (WIA), which differed the most with respect to patient characteristics and risk factors from Western series, was also higher. The progressive reduction in the toxic death rate over time was associated with improvements in outcome at the Tata Memorial Hospital,

although there was also some reduction in the relapse rate after 1989. This provides an important lesson. Not only the treatment itself and the population being treated, but also the ability to support the patient, and, quite likely, the degree of adherence to the planned treatment (by both physician and patient) are likely to influence risk factor analyses. Some of these factors vary with time, including the extent of disease at presentation, which is, in part, a reflection of socioeconomic and educational levels, such that the relative importance of various risk factors may also vary with time. However, it is likely that even if improvements continue to occur, higher toxic death rates than seen in Western countries will be a feature of ALL therapy in India for some time to come, particularly outside the major centres. This is because of the existence of significant co-morbidities, as well the generally poorer hygienic conditions, more limited access to care, lower levels of education and the impact of these on patient compliance. Toxic death rates are also influenced by the overall quality of care, which is determined not only by the quality of the staff, facilities, microbiological and transfusion services and the availability of particular antibiotics and policies regarding their use [29], but also by the patient load per staff member – which is much higher than in Western institutions. It is gratifying that in spite of these obstacles to cure, the most recent results from Tata Memorial Hospital are very similar to those obtained in the United Kingdom ALL XI trial, also conducted between 1990 and 1997, in which EFS at 5 years was 63% [1] and relapse rates are comparable to those of several Western series reported in the same era [30]. This suggests that the treatment protocol itself is not inferior.

Factors other than leukaemia cell burden and biology and the toxic death rates are also likely to be relevant to treatment outcome. As yet ill-defined "ethnic factors", for example. Manera and colleagues published an EFS rate of 60.7% at 8 years in Hispanic and Afro-American children treated in the USA – a result that is also very similar to the most recent Tata Memorial Hospital results [31]. Both of the US children's cancer cooperative groups (now merged to form the Children's Oncology Group), have also recently reported that even when treated with the same protocol in the same institution, different populations may have different outcomes [4,32]. In the Pediatric Oncology Group studies ALinC 14 and 15, conducted in the USA between 1986 and 1996, for example, Hispanic patients had a 5-year EFS of 60.7%, African-American, 65.6% and Caucasian, 72.8%. In the Children's Cancer Group, Asian children treated in the USA had a better outcome (75% EFS at 5 years) than Caucasian children (73%), and African-American and Hispanic children significantly lower EFS rates of 61% and 66%, respectively. These differences were not explicable on the basis of clinical features, disease biology (as far as examined), socioeconomic status or treatment era, but could have been related to treatment compliance, or molecular genetic differences in the leukaemic cells or in enzymes relevant to drug pharmacokinetics and pharmacodynamics [33,34]. Clearly, improved survival rates in the more recently published series from, for example, the USA [35,36], do not necessarily only reflect improvements in treatment protocols, but could also reflect changes in the patient populations, as well as in the distribution of ALL subtypes.

There is much to be learned from studying ALL patient populations in different regions of the world. The present study demonstrates that while the general principles learned from Western clinical research provide the current foundation for treatment strategies, differences in the populations treated, both genetic and environmental, differences in leukaemia cell biology and differences in the quality of care received, can be expected to give rise to differences in the results achieved with the same treatment protocols. Thus, the criteria used for risk adaptation of therapy in Western studies may not be appropriate for use in other patient populations and it is essential that therapy is developed on the basis of clinical trials conducted in the relevant populations. Such studies are, in any event, likely to result in immediate patient benefits. It must also be recognised that risk factors may change over time as delivery of therapy and supportive care improve, and as patient populations and the distribution of ALL subtypes change. Finally, it is likely that the study of patient populations from developing countries, including gene expression profiling of leukaemic cells, will provide new insights into genetic and environmental factors relevant both to the pathogenesis of ALL, and to the identification of prognostic factors.

Conflict of interest statement

None declared.

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