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The clinical indications for identical pathogenesis of isolated and non-isolated testicular relapses in acute lymphoblastic leukaemia

K Jahnukainen[1], TT Salmi[1], J Kristinsson[2], J Müller[3], B Madsen[4] and G Gustafsson[5]

On behalf of the Nordic Society of Paediatric Haematology and Oncology (NOPHO), Finland[1], Iceland[2], Denmark[3], Norway[4] and Sweden[5]

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In the present population-based study, we compared the clinical data of testicular relapses with and without concurrent bone marrow relapse and clinical data of the relapses in other locations among boys with acute lymphoblastic leukaemia (ALL), in order to study the possible evidence of early sequestration and local regulation of leukaemic lymphoblast in the testis of humans. The results suggest that the pathogenesis of isolated testicular relapse (T) and testicular relapse with a concurrent bone marrow relapse (T + BM) is likely to be similar. Isolated and non-isolated testicular relapses appeared late after the achievement of remission (T 34 ± 16 months, T + BM 32 ± 15 months) in ALL compared to relapses in other locations (CNS 23 ± 11 months, BM 25 ± 19 months). The better prognosis after testicular relapses (estimated second event free survival probability, 2-EFS: T 0.63, T + BM 0.32) compared to bone marrow relapse (2-EFS: BM 0.13) further suggests that testicular relapse with a concurrent bone marrow relapse possibly originates from the isolated testicular relapse, and that the isolated testicular relapse is a separate entity and not a manifestation of systemic recurrence. Higher frequencies of isolated and non-isolated testicular relapses (T 9%, T + BM 5%) were observed among boys with onset of ALL in early puberty (10–12 y) compared to those among younger (T 4%, T + BM 2%) and older (T 0%, T + BM 0%) boys. The late occurrence, the possible association with hormonal maturation and the good prognosis after testicular relapses suggest a possible local regulation of the residual leukaemic lymphoblast in human testis. □ *Acute lymphoblastic leukaemia, bone marrow, isolated, relapse, testis*

K Jahnukainen, Department of Paediatrics, University of Turku, FIN-20520 Turku, Finland

Among the patients surviving the first 3 y after initial diagnosis of childhood acute lymphoblastic leukaemia (ALL), the testicular relapses account for the major proportion of the worsened prognosis of boys compared with girls (1, 2). Despite the clinical importance of testicular relapse in childhood ALL, its pathogenesis is still controversial. It is not known whether the leukaemic lymphoblasts escape the impact of chemotherapy in the testicular interstitium, or later spread into the testis from a residual disease in other tissues.

The clinical observations of high frequency of systemic relapse following testicular relapse (3–5), as well as concurrent leukaemic infiltration at other extramedullary sites at the time of overt testicular relapse (6), have led to consideration of the isolated relapse as a local manifestation of systemic relapse in ALL. In support of this, recent molecular studies have demonstrated that, at the time of isolated testicular relapse, there is often a subclinical leukaemic involvement in bone marrow (7–9). However, the problem in these studies is that the bone marrow is in most cases studied at the time of overt testicular relapse, and no information about the preceding minimal residual disease in the testicular tissue is provided. Sequential bone marrow samples taken before isolated testicular relapse have in many case failed to show minimal residual leukaemia before isolated testicular relapses (7, 9). The marrow analysis has also proved to be a poor predictor of other isolated extramedullary relapses (9, 10).

The recent studies with an animal model of ALL have shown that many testicular factors like testicular growth factors, testicular endothelium and testicular interstitial cells are able to locally control the penetration of leukaemic cells into the testicular interstitium and the intratesticular proliferation of leukaemic lymphoblasts (11–13). Furthermore, during the untreated course of experimental ALL, leukaemic lymphoblasts have always been seen to spread into the interstitium of the immature testis (11). These observations suggest that before the start of cytotoxic drug treatment the testis has a potential to gather the leukaemic cells in the interstitium and possibly affect the sensitivity of leukaemic cells to the cytotoxic drug by decreasing their proliferation (11, 12).

In the present study we compared clinical data from the testicular relapses with and without concurrent bone marrow relapse and clinical data from other relapses of child male patients with ALL, in order to study whether indications of early sequestration and local regulation of leukaemic lymphoblasts exist in the testis in humans.

Materials and methods

Non B-cell ALL was diagnosed in 886 boys aged 1–14 y between July 1, 1981 and December 31, 1991 in the 5 Nordic countries (Denmark, Finland, Iceland, Norway and Sweden), with a total population of 22 million people and 4.5 million children. The diagnosis of ALL was based on morphological and immunohistochemical analysis of bone marrow blast cells, and the disease was classified as T cell or non-T cell leukaemia. The median age of the patients was 4.5 y (quartiles: 25%, 2.5 y; 75%, 8.0 y). There were 27 patients who did not achieve remission and 6 patients who died in continuous complete remission (CCR). The patients were divided into risk groups according to risk criteria at diagnosis (Table 1). The total patient distribution among the risk groups was 607 in the standard/intermediate risk group and 279 in the high-risk group.

Initial treatment varied, depending on the year of diagnosis, the country and the risk group. Before 1 July 1986, there were two different Nordic standard risk protocols (S + FIN and DK + N + I) (14). Children in the other risk groups were treated according to national protocols (15). From July 1984 the criterion for standard risk was changed when patients with WBC $10–20\times10^9$/l were moved from the standard risk to the intermediate risk group.

After 1 July 1986, the treatment regimens used in different centres were unified. High- and intermediate-risk group patients were treated according to the ALL-BFM 83 protocol (16). The ALL-BFM 83 protocol differed in many details from the protocols used during the earlier treatment period, but two changes were of most importance. Firstly, the late intensification treatment with the same drugs as used in the initial induction of remission was introduced in the intermediate and the high-risk groups and, secondly, two intensive treatment periods of 5 d before the beginning of the induction treatment were introduced in the high-risk group. In the standard risk group, the treatment differed from the ALL-BFM 83. Remission was induced with prednisolone orally and with 6 weekly intravenous injections of vincristine and 3 weekly injections of adriamycin. L-Asparaginase was given intravenously or intramuscularly over 10 d after the last vincristine and adriamycin dose. Immediately after this, three high-dose methotrexate infusions of 1 g/m^2 were given at 3-week intervals. Intrathecal methotrexate was given four times during the induction treatment and with every high-dose methotrexate infusions. Maintenance therapy consisted of daily oral 6-mercaptopurine and weekly oral methotrexate. During the first year of the maintenance treatment a reinduction with high dose methotrexate (1 g/m^2) with a 4-week interval was given alternatively with vincristine and prednisolone. The oral medication continued until the total therapy had lasted 3 y.

Table 1. Risk criteria for children with non-B-ALL >1 y of age.

Risk	Criteria
High-risk (HR)	WBC $>50\times10^9$/l and/or CNS involvement and/or mediastinal mass and/or T-cell ALL
Intermediate risk (IR)	No HR criteria
	Age 2–<10 y and WBC >20–51×10^9/l from July 1984 WBC $10–51\times10^9$/l
	Age <2 y or ≥10 y and WBC $\leq50\times10^9$/l
Standard risk (SR)	No IR/HR criteria
	Age 2–<10 y and WBC $\leq20\times10^9$/l from July 1984 WBC $<10\times10^9$/l

The relapses that were the first sign of recurrence of ALL were considered in the study. The diagnosis of testicular relapse was based on the observation of leukaemic blasts at surgical biopsy or at fine-needle aspiration. Routine testicular biopsies were not generally included in the treatment protocols. Bone marrow relapse was defined as the presence of ≥5% lymphoblasts, and central nervous system leukaemia as >5 leukocytes/μl in the spinal fluid with definite blasts observed on a cytospin preparation. Relapse therapy was more intensive than the first line treatment. However, no uniform reinduction therapy was used in the Nordic countries during the study period (15).

Statistical analyses were performed with SPSS statistical software (17). The statistical methods included χ^2 test, Kruskall–Wallis test and life table analysis according to the Kaplan-Meier method (17). The estimated second event-free survival probabilities (2-EFS) after relapses in different locations were compared using log rank statistics (17). The events were defined as second relapse, death at second relapse or in second remission. The cases were grouped according to the age at the initial diagnosis of ALL, the location of the recurrence and the treatment received. All children were followed up to January 1995 giving a median length of observation for those in the first CCR of 55 months (quartiles: 25%, 27 months; 75%, 98 months), and for those in the second CCR of 42 months (quartiles: 25%, 19 months, 75%, 75 months).

Results

The duration of continuous complete remission before isolated testicular relapse, testicular relapse with a concurrent bone marrow relapse and relapses without testicular involvement

Altogether 41 isolated testicular relapses and 17 testicular relapses with a concurrent bone marrow relapse (non-isolated testicular relapses) appeared during the observation time.

The median and mean times of appearance of relapses according to location in different risk groups are given in Table 2. Notably, isolated bone marrow relapse and CNS relapse appeared rather equally, in the standard/intermediate-risk group 28 months and in the high-risk group 11–12 months after achievement of remission, whereas the median time to testicular relapses was about 10 months later in all risk groups. No statistically significant

difference in median times of appearance was observed between testicular relapses among the different risk groups or in the total material. The median time of appearance of isolated and non-isolated testicular relapse *differed* significantly ($p < 0.01$) from that of isolated bone marrow relapse in each risk group and in the total material. The difference between the median times of appearance of testicular relapse with and without concurrent bone marrow relapse and of CNS relapse was also statistically significant ($p < 0.01$ in standard/intermediate-risk group and in total material, $p < 0.005$ in high-risk group).

Altogether, 21/41 isolated testicular relapses occurred after the cessation of the cytostatic treatment and 20 occurred during the treatment. A slightly larger proportion of the testicular relapses with a concurrent bone marrow relapse (10/17) occurred after the cessation of the treatment.

The frequency of isolated testicular relapse, testicular relapse with a concurrent bone marrow relapse and the other relapses according to the age at the initial ALL diagnosis

Age at the time of the initial diagnosis of ALL was found to be associated with the risk of a testicular relapse later in the course of the disease (Fig. 1). A linear increase in frequencies of isolated and non-isolated testicular relapses was observed from the age of 4 to 12 y. The highest frequency of isolated and non-isolated testicular relapses was observed among the boys with onset of ALL at the age of 10–12 y (9% and 5%, respectively). This was significantly higher ($p < 0.05$) than the frequency of isolated and non-isolated testicular relapses among younger boys (4% and 2%, respectively). No testicular relapses were observed in the group of boys with the onset of ALL at >12 y of age (Fig. 1).

Contrary to this, there was no evidence that testicular relapses were especially prone to appear at puberty. Only a 2-month difference in the mean duration of continuous complete remission before isolated and non-isolated testicular relapses was observed between the age groups under 10 y and 10–12 y at the time of ALL diagnosis (mean ± SD: <10 y; testis 36 ± 16 months, testis + bone marrow 27 ± 14 months; 10–12 y: 34 ± 14 months, testis + bone marrow 25 ± 17 months).

The pre-treatment characteristics and risk groups of boys who later experienced isolated testicular relapses and testicular relapses with a concurrent bone marrow relapse

In the present material, no increased frequency of isolated testicular relapse was observed among those boys who had features of the ALL with unfavourable prognosis, namely a high white blood cell (WBC) count $\geq 50 \times 10^9$/l, mediastinal mass or CNS leukaemia present at the diagnosis or T cell leukaemia (Table 4). Altogether 73% (30 cases) of boys with isolated testicular relapse were initially treated in the standard/intermediate risk group and 27% (11 cases) in the high-risk group (Table 3).

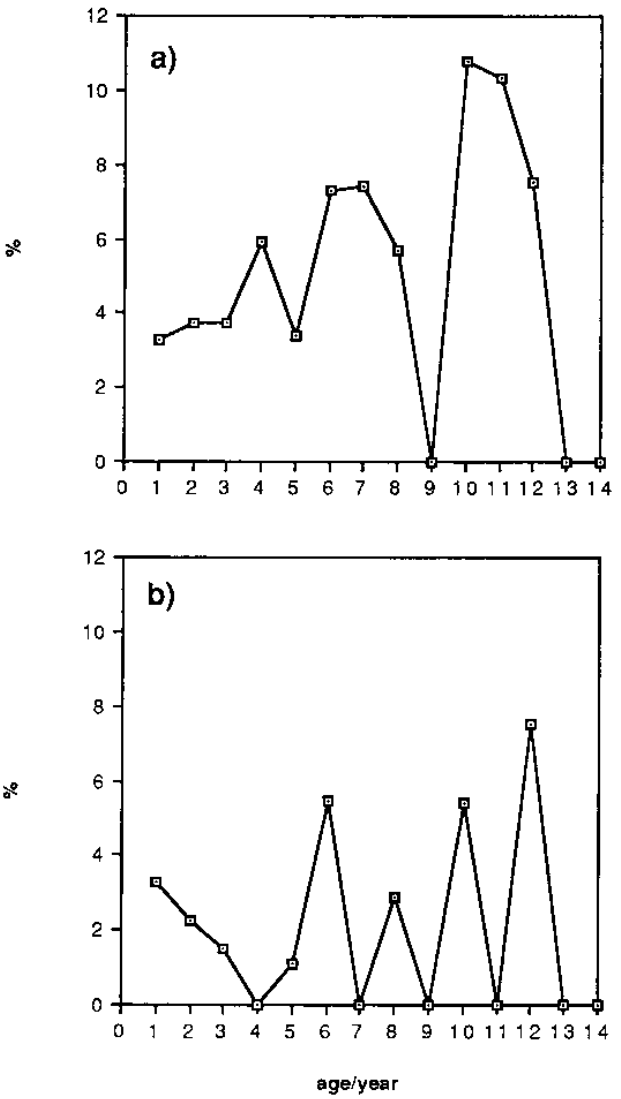

Fig. 1. Percentage proportion of boys with (a) isolated testicular relapse and (b) testicular relapse with concurrent bone marrow relapse, in relation to the total number of boys with ALL grouped according to the age at initial ALL diagnosis.

A significantly larger ($p < 0.01$) proportion of those boys with later non-isolated testicular relapse had WBC counts higher than 100×10^9/l at the time of diagnosis (5/17 cases) compared to boys with isolated testicular relapses (1/41 cases). This was the main reason why 53% (9 cases) of boys with non-isolated testicular relapses were treated in the high-risk group and only 47% (8 cases) in the standard/intermediate risk group (Table 3).

No association was observed between the degree of thrombocytopenia at diagnosis and the later risk of isolated or non-isolated testicular relapse. Not where there any differences in the degree of initial thrombocytopenia among the patients diagnosed at the age of 10–12 y compared to those diagnosed at a younger age.

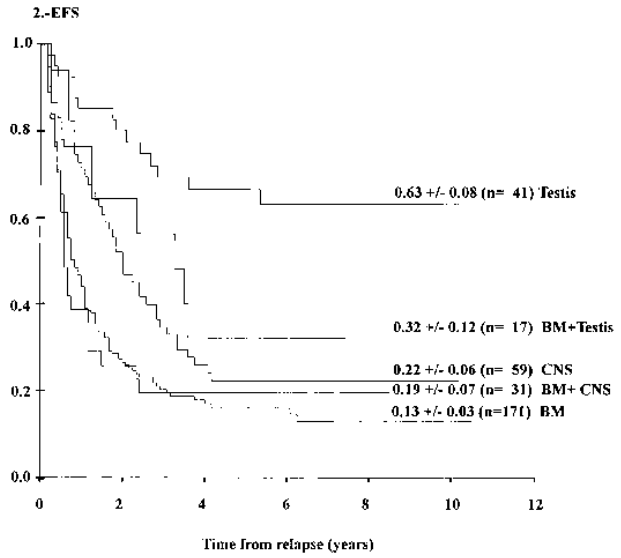

Fig. 2. Estimated second event-free survival probability (2-EFS) ± SD for boys after isolated testicular relapse, testicular relapse with concurrent bone marrow relapse, CNS relapse, CNS relapse with concurrent bone marrow relapse and isolated bone marrow relapse (BM).

The treatment of ALL and the risk of isolated testicular relapse, testicular relapse with a concurrent bone marrow relapse and the relapses without testicular involvement

The effect of intensified systemic control of ALL on the frequency of different testicular relapses and other relapses without testicular involvement were analysed by comparing the two groups with onset of disease before and after changes in treatment protocols on June 1, 1986. A very modest but statistically significant ($p < 0.05$) decrease in the frequency of isolated, and no decrease in the frequency of non-isolated testicular relapse were observed when a more aggressive treatment protocol was introduced. The frequency of isolated testicular relapse in the whole material was 6% before and 4% after July 1986 and, of non-isolated testicular relapse, 3% and 2%, respectively. During the same period, a statistically significant decrease ($p < 0.001$) of CNS and ($p < 0.05$) of bone marrow relapses was observed. The frequency of CNS relapses decreased from 11% to 3% and of bone marrow relapses from 22% to 18%. The impact of high dose methotrexate on the frequency of different testicular relapses could not be estimated since the dose of methotrexate in the standard/intermediate and in the high-risk group differed during the study period.

The prognosis after isolated testicular relapse and testicular relapse with a concurrent bone marrow relapse compared to the prognosis after other relapses

The outcome of boys after isolated testicular relapse (2-EFS 0.63) and testicular relapse with a concurrent bone marrow relapse (2-EFS 0.32) was significantly more favourable ($p < 0.01$) than that of boys after isolated bone marrow relapse (2-EFS 0.13; Fig. 2). There was no statistically significant difference in the prognosis of boys with isolated and non-isolated testicular relapse.

The initial risk groups at the onset of ALL were associated with the prognosis after testicular relapse. The prognosis was most favourable after isolated relapse in the standard/intermediate-risk group patients (2-EFS 0.82), and differed significantly ($p < 0.01$) from that of the high-risk patients (2-EFS 0.27). The boys after non-isolated testicular relapse in standard/intermediate-risk and high-risk groups and after isolated testicular relapses in high-risk group had quite similar prognosis (2-EFS 0.37, 0.27 and 0.27, respectively).

The time of appearance of relapse was also associated

Table 2. The median duration (Md) and quartiles (25%, 75%) in continuous complete remission (months) according to the site of relapse and the initial risk group of ALL.

Group	Isolated testicular relapse	Testicular relapse with bone marrow relapse	Isolated CNS relapse	CNS relapse with bone marrow relapse	Isolated bone marrow relapse
Standard/Intermediate risk					
Md	37	37	28	20	28
25%	26	27	21	15	16
75%	43	50	35	38	39
n	30	8	41	18	101
High-risk					
Md	23	24	11	14	12
25%	13	16	7	7	5
75%	35	39	14	28	23
n	11	9	18	13	70
Total					
Md	36	27	24	18	22
25%	23	21	13	12	10
75%	41	48	31	35	35
n	41	17	59	31	171

Table 3. Clinical and laboratory findings at diagnosis of ALL and risk groups for boys with testicular CNS or bone marrow relapses.Proportions of different findings in each recurrence group are indicated.

	Isolated testicular relapse		Testicular relapse with bone marrow relapse		Isolated CNS relapse		CNS relapse with bone marrow relapse		Isolated bone marrow relapse	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%
WBC/10^9/l										
<50	34	83	10	59	47	80	18	58	119	70
50–100	6	15	2	12	8	13	4	13	19	11
>100	1	2	5	29	4	7	9	29	33	19
Immunophenotype										
T cell	1	3	1	6	6	10	4	13	22	13
Non T cell	37	90	16	94	46	78	24	77	135	79
Unknown	3	7			7	12	3	10	14	8
Age (y)										
1–<5	18	44	7	41	31	52	17	55	67	39
5–<10	13	32	5	29	21	36	11	35	65	38
≥10	10	24	5	29	7	12	3	10	39	23
Risk group										
Standard/Intermediate	30	73	8	47	41	69	18	58	101	59
High	11	27	9	53	18	31	13	42	70	41

with the prognosis after testicular relapse. The prognosis after isolated testicular relapse which appeared later than 24 months into continuous complete remission was significantly ($p < 0.01$) more favourable (2-EFS 0.70) than that which appeared earlier than 24 months (2-EFS 0.45). A similar but not statistically significant difference was observed also in the prognosis after non-isolated testicular relapse (>24 months, 2-EFS 0.42; <24 months, 2-EFS 0.20).

Discussion

The observed similarity in the time of appearance of isolated testicular relapse and testicular relapse with a concurrent medullary relapse suggests that these two types of relapse have the same origin. Because the relapses with a testicular involvement appear late compared to medullary relapse, it seems unlikely that leukaemic lymphoblasts have spread to the testicular interstitium from a residual disease in the bone marrow or other tissues, as has been suggested previously (18, 19). Alternatively, the leukaemic lymphoblasts gather in the testicular interstitium during the early course of ALL and, at the time of relapse, are further infiltrating into the marrow.

The observation that the age at diagnosis but not at the age of relapse affects the risk of testicular involvement further indicates the early sequestration of leukaemic cells to the testicular interstitium. Notably, the frequency of both isolated and non-isolated testicular relapses was high, up to 9% and 5%, respectively, among boys with onset of ALL in early puberty (10–12 y), whereas no testicular relapses were observed among boys with onset later than at the age of 12 y. In this respect, boys over 12 y resembled adult men, among whom the frequency of testicular relapse of ALL is known to be low (20, 21). The observations are comparable to the results in experimental studies where maturation in late puberty is associated with decline in leukaemic infiltration of the testis (11).

In humans, the 10th year of life is known to be the time when adult-type Leydig cells start to develop and daytime levels of plasma testosterone rise (22). The Sertoli cells change their secretory pattern, leading to the initiation of spermatogenesis at the age of 11–13 y (23). Some morphological changes are known to occur in the germ cells even at prepuberty (24). The present observation suggests that these maturational events may be related to the appearance of an increasing tendency for testicular relapses at pre- and early puberty and a sharp decline at the time of spermarche. However, further studies are needed.

The present results confirm the earlier observations of good prognosis after isolated testicular relapse (19, 25). Also the time of appearance of testicular relapse and the initial risk group of ALL were associated with the prognosis after testicular relapses, as in earlier observations (26, 27). Because the material used did not provide information about routine testicular biopsies among patients with testicular relapse, the previously observed more favourable prognosis after late overt testicular relapses compared to that after occult testicular relapse could not be studied (28). It is a new finding that a marrow relapse concurrent with a testicular relapse does not significantly decrease the prognosis of the patients to the unfavourable level associated with bone marrow relapse without testicular involvement. A similar observation on the superior response to chemotherapy after combined compared to isolated bone marrow relapse has earlier been published from ALL material containing both sexes (29).

At the moment, there is no satisfactory explanation of why relapses with testicular involvement are the latest

ones to occur in the course of ALL, or why patients with testicular recurrence fare better than patients with relapse without testicular involvement. However, on the basis of the hypothesis that testicular relapses are related to the intratesticular regulation of leukaemic lymphoblasts (12, 30), the more favourable prognosis and late appearance of testicular relapses are not surprising. Intratesticular regulation is known to be capable of decreasing the proliferation of leukaemic cells (11, 12). Since most of the cytotoxic drugs used in the treatment of ALL have a poor effect on non-dividing cells, these cells, present in the testis early in the course of leukaemia, could be a likely source of late testicular relapses after cessation of leukaemia therapy.

Another possibility is that testicular relapse indicates a more general failure of the treatment of leukaemia. The present observation of an identical interval between diagnosis and relapse for isolated and combined testicular relapse supports this. However, the observed over representation of delayed relapses in the testis compared to bone marrow and other extramedullary sites suggests that, also in this case, some local testicular factors affect the selection of a slowly proliferating leukaemia clone in the testicular interstitium. The present observations of late occurrence, possible association with hormonal maturation and good prognosis after testicular relapses indicate local regulation of residual leukaemic lymphoblasts in human testis.

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