Testicular disease in acute lymphoblastic leukaemia in childhood

Report on behalf of the Medical Research Council's Working Party on Leukaemia in Childhood

Summary and conclusions

In three trials conducted by the Medical Research Council on acute lymphoblastic leukaemia in childhood the incidence of testicular infiltration in relation to presenting features and treatment was analysed. Initial severe thrombocytopenia was associated with an increased incidence. Testicular infiltration was occasionally found during treatment in patients with high blood leucocyte counts at diagnosis but the main incidence in patients with all types of disease occurred within one year of stopping treatment. The incidence may be increased when "second-line" drugs, especially cyclophosphamide, have been used. A reappraisal of the value of additional drugs in maintenance treatment of patients with good prognosis is needed.

Introduction

The incidence of leukaemic infiltration of the testis as a complication of acute lymphoblastic leukaemia (ALL) in childhood has increased as longer remissions from bone-marrow and meningeal disease have been achieved. In this paper we review the incidence and circumstances of testicular disease in three trials conducted by the Medical Research Council.

Patients and methods

The trials concerned (table I) were designed for adolescents and young adults as well as children, but, as testicular disease has not been seen in any patient first diagnosed above the age of 17 years, analysis was confined to patients under the age of 20. All cases entered in the trials up to 31 December 1974 were analysed, but patients entered in pilot trials and those with inadequate records were excluded. Some of the analyses were restricted to UKALL trials I and II because of differences in treatment and inadequate periods of follow-up in UKALL III.

Statistical methods

The figures show actuarial survival curves of relapse of testicular disease, with or without coincident relapse of marrow disease. Thus we regarded a first remission terminated by death or relapse without testicular involvement as a loss. The appreciable difference between the eventual "actuarial" incidence (30%) and the proportion of patients whose first remission was terminated by testicular relapse (15%) reflects the low risk of testicular relapse during maintenance chemotherapy. The former figure is a better estimate of the risk to patients who might otherwise have achieved long remissions. Significance levels are based on the log-rank test.1 2

Results

Sixty cases were observed up to 31 December 1976. These are grouped in table II according to whether testicular disease was present at initial diagnosis of leukaemia (group A; four cases); occurred without previous relapse of marrow disease and either coincided with (or occurred within one month of) first marrow relapse (group B1; 13 cases) or occurred during first remission without other signs of leukaemic recurrence (group B2; 29 cases); or followed relapse of marrow disease and either coincided with subsequent bone-marrow relapse (group C1; two cases) or developed during subsequent remission without other signs of leukaemic recurrence (group C2; 12 cases). All formal analyses were restricted to patients in remission three months from diagnosis and concern groups B1 and B2.

AGE

The age distribution of patients developing testicular disease was not greatly different from the age distribution of all male cases of ALL. This applies to all groups including the important B2 group.

INITIAL PLATELET COUNT

In view of the association between the incidence of meningeal leukaemia and the degree of thrombocytopenia at diagnosis3 we looked for a similar correlation with testicular disease and examined the possible relevance of haemorrhagic signs and symptoms at onset (in UKALL trials I and II). The risk of developing testicular leukaemia was greater in boys with initial platelet counts up to 30 x 10^9 (30,000/mm3) than in those with higher counts (P < 0.05; fig 1). The risk seemed to be highest in boys with thrombocytopenia (< 20 x 10^9), falling as platelet counts increased. Nevertheless, numbers were too small for any more precise statement to be made. Furthermore, the incidence differed little between those with and those without haemorrhagic symptoms or signs at onset.

INITIAL BLOOD LEUCOCYTE COUNT AND MEDIASTINAL ENLARGEMENT

The relations between testicular disease and blood leucocyte count at first diagnosis (mainly the number of leukaemic lymphoblasts) and the presence of a wide mediastinal shadow (presumed to be thymic) are complex. Fig 2 and table III show the relation between time from diagnosis and initial blood leucocyte count for all cases in groups B1 and B2 (those with no previous bone-marrow relapse). Fig 2 also includes the four cases in group A (disease present at or within four weeks of diagnosis); notably, in only one of these was the total count below 50 x 10^9 (an exceptional case, entered but ineligible for the trial since the patient had received steroids for a period of bone-marrow hypoplasia). The proportion of patients with high initial leucocyte counts was also somewhat greater in the C2 group, partly because such patients...
### Table I—Number of male patients aged less than 20 years with acute lymphoblastic leukaemia (ALL) entered in UKALL trials I–III, and type of treatment received

<table>
<thead>
<tr>
<th>Trial</th>
<th>Entry</th>
<th>Cases</th>
<th>Variables</th>
<th>No with WCC &lt; 20 x 10^9</th>
<th>No with WCC &gt; 20 x 10^9</th>
<th>Mediastinal shadow</th>
</tr>
</thead>
<tbody>
<tr>
<td>UKALL I</td>
<td>Aug 1970-Dec 1971</td>
<td>All childhood and adolescent ALL</td>
<td>CNS prophylaxis or not. Six or 12 courses chemotherapy (that is, about 19 or 36 months). Spinal irradiation 2400 or 1000 rads intrathecal methotrexate. Cyclophosphamide or no cyclophosphamide. Eight or 12 courses chemotherapy (that is, about 25 or 36 months).</td>
<td>73</td>
<td>42</td>
<td>10</td>
</tr>
<tr>
<td>UKALL II (main trial)</td>
<td>Jan 1972-Mar 1973</td>
<td>All childhood and adolescent ALL</td>
<td></td>
<td>86</td>
<td>43</td>
<td>11</td>
</tr>
<tr>
<td>UKALL II (modified)</td>
<td>Mar-Sept 1973</td>
<td>All childhood and adolescent ALL</td>
<td>No spinal irradiation. All had cyclophosphamide. Eight or 12 courses chemotherapy (that is, about 25 or 36 months).</td>
<td>35</td>
<td>16</td>
<td>3</td>
</tr>
<tr>
<td>UKALL III (ordinary)</td>
<td>Oct 1973-Dec 1974</td>
<td>Patients with ALL aged &lt;13 and with WCC &lt; 20 x 10^9</td>
<td>Asparaginase timing. Cytosine arabinoside or not (none had cyclophosphamide)</td>
<td>91</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td>UKALL III (intensive)</td>
<td>Oct 1973-Dec 1974</td>
<td>Patients with ALL aged &gt;13 or with WCC &gt; 20 x 10^9</td>
<td>Unrandomised multidrug study (all have cyclophosphamide)</td>
<td>23</td>
<td>47</td>
<td>12</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td>308</td>
<td>148</td>
<td>36</td>
</tr>
</tbody>
</table>

WCC = White cell count.

### Table II—Clinical findings at presentation and treatment allocation in 60 male patients with acute lymphoblastic leukaemia entered in UKALL trials I–III. Patients grouped according to time of testicular relapse

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>At presentation</th>
<th>UKALL trial</th>
<th>Cyclophosphamide treatment</th>
<th>Time (months) from diagnosis of testicular relapse</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>White cells (&lt; 10^10/L)</td>
<td>Platelets (&lt; 10^10/L)</td>
<td>Haemorrhage</td>
<td>Mediastinal shadow</td>
</tr>
<tr>
<td>1</td>
<td>136.0</td>
<td>36</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>57.0</td>
<td>10</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>8.0</td>
<td>60</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>120.0</td>
<td>40</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>3.0</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>12.0</td>
<td>20</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>20.0</td>
<td>10</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>92.0</td>
<td>22</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>9</td>
<td>7.4</td>
<td>80</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*(0) = Randomised but cyclophosphamide not given. (+) = Randomised 0 but cyclophosphamide given. m = Modified trial.
All patients

Total

336

Diagnosis

testicular

Initial leucocyte count. It tended to have earlier marrow relapses. In the clinically more important B1 and B2 groups the incidence of testicular disease during the first two years was almost entirely confined to patients with high initial leucocyte counts; thereafter it was not influenced by the initial leucocyte count.

As most patients with a mediastinal mass had a high initial leucocyte count and there were only three with mediastinal masses out of 34 in groups B1 and B2, we can make no definite statement about the relation between such masses and testicular disease. Nevertheless, earlier testicular infiltration may tend to be associated with mediastinal enlargement at presentation (see table II).

INCIDENCE IN RELATION TO DURATION AND FORM OF TREATMENT

Table III shows in detail the relative risk in successive time intervals from diagnosis in UKALL trials I and II. Patients with a leucocyte
count over \( 20 \times 10^9/1 \) sometimes developed testicular disease during chemotherapy, but in those with lower counts the disease usually occurred after chemotherapy had stopped (at about 19, 25, or 36 months; see table I). This relation becomes clearer if the incidence of testicular disease is plotted from the date of stopping chemotherapy (figs 3 and 4). The rate of relapse was extraordinarily similar whether maintenance lasted two or three years (only in UKALL I was there adequate follow-up beyond 18 months from the end of treatment).

Next it became apparent that the form of treatment might be related to the incidence of testicular disease. Cyclophosphamide came under suspicion. It was not one of the protocol drugs in UKALL I, but in a single deviant case a patient who received cyclophosphamide also developed testicular disease. In UKALL II it was a randomised variable in that half the patients in the main part of the trial were

\[ \text{FIG 1} - \text{Relation of testicular disease to platelet count at first diagnosis of acute lymphoblastic leukaemia in groups B1 and B2.} \]

\[ \text{FIG 2} - \text{Relation of testicular disease to leucocyte count at diagnosis and to duration and form of treatment in groups A, B1, and B2.} \]

\[ \text{FIG 3} - \text{Incidence of relapse of testicular disease in patients randomly allocated to short course of chemotherapy or long maintenance treatment. Patients not in remission at 84 weeks (UKALL I) or 108 weeks (UKALL II) are excluded.} \]

\[ \text{FIG 4} - \text{Incidence of relapse of testicular disease after stopping chemotherapy, by actual duration of treatment. Patients who did not complete their chemotherapy are excluded.} \]

\[ \text{TABLE III} - \text{Incidence of testicular relapse and other relapse or death in first remission in all cases in groups B1 and B2 (UKALL trials I and II) according to leucocyte count at first diagnosis.} \]

<table>
<thead>
<tr>
<th>Initial white cell count</th>
<th>Event terminating first remission</th>
<th>Time from diagnosis (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0-2</td>
<td>2-4</td>
</tr>
<tr>
<td></td>
<td>No of events</td>
<td>Years of observation*</td>
</tr>
<tr>
<td>( \leq 20 \times 10^9/1 ) (169 patients)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Testicular relapse</td>
<td>2</td>
<td>257-4</td>
</tr>
<tr>
<td>Other relapse or death</td>
<td>46</td>
<td>257-4</td>
</tr>
<tr>
<td>Testicular relapse</td>
<td>3</td>
<td>96-0</td>
</tr>
<tr>
<td>Other relapse or death</td>
<td>54</td>
<td>96-0</td>
</tr>
<tr>
<td>All patients (n = 258)</td>
<td>5</td>
<td>353-4</td>
</tr>
<tr>
<td>Testicular relapse</td>
<td>100</td>
<td>353-4</td>
</tr>
<tr>
<td>Other relapse or death</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Total man-years of follow-up in first complete remission.
scheduled to have 600 mg·m² intravenously every 12 weeks for eight or 12 courses. In the subsequent modified arm of the trial all patients were allocated the drug. Fig 5 shows the incidence of relapse of testicular disease in the four allocated treatment groups: UKALL I (no cyclophosphamide); UKALL II (no cyclophosphamide); UKALL II (with cyclophosphamide); and UKALL II (modified; with cyclophosphamide and cranial irradiation only). The incidence of testicular relapse was strikingly higher in patients allocated cyclophosphamide (P < 0.001). Unfortunately, in the randomised part of UKALL II several deviations occurred, so that some patients allocated cyclophosphamide did not receive any and vice versa. Nevertheless, when all four arms of the two trials were analysed, the difference according to actual receipt of cyclophosphamide, although reduced, remained significant (P < 0.01) (fig 6).

These differences, however, should be regarded with caution, and other explanations must be considered. Firstly, the increased incidence of relapse of testicular disease may have been related to the omission of spinal irradiation, being lower in patients in the UKALL II trial who received cyclophosphamide and craniospinal irradiation than in those who received cyclophosphamide and irradiation to the cranium only (P < 0.05). Secondly, a secular change in the incidence of testicular disease may have occurred—perhaps a change in the diagnostic threshold. Such a change would invalidate any inference based on comparison between successive groups of patients, and secure evidence for an effect of cyclophosphamide then rests on the synchronous randomised comparison in UKALL II. When the allocated treatments are compared (fig 5) the difference is significant (P < 0.05), but the significance is lost when actual treatments are analysed (fig 6). A secular trend could also explain the higher incidence in patients in the modified UKALL II trial who received no spinal irradiation.

In UKALL III patients with "poor prognosis" (leucocyte count < 20 × 10⁹/l or aged over 13 years) received cyclophosphamide and "extra" drugs—namely, doxorubicin (adriamycin), cytosine arabinoside (cytarabine; Ara-C), and asparaginase (colaspase)—whereas patients with "good" prognosis were randomised only for extra Ara-C or asparaginase, or both. Analysis of this trial is premature, but the incidence of relapse in the first two years of multidrug treatment was relatively high (fig 2), and among patients with good prognosis six out of eight relapses occurred when treatment included cytosine arabinoside. If Ara-C is under suspicion its omission from UKALL I, where the lowest incidence of testicular relapse was recorded, may be relevant.

Discussion

Although there have been several accounts of testicular disease in acute lymphoblastic leukaemia, there has been no long series analysing the incidence of this complication in relation to age, presenting features, or treatment. Our analysis shows that in young and adolescent boys no age is either exempt or particularly prone to testicular disease. Although immunological typing was not done in our patients it seems certain that T-cell ALL can infiltrate the testis as there were eight cases in all with the typical mediastinal shadow. Most of the remaining cases were probably of the common childhood type of ALL since they mostly had low white cell counts at presentation and reasonably long remissions from bone-marrow disease. Haemorrhage at diagnosis and the occurrence of subsequent relapse of testicular disease were not correlated, but severe initial thrombocytopenia might have been related. As with meningeal disease, the possibility of testicular infiltration being secondary to microhaemorrhages cannot therefore be excluded.

Our main conclusions are that the appearance of overt testicular disease is related to stopping chemotherapy and, perhaps more surprisingly, that the incidence of testicular disease may be increased by some drugs—particularly cyclophosphamide and cytosine arabinoside. Our findings do not absolutely implicate cyclophosphamide, but similar preliminary results have been obtained in a synchronous randomised controlled trial, so that alternative explanations for the combined data, such as a secular trend in the incidence of testicular disease, are improbable. Nevertheless, we believe that a secular trend in the diagnostic threshold might have occurred in the MRC trials. An increased awareness of the possibility of testicular disease will lead to more frequent or earlier biopsy, but if the testicular lesion is not diagnosed early it may be altogether overlooked when marrow relapse supervenes and alternative treatments are begun. This may well account for part of the trend we have observed and does not conflict with the explanation implicating other drugs.

We also examined the possible role of spinal irradiation. Since spinal irradiation was abandoned in March 1973 the incidence of testicular disease seems to have increased, but it seems unlikely that the tiny amount of scattered irradiation could have had a clinically important effect. Even in the youngest children treated with orthovoltage the dose to the gonads could not have exceeded 100 rads, which at most could be expected to eliminate inoqua of eight or nine cells. The effect would be maximal in the youngest children but, in fact, no age-related difference was observed before and after spinal irradiation was stopped.

Paradoxically, cyclophosphamide, which can cause sterility in men, is one of the antileukaemic drugs which definitely crosses the blood-testis barrier. During maintenance treatment with mercaptopurine, methotrexate, vincristine, and prednisolone testicular relapse is uncommon, which suggests that these drugs also cross the barrier to some extent. Possibly the addition of extra drugs (for example, cyclophosphamide or Ara-C) caused the dosage of the other antileukaemic drugs (especially mercaptopurine and methotrexate) to be reduced. In fact, analysis of the prescribed drug dosages in UKALL II shows no reduction when cyclophosphamide was also given, but the dosages actually received by the patient may have fallen short of those prescribed (because of parental intervention, for example) and this may have happened more often when additional drugs were dispensed. Drug antagonism, for which there is some evidence in other systems, is another possibility. Thirdly, these additional drugs might induce neoplastic pro-
gression, engendering a clone of drug-resistant cells from an original testicular infiltrate that would otherwise be susceptible to prolonged treatment with methotrexate and mercaptopurine. Finally, cyclophosphamide-induced destruction of cells within the seminiferous tubules (that is, beyond the blood-testis barrier) may change the microenvironment to allow proliferation of leukaemic cells in that site.

The relation of testicular disease to subsequent relapse of bone-marrow and meningeal disease is considered elsewhere. Meanwhile, the fact that many of the boys with isolated testicular disease had already had bone-marrow relapses indicates the need for further chemotherapy in conjunction with local treatment to the testes and also emphasises the importance of prompt diagnosis and prevention of testicular disease. Exclusion of cyclophosphamide from routine chemotherapy seems to be important, and a full reappraisal of the value of "extra drugs" in maintenance treatment for "good-risk" patients is needed. Routine biopsy of the testes and prophylactic testicular irradiation must be considered.

Treatment of malignant ascitic and pleural effusions with Corynebacterium parvum

H E WEBB, S W OATEN, C P PIKE

Summary and conclusions

Six patients with malignant effusions, five from adenocarcinomas and one from a melanoma, were treated by intrapleural or intraperitoneal Corynebacterium parvum. In each case there was a definite reduction in the effusions with a significant decrease in the number of malignant cells; in most cases the effusions stopped completely.

Although none of the patients lived for more than a year after treatment, they were undoubtedly more comfortable, as they no longer required frequent paracentesis. In some cases the patients lived longer than originally expected in a state in which the quality of life was acceptable.

Introduction

Corynebacterium parvum is now recognised as a possible anticancer agent. A preliminary study on patients with various advanced malignant conditions suggested that C parvum might be beneficial in the routine treatment of patients with malignant ascites. We report here the results of treatment with C parvum in six patients with malignant effusions.

O B Eden is in receipt of a grant from the Leukaemia Research Fund.

References

8. St Jude Children’s Hospital, Memphis. Unpublished results.

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Patients and methods

Corynebacterium parvum strain CN 6134 (batch EZ 174) was obtained from Wellcome Research Laboratories. Preparations consisted of 7 mg dry weight per ml of formalin-killed organisms suspended in 0.01%, thiosulfate. The dose, which was given intraperitoneally, intrapleurally, and occasionally intramuscularly, ranged from 0.5 ml to 2 ml.

Treatment—All patients and their relatives gave informed consent. When it was considered necessary for the patient’s comfort a formal paracentesis was undertaken. Nevertheless, we always left enough fluid behind to mix the C parvum adequately with the effusion. Samples were taken and further injections were given using a 20-ml syringe and a three-way tap with little or no discomfort to the patient. As treatment progressed the fluid became loculated and only small amounts could be withdrawn from any one site. But it was still possible to give the C parvum into areas of loculated fluid. Reactions to the C parvum were nausea, vomiting, fever, and some pain at the site of the injection. These were controlled by analgesics and antieptic. When there was not enough fluid to dilute the C parvum it was given by deep intramuscular injection of 1 ml into the buttocks. No discomfort or inflammation was observed with these injections.

Case reports

Case 1

A 54-year-old woman underwent a subtotal hysterectomy and bilateral salpingo-oophorectomy for a uterine papillary adenocarcinoma. Over the next two years she was treated with chlorambucil, cyclophosphamide, and thiopeta for recurrent ascites, and when she started C parvum treatment she had had about 50 litres of malignant ascitic fluid removed. She was given 2 ml of intraperitoneal C parvum every week for four weeks. Five weeks after the start of treatment no fluid could be detected in the abdomen and it had become concave.