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Paediatric Oncology Update

Acute Lymphoblastic Leukaemia

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MANY ENIGMAS continue to surround childhood acute lymphoblastic leukaemia (ALL). We still do not know what causes it, how long it takes to develop, how best to classify the different subtypes, or how best to use the therapeutic tools we currently have at our disposal. So the "Great Success Story" of paediatric oncology is far from complete. Current uncertainties about the disease can conveniently be considered in the three areas of aetiology, biology and therapy.

AETIOLOGY

The incidence of ALL is not constant throughout the world. It varies from 0.9 to 4.7 per 100 000 children per year. It is high in Europe and North America and lowest in Kuwait and Bombay. In most countries, there is a slight excess of males with a ratio of around 1.2:1. This is not true for infants where there is a female preponderance, and not for T-lymphocyte ALL where the male excess is more pronounced.

The incidence also varies with age. There is a well defined peak between the ages of 2 and 6 years, where, in the United States, the rate rises to around 7 per 100 000 white children [1]. Oddly, this peak is less well defined in American black children, is not evident in developing countries and was not apparent in either the United States or Great Britain until the 1930s [2]. The peak is composed almost entirely of children with 'common' ALL-B-lymphocyte precursor disease, frequently showing hyperdiploidy that responds well to conventional therapy.

That there is no single simple cause of ALL is self evident. Different aetiological factors may be involved in different varieties of the disorder, but what those factors are is far from clear. Much concern has centred around the time-space clusters observed near nuclear installations in the United Kingdom—the Dounreay power station in North Scotland, the Sellafield nuclear waste re-processing plant in North West England, and the Aldermaston nuclear establishment in the South of England. However, suspicion of background radiation as the cause of these clusters has drifted away, despite the Gardner hypothesis of fathers' preconception long-term low-dose exposure being contributory [3]. Rather, Kinlen's theory of population mixing, loss of herd immunity and abnormal response to infection of unusually susceptible children has gained ascendancy [4], fuelled by the compatible ideas of Greaves, who suggests that the risk of 'common' ALL is increased by higher socio-economic status, isolation and other circumstances that may alter the pattern of infection during infancy [5].

These theories leave much unexplained, and only go some way towards recognising a possible contributory factor in some cases of 'common' ALL typically seen in toddlers, playgroup and kindergarten children. Perhaps the infection factor is one of Knudson's 'two hits' where an unrecognised constitutional predisposition is the other, though no germline mutation has yet been identified that predisposes to ALL, other than trisomy 21 associated with Down syndrome. The other 'hit' (or 'hits') would have to be somatic and might, at least in some instances, result in the acquisition of an abnormal fusion gene, such as the recently described TEL/AML1 chimeric transcript found in up to 20% of patients [6].

Apart from environmental ionising radiation and an unusual response to infection(s), other candidate stimuli to somatic mutation of the genome of B-cell precursors have included antenatal X-rays [7] and low frequency non-ionising radiation from high-voltage electricity powerlines and terminals [8], although the evidence for both is very weak, and antenatal radiographs are now rarely taken. In the end, it must be admitted that, despite the best efforts of epidemiologists, scientists and clinicians, the preconditions that allow ALL to develop are still mostly unknown.

BIOLOGY

Morphology

Despite the advent of immunophenotyping with flow cytometry, cytogenetics with digitised karyotype imaging, Southern blotting, the polymerase chain reaction and fluorescence *in situ* hybridisation (FISH), ALL is still usually diagnosed by an experienced morphologist examining a Romanowsky stained bone marrow smear with a high power

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light microscope. Other techniques merely extend the diagnosis, and where there is doubt about the marrow morphology, there is often difficulty in defining an immunophenotype.

Classification schemes based on cell morphology have now been largely superseded by other ways of looking at cells, but the French American British (FAB) system, which defines three categories (L1, L2 and L3), is still widely applied [9]. However, this system is of limited clinical value as the proportions in the three categories are grossly uneven and divide patients into roughly 90%, 9% and 1%, respectively. There is some evidence that L2 ALL is more refractory to therapy, occurs with equal frequency in all ages (i.e. shows no 2–6 year peak), and may have a different immunophenotypic pattern [10]. L3 ALL shows a mature B-cell phenotype, frequently presents as a lymphoma, and does not respond to conventional ALL treatment. The morphological feature that correlates most clearly with responsiveness to conventional treatment—the presence of cytoplasmic vacuoles—was not included by the FAB group. Vacuoles are present in 25–30% of all patients and are associated with a lower presenting white count and the immunophenotype of 'common' ALL [11]. Cytochemistry adds little to morphology in ALL apart from periodic acid-Schiff positivity of a characteristic 'block' pattern being seen in around 15% of cases and correlating with vacuolated classical 'common' ALL, and T-ALL showing strong polar positivity for acid phosphatase [12]

Immunophenotyping

At present, ALL is arguably best classified on the basis of immunophenotyping. Hybridoma technology has produced monoclonal antibodies to many cell clusters of differentiation (CDs) epitopes. Some relate to lymphocyte sublineage (CDs 1–8 mark various stages of T-cell ontogeny, CDs 19–22 and 24 mark B-cells), whereas others, such as CD10 and CD34, mark more primitive features. Other useful immunologically-defined cell characteristics not given CD numbers include cytoplasmic and surface immunoglobulins (found in pre-B and mature B-ALL, respectively), terminal deoxynucleotidyl transferase (TdT, found in immature lymphoid cells) and HLA-DR, a relatively non-specific expression of class II histocompatibility antigens.

Using these tools, it is possible to classify ALL into the major categories of 'common' (around 50%), 'pre-B' (around 25%), 'T' (around 15%), 'null' ALL (around 9%) and 'B' (around 1%). All forms other than T-ALL are considered to be derived from some stage of B-precursor cell, and 'null' ALL is sometimes included as 'early B-precursor ALL' which is a broad term encompassing 'common' and 'null' together. The classification is clinically useful as treatment response and clinical features are different within immunologically defined groups [13].

ALL cells occasionally express cell antigens more usually associated with myeloid differentiation (CD11b, CD13, CD14, CD15, CD33, CD36, CDw12 and CDw65). This may be a more frequent feature of the adult form of the disease. The proportion of ALL children who are My-A (myeloid antigen) positive is somewhere between 7% and 24% [14–17]. The discrepancies may be partly technical as quality control using different reagents is poor [18]. It is not clear whether My-A+ ALL can be recognised by association with other features, but there are data to suggest that it associates with L2 morphology [15] and CD10 negativity [17]. Opinions are divided as to whether My-A expression is relevant in terms of response to therapy, with roughly 50% of investigators claiming the feature to be a highly significant and independent predictor of adverse outcome [15, 16] whilst the other 50% suggest it is of no importance [14, 17].

Cytogenetics and molecular genetics

Clonal chromosome abnormalities are detectable in nearly all cases of childhood ALL. They can be categorised either simply on the basis of the modal number of chromosomes (or ploidy assessment by flow cytometric assessment of DNA index) or in more depth by examining the structural changes and rearrangemetns based on detailed karyotype analysis. Molecular analysis of genes adjacent to the breakpoints of structural anomalies has helped to identify functional lesions in mechanisms regulating cell growth and differentiation, and in some instances has provided a clue to leukaemogenesis.

From a practical point of view, crude assessment of ploidy status is clinically useful, as the 25–30% of children with >50 chromosomes per malignant cell will fare better than the others [19]. More careful karyotype analysis subdivides patients into those with extra normal chromosomes and those with structural rearrangements as well, and it is the former who have the advantage [20]. Hypodiploidy predicts a less favourable response to treatment and is seen in 5–10% of children. A tiny subgroup of children (<1%) with near-haploid karyotypes (<30 chromosomes) fare particularly badly [21].

Most of the more commonly seen karyotypic structural rearrangements have been studied at a molecular level. Some translocations have been found to produce functional fusion genes. One of the most common (5–6% of all cases) is t(1;19)(q23;p13.3), seen in pre-B ALL where the *E2A* gene fuses with *PBX1*. Another clinically important example is the t(9;22)(q34;q11), forming the Philadelphia chromosome and the *BCR-ABL* fusion gene, which arises in 2–5% of all ALLs, and is associated with extreme resistance to therapy. Up to 50% of *BCR-ABL* ALL cases may not be detectable by conventional cytogenetics [22]. Much interest currently centres around rearrangements involving the *MLL* gene on chromosome 11 in the q23 region, found in up to 70% of infants with ALL, and the commonest result is a fusion gene with AF4 on chromosome 4, band q21. The poor outcome of treatment in infants with ALL is largely confined to those with an 11q23 abnormality [23].

Not all structural rearrangemetns identify poor responders. Recently, a rare translocation, t(12;21), cytogenetically detectable in less than 0.1% of children, was cloned and shown to involve fusion of part of the *TEL* gene to the *AML-1* gene. Subsequent FISH studies have shown that conventional cytogenetics cannot detect this abnormality in the majority of cases, but it is present in 16–20% of patients, making it the commonest single genetic lesion so far seen in childhood ALL [6, 24]. Unlike other translocations, it is predictive of a relatively favourable response to therapy.

Other disease characteristics that aid classification or predict response to therapy (risk stratification)

Ever since ALL first responded to chemotherapy, clinicians have been unable to resist attempts to define risk categories based on clinical features, correlated with the likelihood of a child with ALL proving resistant to treatment.

The adverse influence of a high white count at presentation has been recognised for over 40 years [25], and the differing response between age groups and the sexes for over 25 years [26]. However, as Dr Don Pinkel recently re-emphasised treatment itself is, of course, the most important prognostic factor. As therapeutic schedules change and evolve, variation in the apparent predictive importance of other variables waxes and wanes accordingly [27]. Great difficulty has arisen when comparing results between different countries and centres due to the incompatible way in which patients have been stratified for different modalities of therapy based on their locally assigned risk category.

Following an initiative in Rome in 1985, an attempt to bring order to chaos was made at a workshop in 1993, where the various North American collaborative groups met to agree a common approach to risk classification for the purpose of treatment assignment. This meeting concluded that 'standard risk' ALL should be defined as non-T, non-B disease arising in children aged from 1 to 9 years with a diagnostic white count of $<50 \times 10^9$/l. All other patients are high risk. There was some debate about T-ALL and whether the immunophenotype itself carries any sinister significance, or whether it is a more serious disease because it tends to present with a high white count. The matter was left undecided. The workshop also agreed that a common set of variables should be assessed on all patients: DNA index (ploidy), cytogenetics and molecular genetics, an assessment of the speed of response to treatment by analysis of marrow status at day 7 or day 14 of treatment, immunophenotype and the presence and number of blast cells in the cerebrospinal fluid [28].

Several groups have noted that the speed of response to treatment is important. The Berlin:Frankfurt:Munster (BFM) group currently use initial response to steroids to assign risk categories [29], regarding patients that have residual circulating blast cells after 1 week as 'resistant' and, therefore, as candidates for more aggressive therapy. The Children's Cancer Study Group noted that marrow clearance within 14 days was important [30], a point confirmed in a large United Kingdom trial where marrow status at 2 weeks was noted to be the most important prognostic factor after white count, age and gender [31]. Clearance at 7 days might be a more sensitive index than 14 days as less than 20% of children have gross residual disease after 2 weeks, and initial experience would suggest that this is so [32]. Thus, the rate of cytoreduction on any treatment regimen appears to be a powerful prognostic factor.

Whereas well-established CNS involvement at diagnosis is universally regarded as increasing the likelihood of treatment failure, the importance of low numbers of blast cells in the cerebrospinal fluid (<than 5/mm^3) at diagnosis is more a matter of debate. Two studies have suggested that they indicate children at higher risk of subsequent CNS relapse [33, 34], but this has not been confirmed by a third [35]. The number of such patients has varied between studies (from 6 to 18% of the total) perhaps reflecting the difficulty in reliably identifying small lymphoblasts in Romanowsky stained cytospin preparations.

Other disease-related clinical characteristics that have been related to outcome include the presence of gross lymphadenopathy and hepatosplenomegaly ('lymphomatous' features) and the presence of a mediastinal mass. Organomegaly figured in the risk index (RI) used by the BFM group, the RI being expressed as a function of the circulating blast cell count and the size of the liver and spleen [36]. Mediastinal involvement is most frequently seen in T-cell disease, but is also occasionally seen in B-ALL. It is clinically important because of potential anaesthetic difficulties, but has no prognostic importance independent of immunophenotyping.

TREATMENT

Looking back

From the early 1960s to the present day, the outlook for children with ALL has steadily improved and the overall long-term event-free survival is now nudging over 70% [29, 31, 37, 38]. For those in the better prognostic groups, the figure will be higher, but probably not more than 80% [39]. So there is still an elusive 20% of good risk children who unexpectedly relapse.

Over the last half century, there have been three distinct phases of therapy, the first (during the 1950s and 1960s) being the era of drug discovery and defining combined protocols culminating in the concept of 'total therapy'. This was the first attempt at permanent disease eradication [40]. The second phase (during the 1970s and 1980s) was the better application of known drugs, the addition of cytarabine, anthracyclines and epipodophyllotoxins, and the development of the philosophy, pioneered by the BFM group, of intensive re-induction (consolidation) protocols ('blocks'), the attendant increase in toxicity being offset by better disease-free survival [41]. The BFM principle has been amply confirmed in large randomised controlled trials both in the United States [38] and Europe [31]. Since the chemotherapy schedules differed in these randomised studies, it would appear to be the inclusion of intensive blocks of therapy that is important rather than the detail of what they contain. The point was emphasised by a Pediatric Oncology Group study where only high doses of antimetabolites were used in consolidation, avoiding the use of potentially carcinogenic anthracyclines, alkylating agents and epipodophyllotoxins [42]. A Dutch Leukaemia Study Group also showed that excellent results can be obtained in non-high-risk patients without resorting to these drugs [39].

During the last era, the 1990s the mood has changed to emphasise the awareness of late toxic effects of therapy, the difficulty of curing the last 20% of children with low-risk disease, the differing sensitivity to conventional therapy of some ALL subtypes, and the difficulty in developing effective new treatment strategies for high-risk disease. There is frustration at the continuing lack of a clear view of the best use of allogeneic progenitor cell transplantation (PCT). Currently, two parallel therapeutic frontiers are developing—efforts to improve standard therapy in terms of efficacy and long term toxicity, and efforts to develop better strategies for children with more aggressive forms of ALL and those with relapsed disease.

'Standard' therapy for 'standard risk' disease

The 'standard' template of treatment is still in a state of evolution. Although there is little disagreement that the classical triad of vincristine, steroids and L-asparaginase is adequate for remission induction in most circumstances, there is uncertainty over whether dexamethasone is superior to prednisolone in terms of disease control and CNS protection [39]. This question is presently being tested in large multicentre randomised trials. One in the United States closed in 1995, and one in the United Kingdom is about to start. Also different preparations of L-asparaginase have different pharmacokinetics [43] and different toxicity profiles [44], and the best type and dose schedule are still not determined.

The benefit of some sort of intensive re-induction or consolidation schedule or schedules for standard risk patients is generally accepted, but whether anthracyclines, alkylating agents and/or epipodophyllotoxins are needed is still a point of debate (see above) as is the benefit or otherwise of an additional 'late block' of delayed intensification after 6 or 12 months of treatment. The latter question may be answered by large multicentre randomised trials. One such trial in the United States closed in 1993 and one in the United Kingdom has been accruing patients since 1992. The BFM group found no obvious advantage for late intensification in a randomised subgroup of their ALL-BFM 86 study, but the numbers were too small for a firm conclusion to be drawn [29].

The best way to prevent or control CNS disease has not yet been determined. Cranial radiotherapy was originally the standard approach, but because of its potential for damaging intellect and the hypothalamic pituitary axis it is now reserved for children thought to be at especially high-risk of CNS involvement (those with high diagnostic white cell counts or bulky extramedullary disease), or those few who have unequivocal CNS infiltration at diagnosis. For the rest, adequate protection can be achieved by intrathecal therapy either alone or in conjunction with high doses of systemic methotrexate with folinic acid rescue, although it appears to be important to continue intermittent intrathecal injections for a full 2 years [45]. Whether triple intrathecal therapy (adding cytarabine and hydrocortisone to methotrexate) is beneficial is unclear [46] and the question is currently under investigation in a large randomised trial in the United States.

When unexpected late relapse occurs, it has been suggested that the maintenance component of treatment is failing, either through constitutional resistance to antimetabolites shown by some individuals [47] or non-compliance [48]. Other important but neglected factors include nutrition [49], economic status and ethnicity [50]. Current studies are monitoring pharmacokinetics of drugs used during maintenance and doses are being titrated against myelosuppression with a careful eye on compliance. There are some data to suggest that 6-thioguanine may be a better agent than 6-mercaptopurine [51], and this suggestion is currently being tested in three multicentre randomised trials.

'High risk' and relapsed disease

In addition to the problems surrounding the definition of untreated high risk disease, the other main problem concerns the optimal use of PCT. The problem is compounded by the fact that it is virtually impossible to run randomised trials with a transplant option. Observational studies are, therefore, the only source of data comparing PCT with standard chemotherapy. For *de novo* disease, it appears that transplants offer little advantage [52], but with improving techniques it is possible that they may offer a greater chance of cure, at least for the 10–15% of children whose chances of long-term event-free survival on conventional therapy are less than 30–40%.

PCT is widely perceived as an appropriate form of therapy for most relapsed patients, but there are two main points of controversy. First, the confident identification of those children whose chance of successful 'salvage' will not be improved by some form of PCT, and secondly the role of unrelated donor (UD) PCT for children without matched siblings. For the first, isolated marrow or testicular relapse over 12 or 24 months after the completion of therapy may be better treated with chemotherapy alone [53]. For the second, encouraging reports are now appearing to indicate that PCT with marrow from unrelated donors is producing results comparable with those from matched siblings [54, 55].

Cord blood is an alternative. Early experience with sibling placental blood as a source of progenitor cells suggests that results comparable with both marrow can be achieved with a greater tolerance of mismatching [56]. Unrelated cord blood may be equally suitable [57], and cord blood banks, with the advantage of instant availability of material [58], are now being established.

Looking forward

As therapy becomes more successful, the harvest of late side-effects causes growing concern. The worst event that long survivors can suffer is a second malignancy. There is a 20-fold excess of brain tumours amongst those who have had ALL, particularly those who received cranial irradiation before the age of 5 years [59, 60], and exposure to epipodophyllotoxins has produced an increase in secondary acute myeloid leukaemias [61], an otherwise rare event in ALL survivors.

Almost as serious is late anthracycline cardiotoxicity, which has arisen in a few children, manifesting as sudden onset irreversible heart failure [62]. Other late problems are less catastrophic but far from trivial. Children given cranial radiotherapy can have problems with growth due to damage to the hypothalamic–pituitary axis. Those most at risk are patients treated with high doses ($\geq$2400 cGy) at a younger age who may suffer short stature and obesity in later life [63]. Girls are also at risk of precocious puberty, leading to severe curtailment of final height especially if associated with secondary growth hormone deficiency [64]. Whether chemotherapy alone can impair growth to a clinically important degree is less clear. It possibly can [65].

Testicular irradiation renders males sterile and most will need androgen replacement through puberty. Chemotherapy may lead to subfertility which can improve with time [66]. Ovaries are less sensitive and usually function normally unless they have been irradiated. Modest intellectual impairment occurs in some survivors, measurable as a fall in IQ of 10 to 20 points. The cause is not

certain and prospective studies are in progress comparing the outcome of different types of CNS directed treatment.

The quality of life after successful treatment of ALL is generally good, and, while concern over long-term toxicity increases in parallel with the number of long-term survivors, those with unacceptable morbidity are far fewer in number than the children who relapse and cannot be salvaged. There is, therefore, much work yet to be done in making primary therapy more effective for more patients.

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