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ORIGINAL ARTICLE

Acute lymphoblastic leukemia in children and Down syndrome: Analysis of SHOP/ALL-'99 and '05 Protocols*

M. García-Bernal[a], R. Cilveti[a], M. Villa[b], J. Molina[c], R. Fernández-Delgado[d] and I. Badell[e]**

[a] *Pediatrics Department, Hospital Universitari Mútua Terrassa, Terrassa, Barcelona, Spain*
[b] *Pediatrics Department Oncohematology Unit, Hospital Universitario Madrid Montepríncipe, Madrid, Spain*
[c] *Pediatrics Department Oncohematology Unit, Hospital Virgen del Camino de Pamplona, Pamplona, Spain*
[d] *Pediatrics Department Oncohematology Unit, Hospital Clínico de Valencia, Valencia, Spain*
[e] *SHOP Protocols Coordinator, Pediatric Hematology and Hematopoietic Progenitors Transplantation Unit, Hospital de la Santa Creu i Sant Pau, Barcelona, Spain*

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KEYWORDS
Acute lymphoblastic leukemia;
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Abstract
Introduction and objective: Down syndrome bears a known predisposition to childhood leukemia. In regards to acute lymphoblastic leukemia (ALL), most international groups show poorer results when compared to non-Down patients.
Patients and methods: With this study we analyze the results obtained with Down syndrome patients and ALL younger than 18 years who were treated with SHOP (Spanish Pediatric Hematology Society) protocols for the past decade.
Results: Current data obtained from 1000 patients out of 32 centers confirm several aspects: those are related to acute leukemia showing clinical and biological low risk treats, thereof they may be categorized in low risk groups hence receive scheduled chemotherapy of moderate intensity. However, the number of infectious and toxic complications is greater than those for non-Down patients, therefore both overall survival (OS) and event free survival (EFS) are markedly affected.
Conclusions: The future aim is to optimize the knowledge on biological aspects of these leukemia, in order to determine those features to be acted upon to improve their outcome.

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**Autor for correspondence.
E-mail: mgarcia@mutuaterrassa.es; martagbernal@gmail.com (M. García-Bernal).

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PALABRAS CLAVE
Leucemia aguda linfoblástica;
Síndrome de Down;
Leucemia infantil

Leucemia aguda linfoblástica infantil y síndrome de Down: análisis de los protocolos SHOP/LAL-99 y 05

Resumen
Introducción y objetivo: El síndrome de Down (SD) tiene una predisposición conocida a distintos tipos de leucemia infantil. En el caso de la leucemia aguda linfoblástica (LAL), la mayoría de autores refieren peores resultados con respecto a los pacientes no Down.
Pacientes y método: En el presente trabajo analizamos los resultados obtenidos en los pacientes con SD y LAL <18 años tratados según los protocolos del grupo SHOP (Sociedad Española de Hematología Pediátrica) durante la última década.
Resultados: Los datos obtenidos a partir de casi 1.000 pacientes que proceden de 32 centros, confirman diversos aspectos: se trata de leucemias agudas con características clínicas y biológicas de bajo riesgo, por lo que suelen estratificarse en grupos de riesgo bajo y reciben quimioterapias de intensidad moderada. Sin embargo, el número de complicaciones infecciosas y tóxicas es superior al de las de pacientes sin SD, por lo que tanto la supervivencia global (SG) como la supervivencia libre de eventos (SLE) se ven marcadamente afectadas.
Conclusiones: Debemos optimizar el conocimiento de la biología de estas leucemias para interpretar cuáles son los factores sobre los que podemos incidir para mejorar su pronóstico.

Introduction

In children with Down's syndrome (DS), the risk of acute lymphoblastic leukemia (ALL) is increased in comparison with the rest of the pediatric population. Numerous cooperative treatment groups have analysed the response of patients with DS and ALL to their treatment protocols, and most report a clear tendency towards lower survival and greater toxicity in patients with DS. So far there are no clinical, phenotype or genetic findings associating ALL in DS that would clearly account for these unfavourable results in this subgroup.

Objectives

Analysis of the current situation of ALL treatment in children with DS

Revision of current knowledge on the association of DS and ALL in children. We describe the Spanish Cooperative Group SHOP (Spanish Pediatric Hematology Society), dedicated to the study and treatment of leukaemia and lymphomas in children.

Analysis of the results of ALL treatment in patients with DS

This analysis has two main focuses:

- A comparison between DS and non-DS patients included in the SHOP sample.
- Comparison between the subgroup with DS and ALL treated using our protocols and those described in other countries.

Material and method

Retrospective study of data from patients included in the consecutive ALL/SHOP '99 and '05 protocols over the past 10 years.

The patient's families signed an informed consent for the inclusion of clinical, biological and response data in a SHOP group file, according to the Spanish organic law on data protection. For statistical analysis we have used Pearson's χ^2 test, applying Yates' correction when necessary. Survival curves were calculated using the Log-rank test.

History and current state of basic aspects

Down's syndrome and predisposition to cancer

DS is the most frequent chromosome number alteration in humans, with an incidence of approximately 1/700 newborns.

Complete sequencing of chromosome 21 has made it possible to see that there is a critical region corresponding to point 21q22 in which many of the genes involved in the phenotype characteristics of the syndrome are located[1].

Clinical expression of trisomy 21 has a very wide inter-individual variability. Hall has described the characteristic findings in neonates with DS (table 1). All DS neonates have ≥4 of the 10, and 89% have ≥6/10.

Other characteristic clinical manifestations of the syndrome affect different systems: growth, neurocognitive development, cardiovascular system, musculoskeletal system, ears, nose and throat, eyes, gastrointestinal tract, fertility, thyroid and immunity.

With reference to blood disorders, DS patients can present a transient neonatal myeloproliferative syndrome

Table 1 Characteristic phenotype traits in newborns with Down's syndrome[1]

Phenotype traits	Description	%
Craniofacial	Brachycephaly	76
	Flat and broad nasal bridge	61
Eyes	Upward-slanting palpebral fissures	79
	Epicanthus	48
	Brushfield's spots	53
	Strabismus	22
	Nistagmus	11
Ears	Dysplastic ears	53
	Lack of earlobe	70
Mouth	Open mouth	61
	Cleft lip	56
	Tongue protrusion	42
	Macroglossy	43
	Lingual sulcus	61
	Narrow palate	67
Neck	Thick and short	53
Chest	Pectum excavatum	10
	Pectum carinatum	8
	Xyphosis	11
Abdomen	Diastasis recti	82
	Umbilical hernia	5
Hands	Wide and short	70
	Brachydactily	67
	Transverse palmar crease	59
	Clinodactily	62
	Single flexion crease in the fifth finger	20
Feet	Separation between 1st and 2nd toes	50
	Plantar folds	31
Joints	Hyperlaxity	62

and an increase in the incidence of ALL and acute myeloid leukaemia (AML). However, the incidence of solid tumours is less than that of the general population[2,3].

Mean survival has increased markedly from 35 years in 1983 to 49 years in 1997. If there is no severe cardiopathy, life expectancy is currently around 50-60 years of age[1].

Acute lymphoblastic leukaemia in children

ALL is the most common neoplasia in children (20-30%). The improvement in survival seen in recent years in patients with ALL is due to many factors, the most outstanding being advances in risk stratification, protocol based treatments, and optimisation of support treatment. Nowadays, in industrialized countries, 70-80% of the patients with ALL will achieve a long disease free survival (DFS)[3].

In spite of the fact that most patients are cured, it is still difficult to predict individual responses to chemotherapy. At present, one of the main objectives in the treatment of children with ALL is correct risk group stratification. Categories are established according to clinical, cytogenetic and molecular criteria. ALL diagnosis is based on cytology and immunophenotype. Genetic classification of ALL is carried out in different ways. According to the number of chromosomes it can be classified in: hyperdiploid (>46 chromosomes) or diploid (46 chromosomes), with a better prognosis, or hypodiploid (<46 chromosomes), with a worse evolution.

Molecular classification complements cytogenetic studies and is currently indispensable for initial patient assessment. Some alterations are of much worse prognosis such as t(9; 22) or t(4;11) and others have a good or fair prognosis, such as t(12;21). Current ALL treatment protocols are based on therapy adapted to risk: this is a more aggressive form in patients at very high risk of recurrence and, on the other hand, patients with a lower risk benefit from less intensive treatment with fewer sequelae. At the same time, protocol intensification could modify the intrinsic value of a risk factor considered at diagnosis. Among classical risk factors in B-ALL are age and leukocyte count on diagnosis. Therefore, most treatment groups include patients between 1 and 9 years old with a leukocyte count at diagnosis of $<50 \times 10^9$/l in the group at low risk of recurrence. Patients who do not comply with these criteria are in the high risk group. Nowadays, DFS at 5 years in the low risk group is approximately 70-80% and 65% in the high risk group.

Initial response to treatment is one of the risk determining factors, especially early response considered as the percentage of blasts in bone marrow on day +14 of induction[3].

In spite of the advances in ALL treatment in children, the risk of recurrence is about 3%. It is estimated that a patient with ALL in complete remission determined by cytology still has about 10^{10} neoplastic cells. Techniques with greater sensitivity that detect a leukaemia cell in 10^{-4}-10^{-6} cells have generated a new concept of complete remission, since these previously undetected cells are indicators of recurrence. This new concept is known as minimal residual disease (MRD).

Patients with no MRD at the end of induction have a disease free survival >90% at three years. Those who do have MRD will have a DFS of 25% at three years.

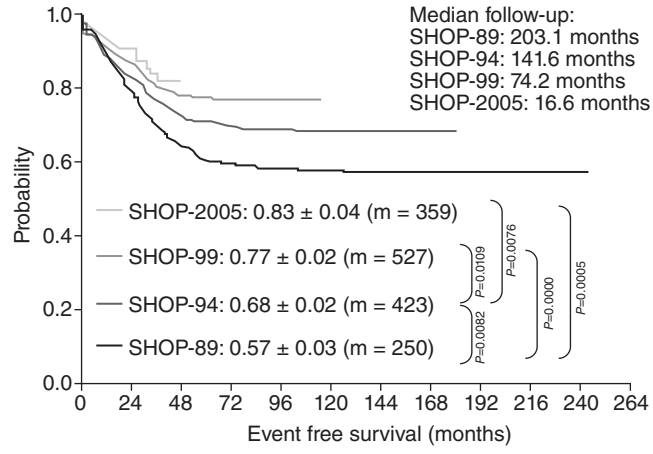

Figure 1 Event free survival (EFS) of the total series of patients in successive protocols.

Table 2 Event predictive factors in ALL-SHOP protocols

	SHOP-89 (n=250)	SHOP-94 (n=423)	SHOP-99 (n=392)
Univariate study	Sex(male) Age ≥10 years Leukocytes ≥20×10⁹/l	Sex(male) Age ≥10 years Leukocytes ≥20×10⁹/l Immunophenotype T Very unfavourable cytogenetics Blasts ≥5% bone marrow day +14	Leukocytes ≥20×10⁹/l Immunophenotype T Very unfavourable cytogenetics Blasts ≥5% bone marrow day +14
Multivariate study	Sex(male) Age ≥10 years Leukocytes ≥20×10⁹/l	Age ≥10 years Leukocytes ≥20×10⁹/l Immunophenotype T Very unfavourable cytogenetics Blasts ≥5% bone marrow day +14	Leukocytes ≥20×10⁹/l Very unfavourable cytogenetic

ALL: acute lymphoblastic leukaemia; BM: bone marrow; SHOP: Spanish Society of Pediatric Hematology.

SHOP Group and ALL/SHOP '99 and '05 protocols

The SHOP Group was founded in 1989 with the aim of achieving homogeneous ALL treatment in children. Currently 32 centres use this protocol to treat their ALL patients.

The preliminary results of the 4 ALL-SHOP protocols developed up to the moment (89, 94, 99 and the current one, ALL-SHOP '05), including more than 1600 patients up to date, have been presented at many meetings[4,5] (figure 1).

With reference to event predictive factors, in table 2 it is possible to see the results obtained with univariate and multivariate analysis of successive protocols. It is necessary to point out that the significance of factors was modified after successive protocol intensifications.

Inclusion and exclusion criteria of the last two protocols, as also the definition of risk groups (standard risk [SR], high risk [HR], very high risk [VHR]), do not differ markedly from those proposed by most international protocols.

Provisional results of the last protocol (2005), with 359 patients included up to the moment, show a DFS of 83%. In the risk group analysis, it is noteworthy that the DFS of the HR group is greater than that of the SR group and that this difference is statistically significant (figure 2A). Since mean follow-up is still 16.6 months and the number of patients will increase before the protocol is closed, it is to be expected that the SR group's DFS will improve.

Factors marked as significant in the multivariate analysis of the last 99 protocol seem to continue being relevant: age, leukocyte count, immunophenotype and response to treatment (figures 2B-D).

Acute leukaemia and Down's syndrome

Characteristics, protocols and results

DS is the most frequent predisposing factor for acute leukaemia in children.

The risk of acute leukaemia, in general, is increased ≥10-20 in children with DS. The risk by ages and subtypes[2,6-8]: ALL risk in <5 year children multiplied by 40; ALL between 5-30 years ×12; AML in <5 year children ×150; in subtype M7 ×500.

There are three relevant blood diseases in DS children:

Neonatal transient leukaemia (NTL)

5%-10% of newborns with DS will present this condition, also known as congenital leukaemia or transient myeloproliferative disorder. It has been described almost exclusively in children with DS or mosaicism[9].

It appears during the first weeks of life as a generally asymptomatic presence of blasts in peripheral blood. The rest of the hemogram is usually normal. Bone marrow exam detects a lower percentage of blast infiltration than that seen in blood (figure 3A). Other clinical symptoms that have been described are: exanthema, bleeding or petechiae, conjugated bilirubin and transaminase elevation, hepatosplenomegaly. This last finding reinforces the hypothesis that this blood condition has its origins in the blood stem cells in the foetal liver. Indeed, almost all cases resolve spontaneously at 3 months, when liver haematopoiesis is replaced by bone marrow haematopoiesis.

The blast population is clonal, normally megakaryoblasts. GATA-1 gene mutations are found in >90% of cases. Up to 20-30% of new borns that overcome NTL will have a true AML M7 before 4 years of age. An identical GATA-1 mutation has been identified in NTL blasts and in subsequent AML-M7 blasts in the same patient. Proof of possible prenatal origin of NTL can be found in the studies carried out using the Neonatal Guthrie Test (early neonatal detection) that identified GATA-1 mutations present in neonates at 48 hours of life[10].

This condition usually resolves spontaneously with minimal clinical symptoms. However, occasionally it can undergo complications and associated conditions such as hyperleukocytosis, cytopenia, effusions or progressive liver failure with fibrosis and disseminated intravascular coagulation that can cause the patient's death in up to 20% of cases[7,11]. As a result, treatment is conservative, but if the newlyborn's life is at risk treatment can be carried out with cytosine arabinoside (ARA-C) at very low doses[9].

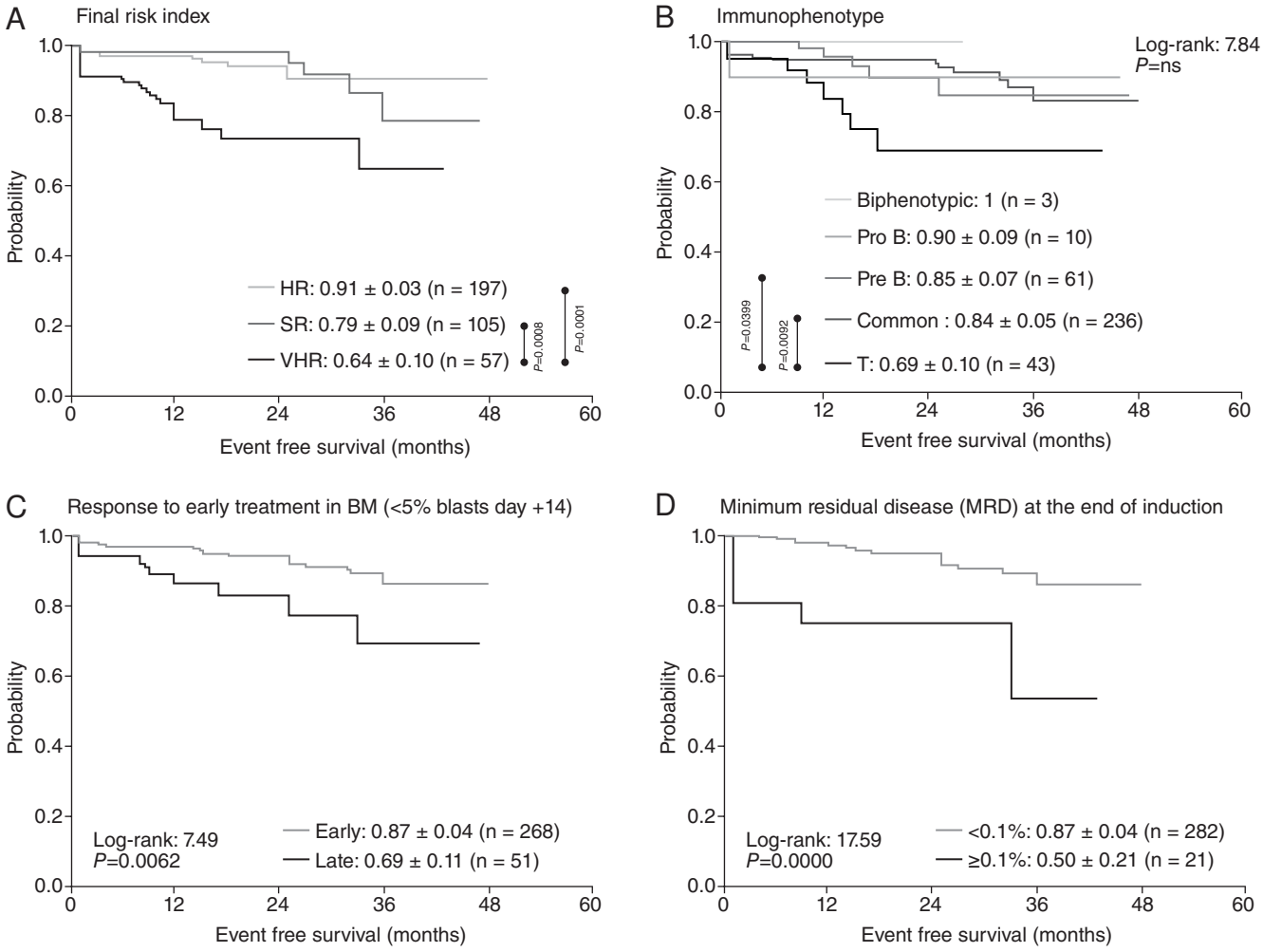

Figure 2 Results of ALL/SHOP-2005 Protocol. A: Event free survival (EFS) according to risk groups. B: EFS according to immunophenotype at diagnosis. C: EFS according to response to early treatment seen by myelopraphy on day +14 of induction. D: EFS according to minimal residual disease (MRD) at the end of induction.

Two current prospective studies (Berlin-Frankfurt-Münster or BFM group and the Dutch Childhood Oncology Group) are addressing the issue of whether systemic treatment of all NTLs would prevent future AMLs[10].

Acute myeloblastic leukaemia

There are two types:

- AML-M7 with GATA-1 mutations frequently becomes NTL and has a good prognosis. It appears before 3-4 years of age and is 65%-80% of AMLs in children with DS[10].
- Another type of AML in >5 year patients, which is not usually M7[12].

We will only comment here on the first type, which is much more frequent.

Blasts express myeloid markers CD33 and/or CD13, CD11b, and are morphologically identical to those of NTL (figure 3B).

From 10%-20% of the AML-M7 had NTL that was self-limiting. Many cases previously presented a myelodisplastic syndrome (MDS), which is expressed as thrombopaenia of variable duration. When bone marrow infiltration reaches 30% it is considered to be AML. Since the progression from MDS to AML is finally inevitable, several authors consider that it would be reasonable to begin chemotherapy even if there is not 30% infiltration[7,9].

The response to chemotherapy is exceptionally good. Disease free survival at 5 years is currently 85%-90%, there are not usually any recurrences and no maintenance treatment is necessary. Treatments are based on the use of ARA-C due to the great sensitivity of blasts to this drug.

Mutations of GATA-1 gene (chromosome X) are characteristic of NTL and AML-M7 in DS. There are >100 different mutations, but they all result in synthesis of the same shortened GATA-1 protein (GATA-1s). GATA-1 mutations are always acquired and are never seen in patients in remission.

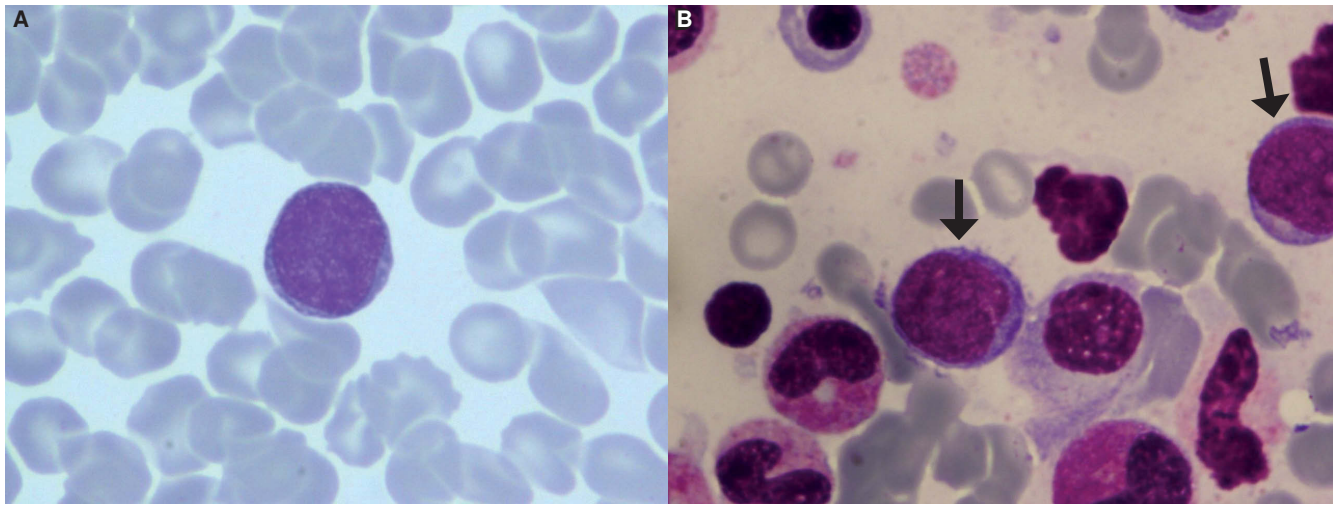

Figure 3 A: May-Grünwald-Giemsa stain of peripheral blood belonging to a neonate with Down syndrome and transient leukemia (NTL). It displays a small blast with dispersed chromatin, nucleolus and sparse basophilic cytoplasm. Magnified ×100. B: May-Grünwald-Giemsa stain of a bone marrow belonging to a patient with Down syndrome and AML-M7. It displays two medium blasts (arrows) with sparse basophilic cytoplasm, no granulation and dispersed chromatin round nucleus with an occasional nucleolus, similar to this in figure 3A. They are surrounded by other myeloid elements with normal appearance and granulation, one lymphocyte and some erythroblasts. Magnified ×1007

All patients with DS have foetal haematopoietic cells with trisomy 21. However, only 10% develop NTL, which almost always (>90%) will have GATA-1 mutations. Of these, only 20%-30% will have a subsequent AML, which will also have these mutations. If it is not acquired, the mutating clone is extinguished, as happens in the case of most NTLs. This is the "multi-step" model proposed to explain the pathophysiology of these processes[7] (figure 4).

An increase of secondary toxicity to chemotherapy is found in these patients with AML and DS, especially in the form of blood toxicity, mucositis and infections[13]. Some cooperative groups such as the German BFM have already established specific protocols for DS AML-M7 which they try to treat with a minimum amount of chemotherapy to minimise toxicity[14].

Acute lymphoblastic leukaemia

From 1.5% to 3.5% of ALL in children are seen in DS[13], but only 1/150 DS will have ALL. Similar to AML, it is proposed that trisomy 21 is the first event to which other genetic alterations are added. Most DS+ALL cases have additional alterations, which affect the same 21 chromosome or other chromosomes (9p, 12p)[12].

Recent studies mention a gene known as JAK2, located in 9p24, whose R683G mutation has been related to ALL+ DS. Patients with this gene have a greater tendency to be <5 years of age on diagnosis and to have a leukocyte count higher than those without the mutation, although their prognosis is similar to these[15]. They can be monitored by PCR and become a way of controlling MRD. Cells with JAK2 mutation are extremely sensitive to JAK1 inhibitors *in vitro.*

In a recent article the high incidence of rearrangement of the CRFL2 gene present in chromosomes X and Y in patients with ALL+DS is noted in comparison with that seen in pre-B ALL in non-DS (56% vs. 3%). The presence of alterations in said gene has been related to mutations in the JAK2 gene, a future field for molecular study[16].

Furthermore, it is still necessary to continue searching for other mutations in ALL-DS when there are no JAK2 mutations (figure 4).

ALL + DS cases usually have a series of common characteristics. They are usually patients between 1 and 9 years of age and no cases have been described in infants. Some studies have reported an increase in frequency of ALL+DS in >10 children[8]. There is a predominance of cases with a leukocyte count on diagnosis of $<50 \times 10^9$/l. They have more anaemia and less thrombocytopenia than non-DS[6,17]. It is extremely rare for the CNS to be affected[9]. No significant differences are seen in comparison with non-DS as to distribution by sex or race, presence of spleno or hepatomegaly or assignation to risk groups, although some studies report a clear decrease of the HRG[8,17]. Only one study reported a predominance of the female sex in their sample[8].

Usually they are B type ALLs, although there may be some T or mature B cell (L3) ALL phenotypes.

50% of cases have a blast karyotype with trisomy 21 with no further alterations. This fact contrasts with the high percentage of genetic alterations in non-DS ALL patients, as if trisomy 21 were an "exclusive" anomaly with a very low tendency to coexist with other anomalies. The most frequent genetic finding is the gain of an extra chromosome 8, 14 or 21 (tetrasomy), as also slight hyperploidy (47-51 chromosomes). The alterations that determine a good prognosis for ALL in the general population [t(12,21), high hyperploidy] are not usually found, but neither are alterations frequent that determine a very poor prognosis, such as t(9;22), t(1;19), t(4;11) and rearrangement of 11q23[9,18]. A discrete increase of t(8;14)[6,12] has been seen.

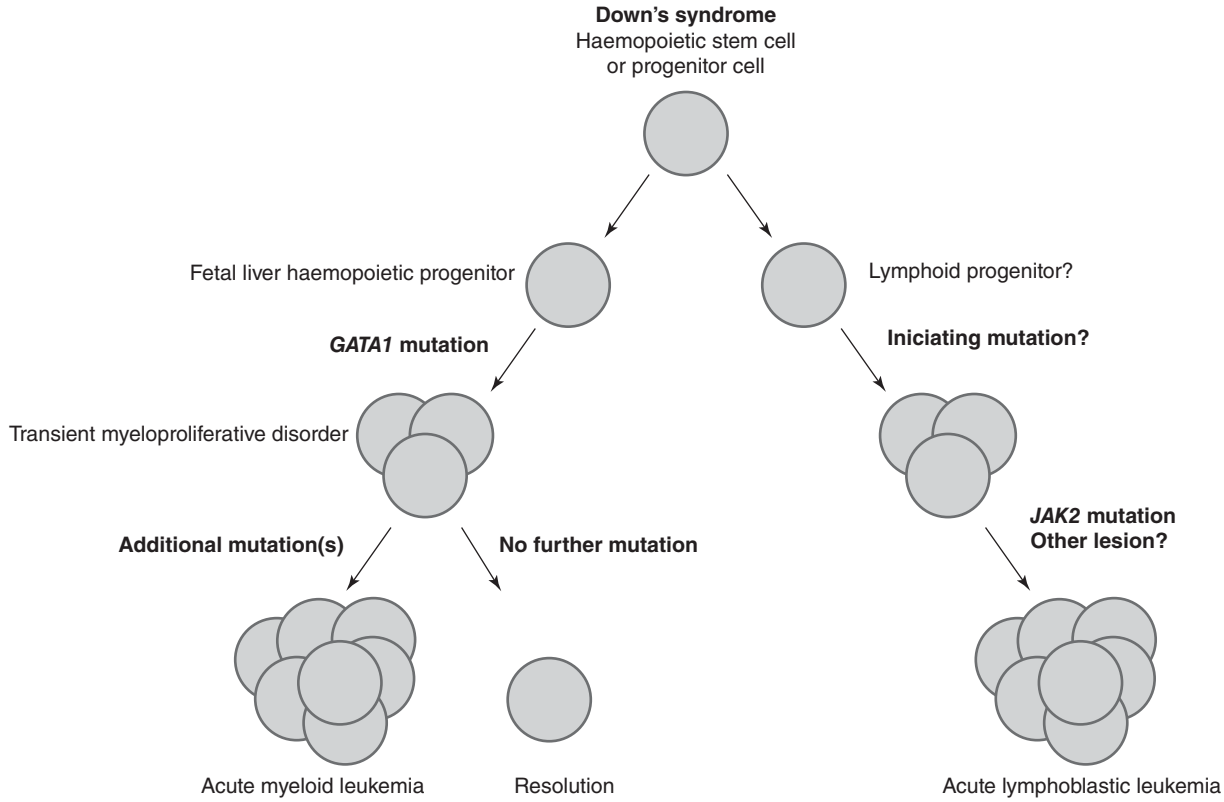

Figure 4 Leukaemogenesis in acute lymphoblastic leukaemia (ALL) and in acute myeloid leukaemia (AML) in Down's syndrome as a model of cooperative processes. From Mulligan CG. JAK2: A new player in acute lymphoblastic leukaemia. Lancet. 2008;372: 1448-50.

Most studies indicate that, with the same protocol and risk groups, general survival and disease free survival are slightly lower in children with DS[8] (DFS in the DS group at 5 years of age was 54%-71% in comparison with 63-82% in the non-DS group).

A slower response to induction, lower percentage of remissions[6,9,17] and increase of deaths during induction have been mentioned[8]. The number of recurrences does not seem to be increased[9,18]. Toxicity is usually increased and there are a greater number of infections, which cause frequent setbacks in maintenance treatment or its decrease.

The increase in the number of reduced folate carrier (RFC) gene copies on chromosome 21 plays an important role in the increase of sensitivity to and toxicity due to methotrexate (MTX). This is a dose-dependent toxicity; therefore differences between DS and non-DS patients are more evident with high doses and prolonged infusions. Depleted folate deposits are explained by an increase in purine synthesis, regulated by a gene also located in chromosome 21. BFM protocols used a decrease in MTX dose of up to 43% in patient with trisomy 21, without obtaining any conclusive results[19].

In vitro studies have shown that DS lymphoblasts have less sensitivity to dexamethasone, asparraginase and ARA-C[20].

Some studies underline the differences in EFS exclusively in the SR group. Given the absence of favourable cytogenetics, maybe many SR patients should really be classified as HR[17]. The Italian and Nordic groups found a lower EFS and a higher recurrence rate in the ALL + DS cases in which treatment had been reduced due to toxicity and infections, in comparison with those whose treatment had not been modified[8,21].

Cardiac toxicity is higher in DS due to a greater frequency of congenital cardiopathies in DS[10].

As to the increase in infections and associated deaths, this is due to multiple factors and is favoured by mucositis secondary to MTX and DS immunological dysfunction[7].

Results

979 patients from 32 centres, registered up to December 2009, were included in the two last SHOP protocols for ALL.

In this sample, 17 patients had DS (1.7% of the general sample), 8 included in protocol of 1999 and 9 in protocol of 2005.

Study of the patients

Clinical and biological characteristics

The group of DS children had a greater frequency of male sex.

Table 3 Clinical and biological characteristics at the time of diagnosis in patients with acute lymphoblastic leukaemia treated with SHOP '99 and '05 protocols

Characteristics at diagnosis	DS (17)	Non-DS (962)	χ^2	p
Sex			0.37	p = 0.53 (ns)
Male	11	551		
Female	6	411		
Age group			2.23	p = 0.32 (ns)
<9 years	12	766		
10-14 years	3	154		
>14 years	2	42		
Leukocytes ($\geq 10^9$/l)			0.13	p = 0.93 (ns)
<20	11	618		
20-50	3	146		
>50	3	198		
CNS infiltration			0.07*	p = 0.79 (ns)
Yes	0	20		
No	17	941		
Testicle infiltration			2.37*	p = 0.12 (ns)
Yes	0	4		
No	11	554		
Inmunophenotype			0.19	p = 0.65 (ns)
No T	16	842		
T	1	120		

*With Yates correction.
CNS: central nervous system; DS: Down syndrome.

The most frequent age of presentation in both groups was 1 to 9 years (minimum age: 2.2 years for non-DS and 1.05 for DS). There has been no infant in any group. A slight increase of the >14 years of age was seen in the non-DS group.

Both the non-DS and the DS group usually had $<20 \times 10^9$/l leukocytes at diagnosis.

There was initial CNS infiltration in 2.1% and testicular infiltration in 0.7% of non-DS patients. No DS patient had CSF or testicular infiltration.

The phenotype was usually pre-B in the DS group, with a single case of a T phenotype.

As can be seen in table 3, none of the clinical-biological variables shows a significant difference when DS and non-DS groups are compared.

- *Cytogenetics* (table 4). Based on the analysis of the 10 samples of the DS group in which a cytogenetic study was performed, this group had a greater prevalence of unfavourable and very unfavourable genetic alterations in comparison with the non-DS group. Neither are there any statistically significant differences between both groups in this aspect.
- *Allocation of risk groups*. In table 5 it is possible to see the final distribution by risk groups, which, in addition to the aforementioned factors, takes treatment into account, especially during the induction phase.

Response to treatment

Early response to treatment = percentage of blasts seen on bone marrow exam on day +14 of induction.

Table 4 Cytogenetics at the time of diagnosis in patients with acute lymphoblastic leukemia

	DS	Non DS	Statistical analysis
No unfavourable (total)	7	320	$\chi^2 = 0.79$; p = 0.67 (ns)
High hiperdiploidy	2	169	
t(12;21) o TEL/AML 1	2	133	
Others	3	18	
Unfavourable (total)	2	183	
Low hiperdiploidy 47-50	0	45	
Hipodiploidy 30-45	0	28	
Almost tetraploidy	0	5	
t(1;19)	0	16	
Others	2	89	
	1	45	
Very unfavourable (total)	1	45	
t(9;22) o BCR/ABL+	1	29	
t(4;11) o MLL+	0	16	

DS: Down syndrome.

The group of patients with DS shows a very good early response, better than that of non-DS patients (table 6). However, due to a higher mortality rate during induction, complete remission (CR) at the end of this phase is lower than in the non-DS group and this difference is statistically

Table 5 Allocation of patients to risk groups according to SHOP protocols

Risk group	DS	Non DS	Statistical analysis
Statistical analysis	8	285	$\chi^2 = 3.55$; p = 0.21 (ns)
High risk	8	513	
Very high risk	1	164	

DS: Down syndrome.

Table 6 Early response to induction treatment in SHOP protocols

	DS	Non DS	χ^2	p
Blasts day +14			2.02	p=0.15 (ns)
<5%	16	768		
≥5%	0	150		

DS: Down syndrome.

Table 7 Current patient status

	DS	Non DS	χ^2	p
Complete remission (CR)	8	809	14.01	p=0.0001
No complete remission	9	153		
Recurrence				
Yes	4	88	2.54*	p=0.11 (ns)
No	13	784		
Death	5	106	3.94*	p=0.04
No death	12	873		

*Yates correction.
DS: Down syndrome.

significant. The number of deaths and recurrences during any phase is also higher in the DS group (table 7), but only the difference in the number of deaths is significant. The EFS and GS curves show that patients with trisomy 21 and ALL have a worse prognosis than other ALL patients (figure 5a-b).

Analysis by risk groups (DS patients)

- *Standard risk (8 patients).* All achieved CR but 4 patients suffered a relapse after treatment (1 early relapse and 3 late ones, 3 bone marrow and 1 testicular). In the 4 cases chemotherapy was begun once more and CR was achieved. The number of SR was clearly greater in the DS group in comparison with the non-DS group. This difference was statistically significant (p=0.05). There was only one death in the SR group due to fatal liver failure in a patient that had achieved CR.
- *High risk (8 patients).* Early response of all patients was excellent. There were 3 deaths before CR, which was only achieved and maintained by 5/8 patients.
  The causes of death were one case of toxicity secondary to chemotherapy (day +25). The other two were due to septic conditions at the end of the induction phase.
- *Very high risk (1 patient).* He had a good early response and achieved CR. The patient was a carrier of a t(9,22) and was therefore submitted to non-related allogeneic transplantation of haematopoietic stem cells. The patient died 10 months after transplantation due to a cerebral haemorrhage.

Protocol based analysis

- *Protocol 99 (8 patients).* 6/8 patients achieved CR. There was a high mortality rate (4/8), two before and two after CR.
  Only one recurrence was seen that responded to rescue treatment and is in CR.
- *Protocol 2005 (9 patients).* 8/9 patients achieved CR, with a single death before CR (toxicity on day +25, HR).

Three patients, all SR, had relapses, but had a good response to renewed chemotherapy, and are currently in CR.

Mortality was, therefore, clearly higher for protocol in 1999 than in 2005, especially in HR and VHR patients. In the ALL/SHOP '99 protocol a dose of iv MTX was given during induction in HR and VHR patients. In the 2005 protocol that extra MTX was excluded and although the consolidation MTX dose was increased from 3 to 5 g/m^2, in DS patients the dose is always 3 g/m^2.

Discussion

The patients in our protocol have similar clinical characteristics to those described in large cooperative studies. They are usually patients 1-9 years of age and there is an increase of patients ≥14 years of age, in comparison with the non-DS group, which was already described in previous studies[8].

The predominance of males in our sample is anecdotal if we take into account the size of the sample.

Most patients with DS and ALL have a B phenotype (14 CALLA+, 2 pre-B), with a single case of T phenotype. We have not found any CNS or testicular infiltration in any DS patient. The SHOP sample has shown a slight increase in patients with unfavourable or very unfavourable genetics, which is in contrast to what has been found in other published studies[9,18]. The number of cases in which genetic studies were performed was small (10/17) which could explain this finding.

Two patients with t(12;21) were found and one with t(9;22), both very unusual in DS+ALL children.

In as far as response to treatment is concerned, we have seen a very good early response, with <5% of blasts in bone marrow on day +14 in 94% of children. However, as can be found in studies of larger samples, the increase in deaths

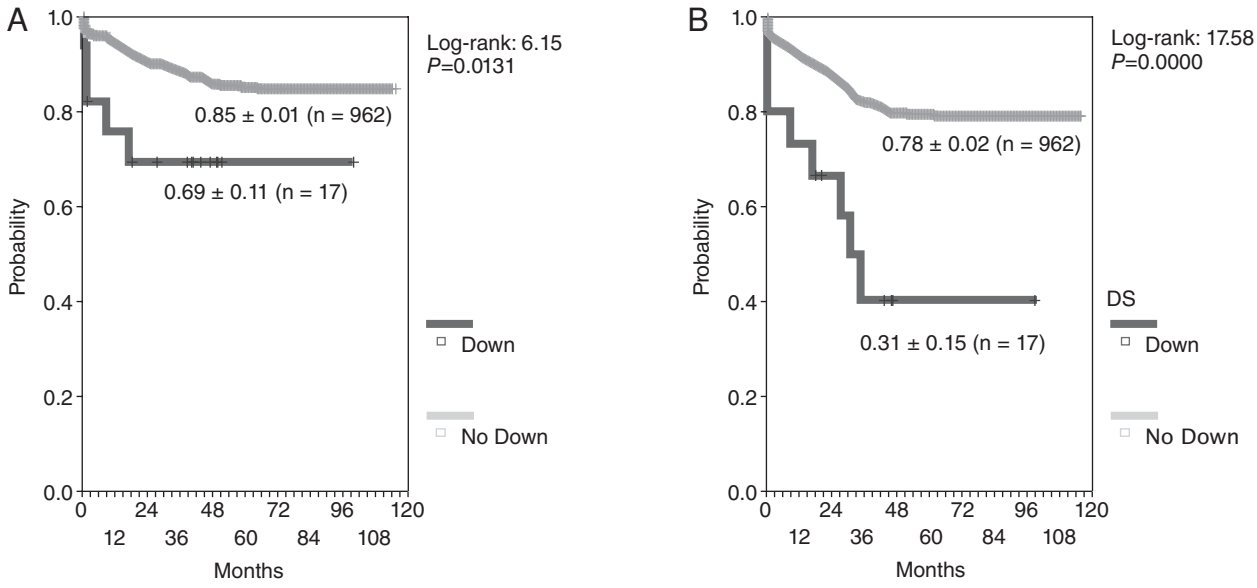

Figure 5 Global survival (A) and event free survival (B) in months of the total patient sample. Updated December 2009.

during induction causes a lower CR rate than that seen in the non-DS group.

General mortality was as high as 30% of patients in our sample. Different studies have provided contradictory results on whether recurrences in DS patients are or not more frequent. In our sample, recurrences in the DS group were greater than in the non-DS group, but only statistically significant in the SR group.

Both the EFS (40%) and GS (69%) are slightly lower in our sample in comparison with most studies. When analysed by risk group, prognosis in the HR and VHR groups was worse than in the SR group, although in this last group 4 recurrences were seen that had a good response to rescue chemotherapy.

Conclusions and proposals for the future

The relationship between ALL and trisomy 21 forms a very wide field of study. We still lack essential knowledge on what part of chromosome 21 or other chromosome regulated by 21 are responsible for increased leukomogenesis in these patients. Probably there are a group of genes that interact in a complex manner with maturation and proliferation mechanisms of haematopoietic stem cells.

The samples from our last two protocols comprised a total of almost one thousand patients, with the usual proportion of DS children (1.7%). In spite of the homogeneous characteristics of the sample and the treatment carried out, there were only 17 patients; therefore all the conclusions based on this study must be treated with caution.

Response to treatment is the most relevant parameter. There is a lower rate of CR in the DS group, in spite of an excellent early response in bone marrow. What happens is that these patients die during induction or just after it due to toxicity and infections.

Some studies have also shown that the differences in EFS are marked when treatment is of less intensity (SR), since recurrences increase. Could this be due to a decreased dose in cases of toxicity in a protocol which is not very aggressive by itself? Due to lack of favourable genetic alterations, especially in t(12,21), which is seen in 20-25% of non-DS ALL, some authors propose classifying all DS patients in the HR group and treating them accordingly.

Given the lower EFS in these patients, the question is to find a balance between reducing the dose to decrease induction-associated mortality and preventing an increase in the number of recurrence that this course of action may cause.

At the same time, it is necessary to study the pharmacogenetics and pharmacokinetics of the drugs used in these patients in greater depth, since they seem to play a key role in response to treatment and toxicity.

The study of JAK2 mutations and the rearrangements of CRFL2 open up a new very important field, since PCR can be used to detect these mutations as an MRD parameter.

It is necessary to search for new mutations associated with trisomy 21 that could affect the other 80% of the patients with ALL and DS that do not have JAK2 mutations. Lastly, it is important to study the role of JAK1 inhibitors on JAK-2 mutations in patient samples and try to determine a possible treatment plan.

Our proposal for the future is to create a specific subgroup within the SHOP protocols specifically for the study and treatment of children with ALL and DS, with the aim of improving the results obtained.

Conflict of interests

The author affirms that they have no conflict of interests.

Collaboration

Dr. Marta García Bernal is enrolled in the Ph.D. program in Pediatrics, Obstetrics and Gynecology at the Universitat Autònoma de Barcelona. This article is within the framework of the childhood leukemia research developed in the Hospital de la Santa Creu i Sant Pau (Barcelona).

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