Acute Lymphoblastic Leukemia

Long-term follow-up of children treated for acute lymphoblastic leukemia and the recovery of β-cell function

We studied the evolution of β-cell function in 32 children treated for acute lymphoblastic leukemia (ALL) through a long-term follow-up after completion of therapy. Our results show that although alterations of the glucose metabolism persists after stop-therapy they are reversible with time.

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Alterations of glucose metabolism commonly occur during the period of treatment for acute lymphoblastic leukemia (ALL).[1,2] However, impaired glucose tolerance can also persist after completion of treatment. These changes have been associated with dysfunction of pancreatic β-cells as a result of islet damage by leukemic cells and/or chemotherapy. Long-term follow-up of glucose metabolism in patients with ALL is still lacking and the evolution of this disturbance is unknown. We conducted a long-term follow-up of glucose metabolism in children treated for ALL after completion of their therapy.

We included all children who had been diagnosed with ALL at the Department of Hematology, University of Chieti, Italy, between December 1996 and December 1999, and who had been off therapy for at least 12 months before entering in the study, to avoid the period of highest risk of relapse.

Eligibility criteria were no first-degree family member with diabetes mellitus, not obese (body mass index SD2 for age), no other endocrine disease and no glucose metabolism alterations during treatment for ALL. We obtained the parents' informed consent, and the study protocol was approved by the institutional review board of the University of Chieti. All participating subjects were in a good health and were not affected by any chronic disease. None was taking any medication or vitamin supplementation. All children had a normally balanced and normocaloric diet and undertook regular physical activity.

All children had been treated in a national, randomized clinical trial, which included the same induction, consolidation and intensification treatment with vincristine, L-asparaginase, prednisone, daunorubicin, doxorubicin, methotrexate, mercaptopurine, cytarabine, cyclophosphamide, and thioguanine. During maintenance treatment, the children had been randomized to receive (arm B) or not (arm A) dexamethasone-vincristine pulses, with mercaptopurine and methotrexate. Furthermore, all patients received intrathecal chemotherapy with methotrexate, cytarabine and methylprednisolone, but not cranial irradiation.

Oral glucose tolerance tests were performed during three follow-up visits, separated by at least 2 years, with serial measurements of plasma glucose and insulin concentrations. Impaired glucose tolerance and diabetes were defined according to WHO criteria.[3]

β-cell function was ascertained by homeostatic model assessment (HOMA β-cell function=[fasting insulin x20]/[fasting glucose-3.5]) and by the insulinogenic index, which was calculated as the ratio of the increment in plasma insulin concentration to that in glucose during the first 30 minutes after ingestion of glucose. Blood samples were also obtained for assessment of glycosylated haemoglobin (HbA1c), C-peptide, insulin autoantibodies and islet cell antibodies. Plasma glucose concentration was measured with a glucose analyzer (YSI, Yellow Springs, OH, USA). Plasma insulin and C-peptide amount were measured by radioimmunoassay. Intra-assay and inter-assay variations were 5% and 7.1% respectively, for insulin, and 3.4% and 1.9% respectively, for C-peptide. In our laboratory, the normal serum insulin level is 4.3-20 μUI/mL and the normal serum C-peptide level is 0.8-4 ng/mL.

HbA1c was measured with high-performance liquid chromatography (HPLC), which separates HbA1c by charge and size from other HbA components in blood. In healthy patients, the HbA1c level is less than 7% of the total hemoglobin. Sera were tested for insulin autoantibodies with a radiobinding assay and for pancreatic islet cell antibodies by indirect immunofluorescence.

We analyzed data with SPSS version 10.0 (SPSS, Chicago, IL, USA). For the analysis, we divided the study population into three groups according to the follow-up visits. Differences in variables between groups were analyzed by one-way ANOVA with Tukey's test for post-hoc comparison of means.

Of 41 children diagnosed in the study period, 32 were elegible for inclusion in the study (18 males and 14 females; mean age 10.5 years [SD 4.1]) (Table 1).

None of the children had insulin autoantibodies or islet cells antibodies at any time. No significant alterations were recorded in HbA1c (5.33±0.42% vs 5.31±0.35% vs 5.05±0.36%) and C-peptide (1.97 ng/mL±1.07 vs 1.34 ng/mL±0.6 vs 1.45 ng/mL±0.67).

The oral glucose tolerance tests performed during the three follow-up visits demonstrated a progressive reduction of the incidence of impaired glucose tolerance (IGT). In fact, in the first evaluation ten children (31.2%) had IGT, while in the second and third evaluations only nine (28.1%) and four (12.5%) children, respectively, had IGT. This was associated with an improvement in β-cell function (Table 2): between follow-up 2 and 3 there was a significant increase in fasting insulin level (p=0.001), and improvements in insulinogenic index (p=0.006) and HOMA β-cell function (p=0.001). No significant difference was found in these indices between follow-up 1 and 2. We also divided the study population according to the time they had been off

Table 1. Anthropometric parameters.

	Follow-up 1	*Follow-up 2*	*Follow-up 3*
Male/Female	18/14	18/14	18/14
Median age (years)	10.06 (4.19)	12.24 (4.29)	15.25 (4.6)
Age range (years)	4.6-19.6	6.6-21.7	8.4-22.8
BMI (median; Kg/m^2)	19.12 (4.4)	20.26 (4.3)	21.96 (4.5)
BMI percentile			
<3rd	1	1	2
3rd-50th	12	9	8
50th-85th	11	12	11
85th-95th	8	10	11
>95th	0	0	0
Weight (Kg)	46.8 (17.79)	49.17 (18.89)	55.71 (19.12)
Height (cm)	145.6 (15.2)	151.73 (16.4)	157.02 (14.16)
Pubertal status			
Tanner 1	20	8	5
Tanner 2	3	11	3
Tanner 3	2	4	10
Tanner 4	2	2	4
Tanner 5	5	7	10

BMI: body mass index.

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Table 2. Indices of glucose metabolism.

	Follow-up 1	*Follow-up 2*	*Follow-up 3*	*p**
Clinical data				
Off therapy (years [SD])	3.6 (2.16)	5.34 (2.22)	6.47 (2.15)	0.001
BMI (median; kg/m^2)	19.12 (4.4)	20.26 (4.3)	21.96 (4.5)	0.05
Metabolic variables				
HbA1c (% [SD])	5.34 (0.4)	5.31 (0.35)	5.21 (0.3)	0.39
C-Peptide (nmol/L[SD])	1.75 (2.59)	1.60 (0.68)	0.89 (0.49)	0.25
Glycemia 120' (mg/dL [SD])	130.62 (27.13)	139.48 (30.75)	105.96 (27.02)	0.001
Indices of β-cell function				
Fasting insulin (mU/L [SD])	8.64 (3.46)	10.56 (5.42)	17.99 (9.5)	0.001
Insulinogenic index (SD)	0.51 (0.36)	0.61 (0.54)	3.89 (7.79)	0.006
HOMA-β cell function (SD)	102.35 (67.38)	104.36 (53.09)	238.35 (146.08)	0.001

*Data are mean (SD). *One-way ANOVA; BMI: body mass index.*

therapy and evaluated the percentage of children with IGT. In the second year off therapy 75% (3 of 4 children) had IGT; in the third and fourth years 33.3% (6 of 18 children), in the fifth year 30% (4 of 13 children), in the sixth 26.6% (4 of 15 children), in the seventh 25% (2 of 8 children), in the eighth 22.2% (2 of 9 children), until in the ninth year off therapy IGT was found in 16.6% (1 of 6 children). Treatment for ALL is associated with IGT, which persists even after therapy has been stopped. These alterations are mainly related to impaired β-cell function. We have previously shown that children treated for ALL have reduced fasting insulin values, a low insulinogenic index and low HOMA β-cell function.[4] However, these alterations seemed to be gradually reversible as they were directly correlated to time off therapy. In fact, in the first study, patients with the shortest period off therapy had the highest incidence of IGT. In the present study we evaluated the incidence of IGT in children up to 9 years after completion of treatment for ALL. We found that the prevalence of IGT decreased with time after stopping therapy, suggesting recovery of β-cell function. This was also nicely demonstrated by the drastic improvement of fasting insulin values, insulinogenic index and HOMA β-cell function over time. In particular, our data showed a sharp improvement of these indices between follow-up 2 and 3, suggesting that β-cell function returns gradually and spontaneously to normal several years after discontinuation of therapy.

The processes underlying impaired β-cell function and its subsequent recovery remain speculative. However, none of our patients had any of the well known risk factors for IGT (obesity, chronic disease) and almost all patients were prepubertal at visit 1 and progressed through puberty during the subsequent follow-ups. This reduces the probability that anthropometric factors might have been implicated in the β-cell dysfunction. Some authors have suggested that leukemic infiltration leukostasis or a direct cytotoxic effect of chemotherapy might be involved. In particular, leukostasis of the pancreatic vessels might interfere with the blood supply to islets resulting in islet ischemia and, together with pancreatic leukemic infiltration, β-cell dysfunction.[5] Moreover, chemotherapy agents, such as L-asparaginase, can interfere with pancreatic function, decreasing serum insulin levels. This decrease may be due to destruction of the insulin molecule, to a reduction in insulin secretion or to interference with insulin synthesis without, however, killing the β-cells.[6,7] The effects of L-asparaginase are, however, short-lasting, leading to a reduction of protein synthesis for a few days. It might be expected that following antileukemic therapy, the effects of leukemic infiltration and chemotherapy would disappear and leukostasis would improve, possibly leading to a recovery pancreatic function. However, this process is surprising slow as we have seen a significant persistence of IGT during the first 5 years off therapy. Further research is, therefore, required to gain a better understanding of the etiology of β-cells dysfunction following treatment for ALL.

In conclusion, our results show that children treated for ALL are at risk of alterations of glucose metabolism immediately after stopping therapy, although β-cell function appears to recover slowly and continuously over time.

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