npg

EDITORIAL

Development of a regional flow cytometry center for diagnosis of childhood leukemia in Central America

SC Howard[1,2,3], D Campana[1,2,3,7], E Coustan-Smith[1], FG Antillon[3,4], M Bonilla[3,5], L Fu[3,6], C-H Pui[1,2,3,7], RC Ribeiro[1,2,3], JA Wilimas[1,2,3] and R Lorenzana[8]

[1]Department of Hematology-Oncology, St Jude Children's Research Hospital, Memphis, TN, USA; [2]Department of Pediatrics, University of Tennessee College of Medicine, Memphis, TN, USA; [3]International Outreach Program, St Jude Children's Research Hospital, Memphis, TN, USA; [4]Unidad Nacional de Oncología Pediátrica, Guatemala City, Guatemala; [5]Hospital Benjamin Bloom, San Salvador, El Salvador; [6]Hospital Escuela, Tegucigalpa, Honduras; [7]Department of Pathology, St Jude Children's Research Hospital, Memphis, TN, USA; and [8]Laboratorio de Patología y Citometría de Flujo, Guatemala City, Guatemala

Assessment of immunophenotype and DNA content of leukemia is important for diagnosis and risk assessment, but requires costly equipment, reagents and technical expertise. Samples from 1118 children in Central America were sent for flow cytometric examination to a reference laboratory in Guatemala City. Consultation with St Jude Children's Research Hospital facilitated quality control. Acute leukemia was diagnosed in 908 cases. Consultation was required in 20% of cases during year 2 and only 3% during year 5. Cost per sample was $240 vs $720 if performed at St Jude Children's Research Hospital. Immunophenotyping in resource-poor areas proved feasible and cost effective.

Immunophenotyping of leukemias is necessary for diagnosis and risk-directed therapy.[1] Otherwise, misdiagnosis (eg confusion of acute lymphoblastic leukemia (ALL) with acute myeloid leukemia (AML)) may occur in as many as 10% of cases.[2–5] Flow cytometry is widely used for immunophenotyping because it allows quantification of antigen expression, rapid analysis of large numbers of cells, and determination of DNA content,[6,7] an important prognostic indicator.[8,9] One-fourth of children with B-lineage ALL have a leukemic cell DNA index (ratio of DNA content to that of normal diploid cells) $\geqslant 1.16$, a favorable prognostic factor allowing use of less intensive chemotherapy.

Immunophenotyping is often unavailable in countries with limited resources. The resulting diagnostic uncertainty and inability to stratify precisely patients based on relapse risk prevents the use of risk-directed therapy and compromises outcomes. Several pediatric oncology programs in Central America previously shipped diagnostic specimens to St Jude Children's Research Hospital (SJCRH) for flow cytometric analysis. However, shipping and processing of samples were expensive, delayed diagnosis, and adversely affected the sample quality. To solve these problems, the SJCRH International Outreach Program collaborated with a pathologist (RL) to establish a flow cytometry laboratory in Guatemala City to serve cancer centers in Guatemala, El Salvador, and Honduras. We analyzed data from the first 1148 samples to evaluate feasibility and cost of referral of specimens to a regional center for immunophenotyping of childhood leukemia.

Correspondence: Dr SC Howard, St Jude Children's Research Hospital, 332 North Lauderdale St., Memphis, TN 38105-2794, USA;
Fax: +1 901 495 5319; E-mail: scott.howard@stjude.org

From April 30, 1998 to December 31, 2002, specimens from pediatric patients with suspected leukemia at the Hospital Escuela (Tegucigalpa, Honduras), the Hospital Benjamin Bloom (San Salvador, El Salvador), and the Unidad Nacional de Oncología Pediátrica (Guatemala City, Guatemala) were analyzed by the Pathology and Flow Cytometry Laboratory in Guatemala City. Specimens from El Salvador and Honduras were shipped by overnight express and processed on the day of arrival.

Flow cytometric analysis was performed by standard methods using commercially available antibodies.[3] Sample processing, antibody staining, and flow cytometric analysis were performed by a pathologist (RL) who had attended Becton Dickinson's standard training course plus an additional week of training at SJCRH in Memphis, TN, USA. Web-based consultation with SJCRH, as described previously, facilitated quality control.[10]

A total of 1148 diagnostic specimens (1089 bone marrow, 59 blood) from 1118 patients were analyzed. Of these, 30 were recollected to replace specimens that could not be evaluated because of nonviable cells due to delayed shipping or processing ($n = 11$), bacterial contamination ($n = 3$), dilution of bone marrow with blood ($n = 6$), or inconclusive findings ($n = 10$). In these cases, a diagnosis was subsequently made from the recollected sample. All but 11 specimens arrived within 72 h of sampling; results were returned by facsimile or e-mail within 48 h.

Of the 1118 evaluable samples, 908 indicated acute leukemia: 650 B-lineage ALL (positive for CD19, CD22, and/or CD79a), 62 T-lineage ALL (positive for CD7 and cytoplasmic CD3), three mixed-lineage ALL (positive for both B- and T-cell markers), 182 AML (positive for CD13, CD33, and/or myeloperoxidase, and negative for lymphoid-associated markers), and 11 unclassifiable (cells expressed lymphoid and myeloid markers or showed no conclusive lineage differentiation). A diagnosis of acute leukemia was ruled out for 210 patients, 11 of whom were subsequently found to have other cancers: chronic myeloid leukemia (8), non-Hodgkin's lymphoma (2), and myelodysplastic syndrome (1).

During year 2 of the program, an on-line system was developed that allowed direct transfer of raw flow cytometry data from the cytometer in Guatemala City to the cytometer at SJCRH. This innovation allowed consultation to clarify the diagnosis or the technical adequacy of results. Consultation was requested in 20% of cases during year 2, but in only 3% during year 5 (Table 1). Consultation led to a change in or refinement of the diagnosis in 4% of all cases in year 2 compared to only 1% in year 5.

Editorial

Table 1 Requests for on-line flow cytometry consultation and the outcome of consultation by year

Year	Total no. of cases evaluated	No. (% of total evaluated) of cases for which on-line consultation was requested	P-value for comparison with the reference group	No. (% of total evaluated) of cases for which diagnosis was refined or altered	P-value for comparison with the reference group
April 30, 1998 to May 15, 1999 (on-line consultation not available)	141	a		a	
May 16, 1999 to December 31, 1999	103	21 (20%)[b]	Reference group	4 (4%)	Reference group
2000	302	14 (5%)	<0.0001	6 (2%)	0.28
2001	332	18 (5%)[b]	<0.0001	7 (2%)	0.30
2002	240	8 (3%)	<0.0001	2 (1%)	0.07

[a]Before on-line exchange of raw data between flow cytometers was possible, data were transferred in 43 cases by faxing or mailing printed results. In these cases, definitive confirmation or modification of the original diagnosis was not possible.
[b]Two of the 21 cases in 1999 and one of the cases in 2001 were considered not evaluable after consultation.

The average cost of analysis per sample, including shipping, antibodies, technician salaries, professional fees, and overhead, was $240 as compared to $720 if the samples were shipped to SJCRH. This difference reflects the lower cost of shipping regionally and the lower salaries paid in Guatemala.

Accurate diagnosis and risk assessment are essential for optimal treatment of childhood leukemia. Immunophenotype and DNA content provide important diagnostic and prognostic information. Risk stratification also ensures cost-effective use of treatment resources.[11] While it is impractical to establish a diagnostic laboratory in each pediatric oncology treatment facility due to the limited number of patients, we developed a regional reference laboratory that provides high-quality testing at relatively low cost. Centralization of testing reduced costs of shipping and laboratory overhead. Ongoing training and quality assurance were accomplished by our innovative use of a web-based system for rapid exchange of flow cytometric data between centers, which proved highly effective.[10] Importantly, in the fifth year of the program, after analysis of hundreds of cases, the reference laboratory rarely required consultation with SJCRH (3% of cases) and the consultations refined or altered the diagnosis in only 1% of all cases (two of eight consulted cases). We consider this program to be a paradigm of technology transfer from developed countries to those with limited resources in a way that builds local capacity and fosters independence – characteristics essential for sustainable development.[12–16] We have previously suggested that low-cost infection control programs, improved supportive care, and prevention of abandonment of therapy are important factors for improving outcomes of children with ALL in countries with limited resources.[17,18] As correct diagnosis and risk classification are essential for appropriate and cost-effective treatment of children with acute leukemia, we would add flow cytometry to this list.

References

1 Pui CH, Evans WE. Acute lymphoblastic leukemia. *N Engl J Med* 1998; **339**: 605–615.
2 Browman GP, Neame PB, Soamboonsrup P. The contribution of cytochemistry and immunophenotyping to the reproducibility of the FAB classification in acute leukemia. *Blood* 1986; **68**: 900–905.
3 Krause JR, Penchansky L, Contis L, Kaplan SS. Flow cytometry in the diagnosis of acute leukemia. *Am J Clin Pathol* 1988; **89**: 341–346.
4 Neame PB, Soamboonsrup P, Browman GP, Meyer RM, Benger A, Wilson WE *et al.* Classifying acute leukemia by immunophenotyping: a combined FAB-immunologic classification of AML. *Blood* 1986; **68**: 1355–1362.
5 Schumacher HR, Shrit MA, Kowal-Vern A, Dizikes G, Radvany RM, Le Beau MM. Acute leukemia and related entities. Impact of new technology. *Arch Pathol Lab Med* 1991; **115**: 331–337.
6 Basso G, Buldini B, De Zen L, Orfao A. New methodologic approaches for immunophenotyping acute leukemias. *Haematologica* 2001; **86**: 675–692.
7 Behm FG, Campana D. Immunophenotyping. In: Pui C-H (ed) *Childhood Leukemias*. New York: Cambridge University Press, 1999, pp 111–144.
8 Look AT, Melvin SL, Williams DL, Brodeur GM, Dahl GV, Kalwinsky DK *et al.* Aneuploidy and percentage of S-phase cells determined by flow cytometry correlate with cell phenotype in childhood acute leukemia. *Blood* 1982; **60**: 959–967.
9 Trueworthy R, Shuster J, Look T, Crist W, Borowitz M, Carroll A *et al.* Ploidy of lymphoblasts is the strongest predictor of treatment outcome in B-progenitor cell acute lymphoblastic leukemia of childhood: a Pediatric Oncology Group Study. *J Clin Oncol* 1992; **10**: 606–613.
10 Lorenzana R, Coustan-Smith E, Antillon F, Ribeiro RC, Campana D. Simple methods for the rapid exchange of flow cytometric data between remote centers. *Leukemia* 2000; **14**: 336–337.
11 Bonilla M, Moreno N, Marina N, deReyes G, Shurtleff SA, Downing JR *et al.* Acute lymphoblastic leukemia in a developing country: preliminary results of a nonrandomized clinical trial in El Salvador. *J Pediatr Hematol Oncol* 2000; **22**: 495–501.
12 Eden T. Translation of cure for acute lymphoblastic leukaemia to all children. *Br J Haematol* 2002; **118**: 945–951.
13 Harris E, Tanner M. Health technology transfer. *BMJ* 2000; **321**: 817–820.
14 Masera G, Biondi A. Research in low-income countries. *Ann Oncol* 1999; **10**: 137–138.

15 Naafs-Wilstra M, Barr R, Greenberg C, Magrath I, Cardenas F, Chesler M *et al.* Pediatric oncology in developing countries: development of an alliance of stakeholders. *Med Pediatr Oncol* 2001; **36**: 305–309.

16 Sitthi-Amorn C, Somrongthong R. Strengthening health research capacity in developing countries: a critical element for achieving health equity. *BMJ* 2000; **321**: 813–817.

17 Metzger ML, Howard SC, Fu LC, Pena A, Stefan R, Hancock ML *et al.* Outcome of childhood acute lymphoblastic leukaemia in resource-poor countries. *Lancet* 2003; **362**: 706–708.

18 Howard SC, Pedrosa M, Lins M, Pedrosa A, Pui CH, Ribeiro RC *et al.* Establishment of a pediatric oncology program and outcomes of childhood acute lymphoblastic leukemia in a resource-poor area. *JAMA* 2004; **291**: 2471–2475.