Central review of cytogenetics is necessary for cooperative group correlative and clinical studies of adult acute leukemia: The Cancer and Leukemia Group B experience

KRZYSZTOF MRÓZEK1, ANDREW J. CARROLL2, KATI MAHARRY1,3, KATHLEEN W. RAO4, SHIVANAND R. PATIL5, MARK J. PETTENATI6, MICHAEL S. WATSON7, DIANE C. ARTHUR8, RAMANA TANTRAVALI9, NYLA A. HEEREMA10, PRASAD R.K. KODURU11, ANNEMARIE W. BLOCK12, MAZIN B. QUUMSIYEH13, COLIN G. EDWARDS1, LISA J. STERLING1, KELSI B. HOLLAND1,3 and CLARA D. BLOOMFIELD1

1Division of Hematology and Oncology, Department of Internal Medicine, Comprehensive Cancer Center, The Ohio State University, Columbus, OH; 2University of Alabama at Birmingham, Birmingham, AL; 3The CALGB Statistical Center, Duke University Medical Center, Durham; 4University of North Carolina at Chapel Hill, Chapel Hill, NC; 5University of Iowa, Iowa City, IA; 6Wake Forest University Medical Center, Winston Salem, NC; 7American College of Medical Genetics, Bethesda, MD; 8National Cancer Institute, Bethesda, MD; 9Dana-Farber Cancer Institute, Boston, MA; 10Department of Pathology, Comprehensive Cancer Center, The Ohio State University, Columbus, OH; 11North Shore University Hospital, Manhasset, NY; 12Roswell Park Cancer Institute, Buffalo, NY; 13Duke University Medical Center, Durham, NC, USA

Received March 21, 2008; Accepted April 18, 2008

DOI: 10.3892/ijo_00000002

Abstract. The Cancer and Leukemia Group B has performed central review of karyotypes submitted by institutional cytogenetics laboratories from patients with acute myeloid (AML) and acute lymphoblastic (ALL) leukemia since 1986. We assessed the role of central karyotype review in maintaining accurate, high quality cytogenetic data for clinical and translational studies using two criteria: the proportion of karyotypes rejected (i.e. inadequate), and, among accepted (i.e. adequate) cases, the proportion of karyotypes whose interpretation was changed on central karyotype review. We compared the first four years during which central karyotype review was performed with a recent 4-year period and found that the proportion of rejected samples decreased significantly for both AML and ALL. However, during the latter period, central karyotype review was performed with a recent 4-year period and found that the proportion of rejected samples decreased significantly for both AML and ALL. Some revisions resulted in changing the patients' assignment to particular World Health Organization diagnostic categories and/or moving patients from one prognostic group to another. Overall, when both data on rejection rates and data on karyotype revisions made in accepted cases were considered together, 32% of AML and 38% of ALL samples submitted were either rejected or revised on central karyotype review during the recent 4-year period. These data underscore the necessity of continued central karyotype review in multi-institutional cooperative group studies.

Introduction

Cytogenetic findings have become an integral part of diagnosis, prognostication and therapeutic stratification of acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Such recurrent chromosome abnormalities and their molecular equivalents as t(8;21)(q22;q22)/RUNX1-RUNX1T1, inv(16)(p13q22)/t(16;16)(p13;q22)/CBFB-MYH11, t(15;17)(q22;q12-21)/PML-RARA, and balanced abnormalities involving band 11q23 and the MLL gene are now included in the World Health Organization (WHO) classification of AML, and together with morphology, immunophenotype and clinical features are being used to define distinct disease entities (1). Notably, the role of cytogenetics will be substantially increased in the 2008 revision of the WHO classification (2).

Moreover, pretreatment cytogenetic findings constitute one of the most important prognostic factors in both AML (3-10) and ALL (7,11-13), are used to determine post-remission therapy (14-17), and are important for molecular genetic studies investigating mutations (18-29) and changes
in expression of specific genes (30-32), as well as gene- (33-37) and microRNA-expression (38,39) profiles in patients with acute leukemias. Importantly, the National Comprehensive Cancer Network (NCCN) Clinical Practice Guidelines in Oncology specify that cytogenetic analysis is an obligatory part of the diagnostic workup in AML (40). Likewise, cytogenetic analysis is one of the minimum laboratory requirements for the diagnosis of AML according to the ‘Guidelines on the Management of Acute Myeloid Leukaemia in Adults’ issued by the British Committee for Standards in Haematology (41). Consequently, the Cancer and Leukaemia Group B (CALGB) now mandates cytogenetic analyses for adult AML and ALL.

For each specimen, two karyotypes and metaphase spreads were submitted with the data on processing and processing. For revisions made by central karyotype review in the submitted karyotypes that were accepted or borderline accepted during eight recent central karyotype review sessions. The reasons for revision were divided into the following categories: i) major errors in karyotype interpretation, such as failure of the submitting laboratory to recognize a clonal abnormality, identification of an abnormality found on central karyotype review not to be present, and incorrect interpretation of an abnormality; ii) the need for refinement of breakpoint assignment in structural abnormalities properly recognized by the submitting laboratory, iii) misidentified or upside down chromosomes, and iv) incorrect use of the ISCN (1995) nomenclature (47). In this study, we excluded samples analyzed cytogenetically during complete remission, because these samples differ from pretreatment and relapse samples in that they rarely contain leukemic cells and are usually karyotypically normal (48). The rejection rates between the first and the recent 4-year periods (Table I) have been compared using the Fisher’s exact test. All analyses were performed by the CALGB Statistical Center.

Results and Discussion

Table I shows rejection rates and reasons for rejection for AML and ALL specimens that underwent central karyotype review during the entire period between 1986 and 2006. Overall, 12% of 4991 AML and 23% of 1177 ALL karyotypes submitted were rejected. The most common reason for rejection was inadequate banding quality, which accounted for rejection of 53% (325 of 612) of AML and 46% (123 of 270) of ALL inadequate cases. When we compared rejection rates between the first four years during which central karyotype review was performed and the recent 4-year period, we found a significant improvement in the quality of submitted specimens in both AML (P<0.0001) and ALL (P<0.001). This was mainly due to the significant decrease in the numbers of karyotypes with inadequate banding quality (AML, P<0.0001 and ALL, P=0.02). Nevertheless, the recent central karyotype reviews still found 8% of AML and 16% of ALL samples inadequate.

Another indication of the quality improvement over time was an increase in the proportion of abnormal karyotypes pretreatment, from 52% in the first 4 years to 57% in the recent 4 years (P=0.05) in AML and from 59 to 75% in ALL.
These improvements in quality can be attributed to many factors, including progress in cell culturing, harvesting and banding techniques, as well as increase in proficiency in analyzing specimens from patients with acute leukemias. The latter has been achieved in part through feedback provided to cytogeneticists from the submitting laboratories after central karyotype review sessions and through educational workshops organized and conducted by the members of the Karyotype Review Committee during CALGB Group Meetings.

Among karyotypes deemed to have adequate banding at 8 recent Central Karyotype Review sessions, changes in karyotype interpretation were made in 26% of both AML and ALL cases (Table II). The revisions included identification of an unrecognized chromosome abnormality or reinterpretation of an abnormality incorrectly interpreted by the submitting laboratory (44% of all revisions), reassignment of breakpoints in structural aberrations (22%), correction of misidentified or upside-down chromosomes (22%), and correction of errors in the ISCN (1995) nomenclature (12%).

(P=0.007). These improvements in quality can be attributed to many factors, including progress in cell culturing, harvesting and banding techniques, as well as increase in proficiency in analyzing specimens from patients with acute leukemias. The latter has been achieved in part through feedback provided to cytogeneticists from the submitting laboratories after central karyotype review sessions and through educational workshops organized and conducted by the members of the Karyotype Review Committee during CALGB Group Meetings.

Among karyotypes deemed to have adequate banding at 8 recent Central Karyotype Review sessions, changes in karyotype interpretation were made in 26% of both AML and ALL cases (Table II). The revisions included identification of an unrecognized chromosome abnormality or reinterpretation of an abnormality incorrectly interpreted by the submitting laboratory (44% of all revisions), reassignment of breakpoints in structural aberrations (22%), correction of misidentified or upside-down chromosomes (22%), and correction of errors in the ISCN (1995) nomenclature (12%).

In several instances, major changes in karyotype interpretation made on central karyotype review resulted in moving patients from one diagnostic category in the current
WHO classification (1) to another. For example, AML patients whose karyotypes submitted as normal were revised to abnormal ones that harbored t(9;11)(p22;q23), t(11;19)(q23;p13.1) or inv(16)(p13q22), as well as patients whose submitted karyotype was changed from 46,X,Y,+8 to 46,X,Y,+8(16)(p22;q23), from 46,XX,t(10;11)(p14;q13) to 46,XX,ins(10;11)(p12;q23), from 46,XY,del(5) q(13q33),del(11)(q23) to 46,XY,del(5)(q13q33),del(6)(11) q(27q23), and from 46,XY,del(3)(p13)add(11)(q23) to 46, XY,del(3)(p13)add(11)(q23) and inv(16)(p13q22), and from 46,XY,t(3;11)(p13q23),add(18)(q21)-21,mar to 46,XY,t(3;11)(p13q23),add(18)(q21)-21 would be reclassified from having ‘AML not otherwise categorized’ to ‘AML with recurrent cytogenetic abnormalities’. Conversely, the patient whose submitted complex karyotype included an inv(16)(p13q22) that was revised on central karyotype review to der(16)add(16)(p13)del(16)(q22) would be reclassified from having ‘AML with recurrent cytogenetic abnormalities’ to ‘AML not otherwise categorized’. There would be multiple additional changes in diagnostic categories once the new 2008 edition of the WHO classification (2) is published, including, for example, a reclassification from ‘AML with myelodysplasia related changes’ to ‘AML with recurrent genetic abnormalities’ for the patient whose karyotype submitted as normal was found on central karyotype review to contain an inv(3)(q13q26).

Many of both the aforementioned and other karyotype revisions made on central karyotype review resulted in changes in prognostic group assignment of the patients. For instance, revisions from a normal karyotype to a karyotype containing inv(3)(q21q26) or t(11;19)(q23;p13.1), or from del(11)(q23) to t(6;11)(q27q23) meant that these patients were no longer classified as having an intermediate risk but were included in the adverse risk category instead (5). Poor prognosis associated with an isolated trisomy of chromosome 13 (49) was no longer predicted in a patient whose karyotype was revised from 47,XY,+13 to 47,XY,+15 when the reviewers discovered that an extra chromosome submitted as chromosome 13 was in fact an extra chromosome 15 placed upside-down in the karyotype of borderline quality. To date, prognostic significance of isolated trisomy 15 is unclear and it has been suggested to represent a benign, age-related abnormality in older male patients (50,51). Moreover, identification of an initially unrecognized inv(16) alters the patient’s risk assignment from intermediate to favorable, whereas revision of the submitted inv(16) to der(16)add(16)(p13)del(16)(q22) as part of a complex karyotype means that the patient’s risk-group assignment is no longer favorable but becomes adverse. Importantly, documentation of the presence or absence of inv(16) is of major clinical importance (52) because the postremission therapy of AML patients with inv(16) on CALGB protocols is administered in a risk-adapted fashion and includes three courses of high-dose cytarabine, whereas patients without inv(16) or (8;21) receive autologous peripheral stem-cell transplantation (15).

Finally, we evaluated both the data on karyotype revisions made in the accepted cases and data on rejection rates during the recent 4-year period. Overall, 32% of AML and 38% of ALL samples submitted were either rejected or revised on central karyotype review.

While several studies have addressed the issue of proficiency testing and quality control in clinical cytogenetics (53-55), to our knowledge only one other study (from the Children’s Oncology Group), published in abstract form, has reported the cooperative group experience concerning central review of karyotypes from acute leukemia patients (Heerema, et al., Cyto.genet Genome Res 106: 136, 2004). Results of this study are remarkably similar to ours, showing that the karyotype was either changed or rejected in 31% of AML and 49% of ALL pediatric cases submitted. Therefore, we conclude that central karyotype review plays a vital role in ensuring the validity of the clinical trials and correlative studies conducted by both adult and children’s cooperative groups.

Acknowledgments

This study was supported in part by National Cancer Institute, Bethesda, MD grants CA77658, CA101140, CA31946, CA16058, CA47545, CA47559, CA47642, CA77440, CA16450, CA32291, CA35279, CA47757 and CA02599, and The Coleman Leukemia Research Foundation.

References


Appendix

The following Cancer and Leukemia Group B institutions, principal investigators, and cytogeneticists participated in this study: Wake Forest University School of Medicine, Winston-Salem, NC: David H. Durd, Harold O. Goodman, P. Nagesh Rao, Wendy L. Flejter and Mark J. Pettenati (grant no. CA03927); North Shore University Hospital, Manhasset, NY: Daniel R. Budman, Natalie B. Kardon and Prasad R.K. Koduru (grant no. CA35279); Roswell Park Cancer Institute, Buffalo, NY: Ellis G. Levine, Avery A. Sandberg and AnneMarie W. Block (grant no. CA02599); The Ohio State University Medical Center, Columbus, OH: Clara D. Bloomfield, Karl S. Theil, Diane Minka and Nyla A. Heerema (grant no. CA77658); Dana-Farber Cancer Institute, Boston, MA: Eric P. Winer, Ramana Tantravahi, Cynthia C. Morton, Leonard L. Atkins and Paola Dal Cin (grant no. CA32291); Duke University Medical Center, Durham, NC: Jeffrey Crawford, Sandra H. Bigner, Mavin B. Qumsiyeh and Barbara K. Goodman (grant no. CA47577); Weill Medical College of Cornell University, New York, NY: John Leonard, Ram S. Verma’ Prasad R.K. Koduru, Andrew J. Carroll and Susan Mathew (grant no. CA07968); University of Maryland Cancer Center, Baltimore, MD: Martin Edelman, Joseph R. Testa, Deana Hallman, Stuart Schwartz, Maimon M. Cohen, Judith Stumberg and Yi Ning (grant no. CA31983); University of Iowa Hospitals, Iowa City, IA: Gerald H. Clamon and Shivanand R. Patil (grant no. CA74642); University of Chicago Medical Center, Chicago, IL: Gini Fleming, Diane Roulston, Katrin M. Carlson, Yanming Zhang and Michelle M. Le Beau (grant no. CA14287); University of North Carolina, Chapel Hill, NC: Thomas Shea and Kathleen W. Rao (grant no. CA47559); Washington University School of Medicine, St. Louis, MO: Nancy L. Bartlett, Christine G. Janney, Eric C. Crawford, Jaime Garcia-Heras and Michael S. Watson (grant no. CA77440); Dartmouth Medical School, Lebanon, NH: Marc S. Ernstoff, Doris H. Wurster-Hill’ and Thuluvancheri K. Mohandas (grant no. CA04326); Rhode Island Hospital, Providence, RI: William Sikov, Teresita Padre-Mendoza, Jennifer A. Ahearn, Philip L. Townes, Hon Fong L. Mark, Shelly L. Kerman and Aurelia Meloni-Ehrig (grant no. CA08025); University of Alabama at Birmingham: Robert Diasio and Andrew J. Carroll (grant no. CA47545); SUNY Upstate Medical University, Syracuse, NY: Stephen L. Graziano, Navniti S. Mitter, Edward J. Hallinan, Lawrence P. Gordon and Constance K. Stein (grant no. CA21060); Christiana Care Health Services, Inc., Newark, DE: Stephen S. Grubbs, Digamber S. Borgaonkar, Jeanne M. Meck and Kathleen Richkind (grant no. CA45418); University of Missouri/Ellis Fischel Cancer Center, Columbia, MO: Michael C. Perry, Judith H. Miles, Jeffrey R. Sawyer, Tim H. Huang and Linda M. Pasztor (grant no. CA12046); Long Island Jewish Medical Center CCOP, Lake Success, NY: Kanti R. Rai, Alan L. Shanske and Prasad R.K. Koduru (grant no. CA11028); Minneapolis VA Medical Center, Minneapolis, MN: Vicki A. Morrison and Sugandhi A. Tharapel (grant no. CA47555); University of California, San Diego, CA: Barbara A. Parker, Oliver W. Jones, E. Robert Wassman, Renée Bernstein’ and Marie L. Dell’Aquila (grant no. CA11789); Walter Reed Army Medical Center, Washington, DC: Thomas Reid, Rawatmal B. Surana, Syed K. Rafi, Doris H. Wurster-Hill’, Digamber S. Borgaonkar, Karl S. Theil, Diane Minka, Nyla A. Heerema and Kathleen E. Richkind (grant no. CA26806); Parkview Hospital, Ft. Wayne, IN: Sreenivasa Nattam and Patricia I. Bader; University of Minnesota, Minneapolis, MN: Bruce A. Peterson, Betsy A. Hirsch and Diane C. Arthur (grant no. CA16450); Mount Sinai School of Medicine, New York, NY: Lewis R. Silverman and Vesna Najfeld (grant no. CA04457); University of Massachusetts Medical School, Worcester, MA: William W. Walsh, Philip L. Townes, Vikram Jaswaney Kathleen E. Richkind, Michael J. Mitchell and Patricia Miron (grant no. CA37135); Massachusetts General Hospital, Boston, MA: Jeffrey W. Clark, Cynthia C. Morton, Paola Dal Cin and Leonard L. Atkins (grant no. CA12449); Eastern Maine Medical Center, Bangor, ME: Harvey M. Segal and Laurent J. Beaurgard (grant no. CA35406); University of Vermont, Burlington, VT: Hyman B. Muss, Elizabeth F. Allen and Mary Tang (grant no. CA77406); Western Pennsylvania Hospital, Pittsburgh, PA: Richard K. Shaddock and Gerard R. Diggans; University of Illinois, Chicago, IL: David J. Peace, Maureen M. McCorquodale, Kathleen E. Richkind and Valerie Lindgren (grant no. CA74811); Medical College of Virginia/Virginia Commonwealth University, Richmond, VA: John D. Roberts and Colleen Jackson-Cook (grant no. CA52784); Columbia-Presbyterian Medical Center, New York, NY: Rose R. Ellison and Dorothy Warburton (grant no. CA12011); Finsen Institute, Copenhagen, Denmark: Nis I. Nissen and Preben Philip; McGill Department of Oncology, Montreal, Quebec: J.L. Hutchison and Jacqueline Emond (grant no. CA31809); University of Puerto Rico School of Medicine, San Juan, PR: Eileen I. Pacheco, Ramana Tantravahi, Cynthia C. Morton, Paola Dal Cin and Leonard L. Atkins; Georgetown University Medical Center, Washington, DC: Minnetta C. Liu and Jeanne M. Meck (grant no. CA77597); SUNY Maimonides Medical Center, Brooklyn, NY: Sameer Rafia and Ram S. Verma’ (grant no. CA25119); University of Nebraska Medical Center, Omaha, NE: Anne Kessinger and Warren G. Sanger (grant no. CA77298); Medical University of South Carolina, Charleston, SC: Mark R. Green, Eduardo S. Cantu, G. Shashidhar Pai and Daynna J. Wolff (grant no. CA03927); University of California, San Francisco, CA: Charles J. Ryan, Athena M. Cherry and Kathleen E. Richkind (grant no. CA60138); Southern Nevada Cancer Research Foundation CCOP, Las Vegas, NV: John Ellerton, Oliver W. Jones, E. Robert Wassman, Renée Bernstein’ and Marie L. Dell’Aquila (grant no. CA35421); University of Cincinnati Medical Center, Cincinnati, OH: Orlando J. Martelo and Ashok K. Srivastava (grant no. CA47515); Kansas City CCOP, Kansas City, MO: Rakesh Gaur and Linda Cooley; South East Cancer Control Consortium, Winston-Salem, NC: James N. Atkins and Mark J. Pettenati (grant no. CA45808). ‘Deceased.