Clinicopathologic Analysis of Acute Myeloid Leukemia in a Single Institution: Biphenotypic Acute Myeloid Leukemia May Not Be an Aggressive Subtype

Ming-Yuan Lee[1,4]*, Tran-Der Tan[2], An-Chen Feng[3]

[1]*Department of Pathology and Laboratory Services, [2]Department of Hemato-oncology, and*
[3]*Clinical Protocol Office, Koo Foundation Sun Yat-Sen Cancer Center, Taipei, and*
[4]*National Yang-Ming University School of Medicine, Taipei, Taiwan, R.O.C.*

Background: Most acute leukemias are classified as lymphoid or myeloid lineages by standard microscopic morphology, cytochemistry and a panel of immunologic markers. The World Health Organization classification of acute leukemia incorporates morphologic, cytogenetic, immunologic and clinical features to define the entities that are biologically homogeneous and that have clinical relevance. The purpose of this study was to determine the clinicopathologic characteristics of acute myeloid leukemia (AML) in Taiwan.
Methods: Archival tissues from 70 AML patients during the period of 1995 to 2003 were retrieved. Histologic subtype was classified, defined by World Health Organization classification. Clinical data, including age, gender, treatment and outcome, were scrutinized.
Results: There were 37 males and 33 females. The median age at onset of disease was 49 years (range, 2–78 years), which was younger in biphenotypic AML (23.5 years) and older in multilineage dysplasia-related AML (61 years). There were 9 cases (13%) with recurrent cytogenetic abnormality, 7 (10%) multilineage dysplasia-related, 7 (10%) therapy-related, 39 (56%) not other categorized and 8 (11%) of ambiguous lineage. The 2- and 5-year overall survival rates of AML were 26.5% and 20.6%, respectively. Histologic subtype was a significant parameter to determine survival ($p < 0.05$). The median survivals of therapy-related, multilineage dysplasia-related and biphenotypic AML were 2 months, 9 months and 30.5 months, respectively.
Conclusion: This was a clinicopathologic study of AML in Taiwan. Histologic subtype plays a significant prognostic role. Multilineage dysplasia- and therapy-related AML have worse prognosis. Biphenotypic AML may not be an aggressive subtype. [*J Chin Med Assoc* 2007;70(7):269–273]

Key Words: acute myeloid leukemia, biphenotypic, multilineage dysplasia, therapy-related, WHO classification

Introduction

Most acute leukemias are classified as lymphoid or myeloid lineages by standard microscopic morphology, cytochemistry and a panel of immunologic markers.[1–3] The World Health Organization (WHO) classification of acute leukemia incorporates morphologic, cytogenetic, immunologic and clinical features to define the entities that are biologically homogeneous and that have clinical relevance.[4] The WHO classification of acute myeloid leukemia (AML) encompasses 5 major categories: (1) AML with recurrent cytogenetic abnormalities; (2) AML with multilineage dysplasia; (3) AML, therapy-related; (4) AML, not other categorized (NOS); and (5) acute leukemia of ambiguous lineage. Different subtypes play important prognostic roles. For example, AML with recurrent favorable cytogenetic abnormality generally has a good prognosis. In contrast, multilineage dysplasia-related or therapy-related AML usually carries poor clinical outcomes.

The purpose of our study was to characterize AML in Taiwan according to the WHO classification and demonstrate their clinicopathologic features with respect to demographics, histologic subtypes and clinical outcomes.

Methods

A total of 70 cases of AML, diagnosed and documented at the Department of Pathology at Koo Foundation Sun Yat-Sen Cancer Center in Taiwan during the period of 1995 to 2003, were retrieved. Of these cases, 4 (6%) came from consultation files. The cases included in this study were collected covering all regions of Taiwan. For comparison, 15 cases of acute lymphoblastic leukemia (ALL) were also included for analysis.

Bone marrow aspiration specimens, including cytochemical studies of myeloperoxidase, nonspecific esterase and periodic acid-Schiff, were reviewed. Bone marrow biopsy specimen was fixed in 10% formalin or B5 solution, routinely processed and embedded in paraffin, then sectioned at 5-µm thickness and stained with hematoxylin and eosin (H&E). Immunohistochemically, basic B- and T-cell markers (CD20/CD79a and CD3) were studied. Additional studies for CD4, CD8, CD10, CD117, MPO or TdT were performed in selected cases. Cytogenetic study and flow cytometry study were also performed in 29 and 41 cases, respectively. A panel of immunophenotypic markers was studied in flow cytometry analysis, including immunoglobulin kappa/lambda light chain, CD20, CD19, CD5, CD23, CD10, CD4, CD8, CD7, CD3, CD15, CD34, CD13, HLA-DR, CD33, CD14, CD64, CD16, CD56, CD71, CD11b, CD38, CD117 and CD45. The histologic subtypes were defined and categorized into 5 groups, defined by the WHO classification.

All patients were treated with a curative chemotherapy, mainly of idarubicin and cytarabine-containing regimen. Ten patients were also given peripheral blood autologous stem cell transplantation (5 biphenotypic AML, 1 AML with prior multilineage dysplasia, 1 AML of NOS) or allogeneic bone marrow transplantation (2 biphenotypic AML and 1 AML of NOS). The AML with recurrent cytogenetic abnormality of t(15;17) (q22;q12)(PML/RARα) and variants was primarily given all-trans retinoic acid.

The period of follow-up was assessed from the first day of diagnosis to the last day of clinical follow-up recorded in chart or by telephone. The overall survival was determined by Kaplan–Meier analysis. Statistical analysis indicated significant difference when p value was less than 0.05.

Results

There were 37 males and 33 females with no sex predominance. The median age at onset of disease was 49 years, ranging from 2 to 78 years. The median age was younger in biphenotypic AML (23.5 years) and older in AML with multilineage dysplasia (61 years). Twenty (69%) of 29 cases receiving chromosome study had cytogenetic abnormalities, including t(8;21) in 2, t(15;17) in 6, 11q23 in 1, trisomy in 3, complex abnormalities in 5, and others in 3. For histologic subtypes, there were 9 cases (13%) with recurrent cytogenetic abnormality, 7 (10%) multilineage dysplasia-related, 7 (10%) therapy-related, 39 (56%) NOS and 8 (11%) of ambiguous lineage. All 8 cases with AML of ambiguous lineage were of biphenotypic AML (BAL).

In 7 cases of AML with multilineage dysplasia, all had antecedent myelodysplastic syndrome (MDS). Regarding these patients of AML with prior MDS, 5 (71%) of 7 cases had high-grade MDS with blasts >10% in bone marrow. The other 2 cases had refractory anemia. The median time to evolve into AML in these patients was 8 months.

Of 7 cases of therapy-related AML, 4 had prior breast cancers; the other pre-existing malignancies in the remaining patients were ovarian cancer, malignant fibrous histiocytoma and osteogenic sarcoma. Five patients received both alkylating agent- and topoisomerase II inhibitor-containing chemotherapy. The other 2 cases received either alkylating agent or topoisomerase II inhibitor. The blasts in 4 cases with therapy-related AML showed evidence of monocytic differentiation. The median time to develop to AML in this group was 50 months.

In 8 cases with BAL, the detailed results of clinical features, cytochemical staining, immunophenotype and chromosome analysis are listed in Table 1.

The duration of follow-up ranged from 1 to 102 months (median, 10.5 months; mean, 17.3 months). The 2- and 5-year overall survivals were 26.5% and 20.6%, respectively. Histologic subtype was a significant prognostic factor ($p=0.04$) (Figure 1). BAL had better 5-year overall survival (54.7%), followed by AML with recurrent abnormalities (47.6%) and AML of NOS (15.4%). Therapy-related and multilineage dysplasia-related AML had poor prognosis, with median survival times of 2 months and 9 months, respectively. All patients with therapy-related AML died within 2 years of diagnosis. Five of 7 patients with multilineage dysplasia-related AML died within 1 year of diagnosis. The remaining 2 cases were uneventful at 12 and 22 months after diagnosis.

Table 1. Clinical characteristics in 8 patients with biphenotypic acute leukemia

Case	Gender/Age	Cytochemistry	Immunophenotype	Chromosomal study
1	M/26	MPO+	CD33+/HLA-DR+/CD19+/CD10+	del(11)(q23)
2	M/8	MPO–/PAS+	CD33+/CD117+/CD3+/CD7+	NA
3	F/47	MPO–/PAS–NSE–	CD33+/CD13+/CD5+/CD10+/CD7+	46xx
4	F/30	NA	CD33+/CD13+/CD19+/CD79a+/CD10+	t(9;22)(q34;q11)
5	M/22	MPO–	CD33+/CD13+/CD19+/CD10+	NA
6	M/2	MPO–	CD33+/CD13+/CD19+/CD10+	NA
7	M/17	MPO–/PAS+/NSE–	CD33+/CD13+/CD117+/CD19+	t(10;11)(p14;q21)
				t(2;13)(31;q14)
8	F/25	MPO+/PAS+	CD3+/TdT+	NA

MPO = myeloperoxidase; NSE = nonspecific esterase; PAS = periodic-acid Schiff.
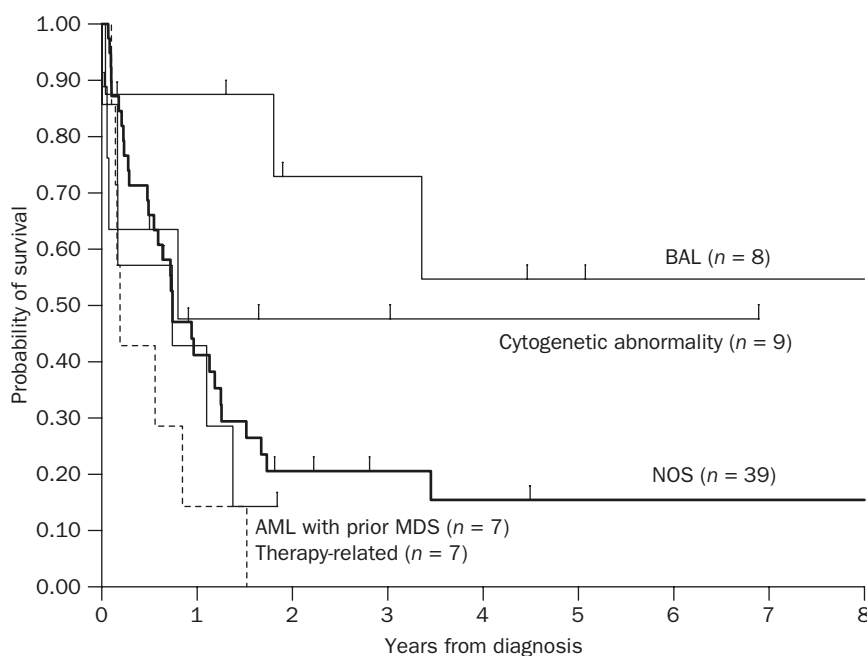
Figure 1. Overall survival of histologic subtypes of 70 patients with acute myeloid leukemia, defined by WHO classification ($p = 0.04$).

Fifteen cases of ALL were also analyzed for comparison. BAL had better 5-year overall survival of 54.7%, compared to non-BAL AML (16.1%) and ALL (23.9%) ($p = 0.04$) (Figure 2).

Discussion

This was a large study to characterize AML in Taiwan according to the WHO classification and demonstrate their clinicopathologic features with regard to aspects of demographics, histologic subtypes and clinical outcomes.

The Medical Research Council AML 10 trial reported the importance of diagnostic cytogenetics as an independent prognostic factor in AML.[5] They demonstrated that AML associated with t(8;21),

t(15;17), or inv(16) predicted relatively favorable outcomes, whereas in patients lacking these favorable changes, the presence of complex karyotypes, -5, del(5q), -7, or abnormalities of 3q defined a group associated with relatively poor prognosis. The remaining group of patients, including those with 11q23 abnormalities, +8, +21, +22, del(9q), del (7q), or other structural or numerical defects not encompassed by the favorable or adverse risk groups, were found to have intermediate prognosis. Our study was limited by relatively small sample size, so the implication of specific cytogenetic abnormalities could not be asserted. In addition, a low number of AML patients with recurrent cytogenetic abnormality was observed in the present study, compared to other studies in Taiwan (13% *vs.* ~30%).[6–8] This may be a consequence of referral status and the relatively low number of pediatric patients in
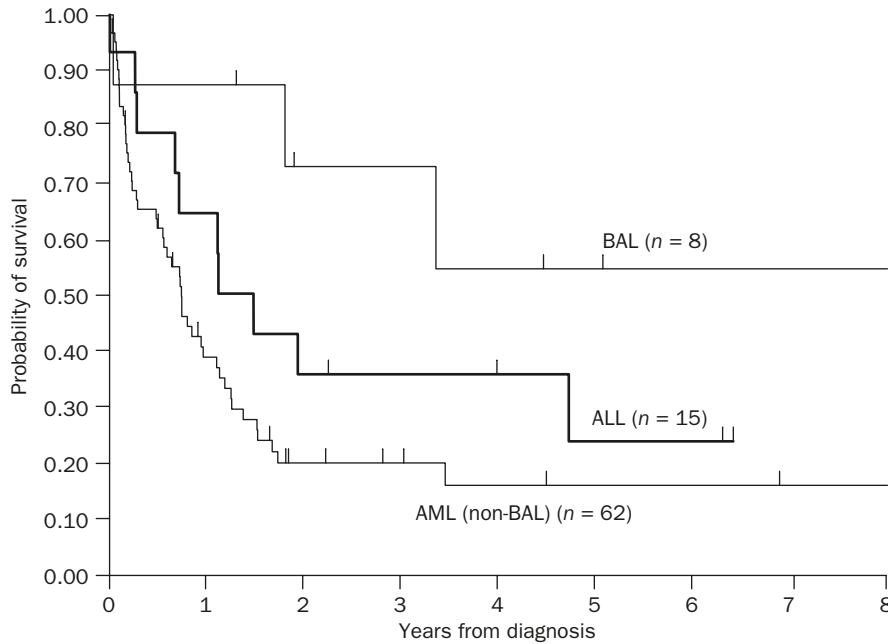
Figure 2. Comparison of overall survival of 8 biphenotypic AML (BAL), 62 non-BAL AML and 15 acute lymphoblastic leukemia (ALL) ($p = 0.04$).

our center. However, we observed a trend of good prognosis in the group of patients with favorable risk (data not shown). In addition, Teng et al[9] reported that lactate dehydrogenase (LDH) correlated to bone marrow vascularity in AML, suggesting that LDH can be used as a prognostic parameter. This may need further study for our patients.

MDS is a heterogeneous group of hematologic diseases. The international scoring system of MDS provides an improved method for evaluating prognosis.[10] According to the 3 variables of the percentage of blasts, number of cytopenias and cytogenetic subgroups, MDS can be classified into 4 groups, of low, intermediate-1, intermediate-2 and high risk. Seven (27%) of 26 cases with prior MDS developed to multilineage dysplasia-related AML in our study. The median time to evolve to AML was 8 months. Thus, close follow-up of these patients is mandatory, particularly for high-grade MDS. Multilineage dysplasia-related AML usually has worse prognosis; in the present study, the median survival in this group was only 9 months.

Therapy-related AML is a complication of intensive chemotherapy regimens, which accounts for 10–20% of all AML cases.[11] The risk is related to total accumulated dose and age. This group usually has high incidence of clonal cytogenetic abnormality. Patients exposed to alkylating agents or topoisomerase II inhibitors are reported to develop secondary AML.[12,13] Therapy-related AML usually develops 5–6 years after exposure to alkylating agent. Nevertheless, topoisomerase II

inhibitor-related AML has shorter latency, of a median of 33 months. Our cases demonstrated a latency of 50 months and a poorer prognosis, with median survival of 2 months. This group often has high significant monocytic component.[12,13] Four (57%) of 7 cases with therapy-related AML had associated monocytic component in the present study.

BAL is an uncommon type of leukemia, which probably arises in a multipotent progenitor with capability of differentiating along both myeloid and lymphoid lineages. According to the WHO classification, BAL is categorized to AML of ambiguous lineage. The incidence of BAL ranged from 3.6% to 10%.[14–16] The diagnosis of BAL is based on comprehensive immunophenotyping. Matutes et al[17] proposed a scoring system to define biphenotypic acute leukemia. According to the definition and scoring system, a case is considered biphenotypic when point values are greater than 2 for the myeloid and 1 for the lymphoid lineages. The most common type is myeloid and B-lymphoid followed by myeloid and T-cell lineage. Cases involving both B- and T-lymphoid lineage are rare; cases with markers for 3 lineages are even rarer. There were 5 cases with markers of myeloid and B lineage and 3 cases with myeloid and T lineage in the current study. Neither trilineage differentiation nor expression of both T and B markers was observed. If involving B-cell lineage, BAL often obtains CD10 expression.[17] Four (80%) of 5 BAL of myeloid and B-cell lineages coexpressed CD10 in this study. The role of CD10 in BAL is still

unknown; this needs further investigation to elucidate. Previous report demonstrated that BAL had poor prognosis with 2-year survival of 39.4%, and thus was likely to require intensive treatment to achieve long-term complete remissions.[15] This may be related to the underlying chromosome abnormality. In addition, the poor prognosis was strongly related to the presence of Philadelphia chromosome, age < 15 years and CD34 phenotype. Legrand et al[16] also showed poor prognosis for BAL, when compared with AML and ALL, in terms of complete remission ($p=0.006$) and 4-year survival ($p=0.003$). On the contrary, patients with BAL had better prognosis in our study, with 2-year survival of 72.9%. The low incidence of cytogenetic changes in the unfavorable group (25%), older age (age > 15 years in 6 patients) and smaller sample number of BAL may explain this discrepancy. Also, 5 patients (63%) received peripheral blood stem cell or bone marrow transplantation treatment, which may also have improved the survival rate. However, extensive data on response to therapy and clinical outcomes are not available. A larger study may be needed for further investigation to elucidate the nature and clinical characteristics of BAL. Furthermore, patients with BAL should have risk stratification with treatment tailored to their prognostic factors. According to relatively better prognosis in BAL in the current study, BAL may not be an aggressive variant and need aggressive treatment to achieve complete remission.

In conclusion, the current data indicate that WHO histologic subtype is a significant parameter in determining the survival rate. Therapy-related and multilineage dysplasia-related AML has worse prognosis. Biphenotypic AML may not be an aggressive subtype.

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