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ARTICLE

Clinical, immunophenotypic, and genomic findings of acute undifferentiated leukemia and comparison to acute myeloid leukemia with minimal differentiation: a study from the bone marrow pathology group

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Abstract

Acute undifferentiated leukemia is a rare type of acute leukemia that shows no evidence of differentiation along any lineage. Clinical, immunophenotypic and genetic data is limited and it is uncertain if acute undifferentiated leukemia is biologically distinct from acute myeloid leukemia with minimal differentiation, which also shows limited myeloid marker expression and has been reported to have a poor prognosis. We identified 92 cases initially diagnosed as acute undifferentiated leukemia or acute myeloid leukemia with minimal differentiation from pathology databases of nine academic institutions with available diagnostic flow cytometric data, cytogenetic findings, mutational and clinical data. Outcome analysis was performed using Kaplan Meier test for the 53 patients who received induction chemotherapy. Based on cytogenetic abnormalities ($N = 30$) or history of myelodysplastic syndrome ($N = 2$), 32 cases were re-classified as acute myeloid leukemia with myelodysplasia related changes. The remaining 24 acute undifferentiated leukemia patients presented with similar age, blood counts, bone marrow cellularity, and blast percentage as the remaining 30 acute myeloid leukemia with minimal differentiation patients. Compared to acute myeloid leukemia with minimal differentiation, acute undifferentiated leukemia cases were characterized by more frequent mutations in *PHF6* (5/15 vs 0/19, $p = 0.016$) and more frequent expression of TdT on blasts ($p = 0.003$) while acute myeloid leukemia with minimal differentiation cases had more frequent CD123 expression ($p = 0.042$). Outcome data showed no difference in overall survival, relapse free survival, or rates of complete remission between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation groups ($p > 0.05$). Acute myeloid leukemia with myelodysplasia-related changes patients showed shorter survival when censoring for bone marrow transplant as compared to acute undifferentiated leukemia ($p = 0.03$) and acute myeloid leukemia with minimal differentiation ($p = 0.002$). In this largest series to date, the acute undifferentiated leukemia group shows distinct characteristics from acute myeloid leukemia with minimal differentiation, including more frequent *PHF6* mutations and expression of TdT.

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Introduction

The WHO classification of myeloid neoplasms relies on the morphological and immunophenotypic features of the neoplastic cells to establish their lineage and degree of maturation. Most case of acute leukemia can be assigned to a myeloid or lymphoid lineage using immunophenotype by multiparameter flow cytometry. In a small number of cases, lineage attribution is problematic. These cases, currently termed "acute leukemias of ambiguous lineage", either show no clear evidence of differentiation along any hematopoietic lineage or show blasts with mixed lineage characteristics (e.g., coexpression of myeloid and T or B-cell antigens). The first consensus lineage classification for leukemias with ambiguous immunophenotypes was first proposed by the European Group for Immunophenotyping of Leukemia and assigned a numerical value, ranging from 0.5 to 2, for each individual myeloid-associated or lymphoid-associated marker expressed by the blasts; a score of >2 points needed to be established for each lineage [1]. In 2008, the WHO classification proposed a simpler diagnostic algorithm to define lineage for the purposes of establishing a diagnosis of mixed-phenotype acute leukemia, which relies on fewer markers that are more lineage specific [2]. In the setting of a mixed phenotype acute leukemia, the assignment of myeloid-lineage requires the presence of myeloperoxidase as detected by flow cytometry, immunohistochemistry or cytochemistry, or evidence of monocytic differentiation with at least 2 of the following markers being positive: non-specific esterase cytochemistry, CD11c, CD14, or CD64. T-lineage is defined by cytoplasmic or surface CD3, with staining as intense as background reactive T-cells in at least a subset of blasts. Multiple antigens are required for B-lineage, including a combination of CD19 with CD79a, cytoplasmic CD22 and/or CD10. If, after a thorough immunophenotypic investigation along the previously mentioned guidelines, none of the lineage-specific markers and less than two myeloid-associated markers (CD13, CD33, and/or CD117) are present, a diagnosis of acute undifferentiated leukemia is made. Thus, in the WHO classification, the term acute undifferentiated leukemia is restricted to a case of leukemia that expresses no markers considered to be specific for lymphoid or myeloid lineages.

Due to its rarity, little is known about acute undifferentiated leukemia, including the optimal number and types of myeloid markers allowed in this diagnosis. In an early study of 9 acute undifferentiated leukemia patients by Cuneo et al, all cases lacked CD13 and CD33; CD117 was only positive in 2 of only 3 cases tested [3]. In a study of 16 acute undifferentiated leukemia cases, Heesch et al. reported that most cases showed expression of CD34, TdT and HLA-DR and lacked lineage specific markers, but made no mention of myeloid marker expression [4]. Kurosawa et al. evaluated 12 acute undifferentiated leukemia patients out of 911 patients with acute leukemia and reported expression of CD13 in 60% of cases [5]. These studies of acute undifferentiated leukemia did not address the number of myeloid markers needed to separate acute undifferentiated leukemia from acute myeloid leukemia with minimal differentiation. Acute myeloid leukemia with minimal differentiation is a subtype of acute myeloid leukemia, not otherwise specified in the WHO classification and roughly correlates with acute myeloid leukemia-M0 in the French-American-British classification. Acute myeloid leukemia with minimal differentiation represents 5% or less of all acute myeloid leukemia cases, is by definition negative for myeloperoxidase, and expresses at least two myeloid marker, usually CD13, CD33 and/or CD117 [2, 5–10].

Genetic data on acute undifferentiated leukemia is also limited. Cuneo et al. reported that del(5q) was seen in 33% of acute undifferentiated leukemia cases, trisomy of chromosome 13 in 33%, and complex karyotype in only one case [3]. Heesch et al. reported an abnormal karyotype in 4/5 acute undifferentiated leukemia cases, although further details were not provided. The incidence of abnormal karyotypes in acute myeloid leukemia with minimal differentiation is also high, ranging from 71–81% and including a high rate of complex karyotypes, abnormalities of chromosome 5 and 7 as well as trisomies of chromosomes 8 and 13 [7]. Molecular genetic studies have shown *RUNX1* mutations in 15–35% of acute myeloid leukemia with minimal differentiation and gene expression profiling suggests that acute myeloid leukemia with minimal differentiation can be divided into two unique subtypes based on the presence or absence of a *RUNX1* mutation [11, 12]. Heesch et al. reported *BAALC*, *ERG* and *MN1* gene expression, but absence of a *WT1* mutation in acute undifferentiated leukemia [4]; further mutation profiling has not been reported in acute undifferentiated leukemia cases.

The 2016 revised WHO classification defines acute myeloid leukemia with myelodysplasia related changes as an acute myeloid leukemia occurring after a prior history of myelodysplastic syndrome or myelodysplastic/myeloproliferative neoplasm, showing a complex karyotype or any of several other myelodysplastic syndrome-associated cytogenetic abnormalities, or showing substantial (>50%) background morphologic dysplasia [13]. This definition is of increasing clinical importance as a new induction therapy, lamellar encapsulated daunorunicin/cytarabine, has been approved for acute myeloid leukemia with myelodysplasia-related changes [14]. Although the clinical significance of morphologic dysplasia in acute myeloid leukemia has been debated, there is general agreement on the poor prognostic impact of a prior myeloid neoplasm or myelodysplastic syndrome-associated cytogenetic aberrations [15]. The current WHO Classification recommends

that cases of acute leukemia with ambiguous lineage (including acute undifferentiated leukemia) that have a history of myelodysplastic syndrome or myelodysplastic syndrome/myeloproliferative neoplasm, or myelodysplastic syndrome defining cytogenetics be classified as acute myeloid leukemia with myelodysplasia-elated changes and that cases with karyotype or molecular genetic findings (*NPM1* or double *CEBPA* mutation) of acute myeloid leukemia with recurrent genetic abnormalities should also be classified as such [13]. Prior studies of acute undifferentiated leukemia included all karyotype abnormalities and did not consider the WHO classification of acute myeloid leukemia with myelodysplasia-elated changes or acute myeloid leukemia with recurrent genetic abnormalities assignment. The goal of this study is to report on a large, multi-institutional series of acute undifferentiated leukemia cases with cytogenetic and molecular findings using the revised WHO classification, and to compare this group with acute myeloid leukemia with minimal differentiation.

Methods

Patients

We searched the databases of multiple institutions for patients with diagnoses of acute undifferentiated leukemia or acute myeloid leukemia with minimal differentiation with available diagnostic flow cytometric data, cytogenetic findings, and clinical data from Brigham and Women's Hospital/Dana-Farber Cancer Institute, Cleveland Clinic, Massachusetts General Hospital, MD Anderson Cancer Center, Stanford Health Care, University of Chicago, University of Pennsylvania, and Weill Cornell Medical Center. We reviewed the pathology reports and ancillary tests including scattergrams and confirmed that all cases met the criteria for acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation based on the WHO 2008 and 2016 classifications. Clinical information and follow up were retrieved from the electronic medical records. This study was approved by the Institutional Review Boards of all participating institutions.

Immunophenotyping

Flow cytometry immunophenotyping was performed at each institution using comprehensive panels for acute leukemia work-up. Although the panels varied among different institutions, all panels were adequate to assess the lineages of leukemic blasts. In the case of cytoplasmic or nuclear staining antibodies, samples were also permeabilized during antibody labeling. The total markers assessed in all institutions included CD34, CD117, CD13, CD33, CD15, CD11b, CD64, MPO, HLA-DR sCD3, cCD3, CD2, CD4, CD5, CD7, CD56, CD1a, CD19, CD20, CD10, CD22, CD79a, CD123, TdT, CD38 and myeloperoxidase (MPO). CD1a, CD79a, CD123 and CD38 were at some, but not all institutions. The percentage of blasts positive for MPO and method of evaluation was also recorded. Additional cytochemical or immunohistochemical stains for lysozyme, non-specific esterase, specific esterase and MPO were also performed in a subset of cases.

Cytogenetic and FISH analysis

Conventional chromosomal analysis was performed on G-banded metaphase cells prepared from unstimulated 24 and 48-h bone marrow aspirate cultures at the time of diagnosis using standard techniques and was documented according to the International System for Human Cytogenetic Nomenclature [16]. The median number of metaphases analyzed was 20 (range, 20 to 23). Fluorescence in situ hybridization (FISH) was performed as a part of the clinical evaluation in some cases to identify common gene rearrangements/fusions, including t(15;17)(q22;q12), t(8;21)(q22;q22), inv(16)(p13.1q22)/t(16;16)(p13.1;q22), t(6;9)(p23;q34), and t(9;22)(q34;q11.2).

Targeted next-generation sequencing

Targeted next-generation sequencing studies were performed to detect gene mutations that are commonly identified in hematolymphoid malignancies as previously described [17]. DNA was prepared from bone marrow or peripheral blood at each institution that included Brigham and Women's Hospital/Dana-Farber Cancer Institute, Cleveland Clinic, Massachusetts General Hospital, MD Anderson Cancer Center, Stanford Health Care, University of Chicago, University of Pennsylvania, and Weill Cornell Medical Center. Specific next-generation sequencing methodologies varied between institution but all included an amplicon based library preparation and sequencing with Illumina MiSeq and panels included ranged from 33 to 103 genes. Specific panels included University of Pennsylvania TruSeq Custom Amplicon panel covering 33 or 68 genes; ARUP myeloid malignancy panel covering 53 genes; Genoptix myeloid molecular profile covering 41 genes; Massachusetts General Hospital Snapshot myeloid malignancy panel covering 103 genes and Brigham and Women's Rapid heme panel Illumina Truseq Custom Amplicon covering 95 genes. The following genes were included in every panel: *FLT3, IDH1, IDH2, JAK2, KIT, NPM1, NRAS* and *TP53*. Additionally, genes present in 6 or 7 of the 8 panels included *ASXL1, BRAF, CBL, DNMT3A, ETV6, EZH2, KRAS, JAK2, PHF6, PTPN11, RUNX1, SETBP1, SF3B1, TET2, U2AF1, WT1* and *ZRSR2*.Statistical Analysis

Fisher's exact test was used to compare categorical variables. Relapse free survival and overall survival from

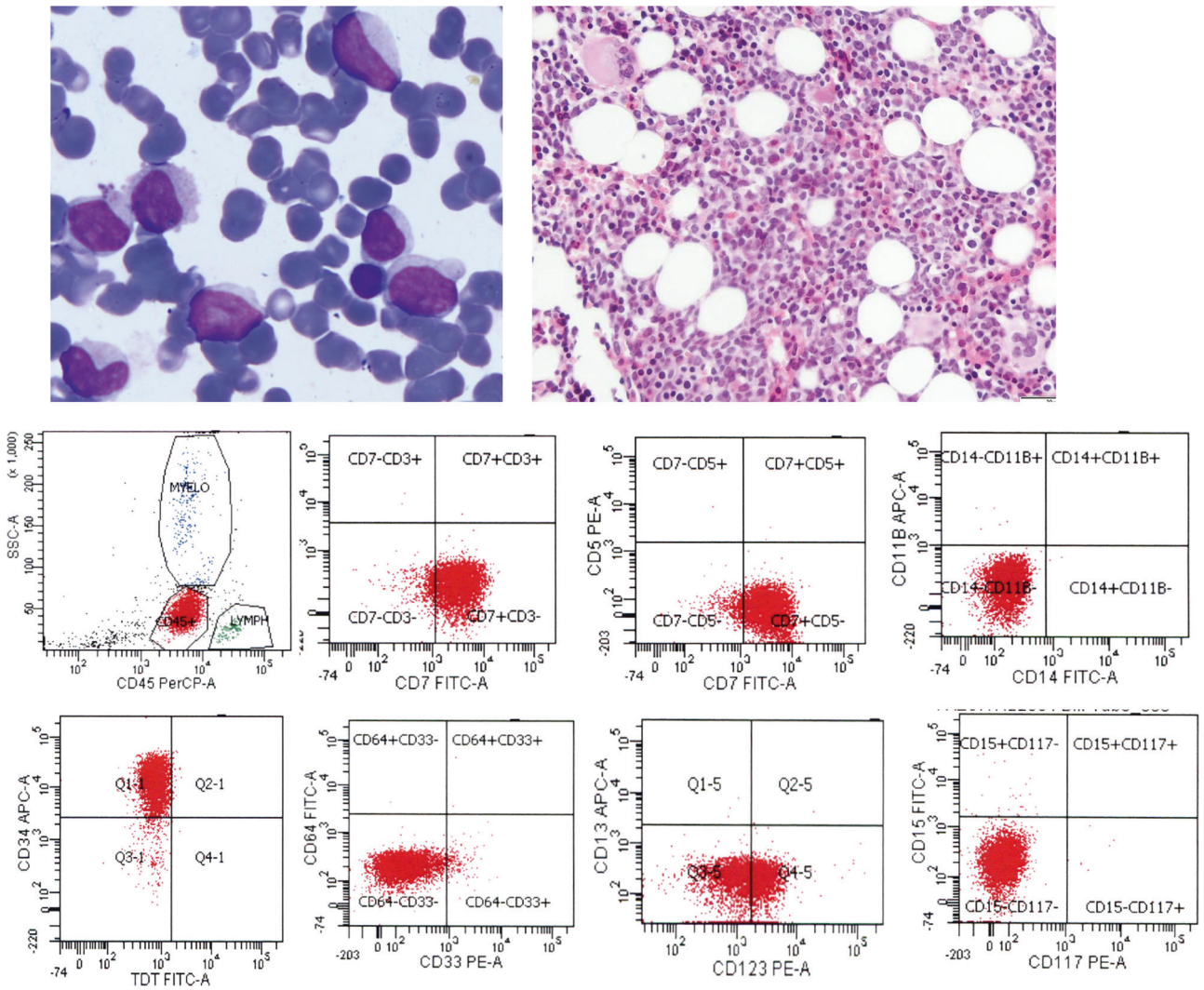

Fig. 1 Example of a typical acute undifferentiated leukemia case with aspirate and biopsy findings composed of predominance of blasts with scant to moderate agranular cytoplasm. Flow cytometry shows a population of blasts expressing CD7 and CD34 and partial CD123 but lacking CD13, CD33, CD64, CD11b, CD14, CD117, and TdT

diagnosis were estimated using the method of Kaplan and Meier. Complete remission was defined according to the criteria of Cheson et al. [18]. A p-value of less than 0.05 was considered to be statistically significant.

Results

Patient cohort

A total of 92 cases fulfilled immunophenotypic features of acute undifferentiated leukemia or acute myeloid leukemia with minimal differentiation. An example of a typical acute undifferentiated acute leukemia from this study is shown in (Fig. 1). 6 cases were excluded from further analysis, as they were classified per the 2016 WHO Classification as acute myeloid leukemia with recurrent genetic abnormalities: t(3;3) (1 case, immunophenotypically acute undifferentiated leukemia) or acute myeloid leukemia with mutated *NPM1* (5 cases, all immunophenotypically acute myeloid leukemia with minimal differentiation). In the remaining 86 cases, 32 had defining cytogenetic features (30 cases) or myelodysplastic syndrome or myelodysplastic syndrome/myeloproliferative neoplasm history (2 cases) consistent with acute myeloid leukemia with myelodysplasia-related changes. The remaining 54 cases comprised 24 assigned to the acute undifferentiated leukemia group and 30 in the acute myeloid leukemia with minimal differentiation group.

Acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation patients

The 24 acute undifferentiated leukemia patients presented with similar age, blood counts, bone marrow cellularity,

and bone marrow and blood blast percentages as the 30 acute myeloid leukemia with minimal differentiation patients (Table 1, all $p > 0.05$). Expression of CD34 was seen in most acute undifferentiated leukemia cases (23/24) and acute myeloid leukemia with minimal differentiation cases (29/30) (Table 2 and Table 3). Six acute undifferentiated leukemia cases showed no myeloid marker expression (CD117, CD13 or CD33), 15 showed partial or full expression of 1 myeloid marker, and 3 showed expression of 1 myeloid marker plus weak/partial expression of another myeloid marker on the blasts (Table 2). Only limited B-cell antigen expression was seen in acute undifferentiated leukemia cases, with no expression of CD19, CD20 or CD10 on blasts; however, 5 of 24 (21%) cases showed partial cCD22 or cCD79a co-expression. None of the cases expressed cytoplasmic or surface CD3, but expression of other T-cell associated antigens was commonly seen on blasts (11/24, 46%) of acute undifferentiated leukemia patients, most often CD7. Monocytic differentiation was not seen any of the cases, with no acute undifferentiated leukemia cases expressing two or more monocytic markers such as CD64, CD11b, CD14 or lysozyme. Two acute undifferentiated leukemia cases showed partial CD11b expression and non-specific esterase was partially positive in 1 of 19 cases tested (Table 2). Comparison of immunophenotypes between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation showed that acute undifferentiated leukemia blasts had more frequent expression of TdT ($p = 0.014$) while acute myeloid leukemia with minimal differentiation cases had more frequent CD123 expression ($p = 0.042$). No differences in B antigen (CD19, CD20, CD10, CD22, CD79a), T antigen (CD2, CD4, CD5, CD7, CD8) or monocytic marker (CD11b, CD64) co-expression on blasts ($p > 0.05$ for all) were seen. In 10 acute undifferentiated leukemia patients who later relapsed, 9 showed an identical immunophenotype to the original disease, while one case showed new expression of CD13 and CD33 that was not seen in the diagnostic sample.

Table 1 Comparison of clinical and genetic features of acute undifferentiated leukemia, acute myeloid leukemia with minimal differentiation and acute myeloid leukemia with myelodysplasia-related changes

	Acute undifferentiated leukemia ($n = 24$)	Acute myeloid leukemia with minimal differentiation ($n = 30$)	Acute myeloid leukemia with myelodysplasia-related changes ($n = 32$)
Age at diagnosis, median (range)	68 (29–86)	61 (23–89)	66 (27–82)
Male:Female	14:10	18:12	19:13
Bone marrow cellularity %, median (range)	90 (20–100)	70 (10–100)	80 (10–100)
Bone marrow blast %, median (range)	75 (20–94)	79 (22–95)	75 (29–95)
White blood count $\times 10^9$/L, median (range)	4.1 (0.3–59.1)	2.3 (0.7–177.3)	2.1 (0.7–63.7)[a]
Hemoglobin (g/dL), median (range)	9.3 (6.7–14.3)	9 (6.2–12.6)	9 (4.7–11.3)
Platelet count $\times 10^9$/L, median (range)	45 (8–167)	70 (9–319)	39 (14–408)[a]
PHF6 mutated, N (%)	5/15 (33%)[b]	0/19 (0%)	1/16 (6%)
SRSF2 mutated, *N* (%)	6/15 (40%)	3/19 (16%)	0/14 (0%)[a]
TP53 mutations, *N* (%)	0/18 (0%)	0/22 (0%)	11/24 (46%)[c]
RUNX1 mutations, *N* (%)	7/15 (46%)	6/20 (73%)	4/25 (16%)
ASXL1 mutations, *N* (%)	6/18 (33%)	5/19 (26%)	1/24 (4%)
Abnormal karyotype, *N* (%)	10 (41%)	13 (43%)	27/28 (96%)[c]
Follow up time, median months (range)	15 (0.2–80)	18 (0.3–79.3)	4.9 (0.7–88)
Complete remission (number of patients)	14/24	19/27	15/23
Bone marrow transplant (number of patients)	11/24	15/27	8/23

[a]Statistically significantly different from acute undifferentiated leukemia

[b]Statistically significant from acute myeloid leukemia with minimal differentiation and acute myeloid leukemia with minimal differentiation

[c]Statistically significantly different from both acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation

Table 2 Immunophenotypic, molecular, clinical and cytogenetic findings of acute undifferentiated leukemia patients

Patient	Diagnosis	Gender	Age	Therapy	RFS (mos)	OS (mos)	CR (Y/N)	Cytogenetics	Pathogenic mutations	CD34	CD117	CD13	CD33	CD15	sCD3	cCD3	MPO flow	CD2	CD4	CD7	CD5	CD56	CD19	CD20	CD10	CD123	CD22	CD79a	CD11b	CD64	TdT
1	AUL	M	69	AML induction	51	52	Y	47,XY,+13[9]/46,XY[cp11]	*ASXL1; IDH2 ; RUNX1; SETBP1; SRSF2*	+	+	-	Subset	-	-	-	-	-	NP	-	-	-	-	-	-	?	+	-	+	-	+
2	AUL	M	80	Hypomethylating	12	12	N	Normal	*ASXL1; KRAS; RUNX1; TET2; TET2*	-	Subset	-	-	-	-	-	-	Subset	NP	+	-	+	-	-	-	+	-	-	-	-	+
3	AUL	F	76	SGI-110 trial	23	5	Y	Normal	*IDH2*	+	Subset	-	-	-	-	-	-	-	-	+	-	-	-	-	-	+	Subset	-	NP	-	Subset
4	AUL	M	31	AML induction	32	26	Y	Normal	*RUNX1*	+	-	-	+	-	-	-	-	-	-	+	-	+	-			-	NP	NP	NP	-	Subset
5	AUL	M	78	Hypomethylating	5	5	N	47,XY,+13[12]/46,XY[8]	*ASXL1; IDH2; KMT2A; KMT2A*	+	-	Subset	-	-	-	-	-	-	+	-	-	-	-		-	+	-	NP	NP	-	+
6	AUL	M	78	Hypomethylating	10	10	N	Normal	negative	-	+	-	Subset	NP	-	-	-	-	-	+	-	-	-	-	-	NP	-	NP	-	-	
7	AUL	F	56	AML induction	6	6	Y	gain of MLL 11q23, gain 13, loss 21	Not done	+	+	-	-	NP	-	-	-	-	-	-	-	-	-	-	-	NP	-	NP	-	-	+
8	AUL	F	66	AML induction	17	17	Y	Normal	Not done	+	Subset	Subset	-	-	-	-	-	-	+	+	-	-	NP	-	-	-	NP	+	-	NP	
9	AUL	F	80	Hypomethylating	12	12	N	46,XX,del(6)(q+3q25)[8]/46,XX[+2]	*ASXL1; BCOR; NRAS; RUNX1; SRSF2*	+	+	-	-	-	-	-	-	-	Subset	+	-	-	-	-	-	+	Subset	Subset	-	-	NP
10	AUL	M	55	AML induction	19	19	Y	Normal	*BCOR; NRAS; U2AF1*	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	Subset	Subset	Subset	-	NP
11	AUL	M	61	AML induction	32	22	Y	Normal	*FLT3*	+	-	+	-	-	-	-	-	-	-	+	-	-	-	-	-	NP	-	-	-	-	Subset
12	AUL	F	78	AML induction	41	39	Y	Normal	*IDH2; PHF6*	+	-	Subset	-	-	-	-	-	-	Subset	-	-	-	-	-	-	NP	-	-	-	-	-
13	AUL	M	65	AML induction	21	21	Y	46,XY,i(21)(q10)[3]/46,XY,+13,-21 [cp3]/46,XY[14]	*BCOR; DNMT3A; RUNX1; SRSF2; STAG2; TET2*	+	-	-	-	NP	-	-	-	-	Subset	-	-	-	-	-	-	NP	-	-	-	-	-	+
14	AUL	F	77	Unknown	1	1	N	47,XX,+13 in 5 of 20 metaphases	*FLT3; RUNX1; BCOR; TET2; PHF6.*	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NP	-	-	NP	-	-	+
15	AUL	M	86	6 MP	3	3	N	46,XY,+13,der(13;22)(q10;q10[12]/47, XY,+8[2]/46,XY[6].	*SRSF2; PTNP11; ASXL1; RUNX1*	+	-	-	-	NP	-	-	-	-	Subset	-	-	-	-	-	-	+	-	-	-	-	-	+
16	AUL	F	56	AML induction	61	61	Y	Normal	Not done	+	-	-	Subset	NP	-	-	-	-	Subset	-	-	+	-	-	-	+	-	-	-	-	+
17	AUL	M	67	AML induction	17	17	Y	46,XY,del(20)(q11.2)[8]/46,XY[12]	*DNMT3; JAK2; SRSF2*	+	-	-	-	-	-	-	-	-	Subset	-	-	-	-	-	-	+	Subset	Subset	-	-	NP
18	AUL	F	29	AML induction	6	6	Y	47,XX,t(10;11)(p13;q21),+mar[8]/46,XX [12]	*PHF6*	+	Subset	-	-	-	-	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	NP
19	AUL	F	86	Hypomethylating	2	2	N	Normal	*CBL; SETBP1; SRSF2; DNMT3A; TET2*	+	Subset	Subset	-	NP	-	-	-	-	-	-	-	-	-	-	-	NP	NP	NP	-	-	NP
20	AUL	M	86	Hypomethylating	15	8	N	Normal	Not done	+	+	-	Subset	Subset	-	-	-	-	-	-	-	-	NP	-	-	-	NP	+	-	NP	+
21	AUL	M	77	Hypomethylating	47	43	N	46,X,-Y[10]/46,XY[10]	Not done	+	+	-	-	NP	-	-	NP	-	-	-	-	-	NP	-	-	-	NP	+	NP	NP	-
22	AUL	M	45	AML induction	45	45	Y	Normal	Negative	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NP	Subset	Subset	-	-	+
23	AUL	F	49	ALL induction	42	10	Y	Normal	Negative	+	Subset	-	-	-	-	-	-	+	-	+	+	-	-		-	-	-	NP	NP	-	+
24	AUL	M	60	AML induction	7	3	N	Normal	*KRAS; PFH6*	+	Subset	-	Subset	NP	NP	-	-	-	-	+	-	-	-	-	-	NP	NP	-	-	-	-

Key

'+' full marker expression on blasts

'−' no marker expression

'Subset' partial marker expression

'NP' marker not performed

AUL acute undifferentiated leukemia

Table 3 Immunophenotypic, molecular and cytogenetic findings of acute myeloid leukemia with minimal differentiation patients

Patient	Gender	Age	Cytogenetics	Pathogenic mutations	CD34	CD117	CD13	CD33	CD15	sCD3	cCD3	MPO flow	CD2	CD4	CD7	CD5	CD56	CD19	CD20	CD10	CD123	HLA-DR	CD22	CD11b	CD64	TdT
1	M	58	Normal	Negative	+	+	+	+	NP	-	-	-	-	-	+	-	-	-	-	-	NP	+	-	-	-	-
2	M	72	Normal	*JAK3; DNMT3A*	+	+	+	-	NP	-	-	-	-	-	-	-	-	-	-	-	NP	+	-	-	-	-
3	F	32	Normal	Negative	+	+	+	+	NP	-	-	-	-	-	-	-	-	Subset	-	-	NP	+	-	-	-	+
4	M	79	Normal	Negative	+	Subset	+	+	NP	-	-	-	-	-	-	-	-	-	-	-	NP	+	-	Subset	-	+
5	M	62	48,XY,+2,+11[18]/46,XY[2]	Negative	+	+	+	-	NP	-	-	Subset	-	-	-	-	-	Subset	-	-	NP	+	-	-	-	-
6	F	89	Normal	Not done	+	+	+	Subset	-	-	-	-	-	+	+	-	-	-	-	-	+	+	Subset	-	-	NP
7	F	59	Normal	Negative	+	+	+	-	-	-	-	-	-	-	-	-	-	+	-	-	+	+	-	-	-	NP
8	M	67	46,XY,t(3;10)(p25;q24)[15]/46,XY[15]	*BCOR; NRAS; FLT3-ITD*	+	+	-	+	-	-	-	-	-	-	+	-	-	-	-	+	+	+	-	-	-	NP
9	F	77	Normal	Not done	+	+	+	-	-	-	-	Subset	Subset	-	+	-	-	-	-	-	NP	Subset	-	-	-	-
10	M	66	47,XY,+mar[4]/46,XY[16]	Not done	+	NP	+	+	NP	-	-	-	NP	-	-	-	NP	-	-	-	NP	Subset	-	-	-	-
11	F	48	46,XX,t(11;19)(q23;p13.1)[9]/46,XX[7]	Not done	+	NP	+	+	Subset	NP	-	Subset	-	NP	+	-	NP	-	-	-	NP	+	-	+	Subset	-
12	M	43	46,XY,der(18)t(8;18)(q21;q23)[20]	Not done	-	NP	+	+	-	-	-	-	-	-	+	-	NP	-	-	-	NP	+	NP	+	-	-
13	M	87	45,X,-Y,add(1)(p36.2)[2]/46,XY,add(1)(p36.2)[1]	*ASXL1; NRAS; FLT3*	+	+	+	-	-	-	-	-	-	-	+	+	+	-	NP	+	+	+	+	NP	-	-
14	M	64	Normal	*IDH2; DNMT3A*	+	+	+	+	-	-	-	-	-	-	-	-	-	-	NP	NP	+	+	-	NP	-	Subset
15	M	70	Normal	*IDH1; DNMT3A; RUNX1; KIT*	+	+	+	+	-	-	-	-	-	-	-	-	-	-	NP	NP	+	+	-	NP	-	-
16	F	72	insufficient yield	*IDH2; RUNX1*	+	+	+	+	-	-	-	-	-	-	-	-	-	-	NP	NP	+	+	-	NP	-	Subset
17	M	53	Normal	*FLT3; RUNX1*	+	+	+	-	+	-	-	-	-	+	-	-	-	-	NP	-	+	+	-	NP	-	+
18	M	59	46,XY,add(2)(q31)[3]/46,XY,del(2)(q31q35),add(3)(q27)[1]/46,XY[16]	*DNMT3A; KRAS ; TET2*	+	+	+	+	-	-	-	-	-	+	+	-	-	-	NP	-	+	+	-	NP	Subset	-
19	M	36	Normal	*RUNX; EGFR*	+	+	+	+	-	-	-	-	-	+	-	-	-	-	NP	-	+	+	-	NP	-	+
20	M	81	47,XY,inv(9)(p12q13),+20[7]/46,XY,inv(9)(p12q13)[13]	*IDH2*	+	+	+	+	+	-	-	-	-	+	-	-	-	-	NP	-	+	+	-	NP	Subset	Subset
21	F	62	47,XX,+13[8];46,XX[12]	Negative	+	+	+			-	-	-	-	+	-	-	-	-	-	NP	NP	+	NP	NP	NP	NP
22	F	59	Normal	*FLT3 (2 mutations); U2AF1*	+	+	+	-	-	-	-	-	-	NP	-	-	-	-	-	-	NP	+	NP	-	NP	NP
23	F	78	47,XX,+10 [1]/46, XX[19]	Negative	+	+	Subset	+	NP	NP	-	-	NP	NP	NP	NP	NP	-	-	NP	NP	+	NP	-	+	NP
24	F	52	46,XX,del(6)(q13q24)[8]/46,XX[12]	Not done	+	Subset	+	Subset	+	NP	NP	-	NP	NP	-	NP	NP	-	-	-	NP	+	NP	Subset	-	+
25	M	69	+13[17]/46,XY[3]	*ZRSR2; SRSF2; RUNX1 (2 mutations); IDH2; ASXL1*	+	+	+	+	-	-	NP	-	-	-	-	-	-	-	-	-	NP	+	-	-	-	NP
26	F	59	Normal	*ASXL1; DNMT3A; CSF3R*	+	+	+	Subset	-	-	NP	-	-	-	-	Subset	+	-	-	-	NP	+	-	-	-	+
27	M	57	Normal	*DDX41 (2 mutations); ASXL1; MIR142*	+	+	+	-	-	-	NP	-	-	-	-	Subset	-	-	-	-	NP	+	NP	-	-	NP
28	F	41	Normal	*RUNX1; PPM1D; DNMT3A; BCOR; IDH1; EZH2; STAG2*	+	+	Subset	+	-	-	NP	-	-	-	-	-	-	-	-	-	NP	+	NP	-	-	NP
29	M	23	Normal	Not done	+	+	+	-	NP	-	-	-	-	-	+	-	-	-	-	NP	-	NP	NP	-	-	-
30	M	61	46,XY,t(1;15)(p36.3;q15)[4]/46,XY[16]	Not done	+	+	+	-	NP	-	-	-	-	-	+	-	-	-	-	NP	-	NP	NP	NP	NP	-

Key

'+' full marker expression

'−' no marker expression

'Subset' partial marker expression

'NP' not performed

Fourteen acute undifferentiated leukemia cases (58%) had a normal karyotype, and of the abnormal karyotypes, 5 had trisomy 13 (55%); the other abnormalities are listed in Table 2. Sixteen (53%) of acute myeloid leukemia with minimal differentiation cases had a normal karyotype; the abnormal karyotypes are listed in Table 2. The frequency of abnormal karyotypes was similar between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation ($p > 0.05$). next-generation sequencing data was available in 19 acute undifferentiated leukemia patients. The most common pathogenic mutations were *PHF6* (5/15), *SRSF2* (6/15), *RUNX1* (6/18), *ASXL1* (5/18) and *BCOR* (4/15). Compared to acute myeloid leukemia with minimal differentiation, acute undifferentiated leukemia cases were characterized by significantly more frequent mutations in *PHF6* (5/15 vs 0/19, $p = 0.016$). *RUNX1* mutation was seen in 6/18 acute undifferentiated leukemia and 6/20 acute myeloid leukemia with minimal differentiation ($p = 0.74$). To explore the significance of myeloid marker expression in acute undifferentiated leukemia, we reassigned the 3 acute undifferentiated leukemia cases with partial expression of a second myeloid marker to the acute myeloid leukemia with minimal differentiation group and found more significant association with *PHF6* mutation in acute undifferentiated leukemia (5/13 vs 0/21, $p = 0.0046$). Acute undifferentiated leukemia cases with no myeloid marker expression also showed borderline more frequent *PHF6* mutations (2/4 vs 3/30, $p = 0.06$) and more frequent *SRSF2* mutations (3/4 vs 6/30, $p = 0.048$) compared to acute myeloid leukemia with minimal differentiation.

Most acute undifferentiated leukemia patients received acute myeloid leukemia-type therapy and only two acute undifferentiated leukemia patients were treated with ALL-type therapy (Table 2). Therapies included standard acute myeloid leukemia induction with $7 + 3$ in 13 (54%) patients, hypomethylating agents in 6 patients, and supportive care in the remaining patients. There was no difference in follow up time, rates of complete remission, or the number of patients who received a hematopoietic stem cell transplant between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation [Table 1]. All of the acute myeloid leukemia with minimal differentiation patients were treated with acute myeloid leukemia-type therapy: 19 (63%) received standard induction with $7 + 3$, 5 received hypomethylating agents, and the rest received supportive care only. Outcome data in the subset of patients who received induction therapy showed no difference in overall survival or relapse free survival between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation patients ($p = 0.45$ and 0.65 respectively). Restricting the definition of acute undifferentiated leukemia to cases with 1 myeloid marker expression or less ($n = 14$) also showed no difference in overall survival or relapse free survival when comparing this group with acute myeloid leukemia with minimal differentiation group (data not shown). Considering the combined acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation groups, patients with *RUNX1*-mutated disease had similar overall survival and relapse free survival as compared to patients without *RUNX1* mutations ($p = 0.67$ and $p = 0.75$, respectively).

Acute myeloid leukemia with myelodysplasia-related changes patients

Using the 2016 WHO classification criteria in this group, acute myeloid leukemia MRC was diagnosed in 32 patients based on myelodysplastic syndrome-related karyotypes or history of myelodysplastic syndrome (Table 4). 10 cases (31%) were immunophenotypically acute undifferentiated leukemia and the rest were immunophenotypically acute myeloid leukemia with minimal differentiation. These 32 acute myeloid leukemia MRC cases presented with lower WBC ($p = 0.026$) and more frequent specifically complex karyotype (20/31) ($p = 0.002$), as compared to acute undifferentiated leukemia cases. CD117 was expressed in all acute myeloid leukemia MRC cases versus 14/24 acute undifferentiated leukemia ($p \leq 0.001$). B-cell antigen expression was similarly infrequent while CD7 expression was similarly frequent (15/27) in acute myeloid leukemia MRC as in acute undifferentiated leukemia. Next-generation sequencing data was available in 28 patients and showed frequent *TP53* mutations (11/28, 39%) and *DNMT3A* mutations (5/28, 18%). Compared with the combined acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation group, more frequent *TP53* mutations were seen in acute myeloid leukemia with myelodysplasia-related changes group ($p = 0.0002$).

Acute myeloid leukemia with myelodysplasia-related changes patients were also treated with acute myeloid leukemia-type therapy: 17 of 32 (50%) received standard induction of $7 + 3$, 10 received hypomethylating agents and the remainder received supportive care. 15 patients relapsed and 18 patients were treated with hematopoietic stem cell transplantation. No difference in overall survival or relapse free survival was seen between acute undifferentiated leukemia, acute myeloid leukemia with minimal differentiation and acute myeloid leukemia MRC groups (all $p > 0.05$). When censoring for hematopoietic stem cell transplant, acute myeloid leukemia with myelodysplasia-related changes showed shorter overall survival when compared to acute undifferentiated leukemia ($p = 0.03$) and acute myeloid leukemia with minimal differentiation ($p = 0.002$) (Fig. 2).

Table 4 Immunophenotypic, molecular and cytogenetic findings of acute myeloid leukemia with myelodysplasia-related patients

Patient	Gender	Age	History of MDS or MDS/MPN	Cytogenetics	Pathogenic mutations	CD34	CD117	CD13	CD33	CD15	sCD3	cCD3	MPO flow	CD2	CD4	CD7	CD5	CD56	CD1a	CD19	CD20	CD10	CD123	HLA-DR	CD22	CD79a	lysozyme	CD11b	CD64	TdT
1	F	36	no	46~47,XX,?t(7;21)(p11.2;?p11.2),-13,del(13)(q12q?21)x2,+mar[cp12]/46,idem,add(19)(p13)[cp2]/46,XX[18]	*EZH2*	+	-	-	Subset	-	-	-	-	-	-	+	-	+	NP	-	-	-	-	-	-	Subset	NP	-	-	-
2	M	29	no	46,XY,add(1)(p36.3),t(10;11)(p13;q21),i(11q)(q10),der(17)t(4;17)(q12;p13),add(21)(p11.2)[13]/46,XY[7].nuc ish(MLLx3)[72/100]	*ATM*	+	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	+	+	-	-	-	-	-	+
3	M	55	no	45,XY,t(3;9)(q12;p13),-7,der(16)t(7;16)(q11.2;q11.2)[cp15]	*DNMT3A; IDH2; TP53; ETV6; SMARCB1*	+	Subset	-	+	-	-	-	-	-	NP	+	Subset	-	-	-	-	-	-	-	-	-	-	-	+	-	+
4	M	57	no	48,XY,+6,add(11)(q23),+21[1]/46,XY[7].	Negative	+	-	-	+	-	-	-	-	+	+	NP	-	+	-	-	-	-	+	+	-	-	+	+	+	-
5	F	57	no	45~47,XX,add(1)(p13),del(2)(q21q25),-4,del(5)(q11q13),del(6)(p22),del(7)(p12),-11,-16,del(16)(p11.2),+19,+20,+22,+0~5mar[cp9]	Not done	-	+	-	-	NP	-	-	-	-	-	+	-	+	-	-		-	+	-	NP	NP	NP	NP	-	-
6	F	51	no	92~97<4n>,XXXX,+4,+4,+6,-7,+10,?t(10;11)(p12~13;q14~21)x2,-13,i(17)(q10),+20[cp4]/46,XX[16] 92~97<4n>,XXXX,+4,+4,+6,-7,+10,?t(10;11)(p12~13;q14~21)x2,-13,i(17)(q10),+20[cp4]/46,XX[16]	*EGFR*	+	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	NP	-	-	-	-	-	NP	NP	-
7	M	28	no	46-48,XY,+1,del(1)(q21)x2,-3,+4,+1-2mar[2]/45-48,idem,del(1)(p34),del(1)(q21),-7[2]/46,XY[16]	Not done	-	+	-	-	NP	-	-	-	-	-	+	-	-	NP	-	-	-	NP	+	-	NP	NP	-	-	-
8	F	69	no	46,XX,-5,+9,add(9)(p12),del(9)(q13),del(16)(q22q24),-18,+r[cp14]/46,XX[6]	*DNMT3A; ETV6; IDH2*	+	+	+	Subset	-	-	-	-	Subset	-	+	-	+	NP	-	-	-	+	+	-	-	Subset	-	-	NP
9	F	27	no	45,XX,-7[15]	*NRAS*	+	-	-	Subset	-	-	-	-	NP	-	+	-	NP	NP	-	-	-	NP	NP	-	-	NP	NP	NP	-
10	M	52	no	44~45,XY,-Y,del(5)(q13q35),-17,+mar[cp6]/44,XY,der(5)t(5;13)(q15;q12), ?dic(10;17)(q22;p11.1),-13,-17[cp18]/46,XY[2]	*IDH1; TP53*	+	+	Subset	+	Subset	-	-	Subset	-	+	+	-	-	NP	-	-	-	NP	Subset	-	-	NP	-	-	-
11	F	64	no	46,XX,add(4)(p16)[2],add(10)(q22)[2],+13,-21[cp8]/46,XX[12]	*RUNX1; BCOR (2 mutations)*	+	Subset	-	+	-	NP	NP	-	NP	NP	NP	NP	-	NP	-	-	+	NP	+	-	Subset	-		-	Subset
12	M	74	no	44,XY,del(5)(q15q35),-13,-15[cp4]/44,XY,add(5)(q15),-13,-15,?del(16)(q2?2)[cp4]/46,XY[12]	*ASXL1; TET2*	+	+	Subset	-	NP	-	-	-	+	+	+	-	+	-	-	-	-	+	+	NP	NP	-	-	-	+
13	F	70	no	46,XX[5%]; 46,XX,dic(5;17)(q11.1;p11.1),+22[5%]; 45,idem,-3,del(9)(q11q34)[64%];90,idemx2,-3,-3,del(9)(q11q34)x2[9%];45,idem,-3,der(4)t(1;4)(p22;q21),del(9)(q11q34)[18%]	*TP53*	+	+	+	NP	NP	-	-	-	NP	NP	+	NP	NP	NP	-	-	NP	NP	+	NP	NP	NP	NP	NP	NP	NP
14	F	56	no	46,XX,inv(3)(q21q26.2)x2[1]/45,XX,idem,-7[14]/46,XX[5]	*PTPN11*	+	+	+	+	-	-	-	-	+	-	-	-	+	NP	-	NP	-	+	+	-	NP	NP	NP	-	Subset
15	M	77	no	43,XY,-3,der(4)t(3;4)(q12;q35),-5,del(6)(q13q26),-7,del(9)(p13),add(17)(p12),dup(18)(q12q23)[12]/43,idem,del(3)(q21)[8]	*NRAS; KRAS; TP53*	+	+	+	+	-	-	-	-	-	+	+	-	+	NP	-	NP	-	+	+	+	NP	NP	NP	-	-
16	M	73	no	43,XY,der(2)t(2;13)(p23;q14),del(3)(p24p25),add(4)(q25),del(5)(q13),del(7)(q22q34),del(12)(p11.2p13),-13,-16,-17,add(21)(p11.2),-22,+mar[17]/46,XY[3]	*DNMT3A; TP53*	+	+	+	+	-	-	-	-	-	+	-	-	-	NP	-	NP	-	+	+	-	NP	NP	NP	-	-
17	M	82	no	46,XY,del(5)(q31q35),del(6)(q13q23),add(11)(q23),del(16)(q11.2),add(21)(p13)[7]/46,XY,idem,del(2)(p13)[3]/46,idem,t(2;8)(p25;q12),del(20)(q11.2q13.1)[2]	*TP53 (2 mutations)*	+	+	+	+	+	-	-	-	-	+	-	-	-	NP	-	NP	-	+	-	-	NP	NP	NP	-	-
18	F	74	no	43,XX,+1,der(1;3)(q10;q10),der(1;5)(p10;p10),add(2)(p21),-7,-17,-18,-19,+2mar[21]	*PTPN11; TP53 (2 mutations)*	+	+	+	+	-	-	-	-	-	-	-	-	+	NP	-	NP	-	+	+	-	NP	NP	NP	Subset	-
19	F	48	no	46,XX,del(5)(q31q35)[12]/46,XX[8]	*NRAS*	+	+	+	+	-	-	-	-	-	+	+	+	+	NP	-	NP	NP	+	+	+	NP	NP	NP	-	-

Table 4 (continued)

Patient	Gender	Age	History of MDS or MDS/MPN	Cytogenetics	Pathogenic mutations	CD34	CD117	CD13	CD33	CD15	sCD3	cCD3	MPO flow	CD2	CD4	CD7	CD5	CD56	CD1a	CD19	CD20	CD10	CD123	HLA-DR	CD22	CD79a	lysozyme	CD11b	CD64	TdT
20	F	80	no	55~58<2n>,-X,del(X)(q21),+1,-2,-3,+4,del(4)(q21q31.3),+5,del(5)(q31q35)x2,+6,+6,del(6)(q13q23),+7,add(7)(p15),+8,add(8)(q24.1),+9,+9,add(9)(p24),+11,add(11)(p15),+12,+12,del(12)(p11.2),+13,+13,+14,add(17)(p11.2),+18,+18,+19,-22,-22,+1~7mar[cp19]/46,XX[1]	*TP53; DNMT3A*	+	+	+	+	-	-	-	-	-	-	-	-	-	NP	-	NP	NP	+	+	-	NP	NP	NP	-	-
21	M	71	no	46,XY,der(10)add(10)(p15)add(10)(q22)[10]/41~43,idem,del(3)(q12),der(7;15)(q10;q10),-11,-13,-14,add(18)(q23),-19,add(21)(p11),der(?)ins(?;11)(?;q23q23),+r,+1~2mar[cp10]	*TP53*	+	+	+	+	-	-	-	-	-	+	-	-	-	NP	-	NP	NP	+	-	-	NP	NP	NP	-	-
22	M	68	no	46,XY,?add(5)(q22~31),add(7)(q31~36),-17,+mar,inc[3]/46,XY[2]	*FLT3; PTPN11*	+	-	+	Subset	+	-	-	-	-	+	-	-	-	-	-	-	-	NP	+	-	-	NP	-	-	Subset
23	F	59	no	44,XX,add(3)(q12),del(5)(q13q31),i(11)(q10),add(12)(q24.1),-17,-18[cp12]/44,idem,add(2)(q11.2)[cp7]/44,XY[1]	*IDH1; TP53*	+	+	+	Subset	-	-	-	-	-	+	-	-	NP	-	-	-	NP	NP	+	-	-	NP	-	-	-
24	M	51	no	83~88,XXYY,del(3)(q12),add(7)(p22),?del(20)(q11.2),+7mar[cp4]/46,XY[9]	*TP53*	+	Subset	Subset	Subset	Subset	-	-	-	-	Subset	+	-	-	NP	Subset	-	-	NP	+	Subset	Subset	NP	-	-	-
25	M	34	no	46,XY,del(5)(q31q35)[cp19]/46,XY[1]	+	+	+	-			-				+			NP	-	-	NP	NP	-	NP	NP	NP	NP	NP	-	
26	M	76	yes	46,XY,-6,12p+,+mar[6]/46,XY[14]	*RUNX1; TET2*	+	Subset	-	-	-	-	-	-	-	-	-	-	-	NP	-	-	-	+	+		-	NP	NP	-	Subset
27	M	81	yes	Normal	*EZH2*	+	+	+	Subset	NP	-	-	-	NP	-	-	-	NP	NP	-	-	-	NP	+	-	-	NP	-	-	-
28	M	72	no	47,X,der(Y)t(Y;1)(q11.23~q12;q12~q21),+8,del(20)(q11.2)[19]/46,XY[3]	*PHF6 ; DNMT3A; IDH1; FBXW7*	+	-	Subset	Subset	-	-	-	-	-	-	-	-	-	-	-	-	NP	-	-	NP	-	-	NP	NP	
29	M	79	no	46,Y, der(X)t(X;12)(p11.2;q23)add(X)(q24),add(12)(q23)[17]/47,idem+9[2]/46	*ASXL1; SRSF2; RUNX1; SETBP1*	+	+	+	+	-	-	-	-	-	-	-	-	-	NP	-	NP	NP	+	+	-	NP	NP	NP	-	+
30	F	55	no	46,XX,der(5)t(5;12)(q22q?15),add(21)(q22)[3]/92,idem x2[14]/46,XX[3]	*IDH2*	+	+	-	Subset	NP	-	-	-	NP	NP	NP	NP	-	NP	-	-	-	NP	Subset	-	-	NP	-	-	Subset
31	M	72	no	49,XY,+Y,+11,+15[4]/51,sl,+Y,+Y[9]/46,XY[7]	*IDH2; JAK2*	+	+	+	+	-	-	-	-	-	-	+	-	-	NP	-	NP	-	+	-	-	NP	NP	NP	-	-
32	M	69	no	46,XY,del(11)(q13q23)[20]	*ASXL1; SRSF2; RUNX1*	+	+	+	-	Subset	-	-	-	-	Subset	-	-	-	-	-	-	-	-	Subset	NP	-	-	NP	NP	-

Key

'+' full marker expression

'−' no marker expression

'Subset' partial marker expression

'NP' not performed

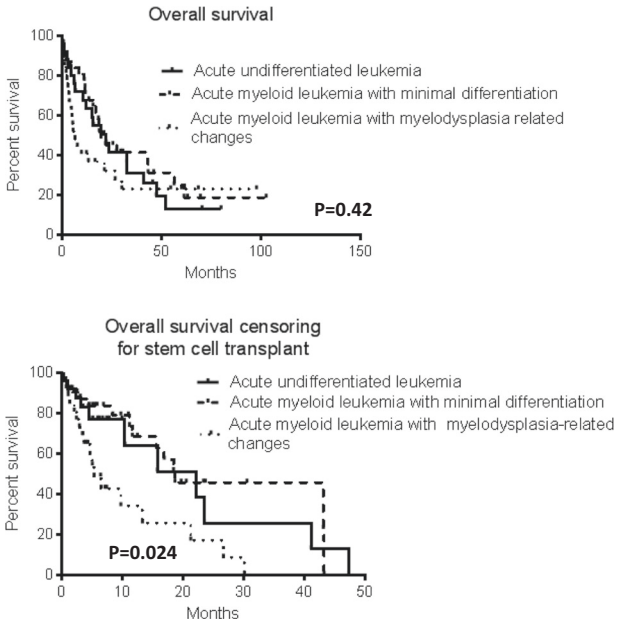

Fig. 2 Kaplan-Meier analysis of outcome of acute undifferentiated leukemia, acute myeloid leukemia with minimal differentiation and acute myeloid leukemia with myelodysplasia-related changes patients. Although there is no difference between three groups in overall survival, when censoring for bone marrow transplant, acute myeloid leukemia with myelodysplasia-related changed has a worse outcome than acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation

Discussion

In this largest to date multi-institution study from eight academic institutions, we identified a total of 92 cases that met immunophenotypic criteria for acute undifferentiated leukemia (33 cases) or acute myeloid leukemia with minimal differentiation (59 cases). Applying the 2016 WHO classification led to re-categorization as categories of acute myeloid leukemia with recurrent genetic abnormalities (6 cases), acute myeloid leukemia with myelodysplasia-related changes (32 cases), acute undifferentiated leukemia [19] and acute myeloid leukemia with minimal differentiation [20]. Many of the acute myeloid leukemia with myelodysplasia-related changes patients showed complex karyotype with frequent *TP53* mutations. Acute myeloid leukemia with complex karyotype constitutes 10–12% of all acute myeloid leukemia patents, frequently occurs with more advanced age, and confers a high risk of treatment failure [21]. Aberrations of the *TP53* gene are seen in high frequency in acute myeloid leukemia with complex karyotype and are associated with even worse outcome [22–25]. Not surprisingly, cases re-classified as acute myeloid leukemia with myelodysplasia-elated changes had shorter survival when compared to acute undifferentiated leukemia (when censoring patients at the time of hematopoietic stem cell transplantation). This finding supports incorporating myelodysplastic syndrome-related cytogenetic findings or clinical history into the final classification of all acute leukemia cases, including acute undifferentiated leukemia.

After applying the WHO classification criteria, we observed an overall lower rate (44%) of abnormal karyotype in acute undifferentiated leukemia as compared to prior studies, which likely reflects reassignment of cases to acute myeloid leukemia MRC. However, similar to prior studies, we found a high frequency of trisomy 13 in acute undifferentiated leukemia. The acute undifferentiated leukemia group showed a similar clinical presentation as acute myeloid leukemia with minimal differentiation but differed from acute myeloid leukemia with minimal differentiation in more frequent expression of TdT. Frequent expression of TdT in acute undifferentiated leukemia cases has also been reported by Heesch et al. [4]. In our study, thirteen cases (48%) of acute undifferentiated leukemia cases lacked expression of CD13 or CD33 and only 6 (22%) cases lacked expression of all myeloid markers including CD117, CD13 and CD33, which highlights the rarity of this stem-cell phenotype. A low rate of aberrant B-cell antigen expression was seen in acute undifferentiated leukemia, while CD7 was frequently co-expressed on blasts of acute undifferentiated leukemia cases with a similar rate as acute myeloid leukemia with minimal differentiation.

Based on these immunophenotypic findings, it is not surprising that most of our acute undifferentiated leukemia patients were treated with acute myeloid leukemia protocols. Therapy regimens in acute undifferentiated leukemia are based on small retrospective studies, reporting variable use of ALL or acute myeloid leukemia treatment protocols [4, 5, 19]. Heesch et al. reported that of 11 acute undifferentiated leukemia patients with available clinical information, 5 received an acute myeloid leukemia protocol, 5 received an ALL protocol and the remaining patient received a mixture of both [4]. In that study, more patients achieved complete remission after ALL protocols as compared to those who received acute myeloid leukemia-type treatment and patients who received hematopoietic stem cell transplantation had a better outcome. Caveats that preclude one from making firm conclusions with regard to therapy include the limited numbers of patients, retrospective nature of the study, and bias related to ability to undergo hematopoietic stem cell transplantation. Kurosawa et al. focused on 10 acute undifferentiated leukemia patients who underwent hematopoietic stem cell transplantation and most of these patients (7/10) were initially treated with an acute myeloid leukemia protocol; the pre-hematopoietic stem cell transplantation karyotype and remission status at the time of hematopoietic stem cell transplantation appeared to influence patient outcome [5]. Clinical outcomes in our study were similar to those of prior studies which utilized both ALL and acute myeloid leukemia regimens. Furthermore,

we find that therapy, including rates of hematopoietic stem cell transplantation, were similar between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation patients.

Little is known of the molecular landscape of acute undifferentiated leukemia from prior studies. Heesch et al. found high frequency of *BAALC, ERG* and *MN1* expression with absence of *WT1* mutations but did not evaluate for presence of other mutations [4]. In our study, we find that acute undifferentiated leukemia patients showed a high frequency of *PHF6* mutations, which were present in 33% present of cases. PHF6 or plant homeodomain finger 6 is a tumor suppressor; mutations of *PHF6* have been reported in 16% of pediatric and 38% of adult T lymphoblastic leukemias [26] but are infrequent in acute myeloid leukemia (3%) [27]. Interestingly, this mutation is also frequently seen in mixed phenotype acute leukemias [28], although our *PHF6* mutated acute undifferentiated leukemia cases did not show a higher frequency of aberrant lymphoid markers as compared to the remaining acute undifferentiated leukemia cases (data not shown). PHF6 appears to modulate chromatin configuration that supports or blocks binding of lineage specific transcription factors and PHF6 loss contributes to treatment resistance in leukemia [26–29]. Thus, one could reasonably speculate that mutations in *PHF6* might contribute to the pathobiology of acute undifferentiated leukemia and the effect of PHF6 may depend on the particular cell of origin harboring the mutation.

SRSF2, one of the common myeloid neoplasm-associated mutations was identified in 40% of the acute undifferentiated leukemia cases as compared to 16% of acute myeloid leukemia with minimal differentiation cases. SRSF2 regulates RNA splicing and mutations of *SRSF2* in acute myeloid leukemia have been associated with secondary acute myeloid leukemia [30]. However, none of *SRSF2* mutated acute undifferentiated leukemia or acute myeloid leukemia with minimal differentiation cases in our study had a clinical history of myelodysplastic syndrome and interestingly, none of the 31 cases re-classified as acute myeloid leukemia with myelodysplasia-related changes bore *SRSF2* mutations. Other frequent mutations found in acute undifferentiated leukemia cases included *RUNX1*, *ASXL1* and *BCOR*. *RUNX1* mutations are present in 10% of acute myeloid leukemia and tend to be associated with older age, male gender, more immature morphology and secondary acute myeloid leukemia evolving from myelodysplastic syndrome or following prior therapy [20]. De novo acute myeloid leukemia with *RUNX1* mutations is a provisional group in the revised WHO classification and thus these cases were not removed from the acute undifferentiated leukemia or acute myeloid leukemia with minimal differentiation categories in this study. We found similar rates of *RUNX1* mutations in both acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation patients and when analyzing the combined *RUNX1*-mutated acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation cases, we did not find a clear difference in outcome from *RUNX1* wild-type acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation cases, although this analysis is limited by a small number of patients. *ASXL1* is mutated in all types of myeloid diseases and often co-occurs with *RUNX1* mutations [31]. *BCOR* mutations are rare in acute myeloid leukemia and account for 4–6% of all cases [32]. The high rate of *RUNX1*, *ASXL1* and *BCOR* mutations in acute undifferentiated leukemia cases (at least one of these being present in 10/19 cases) tends to support a myeloid origin of this disease, as these mutations are rare in lymphoblastic leukemia [32–34].

In summary, we describe the largest series of acute undifferentiated leukemia as defined by the 2016 WHO criteria, which shows this entity exhibits distinct characteristics from acute myeloid leukemia with minimal differentiation. The prognosis of these patients is overall poor and more effective treatments are needed. Albeit with small numbers, we found that clinical outcome was similar between acute undifferentiated leukemia and acute myeloid leukemia with minimal differentiation patients. However, the outcome of patients with acute myeloid leukemia with myelodysplasia-elated changesacute myeloid leukemia with myelodysplasia-related changes was significantly worse than de novo acute undifferentiated leukemia, which supports the WHO classification of cases with history of prior myelodysplastic syndrome and/or myelodysplastic syndrome-type karyotype findings as acute myeloid leukemia with myelodysplasia-related changes.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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