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Immunophenotypic features of acute myeloid leukemia with inv(3)(q21q26.2)/t(3;3)(q21;q26.2)

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Abstract

Immunophenotypic identification of myeloid specific antigens is an important diagnostic tool in the management of patients with acute myeloid leukemia (AML). These antigens allow determination of cell of origin and degree of differentiation of leukemia blasts. AML with inv(3)(q21q26.2)/t(3;3)(q21;q26.2) is a relatively rare subtype of AML. The immunophenotypic characteristics of inv(3) AML patients are somewhat limited. We identified 14 new cases of hematological disorders with increased myeloid blasts carrying inv(3)(q21q26.2)/t(3;3)(q21;q26.2). Also, we identified another 13 cases previously published in the literature, where the immunophenotype of inv(3)(q21q26.2) was documented. As a group, patients with AML with inv(3)(q21q26.2) had high levels of early myeloid (CD13, CD33, CD117 and MPO) and uncommitted markers (CD34, HLA-DR and CD56) and a high rate of monosomy 7 in addition to the inv(3)(q21q26.2). Differential karyotype and expression of certain antigens were noted in patients with *de novo* AML with inv(3)(q21q26.2) vs. those with inv(3)(q21q26.2)-containing blasts.

Keywords

AML; Flow cytometry; Inv(3)(q21q26.2); WHO classification

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Conflict of interest

The authors declare no competing financial interests.

Contributions. BCM and AAA designed the study; BCM, DB, AC, RM, KEK, CAA, SP, HEK, DAA and AAA obtained and analyzed data; BCM and AAA wrote the paper; and all authors read, gave comments, and approved of the final version of the manuscript.

1. Introduction

The revised 2008 World Health Organization (WHO) Classification of Tumours of Hematopoietic and Lymphoid Tissues recognizes acute myeloid leukemia (AML) with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) as a separate entity of AML with recurrent genetic abnormalities of prognostic significance [1]. This genetic abnormality involves *EVI1* at 3q26.2 and *RPN1* at 3q21, leading to the RPN1–EVI1 fusion transcript [2]. AML with inv(3) represents 1–2% of all AMLs and it is associated with normal or elevated platelet counts, atypical bone marrow megakaryocytes and multilineage dysplasia. Clinically, patients with AML with inv(3) carry a poor prognosis, commonly being refractory to conventional chemotherapy, and having an overall short survival [3]. The most frequent concurrent cytogenetic abnormality among patients with inv(3) or t(3;3) is monosomy 7 which carries an even worse prognosis [1].

Immunophenotyping is a major diagnostic tool to assign acute leukemia blast cells to a specific lineage [4]. However, as noted by the 2008 WHO classification, the immunophenotypic features of AML with inv(3)(q21q26.2) are poorly characterized [1,3]. For this reason, we identified 14 cases referred to our institution with inv(3)(q21q26.2) where immunophenotypic characterization was available. Furthermore, we compiled the published immunophenotypic features of all 13 previously described cases with inv(3)(q21q26.2) since its initial description in 1997.

2. Materials and methods

2.1. Patients

A total of 19 patients with inv(3)(q21q26.2) or t(3;3)(q21;q26.2) diagnosed at Stanford University Medical Center between 1989 and 2009 were identified from Stanford Acute Leukemia Database of ~2300 cases. One additional patient was diagnosed at Kaiser Permanente Medical Group in Denver, CO. Clinical parameters, baseline laboratory data, and flow cytometry results at the time of diagnosis were reviewed. Clinical follow-up information was obtained by retrospective review of the electronic charts. Six of 19 patients from our Stanford cohort with incomplete clinical information, including immunophenotypic data, were excluded. Stanford University's Research Compliance Office has approved this study (Stanford IRB ID # 15652).

2.2. Immunologic studies of the leukemic cells

Over the many years expanding the diagnosis of these patients, we performed 2-, 3- or 4-color flow cytometric analysis using a FACScan or FACSCalibur flow cytometer (BD Biosciences, San Jose, CA) on peripheral blood or bone marrow aspirate specimens collected in EDTA. After incubation of the cells with monoclonal antibodies for 10 min at 4 °C, the erythrocytes were lysed with NH_4 Cl for 10 min, followed by two washing steps using phosphate-buffered saline solution. The cells were then resuspended and fixed with 1% paraformaldehyde. A panel containing the following fluorescein isothiocyanate (FITC), or phycoerythrin (PE)-conjugated monoclonal antibodies (MoAb) was used for diagnosis: CD7, CD13, CD38, CD33, CD34, CD36, CD41, CD42, and CD117, CD2, CD4, CD11c,

CD14, HLA-DR, MPO, CD56. CD61, CD64. Cases were considered positive if 20% or more of the cells expressed the specific antigen in the CD45 gate. A similar 4-color flow cytometric analysis was performed on the patient diagnosed at the Denver Institution.

2.3. Selection of studies of immunophenotypic features of inv(3)(q21q26.2) or t(3;3)(q21;q26.2)

Studies were eligible for inclusion if they met all the following criteria. (1) They were published between 1997 and 2009 as original articles written in English and (2) they described the immunophenotype of patients with inv(3)(q21q26.2) and/or t(3;3)(q21;q26.2). Single case reports or small case series were included in this analysis. A computerized literature search of the MEDLINE database was conducted by using the free text search term *AML* AND *inv(3)* or *EVI1* or *t(3;3)* AND *immunophenotype* or *Flow cytometry*, with the publication period limited to between 1997 and 2009, and the language to English. Cited references in selected papers were included in literature review. A total of six manuscripts describing a total of 13 patients were considered to meet all the criteria specified above [3,5–9].

2.4. Statistical analysis

Survival times were calculated from the day of diagnosis until death. The median overall survival was determined by Kaplan–Meyer estimates and compared by log-rank test. Groups were compared with the Pearson Chi-Square or Fisher's Exact tests, as appropriate. All *p*-values are two-sided and only *p*-values < 0.05 were considered statistically significant.

3. Results

3.1. Clinical features of patients with inv(3)

For this analysis patients were divided into two major groups. The first cohort of patients represents those patients who would fulfill diagnostic criteria for AML with inv(3)(q21q26.2) according to the revised 2008 WHO classification [1]. The second group of patients corresponds to patients whose myeloid blasts also carried inv(3)(q21q26.2), but that would fall into separate categories under the WHO classification (i.e. AML with myelodysplasia-related changes or blast phase chronic myeloid leukemia [CML]). Baseline clinical characteristics for the 14 patients presented here and those previously reported in the literature are found in Table 1. For the 15 patients in cohort 1, there were 7 males and the median age was 51 years (range 20–87 years). All patients had *de novo* AML and the FAB classification was known in 11/15 (M0-4; M1-1; M2-1; M4-4). Median white blood cell count and circulating blast count were $9.5 \times 10^9\ L^{-1}$ (range $2.9–438 \times 10^9\ L^{-1}$) and $4.8 \times 10^9\ L^{-1}$ (range $0–221 \times 10^9\ L^{-1}$), respectively. Median hemoglobin and platelet count at presentation were 9.7 g/dL (range 5.6–13.6 g/dL) and $92 \times 10^9\ L^{-1}$ (range $27–903 \times 10^9\ L^{-1}$), respectively. Inv(3) or t(3;3) was the sole abnormality in 4 patients, monosomy 7 in addition to inv(3)(q21q26.2) was found in another 9 patients. One patient had both monosomy 7 and del(5q) and another del(5q) in addition to inv(3)(q21q26.2). Briefly, cohort 2 was comprised of 12 patients with inv(3)(q21q26.2) abnormality. The median age was 57 years (range 21–74 years), including 5 females. Secondary AML (s-AML) was found in 5

patients, CML in blast phase in 3 patients and myelofibrosis (MMM), MDS and therapy-related AML in one patient each.

3.2. Immunologic features

The summarized results of the immunophenotyping on all 27 patients with inv(3) are presented in Table 2. The ratio of positive cases over the total number of cases tested for each specific antigen is shown. In brief, more then 80% of the patients with inv(3)(q21q26)/t(3;3)(q21;q26.2) were positive for CD33, CD13, CD117, CD34 and HLA-DR. Two other antigens were noted in >40% of cases (CD7, CD56). Three different antigens were positive in less than 20% of the cases tested and were not included in Table 2 (CD2—14% [3/22], CD4—19% [4/21], and CD61—5% [1/20]). Furthermore, when patients from cohort 1 were stratified based on the presence or absence of monosomy 7, differential expression of four antigens was noted (MPO—38% vs. 0% ($p = 0.15$), CD38—11% vs. 40% ($p = 0.34$), CD7—60% vs. 20% ($p = 0.28$) and CD56—13% vs. 60% ($p = 0.09$)). When analyzed separately, the 2 cohorts demonstrated non-statistically significant differences in expression of certain markers. For example, CD117 (100% vs. 69%), CD56 (57% vs. 31%) and MPO (57% vs. 23%) expression was significantly more pronounced in cohort 2. Meanwhile, expression of CD7 (47% vs. 32%), CD14 (36% vs. 0%) and especially CD11c (50% vs. 9%) was more commonly found in patients in cohort 1.

3.3. Outcome

AML with inv(3)(q21q26)/t(3;3)(q21;q26.2) is considered to be an aggressive disease with high rates of resistance to chemotherapy and short survival. Although, this was not the primary objective of this study, we noted that all cases presented here either died or relapsed within a median of 9 months from diagnosis (cohort 1—6 months [range 1–23 months]; cohort 2—12 months [range 1–24 months]). Cases with monosomy 7 are felt to carry a worse overall prognosis [10], however on our series; there were no statistically significant differences in the median survival (7 months vs. 9 months) in cases with monosomy 7 when compared to the entire cohort 1 group.

4. Discussion

The present report further elucidates the immunophenotypic features of leukemia blasts in patients with inv(3)(q21q26). The predominant immunophenotype characterized by expression of the pan-myeloid antigens CD33, CD13, CD117 as well as the immature markers CD34 and HLA-DR was noted in >80% of patients. Contrary to the revised 2008 WHO classification, CD38 appears to be expressed in only 28% of cases and megakaryocytic markers (CD61) are extremely uncommon (<10%) [1]. Also, approximately 35–50% of cases aberrantly express CD7, CD56, CD11c and MPO. However, primary cases of inv(3)(q21q26.2) appear to have less pronounced MPO and CD56 expression and express more commonly CD11c and CD7. Lastly, we observed high levels of expression of CD117 in patients with inv(3)(q21q26), a finding not extensively documented in the past [3].

Although the combination of these immunophenotypic markers is not unique enough to allow immunophenotypic distinction of inv(3)(q21q26) cases, the co-expression of early

myeloid markers, such as CD13, CD33, CD117 and less commonly MPO in association with markers of uncommitted cells (CD34, HLA-DR and less commonly CD56), suggests cases of AML with inv(3)(q21q26) derive from relatively undifferentiated hematopoietic precursors [11].

The retrospective nature of our study is a significant limitation. Although careful review of the bone marrow reports and descriptions were conducted, we cannot completely exclude the possibility that dysplastic features were missed and our cases were misclassified as AML with recurrent genetic abnormalities as opposed to AML with MDS-related changes. Nonetheless, the immunophenotypic features reported here suggest that some antigens are commonly expressed in patients with inv(3), irrespective of their origin. Also, our data suggests that perhaps some antigens are more frequently detected in AML with MDS-related changes while others may be more common in primary cases of inv(3)(q21q26.2), although some of these differences, such as the high rate of CD56 and CD11c, could also be due to a more frequent than identified rate of underlying myelodysplasia.

Another important difference noted in these patients is the high rate of monosomy 7 in our cohort. Although previous reports suggest monosomy 7 occurs in approximately 50% of the cases, we found a significantly higher rate of monosomy 7 in cohort 1 (66%). Interestingly, 3 out the 8 new patients from cohort 1 presented here had a monosomy, compared to 7/7 patients previously described patients. Although the cases from cohort 1 with monosomy 7 did not appear to have worse overall survival when compared to the entire group, these patients presented some interesting differences in immunophenotyping compared to patients lacking monosomy 7.

In summary, we confirm patients with inv(3)(q21q26) or t(3;3)(q21;q26.2), present with high platelet count and have a poor prognosis with inferior response to therapy and short overall survival. More importantly, we provide a comprehensive analysis on the immunophenotypic features of patients with this specific entity of AML with recurrent genetic abnormalities.

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References

1. Swerdlow, SH., Campo, E., Harris, NL., Jaffe, ES., Pileri, SA., Stein, H., et al. WHO Classification of tumours of haematopoietic and lymphoid tissues. 4. WHO Press; 2008. p. 439
2. Barjesteh van Waalwijk van Doorn-Khosrovani S, Erpelinck C, van Putten WL, et al. High EVI1 expression predicts poor survival in acute myeloid leukemia: a study of 319 de novo AML patients. Blood. 2003; 101:837–45. [PubMed: 12393383]
3. Shi G, Weh HJ, Dührsen U, Zeller W, Hossfeld DK. Chromosomal abnormality inv(3)(q21q26) associated with multilineage hematopoietic progenitor cells in hematopoietic malignancies. Cancer Genet Cytogenet. 1997; 96:58–63. [PubMed: 9209472]
4. Casasnovas RO, Campos L, Mugneret F, Charrin C, Béné MC, Garand R, et al. Immunophenotypic patterns and cytogenetic anomalies in acute non-lymphoblastic leukemia subtypes: a prospective study of 432 patients. Leukemia. 1998; 12:34–43. [PubMed: 9436918]

5. Fujisawa S, Tanabe J, Harano H, et al. Acute minimally differentiated myeloid leukemia (M0) with inv(3)(q21q26). Leuk Lymphoma. 1999; 35:627–30. [PubMed: 10609803]
6. Glasser L, Meloni-Ehrig A, Greaves W, et al. Synchronous development of acute myeloid leukemia in recipient and donor after allogeneic bone marrow transplantation: report of a case with comments on donor evaluation. Transfusion. 2009; 49:555–62. [PubMed: 19040490]
7. Keung YK, Buss D, Powell BL, Pettenati M. Central diabetes insipidus and inv(3)(q21q26) and monosomy 7 in acute myeloid leukemia. Cancer Genet Cytogenet. 2002; 136:78–81. [PubMed: 12165457]
8. Wieser R, Schreiner U, Wollenberg B, Neubauer A, Fonatsch C, Rieder H. Masked inv(3)(q21q26) in a patient with minimally differentiated acute myeloid leukemia. Haematologica. 2001; 86:214–5. [PubMed: 11224497]
9. Igarashi T, Shimizu S, Morishita K, Ohtsu T, Itoh K, Minami H, Fujii H, Sasaki Y, Mukai K. Acute myelogenous leukemia with monosomy 7, inv(3)(q21q26), involving activated EVI 1 gene occurring after a complete remission of lymphoblastic lymphoma: a case report. Jpn J Clin Oncol. 1998; 28:688–95. [PubMed: 9861236]
10. Secker-Walker LM, Mehta A, Bain B. Abnormalities of 3q21 and 3q26 in myeloid malignancy: a United Kingdom Cancer Cytogenetic Group study. Br J Haematol. 1995; 91:490–501. [PubMed: 8547101]
11. Paietta, E. Immunobiology of acute leukemia. In: Wiernik, PH., et al., editors. Neoplastic diseases of the blood. 4. New York: Churchill Livingstone; 1996. p. 194-231.
12. Schaffer, LG.Slovak, ML., Campbell, LJ., editors. ISCN: an International System for Human Cytogenetic Nomenclature. Basel: S. Karger; 2009.

Table 1

Baseline characteristics of the patients with inv(3)(q21q26) and/or 46,XX,t(3;3)(q21;q26).

#	Age/gender	Diagnosis	WBC	Blast	Hb	Plat	Karyotype [12]	Outcome
WHO 2008 AML with inv(3)(q21q26.2)								
1	55/M	AML-M4	9.8	7	10	197	46,XY,inv(3)(q21q26)[21]	DC/11mos
2	87/M	AML-M0	21.1	15.4	10	64	46,XY,inv(3)(q21q26)[21]	DC/1mos
3	48/F	AML-M4	10.5	7.6	8.9	203	45,XX,inv(3)(q21q26),−7[16]/46,XX[4]	DC/8mos
4	66/F	AML-M0	14	12.6	9.9	92	46,XX,inv(3)(q21q26),del(5)(q13q33)[21]	DC/1mos
5	28/F	AML	3.9	0	8.4	27	46,XY,inv(3)(q21q26.2)[20]	Rel/23 mos
6	39/F	AML	14.2	4.8	13	346	45,XY,inv(3)(q21q26.2),del(5)(q22q31),−7[19]	DC/5mos
7	78/F	AML-M4	95	86	11	45	46,XX,t(3;3)(q21;q26)[15]	DC/1mos
8	51/M	AML-M1	3.8	0	7.5	195	45,XY,t(3;3)(q21;q26),−7[10]	DC/6mos
References								
3	53/F	AML-M2	6.2	1.3	9.5	62	46,XX,inv(3)[4]/45,idem,−7[28]	DC/11mos
3	20/M	AML-M4	438	221	5.6	179	45,XY,inv(3),−7[12]/46,XY[3]	DC/12mos
5	55/M	AML-M0	2.9	0.45	6.1	36	45,XY,inv(3)(q21q26),−7[18]/46,XY[3]	DC/10mos
6	53/M	AML	4	0.42	8.4	58	45,XY,inv(3)(q21q26),−7[20]	DC/1mos
7	31/M	AML	8.8	1.2	13.6	206	46,XXY,inv(3)(q21q26),−7[18]/47,XXY[2]	DC/2mos
8	37/F	AML-M0	9.5	6.7	N/A	903	45,XX,der(6)ins(6;3)(q23;q21q26)inv(3)(q21q26),−7[25]	Rel/2mos
9	48/F	AML-M4	5.8	1.4	9.7	63	46,XX,inv(3)(q21q26)[10]/45,idem,−7[7]/46,XX[3]	DC/11mos
Inv(3)(q21q26.2)-containing blasts								
9	29/M	CML-BP	7.9	0.2	14	449	46,XY,inv(3)(q21q26.2),t(9;22)(q34;q11.2)[20]	DC/9mos
10	70/M	MMM	30.7	6	9.7	61	46,XY,inv(3)(q21q26)[5]	DC/1mos
11	70/F	s-AML	3.5	0.9	9.2	165	45,XX,inv(3)(q21q26),−7[4]/46,XX[1]	DC/7mos
12	62/M	CML-BP	51.5	39	9.6	622	46,XY,inv(3)(q21q26),t(9;22)(q34;q11.2)[3]	DC/12mos
13	21/F	s-AML	1.5	0.3	6.4	108	46,XX,inv(3)(q21q26)[17]/46,idem,−7, +mar[3]	DC/16mos
14	23/M	t-AML	1.8	0.2	13	33	46,XY,inv(3)(q21q26),t(6;12)(q12;q24)[13]/46XY[8]	DC/24mos
References								
3	32/M	s-AML	64.7	39	6.7	477	45,XY,inv(3),−7[21]/46,XY[4]	DC/12mos
3	63/F	s-AML	8.7	8	11	112	45,XY,inv(3),−7[18]/46,XY[2]	DC/5mos
3	52/M	s-AML	22.8	11	10	647	45,XY,inv(3),−7[8]/46,XY[3]	DC/15mos

#	Age/gender	Diagnosis	WBC	Blast	Hb	Plat	Karyotype [12]	Outcome
3	74/M	CML	13.1	1.3	8	179	46,XY,inv(3)[12]/46,XY,idem,t(9;22)[35]	DC/NA
3	73/F	s-AML	1.9	0.2	6.3	181	46,XX,der(3)(q21 → p21::q26 → q21::q27 → q29::p21 → p26) del(5)(q13q33)[13]/46,XX[12]	DC/12mos
6	42/F	MDS	N/A	N/A	N/A	N/A	46,XY,inv(3)(q21q26)[10]/46,XY[3]	DC/20mos

WBC: white blood cell count ($\times 10^9$ L^{-1}), Blast: peripheral blood absolute leukemia blast count ($\times 10^9$ L^{-1}), Hb: hemoglobin (g/dL), Plat: platelet count ($\times 10^9$ L^{-1}), Rel: relapsed, DC: deceased, MDS: myelodysplasia, MMM: myelofibrosis, CMP-BP: blastic phase chronic myelogenous leukemia, t-AML: therapy-related AML, s-AML: secondary AML.

Table 2

Immunophenotypic features of patients with inv(3).

	CD34	HLA-DR	CD38	CD33	CD13	CD117	MPO	CD7	CD14	CD64	CD56	CD11c
Overall patients												
Ratio	22/26	21/26	7/25	25/27	26/27	16/20	7/20	14/27	4/21	4/20	8/20	9/25
%	85	81	28	93	96	80	35	52	19	20	40	36
WHO 2008 AML with inv(3)(q21q26.2)												
Ratio	13/15	11/14	3/14	15/15	15/15	9/13	3/13	7/15	5/14	4/13	4/13	7/14
%	87	79	21	100	100	69	23	47	36	31	31	50
Inv(3)(q21q26.2)-containing blasts												
Ratio	9/11	10/12	4/11	10/12	11/12	7/7	4/7	7/12	0/7	1/7	4/7	1/11
%	82	83	36	83	92	100	57	32	0	14	57	9

AML, acute myeloid leukemia; WHO, World Health Organization; CDs antibodies tested.