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CD34-Negative is Highly Associated with T (15;17), T (V; 11q23) and the NPM1-Mutation Subtypes in 343 Newly Diagnosed Patients with Acute Myeloid Leukemia

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Abstract

Objective: Recent reports found that several cytogenetic or molecular subtypes of acute myeloid leukemia (AML) are associated with CD34-positive. However, the status of CD34-negative in AML needs to be explored. In this study, we aimed to explore the prevalence of the CD34-negative patients and its association with molecular genetics status in a large consecutive AML cohort.

Methods: A group of 343 consecutive newly diagnosed AML patients was retrospectively analyzed in our center. CD34 expression was detected by flow cytometry and considered negative when it was expressed in less than 20% of the bone marrow blast cells. The karyotype was analyzed by the G-banding technique. Leukemic fusion genes and mutated genes were detected by PCR method.

Results: CD34-negative was found in 143 (41.7%) of the 343 patients. According to FAB classification, the percentages of CD34-negative patients were higher in the M3 and M5 (100% and 70%, respectively) and lower in the M2 and M4 subtypes (30.3% and 21.2%, respectively). According to the WHO classification, the percentage of CD34-negative patients was higher in those with t(15; 17), t(v; 11q23), and the NPM1-mutation (100%, n=37; 100%, n=7; and 81.7%, n=71, respectively) and lower in those with t(8;21) and AML with MDS-related changes (8.6%, n=35 and 5.0%, n=20, respectively). The patients with t(15; 17), t(v; 11q23) and the NPM1-mutation consisted of 71.3% (102/143) of the CD34-negative population and 6.5% (13/200) of the CD34-positive population ($p<0.0001$). A CD34-negative phenotype was associated with risk subgroups according to cytogenetics alone and when combining cytogenetics and molecular analysis ($p=0.025$ and $p<0.0001$, respectively). The sensitivity, specificity, positive-predictive value and negative-predictive value of the CD34-negative to t (15;17), t (v; 11q23) and the NPM1-mutation were 88.7%, 82.0%, 71.3% and 93.5%, respectively.

Conclusions: The prevalence of the CD34-negative patients is very common in newly diagnosed AML. CD34-negative is highly associated with t (15;17), t(v; 11q23) and the NPM1-mutation in AML patients, which provide the evidence about the association of immunophenotype and molecular genetics.

Key words:

Acute myeloid leukemia; CD34; t(15;17); NPM1-mutation; MLL-rearrangement; cytogenetics; Molecular analysis

Introduction

Less than half of patients with acute myeloid leukemia (AML) are CD34-negative (<20% blast cells expressing CD34) [1-10]. Earlier studies showed that CD34-negative was most frequently associated with the M3 and M5 subtypes, while CD34-positive was associated with the M0, M1 and M4 subtypes [1,2,5,7]. Most, but not all, previous studies have indicated that CD34-negative AML patients have more favorable outcomes compared to CD34-positive patients [4]; therefore, these inconsistent conclusions need to be further explored.

The cytogenetics and molecular abnormalities are a major prognostic factor for AML [11]; however, other biological and disease-specific risk factors have been postulated [12]. CD34 expression is a frequently studied risk factor, though some information is still debated [1-10]. Recent studies showed that specific AML subtypes were associated with a CD34-negative phenotype, including acute promyelocytic leukemia (APL) and NPM1-mutated AML [13,14]. AML patients with the NPM1-mutation and FLT3-ITD were classified into the poor-risk group, and most of these patients were CD34-negative [14]. Therefore, the prognostic value of CD34 in AML should be explored under cytogenetic and molecular circumstances. Furthermore, the incidence of CD34-negativity among different subtypes described by the 2008 World Health Organization (WHO) classification is scarcely studied [15].

In this study, we performed a systematic retrospective study to determine the relevance of CD34-negative and molecular genetics subgroups in 343 newly diagnosed AML patients in our center, which can be used to better understand the association of immunophenotype and molecular genetics.

Patients and Methods

Patients

From January 2011 until January 2012, 343 newly diagnosed AML patients at Peking University People's Hospital were included in this study. Bone marrow samples were used for diagnostic analysis. The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Peking University People's Hospital

Diagnostic methods and risk status definition

AML diagnosis was based on morphology, immunology, cytogenetics and molecular status as previously described [11,16]. Morphology was identified with a standard technique. Karyotype was analyzed by the G-banding technique. Immunophenotyping was performed by the CD45/SSC gated 4-color flow cytometry as previously described [17], using monoclonal antibodies directed against the following proteins: CD34, CD117, CD33, CD7, CD10, CD19, CD13, CD64, CD11b, CD15, CD123, HLA-DR, CD56, CD9, CD38, CD4, CD2, CD14 and CD45. CD14 and CD7 were purchased from Beckman-Coulter (Hialeah, FL), and the other antibodies were obtained from BD Biosciences (San Jose, CA). An antigen was considered positive when it was expressed by more than 20% of the blast cells. Molecular screening for the leukemic fusion genes and mutated genes was offered to all of the patients as previously described [18,19].

The risk status of AML was according to NCCN Guidelines Version 2.2012 Acute Myeloid Leukemia. According to cytogenetics, AML patients were classified as better-risk (including t (15; 17), t (8;21) and inv (6)/t(16; 16)), intermediate-risk (including normal cytogenetics, +8, t (9; 11) and other non-defined) and poor-risk (including complex cytogenetics, -5, 5q-, -7, 7q-, 11q23 –non t (9; 11), inv (3)/t (3; 3), t (6;9), t (9;22)). According to cytogenetics and molecular abnormalities, AML patients were classified as better-risk (including t (15; 17), t (8;21) and inv (6)/t(16; 16) without the c-KIT mutation, normal cytogenetics with the NPM1-mutation in the absence of FLT3-ITD), intermediate-risk (including normal cytogenetics, +8, t (9; 11) and other non-defined, t (8;21) and inv (6)/t(16; 16) with the c-KIT mutation) and poor-risk (including complex cytogenetics, -5, 5q-, -7, 7q-, 11q23 –non t (9; 11), inv (3)/t (3; 3), t (6;9), t (9;22), normal cytogenetics with FLT3-ITD).

Statistical methods

The comparison of clinical or laboratory parameters between the patient subgroups was performed using the non-parametric Mann-Whitney-U test for continuous variables and the $\chi 2$ test for categorical data. These variables included age, sex, white blood cell (WBC) count, hemoglobin (HGB) and platelets (PLT) subgroups at diagnosis according to the FAB classification and the WHO classification. An effect was considered to be statistically significant if the P value was <0.05. All of the statistical analyses were performed using SPSS version 16.0 (SPSS, Chicago, IL, USA).

Results

Patients

This study involved 343 newly diagnosed AML patients before they started induction chemotherapy. The patient characteristics are listed in Table 1. The median age of these patients was 45 years old, and the ages ranged from 3 to 88 years. Among these patients, 204 were male while 139 were female. The numbers of subgroups for the FAB morphologic classification and the WHO classification are shown in Table 1 and Table 2.

	number	range
Age (yrs)	45	Mar-88
Sex (M/F)	204/139	
WBC (10^9/L)	10.4	0.4-404
HGB (g/L)	81	26-187
PLT (10^9/L)	45	2.5-525
FAB subtypes (n,%)		
M0	3	0.87%
M1	15	4.37%
M2	175	51.02%
M3	37	10.79%
M4	33	9.62%
M5	40	11.66%
M6	20	5.83%
MDS-AML	20	5.83%
Cytogenetics (n, %)		
better-risk	86	25.07%
intermediate-risk	196	57.14%
poor-risk	43	12.54%
unknown	18	5.25%

Table 1: AML patient characteristics.

	CD34-positive	CD34-negative	Total
Classification	n (%)	n (%)	n
FAB classification			
M0	3 (100)	0 (0)	3
M1	7 (46.7)	8 (53.3)	15
M2	122 (69.7)	53(30.3)	175
M3	0 (0)	37 (100)	37
M4	26 (78.8)	7 (21.2)	33

M5	12 (30.0)	28 (70.0)	40
M6	11 (55.0	9 (45.0)	20
RAEB-t	19 (95)	1 (5)	20
Total	200	143	343
WHO classification			
t(8;21)	32 (91.4)	3 (8.6)	35
t(15;17)	0 (0)	37 (100)	37
in(16)/t(16;16)	11 (78.6)	3 (21.4)	14
t(v;11q23)	0 (0)	7 (100)	7
in(3)/t(3;3)	4 (100)	0 (0)	4
mutated NPM1	13 (18.3)	58 (81.7)	71
MDS-related changes	19 (95)	1 (5)	20
Not otherwise specified			
Minimal differentiation	3 (100)	0 (0)	3
without maturation	7 (77.8)	2 (22.2)	9
with maturation	83 (86.5)	13 (13.5)	96
myelomonocytic	7 (87.5)	1 (12.5)	8
monoblastic and monocytic	11 (47.8)	12 (52.2)	23
erythroid	10 (62.5)	6 (37.5)	16
Total	200	143	343

Table 2: Association between CD34 status and subgroups of acute myeloid leukemia.

The association between CD34-negative status and the FAB and WHO subtypes

The percentage CD34-negative patients was 41.7% (102/143) from the entire AML patient group. The association between CD34-negativity and the FAB subtypes is presented in Table 2. The percentage of CD34-negative patients was higher among the M3 and M5 subtypes (100% and 72.5%, respectively) and was lower among the M0, M2 and M4 subtypes (0%, 33.7% and 27.3%, respectively).

The association between CD34-negative patients and the WHO subtypes is presented in Table 2. The percentage of CD34-negative patients was higher among those with t(15; 17), t(v; 11q23) and the NPM1-mutation (100%, 100% and 85.9%, respectively) and lower among those with t(8;21) and AML with MDS-related changes (8.6% and 5.0%, respectively). The patients with t(15; 17), t(v; 11q23) and the NPM1-mutation constituted 71.3% (102/143) of the CD34-negative population but only 6.5% (13/200) of CD34-positive population (p<0.0001) (Figure 1) .

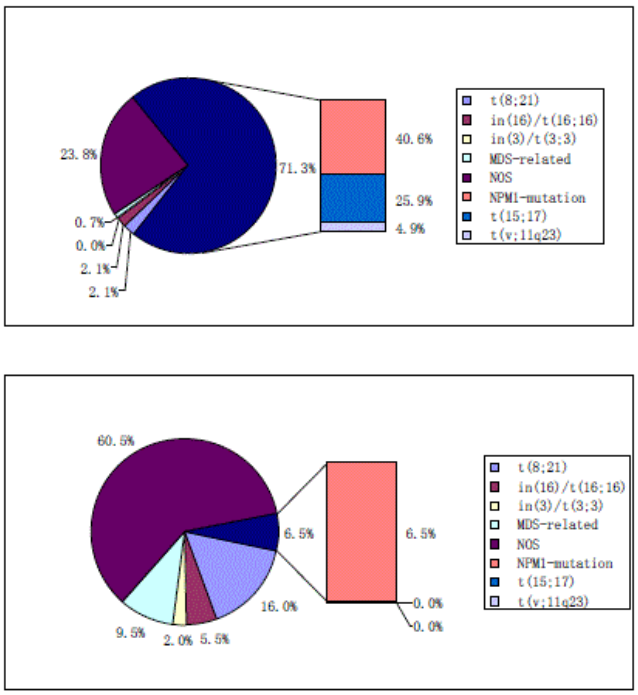

Figure 1: Incidence of AML subtypes in patients with CD34-negativity (upper) and CD34-positivity (lower).

The association between CD34-negativity and the risk status based on cytogenetics alone or combining cytogenetics and molecular analysis

The percentage of CD34-negative patients was significantly different among better-risk, intermediate-risk and poor-risk subgroups according to cytogenetics alone or combining cytogenetics and molecular analysis (p=0.025 and p<0.0001, respectively) (Table 3). According to the at-risk group based on cytogenetics alone (except for patients without available karyotypes, n=18), the percentage of poor-risk patients was lower in the CD34-negative groups compared with the CD34-positive groups (7.7% vs. 17.5%, respectively, p=0.012), while no difference existed between the CD34-negative groups and CD34-positive groups for the better-risk (30.3% vs. 23.5%, p=0.2, respectively) or intermediate-risk groups (62.0% vs. 59.0%, p=0.64, respectively) (Figure 2).

	Total	**CD34+**	**CD34-**	
Risk-stratification	n	n (%)	n (%)	p value
Cytogenetics				0.025
better-risk	86	43 (50.0)	43 (50.0)	
intermediate-risk	196	108 (55.1)	88 (44.9)	
poor-risk	43	32 (74.4)	11 (25.6)	

unknown	18	17 (94.4)	1 (5.6)	
Total	343	200	143	
Cytogenetics and Molecular Analysis				<0.000 1
better-risk	101	33 (32.7)	68 (67.3)	
t(8;21) without KIT-mutation	23	22 (95.7)	1 (4.3)	
t(15;17)	37	0 (0)	37 (100	
in(16)/t(16;16) without KIT-mutation	13	10 (76.9)	3 (23.1)	
NK: NPM1+ without FLT3-ITD	28	1 (3.6)	27 (96.4)	
intermediate-risk	149	101 (67.8)	48 (32.2)	
CBF-AML and KIT-mutation	13	11 (84.6)	2 (15.4)	
poor-risk	75	49 (65.3)	26 (34.7)	
NK and FLT3-ITD+	37	17 (45.9)	20 (54.1)	
unknown	18	17 (94.4)	1 (5.6)	
Total	343	200	143	

Table 3: Associations between CD34 status and the risk-groups of acute myeloid leukemia.

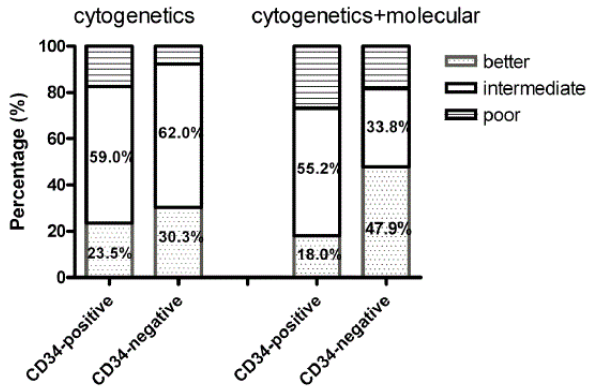

Figure 2: The distribution of the three risk groups according to cytogenetics alone or combined with molecular analyses in CD34-positive and CD34-negative patients.

According to the at-risk group based on combining cytogenetics and molecular analysis, the percentage of the better-risk group was higher (47.9% vs. 18.0%, p<0.001) and the intermediate-risk group was lower (33.8% vs. 55.2%, p=0.0001) in the CD34-negative groups than in the CD34-positive groups, respectively; no difference was observed in the poor-risk groups (26.8% vs. 18.3%, p=0.08, respectively) (Figure 2).

The sensitivity, specificity, positive-predictive value and negative-predictive value of CD34-negativity for t (15;17), t(v; 11q23) and the NPM1-mutation

The sensitivity, specificity, positive-predictive value and negative-predictive value of CD34-negativity for t (15;17), t (v; 11q23) and the NPM1-mutation were 88.7% (102/115), 82.0% (187/228), 71.3% (102/143) and 93.5% (187/200), respectively.

Discussion

In this study, we performed a systematic study to explore the relevance of CD34-negativity in molecular genetics subgroups within 343 AML patients. We found that the prevalence of the CD34-negative patients is very common in newly diagnosed AML. CD34-negativity was highly associated with the subtypes t(15; 17), t(v; 11q23) and the NPM1-mutation. Furthermore, CD34-negativity was associated with risk subgroups according to cytogenetics alone or combining cytogenetics and molecular analyses, which helped reassess the prognostic value of CD34.

Although most AML patients were CD34-positive, the frequency of CD34 expression in particular subtypes of AML was dramatically different [13]. We found that CD34-negativity was presented in 41.7% of the AML patients, and the percentage of CD34-negative patients was higher in the M3 and M5 subtypes and lower in the M0, M2 and M4 subtypes. Our results agree with previous studies, which showed that CD34-positivity is frequently associated with the M0, M1 and M4 subtypes, while the M3 and M5 subtypes are associated with CD34-negativity [1,2,5,7,15].

Recognizing the heterogeneity of the FAB subtypes within AML led to the establishment of the 2008 World Health Organization (WHO) classification mainly based on cytogenetics and molecular abnormalities. The CD34 expression patterns and prognostic values have scarcely been investigated in new diagnosed AML [15]. Although one Chinese study did not find a correlation between CD34 and genetic changes in the cases of 180 AML patients, they did find an obvious correlation between the immunophenotype and cytogenetic abnormalities in t(8;21) and non t(8;21) subtypes [15]. We found that, under the WHO classification, based on cytogenetics and molecular analyses, the percentage of CD34-negative patients was higher with t(15; 17), t(v; 11q23), and the NPM1-mutation and lower with t(8;21) and AML with MDS-related changes. CD34-negativity, as well as HLA-DR- and CD11b-negativity, has been well-recognized as the main immunophenotypic feature of APL [13]. Recently, CD34-negativity was found to be a prominent immunophenotype in patients with the NPM1-mutation[20]. Our results also confirmed that 81.7% of patients were CD34-negative. Furthermore, all seven patients with t(v; 11q23) in our study were CD34-negative, which also agreed with previous studies [21]. Most importantly, we found that patients with t(15; 17), t(v; 11q23) and the NPM1-mutation represented 71.3% of the CD34-negative population and 6.5% of the CD34-positive population (p<0.0001). These findings may lead to improvements in direct molecular screening diagnoses with immunophenotypic results being identified within two hours.

One important finding from our study is that we provided rational explanations for the inconsistent conclusions about the prognostic value of CD34 expression in AML. Although many studies have

demonstrated that CD34-positive patients have a poor outcome compared with CD34-negative patients, this conclusion could not be confirmed by others [4,5]. Kanda et al. performed a meta-analysis in 2488 AML patients and concluded that CD34 expression should not be considered a marker of poor prognosis because of significant heterogeneity between studies [4]. Cytogenetics and molecular abnormalities have been demonstrated as the most powerful prognostic parameters. The prognostic value of CD34 may be affected by certain cytogenetics and molecular abnormalities. When studying the prognostic value of CD34 in AML, the cytogenetics status of the study population significantly skews the results. For example, if more examined cases have favorable cytogenetics, CD34 expression may have no prognostic value. This may be the main reason why the prognostic value of CD34 in AML has been contradictory. Another reason may be due to different treatment protocol used in each study.

In summary, we found that CD34-negative is very common in newly diagnosed AML. CD34-negative is highly associated with t (15;17), t(v; 11q23) and the NPM1-mutation in AML patients, which helps to understand the prognostic value of CD34 in AML.

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