

# Verification of aberrant expression of CD7 in acute myeloid leukemia

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## Introduction

Acute myeloid leukemia (AML) is an aggressive disorder characterized by accumulation of blast cells in bone marrow. The heterogeneous phenotype of AML is based on cytogenetic mutations and molecular aberrations. Immunophenotyping is a convenient method for quick and reproducible diagnosis of most hematological malignancies. The higher frequency of aberrant expression of lymphoid markers in AML may be related to environmental changes and accumulation of biological defects. The CD7, a T-cell antigen, is expressed in a minority of patients with AML, and it is the most common aberrant marker found in AML in most studies.

## Aim

We aimed to determine the frequency of CD7 expression in AML and to verify if this aberrant expression is true or false.

## Patients and methods

The study included 32 newly diagnosed patients with AML. Detection of CD7 in AML was done by using an independent method to check the gene expression, namely, reverse transcriptase-PCR, besides the usually used monoclonal antibody-based flow cytometric measurements.

## Results

The study revealed the frequency of CD7 expression in newly diagnosed AML cases to be 18.1% by flow cytometry. The AML cases with positive CD7 expression by flow cytometry were subjected to reverse transcriptase-PCR to determine gene expression. All cases with positive CD7 expression by flow cytometry were found to be negative for CD7 gene expression by PCR.

## Conclusion

This study raises a reasonable possibility of a false-positive detection of aberrant CD7 expression in AML using immunophenotyping by flow cytometry.

## Keywords:

acute myeloid leukemia, CD7, ectopic expression, flow cytometry, reverse transcriptase-PCR

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## Introduction

Acute leukemias are broadly described by differentiation into myeloid or lymphoid lineages according to the expression of surface and/or cytoplasmic markers associated with their normal myeloid or B-cell and T-cell counterparts [1]. Acute myeloid leukemia (AML) is an aggressive disorder characterized by accumulation of blast cells in bone marrow (BM). AML is the most common acute leukemia affecting adults, and its incidence increases with age. The heterogeneous phenotype of AML is based on cytogenetic mutations and molecular aberrations. Several observations have reported that several T-cell immunophenotypic markers might be aberrantly expressed in AML [2].

Immunophenotyping is a convenient method for quick and reproducible diagnosis of most hematological malignancies [1]. Immunophenotyping data, as for any other clinical or biological characteristics of acute leukemia, cannot be used alone and must be considered together with all parameters of any patient [3].

Aberrant antigen expression was reported to have variable frequency with controversial prognostic and predictive relevance [4,5]. The higher frequency of aberrant expression of lymphoid markers in AML may be related to environmental changes and accumulation of biological defects [6]. In AML, characteristic antigens have been related to particular morphological French-American British Cooperative Group (FAB) subtypes. It was associated with the presence of recurrent genetic abnormalities [7], such as AML-M2 with t (8;21), which shows aberrant expression of lymphoid markers, including CD19 and CD56; another one is co-expression of CD2 in M4E with inv (16) or t (16;16), although not specific for this type of AML [8]. AML-M5 with t (9;11) is reported to have high expression of CD56 [9].

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The CD7, a T-cell antigen, is expressed in a minority of patients with AML, and it is the most common aberrant marker found in AML in most studies [6]. The CD7 gene has been mapped to chromosome 17 by somatic cell hybridization and is formed of four exons that span 3.5 kb [10]. The CD7 may be consistently associated with an early step of myeloid differentiation and perhaps expressed in rare precursors, which is difficult to detect in normal conditions. It was suggested that the CD7 expression must no longer be considered as T-lineage specific antigen because its sensitivity is not absolute, as this antigen can be detected with high frequency in AML [11].

### Aim

The aim of this work was to determine the frequency of CD7 expression in AML flow cytometry and to verify if this aberrant expression is true or false by using an independent method that does not rely on monoclonal antibodies (moAbs) to check the gene expression, namely, reverse transcriptase (RT)-PCR.

### Patients and methods

This study was a cross-sectional study. The study included 32 newly diagnosed patients with AML. The patients were recruited to the Clinical Hematology Unit of Assiut University Hospital, Assiut University, during a 1-year period (2015–2016). The study was approved by the institutional review board, and informed consents were obtained from the patients. This study did not include relapsed or recurrent cases of acute leukemia.

At diagnosis, all patients were subjected to medical history taking (fever, bleeding tendency, therapeutic history, and blood transfusion) and clinical examination (organomegaly and lymphadenopathy). In addition, they underwent the following investigations: full blood picture (CELL-DYN 1700; Abbott Diagnostics, Abbott Park, Illinois, USA), erythrocyte sedimentation rate (ESR), serum calcium level, liver functions test and kidney functions test, coagulation profile, BM examination, and coagulation profile. Flow cytometric immunophenotyping analysis of bone marrow aspirate (BMA) samples was done using 3-color flow cytometer. CD7 expression-positive AML cases were subjected to RT-PCR to determine CD7 gene expression. AML cases included in this study were classified according to FAB criteria.

### Blood samples

The analyzed samples were either of peripheral

blood (PB) or BMA according to availability and presence of blast cells in considerable percentage.

- (1) PB samples: 2 ml of venous blood was withdrawn into EDTA tube for complete blood count.
- (2) BM Samples: BMA sample of 0.5–1 ml was collected in EDTA tube for flow cytometry. Isolation of mononuclear cells from BMA or PB samples of CD7-positive cases within 24 h of sample collection was done for quantitative PCR.

### Morphological examination

For morphologic examination, all BMA/PB smears were air dried and subsequently stained with Leishman's stain to be examined microscopically.

### Enzyme cytochemical analysis

All BMA/PB smears were stained using myeloperoxidase (MPO), periodic acid-Schiff, and nonspecific esterase cytochemical stains.

### Immunophenotyping analysis

Immunophenotyping was done by 3-color flow cytometric analysis of BMA with Becton-Dickinson fluorescence activated cell sorter (BD FACSCalibur, San Jose, California, USA). The BMA samples were lysed by lysing solution (82 g ammonium chloride, 10 g potassium bicarbonate, and 0.37 g disodium EDTA), and washed with phosphate buffered saline until complete RBC lysis and resuspended in appropriate amount of phosphate buffered saline. The BMA cells were stained and studied with different fluorescently labeled moAbs according to the manufacturer's recommendations. Fluorescein isothiocyanate-labeled moAbs included CD45, CD10, CD5, CD7, CD33, HLA-DR, CD41, CD7, CD4, CD8, and MPO (Biotech Spain Company, Barcelona, Spain, ImmunnoStep, Salamanca, Spain). Phycoerythrin-labeled moAbs included CD3, CD19, CD13, CD14, CD10, CD11c (Becton Dickenson), CD61, CD235a, and CD117 (ImmunoStep). Peridinin-chlorophyll protein moAbs included CD34 (Becton Dickenson, Franklin Lakes, New Jersey).

Lysing and staining was done as follows: 10 ml of fluorescein isothiocyanate-conjugated moAb, 10 µl of phycoerythrin-conjugated moAb, and 10 µl of peridinin-chlorophyll protein complex-conjugated moAb was added to 12 × 75-mm tubes, and afterward, 100 µl of whole blood was added in each tube. The mixture was vortexed tenderly and incubated for ~15 min in the dark area at 20–25°C. Then, 3 ml of 1× FACS lysing buffer was added to incubated mixture. Then it was vortexed tenderly and incubated for

15 min in the dark area at 20–25°C again. Thereafter, centrifugation at 2500 rpm for 3 min was done. The supernatant was removed, 3 ml of washing buffer was added, and then centrifuged at 2500 rpm for 3 min. The supernatant was removed, and the cells were resuspended in sheath fluid for immediate analysis or fixed cells can be stored at 2–8°C until analysis.

Acquisition and analysis of stained suspension was performed by FACSCalibur flow cytometer (Becton-Dickinson) acquiring at least 10 000–30 000 cells at a high rate of 400–500 cells/s for each marker. Negative isotype control was run first to identify the position of the negative and the positive populations. At least two plots were drawn during the acquisition of each tube: one of them displayed forward scatter on X-axis versus side scatter on Y-axis to identify the size and granularity of cells and to exclude debris and dead cells, and the second plot displayed the antibody marker on X-axis versus forward scatter or the other marker in case of dual markers on Y-axis. An antigen was considered positive when the expression is at least 20% of the gated cells.

#### Mononuclear cell isolation

The cells were isolated from BMA or PB samples within 24 h after sample collection through Lymphosep, lymphocyte separation media (Biowest). Overall, 3 ml of Lymphosep was transferred to a 15-ml centrifuge tube. BMA sample was mixed with 2 ml of physiological saline at 20–25°C. The diluted blood over 3 ml of Lymphosep was layered and centrifuged at 400g for 30 min at 20–25°C. The top layer of clear plasma was aspirated and discarded. The mononuclear cell layer and about half of the Lymphosep layer below it were aspirated and transferred to a centrifuge tube containing at least two times the volume of physiological saline. Then, it was centrifuged at 150g for 10 min at 20–25°C. The supernatant was aspirated and discarded. The cell pellet was stored at –80°C for later extraction.

#### Total RNA extraction

It was done by using Qiazol lysis reagent (Qiagen, USA). Qiazol lysis reagent is a monophasic solution of phenol and guanidine thiocyanate. It maintains the integrity of the RNA owing to highly effective inhibition of RNase activity during sample homogenization.

Frozen samples were thawed at 20–25°C. Then, 1-ml Qiazol was added to cell pellet. The sample was placed at 20–25°C for 5 min, then 200- $\mu$ l chloroform (Merck, Germany) was added, and the sample was shaken vigorously on vortex for 15 s. The sample was centrifuged at 12 000g for 15 min at 4°C. After that,

the sample was separated into three phases, and the upper, aqueous phase was transferred to a new tube, and then 500  $\mu$ l isopropanol was added and mixed by vortexing and then was placed at 20–25°C for 10 min. The sample was centrifuged at 12 000g for 10 min at 4°C. The supernatant was aspirated and discarded. The RNA pellet was visible at the bottom of the tube. Then, 1 ml of 75% ethanol was added and centrifuged at 7500g for 5 min at 4°C. The RNA was redissolved in 13  $\mu$ l of RNase-free water. The cDNA RT sample has been prepared using the reverse transcription kits (Applied Biosystems, Darmstadt, Germany) and was stored at –70°C until the next step.

#### Reverse transcriptase polymerase chain reaction

For the RT-PCR, complementary DNA load was standardized in preliminary experiments by making serial dilution and amplifying  $\beta$ -actin to produce just detectable signal, thus keeping the PCR condition within the linear range of amplification before reaching saturation. By using KAPA2Gtm Fast PCR kit, duplex PCR reaction assembly was done as follows: 25  $\mu$ l of total volume: 5  $\mu$ l buffer, A 0.5  $\mu$ l dNTP, 1.25  $\mu$ l forward primer of B-actin, 1.25  $\mu$ l reverse primer of B-actin, 1.25  $\mu$ l forward primer of CD7, 1.25  $\mu$ l reverse primer of CD7, 0.1  $\mu$ l fast DNA polymerase, 10.4  $\mu$ l PCR grade water, and 4  $\mu$ l cDNA sample.

The samples were placed in the thermocycler for PCR amplification under the following conditions: 94°C for 5 min for initial melting; 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s for a total of 30 cycles; and 72°C for 10 min for final extension. PCR primers were as follows CD7: forward primer: 5'-TAGACCCAGAGAGGCTCAG-3', and reverse primer: 5'-GGAGACTGCTGCACCTCTTGG-3'. B-actin: forward primer: 5'-ATCTGGCACCACA CCTTCTACAATGAGCTGCG3' and reverse primer: 5'-CGTCATACTCCTGCTTGCTGATCCACAT CTGC-3'. The PCR products were visualized on 2% agarose gel stained by ethidium bromide and photographed under ultraviolet light.

#### Statistical analysis

All statistical analyses were performed using Statistical Package for Sciences Software, version 18 (SPSS 'ver. 21' software; SPSS Inc., Chicago, Illinois, USA). Data were expressed as mean  $\pm$  SD, number, and percentage. A *P* value less than 0.05 was considered statistically significant. Gated cells were analyzed for the expression of CD7 mAb. Cutoff value more than or equal to 20 was reported as positive. Pearson correlation was used to assess the correlation between CD7 and other parameters. *P* value was significant if less than 0.05.



## Results

Of 32 cases of AML, 17 (53.1%) patients were males and 15 (46.9%) patients were females. AML is more common in men than women, with a male to female ratio of 1.5/1.0. Their ages ranged from 17 to 80 years, with mean  $\pm$  SD of  $46.25 \pm 18.19$  years. It was found that six (18.8%) patients had hepatomegaly, 12 (37.5%) patients had splenomegaly, nine (28.1%) patients had lymphadenopathy, 11 (34.4%) patients had bleeding tendency, and five (15.6%) patients had purpuric eruptions. Fever was found in 23 (71.9%) patients. The complete blood count analysis showed a wide range of hemoglobin concentration and platelet and white blood cell (WBC) counts, which varied from normal to anemia, thrombocytopenia to thrombocytosis and leukopenia to hyperleukocytosis. Cytochemistry of the studied patients showed that a total of 22 (68.8%) patients were positive for Sudan black, eight (25%) patients were positive for periodic acid-Schiff stain, and nine (28.1%) patients were positive for nonspecific esterase. In the present study, we found that AML-M4 was the most frequent subtype [12 (37.5%) patients], followed by AML-M2 six (18.8%) patients and M1 four (12.5%) patients. AML-M5 occurred in three (9.4%) patients, whereas AML-M3, M6 and M7 occurred in two (6.3%) patients each. The least frequent subtype was AML-M0, which was presented in only one (3.1%). Immunophenotyping is described in Table 6. It was found that CD7 was aberrantly expressed in six (18.8%) patients: one (25%) patient in AML-M1, two (33.3%) patients in AML-M2, one (8.3%) patient in AML-M4, one (33.3%) patient in AML-M5, and one (50%) in AML-M7. There was insignificant correlation between CD7 and age, WBCs, platelets, reticulocytes, hemoglobin level, total protein, ESR 1, ESR 2, serum urea, serum creatinine, serum calcium, prothrombin time, Prothrombin concentration (PC), international normalized ratio, total bilirubin, direct bilirubin, alanine aminotransferase, albumin, and lactate dehydrogenase, with *P* value more than 0.05. There was a significant positive correlation between CD7 positive and peripheral blast and BM blast ( $r = 0.04/r = 0.03$ ;  $P = 0.82/P = 0.85$ ) (Tables 1-4, Fig. 1).

Electrophoresis on agarose gel shows  $\beta$ -actin expression on lanes 2, 3, 4, 5, 6, 7, and 8 (amplicon size 838 bp). Lane 1 is a negative control, whereas CD7 was observed in lane 2 as a positive control (amplicon size 173) in comparison with 100-bp DNA size marker.

## Discussion

AML is an aggressive disorder characterized by accumulation of blast cells in BM. The heterogeneous phenotype of AML is based on cytogenetic mutations

**Table 1 Age and sex of the studied patients**

	<i>n</i> =32 [ <i>n</i> (%)]
Age (years)	46.25 $\pm$ 18.19
< 40	13 (40.6)
40 to <60	11 (34.4)
$\geq$ 60	8 (25)
Sex	
Male	17 (53.1)
Female	15 (46.9)

**Table 2 Clinical data of the studied population**

	<i>n</i> =32 [ <i>n</i> (%)]
Fever	23 (71.9)
Hepatomegaly	6 (18.8)
Splenomegaly	12 (37.5)
Lymphadenopathy	9 (28.1)
Bleeding tendency	16 (50)

**Table 3 Correlation of CD7 positive with different parameters in the study**

	<i>r</i> ( <i>P</i> )
Age	-0.57 (0.24)
Hemoglobin	0.30 (0.56)
WBCs	0.12 (0.82)
Platelets	0.15 (0.78)
Reticulocytes	-0.65 (0.17)
Peripheral blast	<b>0.82 (0.04)</b>
Bone marrow blast	<b>0.85 (0.03)</b>
Total protein	-0.21 (0.70)
ESR 1	0.57 (0.24)
ESR 2	0.35 (0.50)
Serum urea	-0.74 (0.09)
Serum creatinine	-0.68 (0.14)
Serum calcium	-0.61 (0.20)
PT	-0.55 (0.26)
PC	-0.02 (0.97)
INR	0.71 (0.83)
Total bilirubin	0.12 (0.83)
Direct bilirubin	-0.03 (0.96)
AST	0.78 (0.07)
ALT	-0.15 (0.77)
Albumin	-0.16 (0.77)
LDH	0.42 (0.41)

Data was expressed in form of *r* (strength of correlation) and *P* (significance of correlation that was significant if <0.05).

ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PT, prothrombin time; WBC, white blood cell. Bold: There was significant positive correlation between CD7 positive and Peripheral blast ( $r = 0.82$ ;  $P = 0.04$ ) and bone marrow blast ( $r = 0.85$ ;  $P = 0.03$ ).

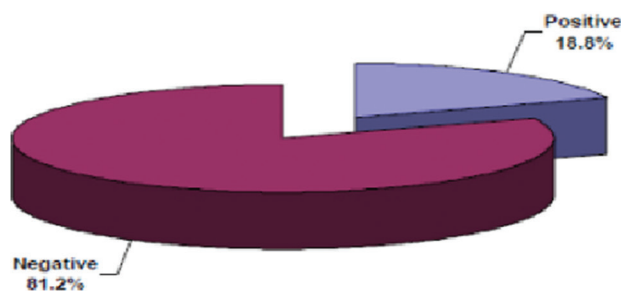
and molecular aberrations. Several observations have reported that several T-cell immunophenotypic markers might be aberrantly expressed in AML [2]. Immunophenotyping data, as for any other clinical or biological characteristics of acute leukemia, cannot be used alone and must be considered together with all other parameters of any patient [3].

Aberrant antigen expression was reported to have variable frequency with controversial prognostic and

**Table 4 Correlation of CD7 negative with different parameters in the study**

	<i>r</i> ( <i>P</i> )
Age	0.32 (0.11)
Hemoglobin	0.16 (0.44)
WBCs	-0.17 (0.40)
Platelets	-0.12 (0.55)
Reticulocytes	-0.12 (0.57)
Peripheral blast	0.10 (0.63)
Bone marrow blast	-0.13 (0.53)
Total protein	-0.23 (0.26)
ESR 1	-0.18 (0.38)
ESR 2	-0.23 (0.27)
Serum urea	0.09 (0.67)
Serum creatinine	0.27 (0.19)
Serum calcium	0.00 (0.99)
PT	-0.25 (0.22)
PC	0.22 (0.29)
INR	-0.35 (0.08)
Total bilirubin	0.04 (0.87)
Direct bilirubin	-0.00 (0.997)
AST	0.31 (0.28)
ALT	0.13 (0.52)
Albumin	-0.25 (0.22)
LDH	0.03 (0.87)

Data were expressed in form of *r* (strength of correlation) and *P* (significance of correlation that was significant if <0.05). ALT, alanine aminotransferase; AST, aspartate aminotransferase; ESR, erythrocyte sedimentation rate; INR, international normalized ratio; LDH, lactate dehydrogenase; PT, prothrombin time; WBC, white blood cell.

**Figure 1**

Frequency of CD7 expression in patients with AML by flow cytometry. AML, acute myeloid leukemia.

predictive relevance [4,5]. The higher frequency of aberrant expression of lymphoid markers in AML may be related to environmental changes and accumulation of biological defects. CD7, a T-cell antigen, is the most common aberrant marker found in AML in most studies [6].

In the present study, we have studied the frequency of CD7 aberrant expression in patients with AML who presented to our hospital during the period from 2015 to 2016 and tried to verify if this aberrant expression is a true or a false one using an independent method that does not rely on the use of moAbs.

In this study, AML was higher in males than females and more common in age below 40 years. This may be caused by action of sex hormones during growth and puberty phases, and work-related biological and environmental processes [12]. Fever and bleeding tendency were the predominant signs in most patients. This may be owing to BM failure, which may lead to increased risk of infections and hyperleukocytosis [13]. The peripheral hemogram of the studied cases showed that the mean hemoglobin was quite low in most patients, and this might be owing to BM infiltration which leads to decreased production of RBCs and might be owing to decreased RBCs life span and autoimmune destruction [14]. Leukocytosis and hyperleukocytosis were observed in most of the cases and could be caused by rapid differentiation and proliferation of leukemic cells. Thrombocytopenia was prominent feature in most patients, and it was associated with bleeding tendency [15].

Immunophenotyping in the present work showed that most patients were positive for HLA-DR, CD34, CD33, CD13, CD45, CD4, and cytoplasmic MPO. Flow cytometric CD7 aberrant expression was observed in six of the 32 studied cases. This aberrant expression was seen in FAB subtypes M1, M2, M4, M5, and M7. Contrary to our expectation, the two M3 cases did not show CD7 expression (Tables 5 and 6). Many studies showed that CD7 was frequently expressed as an aberrant marker in AML with various frequencies ranging from 11 to 40% [1,3–5,16]. Some studies showed that CD7 ectopic expression was observed in the more immature subtypes of AML. This was explained according the notion that CD7 is transiently expressed in the very early stages of myeloid progenitors, and then its expression is downregulated as myeloid progenitors undergo differentiation and maturation [17].

In our study, there was no significant difference between cases with ectopic CD7 and those without regarding age, sex, clinical data, WBC count, and platelets, except PB blast cell count and BM blast cell count. Many other studies showed no correlation between positive CD7 and negative CD7 AML cases according to age, sex distribution, distinctive clinical presentation, CNS involvement, blast count and immaturity of CD7+ leukemic cells with high expression of CD34 [17–26]. In another study, CD7 expression was found to have no direct relation with prognosis [27]. Other studies showed that CD7-positive AML was seen mainly in younger males, with hepatosplenomegaly and CNS involvement [5–15,17–30].

Aberrant CD7 expression in AML has been a matter of controversy in both the frequency of expression

and the trueness of expression. Several studies have discussed these issues with variable explanations; one of them is the use of different moAbs [1,3–6,16]. In other studies, the type of moAbs was the same, but different frequencies of CD7 expression were observed [1,3–6,16]. The analysis of different study populations (children, adults, or both), the stringency of the initial definition of AML (based on morphological appearance or immunophenotyping), and if the examination was performed on PB or BM samples are other factors. Another explanation is the use of flow cytometry, immunofluorescence microscopy, or immunoenzymatic staining, type and number of antigens studied, single or multicolor staining of the malignant cells, differences in the cutoff value levels for the discrimination of positive versus negative results, and analysis of freshly obtained specimens or archived samples [24–35]. Technical errors were another possibility; in this instance, the relevant marker is not actually expressed, and this adds a false signal. The probes used for detection are usually obtained

from commercially available sources, and this may be associated with cross-reactivity with other molecules or epitopes [36].

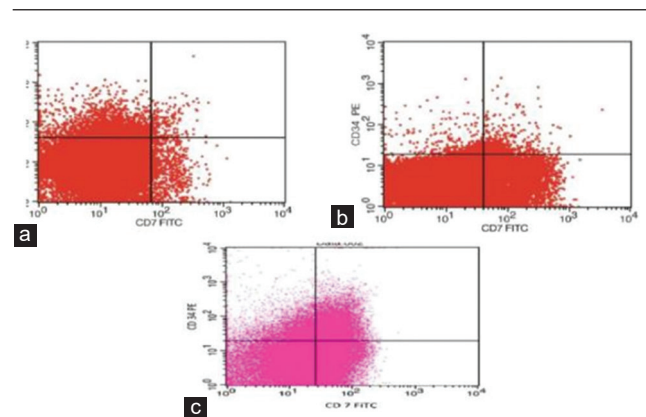
We sought to verify the trueness of CD7 expression in AML using a different approach that does not rely on the use of commercial moAbs. We used RT-PCR to examine the CD7 expression in leukemic cells of AML at the level of gene transcription in the six samples that showed positive expression of the protein. Surprisingly, none of the six cases examined showed mRNA expression (Fig. 2a and b). Given the higher sensitivity of PCR compared with flow cytometry, and the design of a properly controlled experiment of duplex RT-PCR with internal control of the

**Table 5 French-American British Cooperative Group subtypes of the studied patients**

FAB	n=32 [n (%)]
M0	1 (3.1)
M1	4 (12.5)
M2	6 (18.8)
M3	2 (6.3)
M4	12 (37.5)
M5	3 (9.4)
M6	2 (6.3)
M7	2 (6.3)

FAB, French-American British Cooperative Group.

**Figure 2**



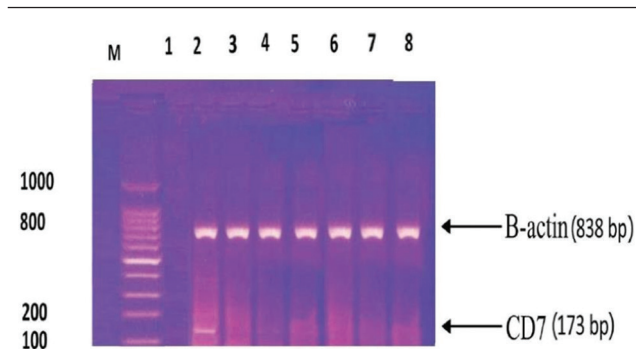
Flow cytometric detection of CD7 expression. (a, b) Flow histograms of AML cases with ectopic CD7 expression with CD7/FITC in AML cases. (c) Flow histogram of ALL case. AML, acute myeloid leukemia; FITC, fluorescein isothiocyanate.

**Table 6 Immunophenotyping in the studied patients**

	M0 (n=1) [n (%)]	M1 (n=4) [n (%)]	M2 (n=6) [n (%)]	M3 (n=2) [n (%)]	M4 (n=12) [n (%)]	M5 (n=3) [n (%)]	M6 (n=2) [n (%)]	M7 (n=2) [n (%)]	Total (n=32) [n (%)]
HLA-DR	1 (100)	3 (75)	6 (100)	2 (100)	10 (83.3)	3 (100)	2 (100)	1 (50)	28 (87.5)
CD34	1 (100)	2 (50)	6 (100)	2 (100)	6 (50)	3 (100)	2 (100)	2 (100)	24 (75)
CD33	0	2 (50)	4 (66.67)	2 (100)	10 (83.3)	3 (100)	1 (50)	1 (50)	23 (71.9)
CD13	1 (100)	4 (100)	6 (100)	2 (100)	11 (91.7)	3 (100)	2 (100)	2 (100)	31 (96.9)
CD45	1 (100)	2 (50)	6 (100)	1 (50)	7 (58.3)	3 (100)	2 (100)	2 (100)	24 (75)
CD19	0	0	0	0	0	0	0	0	0
CD7	0	1 (25)	2 (33.3)	0	1 (8.3)	1 (33.3)	0	1 (50)	6 (18.8)
CD10	0	0	0	0	0	0	0	0	0
CD5	0	0	0	0	0	0	0	0	0
CD8	0	0	0	0	0	0	0	0	0
CD3	0	0	0	0	0	0	0	0	0
CD14	0	0	1 (16.7)	0	5 (41.7)	1 (33.3)	0	0	7 (21.9)
CD41a	0	1 (25)	0	0	1 (8.3)	0	0	0	2 (6.3)
Glycophorin	0	0	0	0	2 (16.7)	0	1 (50)	0	3 (9.4)
Cyto MPO	1 (100)	3 (75)	3 (50)	1 (50)	7 (58.3)	1 (33.3)	2 (100)	2 (100)	20 (62.5)
CD235a	0	0	0	0	1 (8.3)	0	0	0	1 (3.1)
CD11c	0	0	2 (33.3)	0	10 (83.3)	3 (100)	0	0	15 (47)
CD117	1 (100)	0	5 (83.3)	1 (50)	0	2 (66.67)	1 (50)	1 (50)	11 (34.4)
CD4	0	0	2	0	13	2	0	1	0
Cyto CD61	0	0	0	0	0	0	0	2 (100)	2 (6.3)

MPO, myeloperoxidase.

Figure 3



RT-PCR of B-actin and CD7 in patients with AML with positive flow cytometric ectopic expression of CD7. M: DNA size marker (100 bp ladder). Lane 1: negative control. Lane 2: positive control (A case of T-ALL). Lanes 3, 4, 5, 6, 7, and 8. AML cases showed ectopic CD7 expression by flow cytometry. AML, acute myeloid leukemia; RT, reverse transcriptase.

house-keeping gene beta-actin together with external positive (Fig. 2c) and negative controls, makes it a plausible assumption to consider the RT-PCR results to be more reliable than that of flow cytometry in this context. There are several other observations in this study and other studies that support this hypothesis. First is the lack of correlation between ectopic CD7 expression and any of the diagnostic or prognostic parameters of AML. On the contrary, the expression pattern of CD7 observed in cases of AML by flow cytometry was a dim one and very close to the scatter pattern of the negative isotype control, raising the possibility of cross-reactivity and nonspecificity of the observed result (Fig. 3). Moreover, ectopic expression of CD7 would be more expected in tumors harboring cytogenetic rearrangements involving chromosome 17 on which CD7 gene resides. AML-M3 is the subtype of AML which harbors  $t(15; 17)(q22; q21)$ , and the two cases in our study did not show CD7 expression either by flow cytometry or by RT-PCR.

## Conclusion

This study raises a reasonable possibility of a false-positive detection of aberrant CD7 expression in AML using immunophenotyping by flow cytometry and recommends the confirmation of this ectopic expression using an independent technique that does not rely on the use of moAbs. Further detailed molecular studies are required to dissect the underlying mechanisms regulating CD7 gene expression to verify our hypothesis.

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## Conflicts of interest

There are no conflicts of interest.

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