ORIGINAL ARTICLE

# Antigen Expression Pattern of Acute Promyelocytic Leukaemia Cases in Malaysia

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**SUMMARY**

**Introduction: Acute Promyelocytic Leukaemia (APL) is associated with devastating coagulopathy and life threatening condition which requires immediate medical attention. It is crucial to establish an expedited diagnosis as early therapeutic intervention has led to optimal patient management. In this study, we assessed the type and frequency of antigen expressions in APL and correlated these findings with genetic studies.**

**Methods: Multiparametric immunophenotyping was performed on 30 samples and findings were correlated with karyotypes, FISH for t(15;17) translocation and RT-PCR for PML-RARα for detection of breakpoint cluster regions (bcr1,bcr2 and bcr3).**

**Results: On SSC/CD45, APL cells displayed high to moderate SSC, with the expression of CD33 (100%), CD13 (96.8%), cMPO (71%) but lacked CD34 (3.2%) and HLA-DR (9.7%). Aberrant expression of CD4 was seen in 12.9% and CD56 in 6.5% of the cases. A significant association between cumulative aberrant antigen expression and bcr1 were observed bcr1 (X2(2) =6.833,p<.05). However there were no significant association seen in bcr2 and bcr3; (X2(2) =.199,p>.05) and (X2(2)=4.599,p>.05) respectively.**

**Conclusions: Flow cytometry is a rapid and effective tool in detecting APL. It is interesting to note that there is significant association between cumulative aberrant antigen expression and genotype analysis. Further validation is required to corroborate this relationship.**

**KEY WORDS:**
*Antigen expression, flow cytometry, acute promyelocytic leukaemia, PML-RARα, t(15;17)*

## INTRODUCTION

Acute promyelocytic leukaemia (APL) is a distinct subtype of acute myeloid leukaemia (AML) characterized by an arrest in maturation at the promyelocyte stage, leading to inhibition of normal haematopoiesis. Reciprocal chromosomal translocation t (15; 17) which leads to the generation of PML-RARα fusion transcript is the hallmark of APL diagnosis [1,2,3,4,5].

APL constitutes approximately 4% of AML cases in Malaysia (Malaysia Cancer Statistics, 2007) [6]. Although the figure apparently appears small compared to other subtype of acute leukaemia, APL is known to progress aggressively and leads to devastating coagulopathy which can threaten life, and so requires immediate medical attention. It is crucial to establish an expedited diagnosis as early therapeutic intervention has led to optimal patient management in terms of survival and reduced mortality rate. APL is known to be one of the most frequently cured AML subtypes as early introduction of all-trans retinoic acid (ATRA) at the first suspicion of an APL before genetical studies confirmation is critical in patient management.

Traditionally, diagnosis of APL is made based on its morphological features. It is then confirmed by immunophenotyping analysis, detection of t(15;17), and other chromosomal aberrations by conventional karyotyping, fluorescence in situ hybridization (FISH) and reverse transcriptase-polymerase chain reaction (RT-PCR) [7,8,9]. However subjectivity of morphology analysis produces ambiguous results due to poor morphologic features which may be caused by inadequate aspirate smears and presence of morphologic variants. Furthermore, there are notable limitations in the current diagnostic approaches. Conventional cytogenetics consumes much time and can be affected by lack of neoplastic cell growth in the culture medium yielding to poor metaphase spread. Inability to detect cryptic chromosomal abnormalities requires further verification with FISH studies. Additional studies are also required in cases with normal karyotype or a negative FISH as there may be other undetected submicroscopic genetic alterations.

Flowcytometry (FCM) immunophenotyping is considered as a rigorous ancillary tool which aids the diagnosis of APL. Immunophenotypic characteristics of APL is distinct compared to other types of AML which may aid speedy diagnosis. Typically, leukaemic promyelocytes express CD13 and CD33, which are also seen in mature myeloid cells and consistent CD117 expression but lacks the expression of CD34 and HLA-DR. APL displays heterogeneous high scatter and expression of myeloperoxidase is also noted frequently. Other studies also reported that CD10, CD11a, CD11b, CD11c, CD18, CD45RO, CD105 and CD133 are consistently absent or less frequently expressed in APL [1,2,3,4,7,10,11]. Aberrant antigen expression (other than myeloid lineage) such as CD2 (T-cell lineage) and CD56 (NK-cell marker) are related to poor prognosis [7,12].

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However, despite a large number of publications, and in the era of molecular karyotyping and next generation sequencing where FCM has become a secondary diagnostic tool, no standardized panel or consolidated phenotypic profile is available for different AML subtypes specifically. The described phenotypes are characteristic but subjective across the spectrum. The findings are rather ambiguous; some studies reported an adverse prognosis when there is aberrant antigen expression such as CD 2 and CD56 in APL, while others failed to show any significant association [7, 11, 13].

Majority of studies on phenotype of APL and frequency of antigen expressions by FCM are from western countries, Taiwan, Saudi Arabia and Pakistan. In Malaysia such a study has not been reported. Thus, the challenges lie in identifying all APL cases by utilizing an optimal panel of antibodies [1,2,3, 7, 14,15,16,17,18].

In this article, we assessed the type and frequency of antigen expression in APL in Malaysia. The incidence of aberrant phenotypes is still controversial and divergent results have been found by different groups probably due to diversity of monoclonal antibody (MoAbs) panel and sampling size. We explored the occurrence of aberrant phenotypes and correlated these findings with patient's clinical features (demographic data) and genetic studies.

Diagnosis of APL requires a multimodal approach; nevertheless it would be practical to provide a timely diagnosis by utilizing flow cytometric findings based on the widely used findings of CD34- and HLA-DR- cells.

## MATERIALS AND METHODS

Thirty APL cases diagnosed from year 2010-2012 in Clinical Haematology Laboratory, Hospital Ampang were reviewed. Demographic data, morphology studies and immunophenotyping analysis findings were retrieved. The final diagnoses of the cases were based on molecular and cytogenetic analyses. This study has been approved by Malaysian Research Ethics Committee (MREC). (Research ID: NMRR-12-1231-13432)

Morphologic identification of the leukaemic cells in the bone marrow (BM) aspirate or peripheral blood smears (PBS) is crucial in determining the traditional diagnosis of APL. Patients' BM and PBS samples were air dried and subsequently stained with May Grundwald Giemsa and Wright Eosin Methylene Blue respectively.

Flow cytometry immunophenotyping antibody panels were decided based on morphologic examination on bone marrow aspirates and peripheral blood smears.

## FLOW CYTOMETRY IMMUNOPHENOTYPIC METHOD

Eight colour FCM analysis was performed on all samples using a Facs Canto II (Becton Dickinson, San Jose, CA USA). Antibodies in the panel constitute a standard panel for diagnosis of all acute leukaemias. Antigen expressions were assessed at surface and cytoplasmic level. The eight colour panel used in this study is listed in Table I.

### Flow Cytometric Sample Processing Procedure

Briefly, samples were obtained and washed with Phosphate Buffer Saline (PBS) containing 2% of Foetal Bovine Serum (FBS) and centrifuged (3 minutes, 1800g) for 3 times. After incubation with monoclonal antibodies mastermix (titrated and volume of blood is adjusted based on the total white blood cell count),red blood cell lysing was performed using 2 ml of 10% FACS lysing solution (Becton/Dickinson) and re-incubated for 10 minutes in the dark at room temperature. Cells were then centrifuged (5 minutes, 1000g), washed once with 2 ml of FBS (5 minutes at 1000g) and resuspended with 0.5 mL of 0.1 % paraformaldehyde. Samples from January 2012 onwards were processed using Lyse Wash Assistant (LWA) (Becton Dickinson, San Jose, CA USA) with a customized duo lyse procedure.

Cytoplasmic staining was performed using the following protocol: Surface antigen staining was performed for 15 minutes, followed by incubation with IntraStain Reagent A, Fixative (Dako) for 15 minutes. Two ml of PBS was added to each test tube and mixed gently, followed by centrifugation 300 x g for 5 minutes, and then supernatant was aspirated, leaving approximately 50 µL of fluid. One hundred µL IntraStain Reagent B Permeabilization was added, to each test tube. Then an appropriate volume of fluorochrome-conjugated antibody specific for the intracellular antigen to be stained was added. Washing steps were then repeated and pellet was resuspended in 0.1 % paraformaldehyde for flow cytometric analysis.

Sample acquisition was performed in Facs Canto II (Becton Dickinson, San Jose, CA USA) and Facs Diva was used for data analysis. A standardized 50 000 events/ tube were acquired. Instrument calibration was performed using Cytometer Setup and Tracking (Becton Dickinson, San Jose, CA USA). Instrument setting were optimized using a label specific compensation setup and verified daily by running a normal blood with common lymphocyte markers (CD7/lambda+CD56/CD5/CD2/Kappa+CD4/CD3/CD8+CD19/45).

Antigen expression of the leukaemic cells were analysed and reported as: present or absent, fluorescence intensity and expression pattern (homogenous versus heterogeneous). Cluster analysis was performed for all the samples included in the study.

### Molecular and Cytogenetic Studies

Fusion transcripts detection by RT-PCR was done using specific primers for PML-RARα for breakpoint cluster region 1 (bcr1), breakpoint cluster region 2 (bcr2) and breakpoint cluster region 3 (bcr3).G-banding technique was performed for cytogenetic analysis and Fluorescence In-situ Hybridization analysis of the t(15;17) translocation was carried out with PML- RARα probes. For APML cases described in this study, immunophenotypic findings were correlated with morphology, PML/RARα fusion subtypes, and conventional cytogenetics whenever this information was available.

**Table I: Eight Colour Panel for the Diagnosis of Acute Leukaemia in Clinical Haematology Laboratory, Hospital Ampang**

| Tube | FITC | PE | PerCPCy5.5 | PECy7 | APC | APCCy7 | BV421 | V500/KO | Note |
|---|---|---|---|---|---|---|---|---|---|
| 1 | | – | – | – | – | – | – | – | Unstained |
| 2 | cMPO | c79a | CD34(581) | - | c3 | - | - | CD45(J.33) | Cytoplasmic Staining |
| 3 | nTdT | cIgM | CD34(581) | CD10(H110a) | c79a | - | CD19(HIB19) | CD45(J.33) | |
| 4 | CD36 (FA6.152)<br>CD1a(HI149) | CD33(WM53) | CD163(GH1/61)<br>CD3(UCHT1) | CD117(104D2) | CD13(WM15) | CD34(581) | CD14(HCD14) | CD45(J.33) | Surface Staining |
| 5 | CD66c (KOR-SA3544)<br>CD303(AC144) | CD33(WM53) | CD15(W6D3) | CD123(6H6) | CD13(WM15) | CD34(581) | CD19(HIB19) | CD45(J.33) | |
| 6 | CD81(TAPA-1) | CD58(TS2/9) | CD38(HB7) | CD10(H110a) | CD22(HIB22) | CD34(581) | CD20(2H7) | CD45(J.33) | |
| 7 | HLA-DR(L243) | CD56(HCD56) | CD9(M-L13) | CD117(104D2) | CD13(WM15)<br>CD33(WM53) | CD34(581) | CD19(HIB19) | CD45(J.33) | |
| 8 | CD7(MEM186) | Lambda<br>CD56(HCD56) | CD5(L17F12) | CD2(TS1/8) | κappa<br>CD4(SK3) | CD3(SK7) | CD8(RPAT8)<br>CD19(HIB19) | CD45(J.33) | |

cMPO,c79a,c3,nTdT,cIgM refers to cytoplasmic staining.
All letters in bracket refers to antibody clone; e.g. CD36(FA6.152): Flourochrome conjugated antibody against surface antigen CD36, clone FA6.152

**Table II: Demographic data and FBC of patients' with APL**

| | |
|---|---|
| Total patients | 30 |
| Age | 15 to 79 years (Mean,35 years) |
| Sex | |
| Male | 11 (36.7%) |
| Female | 19 (63.3%) |
| Race | |
| Malay | 17 (56.6%) |
| Chinese | 9 (30.0%) |
| Indian | 2 (6.7 %) |
| Others | 2 (6.7%) |

**Table III: Antigen Expression, Cytogenetic and Molecular Characteristics of APL**

| Case No. | Antigen Expression | cytogenetic | Fusion Transcript Detection by RT-PCR (PML-RARα breakpoint) |
|---|---|---|---|
| 1. | CD13+/CD15+/CD33+/CD45+/CD58+/CD123+/CD38+/cMPO-/HLA-DR-/CD117-/CD2-/CD4-/CD56- | t(15;17)(q22;q21) | NA |
| 2. | CD13+/CD15-/CD33+/CD45+/CD58+/CD123+/CD38+/cMPO-/HLA-DR+/CD117+/CD2-/CD4+/CD56- | t(15;17)(q22;q21) | NA |
| 3. | CD13+/CD15-/CD33+/CD45+/CD58+/CD123+/CD38-/cMPO-/HLA-DR-/CD117+/CD2-/CD4-/CD56+ | 46,XX | bcr 1 |
| 4. | CD13+/CD15-/CD33+/CD45-/CD58+/CD123+/CD38-/cMPO-/HLA-DR-/CD117+/CD2-/CD4-/CD56- | t(15;17)(q24;q21) | bcr 1 |
| 5. | CD13+/CD15-/CD33+/CD45+/CD58+/CD123+/CD38+/cMPO-/HLA-DR-/CD117+/CD2-/CD4-/CD56- | t(15;17)(q24;q21) | bcr 1 |
| 6. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38-/cMPO-/HLA-DR-/CD117-/CD2-/CD4-/CD56- | t(15;17)(q22;q21) | bcr 1 |
| 7. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO+/HLA-DR-/CD117-/CD2-/CD4-/CD56- | 46,XY | bcr 1 |
| 8. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO+/HLA-DR-/CD117+/CD2-/CD4-/CD56- | 46,XX | bcr 1 |
| 9. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO+/HLA-DR-/CD117-/CD2-/CD4-/CD56- | 45,X i(xq)(15;17)(q22;q21)<br>t(15;22)(q12,p13)(16)/der 46+15(6) | bcr 1 |
| 10. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO-/HLA-DR-/CD117-/CD2-/CD4-/CD56- | 100/100 (PML-RARα positive) | bcr 1 |
| 11. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO-/HLA-DR-/CD117-/CD2-/CD4+/CD56+ | Der(15)t(15;17)(q22;q21) | bcr 2 |
| 12. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38-/cMPO+/HLA-DR+/CD117+/CD2-/CD4-/CD56- | t(15;17)(q22;q11) | NA |
| 13. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38-/cMPO+/HLA-DR-/CD117-/CD2-/CD4-/CD56- | 46,XY(13) | NA |
| 14. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38-/cMPO-/HLA-DR-/CD117-/CD2-/CD4-/CD56+ | t(15;17)(q24;q21) | bcr 1 |
| 15. | CD13+/CD15-/CD33+/CD45+/CD58-/CD123+/CD38+/cMPO+/HLA-DR-/CD117+/CD2-/CD4-/CD56- | t(15;17)(q22;q21) | bcr2 |

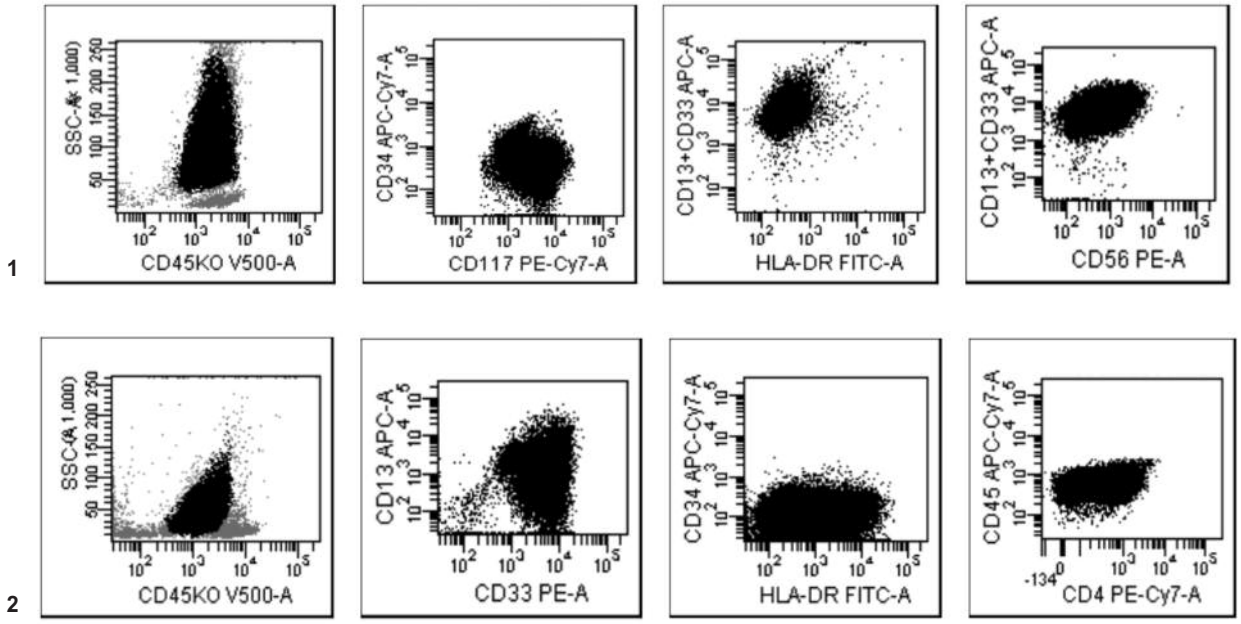


**Fig. :** Immunophenotypic features of APL. (1) APL case with high SSC. 77% cluster of CD45+SSC$^{high}$FSC$^{high}$ cells expressing CD117, CD13, CD33, aberrant CD56 and lacking all other antigens including CD34 and HLA-DR. (2) APL case with moderate SSC.78% cluster of CD45+SSC$^{moderate}$FSC$^{moderate}$ cells expressing CD117, CD13, CD33, HLA-DR partial, aberrant CD4 and lacking all other antigens including CD34.

**Statistical Method**

Relative frequencies of all the phenotypic variables were studied using chi-square test (p values lower than 0.05 were considered to be statistically significant). [Statistical Package for Social Science (SPSS) Version 21]

## RESULTS

A total of 30 APL cases were included in this study based on the morphologic, immunophenotyping findings and genetic studies (RT-PCR and FISH). Eleven patients were male (5 Malays, 3 Chinese, 2 Indians and 1 other race) and 19 were female (12 Malays, 6 Chinese, 1 other race) with a median of median age of the patient was 29 (range, 15-79 years). Patients' demographic data on admission were summarized in Table II.

**Morphologic Findings**

Patients' total white blood cell (WBC) and platelet count at diagnosis ranges from 0.70 - 123.0 X$10^3$/µL and 5 –217 X $10^9$/L respectively. Unfortunately, haemoglobin level was not available during data collection. Bone marrow aspirate and blood film morphological assessment revealed 20 of the cases were typical hypergranular APL and another 10 of the cases were hypogranular respectively. We found that there were no significant association between the morphologic findings (typical hypergranular vs hypogranular) APL with the immunophenotypic features, cytogenetic and molecular findings.

**Immunophenotypic Findings**

Antibody panels for flow cytometry immunophenotyping analysis were selected based on the morphologic findings on all bone marrow aspirates and peripheral blood smears.

On side scatter vs CD45, APL cells displayed primarily high side scatter (Sample I) and cases with moderate SSC expression (Sample II). Analysis of the 30 cases revealed that APL cells typically expressed CD33 (100%), CD9 (100%) and CD13 (96.8%) but lacked CD34 and HLA-DR in 96.8% and 90.3% of the cases respectively. 22 (71 %) of the cases expressed cytoplasmic myeloperoxidase. Expressions of mature myeloid antigen were seen in 16.1% of cases expressing CD15, but was negative for CD10 expression. CD64 and CD117 were expressed in 54.8% and 45.2 % of the cases respectively. Aberrant antigen expressions were also seen in 16% of the cases with 4 cases expressing one aberrant antigen and one case with 2 aberrant antigens. CD4 were expressed in 12.9% of the cases and CD56 were seen in 2 cases (6.5%) as shown in Figure 1.

**Molecular and Cytogenetic Analysis**

Initial diagnosis were made based on the morphology and immunophenotyping and confirmed by conventional karyotyping, RT-PCR and FISH. The PML-RARα gene fusion transcript were analysed by RT-PCR. The long (bcr1) and short (bcr3) forms were detected in 62% (13 cases) and 33% (7 cases). Only one case was reported for bcr2 (variable form).

Complex karyotypes were observed in one third of the APL cases in addition to the pathogonomic t(15;17)(q(22;q21). However we did not observe any significant association on the overall immunophenotypic studies. There was no notable association between the karyotype and aberrant antigen expression observed in this study. We analysed the relationship between aberrant antigen expression with 3 fusion transcripts; bcr1, bcr2 and bcr3. Two aberrant antigens detected in this study are CD4 in 12.9 % and CD56 in 6.5 % of the cases respectively. Selected APL cases with antigenic

profiles, cytogenetic and molecular findings are summarized in Table III.

There was no significant association between CD4 with bcr 1 ($X^2(1)$ =2.062, p>.05), bcr2($X^2(1)$ =.153, p>.05), and bcr 3 ($X^2(1)$ =.015, p>.05). Similar findings were also observed for CD56 ;($X^2(1)$=.057,p>.05), bcr2 ($X^2(1)$=.071,p>.05), and bcr 3 ($X^2(1)$=.919,p>.05).

We then grouped the aberrant antigens expressions (CD4 and CD56) together and performed Chi Square analysis to see if there is any relationship between overall aberrant antigen expressions with any specific breakpoint cluster regions detected in the molecular studies. Surprisingly, there was a significant association between cumulative aberrant antigen expression and bcr1 ($X^2(2)$=6.833,p<.05). However there were no significant association seen in bcr2 and bcr 3; ($X^2(2)$=.199,p>.05) and ($X^2(2)$=4.599,p>.05) respectively. (An alpha level of .05 was adopted for these statistical tests)

## DISCUSSION

In this study, we retrospectively reviewed morphologic features and immunophenotypes of APL and correlated the results with molecular and cytogenetic findings. We found that 100% of the cases were positive for CD33 and CD9, and 96.8% were positive for CD13. CD34 and HLA-DR expression were only seen in 3.2% and 9.7% of the cases respectively. Pei Lin *et al*, Dong Y *et al*, Orfao A *et al* and Gorczyca also reported similar findings in their APL immunophenotypic studies [1,4,7]. Lack of HLA-DR is one of the most sensitive findings associated with APL, even though HLA-DR expression was detected in up to 9% of the cases in this study. However, absence of HLA-DR alone is not sufficient to interpret as APL-specific as similar findings was reported in about 20% of other AML subtypes. It is also interesting to note the expression of CD9 in all cases in this study. Erber WN *et al* also reported expression of CD9 in all of their APL cases[19]. Expression of CD117 was seen in 45.2% of the cases. CD117 is usually indicates myeloid immaturity in APL but it differs among cases; ranging from 50 to 90% as reported by other researchers [1,2,3,4,7].

The "gold standard" for APL diagnosis are detection of t(15;17) and variant RARα translocation. However, FCM remains an indispensable tool in providing rapid diagnosis and early recognition of APL as it helps to resolve uncertainty in the morphologic findings early on and muddles from an initial negative cytogenetics or molecular findings.

Sample size in this study was relatively small to associate the immunophenotypic findings with other available clinical information, so we focused our analysis on laboratory findings. Our study reiterated the notion that immunophenotypic characteristics of APL were independent of the chromosomal abnormalities detected. FCM is capable to be an independent entity in identifying APL with cryptic genomic aberration, otherwise undetectable by conventional cytogenetic methods such as G-banding analysis. The well-defined hallmark, t(15;17)(q22;21), seem to have no impact on the APL immunophenotypic characteristics.

There was no association between the APL immunophenotype antigen expression and 3 fusion transcript type; bcr1, bcr2 and bcr3. There was no significant association between the antigen expression and their molecular counterparts.

The usefulness of aberrant antigen expression in APL as a prognostic factor was assessed. Only two markers of aberrancy were seen in this study; CD4 and CD56. There was no significant correlation between CD4 and CD56 with the karyotypes or molecular findings. This finding matched Di Bona et al on CD56 positivity that did not correlate with age, blast count or karyotype at diagnosis and did not influence the outcomes in terms of complete remission (CR). (12) In another study by Pei Lin et al, aberrant expression of CD2 in APL correlates with short form of PML-RARα transcripts which was associated with poorer prognosis [2,7]. Nonetheless, there was no CD2 aberrancy in APL seen in this study.

The relationships between overall aberrant antigen expressions with any specific breakpoint cluster regions detected in the molecular studies were analysed. We hypothesized that specific genetic abnormalities would be reflected by alteration in the pattern of surface antigen expression. Based on this, we studied if immunophenotypic characteristics of APL could show specificity or sensitivity towards initial screening of PML-RARα gene rearrangements to complement the traditional morphology which would enable increased accuracy and expedite treatment and specific therapy decision. Surprisingly, there is significant association between cumulative aberrant antigen expression and bcr1 transcript (p<.05). Despite showing statistically significant correlation, the usefulness of the association is controversial, and the sample size is relatively small to draw a conclusion.

To recapitulate in short, FCM immunophenotyping is a reliable and powerful tool in identifying APL. It helps in discriminating APL from other AML subtypes. It will be worthwhile to perform prospective studies in larger series of APL patients to corroborate the relationship and correlate the immunophenotype or genotype where new information will probably be discovered to enhance patient care and management.

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## REFERENCES


1. Dong, HY, Kung, JX, Bhardwaj, V, McGill J. Flow cytometry rapidly identifies all acute promyelocytic leukemias with high specificity independent of underlying cytogenetic abnormalities. American J of Path 2011; 135:76-34.
2. Orfao A, Chillon MC, Bortoluci AM, *et al.* The flow cytometric pattern of CD34, CD15 and CD13 expression in acute myeloblastic leukaemia is highly characteristic of the presence of PML-RARα gene rearrangements. Haematologica 1999; 84: 405-12.
3. Kaito K, Kayatama T, Masuoka H, *et al.* CD2+ acute promyelocytic leukaemia is associated with leucocytosis, variant morphology and poorer prognosis. Clin Lab Haem 2005; 27: 307-11.
4. Gorczyca, W. Acute promyelocytic leukaemia: four distinct patterns by flow cytometry immunophenotyping. Pol J Path 2012; 1: 8-17.
5. Stein, E, McMahon B, Kwaan H, Altman JK, Frankfurt O, Tallman MS. The coagulopathy of acute promyelocytic leukaemia revisited. Best Pract Res Clin Haem 2009; 22: 153-63.
6. National Cancer Registry Report 2007 (Malaysia Cancer Statistics – Data and Figure).
7. Lin P, Hao S, Medeiros J, *et al.* Expression of CD2 in acute promyelocytic leukaemia correlates with short form of PML-RARα transcripts and poorer prognosis. American K of Clin Path 2004; 121: 402-7.
8. Melnick A, Licht JD. Deconstructing a disease: RARalpha, its fusion partners and their roles in the pathogenesis of acute promyelocytic leukaemia. Blood 1999; 93: 3167-215.
9. Faretta M, Di Croce L, Pelicci PG. Effects of the acute myeloid leukaemia-associated fusion proteins on nuclear architecture. Semin Hematolol 2001; 38: 42-53.
10. Chopa PC, Gomez J, Vall HG, *et al.* A novel method for the detection, quantitation and breakpoint cluster region determination of t(15;17) fusion transcripts using a one-step real-time multiplex RT-PCR. American J of Clin Path 2003; 119: 137-44.
11. Schabath R, Ratei R, Ludwig WD. The prognostic significance of antigen expression in leukaemia. Best Practice & Research Clin Haem 2003; 16: 613-28.
12. Di Bona, E, Sartori, R, Zambello, R. Prognostic significance of CD56 antigen expression in acute myeloid leukaemia. Hematologica 2002; 87: 250-6.
13. Ossenkoppele GJ, Loosdrecht AA, Schuurhuis GI. Review of the relevance of aberrant antigen expression by flow cytometry in myeloid neoplasms. British J Haem 2011; 153: 421-36.
14. Gujral S, Badrinath Y, Kumar A, *et al.* Immunophenotypic profile of acute leukaemia: critical analysis and insights gained at a tertiary care center in India. Cytometry Part B 1999; 76B: 199-205.
15. El-Sissy AH, El-Mashari MD, Basunni WY, El-Swaayed AF. Aberrant lymphoid antigen expression in acute myeloid leukaemia in Saudi Arabia. J of the Egyptian Nat Cancer Inst Vol 2006; 18(3): 244-9.
16. Khurram MM, Jafri SA, Mannan A, Nadeem A, Jamal A. Frequency of aberrant expression of CD markers in cases of acute leukaemia. Med J of Islamic World Academy of Sciences 2010; 18:2, 55-60.
17. Andoljsek D, Zupan IP, Zontar D, *et al.* Cell markers in the recognition of acute myeloblastic leukaemia subtypes. Cell Mol Bio Letter 2002; (7)2.
18. Hsiao CH, Tang JL, Yao M, *et al.* High incidence of CD 56 expression and relapse rate in acute myeloid leukaemia patients with t(8.21) in Taiwan. J Formos Med Assoc 2002; 101(6) 393-8.
19. Erber WN, Asbahr H, Rule SA, Scott CS. Unique immunophenotype of acute promyelocytic leukaemia as defined by CD9 and CD68 antibodies. Br J Haematol 1994; 88(1):101-4.