

Number of Articles: 17

Editorial Hematopathology

The changes of Coagulation State in Patients Contracted with SARS-COV-19

Anahita Nosrati

Volume 15, Issue 4 , October 2020, Pages 259-260

<https://doi.org/10.30699/ijp.2020.129354.2427>

Abstract Coronaviruses are considered to be one of the most significant human and animal pathogens. In late 2019, a new species of coronavirus was recognized as the cause of some pneumonia cases in Wuhan, China. The disease spread rapidly and made an epidemic in China and subsequently in almost all countries ... [Read More](#)

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Original Research Diagnostic Pathology

Clinical Validation of Eleven Formulas for Calculating LDL-C in Iran

Fereshteh Atabi; Reza Mohammadi

Volume 15, Issue 4 , October 2020, Pages 261-267

<https://doi.org/10.30699/ijp.2020.110379.2174>

Abstract Background & Objective: Concentration of low-density lipoprotein (LDL) is a known risk factor for cardiovascular disease which is routinely measured or calculated as LDL-C in clinical laboratories. In order to decrease the cost, instead of its measuring, it is recommended to calculate it using multiple ... [Read More](#)

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Original Research GI, Liver & Pancreas Pathology

Assessment of p16 and Ki67 Immunohistochemistry Expression in Squamous Intraepithelial Lesion with Cytomorphological Correlation

Apurv Ghosh; M Nirupama; Nandan Padmanabha; Hema Kini

Volume 15, Issue 4 , October 2020, Pages 268-273

<https://doi.org/10.30699/ijp.2020.112421.2208>

Abstract Background & Objective: Cervical cancer is the most common cancer in women worldwide with high mortality, necessitating quicker diagnostic methods. We wish to enhance the existing cervical biopsies of Squamous Intraepithelial Lesions (SIL) using p16 and Ki67 as surrogate markers to assess correlation ... [Read More](#)

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Original Research Molecular Pathology

Investigation of HER-2 expression and its Correlation with clinicopathological parameters and overall survival of esophageal squamous cell carcinoma patients

Mitra Heidarpour; Mehran Taheri; Ali Akhavan; Parvin Goli; Amirhosein Kefayat

Volume 15, Issue 4 , October 2020, Pages 274-281

<https://doi.org/10.30699/ijp.2020.113829.2235>

Abstract Background & Objective: Human epidermal growth factor receptor 2 (HER-2) exhibits a vast range of expression in esophageal squamous cell carcinoma (ESCC) patients as a biomarker. This paper aimed to investigate HER-2 expression and clinicopathological parameters of esophageal SCC. Methods: HER-2 ... [Read More](#)

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Original Research Hematopathology

Overexpression of MicroRNA-506 in Jurkat (acute T Cell Leukemia) Cell Line

Shaghayegh Rostami Yasuj; Narges Obeidi; Gholamreza Khamisipour; Zeynab Gharehdaghi; Zivar Zangeneh

Volume 15, Issue 4, October 2020, Pages 282-291

<https://doi.org/10.30699/ijp.2020.119627.2298>

Abstract Background & Objective: Acute lymphoblastic leukemia (ALL) is a malignant disease that arises from various mutations in B or T-lymphoid progenitors. MicroRNAs (miRNAs) regulate gene expression by binding to the 3' untranslated region of protein-coding genes. Dysregulation of miRNA expression ... [Read More](#)

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Original Research Gynecologic Pathology

Association of Human Papilloma Virus and Epstein-Barr Virus with Ovarian Cancer in Shiraz, Southwestern Iran

Mohammad Reza Shokouh; Akbar Safaie; Afagh Moattari; Jamal Sarvari

Volume 15, Issue 4, October 2020, Pages 292-298

<https://doi.org/10.30699/ijp.2020.119681.2306>

Abstract Background & Objective: Ovarian cancer is one of the most common cancers amongst women. The association of Human papillomavirus (HPV) and Epstein-Barr virus (EBV) with ovarian cancer is inconclusive; therefore, the aims of this study were to evaluate the frequency of HPV and EBV in malignant, ... [Read More](#)

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Original Research Dermatopathology

Thiopurine S-methyltransferase and Pemphigus Vulgaris: A Phenotype-Genotype Study

Maral Mokhtari; Farzaneh Mostanbet; Saideh Nekooee Fard; Golsa Shekarkhar; Mozhdeh Sepaskhah; Maryam Sadat Sadati

Volume 15, Issue 4, October 2020, Pages 299-305

<https://doi.org/10.30699/ijp.2020.121365.2320>

Abstract Background & Objective: Thiopurine drugs are considered as a treatment modality in various autoimmune disorders including pemphigus vulgaris (PV). These drugs are metabolized by an enzyme "Thiopurine S-methyl transferase" (TPMT). Various variants of this enzyme may have decreased ... [Read More](#)

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Original Research Hematopathology

The Association of FLT3-ITD Gene Mutation with Bone Marrow Blast Cell Count, CD34, Cyclin D1, Bcl-xL and hENT1 Expression in Acute Myeloid Leukemia Patients

Paulus Budiono Notopuro; Jusak Nugraha; Budi Utomo; Harianto Notopuro

Abstract Background & Objective: FLT3-ITD has been recently used as a molecular prognostic marker for risk classification in acute myeloid leukemia (AML) therapy. In this study we aimed to investigate the association of FLT3-ITD gene mutation with bone marrow blast cell count, CD34 expression as ... [Read More](#)

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Original Research [Molecular Pathology](#)

Comparison of patient-collected and lab technician-collected nasopharyngeal and oropharyngeal swabs for detection of COVID-19 by RT-PCR

Alireza Abdollahi; Abbas Shakoori; Hoda Khoshnevis; Mohammad Arabzadeh; Seyed Ali Dehghan Manshadi; Esmaeil Mohammadnejad; Dorsa Ghasemi; Maryam Safari Aboksari; Shaban Alizadeh; Vahid Mehrtash; Arezoo Eftekhari-javadi; Masoomeh Safaei

Volume 15, Issue 4, October 2020, Pages 313-319

Abstract Background & Objective: A simple approach to prevent close contact in healthcare settings during the COVID-19 outbreak is to train patients to collect their own nasopharyngeal and oropharyngeal swabs and deliver them to medical laboratories to have them processed. The aim of our study ... [Read More](#)

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Original Research [Molecular Pathology](#)

Genetic Diversity of ctxB Gene Among Classical O1 and El Tor Strains of Vibrio cholerae using High-Resolution Melting Curve Analysis

Mahdiah Mahboobi; Reza Mirnejad; Hamid Sedighian; Vahhab Piranfar; Abbas Ali Imani Fooladi

Volume 15, Issue 4, October 2020, Pages 320-325

Abstract Background & Objective: Vibrio cholerae is a natural inhabitant of the environment and causes severe diarrhea ailments (cholera) that affects thousands of people each year worldwide. The most important virulence factors of this pathogen are cholera toxin (cholera toxin CT) and Type IV ... [Read More](#)

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Original Research [Hematopathology](#)

Matrix metalloproteinase-9 (MMP-9) Expression in Non-Small Cell Lung Carcinoma and Its Association with Clinicopathologic Factors

Amir Hossein Jafarian; Melika Kooshki forooshani; Hossein Reisi; Nema Mohamadian roshan

Volume 15, Issue 4, October 2020, Pages 326-333

Abstract Background & Objective: Matrix metalloproteinases-9 (MMP-9) is one of the most important enzymes to breakdown extracellular matrix which plays a major role in tumor invasion and metastasis. This study aimed to determine tumor MMP-9 expression in non-small-cell lung carcinoma (NSCLC) and ... [Read More](#)

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Case Reports [Gynecologic Pathology](#)

Coexistence of Benign Brenner Tumor with Mucinous Cystadenoma in an Ovarian Mass

Farzaneh Nazari; Zahra Dehghani

Volume 15, Issue 4 , October 2020, Pages 334-337

<https://doi.org/10.30699/ijp.2020.104017.2052>

Abstract Ovarian cancer is the most lethal gynecologic malignancy. The surface epithelial tumor is the most common type of ovarian cancer. Among these, the mucinous tumors account for 10-15% of ovarian tumors. Mucinous ovarian tumors are among the most difficult ovarian neoplasms for surgical pathologists to ... [Read More](#)

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Orthotopic Liver Transplantation for Etanercept-Induced Acute Hepatic Failure; a case report

Dorsay Sadeghian; Farid Azmoudeh-Ardalan; Simin Dashti-Khavidaki; Nasir Fakhar

Volume 15, Issue 4 , October 2020, Pages 338-341

<https://doi.org/10.30699/ijp.2020.117000.2269>

Abstract The occurrence of hepatotoxicity following etanercept (tumor necrosis factor-alpha antagonist) prescription, has been studied well. However, an acute hepatic failure leading to liver transplant as an adverse effect of this drug has not been reported in the literature. In this article, we are going to ... [Read More](#)

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A Rare Case of Metastasis of Renal Clear Cell Carcinoma to the Thyroid Gland, Presenting as a Goiter Nodule, Three Years After Nephrectomy.

Hedieh Moradi Tabriz; Arezoo Eftekhar-Javadi; Atiieh Zandnejadi

Volume 15, Issue 4 , October 2020, Pages 342-345

<https://doi.org/10.30699/ijp.2020.117839.2282>

Abstract Thyroid gland metastatic tumors are rare in clinical practice. Clear cell RCC is one of common metastatic tumors to thyroid. We here reported a case of incidentally found clear cell renal carcinoma metastasis to the thyroid gland 3 years after nephrectomy, in the thyroidectomy procedure performed for ... [Read More](#)

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Intravascular Large B-cell Lymphoma: A Report of Two Cases

Fereshteh Ameli; Fatemeh Nili; Hana Saffar

Volume 15, Issue 4 , October 2020, Pages 346-350

<https://doi.org/10.30699/ijp.2020.119590.2299>

Abstract One of the rare variants of extranodal large B-cell lymphoma is intravascular large B-cell lymphoma (IVLBCL). Characteristics of IVLBCL include intraluminal selective proliferation of atypical lymphoid cells in small to medium-sized vessels. The etiologic of IVLBCL is unknown, but due to the growth pattern ... [Read More](#)

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Is Micronucleus Assay Suitable for Cytogenetic Biomonitoring the Different Ways to Smoke?

Daniel Ribeiro; Ingra Malacarne; Daniel Souza

Volume 15, Issue 4 , October 2020, Pages 351-351

<https://doi.org/10.30699/ijp.2020.125206.2370>

Abstract The aim of the letter to editor is to provide some comments regarding the manuscript of DehghanNezhad et al.(1) recently published in the Iranian Journal of Pathology titled “Micronucleus Assay of Buccal Mucosa Cells in Waterpipe (Hookah) Smokers: A Cytologic Study.” In this article, the ... [Read More](#)

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Response to letter to the editor:” Is micronucleus assay suitable for cytogenetic biomonitoring the different ways to smoke? ”

Noushin Jalayer naderi

Volume 15, Issue 4 , October 2020, Pages 352-354

<https://doi.org/10.30699/ijp.2020.127517.2390>

Abstract Dear Editor, I was very pleased to find that the article” Micronucleus Assay of Buccal Mucosa Cells in Waterpipe (Hookah) Smokers: A Cytologic Study “(1) has attracted the attention of some readers and has created a few points for them. In my opinion, these points are good bases to overcome ... [Read More](#)

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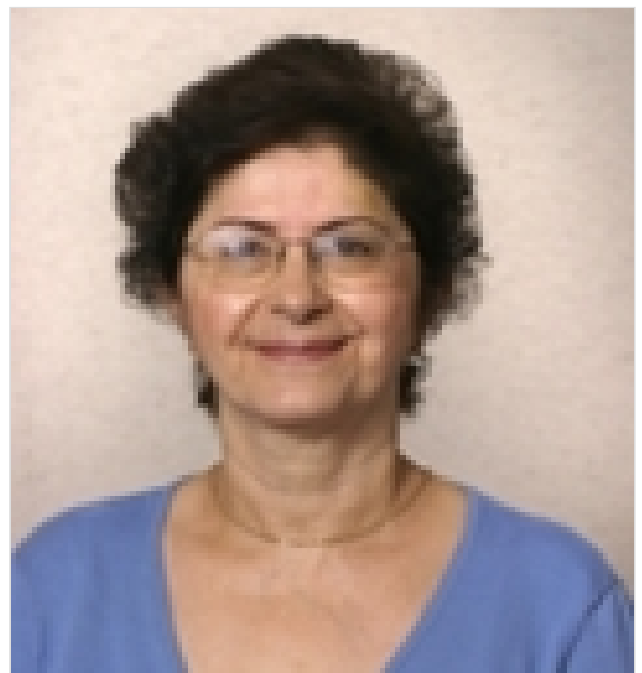


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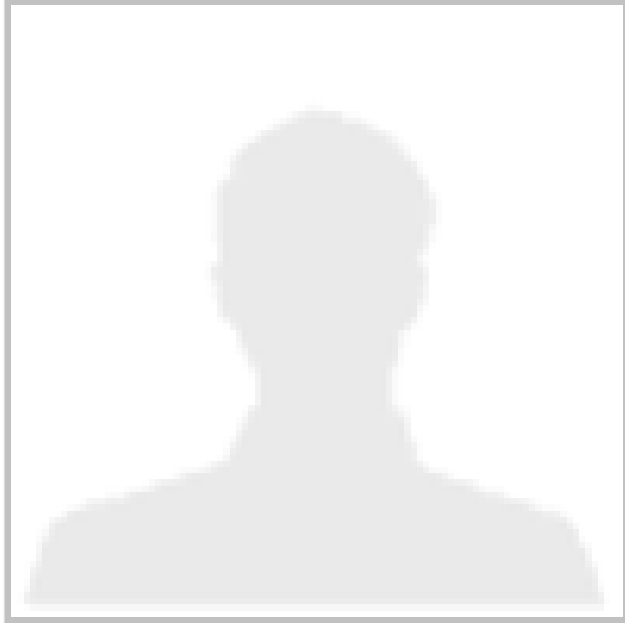
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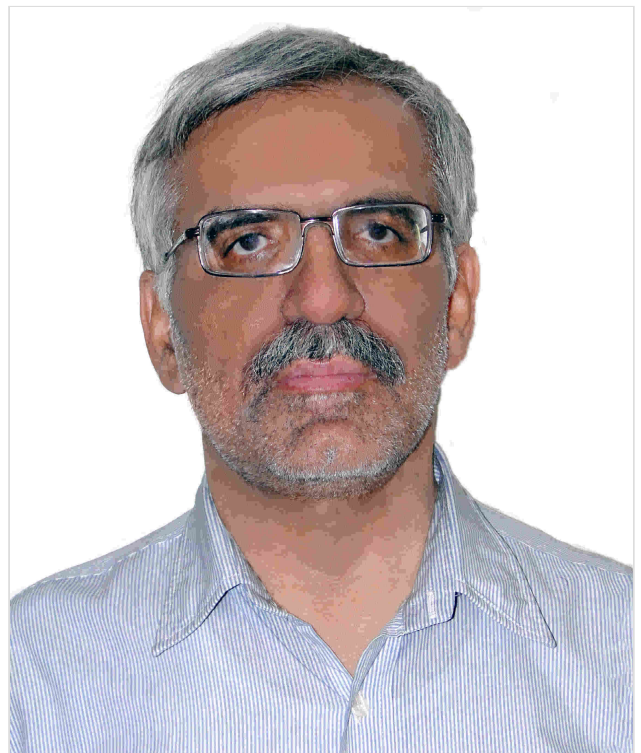
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The Association of *FLT3*-ITD Gene Mutation with Bone Marrow Blast Cell Count, CD34, Cyclin D1, Bcl-xL and hENT1 Expression in Acute Myeloid Leukemia Patients

Paulus Budiono Notopuro^{1*} , Jusak Nugraha² , Budi Utomo³ , Harianto Notopuro⁴ 

1. Faculty of Medicine, Airlangga University, Jawa, Indonesia
2. Department of Clinical Pathology, Faculty of Medicine, Airlangga University, Jawa, Indonesia
3. Department of Public Health, Faculty of Medicine, Airlangga University, Jawa, Indonesia
4. Department of Biochemistry and Molecular Biology, Faculty of Medicine, Airlangga University, Jawa, Indonesia

KEYWORDS

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ABSTRACT

Background & Objective: *FLT3*-ITD has been recently used as a molecular prognostic marker for risk classification in acute myeloid leukemia (AML) therapy. In this study we aimed to investigate the association of *FLT3*-ITD gene mutation with bone marrow blast cell count, CD34 expression as malignant cell burden, cyclin D1 and Bcl-xL expressions as indexes of cell proliferation and anti-apoptosis and human equilibrative nucleoside transporter 1 (hENT1) expression as cytarabine transporter during AML treatment.

Methods: We investigated *FLT3*-ITD mutations, bone marrow blast cell count, CD34, cyclin D1, Bcl-xL and hENT1 expression in bone marrow aspirates from 22 de novo AML patients in a cross sectional study.

Results: *FLT3*-ITD mutations were observed in 5 out of 22 de novo AML patients (22.7%). Patient with *FLT3*-ITD mutations had higher blast cell counts (79.5% vs 56.1%, $P=0.004$). In patients with *FLT3*-ITD mutations, CD34 and cyclin D1 expressions were higher (MFI 328.80 vs 25.78, $P=0.003$ and MFI 74.51 vs 57.15 $P=0.005$) than the patients without mutations. hENT1 expression in AML with *FLT3*-ITD mutation was lower (MFI 29.64 versus 56.32, $P=0.0000$) than in mutation-free AML. There was no significant difference in Bcl-xL expression between patients with and without mutations ($P=0.61$).

Conclusion: A significant association was found between *FLT3*-ITD gene mutations in AML patients with bone marrow blast cell count, CD34, cyclin D1 and hENT1 expressions, however no association was obtained with Bcl-xL expression. These findings support the role of such mutation in pathogenesis of AML and its contribution in rearrangement of standard therapy with cytarabine in management of AML.

Corresponding Information: Notopuro Harianto, Professor in Biochemistry and Molecular Biology, Faculty of Medicine, Airlangga University, Indonesia. Email: paulusbudiono77@gmail.com

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Introduction

Acute myeloid leukemia (AML) is a hematologic malignancy with clonal abnormalities in hematopoietic stem cells with heterogeneous clinical features and basic genetic aberrations. The incidence of AML is 4.2/100.000 persons/year globally and its mortality rate is 2.8/100.000 persons/year (1). Recently, it has been proposed that AML prognosis and risk stratification was determined based on the cytogenetic and molecular findings. The findings in favorable risk cases include *inv16*, *t(16,16)*, *t(8,21)*, *t(15,17)*, normal cytogenetics with *CEBPA* biallelic gene mutation and *NPM1* gene mutation without *FLT3*-ITD mutation. Findings in intermediate risk cases include

normal cytogenetics, *+8* and *t(9,11)* and the findings in poor risk cases include complex cytogenetic abnormalities (≥ 3 abnormalities), *-5/5q-*, *-7*, *7q-* and *11q23* rearrangement except *t(9,11)*, *inv(3)* or *t(3,3)*, *t(6,9)*, *t(9,22)* and normal cytogenetics with *FLT3*-ITD gene mutation (2).

There are various clinical pictures and different treatment outcomes in more than 50% of AML patients with normal cytogenetics (3). *FLT3*-ITD gene mutation has been reported to be a strong factor in newly diagnosed AML patients with normal cytogenetics and an independent prognostic factor that influences the

treatment outcomes, overall survival and disease free survival. The frequency of *FLT3*-ITD gene mutation in AML patients is about 30% and varies around the world. Its frequency in de novo AML is higher than in secondary AML. *FLT3*-ITD gene mutation causes ligand independent dimerization and activation of FLT3 receptor. Autoactivation of FLT3 receptor leads to unregulated cell growth through excessive cell proliferation and anti-apoptotic activity (4,5).

CD34+ cells are poor prognostic factors for complete remission achievement after induction therapy (6,7). Blast cell count and CD34+ cells reflect tumor burden and depend on cell proliferation and anti-apoptotic activity (8). *FLT3*-ITD gene mutation was reported to affect the human equilibrative nucleoside transporter 1 (hENT1) expression in children with acute lymphoblastic leukemia (9). hENT1 has an important role as a cytarabine transporter. Cytarabine is a major induction and consolidation backbone in AML treatment (10).

In this study, we investigated the association of *FLT3*-ITD gene mutation with bone marrow blast count, CD34, Cyclin D1 (a cell proliferation marker), Bcl-xL (an anti-apoptotic agent) and hENT1 (a cytarabine transporter) expressions that lead to poor prognosis and determine treatment outcomes in AML patients with this mutation.

Materials and Methods

This study was a cross sectional study and was ethically approved by the ethical committee, faculty of medicine, Airlangga University. Appropriate informed consents were obtained before samples procurement. Bone marrow aspirates were collected from 22 newly diagnosed AML patients from August 2019 – January 2020 from a private hospital around Surabaya, Indonesia. All bone marrow samples investigated in the study were obtained at the time of first diagnosis. The laboratory examination was performed in department of biochemistry – molecular biology and clinical pathology, faculty of medicine, Airlangga University, Dr. Soetomo general hospital Surabaya.

Diagnosis of AML was established based on bone marrow aspirate cytology analysis. Diagnosis of AML was established observing more than 20% blast cell count as cut off point. Subtypes of AML were classified based on French American British (FAB) criteria. Bone marrow blast cells were counted among 500 of nucleated cells. The expressions of CD34, hENT1, Bcl-xL and cyclin D1 were examined with BD FACSCalibur® using monoclonal antibody anti-CD45 conjugated to peridinin chlorophyll protein (PerCP), anti-CD34 conjugated to phycoerythrin (PE) from Becton Dickinson®, anti-hENT1 conjugated to fluorescein isothiocyanate (FITC), anti-Bcl-xL conjugated to PE and anti-cyclin D1 conjugated to FITC provided from Santa Cruz Biotechnology Inc®.

The PCR investigation for *FLT3*-ITD gene mutation was done with bone marrow specimen. DNA was extracted and purified using QIAmp DNA blood mini kit Qiagen®. In brief, 1 µL of DNA was mixed with 10 µL of PCR kit Go Taq 2x PCR master mix solution (Promega®),

1 µL of forward and 1 µL of reverse primer in a total volume of 20 µL. The forward *FLT3*-ITD primer was 14F: 5'-GCA-ATT-TAG-GTA-TGA-AAG-CCA-GC-3' (23mer) and the reverse primer was 15R: 5'-CTT-TCA_GCA-TTT-TGA-CGG-CAA-CC-3' (23mer). The amplification (resulting in 329 bp target amplicon) was achieved after following steps: hot start (for 3 min at 95°C), 40 cycles of denaturation (for 30 sec at 95°C), annealing (for 45 sec at 56°C), extension (for 30 sec at 72°C) and finally extension (for 10 min at 72°C). PCR products were run on 3% agarose gel and 100 V electrophoresis was done with a 100 bp DNA ladder (Promega®). The presence of *FLT3*-ITD mutation was determined by visualization of 329 bp wild type gene fragment and a fragment larger than 329 bp.

Of bone marrow aspirate with EDTA, 2 mL was examined for flowcytometry analysis. In brief, 50 µL of homogenous bone marrow aspirate and phosphate buffer saline (1:1) mixture was added to 2, 5 µL of PerCP-labeled anti-CD45, 2, 5 µL of PE-labeled anti-CD34 and FITC-labeled anti-hENT1 (pretreated with 1 mL of lysing solution, 250 µL of cytofix/cytoperm and 1 mL of perm wash reagent from Becton Dickinson®) in the first tube and 2, 5 µL of PerCP-labeled anti-CD45, 2, 5 µL of anti-Bcl-xL and 2, 5 µL of anti-cyclin D1 (pretreated with 1 mL of lysing solution, 250 µL of cytofix/cytoperm and 1 mL of perm wash reagent from Becton Dickinson®) in the second tube. Blast gating strategy was used to evaluate CD34, hENT1, Bcl-xL and cyclin D1 expressions in the blast cells. Median Fluorescent Intensity (MFI) was applied to examine CD34, hENT1, Bcl-xL and cyclin D1 expressions (11).

Statistical Analysis

Independent sample t-test and Mann-Whitney U test were performed to compare quantitative data between AML patients with and without mutation. P-values less than 0.05 were considered statistically significant. All calculations were performed using the SPSS 22 (SPSS, Chicago, IL., USA).

Results

We analyzed bone marrow specimens obtained from 22 newly diagnosed AML patients for the presence of *FLT3*-ITD gene mutation, blast cell count, CD34, Bcl-xL, cyclin D1 and hENT1 expressions. Total AML patients included 13 males and 9 females. Both adult and pediatric AML cases (ages 4–84) were included in this study. According to FAB classification for AML, 6 cases were diagnosed with AML M1 (27.3%), 5 cases were diagnosed with AML M2 (22.7%), 5 cases were diagnosed with AML M3 (22.7%), 1 case was diagnosed with AML M4 (4.5%) and 5 cases were diagnosed with AML M5 (22.7%).

Mutations in *FLT3*-ITD were found in 5 (22.7%) AML patients based on the detectable amplicon with 329 bp and larger than 329 bp in 3% agarose gel electrophoresis. All of those patients were classified as *FLT3*-ITD mutants. The cases result of gel electrophoresis is presented in Figure 1.

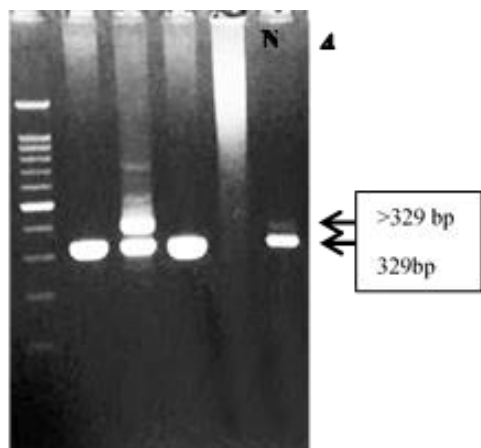


Fig. 1. PCR results for detection of *FLT3*-ITD gene mutation. LD: DNA ladder 100 bp, NA: negative control, positive results of heterozygote mutant *FLT3*-ITD in patient number 2 (2 bands of 329 and > 329 bp), negative result (wild type) in patient number 1,3,4 (single band of 329 bp).

Table 1. Values of blast cell count, expression of CD34, hENT1, cyclin D1, and Bcl-xL in AML patients with and without *FLT3*-ITD gene mutation

Parameter	AML with <i>FLT3</i> -ITD gene mutation (n = 5)	AML without <i>FLT3</i> -ITD gene mutation (n = 17)	P-value
Blast cell count - % mean \pm SD	79.5 \pm 12.9	56.1 \pm 14.2	0.004*
CD34+ expression (MFI) median	328.8	25.78	0.003*
hENT1 expression (MFI) mean \pm SD	29.64 \pm 5.10	56.32 \pm 12.81	0.0000*
Cyclin D1 expression (MFI) mean \pm SD	70.11 \pm 15.41	57.15 \pm 11.49	0.036*
Bcl-xL expression (MFI) median	208.28	188.7	0.61

* P-value <0.05

The mutation of *FLT3*-ITD was frequently found in AML-M2 patients (3 out of 5 patients). The other subtypes of AML patients with *FLT3*-ITD mutations were AML-M3 (1 out of 5 patients) and AML-M5 (1 out

of 5 patients). This mutation is found in 1 pediatric 5-year-old patient. The flowcytometry analysis of AML cases with and without *FLT3*-ITD gene mutation are presented in [Figures 2](#) and [3](#).

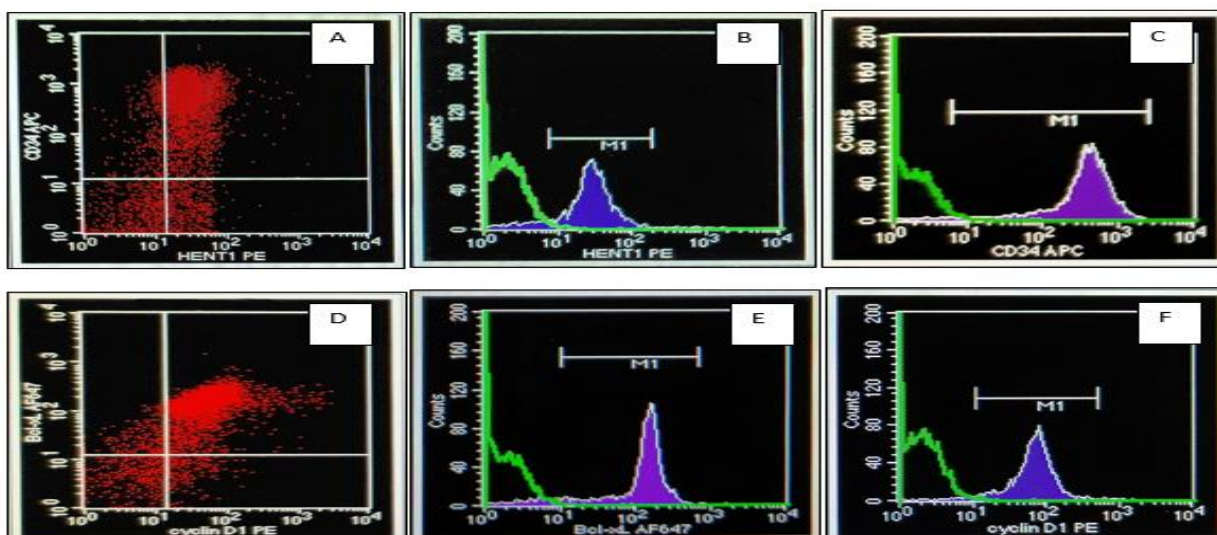


Fig. 2. Flow cytometry analysis for the expression of CD34, Bcl-xL, cyclin D1, hENT1 in an AML patient with *FLT3*-ITD mutation. 2A and 2D) were the scattergrams for CD34, hENT1, Bcl-xL and cyclin D1 expression. 2B, 2C, 2E, 2F) were the histograms for CD34, hENT1, Bcl-xL and cyclin D1 expression (MFI analysis).

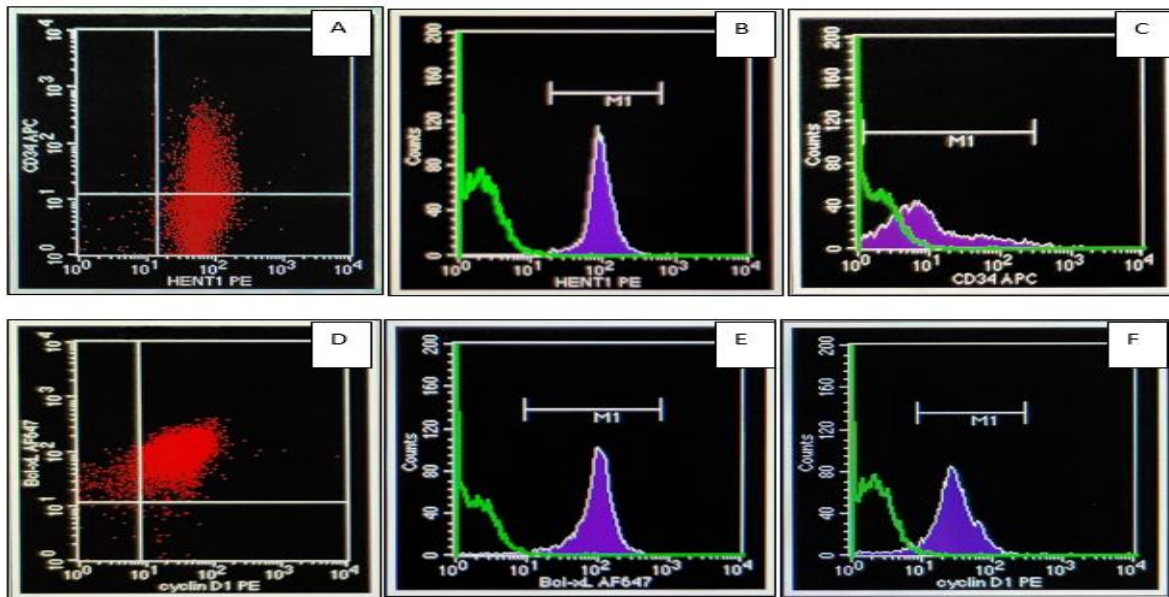


Fig. 3. Flow cytometry analysis for the expression of CD34, Bcl-xL, cyclin D1, hENT1 in an AML patient without FLT3-ITD mutation. Figure 3A and 3D were the scattergrams for CD34, hENT1, Bcl-xL and cyclin D1 expression. Figure 3B, 3C, 3E, 3F were the histograms for CD34, hENT1, Bcl-xL and cyclin D1 expression (MFI analysis). Flow cytometry analysis for the expression of CD34, Bcl-xL, cyclin D1, hENT1 in an AML patient without FLT3-ITD mutation. Figure 3A and 3D were the scattergrams for CD34, hENT1, Bcl-xL and cyclin D1 expression. Figure 3B, 3C, 3E, 3F were the histograms for CD34, hENT1, Bcl-xL and cyclin D1 expression (MFI analysis).

The blast cell count in AML patients with *FLT3*-ITD gene mutation was significantly higher than mutation-free AML patients (79.5% vs 56.1%, $P=0.004$). The expressions of CD34 and cyclin D1 in AML cases with *FLT3*-ITD mutation were higher than in AML cases without this mutation (MFI 328.8 vs 25.78, $p=0.003$ and MFI 74.51 vs 57.15, $P=0.005$). hENT1 expression in AML with *FLT3*-ITD mutation was lower (MFI 29.64 vs 56.32, $P=0.0000$) than mutation-free AML. The Bcl-xL expression didn't have a significant difference between AML patients with and without *FLT3* mutation ($P=0.61$). The analytical data are summarized in [Table 1](#).

Discussion

FLT3 is a tyrosine kinase receptor involved in hematopoiesis and commonly mutated in AML. There are two common mutations in *FLT3*. The first mutation is internal tandem duplication (ITD) in juxtamembrane (JM) domain and a point mutation in tyrosine kinase domain. *FLT3*-ITD gene mutation has been known as a strong prognostic factor in AML patients and it is related to disease progression, high relapse rate and low overall survival for 10 months (4,12,13). The frequency of *FLT3*-ITD gene mutation in AML is 21-24%. Its frequency is lower than *NPM1* gene mutation which is the most frequent mutation found in AML with the frequency of 35% in de novo AML and 45% in AML with normal cytogenetics. *FLT3*-ITD gene mutation in AML is definitively related to poor prognosis (5,14,15).

In the current study, we determined the frequency of *FLT3*-ITD gene mutation and its association with blast cell count, CD34, cyclin D1, hENT1 and Bcl-xL expressions. The frequency of *FLT3*-ITD gene mutation

in this study was 22.7% (5 out of total 22 AML patients). Our result is slightly higher than the published studies in Asian populations in which the frequency of *FLT3*-ITD gene mutation varies between 10 to 21% (13,16-18). The difference in mutation frequency could be due to differences in sample size, ethnicity, environmental factors and patient selection. The small sample size in this study does not represent the true frequency in population. In this study, the mutation is also more frequent in adult AML patients (80%) than in children, this result is similar to some previous studies (16,18).

Among various subtypes of AML based on FAB classification, in this study *FLT3*-ITD gene mutation is more frequent in AML-M2 patients (60%, 3 out of 5 AML patients). The other AML subtypes with *FLT3*-ITD gene mutation include AML-M3 (1 patient) and AML-M5 (1 patient). This result is different from the result of a large study in Germany. Thiede C. *et al.* reported that AML-M5 is the most common FAB subtype in AML with *FLT3*-ITD gene mutation (19). A study in a Chinese population showed that AML-M2 was the most frequent subtype in AML patients with *FLT3*-ITD gene mutations, while a study in a Thai population showed that AML-M3 was the most frequent subtype (16,17). The prognostic significance in AML-M3 patients with t(15,17) was still controversial (16,20).

Mutant *FLT3* receptor inhibits the function of silencing mediator of retinoic acid and thyroid hormone receptor (SMRT), a co-repressor that interacts with promyelocytic leukemia zinc finger (PLZF) and eight twenty one protein (ETO) that are related to the cell proliferation blockade (4). Mutated receptor activates signal transducer and activator of transcription 5 (STAT5) that plays a critical role in cell proliferation and

anti-apoptotic function. Leukemic cells harboring *FLT3*-ITD have high levels of STAT5 phosphorylation and increased bonding of this transcription factor to DNA. Activation of STAT5 plays a critical role in cell growth that is related to mitogen activated protein kinase (MAPK) and the regulation of cyclin D1, Bcl-xL, c-MYC, PIM serine-threonine kinase and *p21*^{WAF1/CIP1} (inhibitor of cyclin dependent kinase) transcription (4).

FLT3-ITD mutation induces constitutive receptor activation, ligand independent dimerization and autophosphorylation which supports uncontrolled leukemic cells proliferation and apoptosis. The leukemic cells burden is the resultant of uncontrolled proliferation and apoptotic-antiapoptotic activity (4,21). In this study, we examined cyclin D1 as a cell proliferation marker and Bcl-xL as an anti-apoptosis activity marker of leukemic cells (22,23). AML patients with *FLT3*-ITD mutation had higher bone marrow blast cell count and CD34 expression level than mutation-free patients. Blast cell count and CD34 expression had significant association with the *FLT3*-ITD gene mutation in AML. Some studies have also reported that the presence of *FLT3*-ITD gene mutation is significantly associated with the higher blast cell count and CD34 expression (7,17,18,24). Normal *FLT3* and negative expression of CD34 predict a longer survival (25).

In this study, cyclin D1 expression was significantly higher in AML with *FLT3*-ITD mutation than in patients without mutation. Cyclin D1 expression had a significant association with this mutation. This result supports the excessive leukemic cells proliferation in the pathogenesis of AML with *FLT3*-ITD mutation (4,22). *FLT3*-ITD gene mutation impairs the auto-inhibitory function in JM domain, causing ligand independent activation of the receptor and the receptor related pathway. The expression of Bcl-xL as the marker of anti-apoptotic activity was not significantly different in AML cases with and without *FLT3*-ITD gene mutations. The *FLT3*-ITD mutation was not associated with the expression of Bcl-xL. Based on this study, the excessive proliferation activity is the prominent pathogenesis of the AML with *FLT3*-ITD gene mutation.

AML patients with *FLT3*-ITD mutation have poor prognosis with high relapse rate, low overall survival (5,26). Our study showed that *FLT3*-ITD gene mutation had significant association with expression of hENT1, the important cytarabine transporter. AML patients with *FLT3*-ITD gene mutation had significantly lower expression level of hENT1 than mutation-free patients. Previous study proposed that *FLT3*-ITD mutation in AML cell lines had association with hENT1, an important cytarabine transporter in AML therapy (10). This finding suggested that *FLT3*-ITD specifically induced cytarabine resistance in leukemic cells through repression of hENT1 expression. Its mechanism might be due to upregulation of HIF-1 α (10,27). Cytarabine is one of the standard chemotherapy drugs for AML (27,28). Our result supports the link between *FLT3*-ITD gene mutation and the resistance to cytarabine, one of the essential chemotherapy drugs in the current strategy

of AML treatment in vivo. hENT1 is responsible for transporting cytarabine to the leukemic cells with up to 80% influx. Intracellular concentrations of cytarabine depend on the uptake process by hENT1 in AML induction treatment with standard doses of cytarabine (29-35).

Conclusion

FLT3-ITD gene mutation in AML patients was associated with blast cell count, CD34 and cyclin D1 expression, therefore it supports the role of *FLT3*-ITD gene mutation in excessive proliferation activity responsible for the pathogenesis of AML. This mutation was not associated with Bcl-xL expression.

FLT3-ITD gene mutation in AML is associated with hENT1 expression. The expression of hENT1 in AML patients with *FLT3*-ITD mutation is significantly lower than in mutation-free AML patients. This phenomenon supports the pathogenesis of cytarabine resistance in AML with mutated *FLT3*-ITD during AML induction therapy. It is important that either addition of FLT3 inhibitor in standard induction therapy and resetting cytarabine doses or development of novel therapeutic approaches should be considered in AML patients with *FLT3*-ITD gene mutation.

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Conflict of Interest

The authors confirm that there are no known conflicts of interest associated with this publication, and there has been no significant financial support for this work that could have influenced its outcome.

References

1. Seer cancer statistic review (CSR) 1975 - 2014 [Internet]. National Cancer Institute. 2017 [cited September 29, 2017]. Available from: <http://seer.cancer.gov/csr/1975-2014/>.
2. Yohe S. Molecular genetic markers in acute myeloid leukemia. *J Clin Med*. 2015;4:460-78. [DOI:10.3390/jcm4030460] [PMID] [PMCID]
3. Ferrara F, Palmieri S, Leoni F. Clinically useful prognostic factors in acute myeloid leukemia. *Crit Rev Oncol Hematol*. 2008;66:181-93. [DOI:10.1016/j.critrevonc.2007.09.008] [PMID]
4. Grafone T, Palmisano M, Nicci C, Storti S. An overview on the role of FLT3-tyrosine kinase receptor in acute myeloid leukemia : biology and treatment. *Oncol Rev*. 2012;6(1):64-74. [DOI:10.4081/oncol.2012.e8] [PMID] [PMCID]
5. Patnaik MM. The importance of FLT3 mutational analysis in acute myeloid leukemia. *Leuk Lymphoma*. 2018;59:1-15. [DOI:10.1080/10428194.2017.1399312] [PMID]
6. Schuurhuis GJ, Kedler A, Terwijn M, Rutten AP, Smit L, Zweegman S, et al. The prognostic value of CD34 expression in acute myeloid leukemia. A mystery solved. *Blood*. 2010;116:2725. [DOI:10.1182/blood.V116.21.2725.2725]

7. Zhu HH, Liu Y-R, Jiang H, Lu J, Qin Y-Z, Jiang Q, et al. CD34 expression on bone marrow blast is a novel predictor of poor prognosis independent of FLT3-ITD in acute myeloid leukemia with NPM1 mutation. *Jleukres*. 2013;37(6):624-30. [DOI:10.1016/j.leukres.2013.02.007] [PMID]
8. Haferlach T, Bacher U, Alpermann T, Haferlach C, Kern W, Schittger S. Amount of bone marrow blasts is strongly correlated to NPM1 and FLT3-ITD mutation rate in AML with normal karyotype. *JLeukres*. 2012;36:51-8. [DOI:10.1016/j.leukres.2011.04.026] [PMID]
9. Catala A, Pastor-Anglada M, Caiedes-Cardenas L, Malatesta R, Rives S, Vega-Garcia N, et al. FLT3 is implicated in cytarabine transport by human equilibrative nucleoside transporter 1 in pediatric acute leukemia. *Oncotarget*. 2016;7(31):49786-99. [DOI:10.18632/oncotarget.10448] [PMID] [PMCID]
10. Jin G, Matsushita H, Asai S, Tsukamoto H, Ono R, Nosaka T, et al. FLT3-ITD induces Ara-C resistance in myeloid leukemic cells through the repression of the ENT1 expression. *BiochemBiophysResCommun*. 2009;390:1001-6. [DOI:10.1016/j.bbrc.2009.10.094] [PMID]
11. Gorczyca W, Emmons FN. Immunophenotyping. In: Gorczyca W, Emmons FN, editors. *Atlas of differential diagnosis in neoplastic hematopathology*. 2nd ed. UK: Informa UK Ltd; 2008. p. 42-9. [DOI:10.3109/9781439806487]
12. Licinio MA, Silva MCSd. Importance of detecting FLT3 and NPM1 gene mutations in acute myeloid leukemia - World Health Organization classification 2008. *Rev Bras Hematol Hemoter*. 2010;32(6):476-81. [DOI:10.1590/S1516-84842010000600012]
13. Sarojam S, Vijay S, Raveendran S, Sreedharan J, Narayanan G, Sreedharan H. FLT3 Mutation as a Significant Prognostic Marker in de novo Acute Myeloid Leukemia Patients: Incidence, Distribution and Association with Cytogenetic Findings in a Study from South India. *MEJC*. 2014;5(4):185-96.
14. Rombouts E. *Internal tandem duplications in the Flt3-gene in human acute myeloid leukemia*. Rotterdam: Erasmus Medical Center; 2004.
15. Notopuro PB, Notopuro H, Budiwijono I, Adhipireno P. The difference in initial leukocyte count, bone marrow blast cell count, and CD34 expression in patients with acute myeloid leukemia with and without NPM1 gene mutation. *IJBC*. 2017; Jun 10;9(2):44-7
16. Kumsaen P, Fucharoen G, Sirijerachai C, Chainansamit S-o, Wisanuyothin N, Kuwatjanakul P, et al. FLT3-ITD Mutations in Acute Myeloid Leukemia Patients in Northeast Thailand. *Asian Pac J Cancer Prev*. 2016;17(9):4395-9.
17. Wang L, Xu Wl, Meng Ht, Qian Wb, Mai Wy, Tong Hy, et al. FLT3 and NPM1 mutations in Chinese patients with acute myeloid leukemia and normal cytogenetics. *J Zhejiang Univ-Sci B (Biomed & Biotechnol)*. 2010;11(10):762-70. [DOI:10.1631/jzus.B1000052] [PMID] [PMCID]
18. Yunus NM, Johan MF, Al-Jamal HAN, Husin A, Hussein AR, Hassan R. Characterization and Clinical Significance of FLT3-ITD and non-ITD in Acute Myeloid Leukemia Patients in Kelantan, Northeast Peninsular Malaysia. *Asian Pac J Cancer Prev*. 2015;16(12):4869-72. [DOI:10.7314/APJCP.2015.16.12.4869] [PMID]
19. Thiede C, Studel C, Mohr B, Schaich M, Schakel U, Platzbecker U, et al. Analysis of FLT3-activating mutations in 979 patients with acute myelogenous leukemia: association with FAB subtypes and identification of subgroups with poor prognosis. *Blood*. 2002;99:4326-35. [DOI:10.1182/blood.V99.12.4326] [PMID]
20. Molica M, Breccia M. FLT3-ITD in acute promyelocytic leukemia: clinical distinct profile but still controversial prognosis. *Jleukres*. 2015;39(4):397-9. [DOI:10.1016/j.leukres.2015.01.004] [PMID]
21. Alejandro F, Rangel L, Chavez-Valencia V. FLT3-ITD and its current role in acute myeloid leukemia. *Med Oncol*. 2017;34:114. [DOI:10.1007/s12032-017-0970-x] [PMID]
22. Aref S, Mabed M, El-Sherbiny M, Selim T, Metwaly A. Cyclin D1 expression in acute leukemia. *Hematology*. 2006;11(1):31-4. [DOI:10.1080/10245330500322321] [PMID]
23. Pallis M, Zhu Y-M, Russell N. BCL-XL is heterogeneously expressed by acute myeloblastic leukaemia cells and is associated with autonomous growth in vitro and with P-glycoprotein expression. *Leukemia*. 1997;11:945-9. [DOI:10.1038/sj.leu.2400705] [PMID]
24. Elyamany G, Awad M, Fadalla K, Albalawi M, Shahrani MA, Abdulaaly AA. Frequency and prognostic relevance of FLT3 mutations in Saudi acute myeloid leukemia patients. *Adv Hematol*. 2014;2014:1-7. [DOI:10.1155/2014/141360] [PMID] [PMCID]
25. Ibrahim EK, Assem MM, Amin AI, Kamel M, Meligui YME, Metwaly AM. FLT3 internal tandem duplication mutation, cMPL and CD34 expressions predict low survival in acute myeloid leukemia patients. *Ann clin lab sci*. 2016;46(6):592-600.
26. Kottaridis PD, Gale RE, Frew ME, Harrison G, Langabeer SE, Belton AA, et al. The presence of a FLT3 internal tandem duplication in patients with acute myeloid leukemia (AML) adds important prognostic information to cytogenetic risk group and response to the first cycle of chemotherapy: analysis of 854 patients from the United Kingdom Medical Research Council AML 10 and 12 trials. *Blood*. 2001;98(6):1752-61. [DOI:10.1182/blood.V98.6.1752] [PMID]
27. Anglada MP, Torras SP. Nucleoside transporter proteins as biomarkers of drug responsiveness and drug targets. *Front Pharmacol*. 2015;6(13):1-14. [DOI:10.3389/fphar.2015.00013] [PMID] [PMCID]
28. Chabner BA. Antimetabolites: fluoropyrimidines and other agents. In: Chabner BA, Lynch TJ, Longo DL, editors. *Harrison's Manual of Oncology*. United States of America: McGraw-Hill Companies Inc; 2008. p. 1-17.
29. Zhang J, Visser F, King KM, Baldwin SA, Young JD, Cass CE. The role of nucleoside transporters in cancer chemotherapy with nucleoside drugs. *Cancer Metast Rev*. 2007;26:85-110. [DOI:10.1007/s10555-007-9044-4] [PMID]
30. Safaie A, Monabati A, Mokhtari M, Safavi M, Solhjoo F. Evaluation of the CD123 Expression and FLT3 Gene Mutations in Patients with Acute Myeloid Leukemia. *Iran J Pathol*. 2018;13(4):438-46.
31. Aleem E, Arceci RJ. Targetting cell cycle regulators in hematologic malignancies. *Front Cell Dev Biol*. 2015;3(16):1-22. [DOI:10.3389/fcell.2015.00016] [PMID] [PMCID]
32. Eltzshig HK, Abdulla P, Hoffman E, Hamilton KE, Daniels D, Schonfeld C, et al. HIF-1 dependent repression of equilibrative nucleoside transporter (ENT) in hypoxia. *J Exp Med*. 2005;202(11):1493-505. [DOI:10.1084/jem.20050177] [PMID] [PMCID]

33. Huang W, Zeng X, Shi Y, Liu M. Functional characterization of human equilibrative nucleoside transporter 1. *Protein Cell*. 2017;8(4):284-95. [[DOI:10.1007/s13238-016-0350-x](https://doi.org/10.1007/s13238-016-0350-x)] [[PMID](#)] [[PMCID](#)]
34. Mehrazma M, Rafsanjani KA, Torkamanipoor B. Correlation between soluble vascular endothelial growth factor A and its receptor 1 with response to chemotherapy in acute leukemia in children. *Iran J Pathol*. 2008;3(4):179-82.
35. Wan H, Zhu J, Chen F, Huang H, Han X, Zhong L, et al. SLC29A1 single nucleotide polymorphisms as independent prognostic predictors for survival of patients with acute myeloid leukemia: an in vitro study. *J Exp Clin Cancer Res*. 2014;33(90):1-10. [[DOI:10.1186/s13046-014-0090-9](https://doi.org/10.1186/s13046-014-0090-9)] [[PMID](#)] [[PMCID](#)]

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