Original Article

Assessment of Soluble Natural Killer Group 2D Ligand (MHC Class I A and UL16 Binding Protein 1) in Iraqi Patients with Acute Myeloid Leukemia

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Abstract

Background: Acute myeloid leukemia (AML) is "a heterogeneous disease," defined by a wide range of genetic alterations and molecular mutations that have an effect on clinical outcomes and could be used to develop new drugs. In AML, the immune system is tricked and actively suppressed by leukemia itself and by mechanisms that leukemia picked up through further mutations under suppression of selection. Myeloblasts in Acute myeloid leukemia can evasion the naturak killer cell killing by many ways , one of the these way ,the myelocblast cells shed NKG2D soluble ligand (MIC A/B and or ULPB 1-6) in blood and bound to NKG2D activation receptor which lead to inhibit activation of NK cells. **The Aim of Study:** The aim of this study assessment of Soluble ligand (MICA and ULPB-1) in patients with AML. **Patients and Methods:** Thirty patients newly diagnosed as AML were enrolled in this study, 24 patients out of 30 were follow up after 14 days of tratment. after 30 days of treatment we get result of therapy. twenty healthy looking persons were considered as control subjects. We used ELISA technique to detection the level of soluble legand (MICA and ULPB-1). **Results:** The study showed that in order level of sMICA, there were significant differences in AML patients at diagnosis and after 14 days of treatment in comparison to control subjects while there were no significant differences in the level of sULPB1 between AML patients at diagnosis and after 14 days of treatment in comparison to control subjects. **Conclusion:** This study showed that there was an elevated level of sMICA in AML patients at diagnosis and 14 days to treatment while there was no elevated level of sULPB1 in comparison to the control group.

Keywords: Acute myeloid leukemia, natural killer, soluble MHC class I A, soluble natural killer group 2D ligand, soluble UL16 binding protein 1

Introduction

Acute myeloid leukemia (AML) is a kind of leukemia in which aberrant, proliferative myeloid precursors infiltrate the bone marrow, blood, and other tissues.[1] The French-American-British Cooperative Leukemia Working Group established the most important diagnostic criteria for AML, there are at least 20% blasts in the aspirate, or CD34 or CD117 immunostains show that immature precursors are in an unusual place on the biopsy section.[2]

AML is a very variable illness that can manifest as a *de novo* or secondary disease "therapy-related or postantecedent hematologic condition." The incidence of onset rises with age, and age is also linked to a larger frequency of high-risk cytogenetic and molecular abnormalities.[3] Although AML accounts for only about 1% of all cancers with an incidence of 4 per 100,000 a year, it is the most common acute leukemia in adults.[4-6] AML is often associated with elderly persons and is uncommon before the age of 45, while it can occur at any age. The typical age upon diagnosis is 68 years, with most

Access this article online

Quick Response Code:

Website: http://www.mmjonweb.org

DOI: 10.4103/mj.mj_29_22

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Submitted: 02-Aug-2022 **Revised:** 19-Aug-2022 **Accepted:** 21-Aug-2022 **Published:** 02-Jan-2023

How to cite this article: Mtashar BA, Ashoor ZF, Shabeeb ZA, Matti BF. Assessment of soluble natural killer group 2d ligand (MHC Class I A and UL16 Binding Protein 1) in Iraqi patients with acute myeloid leukemia. Mustansiriya Med J 2022;21:171-7.

individuals diagnosed between the ages of 65 and 74[7] and predominant in males more than females.[8,9]

The idea of using the immune system to fight cancer comes from cellular immunology, but until recently, it was thought that cancer cells were not very immunogenic and that the immune system did not tumor cells.[10]

It has been observed that adaptive and innate immune system features, such as the number of various kinds of immune cells in tumor tissue, have a significant impact on the clinical prognosis of AML.[11] Immune surveillance mechanisms comprising adaptive and innate immune systems are naturally designed to eliminate AML development. However, leukemic cells apply various immune evasion mechanisms to deviate host immune responses resulting in tumor progression. One of the recently well-known immune escape mechanisms is the overexpression of immune checkpoint receptors and their ligands. The introduction of blocking antibodies targeting co-inhibitory molecules achieved invaluable success in tumor-targeted therapy. Moreover, several new co-inhibitory pathways are currently studying for their potential impacts on improving anti-tumor immune responses.[12]

The equilibrium between the expression of activating and inhibiting receptors mediates and controls nature killer (NK) cell functioning.[13] Natural cytotoxic receptors "(NKp30, NKp46, NKp44), natural killer group 2D (NKG2D), and DNAX accessory molecule-1 (DNAM-1)" are the primary activating receptors, while the killer inhibitory receptor (KIR) family and natural killer group 2A are inhibitory receptors. In a process known as "licensing" or "education" of NK cells, inhibitory receptors (mostly KIRs) engage with self-molecules (self-MHC class I [MICA]) to prevent the detection of self-cells.[14] The uneven expression of these receptors and their ligands impedes the identification of malignant cells and facilitates the growth of tumors. In a large proportion of AML patients, the expression levels of several activating receptors "(NKG2D, NKp30, NKp46, and DNAM-1)" are either null or very low.[15] The negative association between DNAM-1 expression levels and one of its ligands (CD112) in AML patients implies that activating receptor expression is dependent on the presence of their ligands on the surface of AML cells.[16] AML also modifies the expression of the activating receptors' ligands. NKG2D ligands "(NKG2DL, MICA/MICB, and ULBPs1-3)" are missing or expressed at extremely low levels on the cell surface of myeloid blasts, as shown by many research groups.[17] Expression of these ligands is dependent on the cellular subtype, with little or no expression in myeloblastic cells and a high level of expression in monoblastic cells, indicating that NKG2DL is acquired during the last phases of maturation.[18] The release of these ligands in a soluble form from the cell surface by metalloprotease cleavage or into exosomes is an essential technique evolved by AML cells to elude immune identification. Two investigations have shown that these ligands are often found in the serum of AML patients, resulting in the downregulation of NKG2D and the loss of NKs' cytotoxic potential.[19] Therefore, AML patients with decreased levels of soluble MICA (sMICA), MICB, and ULBP1 had a greater chance of achieving complete remission (CR) and surviving more than a year.[19]

Aim of study

Assessment of NKG2D ligand (MICA and ULPB-1) in patients with AML.

Patients and Methods

Study design

A cohort study designed 30 patients who were diagnosed with AML and follow-up on day 14 and day 30 at treatment collected from Baghdad Medical City, National Center of Hematology, from February 2021 to November 2021.

Patients

Thirty persons were diagnosed as AML, 19 male and 11 female. 24 ppatient out of 30 were follow up after 14 days of tratment. The patients collected from Baghdad medical city, Hematology center from February 2021 to November 2021. Twenty healthy-looking persons were considered control subjects.

Inclusion criteria

New diagnosed AML patients with all types of AML (M0 - M7), axcept AML-M3 all patients before starting tratment collected from them Bone maroow asperate and diagnosed by immunophynotyping study with multicolor Flowcytometry, of any sex, age 18–65 years. The treatment is a 7 + 3 drug regimen.

Exclusion criteria

The Age under 18 years , AML patients wit M3 type and the patients did not trated with 7+3 drug regimen.

Methods

In this study detected level of MICA and ULPB-1 using Sandwich ELISA techniques (Sunlong Biotech, china). After prepered serial diluted standared, 50 microliter of standered dilutiens were added to the microtitter wells. First, micro-ELISA stripplets leave a well empty as blank control. In sample wells, 40 µl sample dilution buffer and 10 µl sample were added (dilution factor is 5) the microtitter plate were mixed and cover by plate membrane. Micro-ELISA stripplets were incubated for 30 min at 37°C. the micro-ELISA plate were washed by washing buffer 5 times by asperated and refilled the plate with washing buffer and hold for 30 sec. HRP conjugate were added to each wells accepted blank conjugate) reagent to each accept the blank control well. Incubation was described previously. Microplate was washed as described. 50 microliter of chromogen reagent A and 50 microleter of Chromogen B were added to each well, then gently shacked and incubated for 15 min in 37 C in dark place. Termination: 50 µl of stop reagent was added to each well to terminate the reaction. The color in the well changed from blue to yellow. The microplate

was read absorbance OD 450 nm using a microtiter plate reader. The OD value of the blank control well is set as 0.

Calculation of the results

The results of sMICA and sULPB-1 detection level used logaretmic Standered curve. the serial dilution of standered with known concentration were plotted on X - axis and the Optecal dencity of standared dilution plotted on Y - axis ULPB1 (pg/ml) and sMICA (ng/L) in samples were determined by plotting. By plotting the sampple's OD on the y-axes. Then, the original concentration was calculated by multiplying the dilution factor.

Statistical analysis

Statistical analysis was performed using the "Statistical Package for the Social Sciences SPSS software version 23.0.0. Analytic statistics were carried out using a *t*-test, with $P < 0.05$ which was considered statistically significant. Descriptive statistics were presented in tables, bar charts, stem and leaf plots, scatter plots, and trend line mainly in the form of a range, mean, and standard deviation.

Results

30 patients were diagnosed as AML, 24 patients ourt of 30 were followed up after 14 days of tratment. 19 AML patients were male while 11 was female with ratio male: female (1.72: 1) [Table 1 and Figure 1]. 6 patients out of 30 were died. Twenty age- and gender-matched healthy-looking subjects were considered a control group.

The mean age of patients with AML was 40.2 ± 15.2 years and ranged from 18 to 65 years, while the mean for the healthy-looking subjects was 41.1 ± 13.3 years and ranged from 24 to 64 years [Table 2 and Figure 2].

Hematological remission with a patient of acute myeloid leukemia

Hematological remission is defined as an absence of clinical evidence of leukemia with bone marrow blast cells <5%, absence of Auer rods, neutrophil count ≥1 × 10^9/l, and platelet count ≥100 × 10^9/l.[20] Fifteen (62.5%) out of 24 patients with AML at day 30 after treatment were in remission, and 9 (37.5%) out of 24 patients were not in remission [Table 3 and Figure 3].

According to the data represented in Table 4, there are no statistical differences between AML at diagnosis and AML 14 days after treatment in the serum level of sULPB1 and sMICA [$P = 0.593$ and 0.128, respectively; Figures 4 and 5].

With $P = 0.001$, there are significant statistical differences between AML at diagnosis and control subjects' serum levels of sMICA. On the contrary, there were no statistically significant differences in the sULPB1, $P = 0.942$.

Table 1: Sex distribution of patients with acute myeloid leukemia

Sex group	Patients ($n=30$), *n* (%)	Control subjects ($n=20$), *n* (%)
Males	19 (63)	13 (65)
Females	11 (27)	7 (25)
Male:female	1.72:1	1.86:1

Table 2: Age distribution of patients with acute myeloid leukemia

Age group (years)	Patients ($n=30$), *n* (%)	Control subjects ($n=20$), *n* (%)
16–25	8 (26.6)	2 (10)
26–35	4 (13.3)	6 (30)
36–45	7 (23.4)	6 (30)
46–55	5 (16.7)	2 (10)
56–65	6 (20)	4 (20)
Range	18–65	24–64
Mean±SD	40.2±15.2	41.1±13.3

SD: Standard deviation

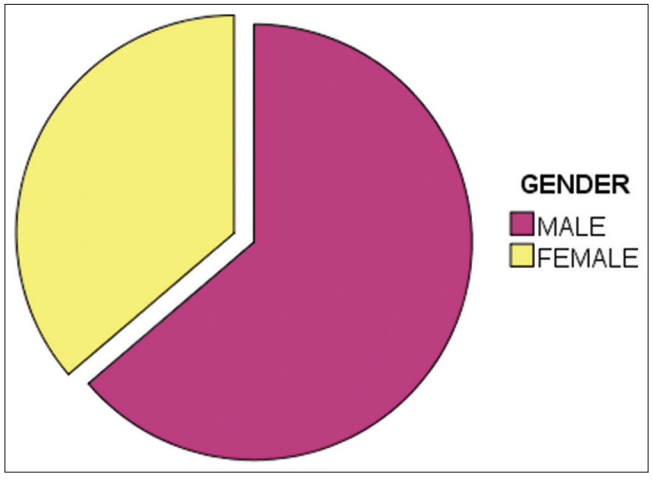

Figure 1: Sex distribution of patients with acute myeloid leukemia

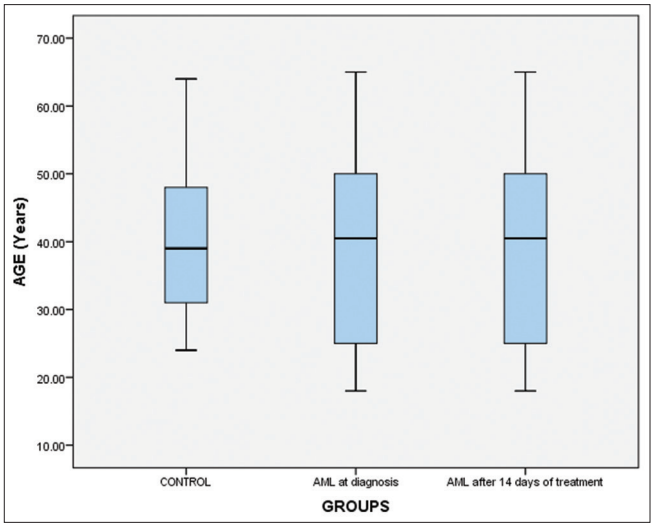

Figure 2: Age distribution of patients with acute myeloid leukemia at diagnosis and 14 days after treatment

In the comparison between AML at 14 days after treatment and control subjects, there were no statistically significant differences in serum levels of sULPB1 with $P = 0.079$ while there were statistically significant differences in serum levels (sMICA) with $P = 0.001$.

According to mortality, there were no significant differences between alive patients with AML and deceased in serum levels of sULPB1 and sMICA [$P = 0.421$ and 0.27, respectively; Table 5].

Concerning remission after 30 days of treatment, there are statistically significant differences in serum level of sMICA, $P = 0.041$, while there are no statistically significant differences between remission and no remission in serum level of sULPB1, $P = 0.767$ [Table 6].

Concerning remission after 14 days of treatment, there was no statistically significant difference between remission and no remission in serum level of sULPB1 and sMICA [$P = 0.218$ and 0.114, respectively; Table 7].

Discussion

AML is a cancer of the bone marrow that is caused by myeloid progenitor cells that grow in clones and stop differentiating. Age-adjusted rates of AML in the US are 4.3 per 100,000 people/year.[21] In Iraq, Abdulridha *et al.* studied 3102 leukemia patients in the Iraqi Center for Hematology in the City of Medicine in Baghdad between January 2018 and December 2019, AML was 37% of total leukemia in 2018, while in 2019, AML was 40.8% of total leukemia.[22] In Iraq, sperated studies in Baghdad , Karbala and Sulaymaniyah demonstrated that the incidence of AML among other type of leukemia was 24.85%, in Baghdad between 2018 and 2019,[22] in Karbala the incidence of AML in 2019 out of 402 leukemic patients 19.2%.[23] In research conducted at the Hiwa Hospital in Sulaymaniyah between 2009 and 2013, 17% of 570 leukemia patients had AML.[24]

AML may affect people of all ages, from infants to the elderly.[25,26] AML is thought to be a disease of the elderly. In the United States, the median age of AML diagnosis ranged from 62 to 68 years.[27] Similar variations were seen in Europe and Canada.[28,29] In Saudi Arabia, the median age at AML diagnosis seems to be lower than that reported for the United States and Europe, even though there are minimal data to support this notion. The current research, which included 30 patients with AML at the time of diagnosis and followed up 24 of those patients after 14 days of treatment, found that the mean age of patients with AML was 40.2 ± 15.2 years, ranging from 18 to 65 years according to the exclusion criteria.

The AML patients in the current study were 19 males and 11 females with a male: female ratio of 1.72:1. This result agrees with Juliusson *et al.,* as with most leukemia, AML is a hematological malignancy which is more common in males than in females.[25]

According to the data represented in Table 5, there were no statistically significant differences between AML at diagnosis and AML 14 days after treatment in the serum levels of sULPB1 and sMICA ($P = 0.593$ and 0.128, respectively). There were

Table 3: Hematological remission of patients with acute myeloid leukemia at day 30 after treatment (n=24)

Patients	*n* (%)
Remission	15 (62.5)
No remission	9 (37.5)

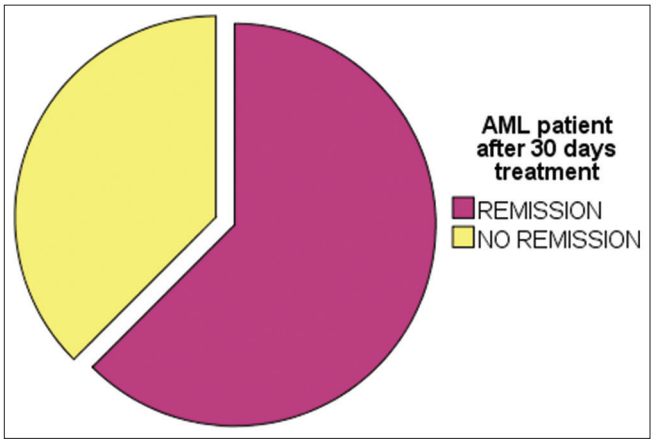

Figure 3: Hematological remission of patients with acute myeloid leukemia at day 30 after treatment

Table 4: Serum level of soluble UL16 binding protein 1 and soluble MHC class I A in patients with acute myeloid leukemia

Parameter/ serum level	Range (mean±SD)					
	Patients		Control subjects	*P*-value compare		
	At diagnosis	At 14 days after treatment		At diagnosis and at 14 days after treatment	At diagnosis and control subjects	At 14 days after treatment and control subjects
sULPB1 (pg/mL)	190–250.0 (193.37±49.69)	161.0–215.0 (199.31±11.7)	180.0–225.0 (192.9±11.79)	0.593	0.942	0.079
sMICA (ng/L)	30.0–51.0 (33.68±2.04)	30.0–35.0 (32.83±1.34)	25.0–32.0 (30.3±1.50)	0.128	<0.001**	0.001**

**Highly significant P<0.01. SD: Standard deviation, sULPB1: Soluble UL16 binding protein 1, sMICA: Soluble MHC class I A

Table 5: Serum level of soluble UL16 binding protein 1 and soluble MHC class I A in patients with acute myeloid leukemia at diagnosis in relation to mortality

Parameter/serum level	Range (mean±SD)		
	Alive (*n*=24)	Deceased (*n*=6)	*P*
sULPB1 (pg/mL)	190.0–250.0 (197.45±40.52)	191.5–245.5 (178.83±80.21)	0.421
sMICA (ng/L)	31.0–38.5 (33.43±2.10)	32.0–39.0 (34.66±3.43)	0.27

SD: Standard deviation, sULPB1: Soluble UL16 binding protein 1, sMICA: Soluble MHC class I A

Table 6: Serum level of soluble UL16 binding protein 1 and soluble MHC class I A in patients with acute myeloid leukemia at diagnosis, concerning remission at 30 days after a treatment

Parameter/serum level	Range (mean±SD)		
	Remission (*n*=15)	No remission (*n*=9)	*P*
sULPB1 (pg/ml)	190.0–250.0 (195.49±51.41)	190.0–213.0 (200.7±8.68)	0.767
sMICA (ng/L)	31.5–35.0 (32.36±1.17)	31.0–38.5 (34.55±2.90)	0.041*

SD: Standard deviation, sULPB1: Soluble UL16 binding protein 1, sMICA: Soluble MHC class I A

Table 7: Serum level of soluble UL16 binding protein 1 and soluble MHC class I A in patients with acute myeloid leukemia at diagnosis, concerning remission at 14 days after a treatment

Parameter/serum level	Range (mean±SD)		
	Remission (*n*=15)	No remission (*n*=9)	*P*
sULPB1 (pg/mL)	161.5–213.0 (196.36±13.23)	193.0–215.0 (204.2±6.75)	0.218
sMICA (ng/L)	30–34.0 (32.56±1.17)	30.5–35.0 (33.27±1.56)	0.114

SD: Standard deviation, sULPB1: Soluble UL16 binding protein 1, sMICA: Soluble MHC class I A

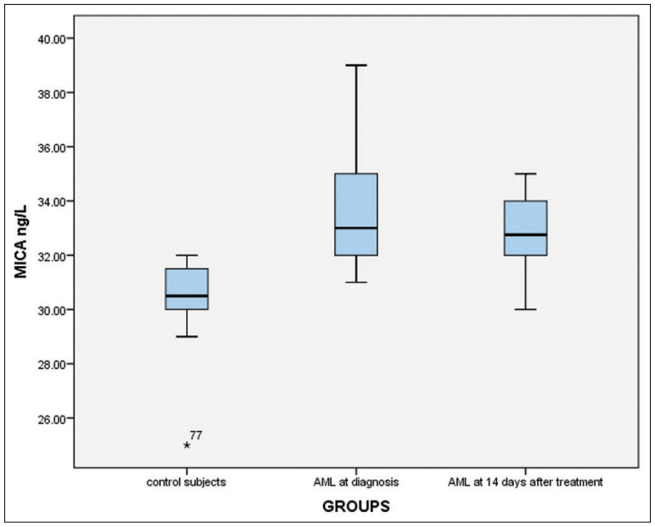

Figure 4: Concentration of soluble MHC class I A (ng/L) in patients with acute myeloid leukemia at diagnosis and 14 days after treatment in comparison to control subjects

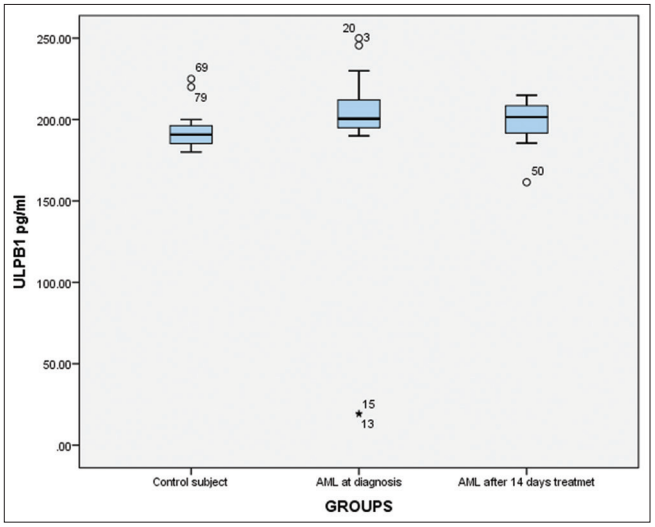

Figure 5: Concentration of soluble UL16 binding protein 1 (pg/mL) in patients with acute myeloid leukemia at diagnosis and 14 days after treatment in comparison to control subjects

significant statistical differences in serum level sMICA between AML at diagnosis and control subjects, with $P = 0.001$. On the contrary, there were no statistically significant differences in the ULPB1, $P = 0.942$. Compared to a control subject, AML patients after 14 days of treatment showed no statistically significant differences in serum levels of ULPB1 ($P = 0.079$), but there were statistically significant differences in serum levels of sMICA ($P = 0.001$) ligands (MICA and ULPB1). MICA/B and ULBP ligands interact with the activating receptors NKG2D to facilitate their capacity to remove altered or virally infected cells. The NK cells considered effective cells to kill tumor cells and viruses thus these cells developed a varaity of protection mechanisms to avoied natural killer lysis by production different typpes of NKG2D ligands. Targeted cells may degrade ligand transcripts through microRNAs or alter them at the protein level to prevent their appearance at

the cell surface via shedding, with the added benefit that shed ligands desensitize NKG2D receptors and prevent destruction by NK cells.[18,30] The present study demonstrates an increase in soluble ligand MICA in AML patients at diagnosis and after 14 days of therapy, which is consistent with previous research versus ULPB1, whose level decreased; this may be due to the rise in another form of soluble legends, such as ULPB2, ULPB3, ULPB4, ULPB5, and ULPB6.[31,32] Consistent with the hypothesis that NKG2DL releasing is a tumor escape mechanism, higher levels of soluble NKG2DL have been found in the serum of malignancies patients, and associations between soluble NKG2DL and tumor stage and/or progression have been reported.[32] The soluble NKG2D-L may bind to NKG2D, induce the downregulation of NKG2D expression on NK cells and CD8 + T-cells, and decrease the concentration of NKG2D-L on the surface of tumor cells, therefore inhibiting NKG2D-mediated immune recognition and elimination.[33] It has been hypothesized that adsorption apheresis may be used to remove soluble NKG2D-L from patients' plasma.[34] Accumulating data indicate that soluble NKG2D-L, as a biomarker, may restrict immune checkpoint blockage.[35,36] Some clinically used chemotherapeutic medicines (e.g., Epirubicin) block sMICA shedding by downregulation of A disintegrin and Metalloprotinase 10 (ADAM10), resulting in decreased sMICA levels *in vivo*. In some circumstances, soluble NKG2DL in the serum of cancer patients is functionally active and able to downregulate NKG2D expression and suppress NKG2D-dependent cytotoxicity[37] and also low level of NKG2D soluble ligand in CR gives chance for survival more than 1 year.[38]

Conclusion

The level of sMICA was elevated in AML patients practically increasing in no remission patients and decreasing in remission patients, while sULPB1 is not significantly different among AML patients at diagnosis, patients at 14 days of treatment, and controls. Therefore, sMICA could be a prognostic biomarker for AML.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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