T-cell immunoglobulin mucin-3 expression in Acute Myeloid Leukemia: a cross-sectional study

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Abbreviations:

TIM3: T-cell immunoglobulin mucin-3; **AML**: acute myeloid leukemia; **FAB:** French-American-British.

Abstract

Background and aim: As acute leukemia's are characterized by abnormal proliferation of precursor cells simulating normal hematopoietic stem cells (HSC.) It is critical to isolate acute myeloid leukemia stem cells (AML LSCs) from normal HSCs in order to eradicate the AML LSC without killing normal HSCs. This can be done by identifying a molecule that is expressed or functions specifically at the AML LSC stage. This paper is aimed to investigate the role of T-cell immunoglobulin mucin-3 (TIM3) expression on leukemic stem cells of AML patients and to correlate its level with the outcome of the disease. TIM3 was assessed by Flow-cytometer technique

Patients and methods: Our study included 30 patients newly diagnosis as acute myeloid leukemia between March 2016 and December 2016. Age ranged from 20 to 68 years old with median age was 32 years. Seventeen cases were males, and 13 cases were females. The Ethical and Research committees approved this study in the South Egypt Cancer Institute. An informed written consent in accordance was taken from all cases.

Results: mean expression of TIM3 on CD34+ CD38- LSCs was 48.80 ± 29.70 % with range of 30% to 97% while expression of TIM3 on CD34+ CD38+ LBs was 25.29 ± 18.34 with range of 26% to 67%. Remission occurred in 18 cases (60%) based on morphologic assessment of peripheral blood and bone marrow aspirates. Patients who did not achieve complete remission had significantly higher expression of TIM3 on leukemic stem cells in comparison to those who achieved remission (64.18 ± 20.95 versus 38.55 ± 30.69. Disease-free survival was higher in those who had remission in comparison to those patients who failed to achieve remission (14 months versus 12 months).

Conclusion: lower T-cell immunoglobulin mucin-3 expression may improve outcome and survival of patients with acute myeloid Leukemia but further large control study studies are recommended.

Keywords:

Acute myeloid leukemia, T-cell immunoglobulin mucin-3

Background

Acute myeloid leukemia (AML) is a clonal malignant disorder derived from a small number of leukemic stem cells (LSCs). LSCs are self-renew and generate leukemic progenitors that actively divide to produce a

Large number of the immature clonogenic leukemic blast **(Jone et al., 2019).** To eradicate the AML LSC without killing normal HSCs, it is critical to isolate a molecule that is expressed or functions specifically at the AML LSC stage **(Krause and Van Etten, 2007).** T-cell immunoglobulin mucin-3 (TIM3) has been described as a unique acute myeloid leukemia (AML) stem cell antigen that is not present on normal hematopoietic stem cells.TIM3 is also known to be expressed in natural killer (NK) cells, monocytes, and a subset of T cells.**(Jan et al., 2011)**.

TIM3 has been shown to be expressed in most leukemic stem cells (LSC) in the majority of AML subtypes. Although the specific mechanism underlying upregulation is unclear, targeted therapy with an anti-TIM3 monoclonal antibody has shown efficacy in reducing leukemic burden in a mouse model **(Kikushige et al. 2010).**

In AML, a prior functional study focused on natural killer cells (NK), showing enhanced interferon-gamma production by TIM-3 overexpressing NK cells against galectin-9 (Gal-9) positive AML primary tumors. Although this study has important therapeutic implications for AML therapy, it did not address the function of TIM3 expression on leukemic myeloblasts **(Gleason al, 2012)**. The initial studies that have reported TIM3 expression in AML have focused on evaluating TIM3 expression in LSC.

We hypothesized that detection of TIM3 overexpression could aid in the identification of neoplastic myeloblasts. This distinction would be especially diagnostically useful during the post-chemotherapy time interval, in which the differential diagnosis is often between regenerating versus residual leukemic myeloblasts.

Therefore, this study was carried out to investigate the role of TIM3 expression in leukemic stem cells of AML patients and to correlate its level with the course and outcome of the disease.

Patients and methods

Our study included 30 patients newly diagnosis as acute myeloid leukemia (not including AML M3) attending South Egypt Cancer Institute, Assiut University between March 2016 and December 2016. Age of the patients ranged from 20 to 68 years old.

According to sex, 17 cases were males and, 13 cases were females. The study was approved by the Ethical and Research committees in the faculty of medicine. Informed written consent in accordance was taken from all cases. Diagnosis of AML was achieved by morphology, cytochemistry, immunophenotyping and cytogenetic studies.

TIM3 expression was assessed where samples were analyzed using a 4-color approach on a FACS Caliber from Becton Dickinson (BD, San Jose, CA, USA) using Cell Quest software. The monoclonal antibodies used for staining the samples were TIM3 (FITC) (Invitrogen, Code: 11-5870-82), CD 34 (PerCp) (BD, Code: 347222) and CD 38 (PE) (BD, Code: 347687.)

Results

This study was conducted prospectively on 30 patients with AML. The mean age at presentation was 35.2 years; and the age range was from 20 years to 68 years. According to sex; 17 patients (56.7%) were males, and 13 patients (43.3%) were females with male to female ratio was 1.3. As regarding clinical data, 7 out of 30 patients (23.3%) had lymph node enlargement at presentation, 16 of 30 patients (53.3%) had fever, 9 patients (30%) had hepatomegaly and 7 (23.3%) had splenomegaly (table 1).

Table (1): Baseline characteristics of AML patients

Total no of patients= 30		
1. Age:		
Mean	**35.2 years**	
Range	**20 – 68 years.**	
2. Sex		
Male	**17**	**56.7%**
Female	**13**	**43.3%**
3. Clinical Data:		
Lymph node enlargement:	**7/30**	**23.3%**
Fever	**16/30**	**53.3%**
Hepatomegaly	**9/30**	**30%**
Splenomegaly	**7/30**	**23.3%**
4. Hematological data:		
WBC (x 10^9/L)	11.56 x 10^9/L (range 1.45- 61.56 x 10^9/L)	

Hemoglobin (g/dL)	**8.20 ± 1.9**
Platelets (x 10^9/L)	**127.53 ± 134.38**
PB Blasts:	**42.17 ± 30.95**
BM Blasts:	**57.14 ± 24.18**

WBC: White blood cell; **PB**: Peripheral blood; **BM**: Bone marrow

Regarding hematological data at presentation, median WBC count was 11.56 x 10^9/L (range 1.45- 61.56 x 10^9/L), mean hemoglobin (Hb) concentration was 8.20 ± 1.9 g/dL and mean platelet count was 127.53 ± 134.38 x 10^9/L. Regarding platelet count, 4 patients (13%) had normal platelet count (from 150 to 450 x 10^9/L) at presentation and 26 patients (86%) had thrombocytopenia (<150 x 10^9/L). The mean peripheral blast count was 42.17 ± 30.95 and the mean BM blasts was 57.14 ± 24.18 (table 1).The current study showed that expression CD34+CD38-TIM3 (Leukemic stem cells) was significantly higher than expression of CD34+CD38+TIM3 (leukemic blasts) (48.80 ± 29.70 versus 25.29 ± 18.34; *P*= 0.01) (table 2).

Table (2): Leukemic stem cells and Leukemic blasts and expression of TIM3

	Mean ± SD
CD34+CD38-LSC:	**4.743.55**
CD34+CD38+LB:	**94.33 ± 2.78**
CD34+CD38-TIM_3:	**48.80 ± 29.70**
CD34+CD38+TIM_3:	**25.29 ± 18.34**

CD: Cluster of differentiation; **LSC:** Leukemic stem cells; **TIM_3:**T-cell immunoglobulin mucin-3

It was noticed that CD34+CD38+TIM3+ had significant correlations with WBCs, Platelet count, PB blast, BM blast, and CD34, but CD34+CD38-TIM_3+ had insignificant correlations with other laboratory data (table 3 and 4).

Table 3: Correlations of CD34+CD38-TIM3+ and laboratory data

	CD34+CD38-TIM_3+	
	R	**P**
Age	**0.086**	**0.653**
WBCs	**0.263**	**0.161**
Hb	**0.159**	**0.400**
PLT	**-0.216**	**0.252**
P B	**0.310**	**0.096**
BM blast	**0.192**	**0.309**
CD34	**-0.014**	**0.941**

***** Statistically significant correlation (p<0.05). WBC: White blood cell; PB: Peripheral blood; BM: Bone marrow; CD: Cluster of differentiation; TIM_3:T-cell immunoglobulin mucin-3;P:p value; R:correlation coefficient; HB: Hemoglobin

Table 4: Correlations of CD34+CD38+TIM3+_ and laboratory data

	CD34+CD38+TIM_3+	
	R	**P**
Age	**0.158**	**0.404**
WBCs	**0.383**	**0.028***
Hb	**0.149**	**0.432**
Platelet count	**-0.329**	**0.033***
PB blast	**0.440**	**0.017***
BM blast	**0.314**	**0.022***
CD34	**0.416**	**0.013***

*Statistically significant correlation (p<0.05). WBC: White blood cell; PB: Peripheral blood; BM: Bone marrow; CD: Cluster of differentiation; TIM_3:T-cell immunoglobulin mucin-3;P:p value; R:correlation coefficient; HB: Hemoglobin

Table 5 showed that each of expression of CD34+CD38-TIM_3+, and CD34, BM blast, WBCs, the peripheral blast was significantly higher in patients who achieved complete remission, but platelet count and expression of CD34+CD38+LBs were significantly higher in patients who did not achieve complete remission.

Table (5): Characteristics of studied patients based on complete remission

Laboratory data	No complete remission	Complete remission	P. value
Age	**39.58±13.39**	**32.22±14.24**	**0.167**
Peripheral Blast	**76.25±16.45**	**19.44±9.61**	**<0.001****
Platelet	**35.08±24.66**	**189.17±142.67**	**0.001****
WBCs	**83.25±71.54**	**3.92±3.33**	**<0.001****
Hb	**7.92±1.83**	**8.39±1.97**	**0.515**
BM blast	**68.78±25.9**	**49.38±20.09**	**0.029***
CD34	**64±32.98**	**34.75±15.91**	**0.003****
CD34+CD38-LSC	**6.44±3.69**	**3.6±3.05**	**0.029***
CD34+CD38+LBs	**92.98±2.3**	**95.24±2.76**	**0.027***
CD34+CD38-TIM_3+	**64.18±20.95**	**38.55±30.69**	**0.018***
CD34+CD38+TIM_3+	**30.11±20.06**	**22.08±16.92**	**0.247**

Kaplan Meier analysis survival analysis (overall survival) showed that median survival of all studied patients was 11.50 months with 95%CI= 11.11- 15.45. Median survival of those patients who had remission was significantly higher than those failed to achieve remission (11.33 months with 95%CI= 8.98-15.56 versus 9.45 months with 95%CI= 8.90-13.11; *P*= 0.01). Disease- free survival was higher in those who had remission in comparison to those patients who failed to achieve remission (14 months with 95%CI= 10-17 versus 12 months with 95%CI 10- 14; *P*= 0.06) (figure 1-2).

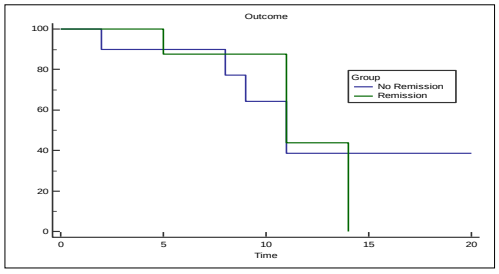

Figure 1: Overall survival in the current study based on occurrence of remission

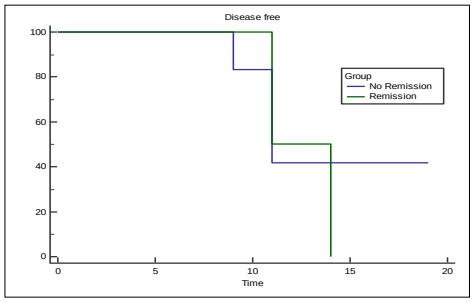

Figure 2: Disease free survival in based on occurrence of remission

Discussion

In the current study, there was slight male predominance. **Shah et al., (2012)** reported that the percentage of males to females was77% to 23%. (**Cheng et al., 2015) Also** found that the male percentage was 54.2% while female percentage was 45.8%. The age at presentation varied from 20 to 68 year, with the mean 35. Similar results were reported by **(El-Sissy et al., 2006)** whose age ranged from 16 to 60 years, with a mean of 32.4 years and median of 35 years. (**Cheng et al., 2015)**, reported that the patients' age was between 10-60 years.

Momani et al., 2016, also reported that the Patient's age ranged from 3 months to 89 years, with a mean age of 44.5 years. The patients were evaluated during their treatment program and assigned into patient who achieved remission (60%) and patient who did not achieve remission (40%) based on morphologic assessment of peripheral blood and bone marrow aspirates after recovery of BM suppression (between 10-20 days after starting chemotherapy).

Many previous studies took about the expression of TIM3 on patients with acute myeloid Leukemia. In the majority of this study a control group of non-hematological was used for comparison with such patients with AML **(Jan et al. 2011; Roth et al., 2013).**

Secondary to ethical and social conflicts in our

locality, it wasn't allowed to use a control group to be subjected to bone marrow study for research issues. To overcome this point, we tried to identify the expression of TIM3 in LSCs (CD34+ CD38-) and LBS (CD34+ CD38+) in patients with AML to correlates its levels with clinical outcome.

We noticed that expression of TIM3 was 48.80 ± 29.70 % and 25.29 ± 18.34 on CD34+ CD38- LSCs and CD34+ CD38+ LBs respectively with significant difference between both subtypes of cells. **Roth et al.2013** reported that expression of TIM3 on myoblast in patients with AML was 71.4% that was significantly higher from the non-neoplastic control group in such study (50.3%). Other study reported that TIM3 expression was significantly higher in LSCs (98%) in comparison to normal hematopoietic stem cell (30%) **(Jan et al., 2011).** So, based on these previously mentioned data, TIM3 expression is higher on LSCs (CD34+ CD38-) than CD34+ CD38+ LBs.

In our study, TIM_3 expression on LSCs was insignificantly correlated with laboratory date as hemoglobin, platelets count, blasts count (either in bone marrow or peripheral blood), white blood cells count and CD34 expression. However there were significant moderate correlations between TIM_3 expression on LBs and white blood cells count, blast count and CD34 expression and significant correlation with platelets count.

Other previous study showed that TIM3 expression was positively correlated with CD34 expression in patients with AML (**Liang Jing et al., 2017**). The current study showed that patients who didn't achieve complete remission had significantly higher expression of TIM3 on LSCs in comparison to those achieved remission (64.18 ± 20.95 versus 38.55 ± 30.69). This may indicate that higher expression of TIM3 may associate with resistance to chemotherapy. **Darwish et al. (2016)** reported that TIM3 expression in patients with AML associated with more aggressiveness and less response to therapy. But in contrast to these results, **Liang Jing et al. (2017)** said that TIM3 expression was significantly higher in those patients with AML and achieved complete remission.

The current study survival analysis (overall survival) showed that the median survival of all studied patients was 11.50 months with 95%CI= 11.11- 15.45. Median survival of those patients who had remission was significantly higher than those failed to achieve remission (11.33 months with 95%CI= 8.98- 15.56 versus 9.45 months with 95%CI= 8.90- 13.11). Disease-free survival in all patients was 12.9 month with 95%CI= 10.8- 14.9. Disease-free survival was higher in those who had remission in comparison to those patients who failed to achieve remission (14 month with 95%CI= 10- 17 versus 12 months with 95%CI 10- 14).

So, patients who achieved complete remission had significantly lower TIM3 expression with more survival and more disease free survival in contrast to those patients who didn't achieved complete remission had significantly higher TIM3 with less survival and less disease free survival. AML is generally regarded as CD34+ve CD 38-ve and those have chemotherapy resistant properties. CD34+ve CD 38-ve stem cell frequency at diagnosis significantly correlates with high minimal residual disease frequency after chemotherapy and short survival.

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