Clinical significance of interleukin 10, interleukin 33, and interleukin 35 on induction chemotherapy in acute myeloid leukemia patients

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Background Acute myeloid leukemia (AML) has been linked to immunological disorders. The immunological milieu associated with AML is still debated, particularly regarding interleukins (IL) linked to T regulatory cells dysregulation and apoptosis. Till now, IL33 has not been studied in AML patients on induction chemotherapy or correlated with AML immunophenotypic markers. This study aimed to investigate the levels of IL10, IL33, and IL35 as possible follow-up markers in AML patients at diagnosis and after chemotherapy induction and to correlate their levels with AML immunophenotypic markers.

Patients and methods In this study, newly diagnosed AML patients were followed up from diagnosis till complete remission or death, and levels of IL10, IL33, and IL35 were detected using enzyme-linked immunosorbent assay.

Results The results revealed that IL10 and IL35 levels were significantly elevated in newly diagnosed AML patients in comparison to the control group (P>0.001). After chemotherapy induction, IL10 and IL35 levels in complete remission in AML patients were significantly reduced (P>0.001). The results of this study reveal, for the first time, a statistically significant association between IL33 level and CD13 prognostic marker.

Conclusion In conclusion, IL10 and IL35 are recommended as follow-up markers in AML patients on induction chemotherapy. In addition, IL10 and IL35 could be related to AML disease progression and clinical outcomes after induction chemotherapy. Moreover, IL33 was correlated with CD13 and could be a novel promising marker in AML.

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Introduction

In acute myeloid leukemia (AML), cytokines mediate activities across the immune system, and when they are produced abnormally, cytokines provide a favorable tumor microenvironment [1]. Interleukin 10 and 35 (IL10 and IL35) are both released from T regulatory cells (Treg) with different actions. IL-35 has a potent potential to inhibit effector T cell activity and proliferation, either directly or via raising the proportion of CD4+ Tregs [2–5]. In AML, IL-35 boosted AML blasts' immune escape and directly increased proliferation and decreased apoptosis [2].

IL10 [6] and transforming growth factor beta [7] are known to be essential for immune homeostasis with anti-tumor effect. In addition, IL10 was shown to be related to immunosuppression and proliferation [8,9]. Moreover, IL10 was reported to be essential in the relation pathway between Tregs and leukemic stem cells (LSC). This validates the importance of targeting LSCs in AML by blocking the communication between Tregs and LSCs [9]. Nowadays, recent immunotherapies for the elevated PD marker [10] applying anti-IL10 and anti-PD-L1 blocking antibodies are being tested to manipulate the inhibitory network, making this type of immunotherapy appealing for patients with AML who are not suitable candidates for the standard chemotherapy and with fewer severe side effects [11].

Concerning cell proliferation and apoptosis, IL33 was found to be related to Treg function and associated with Treg accumulation in the microenvironment [12–14]. At the same time IL33 receptor blockers gave promising results in inhibiting myeloid and leukemic microenvironment, especially against LSCs [15–17]. A study on animal models reported the efficacy of IL33 administration on better antileukemic activity, T-cell activity, and survival [12]. Within a malignant

environment, IL33 expression varies according to many factors, including the disease condition and type of treatment [18]. However, studies demonstrating IL33 serum level in AML patients and its association with disease outcomes, chemotherapy, and other ILs remain to be explained.

A dysregulated cytokine network in AML at least partly contributes to T-cell dysfunction [19]. Besides, response to chemotherapy was associated with immunological markers suggestive of the recovery of T cell activity [20]. Hence, solving the riddles surrounding cytokine interactions will support the development of successful immune therapeutics especially for the investigation of new leukemia therapeutics since the eradication of LSC is needed to cure leukemia [21].

In general, IL10, IL33, and IL35 have a multifaceted and pleiotropic role in AML, affecting AML cell growth, proliferation, and treatment resistance [22]. This raises questions about the importance of correlation between IL10, IL33, and IL35 levels in AML after induction chemotherapy.

Aim

The current study aimed to investigate the levels of IL10, IL33, and IL35 as possible follow-up markers in AML patients at diagnosis and after induction chemotherapy and to correlate their levels with AML immunophenotypic markers. Newly diagnosed AML patients will be followed up from diagnosis till complete remission or death, and levels of IL10, IL33, and IL35 will be detected using enzyme-linked immunosorbent assay (ELISA).

Patients and methods

Study design and setting

This is a prospective cohort study. This study was carried out in the Clinical Pathology Department at South Egypt Cancer Institute (SECI) and the Clinical Hematology Unit, Faculty of Medicine, Assiut University, Egypt. This research included 40 newly diagnosed adult AML patients (27 males and 13 females with a median age of 35.5), 13 AML-IR patients (nine males and four females with a median age of 30), and 12 AML-CR patients (seven males and five females with a median age 29.5) were hospitalized between December 2022 and September 2023. The control group consisted of people of 20 years of age and sex who were matched in good health (14 males and six females with a median age of 33.5). Patients were followed up after chemotherapy until complete remission or death. Samples were taken 4 weeks after the end of the chemotherapy cycle. The Regional Ethical Faculty of Medicine, Assiut University, authorized this study according to the guidelines in the Declaration of Helsinki (number 17200798 on 29-12-2022). Informed consent was obtained from all participants before enrolment. Newly diagnosed AML patients before induction chemotherapy aged from 18 years old were included in the study. Exclusion criteria include AML patients on chemotherapy, AML patients with recurrent AML, AML M3, and AML patients with prior hematologic malignancies such as myelodysplastic syndrome and myeloproliferative neoplasms. The patients were treated with the following chemotherapy regimen: cytarabine (100 mg/m^2/day for 5–7 days) and idarubicin (12 mg/m^2/day for 2–3 days) [23].

Patient diagnosis

The diagnosis was performed based on clinical presentation, complete blood count, differential white blood count (WBC), bone marrow examination, immunophenotyping, and cytogenetic studies. Immunophenotyping using monoclonal antibodies to stain the blast cells using flowcytometry FACS Canto II (Becton Dickinson, USA). Markers were used to prove or disprove the diagnosis of AML including CD2, CD3, CD7, CD13, CD14, CD20, CD33, CD36, MPO, CD45, CD117, CD 41, CD61, and CD64 monoclonal antibodies that were supplied from Coulter, USA. Also, CD4, CD5, CD10, CD19, CD34, and HLA-DR monoclonal antibodies which were brought from Becton Dickinson Bioscience, USA. Using FACS Canto II (BD) flow cytometry, and the FACS Diva software, FACS acquisition, and analysis were carried out. Remission was proven by bone marrow biopsy following induction chemotherapy (range of 21 days without additional therapy). The 2010 European Leukemia Network (ELN) guideline was followed when defining the response to chemotherapy [24]. A blast count in the bone marrow from AML patients after chemotherapy of less than 5% was considered to be in the complete remission stage. The term "incomplete remission" refers to a blast count in the bone marrow from AML patients after chemotherapy of more than 5% [23].

In this study, a total of 85 samples were obtained: 40 samples from newly diagnosed AML (AML-ND) patients, 25 samples from AML patients after induction chemotherapy, and 20 samples from the apparently healthy controls. Blood samples (2 ml blood on sterilized EDTA blood tube) were collected. The samples were centrifuged, and plasma was stored at −20°C for further ELISA procedure. ELISA kits for plasma IL10, IL33, and IL35 were purchased from ELK Biotechnology, China (catalog number: ELK1142, ELK2728, and ELK2745).

Statistical analysis

Median±interquartile range was used to express quantitative data. The Shapiro–Wilk test was utilized to verify the normality of the quantitative data. The Kruskal–Wallis test, pairwise comparison test, and Mann–Whitney *U* test were employed to assess the group differences. Spearman's correlation coefficient test was used for correlation between not normally distributed data. All statistical tests employed in the investigation were conducted at a significance level of 5%. Version 26 of IBM SPSS Statistics for Windows was used to analyze the data.

Results

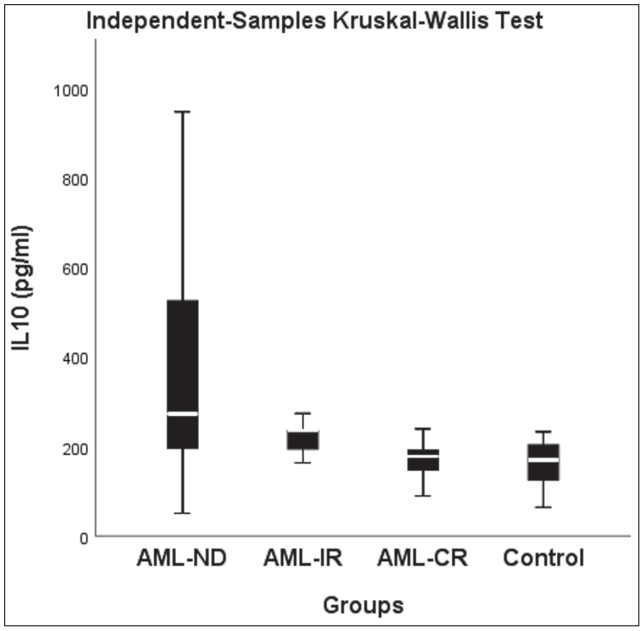
Demographic and laboratory data of patients and controls

At the end of this study, a total of 85 samples were collected: 40 in AML-ND patients (27 males and 13 females with a median age of 35.5), 13 in AML-IR patients (nine males and four females with a median age of 30), and 12 in AML-CR patients (seven males and five females with a median age of 29.5). Twenty controls were enrolled (14 males and six females with a median age of 33.5). Data is shown in Table 1.

Comparison of levels of IL10, IL33, and IL35 in AML-ND, AML-IR, and AML-CR patient groups and controls

Figure 1 and Table 2 show the levels of IL10 between the studied groups. There was a statistically significant increase in IL10 level in the AML-ND group in comparison to the control group with *P* value less than 0.001. In addition, there was a statistically significant decline in IL10 level in AML-CR patients in comparison to the AML-ND group and AML-IR groups with *P* values lesst than0.001 and 0.044, respectively. On the other hand, there was no

Figure 1



Expression of IL10 level within AML-ND, AML-IR, AML-CR, and control groups. The Kruskal–Wallis test and pairwise comparison test were used. **P* value less than or equal to 0.05. AML-CR compared to the AML-ND group. ***P* value less than or equal to 0.05 control compared to AML-ND group. AML, acute myeloid leukemia; IL, interleukin.

Table 1 Demographic and laboratory data of acute myeloid leukemia patients (newly diagnosed and after induction chemotherapy) and controls

Parameters	AML-ND (*N*=40)	AML-IR (*N*=13)	AML-CR (*N*=12)	Control (*N*=20)
Blasts %	68.8 (13.93)	8 (14)	2.7 (0.82)	-
WBCs 10^3/µl	44.75 (67.63)	6.1 (5.19)	6.35 (1.5)	6.95 (2.1)
Platelets 10^3/µl	44.5 (64.33)	294.36 (237)	414 (149.5)	244 (108)
Hemoglobin, g/dl	8.7 (2)	10.4 (2.35)	10.78 (1.42)	14.35 (1.5)
Promyelocytes %	3.18 (2.19)	4 (1.05)	4.7 (2.68)	–
Myelocytes %	3.28 (3.29)	5.1 (3.5)	5.2 (1.57)	–
Neutrophils %	5.65 (3.7)	29.96 (20.5)	32.57 (7.1)	53.75 (15.7)
Lymphocytes %	4 (6)	9 (11.69)	15.57 (8.39)	44.1 (15.63)
Monocyte %	2 (4.3)	2.25 (3.5)	3.85 (1.71)	1 (0.68)
Eosinophils %	5.5 (6.65)	1 (1.25)	0.16 (0.17)	1 (0.25)
Basophils %	3 (4.76)	0 (0.6)	0.0	0
Demographic data of AML-ND (*N*=40)				
Fever		40%		
Hepatomegaly		27.5%		
Splenomegaly		15%		
Lymphadenopathy		15%		
Hepatosplenomegaly		12.5%		
Other		70%		

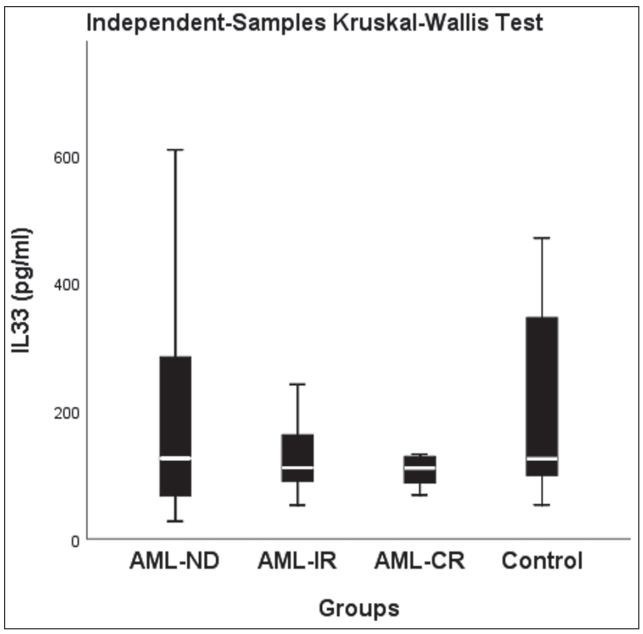
Data expressed in median (interquartile range). Data expressed in percentage.
AML-CR, acute myeloid leukemia-complete remission; AML-IR, acute myeloid leukemia-incomplete remission; AML-ND, acute myeloid leukemia-newly diagnosed; WBC, white blood count.

Table 2 Expression of IL10, IL33, and IL35 levels within AML-ND patients, AML-IR patients, and AML-CR patients groups

	AML-ND (*N*=40)	AML-IR (*N*=13)	AML-CR (*N*=12)	Control (*N*=20)
IL10 (pg/ml)	273 (339.6)	238.86 (135.37) *P*=0.265	180.30 (91.6)* *P*>0.001	185.30 (107.41)** *P*≤0.001
IL33 (pg/ml)	125.73 (223.23)	110.98 (96.95) *P*=0.664	110.38 (47.23) *P*=0.474	125.13 (260) *P*=0.743
IL35 (pg/ml)	584.3 (540.3)	263.3 (323.8)* *P*=0.004	216 (205.11)* *P*>0.001	274.47 (317.22)** *P*=0.001

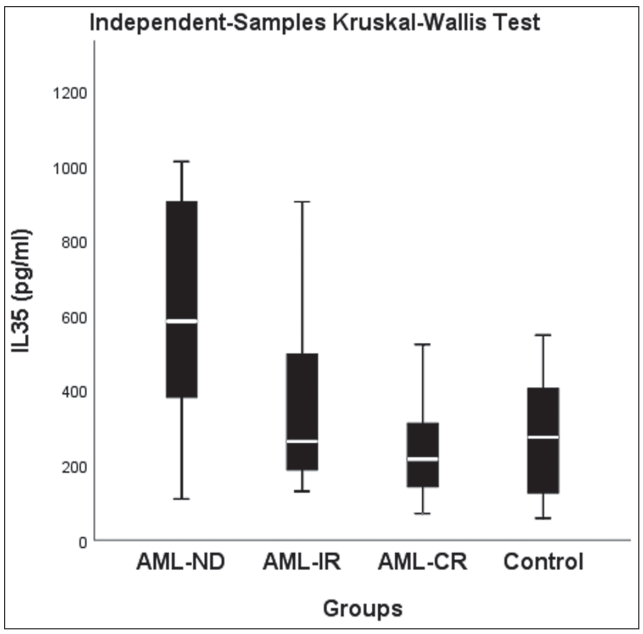
Data expressed in median (interquartile range).
AML-CR, acute myeloid leukemia-complete remission; AML-IR, acute myeloid leukemia-incomplete remission; AML-ND, acute myeloid leukemia-newly diagnosed.
The Kruskal–Wallis test and pairwise comparison test was used.
**P* value less than or equal to 0.05, AML-CR compared to AML-ND group.
***P* value less than or equal to 0.05, control compared to AML-ND group.

Figure 2



Expression of IL33 level within AML-ND, AML-IR, AML-CR, and control groups. The Kruskal–Wallis test and pairwise comparison test were used. AML, acute myeloid leukemia; IL, interleukin.

Figure 3



Expression of IL35 level within AML-ND, AML-IR, AML-CR, and control groups. The Kruskal–Wallis test and pairwise comparison test were used. **P* value less than or equal to 0.05. AML-CR compared to the AML-ND group. ***P* value less than or equal to 0.05 control compared to AML-ND group. AML, acute myeloid leukemia; IL, interleukin.

statistically significant difference between IL10 level AML-IR and AML-ND with *P* value 0.265. Moreover, no statistically significant change was observed in IL10 level in the AML-CR group in comparison to the control group with *P* value of 0.995. A statistically significant increase was observed in IL10 level in the AML-IR group in comparison to the control group with a *P* value of 0.023, as shown in Fig. 1 and Table 2.

Figure 2 and Table 2 show the levels of IL33 within the four groups. Results showed no statistically significant difference within groups. There was the insignificant elevation of IL33 in AML-ND compared to the control group with *P* value of 0.743. In addition, there was no statistically significant difference in the level of IL33 between AML-IR, AML-CR, and both control groups or AML-ND with *P* values of 0.664, 0.474, 0.457, and 0.291, respectively. In addition, there was no statistically significant change in IL33 level between AML-IR and AML-CR groups, with *P* value 0.913 as shown in Fig. 2 and Table 2.

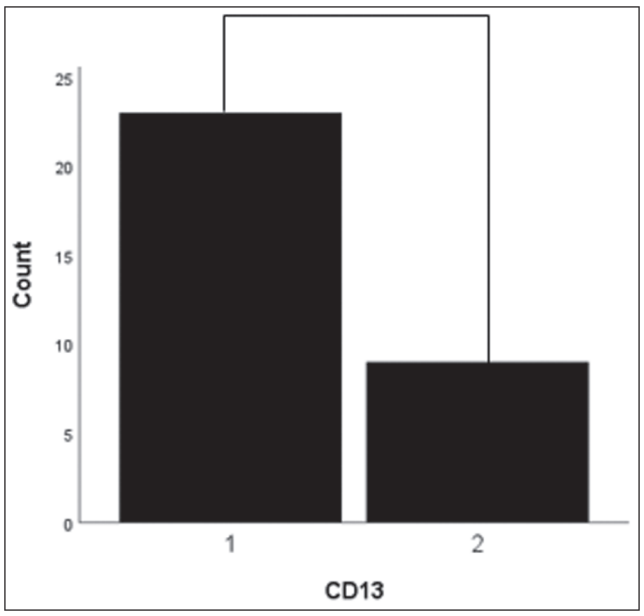
Figure 3 and Table 2 show the levels of IL35 within the four groups. There was a statistically significant increase in IL35 level in the AML-ND group in comparison to the control group with a *P* value 0.001. In addition, there was a statistically significant decline in IL35 level after induction chemotherapy in AML-IR and AML-CR groups in comparison to AML-ND group with *P* value 0.004 and less than0.001, respectively. Also, there was no statistically significant difference in IL35 level between AML-IR and AML-CR groups with *P* value of 0.232. Moreover, no statistically significant change was observed in IL35 level in AML-IR and AML-CR groups in comparison to the control group (*P*=0.552 and 0.593), respectively, as shown in Fig. 3 and Table 2.

Table 3 Expression of IL10, IL33, and IL35 levels within AML-ND patients according to immunophenotypes parameters

Parameter number (positive/negative)	AML-ND IL10	AML-ND IL33	AML-ND IL35
CYTO MPO 30 (25/5)	30 (16.4/11) *P*=0.229	30 (16.36/11.2) *P*=0.251	30 (16.44/10.8) *P*=0.208
CD13 32 (23/9)	32 (15.26/19.67) *P*=0.246	32 (14.17/22.44)* *P*=0.024	32 (18.17/12.22) *P*=0.112
CD33 32 (30/2)	32 (16.6/15) *P*=0.847	32 (16.87/11) *P*=0.444	32 (16.47/17) *P*=1
CD36 26 (12/14)	26 (15.42/11.86) *P*=0.252	26 (13.42/13.57) *P*=1	26 (13.17/13.79) *P*=0.860
CD 11c 25 (15/10)	25 (12.03/14.45) *P*=0.428	25 (12.8/13.3) *P*=0.892	25 (13.67/12) *P*=0.605
CD34 30 (17/13)	30 (15.41/15.62) *P*=0.967	30 (15.76/15.15) *P*=0.869	30 (16.29/14.46) *P*=0592
HLA-DR 26 (21/5)	26 (13.48/13.6) *P*=1	26 (12.26/18.7) *P*=0.09	26 (13.93/11.7) *P*=0.569
CD117 28 (24/4)	28 (14.83/12.5) *P*=0.635	28 (14.29/15.75) *P*=0.776	28 (14.17/16.5) *P*=0.635
CD4 23 (12/11)	23 (11.54/12.5) *P*=0.740	23 (12.33/11.64) *P*=0.833	23 (12.71/11.23) *P*=0.608
CD7 22 (3/19)	22 (7.33/12.16) *P*=0.265	22 (11.67/11.47) *P*=1	22 (11.33/11.53) *P*=1

Data expressed in number (mean of yes/no) AML-ND patient results.
AML-CR, acute myeloid leukemia-complete remission; AML-IR, acute myeloid leukemia-incomplete remission; AML-ND, acute myeloid leukemia-newly diagnosed.
The Mann–Whitney *U* test was used.
*A statistically significance ($P \leq 0.05$).

Figure 4



Association between IL33 level and CD13 positive (1) and CD13 negative (2) patients in the AML-ND group. The Mann–Whitney *U* test was used. *Refers to statistical significance at level ($P \leq 0.05$). AML, Acute myeloid leukemia; IL, interleukin.

Association of IL10, IL33, and IL35 level with immunophenotypes

Figure 4 and Table 3 show a statistically significant association between the expression of CD13 and IL33 in the AML-ND group. There was no significant association between IL10, IL33, and IL35, and other immunophenotyping markers, as shown in Table 3.

Correlation between the level of IL10, IL33, and IL35 among different studied groups

Figure 5 shows a statistically significant correlation between IL10 and IL33 in the AML-ND group (r=0.741, $P \leq 0.001$). No statistically significant correlation between IL10, IL33, and IL35 within other groups.

Correlation between laboratory parameters and level of IL10, IL33, and IL35 in studied groups

Table 4 shows the correlation between IL10, IL33, and IL35 levels with other laboratory markers within AML-ND, AML-IR, and AML-CR groups. Results revealed a statistically significant correlation between IL10 and blast and IL35 and WBCs within the AML-IR group.

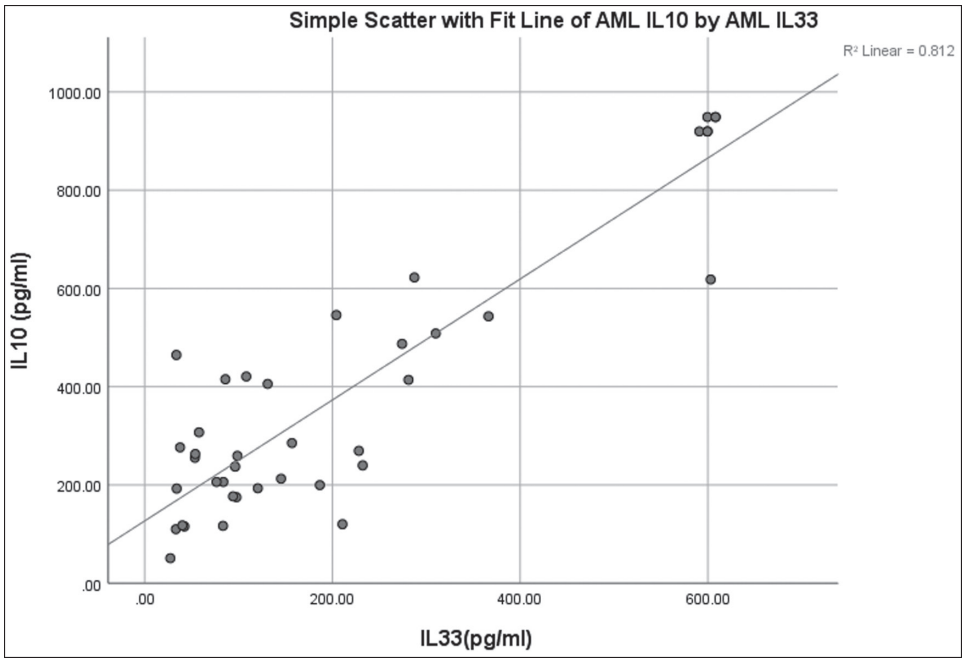
Discussion

It has been demonstrated that immunological disorders are associated with the development of AML. Immunosuppressive conditions in the AML microenvironment help malignant hematopoietic cells survive. Nonetheless, there is still uncertainty surrounding the aberrant immunological milieu associated with AML, particularly the ILs.

This study aimed to investigate the levels of IL10, IL33, and IL35 as possible follow-up markers in AML patients at diagnosis and after chemotherapy induction and to correlate their levels with AML immunophenotypic markers. Newly diagnosed AML patients were followed up from diagnosis till complete remission or death, and levels of IL10, IL33, and IL35 were detected using ELISA.

Concerning demographic data, this study was conducted on 65 AML patients. The ages of included patients ranged from 18 to 61 years (with a mean of 35.5 years). There was a little male preponderance (male to female ratio was 2: 1). This study results were somewhat comparable to those of another study conducted on 82 adult AML patients at the National Cancer Institute in Egypt, which revealed similar epidemiological features. With a median age of 34, their ages varied from 18 to 68. The ratio of men to women was 1.05: 1 [25]. In another study, which was

Figure 5



Correlation between IL10 and IL33 levels within AML-ND patients group. Spearman's correlation coefficient test was used. AML, acute myeloid leukemia.

Table 4 Correlation between IL10, IL33, and IL35 levels and laboratory parameters within AML-ND, AML-IR, and AML-CR patients groups

Parameters	AML-ND (*N*=40)			AML-IR (*N*=13)			AML-CR (*N*=12)		
	IL10	IL33	IL35	IL10	IL33	IL35	IL10	IL33	IL35
Age	*r*=0.044 *P*=0.786	*r*=0.09 *P*=0.579	*r*=0.079 *P*=0.628	*r*=0.07 *P*=0.753	*r*=−0.352 *P*=0.238	*r*=−0.429 *P*=0.143	*r*=−0.188 *P*=0.558	*r*=0.284 *P*=0.371	*r*=−0.095 *P*=0.770
Blasts %	*r*=−0.042 *P*=0.796	*r*=0.158 *P*=0.331	*r*=0.135 *P*=0.406	*r*=0.648* *P*=0.017	*r*=−0.221 *P*=0.469	*r*=0.041 *P*=0.893	*r*=− 0.552 *P*=0.063	*r*=0.415 *P*=0.18	*r*=−0.044 *P*=0.893
WBCs 10^3/ul	*r*=0.172 *P*=0.228	*r*=0.03 *P*=0.855	*r*=−0.165 *P*=0.308	*r*=0.42 *P*=0.153	*r*=−0.374 *P*=0.208	*r*=0.589* *P*=0.034	*r*=−0.163 *P*=0.612	*r*=−0.116 *P*=0.72	*r*=−0.225 *P*=0.482
Platelets 10^3/ul	*r*=−0.173 *P*=0.285	*r*=0.091 *P*=0.576	*r*=0.19 *P*=0.239	*r*=−0.372 *P*=0.21	*r*=0.347 *P*=0.246	*r*=0.143 *P*=0.641	*r*=−0.443 *P*=0.149	*r*=0.442 *P*=0.15	*r*=−0.319 *P*=0.312
Hemoglobin g/dl	*r*=−0.023 *P*=0.886	*r*=−0.101 *P*=0.536	*r*=−0.043 *P*=0.794	*r*=0.227 *P*=0.455	*r*=−0.105 *P*=0.734	*r*=0.072 *P*=0.816	*r*=0.000 *P*=0.1	*r*=0.268 *P*=0.399	*r*=−0.268 *P*=0.399
Promyelocytes %	*r*=−0.211 *P*=0.191	*r*=−0.136 *P*=0.404	*r*=−0.042 *P*=0.797	*r*=−0.357 *P*=0.231	*r*=0.053 *P*=0.863	*r*=0.297 *P*=0.324	*r*=−0.016 *P*=0.96	*r*=−0.087 *P*=0.787	*r*=−0.131 *P*=0.685
Myelocytes %	*r*=−0.263 *P*=0.101	*r*=−0.307 *P*=0.054	*r*=−0.042 *P*=0.797	*r*=−0.443 *P*=0.13	*r*=0.108 *P*=0.724	*r*=0.070 *P*=0.821	*r*=−0.139 *P*=0.668	*r*=0.022 *P*=0.946	*r*=−0.175 *P*=0.587
Neutrophils %	*r*=0.05 *P*=0.76	*r*=0.069 *P*=0.67	*r*=−0.076 *P*=0.639	*r*=−0.54 *P*=0.57	*r*=0.385 *P*=0.194	*r*=−0.363 *P*=0.223	*r*=−0.036 *P*=0.911	*r*=−0.464 *P*=0.129	*r*=0.087 *P*=0.788
Lymphocytes %	*r*=0.227 *P*=0.158	*r*=0.021 *P*=0.897	*r*=−0.08 *P*=0.625	*r*=0.042 *P*=0.892	*r*=0.521 *P*=0.068	*r*=−0.408 *P*=0.167	*r*=0.025 *P*=0.937	*r*=−0.319 *P*=0.312	*r*=0.290 *P*=0.360
Monocyte %	*r*=0.158 *P*=0.331	*r*=0.101 *P*=0.534	*r*=0.122 *P*=0.452	*r*=0.15 *P*=0.625	*r*=0.081 *P*=0.793	*r*=−0.251 *P*=0.409	*r*=−0.322 *P*=0.307	*r*=−0.047 *P*=0.884	*r*=−0.156 *P*=0.628
Eosinophils %	*r*=−0.021 *P*=0.897	*r*=0.033 *P*=0.838	*r*=−0.001 *P*=0.997	*r*=−0.316 *P*=0.293	*r*=−0.003 *P*=0.993	*r*=−0.051 *P*=0.869	*r*=−0.411 *P*=0.184	*r*=0.066 *P*=0.838	*r*=0.358 *P*=0.253

Data expressed in spearman's correlation coefficient (*r*).
AML-CR, acute myeloid leukemia-complete remission; AML-IR, acute myeloid leukemia-incomplete remission; AML-ND, acute myeloid leukemia-newly diagnosed; WBC, white blood count.
Spearman's correlation coefficient test was used.
*A statistical significance (*P*≤0.05).

conducted on 90 AML patients, the ages had a mean of 37.8 years and varied from 18 to 76 years. The ratio of male to female was found to be somewhat in favor of the female gender [26]. A further Upper Egypt study conducted on 170 AML patients reported that the range of ages was 18–69, with a mean age of 49. There

was a minor ratio of 1: 1.36 between males and females [23].

The results of this study revealed a significantly higher IL10 level in the AML-ND group compared to the control group, which was reduced in patients who achieved complete remission after chemotherapy induction (Fig. 1 and Table 2). In addition, the level of IL10 was not statistically significant reduced in patients who did not achieve complete remission (Fig. 1 and Table 2). This result may indicate the association of IL10 with better outcomes in AML patients after induction chemotherapy. This result comes in accordance with previous studies [27–29].

Figure 2 and Table 2 report for the first time that IL33 level was insignificantly elevated in AML-ND compared control groups, with no statistically significant reduction in IL33 level after induction chemotherapy. This can be explained as IL33, which is a resistant marker that is not affected by induction chemotherapy and may be related to chemotherapy resistance and disease relapse in AML patients.

Figure 3 and Table 2 showed that the IL35 level was significantly increased in AML-ND compared to the control group, which was significantly reduced after induction chemotherapy whether AML patients were in complete or incomplete remission with more reduction detected in AML-CR group than AML-IR group. These results indicate that IL35 level can be used as a successful biomarker for treatment response in AML patients, disease prognosis, and possible immunotherapy. These results come in accordance with previous studies [2,27,29–31]. On the contrary, Sun *et al.* [28] stated that no discernible reduction existed at the IL35 level in AML-CR compared to AML-ND. However, this may be due to various demographics and treatment plans.

Data in Table 4 indicated a statistically significant association between IL35 and WBC in the AML-IR group. Previous studies reported that IL-35 was highly expressed and significantly correlated with AML patients' clinical outcomes and could be a sensitive diagnostic marker for de novo AML patients and related to poor prognosis [2,30]. A previous study on pediatric acute lymphoblastic leukemia suggested a role for IL-35 in ALL development and progression [30].

Results of immunophenotypes (Fig. 4 and Table 3) show for the first time a statistically significant association between IL33 level and CD13 prognostic marker. CD13 has been demonstrated as a highly expressed leukemic marker on AML cells that is related to proliferation and apoptosis, and its inhibitors were reported as a targeted therapy in AML [32–35]. This correlation indicates the association of IL33 with cell proliferation, apoptosis, or immune resistance in AML and the possibility of being a promising targeted therapy against leukemic blasts.

Data in Fig. 5 and Table 4 reported a statistically significant correlation between IL10 and IL33 in the AML-ND group. This result comes in agreement with previous studies on Treg, which proved the higher activity of Treg expressing IL33 receptor, which exerts its inhibitory function through IL10 [13]. In addition, ST2-blocking antibodies in AML animal models gave promising results in decreasing LSC and survival rates [17]. Together, IL10 and IL33 are suggested to be responsible for immunosuppressive function exerted by Treg in AML and related to LSC. This study has some limitations because of the small sample sizes in the follow-up AML-IR and AML-CR groups.

Conclusion

Based on the results of this study, IL10 and IL35 are recommended as follow-up markers in AML patients. IL10 and IL35 may be related to AML disease progression and clinical outcomes after induction chemotherapy. Moreover, IL33 is correlated with CD13 and could be a novel prognostic marker in AML patients. More large-scale studies are needed to evaluate the efficacy of IL10, IL33, and IL35 as a targeted immunotherapy, especially against LSC, which is one of the highest obstacles in AML therapy.

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Conflicts of interest

There are no conflicts of interest.

Abbreviations:

AML, acute myeloid leukemia;
IL10, IL33, and IL35, interleukin 10, 33 and 35;
LSC, leukemic stem cells;
Treg, T regulatory cells.

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