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Role of Protein Bax and Bcl2 in The Prognosis of Acute Myeloid Leukemia in Adults

Gehad Hamada Fekry*[1], Mohamed Hamdy[1], Ahmed Samy Elsayd[1], Shereen Abdel Monem Ibrahim[2], Mostafa Kamal El Razazz[1], Heba Mohamed Saber Hafez[1]

Departments of [1]Internal Medicine and [2]Clinical Pathology, Faculty of Medicine, Ain Shams University, Egypt

***Corresponding author:** Gehad Hamada Fekry, **Mobile:** (+20) 01010891794, **E-mail:** gehad _fekry@med.asu.edu.eg

ABSTRACT

Background: Acute myeloid leukaemia (AML) is a fatal hematological disease that occurs due to differentiation arrest and uncontrolled proliferation of immature cells. Malignant cells undergo apoptosis when exposed to some anticancer medications; therapeutic resistance may result from this resistance. In a variety of malignancies, Bax, Bcl-2, and their ratio have all been identified as prognostic indicators. Regarding AML, however, contradictory findings have been documented.

Objective: Our work investigated the association between Bax and Bcl-2 gene expression and their ratios with the therapeutic response in AML.

Patients and methods: Our prospective study included 55 newly diagnosed AML patients, treated at Hematology Department at Ain Shams University Hospital during (January to December) 2023 and followed during the induction chemotherapy for 1 month.

Results: The age of the patients participated in the study ranged from 18-60 years with a mean age of 40.84+/-12.74 years. Our patients were 26 (47.3%) males and 29 females (52.7%). ECOG score ranged from 1-5; one indicated restricted physical activity but ambulatory while five referred to being dead. The cytogenetics of studied patients showed a major dominance for T (8-21) –ve and normal karyotyping of all the participating patients. The study showed that Bax and Bcl-2 and their ratio expression in blood samples correlated with AML more among poor responders and complicated patients during induction and there was statistically significant correlation between their level and the outcome after chemotherapy.

Conclusion: Expression of Bax and Bcl2 and their ratio in AML patients differed significantly as regard outcome after chemotherapy.

Keywords: AML, Bax and Bcl2, Apoptosis, Survival rate and remission.

INTRODUCTION

One kind of malignant hematological illness called AML is brought on by differentiation arrest, unchecked immature cell proliferation, and a reduction in myeloid progenitor cell death. Approximately 80% of all instances of acute leukemia in adults are this type, making it the most prevalent [(1)]. Acute leukaemia is the seventh cancer as regard the incidence (5231) for all cancers in Egypt in 2022 [(2)].

Cytarabine and anthracyclines like daunorubicin are frequently used in chemotherapy, which is the main treatment for acute myeloid leukemia. Two phases are usually included in its administration: an induction phase and a consolidation phase. Between days 21 and 28, the patient's bone marrow and blood are examined for complete remission (CR) following induction chemotherapy [(3)]. Unfortunately, many people do not react to treatment. In addition, AML patients who respond to treatment frequently relapse [(4)].

Relapse is defined as the presence of more than 5% blast cells in bone marrow, the recurrence of blast cells in the blood, or the formation of blasts from locations other than bone marrow following CR. Relapse frequently happens within the first three years after the conclusion of treatment, particularly in young individuals. Relapse rates have varied over the world, ranging from 21% to 39% [(5)].

Thus, resistance to chemotherapy is a regular occurrence and a significant challenge in treating AML patients. Although patients may react to anticancer medications, their overall survival (OS) remains poor[(6)].

Apoptosis, or programmed cell death, is an active process that can be initiated by DNA-damaging substances including ionizing radiation and chemotherapy medicines. Although apoptosis is a complicated sequence of intracellular molecular interactions, cell line studies have shown that, in most systems, changes in the expression of Bcl-2 and associated proteins can have a significant impact on chemosensitivity [(7)].

When a cell enters apoptosis, the B-cell lymphoma protein 2 (Bcl-2)-associated X (Bax) protein triggers a series of events by releasing cytochrome c from the mitochondria. This facilitates the activation of caspases one after another and finally results in cell death [(5)]. Though it is also thought that Bcl-2 helps proliferating cells return to the G0 phase of the cell cycle, it also inhibits Bax's release of cytochrome c, which limits the downstream activation of an apoptotic mechanism that would otherwise result in cell survival [(8)]. Cytoplasmic proteins make up both Bcl-2 and Bax. Cell death is thought to occur automatically when cytochrome c is released from the mitochondrial matrix. DNA fragmentation inhibits Bcl-2 via P53 and promotes Bax [(7)].

One theory for the processes behind multidrug resistance is the dysregulation of cancer cell death.

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There have been contradictory findings from several research that have looked at the role of Bax and Bcl-2 in AML and other malignancies. It has been suggested that a high Bax/Bcl-2 ratio, together with high Bax and/or low Bcl-2, promotes apoptosis and may thus result in a good outcome, while other findings have shown the contrary [(9)].

Therefore, even with continuous study, we still don't fully understand this process and how it affects the result of therapy. The present emphasis on developing anti-Bcl-2 medications and their expected application in cancer treatment further complicates this. Therefore, utilizing peripheral blood samples from newly diagnosed AML patients was used to examine the association between the chemotherapeutic response to Bax and Bcl-2 gene expression and analyze their significance as chemotherapy biomarkers[(5)].

Our work investigated the association between Bax and Bcl-2 gene expression and their ratios with the therapeutic response in AML.

PATIENTS AND METHODS

Patients

Our prospective study included 55 newly diagnosed AML patients, treated at Hematology Department at Ain Shams University Hospital during (January to December 2023) and followed during the induction chemotherapy for 1 month. Our patients were diagnosed and classified according to the French-American-British morphological system along with the WHO immunological classifications [(10)].

All prospective AML patients, including acute promyelocytic leukemia (M3), were recruited into the research. 34 patients underwent an induction chemotherapy consisting of solely the conventional 3+7 regimen (Adriamycin 25 mg/m^2 on days 1-3; cytarabine 200 mg/m^2 on days 1-7), 11 patients received 2+5 regimen (Adriamycin 25 mg/m^2 on days 1–2; cytarabine 200 mg/m^2 on days 1–5), 5 acute promyelocytic leukemia patients received pethema protocol and 5 patients received vidaza+venetoclex protocol. All patients aged 18 years or older.

METHODS

Expression of both BAX (Bcl-2 Associated X protein) and BCL2 were measured using Thermo Fisher Scientific ELISA kits.

The BAX gene encodes a protein that is a member of the Bcl-2 protein family, which regulates apoptosis either positively or negatively. The release of cytochrome c and a decrease in membrane potential are caused by this protein's interaction with an increased opening of the mitochondrial voltage-dependent anion channel (VDAC).

Assay principle

A target specific antibody was pre-coated on ELISA plates. Serum samples and standards were added to each well, followed by the addition of a biotinylated detector antibody. After washing away the unbound biotinylated antibody, horseradish peroxidase (HRP)-conjugated streptavidin was pipetted to the wells. The wells were again washed and a substrate solution was added that reacts with the enzyme-antibody-target complex to produce measurable signal. The intensity of this signal is directly proportional to the concentration of target present in the original specimen Spectrophotometric measurements were made at 450 nm to determine the optical density.

Ethical approval

Ain Shams Faculty of Medicine's Institutional Review Board approved this report. Following receipt of all information, signed consent was provided by each participant. The Helsinki Declaration was adhered to at every stage of the investigation.

Statistical analysis

SPSS 20.0 was used to analyze the data. Numerical data that were not parametric were shown as median and interquartile range, parametric data were shown as mean± SD, and range, and categorical data were shown as frequencies and percentages. To compare medians between two or more groups, the Mann-Whitney U test and the Kruskal-Wallis test with the Dunn-Bonferroni post hoc procedure were employed, respectively. Spearman coefficients were utilized in the correlation process. Statistical significance was defined as P values < 0.05.

RESULTS

The mean age of the patients participated in the study was 40.84 years. 52.7% of the patients were females. ECOG score ranged from 1-5, one indicates restricted physical activity but ambulatory while, five refers to being dead. The cytogenetics of studied patients showed a major dominance of T (8-21) –ve and normal karyotyping of all the participating patients. NCCN risk which mainly based on the cytogenetics showed 36 patients with an intermediate risk. During the induction, 26 patients suffer neutropenic fever. After the induction, 38 patients entered remission (Table 1). CR is characterized by a total leukocyte count (TLC) of at least 1500/L, a platelet count of at least 100,000/L, and less than 1% of blast cells in the peripheral blood and less than 5% in the bone marrow, all without any signs of extramedullary leukemia.

Table (1): Demographic and characteristics of the studied patients

		Total no.=55
Age	Mean±SD Range	40.84 ± 12.74 18 –60
Sex	Female Male	29 (52.7%) 26 (47.3%)
ECOG score	0 1	29 (52.7%) 26 (47.3%)
AML subtype	AML0 AML1 AML2 AML3 AML4 AML5 AML6 AML7	5 (9.1%) 6 (10.9%) 16 (29.1%) 5 (9.1%) 6 (10.9%) 11 (20.0%) 4 (7.3%) 2 (3.6%)
Denovo	Denovo	55 (100.0%)
Cytogenetics	(t 8:21) +ve T (15:17) +ve Inv (16) +ve	9 5(9.1%) 2 (3.6%)
Karyotyping	No abnormality	55 (100.0%)
NCCN Risk assessment	Intermediate risk Favorable risk Adverse risk	36 (65.5%) 11 (20.0%) 8 (14.5%)
Complication during induction	No Yes	2 (3.6%) 53 (96.4%)
Initial response at day 28th	Not in remission Remission Died	10 (18.2%) 38 (69.1%) 7 (12.7%)

TLC median (IQR) was 20 (3 – 68), platelet level median (IQR) was 23 (12 – 44) and peripheral blast% median (IQR) was 34 (20 – 60). Blast% in BM aspiration mean±SD was 70.55 ± 17.15 (Table 2).

Table (2): Results of CBC, peripheral blast%, LDH and BM blast%

		Total no.=55
TLC	Median (IQR) Range	20 (3 – 68) 0.3 –309.5
Hb (g/dL)	Mean±SD	9.13 ± 2.13
LDH (U/L)	Median (IQR) Range	570 (400 – 700) 190 –1400
PLT (mcL)	Median (IQR) Range	23 (12 – 44) 2 –221
Peripheral blast	Median (IQR) Range	34 (20 – 60) 5 – 89
BM Aspirate (blast%)	Mean±SD	70.55 ± 17.15

BAX, Bcl-2, and BAX\Bcl-2 levels are shown in table 3.

Table (3): Serum level of BAX, Bcl-2 and BAX/Bcl-2 ratio

		Total no.=55
BAX	Median (IQR) Range	7.4 (5.54 – 8.72) 4 –17.77
Bcl-2	Median (IQR) Range	7.34 (5.56 – 8.6) 4 –15.2
BAX\Bcl-2	Median (IQR) Range	1.01 (0.99 – 1.02) 0.85 –1.09

Table (4): Correlation of BAX, Bcl-2 and BAX/Bcl-2 ratio levels

	BAX		Bcl-2		BAX\Bcl-2	
	r	P-value	r	P-value	r	P-value
BAX	--	--	**0.983****	**<0.001**	**0.385****	**0.004**
Bcl-2	**0.983****	**<0.001**	--	--	**0.358****	**0.007**
BAX\Bcl-2	**0.385****	**0.004**	**0.358****	**0.007**	--	--

**: Highly significant

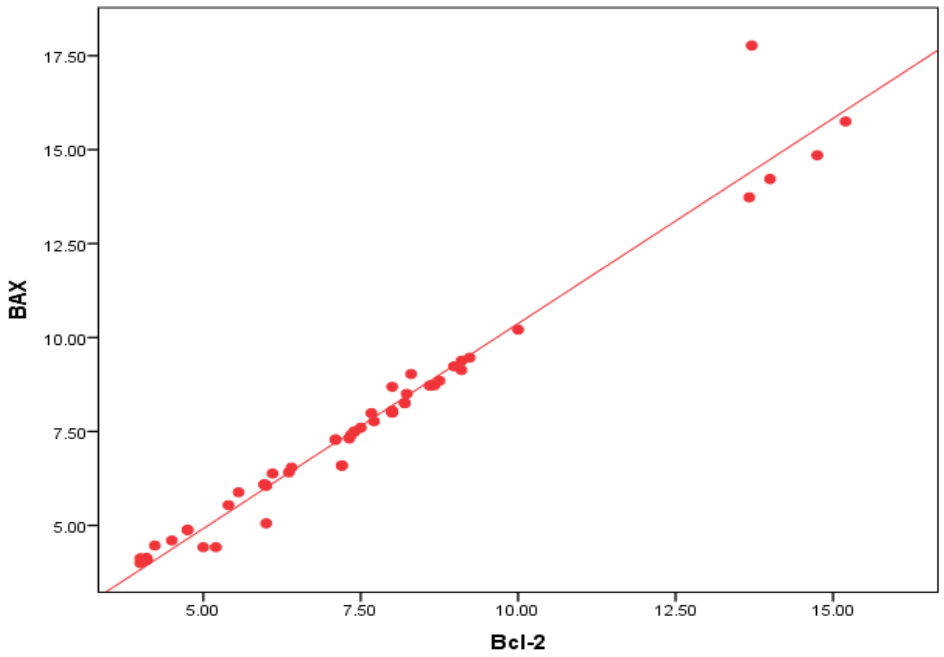

Figure (1): Correlation between BAX and Bcl-2 levels.

From the results above and after the correlation between the expression level of BAX, BCL2 and BAX/BCL2 ratio and other parameters we found that there was no statistically significant difference between the expression level of BAX, BCL2 and BAX/BCL2 ratio and age, sex, ECOG score and hematological parameters (WBC count, hemoglobin, platelets, peripheral blood and bone marrow blasts), AML subtypes and karyotyping results.
However, there was statistically significant relation between BAX and NCCN risk assessment, complications during induction, neutropenic fever with hypokalaemia and initial response after 28 days (Table 5).

Table (5): Relation of BAX with the other studied parameters

		BAX		Test-value	P-value	Sig.
		Median (IQR)	Range			
NCCN Risk assessment	Intermediate risk Favorable risk Adverse risk	7.55 (6.24 – 8.6) 4.6 (4.08 – 6.09) 11.6 (9.05 – 14.54)	4.08 – 15.75 4 – 6.54 4.13 – 17.77	21.327**	<0.001	HS
Complication during induction	No Yes	4.11 (4.08 – 4.13) 7.5 (6.06 – 8.72)	4.08 – 4.13 4 – 17.77	-2.114*	0.035	S
Initial response at day 28th	Not in remission remission died	6.07 (4.6 – 7.99) 7.36 (5.06 – 8.69) 8.73 (8.25 – 14.22)	4.13 – 14.85 4 – 15.75 6.41 – 17.77	7.847*	0.020	S

*: Significant; **: Highly significant

We found also statistically significant relation between BCL2 and NCCN risk assessment, complications during induction, neutropenic fever with hypokalaemia and initial response after 28 days (Table 6 and figures 2 and 3).

Table (6): Relation of Bcl-2 with the other studied parameters

		Bcl-2		Test-value	P-value	Sig.
		Median (IQR)	Range			
NCCN Risk assessment	Intermediate risk Favorable risk Adverse risk	7.45 (6.05 – 8.22) 5 (4.1 – 5.97) 11.45 (8.85– 13.86)	4.1 – 15.2 4 – 6.4 4 – 14.75	20.382**	<0.001	HS
Complication during induction	No Yes	4.1 (4.1 – 4.1) 7.4 (5.97 – 8.6)	4.1 – 4.1 4 – 15.2	-2.114*	0.034	S
Initial response at day 28th	Not in remission Remission Died	5.98 (4.5 – 7.67) 7.33 (5.4 – 8.23) 8.67 (8.2 – 13.71)	4.1 – 14.75 4 – 15.2 6.36 – 114.0	8.282*	0.016	S

*: Significant; **: Highly significant

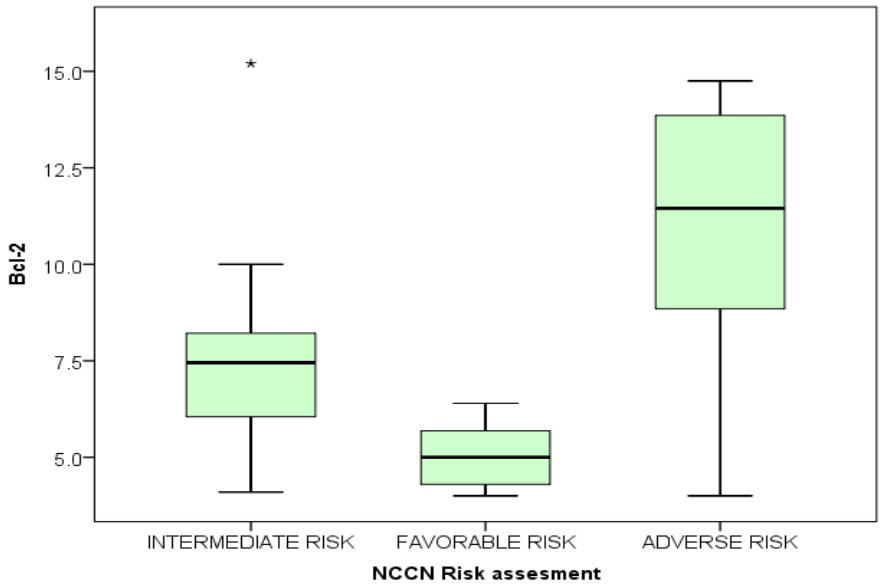

Figure (2): Relation between Bcl2 and NCCN risk assessment

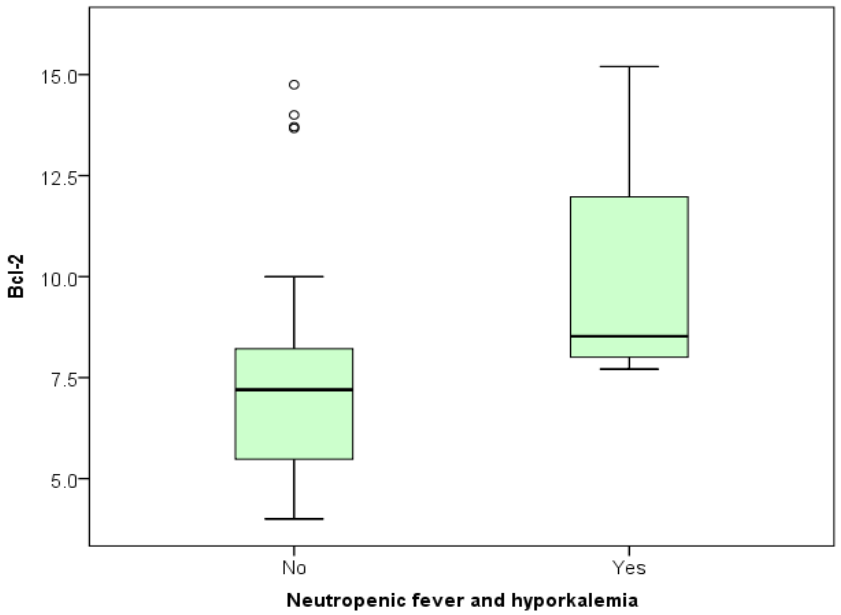

Figure (3): Relation between Bcl2 and neutropenic fever and hypokalaemia.

We found that there was no statistically relation between BAX/BCL2 ratio and complications during induction and initial response after 28 days. But there was highly significant relation between BAX/Bcl2 ratio and NCCN risk assessment (Figure 4).

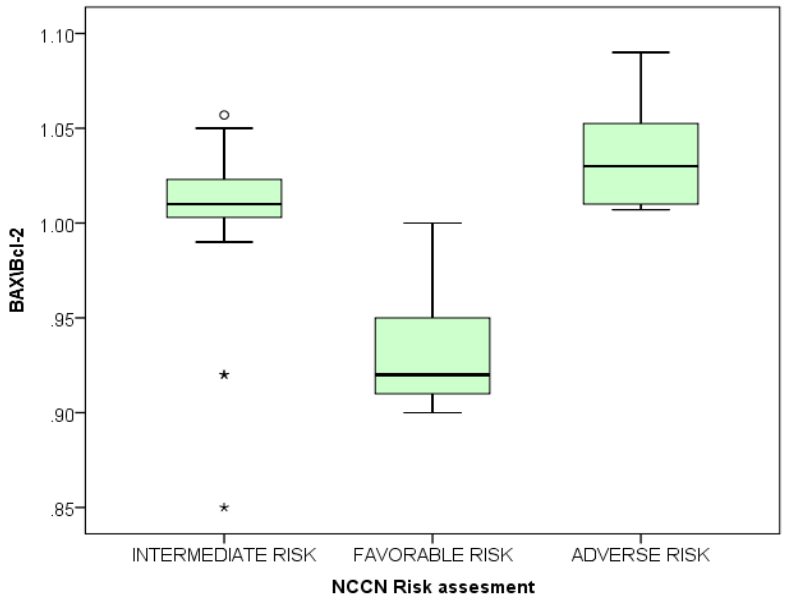

Figure (4): Relation of BAX/Bcl-2 with NCCN risk assessment.

DISCUSSION

The pathogenesis of AML and the majority of malignancies is an imbalance between apoptosis and cell growth. There is significant variation in the pathogenesis of AML. At least two kinds of gene mutations must cooperate for it to occur [(11)].

In order to link the expression levels of these genes with the clinical and laboratory data of the patients as well as their prognosis and patient survival, we assessed the expression of BCL2, BAX, and their ratio in freshly diagnosed peripheral blood samples obtained from adults with acute myeloid leukemia in this study.

Children who lived had a lower Bax/Bcl-2 ratio than those who did not, ranging from 1.74 (SD 1.846) to 3.88 (SD 4.663), but there was no statistically significant difference (p=0.763).

In our investigation, the expression level of BAX did not significantly differ regarding age, sex, ECOG score, hematological parameters (WBC count, Hb, PLT, peripheral blood and bone marrow blasts), AML subtypes, karyotyping, and induction protocols. However, there was statistically significant relation between BAX and NCCN risk assessment, complications during induction, neutropenic fever with hypokalaemia and initial response after 28 days.

Prokop *et al.* [(12)], reported that a greater CR rate (n=34) in AML patients is connected with increased Bax expression, which is an excellent predictor of being a pro-apoptotic marker. In ALL, on the other hand, low Bax expression was linked to recurrence (n=14, immunoblotting).

In the other hand, **Kulsoom *et al.* [(13)]**, reported that in AML patients, they found no evidence of a significant correlation between Bax or Bcl-2 expression and remission, DFS, or OS.

Furthermore, in our investigation, we discovered that there was no statistically significant difference between the expression level of BCL2 and age, sex, ECOG score, and hematological parameters (WBC count, Hb, PLT, peripheral blood and bone marrow blasts), AML subtypes, karyotyping, and induction protocols.

This finding is consistent with research by **Zhou *et al.* [(14)],** which found that while BCL2 overexpression helped identify particular FAB subtypes of AML, it had no effect on prognosis.

Furthermore, **Kulsoom *et al.*[(13)]** research revealed that AML patients' expressions of BAX and BCL2 did not significantly differ in terms of remission, relapse, resistance, overall survival, or disease-free survival; however, they did imply that there was no meaningful correlation between the expressions of these two proteins and their ratio with clinical response. This may be due to the difference in the sample size and site of sample collection.

On the other hand, we found a statistically significant relation between BCL2 and NCCN risk assessment, complications during induction, neutropenic fever with hypokalaemia and initial response after 28 days.

Same as the results mentioned by **Kornblau *et al.* [(15)]**, a higher median survival and a longer duration of remission were linked to increased Bcl-2 expression in the favorable and intermediate cytogenetic groups, but a smaller sample of AML patients (n=198; Western blotting) showed the opposite pattern.

In our study, we discovered that there was no statistically significant difference between the BAX/BCL2 ratio and age, sex, ECOG score, or hematological parameters (WBC count, Hb, PLT, peripheral blood, and bone marrow blasts). Bax and Bcl-2 as a ratio (Bax/Bcl-2 or otherwise) has been extensively studied as a prognostic marker, AML subtypes, karyotyping, induction protocols, complications during induction and initial response after 28 days.

These are same as the results of **Kulsoom *et al.* [13]**, when they reported that APL, an AML subtype with a better prognosis than all other subtypes combined, had a much lower Bax/Bcl-2 ratio than other subtypes (mainly M0, M1, and M2). However, there was no significant difference in Bax/Bcl-2 ratio between excellent and poor responders.

However, we discovered that the BAX/BCL2 ratio and NCCN risk assessment differed in a highly statistically significant way. This goes hand in hand with the finding of **Del Poeta *et al.* [16],** as a greater Bax/Bcl-2 ratio was linked to a higher CR rate, while a lower Bax/Bcl-2 ratio was linked to a poorer result, according to research done on AML patients.

Also, it is similar to the results in another extensive research included AML patients, **Köhler *et al.* [17]**, found that reduced Bax/Bcl-2 levels were linked to reduced OS.

On the contrary, in **Cahyadi *et al.* [18]**, expression of Bcl-2, Bax, and the ratio of the Bax/Bcl-2 protein at the time of diagnosis had no effect on the prognosis for childhood ALL during the induction phase of chemotherapy. It may be due to difference between age groups and type of acute leukaemia.

Furthermore, it appears that there is no meaningful correlation between the expression of BAX and BCL 2 and their ratio with clinical response, as evidenced in one research by **Kulsoom *et al.* [13]**, which demonstrated that expression of BAX and BCL2 does not differ significantly among AML patients in terms of remission, relapse, resistance, overall survival, and disease-free survival.

On the other hand, in a larger sample of AML patients (n=198), **Kornblau *et al.* [15]** found that high Bcl-2 expression was linked to a longer median survival and duration of remission in the poor cytogenetic group, but a shorter median survival in the favorable and intermediate cytogenetic group.

CONCLUSION

Our results indicate that there was a strong connection between Bax and Bcl-2 expression, as well as their ratio, and clinical response after 28 days. We recommend further studies of BAX, BCL2 and their ratio with larger samples of AML patients with longer periods of follow up.

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