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Evaluating the Role of Cytokine Receptor-like Factor 2 and Janus Kinase 2 in Adult Acute Lymphoblastic Leukemia

Naglaa M. Hassan[1], Mona S. El Ashry[1], Mona Abdellateif[2]*, Reem Nabil Hassan[1]

[1]Department of Clinical Pathology, National Cancer Institute, Cairo University, Cairo, Egypt; [2]Department of Medical Biochemistry and Molecular Biology, Cancer Biology, National Cancer Institute, Cairo University, Cairo, Egypt

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***Correspondence:** Mona Abdellateif, Department of Medical Biochemistry and Molecular Biology, Cancer Biology, National Cancer Institute, Cairo University. E-mail: mona.sayed@nci.cu.edu.eg
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Abstract

AIM: The aim of the present study was to assess the diagnostic, prognostic, and predictive roles of the cytokine receptor-like factor 2 (CRLF2) and the Janus Kinase 2 (JAK2) genes expression in adult acute lymphoblastic leukemia (ALL) patients.

METHODS: The expression levels of CRLF2 and JAK2 genes were evaluated in the bone marrow (BM) samples of 105 adult ALL patients, compared to 12 healthy controls. The data were correlated to the patients' relevant clinic-pathological features, response to treatment and survival rates.

RESULTS: There was a significant overexpression of JAK2 in ALL patients compared to the control group [0.04 (0–160.8) and 0.006 (0–0.009), respectively, $p < 0.001$]. Similarly, CRLF2 was overexpressed in ALL patients in comparison to control subjects [0.008 (0–78.2) and 0.0005 (0–0.006), respectively, $p < 0.001$]. The sensitivity, specificity, and the area under curve (AUC) for JAK2 were 78.1%, 81.8%, and 0.796, respectively ($p < 0.001$), and that of CRLF2 were 92.4%, 90.9%, 0.958, respectively ($p < 0.001$). When combining both JAK2 and CRLF2 for the diagnosis of ALL patients, it revealed 90.9% sensitivity, 91.4% specificity, and AUC of 0.957 ($p < 0.001$). The JAK2, CRLF2, or their combined expression associated significantly with the increased expression of MHC-II ($p = 0.015$, 0.001, and 0.004, respectively). However, they had no significant impact on patients' response to treatment, overall (OS), and disease-free survival (DFS) rates ($p > 0.05$ for all).

CONCLUSION: JAK2 and CRLF2 could be a potential useful diagnostic molecular marker for ALL patients, which allow them to be successful targets for ALL therapy.

Introduction

Acute lymphoblastic leukemia (ALL) is a heterogeneous disease characterized by complex molecular changes such as fusion proteins, copy number alterations, and gene mutations. The significant progress achieved in leukemia genomics has led to the recognition of genes and pathways undergoing dysregulation in ALL, and accordingly results in the identification of new modalities for ALL precise treatment [1]. ALL can be divided according to the cell of origin into B-acute lymphoblastic leukemia (B-ALL) which occurs in 85% of ALL cases and T-acute lymphoblastic leukemia (T-ALL) which occurs in 15% of the cases [2].

In the B-ALL subtype, variable genetic abnormalities and chromosomal translocations have been reported to affect the risk stratification for therapy selection [3]. On the contrary, although many molecular abnormalities have been identified in T-ALL, only few genetic aberrations were proved to be of prognostic value, and still, none of them has a beneficial effect regarding the improvement or the reduction of the current treatment tolerability for T-ALL patients [4], [5].

Of interest, rearrangements of cytokine receptor-like factor 2 (CRLF2-R) (IGH-CRLF2 or P2RY8-CRLF2) had been identified in approximately 50% of Ph-like ALL patients resulting in overexpression of CRLF2 with concomitant JAK1/JAK2 mutations in 50% of the CRLF2-rearranged patients [6], [7] that are potentially amenable to treatment with JAK inhibitors [8].

The CRLF2 receptor is a heterodimeric type I receptor complex for thymic stromal lymphopoietin (TSLP), comprised CRLF2 and interleukin-7 receptor alpha (IL-7Rα), where the latter is shared with the cytokine receptor common chain γc to form the heterodimeric IL-7 receptor complex for IL-7. Both CRLF2 and IL-7 can activate the transcription factor STAT5, where IL-7Rα binds to JAK1 and the γc binds to JAK3 on addition of IL-7. In addition, JAK2 has been demonstrated to be involved in STAT5 activation following the binding of TSLP to the CRLF2 receptor complex [9].

Rearrangements of CRLF2, located on chromosome Xp22.3 and Yp11.3 [10], occur by either

translocation of CRLF2 into the immunoglobulin heavy chain enhancer locus (IGH-CRLF2) or through focal deletion of a portion of the PAR1 pseudo-autosomal region of chromosome X/Y, resulting in P2RY8-CRLF2 fusion. CRLF2 expression also can be upregulated by gain of function mutations either in CRLF2 itself or in its partner gene, IL7RA. The CRLF2 overexpression can be assessed either by real-time quantitative polymerase chain reaction (q-PCR) or flow cytometry methods [11], [12], [13], [14].

Importantly, overexpression of CRLF2 is associated with a particularly poor prognosis, as these patients showed significantly worse relapse-free survival relative to patients without CRLF2 overexpression. While the molecular basis for this clinical observation is currently unknown, this suggests that these patients may have an intrinsic resistance to the conventional chemotherapy [7], [15]. B-ALL cell lines with CRLF2 overexpression showed evidence of increased JAK2/STAT5 signaling. The frequency of JAK2 mutations in ALL has been reported to be about 10% in pediatric high-risk ALL and about 20% in Down syndrome ALL [16], [17], [18].

Accordingly, in this study, we aimed at investigation of the different expression patterns of CRLF2 and JAK2 genes in adult ALL cases, with special emphasis on the overexpressed cases to determine the clinical features associated with those cases and their impact on the outcome.

Methods

The present prospective cohort study included 105 newly diagnosed ALL patients who presented to the Clinic of Medical Oncology Department, National Cancer Institute, during the period from August 2018 to December 2020. Control samples were obtained from 12 age- and sex-matched healthy pediatric subjects who were donors for bone marrow transplantation (BMT) in NCI. Control samples were obtained from 12 healthy age- and sex-matched subjects who were donors for BMT in NCI transplantation unit. All patients were subjected to full history taking, clinical, radiological, and laboratory examination for diagnosis of ALL. The diagnosis of ALL was based on morphological examination of the peripheral blood (BP) and BM smears, cytochemistry, immune phenotyping, conventional cytogenetics, and molecular studies of the BM samples.

Response to treatment was evaluated through clinical and by BM examination at day14 and day 28 of induction treatment. Outcome of the induction treatment was assessed at day 28, where patients were categorized into complete remission (CR) or refractory group.

All patients received total XV protocol (modified from St. Jude total XV protocol). The treatment protocol consists of three phases, induction of remission, consolidation, and maintenance [19]. Induction phase (42 days) based on four drug regimens (prednisone, vincristine, doxorubicin, and L-asparaginase), consolidation therapy (8 weeks) consists of 4 cycles of high-dose MTX (HDMTX) and maintenance treatment duration for 120 weeks for females and 146 weeks for males. Patients with t(9;22) [*BCR-ABL1*] started tyrosine kinase inhibitor (imatinib) at a dose of 260 mg/m^2 per day once molecular results were available and continued till the end of treatment. If the patient had the minimal residual disease (MRD) less than <0.1% by flow cytometry at the end of induction, and more than 3 log reduction (major molecular response) MRD by PCR at week 7, he will not be eligible for hematopoietic stem cell transplantation (HSCT). Allogeneic HSCT is indicated for patients with high-risk leukemia (poor response to induction treatment MRD>1%).

Assessment of JAK2 and CRLF2 in ALL patients

Total RNA was extracted from the BM of all the study and healthy control groups using *QIAamp RNA blood Mini Kit* (Qiagen, LOT no. 154013334), according to the manufacturer's instruction. Quantitation and purity assessment for RNA samples were done using the Nano Drop® (ND)-1000 spectrophotometer (Nano Drop Technologies, Inc., Wilmington, USA).

Conversion of RNA to cDNA was done using the Applied Biosystems™ High-capacity cDNA Reverse Transcription Kit (Thermo Fisher Scientific, LOT no. 00716544).

Quantitative reverse-transcription PCR (RT-qPCR) was performed using fluorescent TaqMan Gene Expression Assays (CRLF2: Hs00845692_m1; JAK2: Hs010782136_m1; β-Actin as a reference gene, Thermo Fisher Scientific). Real-time PCR amplification was done using the computerized thermocyclers (ABI step one Applied Biosystems). Data were presented as the fold change in gene expression normalized to an endogenous reference gene and relative to the healthy control, using the $2^{-\Delta\Delta CT}$ method [20].

Statistical analysis

Data management and analysis were performed using SPSS, version 22 (IBM, Armonk, Ny, USA). Qualitative data were presented as numbers and percentages, while quantitative data were described as median and interquartile ranges (IQR) according to the appropriate normality test.

The comparison between groups was performed by Chi-square test and/or Fisher's exact test when appropriate. Mann–Whitney U-test was used for comparing numerical variables between two groups. Spearman correlation coefficients had been used to assess the correlation between two quantitative parameters in the same group. The area under the receiver operating curve (ROC) was calculated to detect the sensitivity and specificity and the best cutoff value for the diagnosis of ALL. Survival analysis was done using the Kaplan–Meier test, and comparison between survival curves was done using the log-rank test. Overall survival (OS) was calculated from the date of diagnosis till the date of death or last follow-up. Disease-free survival (DFS) was calculated from the date of complete remission till the date of relapse, death, or last follow-up. All tests of hypotheses had been conducted at the alpha level of 0.05, with a 95% confidence interval.

Results

Patients' characteristics

The present study included 105 newly diagnosed ALL patients with a median age of 29 (range: 18–74) years old. Males represented 61.9% (65/105), and females were 38.1% (40/105). Seventy-three patients (69.5%) had B-ALL, 26 (24.8%) patients had T-ALL while 6 (5.7%) patients had mixed phenotype acute leukemia (MPAL). Recurrent translocations were identified in 25/85 (29.4%) cases. Sixty-six out of 105 patients (62.8%) achieved CR and 35/105 (33.3%) of them relapsed. At the end of the study, 89 (84.8%) patients died, with 34 (32.3%) of them died before day 28 after treatment. The detailed demographic and clinical features of the patients are illustrated in Table 1.

Table 1: Clinico-pathological characteristics of the assessed ALL patients

Variable	Frequency (%)	Variable	Frequency (%)
Age	29 (18–74)	TLC	45 (1–406)
HB	7.9 (4–13.5)	PLT	48 (4–416)
Initial PB Blast%	50 (5–97)	Initial BM Blast%	85 (11–99)
Blast% day 14	1 (0–97)	Blast% day 28	1 (0–92)
Sex		Hepato/splenomegaly	
Male	65 (61.9%)	Negative	52 (49.5%)
Female	40 (38.1%)	Positive	53 (51.5%)
BM cellularity		FAB	
Hypocellular	9 (7.7%)	L2	97 (92.4%)
Normocelluar	23 (22.1%)	L3	2 (1.9%)
Hypercellular	73 (70.2%)	MPAL	6 (5.7%)
Lymphadenopathy		CD1	
Negative	34 (40%)	Negative	94 (89.5%)
Positive	63 (60%)	Positive	11 (10.5%)
CD10		CD3	
Negative	42 (40%)	Negative	78 (74.3%)
Positive	63 (60%)	Positive	27 (25.7%)
CD7		TdT	
Negative	76 (72.4%)	Negative	93 (88.6%)
Positive	29 (27.6%)	Positive	12 (11.4%)
CD5		CD19	
Negative	83 (79%)	Negative	33 (31.4%)
Positive	22 (21%)	Positive	72 (68.6%)
CD22		CD79a	
Negative	46 (43.8%)	Negative	55 (52.4%)
Positive	59 (56.2%)	Positive	50 (47.6%)
HLADR		MHCII	
Negative	60 (57.1%)	Negative	92 (87.6%)
Positive	45 (42.9%)	Positive	13 (12.4%)
CD33		Cytom	
Negative	93 (88.6%)	Negative	73 (69.5%)
Positive	12 (11.4%)	Positive	32 (30.5%)
CD34		CD13	
Negative	51 (48.6%)	negative	93 (88.6)
Positive	54 (51.4%)	positive	12 (11.4%)
Molecular genetics		CSF	
Negative	60 (70.6%)	Free	90 (96.8%)
t (9;22)	22 (25.9%)	Positive	3 (3.2%)
t (1;19)	2 (2.4%)	Cytogenetics	
t (4;11)	1 (1.2%)	Normal	43 (50.6%)
IPT type		Abnormal	28 (32.9%)
B ALL	73 (69.5%)	Hypodipiody	3 (3.5%)
T ALL	26 (24.8%)	Hyperdiploidy	11 (12.9%)
MPAL	6 (5.7%)		
Patients' response to therapy		Death	
CR	No	13 (12.4%)	
Relapse	Yes	89 (84.8%)	
Refractory	Lost follow up	3 (2.9%)	
Early death	34 (32.3%)		
Lost follow up	3 (2.9%)		

BM: Bone marrow, HB: Haemoglobin: IPT: Immunophenotyping, PB: Peripheral blood, PLT: Platelets, TLC: Total leukocyte count.

Expression levels of JAK2 and CRLF2 in ALL patients

There was a significant overexpression of JAK2 in ALL patients compared to the control group [0.04 (0–160.8) and 0.006 (0–0.009), respectively, $p < 0.001$, Figure 1a]. Similarly, CRLF2 was overexpressed in ALL patients in comparison to control subjects [0.008 (0–78.2) and 0.0005 (0–0.006), respectively, $p < 0.001$, Figure 1b].

Diagnostic value of JAK2 and CRLF2 for ALL patients

The ROC analysis was performed for the identification of ALL patients using JAK2 and/or CRLF2 expression levels. It showed that the sensitivity, specificity, and the area under curve (AUC) for JAK2 were 78.1%, 81.8%, and 0.796, respectively, at a cutoff value of 0.01 ($p < 0.001$, Figure 2a), and that of CRLF2 were 92.4%, 90.9%, and 0.958, respectively, at a cutoff value of 0.0001 ($p < 0.001$, Figure 2b). While when combining both JAK2 and CRLF2 for the diagnosis of ALL patients, it revealed 90.9% sensitivity, 91.4% specificity, and AUC of 0.957 ($p < 0.001$, Table 2, and Figure 2c).

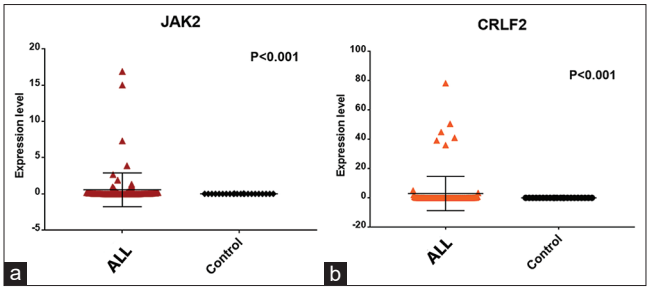

Figure 1: Expression levels of (a) JAK2 and (b) CRLF2 in ALL patients and control subjects

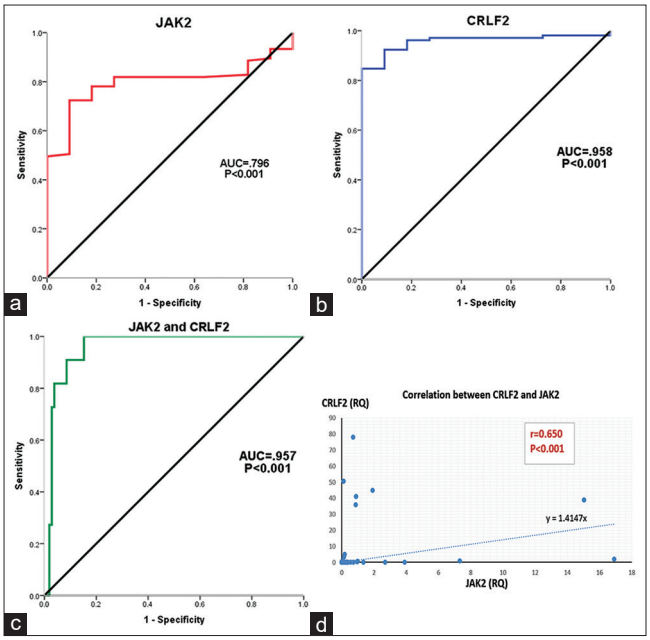

Figure 2: The ROC analysis for diagnosis of ALL patients; (a) JAK2 gene expression level, (b) CRLF2 gene expression level, and (c) combined JAK2 and CRLF2 expression level. (d) Correlation between JAK2 and CRLF2 expression levels in ALL patients

Correlation between JAK2 and CRLF2 expression levels in ALL patients

The median relative quantification (RQ) of JAK2 in ALL patients was 5.31 with a range of 0–21,604, while the median RQ of CRLF2 in ALL patients was 157.26 with a range of 0–91,094.

There was a significant intermediate positive correlation between the median RQ of JAK2 and CRLF2 expressions in ALL patients ($r = 0.650$, $p < 0.001$, Figure 2d).

Patients were classified according to the median RQ expression of CRLF2 and JAK2 into low and high expressors.

Association between JAK2 expression and the clinicopathological features of the ALL patients

There was a significant association between JAK2 low expression and increased both peripheral blood (PB) blast % and BM blast % at diagnosis ($p = 0.006$ and 0.001, respectively). Furthermore, JAK2 low expression associated significantly with positive expression for CD3 and CD7 ($p = 0.046$ and 0.005, respectively). On the other hand, 11 out of 13 MHC-II-positive cases showed JAK2 overexpression revealing a statistically significant association ($p = 0.015$). There was no significant association between JAk2 expression, and the other clinicopathological features assessed of the patients (Table 3).

Table 2: Diagnostic value of JAK2 and CRLF2 for ALL patients

	Area	Cut–off	Sensitivity	Specificity	SE	p value
JAK2	0.796	0.01	78.1%	81.8%	0.040	<0.001
CRLF	0.958	0.0001	92.4%	90.9%	0.018	<0.001
JAK2 + CRLF	0.957	–	90.9%	91.4%	0.018	<0.001

Table 3: Association between JAK2 expression and clinioc-pathological features of the ALL patients

Patients' characteristics	JAK2 expression		p value
	low expression (52)	over expression (53)	
Age	29 (18–74)	29 (18–61)	0.524
TLC	49 (1–406)	46 (4–194)	0.520
HB	8.1 (4–12.9)	7.6 (4–13.5)	0.148
PLT	51 (4–320)	41 (4–416)	0.349
PB blast%	75 (0.7–97)	19 (0.05–95)	0.006
BM blast%	89.5 (0.9–99)	70 (0.3–98)	0.001
Sex			
Male	30 (57.7%)	35 (66.0%)	0.425
Female	22 (42.3%)	18 (34.0%)	
BM cellularity			
Hypocellular	2 (3.9%)	6 (11.3%)	0.260
Normocelluar	10 (19.6%)	13 (24.5%)	
Hypercellular	39 (76.5%)	34 (64.2%)	
FAB			
L2	48 (92.3%)	49 (92.5%)	0.263
L3	0 (0.0%)	2 (3.8%)	
MPAL	4 (7.7%)	2 (3.8%)	
IPT type			
B ALL	32 (61.5%)	41 (77.4%)	0.207
T ALL	16 (30.8%)	10 (18.9%)	
MPAL	4 (7.7%)	2 (3.8%)	
Cytogenetics			
Normal	27 (54%)	16 (45.7%)	0.241
Abnormal	13 (26%)	15 (42.9%)	
Hypo	3 (6%)	0 (0.0%)	
Hyper	7 (14%)	4 (11.4%)	
Molecular genetics			
Negative	36 (75%)	24 (64.9%)	0.188
t (9;22)	9 (18.8%)	13 (35.1%)	
t (1;19)	2 (4.2%)	0 (0.0%)	
t (4;11)	1 (2.1%)	0 (0.0%)	
CD1			
Negative	44 (84.6%)	50 (94.3%)	0.123
Positive	8 (15.4%)	3 (5.7%)	
CD3			
Negative	34 (65.4%)	44 (83.0%)	0.046
Positive	18 (34.6%)	9 (17.0%)	
CD7			
Negative	31 (59.6%)	45 (84.9%)	0.005
Positive	21 (40.4%)	8 (15.1%)	
TdT			
Negative	43 (82.7%)	50 (94.3%)	0.072
Positive	9 (17.3%)	3 (5.7%)	
CD5			
Negative	38 (73.1%)	45 (84.9%)	0.157
Positive	14 (26.9%)	8 (15.1%)	
CD19			
Negative	19 (36.5%)	14 (26.4%)	0.298
Positive	33 (63.5%)	39 (73.6%)	
CD22			
Negative	26 (50.0%)	20 (37.7%)	0.241
Positive	26 (50.0%)	33 (62.3%)	
CD79a			
Negative	29 (55.8%)	26 (49.1%)	0.560
Positive	23 (44.2%)	27 (50.9%)	
HLADR			
Negative	33 (63.5%)	27 (50.9%)	0.238
Positive	19 (36.5%)	26 (49.1%)	
MHCII			
Negative	50 (96.2%)	42 (79.2%)	0.015
Positive	2 (3.8%)	11 (20.8%)	
CD10			
Negative	24 (46.2%)	18 (34.0%)	0.235
Positive	28 (53.8%)	35 (66.0%)	
Cytom			
Negative	39 (75.0%)	34 (64.2%)	0.290
Positive	13 (25.0%)	19 (35.8%)	
CD34			
Negative	28 (53.8%)	23 (43.4%)	0.331
Positive	24 (46.2%)	30 (56.6%)	
CD13			
Negative	45 (86.5%)	48 (90.6%)	0.555
Positive	7 (13.5%)	5 (9.4%)	
CD33			
Negative	47 (90.4%)	46 (86.8%)	0.761
Positive	5 (9.6%)	7 (13.2%)	
CSF			
Free	42 (97.7%)	48 (96.0%)	1.000
Positive	1 (2.3%)	2 (4.0%)	
Hepto/spleno megaly			
Negative	28 (53.8%)	24 (45.3%)	0.666
Positive	24 (46.2%)	29 (54.7%)	
LN			
Negative	23 (44.2%)	19 (35.8%)	0.510
Positive	29 (55.8%)	34 (64.2%)	
Response to treatment			
CR	13 (37.1%)	18 (54.5%)	0.177
Relapse	20 (57.1%)	15 (45.5%)	
Refractory	2 (5.7%)	0 (0.0%)	

BM: Bone marrow, HB: Haemoglobin, CSF: Cerebrospinal fluid, IPT: Immunophenotyping, PB: Peripheral blood, PLT: Platelets, TLC: Total leukocyte count.

Association between CRLF2 expression and the clinicopathological features of the ALL patients

There was a significant association between CRLF2 low expression and increased both PB blast % and BM blast % at diagnosis (p = 0.006 and 0.002, respectively). In addition, CRLF2 low expression associated significantly with positive expression for CD1, CD3, CD7, TdT, and CD5 (p = 0.008, 0.008, 0.002, <0.001, 0.004, and 0.007, respectively), while CRLF2 overexpression associated significantly with the presence of CD22 and MHC-II (p = 0.031 and p = 0.001; respectively). Forty-two out of 52 (80.8%) CRLF2 over-expressers patients had B-ALL phenotype, while T-ALL phenotype associated significantly with low CRLF2 expression (p = 0.028). The other clinical features of the patients assessed showed no significant association with CRLF2 expression level (Table 4).

Association between combined overexpression of JAK2 and CRLF2 with the clinicopathological features of the ALL patients

Patients with combined overexpression of JAK2 and CRLF2 showed a significant decrease of PB blast % and BM blast % at diagnosis (p = 0.009 and 0.001, respectively). Similarly, they showed a significant decrease in the expression levels of CD1, CD3, CD7, and CD5 (p = 0.007, 0.016, 0.010, and 0.013, respectively). On the other hand, the combined overexpression of JAK2 and CRLF2 associated significantly with increased MHC-II expression (10/13 [76.9%] in patients with JAK2 and CRLF2 overexpression, compared to 3/13 [23.1%] in low expressers patients, p = 0.004, Table 5).

Impact of JAK2 and CRLF2 expressions on patients' survival rates

The present data showed that JAK2, CRLF2, or their combined expression have no significant effect on the OS and DFS rates of the assessed ALL patients (p > 0.05 for all, Figure 3).

Discussion

The role of JAK2 and CRLF2 dysregulation in childhood ALL had been identified clearly, however, their diagnostic, prognostic, and predictive role in adult ALL is still a debatable issue.

In our assessed cohort, JAK2 and CRLF2 were significantly overexpressed in ALL patients compared to the control subjects, which indicated their potential diagnostic value for adult ALL. This diagnostic

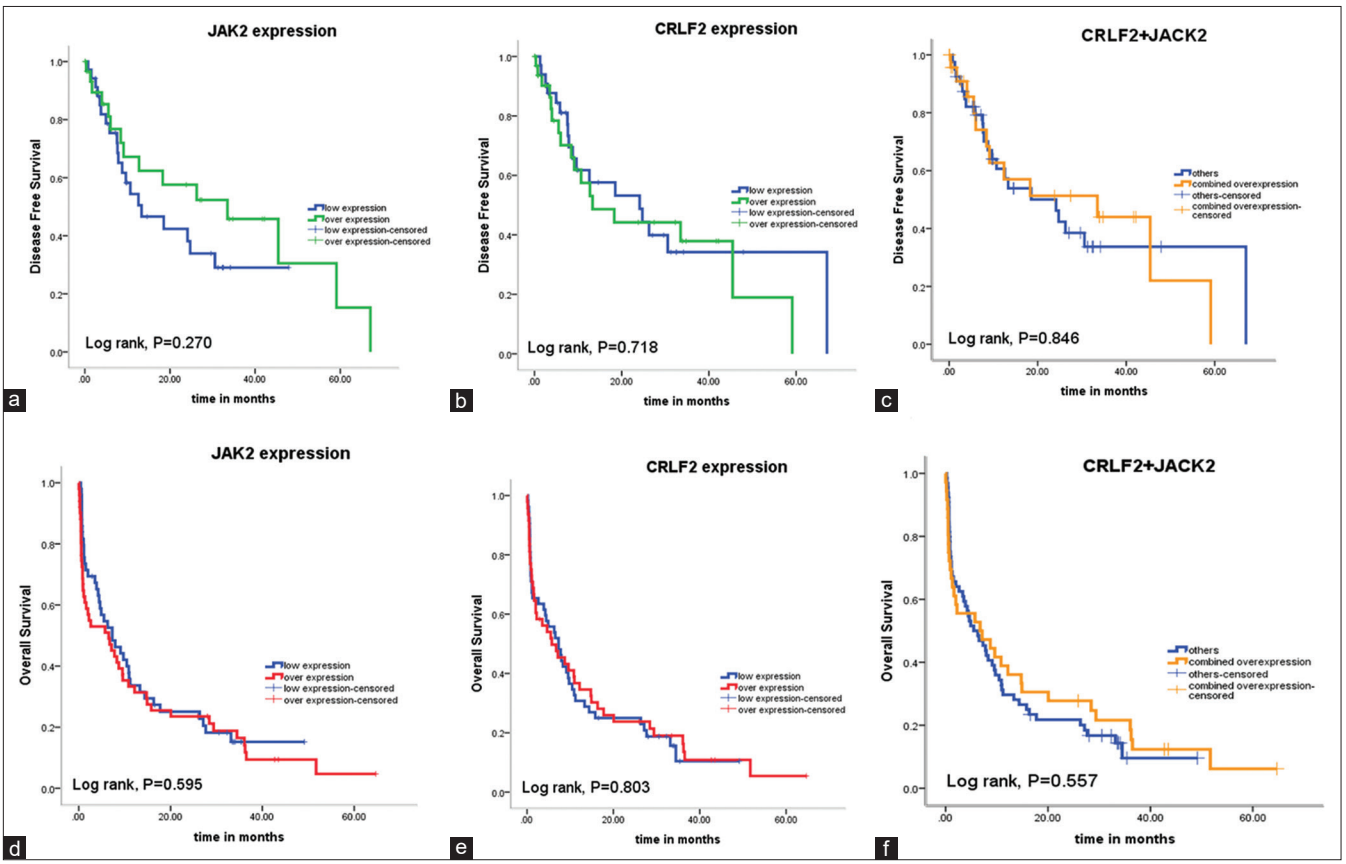

Figure 3: Impact of (a) JAK2, (b) CRLF2, and (c) their combined expression on DFS rates of ALL patients. Impact of (d) JAK2, (e) CRLF2, and (f) their combined expression on OS rates of ALL patients

Table 4: Association between CRLF2 expression and clinic–pathological features of the ALL patients

Patients' characteristics	CRLF expression		p value
	Low expression (53)	Over expression (52)	
Age	28 (18–74)	31 (18–61)	0.177
TLC	47 (1–406)	46 (1–194)	0.856
HB	8.3 (4–13.5)	7.4 (4–12.5)	0.118
PLT	44.5 (5–320)	48 (4–416)	0.595
PB blast%	71.5 (0.1–97)	18 (0.05–96)	0.006
BM blast%	90 (0.3–99)	77 (0.3– 98)	0.002
Sex			
Male	31 (58.5%)	34 (65.4%)	0.548
Female	22 (41.5%)	18 (34.6%)	
BM cellularity			
Hypocellular	2 (3.8%)	6 (11.5%)	0.212
Normocelluar	14 (26.9%)	9 (17.3%)	
Hypercellular	36 (69.2%)	37 (71.2%)	
FAB			
L2	50 (94.3%)	47 (90.4%)	0.353
L3	0 (0.0%)	2 (3.8%)	
MPAL	3 (5.7%)	3 (5.8%)	
IPT type			
B ALL	31 (58.5%)	42 (80.8%)	0.028
T ALL	19 (35.8%)	7 (13.5%)	
MPAL	3 (5.7%)	3 (5.8%)	
Cytogenetics			
Normal	26 (53%)	17 (47.2%)	0.797
Abnormal	14 (28.6%)	14 (38.9%)	
Hypo	2 (4.1%)	1 (2.8%)	
Hyper	7 (14.3%)	4 (11.1%)	
Molecular genetics			
Negative	37 (77.1%)	23 (62.2%)	0.129
t (9;22)	9 (18.8%)	13 (35.1%)	
t (1;19)	2 (4.2%)	0 (0.0%)	
t (4;11)	0 (0.0%)	1 (2.7%)	
CD45			
Negative	24 (46.2%)	20 (43.5%)	0.841
Positive	28 (53.8%)	26 (56.5%)	
CD1			
Negative	43 (81.1%)	51 (98.1%)	0.008
Positive	10 (18.9%)	1 (1.9%)	
CD3			
Negative	32 (60.4%)	46 (88.5%)_	0.002
Positive	21 (39.6%)	6 (11.5%)	
CD7			
Negative	29 (54.7%)	47 (90.4%)	<0.001
Positive	24 (45.3%)	5 (9.6%)	
TdT			
Negative	42 (79.2%)	51 (98.1%)	0.004
Positive	11 (20.8%)	1 (1.9%)	
CD5			
Negative	36 (67.9%)	47 (90.4%)	0.007
Positive	17 (32.1%)	5 (9.6%)	
CD19			
Negative	21 (39.6%)	12 (23.1%)	0.093
Positive	32 (60.4%)	40 (76.9%)	
CD22			
Negative	29 (54.7%)	17 (32.7%)	0.031
Positive	24 (45.3%)	35 (67.3%)	
CD79a			
Negative	32 (60.4%)	23 (44.2%)	0.120
Positive	21 (39.6%)	29 (55.8%)	
HLADR			
Negative	32 (60.4%)	28 (53.8%)	0.557
Positive	21 (39.6%)	24 (46.2%)	
MHCII			
Negative	52 (98.1%)	40 (76.9%)	0.001
Positive	1 (1.9%)	12 (23.1%)	
CD10			
Negative	25 (47.2%)	17 (32.7%)	0.164
Positive	28 (52.8%)	35 (67.3%)	
cytom			
Negative	40 (75.5%)	33 (63.5%)	0.208
Positive	13 (24.5%)	19 (36.5%)	
CD34			
Negative	31 (58.5%)	20 (38.5%)	0.051
Positive	22 (41.5%)	32 (61.5%)	
CD13			
Negative	47 (88.7%)	46 (88.5%)	1.000
Positive	6 (11.3%)	6 (11.5%)	
CD33			
Negative	49 (92.5%)	44 (84.6%)	0.236
Positive	4 (7.5%)	8 (15.4%)	
CSF			
Free	44 (95.7%)	46 (97.9%)	0.617
Positive	2 (4.3%)	1 (2.1%)	
Hepto/spleno megaly			
Negative	29 (54.7%)	23 (44.2%)	0.518
Positive	24 (45.3%)	29 (55.8%)	
LN			
Negative	24 (45.3%)	18 (34.6%)	0.277
Positive	29 (54.7%)	34 (65.4%)	
Response to treatment			
CR	16 (47.1%)	15 (44.1%)	0.357
Relapse	18 (52.9%)	17 (50.0%)	
Refractory	0 (0.0%)	2 (5.9%)	

(Contd...)

Table 4: (*Continued*)

BM: Bone marrow, HB: Haemoglobin, CSF: Cerebrospinal fluid, IPT: Immunophenotyping, PB: Peripheral blood, PLT: Platelets, TLC: Total leukocyte count.

role was confirmed by the ROC analysis that revealed the significant ability of both JAK2 (sensitivity 78.1% and specificity 81.8%) and CRLF2 (sensitivity 92.4% and specificity 90.9%) to identify patients with ALL. While when combining both JAK2 and CRLF2 for the diagnosis of ALL, it revealed 90.0% sensitivity, 91.4% specificity, and AUC of 0.957. These data demonstrated that JAK2 and CRLF2 could be a potential diagnostic molecular marker for ALL patients, which allow them to be a successful target for ALL therapy.

The present data showed also a significant intermediate correlation between CRLF2 overexpression and JAK2 dysregulation in ALL patients. These results are in agreement with Chiaretti *et al*. [12] and Konoplev *et al*. [21] who reported a significant association between CRLF2 overexpressing and JAK2 mutation in adult B-ALL. Many other reports demonstrated that both JAK2 mutations and CRLF2 overexpression display a transforming activity in hematological diseases, especially in pediatric ALL, which results in JAK/STAT pathway activation [22], [23], [24], [25], [26]. Moreover, Tasian *et al*. [27] reported that all JAK-mutated ALL patients overexpress CRLF2 through the P2RY8-CRLF2 fusion or IGH-CRLF2 translocation, which indicating the cooperative nature of these genetic events in leukemogenesis [15], [28].

The present study demonstrated that patients with B-ALL phenotype showed a significant association with CRLF2 overexpression, while T-ALL phenotype is more common in patients with CRLF2 low expression. These data are concordant to that observed by Yoda *et al*. [10] who found significant overexpression of CRLF2 in B-ALL, while low or undetectable CRLF2 expression in T-ALL phenotype. Similarly, many recent studies reported CRLF2 overexpression in B-ALL [11], [12], [21], which explained the important role of CRLF2 in the development, proliferation, and survival of normal B lymphocytes [29]. This finding is supported by the significant association between low expression of CRLF2 or its combination with JAK2 and the positive expression of the T-lineage markers (CD1, CD3, CD7, and CD5), while CRLF2 overexpression associated significantly with the presence of the B-lineage markers (CD22).

The other clinical features of the patients assessed including leukocytosis and thrombocytopenia had no significant association with CRLF2 expression level**.** In line with our results, Yoda *et al*. [10] proposed no significant association between CRLF2 expression

Table 5: Association between combined overexpression of JAK2 and CRLF2 with the clinico–pathological features of the ALL patients

Patients' characteristics	combined overexpression JAK2+CRLF2	Other groups	p value
Age	31 (18–61)	29 (18–74)	0.373
TLC	46.5 (1–194)	45 (1–406)	0.816
HB	7.6 (4–12.5)	8 (4–13.5)	0.423
PLT	40.5 (4–416)	50 (4–320)	0.356
PB blast%	15 (0.05–95)	67 (0.1–97)	0.009
BM blast%	46.5 (0.3–98)	89 (0.3–99)	0.001
Sex			
Male	27 (71.1%)	38 (57.6%)	0.210
Female	11 (28.9%)	28 (42.4%)	
BM cellularity			
Hypocellular	6 (15.8%)	2 (3.1%)	0.062
Normocelluar	7 (18.4%)	16 (24.6%)	
Hypercellular	25 (65.8%)	47 (72.3%)	
FAB			
L2	34 (89.5%)	62 (93.9%)	0.169
L3	2 (5.3%)	0 (0.0%)	
MPAL	2 (5.3%)	4 (6.1%)	
IPT type			
B ALL	31 (81.6%)	42 (63.6%)	0.130
T ALL	5 (13.2%)	20 (30.3%)	
MPAL	2 (5.3%)	4 (6.1%)	
CD45			
Negative	17 (50.0%)	27 (42.9%)	0.528
Positive	17 (50.0%)	36 (57.1%)	
CD1			
Negative	38 (100.0%)	55 (83.3%)	0.007
Positive	0 (0.0%)	11 (16.7%)	
CD3			
Negative	34 (89.5%)	43 (65.2%)	0.010
Positive	4 (10.5%)	23 (34.8%)	
CD7			
Negative	35 (92.1%)	41 (62.1%)	0.001
Positive	3 (7.9%)	25 (37.9%)	
TdT			
Negative	37 (97.4%)	55 (83.3%)	0.052
Positive	1 (2.6%)	11 (16.7%)	
CD5			
Negative	35 (92.1%)	47 (71.2%)	0.013
Positive	3 (7.9%)	19 (28.8%)	
CD19			
Negative	9 (23.7%)	23 (34.8%)	0.275
Positive	29 (76.3%)	43 (65.2%)	
CD22			
Negative	12 (31.6%)	33 (50.0%)	0.100
Positive	26 (68.4%)	33 (50.0%)	
CD79a			
Negative	18 (47.4%)	36 (54.5%)	0.544
Positive	20 (52.6%)	30 (45.5%)	
HLADR			
Negative	21 (55.3%)	38 (57.6%)	0.840
Positive	17 (44.7%)	28 (42.4%)	
MHCII			
Negative	28 (73.7%)	63 (95.5%)	0.004
Positive	10 (26.3%)	3 (4.5%)	
CD10			
Negative	13 (34.2%)	29 (43.9%)	0.408
Positive	25 (65.8%)	37 (56.1%)	
Cytom			
Negative	23 (60.5%)	49 (74.2%)	0.186
Positive	15 (39.5%)	17 (25.8%)	
CD34			
Negative	15 (39.5%)	35 (53.0%)	0.223
Positive	23 (60.5%)	31 (47.0%)	
CD13			
Negative	33 (86.8%)	59 (89.4%)	0.755
Positive	5 (13.2%)	7 (10.6%)	
CD33			
Negative	33 (86.8%)	59 (89.4%)	0.755
Positive	5 (13.2%)	7 (10.6%)	
Molecular Genetics			
Negative	15 (60.0%)	44 (74.6%)	0.222
t (9;22)	10 (40.0%)	12 (20.3%)	
t (1;19)	0 (0.0%)	2 (3.4%)	
t (4;11)	0 (0.0%)	1 (1.7%)	
Cytogenetics			
Normal	12 (48%)	31 (52.5%)	0.623
Abnormal	10 (40%)	18 (30.5%)	
Hypo	0 (0.0%)	2 (3.4%)	
Hyper	3 (12.0%)	8 (13.6%)	
CSF			
Free	35 (97.2%)	55 (96.5%)	0.846
Positive	1 (2.8%)	2 (3.5%)	
Organomegaly			
Negative	18 (47.4%)	34 (49.3%)	0.824
Positive	20 (52.6%)	33 (50.7%)	
LN			
Negative	11 (28.9%)	31 (46.3%)	0.114
Positive	27 (71.1%)	36 (53.7%)	
Response to treatment			
CR	13 (52.0%)	18 (42.9%)	0.465
Relapse	12 (48.0%)	22 (52.4%)	
Refractory	0 (0.0%)	2 (4.8%)	
Death			
No	4 (10.8%)	9 (14.1%)	0.764
Yes	33 (89.2%)	55 (85.9%)	

BM: Bone marrow, CSF: Cerebrospinal fluid, HB: Haemoglobin: IPT: Immunophenotyping, PB: Peripheral blood, PLT: Platelets, TLC: Total leukocyte count.

and patients' age, gender, and white blood cell count. However, Chiaretti *et al*. [12] found leukocytosis and thrombocytopenia in ALL patients who had CRLF2 overexpression.

Regarding the survival rates, the present data showed that JAK2, CRLF2, or their combined expression have no significant effect on the overall and disease-free survival rates of the assessed ALL patients. In agreement with our results, many studies concluded that CRLF2 was not relevant to the pediatric ALL patients' outcome in the context of OS and DFS rates [30], [31], [32], [33]. However, other studies reported a significant association between CRLF2 overexpression and shorter DFS as well as OS rates in either adult ALL [10], [12], [34] or pediatric ALL patients [23], [35]. This could be explained by many reasons, first; the different methods used for its detection, either by rearrangement or by quantitative expression levels [35], [36], [37]. Second; many of the previously mentioned studies included only B-ALL or T-ALL patients in their studies, not both cell lineages of ALL as in our cohort. In addition, many studies excluded the recurrent translocations of B-ALL while detecting the prognostic impact of CRLF2 quantitation in B-ALL patients [12], [31]. Consequently, according to the recent review done by Moorman *et al*., further work is progressively required to determine the true frequency of CRLF2 and JAK2 dysregulation among the different age groups of ALL patients, to achieve better management and prolonged survival of the patients [38].

Of interest, the present data revealed a significant association between the low expression of CRLF2, JAK2, or their combination and increased both PB blast % and BM blast % at diagnosis. These data could be explained by the recent results published by Gu *et al*. [29] who found six types of genetic alterations in CRLF2 among adult ALL patients. These genetic alterations included the R186S type which prompted a better prognosis, while L86I, F232F, and W255C mutations associated with poor prognosis.

Conclusion

The present study provides evidence that both CRLF2 and JAK2 could be considered useful diagnostic markers for adult ALL, however, their impact on patients' response to treatment and survival rates

as well as their clinical outcome could not be well identified in our cohort of the patients, though JAK2 and CRLF2 overexpression associated significantly with the increased expression of MHC-II, which is a marker of a poor clinical outcome [39], [40]. Therefore, the exact role of CRLF2 as a risk factor for patients' outcome is still not clear, especially in adult ALL. Hence, further studies are required to precisely detect the expression levels as well as the type of CRLF2 mutations in adult ALL, and the possibility of incorporating their targeted therapy into the treatment protocols.

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Ethics Statement and Consent to Participate

The study protocol was approved by the Ethical Committee of the National Cancer Institute, Cairo University, according to 2011 Declaration of Helsinki. An informed written consent was obtained from the participants or their relatives before enrolment in the study.

Authors' Contributions

NMH put the idea of the work and revised the final manuscript; MSE shared in the laboratory and the molecular work of the patients; MSA analyzed the data and writing the manuscript; and RM performed the gene expression work.

Availability of Data and Material

All data and materials are available on request.

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