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

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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 clinicopathological 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 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 90.0% 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 day 14 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/m² 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^ΔΔCT 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 translocations were identified in 25/85 (29.4%) cases. Thirty-five patients (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.

**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 0.0011 (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](https://oamjms.eu/index.php/mjms/index)
Assessment of the role of CRLF2 and JAK2 in ALL

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 clinico-pathological 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 clinico-pathological features assessed of the patients (Table 3).

Table 2: Association between JAK2 expression and clinico-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 (1–499) | 46 (4–194) | 0.520 |
| HB                      | 8.1 (4–12.9) | 7.6 (4–13.5) | 0.148 |
| PLT                     | 51 (4–320) | 41 (4–146) | 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 (57.5%) | 36 (60%) | 0.425 |
| Female                  | 22 (42.5%) | 18 (39.5%) |          |
| BM cellularity          |                |          |
| Hypocellular            | 2 (3.9%) | 6 (11.3%) | 0.260 |
| Normal                  | 10 (19.6%) | 13 (24.5%) |          |
| Hypercellular           | 39 (76.9%) | 34 (64.2%) |          |
| FAB                     |                |          |
| L2                      | 48 (92.3%) | 49 (92.5%) | 0.263 |
| L3                      | 0 (0%) | 2 (4.0%) |          |
| 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%) |          |
| Abnormal                | 13 (26%) | 15 (42.9%) |          |
| Hypo                    | 3 (6%) | 0 (0%) |          |
| Hyper                   | 7 (14%) | 4 (11.4%) |          |
| Molecular genetics      |                |          |
| Negative                | 36 (75%) | 24 (64.9%) | 0.188 |
| Positive                | 19 (35.5%) | 16 (45.7%) |          |
| t (9;22)                | 9 (18.8%) | 13 (31.5%) |          |
| t (1;19)                | 2 (4.0%) | 0 (0%) |          |
| t (4;11)                | 1 (2.1%) | 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%) |          |
| CD10                    |                |          |
| Negative                | 43 (82.7%) | 50 (94.3%) | 0.072 |
| Positive                | 9 (17.3%) | 3 (5.7%) |          |
| CD19                    |                |          |
| Negative                | 38 (73.1%) | 45 (84.9%) | 0.157 |
| Positive                | 14 (26.9%) | 8 (15.1%) |          |
| CD22                    |                |          |
| Negative                | 19 (36.5%) | 14 (26.4%) | 0.298 |
| Positive                | 33 (63.5%) | 39 (73.6%) |          |
| CD78a                   |                |          |
| Negative                | 26 (50.0%) | 20 (37.7%) | 0.241 |
| Positive                | 26 (50.0%) | 33 (62.3%) |          |
| HLA DR                  |                |          |
| Negative                | 29 (55.8%) | 26 (49.1%) | 0.560 |
| Positive                | 23 (44.2%) | 27 (50.9%) |          |
| MHCI1I                  |                |          |
| Negative                | 33 (63.5%) | 27 (50.9%) | 0.238 |
| Positive                | 19 (36.5%) | 26 (49.1%) |          |
| CD10                    |                |          |
| Negative                | 50 (96.2%) | 42 (79.2%) | 0.001 |
| Positive                | 2 (3.8%) | 11 (20.8%) |          |
| CD11                    |                |          |
| Negative                | 24 (44.2%) | 18 (34.0%) | 0.235 |
| Positive                | 28 (55.8%) | 35 (66.0%) |          |
| CD12                    |                |          |
| Negative                | 39 (75.5%) | 34 (64.2%) | 0.290 |
| Positive                | 13 (24.5%) | 19 (35.8%) |          |
| CD13                    |                |          |
| Negative                | 28 (53.8%) | 23 (44.2%) | 0.331 |
| Positive                | 24 (46.2%) | 30 (56.0%) |          |
| CD14                    |                |          |
| Negative                | 45 (85.5%) | 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%) |          |
| Hepatospleno megal        |                |          |
| 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%) | 9 (26.3%) |          |

BM: Bone marrow; HB: Haemoglobin; CSF: Cerebrospinal fluid; IPT: Immunophenotyping; PB: Peripheral blood; PLT: Platelets; TLC: Total leucocyte 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 overexpressers 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

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**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 | CRLF2 expression | p value |
|---------------------------|------------------|---------|
|                           | Low expression (53) | Over expression (52) |
| Age                       | 28 (18–74)       | 31 (18–61)       | 0.177 |
| TCL                       | 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 |
|                           | Normocellular    | 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%)   |
|                           | PT type          |                   |           |
|                           | B-ALL            | 31 (58.5%)       | 42 (80.8%) | 0.028 |
|                           | T-ALL            | 19 (35.8%)       | 7 (13.5%)  |
|                           | BM               | 3 (5.7%)         | 3 (5.8%)   |
| Cytogenetics              | Normal           | 26 (53.3%)       | 17 (47.2%) | 0.797 |
|                           | Abnormal         | 14 (26.9%)       | 14 (38.9%) |
|                           | Hypo             | 2 (4.1%)         | 1 (2.8%)   |
|                           | Hyper            | 7 (14.3%)        | 4 (11.1%)  |
| Molecular genetics        | Negative         | 37 (77.1%)       | 23 (42.2%) | 0.129 |
|                           | Positive         | 16 (32.9%)       | 27 (57.8%) |
| CD1                       | Negative         | 43 (81.1%)       | 51 (98.1%) | 0.008 |
|                           | Positive         | 10 (18.9%)       | 1 (2.5%)   |
| CD3                       | Negative         | 32 (60.4%)       | 46 (88.5%) | 0.002 |
|                           | Positive         | 21 (39.6%)       | 6 (11.5%)  |
| CD7                       | Negative         | 20 (54.7%)       | 47 (90.4%) | <0.001 |
|                           | Positive         | 24 (45.3%)       | 5 (9.6%)   |
| TCR                       | Negative         | 42 (79.2%)       | 51 (98.1%) | 0.004 |
|                           | Positive         | 11 (20.8%)       | 1 (2.5%)   |
| 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 (88.1%)       | 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%) |
| CD12                      | 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         | 4 (7.5%)         | 8 (15.4%)  |
| CSF                       | Free             | 44 (89.5%)       | 46 (97.9%) | 0.617 |
|                           | Positive         | 2 (4.3%)         | 1 (2.1%)   |
| Hleptop/pleno megaly      | Negative         | 29 (54.7%)       | 23 (44.2%) | 0.518 |
|                           | Positive         | 24 (45.3%)       | 29 (55.8%) |
| AML                       | Negative         | 24 (45.3%)       | 18 (34.6%) | 0.277 |
|                           | Positive         | 29 (54.7%)       | 34 (65.4%) | 0.006 |

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

| Patients’ characteristics | combined overexpression | Other groups | p value |
|--------------------------|-------------------------|-------------|---------|
| Age                      |                         | 31 (18.4%)  | 28 (16.7%) | 0.374 |
| TLC                      | 46.5 (1–194)            | 45 (1–196) | 0.816 |
| HB                       | 7.6 (4–12.5)            | 8 (6–13.5) | 0.423 |
| PLT                      | 40.5 (4–416)            | 50 (4–220) | 0.356 |
| PB blast%                | 15.0 (0.05–95)          | 67 (0–97)  | 0.009 |
| BM blast%                | 46.5 (0.3–98)           | 89 (0.3–99) | 0.001 |
| Sex                      |                         |             |         |
| Male                     | 27 (71.1%)              | 38 (75.5%) | 0.210 |
| Female                   | 11 (28.9%)              | 12 (24.5%) |         |
| BM cellularity           |                         |             |         |
| Hypocellular             | 6 (15.8%)               | 2 (5.1%)   | 0.062 |
| Normocellular            | 7 (18.4%)               | 16 (24.6%) |         |
| Hypercellular            | 25 (65.8%)              | 47 (72.3%) |         |
| FAB                      |                         |             |         |
| L1                      | 34 (89.5%)              | 62 (93.9%) | 0.169 |
| L3                      | 2 (5.3%)                | 0 (0.0%)   |         |
| 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                 | 1 (0.5%)                | 24 (34.8%) |         |
| CD7                      |                         |             |         |
| Negative                 | 35 (92.1%)              | 41 (62.1%) | 0.001 |
| Positive                 | 3 (7.9%)                | 25 (37.9%) |         |
| TdT                      |                         |             |         |
| Negative                 | 37 (94.7%)              | 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                 | 20 (51.3%)              | 36 (51.2%) |         |
| CD22                     |                         |             |         |
| Negative                 | 12 (31.6%)              | 33 (50.0%) | 0.100 |
| Positive                 | 8 (21.6%)               | 33 (50.0%) |         |
| CD77a                    |                         |             |         |
| 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%) |         |
| MNCHII                   |                         |             |         |
| Negative                 | 28 (73.7%)              | 63 (95.5%) | 0.004 |
| Positive                 | 10 (26.3%)              | 4 (4.5%)   |         |
| CD10                     |                         |             |         |
| Negative                 | 13 (34.2%)              | 29 (43.9%) | 0.408 |
| Positive                 | 25 (65.8%)              | 37 (56.1%) |         |
| Cytoe                   |                         |             |         |
| 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%) |         |
| CD15                     |                         |             |         |
| Negative                 | 33 (86.8%)              | 59 (89.4%) | 0.755 |
| Positive                 | 5 (13.2%)               | 7 (10.6%)  |         |
| Molecular Genetics       |                         |             |         |
| Negative                 | 15 (60.0%)              | 44 (74.8%) | 0.222 |
| 1 (0.22)                 | 10 (40.0%)              | 12 (22.0%) |         |
| t (11:19)                | 0 (0.0%)                | 2 (3.4%)   |         |
| t (1:11)                 | 0 (0.0%)                | 1 (1.7%)   |         |
| Cyogenetics              |                         |             |         |
| 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%)   |         |
| Organogenegy             |                         |             |         |
| 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%) |         |

Table 5: (Continued)

| Response to treatment | combined overexpression | Other groups | p value |
|-----------------------|--------------------------|-------------|---------|
| 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 (92.2%)               | 55 (85.9%)  |         |

BM: Bone marrow, CSF: Cerebrospinal fluid, IPT: Immunophenotyping, PB: Peripheral blood, PLT: Platelets, TLC: Total leucocyte 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 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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ΔΔ . CRLF2-positive B-cell acute lymphoblastic leukemia in Long-term results of St Jude total Aberrant STAT5 and PI3K/mTOR pathway outcomes of children with BCR-ABL1-like Deregulated expression of cytokine Treatment outcome of CRLF2-rearranged JAK mutations in high-risk Independent prognostic Mutations of JAK2 in acute lymphoblastic Genetic alterations activating kinase and cytokine.

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