Role of interleukin-8 as a molecular indicator in the pathogenesis of T-cell acute lymphoblastic leukemia (T-ALL)

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Research Article

Keywords:

Posted Date: March 15th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1426745/v1

Abstract

Inflammation plays a critical role in the pathogenesis of a large number of diseases, such as atherosclerosis myocardial infarction (MI), sepsis, rheumatoid arthritis, and cancer. The present study aimed to investigate the association of IL-8 in T-ALL patients. IL-8 levels have been estimated in 52 individuals, out of which 26 were healthy and disease-free volunteers. IL-8 was found to be relatively higher in the T-ALL patients than in the healthy volunteers. Here, the results revealed that IL-8 is positively correlated with T-ALL patients at the genomic and proteomic levels. Elevated serum IL-8 levels were related to the highly advanced disease stage of the clinicopathological parameters. Our results indicate that monitoring IL-8 has a role in modulating disease sensing in T-ALL and may hold the key for targeting innovative diagnostic and advanced therapeutic strategies.

Introduction

T-cell acute lymphoblastic leukemia (T-ALL) is a blood-related aggressive malignancy that represents ~ 25% of adults and ~ 15% of the childhood population. It arises from the clonal proliferation of bone-marrow-derived circulating T lymphocytes that are home to the thymus. It mainly affects children and young adults and is associated with poor outcomes of the disease. Although in general T-ALL cells remain confined to the thymus, in most cases, leukemia cells can migrate to the bone marrow [1]. The expression of IL-8 has been found to be significantly higher in a wide range of cancers than in normal tissues [2]. Consistent with this, a high concentration of IL-8 has been identified in the serum of cancer patients and correlated with the progressive size of the tumor, stages, and prognosis [3–6]. It has been recently found that the gene that codes for interleukin-8 (CXCL8) is highly expressed in chemorefractory leukemia cells (T-ALL) and has also been proposed to play several functions in T-ALL cells [7]. Interleukin-8 (CXCL8) is a polypeptide cytokine with a molecular mass of KDa and is a specified chemoattractant for neutrophils. There is the possibility that it plays an important role in the inflammation associated with a vast range of illnesses, such as myocardial injury, shock, pneumonia, sepsis, rheumatoid arthritis, and respiratory distress syndrome (RDS), in newborns [8]. Some previous reports revealed that IL-8 is not produced constitutively but is secreted only in response to specific stimuli, such as lipopolysaccharide stimulation of human monocyte-macrophage cells, endothelial cells, TNF, and IL-1 stimulation of fibroblasts, epithelial cells, synovial cells, and mesothelial cells [7–11]. However, Brennan F et al. showed that IL-8 is constitutively produced in the synovial cells of rheumatoid arthritis patients [12] but human B and T-cells have also not been reported to produce IL-8. Kaashoek J and Yoshida M found the constitutive production of IL-8 in the human bladder carcinoma cell line 5637 [13] and thyroid carcinoma cell line KHM-5M [14] respectively. The present research aimed to investigate the constitutive production of IL-8 by leukemia (T-ALL) cells.

Methods

Patient samples

Human T-ALL samples (N = 26) and healthy control samples (N = 26) were obtained from the Department of Pathology and Biochemistry at King George's Medical University after informed consent was obtained from all the patients enrolled in the study. The clinical diagnosis of T-ALL was based on the patient's presentation, morphology on a peripheral blood smear, and bone marrow by using Leishman staining. Further immunophenotyping of blast cells was performed by flow cytometry by taking markers such as CD34, CD33, CD14, CD20, CD10, CD19, HLA-DR, TdT, CD2, CD3, CD5, CD7, CD13, CD19, CD20, CD23, CD45, CD64, CD79a, CD117, and CD 200. The characteristic features of the patients are presented in Table I. Most of the experiments were performed immediately after taking samples in the Department of Biochemistry.

The written informed consent form (ICF) of all the patients was taken before peripheral blood collection. Serum samples from all the blood samples were separated by centrifugation and stored at − 80°C until further processing and analysis. Serum IL-8 levels were determined by enzyme immunoassay (EIA) using commercially available kits from Abcam, according to the manufacturer's instructions and protocol. The detailed characteristics of T-ALL patients, including clinical and histopathological reports, were collected from case files maintained at the pathology department, King George's Medical University Lucknow.

Peripheral blood smear and bone marrow staining

Leishman dye was prepared in methanol (0.2 g of Leishman powder and dissolved in 100 mL of methanol). After preparing blood films, they were allowed to air dry. The slides were flooded with Leishman stain for 2 minutes and washed in a stream of buffered water for 2 minutes to acquire a pinkish tinge. In cases of suspicion of leukemia, blood films made from buffy coat preparations were stained with Leishman's stain.

Blast cell conformation by flow cytometry

The combinations of monoclonal antibodies used were labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), pyridinyl chlorophyllin (PerCP), and phycoerythrin-Cy7 (PE-Cy7). Anti-CD45 V500-A, anti-CD34 PercP-A, anti-CD33 APC-A, anti-CD79a, APC-A, anti-CD13-PE-A, anti-CD19-PE, anti-CD25-FITC-A, anti-CD20-V450-A, anti-CD7-FITC-A, anti-CD10-PE, anti-CD19-PE, anti-CD20-V450-A, anti-CD14-APC, anti-CD20-V450-A, anti-CD14-APC, anti-CD-64 FITC-A, anti-CD117-PE, human leukocyte antigen (HLA)-DR-APC, anti-CD14-APC, and many more antibodies were procured from BD Biosciences (San Jose, CA 95131, California, United States). We followed the stain lyse wash method; the appropriate number of FACS tubes was labeled for the name of the patient and the combination of fluorochrome-conjugated monoclonal antibodies. In each tube, 100 µL of the sample (whole blood) was pipetted into the tube and poured with 20 µL of antibody/antibody cocktail in the respective tubes, which was incubated in the dark for 10–15 minutes. After incubation, 2 mL of diluted FACS lysis solution was added to each tube. The samples were centrifuged at 200–300 g for 3–5 minutes. The supernatant was discarded, the pellet formed at the bottom of the tube was broken, and the remaining cells were washed 2–3 times with shealth fluid. The cells were resuspended again in approximately 0.5 mL of shealth fluid and run on a precalibrated flow cytometer. Data acquisition was performed by using FACSCanto (BD Biosciences).

RT-PCR

Total mRNA from blood cells was isolated by following the TRIzol method. The concentration of mRNA and its structural integrity were confirmed by using a Nanodrop spectrophotometer from Thermo Scientific (2000 UV–Vis). Only RNA with ratios from 1.9- 2.0 of absorbance at 260/280 nm was used. According to the protocol, the isolated mRNA was reverse-transcribed using the High-Capacity cDNA Reverse Transcription Kit (4368814). Quantitative reverse transcription-PCR (qRT-PCR) was performed by using PowerUp SYBR green master mix (ABI- A25741) on a 7500 Fast Real-Time PCR system (Applied Biosystem, Thermo Scientific). Quantification was performed with the ΔΔ Ct method with β-actin serving as a reference gene, and the RT-PCR results were analyzed by DataAssist software (Thermo Scientific). The oligonucleotide primers used in the study are listed in Table S1. All primers were analyzed with positive controls by executing melting profiles following qRT-PCR, and the sizes of products were checked by gel electrophoresis (2.2% agarose). The conditions for performing PCR were as follows: 40 cycles of 15 seconds at 95°C, 15 seconds at annealing temperature (60°C for all other genes), and 15 seconds at 72°C. Exemplars were investigated in duplicate for a minimum of three sets of individualistic experiments as indicated.

Western blotting

Blood was harvested, the proteins were isolated by RIPA lysis buffer, and the protein concentration was measured by the BCA method (bicinchoninic acid assay) with a spectrophotometer from Thermo Scientific at 562 nm. Proteins were separated by 15% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto PVDF membranes (Bio-Rad). Protein was incubated overnight at 4°C with primary antibodies to form immune complexes. Blots were washed 2–3 times properly and incubated with HRP-conjugated secondary antibodies for one hour. Bands of the immunoreactive protein were visualized and identified with the help of a scanning system machine named Odyssey LI-COR.

Statistical analysis

All the data were analyzed by using GraphPad Prism-9, SPSS 16.0 version, from which we performed Student's t-test and analysis of the ROC curve (Metaboanalyst version 5.0). All comparisons were made relative to healthy controls (noncancerous), and significance of the difference is indicated as *$p < 0.01$, **$p < 0.001$, ***$p < 0.0001$, ****$p < 0.00001$. All the presented quantitative data are presented as the mean ± SD from a minimum of 3 samples per data point.

Results

Clinical diagnosis of human chronic myeloid leukemia (CML)

In the present study, we included the peripheral blood of T-ALL patients. The morphology of blast crisis cells from peripheral blood smears, aspirates from the smears of bone marrow, and bone marrow trephine biopsy specimens, along with immunophenotypic findings and flow cytometry, are shown in

Fig. 1 (a, b), which shows that blast cells were stained with Leishman dye. Furthermore, flow cytometry results helped identify phenotypes with different biomarkers, such as CD34, CD33, CD14, CD20, CD10, CD19, HLA-DR, TdT, CD2, CD3, CD5, CD7, CD13, CD19, CD20, CD23, CD45, CD64, CD79a, CD117, and CD 200. Blast cells were gated on CD45 vs side scatter. The expression of myeloid markers (CD13, CD33, CD117, CD14, CD64, cMPO), B-lymphoid markers (CD19, CD20, CD79a, CD10), T-lymphoid markers (CD3, cytoplasmic CD3, CD2, CD5, CD7) and immaturity markers (CD34, TdT, HLA-DR) was studied. We performed flow cytometry analysis of different patients, as shown in Fig. 1 (c-e). The results are summarized in Table I, and the extension is shown in Table S3. Ninety percent of blast cells gated on dim CD45 and extended to the monocytic region on CD45 versus the side scatter plot, as shown in Fig. 1 (g). A reliable number of CD45 events (20,000) were measured, and we found that 95% of patients had approximately 15,000 blast cells in 20000 events. Flow cytometry data confirmed the presence of blast cells in CML patients.

Molecular Study

The expression levels of serum IL-8 were elevated in all patients (19 ± 2.3, $p < 0.0001$) with T-ALL compared to healthy individuals (4.8 ± 2.2). IL-8 levels at the genomic and proteomic levels in T-ALL (RQ 7.8 ± 1.2 $p < 0.0001$) patients were higher in different histopathological subgroups than in healthy individuals (1.542 ± 03342). Analysis of the ROC curve (Fig. 2) indicated that both IL-8 showed good discriminative efficiency among the healthy volunteers and the total patients with leukemia/T-ALL (IL-8: AUC-0.93553, $p = 5.0815e\text{-}9$). Moreover, the ROC curves suggested that IL-8 possesses good sensitivity and specificity to differentiate healthy volunteers from T-ALL patients.

Discussion

The presented results showed the substantial constitutive secretion of IL-8 by T-ALL cells. Previous studies have reported that IL-8 production occurs mostly through a malignant origin, which may contribute to IL-8-mediated processes such as the chronic inflammatory disease state. Some literature has explored whether IL-8 expression in T-ALL cells is regulated by the bone marrow microenvironment. The bone marrow microenvironment has emerged as a critical player in cancer biology. However, most isolated parts of the bone marrow have been investigated in the case of acute myeloid leukemia (AML), e.g., either mesenchymal stromal cells [15, 16] or bone marrow hypoxia [17, 18] and their consequences on blasts of AML cells. CXCL8 is a well-identified proinflammatory cytokine and a strong chemoattractant for neutrophils [19]. In addition to physiological function, a few environmental stresses, such as acidosis, hypoxia, chemotherapy, etc. have also been attributed to the induction of IL-8 in tumor tissue [20]. In addition, constitutively elevated levels of IL-8 have been observed in a vast range of cancers, such as prostate cancer [21], colorectal cancer [22], and non-small cell lung cancer (NSCLC) [23].

Conclusion And Future Perspectives

Our results are consistent with previous reports demonstrating that the level of IL-8 was relatively positive in the ill stage and that the increased IL-8 was primarily correlated with the advancement of the T-ALL condition. Thus, IL-8 is considered to have a role in the pathogenesis of T-ALL, and quantification of IL-8 levels in leukemia conditions might be more useful and feasible in the clinical setting for the prediction of drug responses, where it may represent a presumptive target for innovative diagnostic and effective therapeutic approaches. However, further research explorations are needed, including a greater number of patients with T-ALL, and estimating the IL-8 levels in leukemia patients may hold the key for the additional predictive values on the recurrence of the disease and its prognosis.

Declarations

Ethics Approval

The ethics committee of King George's Medical University, Lucknow, with reference code 96th ECM II A/P22, approved this study. Written informed consent and diagnosis forms were collected from the Department of Pathology, King George's Medical University, Lucknow.

Competing Interest

The authors have declared that they have no competing interests.

Authors' Contributions

Vivek Singh and Ranjana Singh designed the research, performed the experiments, and acquired and analyzed the data; Rashmi Kushwaha, Shailendra Prasad Verma and Anil Kumar Tripathi helped in clinical diagnosis; Sandeep Pandey and Nimra Habib also helped in acquiring the literature; Vivek Singh and Ranjana Singh drafted the article; Ranjana Singh and Abbas Ali Mahdi revised the manuscript; Vivek Singh and Ranjana Singh performed the statistical analysis and conceived the grants.

Acknowledgements

The work was supported by Department of Biotechnology (BT/IN/Indo-US/Foldscope/39/2015) and ICMR (45/3/2019-Hae/BMS), and (56/6/2019-HAE/BMS) India. The English editing was done by AJE.

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Table

Table I: Haematological Examination Report of T-ALLPatients

S. No.	Characteristics of Patients	Examination Report
1.	Number of Patients	26
2.	Healthy Control	26
3.	Male/Female	36/16
4.	Specimen	Peripheral Blood Sample
5.	Age at diagnosis, year median (range)	21.375 (4-40)
6.	Haemoglobin	6.8875 gm/dl (Average)
7.	Total Leucocyte Count	7.8812.5 cells/cumm (Average)
8.	RBC	2.158×10^{12}/L (Average)
9.	Blast cells range	79.8 (Average)
10.	Markers Combination	CD2, CD3, CDE, CD4, CD5, CD7, CD8, CD10, CD13, CD33, CD34, CD38, CD99, CD117, cCD3, TdT

Supplementary Tables

Supplementary Tables are not available with this version

Figures

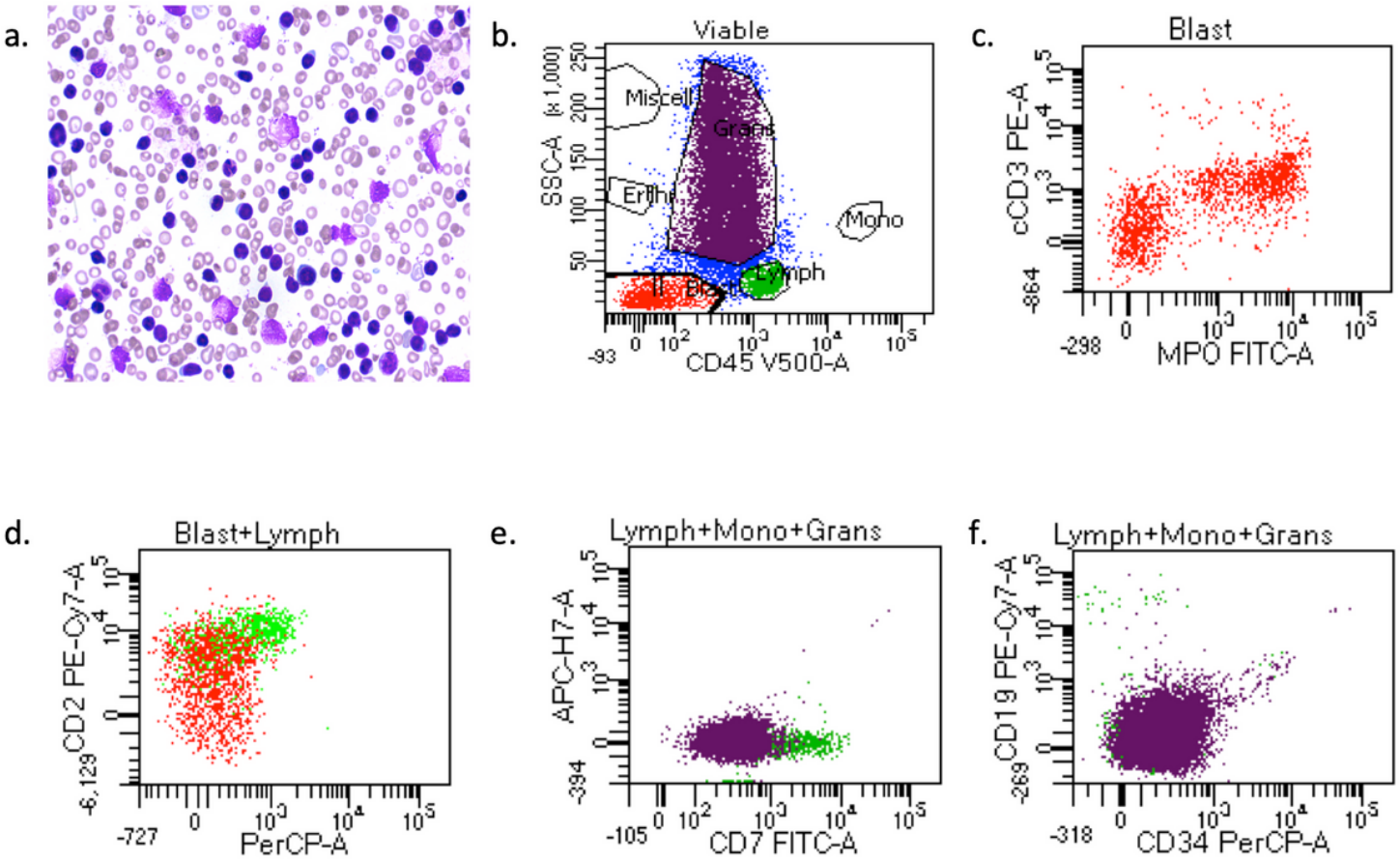

Figure 1

Clinical diagnosis was confirmed on a peripheral blood smear (PBS) and bone marrow aspiration. (a) Representative images of Leishman staining of the blood of T-ALL patients. (b, f) Flow cytometry results show all kinds of cells in T-ALL patients, which were plotted on CD45 with side scatter and plotted on the marker and marker vs marker for the confirmation of T-ALL.

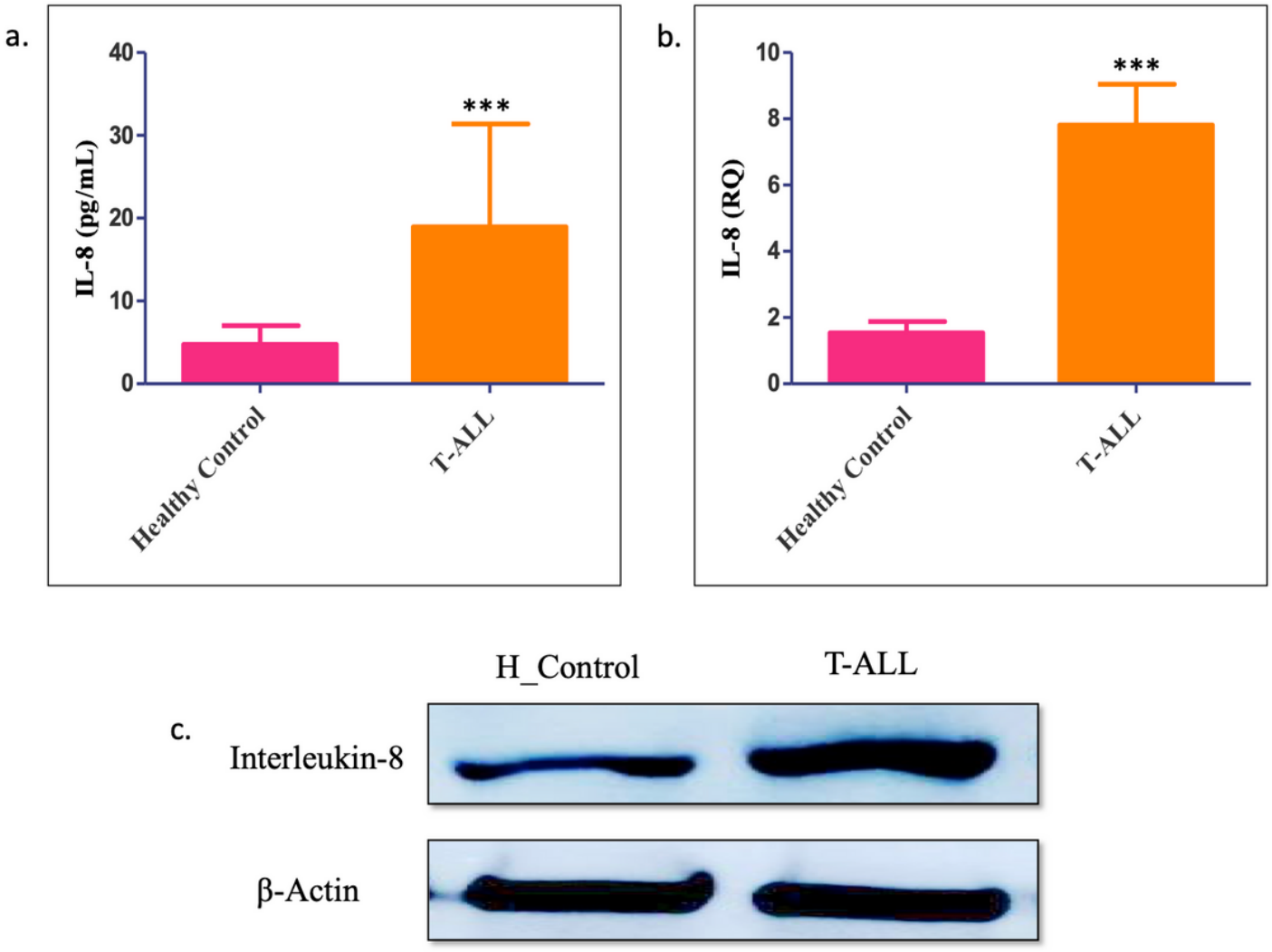

Figure 2

The expression of IL-8 was measured by ELISA, RT-PCR, and Western blotting in T-ALL. (a-c) The IL-8 expression level was estimated in T-ALL patients at the serum, genomic, and proteomic levels. All the results follow the same trajectory, as shown in the figure. All quantitative data are the mean ± SD, ****p<0.00001, Student's t-test (paired).

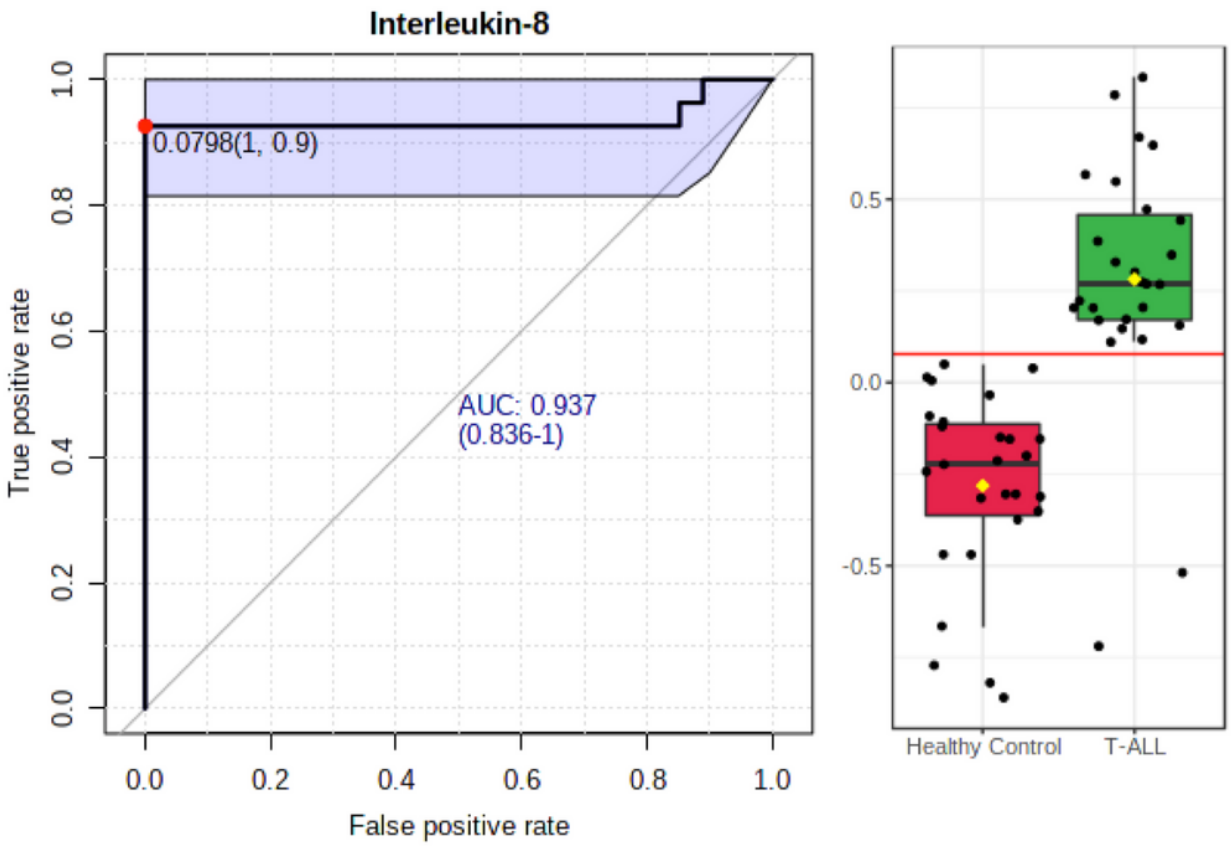

Figure 3

ROC curve for IL-8 in T-ALL. AUROC analysis showed that IL-8 (AUC: 0.937; 0.836-1) had a cutoff value (0.0798; 1, 0.9).