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# Prognostic value of soluble angiotensin II receptor 1 and soluble angiotensin converting enzyme (CD 143) in patients with acute leukemia

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**Abstract**

Background: The renin-angiotensin system (RAS) is a bioenzymic cascade that plays an integral role in cardiovascular homoeostasis by influencing vascular tone, fluid and electrolyte balance and the sympathetic nervous system. RAS was viewed as a circulating endocrine system, whereby renin released from the juxtaglomerular cells of the kidney cleaves the liver-derived macroglobulin precursor angiotensinogen, to produce the inactive decapeptide angiotensin I, which is then converted to the active octapeptide Ang II by angiotensin converting enzyme (ACE) within the pulmonary. There is increasing evidence that Ang II, a major regulator of blood pressure and cardiovascular homeostasis, is involved in the regulation of cell proliferation, angiogenesis, inflammation and tissue remodeling, which suggests that this peptide might also play a role in cancer. Ang II is the main effector of the RAS and it alternatively binds to either Ang II T1R or Ang II T2R. The Ang II T1R and Ang II T2R can act as antagonists, and mediate effects on cell migration and proliferation of metastatic cancer cells and hemopoietic stem-progenitor cells. Components of the RAS are frequently differentially expressed in various cancers in comparison with their corresponding non- malignant tissue. Yet, the RAS has not been fully elucidated in patients with acute leukemia. Objective: The aim of the present work was to study serum level of Angiotensin II receptor type 1 and the soluble angiotensin converting enzyme (CD143) in patients with acute leukemia in order to extrapolate their possible prognostic value.
Subjects and Methods: The present study included 20 healthy volunteers clinically free from hypertension or sarcoidosis, 20 patients of newly diagnosed AML and 20 patients of newly diagnosed ALL. Blood samples were collected from all subjects and the level of serum ACE and serum Ang IIT1R were measured by enzyme linked immunossorbent assay.
Results: The activity of ACE (U/L) and the concentration of Ang IIT1R (U/L) in patients groups with either AML or ALL before therapy were significantly higher than in control group. After therapy, the activity of the enzyme and its receptor concentration in both groups of patients were significantly decreased but still significantly higher than in normal control subjects.
Conclusions: Estimating the serum level of ACE and soluble Ang IIT1R is of informative diagnostic and prognostic value. Estimation serum level of ACE and Ang IIT1R levels in patients with either AML or ALL is of value in deciding the treatment protocol.



**Keywords:**
renin-angiotensin system, angiotensin converting enzyme, leukemia

## Introduction

There is increasing evidence that angiotensin II (Ang II), a major regulator of blood pressure and cardiovascular homeostasis, is involved in the regulation of cell proliferation, angiogenesis, inflammation and tissue remodeling, which suggests that this peptide might also play a role in cancer [1]. The renin-angiotensin system (RAS) has been characterized extensively in hypertension and atherosclerosis, but recently its relevance to the fields of oncology and hematology are being studied [2, 3].

The RAS pathway mediates its effect primarily via angiotensinogen, angiotensin I (Ang I), and Ang II. The enzyme renin converts angiotensinogen to the decapeptide Ang I, which is transformed into the octapeptide Ang II by the exopeptidase angiotensin I-converting enzyme (ACE) [4]. Components of RAS are expressed in several adult organs including the liver, kidney, pancreas, brain and reproductive organs. It is the paracine mechanisms of locally expressed RAS, not in its circulating counterpart, that appear important for tumourigenesis [5, 6]. Ang II is the main effector of the RAS and it alternatively binds to either Ang II-type 1 receptors (AGTR1) or Ang II-type 2 receptors (AGTR2). The AGTR1 and AGTR2 can act as antagonists, and mediate effects on cell migration and proliferation of metastatic cancer cells and hemopoietic stem-progenitor cells [7]. Components of the RAS are frequently differentially expressed in various cancers in comparison with their corresponding non-malignant tissue. In particular, over-expression of AGTR1 [3].

Studies of knockout mice for ACE as well as other RAS components such as angiotensinogen, renin, AGTR1 and AGTR2 have further implicated a regulatory role for the RAS in hematopoiesis [8]. These mice have exhibited not only phenotypes related to blood pressure, but also demonstrated defects in development and in hematopoietic system [9]. ACE null mice are mildly anemic, so it is presumed that the lack of systemic or local production of Ang II has a detrimental effect on erythropoiesis [10]. The AGTR1 belong to the G protein-coupled receptor family and typically activates phospholipase C (PLC). It maps to chromosome 3 [11].

Acute leukemias are hemopoietic neoplasms characterized by accumulation of blast cells in the bone marrow and peripheral blood [12]. Acute lymphoblastic leukemia (ALL) is a malignant disease characterized by the accumulation of lymphoblasts and it may be B

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or T lineages and the first attempt at classifying ALL was the French-American-British (FAB) morphological criteria that divided ALL into 3 subtypes (L1, L2 and L3) based on cell size, cytoplasm, nucleoli, vacuolation and basophilia. In 1997, the World Health Organization proposed a composite classification in an attempt to account for morphology and cytogenetic profile of the leukemic blasts and identified three types of ALL: B lymphoblastic, T lymphoblastic and Burkitt-cell leukemia. Later revised in 2008, Burkitt-cell leukemia was eliminated as it is no longer seen as a separate entity from Burkitt lymphoma, and B-lymphoblastic leukemia was divided into two subtypes: B-ALL with recurrent genetic abnormalities and B-ALL not otherwise specified. B-ALL with recurrent genetic abnormalities is further delineated based on the specific chromosomal rearrangement. In 2016, two new provisional entities were added to the list of recurrent genetic abnormalities and the hypodiploid was redefined as either low hypodiploid or hypodiploid with *TP53* mutations. In adults, B-cell ALL accounts for ~75% of cases while T-cell ALL comprises the remaining cases [12, 13]. Acute myeloid leukemia (AML) is a malignant clonal disorder of immature cells in the hemopoietic hierarchical system and is confirmed by an excess of primitive blast cells in the bone marrow required to be at least 20% and there is 11 subtypes of AML [14, 15]. Diagnosis is established by the presence of 20% or more lymphoblasts in the bone marrow or peripheral blood. Evaluation for morphology, flow cytometry, immunophenotyping and cytogenetic testing is valuable both for confirming the diagnosis and risk stratification. Lumbar puncture with CSF analysis is standard of care at the time of diagnosis to evaluate for CNS involvement. If the CNS is involved, brain MRI should be performed. Other evaluation includes complete blood count with differential and smear to evaluate the other hematopoietic cell lines, coagulation profiles and serum chemistries. Baseline uric acid, calcium, phosphate and lactate dehydrogenase should be recorded to monitor for tumor lysis syndrome. Morphology remains the method by which acute leukemia is initially detected and is the major aid with cytochemical reaction in distinguishing between acute lymphoblastic leukemia and acute myeloid leukemia [15]. Yet, the RAS has not been fully elucidated in patients with acute leukemia.

The present study aimed to study serum level of angiotensin II receptor type 1 and the soluble angiotensin converting enzyme (CD143) in patients with acute leukemia in order to extrapolate their possible prognostic value. Individuals submitted to this study were divided into three groups: group I – involved 20 healthy volunteers clinically free from hypertension or sarcoidosis (control group), their mean age was 33.25 years and were chosen from the stuff members of MRI, Alexandria University, and clinical research center, Faculty of Medicine, Alexandria University and their relatives, group II – involved 20 patients of newly diagnosed AML and group III – involved 20 patients of newly diagnosed ALL. Patients in group II and III were of matched age as the control group and were recruited from Hematological Department, MRI, Alexandria University and clinical research center, Faculty of Medicine, Alexandria University. An informed consent was taken from all contributors in this study.

Patients with AML received 3 + 7 protocol of induction including: daunomycin 45 mg/m$^2$ for 3 days, cytosine arabinoside 100 mg/m$^2$ × 2/day for 7 days. Patients with ALL received induction protocol as follows: vincristine 1.4 mg/m$^2$ days 1, 8, 15, 22, prednisolone 1 mg/kg/day × 28 days, doxorubicin 25 mg/m$^2$ days 1, 2, 3. After completion of the cycles and restoration of bone marrow cellularity, bone marrow aspiration was done. Patients who achieved complete remission had a BM blasts less than 5%. Those who did not achieve complete remission received a 2nd induction cycle.

To all patients the following investigations were done:

1 – full history recording, 2 – thorough clinical examination, 3 – routine laboratory investigations including: complete blood picture, liver and kidney functions, 4 – radiological investigations including: chest x ray, abdominal ultrasound and ECG study, 5 – bone marrow examination [16], 6 – determination of serum human ACE by ELISA method [17] and 7 – determination of serum human Ang IIT1R by ELISA method [17].

## Statistical analysis

Statistical analysis was carried out using SPSS statistics software version 20. Quantitative data were tested for normality using Kolmogorov-Smirnov test. Abnormally distributed data was given as range (minimum-maximum). Non-parametric statistical tests of significance were applied; Mann-Whitney test was used to compare two independent groups. All applied statistical tests of significance were two-tailed. Receiver operating characteristic (ROC), carried out using MedCalc statistical software, was used to evaluate the diagnostic and prognostic accuracy of a test to correctly pick cured and non-cured subjects. The larger the area under the curve (AUC) i.e. closer to 1, the better the performance of a diagnostic test. Youden index was used to find the cut-off point i.e. the point that gives maximum correct classification. At this cut-off point, sensitivity; defined as the probability that the test is positive in patients with the disease and specificity; defined as the probability that the test is negative in patients without the disease were determined. Also, positive predictive value; defined as probability that the patient has the disease when the test is positive and negative predictive value; defined as probability that the patient will not have the disease when the test is negative were identified.

## Biochemical Results

### *ACE activity (U/L) in normal control subjects and patients groups with either AML or ALL before and after therapy*

As presented in table I, the statistical analyses of these results revealed that the activity of ACE (U/L) in patients groups with either AML or ALL before therapy was significantly higher than in control group. After therapy, the activity of this enzyme in both groups of patients was significantly decreased but still significantly higher than in normal control subjects.

### *Ang IIT1R level (U/L) in normal control subjects and patients groups with either AML or ALL before and after therapy*

As presented in table II, the statistical analyses of these results revealed that the level of Ang IIT1R (U/L) in patients groups with either AML or ALL before therapy was significantly higher than in

***Table I. Statistical analysis of serum ACE activity (U/L) in normal control subjects and patients groups either with AML or ALL before and after therapy***

| | Normal control (n = 20) | Patients with AML (n = 20) | | Patients with ALL (n = 20) | |
|---|---|---|---|---|---|
| | | Before therapy | After therapy | Before therapy | After therapy |
| **ACE (U/L)** | | | | | |
| Range | 43.0-47.25 | 48.25-71.0 | 43.0-65.0 | 49.25-69.0 | 43.75-63.0 |
| Mean ± SE | 45.51 ± 0.29 | 53.56 ± 1.20 | 48.12 ± 1.02 | 53.10 ± 1.81 | 49.20 ± 1.61 |
| $p_1$ | | < 0.001* | 0.031* | < 0.001* | < 0.001* |
| $p_2$ | | < 0.001* | | 0.005* | |

$p_1$: p comparing mean values with control group; $p_2$: p comparing mean values before and after therapy
*: Statistically significant at p ≤ 0.05

***Table II. Statistical analysis of serum Ang IIT1R concentration (U/L) in normal control subjects and patients groups either with AML or ALL before and after therapy***

| | Normal control (n = 20) | Patients with AML (n = 20) | | Patients with ALL (n = 20) | |
|---|---|---|---|---|---|
| | | Before therapy | After therapy | Before therapy | After therapy |
| **AngIIT1R (U/L)** | | | | | |
| Range | 324.0-360.0 | 370.0-520.0 | 336.0-400.0 | 378.0-550.0 | 338.0-392.0 |
| Mean ± SE | 344.60 ± 1.61 | 418.33 ± 7.39 | 358.81 ± 3.50 | 432.0 ± 18.14 | 361.40 ± 5.66 |
| $p_1$ | | < 0.001* | 0.001* | < 0.001* | 0.016* |
| $p_2$ | | < 0.001* | | 0.004* | |

$p_1$: p comparing mean values with control group; $p_2$: p comparing mean values before and after therapy
*: Statistically significant at p ≤ 0.05

control group. After therapy, it was noticed that the level of this receptor in both groups of patients was significantly decreased than in their corresponding values before therapy and still significantly higher than in normal control group.

### *Liver function levels in normal control subjects and patients groups with either AML or ALL*

As presented in table III, the statistical analyses of these results revealed that the mean values of indirect bilirubin (mg/dl), total bilirubin (mg/dl) and SGPT (u/l) concentrations in both groups of patients were significantly higher than normal control group. In addition, the concentrations of direct bilirubin (mg/dl) as well as SGOT (u/l) in patients with ALL were significantly higher than normal control group. On the other hand, serum albumin (mg/dl) concentrations in both groups of patients were significantly lower than in normal subjects. Kidney function levels in normal control subjects and patients groups with either AML or ALL.

As presented in table IV, the statistical analyses of these results revealed that in both groups of patients, the mean values of creatinine (mg/dl) concentrations were significantly lower than in normal control group.

### *Sodium, Potassium, Calcium and Phosphorus levels in normal control subjects and patients groups with either AML or ALL*

As presented in table V, the statistical analyses of these results revealed that the mean values of sodium, potassium, calcium and phosphorus concentrations in both groups of patients were significantly lower than in normal control group.

## Hematological Results

### *Mean values of WBC count (x10e9), Platelets count (x10e9) and Hemoglobin concentration (g/dl) in normal control subjects and patients groups with either AML or ALL*

As presented in table VI, the statistical analyses of these results revealed that the mean values of WBCs count in AML and ALL patients were higher than in control group. On the other hand, Platelets count and Hb concentration in both groups of patients were significantly lower than in normal subjects.

### *Mean values of blasts cells% in normal control group and patients group with either AML or ALL before and after therapy*

As presented in table VII, the statistical analyses of these results revealed that the blast cells % in patients with either AML or ALL before therapy was significantly higher than in control group. After therapy, the % of blast cells in both groups of patients were significantly decreased compared to their corresponding values before therapy and still significantly higher than in control group.

### *Correlation between ACE activity (U/L) and all studied parameters*

As presented in table VIII, ACE activity (U/L) in serum of AML patients group showed a significant positive correlation with blast cells % at presentation.

*Table III. Statistical analyses of liver function in normal control subjects and patients groups either with AML or ALL*

| | Normal control (n = 20) | Patients with AML (n = 20) | Patients with ALL (n = 20) |
|---|---|---|---|
| **Direct bilirubin (mg/dl)** | | | |
| Range | 0.10-0.20 | 0.10-0.40 | 0.10-1.0 |
| Mean ± SE | 0.12 ± 0.01 | 0.16 ± 0.02 | 0.33 ± 0.09 |
| **P1** | | $p_1 = 0.347$ | $p_2 = 0.008^*$ |
| **Indirect bilirubin (mg/dl)** | | | |
| Range | 0.15-0.30 | 0.10-0.50 | 0.10-0.60 |
| Mean ± SE | 0.23 ± 0.01 | 0.36 ± 0.02 | 0.34 ± 0.05 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 = 0.011^*$ |
| **Total bilirubin (mg/dl)** | | | |
| Range | 0.30-0.40 | 0.30-0.80 | 0.30-0.90 |
| Mean ± SE | 0.35 ± 0.01 | 0.52 ± 0.03 | 0.58 ± 0.07 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 < 0.001^*$ |
| **Total serum protein (mg/dl)** | | | |
| Range | 6.80-7.50 | 1.90-9.50 | 2.0-8.30 |
| Mean ± SE | 7.21 ± 0.04 | 6.87 ± 0.32 | 6.52 ± 0.55 |
| **P1** | | $p_1 = 0.640$ | $p_2 = 0.411$ |
| **Albumin (mg/dl)** | | | |
| Range | 3.90–4.60 | 1.80-4.30 | 2.40-3.50 |
| Mean ± SE | 4.29 ± 0.04 | 3.09 ± 0.12 | 3.07 ± 0.14 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 < 0.001^*$ |
| **SGOT (u/l)** | | | |
| Range | 12.0-25.0 | 11.0-55.0 | 12.0-141.0 |
| Mean ± SE | 17.45 ± 0.82 | 21.67 ± 1.96 | 46.0 ± 12.83 |
| **P1** | | $p_1 = 0.133$ | $p_2 = 0.010^*$ |
| **SGPT (u/l)** | | | |
| Range | 14.0-25.0 | 10.0-82.0 | 7.0-89.0 |
| Mean ± SE | 18.35 ± 0.74 | 31.11 ± 3.60 | 46.90 ± 8.36 |
| **P1** | | $p_1 = 0.002^*$ | $p_2 = 0.005^*$ |

$p_1$: p comparing mean values with control group; *: Statistically significant at $p \leq 0.05$

*Table IV. Statistical analyses of kidney function in normal control subjects and patients groups either with AML or ALL*

| | Normal control (n = 20) | Patients with AML (n = 20) | Patients with ALL (n = 20) |
|---|---|---|---|
| **Alkaline phosphatase (U/L)** | | | |
| Range | 65.0-85.0 | 40.0-113.0 | 40.0-92.0 |
| Mean ± SE | 76.35 ± 1.15 | 79.52 ± 3.18 | 69.30 ± 5.49 |
| **P1** | | $p_1 = 0.440$ | $p_2 = 0.193$ |
| **Uric acid (mg/dl)** | | | |
| Range | 2.30-3.50 | 1.90-5.10 | 1.30-6.0 |
| Mean ± SE | 2.87 ± 0.09 | 2.78 ± 0.18 | 2.89 ± 0.43 |
| **P1** | | $p_1 = 0.076$ | $p_2 = 0.414$ |
| **Urea (mg/dl)** | | | |
| Range | 22.0-35.0 | 13.0-42.0 | 24.0-35.0 |
| Mean ± SE | 27.90 ± 0.88 | 26.74 ± 1.33 | 28.56 ± 1.38 |
| **P1** | | $p_1 = 0.531$ | $p_2 = 0.673$ |
| **Creatinine (mg/dl)** | | | |
| Range | 1.20-1.60 | 0.40-1.90 | 0.50-1.40 |
| Mean ± SE | 1.38 ± 0.03 | 1.03 ± 0.09 | 0.97 ± 0.10 |
| **P1** | | $p_1 = 0.003^*$ | $p_2 < 0.001^*$ |

$p_1$: p value for comparing between control and patients; *: Statistically significant at $p \leq 0.05$

***Table V. Statistical analyses of Sodium, Potassium, Calcium and Phosphorus in normal control subjects and patients groups either with AML or ALL***

| | Normal control (n = 20) | Patients with AML (n = 20) | Patients with ALL (n = 20) |
|---|---|---|---|
| **Sodium (meq/l)** | | | |
| Range | 130.0-145.0 | 114.0-139.0 | 120.0-135.0 |
| Mean ± SE | 138.95 ± 0.75 | 132.48 ± 1.15 | 128.60 ± 1.90 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 < 0.001^*$ |
| **Potassium (meq/l)** | | | |
| Range | 3.50-4.90 | 2.80-5.10 | 3.30-5.10 |
| Mean ± SE | 4.33 ± 0.08 | 3.69 ± 0.12 | 3.72 ± 0.19 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 ^*= 0.003^*$ |
| **Calcium (meq/l)** | | | |
| Range | 9.50-10.60 | 7.10-9.60 | 4.30-9.70 |
| Mean ± SE | 10.20 ± 0.07 | 8.53 ± 0.14 | 8.28 ± 0.49 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 < 0.001^*$ |
| **Phosphorus (meq/l)** | | | |
| Range | 4.80-5.30 | 2.70-5.10 | 2.50-4.60 |
| Mean ± SE | 5.04 ± 0.03 | 3.95 ± 0.10 | 3.90 ± 0.22 |
| **P1** | | $p_1 < 0.001^*$ | $p_2 < 0.001^*$ |

$p_1$: p value for comparing between control and patients *: Statistically significant at $p \leq 0.05$

***Table VI. Statistical analyses of hematological parameters in normal control subjects and patients groups either with AML or ALL***

| | Normal control (n = 20) | Patients with AML (n = 20) | Patients with ALL (n = 20) |
|---|---|---|---|
| **WBCs count (x10e9)** | | | |
| Range. | 3.50-10.0 | 0.41-86.20 | 1.03-86.16 |
| Mean ± SE | 7.21 ± 0.40 | 15.40 ± 4.19 | 22.58 ± 9.95 |
| **P1** | | 0.208 | 0.397 |
| **Platelets count (x10e9)** | | | |
| Range | 127.0-328.0 | 4.0-66.0 | 8.0-44.0 |
| Mean ± SE | 233.0 ± 11.96 | 22.42 ± 2.76 | 28.34 ± 3.75 |
| **P1** | | $< 0.001^*$ | $< 0.001^*$ |
| **HB concentration (g/dl)** | | | |
| Range | 12.40-15.50 | 4.40-11.20 | 5.40-10.0 |
| Mean ± SE | 13.23 ± 0.18 | 8.11 ± 0.26 | 7.71 ± 0.49 |
| **P1** | | $< 0.001^*$ | $< 0.001^*$ |

$p^1$: p comparing mean values with control group *: Statistically significant at $p \leq 0.05$

***Table VII. Statistical analyses of blasts cells % in normal control subjects and patients groups either with AML or ALL before and after therapy***

| | Normal control (n = 20) | Patients with AML (n = 20) | | Patients with ALL (n = 20) | |
|---|---|---|---|---|---|
| | | Before therapy | After therapy | Before therapy | After therapy |
| **Blast cells %** | | | | | |
| Range | 1.0-3.0 | 10.0-96.0 | 1.0-60.0 | 15.0-90.0 | 2.0-20.0 |
| Mean ± SE | 1.45 ± 0.14 | 73.93 ± 3.68 | 8.81 ± 2.34 | 70.90 ± 6.73 | 10.50 ± 2.27 |
| **$p_1$** | | $< 0.001^*$ | $< 0.001^*$ | $< 0.001^*$ | $< 0.001^*$ |
| **$p_2$** | | $< 0.001^*$ | | $0.005^*$ | |

$p_1$: p comparing mean values with control group $p_2$: p comparing mean values before and after therapy
*: Statistically significant at $p \leq 0.05$

*Table VIII. Correlation between ACE activity (U/L) and all studied parameters*

| ACE activity (U/L) | AML patients | | ALL patients | |
|---|---|---|---|---|
| | $r_s$ | p | $r_s$ | p |
| Blast cells % | 0.797* | < 0.001 | 0.689* | 0.028 |
| WBCs count (x10e9) | 0.073 | 0.718 | -0.237 | 0.510 |
| HB concentration (g/dl) | 0.006 | 0.978 | 0.670* | 0.034 |
| Platelets count (x10e9) | 0.044 | 0.829 | -0.535 | 0.111 |
| Direct bilirubin concentration (mg/dl) | 0.247 | 0.215 | -0.430 | 0.251 |
| Indirect bilirubin concentration (mg/dl) | -0.376 | 0.053 | -0.405 | 0.246 |
| Total bilirubin concentration (mg/dl) | -0.147 | 0.464 | -0.165 | 0.648 |
| Total serum protein concentration (mg/dl) | -0.036 | 0.857 | -0.210 | 0.561 |
| Albumin concentration (mg/dl) | -0.159 | 0.428 | 0.198 | 0.583 |
| SGOT concentration (u/l) | 0.117 | 0.562 | 0.598 | 0.068 |
| SGPT concentration (u/l) | -0.083 | 0.680 | 0.615 | 0.059 |
| Alkaline phosphatase concentration (u/l) | -0.324 | 0.099 | -0.379 | 0.280 |
| Uric acid concentration (mg/dl) | 0.203 | 0.311 | 0.232 | 0.519 |
| Urea concentration (mg/dl) | -0.162 | 0.421 | 0.518 | 0.125 |
| Creatinine concentration (mg/dl) | -0.058 | 0.773 | -0.117 | 0.748 |
| Sodium concentration (meq/l) | 0.032 | 0.874 | -0.437 | 0.207 |
| Potassium concentration (meq/l) | -0.220 | 0.271 | 0.022 | 0.952 |
| Calcium concentration (meq/l) | 0.196 | 0.328 | 0.257 | 0.474 |
| Phosphorus concentration (meq/l) | 0.069 | 0.733 | -0.659* | 0.038* |

$r_s$: Spearman coefficient; *: Statistically significant at $p \leq 0.05$

In ALL patients groups, it was noticed that there was a significant positive correlation between ACE activity (U/L) with blast cells % and Hb concentration (g/dl), and was reversely correlated with Phosphorus concentration (meq/l).

### *Correlation between Ang IIT1R concentration (U/L) and all studied parameters*

As presented in table IX, Ang IIT1R concentration (U/L) in serum of AML patients group showed a significant positive correlation with blast cells % and urea concentration (mg/dl) at presentation.
In ALL patients groups, it was noticed that there was a significant positive correlation with blast cells %, and was reversely correlated with WBCs count (x10e9) and Sodium concentration (meq/l).

### *Comparison between the values of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) as diagnostic markers for acute leukemia*

As presented in table X and fig. 1, serum ACE activity (U/L) showed significant AUC (100%, $p < 0.001$) with sensitivity 100% and specificity 100% at a cut off value (47.25 U/L).
Serum Ang IIT1R concentration (U/L) showed significant AUC (100%, $p < 0.001$) with sensitivity 100% and specificity 100% at a cut off value (360 U/L).

### *Prognostic values of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) in acute leukemia patients*

As presented in table XI, XII and fig.2, fig.3, Kaplan-Meier DFS curves for acute leukemia patients groups revealed that, patients with elevated levels of serum ACE activity (U/L) and Ang IIT1R concentration (U/L) than their corresponding cut off points were

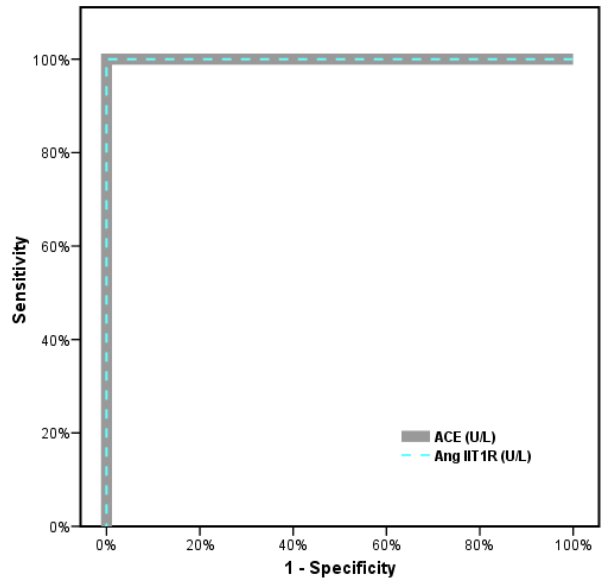


*Fig. 1. Graphical representation of the ROC curves for serum ACE activity (U/L) and Ang IIT1R concentration (U/L) in acute leukemia patients before therapy*

*Table IX. Correlation between Ang IIRT1 concentration (U/L) and all studied parameters*

| AngIIT1R concentration (U/L) | AML patients | | ALL patients | |
|---|---|---|---|---|
| | Coff. | p | Coff. | p |
| Blast cells % | $r_s = 0.873^*$ | < 0.001 | $r_s = 0.681^*$ | 0.030 |
| WBCs count (x10e9) | $r_s = 0.094$ | 0.641 | $r_s = -0.806^*$ | 0.005 |
| HB concentration (g/dl) | r = 0.216 | 0.279 | r = 0.029 | 0.938 |
| Platelets count (x10e9) | $r_s = -0.052$ | 0.798 | $r_s = -0.512$ | 0.130 |
| Direct bilirubin concentration (mg/dl) | $r_s = 0.056$ | 0.781 | $r_s = -0.365$ | 0.299 |
| Indirect bilirubin concentration (mg/dl) | $r_s = -0.027$ | 0.895 | $r_s = -0.279$ | 0.435 |
| Total bilirubin concentration (mg/dl) | $r_s = 0.019$ | 0.924 | $r_s = -0.081$ | 0.825 |
| Total serum protein concentration (mg/dl) | $r_s = -0.180$ | 0.370 | $r_s = -0.080$ | 0.827 |
| Albumin concentration (mg/dl) | $r_s = 0.063$ | 0.755 | $r_s = 0.129$ | 0.722 |
| SGOT concentration (u/l) | $r_s = 0.253$ | 0.204 | $r_s = -0.115$ | 0.751 |
| SGPT concentration (u/l) | $r_s = -0.226$ | 0.257 | $r_s = -0.401$ | 0.250 |
| Alkaline phosphatase concentration (u/l) | $r_s = -0.328$ | 0.095 | $r_s = 0.201$ | 0.578 |
| Uric acid concentration (mg/dl) | $r_s = -0.116$ | 0.563 | $r_s = -0.018$ | 0.960 |
| Urea concentration (mg/dl) | $r_s = 0.428^*$ | $0.026^*$ | $r_s = 0.073$ | 0.841 |
| Creatinine concentration (mg/dl) | $r_s = 0.135$ | 0.502 | $r_s = -0.140$ | 0.699 |
| Sodium concentration (meq/l) | $r_s = -0.248$ | 0.212 | $r_s = -0.838^*$ | $0.002^*$ |
| Potassium concentration (meq/l) | $r_s = -0.244$ | 0.220 | $r_s = 0.332$ | 0.348 |
| Calcium concentration (meq/l) | $r_s = 0.073$ | 0.716 | $r_s = -0.079$ | 0.828 |
| Phosphorus concentration (meq/l) | $r_s = 0.099$ | 0.624 | $r_s = 0.215$ | 0.550 |

$r_s$: Spearman coefficient *: Statistically significant at $p \leq 0.05$

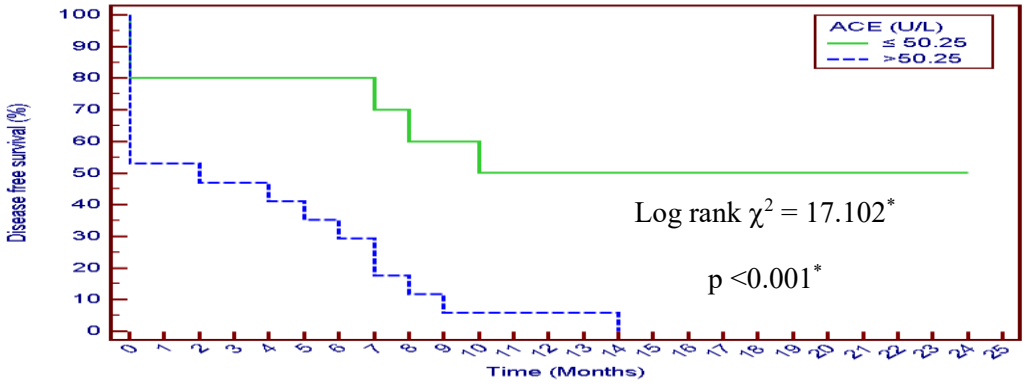


*Fig. 2. Kaplan-Meier disease free survival of serum ACE activity (U/L) for acute leukemia patients*

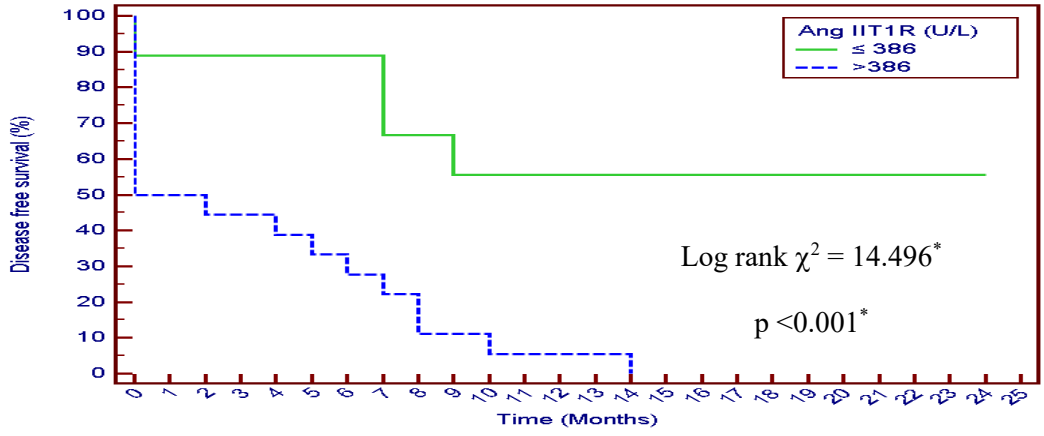


*Fig. 3. Kaplan-Meier disease free survival of serum Ang IIT1R concentration (U/L) for acute leukemia patients*

*Table X. The area under the ROC curves, sensitivity and specificity for serum ACE activity (U/L) and Ang IIT1R concentration (U/L) in acute leukemia patients before therapy*

| Before therapy | Area under the curve | Asymptomatic significance | Cut off values | Sensitivity % | Specificity % |
|---|---|---|---|---|---|
| ACE activity (U/L) | 100% | < 0.001 | 47.25 | 100.0 | 100.0 |
| Ang IIT1R concentration (U/L) | 100% | < 0.001 | 360 | 100.0 | 100.0 |

*Table XI. Test of significance of disease free survival of serum ACE activity (U/L) in acute leukemia patients*

| ACE (U/L) | Mean | % | Log rank | |
|---|---|---|---|---|
| | | | $\chi^2$ | p |
| ≤ 50.25 | 14.75 | 50.0 | 17.102* | <0.001* |
| > 50.25 | 2.84 | 0.0 | | |

*Table XII. Test of significance of disease free survival of serum Ang IIT1R concentration (U/L) in acute leukemia patients*

| Ang IIT1R (U/L) | Mean | % | Log rank | |
|---|---|---|---|---|
| | | | $\chi^2$ | p |
| ≤ 386 | 15.18 | 54.5 | 14.496* | < 0.001* |
| > 386 | 3.12 | 0.0 | | |

significantly different from those with low levels according to log rank test ($p < 0.001^*$ and $p < 0.001^*$ respectively).

## Discussion

The physiology of angiotensin II continues to be a major field of investigation. Recently reported mechanisms suggest that it has growth-promoting factor and cytokine like properties in addition to its vasoconstrictor actions.

Some of the pathophysiological effects of Ang II may be mediated through activation of the transcription factor nuclear factor KB (NF KB) which participates in a variety of inflammatory responses as well as neoplasia [18].

In addition to ACE dependent pathways of Ang II formation, non ACE pathways have been demonstrated, namely chymase which is a chymotypsin-like serine protease which may represent an improtant mechanism for the conversion of Ang I to Ang II in tissues and in vasculature. In this regard, mast cell chymase immunostaining could identify acute myeloid leukemia [19].

The Ang II T1R has recently been identified, and its mechanisms of action continue to be elaborated.

On the other hand, Ang II T1R mediated over-production of ROS has potent growth-promoting actions by exerting positive feedback effects that amplifies its signaling in leucocytes and monocytes. Agonist-induced activation of the Ang II T1R participates in promotion of tumor progression and metastasis though its growth-promoting and proangiogenic Ang II T1R [20].

In the present study, the median serum level of ACE was significantly higher in leukemic patients before therapy compared to the control. In the meantime, the level significantly declined post chemotherapy compared to the pre-treatment levels, yet it did not reach the control level in some patients. This reflects the leukemogenic effect of this enzyme especially in patients with AML, this also proved the positive correlation between serum level of ACE and blast percent denoting that these leukemic blasts are incriminated in its production. Moreover, an interindividual variability in the serum level of the enzyme was observed in leukemic patients which could be attributed to the possible interplay of ACE gene polymorphism. This is in agreement with Angela et al. [21] who reported that ACE insertion/deletion (I/D) polymorphism plays important role in breast cancer risk and disease-free survival in Caucasian postmenopausal women, the D/D carries having an increased risk of developing breast cancer. The ACE gene is located in chromosome 17 q 23 and has many polymorphisms. The most commonly studied is a 287 bp insertion/deletion polymorphism in intron 16 that accounts for 50% of the variability in circulating ACE [22, 23].

Moreover, several studies have shown that Ang II acts as a growth factor in normal and breast cancer cells through phospholipase C activation [24]. The deletion polymorphism of ACE gene leads to increased serum levels [25]. Alternatively, other as yet unidentified gene loci in linkage disequilibrium with the ART1 1166 variant may account for the different serum levels of the enzyme.

In the present study, we observed that patients with the maximal level of ACE before therapy did not reach a level nearing with the control, but the level remained higher than the control reflecting a state of failure of induction chemotherapy in reducing the burden of leukemic cells. This denotes a more aggressive leukemic blasts possibly bearing the CD34+ immunophenotype and necessitating a more aggressive induction chemotherapy protocol.

Our finding confirms the study of Jokubaitis et al. [26] who reported that ACE (CD143) marks hemopoietic cell with hemopoietic stem cell characteristics in adult haemopoietic tissues.

The present study is also supported by the report of Aksu et al. [27] who demonstrated that AML cells overexpress ACE (CD

143), an observation that explains, in part, the relative refractoriness of leukemic cells to the inhibitory effects of chemotherapeutic drugs. Their group hypothesized the presence of a local bone marrow renin-angiotensin system affecting physiological and neoplastic blood cell formation. The local RAS has been defined as an autocrine-paracrine system within the hematopoietic lineage and marrow stromal cells [28] that may be involved in neoplastic hematopoiesis and leukemogenesis [29, 30, 31].

In the present study, a positive correlation was found between ACE levels and bone marrow blasts, a finding which is congruent with Aksu et al. [27]. Moreover, several authors reported that renin mRNA was found in leukemic blast cells [29, 30].

Our results were as well explained by Abali et al. [29] who stated that ACE degrades a tetra-peptide called ACSDKP (goralatide), a negative hematopoietic regulator, hence while peripheral blood ACE levels increase, blast percentages in the bone marrow accumulate and migrate to the circulation. Therefore, ACE hyperfunction may lead to the enhanced goralatide metabolism, which in turn lowers its level in the bone marrow micro-environment, abolishing the anti-proliferative effect of the peptide on the hematopoietic cells and leukemic blasts [30].

Leukemogenesis is a multistep and multifactorial process which includes activation of oncogenic mutations in signal transduction pathways, conferring a proliferative survival advantage on leukemic cells over normal hematopoietic profanities. Angiogenesis which is neovascularization is one of these crucial pathways in the biology of acute leukemia. In addition to conversion of Ang I to Ang II, ACE breaks down bradykinin (BK) into inactive peptides. Several lines of evidence underline the putative role of BK in the modulation of angiogenesis, hence BK activates angiogenesis independent of VEGF pathway [32].

Similarly, it has been reported that AngII activates the mitogen-activated protein kinase pathway (MAPK), which has a mitogenic effect [27].

On the other hand, recent studies have shown that Ang II and Ang (1-7) also have regulatory effect on tissue regeneration, cellular proliferation and growth factor release [33, 34]. In vivo studies have shown that Ang II and Ang (1-7) peptides increased hematopoietic recovery after myelosuppression on multiple blood cell lineages [35, 36]. This could explain the post chemotherapy raised level of ACE despite being lower than pre-treatment level. In our study, the enhanced enzyme activity could be beneficial in terms of bone marrow regeneration after the nadir induced by induction chemotherapy. This key component in the renin-angiotensin system, ACE, in the normal human hemangioblast, supports the critical nature of this enzyme in hematopoietic development [37].

In support of this finding, Shen et al. [37] working on ACE knockout mice, stated that these mice have hematologic developmental defects. They added that an animal lacking all ACE is very different from a wild type animal and can be modeled as representing an extreme complicated phenotype with cardiovascular, reproductive, hematologic and developmental defects.

Another possibility is that post chemotherapy production of ACE might be enhanced by cells other than leukemic blasts. These cells are namely monocytes and macrophages. In this context, Shen et al. [37] forwarded substantial evidence that tumor resistance of ACE Knockout mice exhibits a different immune response as compared to wild type animals. Their data include the finding that their knockout mice develop greater numbers of CD8+ cytotoxic T lymphocytes directed at tumor antigens. In addition, the cytokine levels in these mice are different from those in wild type animals.

Similarly, macrophages express Ang II T1R and release angiogenic cytokines, including VEGF, which promote angiogenesis [38]. In this regard, it has been reported that pharmacological blockade of Ang II T1R also reduced tumor angiogenesis, growth and metastasis [39, 40].

Moreover, it has been reported that mechanisms of Ang IIT1R activation, such as receptor transactivation of tyrosine kinase receptors and stimulation of ROS production suggest that Ang II has growth factor and cytokine-like properties. Receptor transactivation may be defined as that process whereby ligand stimulation of one receptor leads to activation of another distinct receptor.

In the present study, a significant decline in serum levels of Ang IIT1R in patients after therapy, approximately reaching the control level was observed. This could be explained by the decrease in blast percentage by effective chemotherapy at one hand, and by the possibility that the soluble form of the receptor formed a ligand to the remaining receptors expressed on remaining blasts or leukocytes. Hence, they block the receptors leading to abrogation of the signal transduction pathways and dampening of their activation.

In the current study we examined the diagnostic significance of ACE activity (U/L) and Ang IIT1R concentration (U/L) by ROC curve. The curve showed that both parameters were excellent diagnostic markers of acute leukemia as was indicated by asymptomatic significance and very high area under the curve (100%, 100% respectively). The optimum cut off value of ACE activity (U/L) and Ang IIT1R concentration (U/L) were with a corresponding sensitivity 100% and specificity 100% for both parameters.

For the first time, we could define a prognostic value for estimating the levels of ACE activity (U/L) and Ang IIT1R concentration (U/L) in patients with acute leukemia. The Kaplan-Meier disease free survival curve specified a cut off level for ACE < or > than 50.25 U/L with statistical significance ($p < 0.00$). This was true for Ang IIT1R as a cut off value of below or above 386 U/L could discriminate between a higher mean disease free survival of 24 months compared to 14 months in patients with higher level above the cutoff point and the difference was statistically significant ($p < 0.001$).

In the present study, a lower serum potassium was observed in patients with acute leukemia, whether ALL or AML, yet AML patients had even lower levels in AML reflecting hypokalemia. We attributed this decrease in serum potassium to a paraneoplastic syndrome or disturbed electrolytes in these patients.

In accordance to our findings, Wulf et al. [41] reported on paraneoplastic hypokalemia in AML. They stated that it was due to enhanced renin activity in AML blast cells.

As regards serum albumin in level, it was significantly decreased in patients with both types of acute leukemia denoting liver dysfunction due to leukemic infiltration. Another possibility is that patients with acute leukemia are cachectic and suffer from a negative protein balance, especially with the presence of fever and loss of weight.

In agreement with our results, a low serum albumin is considered as a poor prognostic marker in AML [42].

Regarding serum sodium level, the present study exhibited a significant decrease in serum sodium levels in patients with acute leukemias and the control. This could be ascribed to paraneoplastic production of antidiuretic hormone, a syndrome known as "syndrome of inappropriate antidiuretic hormone" release by the leukemic blasts. This syndrome is sometimes aggravated by chemotherapeutic drugs, especially those used in treating ALL.

The novelty of this study is that it evaluates the impact of induction chemotherapy on serum ACE and soluble Ang II T1R in patients with acute leukemia. Other studies were in-vitro studies.

Hence our study indicates a causal relationship between RAS and acute leukemia imparting that manipulating the renin-angiotensin pathways and targeting its receptors could be exploited as adjuvant therapy in these patients.

## Conclusion

To the best of our knowledge the above study is the first to investigate the levels of serum ACE and its soluble receptor1 in patients with acute leukemia. We may conclude that estimating the serum level of ACE and soluble Ang IIT1R might be of informative diagnostic and prognostic value.

The findings of the present study make AR blockers and ACE inhibitors as a targeted option in the management of acute leukemia.

## Recommendations

From the results of the present study we recommend the following:

1. Estimation of Ang II T1R and ACE levels in acute leukemia is of value in deciding the treatment protocol.
2. Further study on the other components of the RAS system is warranted.
3. Further studies on the impact of RAS on other hemopoietic neoplasms are to be envisaged namely the chronic leukemias and lymphomas.
4. Novel clinical applications of ACE inhibitors and Ang II T1R blockade in the management of acute leukemia is worthwhile.

### *Authors' contributions/Wkład autorów*

SAEME – supervision of biochemical part
NAS – supervision of collecting samples and hematological part
NESZ – supervision of collecting samples and hematological part
SAAEK – supervision of biochemical and hematological practical work, statistical data analysis and writing
HKAEK – participation in practical work and writing

### *Conflict of interest/Konflikt interesu*

We declare no conflict of interest.

### *Financial support/Finansowanie*

There is no financial support.

### *Ethics/Etyka*

The work described in this article has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments involving humans; EU Directive 2010/63/EU for animal experiments; Uniform Requirements for manuscripts submitted to Biomedical journals.

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