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# The indicator of hypoxia in acute leukemia: Ischemia-modified albumin

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**Abstract.**
**BACKGROUND:** Hypoxia plays an important role in the development and progression of hematologic malignancies.
**OBJECTIVE:** This study was intended to investigate the effectiveness of ischemia-modified albumin (IMA) for demonstrating hypoxia in patients with acute leukemia.
**METHODS:** Blood specimens were collected from 132 subjects (44 acute leukemia patients, 40 iron deficiency anemia (IDA) patients and 48 healthy controls). Serum levels of IMA and malondialdehyde (MDA) were analyzed using conventional methods.
**RESULTS:** Serum levels of IMA were higher in patients with acute leukemia than in those with IDA and healthy controls (acute leukemia patients; 0.69 $\pm$ 0.14 ABSUs, IDA patients; 0.61 $\pm$ 0.09 ABSUs, controls; 0.50 $\pm$ 0.09 ABSUs, respectively). There was a negative correlation between serum IMA levels and hemoglobin (Hb) values ($r = -0.312$) and between serum IMA levels and hematocrit (Hct) values, ($r = -0.305$) in patients with acute leukemia. Serum levels of MDA were higher in patients with acute leukemia than in those with IDA. But there was no difference in patients with acute leukemia and IDA compared to healthy controls (acute leukemia patients; 2.23 $\pm$ 1.82 nmol/mL, IDA patients; 1.36 $\pm$ 0.94 nmol/mL, healthy controls; 1.79 $\pm$ 0.78 nmol/mL, respectively).
**CONCLUSIONS:** IMA can be effective for demonstrating hypoxia in patients with acute leukemia.

Keywords: Ischemia-modified albumin, acute leukemia, hypoxia

## 1. Introduction

Acute leukemia is a clonal hematopoietic stem cell disease characterized by accumulation of cells with uncontrolled proliferation and impaired differentiation in the bone marrow, blood or tissue [1]. Hypoxia can develop as a result of anemia and ischemia in acute leukemia. Recent studies have found that the mean partial oxygen pressure ($pO_2$) of normal bone marrow is lower than atmospheric $pO_2$ and referred to as physiological hypoxia [2]. Moreover, low oxygen pressure is determined in the bone marrow of patients with acute leukemia compared to normal bone marrow [3]. But leukemic cells are able to live and grow by adapting to hypoxia [4].

The pathogenesis of acute leukemia includes impairment of programmed cell death, DNA damage, molecular events such as alterations in transcription factors and activating mutations of singal transduction intermediates [5,6]. Also, oxidative stress has been shown to contribute to the pathogenesis of acute leukemia [7–9]. Ischemia-modified albumin (IMA), a marker of oxidative stress, increases in conditions such as hypoxia, acidosis and free radical damage [10,11]. The N-terminus residues of human serum albumin (HSA) are able to bind to transition metals such as

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cobalt, copper and nickel [12,13]. Pathological conditions change irreversibly this terminal peptide to a dysfunctional form, known as IMA [10,11]. Recent studies have determined that serum IMA levels increase in several ischemic states including acute coronary syndrome, ischemic central nervous system disease, lower extremity ischemia and pulmonary embolism [10,14–16]. This study was planned to investigate the relation between hypoxia and serum IMA levels in acute leukemia.

## 2. Materials and methods

Forty-four newly diagnosed patients with acute leukemia, 40 patients with iron deficiency anemia (IDA) and 48 healthy volunteers admitted to the Karadeniz Technical University, Faculty of Medicine, Deparment of Hematology between May 2009 and December 2011 were included. Of the patients with acute leukemia, 38 had acute myeloid leukemia (AML) and 6 had acute lymphocytic leukemia (ALL). All participants were informed of the purpose of the study. The study was approved by the Local Ethical Committee, Karadeniz Technical University, Faculty of Medicine (no. 2013/12) and was conducted in accordance with the Declaration of Helsinki.

All acute leukemia patients were diagnosed according to the World Health Organization classification system and for a diagnosis of acute leukemia, a marrow or blood blast count of 20% or more is required [17]. For a diagnosis of IDA, hemoglobin (Hb) $<$ 130 g/L in men or $<$ 120 g/L in women, a mean corpuscular volume (MCV) $<$ 80 fL and ferritin $<$ 33 pmol/L is required [18].

Patients with ischemic vascular diseases such as acute coronary syndrome, ischemic central nervous system disease, peripheral vascular disease or pulmonary embolism were excluded. In addition, patients with advanced hepatic, renal or cardiac insufficiency, uncontrolled infection, other malignancy, inflammatory condition, endocrine dysfunction and abnormal serum albumin levels ($<$ 35 g/L or $>$ 55 g/L) were also excluded. They also did not smoke or use antioxidative agents (drug, supplements).

Venous blood specimens collected from both patient and control groups were placed into biochemical separator containing tubes. After centrifugation at 3000 rpm for 10 min, serum was separated and then kept at $-80$°C for IMA and MDA assays.

### 2.1. Measurement of IMA

Reduced cobalt to albumin binding capacity (IMA level) was analyzed using the rapid and colorimetric method. Two hundred $\mu$L of heparinised plasma was placed into glass tubes and 50 $\mu$L of 0.1% cobalt chloride (Sigma, $CoCl_2.6H_2O$) in $H_2O$ was added. After gentle shaking, the solution was left for 10 minutes in order to ensure sufficient cobalt albumin binding and then 50 $\mu$L of dithiothreitol (DTT) (Sigma, 1.5 mg/mL $H_2O$) was added as a colorizing agent. After waiting 2 minutes, 1 mL of 0.9% NaCl was added in order to halt the cobalt-albumin binding process. A colorimetric control specimen was prepared for serum samples. For the colorimetric control samples, 50 $\mu$L of distilled water was substituted for 50 $\mu$L of 1.5 mg/mL DTT. Specimen absorbencies were analyzed at 470 nm by a spectrophotometer (Shimadzu UV1601, Australia). The color of the DTT containing specimens was compared with that of the colorimetric control tubes. The results were reported as absorbance units (ABSUs) [13].

### 2.2. Measurement of MDA

Lipid peroxidation in human serum samples was determined as MDA concentration using the method described by Yagi [19]. Briefly, to 0.3 mL of serum was mixed 2.4 mL of 0.042M $H_2SO_4$ and 0.3 mL of 10% phosphotungstic acid. After being allowed to stand at room temperature for 5 minutes, the mixture was centrifuged at 1600 g for 10 minutes. Discard supernatant and sediment was suspended in 4 mL of distilled water. Subsequently, 1 mL of 0.67% solution of ratio 1:1 thiobarbituric acid and acetic acid was added and the mixture was heated in boiling water for 60 minutes. The mixture was centrifuged at 1600 g for 10 minutes. The absorbance of the organic layer was read at 532 nm. Tetramethoxypropane was used as a standard and MDA levels were calculated as nmol/mL.

### 2.3. Statistical analysis

The results were expressed as mean $\pm$ SD. All analyses were performed using the statistical sortware package of SPSS ver. 13.0.1 (SPSS, Chicago, IL; License no: 9069728, KTU, Trabzon, Turkey). Data were assessed for normal distribution using the Kolmogorov-Smirnov test. The comparative analysis of serum levels of IMA and MDA between the three groups was performed using the ANOVA (Tamhane

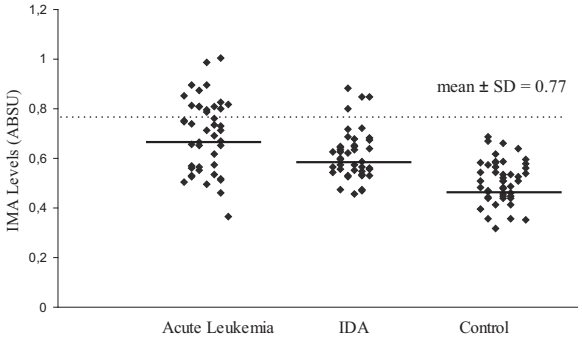



Fig. 1. The distribution of ischemia-modified albumin (IMA) values in acute leukemia, IDA and healthy controls. The dotted line indicates the mean value plus 3 SD of healthy control serum (0.77 ABSU). The central horizontal line indicates the mean value.

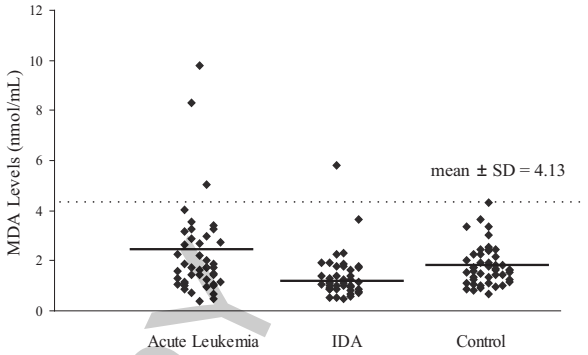



Fig. 2. The distribution of malondialdehyde (MDA) values in acute leukemia, IDA and healthy controls. The dotted line indicates the mean value plus 3 SD of healthy control serum (4.13 nmol/mL). The central horizontal line indicates the mean value.

post hoc test). The association between serum IMA levels and Hb, hematocrit (Hct) values and white blood cell (WBC) count was examined by the Pearson correlation analysis in patients with acute leukemia and IDA. The area beneath the receiver operating characteristics (ROC) curve (AUC) was used to compare the discriminative power of serum levels of IMA and MDA in the diagnosis of acute leukemia and IDA. $P < 0.05$ was considered as statistically significant.

## 3. Results

The study included 132 individuals, 44 patients with acute leukemia, 40 patients with IDA and 48 healthy volunteers. Table 1 shows the general characteristics and laboratory findings for both patients and healthy individuals.

Serum levels of IMA were $0.69 \pm 0.14$ ABSUs in patients with acute leukemia, $0.61 \pm 0.09$ ABSUs in patients with IDA and $0.50 \pm 0.09$ ABSUs in healthy controls (Fig. 1). Serum levels of IMA were significantly higher in patients with acute leukemia than in healthy controls ($p < 0.0001$). In addition to, serum levels of IMA were higher in patients with acute leukemia than in patients with IDA ($p < 0.01$). Also serum levels of IMA of patients with IDA were higher compared to healthy controls ($p < 0.05$).

Serum levels of MDA were $2.23 \pm 1.82$ nmol/mL in patients with acute leukemia, $1.36 \pm 0.94$ nmol/mL in patients with IDA and $1.79 \pm 0.78$ nmol/mL in healthy controls (Fig. 2). Serum levels of MDA were higher in patients with acute leukemia compared to patients with IDA ($p < 0.05$). But there was no difference between patients with acute leukemia and healthy controls for serum levels of MDA ($p > 0.05$). In addition to, no significant difference was observed between patients with IDA and healthy controls for serum levels of MDA ($p > 0.05$).

Negative correlation was determined between serum levels of IMA and values of Hb ($r = -0.312$, $P = 0.039$) and Hct ($r = -0.305$, $P = 0.044$) (Fig. 3). But there was no significant correlation between serum levels of IMA and WBC count in patients with acute leukemia ($r = -0.035$, $P = 0.824$). There was no correlation between serum levels of IMA and values of Hb and Hct in patients with IDA ($r = -0.064$, $P = 0.695$, $r = -0.123$, $P = 0.448$, respectively).

For patients with acute leukemia, AUC for IMA was 0.846 (95% CI: 0.756–0.913). For the optimum diagnostic cut-off value of 0.64 ABSU, the sensitivity and specificity were 65.91% and 91.67%, respectively. For patients with IDA, the AUC for IMA was 0.794 (95% CI: 0.694–0.873). The optimum diagnostic cut-off value was obtained to be 0.52 ABSU, with a sensitivity of 92.5% and specificity of 58.33% for patients with IDA (Fig. 4).

For patients with acute leukemia, the optimal diagnostic cut-off value was 1.67 nmol/mL for MDA (for a sensitivity and specificity of 52.27% and 54.17%, respectively) and the AUC for MDA was 0.527 (95% CI: 0.421–0.632) in patients with acute leukemia. For patients with IDA, the optimal diagnostic cut-off value was 1.35 nmol/mL for MDA (for a sensitivity and specificity of 67.5% and 68.75%, respectively) and the AUC for MDA was 0.721 (95% CI: 0.615–0.811) (Fig. 5).

## 4. Discussion

Oxidative stress resulting from overproduction of reactive oxygen species and suppression of the antioxi-

Table 1
General characteristics and laboratory findings of acute leukemia patients, IDA patients and healthy controls

| Parameter | Acute leukemia (44) | IDA (40) | Controls (48) |
|---|---|---|---|
| Age, years | $44 \pm 16$ | $40 \pm 17$ | $48 \pm 15$ |
| Male/Female | 23/21 | 11/29 | 26/22 |
| Hemoglobin (g/L) | $97 \pm 29^{a}$ | $90 \pm 12^{b}$ | $144 \pm 11^{c}$ |
| Hematocrit (%) | $28 \pm 8.3^{d}$ | $28 \pm 3.6^{e}$ | $41.7 \pm 3.1^{f}$ |
| Leukocyte ($\times 10^9$/L) | $50.7 \pm 49.8^{g}$ | $6.5 \pm 1.9^{h}$ | $6.7 \pm 1.3^{i}$ |
| Platelet ($\times 10^9$/L) | $71.6 \pm 72.2^{j}$ | $306.5 \pm 99.5^{k}$ | $246.5 \pm 57.4^{l}$ |
| Albumin (g/L) | $41 \pm 7^{m}$ | $42 \pm 4^{n}$ | $44 \pm 3^{o}$ |
| Glukoz (mmol/L) | $4.45 \pm 0.4^{p}$ | $4.55 \pm 0.45^{r}$ | $4.5 \pm 0.46^{s}$ |

a–b: NS (not significant), a–c: $p < 0.01$, b–c: $p < 0.01$, d–e: NS, d–f: $p < 0.01$, e–f: $p < 0.01$, g–h: $p < 0.01$, g–i: $p < 0.01$, j–k: $p < 0.01$, j–l: $p < 0.01$, m–n: NS, m–o: NS, m–n: NS, n–o: NS, p–s: NS, p–r: NS, r–s: NS.

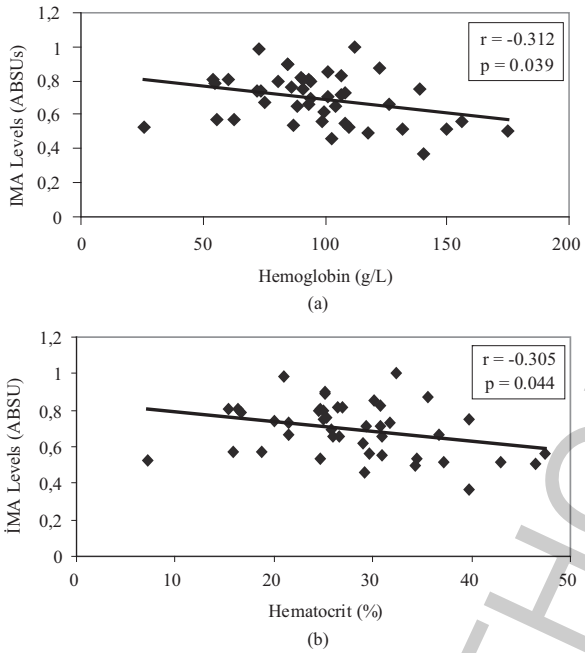
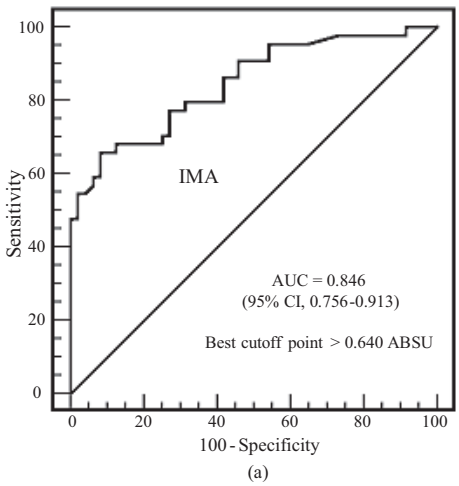



Fig. 3. The linear correlation analysis of serum levels of IMA and values of Hb in patients with acute leukemia (a). The linear correlation analysis of serum levels of IMA and values of Hct in patients with acute leukemia (b). Serum levels of IMA negatively correlated with values of Hb ve Hct.

dant system plays an important role in the development of cancer [20]. Recent studies have shown that oxidative stress increases in various hematologic diseases such as AML, ALL, chronic myeloid leukemia (CML) and myelodysplastic syndrome (MDS) [7,9,21,22]. IMA is a new sensitive biochemical marker for myocardial ischemia and acute coronary syndrome [23]. It is known as an inticator of ischemia and oxidative stress originating as a consequence of tissue hypoxia [24]. Our results showed increased serum levels of IMA in patients with acute leukemia and IDA compared to healthy individuals. Serum levels of IMA were also negatively correlated with Hb and Hct values in patients with acute leukemia. Awadallah et al. determined that serum levels of IMA rised in patients with thalassemic major [25]. Similarly, Cichota et al. showed increased IMA levels in patients with chronic kidney disease [26]. These studies have considered that this elevation of IMA levels could be associated to hypoxia due to low Hb levels. Alike, elevated IMA levels in patients with acute leukemia and IDA were associated with anemia in our study. Anemia may develop in acute leukemia due to suppression of normal bone marrow cells secondary to chemotherapy and infiltration by blastic cells [27]. Hemorrhage, hemophagocytic syndrome, autoimmune hemolytic anemia, microangiopathic hemolytic anemia and bone marrow necrosis may also contribute to anemia [28–31]. Hb levels should be raised above 7 g/dL in order to improve the signs and symptoms of patients and increase the quality of life in hematologic cancers [32]. In conclusion, we wish to emphasize the importance of red blood cell transfusion in order to reduce tissue hypoxia in acute leukemia.

Tumor hypoxia can lead to resistance to apoptosis, inhibition of DNA repair, alter cells metabolism, promote angiogenesis, increase local invasiveness and metastasis [33]. Hypoxia also can cause resistance to radiotherapy and anticancer chemotherapy [34]. Oxygen levels are three times lower in the bone marrow of patients with AML compared to normal individuals [3]. Animal studies have showed that the progression of acute leukemia within the bone marrow is associated with low oxygen levels and expansion of hypoxic bone marrow areas [35]. Some studies of the biology of leukemia have shown that elevated levels of reactive oxygen species (ROS) in leukemic stem cells may trigger differentiation of leukemic blasts [36]. Oltra et al. showed that superoxide dismutase (SOD) and catalase activity decreased in CLL, whereas glutathione peroxidase (GSH-P$_x$) activity increased [37]. He et al. found that SOD and GSH-P$_x$ activity were



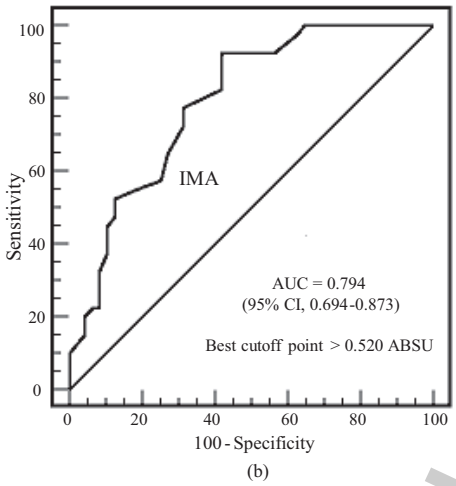
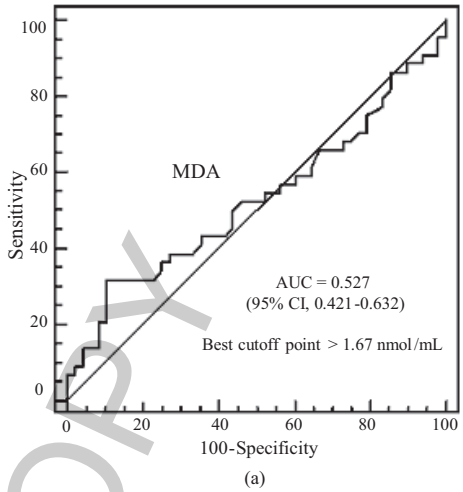




Fig. 4. The ROC curve for IMA in patients with acute leukemia. The optimal diagnostic cut-off value for IMA was 0.64 ABSU (for a sensitivity and specificity of 65.91% and 91.67%, respectively) (a). The ROC curves for IMA in patients with IDA. The optimal diagnostic cutoff value for IMA was 0.52 ABSU (for a sensitivity and specificity of 92.5% and 58.33%, respectively) (b).



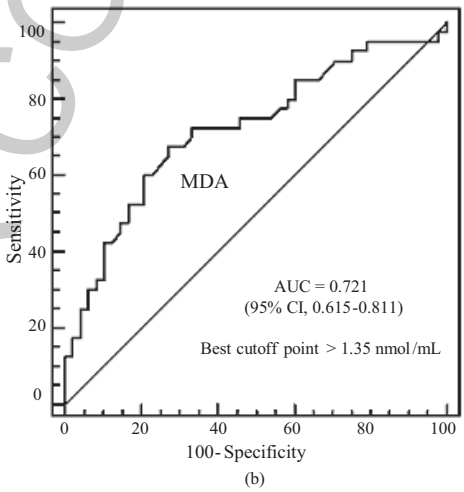




Fig. 5. The ROC curve for MDA in patients with acute leukemia. The optimal diagnostic cut-off value for MDA was 1.67 nmol/mL (for a sensitivity and specificity of 52.27% and 54.17%, respectively) (a). The ROC curve for MDA in patients with IDA. The optimal diagnostic cut-off value for IMA was 1.35 nmol/mL (for a sensitivity and specificity of 67.5% and 68.75%, respectively) (b).

significantly lower in patients with active ALL than in patients with achieved complete remission and normal individuals [38]. Also Zhou et al. demonstrated that the activities of xanthine oxidase and adenosine deaminase were higher in the relaps condition, whereas the activities of GSH-$P_x$, monoamine oxidase (MAO), SOD, and total antioxidant capacity were lower in patients with relapsed AML than in control group [39]. Although levels of Hb were similar in both groups, our study showed higher levels of IMA in patients with acute leukemia compared to patients with IDA. Thus, our results suggested that in addition to anemia, oxidative stress specific factors may contribute to increased IMA levels. The ROC analysis showed that IMA levels $>$ 0.64 ABSU had a sensitivity of 65.91% and specifity of 91.67% in patients with acute leukemia. This study pointed that IMA was a specific diagnostic biomarker for demonstrating tissue hypoxia, although its sensitivity was low.

Hyperleukocytosis results in blockage of small blood vessels by increase in the viscosity of the blood. This complication of acute leukemia contributes to tissue hypoxia [40]. In our study, there was no relationship between serum IMA levels and leukocyte count in patients with acute leukemia. Therefore, our results showed that hyperleukocytosis was not associated serum IMA levels.

MDA, another hypoxia-related markers, is generated via the peroxidation of polyunsaturated fatty acids during in various hypoxic conditions such as metabolic syndrome, cancer, aging and ischemia [41]. Battisti et al. determined that serum levels of MDA and serum protein carbonylation were higher in patients with ALL than in controls [9]. Ahmad et al. also found that there was a significant increase levels of plasma MDA and protein carbonyl in patients with CML compared to healthy subjects [42]. In addition to, Zhou et al. showed that levels of MDA and 8-hydroxydeoxyguanosine were significantly higher in relaps AML patients [39]. In our study, serum levels of MDA were higher in patients with acute leukemia compared to patients with IDA, while there was no statistically significant difference in patients with acute leukemia and IDA compared to healthy controls. Moreover, ROC analysis revealed that the AUC for MDA was 0.527 (95% CI: 0.421–0.632) in patients with acute leukemia and serum levels of MDA $\geqslant$ 1.67 nmol/mL demonstrated 52.27% sensitivity and 54.17% specificity as a diagnostic value in acute leukemia. MDA as a single marker has a low diagnostic value in acute leukemia because of low sensitivity and specifity. We think that IMA may be a more suitable biomarker than MDA as an indicator of oxidative stress.

This study indicated that increased serum IMA levels in patients with acute leukemia could be considered as a sign of increased tissue hypoxia. IMA can be used as a specific, inexpensive and easily detectable biomarker for defining hypoxia in acute leukemia. Anemia and oxidative stress specific factors may contribute to increased IMA levels. Red blood cell transfusion should be used to reduce oxidative stress. In addition to, increased oxidative stress in the leukemic cells is likely to provide new potential therapeutic targets in acute leukemia.

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