RESEARCH ARTICLE

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# The Clinical Relevance and Prognostic Significance of Calcitonin Receptor-Like (*CALCRL*) Gene Expression in AML Patients

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## Abstract

The outcome of acute myeloid leukemia (AML) is heterogeneous, with both patient-related and disease-related factors contributing to an individual patient's likelihood of achieving a therapeutic response and survival. The Calcitonin Receptor-Like (*CALCRL*) gene, which encodes the calcitonin receptor-like receptor, has emerged as a point of interest in studying AML. Its expression levels may hold clinical relevance and contribute to the prognostic assessment of AML patients. In the current study, we evaluated *CALCRL* gene expression levels to verify their possible association with the clinical and laboratory characteristics of AML and to clarify its potential role as a molecular prognostic marker in a cohort of Egyptian AML patients. **Methods:** *CALCRL* gene expression was estimated in 80 newly diagnosed adult Egyptian AML patients by quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR). **Results:** *CALCRL* gene expression in AML cases ranged from 0.11 to 104.11, with a median value of 2.1. It was higher in AML cases compared to controls; however, the difference was not statistically significant. AML cases were stratified into high and low *CALCRL* expression groups. Overall survival (OS) was higher in *CALCRL*-low compared to *CALCRL*-high expressers, yet the difference was not statistically significant. There was no statistical difference between *CALCRL*-high and *CALCRL*-low expressers regarding their complete remission rate (CR) and relapse-free survival (RFS). However, the incidence of relapse was higher in *CALCRL*-low expressers. In our study, the median age of the AML cases was 43 years. OS was significantly longer in *CALCRL*-low expressers, while RFS was significantly longer in *CALCRL*-high expressers younger than 43 years old. **Conclusion:** Studying *CALCRL* gene expression in larger cohorts and over longer follow-up periods is highly recommended to gain deeper insight into its functional role in oncogenesis and chemoresistance, as well as its potential as a molecular prognostic marker and future therapeutic target.

**Keywords:** AML- *CALCRL*- Therapy – response- prognosis

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## Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous haematological malignancy characterized by several recurrent mutations that affect disease biology and phenotype, response to therapy, and risk of subsequent relapse [1-3]. An array of different recurrent genetic alterations has been described in AML, leading to substantial genetic heterogeneity between patients. These recurrent aberrations may act as drivers of leukemogenesis and serve as prognostic markers and/or as targets for rationally designed therapies [4, 5].Calcitonin Receptor Like (*CALCRL*) gene encodes a G-protein-coupled neuropeptide receptor that is part of the bioactive calcitonin gene-related peptide (CGRP) receptor. The two peptides CGRP and adrenomedullin (ADM) exert their versatile functions via the CGRP receptor [6]. *CALCRL* is involved in the functional regulation of the cardiovascular, neurological, and digestive systems [7, 8], angiogenesis, cell proliferation, apoptosis and inflammation [9]. *CALCRL* and its ligands have been implicated in the pathogenesis of various malignant diseases [10]. In the neoplastic tissues, *CALCRL* was predominantly expressed in thyroid carcinomas, parathyroid adenomas, small-cell lung cancers, large-cell neuroendocrine carcinomas of the lung, pancreatic neuroendocrine neoplasms, renal clear-cell carcinomas, pheochromocytomas, lymphomas, multiple myeloma and melanomas [11]. Furthermore, CALCRL protein level was positively associated with the clonogenic capacities of primary AML samples, which implied a critical role of *CALCRL* in AML cell proliferation [12]. Notably, upregulated genes were primarily associated with the function of neutrophils, such

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as neutrophil activation and neutrophil degranulation, which indicated that *CALCRL* depletion may contribute to the differentiation of AML cells [13]. The current work aimed to study the association between *CALCRL* gene expression and the clinicopathological features of the disease as well as its prognostic role in a cohort of newly diagnosed adult Egyptian AML patients.

## Materials and Methods

*Patients and methods*

This study was conducted on 80 newly diagnosed adult Egyptian AML patients recruited from the National Cancer Institute (NCI) between March 2021 to August 2022. They were eighteen females (22.5%) and sixty-two males (77.5%). Their ages ranged between 18 and 72 years with a median age of 43.5 years. Twenty age-gender-matched healthy subjects were included in our study as a control group. The Research Ethics Committee of the Faculty of Medicine, Cairo University, approved the study. Informed written consents were obtained from all participants before enrollment in the study. All procedures performed were following the recommendation of the Declaration of Helsinki the 1964 and its later amendments or comparable ethical standards. Diagnosis of AML was established by thorough clinical examination, and laboratory investigations including bone marrow examination, immunophenotyping (IPT), immunohistochemical staining (IHC), and cytogenetic analysis according to the WHO classification (2016). Patients with acute promyelocytic leukemia (AML-FAB M3), pediatric age group or those who started treatment were excluded from the study.

*Detection CALCRL gene expression*

Bone marrow samples were collected from AML patients under complete aseptic conditions in EDTA vacutainers. Total RNA was extracted (QIAamp RNA extraction kit-QIAGEN) and reverse transcribed using a high capacity cDNA reverse transcription kit (Analysis). Detection of *CALCRL* gene expression was done using step one plus TM Real-Time PCR system (Applied Biosystem, USA). For real-time RT-qPCR, 20 µl reaction volume of 10 µl (2X) TaqMan® Universal Master Mix II, 1 µl (20X) TaqMan® Gene Expression Assay for *CALCRL*, 3 µl cDNA and 6 µl nuclease-free water was prepared. The applied thermal cycler program was 1 cycle at 95°C for 10 min., followed by 40 PCR cycles at 95°C for 15 seconds, and 60°C for 1 min. [14]. *CALCRL* gene expression was normalized to the expression of β-actin (endogenous control) and a calibrator sample using $2^{-\Delta\Delta CT}$ method.

*Chemotherapy regimen*

Patients were treated according to NCI adult protocol: Those < 60 years and fit; received induction with (3+7) regimen consisting of Doxorubicin 45 mg/m$^2$ given as IV bolus for 3 days & Ara-C at a dose 100 mg/m$^2$ as a 24- h infusion daily for 7 days. While frail and elderly patients received induction by LDAC consisting of Ara-C at a dose of 20 mg/12 hours subcutaneously for 10 days. Follow-up bone marrow assessment was performed 7-14 days post-induction to assess remission. If patients did not achieve CR, re-induction was done by HAM regimen with high dose Ara-C (HDAC) 2-3 gm/m2 IV infusion over 3 hours every 12 hours for 3 days and Mitoxantrone 12 mg/m$^2$ 2 hours IV infusion on days 3-5. After the achievement of CR consolidation chemotherapy was performed by HDAC. Patients eligible for transplantation and those who had matched donors were candidates for Allo-SCT.

*Statistical methods*

Data were analyzed using IBM SPSS advanced statistics (Statistical Package for Social Sciences), version 24 (SPSS Inc., Chicago, IL). Numerical data were presented as a range, mean and standard deviation, or median as appropriate, while qualitative data were described as numbers and percentages. The Chi-square (Fisher's exact) test was used to examine the relation between qualitative variables as appropriate. Survival analysis was performed using the Kaplan-Meier method. A comparison between two survival curves was performed using a Log-rank test. The Cox regression model did multivariate analysis to test for independent prognostic effect of statistically significant variables on univariate level with calculating hazard ratio and its 95% confidence interval. Bonferroni corrections of P-value were done to avoid hyperinflation of type 1 error that arises from multiple tests. A P-value less than or equal to 0.05 was considered statistically significant. All tests were two-tailed. Relapse-free survival (RFS) was calculated from the date of complete remission till the date of relapse. Overall survival (OS) was calculated from the date of diagnosis till the date of death or last follow-up.

## Results

*CALCRL* gene expression in AML cases ranged from 0.11 to 104.11 with a median value of 2.1, while its level in healthy controls ranged from 0.26 to 3.44 with a median value of 1.35 with no statistical difference between AML cases and controls (p-value = 0.4) (Table 1). AML cases were stratified into high- and low- expression groups based on median *CALCRL* gene expression according to [14]. To determine the possible association between *CALCRL* expression levels and the clinical/laboratory features or prognosis of AML patients, we compared high and low-expression groups. There were no statistically significant differences encountered between *CALCRL*high and *CALCRL*low groups regarding their demographic data or clinical characteristics. Basic laboratory features

Table 1. *CALCRL* Gene Expression in AML Cases and Controls

| Groups | Minimum | Maximum | Mean±SD | Median | P value |
|---|---|---|---|---|---|
| Controls | 0.26 | 3.44 | 1.72 ± 1.04 | 1.35 | 0.4 |
| AML cases | 0.11 | 104.11 | 11.46 ± 20.39 | 2.1 | |

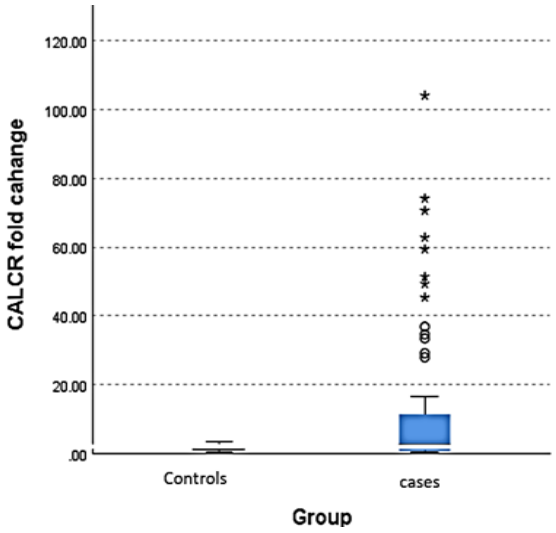


Figure 1. *CALCRL* Gene Expression in AML Cases and Controls

were similar in the two groups, including white blood cell counts, hemoglobin, platelet levels, peripheral blood and bone marrow blast percentages, and molecular and cytogenetic studies. Immunophenotypic analysis revealed that FAB-M2 AML patients had high *CALCRL* gene expression, while FAB-M4 cases had low *CALCRL* gene expression, yet the difference didn't reach a statistical significance (P value = 0.06) (Table 2, 3 and 4).

Regarding the therapeutic regimen, seventy-five patients received induction by 3 and 7 protocol, 3 patients received induction by LDAC and 2 cases died before induction therapy. Early death (death before induction + death before day 28) occurred in 23/78 (29.5%) patients. Complete remission was achieved in 41/78 (52.5%) patients. Twenty-seven patients (34.6%) attained CR after the first course of induction, while 14 patients (17.9%) achieved CR after re-induction. Seventeen patients (21.8%) were refractory to treatment, while twenty-two patients (28.2%) relapsed. Relapse was diagnosed in AML patients

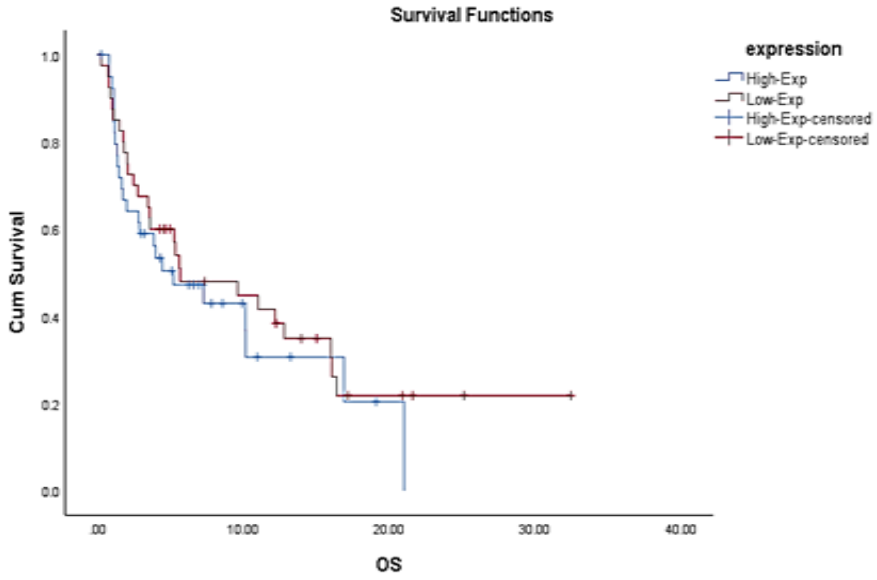


Figure 2. The Relation between *CALCRL* Gene Expression and Overall Survival (OS).

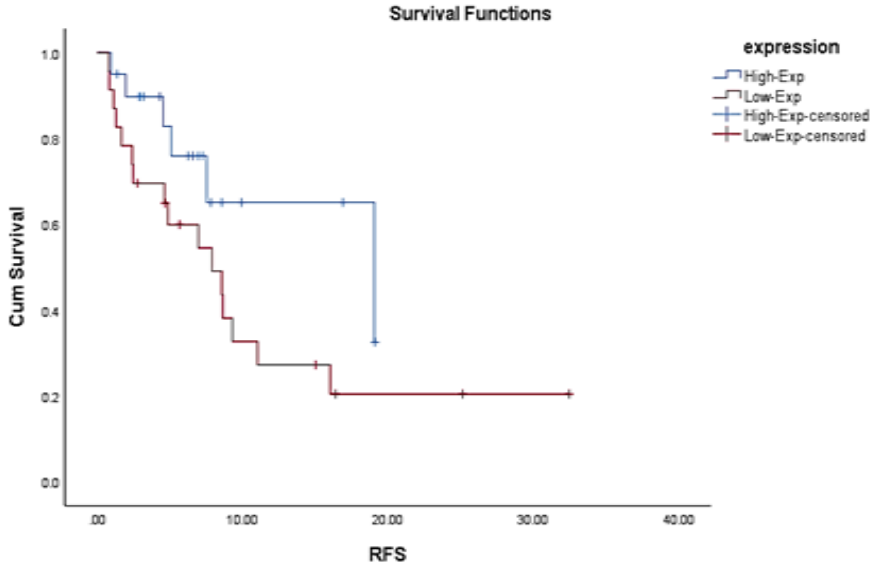


Figure 3. The Relation between *CALCRL* Gene Expression and Relapse-Free Survival (RFS).

Table 2. Comparison between the Clinical Characteristics of *CALCRL* High and Low Expressers

| Item | | *CALCRL*$^{high}$ expressers (n=40) | *CALCRL*$^{low}$ expressers (n=40) | | P value |
|---|---|---|---|---|---|
| Gender (male/Female) | | 32/8 | 30/10 | | 0.59 |
| Hepatomegaly | | 8 (57.1%) | 6 (42.9%) | | 0.56 |
| Splenomegaly | | 7(58.3%) | 5 (41.7%) | | 0.53 |
| Lymphadenopathy | | 8(50.0%) | 8 (50%) | | 1 |
| Diabetes | | 5(50.0%) | 5 (50%) | | 1 |
| Hypertension | | 6 (60.0%) | 4 (40.0%) | | 0.5 |
| B symptoms | | 9 (47.4%) | 10 (52.6%) | | 0.79 |
| Anemic manifestations | | 33 (53.2%) | 29 (46.8%) | | 0.28 |
| Bleeding tendency | | 4 (40.0%) | 6 (60.0%) | | 0.5 |
| Infection | | 2(40.0%) | 3(60.0%) | | 0.64 |
| | | Response to therapy | | | |
| Complete remission (CR) | no | 21 (26.5%) | 18(22.5%) | | 0.82 |
| | yes | 19(23.7%) | 22(27.5%) | | |
| Refractory | no | 6(17.6%) | 7(20.6%) | | 0.9 |
| | yes | 10(29.4%) | 11(32.4%) | | |
| Relapse | no | 13 ( 31.7 %) | 6 ( 14.6 %) | | 0.025 |
| | yes | 6 ( 14.6 %) | 16 ( 39 %) | | |
| | | Overall survival (OS) | | | |
| | Number of events | Cumulative survival at (1 year) | Cumulative survival at (2 years) | Median survival | p-value |
| Total Cohort (n=80) | | 0.325 | 0.1 | 5.6 | |
| *CALCRL*$^{low}$ expressers (n=40) | 27 | 0.416 | 0.218 | 5.67 | 0.49 |
| *CALCRL*$^{high}$ expressers (n=40) | 25 | 0.3 | 0.228 | 5.2 | |
| | | Relapse-free survival (RFS) | | | |
| Total Cohort (n=43) | 22 | 0.385 | 0.248 | 0.83 | |
| *CALCRL*$^{low}$ expressers (n=23) | 16 | 0.272 | 0.204 | 7.9 | 0.11 |
| *CALCRL*$^{high}$ expressers (n=20) | 6 | 0.615 | NA | 19.067 | |

who have achieved CR, then had increased bone marrow blasts to be ≥5%, reappearance of blasts in peripheral blood, or development of extramedullary disease [15]. The median follow-up period of the whole study was 4.2 months ranging from 2 to 24 the median overall survival of the whole group is 5.6 months with cumulative survival of 32.5 % at one year and 10% at 2 years. OS was higher in *CALCRL*low compared to *CALCRL*high expressers, yet the difference was statistically insignificant. There was no statistical difference encountered between *CALCRL*high and *CALCRL*low expressers regarding their complete remission rate (CR), and relapse-free survival.

## Discussion

Acute Myeloid Leukemia is a genetically diverse clonal cancer that arises from clonal hematopoietic stem cells, characterized by chromosomal abnormalities, recurrent gene mutations, epigenetic chromatin alterations, and microRNA deregulations [16]. Among the numerous variables that have an impact on therapy effectiveness for AML patients, genomic heterogeneity, patient individual variability, and gene alterations are a few key roadblocks [17]. Based on gene expression data and their abilities to stimulate proliferation, migration, invasiveness, angiogenesis, and (inhibition of apoptosis) to inhibit apoptosis and antitumor immune responses, *CALCRL* and its ligands (CGRP or ADM) have also been implicated in the pathogenesis of various malignant diseases [10]. Genetic or pharmacologic inhibition of CGRP or ADM signaling reduced tumor-related properties in vitro and in animal models [18, 19]. CGRP stimulated proliferation and inhibited apoptosis of hematopoietic cells in vitro [20]. An increasing number of mRNAs have been demonstrated to be potential prognostic biomarkers in AML. Several studies focused on *CALCRL* gene expression levels and its association with remission rates, and overall, and event-free survival of AML patients [21].

The current work aimed at estimating *CALCRL* gene expression in a cohort of newly diagnosed, adult, Egyptian AML cases to clarify its association with the clinicopathological feature of the disease and to verify its role as a molecular prognostic marker. *CALCRL* gene expression levels in AML patients varied significantly, with a median value of 2.1, compared to healthy controls' median of 1.35. This suggests that *CALCRL* may play a role in the pathophysiology of AML, although the lack of significant statistical difference (p-value = 0.4) indicates that its expression solely may not be a definitive biomarker for diagnosis. Angenendt et al. [9], Wang et al. [14], and

Table 3. Comparison between the Laboratory Data of *CALCRL* High and Low Expressers at Presentation

| Item | | *CALRL*$^{high}$ expressers (no.=40) | | *CALCRL*$^{low}$ expressers (no.=40) | P value |
|---|---|---|---|---|---|
| Age (years) | <43 | 19 | | 21 | 0.66 |
| (median=43.5 ys) | ≥ 43 | 21 | | 19 | 0.66 |
| Hemoglobin level (gm/dL) | Median (mean±SD) | 8.05 (8.28 ± 2.06) | | 7.75 (7.83 ± 1.97) | 0.26 |
| Total leucocytic count (x10$^9$/L) | Median (mean±SD) | 21.5 (47.17 ± 66.73) | | 34.5 (64.88 ± 89.51) | 0.32 |
| Platelet count (x10$^9$/L) | Median (mean±SD) | 39 (51.9 ± 52.24) | | 54.5 (64.9 ± 50.39) | 0.27 |
| Peripheral blood blasts (%) | Median (mean±SD) | 58 (53.55 ± 26.79) | | 58 (53.55 ± 26.79) | 0.32 |
| Bone marrow blasts (%) | Median (mean±SD) | 69 (64.78 ± 20.73) | | 69.5 (63.06 ± 22.65) | 0.83 |
| FAB classification | M0 | 2 | | 0 | 0.06 |
| | M1 | 7 | | 7 | |
| | M2 | 21 | | 13 | |
| | M4 | 7 | | 18 | |
| | M5 | 3 | | 2 | |
| CD13 | Negative | 8 | | 9 | 0.79 |
| | Positive | 32 | | 31 | |
| CD34 | Negative | 15 | | 12 | 0.48 |
| | Positive | 25 | | 28 | |
| HLA-DR | Negative | 14 | | 16 | 0.64 |
| | Positive | 26 | | 24 | |
| CD33 | Negative | 7 | | 3 | 0.18 |
| | Positive | 33 | | 37 | |
| CD4 | Negative | 38 | | 36 | 0.4 |
| | Positive | 2 | | 4 | |
| CD117 | Negative | 10 | | 12 | 0.62 |
| | Positive | 30 | | 28 | |
| CD11C | Negative | 31 | | 25 | 0.14 |
| | Positive | 9 | | 15 | |
| CD64 | Negative | 29 | | 25 | 0.34 |
| | Positive | 11 | | 15 | |
| *FLT3-ITD* mutation (n=58) | Negative | 23 | 19 | | 0.67 |
| | Positive | 7 | 9 | | |
| *NPM1* mutation (n=8) | Negative | 4 | 3 | | 0.55 |
| | Positive | 1 | 0 | | |
| Karyotyping (n=78) | Abnormal | 16 | 18 | | 0.9 |
| | Normal | 23 | 21 | | |
| t(8;21) (n=79) | Negative | 36 | 36 | | 0.59 |
| | Positive | 4 | 3 | | |
| Inv16 (n=79) | Negative | 39 | 38 | | 0.6 |
| | Positive | 1 | 1 | | |

Gluexam et al. [10] reported that *CALCRL* gene expression was significantly higher in AML patients at diagnosis compared to normal BM samples. This could be attributed to difference in sample size, AML subtypes or the ethnic difference between the studied populations.

By stratifying AML patients into high and low-expression groups based on median *CALCRL* expression [14], we aimed to explore correlations between *CALCRL* gene expression and various clinical/laboratory features. However, results showed no statistically significant differences in demographic or clinical characteristics, laboratory data, cytogenetic and molecular results. This agrees with the study of Wang et al. [14] as they could not find a significant association between *CALCRL* gene expression and demographic data, laboratory findings, or *FLT3* mutation status among their cohort. Moreover, Angenendt and colleagues [9] reported that *CALCRL* expression was not associated with age, gender, French-American-British classification type, involvement of the central nervous system, myeloid sarcoma, leucocytic

count, or peripheral blast count. However, they reported that higher *CALCRL* transcript levels were significantly associated with older age, higher BM blast counts, and lower LDH levels.

High *CALCRL* expression was evident among FAB-M2 AML patients, while low *CALCRL* expression was evident among AML cases with monocytic differentiation (FAB M4). This is in agreement with the study of Angenendt and co-workers [9] as high *CALCRL* expression was associated with immature cytomorphology (FAB M0/M1), while low *CALCRL* expression was associated with monocytic differentiation (FAB M4/M5). Their study reported that high *CALCRL* expression correlated with HSC and LSC gene expression signatures, and *CALCRL* levels were higher in immature than in mature myeloid cells. Collectively, these findings point toward the role of *CALCRL* in HSCs and LSCs and suggest that high *CALCRL* expression indicates an AML phenotype at a more undifferentiated stage [9].

As for the molecular and cytogenetic status of the studied cohort, there was no statistically significant difference in the distribution of *FLT3*-ITD and NPM1 mutations between high and low *CALCRL* expressers. This goes with the study of Angenendt et al. [9] who reported that there was no association between *CALCRL* expression and *FLT3*-ITD or NPM1 mutations when analyzed separately. However, NPM1mut/*FLT3*-ITDneg/low status was significantly higher in AML patients with low *CALCRL* levels, whereas higher *CALCRL* levels co-occurred with NPM1wt/ *FLT3*-ITDhigh status. The study of Wang et al. [14] stated that *CALCRL* expression did not associate with the *FLT3*-ITD status of their AML cohort. In the present work, there was no statistical difference in the frequency of t(8;21) and inv (16)/t(16;16) between low and high *CALCRL* expressers. On the other hand, the study of Angenendt et al. [9] revealed that the frequency of t(8;21) and inv (16)/t(16;16) was significantly higher in low expressers compared to intermediate and high expressers. Furthermore, t(8;21) was not detected in high *CALCRL* expressers in their cohort.

The outcome of acute myeloid leukemia is heterogeneous, with both patient-related and disease-related factors contributing to an individual patient's likelihood of achieving a response to therapy and long-term survival (Short et al., 2018). Chemotherapy is the main treatment option for AML, however, acquired resistance of leukemic cells to chemotherapeutic agents often leads to difficulties in AML treatment and disease relapse. In the current study, the survival analysis revealed that OS was higher in *CALCRL*low compared to *CALCRL*high expressers, yet the difference was statistically insignificant. Moreover, there was no statistical difference encountered between *CALCRL*high and *CALCRL*low expressers regarding their complete remission rate (CR), and relapse-free survival. The study of Angenendt and colleagues (2019) stated that high *CALCRL* expression was associated with low complete remission rates, 5-year overall, and event-free survival. Additionally, Wang et al. [14] reported that patients with less *CALCRL* expression achieved CR after the first course of chemotherapy more rapidly and there was a negative correlation between *CALCRL* expression level and patient survival time. Larrue et al. [12] reported that increasing protein levels of *CALCRL* was associated with decreasing complete remission rates, inferior 5-year overall survival, and event-free survival.

Relapse-associated gene expression signature was significantly enriched for gene expression profiles defining AML LSCs and/or linked to poor outcomes in AML, confirming the notion that genes deregulated at relapse are related to stemness and chemotherapy resistance. One of the top up-regulated genes in the relapse signature was *CALCRL* [22]. Drug tolerant/resistant leukemic stem cell (LSC) subpopulations may explain frequent relapses in acute myeloid leukemia (AML), suggesting that these relapse-initiating cells (RICs) persistent after chemotherapy represent bona fide targets to prevent drug resistance and relapse. Larrue et al. [12] stated that *CALCRL* was expressed in RICs and that the overexpression of *CALCRL* and/or of its ligand adrenomedullin (ADM), and not CGRP, correlates to adverse outcomes in AML. Both the prognostic significance of *CALCRL* mRNA and protein expression at diagnosis and the upregulation of *CALCRL* at relapse were confirmed independently and suggest a possible role for this gene in both primary and relapse-associated therapy resistance [1]. Additionally, Grandits et al. [23] confirmed the expression, and the upregulation at relapse, of *CALCRL* in primary human AML cells. On the contrary (Surprisingly), the current study revealed that relapse rate with associated with low *CALCRL* expression.

In our cohort, the median age of AML patients at diagnosis was 43 years. Studying the combined effect of other prognostic markers with *CALCRL* gene expression as patients' age on overall survival (OS) and relapse-free survival revealed that OS was significantly higher among *CALCRL* low expressers, while relapse-free survival was significantly higher in *CALCRL* high expressers younger than 43 years old. The combined effect of *CALCRL* gene expression and gender, laboratory findings, and molecular and cytogenetic results on relapse-free survival were statistically insignificant (P value >0.05).

High *CALCRL* expression is an independent prognostic parameter for poor outcomes of AML in several publicly available data sets comprising patient populations with different genetic and age compositions [1, 9, 24], with some overlap and some divergence regarding the data sets used in each of these studies. *CALCRL* was consistently up-regulated at relapse of AML, a disease stage characterized by increased chemotherapy resistance as compared to its level at diagnosis [1, 9]. Genomic data were translated into precision medicine-based therapeutic approaches for categorizing and treating patients with AML and are driving a deeper evaluation of new therapies targeting specific genetic lesions [25]. Identifying more effective biomarkers predictive of treatment success and failure is essential for informing tailored therapeutic decisions [1]. *CALCRL* may represent a novel therapeutic target in AML as its expression correlates with chemotherapy resistance [10, 24]. Antibodies targeting

*CALCRL* signaling have been approved for the preventive treatment of migraine [26]. Moreover, several clinically fully developed agents are available and tested for their abilities to reduce chemotherapy resistance and stemness in *CALCRL*-positive AML [10]. In conclusion, *CALCRL* gene expression levels may hold clinical relevance and contribute to the prognostic assessment of AML patients. . Further studies with larger cohorts and for longer follow-up periods are more robust are warranted to gain deeper insight into its functional role in leukemogenesis as well as its role as a molecular prognostic marker and a future therapeutic target for AMLThe ongoing exploration of gene expression profiles concerning clinical outcomes may ultimately provide critical insights into improving AML management.

## Author Contribution Statement

All authors contributed equally in this study.

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## References

1-Vetrie D, Helgason G, Copland M. The leukemia stem cell: similarities, differences and clinical prospects in CML and AML. Nat Rev Cancer. 2020; 20: 158–173.

2-Wagner S, Vadakekolathu J, Tasian S.K, Altmann H, Bornh¨auser M, Pockley A.G, Ball G.R, Rutella S. A parsimonious 3-gene signature predicts clinical outcomes in an acute myeloid leukemia multicohort study. Blood Adv. 2019; 3, 1330–1346.

3-Ningombam A, Verma D, Kumar R, Singh J, Shadab Ali M, Pandey A.K, et al. Refinement of Risk-Stratification of Cytogenetically Normal Acute Myeloid Leukemia Adult Patients by MN1 Expression. Asian Pac J Cancer Prev, 2024; 25 (7), 2283-2289.

4- Bohl S.R, Bullinger L, Rucker F. New targeted agents in acute myeloid leukemia: New hope on the rise. Int J Mol Sci . 2019 Apr 23;20 (8):1983.

5- Hauser, A.S. et al. Trends in GPCR drug discovery: new agents, targets and indications. Nat. Rev. Drug Discov. 2017;16, 829–842.

6- Hay DL, Pioszak AA. Receptor activity-modifying proteins (RAMPs): new insights and roles. Annual review of pharmacology and toxicology. 2016 Jan 6;56: 469-87.

7- Russell F.A, King R, Smillie S.J, Kodji X, Brain S.D. Calcitonin gene-related peptide: physiology and pathophysiology. Physiol. Rev. 2014; 94, 1099–1142.

8- Charles A, Pozo-Rosich P. Targeting calcitonin gene-related peptide: a new era in migraine therapy. Lancet. 2019 Nov 9;394(10210):1765-1774.

9- Angenendt L, Bormann E, Dohlich C.P, Schwöppe C, Bohlander S.K, Alla V, et al. The neuropeptide calcitonin receptor-like (*CALCRL*) is a potential therapeutic target in acute myeloid leukemia. Leukemia. 2019; 33:2830–2841.

10- Gluexam T, Grandits AM, Schlerka A, Nguyen CH, Etzler J, Finkes T, Fuchs M, Scheid C, Heller G, Hackl H, Harrer N. CGRP signaling via CALCRL increases chemotherapy resistance and stem cell properties in acute myeloid leukemia. International journal of molecular sciences. 2019 Jan; 20(23):5826.

11- Ostrovskaya A, Hick C, Hutchinson DS, Stringer BW, Wookey PJ, Wootten D, et al. Expression and activity of the calcitonin receptor family in a sample of primary human high-grade gliomas. BMC Cancer. 2019; 19:157. 10.1186/s12885-019-5369-y

12- Larrue C, Guiraud N, Mouchel P.L, Dubois M, Farge T, Gotan`egre M, Bosc C, Saland E, Nicolau-Travers M.L, Sabatier M, Serhan N, Sahal A, Boet E, Mouche S, Heydt Q, Aroua N, Stuani L, Kaoma T, Angenendt L, Mikesch J. H, Schliemann C, Vergez F, Tamburini J, R´echer C, Sarry J.E. Adrenomedullin-CALCRL axis controls relapse-initiating drug tolerant acute myeloid leukemia cells. Nat. Commun. 2021; 12, 422.

13- Huang Z, Zhang H, Xing C, Zhang L, Zhu H, Deng Z, et al. Identification and validation of CALCRL-associated prognostic genes in acute myeloid leukemia. Gene. 2021; 809, 146009.

14- Wang R, Li M, Bai Y, Jiao Y, Qi X. CALCRL Gene is a Suitable Prognostic Factor in AML/ETO+ AML Patients. J Oncol. 2022; 16:3024360. Identification and validation of CALCRL-associated prognostic genes in acute myeloid leukemia.

15- Thol F. & Heuser M. Treatment for Relapsed/Refractory Acute Myeloid Leukemia. HemaSphere. 2021; 5(6):p e572.

16- Moualla Y, Moassass F, AL-Halbi B, Al –Achkar W, Georgeos M, Yazigi H, et al. Prognostic Relevance of DNMT3A, *FLT3* and NPM1 Mutations in Syrian Acute Myeloid Leukemia Patients. Asian Pac J Cancer Prev, 2022; 23 (4), 1387-1395.

17- Döhner H, Estey E, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. Blood, 2017;129, 424-47

18- Vazquez R, Riveiro M, Berenguer-Daize C, et al. Targeting adrenomedullin in oncology: a feasible strategy with potential as much more than an alternative anti-angiogenic therapy. Front Oncol. 2020; 10 589218 [PubMed: 33489885]

19- Dallmayer M, Li J, Ohmura S, et al. Targeting the CALCB/RAMP1 axis inhibits the growth of Ewing sarcoma. Cell Death Dis. 2019; 10: 116. [PubMed: 30741933]

20- Liu C, Chen X, Jin Y, et al. Effects of calcitonin gene-related peptide on apoptosis of peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Clin Exp Dermatol. 2007; 32: 650–653. [PubMed: 17725664]

21- Sha k., Lu Y., Zhang P., Pei R., Shi X., Fan Z., et al. Identifying a novel 5 gene signature predicting clinical outcomes in acute myeloid leukemia. Clinical and Translational Oncology 2020. 23(3):648-656. https://doi.org/10.1007/s12094-020-02460-1

22- Hackl H, Steinleitner K, Lin, K, Hof, S, Tosi, N, Pavlovic S, Suvajdzic N, Si, H, Wie, R. A gene expression profile associated with relapse of cytogenetically normal acute myeloid leukemia is enriched for leukemia stem cell genes. Leuk. Lymphoma 2015, 56, 1126–1128.

23- Grandits AM, Wieser R. Gene expression changes contribute to stemness and therapy resistance of relapsed acute myeloid leukemia: roles of SOCS2, CALCRL, MTSS1, and KDM6A. Experimental hematology. 2021 Jul 1;99:1-1.

24- Tang, Shanhaoa; Li, Shuangyueb; Shi, Xiaoweib; Sheng, Lixiaa; Mu, Qitiana; Wang, Yia; Zhu, Huilinga; Xu, Kaihonga; Zhou, Miaoa; Xu, Zhijuana; Wu, Ana; Ouyang, Guifanga: CALCRL induces resistance to daunorubicin in acute myeloid leukemia cells through upregulation of XRCC5/TYK2/JAK1 pathway. Anti-Cancer Drugs 35(2):p 163-176, February 2024. | DOI: 10.1097/CAD.0000000000001547

25- Bullinger L, Do¨ hner K, Do¨ hner H. Genomics of acute myeloid leukemia diagnosis and pathways. J Clin Oncol.

2017;35(9): 934-946.
26- Yuan H, Spare N, Silberstein S. Targeting CGRP for the prevention of migraine and cluster headache: A narrative review. Headache 2019, 59, 20–32.