TERT rs2853669 as a predictor for overall survival in patients with acute myeloid leukaemia

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Abstract

Introduction: the aim of the study was to investigate the contribution of TERT rs2736100 and rs2853669 gene polymorphisms in defining the genetic predisposition to acute myeloid leukaemia (AML), their association with different prognostic markers, and their impact on survival, outcome, and the prognosis of affected patients. Also, we investigated the association of TERT SNPs in AML in the presence or absence of DNMT3A (R882), NPM1, and FLT3 mutations.

Material and methods: A total of 509 participants were enrolled in our study, consisting of 146 AML patients and 363 healthy participants, with no history of malignancy. TERT rs2736100 and rs2853669 polymorphisms were genotyped by using TaqMan SNP genotyping assay FLT3 (ITD, D835), DNMT3A (R882), and NPM1 c.863_864insTCTG (type A) mutations were analised in each AML case.

Results: TERT rs2736100 and rs2853669 were not associated with AML risk in the codominant, dominant, recessive, or allelic models. Multivariate Cox regression showed that TERT rs2853669 was a significant predictor for overall survival in AML patients. After adjusting for age, gender, cytogenetic risk group, ECOG status, FLT3, DNMT3A, NPM1 mutation, AML subtype, and treatment, the estimated adjusted hazard ratio (HR adjusted = 1.54, 95% CI: 1.01–2.35) showed that the TERT rs2853669 variant genotype had a negative influence on survival time.

Conclusions: TERT rs2853669 and rs2736100 polymorphisms were not risk factors for developing AML in the Romanian population, but the TERT rs2853669 variant genotype had a negative effect on AML patients' overall survival in the presence of other known prognostic factors.

Key words: acute myeloid leukaemia, overall survival, predictor, TERT gene polymorphism.

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Introduction

Acute myeloid leukaemia (AML), the most common acute leukaemia in adults, is associated with short survival and is characterized by specific genomic abnormalities, for instance chromosomal aberrations and DNA mutations with prognostic impact, such as *FLT3* (fms-like tyrosine kinase 3), *NPM1* (Nucleophosmin 1) *CEBPA,* and recently *DNMT3A* (DNA methyltransferase 3a) R882 mutations [1–4].

Telomerase reverse transcriptase (TERT) protein, encoded by the *TERT* gene, plays an important role in maintaining telomere stability and its length, and therefore it helps in preserving chromosomal stability [5]. Associations between telomere length, disease progression, overall survival (OS), and prognosis have already been reported in haematological cancers [5–7].

Previous studies revealed that the presence of single nucleotide functional polymorphisms (SNPs) in the human *TERT* gene may affect its expression and telomerase activity, and may contribute to the development of cancer as well as the outcome of chemotherapy [8–10]. We previously reported that *TERT* rs2736100 predisposes to all BCR-ABL-negative myeloproliferative neoplasms (MPN), regardless of the clinical or molecular subtype [10]. Similarly, some *TERT* promoter SNPs were associated with an increased risk of developing haematological malignancies like acute lymphoblastic leukaemia [11] and chronic lymphocytic leukemia [12]. A meta-analysis showed that rs2853669 alone did not increase or decrease the overall cancer risk or influence the prognosis [13]. Conflicting results in the meta-analyses [9, 13] may be due to ethnicity and pathology selection, as they contained only one or two studies on AML.

Recently, two *TERT* gene SNPs, namely rs2736100 and rs2853669, previously associated with myeloproliferative neoplasms [10, 14–16], emerged as possible predisposing factors to AML and as prognostic markers for survival [17, 18]. In contrast, Krahling *et al.* [19] did not find *TERT* rs2736100 to represent a susceptibility factor for chronic myeloid leukaemia and AML cases in the Hungarian population.

Whereas these studies are the only ones that examined the association between rs2736100 and rs2853669 in *TERT* gene and the risk of AML, additional studies are necessary to investigate this possible association in groups of various ethnic origins.

We aimed to investigate the contribution of *TERT* rs2736100 and rs2853669 SNPs in defining the genetic predisposition to AML in Romanian patients – an Eastern European population – their associations with different prognostic markers, and their impact on survival, outcome, and prognosis of patients. In addition, we analysed the as-sociation of *TERT* SNPs in AML in the presence or absence of *DNMT3A*, *NPM1*, and *FLT3* mutations.

Material and methods

Patients and controls

Our study comprised 509 participants (240 males, 269 females), consisting of 146 AML patients and 363 healthy participants. The present study included consecutive AML patients from the central region of Romania. Samples were collected at the moment of diagnosis at admission in the Haematology Clinics from Tîrgu Mureş, Romania before starting chemotherapy. The inclusion criteria were as follows: patients diagnosed with AML, adults (> 18 years old), and having given written informed consent. Briefly, the therapy protocols used for AML induction were the "5+2", "7+3" regimen and all-trans-retinoic acid (ATRA) for LAM M3 (acute promyelocytic leukaemia). The doses were adjusted mainly according to the patient's age (standard or low dose).

The controls were unrelated healthy individuals, from the same geographic area, without any malignancy, and they were included in control group in parallel with the patients. Controls were referred to the Emergency County Hospital from Tîrgu Mureş for investigation, mainly due to anaemia or leukocytosis. The 2 groups of subjects had a similar gender distribution.

Our study was approved by the Ethical Committee of the University of Medicine and Pharmacy of Tîrgu Mureş, Romania and was performed in accordance with the Declaration of Helsinki.

Methods

Genomic DNA (gDNA) was extracted from fresh blood using DNA Quick-gDNA MiniPrep (ZymoResearch) and PureLink Genomic DNA Mini (Invitrogen) kits.

TERT rs2736100 (c.1574-3777G>T) and rs2853669 (c.-245T>C) polymorphisms were genotyped by TaqMan SNP genotyping assays (c_1844009_10, c_8773290_10) from ThermoFisher Scientific according to the manufacturer's instructions and run on a 7500 Fast Dx Real-Time PCR Instrument (Applied Biosystems).

FLT3 (ITD, D835 c.2503G>A/C/T, c.2504A>C/T), *DNMT3A* (R882 c.2644C>A/G/T and C.2645G>A), and *NPM1* (c.863_864insTCTG, type A) mutation status was analysed in each AML case as previously reported [20–22]. Randomly, in 10% of AML cases, *FLT3* and *DNMT3A* were also investigated by HRM (in-house method) while *NPM1* c.863_864insTCTG mutation was analysed by castPCR.

Statistical analysis

Quantitative continuous variables were expressed as mean (± standard deviation) for Gauss-

ian distribution and as median and interquartile range (percentile 25%; percentile 75%) for data with deviations from normal distribution of probability. Qualitative variables were described by absolute frequencies (number of cases) and relative frequencies (%). The χ^2 test was used in bivariate analysis of associations between qualitative variables. The impact of *TERT* rs2736100 and rs2853669 polymorphisms on AML risk was tested using logistic regression analysis, the size effect of association being quantified by odds-ratio (OR) and 95% confidence level for OR.

The hypothesis of difference in survival time distribution was investigated by non-parametric Kaplan-Meier method while the univariate and multivariate effects of *TERT* rs2736100 and *TERT* rs2853669 SNPs on OS time in AML patients were tested using Cox regression analysis. The effect of the studied SNPs on OS was estimated adjusting for the following covariates: age group, gender, cytogenetic risk group, ECOG status, *FLT3*, *DNMT3A* or *NPM1* mutation, AML subtype (de novo; secondary or therapy-related), and treatment (high dose of chemotherapy, low dose of chemotherapy, or high dose + haematopoietic stem cell transplantation). Because the type of treatment did not accomplish the condition of proportional hazard ratio, we used a stratified Cox regression analysis. The effect size of association between each of the studied SNPs and OS was quantified by hazard ratio (HR) adjusted according to the above-mentioned factors. Candidate predictors included in multivariable Cox regression models were significant univariate predictors along with all important clinical variables [23], so that the number of predictors (*p*) was lower than m/15, where *m* was the number of deaths in the studied sample [24]. Statistical analysis was performed using the advanced environment for statistical computing R version 3.4.4.

Results

The AML group included 74 females and 72 males, while the control group consisted of 195 females and 168 males. In the investigated groups a similar frequency was observed regarding females and males (*p* = 0.535). The mean age ± SD of studied AML patients at diagnosis was 52.65 ±16.7 years (range: 19–84 years old). In the AML group 84 (57.5%) cases were over 50 years of age. The characteristics of the AML cases included in our study, which consist of demographic, clinical, and biological data and AML classification based on WHO recommendations [25] are presented in Table I.

We included our patients in the following AML subtypes: 112 (76.7%) with de novo AML, 30 (20.5%) with secondary AML (sAML, arising from myelodysplastic syndrome MDS or from MPN), and

4 (2.7%) with therapy-related AML (tAML for patients with prior chemotherapy). *FLT3* mutation was present in 28 of our AML cases, as follows: 23 (15.8%) with ITD mutation, while D835 mutation was present in 8 (5.5%) cases, and 5 AML patients presenting both mutations. Among the AML cases, 74 patients reached remission (15 complete remission CR), 55 cases were chemotherapy resistant, while no response or induction death was observed in 17 patients.

In the AML and control groups the TT, TC, and CC *TERT* rs2853669 genotypes were 59 (40.4%), 65 (44.5%), 22 (15.1%) vs. 166 (45.7%), 162 (44.6%), 35 (9.6%), respectively. For TERT rs2736100 SNP the AA, AC, and CC genotype distributions in AML and healthy controls were as follows: 31 (21.2%), 76 (52.1%), and 39 (26.7%) vs. 95 (26.2%), 191 (52.6%), and 77 (21.2%). None of the *TERT* rs2853669 and rs2736100 genotypes demonstrated deviation from the Hardy-Weinberg equilibrium (HWE) in both AML cases and controls (*TERT* rs2736100: $X^2[2] = 1.10$, *p* = 0.295 in controls, and $X^2[2] = 0.29$, *p* = 0.593 in AML group; *TERT* rs2853669: $X^2[2] = 0.25$, *p* = 0.617 for the control group and $X^2[2] = 0.34$, *p* = 0.558 for the AML group).

In the overall analysis, *TERT* rs2736100 and rs2853669 polymorphisms were not associated with AML risk in the codominant, dominant, and recessive models in our population (*p* > 0.05).

We compared the baseline characteristics of AML patients carrying the variant genotypes for *TERT* rs2736100 (AC or CC) and for *TERT* rs2853669 (TC or CC) and patients with wild-type genotypes (Table II). We found no associations between the investigated SNPs and age, gender, white blood cell (WBC) counts, platelets, haemoglobin, lactate dehydrogenase (LDH) levels, percentage of blasts in bone marrow, and cytogenetic risk groups in our cases at diagnosis. Patients with polymorphic *TERT* rs2736100 (AC or CC) frequently had haemoglobin (Hgb) levels below 10 g/dl, but the difference was not statistically significant (*p* = 0.084). No association was observed between the variant genotypes and response to treatment (complete, partial remission, relapse, chemotherapy resistant, or induction death) or AML subtype. Likewise, we noticed no association between the studied SNPs and Eastern Cooperative Oncologic Group Scale (ECOG) status (*p* > 0.05).

As a next step, we analysed the distribution of the two TERT rs2853669 and rs2736100 polymorphisms taking into account the somatic mutations displayed in *NPM1*, *FLT3*, and *DNMT3A* genes in AML patients. Interestingly, we noticed no association between *NPM1*, *FLT3*, and *DNMT3A* mutation status and variant genotypes of the investigated SNPs. We looked for the effect of the variant gen-

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Table I. Characteristics of AML patients

Variables	Absolute frequencies (relative frequencies %)
Age [years]:	
< 65	103 (70.5)
≥ 65	43 (29.5)
Gender:	
Female	74 (50.7)
Men	72 (49.3)
WBC [× 10^3/mm^3]:	
< 50000	123 (84.2)
≥ 50000	23 (15.8)
PLT [× 10^3/mm^3]:	
< 40000	69 (47.3)
≥ 40000	77 (52.7)
Hgb [g/dl]:	
< 10	99 (67.8)
≥ 10	47 (32.2)
LDH level [IU/l]:	
< 600	71 (48.6)
≥ 600	75 (51.4)
Blasts (in bone marrow, %):	
< 70	86 (58.9)
≥ 70	60 (41.1)
Cytogenetic risk:	
Low risk (favourable prognostic)	16 (11.0)
Intermediate	74 (50.7)
High risk (Adverse)	44 (30.1)
Not available (NA)	12 (8.2)
Normal karyotype[a]	62 (42.5)
French-American-British (FAB) classification:	
M0 – minimally differentiated AML	15 (10.3%)
M1 – AML without maturation	25 (17.1%)
M2 – AML with maturation	34 (23.3%)
M3 – acute promyelocytic leukaemia	10 (6.8%)
M4 – acute myelomonocytic leukaemia	27 (18.5%)
M5 – acute monocytic leukaemia	23 (15.8%)
M6 – erythroleukemia, M7 – megakaryoblastic, unclassified leukaemia	12 (8.2%)

Variables	Absolute frequencies (relative frequencies %)
AML classification based on WHO 2016 [18]:	
AML with recurrent cytogenetic anomalies	33 (22.6%)
AML dysplasia related	44 (30.14%)
Therapy-related myeloid neoplasms	4 (2.74%)
AML not otherwise specified	65 (44.52%)
ECOG performance status:	
≤ 1	21 (14.4)
≥ 2	125 (85.6)
ECOG performance status:	
0	0
1	21 (14.4)
2	18 (12.3)
3	65 (44.5)
4	42 (28.8)
FLT3 status (ITD+D835):	
Wild-type	118 (80.8)
Mutant	28 (19.2)
*DNMT3*A R882 status:	
Wild-type	123 (84.2)
Mutant	23 (15.8)
NPM1 c.863_864insTCTG status:	
Wild-type	125 (85.6)
Mutant	21 (14.4)
Treatment:	
High dose	79 (54.1)
Low dose	51 (34.9)
High dose + haematopoietic stem cell transplantation	16 (11.0)
Response:	
Complete remission	15 (10.3)
Partial remission	24 (16.4)
Relapse	35 (24.0)
Resistant	55 (37.7)
No response/Induction death	17 (11.6)

[a]*Patients with normal karyotype were included in the intermediate-cytogenetic-risk group, ECOG – Eastern Cooperative Oncologic Group Scale.*

otype of the investigated SNPs in the presence of at least 1 mutation (*DNMT3A, FLT3, NPM1*), 2 mutations (*FLT3 + NPM1, FLT3 + DNMT3A, DNMT3A + NPM1*), or all 3 somatic mutations in AML cases, but no association was observed ($p > 0.05$ for all comparison performed; data not shown).

Table II. Comparison of clinical data between *TERT* rs2853669 and rs2736100 wild-type and variant genotypes in AML patients

Variables	TERT rs2736100 AA	TERT rs2736100 AC + CC	*P*-value	TERT rs2853669 TT	TERT rs2853669 TC + CC	*P*-value
Age [years]:						
< 65	21 (67.7)	82 (71.3)	0.699	40 (67.8)	63 (72.4)	0.548
≥ 65	10 (32.3)	33 (28.7)		19 (32.3)	24 (27.6)	
Gender:						
Female	14 (45.2)	60 (52.2)	0.488	28 (47.5)	46 (52.9)	0.521
Male	17 (54.8)	55 (47.8)		31 (52.5)	41 (47.1)	
WBC [× 10³/mm³]:						
< 50000	27 (87.1)	96 (83.5)	0.624	50 (84.7)	73 (83.9)	0.892
≥ 50000	4 (12.9)	19 (16.5)		9 (15.3)	14 (16.1)	
PLT [× 10³/mm³]:						
< 40000	13 (41.9)	56 (48.7)	0.503	26 (44.1)	43 (49.4)	0.525
≥ 40000	18 (58.1)	59 (51.3)		33(55.9)	44 (50.6)	
Hgb [g/dl]:						
< 10	17 (54.8)	82 (71.3)	0.084	40 (67.8)	59 (67.8)	0.998
≥ 10	14 (45.2)	33 (28.7)		19 (32.2)	28 (32.2)	
LDH level [IU/l]:						
< 600	17 (54.8)	54 (47.0)	0.436	32 (54.2)	39 (44.8)	0.264
≥ 600	14 (45.2)	61 (53.0)		27(45.8)	48 (55.2)	
Blasts (in bone marrow, %):						
< 70	17 (54.8)	69 (60.0)	0.604	35 (59.3)	51 (58.6)	0.933
≥ 70	14 (45.2)	46 (40.0)		24 (40.7)	36 (41.4)	
Cytogenetic risk:						
Low risk	1 (3.2)	15 (13.0)	0.446	6(10.2)	10 (11.5)	0.845
Intermediate risk	16 (51.6)	58 (50.4)		29 (49.2)	45 (51.7)	
High risk	11 (35.5)	33 (28.7)		20 (33.9)	24 (27.6)	
Not available (NA)	3 (9.7)	9 (7.8)		4 (6.8)	8 (9.2)	
ECOG performance status:						
1	4 (12.9)	17 (14.8)	0.962	7 (11.9)	14 (16.1)	0.793
2	4 (12.9)	14 (12.2)		7 (11.9)	11 (12.6)	
3	15 (48.4)	50 (43.5)		29 (49.2)	36 (41.4)	
4	8 (25.8)	34 (29.6)		16 (27.1)	26 (29.9)	
***FLT3* status (ITD + D835):**						
Wild-type	25 (80.6)	93 (80.9)	0.978	45 (76.3)	73 (83.9)	0.250
Mutant	6 (19.4)	22 (19.1)		14 (23.7)	14 (16.1)	
***DNMT3A* status:**						
Wild-type	26 (83.9)	97 (84.3)	1.00	47 (79.7)	76 (87.4)	0.210
Mutant	5 (16.1)	18(15.7)		12 (20.3)	11 (12.6)	
***NPM1* status:**						
Wild-type	28 (90.3)	97 (84.3)	0.567	51 (86.4)	74 (85.1)	0.815
Mutant	3 (9.7)	18 (15.7)		8 (13.6)	13 (14.9)	

P-value obtained from chi-square or Exact Fisher's tests.

The results of Kaplan-Meier analysis (Table III) showed that the distribution of survival time was different for the following factors: age, cytogenetic risk, treatment, and *FLT3* mutation, respectively. We found no significant differences in survival time regarding *TERT* rs2736100 or rs2853669 genotypes in AML, nor for *NPM1* type A, *DNMT3A* R882 mutation, and AML subtypes (de novo, sAML,

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Table III. Univariate analysis of overall survival time in patients with AML: Kaplan-Meier method

Factors	Median of overall survival time (95% CI)	*P*-value*
Age category:		
< 65 years	9.0 (7.3–10.7)	
≥ 65 years	3.0 (1.6–4.4)	< 0.001
< 50 years	10.0 (8.0–11.9)	
≥ 50 years	5.0 (3.1–6.9)	0.001
Cytogenetic risk:		
Low risk (favourable prognostic)	10.0 (4.7–15.3)	
Intermediate	8.0 (6.2–9.8)	
High risk (adverse)	3.0 (1.5–4.5)	0.001
Treatment:		
High dose	8.0 (6.2–9.8)	
Low dose	4.0 (2.5–5.4)	
High dose + haematopoietic stem cell transplantation	12.0 (9.1–14.9)	< 0.001
FLT3 mutation (ITD + D835):		
Negative	8.0 (6.7–9.3)	
Positive	2.0 (0.0–4.3)	0.001
NPM1 type A mutation:		
Negative	7.0 (5.7–8.3)	
Positive	5.0 (1.9–8.0)	0.310
DNMT3A mutation:		
Negative	8.0 (6.8–9.2)	
Positive	4.0 (2.3–5.7)	0.290
TERT rs2736100:		
AA	8.0 (4.8–11.2)	
AC	5.0 (1.9–8.0)	
CC	8.0 (5.5–8.5)	0.108
TERT rs2853669:		
TT	8.0 (6.4–9.6)	
CT	4.0 (1.6–6.4)	
CC	9.0 (5.8–12.2)	0.315

Estimated significance level obtained from log-rank test.

tAML). Furthermore, we investigated the survival time according to the ECOG status and we noticed a shorter survival in patients with ECOG 4 status, the OS median being 5 months (95% CI: 0.8–9.2).

The results of multivariate Cox regression showed that *TERT* rs2853669 SNP was a significant predictor for overall survival in AML patients (Table IV). After adjusting for the main prognostic factors in AML, such as age, gender, cytogenetic risk group, *FLT3*, *DNM3A* or *NPM1* mutations, AML subtype, and treatment, the estimated adjusted hazard ratio (HR adjusted = 1.54, 95% CI: 1.01–2.35) showed that the *TERT* rs2853669 variant genotype (CC + CT) had a negative influence on survival time. We evaluated the predictive accuracy of Cox-regression-tested models. Based on the concordance index (C-index), all regression-tested models demonstrated a good discriminant accu-

racy, with an estimated value of the concordance index between 0.70 and 0.80.

Discussion

In our study, we analysed the association of rs2853669 and rs2736100 in the *TERT* gene with the risk of developing AML, in relation to clinical and prognostic markers and to OS in AML. Although there was no significant difference between *TERT* rs2853669 and rs2736100 SNPs variant alleles or genotypes in AML cases, an increasing *TERT* rs2853669 homozygous variant frequency trend was noted ($p = 0.067$).

Our results are different from those reported earlier in South-Eastern Swedish AML patients [17], where the variant CC genotype of *TERT* rs2853669 was strongly associated with an increased risk for AML ($p = 0.00015$) and with

Table IV. Associations between *TERT* SNPs and overall survival of AML patients

Gene polymorphisms	Patients	Death	MST	HR[a] (95% CI)	*P*-value[b]	Model performance (C-statistics; likelihood ratio test)
TERT rs2736100:						
AA	31 (21.23)	26	8.00	1.00 (Reference)	–	$C = 0.73$; $X^2(10) = 29.79$; $p = 0.0009$
AC	76 (52.05)	69	5.00	1.40 (0.72–0.83)	0.206	
CC	39 (26.71)	31	8.00	0.99 (0.54–1.84)	0.985	
AC + CC	115 (78.77)	100	7.00	1.27 (0.76–2.10)	0.358	$C = 0.72$; $X^2(9) = 27.48$; $p = 0.0012$
AA + AC	107 (73.29)	95	6.00	1.00 (Reference)	–	$C = 0.72$; $X^2(9) = 27.71$; $p = 0.0011$
CC	39 (26.71)	31	8.00	0.77 (0.48–1,22)	0.264	
TERT rs2853669:						
TT	59 (40.41)	49	8.00	1.00 (Reference)	–	$C = 0.73$; $X^2(10) = 34.32$; $p = 0.0002$
CT	65 (44.52)	61	4.00	1.74 (1.12–2.70)	0.014[+]	
CC	22 (15.07)	16	9.00	1.03 (0.52–2.03)	0.932	
CT + CC	87 (59.59)	77	7.00	1.54 (1.01–2.35)	0.043[+]	$C = 0.72$; $X^2(9) = 31.43$; $p = 0.0002$
CT + TT	22 (15.07)	16	9.00	1.00 (Reference)	–	$C = 0.71$; $X^2(9) = 26.98$; $p = 0.0014$
CC	22 (16.42)	16	8.00	0.77 (0.41–1.56)	0.427	

HR – hazard ratio, MST – median survival time (months); [a]Adjusted for age group (≥ 65 years versus < 65 years), gender, ECOG status, treatment, cytogenetic risk group, presence of at least one FTLT3, DNM3A or NMP1 mutation, AML subtype; [b]p-value obtained from stratified multiple Cox-regression; [+]statistical significance (p < 0.05).

a decreased OS [17]. In the same study, *TERT* rs2736100 showed an association with the risk for developing AML (*p* = 0.03), but without any effect on survival in AML. Regarding the relation between rs2736100 and AML overall survival, we observed partially overlapping results with those reported by Mosrati *et al.* [17].

The study performed by Krahling *et al.* [19] on 308 cases with AML (195 de novo cases, 93 myelo-dysplasia-related AML cases, and 20 therapy-related AML cases) revealed no association between *TERT* rs2736100 SNP and AML susceptibility, similarly to what we noticed but in contrast to the results of Mosrati *et al.* [17]. A possible explanation for these discordant results may be the origin of the studied groups: Mosrati *et al.* [17] investigated cases from Northern Europe while Krahling *et al.* [19] analysed individuals from Eastern Europe, as our study did.

Our association analysis revealed no significant correlations between *TERT* rs2853669 and rs2736100 polymorphisms and the risk of AML in our Romanian population, so our findings do not support the hypothesis of their involvement in the aetiology of AML in the Romanian population.

Furthermore, by applying univariate analysis (Kaplan-Meyer method) we found no association between *TERT* rs2853669 or *TERT* rs2736100 variant genotypes and AML subtype, cytogenetic risk group, OS, as well as response to treatment. We found no significant differences regarding survival time (*p* = 0.504) or AML predisposition (*p* = 0.535) between genders in the AML group.

We investigated the combined effect of both TERT rs2853669 and rs2736100 polymorphisms (the presence of both variant genotypes) and noticed no association with the risk of developing AML, and we considered that this association is not a predictor for AML risk (*p* = 0.671). In addition, our findings revealed that the combined variant genotypes of the investigated SNPs did not modify overall survival in AML patients (*p* = 0.223).

We have investigated the OS time in AML and whether *TERT* rs2853669 and rs2736100 were independent prognostic markers for OS in their AML cohort by performing multivariate analysis using Cox regression tested models. Multivariate analysis showed that the variant rs2853669 CT and CT + CC genotypes were predictors for patient survival in AML cases (*p* = 0.014; *p* = 0.043), after adjusting for the main prognostic factors in AML, such as age, gender, cytogenetic risk group, *FLT3*, *DNM3A*, or *NPM1* mutations. The effect size of association between rs2853669 and OS quantified by hazard ratio was 1.74, 95% CI: 1.12–2.70 for CT genotype and 1.54, 95% CI: 1.01–2.35 for CT + CC (dominant model). Our observations are in line with those reported by Mosrati *et al.* [17], which underlined that the *TERT* rs2853669 CC genotype may represent an independent biomarker associated with poor prognosis in AML. Multivariate Cox-regression analysis performed in our study indicated that *TERT* rs2736100 did not represent a risk factor for AML development. These results are consistent with previously reported data [17].

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Multivariate analysis was not performed by Krahling *et al.* [19] in their investigation of OS time and whether *TERT* rs2853669 and rs2736100 were prognostic markers for OS in their AML cohort.

Taking into account that the Concordance index (C-index or C-statistic) obtained was between 0.70 and 0.80, we consider that we had strong models and that the regression test models used demonstrated good discriminant accuracy.

There are several limitations of our study that should be mentioned: telomerase activity and telomere length were not evaluated in AML cases, the lack of investigation of *CEBPA* and *RUNX1* gene mutations, the relatively small study sample size, and the lack of gene-environment interaction evaluation.

This is the first study focusing on the impact of interaction between *TERT* gene polymorphisms and *DNMT3A* mutations on AML risk. In our AML cohort, *TERT* rs2853669 and rs2736100 variant genotypes were not associated with *DNMT3A* mutations. We observed that *DNMT3A* R882 affected the patients' OS (OS median was 4 months in cases with *DNMT3A* mutation).

Considering the low number of studies that investigated the association between *TERT* polymorphisms and the risk of acute myeloid leukaemia, and the fact that the findings are still preliminary and inconclusive, further research is necessary in other ethnic groups.

In conclusion, our results indicated that *TERT* rs2853669 and rs2736100 polymorphisms were not risk factors for developing AML in the Romanian population, but the presence of *TERT* rs2853669 CT or CT + CC variant genotypes had a negative effect on AML patients' overall survival in the presence of other known prognostic factors.

Acknowledgments

Conflict of interest

The authors declare no conflict of interest.

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