Prognostic value of serum levels of Progranulin in Egyptian Acute Myeloid Leukemia Patients

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List of Abbreviations

Abb.	Full term
AML	Acute myeloid leukemia
AMLSG	German-Austrian AML study group
APL	Acute promyelocytic leukemia
ASXL1	Additional sex comb-like 1 gene
AUC	Area under curve
BSC	Best supportive care
CALGB	Cancer and Leukemia Group B
CBF	Core binding factor
CLL	Chronic lymphocytic leukemia
CN-AML	Cytogenetically normal AML
CR	Complete remission
CRi	CR with incomplete platelet recovery
CTD	Carboxyl terminal domain
DIC	Disseminated intravascular coagulation
DLBCL	Diffuse large B cell lymphoma
DNMT3A	DNA methyltransferase 3A
ECOG	Eastern Cooperative Oncology Group
EGF	Epidermal GF
ELISA	Enzyme-linked immune-sorbent assay
ELN	European Leukemia Net
EPI	European Prognostic Index
ER+	Estrogen receptor positive
ERK	Extracellular regulated kinase
EVI1	Ecotropic virus integration site-
FAB	French-American-British
FAK	Focal adhesion kinase
FGF	Fibroblast GF
FISH	Fluorescent in situ hybridization
FLT3	*FMS*-like tyrosine kinase 3
GF	Growth factor
GO	Gemtuzumab Ozogamicin
GP	Glycoprotein
GP88	Glycoprotein, 88kDa
grn/epi	Granulin-epithelin
GVHD	Graft-versus-host disease
GVL	Graft-versus-leukemia
HCC	Hepatocellular carcinoma
HD-AraC	High-dose cytarabine
HER	Human epidermal GF receptor
HMW	High molecular weight
HSCT	Haemopoietic stem cell transplantation
IDC	Invasive ductal carcinoma
IDH1	Isocitrate dehydrogenase 1 gene
IGF	Insulin-like GF
IGHV	Immunoglobulin heavy chain variable region
ITD	Internal tandem duplications
KIR	Killer-cell Ig-like receptor
LD-AraC	Low-dose cytarabine
LMP	Low malignant potential
LN	Lymph node
MAPK	Mitogen activated protein kinase
MDS	Myelodysplastic syndrome
miRNA	Micro-RNAs
MLL	Mixed lineage leukemia
MMPs	Matrix metalloproteinases
MPN	Myeloproliferative neoplasms
MRC	Medical Research Council
MRD	Minimal residual disease
mTOR	Mammalian target of rapamycin
Negative	-ve
nfK-B	Nuclear factor kappa-B
NPM1	Nucleophosmin 1
NPV	Negative predictive value
NSCLC	Non-small cell lung cancer
O.D.	Optical density
OS	Overall survival
PCDGF	PC-cell-derived GF
PDGF	Platelet-derived GF
Pgrn	Progranulin
PI3K	Phosphatidyl-inositol 3-kinases
PIN	Prostatic intraepithelial neoplasia
PPV	Positive predictive value
PRT	Post-remission treatment
PTD	Partial tandem duplication
P-TEFb	Positive transcription elongation factor b
ROC	Receiver operating characteristic curve
RT	Room temperature
RT-PCR	Reverse transcription-polymerase chain reaction
RUNX1	the runt-related transcription factor 1
SAL	Study Alliance Leukemia
SORT1	Sortilin
SWOG	Southwest Oncology Group
TET2	Tet methylcytosine dioxygenase 2
TGFe	Epithelial TGF
TGF-β	Transforming GF-β
TKI	Tyrosine kinase inhibitor
TNF	Tumor necrosis factor
TP53	Tumor protein p53
VEGF	Vascular endothelial growth factor
WHO	World Health Organization
wt	Wild type

INTRODUCTION

Cancer cells have defects in regulatory mechanisms that usually control cell proliferation and homeostasis. Different cancer cells share crucial alterations in cell physiology, which lead to malignant growth. Tumorigenesis or tumor growth requires a series of events that include constant cell proliferation, promotion of metastasis and invasion, stimulation of angiogenesis, evasion of tumor suppressor factors, and avoidance of cell death pathways. All these events in tumor progression may be regulated by growth factors produced by normal or malignant cells ***(Serrero et al., 2012)***

The growth factor (GF) Progranulin (Pgrn) has significant biological effects in different types of cancer. This protein is a regulator of tumorigenesis because it stimulates cell proliferation, migration, invasion, angiogenesis, malignant transformation, resistance to anticancer drugs, and immune evasion ***(Yamamoto et al., 2014).***

In the extracellular matrix, Progranulin binds to receptors resulting in either activation of a signal transduction pathway or engulfment into the cell. Several studies have shown progranulin involvement in the binding of Sortilin (SORT1) which promotes tumor cell proliferation, migration and survival, and induces drug resistance ***(Edelman et al., 2014).***

Pgrn activity is associated with p44/42 mitogen-activated protein kinase as well as phosphatidyl-inositol 3-kinases (PI3K) signaling pathways. In addition, Progranulin may stimulate the formation of the tumor stroma. Tumor necrosis factor (TNF) and EPH receptor A2 were suggested as potential Pgrn facilitators ***(Bouchet et al., 2015).***

In breast cancer, Pgrn has been implicated in tumorigenesis and resistance to anti-estrogen therapies for estrogen receptor positive (ER+ve) breast cancer. Previous pathological studies showed that Pgrn is expressed in invasive ductal carcinoma (IDC), but not in normal mammary epithelial tissue, benign lesions or lobular carcinoma ***(Serrero et al., 2012).***
In Rheumatoid Arthritis patients, the levels of circulating serum Pgrn have been measured and found to be significantly higher than those in age-matched healthy controls ***(Yamamoto et al., 2014).***
High Pgrn plasma levels were found to be strongly associated with adverse risk factors in chronic lymphocytic leukemia (CLL) patients, including unmutated IGHV (Immunoglobulin heavy chain variable region) status, expression of CD38 and ZAP-70, and poor risk cytogenetics (11q-, 17p-) suggesting that Pgrn is a novel, robust and independent prognostic marker in CLL ***(Göbel et al., 2013).***

The Pgrn levels were significantly higher in the serum of patients with lymphoid malignancies than in healthy controls. High serum Pgrn levels were associated with poor prognosis in patients with diffuse large B cell lymphoma (DLBCL) ***(Yamamoto et al., 2017).***

Aim of the Work

The aim of the study is to measure levels of Progranulin in the serum of adult patients with acute myeloid leukemia and correlate its serum levels with prognosis and clinical outcome.

Acute Myeloid Leukemia

Acute myeloid leukaemia (AML) is a highly heterogenous disorder in terms of clinical course, morphology, cytochemistry of the leukemic population, immunophenotype, cytogenetic, molecular abnormalities and different treatment modalities with an incidence of 3 per 100,000 men and women per year and median age at diagnosis of 67 years ***(Burnett et al, 2011).***

AML is characterized by the accumulation of somatically acquired genetic changes in hematopoietic progenitor cells that alter normal mechanisms of self-renewal, proliferation, and differentiation. Outcome is influenced by various factors, including patient features such as age, comorbidities, and performance status and disease characteristics of which the genetic profile of the disease is the most important ***(Richard and Hartmut, 2013).***

Prognostic factors in malignant diseases are positively or negatively associated with one or several outcome parameters. While prognostic factors are associated with prognosis in the context of a standard treatment, predictive factors assess the success of a specific treatment in a specific disease situation and thus can favor one therapy over another. In AML, correlations have been typically evaluated with the rate of complete remission (CR) following induction treatment, with overall survival (OS), for factors influencing prognosis after CR achievement or factors predictive for post-remission treatment (PRT), with relapse-free survival. Several prognostic factors with independent association with outcome parameters can be incorporated into scoring systems, which enable fine-tuning of the prognostic impact and aiding therapy decisions ***(Liersch et al., 2014).***

Risk factors of AML:

Risk factors can be classified into patient-related and disease related factors

Patient-related factors

Age: Patient age at diagnosis is the strongest patient-related prognostic factor in AML. The influence of age on prognosis is evident from 50 years of age onwards and in AML patients below the age of 30 years ***(Juliusson et al, 2009)***. In adult patients, many factors contribute to an impaired prognosis in elderly AML patients: a higher frequency of comorbidities and contraindications against intensive cytotoxic treatment, a higher incidence of a decreased performance status, a higher incidence of secondary AML following antecedent myelodysplastic syndrome (MDS) or cytotoxic treatment, and a higher frequency of adverse cytogenetic abnormalities with a lower frequency of favorable ones. However, even when taking all these factors into account, patient age remains a strong independent

prognostic factor ***(Krug et al, 2009).*** Patients aged 65 years or older have a dismal prognosis even after intensive chemotherapy, irrespective of their cytogenetic risk ***(Buchner et al, 2009).***

Performance status: Another strong predictor for outcome, especially in elderly patients, is the performance status. Patients with an Eastern Cooperative Oncology Group (ECOG) performance status >2 display a worse prognosis independent of age ***(Juliusson et al, 2009),*** and the combination of age and performance status is highly predictive for early death after induction treatment in elderly patients ***(Appelbaum et al, 2006).***

Geriatric assessment: An impairment in the activities of daily living was independently associated with decreased OS in patients with AML or high-risk MDS treated with either hypomethylating agents or best supportive care (BSC) ***(Deschler et al, 2013).***

Leukaemia-related risk factors

Cytogenetics: Cytogenetic risk is the strongest leukaemia-related risk factor for outcome of patients with AML after intensive chemotherapy. Balanced translocations with involvement of the core binding factor (CBF) transcription factor complex, namely translocation t(8;21), inversion (16) and t(16;16), represent favorable risk aberrations. The following cytogenetic aberrations were identified as high-risk: abn(3q) (excluding t(3;5)(q25;q34)), inv(3)(q21q26)/ t(3;3)(q21;q26), abn(5q)/del(5q), -5, -7, abn(7q)/del(7q), t(11q23) (excluding t(9;11)(p21–22;q23) and t(11;19)(q23;p13)), t(9;22)(q34;q11), -17, abn(17p) and complex cytogenetic aberrations with at least four aberrations excluding good-risk abnormalities ***(Grimwade et al, 2010)***. The more widespread definition of a complex-aberrant karyotype includes patients with at least 3 abnormalities excluding good-risk aberrations ***(Dohner et al, 2010)***. In addition to this risk classification, Dutch-Belgian Hemato-Oncology Cooperative Group (HOVON) identified a monosomal karyotype, defined as the presence of one monosomy and one additional structural aberration or monosomy (not including loss of a genosome), as a strong adverse prognostic factor, independent of the presence of complex cytogenetic abnormalities. Adverse cytogenetic risk is also a negative prognostic factor for palliative therapy with low-dose cytarabine (LD-AraC) ***(Burnett et al, 2013).***

Gene mutations

KIT: In favorable risk patients with t(8;21), inv(16) ot t(16;16), mutations of the KIT gene occur in a frequency of 15–46% and confers an adverse risk, especially activating mutations in the tyrosine kinase domain in exon 8. This negative impact is evident only in leukaemias with a high mutant to wild type (wt) ratio ***(Allen et al, 2013).***

FLT3: Internal tandem duplications (ITD) of the fms-like tyrosine kinase 3

(FLT3) gene occur in approximately 35% of adult patients with AML and confer a negative prognostic impact in patients with favorable or intermediate cytogenetic risk ***(Paschka et al, 2013)***. In patients with intermediate cytogenetic risk, the negative prognostic impact is evident in patients with concomitant Nucleophosmin 1 (NPM1) mutation only when present with a high allelic mutant to wt ratio, while in patients with wt NPM1, the presence of a FLT3-ITD itself confers a negative prognosis ***(Pratcorona et al, 2013)***. ITD size highly correlated with the ITD location within the FLT3 gene ***(Kayser et al, 2009)***. In unselected elderly patients treated with intensive chemotherapy, one study found FLT3-ITD of 34% in patients >55 years of age, whereas 2 recent reports found lower incidences of 14 and 12% in patients aged >60 and ≥65 years, respectively. FLT3-ITD did not confer an independent prognostic effect in these patients, whereas in a study of intensively treated cytogenetically normal AML (CN-AML) patients aged ≥60 years the presence of a FLT3-ITD was an independent adverse risk factor ***(Whitman et al, 2010).*** A clear prognostic impact so far could be demonstrated for ITD, but not for activating tyrosine kinase domain mutations ***(Allen et al, 2013).***

NPM1: The commonest molecular aberration in AML is a mutation of NPM1, found in 46-64% of CN-AML, compared to 9-18% of cyto-genetically abnormal AML. 63-50% of AML with mutated NPM1 also carry FLT3-ITD. Mutated NPM1 confers an independent favorable prognosis for patients with CN-AML and either absence or presence of a FLT3-ITD. In elderly patients with AML receiving intensive treatment, NPM1 mutations were found in 56% of patients with CN-AML ≥60 years of age, where it also was an independent favorable risk factor ***(Becker et al, 2010).***

In unselected patients ≥65 years of age, the incidence of NPM1 mutations was only 16% and had a favorable prognostic impact in the subgroup of AML patients with FLT3 no ITD ***(Daver et al, 2013).***The German-Austrian AML study group (AMLSG) demonstrated a superior OS in patients with PCR negativity for NPM1 both after induction therapy and after completion of therapy, and defined a cutoff level of >2 mutant NPM1 copies/100 ABL1-copies predicting relapse after allogeneic HSCT ***(Kronke et al, 2011)***. The Study Alliance Leukaemia (SAL) group defined a cutoff level of >1 mutant NPM1 copies/100 ABL1-copies after chemotherapy and >10 mutant NPM1 copies/100 ABL1-copies after allogeneic HSCT independently associated with inferior OS ***(Shayegi et al, 2013)***. The utility of NPM1 for MRD detection is hampered by the fact that approximately 10% of patients with initial NPM mutations loose those mutations at relapse ***(Papadaki et al, 2009).***

CEBPA. Mutations of the CEBPA gene confer a good prognosis in

patients with CN-AML. Those aforementioned gene aberrations have already been incorporated into combined cytogenetic and molecular risk classification systems, such as the European Leukaemia Net (ELN) classification ***(Dohner et al, 2010).*** However, the positive prognostic impact of CEBPA mutations is restricted to biallelic mutations ***(Dufour et al, 2010)*** and that this positive prognostic impact can also be found in patients with AML harbouring intermediate risk cytogenetic aberrations, but not in patients with concurrent FLT3-ITD ***(Green et al, 2010).***

MLL-PTD. The mixed lineage leukaemia (MLL) gene locus is frequently involved in balanced translocations, which confer a negative prognosis in majority of cases ***(Dohner et al, 2010)***. In addition, partial tandem duplications (PTD) can be identified in approximately 5% of AML patients. Studies demonstrated an impact on OS ***(Bacher et al, 2005).***
DNMT3A. Mutations of the DNA methyltransferase 3A gene are considered a negative prognostic factor by most study groups ***(Markova et al, 2012)***. Within CN-AML patients, a negative prognostic impact was found in patients with unfavorable molecular risk (CEBPA wt combined with NPM1 wt and/or FLT3-ITD) ***(Gaidzik et al., 2013)***, while a negative prognostic effect was found in patients with favorable molecular risk (CEBPA mutated or NPM1 mutated plus FLT3 no ITD) ***(Renneville et al., 2012).***A possible explanation for this discrepancy is the observation that the prognostic impact of DNMT3A mutations depend on the anthracycline dose administered during induction therapy ***(Patel et al, 2012).*** One report suggests that DNMT3A mutations predict a good response to hypomethylating therapy with decitabine ***(Metzeler et al, 2012).***

TET2. TET2 (Tet methylcytosine dioxygenase 2, previously Tet oncogene family member 2) mutations predicted a higher response rate in patients with high-risk MDS or low blast count AML (MDS/AML with 11–30% bone marrow blasts) upon treatment with 5-azacytidine, albeit with comparable OS rates ***(Itzykson et al, 2011)***. In patients receiving intensive therapy, one group found a negative prognostic effect restricted to patients with CN-AML and favorable genetic aberrations (NPM1 mutated/FLT3 no ITD, or CEBPA mutated) ***(Metzeler et al, 2012).***Another group found TET2 mutations were a negative prognostic factor in patients with intermediate cytogenetic risk. However, it lost its independent significance when additional genetic aberrations were considered ***(Hou et al, 2012).***

WT1. Wilms tumor mutations are detected in approximately 13% of AML and an independent negative prognostic impact, as well as no prognostic impact have been reported in CN-AML patients ***(Damm et al, 2010).***

IDH1/2. Mutations of the isocitrate dehydrogenase 1 gene (IDH1) have been reported as an adverse prognostic factor only in genetically defined subgroups of patients ***(Nomdedeu et al, 2012).***Two meta-analyses with respect to the prognostic impact of IDH1 mutations exist: one covered trials that included AML patients with all cytogenetic groups except APL and found a negative impact of IDH1 mutations on survival restricted to patients with CN-AML harbouring NPM1 mutations and FLT3 no ITD ***(Zhou et al, 2012).*** The second meta-analysis included studies restricted to CN-AML patients and found a small but significant negative prognostic impact on AML patients ***(Feng et al, 2012).*** The prognostic effect of IDH2 mutations is even less clear, with some groups finding a positive impact on survival**,** while others found a negative impact in defined subgroups ***(Ravandi et al, 2012)***. Most groups did not find prognostic impact of IDH2 mutations ***(Renneville et al, 2012).*** While a meta-analysis found a positive prognostic impact of IDH2 mutations on survival ***(Zhou et al, 2012).***

ASXL1. Additional sex comb-like 1 gene mutations were found to be an independent adverse prognostic factor for survival in patients with CN-AML (***Schnittger et al, 2013***) and in older patients with CN-AML and favorable genetic risk. ASXL1 mutations were an adverse factor for survival in a univariate analysis, but not in a multivariate analysis ***(Metzeler et al, 2011).***

TP53. In AML, the presence of tumor protein p53 gene mutations is highly associated with the presence of complex cytogenetic aberrations, and as such does not represent an independent prognostic factor for patients with AML. However, within patients with AML exhibiting a complex karyotype, TP53 alterations (mutations or deletions) are an independent adverse prognostic factor for survival, even outweighing the impact of monosomal karyotype in a multivariate analysis ***(Rucker et al, 2012).***

RUNX1. Mutations of the runt-related transcription factor 1 occur in 6–26% of AML and were independently associated with inferior outcome ***(Schnittger et al, 2011).***

RAS. RAS mutations (NRAS or KRAS) occur in 12–27% of patients with AML. No prognostic impact on survival could be demonstrated. However, one study found RAS mutations to be predictive for prolonged remission duration for CR patients treated with high-dose cytarabine (HD-AraC) compared to patients with RAS wt AML***(Neubauer et al, 2008).***

Micro-RNAs. Micro-RNAs (miRNA) are short, highly conserved, noncoding RNAs involved in post-transcriptional gene silencing. A high expression of MIR181 family members is an independent favorable prognostic factor for CN-AML and cytogenetically abnormal AML. A high expression of MIR155 and of MIR3151 constitutes an independent adverse prognostic factor in CN-AML ***(Marcucci et al, 2013)***. Differential