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Role of GM-CSF in Myeloid Neoplasms

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ABSTRACT

Background: Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF) cytokine stimulates growth, differentiation and function of myeloid progenitors.

Aim: The present work aimed to study the role of GM-CSF gene expression, its protein and antibodies in acute myeloid leukemia/myelodysplastic syndromes (AML /MDS) patients and their correlation to disease behavior and treatment outcome. The study included 50 AML/MDS Egyptian patients in addition to 20 healthy volunteers as controls.

Patients and Methods: Assessment of GM-CSF gene expression was performed by quantitative real-time PCR. GM-CSF proteins and antibodies were assessed by ELISA.

Results: There was significant decrease in GM-CSF gene expression (*p*-value=0.008), increase in serum level of GM-CSF protein (*p*-value=0.0001) and anti GM-CSF antibodies (*p*-value=0.001) in AML/MDS patients in comparison to healthy controls. Also, there was significant negative correlation between serum level of GM-CSF and initial PB blasts percentage as well as response to therapy.

Conclusion: Any alteration in GM-CSF gene expression could have implication in leukemogenesis, also GM-CSF serum level could be used to predict outcome of therapy. GM-CSF antibodies may as well play a role in the pathogenesis of AML/MDS. The use of these GM-CSF parameters for disease monitoring and as markers of disease activity needs further research.

Key Words: *GM-CSF – Gene expression – Protein concentration – Antibodies – AML/MDS.*

INTRODUCTION

Acute Myeloid Leukemia (AML) is a heterogenous group of leukemias that results from a genetic event or series of events occurring in an early hematopoietic precursor that both blocks differentiation and allows uncontrolled proliferation. The abnormally proliferating leukemic cells accumulate in the marrow space, eventually replacing normal marrow progenitors, with consequent diminished production of red cells, white cells and platelets. This, in turn, leads to the common clinical manifestations of AML **[1]**.

The Myelodysplastic Syndromes (MDS) include a large spectrum of clonal hemotopoietic stem cell disorders that are characterized by peripheral cytopenia (s), morphologic dysplasia, ineffective hematopoiesis and a variable propensity to transform to AML **[2]**.

GM-CSF stimulates multipotent progenitor cells depending on its concentration, the proliferation of macrophage progenitors, followed by granulocytes, erythroid, eosinophil, megakaryocyte and multipotent progenitors. It also stimulates the differentiation of myeloid leukemic cells and controls eosinophil function in some instances **[3]**.

Human GM-CSF gene is approximately 2.5 kbp long. The gene is located on the long arm of chromosome 5 (5q21-q32). It has 4 exons that are separated by 3 introns **[4]**. Besides the GM-CSF gene, genes encoding various cytokines like IL4, IL5, IL9, IL12, M-CSF and EGR1 gene are located at the 5q31.1 locus of chromosome 5 **[5]**. The (5q-) syndrome has elucidated the role of these cytokines in development of clonal hematopoietic stem cells **[6]**. The current study aimed to investigate and understand the role of GM-CSF in pathogenesis, progression and response to therapy in Egyptian AML/MDS patients.

SUBJECTS AND METHODS

Study population:

The present study included 50 patients with AML/MDS. Patients were recruited from the outpatient clinic and the inpatient wards of Kasr

Al-Ainy Clinical Hematology Unit, Internal Medicine Department and Clinical Oncology Department, Faculty of Medicine, Cairo University.

Patients in complete remission or those with history of recombinant human GM-CSF intake were excluded. Twenty age and sex matched healthy volunteers were included in the study as a control group. The study was approved by the Research Ethical Committee of Internal Medicine Department, Faculty of Medicine, Cairo University and informed consents were obtained from all participants prior to enrollment in the study.

For both patients and controls, 4ml EDTA blood and serum samples were collected under complete aseptic conditions for routine workup, molecular studies and ELISA techniques respectively.

Quantitative assessment of GM-CSF gene expression:

Extraction of total RNA was performed by QIAamp RNA Blood Mini Kit (Qiagen, Germany) according to manufacturer's instructions. Total RNA was reverse transcribed using random primers with a high capacity cDNA archive kit (Applied Biosystems, Foster city, CA, USA).

GM-CSF gene expression was detected by real-time PCR based on Taqman technology using ABI Prism 7700 (Applied Biosystems, USA).

The primers and probes for GM-CSF as well as the house keeping gene (GAPDH) were provided by Qiagen® (USA). GM-CSF gene: F: 5'-CTGCTGAGATGAATGAAACAG-3'and R:5'-TCCAAGATGACCA TCCTGAG-3', FAM probe:5'-ACTCCCACCATGGCTGTGG-3' (TaqMan GM-CSF, access No: M11220, Applied Biosystem, USA). The thermocycler program conducted was initial denaturation at 50ºC for 2min. followed by 40 cycles of denaturation at 95ºC for 10min., annealing at 95ºC for 0.15min. and extension at 60ºC for 1min. The Relative Quantification (RQ) of gene expression was assessed by $2^{-\Delta\Delta Ct}$ method ($\Delta\Delta Ct$= {[Ct (GM-CSF sample)-Ct (GAPDH sample)]-[Ct (GM-CSF calibrator)-Ct (GAPDH calibrator)]}. The calibrator was the average ΔCt value of 20 controls **[7]**.

Assessment of GM-CSF protein and anti GM-CSF antibodies:

The concentration of GM-CSF was measured in appropriately diluted sera from all AML/MDS patients as well as healthy controls by using a specific ELISA assay (Quantikine human GM-CSF kit, catalog # SGM00, R & D Systems, Inc., Minneapolis, USA). The minimum detectable dose of GM-CSF by this reagent is typically less than 3pg/ml.

The concentration of GM-CSF Abs was measured in appropriately diluted sera from 42AML/MDS patients as well as healthy controls by using a specific ELISA assay (anti GM-CSF Ab kit, catalog # MBS162797, My Biosource, USA). The minimum detectable dose of GM-CSF Abs by this reagent is typically less than 0.52ng/ml.

Treatment regimen and response to therapy:

ALL patients were treated according to the adopted protocol of the Internal Medicine Department, Faculty of Medicine, Cairo University. AML patients (except M3 cases) were subjected to 7-3 protocol for induction of remission. M3 patients were subjected to All-Trans-Retinoic Acid (ATRA). Treatment of AML depends on the fitness of the patient. Fit patients (<60 years) received intensive therapy. Treatment includes induction and postremission therapy (consolidation). Less fit patients (70-75 years and older, or younger patients with significant co-morbidities) receive low-intensity therapy. For induction therapy, combination of cytarabine and anthracycline or anthracenedione is recommended (Cytarabine 100-200mg/m^2 continuous IV infusion for 7 days plus Idarubicin 12 mg/m^2/day for 3 days or Daunorubicin 60-90 mg/m^2/day for 3 days). Follow-up by bone marrow examination to assess remission is typically done 7-14 d after completion of induction chemotherapy. For postremission therapy, all patients should be assessed for risk of relapse. Specific drug regimens are recommended based on a patient's risk of relapse (e.g: High-dose Cytarabine 3g/m^2 IV over 3h every 12h on days 1, 3, and 5 for 4 cycles).

Complete Remission (CR) status was defined by normalization of the neutrophil count (≥1.5/μL) and platelet count (>100X10^3/mm^3), and marrow examination that demonstrates at least 20% cellularity, less than 5% blasts and no Auer

rods, as well as absence of extramedullary infiltration. Resistance to treatment (RD) is defined as more than 25% blasts in the BM, lack of regeneration of normal hematopoiesis, persistence of peripheral blood blasts and/or extramedullary leukemia after induction. Relapse was defined as re-infiltration of the bone marrow by 5% or more leukemic blasts or proven leukemic blasts at any site. Death during induction is defined as death during or after the first course of therapy with aplastic or hypocellular marrow **[8]**.

The only known curative modality for patients with MDS is Stem Cell Transplantation (SCT). Therefore, all appropriate candidates should be considered for SCT. They include patients younger than 70 years, with a reasonable performance status and no significant comorbidity Fig. (1).

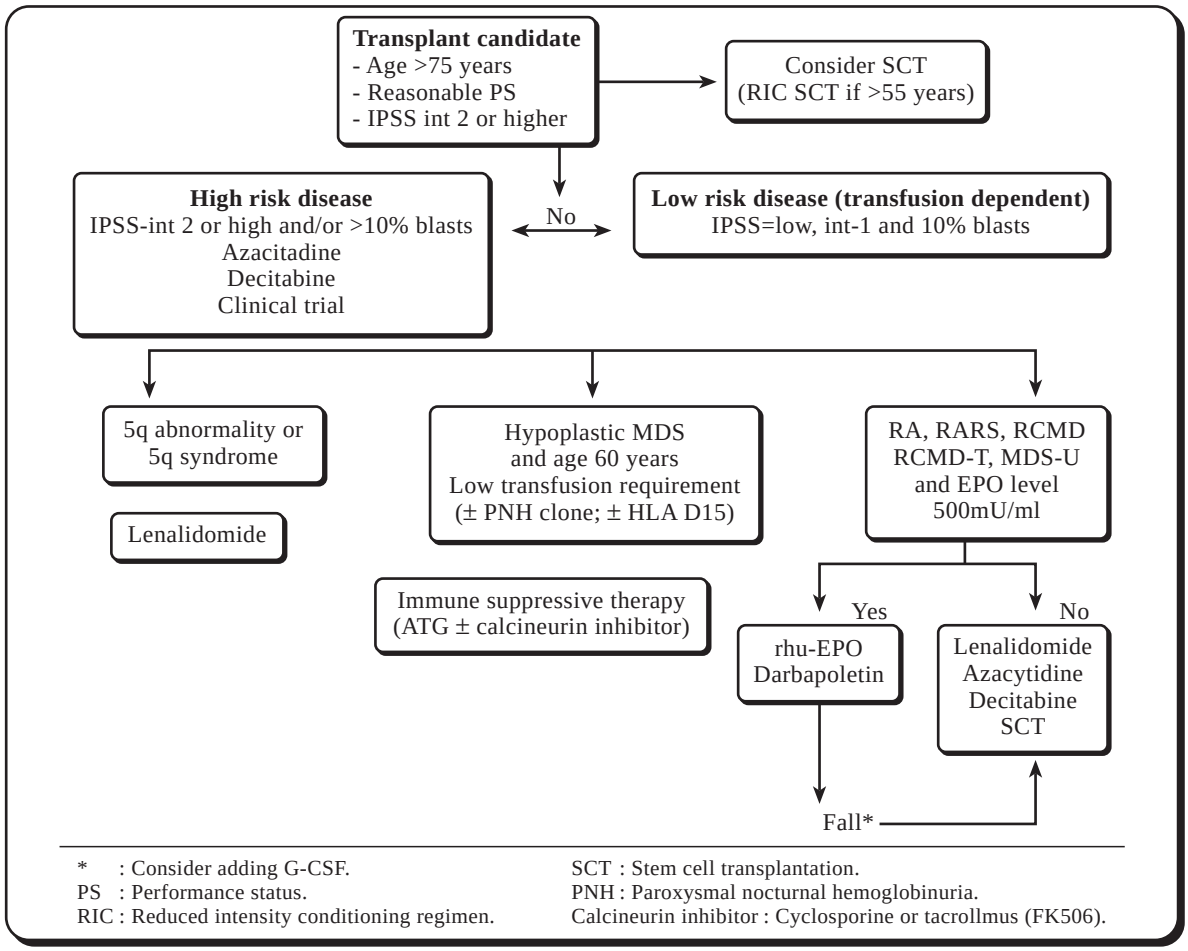

Fig. (1): Algorithm for management of a patient with MDS.

Data analysis:

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as number and percentage. Statistical differences between groups were tested using Chi Square test for qualitative variables. For non parametric quantitative data, comparison between groups was done using Mann-Whitney test and Wilcoxon Signed Ranks Test. Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" on rank of variables was used for pair-wise comparison. Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. A p-value <0.05 was considered significant.

RESULTS

The patients were 27/50 (54%) males and 23/50 (46%) females. Their age ranged between 20 and 91 years with a median of 45.5 years. According to disease subtypes, 44 patients (88%) were AML and 6 patients (12%) were MDS. According to FAB classification of AML, 14 patients (28%) were FAB-M1, 9 (18%) were M2, 3 (6%) were M3, 9 (18%) were M4, 4 (8%) were M5, while 5 patients (10%) were M7.

Patients who did not receive chemotherapy due to bad general condition, poor performance or just refusal were 17. Among patients who received chemotherapy, CR rate was 15/33 (45.4%), failure of induction rate was 6/33 (18.1%) and death rate was 12/33 (36.36%). Out of 15 patients who achieved CR, relapse was reported in 5/15 patients (33.3%) at a follow-up period of 24 months. Overall Survival (OS) ranged between 1 and 24 months with a mean value of 4±4.04 months, while the OS for patients achieving CR ranged between 2 and 12 months with a mean value of 7.6±3.8 months.

GM-CSF gene expression:

GM-CSF gene expression values in the 20 healthy controls ranged between 0.659 and 1.515 with a mean value of 0.62±0.09, while in the 50 AML/MDS patients it ranged between 0.0126 and 3.249 with a mean value of 0.629± 0.09. GM-CSF gene expression levels were significantly lower in AML/MDS patients compared to controls (*p*-value=0.008). We didn't find significant correlation between GM-CSF gene expression and age, gender, clinical data, TLC, initial PB blasts percentage, treatment outcome, period of CR and OS [(Table 1) & Fig. (2)].

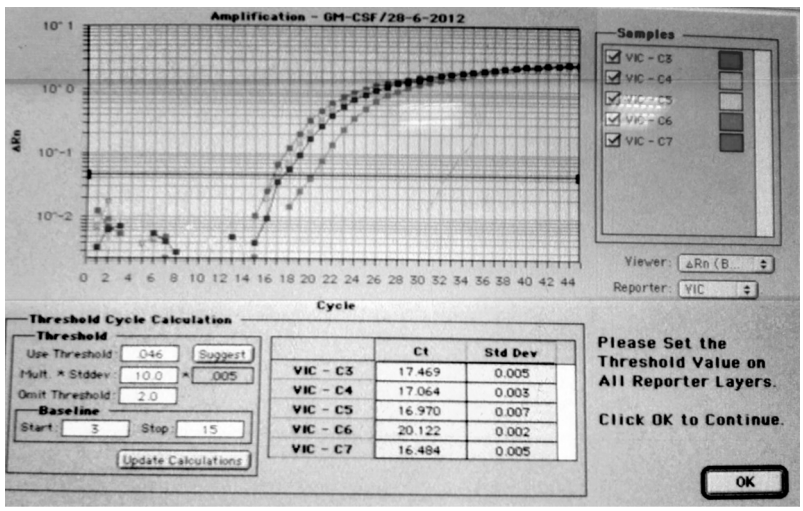

Fig. (2): GM-CSF amplification plot.

GM-CSF protein and anti GM-CSF antibodies:

Serum GM-CSF protein concentration in the healthy controls ranged between 7 and 8.6 pg/mL with a median of 7.8pg/mL, while in the 50 AML/MDS patients it ranged between 12 and 80pg/mL with a mean value of 41.1±18.4 pg/mL. We found higher GM-CSF protein concentration in AML/MDS patients compared to controls (*p*-value=0.0001). We found significant negative correlation between GM-CSF protein concentration and initial PB blasts percentage (*r*=–0.357, *p*-value=0.011), treatment outcome (*r*=–0.339, *p*-value=0.016). Otherwise, we didn't find significant correlation between GM-CSF protein concentration and age, gender, clinical data, other laboratory data, period of CR and OS (Table 1).

Serum anti GM-CSF antibodies in the healthy controls ranged between 10 and 18 ng/mL with a median of 14ng/mL, while in 42/50 (84%) AML/MDS patients it ranged between 37 and 240ng/mL with a mean value of 64.4±42.33ng/mL. We found higher serum anti GM-CSF antibodies in AML/MDS patients compared to controls (*p*-value=0.001). We didn't find significant correlation between serum anti GM-CSF antibodies and age, gender, clinical data, laboratory data, treatment outcome, period of CR and overall survival OS (Table 1).

Table (1): Correlation between GM-CSF and different clinical and laboratory parameters of 44 AML/6 MDS patients.

Parameter	GM-CSF gene expression		GM-CSF protein		Anti GM-CSF antibodies	
	r	*p*	*r*	*p*	*r*	*p*
Age	–0.045	0.755	0.229	0.110	–0.129	0.414
Gender	–0.032	0.825	0.086	0.551	0.117	0.461
TLC	–0.141	0.328	–0.180	0.212	–0.540	0.735
PB blasts	–0.026	0.858	–0.357	0.011*	–0.255	0.102
Outcome	0.163	0.258	–0.339	0.016*	–0.021	0.616
Survival	0.02	0.891	0.072	0.621	0.035	0.828
Period of CR	–0.193	0.491	0.003	0.991	0.102	0.521
GM-CSF protein	–0.197	0.171	–	–	0.052	0.746
Anti GM-CSF antibodies	–0.038	0.813	0.052	0.746	–	–
GM-CSF gene expression	–	–	–0.197	0.171	–0.038	0.813

*Significant correlation.

Response to therapy given to AML/MDS patients:

- After 24 months observation period, the mean survival time was 4±4.04 ranging from 1-24 months, while the mean complete remission period was 7.6±3.8 ranging from 2-12 months.
- In patients who did not receive chemotherapy, the mean survival time was 4.2±2.4 months while in patients who received chemotherapy it was 8.2±4.5 months. There was highly significant association between mean survival time and receiving chemotherapy (p=0.006).

DISCUSSION

Growth and progression of leukemic cells are mediated by alterations in the microenvironment often caused by an aberrant expression of growth factors and receptors **[9]**. GM-CSF is an autocrine/paracrine cytokine which stimulates growth, differentiation and function of normal and leukemic myeloid progenitors. Antibodies to GM-CSF are also implicated in the process of leukemogenesis.

The aim of the current study was to investigate the role of GM-CSF gene expression, serum GM-CSF protein concentration and anti GM-CSF antibodies in AML/MDS patients in relation to treatment outcome and overall survival to help in understanding their impact in the pathogenesis of the disease, and hence predict prognosis as well as response to treatment.

The present study revealed that GM-CSF gene expression levels were significantly lower in AML/MDS patients compared to controls. This is in accordance with previous studies **[10, 11]** who reported that GM-CSF gene expression by RT-PCR was lower in leukemia patients than in healthy volunteers. Also, it has been demonstrated that in vivo autocrine production of GM-CSF is not common in unperturbed AML, but allow the possibility that either autocrine or paracrine GM-CSF activity could be induced in leukemic cells under stress conditions **[12]**. Furthermore, abnormalities in GM-CSF gene expression levels may contribute to the pathogenesis and abnormal proliferation of leukemia **[13,14]**.

In the current study, serum GM-CSF protein concentration was significantly higher in AML/MDS patients compared to controls. This is in accordance with previous reports **[15,16]**. Highly significant increase in serum levels of GM-CSF was previously reported in 14 Egyptian AML patients compared to the reference control group **[17]**. This was denied by another study that reported comparable concentrations of serum GM-CSF in AML and healthy controls **[11]**.

The current finding of negative correlation between serum levels of GM-CSF and initial PB blasts percentage could be correlated to defect in the biological functions of GM-CSF which may be attributed to a functional alteration of GM-CSF receptor or disturbances of signal transduction pathways which needs larger future studies. Furthermore, lower serum level of GM-CSF was associated with better response to therapy which suggests that GM-CSF protein concentration could be used as a biomarker to predict the outcome of therapy in AML/MDS cases.

In the present study, higher GM-CSF antibodies were found in AML/MDS patients compared to controls. Our results are in agreement with a previous study that reported a high prevalence of anti GM-CSF antibodies in patients with myeloid leukemia and MDS and higher titer of GM-CSF antibodies in patients with active disease compared to patients in complete remission **[11]**.

In conclusion, our study results suggest that GM-CSF expression level might have an implication in leukemogenesis. The use of GM-CSF protein level and GM-CSF antibodies as prognostic markers of disease activity needs further investigations.

Conflicts of interest:

The authors declare that they have no conflicts of interest.

REFERENCES

1- Rubnitz JE, Gibson B, Smith Fo. Acute myeloid leukemia. Hematol Oncol Clin North Am. 2010; 24: 35-63.

2- Brunning RD, Orazi A, Germing U. Myelodysplastic syndromes/neoplasms, overview. WHO classification of tumours of hemopoietic and lymphoid tissues. Lyon, France: IARC Press. 2008; 88-107.

3- Nicola NA. Granulocyte colony-stimulating factor and differentiation induction in myeloid leukemia cells. Int J Cell Cloning. 1987; 5: 1-15.

4- Huebner K, Isobe M, Croce CM. The human gene encoding GM-CSF is at 5q21-q32, the chromosome region deleted in the 5q-anomaly. Science. 1985; 230: 1282-5.

5- Gasson JC. Molecular physiology of granulocyte macrophage colony stimulating factor. Blood. 1991; 77: 1131-45.

6- Le Beau MM, Westbrook CA, Diaz MO. Evidence for the involvement of GM-CSF and FMS in the deletion (5q) in myeloid disorders. Science. 1986; 231: 984-7.

7- Wong ML, Medrano JF. Real-time PCR for mRNA quantitation. Biotechniques. 2005; 39: 75-85.

8- Bacher U, Kern W, Schoch C. Evaluation of complete disease remission in acute myeloid leukemia: A prospective study based on cytomorphology, interphase fluorescence in situ hybridization, and immunophenotyping during follow-up in patients with acute myeloid leukemia. Cancer. 2006; 106 (4): 839-47.

9- Witsch E, Sela M, Yarden Y. Roles for growth factors in cancer progression. Physiology (Bethesda). 2010; 25: 85-101.

10- Young DC, Griffin JD. Autocrine secretion of GM-CSF in acute myeloblastic leukemia. Blood. 1986; 68: 1178-81.

11- Sergeeva A, Ono Y, Rios R. High titer autoantibodies to GM-CSF in patients with AML, CML and MDS are associated with active disease. Leukemia. 2008; 22: 783-90.

12- Kaufman DC, Baer MR, Gao XZ. Enhanced expression of the granulocyte macrophage colony stimulating factor gene in acute myelocytic leukemia cells following in vitro blast cell enrichment. Blood. 1988; 72: 1329-32.

13- Lang RA, Metcalf D, Gough NM. Expression of a hemopoietic growth factor cDNA in a factor-dependent cell line results in autonomous growth and tumorigenicity. Cell. 1985; 43: 531-42.

14- Ymer S, Tucker Qi, Sanderson Ci. Constitutive synthesis of interleukin-3 by leukemia cell line WEHI-3B is due to retroviral insertion near the gene. Nature. 1985; 317: 255-8.

15- Stauch M, Fritsche D, Hoffken K. Cytokine serum levels during the course of acute myeloid leukemia. Hamatologie und Bluttransfusion. 1997; 38: 173-84.

16- Tao M, Li B, Nayini J. SCF, IL-1≤, IL-1ra and GM-CSF in the bone marrow and serum of normal individuals and of AML and CML patients. Cytokine. 2000; 12: 699-707.

17- Elbaz O, Shaltout A. Implication of granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin-3 (IL3) in children with acute myeloid leukemia. Hematology. 2001; 5: 383-8.