Combined CD86 Expression & Increase in Soluble Vascular Endothelial Growth Factor Confers Bad Prognosis in Adult Acute Myeloid Leukemia

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Abstract: Acute myeloid leukemia (AML) is a clonal hematological disease with poor clinical outcome despite recent improvements in chemotherapy and stem cell transplantation regimens. Costimulatory molecules such as CD80, CD86 are important regulatory elements in healthy immunological cascade. Angiogenesis plays an important role in the progression of solid tumours and hematologic malignancies. VEGF and its receptors are crucial positive regulators of this process. This study was carried out on sixty patients with de novo AML presented to the outpatient clinic in NCI of EGYPT. They were evaluated for the expression of CD80, CD86 by Flowcytometric Immunophenotyping, and serum level of VEGF using an enzyme linked immunoassay technique. The results of CD80 and CD86 were expressed as percentage of positive cells. Twenty percent (20%) or more was considered positive. All patients were negative for CD80, the mean percentage of positive cells in all patients was 1.95. Thirty two patients (53.3%) were positive for CD86, the mean percentage of positive cells for these patients was 39.72. The expression of both CD80 and CD86 was significantly lower in AML patients compared to the control group ($p<0.001$, $p=0.014$ respectively). CD86 expression was highly heterogeneous in AML blasts of FAB types and was mainly expressed in AML cells having a monocytic component (M4 and M5) ($p=0.002$). CD86 expression was significantly lower in patients who achieved CR compared to patients who did not achieve CR($p=0.021$) and was significantly lower in patients with low risk karyotypes compared to the patients with high and intermediate risk karyotypes ($p=0.015$). The expression of CD86correlated significantly with TLC and percentage of bone marrow blasts($r=0.6$, $p<0.001$, $r=0.3$, $p=0.014$ respectively). Patients, who were CD86–ve, had a longer duration of DFS and OS compared to patients who are CD86+ve($P<0.001$,$P=0.001$ respectively). sVEGF was detected in sera of all AML patients, serum VEGF levels ranged from 230 to 2800 pg/ml with a median of 850 pg/ml and was significantly increased in sera of AML patients compared with the levels found in the normal controls ($P=0.011$). sVEGF levels were significantly increased in AML M4 and M5 patients compared with the levels found in AML MI,M2 and M3 ($P<0.001$). sVEGF was significantly lower in patients who achieved CR compared to patients who did not achieve CR($p=0.003$) and was significantly lower in the sera of patients with low risk karyotypes compared to the patients with high and intermediate risk karyotypes ($p<0.001$). sVEGF levels correlated significantly with TLC, percentage of peripheral blood blasts, and percentage of bone marrow blasts($r=0.9$, $p<0.001$, $r=0.4$, $p=0.003$ and $r=0.4$, $p=0.001$ respectively). Patients with low VEGF expression(<850 pg/dl) had a longer duration of DFS and OS compared to patients with high VEGF expression(>850 pg/dl) ($P<0.001$, $P<0.001$ respectively). When combined high CD86 expression AND increase in sVEGF significant had bad prognosis in terms of DFS &OS($p<0.001$ for both) was obtained. Conclusion: Both CD80 and CD86 costimulatory molecules should be present for an appropriate immune response. We thus concluded that, in Egyptian de novo AML patients, elevated levels of CD86 and sVEGF are associated with a worse outcome concerning disease free and overall survival, as well as being associated with adverse cytogenetics. CD86 and VEGF might contribute to the proliferation and progression of leukemic cells in AML and could be considered as poor prognostic factors particularly when present together.

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Key Words: CD80; CD86**;** $sVEGF_{165}$ and Adult AML**.**

1. Introduction

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disorder characterized by the accumulation of acquired genetic alterations in hematopoietic progenitor cells that disturb normal mechanisms of cell growth, proliferation and differentiation resulting in the accumulation of Leukemic cells in the bone marrow Mrózek *et al.,* (2007).

Costimulatory molecules such as CD80, CD86 are important regulatory elements in healthy immunological cascade Maria de Lourdes Palermo *et*

al. (2012) they are molecules expressed by APCs that play a major role in the outcome of T-cell activation. Although structurally and functionally similar Bhatia *et al.* (2006), they exhibit different immunological properties leading to distinct T-cell functional outcomes. CD86 has a higher relative affinity for CD28, while CD80 has higher affinity for CD152 Collins *et al.* (2005). CD28 is involved in productive T-cell activation, signalling for cellular proliferation, interleukin (IL)-2 secretion and cell survival through enhanced Bcl-2 expression Bour-Jordan *et al.* (2011), while CD152 halts T-cell activation, favours apoptosis and induces either anergy or tolerance Fife & Bluestone (2008). Moreover, while CD86 and CD28 are constitutively expressed by APC and T-cells, respectively, CD80 and CD152 are expressed only following 24-48 h of activation of the APC and T-cell Lenschow et al. 1996, Bour-Jordan *et al.* (2011). A model of T-cell costimulation has thus been proposed in which the distinct structures and binding properties of CD86 and CD80 significantly enhance the activating and inhibitory functions of CD28 and CD152, respectively Collins *et al.* (2002). This seems to be relevant to the regulation of the immune responses in several clinical conditions. Angiogenesis is important forin the progression of hematologic malignancies. VEGF and its receptors are key regulators of this process. VEGF is a sub-family of growth factors, to be specific, the platelet-derived growth factor family of cystine-knot growth factors. They are important signaling proteins involved in both vasculogenesis (the de novo formation of the embryonic circulatory system) and angiogenesis (the growth of blood vessels from pre-existing vasculature) Hoeben *et al.* (2004). VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, and cyclical ovarian angiogenesis tuttenberg *et al.,* (2006)

When VEGF is overexpressed, it can contribute to disease. Solid cancers cannot grow beyond a limited size without an adequate blood supply; cancers that can express VEGF are able to grow and metastasize. Overexpression of VEGF can cause vascular disease in the retina of the eye and other parts of the body Hoeben *et al.* (2004). Many studies have suggested that vascular endothelial growth factor (VEGF) or vascular endothelial growth factor receptor (VEGFR) overexpression correlates with poorer survival in adult patients with acute myeloid leukemia Guo B. *et al.* (2012), Drugs such as bevacizumab can inhibit VEGF and control or slow those diseases.

In ChinaCheng *et.al;* (2009), the Netherlands De Jonge *et.al;* (2009), France Ourouda *et al;* (2009), there is a lot of excitement regarding VEGF 121 & 165 isoforms & their inhibition using natural inhibitors.

2. Materials & Methods

This study was carried out on sixty patients with de novo AML presented to out patient clinic in NCI. They were evaluated for the expression of CD80, CD86 by Flowcytometric Immunophenotyping, and serum level of VEGF using an enzyme linked immunoassay technique.

3. Result

The expression of both CD80 and CD86 was significantly lower in AML patients compared to the control group (1.95+1.86 and 23.85+21.49 Vs 12.68+8.05 and 43.5+21.27) with a p value of $p<0.001$ and $p=0.014$ respectively.

In M4 and M5, the level of CD86 expression was significantly higher than the other FAB subtypes (45+22.9 Vs 14.8+12.9) with a p value of $p<0.001$.

The level of CD86 was significantly lower in patients with low risk karyotypes as compared to patients with high and intermediate risk karyotypes (15.08+16.54 Vs 27.32+22.39) with a p value of $p=0.015$.

A cut-off point of the median value for VEGF was used to define patients into high (>850pg/ml) and low (<850pg/ml) expressors and a cut-off of 20% was used to define CD86 patients as positive (>20%) or negative (<20%).

Soluble VEGF levels were significantly increased in the sera of AML patients compared to the control group (933.2+667.5 Vs 256.5+17.8) with a p value of $p=0.011$.

Soluble VEGF levels were significantly increased in M4 and M5 compared to the other Fab subtypes (1636.1+726.3 Vs 631.9+335.6) with a p value of $p<0.001$.

Serum VEGF was significantly higher in patients with high and intermediate risk karyotypes compared to those with low risk karyotypes (1137.1+676.2 Vs 417.4+220) with a p value of $p<0.001$.

There was a significant positive correlation between CD86 expression and both TLC and the percentage of bone marrow blasts and a significant negative correlation with both disease free and overall survival (p0.001, $p=0.014$, $p<0.001$ and $p=0.001$ respectively).

There was a significant positive correlation between sVEGF level and each of TLC, percentage of peripheral blood blasts, percentage of bone marrow blasts and age and there was a negative correlation with both disease free survival and overall survival ($p<0.001$, $p=0.003$, $p=0.001$, $p<0.001$, $p<0.001$ and $p<0.001$ respectively).

There was a significant positive correlation between the expression of sVEGF and CD86 ($p<0.001$).

CD86 positive AML had a significantly higher TLC and bone marrow blast percentage compared to CD86 negative blasts ($p=0.005$ and $p=0.004$ respectively).

VEGF was higher in CD86 positive AML versus CD86 negative AML ($p=0.003$).

The combined CD86 and VEGF elevation correlated with worse cytogenetic risk with a p value of $p=0.003$ (table 1)

Correlation with survival:

CR was achieved in 40 out of 60 patients (66.7%), while 11 patients (18.3%) failed to achieve CR after the second course of induction therapy. 9 patients (15%) died early within first 2 weeks of induction therapy and were excluded from the analysis.

42.5% of patients who achieved CR were CD86+ve, while 57.5% were CD86-ve. 81.1% of the patients who failed to achieve CR were CD86+ve, while 18.2% were CD86-ve with a p value of $p=0.021$ between the two groups.

40% of patients who achieved CR had high VEGF levels in their sera, while 60% had low VEGF levels. 90.9% of the patients who failed to achieve CR were VEGF high expressors, while 9.1% were low expressors with a p value of $p=0.003$.

Table 1. Presenting characteristics of studied AML patients

Number of patients	60
Male/Female	34/26
Age in years, mean ± SD (range)	38.9 ± 13.8 (18-72)
TLC mean ±SD (range) (109/L)	29.5 ± 19 (0.6-113)
Hb mean ±SD (range) (gm/dl)	6.7 ± 1.8 (3.5-11)
Plt mean ±SD (range) (109/L)	47.8 ± 24.8 (6-95)
BL-PB mean ±SD (range)	41.8 ± 22.7 (0-98)
BL-BM mean ±SD (range)	51.9±27 (0-93)
B.M cellularity Frequency (%) Normocellular Hypercellular	 11 (18.3%) 49 (81.7%)
FAB classification Frequency (%) M1 M2 M3 M4 M5a M5b	 14 (23.3%) 21 (35.0%) 7 (11.7%) 15 (25.0%) 1 (1.7%) 2 (3.3%)

Table 2: Presenting karyotypic characteristics of studied AML patients

Karyotyping Frequency(%) **Normal karyotype**	39 (65%)
t(8;21)	9 (14.9%)
t(15;17)	7 (11.6%)
47xy;t(8;21) add19	1 (1.7%)
47xy;+8	1 (1.7%)
46xy;-3,+8	1 (1.7%)
Monosomy 7	1 (1.7%)
Complex karyotyping	1 (1.7%)
Karyotyping risk group Frequency (%) low risk intermediate risk **high risk**	17 (28.3%) 41 (68.4%) 2 (3.3%)

Table 3: Comparison of the studied markers in AML patients and in the control group.

	%positivity	AML(n=60) mean±SD	Control(n=10) mean±SD	P value
CD80	0/60(0%)	1.95 ± 1.86	12.68 ± 8.05	**p<0.001**
CD86	32/60(53%)	23.85 ± 21.49	43.5 ± 21.27	**p=0.014**
Soluble VEGF		933.2 ±667.5	256.5±17.8	**P=0.011**

Table 4: CD86 & VEGF level in different karyotype risk groups

	Low risk karyotype	**Intermediate and high risk karyotype**	**P value**
CD86	**15.08±16.54**	**27.32±22.39**	**P=0.015**
VEGF*	**417.4±220**	**1137.1±676**	**P<0.001**
Combined +(22)	**2/22**	**20/22**	**P<0.001**
Combined –(19)	**10/19**	**9/19**	**NS**
Either +(19)	**5/19**	**14/19**	**p<0.001**

*VEGF cutoff 650 was used for statistical comparison

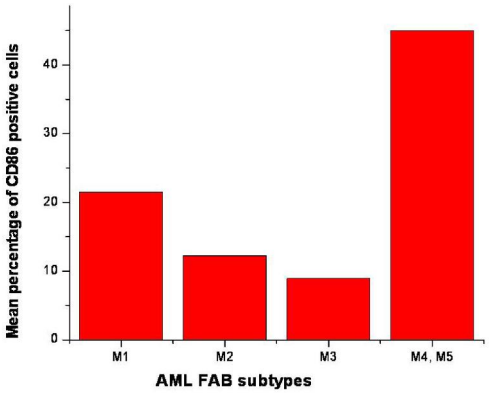

Figure 1: Comparison of Mean percentages of CD86 positive cells between different FAB subtypes.

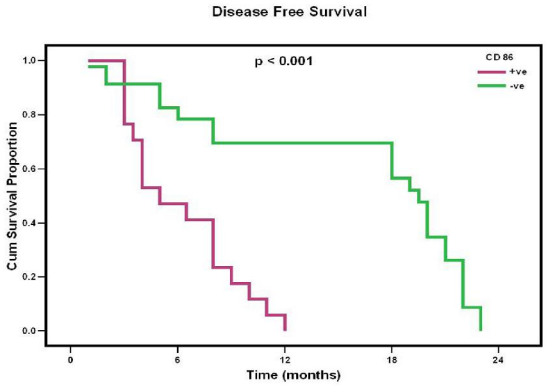

Figure 2:Disease free survival and CD 86 in AML patients.

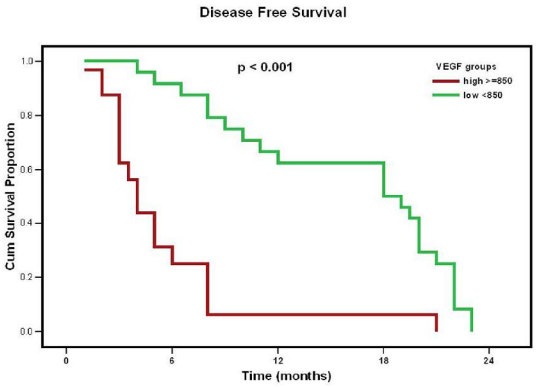

Figure 3 : Disease free survival and VEGF level in AML patients.

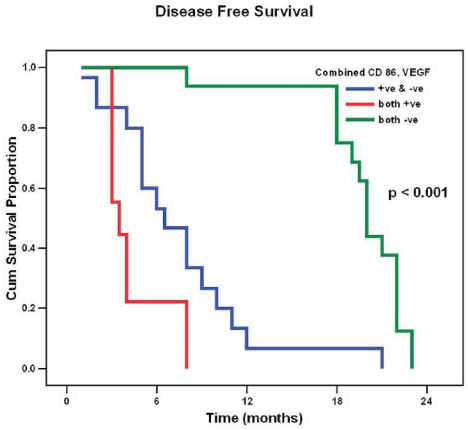

Figure 4 : Disease free survival and Combined VEGF level & CD86 in AML patients.

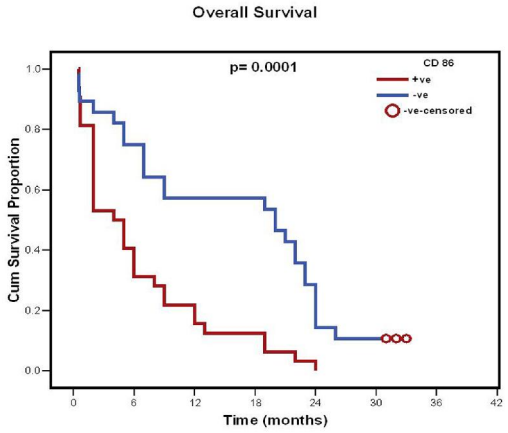

Figure 5 : Overall survival and CD86 level in AML patients.

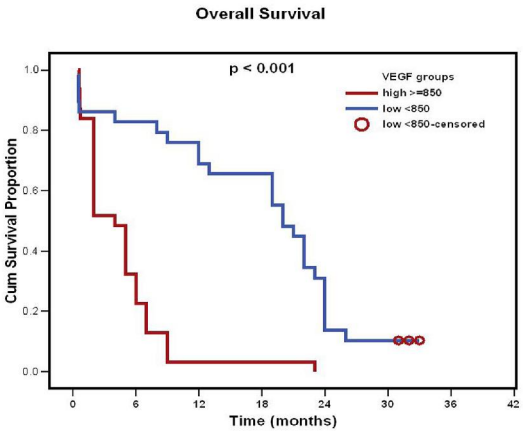

Figure 6: Overall survival and VEGF level in AML patients.

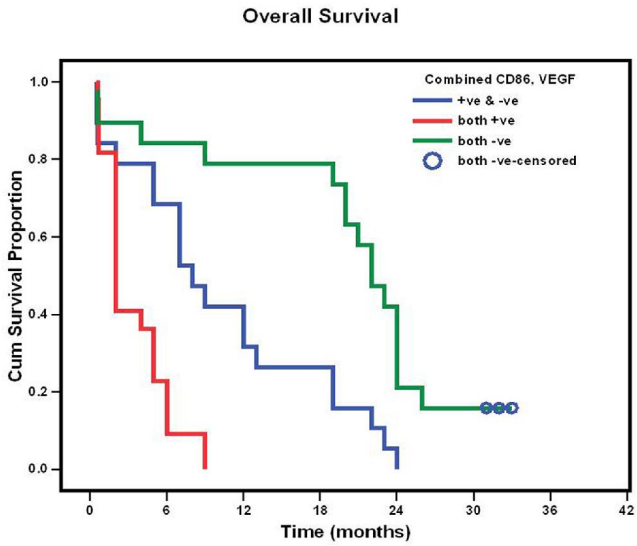

Figure 7: Overall survival and combined VEGF level & CD86 in AML patients.

4. Discussion

The present study was carried out to clarify the significance of CD80, CD86 and serum VEGF expressions in Egyptian de novo adult AML patients. All the examined patients were negative for CD80, in agreement with Vollmer *et al.* (2003), suggesting an impaired immune response in AML; a condition which might hamper the stimulation of T-cell responses. 32 out of the 60 examined cases (53.3%) were positive for the expression of CD86. Our results in this aspect agreed with those of Re *et al.* (2002) and Whiteway *et al.* (2003) who reported 54% and 57% positivity for CD86 respectively. In fact, the absence of CD80 could hinder the function of an expressed CD86, helping the leukemic blasts to escape immune surveillance mechanisms. Our results revealed also higher CD86 expression in AML blasts of FAB subtypes M4 and M5 compared to the other FAb subtypes ($p<0.001$), similar to the results of Notter *et al.* (2001) and whiteway *et al.* (2003). The former suggested that this reflects the biology of their non malignant cellular counterparts (the monocytes), which constitutively express CD86. The least CD86 expression was observed in M3, with a mean of 8.92% positive cells, in agreement with Tamura *et al.* (2005). The expression of CD86 was significantly lower in patients with low risk cytogenetic abnormalities compared to those with high and intermediate risk cytogenetic abnormalities ($p=0.015$).

Considering the cut-off of 20%, CD80 was negative in all patients, however, the mean CD80 percentage positivity was significantly higher in low risk versus high and intermediate risk cytogenetic abnormalities, even when both groups are in the negative category ($p<0.001$).

CD86 high expression was associated with failure of response to induction therapy with a pa value of $p=0.021$ between responders (low CD86 expressors) and non responders (high CD86 expressors). Also, high CD86 expression correlated with shorter disease free survival ($p<0.001$) as also mentioned by Graf *et al.* (2005) and shorter overall survival ($p=0.001$) as mentioned by Tamura *et al.* (2005), who suggested that the expression of CD86 on leukemic cells enhances the production of IL-4 and IL-10 which might inhibit tumor specific TH1 cell differentiation and CTL activity, resulting in the creation of a favourable environment for the growth of leukemic cells. Alos, Hock *et al,* (2006) suggested that sCD86 released provides a mechanism by which leukemic cells inhibit the immune response.

Our results also revealed a significantly increased sVEGF level in M4 and M5 subtypes compared to the other FAB subtypes with a p value of $p<0.001$, in agreement with Aref *et al.,* (2002) who suggested that M5 subtype is the commonest AML type associated with extramedullary infiltration, which might be due to the result of direct stimulation of monocytes by sHGF and sVEGF. In contrast to the results of Wierzbowska *et al.* (2003) and Aguayo *et al.* (2002) who reported no significant difference in sVEGF levels between low risk and high risk groups of cytogenetic abnormalities, we found significantly higher sVEGF levels in patients with high and intermediate risk cytogenetic abnormalities compared to those with low risk cytogenetic abnormalities ($p<0.001$). The level of sVEGF directly correlated to TLC and the percentages of peripheral blood and bone marrow blasts, as also reported by Aref *et al.* (2005) and Xie and Qi, (2003), who reported that this observation suggests that high levels of sVEGF

reflect increased blasts cell proliferation and mobilization and subsequently increased blast cell mass. It thus reflects blast cell mass, in spite of the fact that it was not estimated by flowcytometry. The levels of sVEGF in patients who entered into complete remission after induction chemotherapy were significantly lower than in patients who didn't enter into complete remission ($p=0.001$), in agreement with Aref *et al.,* (2005) and Wang *et al.,* (2004), who stated that VEGF has an impact on the effectiveness of some therapies, as it was reported to inhibit apoptotic death in hemopoietic cells after exposure to chemotherapeutic drugs by inducing MCL1, which acts as an anti-apoptotic factor. Higher levels of sVEGF associated with shorter disease free and overall survival ($p<0.001$ for both), as also reported by Aref *et al.,* (2005).

The combined elevation of CD86 and sVEGF correlated negatively with cytogenetic risk groups, as they were both higher in high and intermediate risk groups compared to the low risk group ($p=0.003$).

Conclusion

Absence of CD80 on AML blasts might be the cause of bad prognostic influence of CD86 costimulatory molecule. Both CD86 and VEGF might contribute to the proliferation and progression of leukemic cells in AML.

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