Journal of the Egyptian Society of Haematology & Research, Vol. 2, No. 2, September: 135-143, 2006

Clinical Relevance of Death Receptors (TNFR1, TNFR2 and CD95) and the Chemokine Receptor CXCR4 Expression in Childhood Acute Leukaemia

MAHA SALEH MADBOULY, M.D.*; GHADA MOHAMMED EL SAYED, M.D.*;
MANAR MOHAMAD ISMAIL, M.D.*; EMAD EBEID, M.D.**;
FATEN HAFEZ EL MAHALAWY, M.D.* and LOBNA MOSTAFA ABD EL MEGUID, M.D.*

The Departments of Clinical Pathology and Paediatirc Onclology**, National Cancer Institute, Cairo University.*

ABSTRACT

Introduction: Death receptors, such as TNF and FAS, play an important role in activating intracellular proteases leading to apoptosis. Some tumor cells such as T- & B-cell leukaemias, and acute myeloid leukemia (AML) over- and under-express FAS. These abnormal regulations of FAS suggest a significant association between tumor and host, namely tumor elimination by apoptosis and tumor survival or progression by escape from apoptosis. Recent studies have shown that CXCR4 is expressed on CD34+ haemopoietic progenitors and also variably on malignant CD34+ myeloid precursors (e.g. AML), as well as adult acute and chronic lymphoblastic leukaemias. A potential mechanism for trafficking of leukemia cells is the interaction of the chemokine receptor CXCR4 and its ligand SDF-1.

Aim of the Work: This study was conducted to evaluate the clinical relevance of expression of these molecules (TNFR1, TNFR2, CD95, and CXCR4) in acute leukaemia.

Material and Methods: We analyzed a cohort of 68 childhood acute leukemia patients (30 AML and 38 ALL) treated at Paediatric Oncology Department, National Cancer Institute, Cairo University, for the expression of the death receptors (FAS and TNFR1 & TNFR2) as well as the chemokine receptor CXCR4 by flow cytometry. We correlated the expression patterns with French-American-British (FAB) subclasses in AML and immunophenotypes of ALL and studied the relationship between expression levels of these molecules and survival.

Results: TNFR2 was expressed in 46.7% of AML and in 18.4% of ALL (p=0.012) while CD95 was expressed in 90% of AML and in 52.6% of ALL (p<0.001). The median expression of TNFR1 was observed to be higher in M4 & M5 than other AML FAB subtypes. The median expression of CD95 was 31 for T-ALL as compared to 12 for C-ALL, and 8 for pre-B ALL (p=0.04). In AML, no correlation between CD34 and CD95 expression was found, however, there was a significant association between extramedullary infiltration of malignant cells and expression of CXCR4 (p=0.05). No significant association was encountered between the studied parameters and response to induction chemotherapy in either ALL or AML. Survival analysis revealed that TNFR1 expression was significantly associated with both overall survival (OS) and disease-free survival (DFS) while expression of TNFR2 was significantly associated with OS in ALL.

We conclude that death receptors and CXCR4 can not be used to predict response to induction chemotherapy in paediatric acute leukaemia, however, TNFR-1 and -2 can predict survival. Since Fas expression is enhanced during maturation of the normal myeloid series and since we found no correlation between Fas expression and CD34 in AML, a possible interpretation is that Fas might be induced via several other pathways e.g. cytokines or cell to cell contact.

Key Words: *Death receptors - CXCR4 - Paediatric acute leukaemia.*

INTRODUCTION

Death receptors, such as TNF and FAS, play an important role in activating intracellular proteases leading to apoptosis. It has been demonstrated that T-cells can kill their target tumor cells using FAS and/or TNF receptors **[1,2]**. However it is less clear whether expression levels of death receptors may play a role in the susceptibility of the tumor to chemotherapy **[3,4]**.

Fas (APO-1/CD95) is a 45kd-membrane protein that belongs to the tumor necrosis factor (TNF)-nerve growth factor receptor family, a group of type 1 transmembrane receptors. Mutational analysis of Fas and the human TNF receptor (TNFR-1) proteins demonstrated that the cytoplasmic domains share a homologous

region necessary to induce the apoptotic signal. This conserved region of approximately 70 amino acids was, therefore, designated as the death domain (DD) **[5]**.

Based on the concept of activation-induced death in T-cells, the cytotoxicity of anti-cancer treatment using cytotoxic drugs or γ-irradiation has been studied with respect to involvement of CD95 receptor ligand interaction. In human T-cell lines, doxorubincin and other cytotoxic drugs used in chemotherapy of leukemias were found to induce CD95L expression **[4]**.

Some tumor cells such as T- & B-cell leukaemias, acute myeloid leukaemia (AML) over- and under-express FAS. These abnormal regulations of FAS suggest a significant association between tumor and host, namely tumor elimination by apoptosis and tumor survival or progression by escape from apoptosis. Indeed, down-modulation or abrogation of FAS on tumor cells such as hepatocellular carcinoma cells, cancer colon cells and chronic lymphocytic leukaemia cells whose Fas is constitutively expressed before transformation has been reported to be a selective advantage and to result in escape from Fas-mediated apoptosis. Fas also has been indicated as a surrogate marker of malignant behavior in T cell leukemia, implying that poor prognosis can be predicted based on the FAS expression levels **[6]**. In AML, some reports have suggested a correlation between FAS expression and complete remission rate and survival after chemotherapy treatment **[7,8]** although others have not confirmed these findings **[9,10]**.

Chemokines are chemotactic cytokines that coordinate development, differentiation, anatomic distribution and trafficking of leucoctyes during innate and adaptive immune reactions and appear to play an important role in tumour growth and expansion through autocrine or paracrine amplification mechanisms. The accumulation of malignant cells can indeed be favored by the production of chemokines by tumor cells themselves and/or surrounding nontumoral cells **[11]**. The stromal cell-derived factor 1 (SDF-1) plays an important role in leucocyte trafficking. It belongs to the CXC chemokine family, which is characterized by intervening residues within a conserved motif. In contrast to other members of the CXC chemokine family that are produced upon cytokine stimulation (e.g. increased interleukin 8 expression during inflammation), SDF-1 is constitutively produced by stromal cells. SDF-1 is not only restricted to the bone marrow, but also found in other tissues such as lymph nodes, liver, spleen, and brain. SDF-1 signals through a G protein-coupled receptor named CXCR4. Recent studies have shown that CXCR4 is expressed on CD34+ haemopoietic progenitors and also variably on malignant CD34+ myeloid precursors (e.g. AML), as well as adult acute and chronic lymphoblastic leukaemias **[12,13,14]**. A potential mechanism for trafficking of leukemia cells is the interaction of the chemokine receptor CXCR4 and its ligand SDF-1.

To evaluate the clinical relevance of expression of these molecules in paediatric acute leukemia, we analyzed a cohort of 68 childhood acute leukaemia patients presented to Paediatric Oncology Department, National Cancer Institute, Cairo University, for the expression of CXCR4 chemokine receptor as well as the death receptors FAS and TNFR1, and TNFR2. We correlated the expression patterns with French-American-British (FAB) subclasses in AML and immunophenotypes of ALL and studied the relationship between expression levels of these molecules and survival.

PATIENTS AND METHODS

Patients:

Sixty eight patients with newly diagnosed childhood acute leukaemia presented to the Paediatric Oncololgy Department, National Cancer Institute, Cairo University were included in the study following informed consent. Thirty patients were AML and 38 were ALL. The patients' characteristics are shown in Tables (1,2). Significant organ infiltration at initial diagnosis in children was defined by ultrasonografic extension of the liver edge ≥2cm below the right costal margin in the right midclacvicular line or the spleen edge ≥2cm below the left costal margin in the left midclacvicular line.

Methods:

The diagnosis of acute leukaemia was based on routine morphologic evaluation and cytochemical smears, as well as immunophenotyping according to the criteria of European Group of the Immunological Characterization of Leukemia (EGIL) **[15]**. B-cell acute lympho-

blastic leukemia (ALL) were subclassified into pro-B ALL, common ALL (n=15), pre-B ALL (n=9) and mature B ALL (n=1). T-cell ALL (n=13) were subclassified into early-T ALL, intermediate-T, and mature T ALL One case mature B ALL and one biphenotypic (B/T) were excluded for statistical reasons. Fresh bone marrow samples were obtained and processed using the whole blood lysing technique and two color staining methods using directly labeled monoclonal antibodies (MoAbs) against the myeloid and lymphoid-associated antigens. TNFR 1, TNFR2, CD95 and CXCR4 antigens were detected using fluorescin isothiocyanate (FITC) conjugated anti-TNFR1, TNFR2, CD95 and CXCR4, conjugated monoclonal antibodies (sc-12746, sc-12750, BD-555674, sc-12764#). FITC isotype matched mouse monoclonal antibodies were used as a negative control for expression analysis. Immunophenotype expression was measured by (FACVantage SE): Becton Dikinson, San Diego, USA) using the CellQuest software programe (Becton Dikinson). The blast region was gated by forward and right-angle light scatter parameters or CD45 antigen versus right-angle light scatter. The result of each antigen was expressed as percent positivity stained cells within the blast population. The antigen was considered positive when 20% or more cells in the blast region expressed the antigen **[16]**.

STATISTICAL METHODS:

Data management and analysis were performed using Statistical Analysis Systems. The graphs were done using Microsoft Word.

Numerical data were summarized using means and standard deviations. Categorical data were summarized as percentages. Comparisons between two groups with respect to numeric variables were done using the Mann-Witney nonparametric test, Kruskal-Wallis test was used for more than two groups. Comparisons between categorical variables were done by the chi square test or Fisher's exact for small sample size.

Overall survival was defined as the time from the date of diagnosis to the last date seen. Disease free survival was defined as the time from the end of treatment to the date of first relapse. Survival estimates were calculated using the Kaplan and Meier procedures. Comparisons of the survival time between the different groups were performed by the Log rank test **[17]**.

All *p*-values are two-sided. *p*-values less than or equal to 0.05 were considered significant.

RESULTS

Patients characteristics:

Clinical criteria of the studied AML and ALL patients are shown in Tables (1,2) respectively.

Expression of death receptors (TNFR1, TNFR2 and CD95):

TNFR2 was expressed in 14/30 (46.7%) and in 7/38 (18.4%) of AML and ALL respectively (p=0.012). On the other hand, CD95 was expressed in 27/30 (90%) of AML and in 20/38 (52.6%) of ALL (p<0.001) (Table 3).

In ALL, the median expression of TNFR1 & TNFR2 were not significantly different among ALL immunophenotypes (p=0.430 & 0.208 respectively). However, the median expression of CD95 was significantly higher in T-ALL than c-ALL or pre B-ALL (p=0.043). (Fig. 1, Table 4).

In AML, the median expression of TNFR1 in M4+M5 group was higher than in M3 or in M1+M2 groups. Such higher expression did not reach a statistical significance due to the small sample size. (Fig. 2, Table 5).

Expression of CXCR4 receptor:

CXCR4 expression did not differ between ALL and AML (p=0.4), among ALL immunophenotypes (p=0.779) or among AML FAB subtypes. (Tables 3,4,5).

Extramedullary infiltration and CXCR4 expression:

Patients were divided in two groups according to the presence or absence of splemomegaly and/or hepatomegaly at diagnosis.

In AML, there was a statistically significant association between CXCR4 expression and extramedullary involvement regardless of the FAB subtype (p=0.05). Patients with organ infiltration had a significantly higher expression of CXCR4 than those without organ infiltration, median 33.5 (9-82) and 13.5 (8-79) respectively.

Table (1): Clinical characteristics of AML patients at diagnosis (N=30).

Characteristic	
Male/female	1.5/1.0
Age years median (range)	8.8 (1.0-17.0)
WBC $X10^9$/L median (range)	16.4 (3.4-335.5)
HGB g/dl median (range)	7.3 (4.0-11.3)
PB blasts median (range)	57.0 (8.0-90.0)
BM blasts median (range)	71.5 (20.0-89.0)
Platelets $X10^9$/L median (range)	33.0 (5.0-84.0)
LDH I U/L median (range)	755 (335-2838)
FAB subtypes (No, %):	
M1 + M2	20 (66.7)
M3	4 (13.3)
M4 + M5	5 (16.7)
M6	1 (3.3)
Hepatomegaly (No, %)	9 (30)
Splenomegaly (No, %)	10 (33.3)
Lymphadenopathey (No, %)	6 (20)
CNS disease (No, %)	2 (6.6)
Response to induction chemotherapy (n, %):	
1- Complete remission	26 (86.7)
2- No response	4 (13.3)

Table (2): Clinical characteristics of ALL patients at diagnosis (N=38).

Characteristic	
Male/female	25/13
Age years median (range)	9 (1.0m-18.0)
WBC $X10^9$/L median (range)	26.6 (1.1-421.0)
HGB g/dl median (range)	7.7 (3.2-16.2)
PB blasts median (range)	34 (0.0-99.0)
BM blasts median (range)	87.0 (20.0-99.0)
Platelets $X10^9$/L median (range)	47.0 (10.0-329.0)
LDH I U/L median (range)	1074 (342-3848)
Immunophenotypes (No, %):	
B-ALL	25 (65.7)
T-ALL	13 (34.2)
Hepatomegaly (No, %)	18 (47.3)
Splenomegaly (No, %)	24 (63.1)
Lymphadenopathey (No, %)	17 (44.7)
CNS disease (No, %)	1 (2.6)
Response to induction chemotherapy (No, %):	
1- Complete remission	26 (68.4)
2- No response	12 (31.5)

Table (3): Expression of the studied markers on AML and ALL.

*Measurement	ALL N=38	AML N=30	*p* value
TNFR1	12 (31.6)	9 (30)	0.889
TNFR2	7 (18.4)	14 (46.7)	0.012
CD95	20 (52.6)	27 (90)	<0.001
CXCR4	18 (47.4)	17 (56.7)	0.446

* No (%).

Table (4): Expression levels of the studied markers among ALL immunophenotypes.

*Measurement	C-ALL N=15	Pre-B N=9	T-ALL N=13	*p* value
TNFR1	11 (0-82)	4 (0-32)	7 (0-30)	0.430
TNFR2	12 (0-72)	4 (1-34)	3 (0-18)	0.208
CD95	12 (1-79)	8 (2-41)	31 (2-82)	0.043
CXCR4	52 (1-87)	10 (2-67)	11 (1-96)	0.779

* Median (range).

Table (5): Expression of the studied markers among AML FAB subtypes.

*Measurement	M1+M2 N=20	M3 N=4	M4+M5 N=5
TNFR1	6.5 (1-34)	27 (3-39)	34 (2-82)
TNFR2	8.5 (1-68)	49 (2-77)	41 (1-77)
CD95	74 (10-92)	83 (36-93)	40 (11-70)
CXCR4	18 (8-82)	39 (9-55)	32 (8-79)

* Median (range).
p values not valid due to the small sample size.

In ALL, no statistically significant difference was found between the two groups (p=0.467).

Association between CD34 and the studied markers in AML:

TNFR1 was positive in 1/12 (8.3%) of the CD34 positive group as compared to 8/18 (44.4%) of the CD34 negative group with a significant difference between the two groups (p=0.049). On the other hand, expression of TNFR2, CD95, and CXCR4 was not different between CD34 positive and CD34 negative groups (p=0.072, 0.255, and 0.176 respectively). (Table 6).

Response to induction chemotherapy:

In AML, there was no significant association between TNFR1, TNFR2, CD95 and CXCR4 expression and response to induction chemotherapy (p=1.0 for all).

Similarly, in ALL, no significant association between TNFR1, TNFR2, CD95 and CXCR4

expression and response to induction chemotherapy was encountered (p=0.060, 0.075, 0.633, and 0.825 respectively).

Survival analysis:

Overall survival (OS):

One year OS in ALL was 100% for TNFR1 positive patients and 48% for TNFR1 negative patients with a significant difference between the two groups (p=0.016). The OS for TNFR2 was 100 versus 57% for patients with positive expression versus negative expression respectively with a significant difference between the two groups (p=0.042). (Table 7).

Disease free survival (DFS):

The median follow-up duration was 12 months ranging from 1.5 to 18 months. In ALL, DFS was 100% versus 59% for patients positively expressing TNFR1 versus those with negative expression with a significant difference between the two groups (p=0.005). (Table 8).

Table (6): Association between CD34 expression and the studied markers in AML.

*Measurement	CD34+ N=12	CD34– N=18	*p* value
TNFR1	1 (8.3)	8 (44.4)	0.049
TNFR2	3 (25.0)	11 (61.11)	0.072
CD95	12 (100)	15 (83.33)	0.255
CXCR4	5 (41.67)	12 (66.67)	0.176

* No (%).

Table (7): Impact of the studied markers on overall survival among ALL and AML.

	ALL			AML		
	Survival %	No.	*p* value	Survival %	No.	*p* value
TNFR1:						
Positive	100	12	0.016	88	9	0.808
Negative	48	26		83	21	
TNFR2:						
Positive	100	7	0.042	83	14	0.868
Negative	57	31		85	16	
CD95:						
Positive	73	20	0.246	86	27	0.270
Negative	61	18		66	3	
CXCR4:						
Positive	75	18	0.164	80	17	0.494
Negative	61	20		90	13	

Table (8): Impact of the studied markers on disease-free survival among ALL and AML.

	ALL			AML		
	Survival %	No.	*p* value	Survival %	No.	*p* value
TNFR1:						
Positive	100	11	0.005	83	6	0.837
Negative	59	15		78	14	
TNFR2:						
Positive	100	7	0.084	75	8	0.653
Negative	67	19		83	12	
CD95:						
Positive	84	13	0.176	82	17	0.428
Negative	61	13		66	3	
CXCR4:						
Positive	75	14	0.723	66	9	0.219
Negative	78	12		90	11	

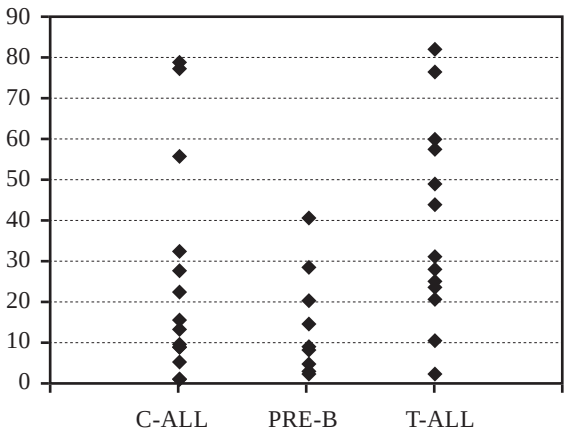

Fig. (1): Expression of CD95 among ALL immunophenotypes.

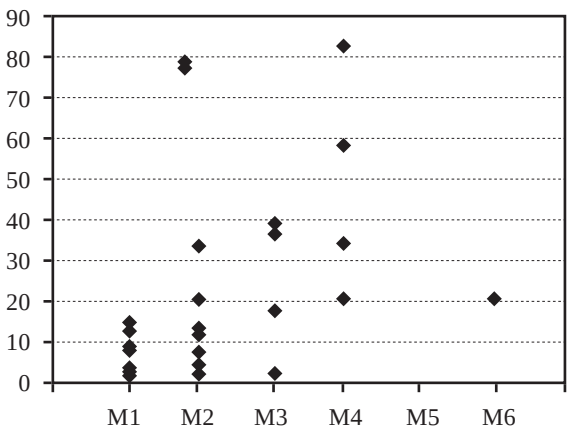

Fig. (2): Expression of TNFR1 among AML FAB subtypes.

DISCUSSION

Death receptors, such as TNF and FAS, play an important role in activating intracellular proteases leading to apoptosis. Some tumor cells such as T- & B-cell leukaemias, and acute myeloid leukemia (AML) over- and under-express FAS. These abnormal regulations of FAS suggest a significant association between tumor and host, namely tumor elimination by apoptosis and tumor survival or progression by escape from apoptosis. Recent studies have shown that CXCR4 is expressed on CD34+ haemopoietic progenitors and also variably in malignant CD34+ myeloid precursors (e.g. AML), adult acute and chronic lymphoblastic leukaemias. A potential mechanism for trafficking of leukemia cells is the interaction of the chemokine receptor CXCR4 and its ligand SDF-1 **[4]**.

To determine the clinical relevance of the expression of various death receptors (TNFR1, TNFR2, CD95) and the chemokine receptor CXCR4 on childhood AL cells, we performed a phenotypic analysis on leukaemia cells from 30 patients with AML and 38 patients with ALL. We also analyzed the relationship between expression levels of these molecules and OS as well as DFS rates.

In the current study, Fas was expressed with variable percentage (11-93%) in all AML cases and 90% were positive for FAS expression. Similarly, Iijima et al. and Munker et al. **[8,9]** found that Fas expression was quite variable in AML reflecting its heterogeneity.

In our AML cases, Fas was positive in 12 cases (100%) of the CD34+ group as compared to 15 cases (83.3%) of the CD34- group (p=0.2). Our result is similar to Iijima et al. **[8]** who found no correlation between Fas expression and CD34 expression. In normal haemopoesis, immature cells do not express a significant level of Fas. Fas expression becomes enhanced with the maturation pathway of myeloid series. Thus an alternative interpretation is that FAS might be induced via several pathways such as cytokines and cell-cell interactions. Previous studies have shown that INF γ and TNFα can induce FAS on normal and tumor cells **[8]**.

In our ALL, FAS expression was significantly lower (p<0.001) than in AML cases and this finding was supported by the previous study of Tsuruda et al. **[6]**. Despite this lower expression, CD95 was expressed in all cases of ALL with expression percentages varying between very low (1%) and high (89%). A comparison of B and T lineage ALL revealed a significantly higher CD95 expression in T-lineage ALL (p=0.04). Our results are similar to the previous studies **[6,18,19]**.

In this work, we found no association between Fas expression and response to induction chemotherapy in ALL. Similar data were also reported by Wuchter et al. **[19]**. The fact that, compared to AML, CD95 expression and function is constitutively low in ALL might explain the lack of its prognostic relevance in childhood ALL **[18]**. Also no significant association could be reached between CD95 expression and response to induction chemotherapy in AML. In contrast, CD95 expression was shown to correlate with response to induction chemotherapy in AML **[7,8]**.

Reports on FAS expression and prognosis are conflicting, showing no relationship **[9]** or a relationship with resistant disease **[7,8]** although no correlation between FAS expression and apoptosis could be proven. In the current study we did not find a significant relationship between CD95 expression and overall survival in AML or ALL. Our result may be supported by the previous observations that the expression of CD95 on the surface of leukaemic cells alone is not sufficient for CD95 induced apoptosis **[18]**. Although most of malignant cells expressed CD95 they failed to undergo CD95 mediated apoptosis showing low susceptibility to CD95 triggering.

Data regarding expression of TNFR1 and TNFR2 in AML are very scarce and no reports, so far, regarding their expression in ALL was found. In our work, TNFR1was significantly higher in AML than ALL (p=0.01) while TNFR2 was not different between both groups of AL (p=0.9). Both TNFR1 and TNFR2 expression was not significantly different among ALL immunophentotypes (p=0.4 & 0.2 respectively). Brouwer et al. **[16]** reported higher levels of TNFR1 and TNFR2 in AML-M4 & M5 FAB subtypes; our study only showed a higher median expression of TNFR1 in M4 & M5. Statistical analysis could not be done due to the small sample size. It is worth mentioning that, at the

individual case level, M1 showed the lowest values followed by M2. In ALL, we found a positive correlation between TNFR1 and TNFR2 expression and OS (p=0.016, and 0.04) respectively and a significant correlation between TNFR1 expression and DFS (p=0.005). TNFR1 may induce pro- and anti-apoptotic signals resulting in variable net effect **[20]**, whereas the role of TNFR2 is unknown. The relatively higher expression of death receptors in AML cells could imply that these cells are prone to apoptosis **[21]** but to date, there are no convincing data that show a clear correlation between high levels of FAS and TNFR and susceptibility to T, NK cell or chemotherapy mediated lysis **[4,22]**.

In our work, CXCR4 was expressed in all AML FAB subtypes with no observed difference between the groups. Our results are similar to the few available reports of Cignetti et al. and Mohle et al. **[11,12]** who found that all AML cases, regardless of the FAB subtype, express CXCR4. Although Brouwer et al. **[16]** found that FAB M4 and M5 show a significantly higher expression of CXCR4 than other FAB subtypes, our results could not support this finding. Expression of the chemokine receptor CXCR4 by the myeloid leukaemic cells suggests that such expression upon interaction with its ligand SDF-1 might mediate migration of leukaemic blasts across bone marrow endothelium.

In agreement with Mohle et al. **[13]**, we found a significant association between extramedullary organ infiltration of leukemia blasts and CXCR4 expression in AML. In our ALL, on the other hand, no significant association could be reached. This contrasts with the previous studies of Crazzaolara et al. **[23]** who found an association between extramedullary organ infiltration of leukemia blasts and CXCR4 expression. The reason for the discrepancy between the two studies could be attributed to small number of patients with mature B-ALL in our study (one patient) as it has been reported that the strongest expression of CXCR4 in ALL was found in mature B-cell ALL which is generally characterized by a high incidence of extramedullary bulky disease **[23]**.

In ALL patients CXCR4 expression was found in 47.3% of cases which was lower than the 91.9% observed by Crazzaolara et al. **[23]**. Their higher result could be due to the fact that they measured CXCR4 using mean fluorescence intensity (MFI) rather than percent expression as in our study thus allowing detection of low level of expression of the CXCR4 **[24]**.

The presumed normal counterparts of malignant acute lymphoblastic B-cells, have been studied for the expression and function of CXCR4 **[25,26]**. Expression of the chemokine receptor was found to be sinusoidal: Highest expression on pro-B cells, decreased as cells develop into immature B cells, and then increased again upon transition to mature B cell stage. In our study we found a similar expression pattern on lymphoblastic subpopulations: Highest expression in the c- ALL (median 52) with lower expression on pre-B ALL (median 10). Our results are supported by the finding of Crazzaolara et al. **[23]**.

In this work, no statistical difference in CXCR4 expression on lymphoblasts between B-and T-ALL (p=0.8). Similar observation was reported by Crazaolara et al. **[23]**.

In this study, no correlation was encountered between CXCR4 expression and either OS or DFS in ALL. Our result is not in agreement with Schneider et al. **[27]** who found that children with DFS under 24 months had a significantly higher expression of CXCR4 than children in first remission at 24 months. CXCR4 analysis in acute leukemia cells could be of value. However, its prognostic relevance requires further evaluation in large prospective studies of homogeneously treated patients.

In conclusion, TNFR2 and CD95 were significantly higher in AML than ALL while TNFR1 showed a higher median expression in M4 & M5 FAB subtypes. Since Fas expression is enhanced during maturation of the normal myeloid series and since we found no correlation between Fas expression and CD34 in AML, a possible interpretation is that Fas might be induced via several other pathways e.g. cytokines or cell to cell contact. There was a significant association betwee CXCR4 expression and extramedullary infiltration in AML. No correlation was found between expression of the studied molecules and response to induction chemotherapy in either ALL or AML. TNFR1 correlated significantly with both OS and DFS while TNFR2 correlated significantly with OS

in ALL. Expressopm of CXCR4 correlates with extramedullary infiltraion in AML. Death receptors and CXCR4 can not be used to predict response to induction chemotherapy in paediatric acute leukaemia, however, TNFR-1 and -2 can be used to predict survival in this group of patients.

REFERENCES

1- Nagata S. Apoptosis by death receptors. Cell. 1997, 88: 355-365.

2- Henkart PA, Sitkovsky MV. Cytotoxic lymphocytes. Two ways to kill target cells. Current Biololgy. 1994, 4: 923-925.

3- Findly HW, Zhou M. The clinical significane of Fas expression in leukemia. Questions and controversies. Leukemia. 1999, 13: 147-149.

4- Kaufmann SH, Earnshaw WC. Introduction to apoptosis by cancer chemotherapy. Experimental Cellular Reach. 2000, 256: 42-49.

5- De Thonel, Bettaib Ali, Jean C, Laurent G, Quillet-Mary A. Role of protein kinase C, isoform in Fas resistance of immature myeloid KGia leukemic cells. Blood. 2001, 98: 3770-3777.

6- Tsuruda K, Yamada Y, Hirakata Y, Sugahara K, Meda T, Atogma S, Tomonaga M, Kamihira S. Qualitative and quantitative characterization of Fas (APO-1/CD95) on leukemic cells derived from patients with B-cell neoplasms. Leukemia Research. 1999, 23: 159-166.

7- Min YH, Lee S, Lee JW, Chong SY, Hahn JS, Ko YW. Expression of FAS antigen in acute myeloid leukemia is associated with therapeutic response to chemotherapy. British Journal of Hematology. 1996, 93: 928-930.

8- Iijima N, Miyamura K, Itou T, Tanimoto M, Sobue R, Saito H. Functional expression of FAS (CD95) in acute myeloid leukemia cells in the context of CD34 and CD38 expression: Possible correlation with sensitivity to chemotherapy. Blood. 1997, 90: 4901-4909.

9- Munker R, Lubbert M, Yonehara S, Tuchitz A, Mertelsmann R, Wilmanns W. Expression of the FAS antigen on primary human leukemia cells. Ann Hematol. 1995, 70: 15-18.

10- Lewis NR, Pallis M, Russel NH. Fas receptor-Fas ligand system independent of both CD34 status and chemosensitivity in acute myeloid leukemia. Experimental Hematology. 2000, 28: 532-542.

11- Cignetti A, Vallario a, Roato I, Circosta P, Strola G, Scielzo C, Allione B, Garetto L, Caligaris-Cappio F, Ghia P. The characterization of chemokine production and chemokine receptor expression reveals possible functional cross-talks in AML blasts with monocytic differentiation. Experimental Hematology. 2003, 31: 459-503.

12- Mohle R, Bautz E, Rafii S, Moore MA, Brugger W, Kanz I. The chemokine receptor CXCR4 is expressed on CD34 hematopoietc progenitors and leukemic cells and mediates transendothelial migration induced by stromal cell-derived factor-1. Blood. 1998, 91: 4523-4530.

13- Mohle R, Bautz F, Rafii S, Moore MAS, Buugger W, Kanz L. Regulation of transendotherlial migration of hematopoietic progenitor cells. Annal of the New York Acadamy of Sciences. 1999a, 872: 176-186.

14- Mohle R, Schittehelm M, Failenschmid C, Butzf, Kratz-Albess K, Serve H, Brugger W, Kanz L. Functional response of leukemia blasts to stromal cell-derived factor-1 correlates with preferential expression of the chemokine receptor CXCR4 in acute myelomonocytic and lymphoblastic leukemia. Br J hematol. 2000, 563-572.

15- Bene MC, Castoldi G, Knapp W, Ludwig WD, Matutes E, Orfao A, Vannt Veer MB. Proposal for immunological classification of acute leukemias. Europian Group for the Immunological characterization of Leukemias. EGIL Leukmia. 1995, 9: 1783.

16- Brouwer R, Hoefnagel J, Broger Van Der Burg B, Jedema I, Zwinderman KH, Starrenburg I, Kulin-Nelenmans H, Barge R, Willemze R, Falkenburg J. Expression of co-stimulatory and adhesion molecules and chemokine or apoptosis receptor on acute myeloid leukemias: High CD40 and CD11a expression correlates with poor prognosis. British Journal of Hematology. 2001, 115: 298-308.

17- Dawson B, Trapp GT. Basic and Clinical Biostatistics, 3rd edition, Lange medical book, Appleton & Lange, Norwalk, Connecticut, 2001.

18- Karawajew L, Wuchter C, Ruppert V, Drexler H, Grudd H-J, Dorken B, Ludwig W-D. Differntial CD95 expression and function in T and B lineage acute lymphoblastic leukemia cells. Leukemia. 1997, 11: 1245-1252.

19- Wuchter C, Karawajew L, Ruppert V, Schrappe M, Harbott J, Ratei R, Dorken B, Ludwig WD. Constitutive expression levels of CD95 and Bcl2 as ell as CD95 function and spontaneous apoptosis in vitro do not predicts the response to induction chemotherapy ad relapse rate in childhood acute lymphoblastic leukemia. British Journal of Hematology. 2000, 110: 154-160.

20- Delwel R, Van Buitenen C, Lowenberg, Touw I. Involvement of tumor necrosis factor receptor p55 and p 75 in TNF responses of acute myeloid leukemia blasts in vitro. Blood. 1992, 80: 1798-1803.

21- Santini V, Gozzini A, Scappini B, Rossi FP. Maturation and apoptosis of primary human acute myeloblastic leukemia cells are determined by TNF-alpha exclusively through CD120A stimulation. Hematologica. 1999, 84: 291-297.

22- Ashenazi A, Dixit VM. Apoptosis control by death and decoy receptors. Current Opinions in Cellular Biology. 1999, 11: 255-260.

23- Crazzolara R, Krecky A, Mann G, Heitger A, Eibl G, Fink FM, Mohle R, Meister B. High expression of the chemokine receptor CXCR4 predicts exramedul-

lary organ infiltration in childhood acute lymphoblastic leukemia. Hematology. 2001, 15: 545-553.

24- Sharpio HM: Practical flow cytometry 3rd ed, Wiley liss John Wiley and sons INC publication New York. 1994. pg 192.

25- Fedyk ER, Ryyan DH, Ritterman I, Springer TA. Maturation decreases responsiveness of human bone marrow B lineage cells to stromal-derived factor 1 (SDF-1). Journal of Leucocyte Biology. 1999, 66: 667-673.

26- Honczarenko M, Douglas RS, Mathias C, Lee B, Ratajczak MZ, Silbertien LE. SDF-1 responsiveness does not correlate with CXCR4 expression levels of developing human bone marrow B-cells. 1999, 94: 2990-2998.

27- Schneider P, Vasse M, Al Bayati A, Leonormand B, Vannier JP. Is high expression of the chemokine receptor CXCR4 of predictive value for early relapse in childhood acute lymphoblastic leukemia. British Journal of Hematology. 2002, 119: 579-580.