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Prognostic Value of Serum IL-10 and Soluble IL-2 Receptor Level in Aggressive Non-Hodgkin's Lymphomas

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ABSTRACT

Combination chemotherapy has transformed aggressive NHL from a fatal disease into one that is often curable. However, many patients still die of their disease, underscoring the need of more accurate methods of prospectively identifying patients with different long term prognosis. In this study we aim to explore the ability of the combined detection of soluble IL-2 receptor (sIL-2R) and interleukin-10 (IL-10) to predict treatment failure in patients with aggressive non-Hodgkin's lymphoma (NHL). Sixty patients with aggressive NHLs were included in the study. All cases were subjected to history taking, clinical examination, intensive laboratory and radiological investigations and special investigations including ESR, serum LDH, serum IL-10 and serum sIL-2R before and after treatment with 4 doses of CHOP regimen. According to response to treatment they were divided into 3 groups (group I including patients with complete response, group II patients with partial response and group III patients with no response to treatment). The obtained results showed significant difference in the mean values of ESR mm/h in groups I, II and III before and after treatment. The results showed significant difference in the mean values of LDH/L, IL-10 pg/ml and sIL-2R IU/mL in group I before and after treatment with no significant difference before and after treatment in both group II and group III. It also showed significant difference between ESR and IL-10 in relation to clinical stage of the disease, significant difference in sIL-2R between patients with stage II and stage III, IV, a significant difference between ESR, sIL-2R and bulkiness of the tumor and significant difference between IL-10 and bulkiness of the tumor. IL-10 and sIL-2R levels declined to normal range in responding patients whereas they remained elevated in partially responder and non responder patients. Finally the results showed that IL-10 was the only associated risk for failure of treatment induction in univariate analysis but IL-10 and sIL-2R maintained their prognostic significance with multivariate logistic regression analysis. We can conclude that, the detection of pretreatment levels of serum IL-10 and sIL-2R are of prognostic value in patients with aggressive NHLs with high tumor mass who would probably require different strategies and therapeutic reinforcement.

Key Words: *Aggressive NHL - IL-10 - sIL-2R - Prognosis.*

INTRODUCTION

Non-Hodgkin's lymphoma (NHL) comprises a large spectrum of lymphoproliferative disorders with different clinical and biologic features [7].

Although, there has been a dramatic improvement in the ability to therapeutically alter the course of this malignancy, a substantial proportion of patients may either not respond to treatment or experience relapse and eventually die of progressive disease that is not sensitive to further chemotherapy [1] underscoring the need of more accurate methods of prospectively identifying patients with different long term prognosis.

The identification of those at high or low risk would have important therapeutic implications. It would also aid in the design and interpretation of therapeutic trials [9].

Pretreatment soluble IL-2R and IL-10 concentrations have been reported to significantly predict treatment outcome or recurrence of the disease [2].

IL-10 is a cytokine normally originating from monocytes, macrophages and T cells and exerting multiple biological effects on hemopoietic cell lingeages [8]. Although the significance of elevated IL-10 in NHLs was undetermined, studies on B lymphomas and HIV related lymphomas suggest that this cytokine may play a pathogenetic role [2].

Soluble IL-2R is a truncated portion of the alpha chain of the Tac antigen (CD25) capable of efficiently binding IL-2. Besides being expressed by normal lymphoid cells and monocytes upon activation, CD25 has been demonstrated on unstimulated neoplastic cells in many lymphoproliferative disorders [6].

In this study, we aim to use IL-10 and sIL-2R as prognostic indicators and markers to monitor disease activity in patients with aggressive NHLs.

PATIENTS AND METHODS

Patients:

This study was carried out at the Medical Oncology Hematology unit, Internal Medicine Department, Faculty of Medicine, Zagazig University Hospitals between June 1997 and December 1998 where 60 patients with pathologically confirmed aggressive non Hodgkin's lymphomas by the Working Formulation classification were included (Table,1). They were divided into 3 groups according to the response to treatment: group I including patients with complete response (CR), group II patients with partial response (PR) and group III patients with no response (NR).

All patients that fulfilled the following eligibility criteria were included:

1- Age more than 18 years and less than 70 years.
2- Performance status P.S. ≤ 2.
3- Clinical stage II to IV.
4- No previous chemotherapy or radiotherapy.
5- Cardiac, hepatic and renal functions within normal.

Methods:

All patients were subjected to the following: thorough history taking and clinical examination, complete blood count (CBC), bone marrow aspirate and biopsy, liver and kidney function tests, serum uric acid, serum electrolytes, radiological studies including chest X-ray, abdominal ultrasonography and C.T. abdomen and pelvis for proper staging.

All patients were subjected to two blood samples to estimate specific prognostic parameters, including ESR, serum LDH, serum IL-10 (MEDGENIX IL-10 ELISA)$^{T.M}$ and serum IL-2R (PREDICTA) Genzyme corporation USA.

One sample was taken before starting chemotherapy, another sample was taken after 4 cycles of chemotherapy. All patients were treated with CHOP regimen; cyclophosphamide 750 mg/m^2 I.V. day I, adriamycine 50 mg/m^2 I.V. day I, vincristine 1.4 mg/m^2 I.V. day I and prednisone 60 mg/m^2 P.O. daily for 5 days to be repeated every 21 days. All patients were followed up monthly during therapy and at the end of 4 cycles of chemotherapy.

Statistical analysis:

Data were analyzed using Epi-INFO version 6.1 software package [5]. Data were expressed as mean ± standard deviation, *t* test, paired *t* test and Chi-Square test were used for analysis of the results. A *p*-value of ≤ 0.05 was considered as statistically significant.

Logistic regression analysis by the Logress 2 program was used to determine the prognostic factors that predict the achievement of response or conversely, failing of treatment. Continuous variables were dichotomized according to their median into low and high.

RESULTS

Table (2) shows ESR, LDH, IL-10 and sIL-2R changes before and after treatment. Where a significant difference in ESR level before and after treatment was obtained for the 3 groups, most profound in group I and II, while LDH, IL-10 and sIL-2R levels showed significant difference before and after treatment only for group I.

Table (3) shows ESR, LDH, IL-10 and sIL-2R levels in relation to clinical stage of the tumor before treatment. There was no significant difference in LDH in relation to the stage of the tumor, a significant difference in ESR and IL-10 and significant difference in sIL-2R between stage II and stage III and IV.

Table (4) shows ESR, LDH, IL-10 and sIL-2R levels in relation to bulkiness of the tumor before treatment. There was no significant difference between LDH and bulkiness of the tumor while there was significant differences between ESR, sIL-2R and bulkiness of the tumor and high significant difference between IL-10 and bulkiness of the tumor.

Table (5) shows association between pre-

treatment parameters and risk for failure of treatment induction. There was no significant difference in all parameters, except in IL-10 (p = 0.002) in univariate analysis, but IL-10 and sIL-2R maintained their prognostic significance with logistic regression analysis (Table 6).

Table (1): Characteristics of 60 NHL patients.

Parameters	Total	Group I		Group II		Group III	
		No.	%	No.	%	No.	%
Sex:							
Male	35	13	37.1	10	28.6	12	34.3
Female	25	7	28.0	10	40.0	8	32.0
Stage:							
II	13	5	38.5	4	30.8	4	30.8
III	33	12	36.4	13	39.4	8	24.2
IV	14	3	21.4	3	21.4	8	57.1
B-symptoms:							
+ve	28	9	32.1	8	28.6	11	39.3
-ve	32	11	34.4	12	37.5	9	28.1
Bulky disease:							
> 10 cm	26	8	30.8	8	30.8	10	38.5
< 10 cm	34	12	35.3	12	35.3	10	29.4
Histopathology:							
Diffuse mixed small and large cell	20	6	30.0	9	45.0	5	25.0
Diffuse large cell	28	9	32.1	9	32.1	10	35.7
Large follicular	3	1	33.3	0	0.0	2	66.7
Small non cleaved	6	4	66.7	2	33.3	0	0.0
Lymphoblastic lymphoma	2	0	0.0	0	0.0	2	100.0
Anaplastic pleomorphic	1	0	0.0	0	0.0	1	100.0

Group I : Patients with complete response.
Group II : Patients with partial response.
Group III: Patients with no response.

Table (2): ESR, LDH, IL-10 and sIL-2R changes of 60 NHL patients before and after treatment.

Group	ESR			LDH			IL-10			sIL-2R		
	Before treatment Mean ± SD (Range)	After treatment Mean ± SD (Range)	p-value	Before treatment Mean ± SD (Range)	After treatment Mean ± SD (Range)	p-value	Before reatment Mean ± SD (Range)	After treatment Mean ± SD (Range)	p-value	Before treatment Mean ± SD (Range)	After treatment Mean ± SD (Range)	p-value
I	77.05± 24.5 (30-110)	8±3.7 (3-15)	0.000	7329± 273.3 (113-1260)	322.6± 95.7 (100-410)	0.000	351.7±3 87.1 (48-950)	71.2± 13.5 (51-94)	0.000	5753.1± 2474.8 (1843-9360)	1770.5± 549.3 (1185-3085)	0.01
II	88.65± 23.45 (45-135)	52.5± 21.48 (15-90)	0.000	936.1± 252.3 (80-1400)	884.57± 234.4 (510-1300)	0.1	498.5± 337.3 (46-992)	394.45± 206.9 (170-929)	0.06	6203.3± 2446.8 (807-9727)	5301.8± 2266.8 (2283-9670)	0.07
III	98.5± 20.08 (60-130)	89.9± 12.7 (65-110)	0.02	870.5± 189.6 (530-1300)	858.02± 158.2 (600-1200)	0.7	596.2± 281.8 (82-885)	614.1± 181.2 (248-950)	0.74	6657.5± 2554.5 (1115-9560)	5818±1 574.2 (2889-8384)	0.22

Paired t-test was used.
Group I : Patients with complete response.
Group II : Patients with partial response.
Group III: Patients with no response.

Table (3): ESR, LDH, sIL-2R, IL-10 before treatment in relation to stage of the tumor in 60 NHL patients.

Parameters	Stage II No. of cases (13) X±SD (Range)	Stage III, IV No. of cases (47) X±SD (Range)	*p**
ESR (mm/h)	73.3±18.9 (35-105)	91.7±23.9 (30-135)	0.015
LDH (u/mL)	872.1±228.6 (530-1260)	839±258.9 (113-1400)	0.69
IL-10 (pg/mL)	229.6±214.8 (62-520)	246.25±342.8 (46-992)	0.031
sIL-2R IU/mL)	2639.08±1394.1 (1115-6301)	7096.02±1787 (807-9727)	0.000

* *t*-test was used.

Table (4): LDH, IL-10, sIL-2R, ESR before treatment in relation to bulkiness of the tumor in 60 NHL patients.

Parameters	Tumor size > 10 cm No. of cases (26) X±SD (Range)	Tumor size < 10 cm No. of cases (34) X±S (Range)	*p**
LDH (U/L)	830.5±277 (480-1300)	857±235.09 (113-1400)	0.690
IL-10 (pg/mL)	744.3±225.7 (75-992)	294.8±294.8 (46-885)	0.00004
sIL-2R IU/mL)	7226.39±2240.3 (807-7927)	5474±2407.3 (1115-9560)	0.005
ESR (mm/h)	98.9±21.4 (44-135)	80.2±22.9 (30-125)	0.002

* *t*-test was used.

Table (5): Association between pretreatment parameters and risk to fail induction treatment in 60 NHL patients.

Univariate analysis	No. of patient	Responder		Non-responder		*p*-value*
		No.	%	No.	%	
ESR:						0.143
≤ 90 mm/h	31	13	41.9	18	58.1	
> 90 mm/h	29	7	24.1	22	75.9	
Stage:						0.146
II	12	6	50.0	6	50.0	
III	34	12	35.3	22	64.7	
IV	14	2	14.3	12	85.7	
LDH:						0.217
≤ 585 U/L	9	5	55.6	4	44.4	
> 585 U/L	51	15	18.9	36	70.6	
IL-10:						0.002**
≤100 pg/mL	23	13	56.4	10	43.5	
> 100 pg/mL	37	7	18.9	30	81.1	
sIL-2R:						0.34
≤ 3500 IU/mL	11	5	45.5	6	54.5	
> 3500 IU/mL	49	15	30.6	34	69.4	

* Chi-squared (X^2) was the test of significane. ** *p* value was significant.

Table (6): Logistic regression analysis for the value of IL-10, sIL-2R, LDH to predict the risk to fail induction treament in 60 NHL patients.

Logestic model	Beta-coefficient	SE	OR (95% CI)	*p*-value
IL-10	1.329	0.538	2.02 (1.26-3.2)	< 0.01
sIL-2R	1.803	0.630	1.78 (1.14-2.97)	< 0.05
LDH	1.871	0.539	1.73 (1.06-2.63)	< 0.05

DISCUSSION

Over the last decade, new diagnostic and therapeutic developments have resulted in higher cure rate for patients with aggressive non-Hodgkin's lymphoma (NHL). A more precise definition of the variables of prognostic significance has led to a more rational stratification of patients into multiple risk categories for which different therapeutic strategies are required.

Prognostic indicators for clinical use, other than pathological stage, have been found to be

reliable predictors of treatment outcome. They can be divided into those related primarily to the patients (e.g. age) and those related to the tumor itself (histologic subtypes, bulkiness). Laboratory evaluation, particulary serum concentration of LDH and ß$_2$ microglobulin, also have led to improvement in staging systems **[11]**.

Recently, the measurement of initial cytokine and soluble receptor level has been suggested as an additional tool for the clinical assessment of patients with NHLs.

In our study, we found that there was an elevation of pretreatment level of IL-10 which was related to the stage of the disease and highly related to the bulkiness of the disease, but not with other prognostic factors including age, sex, performance status, histopathology, ESR and LDH. This is in line with the studies of Stasi et al. and Blay et al. **[10,3]**.

Also, IL-10 was measured again after treatment and it was found to be markedly reduced down to the normal level in patients who achieved complete remission, while it was still slightly elevated in patients with partial remission and very high or even higher than those at presentation in non responder individuals.

This is in agreement with the study of Blay et al. **[2]** who detected serum IL-10 in half of the patients with active NHLs, whereas it was mostly undetectable in more than 90% of patients who achieved CR as well as in all healthy donors tested.

This is also in line with the study of Stasi et al. **[10]** who found that follow up of patients at diagnosis, remission and relapse highly suggested that the detectable serum IL-10 was associated with the presence of active disease.

sIL-2R level in our study was found to be very high in most cases and was related to stage of the disease as patients in stage III, IV showed higher level of sIL-2R than those in stage II; also it was related to the bulkiness of the tumor. However, it was not related to any other prognostic factor including age, sex, performance status, histopathology, ESR or LDH. This result is in line with Chilosi et al. **[4]**.

In our study, sIL-2R was measured again after treatment and it was found to decrease markedly to a level near that of normal in patients who achieved complete remission and slightly decreased in patients with partial remission, while it was still highly elevated, even higher than pre treatment level, in non responder patients. This is an agreement with the study of Stasi et al. **[10]** who found that sIL-2R was elevated at diagnosis and have proven that it was a reliable marker to monitor disease activity as regression of the disease was associated with progressive decrease of circulating values of this receptor whereas unresponsive cases retained elevated levels.

Although no single factor was associated with response to therapy, we managed to identify a subgroup of non responders, with elevated IL-10 and sIL-2R. This may be justified biologically because, as previously discussed, elevated sIL-2R level reflects tumor bulkiness and high IL-10 level suggests a poor immunological recognition of the tumor. This is in line with Blay et al. **[2]** who found that elevated levels of these two molecules were associated with poor outcome in terms of survival.

The achievement of complete remission is in fact the primary goal of chemotherapy, so early separation of this poor risk category from the more favorable groups will permit the use of intensive therapy for patients with adverse pretreatment characteristics while avoiding undue toxicity for patients who can be cured using the current regimens.

In conclusion, the detection of sIL-2R and IL-10 can be considered as a useful tool in the management of aggressive NHLs, with high tumor mass that may require different strategies and therapeutic reinforcement.

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