6 392-07 6

Med. J. Cairo Univ., Vol. 62, No. 1, March: 1 - 11, 1994

Cytochemical and Immunocytochemical Studies in Some Lymphoproliferative Disorders

AIDA ABDEL AZIME, M.D.; ZEINAB A. ISMAIL, M.D.; ZEINAB ABDEL AAL, M.D.; OSAMA B. SEDIK, M.D. and ESMAT ABDEL AZIZ, M.Sc.

The Clinical Pathology Departments, Faculties of Medicine, Ain Shams, Minia and Assiut Universities.

Abstract

This study was carried out on 40 patients with different lymphoproliferative disorders: 20 patients with acute lymphoblastic leukemia (ALL), 15 patients with non-Hodgkin's lymphoma (NHL), 3 patients with chronic lymphocytic leukemia (CLL) and 2 patients with multiple myeloma (MM). The study showed that about 80 % of BNHL group express CD10 antigen and that T cells sometimes express it. The 2 cases of MM express CD10 and not CD19 although the latter is a B cell restricted antigen. CD3 is expressed on more mature forms of T-lymphocytes than CD7.

Introduction

N recent years immunocytochemical techques for studying cell surface antigens on ıman bone marrow and peripheral blood ells have provided much new information ı white cell populations and their diffcntiation and have also proved of great ılue in the diagnosis and classification of matological malignancies [1].

Lymphocytes and leukaemic cells can classified into different types and subɔes based on specific or selective cell ırkers, the latter may be receptors [2], enmes [3] or maturation and/or differentian linked antigens [4], actual phenotyping aracteristic can be detected by rosette as/s, immunofluorescence and immunoenzymatic methods [5].

At present the use of immunoenzymatic or immunocytochemical tests in the diagnosis of hematological neoplasms shows that these tests are simple and reliable tests and have many practical advantages [6].

The present study tries to use cytochemical tests as markers in the phenotyping of lymphocytes in some lymphoproliferative disorders and the use of Alkaline Phosphatase Anti-Alkaline Phosphatase (APAAP) technique as an immunoenzymatic method in the immunophenotyping of lymphocytes and further immunological classification of the diseased groups, then study the correlation between cytochemical and immunocytochemical results.

Material and Methods

The present study involved 40 patients and were divided into:

Group I (ALL): the group consists of 20 patients, all cases are children except one case aged 70 years, their age ranged from 2 to 70.

Group II (NHL): this group consists of 15 patients, their ages ranged from 6 to 65 years.

Group III (CLL): it consists of 3 patients, their ages ranged from 50 to 60 years.

Group IV (MM): 2 cases aged 45 and 48 years.

Samples: 1 ml. of venous blood was withdrawn under complete aseptic conditions from median cubital vein of each patient on tube containing EDTA.

The following laboratory tests were performed for each patient:

A- Complete blood picture using Coulter counter model T660, the differential leukocytic count was done after staining film with leishman stain and reticulocyte percent after staining with brilliant cresyl blue.

B- Bone marrow aspiration.

C- Cytochemical studies for both peripheral blood and bone marrow and include the following:

1- Acid phosphatase (AP) [7].
2- Periodic acid schiff (PAS) [8].
3- Sudan black B (SBB) [9].
4- Alpha naphthyl acetate esters (α NAE) [10].
5- Oil red O (ORO) [11].
6- Methyl green pyronine (MG) [12].

D- Immunocytochemical tests using APAAP technique [13].

Principle: in the APAAP technique, a primary mouse monoclonal antibody is used to identify cellular antigens (Behringwerke research products). A rabbit anti-mouse immunoglobulin acts as an antibody bridge to connect the primary mouse monoclonal antibody now bound to the antigen with APAAP complexes (mouse monoclonal anti-AP bound to calf intestinal APAAP complexes from Behring). The alkaline phosphatase then hydrolizes the substrate naphthol AS-BI phosphate or naphthol AS-MX phosphate to phosphate and arylnaphtholamide which is coupled to the diazonium dye, Fast Red TR forming an insoluble precipitate.

Results

All the results of the peripheral haemogram are shown in table (1).

In ALL the bone marrow is hypercellular in most cases with predominance of blast and abnormal mononuclear cells. In NHL there is complete replacement of the bone marrow by abnormal mononuclear cells and blast cells, however, in CLL the bone marrow showed high number of small lymphocytes with few immature forms. In MM the bone marrow is normocellular in both cases with infiltration by 85% myeloma and plasma cells in one case and 70% in the other case.

Cytochemistry: AP, PAS and MG gave positive results in some groups as shown in table 2 and Fig. 1a, 1b & 1c while SBB and α NAE and ORO gave negative results in all cases.

Immunocytochemistry: table 3 shows reactivity of monoclonal antibodies in different groups using APAAP technique as shown in Fig. 2a, 2b & 2c which show strong positivity of APAAP reaction in one case of each T NHL(CD7), MM(CD10)

and CLL(CD19) as primary monoclonal antibodies respectively, the results of correlations are shown in table 4.

AP shows high significant positive correlation with both CD3 and CD7 in ALL group (p <0.01), while in NHL the correlation in complete (p = 0).

The correlation between CD3 and CD7 is strong positive in ALL (p <0.001) and complete in NHL (p = 0).

PAS shows significant positive correlation with CD10 in ALL group and with CD19 in NHL group (p <0.05) and the correlation is nonsignificant between PAS and CD19 in ALL (p <0.2) and CD10 in NHL (p <0.4).

The correlation between CD10 and CD19 is negative in ALL (r = -0.459, p <0.05) and weak positive in NHL (p <0.1).

Table (1): Mean ± SD of Peripheral Haemogram in Different Diseased Groups.

Group	ALL	NHL	CLL	MM
Hb in gm/dL (mean ± SD)	5.47± 1.8	7.86 ± 3.5	8.8 ± 1.9	8.5 ± 0.7
RBCs. 10^{12}/L (mean ± SD)	2.135 ± 0.753	2.881 ± 1.35	3.160 ± 0.66	3.3 ± 0.28
Hct % meaan ± SD	17.5 ± 5.4	24.35 ± 10.6	28.6 ± 8.1	35 ± 11.3
Type of anemia	normocytic normochromic	normocytic normochromic	normocytic normochromic	normocytic normochromic
Reticul %	2.5 ± 2	1.82 ± 1.3	2.56 ± 0.6	1.3 ± 0.8
WBCs / Cumm	33.05 ± 30.14	89.94 ± 11.41	117.66 ± 33.23	3.900 ± 0.56
Platelet /Cumm	63.9 ± 4.8	99.6 ± 95	111.66 ± 63.4	125 ± 35.3

Table (2): Results of Cytochemical Tests in Different Diseased Groups.

Cytochemical test	ALL (n = 20)	NHL (n = 15)	CLL (n = 3)	MM (n = 2)
Acid phosphatase				
No. of positive cases	3	3	0	0
% of positive cases	15 %	20%	0	0
Periodic acid schlff				
No. of positive cases	11	7	0	0
% of positive cases	55%	46.6%	0	0
Methyl green-pyronine				
No. of positive cases	1	0	0	0
% of positive cases	5 %	0	0	0

Table (3): Reactivity of Different Monoclonal Antibodles in Different Diseased Groups.

Disease group	CD 3		CD 7		CD 10		CD19	
	No. of + ve cases	%	No. of + ve cases	%	No. of + ve cases	%	No. of + ve cases	%
ALL n = 20	3	15	4	20	17	85	1	5
NHL n = 15	3	20	3	20	10	66.6	12	80
CLL n = 3	0	0	0	0	0	0	3	100
MM n = 2	0	0	0	0	2	100	0	0

Table (4): The Correlation between Cytochemical Tests and Lymphocyte Markers and between Different Markers Together.

	ALL n = 20	NHL n = 15
AP and CD3		
r	0.608	1.0
p	<0.01	0
AP and CD7		
r	0.608	1.0
p	< 0.01	0
CD3 and CD7		
r	0.84	1.0
p	< 0.001	0
PAS and CD10		
r	0.46	0.095
p	< 0.05	0.40
PAS and CD19		
r	0.208	0.468
p	< 0.2	0.05
CD10 and CD19		
r	-0.459	0.354
p	< 0.05	< 0.10

Discussion

Cytochemistry is the application of chemical processes to microscopicaol preparations and by its use an attempt is made to reveal the chemical composition of cells, usually by development of colour reaction without damaging the cells [14]. Our results of AP are in agreement with the results obtained by Telke et al. and Veerman et al. [15,16]. We found a characteristic polar pattern of positivity of both T-ALL and T-NHL groups, three cases of four in T-ALL and all cases of T-NHL (3 cases).

These findings provided the first clear example of a correlation between a cytochemical property and a well defined immunological phenotype.

The small sample size in CLL and MM may explain the cause of our disagreemen with results of Grossi et al. [17] who found AP positive more than negative cases in CLL.

As regard PAS the reaction in our study is positive in 55% of cases of ALL an

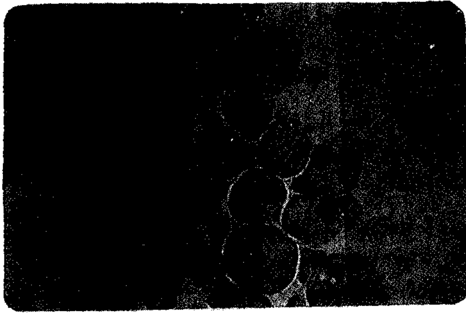


Fig. (1 a): Strong AP positivity (polar pattern) was shown in case with T-ALL (BM film).

Fig. (1 b): Strong PAS positivity was shown in case with B-NHL (BM film).

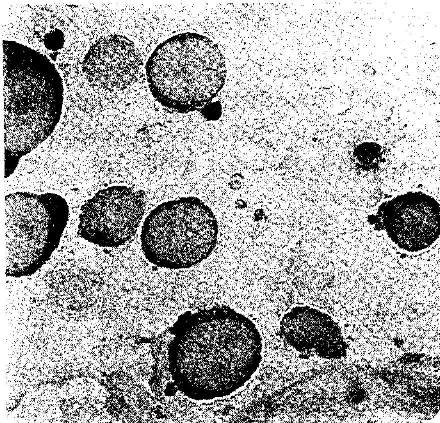
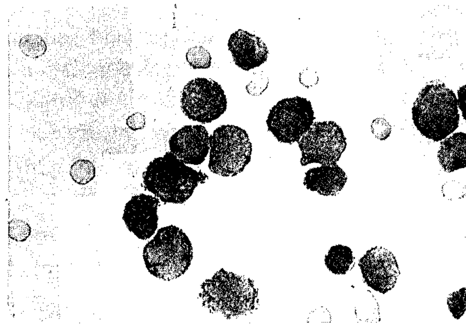


Fig. (1 c): MG with intense pyroninophilia of the cytoplasm was shown in case with L3-ALL (BM film).



F (2 a): Strong positivity of APAAP reaction (diffuse and focal patterns) in case with T-CHL using CD7 as primary McAb (BM film.

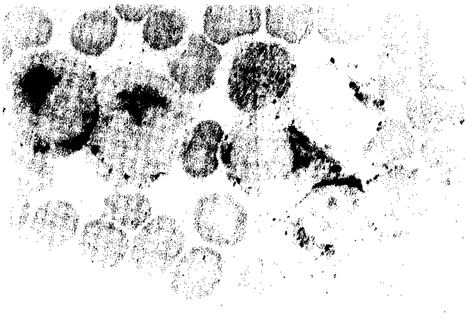


Fig. (2 b): Strong positivity APAAP reaction (diffuse and focal patterns) in case with MM using CD10 as McAb (BM film)

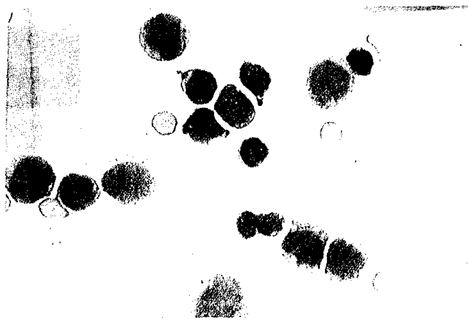


Fig. (2 c): Strong positivity of APAAP reaction (linear pattern) in case with CLL using CD19 as primary McAb (BM film).

46.6% of cases of NHL with the range of positivity from granular to block, this is in agreement with the finding of Catovsky et al. [18] who reported that PAS is the most common positive test in CALL, this block of positivity represents the glycogen content of leukocytes [19,20].

The results of α NAE coincide with that of Veerman et al. [16] and Boesen et al. [21] who could not confirm the activity of it in T and B lymphoproliferative diseases.

As regard SBB no positive cases were found in this study as the finding of significant granular sudanophilia in the cells of lymphoid series is uncommon [22]. Occasional additional sporadic reports of sudanophilia in leukaemic blast cell populations clearly marked as lymphoblastic by immunological reactivity were reported by Savage and Hoffman [23] and Stass et al. [24]. This is due to the staining of neutral fat and phospholipids within membrane bound phagosomes.

The positivity of ORO reaction distinguishing leukaemic lymphoblasts from myeloid precursors since the pattern of positivity in lymphoblasts is globular while in myeloid is fine granular [25]. But the reaction is less valuable in separating the forms of acute leukaemic as found by Shaw and Klemp [26], also we found ORO to be negative in all cases of our study.

As regards MG our results met with results of Bessis [27] who reported that the pyroninophilia of the cytoplasm is more intense in L3 type of ALL, we found only one case with intense pyroninophilia in cytoplasm and this case was diagnosed by FAB morphological classification as L3 and by immunophenotyping as B-cell variant.

The recent development of monoclonal antibodies (McAb) has provided the advantage of large scale production and universal availability and standardized specific reagents for immunophenotype determination [28].

Monoclonal antibody analysis has demonstrated that T-lymphocytes express different surface antigens at successive stages of maturation [29], the terminally differentiated thymocytes (stage III) found in the thymic medulla express CD7, CD2, CD3, the T-antigen receptor and either CD4 or CD8 membrane antigens [30].

Also Vodenlich et al. [31] described CD7 as the reagent of choice in the routine diagnosis of T-ALL and with other marker candidates for the use in the treatment of this disease in the future, also Cantue et al. [32] considered it as the reagent of choice.

Our results concerning CD3 and CD7 as a definite T-lymphocyte marker coincide with previous findings since we found CD7 positive in 4 cases of ALL group and CD3 is positive in 3 cases of these 4 cases as shown in table (2) and also AP with the characteristic pattern in almost all blast and immature form was found in 3 cases CD3 and CD7 positive. So from the previous findings there is a high significant positive correlation between AP positivity in T-ALL and CD3 expression and also with CD7 (p <0.01), while in NHL 3 cases are positive for CD3 and CD7 and there is a complete positive correlation between AP and both CD3 and CD7 ($p = 0$).

Keller et al. [33] recorded no expression of CD3 in B-CLL and reported CD7 expression in few cases, we found no expression of both CD3 and CD7 but this may be due to small sample size we studied.

San et al. [34] studied the immunophen

otyping of MM using a panel of monoclonal antibodies but none of the T-cell markers were included and this is in agreement with our study in which we found no expression of CD3 and CD7 in MM.

CD10 or CALLA has become a standard marker used in the identification of patients with ALL. CALLA is also present in Burkitt lymphoma cells, follicular lymphoma cells and cells from 40% of patients with lymphoblastic lymphoma [35]. In our study we found CD10 to be expressed in 85% of cases of ALL group and this percent agrees with the international recorded results of Chesseles et al. [36] and Richard et al. [37] who found CD10 to be expressed in 70-80% of non T-ALL.

In NHL 12 cases (80%) expressed CD19 included 10 cases expressed CD10 which represented 67% of NHL studied cases since T type of NHL does not express CD19, all these results are in agreement with the recorded studies of Jerom et al. [38] who found CD10 to be expressed in all cases of B-NHL while Anderson et al. [39] found CD10 to be expressed on more than half of his studied cases.

In CLL CD10 is not expressed as this is a definite differentiation between CLL and leukaemic phase of follicular lymphoma where the latter expresses the antigen in more than 50% of cases [40,41].

In the 2 cases of CLL we did not detect the CD10 this is in agreement with the results of Delia et al. [42] and Merle et al. [43], as regard the 2 cases of MM they showed expression of CD10 and this agrees with the results obtained by San Miguel et al. [44].

The correlation between PAS positivity and CD10 expression in ALL is significantly positive (p <0.05), while in NHL group there is no significant correlation (p <0.4).

CD19 is B-specific antigen expressed on early B-cell tumours [45] and it is expressed nearly in all B-cell malignancies except plasma cell [46].

In ALL CD19 is positive in one case only (5%) of our studied cases and this finding agrees with the results of Brouet et al. [47] and Flandrin et al. [48], this case also expressed CD10 which agrees with the results of Craig et al. [49] who found an association of both antigens in B-ALL, the same case is classified as L3 and is MG positive.

The majority of NHL are B-cell as judged by their phenotype as well as expression of surface antigens characteristic of B-cells including CD19 [50]. Our results agree with the previous one as 80% of NHL expressed CD19 (12 cases of the total 15).

All our cases of CLL expressed CD19 while Guillaume et al. [51] found 95% of their cases to be of B-cell type and Nadler [52] found nearly all studied cases to be positive for CD19.

It has already been established that several B-cell specific antigen recognized by monoclonal antibodies including CD19 are gradually lost when the plasma cell morphology is acquired [34,53]. CD19 is never expressed on plasma cells [54] and we also found CD19 not expressed in the two cases of MM.

The correlation between CD19 and PAS is not significant in ALL group (p <0.2) while in NHL is significant (p <0.05).

There is negative correlation between CD10 and CD19 in ALL (p <0.5), while in

NHL the correlation is weakly significant (p <0.1).

We conclude that APAAP is a simple and reliable test for immunophenotyping of haematological malignancies having some practical advantages over conventional immunofluorescence techniques which include the requirement for only small volumes of blood (few drops), the possibility of storing samples as air dried blood smears for long periods of time before analysis, the clarity of the stained films and the fact that separation of mononuclear cells is not required. Also there is saving in the cost on doing samples on batches and in equipment costs since there will be no need for fluorescence microscope.

References

1- MOIR D. J., GHOSN A. K., ABDULAZIZ Z., KNIGHT P.M. and MASON D.Y.: Immunoenzymatic staining of haematological samples with monoclonal antibodies. Br. J. Haematol., 55: 395, 1983.

2- ESBER E.S., BUELL D.N. & LEIKIN S.L.: Insulin binding of acute lymphocytic leukaemia cells. Blood, 48, 33, 1976.

3- BOLLUM F.: Terminal deoxynucleotidyl transferase as a hemopoietic cell marker. Blood, 54, 1203, 1979.

4- FOON K.A., SCHROFF R.W. and GALE R.P.: Cell surface markers on leukaemia and lymphoma cells: Recent advances. Blood, 60: 1, 1982.

5- FOON K.A. and TOOD R.F. III: Immunologic classification of leukaemia and lymphoma. Blood, 68: 1, 1986.

6- ERBER W.N., PINCHING A.J. & MASON D.Y.: Immunocytochemical detection of T and B cell populations in routine blood smears. Lancet, ii, 1042, 1984.

7- GOLDBERG and BARKA: Acid phosphatase activity in human blood cells. Nature, 4838 (July 21): 297, 1962.

8- HAYHOE F.G. and QUAGLINO D.: Refractory sideroblastic anemia and erythaemic myelosis: Possible relationship and cytochemical observation. Brit. J. Haem., 6: 381, 1960.

9- BAILLIF R.N. and KIMBROUGH C.: Studies on leucocyte granules after staining with SBB and May Grunwald Geimsa. J. Lab. Clin. Med., 32, 155, 1947.

10- ROZENSZAJIN L., LEIBOVICH M., SHOHAM D. and EPSTEIN J.: The esterase activity in megaloblasts, leukaemic and normal haemopoietic cells. Br. J. Haematol., 14: 605, 1968.

11- LILLIE R.D.: Various oil soluble dyes as fat stains in supersaturated isopropanol technic. Stain Technology, 19: 55, 1944.

12- KASS L.: Bone marrow interpretation. J.B. Lippincott company, Philadelphia, Toronto, 1979.

13- HANSON C.A., GAJIL-PECZALSKA K.J., PARKIN J.L. et al.: Immunophenotyping of acute myeloid leukaemia using monoclonal antibodies and the APAAP technique. Blood, 70: 83, 1987.

14- HAYHOE F.G. and QUAGLINO D.: Haematological cytochemistry, second edition, Chap. 1 pp. 1, 1988.

15- TELEK B., KISS A., PECZE K. and RAK K.: Differential diagnostic value of acid phosphatase and beta glucuronoidase in acute leukaemia. Blut., 46: 67, 1983.

16- VEERMAN A.J.P., HUISMANS D.R. and VAN ZANK C.H.: Immunological phenotype related to acid α-naphthyl acetate esterase and acid phosphatase in childhood

ALL. Acta Haematologica, 69: 32, 1983.

17- GROSSI C.E. and FERRARINI M.: Morphology and cytochemistry of human lymphocytes, in NK cells and other natural effector cells, edited by RB Herberman, Academic, New York, p. 1, 1982.

18- CATOVSKY D., BERNASCONI C., VERDONCK P.J. et al.: The association of eosinophilia with lymphoblastic leukaemia or lymphoma: A study of seven patients. Br. J. Haematol., 45: 523, 1980.

19- HAYHOE F.G., QUAGLINO D. & DOLL R.: The cytology and cytochemistry of acute leukaemias. Special Report Series of the Medical Research Council No. 304: HMSO: London, 1964.

20- BRITTON S., WESPIC T. & MOLLER G.: Persistence of immunogenicity of two complex antigens retained in vivo. Immunology, 4: 491, 1968.

21- BOESEN A.M., HOKLAND P. & JENSEN O.M.: AP and acid esterase activity in neoplastic and non neoplastic lymphoid cells. Scand. J. Haematol., 32: 313, 1984.

22- HAYHOE F.G. and QUAGLINO D.: Haematological cytochemistry, second edition, chap. 5, pp. 86, 1988.

23- SAVAGE R.A. & HOFFMAN G.C.: Sudan Black positivity in non-myeloid leukaemia. Br. J. Haematol., 54: 494, 1983.

24- STASS S.A., PUI C.H., MELVIN S., ROVIGATTI O., WILLIAMS D., MOTRONI T., KALWINSKY D. and DAHL G.V.: Sudan Black B positive ALL. Br. J. Haematol., 57: 413, 1984.

25- BENNETT J.M., CATOVSKY D., DANIEL M.T., et al.: Proposed revised criteria for the classification of acute myeloid leukaemias. Ann. Intern. Med., 103: 626, 1985.

26- SHAW M.T. and KLEMP V.A.: Cytochemical staining with oil Red and B-glucuronoidase in the diagnosis of acute leukaemia. Am. J. Clin. Pathol., 61: 169, 1974.

27- BESSIS, M.: Living blood cells and their ultrastructure, Springer Verlag, Berlin, p. 435, 1973.

28- ROBERT E. SOBOL, CLARA D. BLOOMFIELD and IVOR ROYSTON: Immunophenotyping in the diagnosis and classification of acute lymphoblastic leukaemia. Clinics in Laboratory Medicine Vol. 8 No. 1, March 1988.

29- REINHERZ EL, and SCHLOSSMAN S.F.: The differentiation and function of human T lymphocytes. Cell, 19: 821-827, 1980.

30- NEIL E-KAY, STEVEN D. DOUGLAS: Morphology and antigenic phenotype of human blood lymphocytes. Haematology, 4 th edit. Vol. I pp. 905, 1990.

31- VODENELICH I., TAX W., BAI Y., PEGRAM S., CAPAL P. and GREAVES M.F.: A monoclonal antibody (WT1) for detecting leukaemias of T-cell precursors (T-ALL). Blood, 62: 1108, 1983.

32- CANTUE-RAJNOLDI, PUTTI, M. SAITTA et al.: Co-expression of myeloid antigens in childhood ALL: relationship with the stage of differentiation and clinical significance. Br. J. Haematol., 79: 40, 1991.

33- KELLER R.H., LIBNOCH J.A., KALLAS G., PATRICK L.C. and PATRICK C.W.: Sequential T-cell immunoregulatory subsets and function in B-CLL, in chronic lymphocytic leukaemia: Recent Progress and Future Direction. New York, NY, Liss, p. 147, 1987.

34- SAN MIGUEL J.F., CABALLERO M.D., GONZALEZ M. ZOLA H. and LOPEZ BORRASCA A.: Immunological phenotype of neoplasms involving the B cell in the last

step of differentiation. Br. J. Haematol., 62: 75-83, 1990.

35- RITZ J., NADLER L.M., BHAN A.K., NOTIS MC CONARTY J., PESANDO J.M. and SCHLOSSMAN S.F.: Expression of common acute lymphoblastic leukaemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. Blood, 58: 648, 1981.

36- CHESSELS J.M., HARDISTY R.M., RAPSON N.T. and GREAVES M.F.: Acute lymphoblastic leukaemia in children: Classification and prognosis. Lancet, 2: 1307, 1977.

37- RICHARD GARAND, JEAN PIERRE VANNIER, MARIE C. BENE, GILBERT C, FAURE and ALAIN BERNARD: CORRELATIONS BETWEEN ACUTE LYMPHOID LEUKAEMIA (ALL) immunophenotype and clinical and laboratory data at presenttion. Cancer, 64: 1437-1446, 1989.

38- JEROME RITZ, LEE M. NADLER, ATUL K. BHAN, JEAN NOTIS MC CONARTY, JOHAN M. PESANDO and STUART F. SCHLOSSMAN: Expression of common acute lymphoblastic leukaemia antigen (CALLA) by lymphomas of B-cell and T-cell lineage. Blood, 56(3), 648, 1981.

39- ANDERSON K.C., BATES M.P., SLAUGHENHOUPT B.L., PINKUS G.S., SCHLOSSMAN S.F. and NADLER L.M.: Expression of human B-cell associated antigens on leukaemias and lymphomas: A model of human B-cell differentiation. Blood, 63: 1424, 1984.

40- KOZINER B., FILIPPA D.A., MERTELSMANN R., GUPTA S., CLARKSON B., GOOD R.A. and SIEGEL F.P.: Characterization of malignant lymphoma in leukaemia phase by multiple differentiation markers on leukaemic cells. Correlation with clinical features and conventional morphology. Am. J. Med., 63: 556, 1977.

41- KOZINER B., KEMPIN S., PASSE S., GEE T., GOOD R.A. and CLARKSON B.D.: Characterization of B-cell leukaemias: A tentative immunomorphological scheme. Blood, 56: 815, 1980.

42- DELIA D., CATTORETTI G., POLLI N., FONTANELLA E., AIELLO A., GIARDINI R., RILKE F., and DELLA PORTA G.: CDlc but neither CDla nor CDlb are expressed on normal activated and malignant human B-cells: Identification of a new B-cell subset. Blood, 72: 241, 1988.

43- MERLE-BERAL H. , BOUMSELL L. , MICHEL A. and DEBRE P.: CDl expression on B CLL lymphocytes. Br. J. Haematol., 71: 209, 1989.

44- SAN MIGUEL J. F., GONAZALEZ M., MORO M.J., et al.: Immunophenotypic heterogeneity of MM: Influence on the biology and clinical course of the disease. Br. J. Haematol., 77:185-190, 1991.

45- NADLER L.M., ANDERSON K.C., MARTI G., BATES M., PARK E., DALEY J.E. and SCHLOSSMAN S.F.: B4, a human B lymphocyte-associated antigen expressed on normal mitogen-activated and malignant B lymphocytes. J. Immunol., 131: 244, 1983.

46- PEZZUTTO A., DORKEN B., MOLDENHOUER G. and CLARK E.A.: Amplification of human B-cell activation by a monoclonal antibody to the B-cell-specific antigen CD22. J. Immunol., 138: 98, 1987.

47- BROUET J.C., VALENSI F., DANIEL M.I. et al.: Immunological classification of acute lymphocytic leukaemias: Evaluation of its clinical significance in a hundered patients. Br. J. Haematol., 33: 319, 1976.

48- FLANDRIN G., BRAUT J.C., DANIEL M.T. and PREUD HOMME T.L.: Acute

leukaemia with Burkitt's tumour cells: A study of six cases with special reference to lymphocyte surface markers. Blood, 45: 813, 1975.

49- CRAIG A. HURWITZ R. LOKEN, MICHAEL J., BOROWITZ O. et al.: A synchronous antigen expression in B lineage ALL. Blood, 72 (No 1), p. 299-307, 1988.

50- SHIMOYAMA M., OTA K., KIKUCHI M., et al.: Major prognostic factors of adult patients with advanced T-cell lymphoma, leukaemia. J. Clin Oncol., 6: 1088, 1988.

51- GUILLAUME D., PHILIPPE T., SYLVIE C. et al.: B-cell CLL: Present status and future directions. Blood, 68: 1901, 1991.

52- NADLER L.M.: B-cell / leukaemia panel workshop: Summary and comments, in leucocyte typing II. New York, NY, Springer-Verlag, 1986.

53- TAZZARI P.L., GOBBI M., DINOTA A., BONTADINI A., GRASSI G., CERATO C., CAVO M., PILERI S., CALIGARIS-CAPPIO F. and TURA S.: Normal and neoplastic plasma cell membrane phenotype, studies with new monoclonal antibodies. Clin. Experiment. Immunol., 70, 192-200, 1987.

54- KURITANI T. & COOPER M.D.: Human B-cell differentiation II: pokeweed responsive B cells belong to a surface immunoglobuline D-negative subpopulation. J. Exp. Med., 155: 1561, 1982.