Journal of Korean Medical Science
Vol. 9, No. 5, 362-368 October 1994

Immunoglobulin and T-Cell Receptor Gene Rearrangement Analysis in Malignant Lymphoid Neoplasms

Chan-Kum Park, M.D., Chul-Woo Kim, M.D.,*
In-Soon Kim, M.D.,** Jung-Dal Lee, M.D.

*Departments of Pathology and Internal Medicine**, College of Medicine, Hanyang University and Seoul National University*, Seoul, Korea*

Gene rearrangement analysis using Southern-blot hybridization technique is a standard method for evaluating clonal receptor gene rearrangement. Both clonality and lineage can be identified in lymphoid neoplasms by the demonstration of one or more rearranged antigen receptor genes of the immunoglobulin supergene family-immunoglobulin and T-cell receptor genes. To evaluate the diagnostic applicability of antigen receptor gene rearrangements in the diagnosis of malignant lymphomas and leukemias, the authors performed a gene rearrangement analysis of 54 cases by southern blot hybridization technique. One or two clonally rearranged bands were detected in the malignant lymphomas and in the lymphoblastic leukemias with a false-negative rate of 13.8%. No clonal, rearranged band was detected in benign reactive hyperplasias, carcinomas or non-lymphocytic leukemias. Rearrangement analysis could resolve the lineage, clonality and stage of differentiation of malignant lymphoid neoplasms.

Key Words : *Gene rearrangement, Immunoglobulin, T-cell Receptor.*

INTRODUCTION

The phenomenon of antigen receptor gene rearrangement (Matthyssens and Rabbits, 1980 ; Ravetch et al., 1981 ; Tonegawa, 1983 ; Malynn et al., 1-987, Schroeder et al., 1988), a series of somatic mutations that occurs during the normal maturation of lymphocytes, provides an opportunity for assessing monoclonality in lymphoid populations (Arnold et al., 1983). Gene rearrangements in neoplastic populations generally correspond to cell lineage. B-cell populations have rearranged immunoglobulin genes and T-cell populations have rearranged T-cell receptor genes (Korsmeyer et al., 1983 ; Sun et al., 1989 ; Cossman et al., 1991 ; Medeiros et al., 1991 ; Trainor et al., 1991). Both clonality and lineage can be identified in lymphoid neoplasms by the demonstration of rearrangements of antigen receptor genes of the immunoglobulin supergene family-immunoglobulin and T-cell receptor genes (Cossman et al., 1988 ; Cossman et al., 1991). Rearrangement analysis is not only useful in differential diagnosis and classification but also serves as a sensitive unique clonal marker to detect early occult recurrences in patients after therapy (Cossman et al., 1988). Southern-blot hybridization technique is a standard method for evaluating clonal receptor gene rearrangement which involves analysis of restriction endonuclease-generated DNA fragments with electrophoretic size separation (Southern, 1975).

Address for correspondence : *Chan-Kum Park, M.D., Department of Pathology, College of Medicine, Hanyang University, Sungdong-gu, Haengdang-dong, 17, Seoul, 133-791, Korea. Tel : (02)293-2111(Ext : 3666).*
This work was supported by grants awarded by the Korea Science & Engineering Foundation(KOSEF-92-29-00-16-03-2).

This method depends on the fact that gene rearrangement may delete endonuclease recognition sites or alter the distance between them, resulting in a change in size of the fragment that contains the gene. A clonal lymphoid population produces a novel fragment of a particular length, resulting in a new band on the Southern blot when it is probed for the gene (Reed et al., 1993). To evaluate the diagnostic applicability of antigen receptor gene rearrangements in the diagnosis of malignant lymphomas and leukemias, the authors performed a gene rearrangement analysis by Southern blot hybridization technique.

MATERIALS AND METHODS

Tissue samples were obtained from 54 patients. They included 39 malignant lymphomas and leukemias. Six examples of non-malignant lymphoproliferative processes, six normal peripheral blood lymphocyte samples, and three carcinomas. A detailed list of the diagnoses is outlined in Table 1.

All samples were analyzed and interpreted as previously described (Jaffe and Cossman, 1986) with the use of immunohistochemical studies for solid tissue and flow cytometry for fluid-phase cell suspensions (peripheral blood and bone marrow). A panel of monoclonal antibodies for CD43, CD45RO, CD20, MB2, CD68 was used for immunohistochemistry and CD3, CD4, CD7, CD8, CD10, CD15, CD19, CD20, κ, λ were used for flow cytometry.

Gene rearrangement analysis

Tissue samples were freshly obtained and immediately frozen and stored at −70℃. High molecular weight DNA was extracted and purified with a conventional phenol-chloroform technique (Pittaluga et al., 1987). All DNA samples were digested with two restriction enzymes : EcoRI and HindIII. Restriction fragments were electrophoretically size separated on 7% agarose gels, transferred to nylon membranes (Hybond N^+ membrane, Amersham) that were prehybrized, and hybridized with P^{32}-radiolabeled DNA probes by the random primer method (Feinberg and Vogelstein, 1983).

Three DNA probes were used in this study and included genomic probes of the human immunoglobulin heavy chain joining region (J_H,5.4-kilobase [kb] fragment, Oncor), kappa light chain joining region(J_K, 1.8-kb fragment, Oncor), and cDNA of the human T-cell receptor beta chain constant re-

Table 1. Distribution of Specimcens According to Diagnosis

Diagnosis	No. of samples
Malignant lymphoid neoplasms	
Acute lymphoblastic leukemia	21
Acute non-lymphocytic leukemia	5
Malignant lymphoma, follicular	
Mixed small cleaved and large cell	1
Malignant lymphoma, diffuse	
Small cleaved cell	1
Small noncleaved cell (undifferentiated Burkitt's)	1
Mixed small and large cell	3
Large cell	5
Large cell, immunoblastic	2
Non-malignant lymphoid disorders	
Reactive hyperplasia	4
Lymphoid hyperplasia	1
Chronic inflammation	1
Normal peripheral blood	6
Carcinoma	
Endometrioid carcinoma, ovary	1
Invasive ductal carcinoma, breast	2

gion gene ($C_T\beta$, 420bp fragment, Oncor). Each gel included one lane containing size markers composed of a 1kb ladder and a lane containing high molecular weight human placental DNA digested with the same restriction enzyme corresponding to that of the other samples in the gel. Hybridizations and washings were performed under stringent conditions, and autoradiographic exposures on Agfa X-ray film were performed for 2 to 7 days at −70℃.

RESULTS

To determine the degree of concordance of gene rearrangement analysis with histologic diagnosis, 54 samples were analyzed (Table 2). Samples were separated into four general diagnostic groups : malignant diagnosis (malignant lymphoid neoplasm), normal or benign histologic diagnosis, acute nonlymphocytic leukemia, and carcinoma. Five of the samples could not be analyzed because the DNAs obtained from these were insufficient in amount. Of the remaining 29 cases with a diagnosis of malignant lymphoma and lymphoblastic leukemia, 25 were found to have one or more clonally rearranged band. This yielded a false-negative rate of 13.8%. Of the false negative cases, two were T lymphoblastic leukemia. There were only decreased intensities of 11 kb band on EcoRI digestion (Fig. 1a) and 3.7 kb band on HindIII digestion (Fig. 1b), respectively, with non-germline band. The remaining cases were one malignant lymphoma showing exuberant granulomatous reactions and one follicular lymphoma of mixed small cleaved and large cell type.

One of six cases with a benign histologic diagnosis was a lymphoid hyperplasia of the esophagus which showed evidence of kappa joining region gene rearrangement without heavy chain joining region gene rearrangement. Actually, it was a "Maltoma" of the gasrointestinal tract. None of the remaining five cases showed evidence of gene rearrangement. Three cases of ovarian and breast carcinomas showed no rearrangements.

Thus, no "false-positive" results were observed

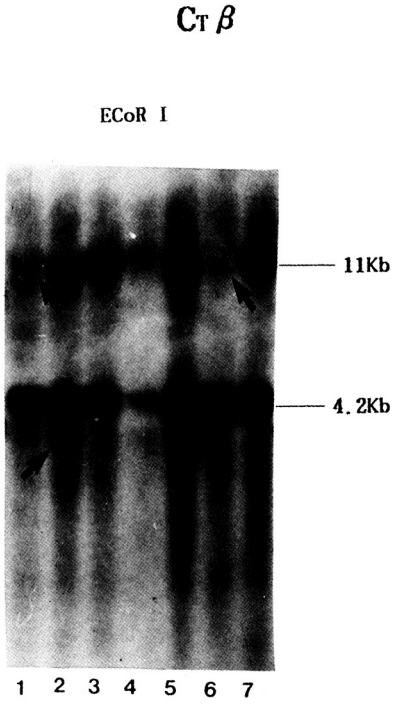

Fig. 1a. Lane 1(Case No. 14, T cell acute lymphoblastic leukemia) and Lane 6 (Case No. 36, T cell malignant lymphoma with leukemic transformation) showing decreased intensity of 11 Kb band with ECoRI digestion and $C_T\beta$ probe(arrow). There was a rearranged band(arrow) of T-cell receptor beta chain gene in a patient with T-cell acute lymphoblastic leukemia(Lane 2, Case No. 16). The genomic DNAs in Lanes 3 to 5 were prepared from B-cell neoplasms : Lane 3 and 4, Common ALL ; Lane 5, Malignant lymphoma, diffuse large cell. Lane 7 : Reactive hyperplasia.

Table 2. Results of Gene Rearrangement Analysis

DNA probe results	Pathologic diagnosis			
	Mal. lymphoid neoplasm	Benign or normal	ANLL	Carcinoma
Rearranged	25	1	0	0
Germline	4	11	5	3
Insufficient DNA	5	0	0	0
Total	34	12	5	3

ANLL : Acute nonlymphocytic leukemia

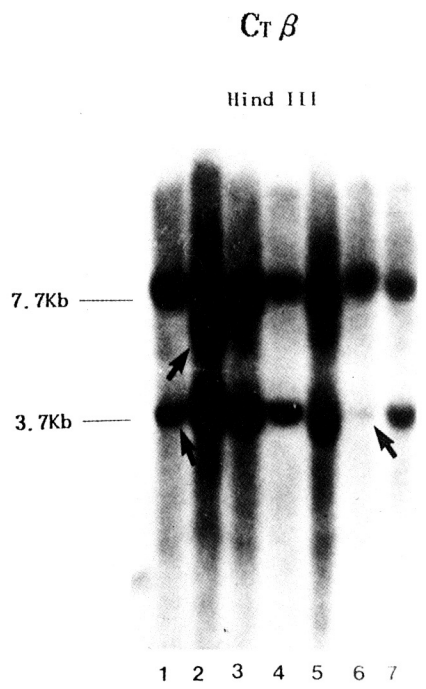

Fig. 1b. The same decreased intensity of 3.7 Kb band was also noted with Hind III digestion and $C_T\beta$ probe in Lane 1 and Lane 6(arrow) The same clonal, rearranged band(arrow) was also noted with Hind III digestion and $C_T\beta$ probe in T-cell acute lymphoblastic leukemia(Lane 2, Case No. 16).

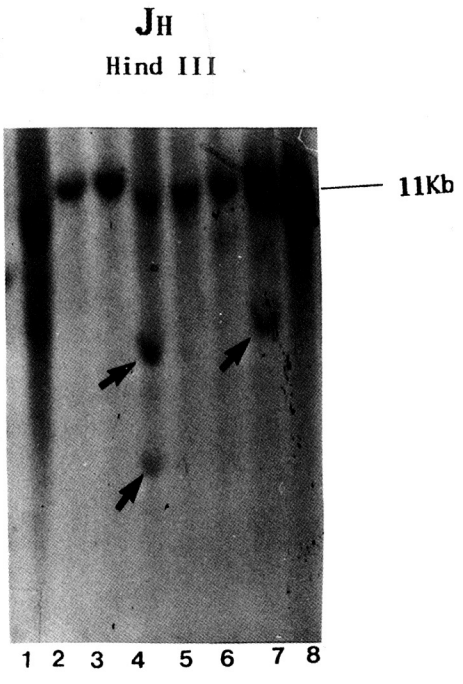

Fig. 2. Genomic DNA was digested with Hind III. The Southern blot was hybridized with J_H probe. There were rearranged bands(arrow) of immunoglobulin heavy chain genes in pre B cell acute lymphoblastic leukemia.
Lanes 1 and 4 to 7 : Pre B All. Lanes 2 and 8 : Normal peripheral blood. Lane 3 : T ALL.

with the use of the gene rearrangement approach in both normal lymphoid cells, benign reactive hyperplasias and non-lymphoid malignancies.

Rearrangement analysis was compared with immunologic phenotyping. Three of 22 cases phenotyped as B cell had T-cell receptor beta-chain gene rearrangement (Table 3).

Of 13 cases immunophenotyped as pre B-cell acute lymphoblastic leukemia (pre B ALL), 12 cases showed immunoglobulin heavy chain gene rearrangement, of which six cases had both immunoglobulin heavy and kappa light chain gene rearrangement (Fig. 2).

Among eight cases of malignant lymphoma immunophenotyped as B cell, five had immunoglobulin heavy chain gene rearrangement (of which three had both immunoglobulin kappa light chain and heavy chain gene rearrangement (Fig. 3).

Table 3. Results of Gene Rearrangement Analysis in B Cell Neoplasia

Immunophenotype	Cases	DNA probe			
		J_H only	J_H and J_K	J_K only	$C_T\beta$
B Cell					
Pre B ALL	13	6	6	0	1
Prepre B ALL	1	1	0	0	1
Mal.lymphoma	8	2	3	2	1
Total	22	9	9	2	3

ALL : Acute lymphoblastic leukemia

Table 4. Results of Gene Rearrangement Analysis in T cell Malignancies

Immunophenotype	Cases	DNA probe		
		J_H	J_K	$C_T\beta$
T cell				
ALL	3	0	0	1
Mal.lymphoma	4	1	0	3
Total	7	1	0	4

Table 5. Results of Gene rearrangement Analysis in Indeterminate Phenotype

Immunophenotype	Cases	DNA probe		
		J_H	J_K	$C_T\beta$
Non-T, non-B ALL	1	1	0	0

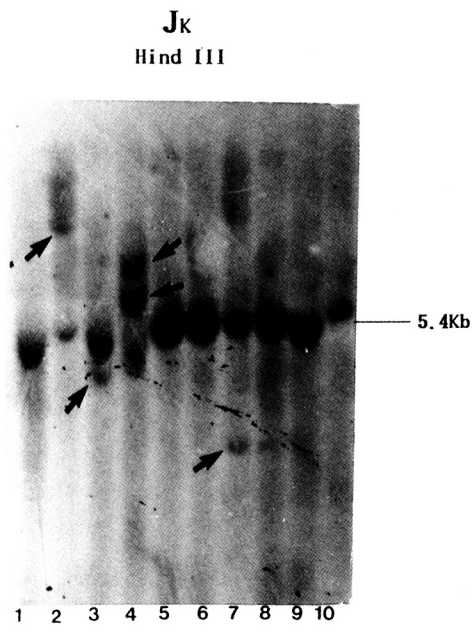

Fig. 3. Genomic DNA was digested with HindIII. The Southern blot was hybridized with the J_K probe. Arrow indicate clonal, rearranged bands.
Lane 1 : Malignant lymphoma, follicular, mixed small cleaved and large cell
Lane 2 : Maltoma of the esophagus
Lanes 3, 5, 7, 8 : Malignant lymphoma, diffuse large cell
Lane 4 : Burkitt's lymphoma
Lane 7 : Malignant lymphoma, diffuse lymphocytic
Lane 9 : ALL
Lane 10 : Placental DNA

Among five cases showing immunoglobulin kappa light chain gene rearrangement, two cases had only immunoglobulin kappa light chain gene rearrangement without immunoglobulin heavy chain gene rearrangement. One prepre B cell acute lymphoblastic leukemia showed rearrangements of both T-cell receptor gene and immunoglobulin heavy-chain gene rearrangement. One of 7 cases phenotyped as T cell had immunoglobulin heavy-chain gene rearrangement (Table 4).

One case of non-T non-B acute lymphoblastic leukemia had immunoglobulin heavy chain gene rearrangement. Gene rearrangement analysis could resolve the lineage (Table 5).

DISCUSSION

In this study of the application of gene rearrangement analysis to the diagnosis of lymphoproliferative processes, we were able to resolve the lineage and clonality of difficult cases of malignant lymphoma and leukemia. When the result of gene rearrangement is compared with histologic diagnosis or phenotypic analysis in malignant lymphoma and leukemia, these tests have shown high concordance (greater than 95%) with histologic characteristics and phenotype. One of six cases with benign histologic diagnosis was found to have immunoglobulin kappa light chain gene rearrangement without immunoglobulin heavy chain gene rearrangement. Original pathologic diagnosis was a lymphoid hyperplasia of the esophagus. It turned out to be a "Maltoma" after gene rearrangement analysis. On careful histologic reviewing, there were diffuse infiltration of atypical lymphocytes in addition to lym-

phoid follicles. In this genotypic analysis, there were two cases of non-Hodgkin's lymphomas characterized by rearrangement of immunoglobulin kappa light chain gene without rearrangement of heavy chain genes. The authors' data confirmed the existence of the only light chain B-cells in the hematopoietic compartment (De Re Valli et al., 1990). One case of common "non-T/non-B" acute lymphoblastic leukemia showed immunoglobulin heavy chain gene rearrangement. This analysis provides a powerful mean to further classify the cases of non-T/non-B acute lymphoblastic leukemia, most of which seem to reside at the early stage along B cell pathway of differentiation (Korsmeyer et al., 1981 ; Bertness et al., 1985).

Immunoglobulin gene rearrangements might occur in an ordered hierarchy. Immunoglobulin heavy chain variable region gene formation precedes that of light chain, in which kappa light chain gene formation precedes that of lambda (Korsmeyer et al., 1981). In eight of 21 cases immunophenotyped as B-cell lymphoma or leukemia, there were both immunoglobulin heavy chain and immunoglobulin kappa light chain gene rearrangement. One case of prepreB-cell acute lymphoblastic leukemia showed both immunoglobulin heavy chain and T-cell receptor gene rearrangement. In about 10% of lymphoid neoplasms, both immunoglobulin heavy chain and T-cell receptor gene rearrangements were found in the same malignant population and a fraction of immature myeloid and undifferentiated leukemias also displayed clonal rearrangements of immunoglobulin heavy chain or T-cell receptor genes (Kitchingman et al., 1985 ; Pelicci et al., 1985 ; Rovigatti et al., 1984 ; Tawa et al., 1985).

In two of four cases of T cell acute lymphoblastic leukemia, there was only decreased intensity of 11Kb band without rearrangement with CT probes. As described by Weiss et al.(1988) certain cases of peripheral T cell lymphoma may lack rearrangements of T-cell receptor genes, particularly those cases expressing restricted numbers of T lineage antigens. In view of these findings, the failure to detect rearrangement of T-cell receptor genes by Southern blot analysis is not necessarily inconsistent with malignant T lymphocytic proliferations. A germ line configuration was present in all patients with non-T cell neoplasms. The analysis of immunoglobulin gene rearrangements offers several advantages over conventional diagnostic methods for lymphomas, including improved sensitivity in detecting minor populations of neoplastic lymphocytes composing as little as 1% of the total cell population. In addition, clonal immunoglobulin gene rearrangements are demonstrable in a subset of lymphomas that lack detectable surface or cytoplasmic immunoglobulin, and thus offers positive evidence for the confirmation of malignancy and B cell origin in particular tumors (Cleary et al., 1984). However, Southern blot analysis suffers from a number of technical disadvantages, including the time necessary to obtain results, the use of radioactive material, and the susceptibility to various artifacts (Bourguin et al., 1990). Since the restriction enzyme-generated fragments are fairly large, Southern-blot hybridization technique requires relatively intact DNA, which can be obtained only from fresh tissue. It also requires a relatively large amount of DNA, making it impractical for fine needle aspiration specimens and even small biopsies. Detection of weak signal may require prolonged radiographic exposure (Reed et al., 1993).

Although there are some serious drawbacks in the gene rearrangement analysis by Southern-blot hybridization technique, it can be used as a diagnostic adjunct to clarify lineage and clonality, and stage of differentiation of malignant lymphoid neoplasms. However, the results of antigen receptor gene rearrangement analysis of neoplastic cell populations should always be correlated with histologic diagnosis and the results of immunophenotypic analysis.

REFERENCES

Arnold A, Cossman J, Bakhshi A, Jaffe ES, Waldman TA, Korsmeyer SJ . *Immunoglobulin gene rearrangement as unique clonal markers in human lymphoid neoplasms. N Engl J Med 1983 ; 309 : 1593-9.*

Bertness V, Kirsch I, Hollis G, Johnson B, Bunn PA . *T-cell receptor gene rearrangements as clinical markers of human T-cell lymphomas. N Eng J Med 1985 ; 313 : 534-8.*

Bourguin A, Tung R, Galili N, Sklar J . *Rapid, nonradioactive detection of clonal T-cell receptor gene rearrangements in lymphoid neoplasms. Proc Natl Acad Sci USA 1990 ; 87 : 8536-40.*

Cleary ML, Chao J, Warnke R, Sklar J . *Immunoglobulin gene rearrangement as a diagnostic criterion of B-cell lymphoma. Proc Natl Acad Sci USA 1984 ; 81 : 593-7.*

Cossman J, Uppenkamp M, Sundeen J, Coupland R, Raffeld M . *Molecular genetics and the diagnosis of lymphoma. Arch Pathol Lab Med 1988 ; 112 : 117-27.*

Cossman J, Zehnbauer B, Garrett CT, Smith LJ, Williams

M, Jaffe ES, Hanson LO, Love J . *Gene Rearrangements in the diagnosis of lymphoma/leukemia. Am J Clin Pathol 1991 ; 95 : 347-54.*

De Re Valli, Carbone A, Dolcetti R, Tirelli U, Folegotto C, Bolocchi M . *Genotypic and immunophenotypic characterization of two human light chain-only B-cell non-Hodgkin's lymphomas. Am J Clin Pathol 1990 ; 94 : 390-6.*

Feinberg AP, Vogelstein B . *A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 1983 ; 132 : 6-13.*

Jaffe ES, Cossman J . *Immunodiagnosis of lymphoid and mononuclear phagocytic neoplasms. In : Rose NR, Frirdman A, Fahey JL, eds. Manual of clinical laboratory immunology. 3rd ed. Washington, DC : American Society for Microbiology, 1986 ; 779-90.*

Kitchingman GR, Rovigatti U, Mauer AM, Melviv S, Murphy SB, Stass S . *Rearrangement of immunoglobulin heavy chain genes in T cell acute lymphoblastic leukemia. Blood 1985 ; 65 : 725-9.*

Korsmeyer SJ, Arnold A, Bakhshi A, Ravetch JV, Siebenlist U, Hieter PA, Sharrow SO, Lebien TW, Kersey JH, Poplack DG, Leder P, Waldmann TA . *Immunoglobulin gene rearrangement and cell surface antigen expression in acute lymphocytic leukemia of B cell and T cell precursor origins. J Clin Invest 1983 ; 71 : 301-13.*

Korsmeyer SJ, Hieter PA, Ravetch JV, Poplack DG, Waldmann TA, Leder P . *Developmental hierarchy of immunoglobulin gene rearrangements in human leukemic pre-B-cells. Immunology 1981 ; 78 : 7096-100.*

Malynn BA, Berman JE, Yancopoulos GD, Bona CA, Alt FW . *Expression of the immunpglobulin heavy-chain variable gene repertoire. Curr Top Microbiol Immunol 1987 ; 135 : 75-94.*

Matthyssens G, Rabbitts TH . *Structure and multiciplicity of genes for the human immunoglobulin heavy chain variable regions. Proc Natl Acad Sci USA 1980 ; 77 : 651-5.*

Medeiros LJ, Lardelli P, Stetler-Stevenson M, Longo DL, Jaffe ES . *Genotypic analysis of diffuse, mixed cell lymphomas ; Comparison with morphologic and immunophenotypic findings. Am J Clin Pathol 1991 ; 95 : 547-55.*

Pelicci PG, Knowles DM II, Dalla-Favera R . *Lymphoid tumors displaying rarrangements of both immunoglobulin and T cell receptor genes. J Exp Med 1985 ; 162 : 1015-24.*

Pittaluga S, Uppenkamp M, Cossman J . *Development of T3/T cell receptor gene expression in human pre-T neoplasms. Blood 1987 ; 69 : 1062-7.*

Ravetch JV, Siebenlist U, Korsmeyer S, Waldmann T, Leder P . *Structure of the human immunoglobulin mu locus ; Characterization of embryonic and rearranged J and D genes. Cell 1981 ; 27 : 583-91.*

Reed TJ, Reid A, Wallberg K, O'Leary TJ, Frizzera, G . *Determination of B-cell clonality in paraffin-embedded lymph nodes using the polymerase chain reaction. Diag Mol Pathol 1993 ; 2 : 42-9.*

Rovigatti U, Mirro J, Kitchingman G, Dahl G, Ochs J, Murphy S, Stass S . *Heavy chain immunoglobulin gene rearrangement in acute nonlymphocytic leukemia. Blood 1984 ; 63 : 1023-7.*

Schroeder HW, Walter MA, Hofker MH, Ebens A, Willems VDK, Liao LC, Cox DW, Milner EC, Perlmutter RM . *Physical linkage of a human immunoglobulin heavy chain variable region gene segment to diversity and joining elements. Proc Natl Acad Sci USA 1988 ; 85 : 8196-200.*

Southern EM . *Detection of specific sequences among DNA fragments separated by gel electrophoresis. J Mol Biol 1975 ; 98 : 503-17.*

Sun T, Eisenberg A, Benn P, Ngu M, Guarino T, Henshall J, Grossman A, Cuomo J, Vinciguerra V . *Comparison of phenotyping and genotyping of lymphoid neoplasms. J Clin Lab Anal 1989 ; 3 : 156-62.*

Tawa A, Hozumi N, Minden M, Mak TW, Gelfand RW . *Rearrangement of the T-cell receptor beta-chain gene in non-T-cell, non-B-cell acute lymphoblastic leukemia of childhood. N Eng J Med 1985 ; 313 : 1033-7.*

Tonegawa S . *Somatic generation of antibody diversity. Nature 1983 ; 302 : 575-81.*

Trainor KJ, Brisco MJ, Wan J-H, Neoh S, Grist S, Morley AA . *Gene rearrangement in B- and T-lymphoproliferative disease detected by the polymerase chain reaction. Blood 1991 ; 78 : 192-6.*

Weiss LM, Picker LJ, Grogan TM, Warnke RA, Sklar J . *Absence of clonal beta and gamma T-cell receptor gene rearrangements in a subset of peripheral T-cell lymphomas. Am J Pathol 1988 ; 130 : 436-42.*