The role of molecular analysis of immunoglobulin and T cell receptor gene rearrangements in the diagnosis of lymphoproliferative disorders

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Abstract

***Aims*—To investigate whether the analysis of immunoglobulin (Ig)/T cell receptor (TCR) rearrangements is useful in the diagnosis of lymphoproliferative disorders.**

***Methods*—In a series of 107 consecutive cases with initial suspicion of non-Hodgkin's lymphoma (NHL), Southern blot (SB) analysis of Ig/TCR rearrangements was performed.**

***Results*—In 98 of 100 histopathologically conclusive cases, Ig/TCR gene results were concordant. In one presumed diffuse large B cell lymphoma (DLCL) and one follicular lymphoma (FL) case no clonality could be detected by SB analysis, or by polymerase chain reaction (PCR) at second stage. In the DLCL, sampling error might have occurred; the FL was revised after an initial diagnosis of reactivity. In many of the histopathologically inconclusive cases Ig/TCR gene SB analysis was helpful, giving support for the histopathological suspicion. However, because of a lack of (clinical) follow up data this could not be confirmed in a few cases.**

***Conclusions*—Experienced haematopathologists or a pathologist panel can diagnose malignant versus reactive lesions in most cases without the need for Ig/TCR gene analysis and can select the 5–10% of cases that might benefit from molecular clonality studies.**

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Keywords: B cell lymphoma; immunoglobulin and T cell receptor genes; clonality analysis; Southern blotting

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Combined histomorphology and immunohistochemistry is often sufficient for a reliable diagnosis of lymphoproliferative lesions. However, the crucial distinction between polyclonality and monoclonality is sometimes difficult to make. In the case of B cell proliferations, immunoglobulin (Ig) (κ or λ) light chain restriction analysis is widely applied for clonality assessment, but this method can be technically difficult in tissue sections. Furthermore, in Ig negative (Ig^-) B cell lesions and T cell proliferations no such strategy is available. For this reason, molecular studies using Southern blot (SB) and/or polymerase chain reaction (PCR) analysis of Ig and T cell receptor (TCR) genes are currently used. Based on the idea that malignancies are clonal proliferations derived from a single transformed cell, the presence or absence of clonal Ig/TCR rearrangements is used to discriminate between neoplastic and reactive proliferations, respectively.

Although molecular analysis of Ig/TCR genes has proved very helpful in selected cases, limited information is available about its applicability in daily practice. Therefore, we conducted a prospective study on 107 consecutive cases selected on the basis of a histopathological suspicion of non-Hodgkin's lymphoma (NHL) or reactive lymphoproliferative disorder. Samples were analysed by standard immunohistochemistry and included in the study if enough frozen material was available for SB analysis. Despite the obvious advantages of PCR based methods, SB is still regarded as the gold standard because of its very low rate of false negativity and false positivity. Therefore, SB was considered the most appropriate method to evaluate the role of molecular techniques in clonality studies for lymphoma diagnosis.

Material and methods

PATIENT MATERIAL

A total of 107 consecutive diagnostic samples (derived from four pathology laboratories—Leiden/The Hague, Dordrecht, and Eindhoven) with an initial histomorphological suspicion of NHL were analysed by standard immunohistochemistry (including Ig light chain restriction). Cases were included based on the availability of frozen tissue for SB analysis. NHL samples were classified according to the REAL classification.[1] Histological slides from all cases were reviewed blindly by a panel of four haematopathologists (JHJMvK, AHM, LWMAV, and PMK). Unexpected findings and/or discrepancies between histomorphology/immunohistochemistry and SB analysis were analysed in a second panel meeting, where a final diagnosis was reached.

SOUTHERN BLOT ANALYSIS

SB analysis of Ig/TCR genes[2] was performed in two molecular diagnostic laboratories (AWL/JJMvD, Rotterdam and ES/PMK, Leiden) with the following probes: IGHJ6, IGKJ5, IGKC, IGKDE, IGLC3, TCRBJ1, TCRBJ2, TCRBC, TCRGJ13, TCRGJ21, TCRDJ1, and TCRDC4 (DAKO Corporation, Carpinteria, California, USA) in combination with the optimal restriction enzyme digests.[3–6] Rearrangements were only regarded as clonal when detected in two distinct digests.

Table 1 Southern blot (SB) analysis of Ig/TCR genes in cases with a clear histopathological diagnosis (n = 100)

Histopathological diagnosis	*No. of samples*	*SB analysis of Ig/TCR genes*	
Haematopoietic			
Diffuse large B cell lymphoma (DLCL)	18	Clonal (Ig)	17
		Non-clonal	1*
Follicular lymphoma (FL)	16	Clonal (Ig)	15
		Non-clonal	1*
Lymphocytic lymphoma (LL)	17	Clonal (Ig)	17
Mantle cell lymphoma (MCL)	7	Clonal (Ig)	7
MALT lymphoma	1	Clonal (Ig)	1
Burkitt's lymphoma (BL)	1	Clonal (Ig)	1
Plasmacytoma (PC)	1	Clonal (Ig)	1
T cell lymphoma (T-NHL)	1	Clonal (TCR)	1
Anaplastic large cell lymphoma (ALCL)	3	Clonal (TCR)	1
		Non-clonal	2
Hodgkin's disease (HD)	4	Non-clonal	4
Acute myeloid leukaemia (AML)	2	Clonal (Ig)	1
		Clonal (Ig+TCR)	1
Non-haematopoietic			
Kaposi's sarcoma	1	Non-clonal	1
Seminoma	1	Non-clonal	1
Reactive lymphoproliferations	27	Non-clonal	27

*Cases are discussed in more detail in table 2.
Ig, immunoglobulin; TCR, T cell receptor.

Results and discussion

Based on haematoxylin and eosin stained sections and immunohistochemistry, a clear histopathological diagnosis was reached in 100 of the 107 samples analysed (table 1). In 98 of these, the results of the blindly performed parallel SB analyses completely matched the histopathological diagnosis, supporting the assumption that for these cases molecular analysis would have been unnecessary in a routine diagnostic setting. One case was originally diagnosed as a reactive lymphoproliferation, but was revised by the pathologists panel as Ig^- follicular lymphoma (FL), and indeed contained clonal Ig rearrangements. Furthermore, two anaplastic large cell lymphomas (ALCLs) did not contain clonal TCR or Ig rearrangements (null-type), whereas in the third one of these lymphomas clonal TCRB (TCR β chain) gene rearrangements were observed. Remarkably, in four mantle cell lymphomas (MCLs) and three lymphocytic lymphomas (LLs), crosslineage TCRB gene rearrangements were observed next to Ig rearrangements. This phenomenon has been described previously and confirms the notion that TCR rearrangements are not proof of the T cell lineage of a tumour.[2 7] Clonal IGH (Ig heavy chain) gene rearrangements were also observed in both acute myeloid lymphoma (AML) cases, one of which also showed TCR rearrangements. This is a remarkable observation, given the low (approximately 15%) frequency of Ig/TCR recombinations in random AML series.[2] Clonal rearrangements were not detected in the reactive lymphoproliferations.

One presumed diffuse large B cell lymphoma (DLCL) and one presumed FL out of the 100 cases showed discordant molecular results (table 1). They are presented in more detail, together with seven cases with inconclusive histopathological diagnosis, in table 2. PCR analysis on paraffin wax and frozen tissue of the discordant cases did not show clonal IGH rearrangements either,[8] although false negative PCR results cannot be excluded. The DLCL case probably represented a sampling error, because the diagnosis was also clear from an earlier biopsy. The presumed FL was initially thought to be reactive, but unfortunately nothing is known from later biopsies.

When the seven inconclusive samples were studied by SB, the molecular data appeared to direct the final diagnosis in virtually all cases. The two suspected B cell NHL (B-NHL) cases and the suspected acute lymphoblastic leukaemia (ALL) case all showed clonal Ig rearrangements, although PCR failed to detect clonality, possibly as a result of incomplete rearrangements or somatic hypermutations. Although clonality detection per se cannot be considered to be proof of malignancy, in the context of a histopathologically suspect lesion it was considered to support the malignant character of these samples. In one suspected B-NHL, this was confirmed by information from a later biopsy. In one of the two cases with features of both B-NHL and Hodgkin's disease (HD), clonal Ig rearrangements were observed by SB analysis. The results implied that one concerned B-NHL (94-105) and the other HD (95-056), although no definite distinction

Table 2 Southern blot analysis of Ig/TCR genes in patients with discordant molecular results (n = 2) or inconclusive histopathological diagnosis (n = 7)

Case	*Category*	*Combined histomorphology and immunohistochemistry*	*SB analysis of Ig/TCR genes*	*PCR analysis of IGH genes (FR3/FR2/FR1)*	*Follow up analysis and clinical data*
767	Discordant	DLCL	Non-clonal	Polyclonal	Diagnosis of B-NHL also based on earlier biopsy; treated for B-NHL
95-040	Discordant	FL (revised; initially reactive)	Non-clonal	Polyclonal	Unknown
759	Inconclusive	Suspected B-NHL (LL)	Clonal (IGH/IGK)	Polyclonal	Unknown
766	Inconclusive	Suspected acute lymphoblastic leukaemia	Clonal (IGH/IGK/IGL)	Polyclonal	Not treated, despite presence of TdT^+ cells in lymph node
818	Inconclusive	Suspected B-NHL	Clonal (IGH/TCRB)	Polyclonal	Diagnosis of DLCL on next biopsy; relapse of DLCL in neck 4 years later
94-105	Inconclusive	Differential diagnosis, B-NHL/HD	Clonal (IGH/IGK)	Clonal (FR2/FR1)	Remission after treatment for T rich B-NHL; 2 years later presentation of lymphocyte rich nodular HD; B-NHL perceived as transformed phase of underlying HD
95-056	Inconclusive	Differential diagnosis, HD/B-NHL	Non-clonal	Polyclonal	Treated for lymphocyte rich nodular HD; follow up 4 years later: no localisation of HD in lymph node
95-054	Inconclusive	Reactive, but suspect	Non-clonal	Polyclonal	Treated for nodular sclerotic HD; follow up 1 year later: reactive lymph node
95-036	Inconclusive	Suspected T-NHL	Non-clonal	ND	Sepsis leading to death; AILD T-NHL at necropsy

AILD, angioimmunoblastic lymphoproliferative disease; B-NHL, B cell non-Hodgkin's lymphoma; DLCL, diffuse large B cell lymphoma; FL, follicular lymphoma; FR, framework region; HD, Hodgkin's disease; IGH, immunoglobulin heavy chain; LL, lymphocytic leukaemia; TCR, T cell receptor; TdT, terminal deoxy nucleotidyl transferase; T-NHL, T cell non-Hodgkin's lymphoma.

could be made, given the description of IGH clonality in HD[9 10] and the variable number of tumour cells in this disease. Follow up analysis revealed that 94-105 was later diagnosed as T rich B-NHL (perceived as transformed phase of underlying HD), whereas in 95-056, no features of HD were found in later biopsies. Finally, one atypical, presumably reactive, but suspect lesion as well as a case with a strong suspicion for T cell NHL (T-NHL) did not contain clonal rearrangements. We regard this as support for the non-malignant character of these lesions, although a small tumour load (< 5%) cannot be fully excluded. Follow up analysis revealed a clear reactive lymph node lesion in the former case, but in the second case an angioimmunoblastic lymphoproliferative disease T-NHL was apparent at necropsy.

Although the results of our study are clear, the role of molecular clonality analysis is still not fully defined. First, although reflecting daily practice in two academic and two non-academic centres, the initial diagnoses were made by a panel of experienced haematopathologists with the availability of frozen tissue for extensive immunohistochemical analysis. Second, in this prospective study, T-NHL and other rare lymphomas were present in only small numbers. Nevertheless, the study does show that SB analysis is of help in diagnosing histopathologically suspect lymphoproliferative lesions, although the true effectiveness of molecular techniques can only be properly assessed with the availability of long term clinical follow up data. Because a panel of pathologists should be able to select the relatively few suspect cases, this further indicates that molecular clonality assessment can only become cost effective when a central facility is organised with enough experience in haematopathology and molecular immunology. Furthermore, quality control studies and interlaboratory standardisation are needed for the routine application of molecular clonality techniques.

The major problem with SB analysis of Ig/TCR gene rearrangements is the need for a sufficient amount of frozen material and the relatively long time taken to obtain results. Therefore, several more rapid and cheaper PCR based techniques are used at present. Although these techniques yield a relatively high number of false negative results,[8] they are very useful as an initial screening tool. However, comparative multicentre studies are essential to establish the real value of PCR techniques. This is the aim of the current BIOMED-2 Concerted Action (PL96–3936) on "Development and standardisation of PCR based clonality studies in lymphoproliferative disorders".

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