



Tissue microarray - a valuable method in diagnosis and prognosis of hematological malignances

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ABSTRACT

BACKGROUND: *The novel technology of tissue microarray (TMA) allows rapid and cost-effective analysis of hundreds of markers on the same set of specimens. Limited amount of tissue that could be analyzed and problem of tissue heterogeneity, are the major drawbacks of TMA technique for immunohistochemical characterization of lymphomas.*

METHODS: *In this paper 65 cases of lymphomas were analyzed using TMA with following panel of antibodies: BOB1, Oct2, Bcl2, Bcl6, CD20, CD21, CD23, CD3, CD5, CD10, CD43, CD79a, CD138, Cyclin D1, Ki67, MUM1, Pax5, p53.*

RESULTS: *In 14 patients with diffuse large B-cell lymphoma (DLBCL), 5 were classified as germinative center and 3 as non-germinative center cases according to the Bcl6, CD10, and MUM1 positivity. Other 2 patients were identified as T cell rich B cell lymphoma based on morphology and Oct2 and BOB1 positivity of pleomorphic B lymphocytes. DLBCL with Bcl6⁺ immunophenotype had better overall survival than Bcl6⁻ cases. All cases of classic mantle cell lymphoma had significantly lower Ki-67 proliferation index than blastoid subtypes. There were 14 cases of chronic lymphocytic leukemia /small cell lymphocytic lymphoma, 6 cases with follicular lymphoma, 5 cases of marginal zone lymphoma, and 7 cases of lymphoplasmacytoid lymphoma. In the indolent lymphoma group, survival of patients with p53^{+/+} was poorer comparing to p53⁻ group.*

CONCLUSION: *We conclude that TMA technique is a valuable method in diagnosis and prognosis of lymphomas, which are considered very heterogeneous group of hematological neoplasms.*

KEY WORDS: *Tissue Array Analysis; Hematologic Neoplasms; Immunohistochemistry; Molecular Diagnostic Techniques; Prognosis; Immunophenotyping*

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INTRODUCTION

The novel technology of tissue microarray (TMA) preparation for high throughput profiling of tumor specimens was originally described in 1998 by Kononen et al. (1). These authors detailed the preparation of paraffin blocks containing up to 1000 cylindrical, 2 mm-diameter core biopsies from archived paraffin blocks of various tumors and normal tissue specimens. The tissue microarray block can be sectioned for morphologic review as well as for standard immunohistochemistry, 1- or 2- color fluorescence in situ hybridization, or mRNA in situ hybridization on consecutive sections. This novel methodology allows for rapid analysis of hundreds of markers on the same set of specimens: up to 200 sections can be cut from each block.

TMA appear to be particularly useful for immunohistochemical characterization of lymphomas. Combining TMA and immunohistochemistry will enable a rapid and cost-effective screening of marker expression. Furthermore, certain molecular markers in lymphoma correlate with differences in course of disease, e.g., p53 over-expression predicts a worse outcome in patients with B-cell chronic lymphocytic leukemia (B-CLL), certain low-grade lymphomas, and mantle cell lymphoma (MCL) irrespective of the stage of disease (2). Markers defining disease subsets with a particularly aggressive course may be rapidly identified

using TMA. Recently, subgroups of diffuse large B-cell lymphoma (DLBCL) have been defined by cDNA microarray (3). The validation of these findings at the post-transcriptional level will be greatly enhanced by combining TMA with immunohistochemistry. In a recent paper from 2004 written by Hans and co workers TMA proved to be useful and accurate in defining more favorable germinal center subtype of DLBCL from activated subtype by using only bcl6, CD10 and MUM1 antigens (4).

Costs and quality control

Several technical issues apparently compensate for some loss of information due to the small tissue size. The staining of a single TMA slide provides a much greater degree of consistency and standardization than the immunostaining of hundreds of individual slides and reduces the amount of antibodies. This significantly reduces high variability of intralaboratory and interlaboratory results, mainly because of interlaboratory differences in antigen retrieval, staining protocols, antibodies used, and in the interpretation of staining results (5). Furthermore, quantitation of immunostainings is markedly easier on arrayed samples than on large sections. Also, this facilitates a reproducible application of the selected scoring criteria because the entire tissue is always used for interpretation and the subjective selection of one tumor area for decision making is avoided. In the future, the TMA technology may

help to optimize and standardize the interpretation of immunostainings, which is currently subjective and poorly reproducible and often leads to major discrepancies in studies investigating clinical associations for novel biomarkers (5).

Advantages and drawbacks of punches with varying diameter

A potential caveat of TM technology is the limited amount of tissue analyzed. The TMA approach has been criticized for its use of small punches of usually only 0.6 mm diameter from tumors with an original size of up to several centimeters in diameter, comprising areas of increased proliferation, apoptosis, matrix remodeling, necrosis, etc. The problem of tissue heterogeneity is maybe the most pronounced in lymphomas. Tumor cells, e.g. in Hodgkin's lymphoma or T-cell-rich B-cell lymphoma, are outnumbered by non-neoplastic background infiltrates and may only be present in very low numbers in TMA punch biopsies. In addition, lymphoma growth may follow lymphoid structures (such as follicles in FL or mantle zone infiltration in MCL), thus, TMA punch biopsies, in contrast to many solid tumors, may not contain relevant tumor areas. Furthermore, prognostic markers such as Ki67 or p53 are not homogeneously expressed, so that the lymphoma punch biopsy may not be representative of the whole neoplasm (6-8). Several experimental and clinicopathological efforts has been made to reduce and even eliminate these concerns. In a recent paper of Hedavat and associates was clearly shown that TMA technique could be used reliably in lymphomas to characterize protein and mRNA expression level (9). In spite of the drawbacks mentioned above, lymphomas can be reproducibly evaluated on TMA (9). Nevertheless, criteria for evaluation of quantitatively expressed markers can strongly influence the rate of concordance. The facts that maximum expression of heterogeneously expressed markers cannot be reliably determined on TMA due to tissue heterogeneity and the limited amount of tissue in punch biopsies do not outweigh the enormous advantages of TMA, namely cost- and time-saving and the mostly homogeneous results of immunohistochemistry (9). Of course it can not be overemphasized in this context that care in the composition of an array and a certain degree of redundancy is essential to minimize TMA sampling drawbacks, because the selection of different tumor areas should be oriented towards the requirements of the investigated tumor entity.

Among alternatives to circumvent these problems is the use of larger punch needles of up to 2 mm diameter. Nevertheless, for the use of TMA in cancer research, no obvious advantage can be seen, because when compared with the original size of a tumor with a diameter of up to several centimeters, an area of about 3 mm² (2 mm diameter) is hardly more "representative" than 0.27 mm² (0.6 mm diameter). In addition, the obvious disadvantage is that instead of several hundreds of tumors on a single slide/section, far fewer than 100 samples can be investigated at the same time. These large punches also cause considerable damage to the donor and acceptor block using conventional paraffin wax blocks. Despite the fact that these arrays might be suboptimal for cancer research, large punch arrays may be preferable for distinct areas of research and perhaps routine practice. In our study we used 2 mm punch needle. The number of core samples per case is also important issue. TMA immunostaining results agree with whole tissue section staining in 86% to 100% of cases and, as the number of core samples increases, the level of agreement also increases (10). In lymphomas was found that added redundancy of 3 cores/case reduced the numbers of cases placed on TMA, but it did not increased the accuracy of a particular stain if tumor cells were present (9). Moreover, in situations with careful core sampling especially in large studies, a single sample from each tumor may often be sufficient to derive information on clinical associations (5).

The aim of this study was to show diagnostic and prognostic significance of TMA based on the experience obtained from our patients.

PATIENTS AND METHODS

All patients with histological diagnosis of B cell non-Hodgkin's lymphoma according to updated Kiel classification criteria as well as those with immunophenotype were considered eligible. Patients diagnosed and treated at the clinic of Hematology and clinical immunology Clinical center Niš between 1991 and 2002 were followed and clinically evaluated using the well established prognostic parameters. From the initial 169 patients biopsy material from only 65 patients were additionally analyzed.

Preparation of "donor" tissue microarray blocks

Areas containing the most characteristic features of pathologic process on hematoxylin-eosin (H&E) stained slides were identified and marked. After putting the marked slides over the surface of paraffin block, arras on the block corresponding to regions on the slide could be identified and marked.

Preparation of "recipient" tissue microarray block

Empty paraffin blocks (recipient block) 30 x 24 mm in diameter and 4 mm deep was prepared with standard mould placed on the standard holder. Block surface was divided on 45 squares (5 columns and 9 rows) by line drawn with marker. Modified kidney biopsy needle 2 mm thick was used to cut out 2 mm wax cylinders from the recipient block, creating 45 holes. Modification was done by cutting and flattening the tip of stylet of the needle.

Construction of the array

Marked region of the donor block was punched out with modified needle and small tissue cylinder was arrayed in to the recipient block. Tissue cylinder was pressed out of the needle gently in to the hole with modified stylet. Procedure has been done repeatedly until complete recipient block was filed. Each sample placed in to the recipient block had to be properly marked in the appropriate table. Recipient blocks ("microarray") were homogenized and embedded. Two such blocks were made.

Secondary preparation of biopsy material and tissue microarray construction was performed at laboratory for histopathology of Prof. Dojčin Dojčinov in Belgrade

Tissue blocks were cut with microtome to obtain sections 4-5 µm thick, which were than mounted onto appropriately electrostatic coated microscope slides "SuperFrost + ®". Approximately 100 sections containing full profiles of all tissue samples were made from each tissue array block. Additional incomplete sections were also placed on slides and stored as a reserve.

Immunohistochemistry

Serial 4-µm sections from TMA blocks were incubated for 12 hours at 55°C and deparaffinized in xylene and hydrated in a graded series of alcohol. Heat-induced antigen retrieval was carried out by microwave pretreatment in the appropriate buffer as indicated for each antibody, according to the manufacturer's instructions. Procedure was performed in automatic processor for immunohistochemistry "DAKO Cytomation" according to standard procedure and instructions of the antibody supplier. Each antigen was stained in triplicate with antigenic controls included. Additional sections were processed for certain markers in case of loss of tissue during the initial staining. List of antibodies used is shown in the table 1. Scoring of intensity of the immunostaining was done semi quantitatively (- = no staining, + = reaction present in most of the cells -at least 2/3, +/- = reaction present in less than 2/3 of tumor cells, -/+ = less than 1/3 of cells stained and focal = staining present only in limited areas of tumor specimen).

Staining was considered weak for CD5, CD43, PAX5, Oct2, and BOB1 in cases of lower intensity of dye in tumor cells comparing to the structures where the expression is normally present. Ki67 proliferative index was expressed in percentages based on comparison with

the sets of visual analogues that are used as a standard for routine diagnostic estimate of proliferative fraction.

Microphotography

All H&E and immunohistochemistry images were analyzed using a Nikon Eclipse 600 microscope (Nikon, Burlingame, CA). Images were photographed with a Nikon DS5M digital camera in resolution 1260x980 pixels. Digitized images were processed using Adobe Photoshop 7 (Adobe Systems, San Jose, CA). Processing consisted of creation of composite photographs, correction of light and contrast, subtraction of background and textual notation of details. Microscopic sections, immunohistochemistry, microphotography, and interpretation of histopathological results were done in cooperation with "All Wales Lymphoma Panel" (Dr. S. Dojčinov) at the department of Pathology of University Hospital Wales (Cardiff, United Kingdom).

Table 1. List of used antibodies and their characteristics

Antibody	Dilution	Antigen Retrieval	Supplier	Supplier code
BOB1	1/400	EDTA	Santa Cruz	SC-955
Bcl2	1/100	EDTA	DAKO	M0887
Bcl6	1/40	High pH	DAKO	M7211
CD3	1/20	EDTA	DAKO	M7254
CD5	1/40	EDTA	Novocastra	CD5-4C7
CD10	1/10	EDTA	Novocastra	CD10-270
CD20	1/400	EDTA	DAKO	M0755
CD21	1/20	Trypsin	DAKO	M0784
CD23	1/40	EDTA	Novocastra	CD23-1B12
CD43	1/100	EDTA	DAKO	M0786
CD79a	1/100	EDTA	DAKO	M7050
CD138	Prediluted	EDTA	DAKO	M7228
Cyclin D1	1/100	High pH	DAKO	M7155
Ki67	1/100	EDTA	DAKO	M7240
MUM1	1/20	EDTA	DAKO	M7259
Oct2	1/800	EDTA	Santa Cruz	233
Pax5	1/100	EDTA	Becton Dickinson	610863
P53	1/200	EDTA	DAKO	M7001

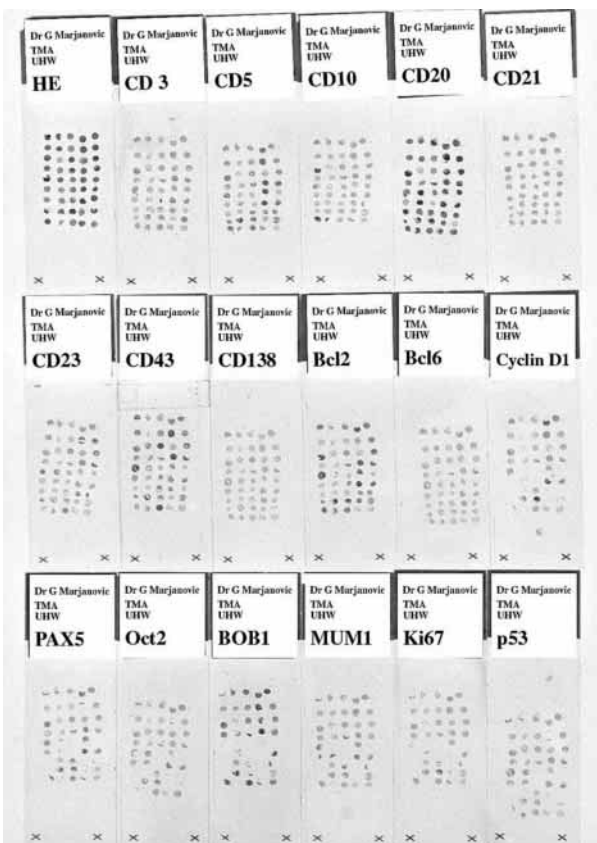


Figure 1. Final look of TMA sections after H&E and immunohistochemistry staining

Statistical analysis

Kaplan-Meier method and log-rank test were used for calculating of survival and univariate differences between groups. $P < 0.05$ was required for statistical significance. Cox regression analysis was used for multivariate analysis. For all calculations a two-sided tests were used.

RESULTS

Immunophenotyping of B cell Non Hodgkin's lymphoma using TMA

Diffuse large B cell lymphoma

Fourteen patients were completely analyzed and all of them were CD20⁺, CD5⁻, CD10⁻, CD23⁻, Oct2⁺, BOB1⁺, Cyclin D1⁻, and CD138⁻. Expression of Bcl2 and Bcl6 was recorded in 6/14 patients, CD43 and MUM1 in 2/14 and p53 in 7/14 patients. MUM1, Bcl6, CD10, and CD138 antigens were used for building and classifying patients according to defined DLBCL subtypes (11) with 5 germinal center type and 3 activated type DLBCL cases. One patient was considered post-germinal center type displaying double positivity for MUM1 and Bcl6 according to criteria given by the same author. Remaining group was classified as complete negative group. Two patients were identified as T cell rich B cell lymphoma according to their morphology, and CD20⁺, Oct2 and BOB1 positivity.

Kaplan Meier analysis showed better prognosis of patients with Bcl6⁺ immunophenotype comparing to Bcl6⁻ cases (two year survival estimate was 19.8% vs. 0; log rank $p = 0.0447$) (Figure 2).

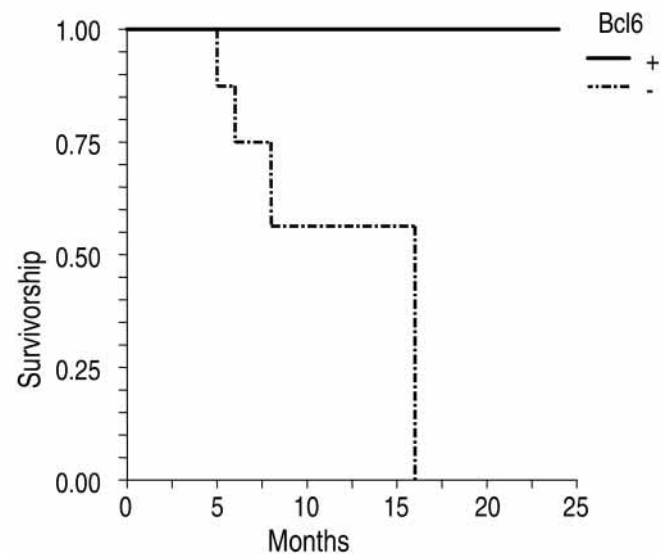


Figure 2. Survivorship of DLBCL patients according to presence of Bcl6 protein

Mantle cell lymphoma

Nine of twelve cases were classic while three were blastoid subtype of mantle cell lymphoma. All cases were CD20⁺, CD5⁺, Cyclin D1⁺, CD3⁻, Bcl2⁺, Oct2⁺, BOB1⁺, Pax5⁺, CD10⁻, CD21⁻, CD23⁻, Bcl6⁻. All cases of classic mantle cell lymphoma had Ki-67 proliferation index <10%, while in blastoid subtype all three had >60% Ki-67⁺ cells.

In 2/5 classic and in 2/3 blastoid cases nuclear P53⁺ was recorded. CD43 was absent in all classic cases while focal positivity was recorded in all blastoid specimens. In classic subtype, both MUM1 and CD138 could not be detected while MUM1 was present in 3/3 blastoid cases.

Indolent lymphomas

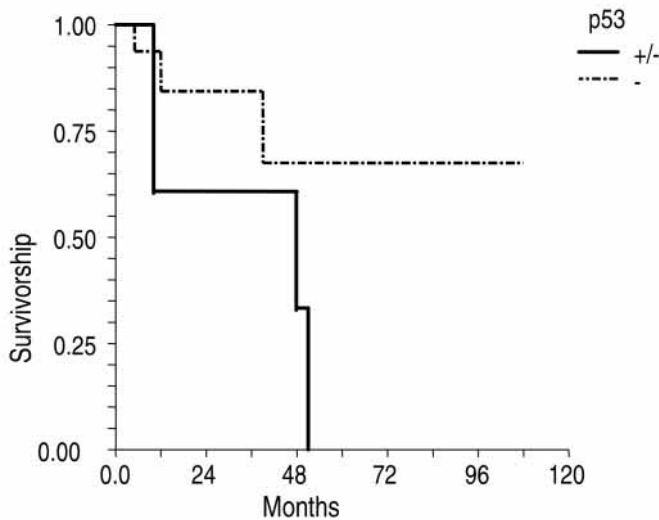
There were 14 cases of chronic lymphocytic leukemia/small cell lymphocytic lymphoma (CLL/SLL), 6 cases with follicular lymphoma (FL), 5 cases of marginal zone lymphoma (MZ), and 7 cases of lymphoplasmacytoid lymphoma (LP). Their immunophenotype was shown in Table 2.

Table 2. Immunophenotype of diagnosed subtypes of indolent lymphomas

Antigen	CLL/SLL +/tested	FL +/tested	MZ +/tested	LP +/tested	Σ +/tested
CD20+	14/14	6/6	5/5	7/7	32/32
CD5+	14/14(+,+/-)	0/6	0/5	0/7	14/32
Cyclin D1+	0/14	0/6	0/5	0/7	0/32
CD3+	0/14	0/6	0/5	0/7	0/32
CD10+	0/14	6/6(nodular)	0/5	0/7	6/32
CD21+	0/14	0/6	0/5	0/5	0/30
CD23+	6/10(+,+/-)	1/5(nodular)	0/5	0/5	7/25
Bcl-2+	6/8	5/6	3/5(+,+/-)	2/5(+,+/-)	16/24
Pax5	6/8	3/4(+,+/-)	3/5(+,+/-)	1/5	13/22
Oct2+	8/8	4/4	4/5(+,+/-)	3/5(+,+/-)	19/22
BOB1+	8/8	4/4	5/5	5/5	22/22
CD138	0/8	0/4	0/5	1/5 (+/-)	1/22
MUM1	3/8 (2 focal)	0/4	0/5	1/5(focal)	4/22
Bcl6+	0/8	4/6	0/5	0/7	4/26
CD43+	7/8 (+,+/-)	0/5	2/5	1/5	10/23
P53+	2/8	1/6(+/-)	1/5	0/6	4/25
Ki-67+	8/8 (0-10%)	6/6 (0-10%)	5/5(0-10%)	6/6(0-30%)	23/23

Abbreviations: CLL/SLL chronic lymphocytic leukemia /small cell lymphocytic lymphoma; FL follicular lymphoma; MZ marginal zone lymphoma; LP lymphoplasmacytoid lymphoma.

In this heterogeneous group of patients Kaplan-Meier 4 year survival estimate in patients with p53^{+/±} was 25% while in p53⁻ group it was 80% (log rank $p=0.0475$) (Figure 3).

**Figure 3.** Survivorship of patients with indolent lymphomas according to presence of p53

DISCUSSION

It is increasingly evident that molecular diagnostics, that is, the use of diagnostic testing to understand the molecular mechanisms of an individual patient's disease, will be pivotal in the delivery of safe and effective therapy for many diseases in the future. A huge body of new information on the genetic, genomic and proteomic profiles of different hematopoietic diseases is accumulating. In this study, we applied high-throughput TMA to study lymphomas and showed that this technique can be used as a reliable method in diagnosis and prognosis of hematological diseases.

In most cases expression of markers used in our study correlates with published results. The characteristic immunophenotype of MCL (CD20⁺, CD5⁺, CyclinD1⁺, CD10⁻, CD23⁻) was conserved in all cases. For markers such as MUM1 defining plasmacytic differentiation range we found positivity in 3/3 blastoid subtype of mantle cell lymphoma. There are no reports in literature concerning this finding to our knowledge. Association of MUM1 protein expression and poor prognosis in blastoid mantle cell lymphoma should be examined. Expression of MUM1 is connected with constant activation of NF- κ B pathway and is already identified as adverse prognostic factor in CLL/SLL (12) and DLBCL(4). In our cohort of

patients with CLL/SLL, MUM1⁺ on TMA could be easily detected as focal in 3/8 cases. In case of CD43 expression, which does not necessarily define the disease class, but is reported to be positive in the majority of CLL/SLLs and mantle cell lymphomas (13) we also found a high rate of positive cases.

Markers of proliferation such as Ki 67 have significant prognostic value in mantle cell lymphomas. Our patients with blastoid subtype had Ki 67 index over 60% on TMA. This is in agreement with Ratty and coworkers who found increased risk for transformation from classic to blastoid subtype with 25% of Ki-67⁺ tumor cells (8, 14). This prognostic cut of point is compatible to results of gene expression profiling analysis of mantle cell lymphomas (15). In case of heterogeneous expression of markers such as Ki-67 and p53 TMA could not be reliably assess the maximal expression. Nevertheless, in case of mean Ki-67 and p53 expression, TMA showed 90 and 92% concordance rate with conventional tissue sections and that could be sufficient for routine practice (16). In our cohort of indolent lymphoma, p53 expression was hallmark of adverse prognosis. Mutations and stabilization of p53 are reported to be related with more aggressive behavior of indolent lymphomas and resistance on chemotherapy, especially in B-CLL, FL and marginal zone lymphomas (17). We report a high rate of Bcl-2 positivity across all NHL types examined. Similar results have been reported by other investigators (9).

Applicability of TMA is best illustrated in group of our patients with DLBCL. Expression of Bcl-6 detected on TMA was found to be associated with better overall survival in our patients. Most studies coincide in showing that a high level of Bcl6 expression, as determined by real-time polymerase chain reaction (PCR) or immunohistochemistry, is a favorable prognostic marker (18, 19). On the other hand, there are studies with opposite results in DLBCL (20,21). The increased expression of Bcl6 was one of the features that made it possible to define a subset of germinal center B-cell-like DLBCLs, characterized by lower aggressivity. Thus, Rosenwald et al. have recently identified 3 different DLBCL subgroups (germinal center B-cell-like-GCB, activated B-cell-like-ABC, and type 3 DLBCL) using gene expression profiling (3). The different clinical behavior of the germinal center B-cell-like group and the fact that some molecular alterations appear to occur exclusively in this concrete subset may support the idea that this DLBCL subgroup actually represents a distinct entity with specific mechanisms of transformation. When TMA and quantitative whole tissue stainings were compared in DLBCL, concordance of 90% was obtained for Bcl6 (9, 22). Furthermore, bcl6, in conjunction with CD10 and MUM1, is a useful marker to identify the GCB phenotype. However, some cases of DLBCL that express bcl6 and MUM1 have an ABC gene expression pattern. The existence of a large group of double bcl6⁺ MUM1⁺ cases demonstrates that the mutual exclusion of these markers, as observed in reactive germinal centers, is not preserved in DLBCLs. We had one such case but there are reports with TMA immunophenotype reaching as high as 47% of DLBCL (22). Although these cases express bcl6, the outcome is most likely to be that of the ABC subtype, and this may explain why there are discrepancies in outcome prediction when using bcl6 expression alone. The cases in some studies were investigated using a polyclonal anti-bcl6 antibody. More recently, a monoclonal antibody (clone PG-B6p), which is also suitable for detection of the bcl6 molecule in routine biopsies, has become commercially available. The monoclonal antibody should facilitate future tissue microarray studies because of its high specificity, absence of background staining, and the good reproducibility of immunostaining results between different centers.

Besides high risk of background staining, TMA proved to be highly compatible with cDNA microarray. Using TMA, Hans and co workers showed that, CD10, Bcl6, and MUM1 can be combined to divide DLBCL into GCB and non-GCB subgroups with an outcome similar to

that predicted by cDNA microarray analysis. In fact, this latter panel of immunostains predicted the cDNA classification in 71% of GCB and 88% of ABC or type 3 cases (4).

BOB1 is a coactivator of the transcription factors Oct1 and Oct2, which regulate transcription of immunoglobulins by binding to the octamer motif of the immunoglobulin gene (both H and L) promoter. Oct2 also participates in the expression of other differentiation and proliferation genes of B-cells including CD20. Bob1 is a co-factor acting as a "clamp" fixing Oct2 to the promoter site (23). The main application of these 2 markers is in diagnosis of Hodgkin's lymphoma (HL) and in particular in the differential diagnosis between classical HL, nodular lymphocyte predominant HL and T-cell rich B-cell lymphoma. In the majority of cases Reed Sternberg cells (RS) are both BOB1⁻ and Oct2⁻ and, when positive, expression is very weak. They are particularly important when RS cells appear CD20⁺ and lack expression of CD15 (23). Majority of cases of T-cell-rich B-cell lymphoma would also be strongly positive in addition to other B-cell lineage markers. Oct2 and BOB1 are of potential use in identifying other CD20 B-cell lymphomas such as plasmablastic lymphoma or plasmacytoma/myeloma

Our results clearly showed the ability of TMA to identify cases of T-cell-rich B-cell lymphoma with Oct2, BOB1, and other cell markers in spite of tissue heterogeneity.

It can be concluded that TMA methodology is highly advantageous in spite of its limitations. This could be evidenced through the logarithmic increase of published papers in which TMA technology was used. The progress in gaining knowledge of the molecular biology of hematolymphoid neoplasms has been substantial, and TMA technology provides a valuable tool to accelerate this process.

REFERENCES

- Kononen J, Bubendorf L, Kallioniemi A, Barlund M, Shraml P, Leghton S, et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. *Nat Med* 1998;4(7): 844-7.
- Hernandez L, Fest T, Cazorla M, Teruya-Feldstein J, Bosch F, Peinado MA, et al. p53 gene mutations and protein overexpression are associated with aggressive variants of mantle cell lymphomas. *Blood* 1996;87(8):3351-9.
- Rosenwald A, Wright G, Chan WC, Connors JM, Campo E, Fisher RI et al. The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N Engl J Med* 2002;346(25):1937-1947
- Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood* 2004;103(1):275-82.
- Torhorst J, Bucher C, Kononen J, Haas P, Zuber M, Kuchli OR, et al. Tissue microarrays for rapid linking of molecular changes to clinical endpoints. *Am J Pathol* 2001;6(159):2249-56.
- Astsaturon IA, Samoilova RS, Iakhnina EI, Pivnik AV, Vorobiov AI et al. The relevance of cytological studies and Ki-67 reactivity to the clinical course of chronic lymphocytic leukemia. *Leuk Lymphoma* 1997;26:337-42.
- Ichikawa A. Prognostic and predictive significance of p53 mutation in aggressive B-cell lymphoma. *Int J Hematol* 2000;71:211-20.
- Raty R, Franssila K, Joesuu H, Teerenhovi L, Elonen E. Ki67 expression level, histological subtype, and the International Prognostic Index as outcome predictors in mantle cell lymphoma. *Eur J Haematol* 2002;69(1):1120.
- Hedvat CV, Hegde A, Chaganti RS, Chen B, Quin J, Filippa DA, et al. Application of tissue microarray technology to the study of non-Hodgkin's and Hodgkin's lymphoma. *Hum Pathol* 2002;10(33):968-74.
- Coiffier B. Diffuse large cell lymphoma. *Curr Opin Oncol* 2001;13(5):325-34.
- Colomo L, Lúpez-Guillermo A, Perales M, Rives S, Martínez A, Bosch F, et al. Clinical impact of the differentiation profile assessed by immunophenotyping in patients with diffuse large B-cell lymphoma. *Blood* 2003;1(101):78-84.
- Chang CC, Lorek J, Sabath DE, Li Y, Chitambar CR, Logan B, et al. Expression of MUM1/IRF4 correlates with clinical outcome in patients with B-cell chronic lymphocytic leukemia. *Blood* 2002;100(13):4671-5.
- Lai R, Weiss LM, Chang KL, Arber DA. Frequency of CD43 expression in non-Hodgkin lymphoma. A survey of 742 cases and further characterization of rare CD43⁺ follicular lymphomas. *Am J Clin Pathol* 1999;111(4):488-94.
- Räty R, Franssila K, Jansson SE, Joensuu H, Wartiovaara-Kautto U, Elonen E. Predictive factors for blastoid transformation in the common variant of mantle cell lymphoma. *EJC* 2003;3(39): 321-9.
- Rosenwald A, Wright G, Wiestner A, Chan W, Connors J, Campo E, et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. *Cancer Cell* 2003;2(3):185-97.
- Zettl A, Meister S, Katzenberger T, Kalla J, Ott MM, Müller-Hermelink HK, et al. Immunohistochemical analysis of B-cell lymphoma using tissue microarrays identifies particular phenotypic profiles of B-cell lymphomas. *Histopathology* 2003;3(43):209-19.
- El Rouby S, Thomas A, Costin D, Rosenberg CE, Potmesil M, Silber R, et al. p53 gene mutation in B-cell chronic lymphocytic leukemia is associated with drug resistance and is independent of MDR1/MDR3 gene expression. *Blood* 1993;82(11):3452-9.
- Offit K, Lo Coco F, Louie DC, Parsa NZ, Leung D, Portlock C, et al. Rearrangement of the bcl-6 gene as a prognostic marker in diffuse large-cell lymphoma. *N Engl J Med* 1994;331(2):74-80.
- Sánchez-Beato M, Sánchez-Aguilera A, Piris MA. Cell cycle deregulation in B-cell lymphomas. *Blood* 2003;4(101):1220-35.
- Akasaka T, Ueda C, Kurata M, Akasaka H, Yamabe H, Uchiyama T, et al. Nonimmunoglobulin (non-Ig)/BCL6 gene fusion in diffuse large B-cell lymphoma results in worse prognosis than Ig/BCL6. *Blood* 2000;96(8):2907-9.
- Barrans SL, Evans PA, O'Connor SJ, Kendall SJ, Owen RG, Haynes AP, et al. Rearrangement of the BCL6 locus at 3q27 is an independent poor prognostic factor in nodal diffuse large B-cell lymphoma. *Br J Haematol* 2002;117(2):322-32.
- Sáez AI, Sáez AJ, Artiga MJ, Pérez-Rosado A, Camacho FI, Díez A, et al. Building an Outcome Predictor Model for Diffuse Large B-Cell Lymphoma. *Am J Pathol* 2004;2(164):613-22.
- Re D, Müschen M, Ahmadi T, Wickenhauser C, Staratschek-Jox A, Holtick U, et al. Oct-2 and Bob-1 deficiency in Hodgkin and Reed Sternberg cells. *Cancer Res* 2001;61(5):2080-4.