J Hematopathol (2008) 1:77–84
DOI 10.1007/s12308-008-0007-7

ORIGINAL ARTICLE

Primary mediastinal B-cell lymphoma: detection of *BCL2* gene rearrangements by PCR analysis and FISH

Cherie H. Dunphy · Dennis P. O'Malley · Liang Cheng · Tina Y. Fodrie · Sherrie L. Perkins · Kathleen Kaiser-Rogers

Received: 29 January 2008 / Accepted: 24 April 2008 / Published online: 18 June 2008

Abstract Primary mediastinal large B-cell lymphoma (PMBCL) has a characteristic clinical presentation, morphology, and immunophenotype, representing a clinically favorable subgroup of diffuse large B-cell lymphoma (DLBCL). By gene expression profiling (GEP), PMBCL shares features with classical Hodgkin lymphoma (cHL). Of further interest, *BCL6* gene mutations and BCL6 and/or MUM1 expression in a number of PMBCLs have supported an activated B-cell (ABC) origin. Several studies, including GEP, have failed to detect *BCL2* gene rearrangements (GRs) in PMBCL. An index case of *t*(14; 18)+ PMBCL prompted our study of the incidence of *BCL2* GRs in PMBCL by polymerase chain reaction (PCR)/fluorescence in situ hybridization (FISH) analyses and its possible clinical impact. Twenty-five retrospectively identified, well-defined PMBCLs (five with cytogenetics) from three institutions were analyzed for a *BCL2* GR by PCR/FISH analyses. The formalin-fixed, paraffin-embedded tissue blocks of 24 available cases were also analyzed by BCL2 immunohistochemistry (IHC). Of the five with cytogenetics, two had a *t*(14; 18) (q32; q21). Of the 25 analyzed by PCR, 2 had no amplifiable DNA (aDNA), including 1 *t*(14; 18)+ case. Of those with aDNA, two showed a *BCL2* GR; by FISH analysis, three demonstrated a *BCL2* GR. BCL2 protein expression by IHC analysis was variably detected in 21 out of 24 (strongly, uniformly expressed: 6, including all with a *t*(14; 18) or a *BCL2* gene rearrangement; moderately weakly expressed in a subset of the malignant cells: 15). Available clinical follow-up of this *BCL2*+ subset showed a similar course to the other PMBCL cases. Our results imply that a subset of PMBCL [(4 out of 24 analyzed) in our series] may be of GC origin. A larger study is necessary to determine any clinical significance.

Keywords Primary mediastinal B-cell lymphoma · *BCL2* gene · PCR and FISH analysis

This manuscript was presented at the annual 2006 USCAP meeting held in Atlanta.

C. H. Dunphy
Divisions of Hematopathology, University of North Carolina, Chapel Hill, NC, USA

D. P. O'Malley · L. Cheng · T. Y. Fodrie
Indiana University,
Indianapolis, Indiana, USA

S. L. Perkins
University of Utah,
Salt Lake City, Utah, USA

K. Kaiser-Rogers
Cytogenetics Laboratory, University of North Carolina,
Chapel Hill, NC, USA

C. H. Dunphy (✉)
CB# 7525 Department of Pathology and Laboratory Medicine,
UNC,
Chapel Hill, NC 27599-7525, USA
e-mail: cdunphy@unch.unc.edu

Introduction

Primary mediastinal B-cell lymphoma (PMBCL) is a diffuse large B-cell lymphoma (DLBCL) first described in 1981 and postulated to arise from noncirculating thymic B lymphocytes [1, 2]. It is recognized as a distinct entity within the World Health Organization (WHO) classification of lymphomas and represents a clinically favorable subgroup of DLBCL [i.e., better 5-year survival rate (64%), than all DLBCLs after therapy (46%)] [3, 4]. It is characterized by a distinctive clinical presentation, morphology, and immunophenotype. Interestingly, by gene-

expression profiling, PMBCL shares features with classical Hodgkin lymphoma [4, 5]. Of further interest, *BCL6* gene mutations and BCL6 and/or MUM1 expression in a number of PMBCLs have supported an activated (or post-germinal center) B-cell (ABC) origin [6]. In addition, several studies, including gene-expression profiling, have failed to detect *BCL2* gene rearrangements in PMBCL [7–9]. An index case of PMBCL associated with a *t*(14; 18) prompted our study of the incidence of *BCL2* gene rearrangements in PMBCL by polymerase chain reaction (PCR) analysis and fluorescent in situ hybridization (FISH) and its possible clinical impact.

Materials and methods

Retrieval of case and clinical follow-ups

Twenty-five consecutive cases, meeting the WHO criteria (definition and description) for a diagnosis of PMBCL (provided below) [3], were retrospectively identified from the three participating academic institutions. The WHO definition and description for PMBCL is stated as follows "a subtype of DLBCL arising in the mediastinum of putative B-cell origin with distinctive clinical, immunophenotypic, and genotypic features. Patients present with localized disease and signs and symptoms relating to large anterior mediastinal masses, sometimes with impending superior vena cava syndrome. When disseminated, other extranodal sites are often involved, such as kidney, liver, skin and brain. The neoplastic cells vary in size and shape. In most cases, the cells have abundant cytoplasm." There is often associated fibrosis in the background. The cells typically express CD19 and CD20, and often lack surface light chain expression by flow cytometry. They may weakly express CD30, either focally or extensively. CD10 and CD5 are absent. The available clinical data regarding presentation, bone marrow staging, and therapeutic follow-up, as well as histologic sections, flow cytometric immunophenotypic data, immunohistochemical immunophenotypic data, and conventional cytogenetic results (available in five cases) were reviewed, and the results supported a diagnosis of PMBCL in each of these cases.

Retrospective analysis of BCL-2 rearrangement by polymerase chain reaction

Tissue samples from 25 patients were qualitatively analyzed for a *BCL2* rearrangement, using a nested PCR assay and gel electrophoresis. Formalin-fixed paraffin-embedded tissue was digested in a proteinase K digestion buffer for 24 h at 56°C and then purified using the QIAamp DNA Mini Kit according to manufacturer's instructions (QIAGEN, Valencia, CA, USA).

A nested PCR assay was performed using a *BCL2* rearrangement assay kit manufactured by InVivoScribe Technologies (San Diego, CA, USA). It involves two nested PCRs, using four sets of primers that target the joining region of the immunoglobulin heavy chain gene and distinct regions of the *BCL2* gene. Two sets of primers were used to identify *BCL2* rearrangements, involving the major breakpoint (Mbr). The second two sets of primers target the minor cluster region (Mcr). The limit of detection, using nested amplifications, is less than one *BCL2*-rearrangement positive cell in ten thousand normal cells (limit of detection $<10^{-4}$). An additional set of primers targeted a HLA class II gene to ensure that the quality and quantity of DNA present was sufficient to generate a valid result. A genomic sequence (Factor V) was also amplified and detected to serve as an additional amplification control for this assay. All PCR reactions were amplified on the GeneAmp PCR System 9700 (Applied Biosystems).

Gel electrophoresis was performed on a 2% agarose gel for 180 min at 70 volts. A DNA size marker (HAE 20 cut p1598, Sigma-Aldrich, St. Louis, MO, USA) was used to determine the presence of the 215-bp product formed when the *BCL2* rearrangement was detected in the major breakpoint region. A 1,000-bp product formation indicated the presence of the *BCL2* rearrangement in the minor cluster region. The absence of the 215- or 1,000-bp product indicated the absence of the *BCL2* rearrangement in the sample. Both positive and polyclonal controls for *BCL2* were analyzed in conjunction with the tissue samples. A "no DNA" control was included as well to ensure the sterility of PCR reagents. Positive and polyclonal controls were included in the *BCL2* rearrangement assay kit provided by InVivoscribe Technologies. Because by a nested PCR assay, rare cells may be detected that carry *BCL2* translocations, all cases positive for a *BCL2* translocation were confirmed, as described below, by FISH analysis.

Retrospective analysis of BCL-2 rearrangement by fluorescent in situ hybridization

Fluorescence in situ hybridization (FISH) was performed on 24 paraffin embedded samples using Vysis' *BCL2* dual color, break apart probe (Abbott Molecular/Vysis Inc., Des Plaines, IL, USA) according to the manufacturer's protocol. For each paraffin-embedded sample, an adjacent hematoxylin- and eosin-stained section was evaluated by an experienced pathologist, and the tumor was marked before FISH analysis. Each FISH slide was scored in a blinded fashion by two independent individuals. For those samples demonstrating a clearly abnormal fluorescence pattern (>20% abnormal cells), consistent with rearrangement of the *BCL2* gene, at least 100 cells were examined. For those

samples demonstrating a fluorescence pattern consistent with the presence of an intact *BCL2* gene (<10% abnormal cells), at least 200 cells were examined. The cutoff values used for the *BCL2* assay were based on our databases for multiple break apart probes, as well as our experience with paraffin samples. In addition, the *BCL2* break apart assay was performed on three known abnormal [three follicular lymphomas with a *t*(14; 18) by routine cytogenetic studies] and six normal paraffin-embedded control samples (all, reactive follicular hyperplasia) in conjunction with this study.

Retrospective immunohistochemical analysis

The formalin-fixed, paraffin-embedded tissue blocks of 24 available cases were also analyzed by BCL2 immunohistochemistry (LSAB2 kit, Dako Corporation, Carpinteria, CA, USA; 1:10 dilution; antigen retrieval: 6.0 pH citrate buffer–steam for 30 min) to determine correlation of a *BCL2* rearrangement with BCL2 protein expression. These blocks were also analyzed by immunohistochemistry for expressions of CD10 (clone 56C6, predilute, Neomarkers, Fremont, CA, USA; antigen retrieval: 10.0 pH citrate buffer–steam for 30 min), BCL6 (clone 4242, Cell Signalling, Danvers, MA, USA; 1:20 dilution; antigen retrieval: 10.0 pH citrate buffer–steam for 30 min), MUM1 (clone MUM1p, Dakocytomation, Carpinteria, CA, USA; 1:50 dilution; antigen retrieval: 6.0 pH citrate buffer–steam for 30 min) and CD138 (CD138/Syndecan-1, Cell Marque, Hot Springs, AR, USA). All of these markers were analyzed by an automated staining and detection system (BenchMark XT, Ventana Medical Systems, Phoenix, AZ, USA), if not performed at the time of initial diagnosis. Cases were classified into three expression patterns: (1) a germinal center (GC) B-cell pattern (expressing CD10 and/or BCL6 but not activation markers: CD138 and MUM1); (2) an activated GC B-cell pattern (expressing at least one of the GC B-cell markers and one of the activation markers); and (3) an activated (post-germinal center) B-cell (ABC) pattern (expressing MUM1 and/or CD138 but not germinal center B-cell markers) [9].

Results

Retrieval of cases

Twenty-five cases retrospectively reviewed from the three perspective academic institutions met the stated general WHO criteria for a diagnosis of PMBCL (Table 1). The patients ranged from 15 to 83 years of age with a female is to male ratio of 1.4:1.0. All patients presented with a primary, large anterior mediastinal mass, and 21 out of 25 initial diagnoses were based on a mediastinal biopsy. The other four diagnostic biopsies originated from supraclavicular lymph nodes (two cases), thoracic lymph nodes (one case), or a lung biopsy (one case). No other sites of lymphomatous involvement were identified, and all staging bone marrows were negative for lymphomatous involvement. All had the typical morphology of PMBCL, characterized by a massive, diffuse proliferation of variably sized cells associated with abundant, pale cytoplasm and variably dense compartmentalizing fibrosis (Fig. 1). Of the 11 cases with available flow cytometric immunophenotypic data, 4 demonstrated lack of any surface light chain expression, which frequently occurs in PMBCL. Of the 23 cases with available immunohistochemical immunophenotypic data at the time of original diagnosis, all were CD45- and CD20-positive. CD30 was expressed in 13 out of 16 analyzed cases, as is also frequently observed in PMBCL. CD30 was expressed in one of the cases with a *BCL2* rearrangement, was negative in one of the cases with a *BCL2* rearrangement, and was not performed in the two remaining cases with a *BCL2* rearrangement. CD10 was not expressed in the majority of cases (18 out of 24); it was weakly to moderately expressed in the remaining cases, only 2 of which had a *BCL2* rearrangement. Although CD10 is stated as being absent in the WHO description of PMBCL, CD10 expression does not exclude a diagnosis of PMBCL. In addition, the two cases with a *t*(14; 18) by conventional cytogenetic studies were CD10-negative. CD23 was expressed in five of six cases tested. The CD23-negative case was not associated with a *BCL2* rearrangement. Two of the 5 CD23+ cases were associated with a *BCL2* rearrangement. Of the five cases with conventional cytogenetic results, two revealed a *t*(14; 18) (q32; q21). Both of these cases were associated with additional abnormalities. The other three cases showed abnormalities as depicted in Table 1. Two of these three cases revealed abnormalities that have been frequently described in PMBCL (i.e., abnormalities of 9p and 6p).

Retrospective analysis of BCL2 rearrangement by PCR

Of 25 cases studied, 2 did not yield amplifiable DNA [including 1 case with a *t*(14; 18) by conventional cytogenetic studies]. Of the 23 cases with amplifiable DNA, a *BCL2* gene rearrangement was detected in 2 cases (Fig. 2). These two cases had not had conventional cytogenetic studies performed.

Retrospective analysis of BCL2 rearrangement by FISH

Nineteen of the 24 analyzed paraffin-embedded samples demonstrated no evidence of a *BCL2* rearrangement; the mean number of cells with a relevant abnormal signal

Table 1 PMBCL cases with cytogenetic, molecular, and immunohistochemical data

Case no.	Age	Sex	Flow	Conv cytogen	PCR bcl-2	FISH bcl-2	IHC bcl-2	IP (GC, AGC, ABC)[a]
1	42	M	NP	83–90 tetra ,−Y, +X, +X dup1q, −1 der/ re9p&11p	Neg	Neg	bcl-2 –	ABC (MUM1+)
2	53	F	NP	NP	Neg	Neg	bcl-2 +++	ABC (MUM1+)
3	83	F	MBC	90–91 tetra num abn incl 2 copies of *t*(14; 18)	NADNA	NR	bcl-2 +++	GC (BCL6+)
4	31	F	NP	NP	Neg	Neg	bcl-2 ++	AGC (MUM1+, BCL6+)
5	41	M	NP	NP	Neg	Neg	bcl-2+++	AGC (MUM1+, BCL6+)
6	56	F	MBC	*t*(1; 4), *t*(5; 8) add 6p, ring	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+)
7	24	F	BC with loss of lt ch exp	del 1p, −Y, −15 *t*(2; 5) (q21; q13.3)	NADNA	NR	bcl-2 v+ (s)	NA
8	35	F	NR	NR	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+, CD10+)
9	44	M	NR	NR	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+)
10	24	M	NP	NP	Neg	Neg	bcl-2 v+ (s)	NR
11	23	M	NP	NP	Neg	NA	NA	AGC (MUM1+, BCL6+)
12	30	F	NP	NP	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+)
13	25	F	MBC	NP	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+, CD10+)
14	23	M	MBC with wk CD10 (s)	NP	Neg	Neg	bcl-2 –	AGC (MUM1+, BCL6+)
15	41	M	BC with loss of lt ch exp	NP	Neg	Neg	bcl-2 v+ (s)	ABC (MUM1+)
16	65	F	MBC with CD10 +	NP	Pos	Pos	bcl-2 +++	AGC (MUM1+, BCL6+)
17	37	M	NR	NP	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+)
18	76	F	BC with loss of lt ch exp	NP	Pos	Pos	bcl-2 v+	AGC (MUM1+, BCL6+, CD10+)
19	20	M	MBC CD22+ CD11c+	NP	Neg	Neg	bcl-2 v+ (s)	ABC (MUM1+)
20	24	M	MBC CD22+	NP	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+, CD10+)
21	15	F	NR	NP	Neg	Neg	bcl-2 v+ (s)	AGC (MUM1+, BCL6+)
22	39	F	NR	NP	Neg	Neg	bcl-2 –	ABC (MUM1+)
23	34	M	NR	NP	Neg	Neg	bcl-2 v+ (s)	ABC (MUM1+)
24	28	F	NP	NP	Neg	Neg	bcl-2 v+ (s)	ABC (MUM1+)
25	65	F	BC with loss of lt ch exp CD10-	Multiple abns with *t*(14; 18)	Neg	Pos	BCl-2+++	GC (BCL6+)

Conv cytogen Conventional cytogenetics, *PCR* polymerase chain reaction, *FISH* fluorescent in situ hybridization, *IHC* immunohistochemical, *IP* immunophenotype, *GC* germinal center, *AGC* activated germinal center, *ABC* activated B- (post-germinal center) cell, *NP* not performed, *MBC* monoclonal B cells, *NADNA* no amplifiable DNA, *NR* no results, *BC* B cells, *lt ch exp* light chain expression, *NA* not available, *abns* abnormalities, *v+* variably positive, *(s)* subset

[a] Indicates only positive markers are listed

pattern was 0.9% (range 0–2.5%) in these specimens. These data did not differ significantly from the six normal control specimens assayed with the *BCL2* break-apart probe. The three remaining samples did reveal evidence of a *BCL2* rearrangement. In one abnormal sample (case no. 16), 66% of the approximate 100 interphase cells examined demonstrated a fluorescence pattern consistent with a rearrangement involving *BCL2*; in the second abnormal sample (case no. 18), 89% of the cells examined demonstrated a *BCL2* rearrangement; and in the third abnormal sample (case no. 25), 86% of the cells examined demonstrated a *BCL2* rearrangement (Fig. 3). Of note, this third abnormal sample did not reveal a *BCL2* gene rearrangement by PCR analysis. Despite several attempts, sufficient probe hybridization for analysis was not able to be obtained from two samples [including the case with a *t*(14; 18) by conventional

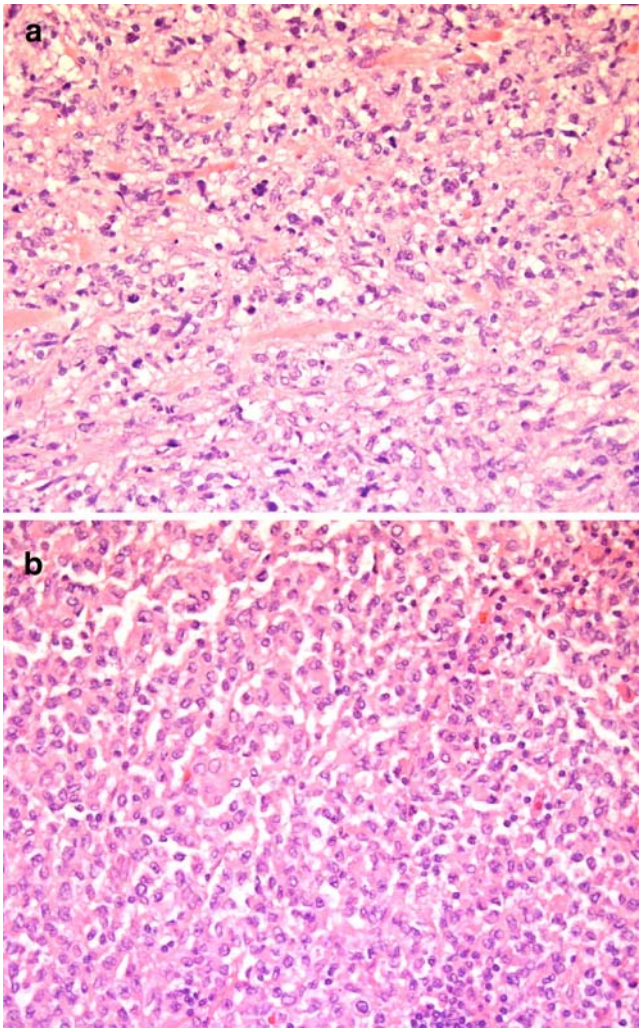

Fig. 1 Sections of the cervical lymph node in this patient with a large anterior mediastinal mass reveal the evident background of fibrosis between the malignant cells (**a** ×400, hematoxylin–eosin stain) that are variable in size with abundant, pale cytoplasm (**b** ×400, hematoxylin–eosin stain)

cytogenetic studies and no amplifiable DNA by PCR analysis]. Of note, a number of the samples demonstrated one to three extra intact *BCL2* signals in a significant portion of the examined cells, suggesting the presence of polysomy 18 and/or a partial aneuploidy involving this locus.

Retrospective IHC analysis

Of the 24 cases analyzed by BCL2 immunohistochemistry, BCL2 protein expression was variably detected in 21 cases (strongly and uniformly expressed in 6 cases, including the 4 with a *t*(14; 18) or a *BCL2* gene rearrangement, and moderately to weakly expressed in a subset of the malignant cells in the remaining 15 cases). The immunohistochemical analysis of the 23 available cases with CD10, BCL6, CD138, and MUM1 revealed a germinal center

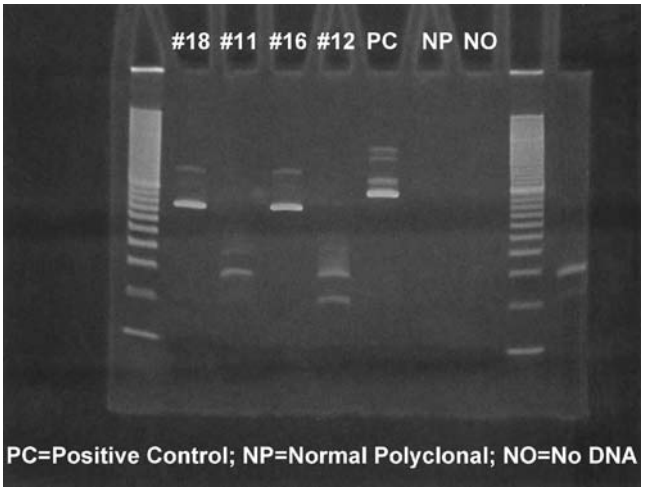

Fig. 2 This image demonstrates the presence of a bcl-2 gene rearrangement in cases nos.18 and 16 compared to the positive control (PC) by PCR analysis. Note the first column represents patient no. 18; the second column, patient no. 11; the third column, patient no. 16; the fourth column, patient no. 12; the fifth column, a PC; the sixth column, a normal polyclonal sample; and the seventh column, a sample with no DNA. The *lower bands* in the columns of case nos. 11 and 12 are not of the appropriate size for interpretation

(GC) immunophenotype in 2 cases [1 with *t*(14; 18) by conventional cytogenetics and 1 with *t*(14; 18) by conventional cytogenetics and FISH alone], an activated GC immunophenotype in 14 cases (the remaining 2 cases with *BCL2* gene rearrangements detected by PCR and FISH) and an activated B-cell (ABC) immunophenotype in the remaining 7 cases, as defined by Chang et al. [10].

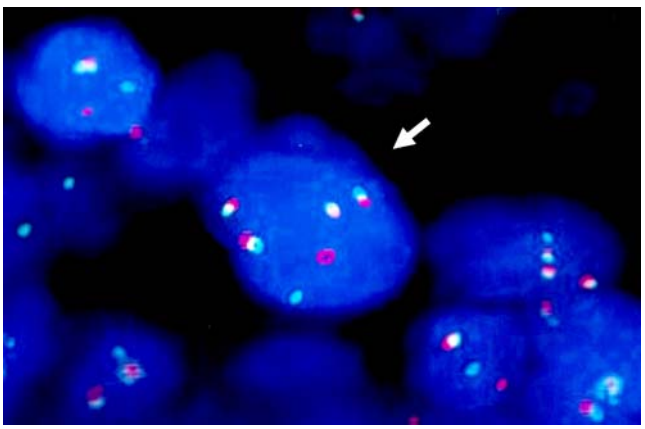

Fig. 3 This image demonstrates fluorescence in situ hybridization results of case no. 25. Shown is interruption of the BCL2 locus in the paraffin-embedded tissue section. The *large arrowed cell* in the center contains four fusion signals (*yellow or overlapped red and green signals*) representing four intact BCL2 loci. Interruption of one BCL2 locus is evidenced by the *single red* (telomeric or 5′ flanking region) *and green* (centromeric or 3′ flanking region) signals in this cell. Each of the other cells in the field also contains individual *red and/or green signals* reflecting interruption of the BCL2 locus

Clinical follow-up

The treatment and clinical follow-up of all available cases (21 of 25 cases) are provided in Table 2. One case with a *BCL2* gene rearrangement by PCR and/or FISH and an activated GC immunophenotype (case no. 18) had a good response to conventional chemotherapy with or without radiotherapy and was in complete remission at last follow-up (2 years after initial diagnosis and subsequent therapy). The case with a *t*(14; 18) by conventional cytogenetic studies and a GC immunophenotype (and no amplifiable DNA by PCR analysis and insufficient probe hybridization by FISH analysis) experienced two recurrences within 2.5 years of initial diagnosis, despite CHOP-Rituxan chemotherapy and consolidative radiotherapy. The case with a *BCL2* gene rearrangement detected by PCR and an AGC immunophenotype (case no. 16) experienced a late relapse in the mediastinum, supraclavicular lymph nodes, and lung and died of disease within 1.5 years. The fourth patient with a *t*(14; 18) by conventional cytogenetic studies and a *BCL2* gene rearrangement by FISH (case #25) had no available clinical follow-up. In comparison to the PMBCL cases without a *t*(14; 18) or a *BCL2* gene rearrangement, there does not appear to be any significant difference in outcome, as there were also relapses and one patient died of disease within 2 years in this group.

Table 2 Correlation of molecular and cytogenetic data with treatment and follow-up

Case no.	Conv cytogen	PCR bcl-2	FISH bcl-2	Treatment and follow-up
1	83–90 tetra, −Y, +X, +X dup1q, −1 der/re9p&11p	Neg	Neg	CHOP and XRT; CR; 6 years
2	NP	Neg	Neg	R-CHOP and XRT; CR; 5 years
3	90–91 tetra num abn incl 2 copies of *t*(14; 18)	NADNA	NR	R-CHOP in CR at 1 year; recurred in right eye at 2 years
4	NP	Neg	Neg	CHOP and XRT; CR; 5 years
5	NP	NEG	Neg	R-CHOP; CR; 4 years
6	*t*(1; 4), *t*(5; 8) add 6p, ring	Neg	Neg	R-CHOP; CR at 1 year; relapsed within 3 years; treated with 2 cycles R-ICE, BEAM chemotx, auto SCT; CR 1 year out
7	del 1p, −Y, −15 *t*(2; 5) (q21; q13.3)	NADNA	NR	R-CHOP; CR; 4 years
8	NR	Neg	Neg	R-CHOP; CR; 3 years
9	NR	Neg	Neg	No follow-up available
10	NP	Neg	Neg	CHOP and XRT; CR; 7.5 years
11	NP	Neg	NA	CHOP and XRT; CR; 6 years
12	NP	Neg	Neg	R-CHOP and XRT; CR; Developed recurrence supraclavicular LN 9 months after tx; received ABMT; CR 3 years after transplant
13	NP	Neg	Neg	R-CHOP and XRT; CR; 4 years
14	NP	Neg	Neg	R-CHOP and XRT; CR; 3 years
15	NP	Neg	Neg	R-CHOP and XRT; CR; local mediastinal relapse at 6 months; treated with ABMT; CR; 2.5 years
16	NP	Pos	Pos	R-CHOP; CR; late relapsed in mediastinum, supraclav nodes, lung; DOD 1.5 years
17	NP	Neg	Neg	R-CHOP and XRT; CR; 2.5 years
18	NP	Pos	Pos	R-CHOP and XRT; CR; 2 years
19	NP	Neg	Neg	CHOP; brain mets treated with Decadron; MTX followed by Vincristine and then Leucovorin; Liver mets treated with ESHAP CR; 5 years
20	NP	Neg	Neg	CHOP and XRT; Rituxan DEPA; at 1 year last follow-up; pulmonary nodules causing at least 50% opacification of thorax; no additional follow-up
21	NP	Neg	Neg	COPADM1, M2, and CYM1; 5961C chemotx and XRT; Rituxan relapse and DOD within 2 years
22	NP	Neg	Neg	R-CHOP; CR at 5 years
23	NP	Neg	Neg	No follow-up available
24	NP	Neg	Neg	No follow-up available
25	Multiple abns with *t*(14; 18)	Neg	Pos	No follow-up available

CR Complete remission, *XRT* radiation therapy, *R* Rituxan, *ABMT* autologous bone marrow transplant, *DOD* dead of disease

Discussion

Primary mediastinal B-cell lymphoma is defined by the WHO classification as a primary large anterior mediastinal mass diffusely infiltrated by variably sized lymphoid cells with abundant, pale cytoplasm associated with compartmentalizing fibrosis. The cells have a B-cell immunophenotype frequently associated with lack of surface light chain expression by flow cytometric immunophenotypic analysis and with variable expression of CD30 by immunohistochemical analysis. Our 25 retrospectively identified cases met the WHO criteria based on clinical presentation, morphology, and immunophenotyping.

As mentioned previously, PMBCL has been postulated to arise from thymic B cells. This postulation has been supported, based on expression of MAL. MAL mRNA was initially identified by a differential screening approach during the search for T-cell maturation-associated cDNAs and has been shown to be associated with the intermediate and later stages of intrathymic T-cell differentiation [11]. Subsequently and interestingly, MAL was shown to be a distinct molecular marker of PMBCL, being demonstrated in 70% of PMBCLs and in only 3% of nonmediastinal DLBCLs, in a study by Copie-Bergman, et al. [12].

Cytogenetic abnormalities that have been described previously in PMBCLs have included frequent observations of genetic gains involving chromosomes 2, 5, 7, 9p, 12, and Xq as well as characteristic abnormalities of 9p in 50% of cases and alterations of chromosome 6q [13–15]. More recently, rare cases of PMBCL with *t*(14; 18) have been described [16]. In our five cases that were analyzed by conventional cytogenetic studies, the three cases that were not associated with a *t*(14; 18) did reveal a derivative and rearrangement involving chromosome 9p (1 case) and an add chromosome (6p; one case).

Although postulated to be of thymic B-cell origin, only relatively recently has there been molecular characterization of PMBCL. In 1996, Tsang et al. analyzed molecular alterations involving *BCL1*, *BCL2*, *BCL6*, c-myc, H-ras, K-ras, N-ras, and p53 genes and for Epstein–Barr virus (EBV) infection in PMBCL [8]. Alterations of *BCL1*, *BCL2*, or ras genes and evidence of EBV infection were not observed; rearrangement of the *BCL6* gene was detected in only one case (16% of cases analyzed).

However, a subsequent molecular study by Pileri et al. demonstrated that more than half of 40 PMBCLs studied displayed *BCL6* gene mutations, usually occurring together with functioning somatic IgV(H) gene mutations, and BCL6 and/or MUM1/IRF4 expression [13]. These findings suggested derivation of PMBCL from either activated GC or activated (post-germinal center) B-cells, as CD10 and/or BCL6, but not activation markers (i.e., CD138 and MUM1), are expressed in a GC pattern, CD10 or BCL6 and CD138 or MUM1 are expressed in an activated GC pattern, and CD138 and/or MUM1, but not CD10 or BCL6, are expressed in the activated (or post-germinal center) B-cell (ABC) pattern [10]. However, as mentioned previously, PMBCL is associated with a better prognosis than non-mediastinal DLBCLs, and of interest, it has been demonstrated that DLBCLs with an activated GC or ABC immunophenotype have worst overall survival and event-free survival than DLBCLs with a GC immunophenotype [10, 17].

Gene-expression profiling studies of PMBCL have in fact supported a strong relationship between PMBCL and classical Hodgkin lymphoma [4, 5]. Over one third of the genes that were more highly expressed in PMBCL than in other DLBCLs were also characteristically expressed in classical Hodgkin lymphoma cells. These studies identified a molecular link between PMBCL and classical Hodgkin lymphoma and a shared survival pathway. Of interest, these gene expression profiling studies identified PMBCL as a clinically favorable subgroup, when compared with GC-like DLBCL and ABC-like DLBCL. Of interest, the PMBCL subgroup was somewhat more related to the GC-like subgroup of DLBCL, than to the ABC-like subgroup of DLBCL, even though PMBCL was clearly distinguishable from both subgroups of DLBCL. Interestingly, *BCL2* gene rearrangements were not described in the gene expression profiling studies of PMBCL.

Our encounter with a case of PMBCL, defined by WHO criteria, associated with a *t*(14; 18) prompted our study of the incidence of this rearrangement by PCR analysis and FISH analysis in this entity. Although our index case did not have amplifiable DNA by PCR analysis and did not have sufficient probe hybridization for FISH analysis, 3 of 23 additional cases of PMBCL with results by PCR and FISH analyses revealed a *BCL2* gene rearrangement by PCR and FISH (2 cases) and by FISH alone (1 case, possibly due to sampling differences, or a different sensitivity by the PCR analysis).

Although our subset of cases with a *BCL2* gene rearrangement may possibly represent non-PMBCL of follicular origin, closely mimicking the clinical and morphologic features of PMBCL, our results also may imply that a subset (4 out of 24) of PMBCL in our series may actually be of GC origin. In particular, two of our cases [those with *t*(14; 18) by conventional cytogenetics and or FISH alone) revealed a GC immunophenotype by immunohistochemical staining. Available clinical follow-up of this subset showed a similar course to the other PMBCL cases. A larger study is necessary to determine a significant clinical impact in this subset. In addition, gene expression profiling of this subset would be interesting to compare to PMBCLs of ABC origin.

References

1. Addis BJ, Isaacson PG (1986) Large B-cell lymphoma of the mediastinum: a B-cell tumour of probable thymic origin. Histopathology 10:379–390
2. Miller JB, Variakojis D, Bitran JD et al (1981) Diffuse histiocytic lymphoma with sclerosis: a clinicopathologic entity frequently causing superior venacaval obstruction. Cancer 47:748–756
3. Jaffe ES, Harris NL, Stein H, Vardiman JW (2001) World Health Organization classification of tumours. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. IARC Press, Lyon, France
4. Savage KJ, Monti S, Kutok JL (2003) The molecular signature of mediastinal large B-cell lymphoma differs from that of other diffuse large B-cell lymphomas and shares features with classical Hodgkin lymphoma. Blood 102:3871–3879
5. Rosenwald A, Wright G, Leroy K et al (2003) Molecular diagnosis of primary mediastinal B cell lymphoma identifies a clinically favorable subgroup of diffuse large B cell lymphoma related to Hodgkin lymphoma. J Exp Med 298(6):851–862
6. Pileri S, Gaidano G, Zinzani PL et al (2003) Primary mediastinal B-cell lymphoma. High frequency of BCL-6 mutations and consistent expression of the transcription factors OCT-2, BOB.1, and PU.1 in the absence of immunoglobulins. Am J Pathol 162 (1):243–253
7. Scarpa A, Moore PS, Rigaud G et al (1999) Molecular features of primary mediastinal B-cell lymphoma: involvement of $p16^{INK4A}$, *p53* and *c-myc*. Br J Haematol 107:106–113
8. Tsang B, Cesarman E, Chadburn A et al (1996) Molecular characterization of primary mediastinal B cell lymphoma. Am J Pathol 148(6):2017–2025
9. Capello D, Vitolo U, Pasqualucci L et al (2000) Distribution and pattern of BCL-6 mutations throughout the spectrum of B-cell neoplasia. Blood 95:651–659
10. Chang C-C, McClintock S, Cleveland RP et al (2004) Immunohistochemical expression patterns of germinal center and activation B-cell markers correlate with prognosis in diffuse large B-cell lymphoma. Am J Surg Pathol 28:464–470
11. Alonso MA, Weissman SM (1987) cDNA cloning and sequence of MAL, a hydrophobic protein associated with human T-cell differentiation. Proc Natl Acad Sci USA 84: 1997–2001
12. Copie-Bergman C, Plonquet A, Alonso MA et al (2002) MAL expression in lymphoid cells: Further evidence for MAL as a distinct molecular marker of primary mediastinal large B-cell lymphomas. Mod Pathol 15(11):1172–1180
13. Pileri SA, Zinzani PL, Gaidano G et al (2003) Pathobiology of primary mediastinal B-cell lymphoma. Leuk Lymphoma 44(Suppl 3):S21–26
14. Rigaud G, Moore PS, Taruscio D et al (2001) Alteration of chromosome arm 6p is characteristic of primary mediastinal B-cell lymphoma, as identified by genome-wide allelotyping. Genes, Chromosomes, and Cancer 31(2):191–195
15. Scarpa A, Moore PS, Rigaud G et al (2001) Genetic alterations in primary mediastinal B-cell lymphoma: an update. Leuk Lymphoma 41(1–2):47–53
16. Palanisamy N, Abou-Elella AA, Chaganti SR et al (2002) Similar patterns of genomic alterations characterize primary mediastinal large B-cell lymphoma and diffuse large-B-cell lymphoma. Genes, Chromosomes, and Cancer. 33:114–122
17. Alizadeh AA, Eisen MB, Davis RE et al (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503–511