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Clinical and pathological features of Burkitt lymphoma showing expression of BCL2 – an analysis including gene expression in formalin-fixed paraffin-embedded tissue

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Summary

The differential diagnosis between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLBCL) can be challenging. BL has been reported to express less BCL2 than DLBCL, but this issue has not been analysed systematically. BL expressing BCL2 can be considered to be MYC/BCL2 co-expressors, a feature that is associated with poorer outcome in DLBCL but that has not been correlated with outcome in BL so far. We analysed the expression of BCL2 in 150 cases of conventionally diagnosed BL using two different BCL2 antibodies. BCL2 expression was detected in 23% of the cases, though the expression varied in intensity and number of positive cells. We did not detect any relevant differences in clinical presentation and outcome between BCL2-positive and BCL2-negative BL in a subgroup of 43 cases for which detailed clinical data were available. An independent cohort of 17 BL with expression of BCL2 were analysed molecularly, with 13 of 17 cases classified as molecularly defined BL (Burkitt Lymphoma) using gene expression profiling on formalin-fixed paraffin-embedded tissues. The four lymphomas diagnosed molecularly as intermediates did not differ in clinical presentation and outcome from molecularly defined BL.

Keywords: NanoString, nCounter, classifier, Burkitt, diffuse large B-cell lymphoma, MYC.

The differential diagnosis of Burkitt lymphoma (BL) versus diffuse large B-cell lymphoma (DLBCL) can be challenging. In daily practice, the diagnosis of BL and DLBCL is based on morphological assessment, immunophenotype and detection of *MYC* translocations and absence of translocations involving the *BCL2* or *BCL6* genes by fluorescence *in situ* hybridization (FISH) (conventionally diagnosed BL). The immunophenotype, which is assessed to distinguish BL from DLBCL, frequently includes BCL2 expression (Cogliatti *et al*, 2006; Kluin & Schuuring, 2011; Salaverria & Siebert, 2011). The former World Health Organization (WHO) classification stated that BCL2 is not expressed in BL (Jaffe *et al*, 2001). However, gene expression profiling identified cases of molecularly defined BL (mBL) with BCL2 expression not caused by translocations of the gene (Dave *et al*, 2006; Hummel *et al*, 2006). These findings led to a new weighting of the immunophenotypic features of BL in the current WHO classification, which describes BCL2 as being expressed in up to 20% of BL cases, predominantly with a weak expression pattern (Swerdlow *et al*, 2008).

BL expressing BCL2 must be distinguished from the group of lymphomas called 'B-cell lymphomas unclassified with features intermediate between BL and DLBCL' (conventionally diagnosed intermediates), which comprises a heterogeneous group of lymphomas. Conventionally diagnosed intermediate lymphomas show at least some features reminiscent of BL, such as a monomorphic cytological picture with small- to medium-sized blasts, a starry sky pattern, high proliferation and frequently translocations involving the *MYC* gene (Swerdlow *et al*, 2008). However, conventionally diagnosed intermediate lymphomas often display immunophenotypic features, such as strong BCL2, expression or genetic features like *BCL2*

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or *BCL6* gene translocations that preclude a BL diagnosis. The presence of both *MYC* and *BCL2* or *BCL6* translocations is called 'double hit' and is usually not compatible with the diagnosis of BL (Boerma *et al*, 2009; Aukema *et al*, 2011, 2014).

Intermediate lymphomas can also be identified by gene expression profiling independent of morphology, immunophenotype and FISH data. Gene expression profiling-based molecular intermediates (m-intermediates) are classified as conventionally diagnosed BL or DLBCL by conventional diagnostics. A subgroup of m-intermediates lack a genetic 'double hit' constellation (Hummel *et al*, 2006). Translocations of *BCL6* and *BCL2*, and thus 'double hit' lymphomas, are exceedingly rare in children and adolescents, but m-intermediates in the young age group can be detected by gene expression profiling (Klapper *et al*, 2008). Similar to 'double hit' lymphomas, m-intermediates identified by gene expression profiling occasionally express BCL2 protein (Klapper *et al*, 2008). Currently, gene expression profiling is not available for routine diagnosis, thus for diagnostic pathologists it is currently unclear whether strong expression of BCL2 justifies the classification of a case with the morphological and immunophenotypical features of a BL as an intermediate lymphoma.

In DLBCL, not only the genetic 'double hit' with *MYC* and *BCL2* translocations but also the co-expression of both MYC and BCL2 proteins ('co-expressor') characterize a subgroup of DLBCL with an aggressive course (Johnson *et al*, 2009; Green *et al*, 2012; Horn *et al*, 2013; Hu *et al*, 2013; Perry *et al*, 2014). In BL, high levels of MYC protein expression are due to *MYC* translocations (Hummel *et al*, 2006). Whether the co-expression with BCL2 indicates inferior outcome in BL has not yet been studied.

To gain a better understanding of the clinical implications, we systematically analysed BCL2 expression in BL. To this end, we screened a large cohort of conventionally diagnosed BL for BCL2 expression using two antibodies that detect BCL2. We correlated the BCL2 expression status with the clinical course in cases with available clinical data. Moreover, we applied digital multiplexed gene expression profiling on formalin-fixed paraffin-embedded (FFPE) tissue specimens from BCL2-positive and BCL2-negative conventionally diagnosed BL cases to determine whether they should be classified as mBL.

Patients and methods

Patients and tissue specimens

BL cases from all age groups that were diagnosed between 2001 and 2014 were identified within the files of the Lymph Node Registry Kiel. Cases with sufficient FFPE tissue were arranged in a tissue microarray (TMA) with duplicate cores 0·6 mm in diameter for each lymphoma. We included only lymphomas with morphological features compatible with the diagnosis of conventionally diagnosed BL as judged on full tissue sections. The lymphomas were composed of dense sheets of monomorphic small- to medium-sized blasts with a narrow rim of cytoplasm showing cohesive growth and a starry sky pattern in areas of good tissue preservation. The TMA cohort comprised a total of 150 cases of conventionally diagnosed BL from all age groups. A subgroup of cases with specimens of sufficient size and a tumour content of at least 70% were selected for molecular analysis ($n = 17$). Clinical data were available for 43 cases.

Immunohistochemistry and fluorescence in situ *hybridization (FISH)*

Analysis of BCL2 expression was conducted using clone 100/D5 (DAKO, Glostrup, Denmark) or clone E17 (Zytomed, Berlin, Germany) (Adam *et al*, 2013). Analysis of CD20, CD10 and Ki67 was performed using an automated stainer (Leica, Wetzlar, Germany). BCL2 immunohistochemistry results were scored by visual inspection in the following categories: negative (no staining in lymphoma cells), 1–25% positive lymphoma cells, 26–50% positive lymphoma cells, 51–75% positive lymphoma cells and >75% positive lymphoma cells. The intensity of BCL2 staining was analysed relative to non-neoplastic T-cells serving as an internal control as weak (weaker than T-cells) or strong (stronger or equal to T-cells). Staining intensity of T-cells also served as a control for immunoreactivity of the tissue. The Ki67 index was assessed in 5% steps, whereas CD20, CD10 and TdT were reported as positive or negative. FISH was conducted as recently described (Ventura *et al*, 2006). Break-apart probes for the *MYC*, BCL2and *BCL6* loci as well as *IGH-MYC* and *IGH-BCL2* fusion probes were applied (Abbott, Abbott Park, Illinois, USA) (Barth *et al*, 2013).

RNA extraction and digital multiplexed gene expression

Formalin-fixed paraffin-embedded material was cut into five 10 μm-thick sections per sample. RNA was extracted according to the manufacturer's instructions (ExpressArt FFPE Clear RNAready Kit, AmpTec, Hamburg, Germany), quantified and checked for quality (Agilent RNA 6000 Nano Chips, Agilent Technologies, Santa Clara, CA, USA) as previously described (Masque-Soler *et al*, 2013). Gene expression analysis was performed using nCounter/Nanostring technology following the previously published protocol (Masque-Soler *et al*, 2013). In five specimens with previously visualized BCL2 expression pattern on FFPE sections, BCL2-positive and -negative areas were analogously labelled on unstained tissue sections, manually dissected and separately processed for RNA extraction and gene expression.

Results

Incidence of BCL2 expression in conventionally diagnosed BL

We analysed 150 cases of conventionally diagnosed BL diagnosed by morphological and immunophenotypical features

as well as the presence of *MYC* translocations. All cases were positive for CD20, CD10 and negative for TdT with a Ki67 index above 90%. All of the samples tested harboured *MYC* (150/150, 100%) translocations but lacked *BCL2* (0/141, 0%) or *BCL6* (0/137, 0%) translocations by FISH. TMAs containing our case cohort were stained by two antibodies directed against BCL2. Applying the most widely used antibody clone, 100/D5, 146 cases yielded an interpretable result. No expression was detected in 113/146 cases (77%). BCL2 expression was detected at various levels in 33/146 (23%) of the cases with interpretable results [1–25% positive lymphoma cells in 16/146 (11%), 26–50% in 6/146 (4%), 51–75% in 6/146 (4%) and >75% in 5/146 (3%)]. The expression intensity was weak (weaker than non-neoplastic T-cells as internal controls) in the majority of cases (Fig 1) and only two cases showed strong staining intensity equal to or stronger than non-neoplastic T-cells (data not shown). To analyse whether BCL2 protein expression might escape detection by the frequently used antibody clone 100/D5, as reported for follicular lymphoma (Adam *et al*, 2013), the cases were additionally analysed by the antibody clone E17. However, none of the conventionally diagnosed BL that lacked BCL2 expression using the clone 100/D5 stained positive with the clone E17 (0/104 of cases with interpretable results, Table S1).

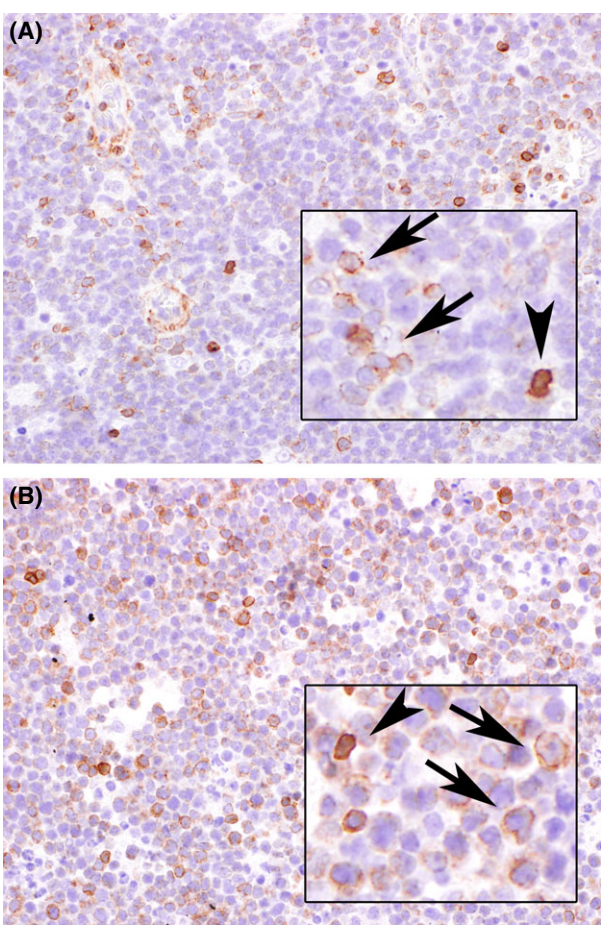

Fig 1. Variable expression of BCL2 in BL. Scattered cells comprising about 10% of all lymphoma cells (A, corresponding to Case 9 in Table I) and diffusely dispersed BCL2-positive lymphoma cells comprising about 40% (B, corresponding to Case 12 in Table I). Insets show high magnifications with arrowheads indicating non-neoplastic T-cells and arrows indicating BCL2-positive lymphoma cells. Original magnification 400x.

Pathological features of conventionally diagnosed BL showing expression of BCL2

A subgroup of 17 patients with conventionally diagnosed BL was further studied on full tissue sections (seven of these patients were also part of the TMA cohort). The patients' clinical features were typical for BL and they were predominantly young (median age 11 years, range 3–24 years) and male (Table I). All but one patient presented with lymphomas with no or limited bone marrow infiltration and they were frequently at a limited stage (Table I). The lymphomas manifested predominantly at extranodal sites with 10/17 in the small or large intestine or as an abdominal or ileocecal mass (Table I).

Histopathology and genetics were compatible with the diagnosis of conventionally diagnosed BL, with small to medium-sized blasts with a narrow rim of cytoplasm. A starry sky pattern was detectable in the majority of cases, at least in areas with good tissue preservation. A plasmacytic differentiation was not detectable. All lymphomas displayed the conventionally diagnosed BL immunophenotype with expression of CD20 and CD10, high proliferation as measured by the Ki67 index and absence of TdT expression (Table I). Only 1/8 cases (13%) was positive for Epstein-Barr virus (EBV) by EBV-encoded small RNA (EBER) *in situ* hybridization (data not shown). The genetic features were compatible with the diagnosis in conventionally diagnosed BL (Table I). The BCL2-expressing cells were either diffusely dispersed over the whole lymphoma/tumour or clustered in restricted areas while leaving other areas BCL2-negative (Fig 2). In cases with spatially divergent BCL2 expression, the phenotype and the morphology did not differ between BCL2-positive and BCL2-negative areas of the lymphoma (Fig 2). Spatially divergent BCL2 expression occurred in 23% of the BCL2 positive cases.

BCL2-positive conventionally diagnosed BL show a molecular profile of mBL or m-intermediate

In order to understand whether the expression of BCL2 in conventionally diagnosed BL truly is a BL, we performed a gene expression analysis using RNA obtained from FFPE tissues and digital multiplexed gene expression technology, as previously published (Masque-Soler *et al*, 2013). Thirteen of 17 conventionally diagnosed BL (76%) were classified as mBL, confirming the clinicopathological-cytogenetic diagnosis (Table I). In five specimens the BCL2-positive and negative areas of the conventionally diagnosed BL were manually

Table I. Clinical, pathological and genetic features of lymphoma cases analysed for molecular diagnosis.

Case	Gender	Age at diagnosis (years)	St. Jude Stage	Relapse	Tumour localization	CD20	CD10	TdT	Ki67 (%)	BCL2% + in lesion	t(8;14)	BCL2-break	BCL6-break	molecular diagnosis by gene expression
1	m	12	II	no	Ileocoecal	pos	pos	neg	>90	60% weak	pos	n.d.	n.d.	mBL
2	m	16	II	no	Mandible	pos	pos	neg	100	60% weak	pos	n.d.	neg	mBL
3	Unknown	12	III	no	ileocoecal	pos	pos	neg	100	30% strong	pos	n.d.	neg	mBL
4	m	4	III	no	Appendix	pos	pos	neg	>95	50% weak	pos	n.d.	neg	mBL
5	m	11	n.d.	no	Meckel-diverticulum	pos	pos	neg	100	5% weak	pos	n.d.	neg	intermediate
6	m	3	III	no	colon	pos	pos	neg	95	80% strong	pos	neg	neg	intermediate
7	m	14	III	no	abdomen	pos	pos	neg	n.d.	20% weak	n.d.	n.d.	n.d.	mBL
8	m	3	II	no	small intestine	pos	pos	neg	>90	30% weak	pos	neg	neg	mBL
9	m	3	I or II	no	unknown	pos	pos	neg	>90	10% weak	n.d.	n.d.	n.d.	mBL
10	m	16	III	no	soft tissue	pos	pos	neg	>95	50% weak	pos	neg	neg	mBL
11	m	24	n.d.	n.d.	small intestine	pos	pos	neg	100	70% weak	pos	neg	neg	mBL
12	m	22	n.d.	n.d.	cervical lymph node	pos	pos	neg	>95	40% weak	pos	neg	neg	mBL
13	m	16	III	no	ileocoecal	pos	pos	neg	100	40% weak	pos	neg	neg	intermediate
14	unknown	8	IV	no	maxilla	pos	pos	neg	100	20% weak	neg*	n.d.	neg	mBL
15	m	9	II	no	ileum	pos	pos	neg	100	20% weak	pos	neg	neg	mBL
16	m	6	n.d.	n.d.	cervical lymph node	pos	pos	neg	95	40% weak	pos	neg	neg	intermediate
17	m	10	B-ALL	no	liver	pos	pos	neg	100	90% strong	pos	neg	neg	mBL

**MYC*-translocation with an undetermined translocation partner. Pt = patient number, m = male, n.d. = not determined, pos = positive, neg = negative, mBL= molecular Burkitt Lymphoma, intermediate = intermediate between Burkitt and diffuse large B-cell lymphoma. Cases 10, 11, 12, 13, 15, 16 and 17 are also part of the TMA cohort.

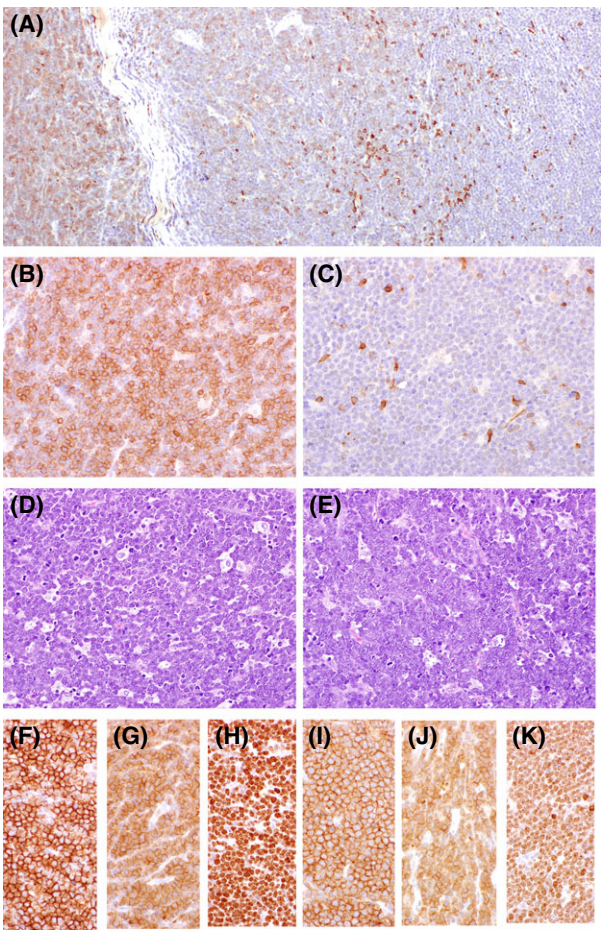

Fig 2. Example of a case of BL with differential spatial expression of BCL2. The specimen was obtained from an ileocecal mass in a 12-year-old male child (Case 1 in Table I). The tumour displays heterogeneous expression of BCL2 (A, overview; B and C high magnification). Both areas with and without BCL2 expression show the typical morphology and immunophenotype of BL. BCL2-positive areas: B, BCL2, D, haematoxylin and eosin, F, CD20, G, CD10, H, Ki67. BCL2-negative areas: C, BCL2, E, haematoxylin and eosin, I, CD20, J, CD10, K, Ki67. Original magnification 100x in A and 400x in B-K.

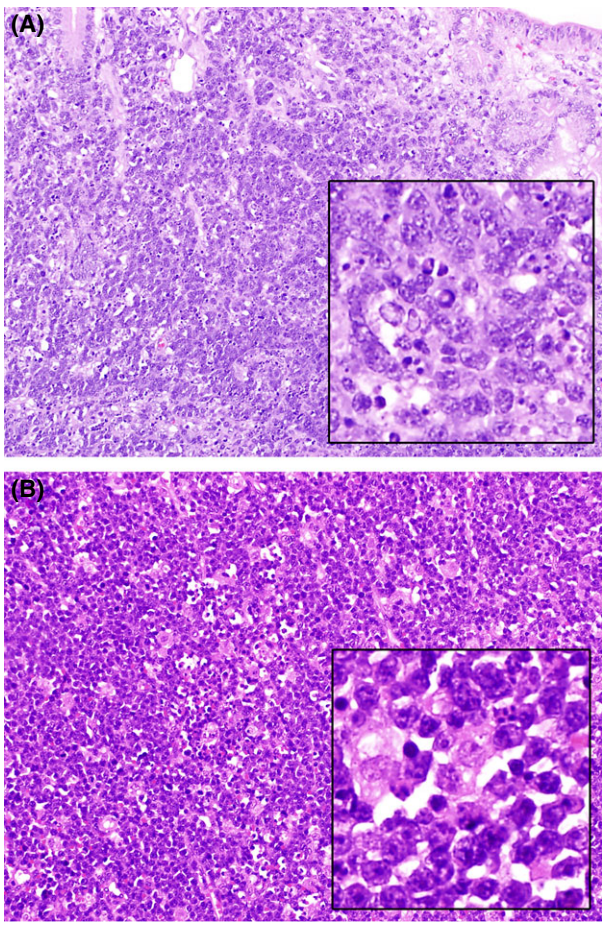

Fig 3. Two lymphomas diagnosed as BL by means of morphology, immunophenotype and genetics that were classified as intermediate BL by gene expression profiling. Panel A corresponds to Case 5 in Table I; panel B corresponds to Case 16 in Table I. All panels are stained with haematoxylin and eosin. Original magnification 200x.

dissected and analysed separately for gene expression. All of these were classified as mBL in both the BCL2-negative and the BCL2-positive areas of the tumour (cases 1, 2, 3, 4 and 11 in Table I).

Interestingly, 4/17 conventionally diagnosed BL (24%) were classified as lymphomas intermediate between mBL and non-mBL (m-intermediate, cases 5, 6, 13 and 16 in Table I, Fig 3). All m-intermediate lymphomas showed typical features of conventionally diagnosed BL. The patients were under the age of 16 and 3 patients presented with an abdominal mass (Table I). Although the morphology of these lymphomas was ambiguous due to poor tissue quality (Fig 3), all lymphomas were positive for t(8;14) and lacked *BCL6* breaks. There were no *BCL2* breaks in two cases with available data. The patients were treated according to the protocols for BL and did not suffer a relapse.

BCL2 expression in paediatric conventionally diagnosed BL is not associated with unfavourable outcome

The co-expression of BCL2 and MYC is associated with poorer outcome in DLBCL. In our cohort of 13 mBL, based on gene expression profiles and available clinical data, the outcome was excellent and none of the patients for whom a molecular diagnosis was available in the current study showed a relapse (Table I). We thus analysed a larger number of conventionally diagnosed BL treated according to protocols of the Berlin-Frankfürt-Münster Non-Hodgkin lymphoma (BFM-NHL) study group. Forty-three patients for whom BCL2 expression status based on whole tissue section staining was available (including three patients that were analysed by gene expression profiling), were registered in the BFM-NHL database, thus providing us with complete clinical data. Seventeen of 43 cases of conventionally diagnosed BL (40%) were reported to show BCL2 expression. There was no major difference in clinical variables between the group with and the group without known BCL2 expression status, with the exception that a lower

number of cases with known BCL2-expression status had bone marrow involvement and elevated lactate dehydrogenase (LDH) levels (Table SII). A comparison of the clinical variables, gender, age, stage, bone marrow involvement, central nervous system involvement, lactate dehydrogenase level, B-symptoms, risk groups and event-free survival did not reveal any difference between 26 BCL2-negative and 17 BCL2-positive cases of conventionally diagnosed BL (Table II).

Discussion

BCL2 is a member of the BCL2 family of proteins, which are involved in a variety of cellular processes and predominantly act in an anti-apoptotic manner (Siddiqui *et al*, 2015). BCL2 is a promising therapeutic target in lymphomas that express the protein (Johnson-Farley *et al*, 2015). In this study we showed that BCL2 is expressed in a considerable number of conventionally diagnosed BL cases. The percentage of BCL2-positive conventionally diagnosed BL cases in the BFM-NHL database (39%) was higher than the percentage of conventionally diagnosed BL reported as positive in our tissue microarray study (23%). The difference might be due to the fact that full slides were analysed for BCL2 expression for the BFM-NHL data, which allows areas of local BCL2 reactivity to be detected that might escape identification using a TMA. Translocations involving *BCL2* as a cause of BCL2 expression were ruled out in our study, confirming previous data (Dave *et al*, 2006; Hummel *et al*, 2006; Klapper *et al*, 2008). Whether other genetic aberrations, such as mutations in the promoter region or epigenetic mechanisms in BCL2-positive subclones of conventionally diagnosed BL, cause the protein's expression has not yet been elucidated. It is worth mentioning that the widely used antibody clone 100/D5 (DAKO, Glostrup, Denmark) is sufficiently sensitive to identify BCL2 expression in conventionally diagnosed BL. In contrast to follicular lymphoma (Adam *et al*, 2013), mutations of BCL2 epitopes do not seem to influence immunoreactivity despite the fact that mutations in *BCL2* occasionally occur in conventionally diagnosed BL (Love *et al*, 2012). It is important to mention that BCL2 staining suffers from a high inter-observer variability (de Jong *et al*, 2007).

Table II. Characteristics BCL2-positive and -negative BL patients followed by the BFM-NHL study group.

Characteristic	BCL2 negative (n = 26)		BCL2 positive (n = 17)		P value (Chi)
Gender					
Male	24	92%	15	88%	
Female	2	8%	2	12%	1·00
Age (years)					
<10	13	50%	9	53%	
10–14	9	35%	3	18%	
>14	4	15%	5	29%	0·39
Stage of disease					
I	1	5%	0	0%	
II	5	24%	5	33%	
III	12	57%	8	53%	
IV	1	5%	0	0%	
B-AL	2	10%	2	13%	0·96
BM involvement					
Yes	2	8%	2	12%	1·00
CNS involvement					
Yes	2	8%	1	6%	1·00
LDH (iu/l)					
<500	15	63%	10	59%	
500–1000	6	25%	3	18%	
>1000	3	13%	4	24%	0·74
B symptoms					
Yes	8	31%	3	18%	0·48
Risk group					
R1	0	0%	0	0%	
R2	13	62%	9	60%	
R3	3	14%	2	13%	
R4	5	24%	4	27%	1·00
Event-free survival	96 ± 4%		82 ± 9%		0·15 LR

B-AL, Burkitt-acute leukaemia; BM, bone marrow; CNS, central nervous system; LDH, lactate dehydrogenase; LR, likelihood ratio.

The expression of BCL2 is associated with poorer survival in DLBCL, especially if co-expressed with MYC protein (Horn *et al*, 2013; Perry *et al*, 2014). As all BL cases express high levels of MYC (Dave *et al*, 2006; Hummel *et al*, 2006), the BCL2-positive BL are MYC and BCL2 co-expressors. Our findings show that, in conventionally diagnosed BL or mBL, co-expression of MYC and BCL2 is not associated with a poorer outcome. Certainly, the differences in therapeutic protocols might explain the different prognostic impact of MYC-BCL2 co-expression. All conventionally diagnosed BL and mBL cases in our study were paediatric patients who received intensive chemotherapy according to paediatric protocols. The DLBCL for which the negative prognostic impact of MYC-BCL2 co-expression has been demonstrated were mainly treated with less aggressive immunochemotherapy using a CHOP [cyclophosphamide, hydroxydaunorubicin (doxorubicin), vincristine, prednisolone]-based regimen (Zhou *et al*, 2014). Whether intensified chemotherapy might overcome the negative prognostic impact of MYC-BCL2 co-expression in DLBCL is still uncertain. It is noteworthy that a recent study of a young, high-risk cohort of DLBCL treated with intensified immunochemotherapy identified biological risk factors that differ from cohorts of elderly patients with DLBCL (Horn *et al*, 2015).

Using our recently developed gene expression-based classifier (Masque-Soler *et al*, 2013), we were able to apply nanostring technology to manually dissected areas of the lymphoma. BCL2-positive and BCL2-negative areas of the same lymphomas were independently and consistently classified as mBL, implying that the expression of BCL2 does not indicate a 'transformation' of BL towards an intermediate lymphoma. To our knowledge, this is the first example of gene expression-based molecular diagnosis in independently analysed areas of a lymphoma. The consistency of the results

confirms the reliability of our multiplex assay and suggests that the molecular diagnosis based on our classifier is not influenced by intratumoural heterogeneity.

We have previously shown that m-intermediates in children do not harbour a genetic 'double hit' constellation and show more 'Burkitt-ness' than adult m-intermediates (Klapper *et al*, 2008; Salaverria & Siebert, 2011). This suggests that the majority of m-intermediates in children and young adults are, in fact, BL, which escape classification as mBL due to minor divergence of gene expression or simply the choice of the statistical cut-off in the current algorithms (Klapper *et al*, 2008). This study again identified four m-intermediates among 17 lymphomas that were classified as conventionally diagnosed BL on the basis of morphology and immunophenotype. However, the clinical, pathological and genetic features of the m-intermediates, such as age, localization, site of the lymphoma, morphology, immunophenotype, cytogenetics and outcome, do not differ from mBL in this cohort of children and young adults. This finding raises questions as to the potential advantage of molecular over conventional diagnosis for mature aggressive B-cell lymphomas in children and adolescents. Obviously, m-intermediates in young patients do not present a clinically or biologically relevant subgroup. As our cohort of molecularly classified lymphomas is rather small, molecular diagnosis based on gene expression profiling needs to be evaluated in larger cohorts of lymphomas in children and young adults to understand its value as a diagnostic and prognostic tool.

Our data suggest that BCL2 expression is a feature detected to a variable extent in a considerable number of cases of BL. It is important to mention, that even a high expression intensity or a high number of BCL2 positive lymphoma cells does not preclude the diagnosis of BL if all other features are compatible with this diagnosis, especially if *BCL2* translocations are absent. BCL2 expression in a lymphoma that clinically, morphologically, immunophenotypically and genetically resembles BL is not sufficient to diagnose an intermediate lymphoma, especially if the BCL2 expression is developed only in a subgroup of lymphoma cells (as was the case in most of the specimens analysed in this study).

In summary, more than 20% of lymphomas with the clinical, morphological, immunophenotypical and genetic features of conventionally diagnosed BL express BCL2. This expression is variable in extent and can occur diffusely or be restricted to certain areas of the lymphoma. BCL2 expression in conventionally diagnosed BL is almost always weaker than in non-neoplastic T-cells. Molecular diagnosis of BCL2-positive conventionally diagnosed BL confirms the diagnosis in the vast majority of cases. The clinical significance of m-intermediates in children is uncertain at the current time. BCL2 expression in conventionally diagnosed BL is not associated with poorer outcome in paediatric patients. Diagnostic pathologists must be aware of the BCL2 expression pattern in conventionally diagnosed BL.

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Author contributions

NMS and MS performed DMGE analysis, CK and RS performed bioinformatic analysis, IN, SMA and RSie analysed genetic data and interpreted results. BB provided clinical data and performed statistical tests. WK designed the research, provided funding and wrote the manuscript. All authors have approved the final version of the manuscript.

Conflict of interest

The authors have no conflict of interest to disclose.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. BCL2 expression for $n = 134$ BL in the TMA cohort for which an interpretable result was available for both BCL2 antibodies used in the study.

Table S2. Patient characteristics of patients with versus patients without known BCL2 status.

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