A comprehensive flow-cytometry-based immunophenotypic characterization of Burkitt-like lymphoma with 11q aberration

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We previously described a subset of *MYC* translocation-negative aggressive B-cell lymphomas resembling Burkitt lymphoma, characterized by proximal gains and distal losses in chromosome 11. In the 2016 WHO classification, these *MYC*-negative lymphomas were recognized as a new provisional entity, 'Burkitt-like lymphoma with 11q aberration'. Here we present an immunophenotype analysis of Burkitt-like lymphomas with 11q aberration. Cells were acquired by fine needle aspiration biopsy from 10 young adult patients, 80% of whom presented recurrence-free 5-year survival. Twenty-three *MYC*-positive Burkitt lymphomas, including three carrying both *MYC* rearrangement and 11q aberration, served as controls. By immunohistochemistry, all Burkitt-like lymphomas with 11q aberration were CD20+/CD10+/BCL6+/BCL2 − /MUM1 − /MYC+/EBV − , usually LMO2 +/CD44 − /CD43 − and sometimes CD56+, and showed high proliferation rate. By flow cytometry, Burkitt-like lymphoma with 11q aberration immunophenotypically resembled *MYC*-positive Burkitt lymphoma, except for significantly (adjusted $P < 0.001$) more frequent CD38^{higher} expression in Burkitt lymphoma (91% *MYC*-positive Burkitt lymphoma *vs* 10% Burkitt-like lymphoma with 11q aberration), more frequently diminished CD45 expression in Burkitt lymphoma (74% *vs* 10%), an exclusive CD16/CD56 and highly restricted CD8 expression in Burkitt-like lymphoma with 11q aberration (60% *vs* 0% and 40% *vs* 4%, respectively). We showed high diagnostic accuracy and effectiveness of flow cytometry in Burkitt lymphoma. CD16/CD56 expression without CD38^{higher} and the lack of CD16/CD56 with CD38^{higher} expression proves to be a reliable, fast, and cost-effective method for diagnosing 11q aberration and *MYC* rearrangements in CD10(+) aggressive lymphomas, respectively. In addition, we confirmed a pattern of an inverted duplication with telomeric loss of 11q, as a recurrent 11q abnormality, but one case presented alternative changes, possibly resulting in an equivalent molecular effect. Our findings reveal similarities along with subtle but essential differences in the immunophenotype of Burkitt-like lymphoma with 11q aberration and *MYC*-positive Burkitt lymphoma, important for the differential diagnosis, but also for understanding the pathogenesis of Burkitt-like lymphoma with 11q aberration.
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We have previously described[1,2] a new entity similar to Burkitt lymphoma with recurrent chromosome 11q aberrations and no detectable *MYC* translocation (*MYC*$^-$). By metaphase analyses, these 11q aberrations mostly present as dup(11)(q23q13),[1] and by interphase FISH and high-resolution copy-number arrays, as proximal gains and telomeric losses in 11q.[2] A similar pattern of 11q aberrations was observed in *MYC*-negative post-transplant Burkitt lymphoma.[3] Remarkably, gene expression analyses of mRNA and miRNA showed major similarities in transcriptional profiles to *MYC*-positive Burkitt lymphoma.[2,4] Consequently, in the updated 2016 WHO classification, these lymphomas were recognized as a provisional entity *MYC*-negative 'Burkitt-like lymphoma with 11q aberration'.[5]

MYC-positive Burkitt lymphoma has typical histopathological and immunohistochemical features (CD20+/CD10+/BCL6+/BCL2 – /MUM1 – /MYC+/CD44 – /CD43+/Ki-67 > 95%) in most cases, enabling differentiation from other, more common aggressive B-cell lymphomas.[6–8] Recently, lack of LMO2 expression by immunohistochemistry was found to be significantly associated with *MYC* translocations in CD10(+) aggressive B-cell lymphomas, including *MYC*-positive Burkitt lymphoma,[9] which is consistent with gene expression profiling studies, that have shown low levels of *LMO2* gene expression in *MYC*-positive Burkitt lymphoma.[8] Rare Burkitt-like lymphoma with 11q aberration have recently been characterized with the use of immunohistochemistry,[1–4] but a detailed flow cytometry analysis of these cases compared to *MYC*-positive Burkitt lymphoma is missing.

In the current study, we present patients with highly aggressive Burkitt-like lymphoma with 11q aberration, characterized by a spectrum of histological features consistent with *MYC*-positive Burkitt lymphoma and a recurrent 11q aberrations, diagnosed before the 2016 update of WHO classification, mostly as *MYC*-negative Burkitt lymphoma and treated according to *MYC*-positive Burkitt lymphoma regimens, at a single institution. We show here that Burkitt-like lymphoma with 11q aberrations is characterized by a distinct flow cytometry and immunohistochemical immunophenotype.

Materials and methods

Patients

Among 82 consecutive adult Burkitt lymphoma patients diagnosed at Maria Sklodowska-Curie Institute—Oncology Center between 2000 and 2014 by the standard histopathology and immunohistochemistry, flow cytometry, conventional cytogenetics, and FISH; 10 cases (12%), 9 male/1 female, median age 27 (18–62) years, HIV/EBV-negative, without bone marrow and cerebrospinal fluid involvement, had no *BCL2*/*BCL6*/*MYC* rearrangements, but displayed a typical 11q aberration pattern. The diagnostic material for cytological smears, flow cytometry, conventional cytogenetics, FISH, and copy-number arrays was obtained by fine needle aspiration biopsy as described in Supplementary Methods. Patients were treated with either modified R-CODOX-M/R-IVAC regimen[10] (Rituximab, fractionated cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate alternating with fractionated ifosfamide, etoposide and high-dose cytarabine, along with intrathecal methotrexate and cytarabine) or GMALL-B-ALL/NHL2002 protocol[11] (Rituximab, fractionated cyclophosphamide (or ifosfamide), vincristine, methotrexate, cytarabine, teniposide, and prednisone or doxorubicin). At the last follow-up, 8 out of 10 Burkitt-like lymphoma with 11q aberration patients were alive (Figure 1). Median follow-up was 54 months (range 0–134), and 5-year Overall Survival was 80% (95% confidence interval (55%,100%)). Patient and tumor characteristics are shown in Table 1. Twenty-three *MYC*-positive Burkitt lymphoma patients with simple karyotypes and *MYC* rearrangement detected by FISH (19 male/4 female, median age 35 (18–63) years, HIV-negative), including three also carrying 11q aberration served as immunophenotype controls. Two out of the three patients with Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration are alive (Figure 1).

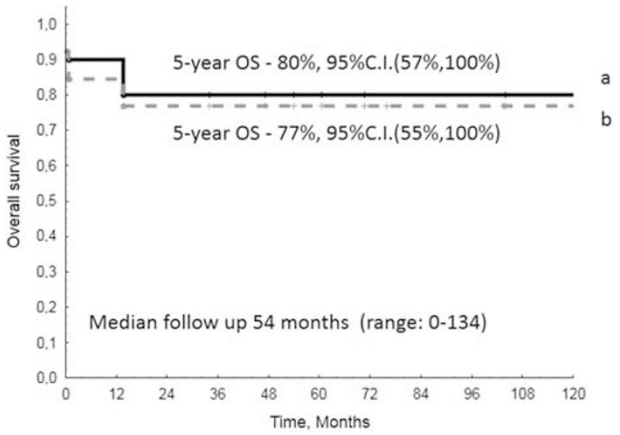


Figure 1 Overall survival from diagnosis, estimated by the Kaplan–Meier method. (**a**) (continuous line) *MYC*$^-$Burkitt-like lymphoma with 11q aberration patients, ($n = 10$); (**b**) (dotted line) All patients with chromosome 11q aberrations: *MYC*$^-$ and *MYC*$^+$ lymphomas ($n = 13$).

Morphological and Immunohistochemical Characterization

Based on histopathology, all 10 patients were morphologically diagnosed with Burkitt lymphoma. To evaluate cytomorphology of lymphoma cells, cytological smears from fine needle aspiration biopsy were used similarly as in histopathological examination.[4] Immunohistochemistry and FISH for the Epstein–Barr virus-encoded small RNA (EBER) were performed on

Table 1 Patient and tumor characteristics

No./A/G	*PS*	*CS*	*B*	*BM/ CNS*	*Bulk*	*Site of involvement*	*LDH > UNV*	*IPI*	*Treatment*	*Response*	*Status/Last FU (mo)*
1/18/M˜	1	I	No	No	No	LNc[1]	No	1	GMALL-B-ALL/NHL2002	CR	ANED (104)
2/25/M˜	2	IV	Yes	No	Yes^	LN/ab[1]	Yes	3	CODOX-M/IVAC	PRpd	DOD (14)
3/37/F˜	1	I	No	No	Yes	LNc[1]	No	0	GMALL-B-ALL/NHL2002	CR	ANED (131)
4/23/M	0	I	No	No	No	T[1]	No	0	GMALL-B-ALL/NHL2002	CR	ANED (54)
5/22/M˜	1	I	No	No	Yes	LNinq[1]	Yes	1	R-CHOP, RT; CODOX-M/IVAC	PRpd; CR	ANED (134)
6/32/M	1	I	No	No	No	T[1]	No	0	GMALL-B-ALL/NHL2002	CR	ANED (34)
8/29/M	0	I	No	No	No	LNc[2]	No	0	R-CODOX-M/R-IVAC, ESHAP	CR	ANED (71)
9/62/M	1	IV	No	No	Yes^	LN/ab[2]	Yes	3	GMALL-B-ALL/NHL2002	CR	ANED (47)
10/40/M	1	IV	Yes	No	Yes^	LN/ab[1]	Yes	2	GMALL-B-ALL/NHL2002	CR	ANED (60)
11/20/M	4	IV	No	No	Yes^	LN/ab[1]	Yes	4	GMALL-B-ALL/NHL2002	CR	TRM, Autopsy (1)
12/20/M	1	I	No	No	No	LNc[1]	Yes	1	GMALL-B-ALL/NHL2002	CR	ANED (103)
13/48/F	2	IV	No	No	Yes	LN/ab[2]	Yes	4	GMALL-B-ALL/NHL2002	PR	TRM, Autopsy (1)
14/25/M	0	I	No	No	No	T[1]	Yes	1	GMALL-B-ALL/NHL2002	CR	ANED (76)

No., case number; A/G, Age (years)/Gender—M, male; F, female; ˜ Patients No. 1, 2, 3, and 5 were previously reported cases, and correspond to cases 1, 2, 3, and 4 in Pienkowska-Grela *et al*,[1] and cases 4, 5, 6 and 7 in Salaverria *et al*,[2] respectively; *Patient No. 7 was withdrawn due to unusual 11q aberration; Patients were HIV/HCV/HBV negative; PS, Performance Status; CS, Ann Arbor Stage of disease; B, B symptoms; BM, Bone Marrow involvement; CNS, Central Nervous System involvement; Bulk, tumor >7 cm in the greatest dimension, ^tumor >20 cm in the greatest dimension; LN, lymph node: c, cervical; ing, inguinal; ab, abdominal presentation of disease; T, tonsil; [1]one enlarged lymph node/tumor, [2]a few neighboring, enlarged lymph nodes/tumors; LDH>UNV, lactate dehydrogenase elevated above the upper normal value; IPI, International Prognostic Index score; GMALL-B-ALL/NHL2002 regimen (GMALL—German Multicenter Adult ALL Study Group), the alternate use of drugs (Rituximab, fractionated cyclophosphamide (or ifosfamide), vincristine, methotrexate, cytarabine, teniposide, and prednisone or doxorubicin (CNS prophylaxis consisted of triple intrathecal methotrexate, cytarabine, and dexamethasone); CODOX-M/IVAC regimen, fractionated cyclophosphamide, vincristine, doxorubicin, and high-dose methotrexate alternating with fractionated ifosfamide, etoposide, and high-dose cytarabine, along with intrathecal methotrexate and cytarabine; R, Rituximab; RT, Radiation therapy; ESHAP, Etoposide, methylprednisone, cytarabine, cysplatin; R-CHOP, Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone; IVAC, fractionated ifosfamide, etoposide, and high-dose cytarabine; PD, Progressive Disease; CR, Complete Remission; PRpd, Partial Remission followed by PD; PR, Partial Remission; ANED, Alive, no evidence of disease; DOD, Died of Disease progression; TRM, Treatment Related Mortality; FU, follow-up (months after the final diagnosis or to death); Horizontal line separates cases into: *MYC*(-) (above) and *MYC*(+) lymphomas (below). CS and IPI score were evaluated by standard criteria.

formalin-fixed, paraffin-embedded tissues, as described by Zajdel *et al*.[4] and in Supplementary Methods. Immunohistochemistry was performed using the EnVision Detection Systems FLEX kit (Dako, Carpinteria, CA, USA, code K 8000) or ultraView Universal DAB Detection kit (Ventana Medical Systems, Tucson, USA, catalog no. 760-500) and, if necessary, antigen-retrieval technique was applied for each monoclonal antibody specific for: CD20, CD10, BCL6, BCL2, LMO2, MYC, MUM1, CD43, CD44, CD56 (two different clones of monoclonal antibodies, mouse and rabbit), Ki-67, cyclin D1, CD3, CD5, and TdT (Supplementary Table 1).

Flow Cytometry Immunophenotyping, Cytology, and Cell Staining

Immunophenotype was also determined by flow cytometry of cellular suspension obtained by fine needle aspiration biopsy or by ultrasound-guided fine needle aspiration biopsy (cases with bulky abdominal mass, stomach, and abdominal lymph node involvement) of the involved lymph nodes, tonsils, and extranodal tumors performed by a hematopathologist. Cells from patients with histopathologically confirmed or suspected of Burkitt lymphoma, were incubated with a panel of monoclonal antibodies[1,4,12] (for staining procedure and a list of antibodies see Supplementary Methods and Supplementary Table 2). Antigen expression (Figures 2 and 3, and Supplementary Table 4) was quantified by FACSCalibur and FACSCanto II cytometers (Becton Dickinson, BD), and was categorized according to the percentages of positive cells into three groups, marked: '(–)' – no expression ($<20\%$ of neoplastic cells), '(+/–)' – expression in $>20\%$ but $<100\%$ of cells, and '(+)' – in 100% of lymphoma cells. A quantitative expression of CD (19/20/22/23/52/79β/81), FMC7, HLA-DR, BCL6, and CD(5/25/38/43/44/45/52/62L/71/200), BCL2, and CD(16/56 and 56) in neoplastic cells was evaluated as median fluorescence intensity value related to the median fluorescence intensity of these antigens on B-, T-, and NK-lymphocytes, respectively (a representative example of CD38 expression is shown in the Figure 2b, A, B). This approach enables to quantify the expression of a given antigen as higher (+)↑ or weaker (+)↓ in Burkitt-like lymphoma with 11q aberration and in Burkitt lymphoma cells than in control lymphocytes, as well as to compare the expression of pan-B antigens in Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma cells (ie, CD19 *vs* CD20 *vs* CD22, using monoclonal antibodies conjugated with the same fluorochrome), as described.[1,4,12] 'Dim' (lower and heterogeneous) or 'bright' (higher and homogeneous) expression was defined as previously.[4,12] Simultaneously, cytological smears were stained with a hematoxylin-eosin and May-Grünwald-Giemsa for morphological evaluation (Figure 2a).

Conventional Cytogenetics and FISH

For conventional cytogenetics, fresh fine needle aspiration biopsy samples were fixed directly or following standard cultures.[1,4,12] Karyotypes were classified according to the ISCN 2016.[13] To study the presence of *MYC*/*BCL2*/*BCL6* translocations (MYC/BCL2/BCL6 break apart probes) and to assess the number of copies, precise positions, possible inversions of *CCND1*/*ATM*/*KMT2A* loci and terminal 11q deletions (CCND1/ATM/KMT2A/D11S1037/CEP11 locus-specific probes), FISH was performed using commercial probes (Vysis Abbott Molecular, Downers, Grove, IL, USA), as previously described.[1,2,4] Representative karyotype and FISH results are shown in Figure 4.

Copy-number Analysis

Copy number and copy-number-neutral loss of heterozygosity were analyzed in Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration cases using CytoSure Haematological Cancer and SNP Array (8×60 k) (Oxford Gene Technology (OGT), Yarnton, UK), as previosuly described.[14] Copy-number data have been deposited at the Gene Expression Omnibus (GEO) database (GSE93002).

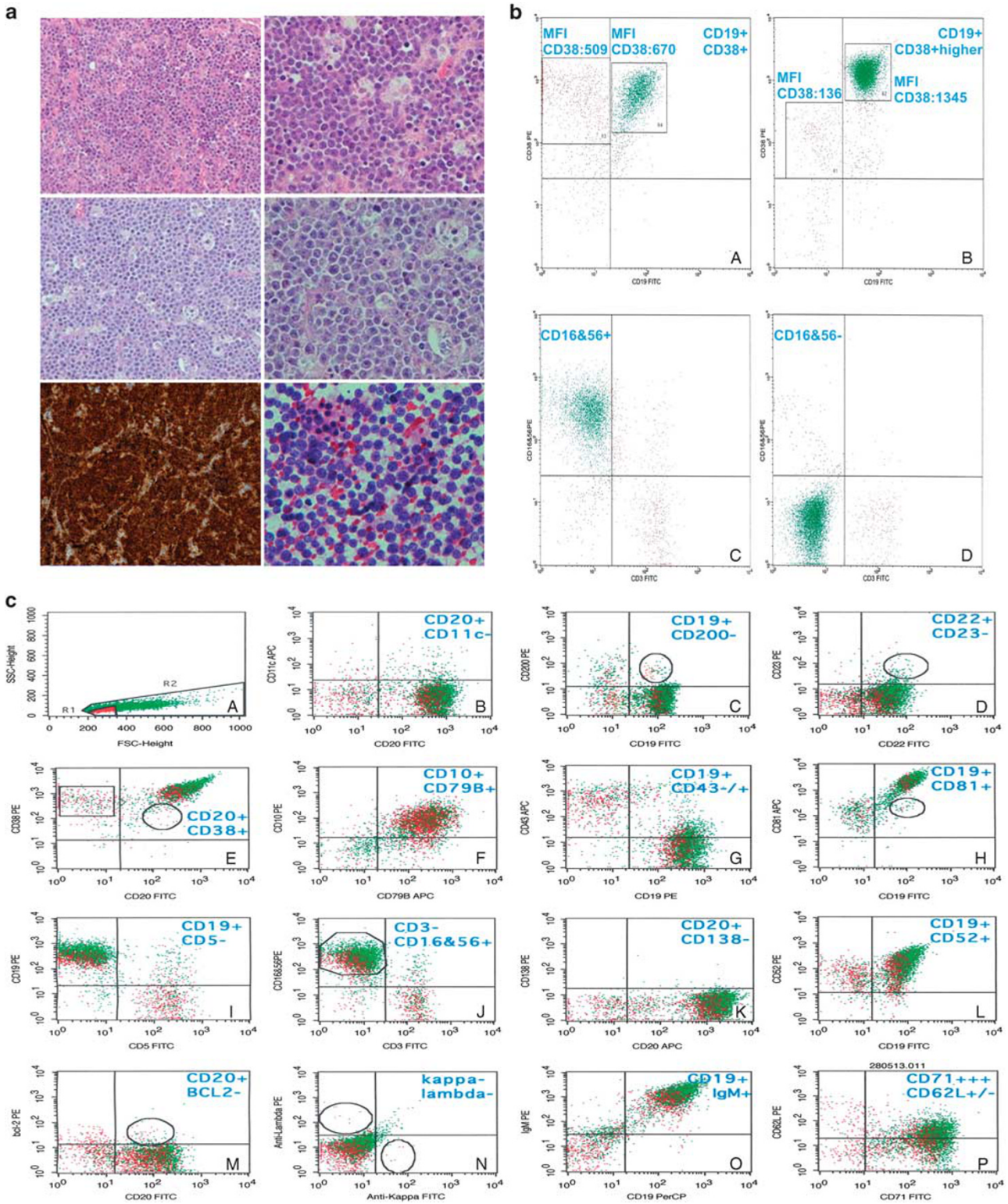
Statistics

Fisher's exact test was used to measure associations between categorical variables using R software (3.2.3 version). *P*-values <0.05, adjusted using the Benjamini–Hochberg method, were considered as statistically significant. Overall Survival was estimated by the Kaplan–Meier method (STATISTICA v.9.1, Statsoft).

Results

Morphologically, all cases of Burkitt-like lymphoma with 11q aberration showed a diffuse lymphoid infiltration, 4 exhibited a classical starry-sky pattern, typical of Burkitt lymphoma (Figure 2a), while the other 6 slightly differed from classical Burkitt lymphoma features by the reduced number of macrophages and apoptotic bodies. In most tumors, the cell size was uniformly medium, with round nuclei and a few small nucleoli, typical of Burkitt lymphoma. However, the lack of a jigsaw puzzle effect of cytoplasmic borders and a mild degree of irregular nuclear contours were noted in some tumors (Supplementary Table 3).

The immunohistochemistry of Burkitt-like lymphoma with 11q aberration was characteristic of Burkitt lymphoma. All 10 cases expressed a homogeneous phenotype of germinal center origin (CD10 +/BCL6+/MUM1 – /usually CD44 –), with a high Ki-67 proliferation index, always $>95\%$ (100% and



>95% in 8 and 2 cases, respectively) and were negative for BCL2/MUM1/cyclin D1/CD3/CD5/TdT/EBER expression. By immunohistochemistry, these tumors expressed LMO2/CD43/CD44 in 70%/30%/20% of cases, respectively (Supplementary Table 3). The two examined cases with Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration were negative for LMO2. Independent of *MYC* status at gene level, the percentage of MYC-expressing cells (monoclonal antibody clone Y69) exceeded the 2016 WHO threshold of 40% of immunohistochemical positivity.[5] A strong CD56

Figure 2 Pathomorphological diagnosis of Burkitt-like lymphoma with 11q aberration. (**a**) Histopathological, immunohistochemical, and cytopathological features of Burkitt-like lymphoma with 11q aberration. Classical Burkitt lymphoma histopathology of Burkitt-like lymphoma with 11q aberration cases no.1 (upper row) and 9 (middle row), a cytopathology (obtained by fine needle aspiration biopsy of abdominal tumor) of case 9, and CD56 positive immunohistochemical reaction of case no.8 (lower row). Diffuse growth is composed of medium-sized lymphoid cells showing jigsaw puzzle effect of cytoplasmic borders with a starry pattern due to admixed phagocytic macrophages. The nuclei are similar in size and shape (paraffin section stained with hematoxylin and eosin). Upper and intermediate panels, original magnification ×200 (left panel) and ×400 (right panel); in cytopathological smears and immunohistochemical reaction, original magnification ×400 (lower row); (**b**) flow-cytometry-based analysis of median fluorescence intensity of CD38 expression (A, B) and CD16/CD56 (C, D) in Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma. Median fluorescence intensity (MFI) of CD38 expression on Burkitt-like lymphoma with 11q aberrations is similar to normal T lymphocyte expression – CD38(+) (plot A), and in Burkitt lymphoma it is higher – CD38(+)higher (plot B). The absence of CD38higher (plot A) and CD16/CD56+ (plot C) characterized Burkitt-like lymphoma with 11q aberration. CD38higher (plot B) and the lack of CD16/CD56 (plot D) characterized Burkitt lymphoma; (**c**) fine needle aspiration biopsy/flow cytometry analysis of Burkitt-like lymphoma with 11q aberration (case 9). Forward scatter/side scatter dot plots presenting both small normal T/B-lymphocytes (red cells) and larger lymphoma cells (green cells). Burkitt-like lymphoma with 11q aberration express: CD20/CD19/CD22 (median fluorescence intensity CD20 > CD19 > CD22)/CD38/CD10/CD79β/CD81higher/CD52/IgM/CD16/CD56/CD71 as well as CD43/CD62L of weak intensity, on a small subpopulation of cells, and are negative for CD5/CD11c/CD23/CD138/CD200/BCL2/κ and λ. Plots E and J show a similar level of CD38 expression in comparison to normal T-lymphocytes CD38 (in the box) and CD16/CD56+ (in the box) on 'Burkitt-like lymphoma with 11q aberration' cells. Antigen expression of a small number of normal B-lymphocytes is marked by circles.

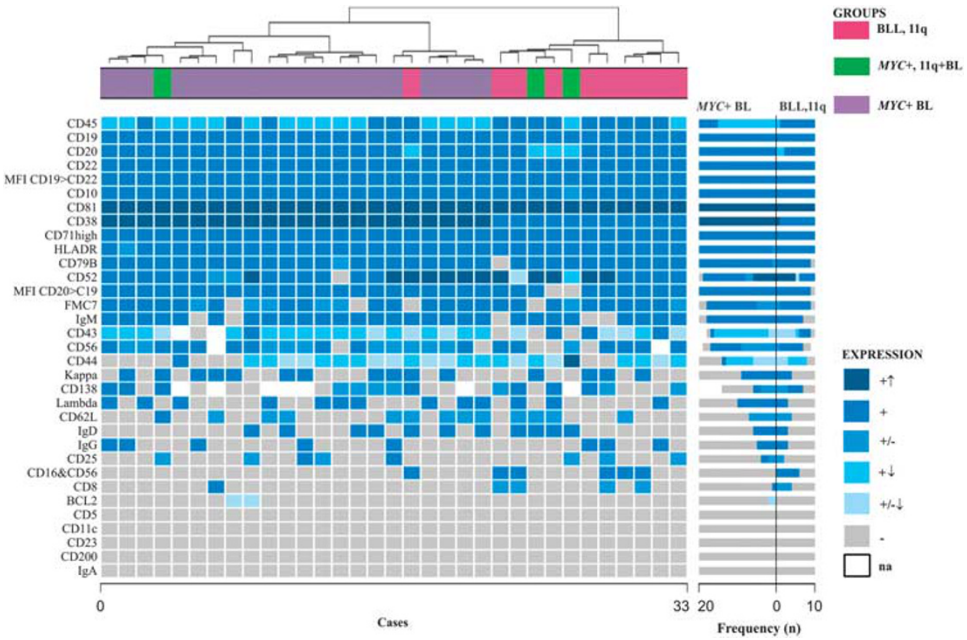


Figure 3 Cluster (variables and cases) analysis of 10 Burkitt-like lymphomas with 11q aberration (BLL, 11q), *vs* 23 *MYC*-positive Burkitt lymphomas (*MYC*$^+$BL), including three cases with an additional 11q aberration (*MYC*$^+$, 11q$^+$BL). Expression: (+)↑, an antigen with higher expression in Burkitt-like lymphoma with 11q aberration cells compared to normal B/T/NK-lymphocytes in 100% of cells; (+), positive in 100% of cells; (+)↓, an antigen with weaker expression in Burkitt-like lymphoma with 11q aberration cells compared to normal B/T/NK-lymphocytes in 100% of cells; (+/–), positive in >20% to <100% of cells; (+/–)↓, an antigen with weaker expression in Burkitt-like lymphoma with 11q aberration cells compared to normal B/T/NK-lymphocytes in >20% to <100% of cells; (–), no expression (i.e. expression in <20% cells); nd, not done.

staining was found in 30% and 40% of cases of Burkitt-like lymphoma with 11q aberration, using mouse and rabbit monoclonal antibodies, respectively.

In the majority of Burkitt-like lymphomas with 11q aberration, flow cytometry analysis showed >85% of lymphoma cells and few normal T-lymphocytes. Forward and side scatter dot-plot showed two distinct cell clusters, small T/B-lymphocytes and slightly larger, homogenous neoplastic cells (Figure 2c). All cases were CD45/CD19/CD20/CD22/FMC7/CD81^{higher}/BCL6/CD10/CD52/HLA-DR-positive with median fluorescence intensity of CD19 higher than that of CD22, while negative for CD5/CD11c/CD23/CD200/BCL2. The median fluorescence intensity of CD20 expression was higher than that of CD19 in 90% of Burkitt-like lymphomas with 11q aberration and 100% of *MYC*-positive Burkitt lymphoma cases. The diminished expression of CD45 was less frequent in Burkitt-like lymphoma with 11q aberration than in *MYC*-positive Burkitt lymphoma (10% *vs* 74%, adjusted $P = 0.0077$), while CD38^{higher} expression[4,15–17] (CD38, PE-conjugated HB7 clone, Figure 2b, A, B) was more frequent in *MYC*-positive Burkitt lymphoma than in Burkitt-like lymphoma with 11q aberration (91% *vs* 10%, adjusted $P < 0.001$). Two out of 3 cases of Burkitt lymphoma carrying both *MYC* rearrangement and

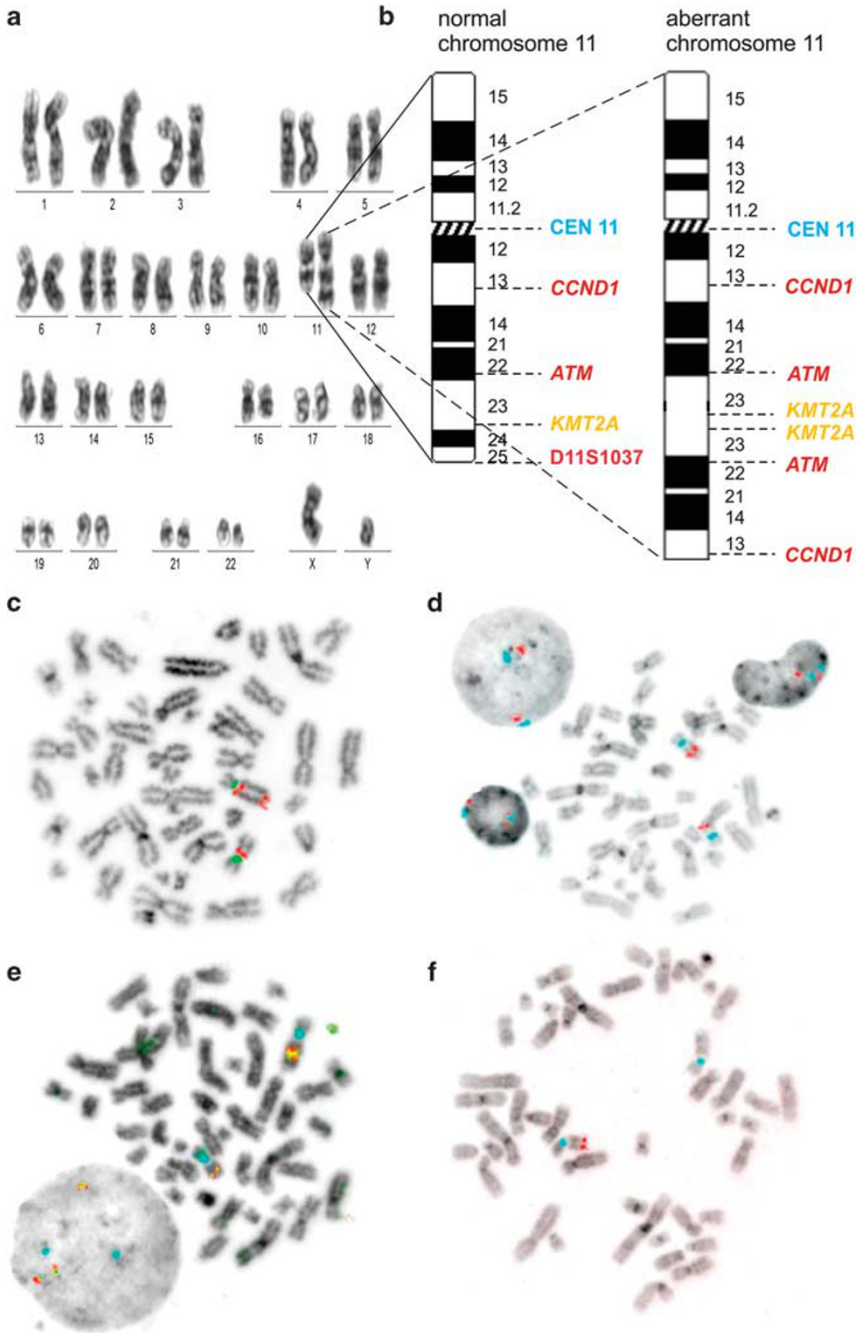


Figure 4 Conventional cytogenetic diagnosis of Burkitt-like lymphomas with 11q aberration. (**a**) Simple karyotype with 11q aberration, 46, XY,dup(11)(q23.3q13.1), as a sole abnormality in case no.1; (**b**) FISH probe positions on normal chromosome 11 and on aberrant chromosome 11, with duplication and inversion of 11q, which explains the results shown on the subsequent FISH analysis; (**c–f**) FISH analysis with chromosome 11 specific probes on metaphases. Each metaphase shows one normal copy of chromosome 11 and one copy of 11q aberration; (**c**) CEP-11 Spectrum Green, CCND1 Spectrum Orange. Big distance between two red signals in 11q aberration indicates an inversion of duplicated region (case no.4); (**d**) CEP-11 Spectrum Aqua, ATM Spectrum Orange. Small distance between two red signals in 11q aberration designates an inversion of the duplicated region. A similar pattern of signals is visible in interphase nucleus (case no.9); (**e**) CEP-11 Spectrum Aqua, KMT2A BAP. Colocalization and multiplication of red-green signals point to 11q inversion with multiplication of the region harboring *KMT2A*. A similar signal pattern is visible in interphase nucleus (case no.9); (**f**) CEP-11 Spectrum Aqua, D11S1037 Spectrum Orange. One red 11q telomeric signal indicates normal chromosome 11, no red signal in 11q aberration confirms terminal deletion (case no.4).

11q aberration also showed $CD38^{higher}$ expression. Other markers in Burkitt-like lymphoma with 11q aberration were expressed as follows: CD79β (90%), CD43 (90%), CD44 (80%, but expression in each case was always weaker than on T lymphocytes), CD56 (78%), CD16/CD56(60%), CD138(70%), CD62L (40%, but usually as a weak expression in slightly over 20% of tumor cells, Figure 2c, P), CD25(20%)

and CD8^{weak}(40%), with a diverse intensity (Figure 3, Supplementary Table 4). The expression of all those antigens was similar in *MYC*-positive Burkitt lymphoma and Burkitt-like lymphoma with 11q aberration, except for CD16/CD56 (containing PE-labeled CD16, clone B73.17-9, and PE-labeled CD56, clone MY31,10, Figure 2b, C, D) and CD8 that were not expressed on *MYC*-positive Burkitt lymphoma (0% *vs* 60% cases, adjusted $P=0.0019$, and 4% *vs* 40% cases, adjusted $P=0.018$, respectively), as well as CD43, expressed in all (+,+↓) Burkitt lymphoma cells (71.4% *vs* 40% cases, adjusted $P=0.0178$). In addition, CD71 (+++) expression, representing proliferative activity, was always detected in 100% of cells in both Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma. Monoclonal light chain expression of moderate intensity was found in 90%, and no κ/λ expression in 10% of cases. IgH (monoclonal heavy immunoglobulin chain) expression included: IgD (+)/IgM(+) in 30%, IgM(+)/IgG(+) in 10%, IgG(+) in 20%, IgM(+) in 30%, and IgH(−) in 10% of cases. Detailed flow cytometry characteristics of Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma are shown in Supplementary Tables 4 and 5, respectively.

Copy-number analysis was performed, if fresh material was available, ie, in nine cases of Burkitt-like lymphoma with 11q aberration (Figure 5a) and two of Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration (Figures 5a and b). Both groups showed similar levels of genetic complexity with the mean number of 5.5 alterations per case, as previously described,[2] and usually a simple (Figure 4a) or low complex karyotype. Nine out of 10 cases of Burkitt-like lymphoma with 11q aberration showed a recurrent pattern of gain/loss (Figures 5a and b), consistent with the copy-number profile of cases 1–3 previously analyzed by Agilent 244k array.[2] In case 10, a 774 Kb homozygous deletion at 11q24.3 was detected. This alteration targeted *ETS1, FLI1, KCNJ1, KCNJ5, C11orf45* and *TP53AIP1* (Figure 5b). In case 8, 11q duplication was not accompanied by a terminal deletion, but the analysis of single-nucleotide polymorphisms revealed a stretch of copy-number neutral loss of heterozygosity at 11q24.1-q25 (Figure 5c). As shown by FISH, the duplicated area in all cases comprised an inversion of the fragment that was gained, with a terminal deletion of 11q (Figures 4b–f and 5a).

Discussion

We characterized 10 cases of Burkitt-like lymphoma with 11q aberration identified among adult BL patients (10 out of 82, 12%), originally diagnosed and treated as *MYC*-negative Burkitt lymphoma at a single institution. Salaverria *et al*,[2] in a series including both children and adults, identified 3% of all molecularly defined Burkitt lymphoma to be Burkitt-like lymphoma with 11q aberration.

Burkitt-like lymphoma with 11q aberration shows a number of clinicopathological and molecular similarities to *MYC*-positive Burkitt lymphoma.[1–4,18] Typical of Burkitt-like lymphoma with 11q aberration, as we have shown here and previously,[1,2,4] was one peak incidence in HIV/EBV-negative young adult males. The typical presentation, predominantly nodal/tonsilar, was a single bulky lymph node/tumor, or less frequently, with a few contiguous lymph nodes/tumors involved, without bone marrow and central nervous system involvement, while in the Salaverria series,[2] there were children with bone marrow involvement. This may be due to lack or very weak expression of CD62L (L-selectin) on a small subpopulation of cells of adult Burkitt-like lymphoma with 11q aberration. CD62L is a cell adhesion molecule, playing an important role in lymphoma dissemination.[19]

A cohort of patients with Burkitt-like lymphoma with 11q aberration had a similar relapse-free outcome to that in patients with *MYC*-positive Burkitt lymphoma, if treated with Burkitt lymphoma-directed regimen,[10,11,20] in contrast to patients with Burkitt-like lymphoma with 11q aberration treated with R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), who tend to relapse,[21] like one of our patients, originally misdiagnosed as diffuse large B-cell lymphoma and treated accordingly at another hospital. By now, 10 patients presented here achieved a sustained complete remission, and 8 are still alive. In the above-mentioned retrospective study by Sevilla *et al*,[21] adults with *MYC*-positive Burkitt lymphoma had longer survival than patients with *MYC*-negative Burkitt lymphoma, but were treated according to *MYC* status, and most of the adult *MYC*$^-$ patients (that could include the currently recognized Burkitt-like lymphoma with 11q aberration entity) received the designed for diffuse large B-cell lymphoma regimens, that seem suboptimal to cure Burkitt-like lymphoma with 11q aberration. Five-year overall survival of patients in our series of Burkitt-like lymphoma with 11q aberration was 80%, similar to that of adult *MYC*-positive Burkitt lymphoma.[20] Nevertheless, after initial excess of non-relapse mortality, the survival curves of the patients with Burkitt-like lymphoma with 11q aberration reached a plateau, which is typical of *MYC*-positive Burkitt lymphoma.[10,11,20]

Tumors of Burkitt-like lymphoma with 11q aberration may have diverse morphology, mainly of Burkitt lymphoma, sometimes of B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, and sporadically of diffuse large B-cell lymphoma.[4,5] As we show here, Burkitt-like lymphoma with 11q aberration have immunophenotype characteristics by immunohistochemistry similar to *MYC*-positive Burkitt lymphoma, with the exception of CD43,

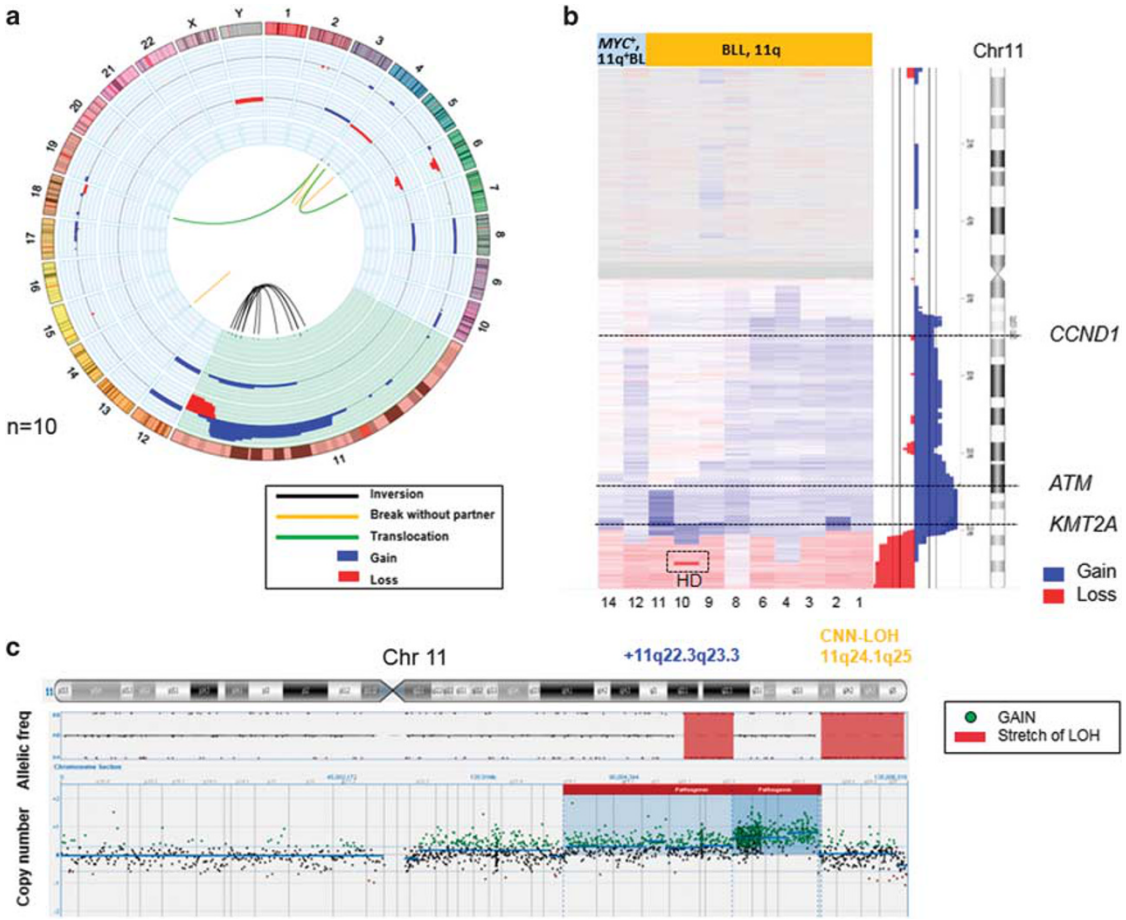


Figure 5 Molecular and cytogenetic analysis of Burkitt-like lymphomas with 11q aberration. (**a**) Circular representation of copy number and structural variants in 10 Burkitt-like lymphoma with 11q aberration cases. Chromosomes are represented in the outer layer, the percentage of regions of loss (red) and gain (blue) are shown in the inner layers (outer layer, CN array data, $n=9$, and inner layer, karyotype data, $n=10$). The internal arcs represent interchromosomal translocations detected by conventional cytogenetics and are marked black (inversion), green (translocations) and orange (break without partner); (**b**) a view of chromosome 11 analyzed by copy-number array (8 × 60 k) in 9 Burkitt-like lymphomas with 11q aberration (BLL, 11q) and 2 cases of Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration (*MYC*$^+$, 11q$^+$BL); HD, a 774 Kb homozygous deletion at 11q24.3 in case 10; (**c**) 11q aberration pattern in case 8, dot plots present large 11q duplication with amplification region, without terminal deletion. In the allelic frequency diagram, the dark red block indicates a stretch of copy-number neutral loss of heterozygosity at 11q24.1q25.

LMO2, and CD56 expression. In this study, LMO2 was expressed in 70% of cases of Burkitt-like lymphoma with 11q aberration and in none of Burkitt lymphoma carrying both *MYC* rearrangement and 11q aberration. Colomo *et al*,[9] in a series including molecularly defined BL, identified 45/46 (98%) *MYC*-positive Burkitt lymphoma cases as LMO2 negative. Taken together, these findings confirm that LMO2 expression may be a good predictor of *MYC* translocation, and may contribute to differential diagnosis of Burkitt lymphoma. The lack of LMO2 expression is typical of *MYC*-positive Burkitt lymphoma and LMO2 is usually expressed in Burkitt-like lymphoma with 11q aberration.

We have previously described flow cytometry immunophenotype of Burkitt lymphoma.[18,20,22] In the current study, antigen expression patterns in all Burkitt-like lymphomas with 11q aberration are similar to that of Burkitt lymphoma, but with some statistically significant differences. First, we found CD16/CD56 expression in 60% of Burkitt-like lymphomas with 11q aberration and in none of Burkitt lymphomas carrying both *MYC* rearrangement and 11q aberration, or *MYC*-positive Burkitt lymphoma. CD16/CD56 is an indicator of NK differentiation, but CD16/CD56 antibody is not currently available for immunohistochemistry. There are two types of CD16, CD16A, which is a transmembrane protein found on NK cells, and CD16B found on neutrophils. Most CD56^{bright} NK cells in the peripheral blood express little to no CD16A. In contrast, the majority of CD56^{dim} cells uniformly express high levels of CD16A,[23] detectable by CD16/CD56 antibody. By flow cytometry, we identified CD56 expression in 78% of Burkitt-like lymphomas with 11q aberration and 89% of *MYC*-positive Burkitt lymphomas. Interestingly, a proportion of Burkitt-like lymphomas with 11q aberration, but none of Burkitt lymphomas, also expressed neural cell adhesion molecule (CD56) by immunohistochemistry, which indicates that it

would be desirable to include immunohistochemistry for CD56 along with LMO2,[9] in the extended diagnostic panel for Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma. CD56 expression is an unusual feature in B-cell lymphomas, with 0.5 to 5.5% expression rate.[24] Therefore, commercially available CD16/CD56 antibody mixture for flow cytometry (containing PE-labeled CD56, clone MY31,10, which seems to be specific for 11q aberration—data not shown) currently presents the best sensitivity for the detection of 11q aberration. Second, we found a significant difference in CD45 expression between Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma, with a decreased expression of this antigen in Burkitt lymphoma. Of note, CD16A (*FCGR3A*) and CD45 (*PTPRC*) loci, the 1q23 and 1q31.3-q32.1, are typically gained in *MYC*-positive Burkitt lymphoma,[25] whereas *CD56* (*NCAM1*) lies at 11q23, the region where duplication 11q takes place in most cases of Burkitt-like lymphoma with 11q aberration.[1,2] It cannot be excluded that differences in CD16/CD56 and CD45 expression between Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma result from duplication of the above-mentioned regions. As shown by CD71 expression in flow cytometry and by Ki-67 index in immunohistochemistry, all cells of Burkitt-like lymphoma with 11q aberration presented high proliferative activity, typical of Burkitt lymphoma.[26] Notably, there is another ontogenetic link between Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma, i.e. heavy immunoglobulin chain cell surface expression pattern that in Burkitt-like lymphoma with 11q aberration is most commonly IgD(+)/IgM(+), followed by IgM(+), while in Burkitt lymphoma most frequent IgM(+) is followed by IgD (+)/IgM(+). Third, we demonstrated the absence of $CD38^{higher}$ expression in Burkitt-like lymphoma with 11q aberration (where CD38 expression comparable to that on T lymphocytes was seen), while $CD38^{higher}$ was typical of Burkitt lymphoma. Therefore, detection of $CD16/CD56^{+}$ and the absence of $CD38^{higher}$ expression by means of fine needle aspiration biopsy/flow cytometry procedure, completed within 1.5 h, appears to be a reliable, fast, easy, and cost-effective method for diagnosing 11q aberration. Importantly, we show that flow cytometric $CD38^{higher}$ expression, consistently present in *MYC*-positive lymphomas,[4,12,15–17,22,27] is a more reliable and quickly detectable indicator of *MYC* translocation in CD10(+) aggressive lymphomas,[15,16] compared to MYC immunohistochemical staining, since MYC expression is highly variable, and not always correlates with *MYC* rearrangements.[3,5,28] Moreover, a recent study that compared LMO2 with MYC protein staining by immunohistochemistry showed that the lack of LMO2 expression is a better surrogate of *MYC* translocation status than the immunohistochemical MYC expression, indicating an advantage for LMO2 immunohistochemistry, particularly in the CD10(+) subgroup. [9]

Compared to histopathology and immunohistochemistry, flow cytometry allows for a closer examination of tumor heterogeneity and identification of subtle differences with regard to antigen expression. Also, fine needle aspiration biopsy proved to be highly efficient way of obtaining tumor cells for karyotype, FISH, and molecular studies. Characteristic 11q aberrations, predominantly dup (11)(q23q13), is visualized by karyotyping. Based on our experience with routine histopathology and immunohistochemical examination performed simultaneously with fine needle aspiration biopsy/flow cytometry/conventional cytogenetics/FISH, we postulate that in specialized hematology–oncology centers, combination of these two diagnostic

Table 2 Flow cytometry/immunohistochemistry-based approach to differential diagnosis of Burkitt lymphoma *vs* Burkitt-like lymphoma with 11q aberration

Burkitt lymphoma	*Burkitt-like lymphoma with 11q aberration*
Antigen expression by flow cytometry in fine needle aspiration biopsy samples	
CD45(+)^{weaker}/CD38(+)^{higher}/ CD16/CD56(–) /CD8(–) /CD43(+) or* CD43(+/–),	**CD45(+)^{bright}/CD38(+)/CD16/CD56(+) or* CD16/CD56(–) /CD8(+) or* CD8(–)/ CD43(+/–) or* CD43(+) or* CD43(–),**
MFI_CD20(+)^{bright} >MFI_CD19(+)$^{brigh\ t}$> MFI_CD22(+),	MFI_CD20(+)^{bright} >MFI_CD19(+)^{bright} > MFI_CD22(+),
CD10(+)/BCL6(+)/CD81(+)^{higher}/BCL2(–),	CD10(+)/BCL6(+)/CD81(+)^{higher}/BCL2(–),
CD44(+↓) or* CD44(+/–) or* CD44(–),	CD44(+↓) or* CD44(+/–) or* CD44(–),
CD56(+/–) or* CD56(+) or* CD56(–)/ CD138(–) or* CD138(+/–) or* CD138(+),	CD56(+) or* CD56(–) or* CD56(+/–)/ CD138(+) or* CD138(+/–) or* CD138(–),
CD79β(+)/FMC7(+) or* FMC7(+/–) or* FMC7(–)/λ(+) or* κ(+),	CD79β(+)/FMC7(+)/κ(+) or* λ(+),
IgM(+) or* IgM(+)/IgD(+) or* IgM(+)/IgG(+) or* IgG(+) or* IgH(–),	IgM(+)/IgD(+) or* IgM(+) or* IgG(+) or* IgM(+)/IgG(+) or* IgH(–),
CD5(–)/ CD11c(–)/CD23/(–)/CD25(–) or* CD25(+/–)/CD62L(–) or* CD62L(+/–)/ HLADR(+)/CD200(–) CD52(+),	CD5(–)/CD11c(–)/CD23(–)/CD25(–) or* CD25(+/–)/CD62L(–) or* CD62L(+/–)/ HLADR(+)/ CD200(–) CD52(+),
100% of cells positive for CD71(+++).	100% of cells positive for CD71(+++)
Antigen reaction by immunohistochemistry	
LMO2(–)^/CD56(–)/CD43(+)^	LMO2(+)^/CD56(+/–)#/CD43(+/–)#

Bold letters mark significant statistical differences between Burkitt-like lymphoma with 11q aberration and typical *MYC*-positive Burkitt lymphoma, which may be useful in the differential diagnosis; *alternative expressions are ordered from more to less frequent; MFI: median flourescence intensity; immunohistochemical staining: ^usually negative or positive; (+/–)#sometimes positive.

approaches is a reliable method for credible diagnosis of Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma. Considering these results, as well as previous data,[9,18,20,22] we propose a practical flow cytometry and immunohistochemistry-based approach to the diagnosis of Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma, as summarized in Table 2.

In the current study, we confirm a pattern of an inverted duplication of a part of the long arm of chromosome 11[1] with mono- or biallelic telomeric loss of 11q,[2,4] which we have previously referred to as a critical set of 11q aberrations,[18] as a recurrent 11q abnormality. One case that presented no 11q deletion detected by single-nucleotide polymorphism analysis/array-comparative genomic hybridization and FISH showed a uniparental disomy in the critical 11q24.1q25 deletion region. We find the telomeric stretch of copy-number neutral loss of heterozygosity (often referred to as uniparental disomy) in this case, to be an alternative genetic event to the terminal deletion, possibly resulting in an equivalent molecular effect. Inverted duplication of 11q is not included in the latest update of 2016 WHO classification,[5] still we found the inversion in all cases with Burkitt-like lymphoma with 11q aberration.

In conclusion, we show significant differences between Burkitt-like lymphoma with 11q aberration and *MYC*-positive Burkitt lymphoma in the expression of CD16/CD56/CD38/CD45/CD8/CD43 by flow cytometry and CD43/LMO2/CD56 by immunohistochemistry, that may contribute to the differential diagnosis between Burkitt-like lymphoma with 11q aberration and *MYC*-positive Burkitt lymphoma, and may help to reveal the pathogenetic mechanisms differentiating these entities. Flow-cytometry immunophenotype of Burkitt-like lymphoma with 11q aberration also presents similarities to that of *MYC*-positive Burkitt lymphoma, as is mRNA and miRNA expression profiles. Given the *MYC*-related genetic differences between these Burkitt lymphoma subtypes, probably correlated to the observed subtle but essential phenotype diversity, the similarities observed on various expression levels might suggest their common cell-of-origin following different mutational pathways. The question of mutational pathways in Burkitt-like lymphoma with 11q aberration will be the subject of our another paper (Wagener *et al*, in preparation). However, cases of lymphomas with 11q aberrations that simultaneously presented *MYC* rearrangements suggest a possible link between mutational pathways in Burkitt-like lymphoma with 11q aberration and Burkitt lymphoma.

Finally, to identify cases with a high probability of 11q aberration and *MYC* translocation, we suggest strategies based on an initial CD16/CD56 and CD38 flow cytometry immunophenotypic analysis and LMO2/CD56 immunohistochemical reaction, which may be very useful in the routine diagnostics of CD10(+) aggressive B-cell lymphomas.

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Author contributions

GR was the principal investigator, diagnosed cases by histopathology and immunohistochemistry, and flow cytometry, performed fine needle aspiration biopsy, drafted the manuscript, reviewed literature; BG, KB, MZ, ZB, RW, PS, and JR did the laboratory work for this study; ZB, FN, and DMG helped with presentation of the results; GR, IS, and RS evaluated and interpreted the results; KD-C, JR-J, and JW treated the pts; GR, MCh, MP-S BP-G, JW, JKS, IS, and RS, contributed to finalizing the manuscript. MCh and GR were heavily involved in writing and editing of the manuscript.

Disclosure/conflict of interest

The authors declare no conflict of interest.

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