

# Immunophenotypic analysis of childhood Burkitt's lymphoma in the West Midlands 1957-1986

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## Abstract

**Aims**—To analyse the immunophenotype of a large number of non-endemic Burkitt's lymphomas to determine whether a B cell phenotype is consistently recognisable using formalin fixed, paraffin wax embedded archival material and a standard panel of commercially available antibodies.

**Methods**—Archival material was obtained from 30 cases of childhood Burkitt's lymphoma registered with the West Midlands Regional Children's Tumour Research Group. These were analysed by a standard avidin biotin complex immunoperoxidase method using antibodies to CD45, CD43, CD30, CD20, CD15, and immunoglobulin heavy and light chains.

**Results**—There was a high incidence of the CD45RB and CD20 immunophenotypes, with a clearly recognisable B cell lineage even in archival material. IgM was identifiable in 13 of the 23 (56.5%) cases tested. Only three of 17 (18%) cases expressed CD30. Positive membrane staining with CD45RO was observed in two (6.7%) cases.

**Conclusions**—A B cell lineage can be identified in Burkitt's lymphoma in formalin fixed, paraffin wax embedded material, even in archival tissue. There was a low incidence of membrane staining with CD45RO which is a potential source of diagnostic confusion.

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**Keywords:** Burkitt's lymphoma, immunophenotypic analysis, B cell phenotype.

Burkitt's lymphoma was first described in Central Africa in 1958.<sup>1</sup> Patients presented at a median age of 17 years with tumours principally affecting the jaw and abdomen. Since then, similar tumours have been described in Europe,<sup>2,3</sup> North America,<sup>4</sup> the Middle East,<sup>5</sup> and North Africa.<sup>6</sup> Burkitt's lymphoma cells typically have round nuclei with three to five small basophilic nucleoli. A starry-sky pattern of tingible body macrophages amid sheets of tumour cells is also a characteristic feature. Tumours with an identical mode of presentation but with differing nuclear morphology with more pleomorphic nuclei and large, often central, nucleoli have been labelled Burkitt-like or non-Burkitt's lymphoma. The relevance of this distinction has been disputed<sup>7-14</sup> and for the purpose of this study, both Burkitt's and Burkitt-like (non-Burkitt's) lymphomas were analysed as a single group.

Immunophenotypic analysis of Burkitt's lymphoma has previously been carried out on either cell suspensions, cryostat sections, or using a limited antibody panel,<sup>7 10 15-20</sup> although more recent analyses have been performed on paraffin wax sections using a larger antibody series.<sup>21-26</sup> Most of these studies have indicated that a B cell phenotype is present; childhood lymphomas, however, have not been as exhaustively studied, although there has been one study on endemic cases.<sup>27</sup> Another study reported two Burkitt-like lymphomas with possible T cell phenotypes.<sup>28</sup> A further study<sup>19</sup> found a high incidence of the OKT10 marker in otherwise typical cases of Burkitt's lymphoma which also expressed immunoglobulin.

## Methods

The West Midlands Regional Health Authority has a child population of roughly 1.17 million. Details of all cases of childhood malignancy diagnosed within this population since 1957 have been obtained by the West Midlands Regional Children's Tumour Research Group. Clinical data have been extracted from the patients' clinical records. The original biopsy specimen and any subsequent necropsy material were obtained. An ongoing review of all cases of Hodgkin's and non-Hodgkin's lymphoma registered by this group produced 30 cases of Burkitt-like and Burkitt's lymphoma.

Unstained paraffin wax sections were obtained either from the referring institution or from the files of the Children's Hospital, Birmingham, where a large number of the

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Table 1 Antibodies used to determine the immunophenotype in paraffin wax embedded material

Antibody	Lymphoid specificity	Source	Antigen molecular weight
PD7/26 (CD45RB)	Leucocyte common	Dr DY Mason	220,205,190
MB1 (CD45R)	Mainly B cells	Eurodiagnostics	200,110,100
MB2	B cells, some macrophages and epithelia	Eurodiagnostics	28
MT1 (CD43)	T cells, some B cells, myeloid cells	Eurodiagnostics	190,110,100
L26 (CD20)	B cells	Dako	43,35,32
4KB5 (CD45R)	B cells, some T cells	Dako	220,205
UCHL1 (CD45RO)	T cells, myeloid cells	Dako	180
Ki1 (CD30)	Ki1 activation antigen	Dako	110
LeuM1 (CD15)	RS cells, myeloid and monocytic cells	Dako	
IgG		Dako	
IgA		Dako	
IgM Light chain		Dako	
λ		Dako	
κ		Dako	

Source: Dr DY Mason, Oxford, UK; Eurodiagnostics, Solihull, UK; Dako, High Wycombe, UK.

Table 2 Antibody reactivity

	Antibody								
	PD7/26	MB1	MB2	MT1	UCHL1	L26	4KBS	Ki1	LeuM1
No. tested	22	30	30	30	30	30	29	17	9
No. positive	20	18	19	9	2	28	12	3	0
Per cent positive	91	60	63	30	6.67	93	41	18	0

cases were diagnosed initially. These were stained with haematoxylin and eosin, periodic acid-Schiff (PAS), and the Gordon and Sweet stain. Stained sections (2 μm) were available from the Children's Hospital cases. Children were diagnosed as having Burkitt's lymphoma if they had the following features: nuclear size less than that of the accompanying histiocytes, round nuclei, and three to five basophilic nucleoli. Cases diagnosed as having Burkitt-like lymphoma were also included in this study.

The antibodies used are presented in table 1. Paraffin wax embedded material was used in all instances as cryostat sections were not available because of the retrospective nature of the study. The sections were stained using a standard avidin biotin complex immunoperoxidase technique consisting of dewaxing, inhibition of endogenous peroxidase activity with hydrogen peroxide in methanol, incubation with 3% bovine serum albumin, and then with the primary marker. Sections were then incubated with biotinylated rabbit antimouse conjugate and avidin biotin complex followed by diaminobenzidine. A light Mayer's haematoxylin was used as the nuclear counterstain. Positive staining for each antibody was as previously described in a large series of adult lymphomas.<sup>29</sup> In some cases insufficient material was available for the full range of antibodies to be used.

**Results**

The median age of the cases included in this study was eight years with an evenly distributed range of two to 14 years. Twenty five patients were male and five female.

The primary sites of the tumours were as follows: 23 intra-abdominal (nine ileal, four ileocaecal, four caecal, one in the mesenteric nodes, one mesentery, one ovary, one liver

and pancreas, and not stated in two cases); two peripheral lymph node based (one cervical and one axillary); two tonsillar; one in the postnasal space; one in the right lobe of thyroid, and one mandible.

The results obtained and the antibody panel used are presented in tables 2, 3, and 4.

Table 3 Immunoglobulin type

	Immunoglobulin				
	G	A	M	κ	λ
No. tested	19	11	23	22	19
No. positive	4	0	13	5	10
Per cent positive	21	0	56.5	22.7	52.6

Table 4 Site of immunoglobulin expression

	G	A	M	κ	λ
Membrane	0	0	9	2	2
Cytoplasmic	0	0	1	1	4
Both	4	0	3	2	4

IgMκ 4; IgMλ 5; IgGκ 2; IgGλ 1.

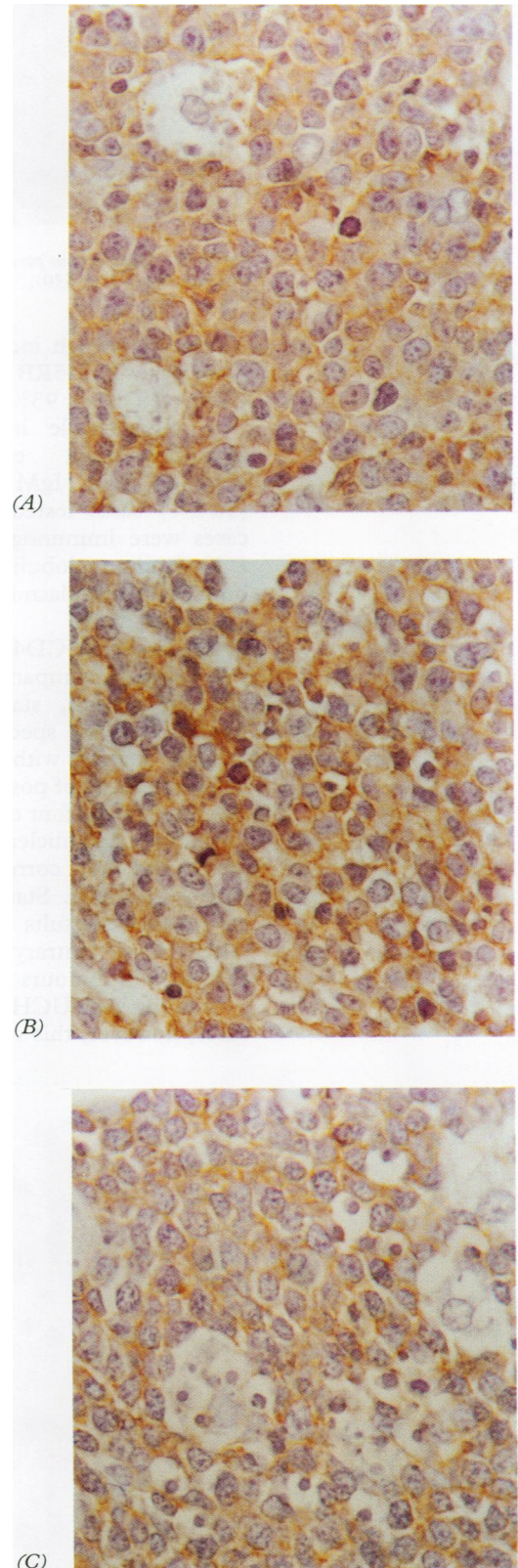


Figure 1 Immunophenotype in Burkitt's lymphoma. On staining, there was a high incidence of the following immunophenotypes: (A) CD45RB (PD7/26); (B) CD20 (L26); and (C) CD45R (MB1) (all × 630).

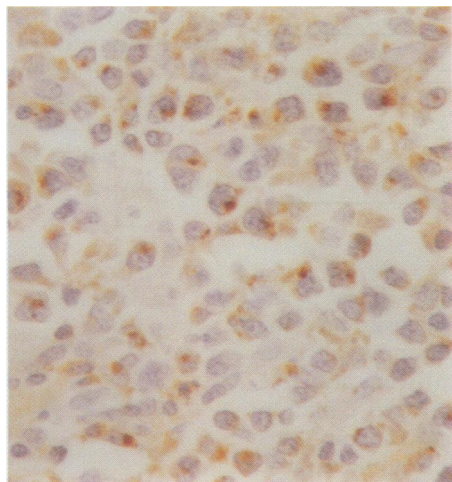


Figure 2 Conspicuous perinuclear cytoplasmic dot positivity (MB2;  $\times 630$ ).

There was a high incidence of the immunophenotype CD45RB (20 of 22; 91%) and CD20 (28 of 30; 93%) with the B cell phenotype recognisable in most cases (fig 1). Immunoglobulin expression was also observed, with IgM present in 13 of 23 (56.5%) cases tested. Both CD20 negative cases were immunoglobulin positive (IgG1,  $\lambda$  1). Immunoglobulin was expressed within both the cytoplasmic membrane and the cytoplasm itself.

Expression of CD45R recognised by 4KB5 was always accompanied by staining for L26 and, in general, staining with 4KB5 was weaker and less specific than that for L26. Overall, staining with MB1 and MB2 gave a similar number of positive results (18 and 19, respectively). In four cases MB2 staining had a distinctive paranuclear dot pattern (fig 2), which did not correlate with staining for immunoglobulin. Staining with Ki1 (CD30) gave positive results in three of 17 sections tested; this is contrary to the results previously described in tumours from endemic areas.<sup>27</sup>

Staining with UCHL1 occurred in 18 of 30 cases; however, this was predominantly cyto-

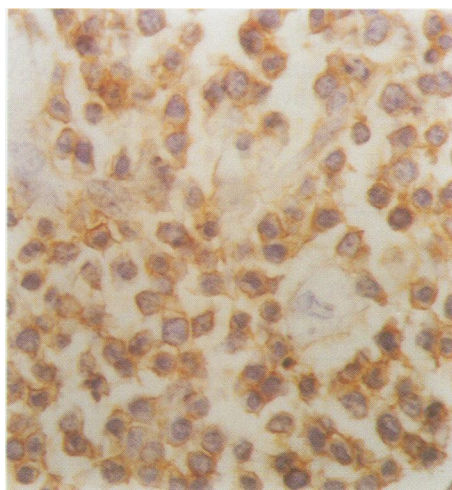


Figure 3 True membrane positivity, rather than non-specific cytoplasmic reactivity, was present (CD45RO (UCHL1)  $\times 630$ ).

plasmic and non-specific, although in two cases membrane staining appeared to be present (fig 3). Interpretation of the staining pattern with this antibody was often hampered by autolysis caused by delay in fixation in the original specimen.

### Discussion

In this study immunophenotypic analysis has shown a clear B cell lineage, identifiable on routinely processed material. Earlier studies using cell suspensions<sup>10 15-17</sup> highlighted the presence of surface immunoglobulin, mainly IgM. Other authors subsequently confirmed these findings using immunofluorescence<sup>20</sup> and immunoperoxidase studies on cryostat sections.<sup>19</sup> Using immunofluorescence, 82% of 114 biopsy specimens were IgM positive; with the immunoperoxidase technique, 16 of 18 cases expressed IgM, with 13 also positive for the  $\lambda$  light chain. A more recent study has confirmed that a B cell immunophenotype can be recognised on paraffin wax sections of Burkitt's lymphoma, although smaller numbers were analysed.<sup>26</sup>

We have shown the presence of both membrane and cytoplasmic immunoglobulin in non-endemic Burkitt-like and Burkitt's lymphoma and confirmed that this is usually IgM, with a smaller number expressing IgG. We have also confirmed the high incidence of  $\lambda$  light chain expression, which was present in 10 of 19 cases tested. The significance of this finding is uncertain but may be caused by a t(8;22) translocation as the  $\lambda$  gene is located on chromosome 22<sup>30</sup>; however, this translocation is present in only 5% of Burkitt's lymphomas and therefore cannot be the complete explanation.

Studies on endemic cases show a similar high incidence of CD20 expression to that observed here.<sup>27</sup>

As yet, only one study has analysed the incidence of leucocyte common antigen (LCA) in Burkitt-like lymphoma compared with lymphoblastic lymphoma, the results of which showed consistent LCA expression in Burkitt-like lymphoma along with the expression of one or more B cell markers.<sup>24</sup> A further study found a high incidence of LN-1 and LN-2 expression in Burkitt-like and Burkitt's lymphoma sections fixed in B5.<sup>25</sup>

Another study<sup>19</sup> has shown a high incidence of OKT10 in Burkitt's lymphoma, which was present in 14 of 17 cases on examination of cryostat sections; other authors<sup>28</sup> have reported two cases with a possible T cell phenotype. This study also used frozen material only and the morphology was different from that usually associated with Burkitt's lymphoma as high numbers of eosinophils and myelomonocytic cells were present; the Ki67 count in these cases was also lower than that usually found in Burkitt's lymphoma (<25% compared with >80%). In this study positive staining with UCHL1 was predominantly cytoplasmic and analysis was limited by autolysis; however, two cases showed true membrane staining as has been reported in other B cell lymphomas.<sup>22</sup>

The low incidence of CD30 staining in this study may be related to the poor correlation with Epstein-Barr virus in the non-endemic tumours<sup>31,32</sup>; this antibody is strongly expressed in the African endemic type.<sup>27</sup>

In conclusion, we have shown that a B cell lineage can be identified in Burkitt-like and Burkitt's lymphoma, that there is a low incidence of membrane staining with UCHL1, and a higher incidence of non-specific cytoplasmic staining, which is a potential source of confusion.

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