Among diffuse large B-cell lymphomas, T-cell-rich/histiocyte-rich BCL and CD30+ anaplastic B-cell subtypes exhibit distinct clinical features

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Summary

Background: The EORTC clinical trial 20901, activated in 1990, was designed to treat non-Hodgkin's lymphomas (NHL) of intermediate/high-grade malignancy according to the Working Formulation. Established in 1994, the R.E.A.L. Classification on NHL has now replaced all former classifications.

Patients and methods: We reanalysed all cases (n = 273) documented by material available for review according to the R.E.A.L. Classification. In addition, we subdivided cases recognised as diffuse large B-cell lymphoma (DLBCL) into three morphologically distinct categories, namely, large cleaved DLBCL (LC-DLBCL), T-cell-rich/histiocyte-rich B-cell lymphoma (T-cell-rich/histiocyte-rich BCL) and CD30+ DLBCL with anaplastic cell features (CD30+ DLBCL). Finally, T/NULL anaplastic large-cell lymphoma (ALCL) cases were subdivided into ALK+ and ALK− lymphomas. Review was performed independently by two pathologists from two different centres.

Results: DLBCL (61%), T/NULL ALCL (15%) and mantle-cell lymphoma (MCL, 5%) were the main NHL categories represented in the study. Fifty-seven of one hundred sixty DLBCL cases were further subclassified as LC-DLBCL (33 cases), T-cell-rich/histiocyte-rich BCL (13 cases) or CD30+ DLBCL (11 cases). The remaining cases were indicated as unspecified DLBCL. A clinico-pathological correlation confirmed the findings of previous studies suggesting that MCL, DLBCL and ALCL represent distinct entities with MCL being characterised by a short survival, in contrast with the longer survival and less frequent progression typical of ALK+ compared to ALK− ALCL. Within DLBCL, T-cell-rich/histiocyte-rich BCL showed distinctive features at presentation whereas CD30+ DLBCL showed a trend towards a more favourable prognosis, that might be comparable to that of ALK+ ALCL.

Conclusions: Our data further support the usefulness of the R.E.A.L. Classification and illustrate the feasibility of DLBCL subtyping. Moreover, our results demonstrate the distinct clinical characteristics of T-cell-rich/histiocyte-rich BCL and CD30+ DLBCL with anaplastic cell features suggesting that they may represent clinico-pathologic entities.

Key words: anaplastic large cell lymphoma, diffuse large B-cell lymphoma, EORTC, morphology, R.E.A.L. Classification, T-cell-rich/histiocyte-rich BCL

Introduction

In 1994 the International Lymphoma Study Group (ILSG), introduced the Revised European–American Lymphoma (R.E.A.L.) Classification for non-Hodgkin's lymphomas (NHL) [1]. It comprises a list of 'real' lymphoma entities that could be defined at that time, using morphologic, immunologic and genetic techniques. Subsequently, various large retrospective studies were performed in order to evaluate the diagnostic reproducibility and the clinical validity of the R.E.A.L. Classification. These studies have demonstrated the clinical relevance of defining new R.E.A.L. entities (e.g., mantle-cell lymphoma, marginal zone cell lymphoma) and the high diagnostic accuracy, which was shown to be additionally improved by the use of immunophenotyping [2-5]. As such, they have refuted the criticising reactions on the proposal and confirmed the usefulness of the R.E.A.L. Classification. Its principles have essentially been adopted in the forthcoming WHO Classification [6].

However, to be able to reach their conceptual goal of listing only well identifiable entities with a significantly distinctive clinical behaviour, the ILSG chose to lump together all cases of diffuse large B-cell lymphoma (DLBCL) in one category. Only one subtype, primary mediastinal (thymic) large B-cell lymphoma, was recognised as a separate entity based on its characteristic clinico-pathological features [1].

Despite the grouping of all DLBCL into one R.E.A.L. category, various histological identifiable subtypes have been described in the past, for which often a distinct clinical course was suggested. The Kiel Classification

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distinguished immunoblastic from centroblastic lymphoma based on the presence of over 90% immunoblasts [7]. Recently, it has been suggested that this particular morphological representation indicates an adverse prognosis when compared with classical centroblastic lymphoma [8].

In the early nineties, our group described the T-cell-rich/histiocyte-rich B-cell lymphoma (T-cell-rich/histiocyte-rich BCL) and noticed that this DLBCL subtype is associated with a characteristic clinical presentation and a particularly aggressive course [9].

Large cleaved DLBCL (LC-DLBCL) was first identified by Lukes and Collins [10] and was maintained by the Working Formulation as a subcategory 'of interest but of uncertain clinical importance' [11]. Whereas it was generally accepted that its characteristic nuclear morphology as well as the prominent sclerosis enabled its recognition, the actual clinical relevance of this subcategory has remained controversial, some investigators showing a favourable outcome [12–16], while others failed to demonstrate any clinical distinctiveness [17–23].

The implications of CD30+ immunopositivity in large B-cell lymphomas is enigmatic as it has received relatively little attention compared with CD30 expression in T/null cell lymphomas [24]. CD30 expression in DLBCL may be correlated with anaplastic cell features, as such delineating another histological identifiable DLBCL subtype, which may be worthwhile to investigate in view of the R.E.A.L. principles.

All available diagnostic biopsies of patients included in EORTC trial 20901 were reviewed. Firstly, all cases were reclassified according to the R.E.A.L. Classification. Secondly, it was decided to subdivide T/NULL anaplastic large-cell lymphoma (ALCL) cases into an ALK + and an ALK negative group, as recent studies suggested that ALK immunostaining in ALCL may provide crucial prognostic information [25, 26]. Finally, we tried to recognise the 4 morphologically subcategories of DLBCL mentioned above (immunoblastic DLBCL, LC-DLBCL, T-cell-rich/histiocyte-rich BCL and CD30+ DLBCL) and evaluated both the diagnostic applicability and the clinical significance of morphologically subgrouping DLBCL.

Patients and methods

Patients

The EORTC clinical trial 20901 included newly diagnosed patients age 15–60 years with stage II–IV NHL. In 1997, the upper age limit was increased to 65 years. The NHL was to fulfil the criteria of intermediate grade histology according to the Working Formulation. In addition, patients with stage I bulky or stage II–IV of the following high grade entities were accepted as well: diffuse large-cell immunoblastic, anaplastic large-cell lymphoma, large-cell and small-cell (if containing numerous blasts) pleomorphic T-cell lymphoma and AILD-like T-cell lymphoma. Low-grade NHL, lymphoblastic NHL and Burkitt's lymphoma were excluded. A complete staging evaluation was performed. Only patients with a performance status of WHO 0, 1 or 2 in the absence of severe cardiac, pulmonary, neurologic or metabolic dysfunction were included

Patients were randomised to two different treatment arms, as described elsewhere [27]. A CHOP-like regimen, CHVmP/BV chemotherapy was given. Briefly, patients were randomised after the first three CHVmP/BV cycles (reaching a complete or partial remission (CR/PR) with a histologically proven negative bone marrow, and no contraindications for bone marrow ablative chemotherapy), between the ABMT arm (a further 3 cycles CHVmP/BV followed by BEAC chemotherapy and autologous stem-cell rescue) or the control arm, with further five cycles CHVmP/BV. The protocol also recommended radiotherapy for all PR patients

Pathology review

Out of 311 cases included in the trial, 273 were available for the present study. Out of these 273 cases, 10 cases were excluded from the study due to the insufficient size or quality of the diagnostic biopsy specimen.

The biopsy was taken from a nodal site in 78% of cases (n = 205) and from an extranodal site in 22% of cases (n = 58). All cases were independently evaluated by two pathologists (C. de Wolf-Peeters, A. Carbone) and subtyped according the R.E.A.L. Classification [1]. In addition, DLBCL cases were further classified based on the morphologic criteria described below.

Immunophenotypic data (including CD20, CD3, CD30, CD15, CD5 and bcl-2) were known from the local pathology form in the vast majority of cases Since ALK staining was not included in the immunophenotypic panel performed at diagnosis, ALK staining was additionally performed for the T/NULL ALCL cases and the CD30+ DLBCL cases for which unstained sections were available (25 out of 38 cases and 9 out of 11 cases, respectively).

DLBCL cases characterised by neoplastic cells showing an irregular, typically indented or cleaved nucleus with evenly dispersed chromatin and by a compartmentalising fibrosis were further subtyped as LC-DLBCL [10]. Mitotic figures, apoptotic cells, foci of necrosis and an accompanying reactive infiltrate composed of small lymphocytes and histiocytes were variable features of these cases. DLBCL cases with strikingly scarce neoplastic large B-cells, but rich both in small reactive T lymphocytes and in histiocytes were specified as T-cell-rich/histiocyte-rich BCL. These DLBCL are to be distinguished from nodular paragranuloma, by the uniform distribution of large cells throughout the neoplasm and by the characteristic reactive background dominated by small T cells and histiocytes rather than B cells [9]. DLBCL cases exhibiting both anaplastic cell morphology and CD30 expression were identified as CD30+ DLBCL. Anaplastic cell features included large or very large cells with abundant cytoplasm, with large, often reniform or indented nuclei, and usually multiple nucleoli [24]. DLBCL cases characterised by the predominance of immunoblasts, e.g., more than 90% as defined by the Kiel group [7], were classified as immunoblastic DLBCL. DLBCL cases failing to answer the criteria to be included into one of these subgroups were left unspecified.

Statistical analysis

All cases analysed were equally distributed over both therapeutic arms. Only the cases that were classified under both schemes are included in the analysis.

Progression-free survival (PFS) was calculated as the time interval between the date of randomisation and the date of disease progression or death, whichever came first. If neither event had been observed, then the patient was censored at the date of the last follow-up.

Overall survival (OS) was calculated as the time interval between the date of randomisation and the date of death due to all causes. Patients who were still alive when last traced are censored at the date of last follow-up. Survival curves were estimated using the Kaplan–Meier method. Due to small numbers, not all subtypes could be analysed so comparisons are only visually allowed.

Table 1 Baseline characteristics of patients subtyped as MCL, DLBCL or ALCL.

	MCL	DLBCL	ALCL	ALK+	ALK−
n (%)	13 (5)	160 (61)	38 (15)	11 (29)	14 (37)
Age (years)					
Range	32–60	18–64	16–58	16–45	17–43
Median	51	45	30	28	30
Sex ratio (M : F)	1.6	1 6	1.6	4.5	0.7
Ann Arbor stage (%)					
I	8	9	3	0	7
II	15	32	47	27	64
III	8	21	28	46	7
IV	69	38	22	27	22
Bone marrow involvement (%)					
Negative	38	81	92	91	93
Positive	54	16	8	9	7
Unknown	8	3			
Systemic symptoms (%)					
Absent	61	65	53	64	50
Present	39	34	47	36	50
Unknown		1			
Hepatomegaly (%)					
Absent	92	84	91	91	93
Present	8	10	3	9	0
Unknown		6	6		7
Splenomegaly (%)					
Absent	38	76	86	82	93
Present	62	20	11	18	7
Unknown		4	3		

Results

Pathology review

Full agreement among both pathologists was reached for R.E.A.L. subtyping and for further subdividing DLBCL cases, in all except two cases, which were excluded from further analysis.

The R.E.A.L. subtypes mainly represented in the study were DLBCL (61%), T/NULL ALCL (15%) and MCL (5%). The remaining cases were either follicle centre cell lymphoma (4.7%), marginal zone cell lymphoma (3.5%), Burkitt's or Burkitt's like lymphoma (2%), T-cell lymphoma (peripheral, not otherwise specified or angioimmunoblastic) (3%), primary mediastinal large B-cell lymphoma (2%) and chronic lymphocytic leukemia (3.8%).

Based on the morphologic features as described above (methods section), the group of DLBCL cases (n = 160) comprised 11 CD30+ DLBCL cases (7%), 13 cases of T-cell-rich/histiocyte-rich BCL (8%) and 33 LC-DLBCL cases (21%). None of the CD30+ DLBCL cases for which ALK staining was performed (9 of a total of 11 cases) showed ALK expression. No more than two cases were composed of a sufficient number of immunoblasts to qualify as immunoblastic DLBCL. These two cases were analysed together with the remaining unspecified DLBCL subgroup (103 cases).

For the 38 cases diagnosed as T/NULL ALCL, ALK immunostaining was positive, negative or not done in respectively 11, 14 and 13 cases.

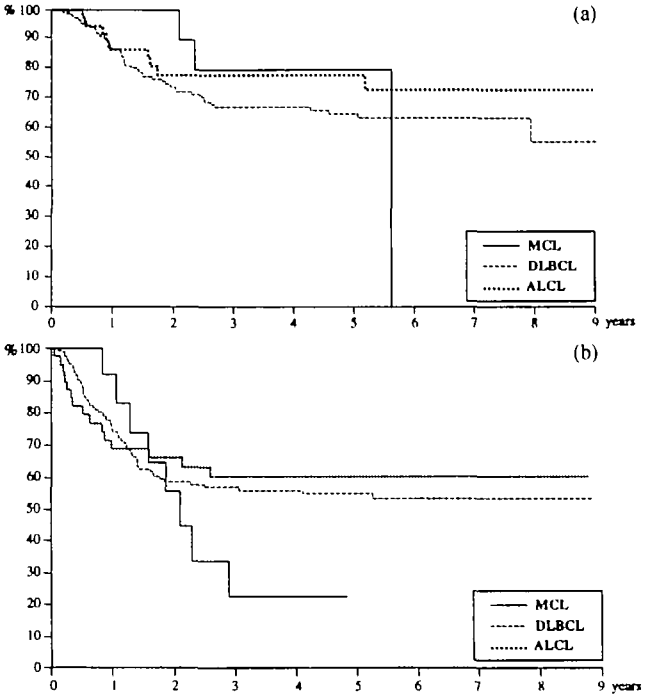

Figure 1. Overall survival (a) and progression-free survival (b) comparison of MCL patients (n = 13), DLBCL patients (n = 160) and ALCL patients (n = 38).

Clinical data

Clinical characteristics at patient entry of the three mainly represented R.E.A.L. categories (MCL, DLBCL, T/NULL ALCL) are summarised in Table 1. The ALCL group included patients on the average 15–20 years younger when compared with MCL and DLBCL (median age 30 years vs. 51 and 45 years). Ann Arbor stages III and IV were observed in 77% of MCL cases compared to 59% and 50% of respectively DLBCL and ALCL cases. Bone marrow involvement was found in more than half of the MCL cases (54%) and was rare in DLBCL and ALCL (respectively, 16% and 8%). Systemic symptoms were more frequently present in ALCL (47%) compared to MCL (39%) and DLBCL (34%). Moreover, besides the important differences in clinical presentation, these three lymphomas appear to behave differently, MCL displaying a tendency towards an inferior prognosis in terms of progression-free survival (Figures 1a and b).

ALK staining divides T/NULL ALCL in two distinct subentities in terms of prognosis as demonstrated by a different overall survival and progression-free survival (Figures 2a and b). Moreover, the ALK positive and the ALK negative subgroups show distinctive clinical characteristics at entry, with a marked male predominance and a higher incidence of aggressive stages III and IV among ALK + ALCL (Table 1).

Among the DLBCL subgroups, T-cell-rich/histiocyte-rich BCL shows highly characteristic clinical features at presentation as shown in Table 2. A clear male prepon-

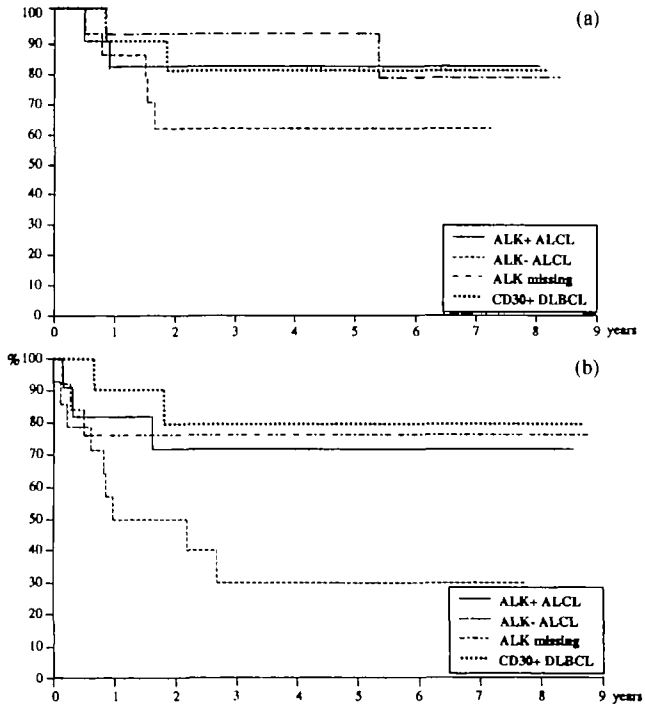

Figure 2 Overall survival (a) and progression-free survival (b) of CD30 + DLBCL patients (n = 11), compared to ALCL patients, divided into ALK+ (n = 11), ALK− cases (n = 14) and cases without available ALK staining (n = 13).

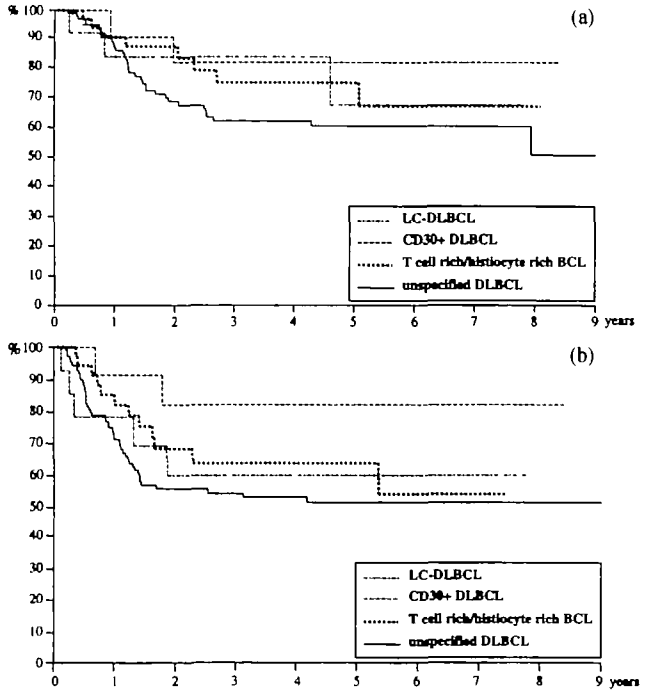

Figure 3. Overall survival (a) and progression-free survival (b) of DLBCL patients, divided into LC-DLBCL (n = 33), T-cell-rich/histiocyte-rich BCL (n = 13), CD30+ DLBCL (n = 11) and unspecified DLBCL (n = 103)

derance (ratio M : F = 5.5) was noted in this category, compared to an almost equal distribution between both sexes in the other DLBCL. T-cell-rich/histiocyte-rich BCL patients presented more often with advanced Ann Arbor stages (III and IV) (84% vs. 55%, 45% and 62% in

Table 2. Baseline characteristics for DLBCL subtypes.

	CD30+ DLBCL	T-cell-rich/ histiocyte-rich BCL	LC-DLBCL	Unspecified DLBCL
n (%)	11 (7)	13 (8)	33 (21)	103 (64)
Age (year)				
Range	18–52	24–52	19–64	18–64
Median	41	41	44	47
Sex ratio (M : F)	1.7	5.5	1.3	1.3
Ann Arbor stage (%)				
I	18	8	12	7
II	27	8	43	31
III	18	15	12	24
IV	37	69	33	38
Bone marrow involvement (%)				
Negative	90	54	82	82
Positive	10	38	15	15
Unknown	0	8	3	3
Systemic symptoms (%)				
Absent	73	46	67	65
Present	27	54	33	34
Unknown	0	0	0	1
Hepatomegaly (%)				
Absent	73	69	94	84
Present	9	23	0	12
Unknown	18	8	6	4
Splenomegaly (%)				
Absent	82	30	82	79
Present	18	62	9	19
Unknown	0	8	9	2

CD30+ DLBCL, LC-DLBCL and unspecified DLBCL, respectively). Approximately 40 % of patients presented with bone marrow invasion at diagnosis, which is rather rare in the other subtypes and in the unspecified DLBCL. In addition, hepatosplenomegaly and systemic symptoms were more frequent findings in T-cell-rich/histiocyte-rich BCL at presentation. The survival curves of the DLBCL subtypes do not show a clear disadvantage for T-cell-rich/histiocyte-rich BCL patients (Figures 3a and b). The small number of T-cell-rich/histiocyte-rich BCL patients included in the analysis may provide a reasonable explanation for this unexpected observation.

CD30+ DLBCL, on the other hand, can be clearly distinguished from the other subtypes with respect to clinical behaviour. Overall and disease-free survival curves suggest that the clinical course of CD30+ DLBCL may be comparable to that of ALK+ ALCL (Figure 2a and b).

Discussion

The morphological review of cases included in the EORTC trial 20901, allowed to address three issues of special current interest.

Firstly, it was ascertained that the R.E.A.L. Classification [1] defines clinically distinct entities within a subpopulation of patients affected by intermediate/high-grade NHL. DLBCL, ALCL and MCL were the

three major categories represented in the study. These three lymphomas show a distinct clinical behaviour with MCL being the most aggressive. These findings confirm the data of other large studies [3, 4], and underscore that lymphomas that were originally lumped together as 'intermediate/high-grade', according to the Working Formulation, show marked differences in survival.

Despite the low percentage of T-cell lymphoma cases represented in the study, partially due to the inclusion criteria of the trial, a relatively high number of cases diagnosed as T/NULL ALCL was found. Since they represented 38 out of a total of 261 cases analysed (15%), a second issue, the prognostic significance of ALK expression in this particular group of lymphomas, could be investigated. ALK expression results from the translocation t(2;5) or from other chromosomal rearrangements involving the ALK gene [28]. t(2;5) has been demonstrated in 15%–85% of ALCL cases, an impressive variability that has been ascribed to differences in methodology applied, inclusion criteria, patient characteristics and the presence of variant translocations [25, 26, 28–31]. In our study, of 25 cases that were stained for ALK, 11 cases showed positivity. Visual comparison of the survival curves, showed a clearly better clinical behaviour of ALK positive ALCL cases as compared to ALK negative cases, in terms of both overall survival and progression free survival (Figure 2). These results confirm those of Falini et al. who reported a significantly better prognosis of ALK positive cases in a series comprising 96 ALCL [25].

Finally, we decided to assess whether previously described, morphologically distinct DLBCL subgroups may be adopted for subclassification. In the R.E.A.L. Classification, subdividing DLBCL was considered impractical for the lack of both diagnostic reproducibility as well as clinical relevance. Our results show that three previously described DLBCL, being LC-DLBCL, T-cell-rich/histiocyte-rich BCL and CD30+ DLBCL can be recognised and distinguished from one another. In addition, our data indicate that these entities have clinically distinct features, either because of the characteristics at presentation (T-cell-rich/histiocyte-rich BCL), or for prognostic reasons (CD30+ DLBCL). It has to be emphasised that the latter findings merely represent observed trends in an analysis that, due to low patient numbers, lacked the power to reach the level of statistical significance.

For LC-DLBCL, some investigators have suggested a better prognosis compared to unspecified DLBCL [12–16], whereas other could not confirm this finding [17–23]. In the present study, no obvious difference could be observed between the clinical presentation and the survival curves of LC-DLBCL on the one hand and DLBCL with non-cleaved cell morphology on the other, despite its readily identifiable morphology.

Patients affected by T-cell-rich/histiocyte-rich BCL, usually middle-aged men, have been shown to present with advanced stage disease, with involvement of the spleen, the bone marrow and/or the liver, besides multiple peripheral lymph nodes [9]. The present study again highlights, apart from the typical morphology, the specific clinical features of this subcategory at presentation, but, due to small patient numbers, does not allow to adequately judge the patients' prognosis.

Except for one study by Noorduyn et al., CD30+ DLBCL have only been found in small numbers in larger series essentially focusing on T/NULL-ALCL [24]. Noorduyn et al. suggested that CD30 expression is not restricted to DLBCL with anaplastic morphology. Moreover, they failed to demonstrate any correlation between survival on the one hand and CD30 expression and/or anaplastic morphology on the other. Our approach essentially differs from the one applied by Noorduyn et al. in that we required both anaplastic features and CD30 expression to delineate the particular DLBCL subtype. However, the observed trend towards a better prognosis comparable with ALK+ ALCL, requires confirmation in a larger study.

The low number (no more than two) of immunoblastic DLBCL cases identifiable in our study, corroborates the forthcoming WHO Classification that will not incorporate this subtype. It was believed that neither reliable pathological or biological criteria for subclassification, nor distinctive therapies are available at this time to justify a distinction between immunoblastic and centroblastic lymphoma [11].

The WHO Advisory Committee has recommended to lump all DLBCL together [11], in the forthcoming classification of NHL. Nevertheless, it was acknowledged that a further subclassification is mandatory, in order to identify subgroups of patients that might benefit from alternative therapies. Our data support the feasibility of subtyping DLBCL according previously described morphological entities. They further indicate that T-cell-rich/histiocyte-rich BCL and CD30+ DLBCL with anaplastic cytology may have clinical distinct features.

Recently DLBCL have been divided into two prognostically distinct subgroups (germinal centre like DLBCL and activated B-cell like DLBCL) using a DNA microarray for the analysis of lymphoid cells (Lymphochip) [32]. These techniques should be applied to DLBCL subtypes identified by morphology, in order to investigate the correlation between immunomorphological findings and molecular data. Such analysis will hopefully result in less complicated utilities to be used in daily practice.

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