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The Reproducibility of the Lukes–Collins Classification of non-Hodgkin's Lymphomas in a Retrospective Reclassification*

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Abstract—*In a retrospective reclassification of* 148 *non-Hodgkin's lymphomas the reproducibility of the Lukes–Collins classification used was tested by comparing a joint diagnosis with that of each author separately. Thirty-six percent of the lymphoma cases were follicular center cell lymphomas, the figure being clearly lower than that of materials published previously. The relatively high portions of small lymphocytic and mycosis fungoides lymphomas* (20 *and* 10% *respectively) were also deviant findings in comparison to formerly published retrospective materials. In this reproducibility study the cases of disagreement were usually proved to have happened within lymphoma groups with an equivalent prognosis. An exception was the relatively high disagreement rate between the subclasses of small lymphocytic and small non-cleaved lymphomas, and this represents the most important finding clinicopathologically. The results of the study indicate that the Lukes–Collins classification as such does not cover the whole spectrum of non-Hodgkin's lymphomas. The studies published already give some clues as to the fact that distribution and cell types of non-Hodgkin's lymphomas vary in different areas of the world.*

INTRODUCTION

IN THE middle of the 1970s new immunological classifications based on the transformation theory of lymphocytes were published, the most important of these being the systems of Lukes and Collins, and Lennert [1–4]. Both systems have their basis in immunobiology and the transformation theory of lymphatic cells. These new classifications have also been utilized in everyday diagnostic pathology. The published studies show that it is possible to reclassify non-Hodgkin's lymphomas by using paraffin-embedded, formalin-fixed material [5–9]. The cytomorphological criteria are well documented and in order to be able to successfully reclassify non-Hodgkin's lymphomas one has to be deeply familiar with the cytomorphological basis of the classifications and the subclasses to understand the importance of clinical findings and their relationship to histology [3, 4, 10]. Successful completion of reclassification also requires good laboratory technique, utilizing excellent microscopic slides and special stainings.

There are only a few publications dealing with the reproducibility of the classifications of non-Hodgkin's lymphomas. The largest of these was the project arranged by the National Cancer Institute (NCI), the goal of which was to create a compromise system based on the six well-known non-Hodgkin's lymphoma classifications [11–18]. One of the project's aims was to test the reproducibility of the different classification systems. This area of the project was very carefully and reliably designed and arranged. So far the results of this part of the project have not been published. The report included only a comment that "reproducibility was adequate in all systems" and "no one system of classification was superior" [11]. The results would have been most interesting as most of the reviewers were among the best-known hematopathologists. Other publications concerning the reproducibility problem are based on the Rappaport classification of non-Hodgkin's lymphomas [8, 19–22]. The results of

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these published reproducibility tests are contradictory. For instance, in their report Straus *et al.* state that the Rappaport classification is poorly reproducible [9].

MATERIALS AND METHODS

The study was conducted from 1975 to 1979 and the material consists of all the non-Hodgkin's lymphoma cases diagnosed in the area of the Oulu University Central Hospital between 1963 and 1975. The primary material was made up of 417 diagnosed or suspected lymphoma cases. After preliminary checking of the field microscopic slides 329 cases were selected, 216 of which were surgical biopsies and 26 autopsy cases. Tissues with reactive lymphatic hyperplasia and non-Hodgkin's disease were omitted. Atypical lymph node hyperplasias were kept in the material. In these 329 cases the praffin-embedded material was cut and stained onto new microscopic slides consisting of, in addition to the routinely used hematoxylin–eosin staining, five different special stainings: Herovici, for collagen fibers; hematoxylin–PAS, with and without diastase; Gomori's reticulin staining; Giemsa; and methylgreen-pyronin staining. The tissues had originally been fixed in 10% neutral buffered formalin. Out of this material 148 non-Hodgkin's lymphoma cases with adequate pretreatment tissue material were selected. The reasons for the big rejection percentage included autolysis, inadequate material, hyperplastic as opposed to neoplastic lymph node lesions, myeloid diseases, etc. Of the selected 148 cases 122 were surgical biopsies and 26 autopsies.

The reclassification was performed by the authors, the senior and junior pathologists of the Oulu University Central Hospital. The final management of the material was divided into two phases. In the first phase both pathologists classified the cases together according to the Lukes–Collins classification. Concerning unclear or borderline cases, the naphthol-AS-D-chloroacetate estrase reaction and the peroxidase–anti-peroxidase method for demonstration of intracytoplasmic muramidase activity were performed to detect myeloid and monohistiocytic malignancies [23–27]. The patient files were studied and applicable forms—designed for this study to record the clinical and morphological findings—were filled. The diagnoses were made together, in some cases after discussions. All possible background information of the patients was thus available to the investigators. In the second phase of the study both pathologists classified the cases independently using only the microscopic slides. In this second phase the clinical records were not available and microscopic slides were examined only once by both investigators.

RESULTS

The number of the reclassified cases was 148. Of those 122 were surgical biopsies and 26 autopsy cases. There were 86 nodal lymphomas, in 28 of which leukemia was also present. The material included 17 gastrointestinal, 7 Waldeyer's ring and 17 skin lymphomas. The remaining 21 patients had lymphomas present in other sites. Age and sex distribution of the material is presented in Table 1. Of the patients 100 were males (68%) and 48 females (32%).

In Table 1 the distribution of the cases into the Lukes–Collins classification of non-Hodgkin's lymphomas is presented. It can be detected that some parameters differ from earlier published findings from Finland [28]. The most remarkable finding is the low percentage of follicular center cell (FCC) lymphomas (36%) compared to that presented in the material of Alavaikko and Aine in South-western Finland (54%) [28]. In Finland

Table 1. Distribution of 148 non-Hodgkin's lymphomas according to the Lukes–Collins classification

Cytologic type	Abbreviation	*n*	%	Males	Females	Mean age
Small lymphocytic	SL	29	20	21	8	61
Plasmacytoid lymphocytic	PL	13	9	6	7	62
Follicular center cell types	FCC	53	36	35	18	
Small cleaved	SC	5	4	4	1	71
Large cleaved	LC	9	6	6	3	48
Small non-cleaved	SNC	24	16	14	10	43
Large non-cleaved	LNC	15	10	11	4	64
Immunoblastic sarcoma	IBS	14	9	8	6	57
Convoluted lymphocyte	Con	8	5	4	4	16
Mycosis fungoides	MF	15	10	14	1	66
Hairy cell leukemia	HCL	1	1	1	0	54
Unclassified	U	15	10	11	4	43
Total		148	100	100	48	

the figures differ clearly in this respect from those published, for instance, for the U.S.A. The NCI lymphoma project consisted of, among others, 724 non-Hodgkin's lymphomas classified according to the Lukes–Collins classification; 83% of these were FCC lymphomas and 46% showed different degrees of follicularity (nodularity) [11]. Connected to the low percentage of the FCC lymphomas in our material from Northern Finland, occurrence of follicular lymphomas was also low (14 cases, 10%). In comparison, the percentages of follicular lymphomas were 16, 54 and 44 in the materials of Alavaikko and Aine, Nathwani *et al.* and Jones *et al.* respectively [28, 6, 5].

The most common subtype was small lymphocytic lymphoma (SL) (20%), the percentage of which is higher than that in other Finnish material (13%) [28]. In this respect the Finnish figures seem to be higher than the formerly mentioned NCI material, in which the percentage was 5%, including plasmacytoid lymphocytic (PL) cases [11]. With the FCC lymphomas one must pay attention to the scarcity of the small cleaved (SC) group (4%). In both sets of Finnish material SC lymphomas occur much less frequently than the 50% of the NCI material [11]. The large non-cleaved (LNC) group's percentage is also low (10%) compared to earlier material published from Finland (20%) [28]. In Lukes' material the percentage of LNC lymphomas was 4% whereas in the NCI material it was 10% [11, 29]. There are no big differences concerning the number of immunoblastic sarcoma (IBS) cases, but convoluted lymphomas (Con) seem to be slightly more frequent in Northern than in South-western Finland. The percentage is, however, similar to the NCI material (4%) [11, 28]. The biggest difference is in the number of mycosis fungoides (MF) patients compared to the previously mentioned reports. The MF cases reach the limit of 10% of this material compared to, for instance, 1% of the non-Hodgkin's lymphomas published from South-western Finland [28]. The portion of unclassifiable (U) cases remained at 10%, which is quite a moderate result in view of the fact that almost 18% of this study's non-Hodgkin's lymphoma cases were autopsies. However, the tissue material was found to be adequate and preserved well enough. There was no attempt to divide the cases into B- and T-cell-derived lymphomas by morphological means.

The results referred to here create a background for the ultimate goal of this project—for the study of reproducibility of the Lukes–Collins classification of non-Hodgkin's lymphomas. The results concerning this area are presented in Table 2. Concerning reproducibility, the subclasses are presented in order of superiority. The study of reproducibility was conducted by using only microscopic slides without clinical records. Both investigators examined the slides only once independently. The results are given in absolute percentages. The 'right' or consensus diagnosis was reached by both of the investigators in 91 cases (62%). Thus the diagnosis was changed by either of the investigators in 57 cases (38%). The study was not designed to measure differences in diagnostic skill between the investigators. The reproducibility was very good in the MF and the

Table 2. The results of the reproducibility study of the Lukes–Collins classification (subclasses appear in order of superiority)

Consensus (right) diagnosis*	*n*	Reproducibility (%) Both right diagnosis	One right diagnosis	Both the same 'wrong' diagnosis	Both different 'wrong' diagnosis
MF	15	100			
SL	29	73	17	3	7
SNC	24	67	17	4	12
IBS	14	65	7	21	7
LC	9	56	33	0	11
LNC	15	53	20	7	20
Con	8	50	13	12	25
SC	5	40	40	20	0
PL	13	39	15	15	31
U	15	38	25	25	12
HCL	1			100	
Total	148				

*MF = mycosis fungoides; SL = small lymphocytic; SNC = small non-cleaved; IBS = immunoblastic sarcoma; LC = large cleaved; LNC = large non-cleaved; Con = convoluted lymphocyte; SC = small cleaved; PL = plasmacytoid lymphocytic; U = unclassified; HCL = hairy cell leukemia.

SL groups, where the percentages of right diagnoses were 100 and 73% respectively. The results were also good concerning the small non-cleaved (SNC) and the IBS subclasses, where the corresponding figures were 67 and 65% respectively. The correctness of the diagnoses was moderately good in the subclasses of large cleaved (LC), LNC and Con (56, 53 and 50% respectively). The results were not satisfactory, however, in the groups of SC and PL (40 and 30% respectively). The SC subclass was very small (only 5 cases), which must have had an influence on the low percentage of agreement. The PL group, on the other hand, is one in which the investigator may use clinical information when making diagnoses. Cross-overs of the diagnoses between the adjoining subclasses are presented in Table 3. By inspecting the different groups, the shifts in the borderline cases can be relatively well explained.

DISCUSSION

To our knowledge, no studies have been published on reproducibility of the new non-Hodgkin's lymphoma classifications. In this discussion we concentrate on those facts that influence the reproducibility of the subclasses. Lymphoma diagnostics is never based solely on tissue examinations and cytomorphology. In this particular study of the reproducibility of the Lukes–Collins non-Hodgkin's lymphoma classification we wanted to test especially cytomorphology and its role as a basis of classification. Therefore, while reclassifying independently we used only microscopic slides.

There are implications that differences exist in distribution and occurrence of non-Hodgkin's lymphomas in different areas of the world as well as within individual countries. This study also reveals that the distribution of the subclasses of the Lukes–Collins classification differs in certain respects in the material from Finland and the U.S.A. The clearest difference is the higher percentage of SL lymphomas and the lower percentage of FCC lymphomas in Finland [11, 28, 29]. The results of this study also indicate that it is possible that the known non-Hodgkin's lymphoma classifications do not cover the whole spectrum of malignant lymphomas in the world [28]. Particularly, Japanese investigators have paid attention to this consideration [22].

When interpreting the results of this kind of reproducibility study, certain facts influencing the results should be kept in mind. Successful reclassification of non-Hodgkin's lymphomas requires, in addition to theoretical knowledge and skill, ideal fixation, excellent microscopic preparations and the necessary special staining techniques. In management of retrospective material the investigators have to be content with filed paraffin-embedded material. There is no way to further influence the representativeness of material. The cytomorphological principles of the Lukes–Collins classification are well documented [5–9]. The investigator must, however, review an appropriate number of microscopic slides of every subclass in order to be able to apply his knowledge. The results are thus influenced by the experience of the investigator. It should also be remembered that a consensus diagnosis may be merely a compromise diagnosis that will perhaps change when either one of the investigators works independently. Clinical findings are important in the interpretation and understanding of classifications. In this study attention was paid only to cytomorphology and information received using cytomorphological methods.

The discrepancies of the diagnoses between the adjoining subclasses are presented in Table 3. Problem situations in the Lukes–Collins classification are found among both small and large cell lymphomas. Concerning small cell lymphomas, borderline cases are found between the SL and the SNC subclasses. This was noticed in the recent study published by Alavaikko and Aine [28]. Certain small cell non-Hodgkin's lymphomas seem to represent a cell type that is between these two categories. This is proved especially in the reproducibility study, where it is quite arbitrary as to into which group—SL, SNC or U—these cases will be put. The consensus diagnoses of these cases have been compromises in many situations. Among small cell lymphomas there is another problem class in this kind of reproducibility study based purely on cytomorphology. Clinical records are a valuable aid in the recognition of the PL subclass. The lymphomas of this category may be associated with an increase in monoclonal immunoglobulins in the blood. These cases are in essence identical to Waldenström's macroglobulinemia. The availability of clinical files are also helpful in the diagnoses of convoluted lymphomas. The patients of this subclass are predominantly in the adolescent age groups and commonly suffer from a mediastinal mass [3]. Among the large cell lymphomas the problem group consists of the LNC and the IBS subclasses. The cytomorphological criteria are not always unambiguous and easy to apply to all cases, and the choice of subclass can be a matter of opinion.

In any reproducibility study the major difficulty is in the borderline cases, the diagnoses of which are easily changed between the subclasses. It is also true that cytomorphologically pure lymphomas are rare and diagnoses

Table 3. The results of the reproducibility study (in 57 cases the two reviewers did not agree on the diagnosis)

Case No.	Consensus ('right') diagnosis	Failing in reproducibility* One right diagnosis	Both the same 'wrong' diagnosis	Both different 'wrong' diagnosis
33	SL	U, SL		
41	SL	SL, SNC		
42	SL	SL, SNC		
102	SL	SNC, SL		
122	SL	SL, PL		
118	SL		U, U	
5	SL			U, SNC
14	SL			Reactive, SC
9	SC	SL, SC		
19	SC	U, SC		
71	SC		LC, LC	
49	LC	Reactive, LC		
106	LC	LC, U		
114	LC	LC, SC		
123	LC			SC, SL
12	SNC	SNC, SL		
64	SNC	U, SNC		
93	SNC	SNC, SL		
103	SNC	U, SNC		
10	SNC		U, U	
72	SNC			LNC, IBS
125	SNC			LNC, IBS
131	SNC			U, SL
35	LNC	LNC, IBS		
37	LNC	LNC, IBS		
58	LNC	LNC, LC		
6	LNC		SNC, SNC	
65	LNC			SC, PC
67	LNC			SNC, IBS
104	LNC			SL, IBS
75	PL	SNC, PL		
127	PL		PL, SL	
46	PL		SL, SL	
68	PL		SL, SL	
73	PL			SL, U
96	PL			U, SL
113	PL			Reactive, SL
114	PL			SL, Hodgkin
132	Con	Con, LC		
86	Con		SNC, SNC	
31	Con			U, SNC
57	Con			LNC, IBS
129	IBS	IBS, LNC		
44	IBS		LNC, LNC	
54	IBS		LNC, LNC	
56	IBS		LNC, LNC	
59	IBS			LNC, U
24	U	U, SNC		
121	U	LNC, U		
124	U	U, SL		
138	U	SL, U		
69	U		SNC, SNC	
80	U		LC, LC	
137	U		Con, Con	
48	U			LNC, LC
53	U			PC, SL
108	HCL		SL, SL	

*SL = small lymphocytic; PL = plasmacytoid lymphocytic; SC = small cleaved; LC = large cleaved; SNC = small non-cleaved; LNC = large non-cleaved; IBS = immunoblastic sarcoma; Con = convoluted lymphocyte; MF = mycosis fungoides; HCL = hairy cell leukemia; U = unclassified.

should be based on harmony of clinical findings and prevailing cell type.

In the interest of clinicopathologic relevance it is important in this kind of study to elucidate whether or not disagreements remain within the frames of lymphoma groups with an equivalent prognosis. Regarding the prognostic significance of the Lukes–Collins classification, we are relying upon a recent study by Aine *et al.* of non-Hodgkin's lymphomas in South-western Finland [30]. In this study two large groups with different prognoses emerged: (1) a group with a favorable outlook, including: plasmacytoid lymphocyte, small lymphocyte and small cleaved follicular center cell types with a 5-yr relative survival rate of 51–74%; and (2) a group with an unfavorable outlook, consisting of: small non-cleaved follicular center cell, large non-cleaved follicular center cell and immunoblastic types. Mortality due to these types manifested itself at a level of 65% in the first 2 yr. In the above study the number of convoluted lymphocyte types was very small, but other studies have shown this type to be extremely fatal [31].

In the working formulation of non-Hodgkin's lymphomas presented by the NCI study group for clinical use, lymphomas were divided into three major survival groups [11]. It can be roughly stated that in that proposal, low-grade lymphomas are of small cell type, intermediate-grade lymphomas consist of the varying groups from small cell to large cell subclasses and high-grade lymphomas are of large cell type. With respect to prognostic groups the results of this study were satisfactory and comforting in that the discrepancies between groups in the reproducibility experiment were not astonishingly large. Besides the difficulty in some cases of SL/SNC groups, no real disagreements between the groups of low and high malignancy occurred.

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