

## ORIGINAL PAPER

# CD30 expression utilization for the accuracy of classical Hodgkin's lymphoma staging

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### Abstract

**Introduction.** The presence of Reed–Sternberg malignant cells is absolutely necessary for Hodgkin's lymphoma diagnostic, but it is not always sufficient because can be observed Reed–Sternberg-like cells in other malignant and benignant diseases, too. The CD30 expression at Hodgkin and Reed–Sternberg level can give us supplementary information in differential diagnostic and can be used as progressive disease factor. **Material and methods.** Our study was composed from 63 cases histopathological diagnosed with Hodgkin's lymphoma and hospitalized in Hematology Department of County Hospital Timișoara. CD30 expression was immunohistochemical semi-quantitative evaluated using clone BerH2 as primary antibody and APAAP–New Fuchsin as visualization system. **Results and discussions.** The increasing of CD30 expression occurs in the same time with advanced stages and the disease progression ( $p \leq 0.001$ ). For I and II stages CD30 expression does not overcome (-/+) category while the III and IV stages, all the cases are situated in (+/-) and (+) categories. No connection can be noticed between histological type and CD30 expression ( $p \leq 1$ ). We consider that using this staining, although less used in Romania, must be done in all Hodgkin's lymphoma and Hodgkin's lymphoma-like cases. We say that because the main cause of relapses is represented by inadequate clinical staging and diagnostic. **Conclusions.** In our study, the increasing of CD30 expression is associated with advanced disease stage. We recommend reinvestigating and restaging all cases that was included into an incipient stages and they have a CD30 expression situated in (+/-) and (+) intervals because some lymph nodes could be overlooked.

**Keywords:** Hodgkin's lymphoma, CD30, tumoral progression.

### Introduction

The Hodgkin's lymphoma diagnostic is mainly a histological diagnostic based by Reed–Sternberg multinucleated cells recognizing surrounded by an inflammatory reaction composed from lymphocytes, granulocytes, plasma cells and fibroblasts. Even if the Reed–Sternberg cell presence is necessary for Hodgkin's lymphoma diagnostic, it is not specific, occurred in some benignant disease (e.g. in infectious mononucleosis), in some malignant lymphomas (e.g. in anaplastic large cell lymphoma) and in some carcinomas. The cellular inflammatory reaction aspect is important in diagnostic establish, too, that representing the host answering at tumoral invasion [1–3].

CD30 surface antigen was identified for the first time in Hodgkin and Reed–Sternberg cells from Hodgkin's lymphoma. It was demonstrated that antibody it is not so specific to Reed–Sternberg cells that believed, it noticed into a non-Hodgkin's lymphomas, especially in anaplastic large cell lymphoma. In Hodgkin's lymphoma, CD30 expression on Reed–Sternberg cells surface is associate with size and invasively of tumor, thus representing a possible indicator of disease aggressively independent by age, race and symptoms [4–7].

The aim of our study is the utilization of CD30 monoclonal antibody (clone Ber-H2) for accuracy of Hodgkin's lymphoma staging. On the other hand, this expression has a very important contribution to Hodgkin's lymphoma diagnostic.

### Material and methods

We had 63 patients hospitalized into the Hematology Department of the County Hospital Timișoara, between January 2000 and June 2004. After the histopathological examination into the Pathologic Anatomy Laboratory, these patients were diagnosed as suffering from classical Hodgkin's lymphoma.

All the lymph nodes were formalin fixed (10%) and paraffin embedded, sectioned at 4  $\mu$ m. They were offered kindly by Professor Elena Lazăr. Usual staining was performed with Hematoxylin–Eosin and PAS reaction.

We used DAKO reagents for immunohistochemical reactions. The visualization was performed by APAAP system using New Fuchsin as chromogen. For antigen retrieving we used Target Retrieval Solution (DAKO). The dilution for primary monoclonal antibody (clone BerH2) was 1/50. Levamisol adding is absolutely necessary for endogen alkaline phosphatase inactivating.

CD30 samples were appreciated semi-quantitative as follows: expression (+) – all the malignant cells positive; (+/-) – positive malignant cells 50–95%; (-/+) – positive cells under 50%; (-) – all the malignant cells negative.

## Results

From the whole 64 patients, 42 (66%) were females and 22 (34%) were males.

The age repartition is:

- 11–20 years, 15 cases (24%);
- 21–30 years, 13 cases (21%);
- 31–40 years, nine cases (14%);
- 41–50 years, 11 cases (17%);
- 51–60 years, seven cases (11%) and
- over 60 years, eight cases (13%).

We can observe that 45% (almost a half) from whole patients are young persons under 30 years.

From the clinical point of view, we performed the staging according to Costwolds classification. The majority of the patients, 36 cases (56%), were diagnosed in the stage III, 12 cases (19%) in the stage II, 12 cases (19%) in the stage IV, and only three (6%) in the stage I.

Histopathological diagnostic was performed using REAL/WHO classification. Using usual staining the lymph node architecture, we observed also the malignant cells morphology and characteristic of the reactive cellular background. Classical Hodgkin's lymphoma exhibited a diffuse pattern of growth, some atrophic or residual lymphoid follicles. Reed–Sternberg cells were clearly visible in all cases.

The cellular background was composed of small lymphocytes and a variable proportion of histiocytes, plasma cells and eosinophils. We could not correlate tissue eosinophilia abundance with clinical status because majority MC–CHL and NS–CHL cases revealed rich eosinophils and plasma cells infiltrate. Also, we could not identify Hodgkin and Reed–Sternberg cells in bone marrow aspirate.

From the whole patients, 32 cases (50%) were mixed cellularity (MC–CHL), 12 cases (19%) were nodular sclerosis (NS–CHL), 19 cases (29%) were lymphocyte depletion (LD–CHL); lymphocyte rich classic Hodgkin's lymphoma was not met.

Differential diagnostic with anaplastic large cell lymphoma was performed on histopathological aspect and CD30 expression. In Hodgkin's lymphoma was occurs a cytoplasmatic and Golgi area intense staining and in anaplastic large cell lymphoma (ALCL), the Reed–Sternberg-like cells was observed a cytoplasmatic diffuse staining (Figures 1 and 2).

Some of the Hodgkin's and Reed–Sternberg-like cells display an intense cytoplasmatic and Golgi area staining but differential diagnostic can be easy performed due to specific aspect and expression pattern of both lymphomas. In addition, the typically cohesive pattern of ALCL tumor cells can be better evidenced using CD30 expression. All these anaplastic large cell lymphoma cases were negative for CD15.

CD30 expression function to clinical stage was

contented by us in (+) interval for main of the cases with stage IV. The data from Table 1 show a CD30 expression increasing according to disease progression.

Thus, for stage I, the CD30 expression does not overcome (-/+) interval, majority as (-) and only a single case being in (-/+) interval.

For stage II, the CD30 expression extended to (+) interval in a low percentage (16%), predominating into (-/+) and (+/-) intervals.

For stages III and IV, the CD30 expression occurred only in (+/-) and (+) intervals, a single case with stage III being in (-/+) interval (Table 1).

**Table 1 – CD30 expression functions to the disease stage**

Clinical stage	CD30 expression			
	No. of cases (+)	No. of cases (+/-)	No. of cases (-/+)	No. of cases (-)
Stage I	–	–	1	2
Stage II	2	5	5	–
Stage III	11	24	1	–
Stage IV	7	5	–	–

Statistical analysis showed a significant distribution: degrees of freedom: 9,  $\chi^2 = 62.8676470588235$ ;  $p \leq 0.001$ .

All cases from (+) and (+/-) interval displayed an intense Hodgkin and Reed–Sternberg's staining, especially at Golgi area level.

Cases from (-/+) interval have few malignant cells from with intense CD30 expression but majority have a weak expression.

All cases from (-) interval display a weak CD30 expression in Hodgkin and Reed–Sternberg's cells.

From the histological point of view, we can observe that in CHL have not significant differences of one histological type in CD30 expression.

MC–CHL displayed a highest percent in (+/-) interval followed by (+) interval.

NS–CHL cases was predominated in (+) interval and LD–CHL in (+/-) interval.

Thus, we could not observe any association between the histological type and the CD30 expression (Table 2).

**Table 2 – CD30 expression functions to the histological type**

Histological type	CD30 expression			
	No. of cases (+)	No. of cases (+/-)	No. of cases (-/+)	No. of cases (-)
MC–CHL	9	21	2	–
LD–CHL	6	8	4	1
NS–CHL	5	5	1	1

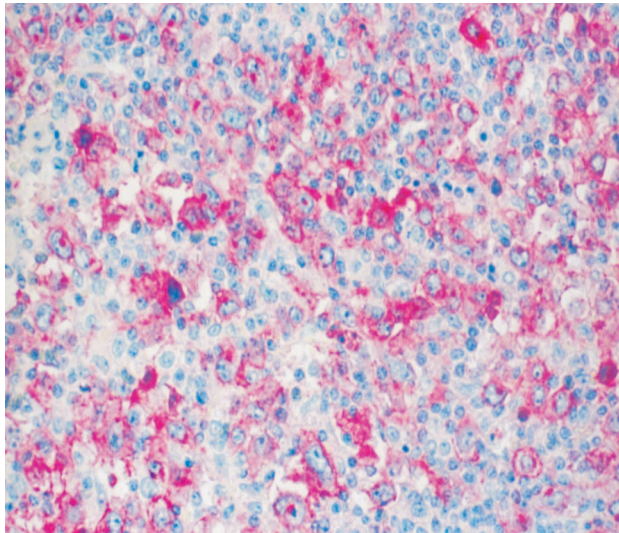
$\chi^2$  test showed a distribution that is not statistic significant: degrees of freedom: 6,  $\chi^2 = 6.87882643188854$  (for significance at the 0.05 level,  $\chi^2$  should be greater than or equal to 12.59);  $p \leq 1$ .

CD30 expression is displayed in the Figures 3–6.

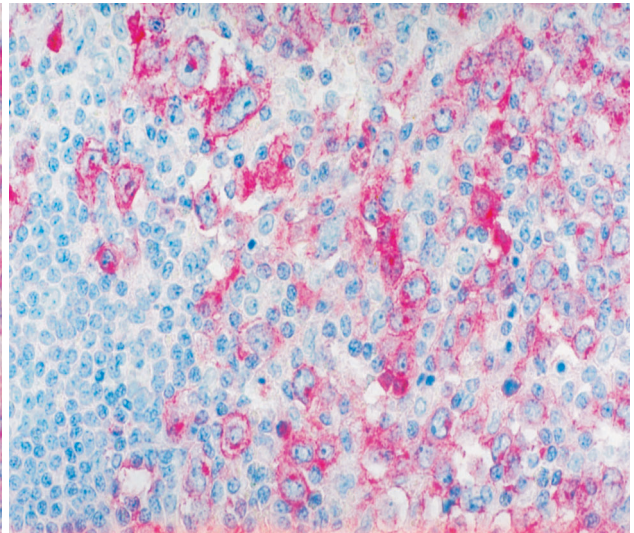
## Discussions

In the Vermeer MH *et al.* [8] study neither classical Hodgkin's disease does belong to (-) category and cases were only MC–CHL and NS–CHL from histological point of view.

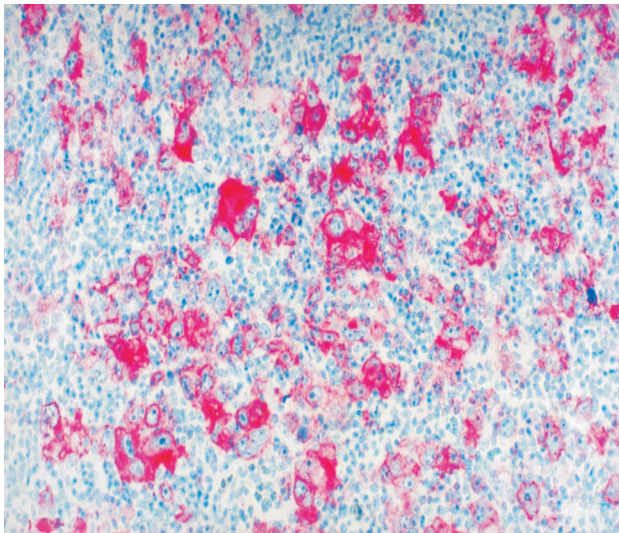




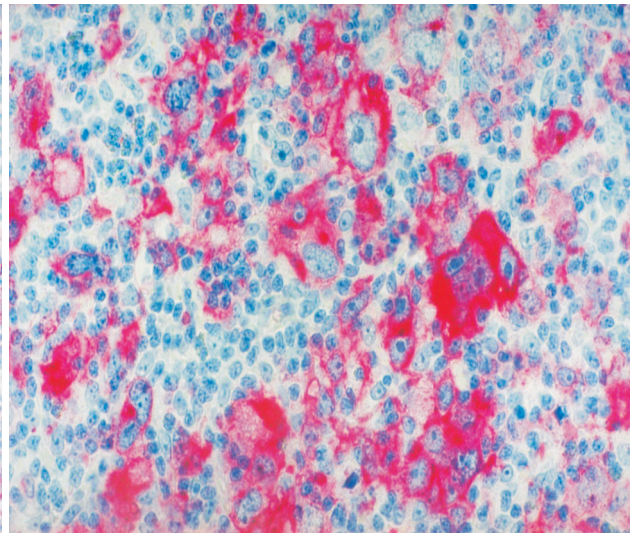
**Figure 1 – ALCL, CD30 expression intense positive at Reed-Sternberg-like malignant cells level (APAAP–New Fuchsin technique, ×400; private collection)**



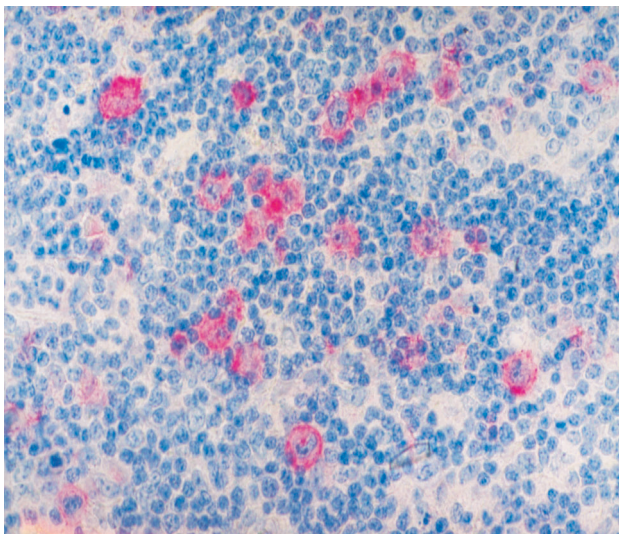
**Figure 2 – ALCL, CD30 expression intense positive at Reed-Sternberg-like malignant cells level (APAAP–New Fuchsin technique , ×400; private collection)**



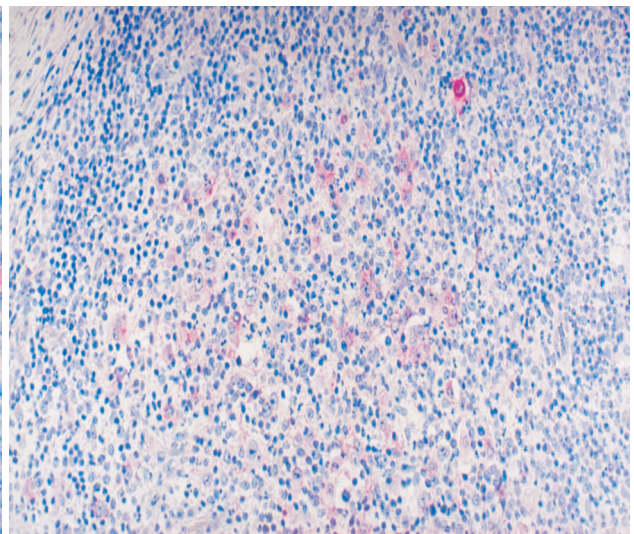
**Figure 3 – LD-CHL, with a (+) CD30 expression (APAAP–New Fuchsin technique, ×200; private collection)**



**Figure 4 – LD-CHL, with a (+) CD30 expression (APAAP–New Fuchsin technique, ×400; private collection)**



**Figure 5 – MC-CHL, with a (+/-) CD30 expression (APAAP–New Fuchsin technique, ×400; private collection)**



**Figure 6 – MC-CHL, with a (-/+) CD30 expression (APAAP–New Fuchsin technique, ×200; private collection)**



Watanabe K *et al.* [9] studied 51 classical Hodgkin's lymphoma cases, and they could not do any association in their study between histological type and CD30 expression because majority of cases are MC-CHL.

Regarding histological type, no connection we can be noticed between histological type and CD30 expression because we have not a significant predominance of one of them into one of the intervals.

Wasielowski S *et al.* [10] reported almost all studied cases with NS-CHD as CD30 positive (99% from cases) and they could not observe any correlation between this expression and disease evolution.

In our study, the CD30 expression increasing is associated with an advanced disease stage (stage III or IV).

Some authors [11, 12] explain the CD30 expression increasing in the same time with disease progression by capability of Hodgkin and Reed-Sternberg cells to inhibit activate T cells proliferation by CD30 interaction. The result of this interaction is an inefficient antitumoral immunity which promotes tumoral cells development and surviving.

Berge RL *et al.* [13] conclude that the percentage of activated cytotoxic T-lymphocytes present in tumor biopsies of CHL patients is a strong prognostic marker for unfavorable clinical outcome, independent of histological diagnosis or clinical characteristics.

Other authors [14–16] identified c-FLIP supra-expression (an apoptosis inhibitor mediated by FAS transmembranar receptor) in Hodgkin and Reed-Sternberg cells which have an intense CD30 expression. In their opinion, c-FLIP has a protective effect against apoptosis for these tumoral cells.

On the other hand, interaction between CD30 and its ligand (CD30L) seems to be crucial in proliferation process of Hodgkin and Reed-Sternberg cells [4].

CD30 binding to CD30L can induced malignant cell proliferation as well as initiate an antiapoptotic signal in these cells [17].

Also, we identified in many cases, mummified Hodgkin cell with an intense CD30 expression. These cells were described by Lorenzen J *et al.* [18] but they could not observe CD30 expression in malignant cells with classical signs of apoptosis.

We proposed for the patients which have a CD30 expression situated in (+/-) and (+) intervals and they are considered in incipient stages, should be reexamined and restaged because some lymph nodes from less accessible imagistic methods areas could be missed. Also, we recommend histopathological reexamination of them besides using other immunohistochemical markers for a differential diagnostic for the cases which we could not observe the CD30 expression but that have bulky lymph nodes above and beneath the diaphragm, with other organs invasion.

In our opinion, the CD30 expression must be investigate in all Hodgkin's disease cases for evaluate the disease progression. We say that because the main cause of relapses is inadequate staging, independent by clinical hematologist experience.

Although the co-expression of CD30 and CD15 is typical for CHL, it is also present in a subset of

peripheral T-cell lymphomas, including ALCL. Gorczyca W *et al.* study [5] found nine of 56 cases (16%) in their series showed a strong expression of CD15 and this subset of ALCL may be misdiagnosed as CHL, especially in small specimens. They recommend using of a broad of immunophenotypic panel in cases with limited material and/or those overlapping histological patterns which will best discriminate between CHL and ALCL in atypical large cell lymphoid neoplasms [5].

Society of Hematopathology and European Association of Hematopathologists recommends term "unclassifiable Hodgkin's lymphoma" when could not be differentiated a Hodgkin's lymphoma by anaplastic large cell lymphoma [19].

Because this expression was performed before the beginning of the treatment, our results values are independent by treatment schemes and depend only by tumor and by tumor effects against organism. In addition, 45% from our patients are young person under 30 years. That is why especially in this group, must be done a complex immunohistochemical examination for tumor aggressiveness before therapy starting.

## Conclusions

The increasing of CD30 expression occurs in the same time with the disease progression.

Due to the fact that the increasing of CD30 expression is associated with advanced disease stage, the cases that was included into an incipient stages and they have an CD30 expression situated in (+/-) and (+) intervals should be reinvestigated and restaged.

We recommend that because some lymph nodes could be overlooked and therapeutically attitude is different in advanced stages comparative with incipient stages.

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