# Reed-Sternberg cells in classical Hodgkin lymphoma in children seem to be predominantly oestrogen receptor α negative and oestrogen receptor β positive

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Oestrogen receptor α (ERα) is responsible for activation of gene transcription, while oestrogen receptor β (ERβ) serves as a negative regulator of ERα function. Since ER status is a prognostic and predictive factor in some cancers, we analysed the immunohistochemical expression of ERα and ERβ in Reed-Sternberg (RS) cells in paraffin-embedded lymph node specimens from 27 children with classical Hodgkin lymphoma (HL) in relation to histological type, clinical stage, age, and gender. Percentage of RS cells with positive nuclear reaction for the presence of ERα and/or ERβ was assessed. ERα positive RS cells were present in 11% (3/27) of lymph nodes (range 1-8%, mean 0.4%) whereas ERβ positive RS cells were detected in 96% (26/27) of lymph nodes (range 1-97.5%, mean 61.8%). The highest percentage of ERβ positive RS cells was observed in patients with the most advanced (IVB) disease as compared to patients with lower stages (90.3% vs. 56.9% respectively, $p = 0.004$). To the best of our knowledge this is the first report on the expression of ERβ in RS cells in children. We conclude that RS cells in classical HL in children seem to be mainly ERβ positive and ERα negative.

**Key words:** Hodgkin lymphoma, Reed-Sternberg cells, oestrogen receptor α and β.

## Introduction

Hodgkin lymphoma (HL) has an incidence of 5 per 100 000 children younger than 15 years of age and comprises 7% of all paediatric cancers in Poland [1]. Hodgkin lymphoma is divided into nodular lymphocyte predominance HL and classical HL type that is further subdivided into nodular sclerosis (NS), lymphocyte-rich (LR), mixed cellularity (MC) and lymphocyte depletion (LD) subtypes. Hodgkin lymphoma is characterized by the presence of diagnostic Reed-Sternberg cells (RS cells). RS cells are large with multiple nuclei or a single multilobulated nucleus, with large nucleoli about the size of a small lymphocyte. Several RS cell variants are recognized: mononuclear cells, lacunar cells, lymphohistocytic variants and pleomorphic RS cells [2].

Oestrogen receptors (ERs) are nuclear receptors. Two different ERs exist, namely ERα and ERβ, which show significant sequence homology. There is evidence that ERα is responsible for activation of gene transcription, while ERβ serves as a negative regulator of ERα function [3-6].

Both ERs are expressed in many normal tissues as well as cancers [7-10]. ERβ is the predominant ER in human leukocytes from peripheral blood, spleen and in leukocytes infiltrating cancers in both males and females. In tonsils, ERβ is expressed in lymphocytes of germinal centres and the follicular mantle zone as well as in granulocytes, while ERα is expressed only in activated germinal centres [8].

To date studies of ER expression in HL have been mostly limited to analysis of cell lines [8]. To the best

of our knowledge there is only one report on the analysis of expression of ERα but not ERβ in HL [11]. Since ER status is regarded as a prognostic and predictive factor in some cancers such as breast carcinoma, the purpose of this study was to analyse the immunohistochemical expression of ERα and ERβ in RS cells in paraffin-embedded lymph node biopsy specimens from children with classical HL in relation to parameters of known prognostic significance such as histological subtype, clinical stage as well as with age and gender of patients with classical HL.

## Material and methods

### Patients

This study included 27 patients (10 girls, 17 boys) aged 36–216 months (mean 148.4 months, median 156 months), diagnosed with *de novo* classical HL in the Clinic of Paediatrics, Haematology and Oncology, Pomeranian Medical University, Szczecin, Poland between January 2000 and December 2010. Diagnosis was based on histological examination of lymph nodes stained with HE, and confirmed by immunohistochemistry (IHC) using anti-CD15, anti-CD30, anti-CD45 and anti-CD20 antibodies. Clinicopathological characteristics of patients are given in Table 1. Twelve patients were diagnosed in stage II, 8 in stage III and 7 in stage IV. B symptoms were present in 14 patients. Histological subtypes included NS (n = 21) and MC (n = 6). All children were treated according to the HD97 Protocol modified by the Polish Pediatric Leukemia/Lymphoma Study Group [12].

### Immunohistochemistry

Formalin-fixed, paraffin-embedded 5 μm sections from lymph nodes were deparaffinized, rehydrated and immersed in pH 6.0 buffer. Heat-induced antigen retrieval was performed in a pressure cooker (Pascal, Dako, Denmark) at 120°C for 3 minutes. Slides were incubated with primary antibodies for 30 minutes at room temperature and immunostained with a Dako Envision+ kit for 30 minutes, AEC+ as a chromogen, haematoxylin as counterstain. Mouse monoclonal anti-ERα antibody (clone ID5, IgG1 isotype; dilution 1 : 50, DAKO) and anti-ERβ antibody (clone PPG5, Ig2a isotype; dilution 1 : 50, DAKO) were applied.

Normal mouse immunoglobulins were substituted for primary antibody as negative controls.

ERα and ERβ positive breast cancers served as positive controls.

RS cells were selected for analysis based on their characteristic morphological features. The percentage of RS cells with a positive nuclear reaction for the presence of ERα and/or ERβ was estimated in the entire slide using 400× magnification. Tumours were considered as ERα or ERβ positive if staining was detected in ≥ 1% of nuclei.

### Statistical analysis

Shapiro-Wilk test was used to evaluate the distribution of the percentages of ERα and ERβ positive RS cells. Since the ER distribution was abnormal non-parametric Mann-Whitney U test served to assess the relation between the percentages of ERα and ERβ positive RS cells and patients' age and gender as well as histological subtype and clinical stage of the disease. A p-value ≤ 0.05 was considered significant. Statistical analyses were performed with Microsoft Office Excel 2007 and Statistica 6.0 StatSoft Inc. software.

## Results

Table 1 lists the clinicopathological details of 27 lymph nodes and patients. IHC nuclear staining was assessed although in the majority of RS cells both nuclear and cytoplasmic expression was seen (Fig. 1).

ERα positive RS cells were present in 11% (3/27) of lymph nodes (range 1-8%, mean 0.4%). ERβ positive RS cells were found in 96% (26/27) of lymph nodes (range 1-97.5%, mean 61.8%).

In the vast majority of lymph nodes (22/27 = 81%) ERβ expression was found in over 10% of RS cells (Table 1).

In the group of patients with the most advanced (stage IVB) disease the mean percentage of ERβ positive RS cells was significantly higher than in the remaining patients (90.3 vs. 56.9; p = 0.004). The only patient with relapsed classical HL (patient no 12) was characterized by the highest (97.5%) percentage of ERβ positive RS cells. The expression of ERβ in RS cells did not correlate with age, gender, histological subtype and clinical stage (including the presence of clinical symptoms B) of the disease (Table 2).

## Discussion

We found ERβ positive RS cells in 96% of patients with classical HL. The percentage of ERβ positive RS was high, ranging from 1% to 97.5% (mean 61.84%). To the best of our knowledge this is the first report documenting the high percentage of ERβ positive RS cells in children with classical HL. On the other hand ERα positive RS cells were found in 11% of classical HLs, but the percentage of ERα positive RS cells was low (1-8%), and this is in agreement with a previously published study, revealing ERα expression in RS cells in 4 out of 41 (9.7%) adult cases [11].

**Table I.** Clinicopathological characteristics of 27 patients and expression of ERα and ERβ in Reed-Sternberg (RS) cells

| Patient No. | Gender | Age (years) | Histological type | Clinical stage | General symptoms | ERα(+) RS cells (%) | ERβ(+) RS cells (%) | Follow-up |
|---|---|---|---|---|---|---|---|---|
| 1. | M | 11 | NS | II | A | 0.00 | 1.00 | 1st remission |
| 2. | K | 9 | NS | II | A | 0.00 | 1.00 | 1st remission |
| 3. | M | 9 | NS | II | A | 0.00 | 37.21 | 1st remission |
| 4. | M | 12 | MC | II | A | 2.70 | 75.67 | 1st remission |
| 5. | K | 11 | NS | II | A | 0.00 | 84.00 | 1st remission |
| 6. | M | 17 | NS | II | A | 0.00 | 95.00 | 1st remission |
| 7. | M | 12 | NS | II | A | 1.00 | 97.30 | 1st remission |
| 8. | M | 11 | NS | II | B | 0.00 | 19.74 | 1st remission |
| 9. | M | 8 | MC | II | B | 0.00 | 34.61 | 1st remission |
| 10. | K | 16 | NS | II | B | 0.00 | 82.35 | 1st remission |
| 11. | M | 14 | NS | II | B | 0.00 | 88.88 | 1st remission |
| 12. | M | 14 | NS | II | B | 0.00 | 97.50 | relapse |
| 13. | K | 17 | NS | III | A | 0.00 | 0.00 | 1st remission |
| 14. | K | 7 | NS | III | A | 8.06 | 1.00 | 1st remission |
| 15. | M | 12 | NS | III | A | 0.00 | 59.09 | 1st remission |
| 16. | M | 3 | MC | III | A | 0.00 | 75.34 | 1st remission |
| 17. | M | 15 | NS | III | B | 0.00 | 15.00 | 1st remission |
| 18. | M | 16 | MC | III | B | 0.00 | 87.69 | 1st remission |
| 19. | K | 14 | NS | III | B | 0.00 | 90.63 | 1st remission |
| 20. | K | 17 | NS | III | B | 0.00 | 93.59 | 1st remission |
| 21. | M | 13 | NS | IV | A | 0.00 | 26.04 | 1st remission |
| 22. | K | 13 | NS | IV | A | 0.00 | 63.27 | 1st remission |
| 23. | K | 14 | NS | IV | A | 0.00 | 82.61 | 1st remission |
| 24. | M | 10 | MC | IV | B | 0.00 | 83.72 | 1st remission |
| 25. | M | 13 | NS | IV | B | 0.00 | 88.24 | 1st remission |
| 26. | K | 15 | NS | IV | B | 0.00 | 94.12 | 1st remission |
| 27. | M | 11 | MC | IV | B | 0.00 | 95.00 | 1st remission |

Classical HL is a lymphoid neoplasm. It has been shown that RS cells of classical HL originate from a germinal centre or post-germinal centre B cells [13] although they do not express most B-cell specific genes. Germinal centres are characterized by expression of both ERα and ERβ [8]. It is well known that the phenotype of RS cells in classical HL does not resemble any normal cell type in the body mainly because RS cells have lost expression of many B cell markers (despite their germinal centre B cell origin), and have acquired markers typical of myelocytic differentiation such as CD15, cytotoxic T cell/NK cell differentiation such as granzyme B, and dendritic cell differentiation such as specific chemokine TAR [14]. Taking into account the abnormal expression of antigens by RS cells, the prevailing ERβ expression in RS cells might be considered as an additional aberrant feature present in these cells [8, 13]. The effect of oestrogens on the immune system is determined by a balance between ERα and ERβ signalling [9]. Activation of ERα promotes proliferation by regulating numerous cell cycle genes whereas activation of ERβ inhibits proliferation, and promotes apoptosis and differentiation [9, 15]. It is known that activation of ERβ represses growth of the immune system by the following mechanisms: ERβ is a negative regulator of B cell lymphogenesis in bone marrow [8] and ERβ is required for oestrogen-mediated thymic cortex atrophy [16]. Oestrogen treatment of human monocytes, which only express ERβ, induces apoptotic cell death [8]. However, the presence of ERβ in RS cells in our study as well as in human lymphoma cell lines including HL (L428, L540, L1236), Burkitt lymphoma, and multiple myeloma (LP-1) which are ERβ positive and ERα negative [8], suggests that ERβ may promote cell growth. It has been observed that approximately 50% of ERα negative breast cancers express ERβ. In contrast to ERα positive breast cancers where ERβ has anti-proliferative activity, in ERα negative breast cancers ERβ positivity seems to cor-

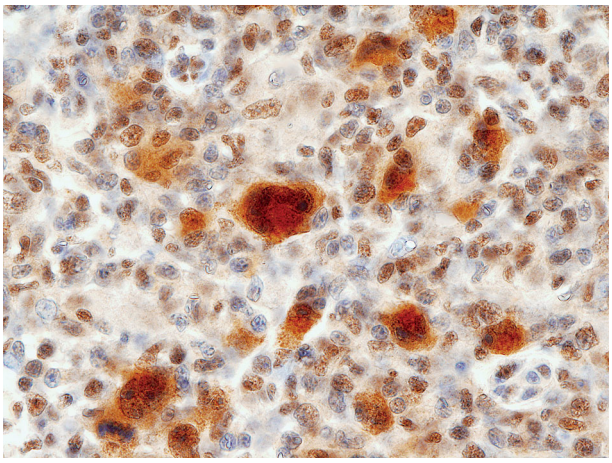

**Fig. 1.** Immunohistochemical expression of ERβ in RS cells. Note the nuclear and cytoplasmic positive staining in RS cells. Some mononuclear cells in the background also reveal ERβ nuclear expression

**Table II.** Expression of ERβ in RS cells in relation to age, gender, histological type and clinical stage and clinical symptoms

| Parameter | ERβ positive RS cells (mean %) | p |
|---|---|---|
| Age | | |
| < 14 years | 52.6 | NS |
| ≥ 14 years | 75.2 | |
| Gender | | |
| F | 59.3 | NS |
| M | 63.4 | |
| Histological type | | |
| NS | 57.9 | NS |
| MC | 75.3 | |
| Clinical stage | | |
| II | 59.5 | NS |
| III | 52.8 | |
| IV | 76.1 | |
| Clinical stage | | |
| IVB | 90.3 | |
| Remaining patients | 56.9 | 0.004 |
| Clinical symptoms | | |
| A | 49.9 | NS |
| B | 74.7 | |

relate with the proliferation marker Ki-67 [15]. The biological explanation for this discrepancy is not clear. It is proposed that mitogen-activated protein kinases phosphorylate the ERβ AF-1 domain which increases the recruitment of steroid receptor co-activator 1 (SRC-1) thereby causing enhanced transcriptional activity [14]. However, alterations in the interaction between ER and the transcription factor NFκB signalling pathways may also be involved, because activation of NFκB is a common event in classical HL. Reciprocal inhibitory cross-talk exists between NFκB and ER [17, 18] and DNA-binding ability of NFκB is affected differently by ERα and ERβ [19]. The relationship between ER and NFκB may be cell-type specific and positive as well as negative interactions between ER and NFκB signalling pathways may exist [20]. Clearly, further studies are necessary to determine the molecular background of the interactions between ER and NFκB pathways in RS cells.

We conclude that RS cells in classical HL are predominantly ERβ positive and ERα negative. However, it is worth noting that the mean percentage of ERβ positive RS cells in lymph nodes of all four patients with stage IVB disease was significantly higher than in the remaining patients. Moreover, the only patient with classical HL who relapsed had the highest percentage of ERβ positive RS cells even though he presented with less advanced (stage IIB) disease at diagnosis. These observations may suggest an association of high percentage of ERβ positive RS cells with poor prognosis of children with classical HL. However, further studies on large groups of patients are needed to reveal the clinical utility of the results of our study, i.e. the assessment of the impact of antioestrogenic drugs on RS cells in tissue culture and subsequent prospective studies on ER targeted therapy in patients with relapsed or refractory classical HL.

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