Haematologica 1999; 84:298-305 original paper

# Genomic instability and recurrent breakpoints are main cytogenetic findings in Hodgkin's disease

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ABSTRACT

***Background and Objective.*** **Successful cytogenetic studies in Hodgkin's disease (HD) are rare, and, except for hyperdiploidy, no chromosome changes typical for this disorder have been described. The purpose of this study was to collect cytogenetic information from a new series of lymphoid neoplasms diagnosed either as classical HD or as Hodgkin's-like anaplastic large cell lymphoma (HD-like ALCL), according to the REAL Classification.**

***Design and Methods.*** **We studied 27 cases of HD and 10 cases of HD-like ALCL. Cytogenetic investigations were performed on lymph nodes (35 cases), bone marrow or pleural effusion. A large screening of slides was performed to detect abnormal metaphases despite the low mitotic index of Reed-Sternberg cells. In addition to ours, available published data were analyzed in detail to identify recurring cytogenetic events.**

***Results*****. Metaphases which could be analyzed were obtained in 86.5% of cases, with 59.4% showing abnormal clones. We found a peculiar kind of cytogenetic instability in which, despite variations in the type of structural rearrangements, chromosome breakpoints were non-randomly distributed. Moreover, from our data plus those collected from literature on HD (total 177 cases), the number of breakpoints was higher in patients in a more advanced clinical stage.**

***Interpretation and Conclusions.*** **Cytogenetic studies in HD are highly informative regarding clonality, provided large numbers of metaphases are examined. Based on karyotype, genetic changes in HD and HD-like ALCL are similar. Results are consistent with a high degree of chromosomal instability and predominance of hyperdiploid complex karyotypes. Chromosome breakpoints are non-randomly distributed and more numerous in advanced clinical stages.**


Keywords: Hodgkin's disease, Hodgkin's-like ALCL, chromosome breakpoints, clonality, staging

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Hodgkin's disease (HD) is a disorder well characterized in terms of clinical and histopathologic findings. Some biological aspects, however, are still lively debated. For example, clonal identification as well as the lineage affiliation of the typical neoplastic element, i.e., the Reed-Sternberg cell, have not yet been definitely established. Results on immunoglobulin gene rearrangements using single cell PCR are controversial, in that mono-, oligo-, and polyclonality of Reed-Sternberg cells have been shown.[1-3] An interesting approach combining FISH and mutation analysis of p53, strongly supported the monoclonal nature of Reed-Sternberg cells obtained from different tissues of the same patient.[4] Classical cytogenetics has not contributed much in the past, mainly because of difficulties in obtaining a sufficient number of mitoses from Reed-Sternberg cells. After a careful search of the literature starting from 1976, we found 163 documented cases of HD with clonal karyotypic aberrations (the complete list of references is available upon request to the authors). These figures are strikingly low especially when compared with those of non-Hodgkin's lymphomas from which thousands of cases are available. Nevertheless, recent papers[5-7] suggested a clustering of chromosome breakpoints in Hodgkin's disease, and also confirmed earlier observations on the high frequency of polyploidy among the abnormal karyotypes. Refined analysis by FISH also focused on increased chromosome content in cells expressing the CD30 antigen, namely Reed-Sternberg cells.[8,9]

The aim of our study was to add information on the cytogenetics of Hodgkin's disease by setting up short term cultures of infiltrated tissues and performing a large screening of slides to check clonality despite a low mitotic index. Since typical HD and the so-called HD-like anaplastic large cell lymphomas (HD-like ALCL) were kept separate in the recently formulated REAL classification,[10] we analyzed the cytogenetic results of these two groups separately, in order to investigate whether cytogenetic changes are helpful in identifying distinct entities.

## Design and Methods

### Selection of cases

Thirty-seven cases with a histologically confirmed diagnosis of classical HD (27 cases) or HD-like ALCL (10 cases) were selected from 201 consecutive specimens investigated cytogenetically in our laboratory from January 1991 to May 1995.

Fresh material was divided into pieces for histologic, immunohistochemical, and cytogenetic investigations. Staging was made according to the Ann Arbor criteria.

### Histopathology

Tissue sections were stained with hematoxylin-eosin for histologic evaluation. Immunophenotyping was performed on paraffin embedded material and additional cryostatic tissue sections by means of an alkaline phosphatase anti-alkaline phosphatase immunostaining method (APAAP). Monoclonal antibodies were applied to detect the expression of activation antigens (CD30, CD25), B (CD19, CD20, CD22) and T (CD3, CD4, CD8) lymphoid antigens, the granulocyte-associated antigen (CD15), the leukocyte common antigen (CD45), macrophage markers (BeMac3, CD68) and the proliferation associated antigen Ki-67.

### Cytogenetic studies

Lymph node biopsies were minced in a Petri dish using a sterile scalpel. The samples available were bone marrow in one case (case #27) and pleural effusion in another (case #6). Cell suspensions were incubated for 16-24 hours at 37°C in RPMI 1640 medium supplemented with 20% fetal bovine serum, 1% L-glutamine, and antibiotics. Direct preparations were also set up when material was available. Before harvesting, cells were exposed for 10 min to colcemid.

Metaphases were G banded with Wright's stain and karyotyped according to ISCN (1995).[11]

### Data analysis

Clinical and cytogenetic data obtained from our HD patients were included in a database and pooled with HD cases with a documented karyotype available in the literature from 1976. A karyotype was assumed to have been documented when published as a figure, or described in detail in tables or text. Numerical chromosome gains and losses were considered only from near diploid (up to 58 chromosomes) cells. Clonal and non-clonal structural aberrations were recorded separately. Following karyotypic description, breakpoints involved in translocations, deletions, inversions, and duplications were attributed to the p or the q chromosome arms, and to identified chromosome bands. Isochromosomes, as well as derivative chromosomes with no further detail were included in the database, but those abnormalities were not assigned to a specific chromosome arm or band. Unidentified marker chromosomes were not computed. Identical chromosome breakpoints involved twice or more in complex karyotypic aberrations were computed only once per case.

## Results

### Selection of cases

We analyzed samples from 26 males and 11 females (M:F ratio = 2.36), aged between 9 and 85 years (mean age = 33). The patients' data and clinical stage are shown in Table 1 and Table 2.

The majority of cases were investigated at diagnosis. However, in the Hodgkin's group all three lymphocyte predominant (LP) patients (#10, #21, #23), one case (#34) of nodular sclerosis (NS) and one (#27) of mixed cellularity (MC) subtypes were studied at "relapse", occurring 10, 28, 26, 1, and 15 years after initial diagnosis, respectively.

In the HD-like group, four patients (#6, #18, #20, #30) were investigated in the early phase of disease (12-18 months after diagnosis), whereas one patient (#3) was studied 33 years after his Hodgkin's disease had been diagnosed.

### Histopathology

Twenty-seven lymph nodes showed features of one of the classical HD subtypes: LP (3 cases), NS (14 cases), MC (10 cases).

Ten lymph node specimens showed lymphocyte-depleted areas with an abundant and/or pleomorphic tumor cell population resembling RS-cells in confluent sheets with a cohesive growth pattern. Hence, showing intermediate characteristics between HD, mainly nodular sclerosis, and ALCL, these cases were considered as HD-like ALCL.

In every case abnormal cells clearly showed CD30 positivity. CD25 was tested and positive in cases #3, #8, #13, #14, #15, #16, and #22. CD68 and BerMac3 expression was never detected.

A B-cell phenotype was found in cases #10 and #23 with LP subtype, in the NS case #36, in MC cases #17 and #35, and in case #6 of the HD-like group. All other cases showed a null phenotype.

### Cytogenetics

The results are shown in Tables 1 and 2.

Karyotypic investigations were successful in 32 cases (86.5%), while in one MC (#8), two NS (#14; #36), and two HD-like ALCL (#2; #18) samples no mitoses were found.

Apart from HD case #23 and HD-like ALCL cases #3, #6, #15, normal cells always exceeded the abnormal ones. Only normal karyotypes were found in one sample of NS (#13) and one sample of MC (#1) with few available metaphases (5 and 10, respectively).

Clonal aberrations were found in nineteen biopsies (59.4%); 3/3 LP, 5/9 MC, 6/12 NS and 5/8 HD-like ALCL cases.

The mean number of cells analyzed was 58.4 (range 15-170) in HD and 38.4 (range 12-117) in HD-like ALCL specimens. In eleven specimens (34.3%), com-

**Table 1. Clinical and cytogenetic results in cases of Hodgkin's disease.**

| *Case* | *Age/Sex* | *Stage* | *Ploidy* | *Karyotype** |
|---|---|---|---|---|
| | | | | **Lymphocytic predominance** |
| 10 | 60/M | IVA | 2n | **46,XY,del(2)(p21p24) [2]** /<br>46,XY [25] |
| 21 | 43/M | IIA | 2n/4n+/2n | **46,XY,t(1;3)(p22;q13) [1] 96,XXYY,t(1;3)(p22;q13)x2,+4mar [2] /**<br>46,XY,del(10)(q24) [1] 46,XY,add(2)(q37),t(9;10)(p22;q24) [1] 46,XY,-15,add(17)(p11),+mar [1] ¬<br>46,XY,del(17)(p11) [1] 46,XY,t(5;19)(p13;q13) [1]<br>46,XY,t(1;14)(p11;p11),del(12)(p12) [1] 46,XY,t(2;12)(q33;q22) [1] 46,XY,t(11;14)(q23;q24) [1] ¬<br>46,XY,t(6;13)(q21;p11) [1] 46,XY,t(8;22)(p11;q13) [1]<br>46,XY [30] |
| 23 | 43/M | IA | 2n+/3n+/2n- | **47-57,XY,+X,i(1)(q10),+2,t(3;**22)(**q27**;q11),**dup(7)(q11q22),trp(7)(q11q22),+8,+11,+12,**add(15)(p?), ¬<br>**+20,**add(20)(p13),**+21,+22,+mar [cp12]**<br>**62-80,XXY,+Y,+1,i(1)(q10)x2,+2,**del(2)(q11),**t(3;5)(q27;q15)x2,-4,del(4)(q24),+5,**del(5)(q14q32),¬<br>**del(6)(q?),del(7)(q32),trp(7)(q11q22),add(10)(q23),+11,**del(11)(q23),**+12,+13,+16,+17,+19,+20,+21, ¬<br>**+22,+mar [cp8]** / 45,XY,der(?)t(12;13)(q?;q?) [1]<br>46,XY [2] |
| | | | | **Nodular sclerosis** |
| 5 | 18/M | IIA | 4n- | **80-82,XXYY,-1,+der(2)t(2;?;12)(q32;?;24),**del(3)(q21),-4,add(5)(p15),del(5)(q31q34),**-6,dic(6;9)(q15;q22),** ¬<br>**del(6)(q15),+del(7)(q22q32),-8,-8,i(8)(q10),-9,**dic(**9**;16)(**q22**;q22),add(10)(p13),**-11**,add(12)(q22), ¬<br>**der(12)t(2;?;12)(q32;?;24),-13,**add(13)(p?),**-14,add(15)(p11),-16,-17,-18,-20,-21,+mar [cp6]** /<br>46,XY [18] |
| 9 | 19/F | IA | 2n/4n+ | 46,XX,add(4)(p?) [1] 93,XXXX,-2,-6,-6,-10,+21,+4mar [1]<br>95,XXXX,+X,+X,t(2;4;10)(q14;q24;q25)x2,-13,add(13)(p11),der(14)t(14;19)(p11;q11),¬<br>+16,+18,+18,-19,+20,-22 [1]<br>46,XX [14] |
| 12 | 17/M | IIA | 2n+/4n- | **55,XY,+1,add(1)(p36),**+der(1),-6,**+7**,+7,**inv(7)(q31q34),**-9,**+der(11)t(6;11)(q15;q23),der(11)t(11;13)** ¬<br>**(q23;q15),**-13,+14,-15,-15,+16,+16,+16,+17,+19, +20,+21,+22 **[1]**<br>**82,XXYY,**-1,**add(1)(p36)**,-6,**+7,inv(7)(q31q34)**,-8,-8,+9,**der(11)t(6;11)(q15;q23),der(11)t(11;13)(q23;q15)**, ¬<br>-12,-12,-13,-13,-13,-14,-14,add(17)(p?)x2,-21 **[1]** /<br>46,XY [13] |
| 13 | 35/M | na | | 46, XY [5] |
| 14 | 19/M | IIIA | | no mitoses |
| 16 | 22/F | IIIB | 2n/2n/4n- | **46,XX,**add(1)(p22),**del(4)(q25q33),add(20)(q13) [cp5]** / 46,XX,del(10)(q22) [1] 91,XXXX,¬<br>-1,+2,-3,-4,+6,-8,-8,-10,+11,+12,-14,-15,+16,+19,-21,-22,+3mar [1]<br>46,XX [21] |
| 19 | 44/F | IIA | 2n/3n+ | 46,X,-X,+mar1 [1] 46,XX,-10,+mar2 [1] 46,XX,del(6)(q?) [1] 72,XX,-X,add(1)(p36),+4mar [1]<br>46,XX [18] |
| 22 | 16/F | IIA | 2n+/2n+ | **47,XX,+X,** del(6)((q13q16),del(16)(p11) **[cp2]** / 47,XX,+mar [1]<br>46,XX [62] 92,XXXX [1] |
| 25 | 25/M | IIA | 2n/4n- | 46,XY,add(4)(q?)1 [1] 46,XY,add(4)(q?)2 [1] 89,XXYY,+12,-15,-17,-22,-22,-22, +mar [1]<br>46,XY [23] |
| 26 | 22/M | IIIA | 2n+/4n-/2n- | **48,XY,del(1)(q11),+i(1)(q10)x2 [1]**<br>**91,XXYY,+del(1)(q11),i(1)(q10)x2**,+2,+2,del(2)(p?)x3,add(2)(p?)x2,-3,del(3)(q11)x2,add(4))(q?),del(4)(q?), ¬<br>+add(9)(p12)x2,-11,-11,-12,-12,-13,-19, -19,add(19)(p?),-20,--21,-22,-22,+6mar [1] / ¬<br>39,Y,-X,der(1;?;3)(1pter->q31::?::3q21->3pter),-4,-9,-15,-19,-21 [1] 45,X,-Y,-5,+7,+7,-8,-20,+mar [1]<br>46,XY [37] |
| 31 | 12/M | IIB | 2n | 46,XY,del(6)(q?) [1]<br>46,XY [59] |
| 34 | 42/M | IVB | 2n+ | 48,XY,+2,+5 [1]<br>46,XY [79] |
| 36 | 67/M | IVB | | no mitoses |
| 37 | 18/F | IIIA | 3n-/4n- | **59-83,XXX,-X,i(X)(p10),-1**,del(1)(q?),**+2,add(2)(q37)x3,+3**,del(3)(q26),**-6,del(7)(q12q22),**del(7)(q35),**-8,** ¬<br>**del(8)(q24),-9,-10,-11,-11,del(11)(q12q13),+12,-13,-13,-14, -15,-16,-17,-17,-18,+20,del(20)(q11q13),** ¬<br>**add(20)(q13),-21,+4mar [cp6]** /<br>46,XX [164] |

*M, male; F, female; na, not available. *clonal aberrations are typewritten in **bold** characters.* *(continued on next page).*

**Table 1. (continued) Clinical and cytogenetic results in cases of Hodgkin's disease.**

| *Case* | *Age/Sex* | *Stage* | *Ploidy* | *Karyotype** |
|---|---|---|---|---|
| | | | | **Mixed cellularity** |
| 1 | 55/M | IVB | | 46,XY [10] |
| 4 | 44/F | IIIA | 2n-/2n/2n+ | **45,XX,-22 [3]/ 46,XX,dupcen(9) [2] / 47,XX,add(9)(p?),+del(17)(p?) [3] /** 46,XX [13] |
| 8 | 22/M | IIA | | no mitoses |
| 17 | 42/M | IVB | 3n+ | **66-78,XX,-Y,del(X)(q25),+1,del(1)(p21)x2,+2,+2,add(2)(q?)x2,**del(2)(p11),**add(3)(p?),add(3)(q?),der(3),** ¬ **+add(4)(q?),del(4)(q?)x2,+5,add(5)(p15)x2, der(6)t(6;11)(q21;q13)x2,add(7)(p?),**add(7)(q?),**+der(7;?5;10),** ¬ **del(7)(q?),der(7)t(5;7)(q11;q31),-8,**+9,dic(9;16)(q22;p12),**der(9)t(9;?;21)(q31;?;q11)**,i(9)(q10),+10, ¬ **add(10)(q25),add(10)(p?),add(11)(q23),del(11)(q?),add(11)(p?),add(12)(p13),+i(12)(p10),+13,** ¬ **add(13)(p11),add(14)(p11)x2, der(15)t(9;15)(p10;p10),+der(15)t(12;15)(p10;p10),+add(16)(q21),** ¬ **der(17)t(3;17)(p11;p11)add(17)(p13),add(17)(q25)x2,+18,**del(18)(p?),**add(18)(p11),+19,**+20,**-21,-22 [cp7]** / 46,XY [22] |
| 24 | 24/M | IVB | 2n/2n+/3n+/4n- | **47,XY,del(6)(q?),+8,-9,+20 [cp5]** **74-91,X,-X,-X,-Y,+del(2)(p11),-3,del(4)(q27q33),i(4)(p10),+del(5)(q33),** del(6)(q?),**der(8)t(8;15)(q11;q11),**¬ **+9,-11,+del(12)(q11),-13,+14,add(16)(q13), -18,-19,+3mar [cp2]** / 45,XY,+der(2),-8,-12 [1] ¬ 46,XY,t(X;12)(q11;q11),del(3)(p?) [1] 46,XY,-8,+mar [1] 47,XY,+3 [1] 46,XY,add(11)(q?) [1] 46,XY [52] |
| 27 | 50/M | IIIA | 2n/3n- | 46,XY,i(1)(q10) [1] 46,XY,add(10)(q24) [1] 63,too complex [1] 46,XY [110] 92,XXYY [1] |
| 28 | 21/M | IIIA | 2n+/2n | **47,XY,+del(7)(q?)** [6] / 46,XY,del(1)(q11) [1] 46,XY [23] |
| 32 | 15/M | IIIA | 2n/2n+/4n- | 46,XY,t(2;14)(p11;q32),del(13)(q?) [1] 48,XY,-2,+der(2)t(2;2)(p11;q12),add(7)(q36),+8,del(11)(q14q23), ¬ -14,add(15)(p11),-16,del(17)(p?), +3mar [1] 89,XXYY,del(2)(p11),poor quality [1] / 46,XY,add(1)(p36) [1] 46,XY [155] |
| 33 | 59/M | IIA | 4n- | 87,XXY,-Y,+2,-3,-4,-5,-5,+8,del(12)(q11),-14,-15,-15,add(15)(p11)x2,add(17)(q?),-18,-21,-21,-22,+5mar [1] ¬ 46,XY [72] 92,XXYY [2] |
| 35 | 85/F | IIIA | 2n-/4n-/2n | **45,XX,add(1)(p36),**+11,-16,**-19** [1] **79-82,XXX,-X,add(X)(q26),+1,add(1)(p36)x2,del(3)(q?),add(3)(q?)x2,**del(4)(q?),add(5)(q35),**-6,del(6)(q21q25),** ¬ **del(7)(q?)x2,+8,**del(8)(p12),**-9**,del(9)(q11)x2,i(9)(q10),**-11,+del(12)(q24),**+i(12)(p10),**add(13)(p11)x2,-14,** ¬ **-14,+der(14;15)(p10;p10),-15,-15,**add(15)(p11),**-17,-18,-19,**del(20)(q?),**-21,-22,-22 [cp4]** / ¬ 46,XX,del(3)(q21) [1] 46,XX,t(2;14)(p24;q11) [1] 46,XX,add(5)(p15) [1] 46,XX [144] |

*M, male; F, female; na, not available. *clonal aberrations are typewritten in **bold** characters.*



prising three MC, five NS, and three HD-like ALCL cases, only non clonal abnormalities were present.

Some patients had been previously treated: 3/14 HD (#10, #21, and #23) and 2/5 HD-like ALCL cases (#3 and #6) with clonal aberrations as well as 1/8 HD (#34) and 2/5 HD-like ALCL (#20 and #30) cases with single cell aberrations.

*Hodgkin's disease*. Of the fourteen cases with clonal changes, seven had a diploid or hyperdiploid modal chromosome number and five were tri-tetraploid. The modal number could not be defined in two other cases (#12 and #26) with an equal mixture of hyperdiploid and polyploid cells.

Numerical aberrations were mostly gains. Trisomies for chromosome X, 8, and 20 each occurred in two cases. Unrelated clones were detected in one MC case (#4), one of which exhibited monosomy 22 as the only aberration.

Concerning structural abnormalities, 12/14 HD cases showed deletions, 10/14 translocations, 6/14 isochromosomes, and 2/14 other aberrations (inversions, duplications). All chromosomes except Y, 19, and 22 were involved. The most frequent structural changes were deletions at 7q and 6q. Recurrent breakpoints were identified in three cases at bands 7q22 and 15p10-11, while bands 1p36, 4q33, 6q15, 6q21, 7q31, 7q32, 11q13, 11q23, 12q24, 13p11, 14p10-11, 17p11, and 20q13 were involved twice. Diploid and polyploid metaphases displaying identical structural changes were observed in each of six cases (#12, #21, #23, #26, #24, and #35). In case #37 a del(7)(q12q22) was found in near-triploid and near-tetraploid cells.

**Table 2. Clinical and cytogenetic results in cases of Hodgkin's-like ALCL.**

| *Case* | *Age/Sex* | *Stage* | *Ploidy* | *Karyotype** |
|---|---|---|---|---|
| 2 | 17/M | IIA | | no mitoses |
| 3 | 46/M | IVB | 2n+/3n+/2n | **56,XY,**+1,**del(1)(p32),add(2)(p23),**+3,**+3,t(3;7)(p21;q22),add(4)(q?)**,-5,**+6**,+7,-8,**-8,+9**,+10,**+del(11)(q12),** ¬ **add(11)(q24),**+14,**-15**,+17,+20,**del(20)(q11),+r,+2mar [1]** **65-78,XXY,+Y,del(1)(p32),add(2)(p23)x2,+3,t(3;7)(p21;q22), add(4)(q?),+del(4)(q13),+add(5)(q35),** ¬ add(5)(p15),**+6,del(7)(q11),-8,-8,+i(8)(q10),+9,+add(9)(q22),+del(11)(q12),add(11)(q24),-13,-15,** ¬ **del(16)(p12),del(20)(q11),+r,+3mar [cp8]** / 46,XY,del(1)(q21q32) [1] 46,XY [8] |
| 6 | 19/M | IVB | 2n-/2n+/4n+ | **45-51,XXY,+X,**+1,add(2)(q37),**add(2)(q?),i(7)(q10)**,add(7;)(q?),add(8)(q24),+12,add(12)(q24), del(16)(q13q22), ¬ **del(20)(q?),+2mar [cp4]** **87-99,XXXYY,+X,**add(1)(p36),del(2)(p?),del(2)(q23),**add(4)(q34q35)x3**,+5,**-6,+i(7)(q10)x2,**del(7)(q11),**+8**, ¬ t(9;9)(q22;q34), **+i(10)(q10),**+11,**del(11)(q21q23),add(11)(p13),+12,**+del(12)(q15q22),**-16**,del(16)(p?), ¬ +del(17)(p?),**+add(17)(q?),add(18)(q?),-19,-19,+del(20)(q?)x2,-21,+3mar [cp8]** / 46,XY [2] |
| 7 | 20/F | IIB | 3n- | **63-66,XX,-X,del(1)(p13),del(1)(q32q43),add(2)(q32)**,add(3)(p27),-4,-7,der(15),del(22)(q11) **[cp2]** 46,XX [25] |
| 11 | 73/F | IVB | 2n+ | 47,XX,+18 [1] 46,XX [29] |
| 15 | 25/M | IVB | 2n+/4n-/4n+/2n | **46-48,X,-Y,+2,+del(6)(q25),+9,**add(9)(p?),**+15,add(17)(q?),**i(17)(q10),**+19 [cp6]** **85-97,XXY,-Y,+2,del(2)(p24),**+5,**+del(6)(q25),+9,+9,**add(9)(p?),**add(17)(q?),+19 [cp4]** / 46,XY,add(9)(p?) [1] 46,XY [7] |
| 18 | 9/M | IIB | | no mitoses |
| 20 | 25/M | IIIB | 2n-/2n/2n+ | 43,XY,add(3)(q2?1),-12,-19,-20 [1] 45,XY,add(2)(q?),-18,del(22)(q13) [1] 45,XY,add(15)(q26),-17 [1] 45,XY,der(3), ¬ -10 [1] 46,XY,t(3;14)(q25;q11) [1] 46,XY,add(15)(p11) [1] 46,XY,del(9)(p22) [1] 46,XY,del(19)(q?) [1] 46,XY,der(5)t(1;5)(q25;q31) [1] 47,XY,+mar [1] 46,XY [61] |
| 29 | 23/F | IIA | 2n+ | **48,XX**,del(5)(q?),add(9)(p?),**del(11)(q21q23),+18,**+mar [4] / 46,XX [113] |
| 30 | 27/F | IVB | 2n/4n- | 46,XX,-9,+mar [1] 46,XX,+11,-21 [1] 86,XX,-X,-X,del(1)(p13),-2,del(3)(p?),add(4)(q?)x2,add(5)(q35),-6,add(7)(p?),-8,del(8)(q22),-9,-9,-10,add(10)(q26), ¬ del(11)(q21q23)x2,+del(11)(q21q23)x3, del(12)(q24),-13,del(13)(q12q14),-14, add(14)(q32)x2,-15,-15,-16, ¬ del(16)(p?),del(16)(q?),-21,-22,-22,+9mar [1] 46,XX [9] |

*M, male; F, female; na, not available. *clonal aberrations are typewritten in* ***bold*** *characters.*



In two cases (#21, #24) a different type of structural rearrangement was observed in single cells, however those dissimilar aberrations shared at least one identical chromosome breakpoint. In case #21 a breakpoint at 10q24 was affected by a single deletion in one cell and by a reciprocal translocation in another one. Furthermore chromosome 17, at band p11, was deleted in one cell and was involved in a translocation in a second cell. In case #24 band 12q11 was involved in a translocation with chromosome X in a diploid cell and affected by a deletion in tri-tetraploid cells. Another case (#32), not fulfilling standard criteria for clonality, showed the same band 2p11 being implicated in a so-called *jumping translocation* with chromosomes 2 and 14 in two diploid cells, and in a deletion in a near-tetraploid cell. Other jumping translocations were seen in cases #5, #17, and #23, involving 9q22, 15p10 and 3q27, respectively.

*HD-like ALCL.* Five cases with clonal chromosome changes were identified. Karyotypes exhibited a diploid modal number in two cases and polyploid in three. Among numerical aberrations, trisomy 6 and trisomy 9 were observed twice. All cases with abnormal clones showed deletions, four out of five translocations, and two cases presented isochromosomes.

Structural clonal abnormalities did not involve chromosomes X, Y, 12, 19 or the acrocentrics (#13, #14, #15, #21, #22).

In cases #6, #15, and #3 identical clonal aberrations were present in diploid, near-diploid, and polyploid karyotypes. Chromosome 11, at band q23, was affected in two patients (#6 and #29).

### *Data analysis*

Cytogenetic and available clinical data from 177 HD cases were examined. The 177 cases comprised the 14 classical HD from this series and 163 cases

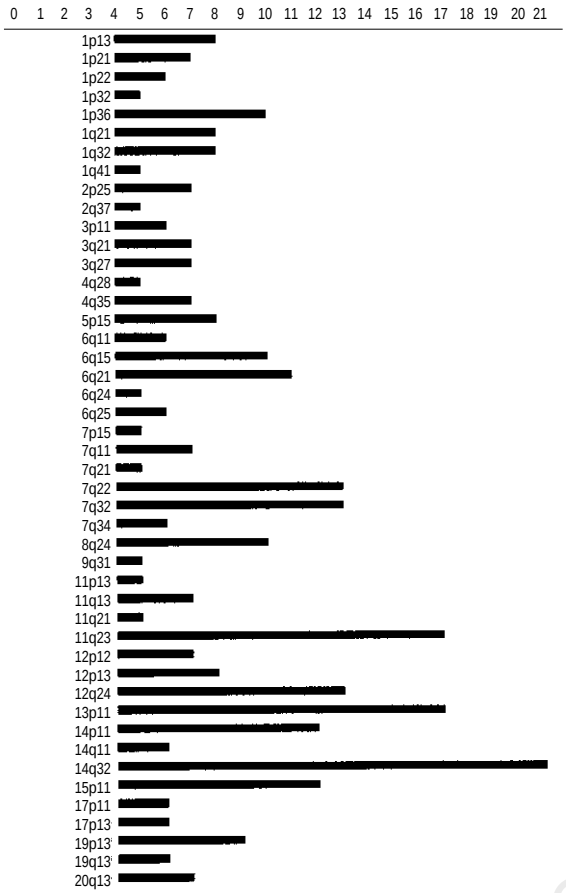


**Figure 1. Schematic representation of distribution of breakpoints in 177 cases of Hodgkin's disease. Bands involved by clonal aberrations in more than 4 cases are reported on the X axis, number of cases is indicated on the Y axis of the histogram.**

published in the literature.

A non random distribution of chromosome abnormalities emerged, as represented in Figure 1.

Staging was available in 88 cases showing clonal structural aberrations (7, 30, 28, 23 cases corresponding to stages I, II, III, and IV, respectively). An increasing number of chromosome breakpoints was shown in the most advanced clinical stages. This phenomenon was striking when complex karyotypes with more than 10 breakpoints were considered. Figure 2 shows this progression from absence of very complex cases in stage I to around 40% of such cases in stage IV.

## Discussion

This study shows that successful cytogenetic analysis can be carried out on tissue samples in a high percentage of HD cases, and cytogenetic clonal aberrations may be detected, provided large screening for metaphases is performed. Polyploidy, especially tetraploidy, was present in 9 out of 14 cases of Hodgkin's disease, as expected in typical multinucleated Reed-Sternberg cells.[8,9] Similarly, in the group of Hodgkin-like ALCL cases, three out of five cases with an abnormal clone had a polyploid number of chromosomes and complex rearrangements. Moreover, in this study six out of fourteen cases of HD (cases #12, #21, #23, #24, #26, #35) and three out of five HD-like lymphoid neoplasms (cases #3, #6, #15) showed the same chromosome aberration in both diploid and polyploid cells. This observation strongly supports a common clonal affiliation of the two cellular populations despite differences in ploidy. Combination of immunophenotype with karyotypic analysis[7,12] and, more recently, with FISH[8] has been helpful in attributing hyperdiploid karyotypes to Reed-Sternberg cells. Whether diploid abnormal karyotypes belong to the Reed-Sternberg elements or to malignant elements among mononuclear infiltrating cells could not be determined in our study. Recently published results by Jansen *et al.*,[13] however, suggested that morphologically normal cells may belong to the malignant population in Hodgkin's disease.

A peculiar observation in this series is that in HD two or more single abnormal cells from the same patient may show structural rearrangements which are different, but which involve the same chromosome breakpoint. These findings (cases #21, #24, #32 of Table 1) could reflect a common origin of karyotypes with different structural changes from cells vulnerable at a given chromosome breakpoint.

It is remarkable that in HD consistent structural changes have not been identified, while by summing up our own HD cases plus those available in the literature, a non-random distribution of chromosome breakpoints clustering within distinct chromosome bands, was found (Figure 1). Interestingly, the chromosomal bands most frequently involved in HD belong to the list of well established breakpoints in lymphoid malignancies. Thus, changes within 14q32 at the IgH locus and 8q24 have been emphasized in early studies[14] as support for the B lymphoid nature of Hodgkin's disease. Band 11q23 contains a number of candidate genes relevant to lymphoma genesis, e.g. BOB-1 gene,[15] the ATM gene of ataxia-telangiectasia,[16] a disorder strongly associated with development of lymphomas; LPC, a proprotein convertase gene, cloned from a t(11;14)(q23;q32) translocation.[17] Translocations and deletions at bands 7q22, 7q32, as well as at 6q15-21, are significantly associated with non-Hodgkin's lymphomas and chronic B-cell proliferations.[18,19] Band q24 of chromosome 12 contains at least two genes of potential interest in Hodgkin's disease, i.e., the gene encoding restin, an intermediate filament protein typical of Reed-Sternberg cells,[20] and BCL7.[21] Band 1p36 is the site of a number of genes involved in tumorigenesis, including neuroblastoma candidate genes,[22,23] PAX7,[24] AML2,[25] and, of interest in the context of HD, the gene encoding for CD30,[26]

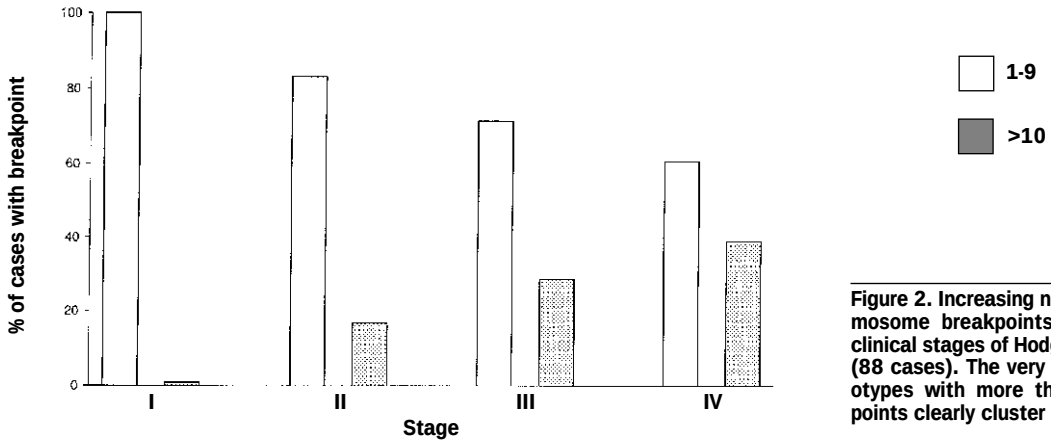


**Figure 2. Increasing number of chromosome breakpoints in advanced clinical stages of Hodgkin's disease (88 cases). The very complex karyotypes with more than 10 breakpoints clearly cluster in stage IV.**

which is typically expressed by Reed-Sternberg cells. In keeping with previous observations,[5,6] the short arms of #13, #14, and #15 were also consistently involved in structural rearrangements in HD in our study. These acrocentric chromosomes contain the so-called nucleolus organizing regions, which have often been implicated in chromosome recombinations in human lymphomas.[27] Altogether these data suggest that in HD, despite the fact that chromosome instability often results in complex abnormal karyotypes that may blur the picture, genomic recombinations do occur at specific chromosomal sites.

It is accepted that, among HD subgroups, LPNP is a distinct entity.[10] All three cases of LP included in the present work showed clonal karyotypic rearrangements. To our knowledge only five LPNP cases with identification of clonal structural aberrations have been previously described.[28-32] Clonality and intraclonal variation in LPNP were recently shown using an elegant PCR approach to detect immunoglobulin gene rearrangement in single neoplastic cells.[33,34] In this study case #21 with a t(1;3), and case #23 with iso(1q) and duplication of 7q showed the same changes in both near-diploid and near-tetraploid cells. These data are in keeping with the definition of LP as a clonal malignancy in which the genomic asset of typical L+H cells is similar to that of Reed-Sternberg cells.[35]

Correlations between chromosomal anomalies and histopathologic subgroups have never been reported in HD. An absence of specific cytogenetic-pathologic associations was also confirmed in this study. However, when chromosomal breakpoints were analyzed with respect to the clinico-pathologic stage of disease (Figure 2), it emerged that very complex abnormal karyotypes (with more than 10 breakpoints) were clustered in samples from patients in the most advanced stages of the disease. If this observation were to be confirmed in larger series of patients, it would be helpful to address studies on earlier and later pathogenetic events in Hodgkin's disease.

### Contributions and Acknowledgments

*DF realized and wrote study, and was responsible for analysis and interpretation of data. BC and CM contributed to producing the cytogenetics results. BF took care of the histopathology of cases entered in the study. MFM and HVDB gave major contributions to the writing of the paper. CM was responsible for the conception of the study, its design, funding, direct supervision, and writing of the paper.*

### Disclosures

*Conflict of interest: none.*

*Redundant publications: no substantial overlapping with previous papers.*

### Funding

*CM was supported by A.I.R.C., Italy.*

### Manuscript processing

*Manuscript received November 19, 1998; accepted January 15, 1999.*

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