Research Paper

Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179

Clinicopathological Profile of Pediatric Classical Hodgkin Lymphoma in Egypt and Role of CD20 Expression

Medical Science

KEYWORDS : Classical Hodgkin Lymphoma – CD20

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ABSTRACT

Background

Although CHL is genotypically considered a B-cell lymphoma, CD20 positive Hodgkin Reed Sternberg (HRS) cells have been reported in 20% to 80% of cases. The relation between CD20 expression in (HRS) cells and prognosis of CHL is still controversial. We conduct this study to determine the profile of pediatric Classical Hodgkin Lymphoma and prevalence of CD20 expression as well as its significance.

Patients and methods

It is a retrospective study that carried out on 70 cases diagnosed as CHL at the National Cancer Institute, Cairo University during the period from 2005-2011 and subjected to CD20 immunostaining. Correlation with disease free survival (DFS) was done.

Results

Nodular sclerosis was the dominant subtype encountered being detected in 43 cases (61.4%) followed by mixed cellularity (21 cases, 30%) then lymphocyte rich (6 cases, 8.6%). Regarding nodular sclerosis cases; 72.1% of cases were grade I and 27.9% were grade II according to BNLI system. CD20 expression was detected in eleven (15.7%) out of all seventy cases. The 2-year disease free survival was 84.4%. Early stage (I and II), and negative bone marrow were significantly associated with a better 2-year DFS. Regarding nodular sclerosis cases, grade I cases according to BNLI grading system showed higher two year DFS than grade II, 89.3% versus 65.6% with significant difference. Despite that the 2-year DFS was slightly better in CD20 positive patients than in CD20 negative patients; yet, the statistical difference was not significant.

Conclusion

BNLI grading system for nodular sclerosis, CHL, is more beneficial for predicting patient's outcome. CD20 expression is found in in a proportion of CHL cases, but no significant relationship could be established with prognosis.

Introduction

Classical Hodgkin's lymphoma (CHL) is a lymphoid neoplasm which is potentially curable with distinct histology, biologic behaviour and clinical characteristic. It accounts for approximately 7% of childhood cancers and 1% of childhood death in the United State**(Jemal, Siegel & Ward, 2009)**[1].

Although 80% of CHL cases can be cured, the other side of high cure rate is late side effect of receiving anti-neoplastic radio-chemotherapy. The identification of clinical and biological factors that control the outcome of patients can allow discrimination of patients who may get benefit from certain regimen in the treatment, aiming at reduction of late side effect**(Barros,Morais&Morais, 2010)**2.

Although CHL is genotypically considered a B-cell lymphoma, CD20 positive Hodgkin Reed Sternberg (HRS) cells have been reported in wide range of cases; 20% to 80% of cases**(Jaffe, Harris, Vardiman, Campo, Arber et al., 2011)**3. Since there is a significant difference in the percentage of CHL cases expressing CD20 in (HRS) cells, the relation between CD20 expression in (HRS) cells and prognosis of CHL is still controversial. Most of studies show that CD20 is not a prognostic factor in Classical Hodgkin lymphoma (CHL) with no correlation between number of CD20positive HRS cells and patient's outcome**(Steidl& Lee, 2010)**4. On the other hand, **Tzankov, Krugman &Fend, 20035,** found that the presence of more than 10 percent of CD20positive HRS cells was associated with better patient outcomes. Other researchers; Portlock and Donelly found an inferior clinical outcome in CD20-positive CHL patients**(Portlock& Donnelly, 2004)**6.

The anti-CD20 mAb (Rituximab) has demonstrated a good safety profile and clinical activity in B-cell lymphomas. In classical Hodgkin lymphoma, emerging data have suggested that Rituximab may also have therapeutic value in patients with CD20 positive HRS cells**(Younes, Oki, Mclaughlin, et al., 2012)**7.

Patients and Methods

I. Retrieval of cases

This is a retrospective study that carried out on seventy cases diagnosed as classical Hodgkin lymphoma at the Pathology Department, National Cancer Institute (NCI), Cairo University during the period from January 2005 to December 2011. Data were collected from pathology reports regarding personal data (age, gender), clinical data (type and date of diagnosis), and pathologic data (site of the affected lymph node, multiplicity, histologic type, histologic grade, and CD15/CD30 status).Clinical data were retrieved from the clinical files of patients (obtained from Radiotherapy Department and Department of Statistics and Epidemiology, NCI) regarding date of diagnosis, stage at presentation (according to the Ann Arbor classification), clinical presentation, presence or absence of B symptoms, initial therapy and therapeutic response, relapse and subsequent therapies and follow-up date.

II. Histopathologic and immunohistochemical assessment of cases

Retrieval of HX&E stained slides as well as immunostained slides of CD15 and CD30 was done. Cases were diagnosed according to criteria described by World Health Organization**(Swerdlow, Campo & Harris, 2008)**[8]Grading of classical Hodgkin lymphoma, nodular sclerosis subtype was done according to British National Lymphoma Investigation (BNLI) and von Wasielweski grading system**(Stein, Delsol, Pileri, Weiss, Poppoma, Jaffe, et al., 2008 and Von**

Wasielewski, Franklin& Fischer, 2003)9,10. Paraffin sections were made at 4 microns thickness and mounted on positive charged slides.Immunostaining for CD20 was done for all cases using BenchMark ^{XT} (Ventana) autostainer and the following steps occurred automatically: deparaffinization, cell conditioning (standard conditioning for 80 minute), application of 100µ of ready to use CD20 mouse monoclonal antibody (clone L26, Cell Marque) under incubation temperature at 42ºC for 32 minutes, application of DAB, Counter stain with Hematoxylin for 8 minutes and post counter stain with bluing reagent for 4 minutes. Slides were washed in tap water and soap for 5 minutes, dehydrated in the ascending grades of alcohol for 5 minutes in each container, cleared in xylene, and then cover slips were applied.Assessment of CD20 immunostaining in HRS cells was performed using Olympus light microscope (BX 51) and the percentage of positively stained cells was recorded by dividing the number of those cells by the total number of HRS in five representative fields at X400 magnification**(Xiao-Hong, Wang, Huang, Xiao, et al., 2008)**11. The staining of small B lymphocytes within the CHL infiltrates served as internal control.Presence of membranous staining of > 10% of the HRS cells was considered positive**(Tzankov et al., 2003 and Xiao-Hong, et al., 2008)**11, 5.

III. Clinical assessment of cases

Patients were assigned a clinical stage according to the Ann Arbor Staging system**(Edge, Byrd& Compton, 2010)**[12]. Recording date of diagnosis, date of remission and relapse and date of last visit was done and subsequently, disease free survival (DFS) was calculated since date of remission till date of relapse or last visit.

IV. Statistical methods

Data were analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. A p-value < 0.05 was considered significant. Multivariate analysis was done using Cox-regression method for the significant factors affecting survival on univariate analysis. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation.

Results

Clinical Characteristics

The study included forty four male and twenty six female with slight male predominance; male to female ratio 1.7:1. Age ranged from 3-18 years with a mean of 9.2±4.1 years and median 9 years. Fifty two cases (74%) presented by supra-diaphragmatic lymphadenopathy, followed by sixteen cases (23%) presented by both supra and infra-diaphragmatic lymphadenopathy. Infra-diaphragmatic lymphadenopathy alone occurred in only two cases (3%). Extranodal involvement was detected in thirteen cases (spleen; 5 cases, liver; 3 cases, bone; 3 cases, lung; 1 case, and nasopharyngeal; 1 case).The commonest encountered stagewas stage II; 33 cases (47.1%), followed by stage III; 20 cases (28.6%), stage IV; 9 cases(12.9%) and lastly stage I; 8 cases (11.4%). Bone marrow involvement was detected in 5 cases after start of chemotherapy. B symptoms were recorded in 21 cases (30%).

All cases received adjuvant chemotherapy in the form of ABVD, ICE, EOPA and ECOP. Sixty five cases received ABVD constituting (92.9%), four cases received both ABVD and ICE constituting (5.7%). Only one case received both EOPA and ECOP constituting (1.4%). Cycles for ABVD ranged from 2-8, for ICE ranged from 1-8 cycles and 2 cycles for each EOPA and ECOP. One case received palliative chemotherapy in the form of VP16. Fifty four cases received adjuvant radiotherapy constituting (77.1%) with doses ranging from 15 to 3000Gy. In addition two cases received palliative radiotherapy.

Pathologic Features

Nodular sclerosis was the dominant subtype encountered being detected in 43 cases (61.4%) followed by mixed cellularity (21 cases, 30%) then lymphocyte rich (6 cases, 8.6%).Regarding nodular sclerosis cases; 72.1% of cases were grade I and 27.9% were grade II according to BNLI system, while according to Von Wasielweski system; 51.2% of cases werelow risk and 48.8% were high risk. CD20 expression was detected in eleven (15.7%) out of all seventy cases using cutoff value ≥10 **(figure 1)**. The expression of CD20 in classical Hodgkin lymphoma varied considerably between tumors in terms of percentage of positive cells that range from 10% to 90%.Our study showed that age was significantly associated with CD20 expression. CD20 positive cases were older than CD20 negative cases (p=0.011). In CD20 positive cases, free bone marrow was significantly higher than its involvement (p=0.025). Although CD20 expression was more prevalent in both mixed cellularity and lymphocyte rich subtypes than nodular sclerosis, the difference was statistically of borderline significance (p=.063). Other clinical variables including gender, B symptoms, and clinical stage are statistically insignificant as shown in **(table 1)**.Table 1 showed the relation between CD20 and clinicopathologic profile of patients.

Patients' Outcome

All patients were followed for a period ranged from three months to 14.2 years (median 2.7 years).All cases entered in remission, but 12 cases (17.1%) relapsed later. Chemotherapy given to relapsed cases included (ABVD, ICE, DHAP, PLAT/Geimsar and VP16) with number of cycles ranged from two to eight cycles. All relapsed cases entered in second remission but second relapse occurred in two out of those twelve cases. One case received three cycles of DHAP. The other case received six cycle of ICE in addition to palliative chemo and radiotherapy. At the end of study, 67 cases were alive and three cases died out of the disease. One case was nodular sclerosis; stage IV with positive bone marrow involvement. Second case was lymphocyte rich; stage II with history of relapse and the last case was mixed cellularity; stage III with positive bone marrow involvement. The Kaplan–Meier method was used to estimate the disease free survival rate and showed that the 2-year disease free survival was 84.4%. In univariate analysis, younger age (<10 years), early stage (I and II), and negative bone marrow were significantly associated with a better 2-year DFS (p=0.002, 0.011, and 0.001; respectively) as shown in **table2**. Table 2 showed the two year DFS in relation to clinicopathologic parameters.Regarding nodular sclerosis cases, grade I cases according to BNLI grading system showed higher two year DFS than grade II, 89.3% versus 65.6% with significant difference (p=0.05). On the other hand, grading according to von Waseilweski system failed to show any significant difference. Absence of B symptoms were associated with better 2-year DFS, however, this was not statistically significant (p= 0.071). Despite that the 2-year DFS was slightly better in CD20 positive patients than in CD20 negative patients, yet, the statistical difference was not significant (p=0.537). In multivariate analysis, the only independent factor that significantly affect the DFS was BM status (p = 0.004) with an odds ratio (probability of relapse of positive cases) of 12.8 times as negative cases (95% confidence interval 2.3-70.9).

Discussion

Classical Hodgkin lymphoma is defined by the World Health Organization (WHO) as monoclonal lymphoid neoplasm (in most instances derived from B cells) composed of mononuclear Hodgkin cells and multinucleated Reed-Sternberg (R-S) cells residing in an infiltrate containing a variable mixture of non-neoplastic

small lymphocytes, eosinophils,neutrophils, histiocytes, plasma cells, fibroblasts and collagen fibers. Based on the characteristics of the reactive infiltrate and to a certain extent on the morphology of the HRS cells, four histological subtypes have been distinguished**(Stein et al., 2008)**[9] and in our study, nodular sclerosis is the dominant histologic subtype (61.4%) followed by mixed cellularity (30%) and lymphocyte rich (8.6%).

Although CHL is genotypically considered a B-cell lymphoma, the classical B-cell marker CD20 positivity of HRS cells has been reported in less than 20% to 80% of cases with the majority of reports being in the 20% to 40% range**(Jaffeet al., 2011)**3which makes the relation between CD20 expression in (HRS) cells and prognosis of CHL still controversial.In this study, we reported CD20 expression in 15.7% of cases. CD20 expression in relation to histologic subtypes was found to be heterogeneous among different studies. While we found predominance of CD20 expression in mixed cellularity subtype, other researchers found predominance of CD20 expression in lymphocyte rich cases**(Xiao-Hong et al., 2008)**11, or in nodular sclerosis cases**(Rassidakis&Medeiros, 2002,Tzankov et al., 2003, and Portlock& Donnelly, 2004)**13,5,6. No significant association could be obtained in these studies including ours concluding that CD20 expression is a finding that could occur in CHL cases disregarding the histologic subtype.

CHL has a favourable outcome in most patients; however, there is a proportion of patients who do not respond to standard treatments. Different research groups have explored the potential use of biologic markers as determinants of clinical outcome in adult CHL, but this scenario remains still poorly studied in the pediatric counterpart**(Chabay, Pesce, De Matteo, Lombardi, Rey, Preciado et al., 2006)**14. All our cases entered in remission, only twelve cases relapsed later. Our relapse rate (17.1%) and 2-year DFS (84.4%) were matched with other researchers**(Smith, Chen, Hudson, Link, Kun, Weinstein, Billett, Marcus, Tarbell, Donaldson et al., 2003)**15 being 15% and 83%.

In our study, univariate analysis showed a significantly better two year disease free survival among patients younger than 10 years, with early stage (I,II) and free bone marrow. Influence of stage on prognosis of CHL patients was also approved by other researches**(Portlock& Donnelly, 2004 and Xiao-Hong et al., 2008)**6,11. Our study showed that 2-year DFS among nodular sclerosis grade I cases was significantly higher than nodular sclerosis grade II (89.3% versus 65.6%) according to BLNI system. Other study**(Maclennan, Bennett &Tu, 1989)**16 also documented better DFS in grade I than grade II nodular sclerosis cases. On the other hand, no consensus was achieved regarding grading according to Von Waseilweski system.

It has been reported in the literature**(Rassidakis&Medeiros, 2002,Tzankov et al., 2003, and Portlock& Donnelly, 2004)**5,6,13 that CD20 is expressed in 5-58% of CHL cases. We found CD20 expression in 15.7% of cases. This finding supports the published molecular single cell line and the immunohistochemical studies, suggesting that the HRS cells arise from germinal centre B-cells with rearranged immunoglobulin genes. We, like other researchers**(Rassidakis&Medeiros, 2002 and Von Wasielewski et al., 2003)**10,13, could not find any relationship between CD20 expression and DFS in CHL patients. Others**(Tzankov et al., 2003)**5 could conclude that CD20 positive CHL cases showed a trend towards a better DFS. They claimed that CD20 resemble a ca2+ ion channel**(Bubien,Zhou, Bell, Frizzell, Tedder et al., 1993)**17and an increase in ca2+ permeability in HRS cells along with CTH and RTH might decrease apoptotic resistance or even activate programmed cell death**(Dillman, 2001)**18. One of the most important reasons for the inconsistency in the relationship between CD20 expression and its prognostic significance is lack of uniform criteria for CD20 expression with existence of different cut off values. Thus we believe that a more definite conclusion could be achieved upon pooled study to obtain large sample in addition to unify evaluation criteria for CD20 expression in addition to standardized immunohistochemical protocols. CD20 expression analysis in CHL gains its value form the expected benefit of using targeted therapy; Rituximab, which is a chimeric (human-mouse) anti-CD20 monoclonal antibody. Vitro experiments**(Cartron, Watier, Golay,et al., 2004)**19 showed that it can reduce tumor size through inducing apoptosis and killing CD20-expressing HRS cells as well as reactive B-lymphocytes in the environment via its complement-dependant and antibody dependant cellular cytotoxicity.

Currently, the clinical application of Rituximab in CHL is found in phase II clinical studies**(Youneset al., 2012)**7.A study from MD-Anderson cancer centre**(Younes, Romaguera,Hagerneister, et al., 2003)**20 shows that Rituximab is also effective in patients with CD20-negative HL. This is because Rituximab can make HRS cells lose their growth environment and therefore reduce tumor size through depleting normal B-cells and decreasing the levels of serum cytokines since tumor infiltrating B-cells can support the growth of HRS cells through regulating the expression of cytokines and chemokines in vivo.

We conclude that BLNI grading system for CHL, nodular sclerosis cases, is a promising grading system for predicting patient outcome. We found CD20 expression in a proportion of CHL cases which reflect the theory that HRS arise from germinal B-lymphocytes; however, we could not obtain any association between its expression and prognosis.

Acknowledgment
We thank Pathology Department and Pediatric Oncology Department, National Cancer Institute, Cairo University in helping us in providing us with cases, paraffin blocks, chemicals and instruments upon which this work has been conducted.

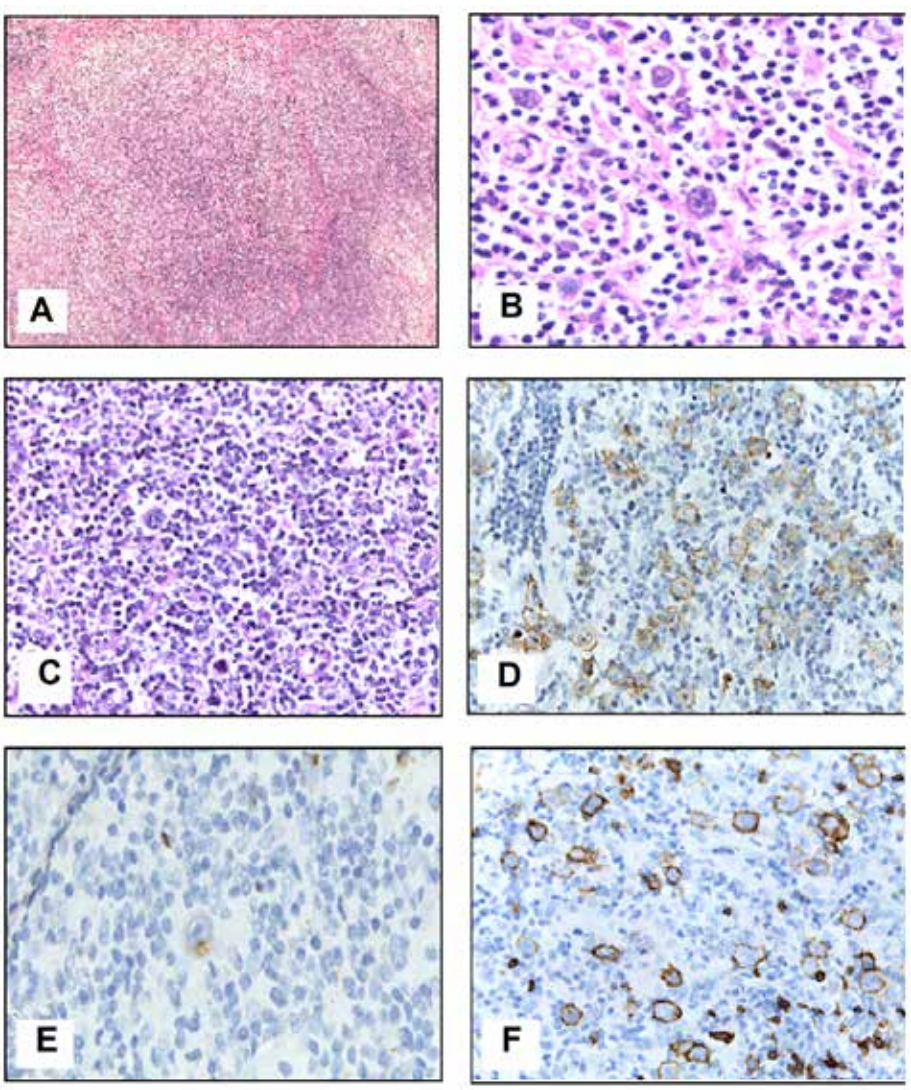
Table (1): Relation between CD20 expression and clinicopathologic parameters

Characteristics	CD20 positive (n=11)	CD20 negative (n=59)	p-value
Age (yrs) • mean±SD	12±3	8.7±4.1	0.011*
Sex (n, %) • Male • Female	7 (15.9%) 4 (15.4%)	37 (84.1%) 22 (84.6%)	0.954
B symptoms • Present • Absent	3(14.3%) 8(16.3%)	18 (85.7%) 41 (83.7%)	0.830
Bone marrow status • Positive • Free	3 (60%) 8 (12.3%)	2 (40%) 57 (87.7%)	0.025*
Histologic subtype • Mixed cellularity and lymphocyte rich • Nodular sclerosis	7 (25.9%) 4 (9.3%)	20 (74.1%) 39 (90.7%)	0.063
Stage • I-II • III-IV	4 (9.8%) 7 (24.1%)	37 (90.2%) 22 (75.9%)	0.103

Table (2): Two-years DFS in relation to clinico-pathologic parameters

Characteristic	No. of cases	2 years DFS	P value
Total	70	84.4%	
Age <10 ≥10	 38 32	 97.2% 68.2%	 0.002*
Gender Female Male	 26 44	 87.5% 82.8%	 0.983
Clinical stage I, II III, IV	 41 29	 89.8% 77.0%	 0.011*
B symptoms Yes No	 21 49	 79.1% 87.1%	 0.071
Bone marrow Positive Free	 5 65	 53.3% 86.6%	 0.001*
Histologic subtypes Lymphocyte rich and mixed cellularity Nodular sclerosis	 27 43	 88.2% 82.4%	 0.923
BLNI grading Grade I Grade II Von Waseilweskin grading Low risk High risk	 31 12 22 21	 89.3% 65.6% 84.4% 80.4%	 0.05* 0.664
CD20 positive CD20 negative	 11 59	 90.9% 83.3%	 0.537

Figure (1):Three cases of classical Hodgkin lymphoma, (A) nodular sclerosis showing nodular pattern, (B) mixed cellularity shoeing mononuclear and binucleated HRS cells, and (C) lymphocyte rich showing background rich in lymphocytes (Hx&E; original magnification X200 in A and x400 in B &C)(D & E) The neoplastic HRS cells showed positive membranous reaction to CD30 (D) and positive paranuclear dot to CD15 (Immunoperoxidase with hematoxylin counter stain; original magnification X400) (F) CD20 immunostaining of the previous case revealed strong positive membranous reaction in about 90% of HRS cells (Immunoperoxidase with hematoxylin counter stain; original magnification X400)



Volume : 3 | Issue : 12 | December 2014 • ISSN No 2277 - 8179

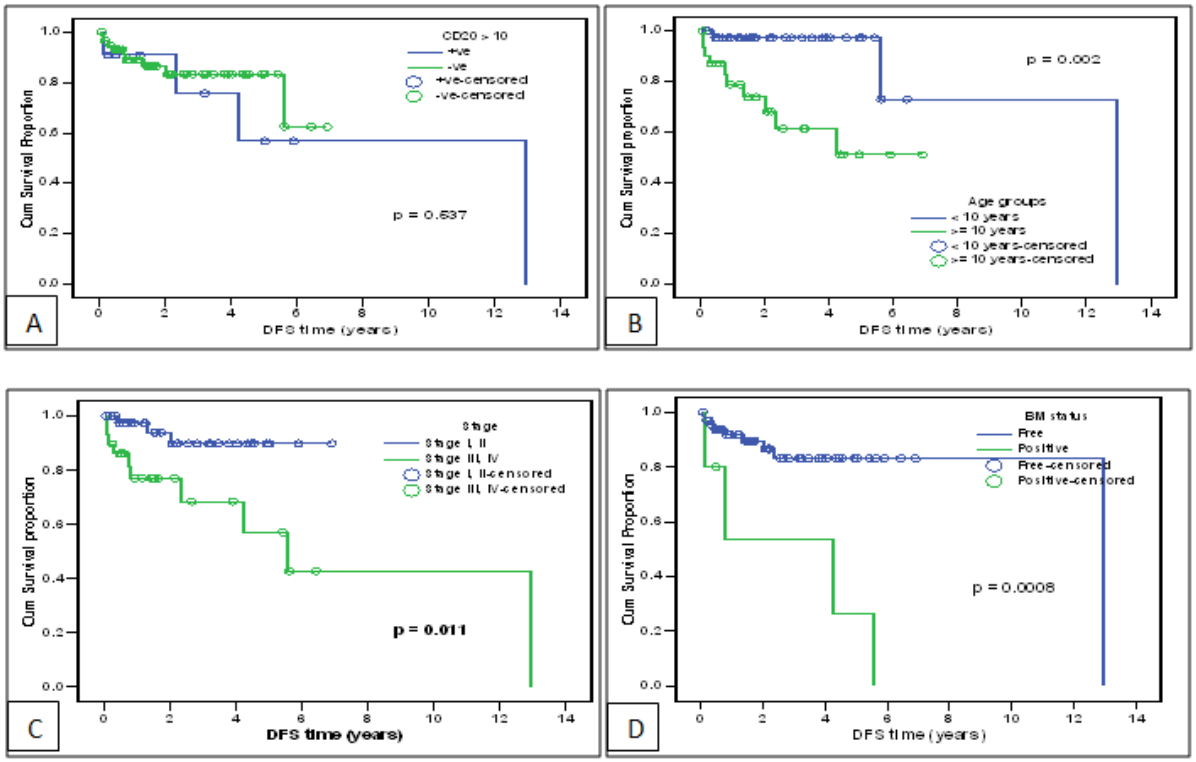


Figure 2: Two-year disease free survival in relation to CD20 expression (A), age (B), stage (C) and bone marrow status (D), Kaplan-Meier curves

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