

Does Immunohistochemically CD5 Positivity Matter In Diffuse Large B-Cell Lymphoma Patients? Turkish Multicenter Study

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Abstract

Background: Diffuse large B-cell lymphoma (DLBCL) is the most common sub-type of Non-Hodgkin lymphomas (NHL) and it is a very heterogeneous group of diseases. CD5 positivity is profound in a substantial patient population in DLBCL. In this population, female patient distribution is higher, LDH elevation, extra nodal involvement, bone marrow involvement, ECOG (Eastern Cooperative Oncology Group) performance score elevation and high international prognostic score (IPI > 2) are more frequent for CD5 - DLBCL. Also, it was observed that prognosis of CD5 + DLBCL was worse than CD5 - DLBCL. We aimed to determine the frequency of CD5 positivity in DLBCL in Turkey with this multicenter study.

Methods: The study, in order to get the overall parts from Turkey, including from each geographical area, 16 center and 9 cities were included. Each center retrospectively scanned the diagnostic pathology reports of DLBCL patients between January 2015-2021.

Results: Two thousand four hundred sixty-nine DLBCL patients were screened retrospectively. CD5 positivity was detected in 169 patients (6.84%). The average age was 59 and 55.7% of the patients were male. Overall survival was 29.8 months. When evaluated with historical data in this group of patients, poor prognostic factors were found to be more common than CD5 - DLBCL patients.

Conclusion: Due to its prognostic effect, immunohistochemically, the presence of CD5 in pathological samples of all DLBCL patients should be checked. The effect on prognosis in this patient group should not be forgotten while treatment and follow-up.

Study Highlights

- Considering its effect on patient survival and its frequency, it should not be underestimated;
- Due to its prognostic effect, CD5 must be applied immunohistochemically in pathological samples of all DLBCL patients
- GCB / non-GCB status should be checked in all DLBCL patients in the presence of Hans algorithm. Double / triple expressor distinction must be made.
- In all patients, FISH should be evaluated in terms of DHL / THL. Both the application of the Hans algorithm to each patient and the inability to evaluate DHL / THL for each patient with FISH were two aspects we had to develop.
- The awareness of the importance of CD5 presence among Hematology and Pathology physicians be increased.
- Although several studies are discussing the role of CNS prophylaxis in DLBCL, patients with CD5 + status may be defined as a CNS relapse risk. In our study number of patients received CNS prophylaxis were few but none of the patients with CNS relapse had previously received prophylaxis.
- Considering the data in the literature and the data in our study, the different evaluation of CD5 + patients, as in DHL / THL patients, seems to be an indisputable fact. The low survival rates with

current primary care support this.

- With the survival and literature information, we think there is an unmet need for this subgroup of patients with low survival expectancy. Thus, we need new studies with novel treatment modalities to be combined or to replace the standard of care therapies.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is the most common sub-type of Non-Hodgkin lymphomas (NHL), which constitutes 40% of patients. This group of diseases can be defined as an "umbrella entity" due to their heterogeneous nature. The fact that a 60% cure^{1,2,3} can be achieved after administration of first-line treatment makes it more important to elucidate this heterogeneity and identify high-risk patients with low response rates. The increase in the number of subgroups demonstrating this heterogeneity in the 2016 WHO Lymphoma classification⁴ has gained relevance and importance in clinical approaches. Double / Triple Hit (DHL/THL)^{5,6} is the most contemporary among these groups and highlights the importance of implementing risk-stratified treatment, which is the current treatment approach.

Another clinically remarkable group is immunohistochemically CD5 positivity in DLBCL, which is profound in a substantial part of the patient population. De novo DLBCL can be found CD5 positivity between 5% and 22%⁷. As a pan T-cell marker, there are many properties that make CD5 positivity important. In this population, unlike the general population of DLBCL patients, female patient distribution is higher, LDH elevation, extra nodal involvement, bone marrow involvement, ECOG (Eastern Cooperative Oncology Group) performance score elevation and high international prognostic score (IPI > 2) are more frequent. A poorer prognosis was observed in patients with CD5 positivity (+) DLBCL, as well as a higher relapse rate, versus CD5 negative (-) DLBCL^{7,8}. According to these clinical findings, there are studies investigating more intensified treatment options other than R-CHOP (Rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone), which is the standard treatment option in DLBCL.

Because CD5 is a T lymphocyte marker and thus not essential in the diagnosis of DLBCL, it is not always examined immunohistochemically by pathologists. Therefore, different ratios in different studies can be seen. In addition, when we reviewed the literature, studies demonstrating its prevalence seem to be predominantly from Asian countries. Our aim was to determine the frequency of CD5 positivity in DLBCL, and increase the awareness in Turkey, with this multicenter study. Our second aim was to investigate the presence and effect of other factors that may affect overall survival in these patients.

Material / Methods

The study was conducted together with the Turkish Society of Hematology, Lymphoma Scientific Subcommittee. This study included 16 centers and 9 cities that represent multiple geographic areas from all over Turkey. Each center retrospectively scanned the diagnostic pathology reports of DLBCL patients dating between January 2015-2021. Treatment and management data of patients with immunohistochemically CD5 positive DLBCL were evaluated by the centers. Pathology results without

CD5 immunohistochemical staining were accepted as CD5 negative. In addition, the centers stated the total number of DLBCL patients diagnosed during this period. The total rate was calculated for comparison with historical data.

Primary central nervous system (CNS) lymphoma patients were excluded due to poor prognosis and different treatment regimens than R-CHOP. In the centers participating in the study, between January 2015 and 2021, the total number of newly diagnosed DLBCL patients examined were 2469. CD5 positivity was detected immunohistochemically in 169 patients. Data of 131 patients were available. Patients' age, gender, date of diagnosis, stage at diagnosis (Ann-Arbor). ECOG performance scales, LDH levels, R-IPI and NCCN-IPI scores, extra nodal involvement, bone marrow involvement, treatments modalities, post-treatment responses and follow-ups were retrospectively revealed from the patient files.

The study was conducted in accordance with the Declaration of Helsinki, Ethical Principles for Medical Research. This study was approved by Trakya University Faculty of Medicine Ethical Committee (TUTF-BAEK2020/439).

Pathological Findings

Pathological findings were retrieved from pathology reports of the participating centers. All of the centers performed immunohistochemical studies on formalin-fixed, paraffin-embedded tissue sections. A panel of selected antibodies was used by the pathologists: CD20, CD3, CD5, CD10, BCL-2, BCL-6, MUM1, FOXP-1, Ki-67, and MYC proto-oncogene protein (MYC). A cutoff level for dim to strong expression of CD5 was $\geq 50\%$ of tumor cells for considering as immunopositivity. The cutoffs for positivity were $\geq 30\%$ for CD10, BCL-6, and MUM-1 to classify these tumors as germinal center (GCB) or non-germinal center (non-GCB) cell-like immunophenotype according to Hans algorithm⁹. The positive cutoff for FOXP1 was $\geq 60\%$ as proposed by Visco-Young algorithm¹⁰. The cutoffs for positive MYC or BCL-2 results were $\geq 40\%$ and $\geq 50\%$ of cells, respectively¹¹ (Figure– 1).

Fluorescence in situ hybridization (FISH) was performed on cases using LSI dual-color break-apart probes for MYC and BCL6, and dual-color, dual-fusion probe for BCL2/IGH. Positive cases for rearrangements had positive signal in $\geq 15\%$ of nuclei examined. Cases were designated as double-hit lymphoma if concurrent MYC and BCL2 or BCL6 were rearranged.

Statistics

Normality assumption was checked using Shapiro-Wilk test. Student T test and one-way analysis of variance were used to compare independent groups. Tukey Post-Hoc test was used to perform multiple comparisons. The log-rank test using the Kaplan-Meier method was performed for univariate survival analysis. The Cox Proportional-Hazards model with a forward selection method was used to identify the prognostic significance of risk factors on survival. Descriptive statistics were expressed as mean and

standard deviation for numerical variables and frequency and percentage for categorical variables. A p value less than 0.05 considered as statistically significant. All statistical analyses were performed using SPSS version 20 (SPSS software, IBM Corporation, Armonk, NY).

Results

Two thousand four hundred sixty-nine (2,469) DLBCL patients were screened retrospectively. CD5 positivity was detected immunohistochemically in 169 patients (6.84%). Data of 131 patients were available. The average age was 59 (18 vs 88). 55% of the patients were 60 years and older. 55.7% of the patients were male. Overall survival was 29.8 months (0.1 vs 72.3 months). Progression-free survival (PFS) was 26.08 months. During their follow-up, 41 of 131 (31.3%) patients died.

Pathology reports of 125 patients could be evaluated in terms of GCB / non-GCB (According to Hans algorithm) ⁹. Seventy-six patients were non-GCB (61.7%), 43 patients were GCB (34.9%) and 4 patients were unclassified between GCB/non-GCB. The average Ki67 was 78.15%. The ECOG score was found to be ≥ 2 in 31.25 % of the patients at the time of diagnosis. 57% patients had B symptoms. LDH was high in 65.4% of the patients. In 84 patients (66.7%), Ann-Arbor was stage 3-4. It was seen that 114 patients were evaluated on the basis of bone marrow involvement. Bone marrow involvement was present in 23% of the patients. 70% patients had 1 or more extra nodal involvement. It was observed that 33 patients (42.8%) had double / triple expressor. R-IPI score was high in 48% of patients, and NCCN-IPI was intermediate-high or high in 44.1% of the patients (Table- 1). It was observed that CNS prophylaxis was not applied frequently in these patients in the centers and there was no standard prophylaxis regimen between centers. It was determined that CNS prophylaxis was applied only to 19 patients. In patients who underwent remission after first-line therapy, 19 patients had relapse, of which 4 were from the CNS. It was observed that none of the patients received CNS prophylaxis during first line treatment.

Evaluation of prognostic factors

Age was found to be a significant variable. Overall survival was found to be significantly shorter in patients older than 60 years old. The mean survival is, statistically, significantly longer in patients under 60 years of age (63,41 vs 42,81 months, $p < 0.01$). There was no difference in survival between genders ($p = 0,891$). Patients with B symptom at the time of diagnosis (57%) had a shorter mean survival ($p = 0,002$) (Figure- 2).

Bone marrow involvement were screened with biopsy in 114 of the patients and revealed that 23% had bone marrow involvement. The mean survival of patients with bone marrow involvement was statistically significantly lower (32.03 – 58.27 months, $p < 0.01$). 113 patients had radiological imaging for staging at the time of diagnosis and 70% of the patients had extra nodal involvement. Mean survival was statistically significantly lower in patients with extra nodal involvement (66,23 vs 46,90 months, $p = 0.02$) (Figure- 3).

We were able to evaluate LDH values in 130 patients. 65.3% of the patients had higher than normal LDH values. Similar to NCCN-IPI scoring, we divided the patients into 3 groups: as normal, up to 3 times as high, and > 3 times as high. We found that high LDH was statistically significant in survival ($p < 0.01$). 27.5% of the patients had a higher than grade 1 ECOG score and survival was shorter in these patients. ($p < 0.01$). R-IPI score was found to be high (> 3) in 47% of the patients. Patients with higher R-IPI scores had a shorter survival rate ($p < 0.01$). Similarly, survival was short in patients with both high-intermediate and high risk NCCN-IPI scores ($p < 0.01$) (Figure- 3-4).

We were able to evaluate 131 patients for double / triple expressor status and found that 54 of the patients did not possess Bcl-2, Bcl6 and/or C-myc markers. In this respect, 33 (42.8%) of 77 patients who were fully evaluated immunohistochemically had a double / triple expressor. If we accept 54 patients whose evaluation is not complete immunohistochemically as no double / triple expressor, we can discuss the presence of double / triple expressor in 24.8% of the patients. Overall survival (OS) was shorter in these patients ($p = 0.095$) (Figure- 4). It was observed that only 10 patients were evaluated in terms of DH/THL with FISH (fluorescent in situ hybridization). Two of these patients were double / triple hit. Additionally, patients were evaluated immunohistochemically according to the Hans algorithm, patients with non-GCB morphology had a shorter mean survival ($p = 0.348$) (Figure- 2-3).

It was observed that the first line treatment of 11 patients was a more intensive treatment (DA-EPOCH-R, R-CHOEP, Hyper-CVAD). The mean survival of these patients compared to patients who received R-CHOP as first-line therapy was similar ($p = 0.997$) (Table- 2).

In terms of overall survival, when multivariate analysis was performed by excluding R-IPI and NCCN-IPI, which are among the other data, it was seen that ≥ 60 years of age, ECOG ≥ 2 and ≥ 1 extra nodal involvement had an effect on survival. The same findings (≥ 60 years of age, ECOG ≥ 2 and ≥ 1 extra nodal involvement) were also valid for PFS in multivariate analysis (Figure- 5-6-7).

Discussion

Interpreted as a pan T-cell marker, CD5 is a monomeric type 1 transmembrane glycoprotein. This protein, which has both an intracellular and extracellular domain, weighs 67 kDa. CD5 gene maps to the chromosome 11q12.2 region. Although it is frequently expressed from T cells, it is also expressed at a low level by a small subset of naive B lymphocytes¹². CD5 inhibits signaling downstream of the B cell receptor (BCR) and modifies intracellular calcium mobilization. It also suppresses the release of IL-2, resulting in increased production of BCR-mediated IL-10, which is an anti-inflammatory marker and a survival factor for B-cells. In addition, there are studies showing that it is associated with the activation of the ERK1 / 2, PI3K, STAT3 and NFAT2 signal pathways in the presence of CD5 positive B cells and is thus related with poor prognosis^{7,12}. Also, CD5 positive B cells can produce autoantibodies, which is also related with autoimmune events in chronic lymphocytic leukemia (CLL) and mantle cell leukemia (MCL)¹³. High LDH, bone marrow involvement, presence of extra nodal involvement, high IPI score, high ECOG performance score and CNS recurrence, which are known as predictive factors and are more

common in the group with CD5 + DLBCL patients. The survival of these patients is also observed to be shorter compared to the CD5 - group⁸.

The frequency of CD5 positivity, which is more observed in the differential diagnosis of CLL and MCL, is variable in de novo DLBCL patients. In a retrospective study by Na H.Y, et al. about the characteristics of CD5 + DLBCL patients in Korea, CD5 positivity was found to be 7.4%¹⁴. In a multi-center study by Yamaguchi et al in Japan, this frequency was found 10%¹⁵. However, in large-scale studies in Japan, prevalence rates of CD5 positivity may vary widely, from 5 to 22%^{7,15}. Besides these, in a multi-center large-scale study with data from Western countries, CD5 positivity ratio in DLBCL is (5.5%) lower than in East Asian countries¹⁶. When we look at the rate of CD5 + DLBCL lymphoma among DLBCL patients, in our study we found that it is 6.84% and this rate is closer to the rate found in Western countries unlike East Asian countries. One of the most important reasons for these differences is that CD5 staining in immunohistochemically, which as a pan-T marker, is not performed in every DLBCL patient in our country. If this evaluation can be performed in all newly diagnosed DLBCL patients in Turkey, we think that these rates may be higher, similar to what is seen in the studies coming from East Asia. When we compare the age distribution with other studies, the number of patients older than 60 and over was more common (≥ 60 age: 55%) or similar. Survival was shorter in this group, as expected. Although, there is a predominance of female patient distribution in CD5 + DLBCL patients in studies from East Asian countries. But in our study, male patients were higher (55.7%) and similar to the data from Western countries.

In DLBCL patients, the chance of obtaining remission with treatment is 50-60% according to the current standard of care. In addition, we know that there are many factors that affect survival. When the patients are evaluated in the presence of clinical scores, the 5-year survival rate in patients with low IPI score is 90%, while this rate decreases to 60% with high IPI score. Also, when evaluated according to the cell of origin, according to Hans algorithm, 5-year OS was 48–56% in non-GCB subtype, while this rate was 73–78% in GCB subtype. The 5-year survival rate in the DHT / THT subtype, which is defined as an aggressive subtype and is found in 6% -14% of DLBCL patients, appears to be very low and nearly 18%^{1,17}. As another variable, when screening the literature regarding the effect of CD5 presence on prognosis, we see that the data are similar in the other studies and the presence of CD5 positivity reduces survival. The 5-year overall survival (OS) is 35.5% in CD5 + DLBCL patients, and it has been found to be shorter than general DLBCL patient population¹². In our study, immunohistochemically, we found that the mean survival was 29.8 months in CD5 + DLBCL patients. During this 5-year follow-up, we found that 31.3% of the patients had died. The study had two important limiting factors. First, we do not have the survival statistics of CD5 - control group. So we had to evaluate only CD5 + DLBCL patients in our study, and then we could make the comparison historically with the CD5 + and - DLBCL groups in the literature. The second important factor is the study was retrospective.

In addition to the shorter OS of CD5 + DLBCL patients, these patients have higher LDH, IPI elevation, advanced Ann-Arbor frequency, poor performance score (ECOG > 1), and bone marrow involvement at the

time of diagnosis compared to CD5 - DLBCL patients. CNS relapse is more common in patients with CD5 positivity. CD5 + DLBCL is more often in the non-GCM / ABC phenotype. Also, the presence of double / triple expressor or double / triple hits (14.4%) is more common in these patients at the time of diagnosis^{12,14,15,16,18}.

The age and gender distribution at the time of diagnosis is variable. In studies from East Asian countries, the frequency of female patients is significantly higher^{12,14,15}. However, there is no difference in gender distribution in Western countries¹⁶. In our data, it was seen that male patients were in the majority (55.7%). Again, in most of the studies, we can say that the group > 60 years was more common in CD5 + patients. Xu-Monette et al. in the study conducted on CD5 + DLBCL patient data from western countries, it is seen that 76.7% of CD5 + patients are over 60 years old¹⁶. But, in a single-center study by Tang H. et al in China, 53.3% of CD5 + DLBCL patients were below the age of 60, in addition, the group over 60 years of age was more common in CD5 - patients¹². In our data, the rate of patients, 60 years and older, are higher (55%) (Table- 3).

Central nervous system relapse is more common in CD5 + DLBCL patients. Its mechanism is still not understood. In the study by Zhang et al, it was observed that CNS involvement in relapsed DLBCL patients was 35% in CD5 + patients, while it was 19% in CD5 -patients¹⁸. Similarly, in the study by Thakral B. et al., CNS relapsed was 33.3% in CD5 + patients and 15.6% in CD5 - patients¹⁹. In our data, it was seen that 19 patients relapsed and only 4 patients relapsed from the CNS region (3% of all patient, 21% of all relapses). When the rate of CNS relapse in examined our CD5 + patients, compared with ratio in the literature, it was seen that although it was higher than in CD5 - patients, it was not as common as in CD5 + patients. Also, we found that not all of these patients received CNS prophylaxis in our study. In addition, we saw that the CNS prophylaxis number and protocol of the centers were not standard.

In our data, 64.6% of the patients had high LDH, and 13.4% had 3-fold or more. Also, 23% of the patients had bone marrow involvement, 70% had extra nodal involvement, 57% of the patients had B symptoms. At 66.6%, Ann-Arbor was seen as stage 3-4. And the presence of these factors had a significant relationship with survival. In addition to the higher frequency of all these factors that have impact on prognosis for CD5 + DLBCL patients. In a study with a small number of patients from Taiwan the presence of CD5 + was seen as the only adverse prognostic factor in multivariate analysis²⁰.

As with DHL/THL, there are studies for intensive treatments for CD5 + patients, which is a poor prognostic factor. Increased overall survival with the introduction of Rituximab seems to be valid also for CD 5 + patients. In a study by Hyo R et al. showed that addition of Rituximab to chemotherapy, similarly increased OS and progression-free survival (PFS) in both CD5 + and CD5-groups²¹. Also, in a study by Miyazaki K. et al revealed that addition of Rituximab to chemotherapy in CD 5 + DLBCL patients, it is seen that the addition of Rituximab to chemotherapy significantly increases OS²². In a retrospective study by Thankral B. et al., in which DLBCL patients treated with R-EPOCH chemotherapy, with a 28.5-month median follow-up, 37.5% of CD5 + patients had died, compared with 9.6% of patients with CD5 -¹⁹. In the

study conducted by Zhang F. et al to evaluate the effect of intensive treatment on survival, the effectiveness of DA-EPOCH-R and R-CHOP treatments were compared. In the study, it was observed that PFS and OS were better in both CD5 + and CD5 - groups in patients who received DA-EPOCH-R in short-term follow-up. In the long-term follow-up, this difference was not seen for OS in CD5 + patients. Also, in another study with a small group of patients with short-term follow-up, we can say that the DA-EPOCH-R combined with high-dose methotrexate regimen had a better survival in the CD5 + patient group²³. In our study, it was observed that the first line treatment of 11 patients was a more intensive treatment (DA-EPOCH-R, R-CHOEP, Hyper-CVAD). The mean survival of these patients compared to patients who received R-CHOP as first-line therapy was similar. The low number of patients receiving intensive treatment seems to be an important limiting factor in this comparison.

Declarations

Funding: There is no additional financial resource for this study.

Conflict of interest: No conflict of interest for all authors.

Ethics approval: TUTF-BAEK2020/439

Informed consent: Informed consent was obtained from all subjects as a retrospective study. Also, informed consent was obtained from a parent and/or legal guardian for patients who died.

Precis for use in the Table of Contents: CD5 + DLBCL has poor prognosis and should be investigated in all DLBCL patients

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Tables

Table -1: Characteristic Findings of the Patients	
Number	131
Age (Mean, Years)	59 (18-88)
Age <60 / (%)	59 / 72 (45 % / 55 %)
Gender (M/F)	73 / 58 55.7 % / 44.3 %
GCB / non-GCB / Nos (%)	43 / 76 / 4 34.9 % / 61.7 % / 3.4 %
Ki67 % (Mean)	78.15 %
ECOG ³2 / <2	40 / 88 (31.25 % / 68,75)
B Symptoms (Y/N)	73 / 55 (57 % / 43 %)
LDH (Normal / High / ³3 fold) (127 patients had LDH data)	Normal: 45 (35.4%) High - <3 fold: 65 (51.2%) >3 fold: 17 (13.4%)
Bone Marrow Involvement (%)	26 patients (23 %)
Extra nodal Involvement (%)	91 patients (70 %)
Ann-Arbor (126 patients had Ann-Arbor data)	
Stage I	20 (15,9 %)
Stage II	22 (17,5 %)
Stage III	26 (20,6 %)
Stage IV	58 (46 %)
Double / Triple Expressor (77 patients had Bcl-2, Bcl6 and or C-myc data)	33 patients (42.8 %)
R – IPI <3, ³3	66 / 61 (52 % / 48 %)
NCCN – IPI ³4 and <6	34 / 127 (26.7 %)

NCCN – IPI ³⁶	22 / 127 (17.3 %)
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Table- 2: Comparison of prognostic markers in CD5 + patients group

Table -2	Overall Survival (months)	95% Confidence Interval	P
Age			
< 60	63.40	57.05 – 69.76	< 0.001
60	42.98	34.74 – 50.87	
B symptoms			
Yes	44.34	36.55 – 52.12	0.002
No	62.44	55.56 – 69.32	
LDH			
Normal	61.58	53.85 – 69.31	< 0.001
Upper - < 3 fold	51.61	43.87 – 59.36	
3 fold	25.94	9.49 – 42.39	
ECOG			
0-1	62.31	57.05 – 67.57	< 0.001
2	29.36	18.71 – 40.02	
R-IPI			
1-2	63.60	57.81 – 69.39	< 0.001
3	38.27	29.33 – 47.22	
NCCN-IPI			
< 4	62.30	56.39 – 68.20	< 0.001
4	42.33	31.66 – 52.99	
6	24.24	11.01 – 37.48	
GCB / non-GCB			
GCB	57.27	48.42 – 66.11	0.348
Non-GCB	53.93	44.41 – 59.60	
Double/Triple expressor			
Yes	43.82	32.28 – 55.37	0.095
No	56.18	47.58 – 64.77	
Bone marrow involvement			
Yes	32.03	19.12 – 44.94	< 0.001

No	58.27	52.30 – 64.23	
Extra nodal involvement			
Yes	46.90	39.87 – 53.93	0.002
No	66.23	60.16 – 72.30	
Treatment			
R-CHOP	54.69	48.94 – 60.44	0.997
Intensive Treatment	40.39	28.34 – 52.44	

Table- 3: Comparison with literature data of presence of prognostic markers in CD5 + and – patients group

Table- 3	Demirci U. et al.	Xu-Monette ZY et al. 2014 from Western countries (16)		Tang H. et al. 2019 from China (10)		Na HY. et al. 2019 from Korea (14)		Hyo R. et al. 2010 from Japan (9)	
Parameter	CD5 + No (%)	CD5 + No (%)	CD5 – No (%)	CD5 + No (%)	CD5 – No (%)	CD5 + No (%)	CD5 – No (%)	CD5 + No (%)	CD5 – No (%)
Age						51.7 %	59 %		
<60	59 (45)	7 (23.3)	225 (44.3)	16 (53.3)	225 (44.3)	> 65 years	> 65 years	5 (26)	55 (40)
≥ 60	72 (55)	23 (76.7)	283 (55.7)	14 (46.7)	283 (55.7)			14 (74)	83 (60)
Gender									
Female	58 (44.3)	14 (45.2)	210 (41.3)	16 (53.3)	38 (34.9)	20 (68.9)	63 (37.9)	11 (57.8)	51 (37)
Male	73 (55.7)	17 (54.8)	298 (58.7)	14 (46.7)	71 (61.5)	9 (31.1)	103 (62.1)	8 (42.2)	87 (63)
IPI	R-IPI								
<3	66 (52)	13 (48.3)	310 (63.4)	9 (30)	54 (49.5)	11 (37.9)	113 (68.1)	4 (21)	69 (50)
≥3	61 (48)	16 (51.7)	182 (36.6)	21 (70)	55 (50.5)	18 (62.1)	53 (31.9)	15 (79)	69 (50)
Stage									
II	42 (33.3)	9 (32.1)	235 (47.8)	4 (13.3)	27 (24.8)	10 (34.5)	97 (58.4)	3 (16)	62 (45)
III-IV	84 (66.7)	19 (67.9)	257 (52.2)	26 (86.7)	82 (75.2)	19 (65.5)	69 (41.6)	16 (84)	76 (55)
LDH									
Normal	45 (35.4)	9 (32.1)	182 (38.8)	7 (29.2)	42 (44.7)	9 (31.1)	78 (47)	1 (5)	39 (27.3)
Elevated	82 (64.6)	19 (67.9)	287 (61.2)	17 (70.8)	52 (55.3)	20 (68.9)	88 (53)	18 (95)	99 (71.7)
ECOG									
0-1	40 (31.2)	17 (60.7)	387 (77.2)	6 (20)	59 (54.1)	18 (62.1)	132 (79.5)	10 (53)	96 (70)
>1	88 (68.8)	11 (39.3)	68 (14.9)	24 (80)	50 (45.9)	11 (37.9)	34 (20.5)	9 (47)	42 (30)

Extra nodal inv.	91 patients have extra nodal inv								
0-1		22 (76.7)	369 (77.2)	23 (76.7)	74 (68.5)	16 (55.1)	30 (18.1)	9 (47)	92 (67)
>1	70 %	8 (23.3)	117 (22.8)	7 (23.3)	34 (31.5)	13 (44.9)	136 (81.9)	10 (53)	46 (33)
B symptoms									
Absent	55 (43)	13 (46.4)	319 (65.9)	15 (50)	67 (61.5)	21 (72.4)	125 (75.3)	6 (32)	93 (67)
Present	73 (57)	15 (53.6)	165 (34.1)	15 (50)	42 (38.5)	8 (27.6)	41 (24.7)	13 (68)	45 (33)
Bone marrow inv.									
Absent	87 (76)	15 (57.7)	399 (91.1)	12 (54.5)	70 (80.5)	18 (62.1)	146 (87.9)	-	-
Present	26 (23)	11 (42.3)	39 (8.9)	10 (45.5)	17 (19.5)	11 (37.9)	20 (12.1)		
GCB	43 (34.9)	7 (23.3)	268 (53.2)	2 (7.4)	25 (31.3)	-	-	-	-
non-GCB	76 (61.7)	23 (76.7)	236 (46.8)	25 (92.5)	55 (68.7)				

Figures

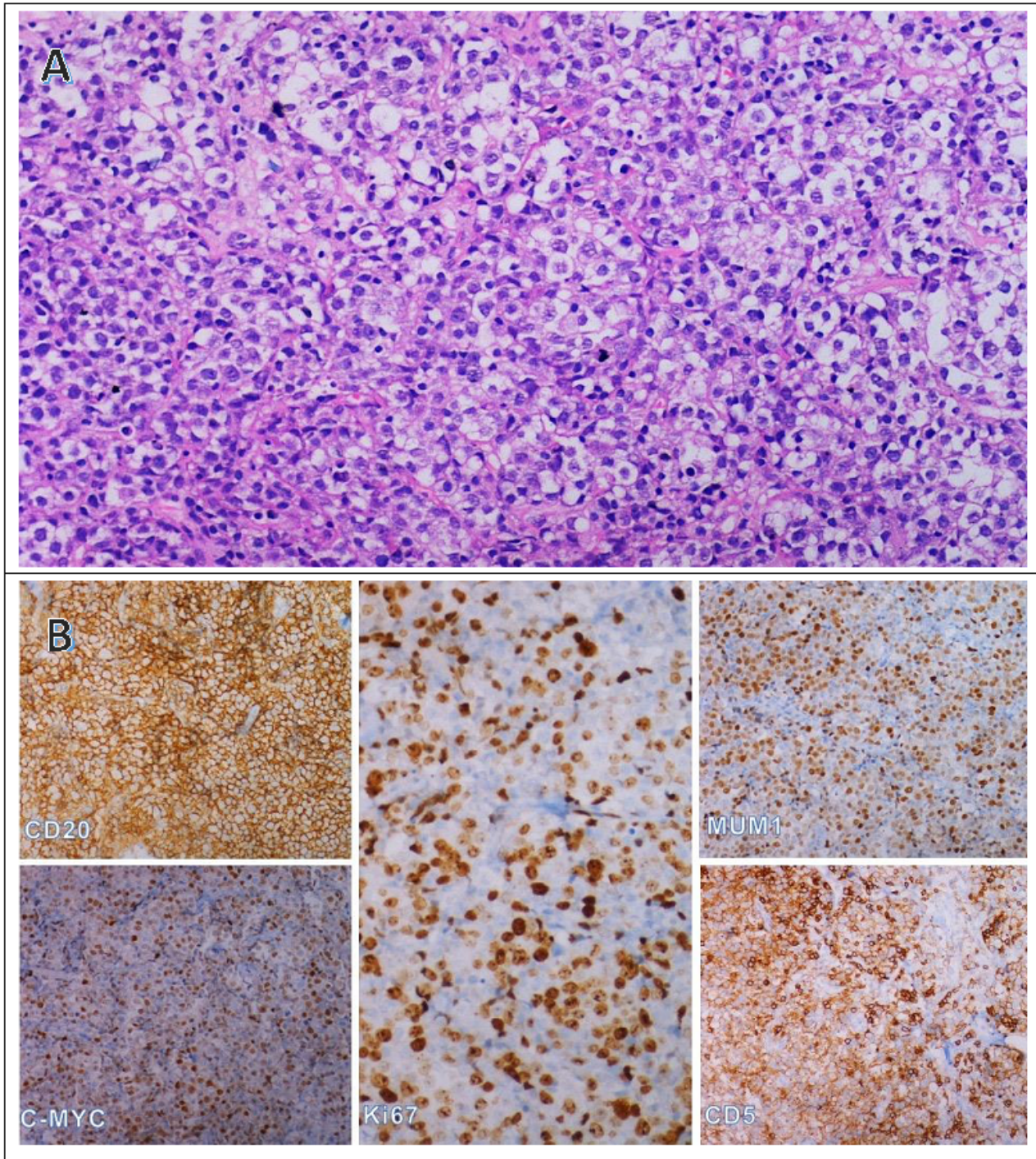


Figure 1

Many of the cases showed large pleomorphic cell groups with vacuolar cytoplasm and vesicular nucleus (A). Tumor cells were positive for CD20, MUM1, C-MYC and CD5. High Ki67 proliferative level was observed on most of the cases (B).

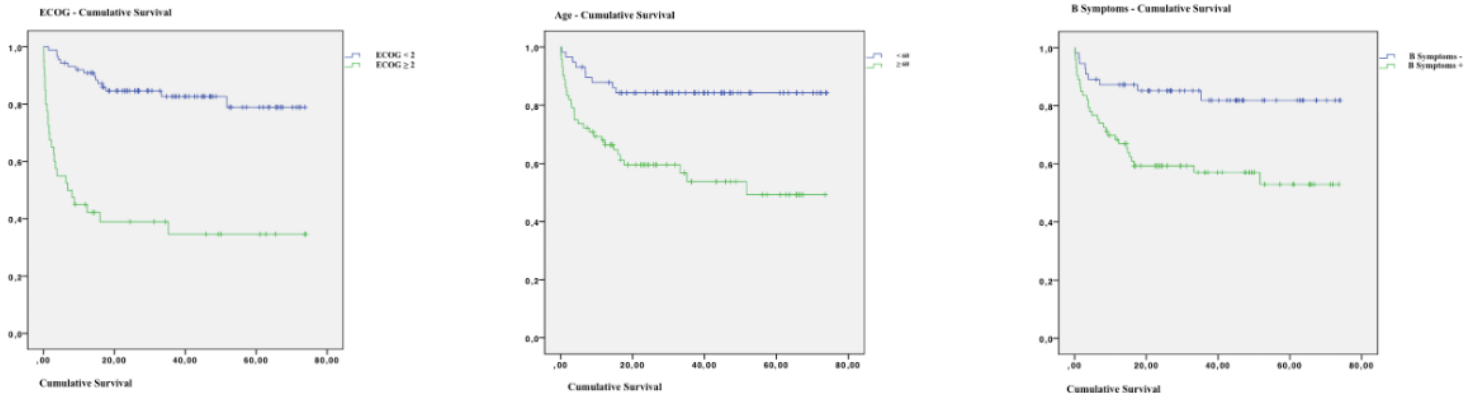


Figure 2

Evaluation of survival with Prognostic factors (ECOG, Age, B symptoms) in CD5 + patients

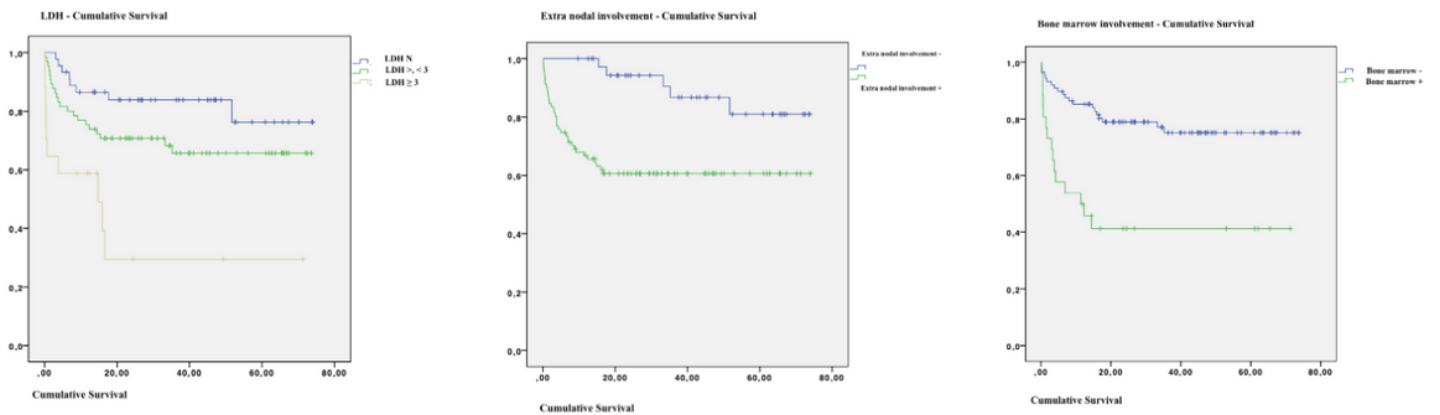


Figure 3

Evaluation of survival with Prognostic factors (LDH, EN, BM) in CD5 + patients

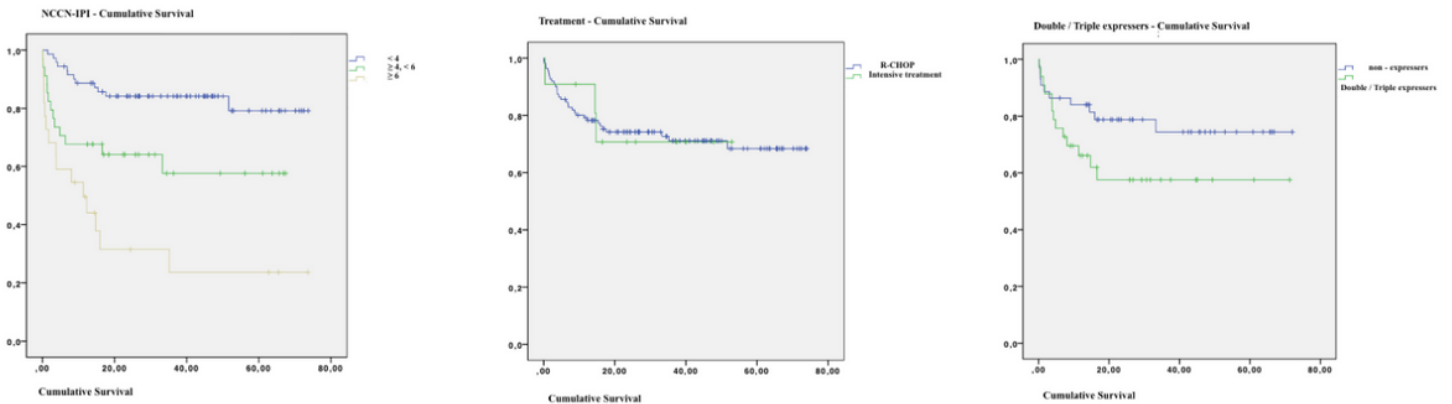


Figure 4

Evaluation of survival with Prognostic factors (NCCN-IPI, Treatment, D/T expr) in CD5 + patients

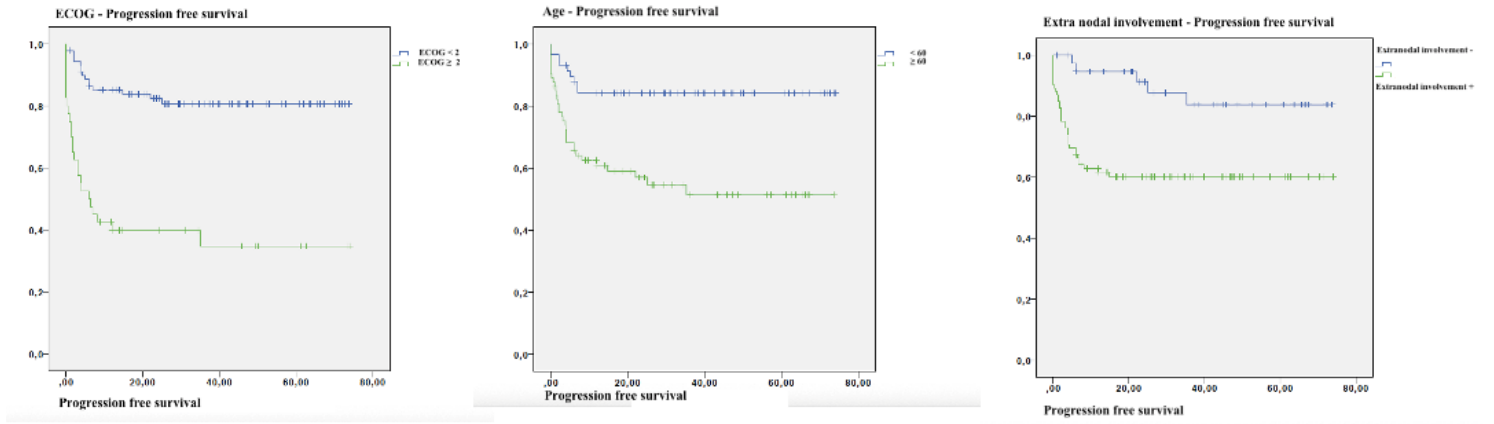


Figure 5

Evaluation of progression free survival (ECOG, Age, EN) with Prognostic factors in CD5 + patients

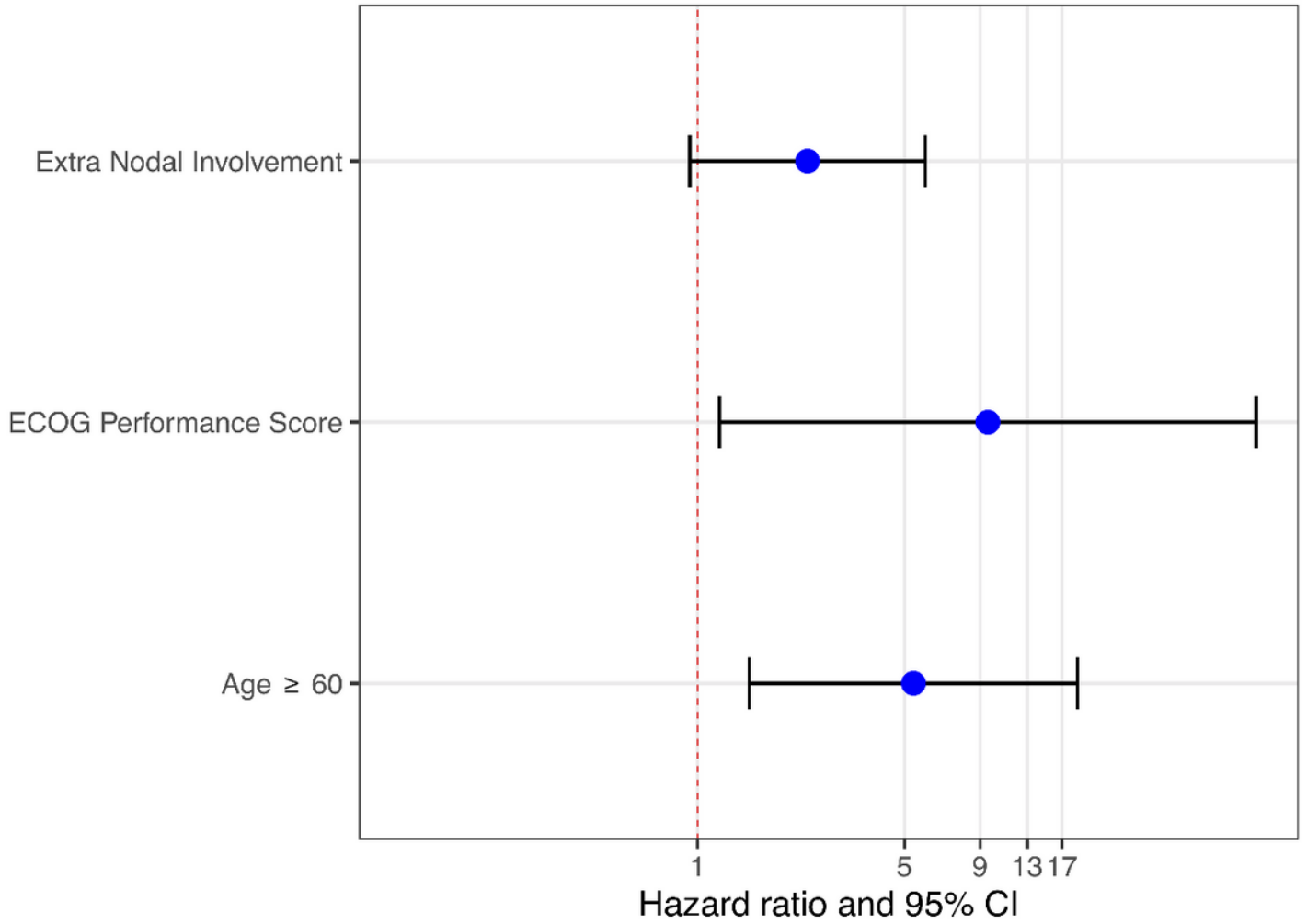


Figure 6

Multivariate analysis and overall survival (Y-axis is given in logarithmic scale)

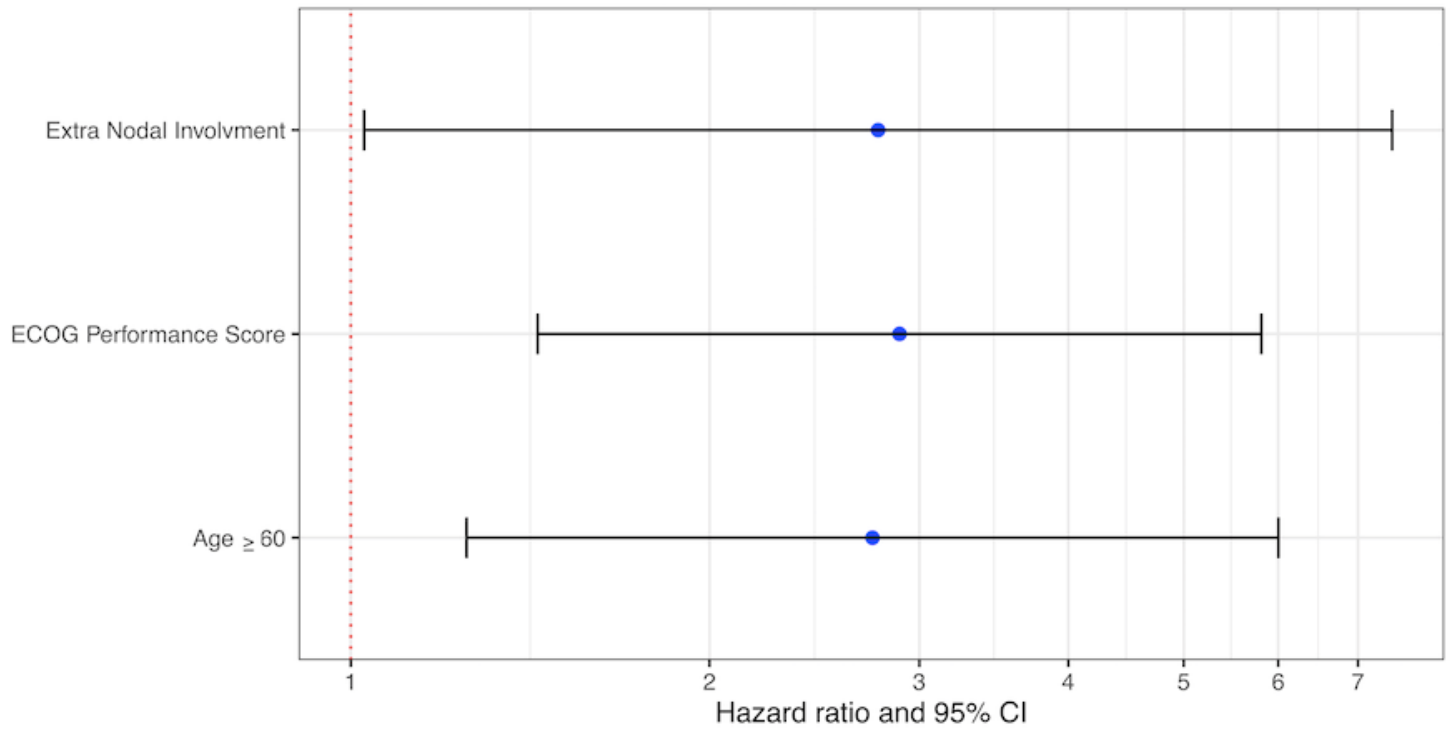


Figure 7

Multivariate analysis and Progression free survival (Y-axis is given in logarithmic scale)