Cytoplasmic expression of C-MYC protein is associated with risk stratification of mantle cell lymphoma

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Aim: To investigate the association of C-MYC protein expression and risk stratification in mantle cell lymphoma (MCL), and to evaluate the utility of C-MYC protein as a prognostic biomarker in clinical practice.

Methods: We conducted immunohistochemical staining of C-MYC, Programmed cell death ligand 1 (PD-L1), CD8, Ki-67, p53 and SRY (sex determining region Y) -11 (SOX11) to investigate their expression in 64 patients with MCL. The staining results and other clinical data were evaluated for their roles in risk stratification of MCL cases using ANOVA, Chi-square, and Spearman's Rank correlation coefficient analysis.

Results: Immunohistochemical staining in our study indicated that SOX11, Ki-67 and p53 presented nuclear positivity of tumor cells, CD8 showed membrane positivity in infiltrating T lymphocytes while PD-L1 showed membrane and cytoplasmic positivity mainly in macrophage cells and little in tumor cells. We observed positive staining of C-MYC either in the nucleus or cytoplasm or in both subcellular locations. There were significant differences in cytoplasmic C-MYC expression, Ki-67 proliferative index of tumor cells, and CD8 positive tumor infiltrating lymphocytes (CD8+TIL) among three risk groups (P=0.000, P=0.037 and P=0.020, respectively). However, no significant differences existed in the expression of nuclear C-MYC, SOX11, p53, and PD-L1 in MCL patients with low-, intermediate-, and high risks. In addition, patient age and serum LDH level were also significantly different among 3 groups of patients (P=0.006 and P=0.000, respectively). Spearman's rank correlation coefficient analysis indicated that cytoplasmic C-MYC expression, Ki-67 index, age, WBC, as well as LDH level had significantly positive correlations with risk stratification (P= 0.000, 0.015, 0.000, 0.029 and 0.000, respectively), while CD8+TIL in tumor microenvironment negatively correlated with risk stratification of patients (P=0.006). Patients with increased positive cytoplasmic expression of C-MYC protein and decreased CD8+TIL appeared to be associated with a poor response to chemotherapy, but the correlation was not statistically significant.

Conclusion: Our study suggested that assessment of cytoplasmic C-MYC overexpression and cytotoxic T lymphocytes (CTLs) by immunohistochemical staining might be helpful for MCL risk stratification and outcome prediction. However, large cohort studies of MCL patients with complete follow up are needed to validate our speculation.

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11   Abstract

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13   (MCL), and to evaluate the utility of C-MYC protein as a prognostic biomarker in clinical practice.

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21   staining of C-MYC either in the nucleus or cytoplasm or in both subcellular locations. There were significant
22   differences in cytoplasmic C-MYC expression, Ki-67 proliferative index of tumor cells, and CD8 positive tumor
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26   significantly different among 3 groups of patients (P=0.006 and P=0.000, respectively). Spearman's rank
27   correlation coefficient analysis indicated that cytoplasmic C-MYC expression, Ki-67 index, age, WBC, as well as
28   LDH level had significantly positive correlations with risk stratification (P= 0.000, 0.015, 0.000, 0.029 and 0.000,
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30   patients (P=0.006). Patients with increased positive cytoplasmic expression of C-MYC protein and decreased
31   CD8+TIL appeared to be associated with a poor response to chemotherapy, but the correlation was not
32   statistically significant.

33   Conclusion: Our study suggested that assessment of cytoplasmic C-MYC overexpression and cytotoxic T
34   lymphocytes (CTLs) by immunohistochemical staining might be helpful for MCL risk stratification and outcome
35   prediction. However, large cohort studies of MCL patients with complete follow up are needed to validate our
36   speculation.

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38

39   **Introduction**

40   Mantle cell lymphoma (MCL) is a less frequent subtype of B cell non-Hodgkin lymphoma characterized by
41   t (11; 14) chromosome translocation and aggressive clinical behavior(Smedby & Hjalgrim 2011). Despite new

42 advances of therapeutic methods in recent years, MCL remains an incurable disease with poor prognosis and
43 most patients eventually succumb to relapse after initial therapy(CheahSeymour & Wang 2016). CHOP
44 (cyclophosphamide, doxorubicin, vincristine, and prednisone)-like chemotherapy with or without rituximab or
45 autologous stem cell transplantation (ASCT) is the most popular regimen applied for the treatment of MCL,
46 with complete remission rates of 20%-50% and a median overall survival of about 3 years(Dreyling 2014; Vose
47 2015). Dose-intensified chemotherapy such as Hyper-CVAD (hyperfractionated cyclophosphamide, vincristine,
48 doxorubicin, and dexamethasone alternating with high-dose methotrexate and cytarabine) with rituximab has
49 achieved higher complete remission rates and 3-year failure-free survival in several clinical studies, however,
50 it has also induced significant toxicity, which made it ineligible for most elderly and frail
51 patients(KhouriRomagueraKantarjianPalmerPughKorblingHagemeisterSamuelsRodriguez & Giralt 2016;
52 RomagueraFayadRodriguezBroglioHagemeisterProMclaughlinYounesSamaniego & Goy 2005). In recent years,
53 with the advance in molecular pathogenesis of MCL, novel small molecular drugs including Bortezomib,
54 Lenalidomide, ibrutinib, and idelalisib have been introduced into treatment of relapsed/refractory MCL and
55 shown promising clinical outcome for part of these
56 patients(InamdarGoyAyoubAttiaOtonTaruvaiCostalesLinPecora & Suh 2016b). Due to genetic, pathological and
57 clinical heterogeneity of MCL (InamdarGoyAyoubAttiaOtonTaruvaiCostalesLinPecora & Suh 2016a), there is a
58 critical need for reproducible biomarkers to guide the decision of individualized risk-adapted treatment for
59 MCL patients.
60   C-MYC is one of the most frequently deregulated oncogenes in human cancer, and C-MYC encoded protein
61 functions as an important transcription factor involved in the regulation of cell growth and cell cycle
62 progression (DangO'DonnellZellerNguyenOsthus & Li 2006; Sears 2004). In malignant cells, genetic alteration
63 on C-MYC gene leads to consistent overexpression of C-MYC protein and promotes tumor progression (Meyer
64 & Penn 2008; Ott 2014). High expression of C-MYC was reported to be associated with poor outcome of a large
65 quantity of malignant diseases including aggressive lymphomas (SewastianikProchorec-SobieszekChapuy &
66 Juszczynski 2014). C-MYC abnormality has also been found in MCL patients with worse prognosis
67 (ChoeYunNaHuhShinKimPaikKimNamJeonPark & Kim 2016;
68 HuMedeirosChenChenLiKonoplevLuPhamYoungWang & Hu 2017). Recently, the immune-modulatory function
69 of C-MYC was identified in tumorigenesis (CaseyBaylot & Felsher 2017;
70 CaseyTongLiDoWalzFitzgeraldGouwBaylotGutgemannEilers & Felsher 2016), which has drawn much attention
71 on the role of oncogenes in tumor microenvironment.
72   Mantle cell lymphoma international prognostic index (MIPI) has been widely used for risk stratification of
73 advanced MCL patients. It combines clinical and laboratory parameters to divide patients into low-,
74 intermediate-, and high-risk groups (HosterDreylingKlapperGisselbrechtvan HoofKluin-
75 NelemansPfreundschuhReiserMetznerEinselePeterJungWormannLudwigDuhrsenEimermacherWandtHasford
76 Hiddemann & Unterhalt 2008; HosterKlapperHermineKluin-NelemansWalewskivan HoofTrnenyGeislerDi
77 RaimondoSzymczykStilgenbauerThieblemontHallekForstpointnerPottRibragDoorduijnHiddemannDreyling &
78 Unterhalt 2014). However, the correlation between C-MYC expression and MIPI, and the clinical value of C-
79 MYC expression in treatment decision for MCL patients are not clear. In this study, we investigated the
80 potential of C-MYC protein level assessed by immunohistochemistry for MCL risk stratification and evaluated
81 its role in individualized therapies for patients.
82

83 **Materials and methods**

84 **Case selection**

85   Cases of MCL diagnosed between 2013 and 2016 in Chongqing Cancer Institute/Hospital were reviewed
86 for complete information including pathological tissues and in-patient history. Sixty-four cases were identified
87 and included in this study. All cases were reviewed by two experienced pathologists according to the criteria
88 of the World Health Organization Classification. Data on clinical and laboratory findings such as staging, white
89 blood cell (WBC) count, renal and liver function tests including serum albumin, globulin and β2-microglobulin
90 (β2M), bone marrow biopsy, and imaging examinations (ultrasonic examination and radiologic examination of
91 brain, chest, abdomen and pelvis) at the time of diagnosis were reviewed. Patients were staged according to
92 Ann Arbor classification, and risk stratification was performed based on MIPI score system. Evaluation of
93 therapy response was based on the Lugano classification for response assessment of Hodgkin and non-Hodgkin
94 lymphoma(ChesonFisherBarringtonCavalliSchwartzZucca & Lister 2014). All the cases were divided into low-,
95 intermediate-, and high-risk groups according to MIPI score, as well as complete remission (CR), partial
96 remission (PR) and progressive disease (PD) groups according to the therapy response. The study was approved
97 by the ethics committees of Chongqing Cancer Institute/Hospital (No. 2017-016).

98

99 **Immunohistochemistry analysis**

100   Immunohistochemical staining was performed using formalin-fixed paraffin-embedded tissue sections
101 from pre-therapeutic samples according to the manufacturer's instructions. Rabbit anti-human C-MYC
102 monoclonal antibody (clone Y69, diluted in 1:150), mouse anti-human Ki-67 monoclonal antibody (clone MIB-
103 1, diluted in 1:200), and rabbit anti-human PD-L1 monoclonal antibody (clone SP142, diluted in 1:150) were
104 purchased from ORIGENE (USA). Rabbit anti-human CD8 monoclonal antibody (clone SP16, diluted in 1:200),
105 mouse anti-human SOX11 monoclonal antibody (clone MRQ-58, diluted in 1:150), and rabbit anti-human p53
106 monoclonal antibody (clone SP5, diluted in 1:200) were purchased from Abcam (USA). GTVision III detection
107 system was purchased from DAKO (USA). Positivity of membranous and cytoplasmic staining pattern was
108 scored by the staining density, ranging from 0 to 3 (0=no staining/-, 1=weak staining/+, 2=moderate
109 staining/++, 3=strong staining/+++). Positivity of nuclear staining pattern was quantified as the percentage of
110 positive MCL cells by manual inspection of stained sections. Tumor-infiltrating CD8 positive T cells were
111 quantified as total counts of CD8 positive lymphocytes per high power field (HPF; 0.2mm^2) by manual
112 inspection of stained sections with at least 10 fields of strong staining density. All the immuno-staining sections
113 were independently determined by 2 experienced pathologists in a blinded fashion.

114

115 **Statistical analysis**

116   All data were analyzed with SPSS 18.0 (IBM Corporation, NY, USA). Categorical variables were compared
117 using chi-square test. The difference between continuous variables was assessed using ANOVA. The role of
118 above clinicopathological parameters for MCL risk stratification and therapy individualization were evaluated
119 based on MIPI score and clinical response respectively. *P* value of less than 0.05 was considered statistically
120 significant. Spearman's rank correlation coefficient analysis was performed to assess the association between
121 clinical factors and immunohistochemical staining results of C-MYC, Ki-67, PD-L1, CD8, SOX11 and p53.

122

123 **Results**

124   **Patient characteristics**

125   There were 51 male (79.7%) and 13 female (20.3%) patients. The median age was 60 years old (range 21-
126   87 years) at the time of diagnosis. The majority of the cases (71.9%) presented as nodal diseases, and 18 cases
127   occurred in primary extra-nodal sites including colon, rectum, ileocecum, oropharynx, and spleen. Sixty-one
128   (95.3%) patients were in Ann Arbor advanced stage (Ⅲ-Ⅳ) and 35 (54.7%) patients presented with B
129   symptoms. Only 4 (6.4%) patients had an ECOG performance status (PS) of 2-4. Thirty-one of the 64 patients
130   had treatment information of at least 2 cycles of chemotherapy for response evaluation. As depicted in
131   supplementary data Table 1, 13 patients received CHOP, 11 patients received rituximab combined with CHOP,
132   3 patients received CHOPE, 3 patients received Hyper-CVAD and 1 patients received GDP treatment. After 2
133   treatment cycles, 5 patients achieved CR, 17 patients achieved PR and 9 patients demonstrated PD.

134

135   **Immunohistochemical study**

136   As shown in Figure 1, SOX11, Ki-67 and p53 presented nuclear positivity of tumor cells, and CD8 showed
137   membrane positivity of T lymphocytes infiltrating the microenvironment. PD-L1 showed membrane and
138   cytoplasm positive pattern mainly in macrophage cells and little in tumor cells. However, C-MYC staining was
139   observed either in the nucleus or cytoplasm or in both subcellular locations.

140   As shown in Table 1, there were significant differences in cytoplasmic C-MYC expression, Ki-67
141   proliferative index of tumor cells, and CD8 positive tumor infiltrating lymphocytes (CD8+TIL) among three risk
142   groups (P=0.000, P=0.037, P=0.020). However, no significant difference existed in the expression of nuclear C-
143   MYC, SOX11, p53, and PD-L1 among MCL patients with low, intermediate, and high risks, respectively. In
144   addition, other clinical parameters including age and LDH level showed significant difference among 3 groups
145   of patients as defined by MIPI score   (P=0.006, P=0.000).

146   Spearman's rank correlation coefficient analysis was performed to evaluate the relationship between
147   clinicopathologcial parameters and prognosis or therapy response. As shown in Table 1, patients' age, WBC,
148   LDH level, as well as cytoplasmic C-MYC expression and Ki-67 index demonstrated a significantly positive
149   correlation with risk stratification (P=0.000, 0.029, 0.000, and 0.000, respectively), while CD8+TIL in tumor
150   microenvironment negatively correlated with risk stratification of patients (P=0.006). Increased positive
151   cytoplasmic expression of C-MYC protein and decreased CD8+TIL were associated with a poor response to
152   chemotherapy, but the correlation did not reach statistical significance (Table 2 and Table 3).

153

154

155   **Discussion**

156   Assessment of C-MYC oncogene condition is critical for differentiating diagnosis and predicting prognosis
157   in Burkitt lymphoma and diffuse large B cell lymphoma harboring a C-MYC translocation(Dalla-
158   FaveraBregniEriksonPattersonGallo & Croce 1982; OttRosenwald & Campo 2013; SavageJohnsonBen-
159   NeriahConnorsSehnFarinhaHorsman & Gascoyne 2009). As a potent nuclear transcription factor, C-MYC
160   protein   overexpression   has   been   typically   found   in   the   nucleus   of   lymphoma
161   cells(ChoeYunNaHuhShinKimPaikKimNam   &   Jeon   2016;
162   OberleyRajguruZhangKimShawGrindleKahlKanughLaffin & Yang 2013). However, in this study, we observed
163   three patterns of C-MYC expression including nuclear, cytoplasmic, and both nuclear and cytoplasmic
164   localization in mantle cell lymphoma (Figure 1), which was different from the results reported by Matthew J.

165   et al using commercially available C-MYC monoclonal antibody (clone number: Y69). The
166   immunohistochemistry staining procedures have been independently validated by two experienced
167   pathologists to exclude false positive or non-specific results. We searched a few studies that reported
168   cytoplasmic expression of C-MYC in leukemia cell line, endometrial carcinoma, and high grade B cell
169   lymphomas (CraigBuchanCivin & Kastan 1993; GeislerGeislerManahanMillerWiemannZhou & Crabtree 2004;
170   RuzinovaCaron & Rodig 2010), but all the authors defined the cytoplasmic C-MYC status as negative or positive,
171   which was different with our four-categories evaluation methods (0, +, ++, +++). The mechanism and biologic
172   importance for the cytoplasmic overexpression of C-MYC was unclear. Since C-MYC needs to dimerize with
173   Max to bind the E-box to activate its downstream genes in transformed cells(Dang et al. 2006), accumulation
174   of C-MYC protein in the cytoplasm might suggest an unknown deregulated pathway synergized with other
175   pathways in promoting tumor growth. Therefore, the cytoplasmic pattern of C-MYC expression might indicate
176   a worse prognosis compared with the typical nuclear pattern. In this study, we found a positive correlation
177   only between cytoplasmic C-MYC level and risk stratification of MCL, but not between nuclear C-MYC level and
178   risk stratification of MCL, suggesting the potential clinical application of C-MYC immunohistochemical staining
179   in determining prognosis and treatment of MCL patients.

180   PD-L1 is an important immune checkpoint molecule in tumor microenvironment, which is associated with
181   immune evasion in a variety of malignancies (Dong & Chen 2003; JanakiramPareekChengNarasimhulu & Zang
182   2016; WlasiukPutowski & Giannopoulos 2016). Prevalence of Th1 type lymphocytes such as CD8+ T
183   lymphocytes usually predicts good prognosis of cancer patients (DonnemHaldPaulsenRichardsenAl-
184   SaadKilvaerBrustugunHellandLund-IversenPoehlOlsenDitzelHansenAl-
185   ShibliKiselevSandangerAndersenPezzellaBremnes & Busund 2015; Hanahan & Weinberg 2011). Expression of
186   PD-L1 and infiltration of cytotoxic T lymphocytes (CTLs) in tumor microenvironment are reported to be the
187   prerequisite for effective response of PD-L1 pathway blockade therapy in many cancer patients
188   (OckKeamKimLeeKimKimJeonKimChung & Heo 2016; TengNgiowRibas & Smyth 2015). High level of PD-L1
189   expression has been observed in some types of lymphomas including a subset of aggressive B cell lymphomas
190   and   EBV-associated   malignancies   such   as   classical   Hodgkin   lymphoma
191   (CHL)(BerghoffRickenWidhalmRajkyHainfellnerBirnerRaderer   &   Preusser   2014;
192   ChenChapuyOuyangSunRoemerXuYuFletcherFreemanShipp   &   Rodig   2013;
193   KwonKimKimGoNamPaikKimKimHeoKim & Jeon 2016). In our study, positive PD-L1 staining was found in
194   68.75% (33/48) MCL patients but only 1.04% (5/48) cases were recorded as moderate to strong positivity. In
195   addition, PD-L1 positive cells were mainly macrophages in the microenvironment. There was no significant
196   difference in PD-L1 expression among various risk groups, but the density of CD8+ T lymphocytes showed a
197   negative correlation with risk stratification, suggesting that CD8+ T lymphocytes might be a useful prognostic
198   biomarker for risk stratification of MCL patients, consistent with other previous studies
199   (NygrenWasikBaumgartner-WennerholmJeppsson-
200   AhlbergKlimkowskaAnderssonBuhrkuhlChristenssonKimbyWahlin & Sander 2014). Extensive future study of
201   PD1/PD-L1 pathway in a large cohort of MCL patients is also warranted.

202   Ki-67 as an independent, significant prognostic factor for MCL has been proved in many clinical studies
203   and integrated into MIPI score system as a combined biological index (MIPI$_b$)(Hoster et al. 2008). The
204   significance of Ki-67 was also confirmed in our study. We similarly assessed the expression of another two
205   important biomarkers, SOX11(EkDictorJerkemanJirstrom & Borrebaeck 2008; Xu & Li 2010) and p53

206 (NordstromSernboEdenGronbaekKolstadRatyKarjalainenGeislerRalfkiaerSundstromLaurellDelabieEhingerJerk
207 eman & Ek 2014; TessoulinEveillardLokChironMoreauAmiotMoreau-AubryLe Gouill & Pellat-Deceunynck
208 2017), in MCL cases but found no significant difference among low, intermediate, and high risk group of MCL
209 patients.
210   We also noted that patients with increased positive cytoplasmic expression of C-MYC protein and
211 decreased CD8+TIL were associated with poor response to chemotherapy, but the correlation did not reach
212 statistical significance, probably due to the loss of follow-up date in 33 of 64 patients in our cohort.
213   Limitations of the study included small cohort of cases and insufficient survival data because of the
214 difficulties in follow-up for most lymphoma patients of this area. In addition, the systematic bias including
215 tissue fixation and observation might influence the accuracy of assessment for protein biomarkers in
216 immunohistochemistry studies (TorlakovicRiddellBanerjeeEl-
217 ZimaityPilavdzicDaweMaglioccoBarnesBerendtCookGilksWilliamsPerez-
218 OrdonezWehrliSwansonOtisNielsenVyberg & Butany 2010). Nevertheless, this is the first report on the
219 association of aberrant expression of C-MYC protein, CD8+TIL, and Ki-67 with risk stratification of MCL
220 patients.
221   In conclusion, our work suggested that assessment of cytoplasmic C-MYC overexpression, CD8 positive
222 CTLs, and Ki-67 by immunohistochemical staining might be helpful for risk stratification and prognosis of MCL
223 patients. Large cohort studies of MCL patients with complete follow up are needed to further examine the
224 potential of these biomarkers being integrated into routine pathological work.
225
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227

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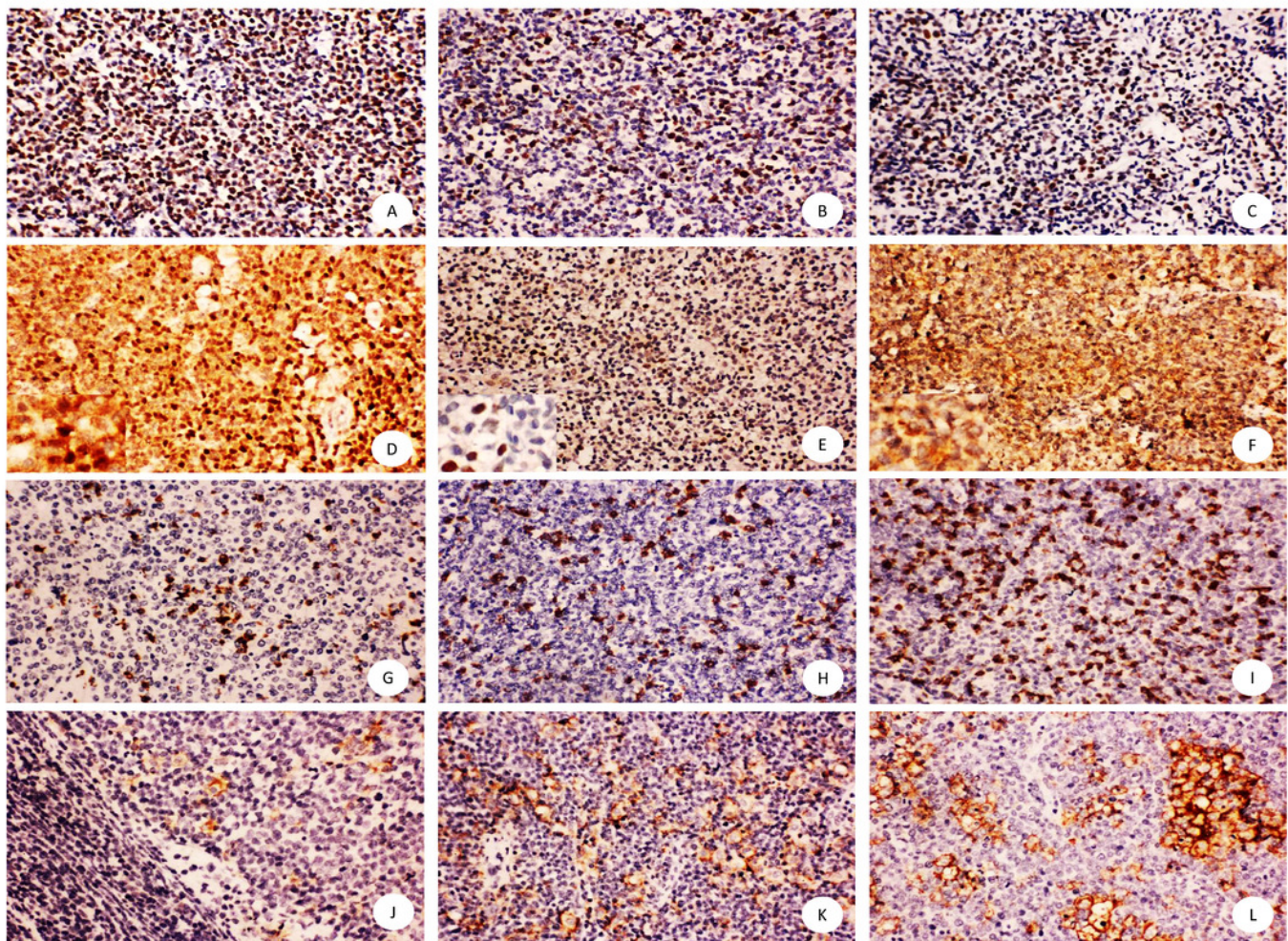
Figure 1

Immunohistochemical staining of mantle cell lymphoma using anti-SOX11, Ki67, p53, C-MYC,CD8, PD-L1

Shown are representative staining patterns of SOX11 (A), Ki-67 (B), p53 (C), C-MYC (D-F),CD8 (G-I), PD-L1 (J-L). Original magnification, ×200. Inserts: typical cytoplasmic and nuclear staining of C-MYC (D), nuclear staining of C-MYC (E), and cytoplasmic staining of C-MYC (F), Original magnification, ×400.

Clinical and immunohistochemical staining characteristics of patients grouped by risk

Note: * *P*<0.05, ** *P*<0.01.

Variable	n	Low risk	Intermediate risk	High risk	P value
Sex	64				.117
Male	51 (79.7%)	15 (23.4%)	27 (42.2%)	9 (14.1%)	
Female	13 (20.3%)	5 (7.8%)	3 (4.7%)	5 (7.8%)	
Age (years)	64	54.10±8.60	61.77±7.77	64.86±15.39	.006**
WBC (10⁹/L)	64	6.50±2.17	8.07±3.69	8.93±3.19	.078
LDH (U/L)	64	190.03±74.84	341.35±226.03	521.15±266.44	.000**
ECOG performance Status	64				0.112
0	46 (71.9%)	16 (25.0%)	22 (34.4%)	8 (12.5%)	
1	14 (21.9%)	4 (6.3%)	7 (10.9%)	3 (4.7%)	
2	4 (6.2%)	0 (0.0%)	1 (1.6%)	3 (4.7%)	
Ann Arbor stage	64				.302
II	3 (4.69%)	2 (3.1%)	1 (1.6%)	0 (0.0%)	
III	32 (50.0%)	11 (17.2%)	12 (18.8%)	9 (14.1%)	
IV	29 (45.31%)	7 (10.9%)	17 (26.6%)	5 (7.8%)	
Location	64				0.589
Nodal	46 (71.9%)	16 (25.0%)	20 (31.3%)	10 (15.6%)	
Extra-nodal	18 (28.1%)	4 (6.3%)	10 (15.6%)	4 (6.3%)	
B symptoms	64				.363
No	29 (45.3%)	10 (15.6%)	15 (23.4%)	4 (6.3%)	
Yes	35 (54.7%)	10 (15.6%)	15 (23.4%)	10 (15.6%)	
Response	31				.883
CR	5 (7.8%)	2 (6.5%)	3 (9.7%)	0 (0.0%)	
PR	17 (26.56%)	6 (19.4%)	8 (25.8%)	3 (9.7%)	
PD	9 (14.1%)	3 (9.7%)	5 (16.1%)	1 (3.2%)	
Cytoplasmic C-MYC	64				.000**
-	19 (29.7%)	11 (17.2%)	7 (10.9%)	1 (1.6%)	
+	25 (39.1%)	6 (9.4%)	17 (26.6%)	2 (3.1%)	
++	20 (31.2%)	3 (4.7%)	6 (9.4%)	11 (17.2%)	
Nuclear C- MYC (%)	64	16±20	11±13	16±19	.537
P53 (%)	61	19±21	13±17	30±31	.082
CD8+TIL(/0.2mm²)	61	160.00±55.52	132.07±54.60	105.71±45.69	.020*
PD-L1	48				.599
-	15 (31.3%)	6 (12.5%)	6 (12.5%)	3 (6.3%)	
+	28 (58.3%)	4 (8.3%)	17 (35.4%)	7 (14.6%)	
++	4 (8.3%)	1 (2.1%)	2 (4.2%)	1 (2.1%)	
+++	1 (2.1%)	0 (0.0%)	1 (2.1%)	0 (0.0%)	
SOX11 (%)	64	33±31	29±31	29±27	.887

Ki67 (%)	64	31±16	45±25	50±26	.037[*]

Clinical and immunohistochemical staining characteristics of patients grouped by treatment response

Note: * $P<0.05$.

Variable	n	CR	PR	PD	*P* value
Sex					.117
Male	23 (74.2%)	5 (16.1%)	10 (32.3%)	8 (25.8%)	
Female	8 (25.8%)	0 (0.0%)	7 (22.6%)	1 (3.2%)	
Age (years)	31	52.60±3.91	56.24±12.73	56.89±8.89	.760
WBC (10^9/L)	31	10.06±3.61	7.68±3.78	7.54±3.43	.407
LDH (U/L)	31	334.82±243.60	333.21±269.65	273.31±95.30	.804
ECOG performance Status					0.130
0	21 (67.7%)	4(12.9%)	13 (41.9%)	4(12.9%)	
1	8 (25.8%)	1(3.2%)	2 (6.5%)	5 (16.1%)	
2	2 (6.5%)	0 (0.0%)	2 (6.5%)	0 (0.0%)	
Ann Arbor stage					.905
II	1 (3.2%)	0 (0.0%)	1 (3.2%)	0 (0.0%)	
III	16 (51.6%)	3 (9.7%)	8 (25.8%)	5 (16.1%)	
IV	14 (45.2%)	2 (6.5%)	8 (25.8%)	4 (12.9%)	
Location					.437
Nodal	23 (74.2%)	3 (9.7%)	12 (38.7%)	8 (25.8%)	
Extra-nodal	8 (25.8%)	2 (6.5%)	5 (16.1%)	1 (3.2%)	
B symptoms					.844
No	15 (48.4%)	3 (9.7%)	8 (25.8%)	4 (12.9%)	
Yes	16 (51.6%)	2(6.5%)	9 (29.0%)	5 (16.1%)	
Risk group					.720
low	11 (35.5%)	2 (6.5%)	6(19.4%)	3 (9.7%)	
intermediate	16 (51.6%)	3 (9.7%)	8 (25.8%)	5 (16.1%)	
high	4 (12.9%)	0 (0.0%)	3 (9.7%)	1 (3.2%)	
Cytoplasmic C-MYC					.031*
-	11 (35.5%)	2 (6.5%)	8 (25.8%)	1 (3.2%)	
+	11 (35.5%)	3 (9.7%)	2 (6.5%)	6 (19.4%)	
++	9 (29.0%)	0 (0.0%)	7 (22.6%)	2 (6.5%)	
Nuclear C- MYC (%)	31	17±12	15±17	6±7	.227
P53 (%)	31	16±21	20±23	20±23	.950
CD8+TIL(/0.2mm²)	31	154.00±35.78	145.00±55.74	111.11±64.51	.277
PD-L1					.352
-	11 (45.8%)	1 (4.2%)	5 (20.8%)	5 (20.8%)	
+	11 (45.8%)	3(12.5%)	6 (25.0%)	2 (8.3%)	
++	2 (8.3%)	0 (0.0%)	2 (8.3%)	0 (0.0%)	
SOX11 (%)	31	36±45	26±30	34±31	.757
Ki67 (%)	31	38±16	50±27	38±30	.480

Correlation coefficient analysis for different parameters with risk group

Note: * *P*<0.05, ** *P*<0.01.

Variable	n	Spearman correlation coefficient with risk group	P value
Sex	64	0.055	0.669
Age	64	0.454**	0.000
WBC	64	0.273*	0.029
LDH	64	0.593**	0.000
ECOG performance status	64	0.207	0.101
Ann Arbor stage	64	0.078	0.542
Location	64	0.085	0.502
B symptoms	64	0.138	0.278
Response	31	0.058	0.755
Cytoplasmic C-MYC	64	0.496**	0.000
Nuclear C- MYC	64	0.005	0.968
P53	61	0.079	0.544
CD8+TIL	61	-0.351**	0.006
PD-L1	48	0.163	0.268
SOX11	64	-0.020	0.873
Ki67	64	0.303*	0.015