

## RESEARCH ARTICLE

# Ki-67 Labeling Indices in 'Classic' versus 'Blastoid' Mantle Cell Lymphomas - Proposed Cutoff Values for Routine Diagnostic Workup

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## Abstract

**Background:** Mantle-cell lymphoma (MCL) is a unique entity of peripheral B-cell lymphoma that has a discrete morphologic, immunologic, and genetic phenotype, with more common 'classic' and less frequent 'blastoid' and 'pleomorphic' variants, associated with an aggressive clinical course. The aim of this study was to analyze proliferation (Ki-67) indices of 'classic' (c-MCL) and 'blastoid' (b-MCL) variants of a cohort of MCL and to suggest cut off values for the Ki-67 proliferation index in these two subsets. **Materials and Methods:** MCL cases diagnosed over 4 ½ years at Section of Histopathology, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi were retrieved and reviewed. Ki-67 labelling was scored and analysed. **Results:** A total of 90 of cases of MCL were scrutinized. Mean age  $\pm$  SD was  $60.2 \pm 12.5$  years and the male to female ratio was 4:1, with 67 (75%) cases of c-MCL and 23 (25%) cases of b-MCL. Most samples were lymph node biopsies (n=68), whereas the remainder were from various extranodal sites. The mean Ki-67 proliferation index was  $29.5\% \pm 14.4\%$  in classic variants and  $64.4 \pm 15.2\%$  for the blastoid variant, the difference being statistically significant ( $p = 0.029$ ). **Conclusions:** It was concluded that differential cut-off values of Ki-67 labeling may be used in more objective way to reliably classify MCL into classic or blastoid variants by diagnostic pathologists. We propose a  $< 40$  proliferative index to be suggestive of c-MCL and one of  $> 50$  for the blastoid variant.

**Keywords:** Mantle cell lymphoma - classic - blastoid - Ki-67 labelling index

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## Introduction

Mantle cell lymphoma (MCL) is a distinct type of B cell non-Hodgkin lymphoma (B-NHL) that accounts for approximately 3-10% of all non-Hodgkin lymphomas (NHL), (Swerdlow et al, 2008; Sader-Ghorra et al, 2014). Patients with MCL show a relatively poor prognosis, with a median survival of about 5 yr. The disease show a predilection for older males, and patients typically present at an advanced stage with frequent extranodal involvement (Mushtaq et al, 2008; Haroon et al, 2014).

MCL is strongly associated with chromosomal abnormalities in particular the translocation t(11;14) and deletion of 11q22-23. A more aggressive form, the blastoid-variant MCL (b-MCL), presents at an earlier age with significant short survival i.e. Mean survival of less than 20 months. The fact that patients with b-MCL show a higher proliferative rate warrants that this variant should be reliably categorized as an aggressive lymphoma (Klapper et al, 2009; Vose 2013).

Diagnostic utility of Ki-67 labeling is well established in variety of hematological and non-hematological malignancies with objective guidelines which are very useful to diagnostic pathologist in their routine practice

(He et al, 2014). Ki67 labeling index is also an important independent prognostic marker in MCL (Katzenberger et al, 2006; Determann et al, 2008; Blaker et al, 2015). The MCL international prognostic index (MIPI) is the prognostic model most often used and incorporates Eastern Cooperative Oncology Group (ECOG) performance status which usually includes age, leukocyte count, and lactic dehydrogenase. A modification of the MIPI also adds the Ki-67 proliferative index if available (Hoster et al, 2008). However no clear or objective guidelines are available for the diagnostic pathologists emanating from robust studies and analyses to confidently use in routine practice. Objective of this study was therefore to assess Ki-67 labeling index of c-MCL and b-MCL variants in a cohort of MCL diagnosed cases and suggest (if any) statistically significant values.

## Materials and Methods

### Case selection

All MCL cases diagnosed between during 4 ½ yrs at Section of Histopathology, Department of Pathology and Laboratory Medicine, Aga Khan University Hospital, Karachi were included. All cases were initially diagnosed

on the basis of morphological and immunophenotypic findings according to WHO classification of Neoplastic Diseases of Hematopoietic and Lymphoid Tissue (2008) and were reviewed again by histopathologist with haematopathology sub-specialty interest and training.

**Histology and immunohistochemistry**

All specimens were fixed in 10% neutral buffered formalin, embedded in paraffin, 4 μm sections were cut and stained with Hemotoxylin and Eosin (H&E) for histological evaluation. Immunohistochemical staining was performed using formalin-fixed, paraffin-embedded tissue sections. Briefly, tissue sections were deparaffinized in xylene, rehydrated in graded alcohols. For antigen retrieval, tissue sections were immersed in Target retrieval solution (pH 9.0) as appropriate for each antibody used and endogenous peroxidase was blocked with 3% hydrogen peroxide. After the sections were rinsed with phosphate-buffered saline (PBS), immunohistochemical analysis was performed using antibodies by Dako against LCA (clone 2B11+PD7/26, dil 2: 1000), CD20 (clone L26, dil 2: 1000), CD3 (polyclonal, dil 10: 1000), CD5 (clone 4C7, prediluted), CyclinD1 (clone SP4, prediluted) and Ki-67 (clone Mib-1, dil 5: 1000). Then it was treated with secondary antibody i.e. Flex HRP and in the end chromogen DAB was used to detect the reaction. These stains were performed on an automated Dako immunostainer.

Appropriate positive and negative controls were run with each batch. The Ki-67 proliferation index was assessed by counting at least 300 tumor cells in at least 2 representative areas of the lymphoma on Ki-67-stained sections as per consensus recommendation. (Ek et al., 2004; Todorovic et al., 2011)

**Statistical analysis**

SPSS statistical software (SPSS Inc., Chicago IL, USA) was used for the statistical analyses. Associations in 2 × 2 tables were evaluated with Fisher’s exact test. Correlations were assessed by Pearson’s or Spearman’s correlation analysis.

**Results**

**Clinico-pathological features**

A total of 90 cases were included. Mean age ± SD was 60.2 ± 12.46 years. Median age was 60 years. Of all the patients included in the study, 72 were males and 18 females (M: F; 4:1). Most tissue samples were lymph nodes biopsies (n=68), whereas the remainder were derived from various extranodal sites (10 from GIT, 3 from bone, 2 from spleen and one each from parotid, bronchus and testis). B symptoms were present in 41 (45.5%) patients. All cases showed strong CyclinD1 immunostaining and a phenotype and morphology consistent with MCL. Sixty seven (74.5%) cases were classified as classic and 23 (25.5%) as blastoid subtype of MCL. Median age was higher in c-MCL i.e. 62 years and lower in b-MCL i.e. 45 years.

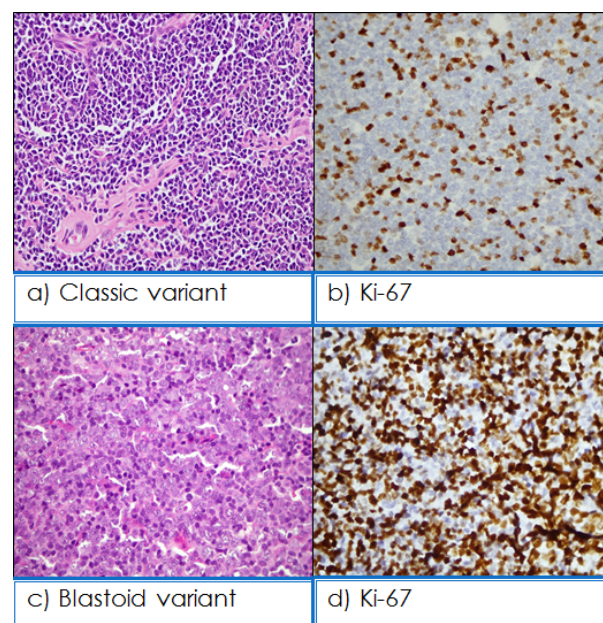
**KI-67 Proliferation index**

The mean proliferation index, as assessed by Ki-67 staining, was 29.5% ± 14.4% in ‘classic’ variants and 64.4 ± 15.2% for the ‘blastoid’ variant. The mean difference between the Ki-67 indices of both groups was 34.87 which proved to be statistically significant when independent sample T-test was applied (P<0.0001 and 95%CI= 27.86 to 41.88). All 23 cases (100%) of ‘blastoid’ MCL showed high proliferation > 30 % as opposed to 11/67 (16%) of the classic variant (P<0.0001) when Pearson Chi-Square test was applied. None of the ‘classic’ variant revealed >50% Ki 67 proliferation index and none of ‘blastoid’ variant showed <40% Ki 67 staining (Figure 1, 2).

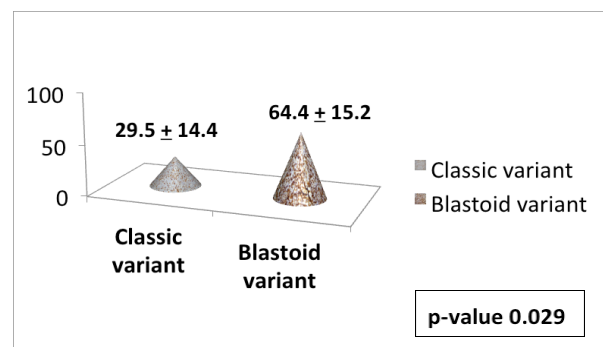
**Correlation between Ki-67 and morphology**

In the univariate analysis, a high Ki-67 expression level of >50% was the significant factor for predicting blasoid morphology (p<0.001). The 50% Ki-67 cutoff point had the largest sum of sensitivity (78.0%) and specificity (82.0%) for the blastoid morphology.

Using the Receiver Operating Characteristic (ROC)



**Figure 1. The Two Variants of Mantle Cell Lymphoma with the Ki-67 (Mib-1) Immunostaining.** A & B) Classic mantle cell lymphoma showing small cell population having irregular nuclei with 10% Ki-67 proliferation index (x20). C & D) Blastoid mantle cell lymphoma with enlarged rounded nuclei and 70% Ki-67 proliferation index (x20)



**Figure 2. The Mean Values of Ki-67 index in Classic and Blastoid Variant**

curve method, the best cut-off value of Ki67 that best predicts classic morphology in the current study was 40% with a sensitivity of 73% and a specificity of 84%, having largest area under curve.

## Discussion

MCL is a unique entity among mature B-cell lymphomas with a unique translocation (11; 14). Cyclin D1 staining is used as a surrogate marker for t(11; 14) and is positive in almost all cases of MCL. Besides MCL Cyclin D1 positivity may also be seen in 'Hairy Cell Leukemia (HCL)' albeit weakly in most cases. Therefore it is important that if CD5 is negative in a Cyclin D1 positive small B-cell lymphoma, differential diagnosis of HCL should be considered. MCL patients usually present with lymphadenopathy as was depicted in our study and may commonly have involvement of the extranodal tissue like GIT, bone, spleen and other organs (Haroon et al, 2014; Mushtaq et al 2008; Swerdlow et al, 2008). In Mid-1970s Rappaport classification system classified it as diffuse or vaguely nodular low-grade lymphoma of intermediate differentiation. With the Kiel classification system and Working formulation system's definitions, the evolutionary process led Banks and colleagues to coin the term 'Mantle Cell Lymphoma' for the first time in 1992 and finally this was included in World Health Organization (WHO) classification system in 2001 and 2008 (Swerdlow et al, 2008)

Morphologically, MCL is composed of sheets of monotonous population of small discohesive B cells with angulated irregular nuclear contours and moderate amounts of cytoplasm. Nucleoli are inconspicuous while immunoblasts and paraimmunoblasts typically seen in Small Lymphocytic Lymphoma (SLL) are absent. Starry sky pattern may be seen. MCL most often arises from a CD5+ B-cell and hence express CD5 as well. These usually grow with a diffuse, nodular, or mantle zone pattern. Blastoid variant show irregularly shaped pleomorphic nuclei with clumped granular chromatin having increased nuclear to cytoplasmic ratio. Apoptotic tumor cells and mitoses are more frequent (Swerdlow et al, 2008). At times morphology of b-MCL may closely mimic Precursor Lymphoblastic lymphoma/leukemia. Indeed cases with suspected lymphoblastic morphology and B immunophenotype, if Tdt negative shall prompt the suspicion of b-MCL and cyclin D1 shall be included in the panel.

The genetic hallmark of MCL, the translocation t(11;14)(q13; q32), juxtaposing the IGH on 14q32 with the BCL1/CCND1 gene on 11q13, results in statutory overexpression of CCND1 and cell cycle deregulation, in essentially all cases (Ek et al, 2004). MCL is typically positive for B-cell associated markers in addition to Bcl-2, Cyclin D1, CD5, and/or CD43 and is negative for Bcl-6, CD10, and CD23 on immunohistochemistry. Attempts to predict the prognosis for patients with MCL using clinical and laboratory parameters led to development of MCL IPI (MIPI), which have proved to be more useful than other parameters (Raty et al, 2003). ECOG performance status typically includes age, leukocyte count and lactic

dehydrogenase. A modification of the MIPI also adds the Ki-67 proliferative index if available (Salek et al, 2014).

The Ki-67 protein is present during all active phases of the cell cycle G1, S, G2 & mitosis, but is absent from resting cells (Go). The fraction of Ki-67-positive tumor cells (the Ki-67 labeling index) is often correlated with the clinical course of the disease. Ki-67 is an indicator of how fast cells mature and is expressed in a range of percentages (Katzenberger et al, 2006; Tiemann et al, 2005). The lower the percentage, lower the speed of maturity hence more indolent the disease and vice versa. However highly subjective assessment of Ki-67 labeling in routine diagnosis workup as low vs. high pose a serious flaw without defining cut-off values, albeit arbitrarily, as grey zones always exist.

The recognition of b-MCL is important. Besides morphological criteria inclusions, reporting of Ki-67 labeling index is a routine in the reporting of all NHLs including MCL, however very little is currently available in literature about mean and median cut-off values in these two variants (Raty et al, 2002; Salek et al, 2014; Todorovic et al, 2012; Yngvild et al, 2015). We found the mean of Ki-67 in c-MCL was 29.5% ± 14.4% vs. 64.4 ± 15.2% in b-MCL, which was highly significant. None of b-MCL showed <40% ki-67 labeling while no c-MCL showed >50% labeling. A grey zone however did exist for a small number of cases where it was not possible to draw a line based on labeling ki-67 index alone.

In conclusion, in this study Ki-67 index showed statistically significant difference between Classic & Blastoid variants. We therefore propose <40 Proliferation index to be suggestive of Classic while >50% suggestive of Blastoid variant.

Future larger studies are however still required to validate our study and come up with even better refined cut-off values.

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