Prognostic Value and Computer Image Analysis of p53 in Mantle cell Lymphoma

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Abstract

Background: P53 has different prognostic cutoff values in different mantle cell lymphoma (MCL) studies, and p53 immunohistochemistry (IHC) interpretation is still based on semiquantitative estimation, which might be inaccurate. This study aimed to investigate the optimal cutoff value of p53 for predicting prognosis and the possible use of computer image analysis to identify the positive rate of p53 in patients with MCL.

Methods: We used QuPath software to determine the p53 positive rate and compared it to the data obtained by manual counting and semiquantitative estimation. Using the Youden index and Kaplan–Meier survival curve analysis, we generated survival curves. The chi-squared (χ^2) test was used to compare MCL cell morphology with p53. Spearman rank correlation test and Bland–Altman analysis were used to compare manual counting, computer image analysis and semiquantitative estimation.

Results: The optimal cutoff value of p53 for predicting prognosis was 20% in MCL patients. Patients with $p53 \geq 20\%$ had a significantly worse overall survival (OS) than those with $p53 < 20\%$ ($P < 0.0001$). MCL patients with blastoid/pleomorphic variant cell morphology had more $p53 \geq 20\%$ than the classical type ($P < 0.0001$). There was a strong correlation between computer image analysis and manual counting of p53 from the same areas in MCL tissues (Spearman's $\rho = 0.966$, $P < 0.0001$).

Conclusions: MCL patients with $p53 \geq 20\%$ have a shorter OS and a tendency toward the blastoid/pleomorphic variant. Computer image analysis could determine the actual positive rate of p53 and is a more attractive alternative than semiquantitative estimation in MCL.

Introduction

Mantle cell lymphoma (MCL) is an aggressive B-cell non-Hodgkin lymphoma (NHL) and comprises approximately 3–10% of NHLs^{1–3}. The clinical prognoses of MCL are heterogeneous, and risk stratification is based on the clinical indices comprising the Mantle Cell Lymphoma Prognostic Index (MIPI) and a combined biological MIPI, including Ki67^{4–6}. However, risk stratification might be further improved by biological markers, such as p53. P53 protein expression might serve as an alternative marker for *TP53* gene mutations^{7–9} and has been confirmed to be of prognostic value independent of the biological MIPI score in multiple MCL studies^{7,10–12}. However, the prognostic cutoff values of p53 differ in different studies; thus, its use is still controversial. Furthermore, p53 immunohistochemistry (IHC) interpretation is still based on semiquantitative estimations that are inaccurate because of more considerable interobserver variability. Therefore, practical and efficient means for precisely interpreting the positive rate of p53 with less interobserver variability would be of great value for diagnosing MCL.

In the present study, we aimed to investigate the prognostic cutoff value of p53 in MCL. Moreover, we applied computer image analysis software to calculate p53 in MCL patients and compared the data to the results obtained from manual counting and semiquantitative estimation in routine clinical diagnostics.

Results

Patient characteristics

The cohort included 65 MCL patients. The male to female ratio was 2.61:1, and the median age was 63. Thirty-eight (58%) patients were at Ann Arbor stage I–II and 27 (42%) were at stage III–IV. From these patients, 43 (66%) tumor tissue specimens were derived from lymph nodes, and others were derived from the gastrointestinal tract, tonsil, nasopharynx, bone marrow, etc. The tumor cell morphology of 36 (55%) patients was the classical type, and 29 (45%) patients had blastoid/pleomorphic variants. Follow-up data were obtained for all 65 patients, and 51 (78%) patients with MCL had died (Table 1).

Table 1
Clinical and pathological features of MCL patients.

Category		n	%
Sex	Male	47	72
	Female	18	28
Age [median (range)]		63 (38-84)	NA
Ann Arbor stage	I-II	38	58
	III-IV	27	42
Tumor tissue type	Lymph node	43	66
	Gastrointestinal tract	9	14
	Tonsil	5	8
	Nasopharynx	2	3
	Bone marrow	2	3
	Others	4	6
Cell morphology	Classical type	36	55
	Blastoid/pleomorphic variant	29	45
Survival state	Surviving	14	22
	Deceased	51	78
MCL, mantle cell lymphoma; NA, not applicable.			

Manual counting, computer image analysis and semiquantitative estimation of p53 and their correlation with survival and cell morphology

Figure 1 shows images based on manual counting (Figure 1A) and the computer image analysis (Figure 1B) of identical images from a representative region. The median p53 assessed by manual counting of at least 1000 tumor cells was 43.1%, and the mean was 45.0% (range 0-100%). The median p53 calculated by computer image analysis of the same areas by manual counting was 53.3%, with a mean of 48.2% (range 0.1-100%). The p53 acquired by semiquantitative estimation displayed a median of 40% and a mean of 39.4% (range 0-100%).

Manual counting, computer image analysis and semiquantitative estimation of p53 can predict OS. Each of these methods showed an AUC > 0.85 on ROC curves ($P < 0.0001$; Figure 2, Table 2). The optimal p53 cutoff for predicting OS were determined for each method. For manual counting, the cutoff value was 16.20%, sensitivity was 80.39%, and specificity was 100%. For computer image analysis, the cutoff value was 17.66%, sensitivity was 74.51%, and specificity was 100%. For semiquantitative estimation, the cutoff value was 5.00%, sensitivity was 76.47%, and specificity was 92.86% (Table 2). As manual counting and computer image analysis are more precise than semiquantitative estimation, we used the approximate value of manual counting and computer image analysis, 20%, as the cutoff value of p53 for subsequent analysis.

Table 2
Diagnostic performance of p53 as measured by different methods.

	AUC (95% CI)	P	Youden index	Optimal cutoff	Sensitivity (95% CI)	Specificity (95% CI)
Manual counting	0.873 (0.767 - 0.943)	<0.0001	0.8039	16.20%	80.39% (66.9% - 90.2%)	100% (76.8% - 100%)
Computer image analysis	0.853 (0.743 - 0.929)	<0.0001	0.7451	17.66%	74.51% (60.4% - 85.7%)	100% (76.8% - 100%)
Semiquantitative estimation	0.856 (0.747 - 0.931)	<0.0001	0.6933	5.00%	76.47% (62.5% - 87.2%)	92.86% (66.1% - 99.8%)
AUC, area under curve; CI, confidence interval.						

To assess the prognosis of these three methods, we performed survival analysis. Kaplan–Meier survival curve analysis showed that patients with p53 < 20% had a significantly longer OS than patients with p53 ≥ 20% (P < 0.0001, Figure 3A-C). Moreover, patients with blastoid/pleomorphic MCL had shorter OS than those with classical MCL (P < 0.0001, Figure 3D). Blastoid/pleomorphic variant MCL patients had more p53 ≥ 20% than classical type patients (P < 0.0001, Table 3).

Table 3
Correlation analysis of p53 and cell morphology.

Cell morphology	P53 by manual counting				P53 by computer image analysis				P53 by semiquantitative estimation			
	< 20%	≥ 20%	χ ²	P	< 20%	≥ 20%	χ ²	P	< 20%	≥ 20%	χ ²	P
Classical type	23	13	21.703	< 0.0001	23	13	21.703	< 0.0001	26	10	27.523	< 0.0001
Blastoid/pleomorphic variant	2	27			2	27			2	27		
CI, confidence interval.												

Correlation between manual counting, computer image analysis and semiquantitative estimation

There was a strong correlation between manual counting and the computer image analysis of p53 in identical areas (Spearman's rho = 0.966, P < 0.0001, Figure 4A). There was a significant correlation between manual counting and semiquantitative estimation (Spearman's rho = 0.938, P < 0.0001, Figure 4B), albeit weaker than the correlation between manual counting and computer image analysis. Moreover, we found a significant correlation between semiquantitative estimation and computer image analysis (Spearman's rho = 0.898, P < 0.001, Figure 4C).

Bland–Altman plots comparing manual counting with the computer image analysis of identical areas revealed a tendency toward a higher evaluation of p53 by computer image analysis (Figure 4D), and comparing manual counting p53 with semiquantitative estimation revealed a tendency toward a lower evaluation of p53 by semiquantitative estimation (Figure 4E). We also used the Bland–Altman plot to compare computer image analysis with the semiquantitative estimation of identical areas. The results revealed a tendency toward a lower evaluation of p53 by semiquantitative estimation (Figure 4F).

Discussion

In this study, we demonstrated that 20% could be used as the optimal cutoff value for determining the prognosis of MCL. MCL patients with p53 ≥ 20% had a shorter OS than patients with p53 < 20%. In addition, patients with blastoid/pleomorphic variants of MCL tended to have p53 ≥ 20%. We also verified the possibility of computer image analysis software to count p53 in patients with MCL.

IHC staining for p53 is a promising tool for predictive purposes, as it serves as an alternative marker for *TP53* mutations and 17p deletions^{7-9,13}. In MCL, the prognostic significance of the immunohistochemical expression of p53 has been studied (Table 4). Rodrigues et al. also used manual counting and computer image analysis for the immunohistochemical counting of p53 and prognosis analysis⁷. They defined

30% as the prognostic cutoff value of p53, which is based on a previous publication ¹¹ and the routine clinical practices at Lund University Hospital. However, the prognostic cutoff values of p53 in MCL in other studies are controversial, ranging from 1%-50%, and the criteria for defining these cutoff values are unclear ^{10-12,14}. We used manual counting and computer image analysis to count the positive rate of p53 and calculated the cutoff values for predicting OS with the optimal specificity and sensitivity through the Youden index. Manual counting was 16.20%, and computer image analysis was 17.66%. To facilitate clinical application, we used 20% as the cutoff value of p53 for survival analysis, and the results revealed that MCL patients with p53 $\geq$ 20% had a worse prognosis than those with p53 < 20%. In addition, we also found that p53 $\geq$ 20% was associated with blastoid/pleomorphic morphology, which is consistent with previous studies ^{12,13,15}.

Table 4
Summary of p53 cutoff values and the prognostic significance of MCL cases from the literature and our study.

Author	Cases	P53 immunohistochemistry		
		Cut off value	Positive rate	Prognostic significance
Our study	65	20%	40/65	Worse
Aukema SM ¹⁰	365	50%	16/365	Worse
Rodrigues JM ⁷	317	30%	42/317	Worse
Nordström L ¹¹	93	30%	10/93	Worse
Choe JY ¹²	62	30%	-	Worse
Abrisqueta P ²¹	40	1%	5/40	Worse
-, not mention.				

Manual counting is very time-consuming and probably not applicable in routine clinical tests. Thus, we compared three methods of p53 counting to define a simple and accurate method. We confirmed that p53 by computer image analysis was more precise for prognosis prediction and had a stronger correlation with manual counting than semiquantitative estimation. Computer image analysis of p53 could count larger tumor regions than manual counting, making it more representative in assessing the percentage of p53-positive cells within the tumor. Additionally, computer image analysis is faster than manual counting and creates repeatable results for retrospection.

The computer image analysis revealed a tendency toward slightly higher estimates of p53 compared to manual counting. Potential reasons for the overestimation of p53 may include positively-stained background cells that were miscalled tumor cells and debris that was miscalled positive staining ¹⁶. Therefore, computer image analysis could possibly benefit from strict quality control of the IHC staining method.

The limitation of computer image analysis is the inability to distinguish between different cell types such as normal cells and tumor cells ¹⁷. Consequently, representative regions of lymphoma must be chosen carefully by pathologists. While pathologists choose the representative region of lymphoma, this would not be a disadvantage in MCL because the reactive cell number is low in MCL ¹⁸.

In conclusion, we demonstrated that MCL patients with p53 $\geq$ 20% had a shorter OS and a tendency for the blastoid/pleomorphic variant. Furthermore, computer image analysis of p53 is more precise than semiquantitative estimation and can be applied to the interpretation of p53 IHC staining in patients with MCL.

Methods

Patients

A total of 65 MCL specimens was obtained from the Department of Pathology, West China Hospital of Sichuan University from 2014 to 2021. All cases were newly diagnosed and reviewed by trained pathologists according to the 2016 World Health Organization (WHO) classification for tumors of hematopoietic and lymphoid tissues ¹⁹. Anonymous data regarding sex, age, Ann Arbor stage, tumor tissue type, tumor cell morphology, and survival time were obtained retrospectively from the patients' medical records and telephone follow-ups. All patients were followed up from the date of diagnosis to July 31, 2021.

The Institutional Review Board of West China Hospital of Sichuan University approved this study (registration number: WCH2021-00333). All recruited patients gave written informed consent following the Declaration of Helsinki.

Immunohistochemistry staining of p53

The EliVision method was used for IHC staining of p53²⁰. Briefly, the paraffin blocks were cut into 4 µm sections and pretreated in ethylenediaminetetraacetic acid (EDTA) buffer at pH 9.0 for 20 minutes. Then, they were incubated with p53 at a dilution of 1:200 (clone DO-7; MXB Biotechnologies, Fuzhou, China) and 3,3'-diaminobenzidine (DAB) chromogen. Finally, they were counterstained with hematoxylin and rinsed with deionized water. The expression of p53 was detected in the tumor cell nucleus. Each slide was scanned by the Hamamatsu Digital Slide Scanner NanoZoomer 2.0-HT C9600-13.

Manual counting, computer image analysis and semiquantitative estimation of p53-positive cells

Manual counting and computer image analysis of each image were performed using QuPath software (version 0.1.2)^{21,22}. Representative regions from each slide were annotated by an experienced pathologist and a Ph.D. student. Regions involving a high density of lymphoma cells were chosen, and hotspot areas were avoided. Images were extracted from the typical region, and identical areas were selected for random manual counting and computer image analysis. When manually counting the selected images, at least 1000 tumor cells were acquired in each slide. Hematopathologists chose the representative areas and obtained semiquantitative estimates of p53.

Statistical analysis

Overall survival (OS) was defined as the number of days from diagnosis to the date of death or final follow-up. The sensitivity and specificity of p53 were tested by assessing the area under the ROC curve (AUC). Furthermore, the Youden index and optimal cutoff value were calculated using the DeLong test²³. Survival curves were generated using the Kaplan–Meier survival analysis method, and the log-rank test was used to examine differences in OS. Comparisons of cell morphology and p53 were conducted using the chi-squared (χ^2) test. The Spearman rank correlation test was used to calculate the correlation between manual counting, computer image analysis, and semiquantitative estimation. Bland–Altman analysis was used for comparison of manual counting, computer image analysis and semiquantitative estimation^{24,25}. Statistical analyses were performed using MedCalc 20.009 software (MedCalc Software Ltd), and $P < 0.05$ was considered statistically significant.

Abbreviations

MCL: mantle cell lymphoma; OS: overall survival; χ^2 : chi-squared; NHL: non-Hodgkin lymphoma; MIPI: Mantle Cell Lymphoma Prognostic Index; IHC: immunohistochemistry; WHO: World Health Organization; EDTA: ethylenediaminetetraacetic acid; DAB: diaminobenzidine; AUC: area under the ROC curve; NA: Not Applicable; CI, confidence interval.

Declarations

Acknowledgements

Not applicable.

Author contributions

YHZ, LMG and WPL contributed to the conceptual design of the study. YHZ and XYX were involved in data acquisition. YHZ, WYZ and LMG were involved in data analysis and interpretation. YHZ and LMG were involved in writing and editing the manuscript. YHZ, XYX, WYZ, LMG and WPL reviewed the manuscript. This study was supervised by WPL and LMG.

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Competing interests

The authors declare that they have no competing interests related to the present study.

Ethics approval and consent to participate

The present study was approved by the Institutional Review Board of West China Hospital of Sichuan University (WCH2021-00333). All subjects signed informed consent forms before participating.

Consent for publication

All subjects signed informed consent forms before participating.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

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Figures

Figure 1

Image analysis of p53-positive tumor cells in identical areas of a randomly chosen specific region in one case. (A) Image-based manual counting. Positively stained (brown) and negative nuclei (blue) are marked with red and blue circles. (B) Image-based computer image analysis. Positively stained (brown) and negative nuclei (blue) are marked with red and blue irregular circles.

Figure 2

Discriminatory power of p53 for predicting overall survival (OS).

Figure 3

Overall survival (OS) according to (A) p53 by manual counting, (B) p53 by computer image analysis, (C) p53 by semiquantitative estimation, and (D) classical type versus blastoid/pleomorphic variant cell morphology.

Figure 4

Correlation between (A) computer image analysis and manual counting of p53, (B) semiquantitative estimation and manual counting of p53, and (C) semiquantitative estimation and computer image analysis of p53 in identical areas. Bland–Altman plots comparing p53 by (D) computer image analysis with manual counting, (E) semiquantitative estimation with manual counting, and (F) semiquantitative estimation with computer image analysis of identical areas.

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