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Detection of MYC rearrangement in high grade B cell lymphomas: correlation of MYC immunohistochemistry and FISH analysis

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Summary

The aim of this study was to analyse sensitivity, specificity and predictive values of recently available MYC immunohistochemistry (IHC) against the currently standard diagnostic method, fluorescence *in situ* hybridisation (FISH) analysis. MYC IHC and FISH analyses were performed on 30 cases of diffuse large B cell lymphoma (DLBCL) with 80% or more Ki-67 index, one case of DLBCL transformed from follicular lymphoma, three cases of B cell lymphoma intermediate between DLBCL and Burkitt lymphoma (IM), six cases of Burkitt lymphoma (BL) and one case of reactive lymph node. The inclusion criteria of high Ki-67 index, more than 80%, was imposed to exclude dependence of MYC positivity on Ki-67 positivity. The indices of specificity and positive predictive value (PPV) were low and varied widely with different thresholds of IHC positivity in percentage. At the threshold of 40% IHC positivity, specificity index was 0.45 and PPV was 0.37. At the threshold of 50% and 70% IHC positivity, specificity indices were 0.61 and 0.84, and PPVs were 0.45 and 0.67, respectively. Good sensitivity and negative predictive value (NPV) were maintained at all different thresholds. A heterogenous staining pattern of IHC was also noted. The heterogeneous IHC staining pattern observed warranted caution in interpretation and counting of IHC positive cells. MYC protein expression detected by IHC was more common than MYC translocation detected by FISH analysis. As a result, low specificity and PPV of MYC IHC, in relation to FISH analysis, were observed despite good sensitivity and NPV.

Key words: Burkitt lymphoma, diffuse large B cell lymphoma, fluorescence *in situ* hybridisation, FISH, immunohistochemistry, IHC, MYC proto-oncogene, unclassifiable.

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INTRODUCTION

MYC proto-oncogene, located on chromosome 8q24, has been shown to have a direct role in the control of DNA replication.[1] It encodes a transcription factor that integrates and accelerates cell metabolism and proliferation.[2] Translocation of MYC gene to IGH@ locus and, less frequently, variant translocations involving one of the immunoglobulin light chain loci are seen in virtually all Burkitt lymphomas (BL) and 5–10% of diffuse large B cell lymphomas (DLBCL).[3–4] MYC translocation has also been reported in other non-Hodgkin lymphomas such as myeloma, T cell lymphoblastic leukaemia, mantle cell lymphoma and follicular lymphoma.[2,4–7] The World Health Organization (WHO) 2008 classification introduced a new category of B cell lymphoma, unclassifiable with features intermediate between DLBCL and BL (IM). This new category incorporates a spectrum of different entities, including double hit lymphomas in which the neoplastic cells harbour both MYC and BCL-2 translocations and high grade lymphomas demonstrating overlapping morphologies of BL and DLBCL and high proliferation indices.[8] These lymphomas with MYC rearrangement carry poor prognosis and poor response to CHOP chemotherapy and R-CHOP combination therapy.[9–16] Hence, the detection of MYC translocation has become pivotal in defining patient management.

Fluorescence *in situ* hybridisation (FISH) is the commonly used standard test for detecting MYC gene translocation. This test is not currently available in most diagnostic pathology laboratories, therefore the tumour tissue has to be sent to specialised laboratories, causing extra cost and delay in establishing reports. In many examples of routine anatomical pathology practice, using immunohistochemistry (IHC), which is available in many diagnostic pathology laboratories, as a surrogate marker and/or screening test to detect underlying mutation has been proven to save cost and promote early and effective patient management. Therefore, its role in detection of MYC translocation has drawn the attention of many haematopathologists.[17] MYC IHC marker has been made commercially available only recently and there were a few correlation studies between MYC IHC and translocation as detected by FISH.[18–20] In contrast to some findings described in these studies, we identified some discrepancies when we performed MYC IHC as part of our routine practice. A heterogeneous staining pattern of MYC IHC expression was seen even in cases where MYC translocation was demonstrated by FISH analysis, and IHC positivity was not limited to cases where MYC translocation was demonstrated by FISH analysis. The latter finding, which we propose is possibly due to alternative mechanisms of MYC up-regulation, was also demonstrated in recent studies by Johnson *et al*., Green *et al*., and Hu *et al.*[14,16,21] Their studies also confirmed that increased MYC protein expression in the presence of bcl-2 coexpression, with or without MYC or bcl-2 gene rearrangements, was associated with aggressive clinical course and inferior prognosis. Herein, we report a series of our experience, demonstrating the sensitivity, specificity and predictive values of MYC IHC against FISH analysis.

MATERIALS AND METHODS

We included a total of 41 cases (all adult patients with age ranging between 29 and 90 years) which had sufficient materials for both immunohistochemical staining and FISH testing in the same block. Thirty-seven cases were from the

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archives of the Anatomical Pathology Department, ICPMR, Westmead Hospital, Sydney, Australia, and four cases were referral cases. WHO 2008 diagnostic criteria were followed in making the diagnoses in all of these cases.[8] The study comprised 31 DLBCLs, three IM, six BL and one case of reactive lymph node. Thirty of 31 DLBCL cases met the inclusion criteria of a high Ki-67 index of more than 80%, which was imposed to exclude dependence of MYC positivity on Ki-67 positivity. One remaining case of DLBCL was a transformed DLBCL with 40% Ki-67 index, which progressed from follicular lymphoma. Of these DLBCL cases, 19 cases had germinal centre immunophenotype (GCB) and 12 cases had activated B cell immunophenotype (ABC) according to Hans' algorithm.[22] Two IM were double hit lymphoma with proven MYC/IGH fusion and IGH/BCL2 fusion by FISH study, and one IM had overlapping morphologies between DLBCL and BL, 99% Ki-67 index and negative EBV ISH. All three IM showed germinal centre phenotype according to Hans' algorithm.[22] One case of reactive lymph node was included as a control. This study was conducted with human ethics review committee approval.

FISH

MYC rearrangement was evaluated in 25 cases during the diagnostic work-up at the Cytogenetic Department, The Children's Hospital at Westmead, Sydney, Australia, where routine karyotyping was performed on all cases. In some cases where MYC rearrangement was not identified in karyotyping but was suspected based on clinical behaviour or high Ki-67 index, FISH analysis was performed on metaphase spread from the cell suspension. The remaining 16 cases were sent to the Cytogenetics/FISH laboratory, SydPath, St Vincent's Hospital, Sydney, Australia. Using 4 μm sections from the formalin fixed, paraffin embedded (FFPE) tissue blocks, each case was analysed by FISH using commercial dual-coloured MYC Break Apart rearrangement, IGH@-MYC dual-coloured dual fusion translocation and IGH/Bcl-2 dual-coloured dual fusion translocation probes (Abbott Molecular, USA) according to the manufacturer's instructions. The images were captured on a Zeiss Axio Imager and analysed with MetaSystems ISIS image analysis software.

IHC

Sections 4 μm thick were prepared from FFPE blocks for each case. IHC for MYC protein (clone Y69, 1:200 dilution; Biocare Medical, USA) was performed using a fully automated BenchMark Ultra immunostainer (Ventana Medical Systems, USA). Individual cases were stained as routine diagnostic cases following the manufacturer's protocol. To avoid any pre-analytic variation, the same protocol was used and each IHC slide was matched with positive control in all cases involved in this study.

The areas with the highest amount of IHC positive staining were identified at scanning magnification (40×) in all slides. These areas were photographed at high magnification (400×). The same image of each case was evaluated by two observers independently (KL and JR), who were blinded to all the clinical, histological, immunohistochemical and FISH results. Only tumour cells were scored using Image J image processing and analysis program downloaded from the National Institutes of Health website (http://rsbweb.nih.gov/ij/). The positive staining was further discriminated into weak nuclear staining and moderate-intense nuclear staining. Non-specific blush-staining of the nuclei and any cytoplasmic staining were regarded negative. The difference in scores over 5% was regarded as discrepant. The discrepant cases were recounted and second time discrepancies were resolved by both observers reviewing the photomicrograph simultaneously. The average between two concordant scores, which were less than 5% interval, was taken as the final score.

RESULTS

The two independent observers were concordant in MYC immunohistochemical staining in all 41 cases, although there were initially discrepant scores in four cases, three of which became concordant in recounting and one of which required simultaneous review of both observers. These four cases had 40% or less MYC positivity.

Ten of 41 cases showed MYC gene rearrangement with IGH@-MYC fusion translocation probe, which was again confirmed by MYC Break Apart probe. These 10 cases included six cases of BL, two cases of IM and two cases of DLBCL. These 10 MYC translocated cases showed MYC IHC moderate to strong positivity in 70–100% of tumour nuclei (for example, Fig. 1).

In the remaining 31 cases where MYC gene rearrangement was negative in FISH analysis, five cases of DLBCL had MYC IHC positivity in 70–93% of tumour nuclei (three cases showed a uniformly strong positive staining pattern in 86–93% of tumour cells (for example, Fig. 2); two cases showed 70% and 80% positivity with heterogeneous staining pattern ranging from weak to strong positivity (for example, Fig. 3). Four other cases showed nuclear positivity in 62–67% of tumour cells (3 cases with strong positive staining pattern and 1 case with heterogeneous positive staining pattern). Three other cases including two DLBCL and one IM showed 50–55% positivity with heterogeneous intensity. The remaining cases showed less than 50% nuclear staining with heterogeneous intensity, including the reactive lymph node which had 16% positive nuclear staining and the case of DLBCL transformed from follicular lymphoma which had no positive staining (Table 1).

Against the translocation status identified by FISH study, the sensitivity, specificity, PPV and NPV were calculated at

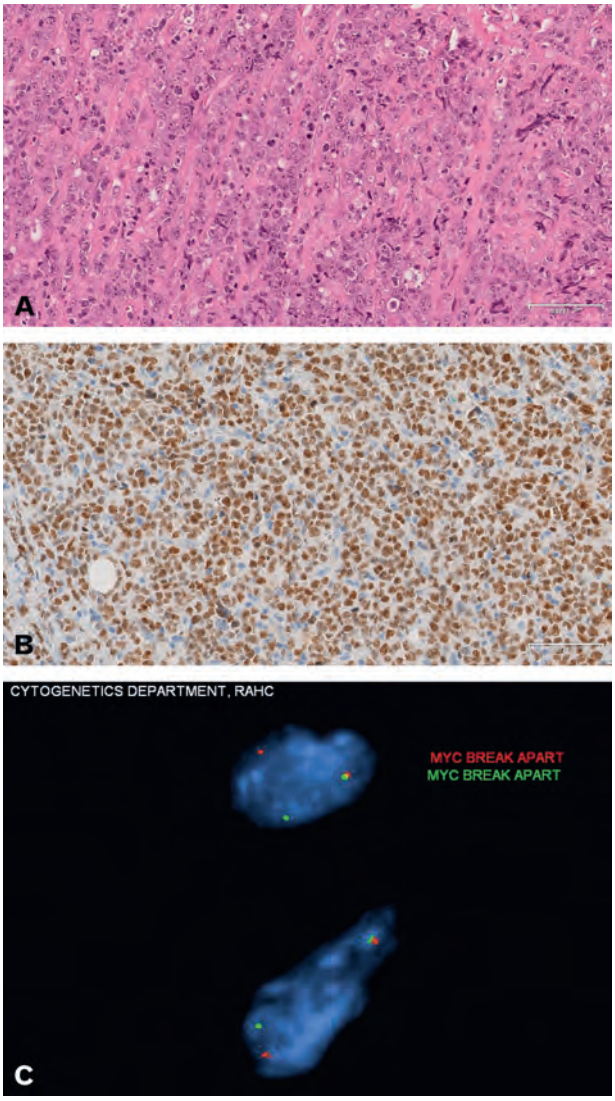

Fig. 1 B cell lymphoma unclassifiable with features intermediate between DLBCL and BL, which showed MYC translocation in FISH analysis and 88% MYC immunohistochemical stain positivity. (A, H&E; B, MYC immunohistochemical stain; C, FISH, dual-coloured MYC Break Apart probe).