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DETECTION OF MYC REARRANGED BY FLUORESCENCE IN SITU HYBRIDIZATION FISH: A DIAGNOSTIC TOOL

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Abstract: *MYC is a potent oncogene and its activation is frequently due by direct gene alteration, such as traslocation and amplification.Originally* MYC *chromosome translocation t(8;14)(q24;q32) has been considered an hallmark of of Burkitt lymphoma, but later it have been identified in other mature B-cell neoplasms. Detection of translocations involving* MYC *at 8q24.1 in aggressive B-cell lymphomas is important for diagnostic and prognostic purposes. Therefore, we analyze the genetic aberrations could be applied to a useful Fluorescent In Situ Hybridization (FISH) approach in the diagnosis of these lymphomas.*

KEY WORDS: *t(8;14) rearrengements, Methods to detect MYC, FISH*

INTRODUCTION

C-MYC is a pleiotropic transcription factor belonging to a transcription factors family that includes MYCL (L-Myc) and MYCN (N-Myc). *MYC* gene is located on 8q24 chromosome and is composed by three exons. It is involved in the regulation of many biological activities, such as differentiation, cell adhesion, apoptosis, angiogenesis, telomerase activity and cell metabolism[1,2]. Frequently, *MYC* oncogenetic deregulation in cancer is linked to uncontrolled cell proliferation, genomic instability, apoptosis, escape of immune surveillance and cell immortalization[3,4]. The oncogenic activation of MYC may occur by direct gene alteration, such as traslocation and amplification, or by dysregulation of upstream signaling pathways[5]. Particularly chromosomal translocations involving *MYC* and the immunoglobulin genes are a recurrent genetic alteration in aggressive B cell-lymphoma such as Burkitt lymphoma (BL), diffuse large B-cell lymphoma (DLBCL) and B-cell lymphoma, unclassifiable (BCLU). Notably, differential diagnosis between this categories, often results difficult and the pathologist requires a molecular approach to support the diagnosis[6,7]. In the adult, it's very important distinguish BL from other aggressive B cell-lymphoma, because are clinically different[8,9].

The translocation t(8;14) has been described as the most frequent aberration involving *Myc* gene in BL with the immunoglobulin heavy chain (IGH) gene as partner. Less common aberration involves light chain immunoglobulin genes (IGL or IGK) in the translocations t(2;8) and t(8;22)[10]. In addition, the activation of the M*yc* gene at 8q24 is considered the main pathogenetic feature of BL (90%), but the contribution of other genetic mutations to the disease is an important developing point[10]. In addition *MYC* translocation is not only specifically observed in BL but it was observed in 5-10% of diffuse large B-cell lymphomas and up to 50% of high-grade B-cell lymphomas other than Burkitt lymphomas[11]. In these tumours, *Myc* translocations can also involve non-*IG* partners[12].

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Several studies have been demonstrated distinctive complex karyotypes (CK) in BL and DLBCL but *MYC* translocation remains the main cytogenetic signature of BL as shown by its routinely use in several diagnostic algorithms. This investigation is fundamental in differential diagnosis with other lymphomas morphologically similar to BL but with atypical immunophenotype or genetic signatures [13-15].

Salaverria et al. proposed a genetic model of pathogenesis of high-grade B cell lymphomas "gray zone", related to genetic aberration and age. They assessed that real adult BLs was very rare and the BL with more genetic alteration is extremely difficult to find. The data show that the distinctive Burkitt feature is represented by its low genomic complexity and by the presence of *IG-MYC* translocation like a primary event (Single-hit) [16].

Hummel et al. proposed a "BL similarity index", based on the analysis of 58 genes that divided B aggressive Lymphomas in three categories: molecular BL (mBL), intermediate cases, and non molecular Burkitt[17,18] .Through this index yet not all cases with morphologic or immunophenotypical features of Burkitt's lymphoma were classified as mBL. They emphasized that in mature aggressive B-cell lymphomas a *MYC*-simple group characterized by *IG-MYC* fusion and a low number of chromosomal imbalances is overlapped with the molecular BL and associated with a favorable clinical outcome. Instead a *MYC*-complex status is associated with a poor outcome, independently of age and clinical stage corresponding to the intermediate group. Finally, *MYC* negative group including non molecular Burkitt cases [18].

Naresh et al. proposed a diagnostic approach based on immunohistochemistry and FISH scoring system. Particularly, this method included 3 phases: in the first phase, the scoring is based on morphological features and a small immunohistochemistry panel (BCL2 and CD10). In the second phase, the unresolved cases with intermediate score, should be further scored through a larger panel of immunohistochemistry, such as ki67, CD38 and CD44. Finally, If this phase is uncertain, FISH analysis, including *IG-MYC* translocation and rearrangements of BCL2 and BCL6 should be crucial to complete the third phase. Through this approach is possible enable to give lead to a precise diagnosis of BL in more than 90% cases [19].

On the basis of current literature, Bellan et al. suggested a practical approach to distinguish among BL, DLBCL and BCLU. They proposed an algorithm for diagnosis of cases with intermediate morphology and CD10\BCL6 expression. In particular, FISH analysis was performed to detect the translocations involving *MYC BCL2* and *BCL6* through commercially available probes. They argued that BL diagnosis is favored by the presence of *IG-MYC* rearrangement (simple karyotype). BCLU diagnosis is favored by the presence both MYC and BCL2 rearrangement (complex karyotype), instead the diagnosis of DLBCL is favored by BCL2 and BCL6 rearrangement and *MYC* negativity[20].

MYC ASSAY IN CLINICAL SETTING

Moreover, *MYC* rearrangement is associated with unfavorable progression-free survival and overall survival and its identification could stratify a subset of patients who may benefit from alternative treatment strategies[5,21-24]. In addition, recently Rituximab has also been introduced for treatment of BL and B aggressive lymphomas. However, several studies have showed that the presence of *MYC* abberations identifies a patient subset that requires more aggressive therapy[25,26]. Thus, a correct characterization is very important because *Myc* translocation has not only diagnostic value but it is also a powerful prognostic indicator in several lymphomas.

METHODS TO DETECT MYC TRANSLOCATION

Currently the detection of *MYC* translocation is performed by several methods such as conventional cytogenetics, Southern blot, and polymerase chain reaction but all these methods can fail to detect *IG-MYC* fusions[27].

Recently, a novel monoclonal antibody that targets the N terminus of the MYC protein was shown to provide sensitive and specific staining of nuclear MYC in paraffin embedded tissue[28,29].

However, the FISH represents the most robust and reliable method. Therefore, we recommend a FISH approach through four important steps to integrate histological diagnosis. Initially *MYC* Break Apart probe should be performed on lymphoma cases with increased (>90%) Ki67, to identify all *MYC* rearranged samples and BCL2 and BCL6 Break Apart probes, will be performed on all negative samples. Afterwards on the Myc positive specimens should be evaluated also the presence of *IG-MYC* translocation, through the use of a Dual colour dual fusion *IGH-MYC* probe and IGK and *IGL* Break Apart probes to distinguish *IG- MYC* from non *IG-MYC* translocation. Finally, BCL2 and BCL6 status should be investigated to identify a complex karyotype and additional chromosomal translocation [30]. (Figure1)

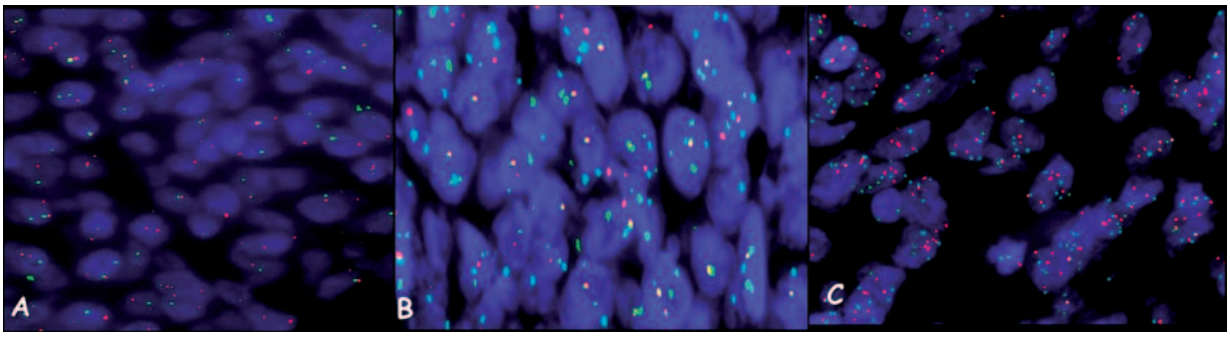

Figure 1: FISH assay shows in **A** the MYC locus rearrangement with a break-apart probe (Vysis LSI MYC Dual Color Break Apart Rearrangement Probe Kit). In **B** IGH/MYC rearrangement with a Dual color dual fusion probe (Vysis IGH/MYC/CEP 8 Tri-Color DF FISH Probe Kit); in **C**. The amplification of MYC (Vysis IGH/MYC/CEP 8 Tri-Color DF FISH Probe Kit).

FISH is suggested to avoid misdiagnosis but it is reccomanded to integrate with morphologic and immunophenotypic evaluation.

FISH still represents a time-consuming and expensive method instead MYC protein expression by immunohistochemistry could be easily performed in every laboratories. However, not all *MYC* traslocated aggressive B lymphomas samples showed a significant (>40%) MYC protein staining pattern [31,32]. Moreover, FISH is unable to detect genetic deregulation that affects gene expression on the transcriptional and translational levels unlike immunohistochemical analysis[28,29]. Consequently the immunohistochemical evaluation of MYC expression and its therapeutically role should be established through more trials. Although the FISH-based algorithmic approach results an important tool for BL diagnosis, it is not easily accessible in most of the pathology laboratories because it is an expensive and time-consuming method.

CONCLUSION

Currently, *MYC* antibody is not routinely applied although several large B-cell lymphomas have MYC protein up-regulation independent of gene alterations. In the future, a combined FISH\Immunoistochemical score could be introdueced in a novel diagnostic alghorithm for agressive B-Cell Lymphoma.

Conflict of Interest:
None declared.

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