Research Article

BIOMEDICAL Journal of Scientific & Technical Research

ISSN: 2574 -1241

DOI: 10.26717/BJSTR.2021.39.006318

Molecular Detection of BCL2/IGH Rearrangement in Follicular Lymphoma in Low Resource Settings: A Phase III Diagnostic Accuracy Study

Maryam Etebari[1,2], Serah Kaggia[3], Emily Rogena[3], Nicholas A Abinya[4], Mercy Gatua[1,4], Noel Onyango4, Shaymaa Khattab[5], Ashraf Elghandour[5], Manal Elsorady[5], Mohsen Navari[6-8] and Pier Paolo Piccaluga[1,3,4,9]*

[1]*Department of Experimental, Diagnostic, and Specialty Medicine, University of Bologna School of Medicine, Bologna, Italy -Institute of Hematology and Medical Oncology "L. and A. Seràgnoli", Italy*

[2]*Department of Medical Genetics, Faculty of Medicine, Mashhad University of Medical Sciences, Iran*

[3]*Department of Pathology, School of Medicine, Jomo Kenyatta University of Agriculture and Technology, Kenya*

[4]*Nairobi Hospital and University of Nairobi, Kenya*

[5]*Hematology Unit, internal medicine department, Faculty of Medicine, Alexandria University, Egypt*

[6]*Department of Medical Biotechnology, School of Paramedical Sciences, Torbat Heydariyeh University of Medical Sciences, Iran*

[7]*Research Center of Advanced Technologies in Medicine, Torbat Heydariyeh University of Medical Sciences, Iran*

[8]*Bioinformatics Research Group, Mashhad University of Medical Sciences, Iran*

[9]*Istituto Euro-Mediterraneo di Scienza e Tecnologia (IEMEST) Palermo, Italy*

***Corresponding author:** Pier Paolo Piccaluga, Department of Experimental, Diagnostic, and Specialty Medicine University of Bologna, Italy; Institute of Hematology and Medical Oncology "L&A Seràgnoli", Via Massarenti, 9 – 40138 Bologna, Italy

ARTICLE INFO

Received: October 11, 2021

Published: October 21, 2021

Citation: Maryam Etebari, Serah Kaggia, Emily Rogena, Nicholas A Abinya, Pier Paolo Piccaluga, et al., Molecular Detection of *BCL2/IGH* Rearrangement in Follicular Lymphoma in Low Resource Settings: A Phase III Diagnostic Accuracy Study. Biomed J Sci & Tech Res 39(3)-2021. BJSTR. MS.ID.006318.

ABSTRACT

Follicular lymphoma (FL) is the second commonest lymphoma worldwide. Despite an overall indolent behavior, the disease can be rarely cured with conventional chemotherapy. The disease, in fact, after initial clinical remission tends to relapse, progressively acquiring drug resistance, and eventually transform in some cases into a secondary diffuse large B-cell lymphoma. Detection of the minimal residual disease (MRD) is clinically meaningful in FL patients. To this aim, either the BCL2/IGH fusion gene, derived from t(14;18) (q32;q21), or the specific IGVH rearrangement can be studied, the first option currently representing the gold standard.In this study we compared the two most diffused methods for MRD detection in FL, namely nested PCR and quantitative real time PCR (qPCR), in order to determine their feasibility in low resources environments like Kenyan Hospitals. We studied 22 patients for a total of 145 tests in a phase 3 diagnostic accuracy study. We found that qPCR, in this peculiar setting, was more reliable in terms of reproducibility and more effective in terms of sensitivity (up to 10-5). Furthermore, the costs per sample (34 vs 126 €) and consumed time (3.5 vs. 18.5 hours) were inferior to nested PCR. We concluded that qPCR, despite a higher initial investment for machinery, is probably preferrable in laboratories with limited resources. Adequate training and continuous standardization process are warranted.

Keywords: Follicular Lymphoma; BCL2; Immunoglobulin; BCL2/IGH; Nested PCR; Quantitative RT-PCR; Next Generation Sequencing; Digital PCR; Minimal Residual Disease; Evidence-Based Medicine; Diagnostic Accuracy: STARD

Introduction

Follicular lymphoma (FL) is the second commonest non-Hodgkin lymphoma (NHL) subtype worldwide and the commonest in certain regions like USA [1]. FL has generally an indolent clinical course, somehow influenced by the cytological grading that is not, however, of prognostic relevance [2]. Conventional chemo-immunotherapy can induce initial remissions; nonetheless, cure is still not common [3]. In fact, relapses do occur, characterized by progressive chemoresistance development. In a percentage of cases, relapsing is also associated with histological transformation to secondary DLBCL [2]. The source of relapse in patients who initially achieve complete clinical remission are residual neoplastic cells representing the so called minimal residual disease (MRD). MRD can be detected either in bone marrow and blood by molecular methods and/or in tissues (mainly lymph nodes) by PET scan [4]. The t(14;18)(q32;q21) is molecular hallmark of FL. This translocation joins the BCL2 gene located on chromosome 18q21 with the immunoglobulin heavy chain locus (IGH) on chromosome 14q32, leading to the inappropriate expression of BCL2 protein, known to be a potent apoptosis inhibitor [5,6]. Detection of the BCL2/IGH rearrangement can be clinically useful for diagnostic purposes (using fluorescence in situ hybridization on tissues), but also for staging and MRD monitoring (using molecular techniques on blood and marrow) in FL patients [3,7,8].

Different techniques can be currently applied for the molecular detection of MRD, including more conventional ones (nested-PCR and quantitative Real-Time PCR, qPCR) and more innovative like digital PCR and next generation sequencing based ones [9,10]. Despite all of them have been demonstrated to be highly effective and overall reproducible and comparable [7-10], in low resource settings it is still debated whether to routinely test, due to costs, and which technique to prefer, due to technologies availability. In this study, we performed a phase 3 diagnostic accuracy study aiming to compare the two most conventional molecular techniques for MRD detection in FL, namely nested-PCR (used as test technique) and qPCR (used as golden standard) for BCL2/IGH detection. The two approaches were chosen as the only currently available in many referral centres even with limited resources.

Material and Methods

Twenty-two FL patients for which biological samples, complete clinical information, and long-term follow up were included. All patients were at diagnosis, and samples were taken before treatment initiation as well as after CHOP-R induction therapy, and after zevalin consolidation treatment at specific time-points (+3, +6, +12, +24, +30 months) [11]. Genomic DNA was extracted from mononuclear cells of peripheral blood (PB) and bone marrow aspirate (BM) as previously described [12]. The nested-PCR and the qPCR based on TaqMan technology [ABI PRISM 7900HT Fast Real-Time PCR System (Applied Biosystem)] were performed as previously reported [3,13,14]. As for BCL2/IGH PCR assays, primers were used according to previous Italian experiences [Ladetto 2001] (Tables 1-2). GAPDH was used as control gene for qPCR. Conversely, AF4 was chosen as control gene and was amplified according to BIOMED2 protocols for nested PCR [15]. All samples were tested by both techniques in triplicate.

Table 1: Primers sequences for nested PCR (BCL2/IGH).

	BCL2/IGH	Sequence
I STEP	Primer Forward MBR out	5'CAGCCTTGAAACATTGATGG3'
	Primer Forward mcr out	5'CGTGCTGGTACCACTCCT3'
	Primer Reverse JH out	5'ACCTGAGGAGACGGTGACC3'
II STEP	Primer Forward MBR in	5'ATGGTGGTTTGACCTTTAG3'
	Primer Forward mcr out	5'GGACCTTCCTTGGTGTGTTG3'
	Primer Reverse JH in	5'ACCAGGGTCCCTTGGCCCCA3'

Table 2: Primers sequences for nested PCR (BCL2/IGH).

BCL2 Gene	Sequence
Primer Forward	5'CTATGGTGGTTTGACCTTTAGAGAG3'
Primer Reverse	5'ACCTGAGGAGACGGTGACC3'
Probe	5'-FAM-CTGTTTCAACACAGACCCACCCAGAC-TAMRA-3'

Calculations of sensitivity (ST), specificity (SP), positive predictive value (PPV), negative predictive value (NPV), were made by CATmaker software (Centre for Evidence Based Medicine, Oxford University, http://www.cebm.net). The limit of significance for all analyses was defined as $P<0.05$. The study was approved by the local Ethical Committee and was developed and conducted in respect of the Helsinki Declaration. The study was designed and conducted according to the evidence-based medicine rules, respecting the STARD requirements.

Results and Discussion

All the enrolled patients could be studied for MRD. In total, 145 tests were performed. In fact, other than the expected 132 (22 cases by 6 timepoints), additional 13 were available from patients with longer clinical CR duration. Overall, we observed good concordance between "qualitative" nested-PCR and quantitative real-time PCR (80,86 %), in detecting MRD. The absolute sensitivity of the qPCR was in line with previously reported data [7]. Particularly, by evaluating serial dilutions of t(14;18)-positive cells into t(14;18)-negative cells, the relative sensitivity of our qPCR assay of about 10−5 resulted greater than the nested-PCR one (10-4), with an enhanced quantitative potential. This is overall in line with most studies. In terms of reproducibility, the precision of qPCR was determined by repeatability *intra*-assay and *inter*-assay; both the tests gave results of high reproducibility, above 95% considering 3 replicates. In contrast, the nested-PCR has given a lower reproducibility with discordant data and the need of additional repetitions to achieve a

uniform result (three nested-PCR in mean). Overall, this is in line with previous works on qPCR. By contrast, nested PCR seemed to be "technically" more complicated and probably requiring more experienced personnel, to be consistently performed. This fact, further stress the need for adequate training and standardization processes when MRD is studied, in order to ensure the requested clinical consistency.

Consistency between the two was evaluated in terms of sensitivity and specificity. Overall, this analysis confirmed what observed in terms of reproducibility, i.e. a significantly higher efficacy of qPCR. Among 145 performed tests, 85 were concordant between the two techniques, while 59 were not (59% overall accuracy). Particularly, among 103 tests turned out to be negative by nested PCR, only 46 were instead positive by qPCR (45%). Conversely, among the 46 that resulted positive at nested PCR, only 13 were discordant and 33 consistent (72%). This was translated into remarkable specificity but low sensitivity of nested PCR (Figure 1).

		TARGET DISORDER qPCR		
		Present	Absent	
TEST	Positive	33 a	13 b	
nested-PCR	Negative	46 c	57 d	95% Confidence Intervals
SENSITIVITY	a / (a+c)	42 %		31 to 53
SPECIFICITY	d / (b+d)	81 %		72 to 91
Pre-test Probability ("Prevalence"):	(a+c)/(a+b+c+d)	53 %		45 to 61
Positive Predictive Value:	a / (a+b)	72 %		59 to 85
Negative Predictive Value:	d / (c+d)	55 %		46 to 65
LIKELIHOOD RATIO +	sens / (1 - spec)	2.25		1.29 to 3.92
LIKELIHOOD RATIO –	(1 - sens) / spec	0.72		0.58 to 0.89

Figure 1: Diagnostic accuracy analusis of qPCR vs Nested PCR (Catmaker, Oxford, UK).

Lastly, analysis of costs and practical feasibility in reduced laboratories was performed. The expenses for reagents, consumables and labor employed for the TaqMan assay was calculated about 34,00€ (4,443 KES) per sample when testing the maximum number of 5 samples in triplicate in 96 well-plates. Conversely, the analysis of 5 sample by nested-PCR has a total amount of 126,00€ (16,466 KES). This calculation was obviously optimized for running a complete TaqMan plate. By reducing the number of available samples, the cost would progressively increase. This implies that referral labs centralizing the activities are advised, particularly when resources are limited, also considering the highest initial investment for machinery. The shortest test duration of 3 hours and 14 minutes was found for the real time PCR while 18 hours and 30 minutes were needed to perform a complete nested-PCR analysis (including gene control PCR, the nested-PCR repeated for three times in mean, post PCR manipulation)

Conclusion

The present study, though based on a limited series, highlights the relevance of using a qPCR-based method to detect BCL2/IGH rearrangements in FL patients in laboratories with limited resources. The use of TaqMan detection system was shown to be a sensitive, reproducible, and economical tool for MRD monitoring in FL. It allowed a relative sensitivity of about 10-5 providing a more accurate prognostic information [16]. Finally, the Taq Man approach in comparison with nested-PCR showed the simplest and shortest workflow sequence with a considerable gain of time and money, the average cost of 34€ per samples makes it feasible also in low resource Countries. Adequate programs of training and standardization should be then planned accordingly.

Acknowledgment

This work was supported by BolognAIL (2020, Prof. Piccaluga), RFO DIMES (2018, Prof. Piccaluga), FIRB Futura 2011 RBFR12D1CB (Prof. Piccaluga). The Authors are grateful to Dr. Maria Rosaria Sapienza for the skilled technical assistance.

References

1. Jaffe E, Harris N, Swerdlow S (2017) Follicular lymphoma. In WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 2017. IARC, Lyon.
2. Piccaluga PP, Pileri SA, Agostinelli C, Klapper W, Lennert K (2011) Follicular lymphoma: still Six characters in search of an author? Leuk Lymphoma 52(9): 1655-1667.

3. Sara Galimberti, Stefano Luminari, Elena Ciabatti, Susanna Grassi, Francesca Guerrini, et al. (2014) Minimal residual disease after conventional treatment significantly impacts on progression-free survival of patients with follicular lymphoma: the FIL FOLL05 trial. Clin Cancer Res 20(24): 6398-6405.

4. S Luminari, I Biasoli, A Versari, S Rattotti, C Bottelli, et al. (2014) The prognostic role of post-induction FDG-PET in patients with follicular lymphoma: a subset analysis from the FOLL05 trial of the Fondazione Italiana Linfomi (FIL). Ann Oncol 25(2): 442-447.

5. Tsujimoto Y, Gorham J, Cossman J, Jaffe E, Croce CM (1985) The t(14;18) chromosome translocations involved in B-cell neoplasms result from mistakes in VDJ joining. Science 229(4720): 1390-1393.

6. Korsmeyer SJ (1999) BCL-2 gene family and the regulation of programmed cell death. Cancer Res 59(7 Suppl.): 1693-1700.

7. Irene Della Starza, Marzia Cavalli, Ilaria Del Giudice, Daniela Barbero, Barbara Mantoan, et al. (2014) Comparison of two real-time quantitative polymerase chain reaction strategies for minimal residual disease evaluation in lymphoproliferative disorders: correlation between immunoglobulin gene mutation load and real-time quantitative polymerase chain reaction performance. Hematol Oncol 32(3): 133-138.

8. Irene Della Starza, Marzia Cavalli, Lucia Anna De Novi, Elisa Genuardi, Barbara Mantoan, et al. (2019) Minimal residual disease (MRD) in non-Hodgkin lymphomas: Interlaboratory reproducibility on marrow samples with very low levels of disease within the FIL (Fondazione Italiana Linfomi) MRD Network. Hematol Oncol 37(4): 368-374.

9. Marzia Cavalli, Lucia Anna De Novi, Irene Della Starza, Luca Vincenzo Cappelli, Vittorio Nunes, et al. (2017) Comparative analysis between RQ-PCR and digital droplet PCR of BCL2/IGH gene rearrangement in the peripheral blood and bone marrow of early-stage follicular lymphoma. Br J Haematol 177(4): 588-596.

10. Lay L, Stroup B, Payton JE (2020) Validation and interpretation of IGH and TCR clonality testing by Ion Torrent S5 NGS for diagnosis and disease monitoring in B and T cell cancers. Pract Lab Med 22: e00191.

11. Zinzani PL, Tani M, Pulsoni A, Gobbi M, Perotti A, et al. (2008) Fludarabine and mitoxantrone followed by yttrium-90 ibritumomab tiuxetan in previously untreated patients with follicular non-Hodgkin lymphoma trial: a phase II non-randomised trial (FLUMIZ). Lancet Oncol 9(4): 352-358.

12. Mannu C, Gazzola A, Ciabatti E, et al. (2016) Comparison of different DNA extraction methods from peripheral blood cells: advice from the Fondazione Italiana Linfomi Minimal Residual Disease Network. Leuk Lymphoma 57(2): 400-410.

13. JG Gribben, As Freedman, S D Woo, K Blake, RS Shu, G Freeman, et al. (1991) All advanced stage non-Hodgkin's lymphomas with a polymerase chain reaction amplifiable breakpoint of bcl-2 have residual cells containing the bcl-2 rearrangement at evaluation and after treatment Blood 78: 3275-3280.

14. M Ladetto, S Sametti, J W Donovan, D Ferrero, M Astolfi, et al. (2001) A validated real-time quantitative PCR approach shows a correlation between tumor burden and successful ex vivo purging in follicular lymphoma patients. Experimental Hematology 29(2): 183-193.

15. JJ van Dongen, E A Macintyre, J A Gabert, E Delabesse, V Rossi, G Saglio, et al. (1999) Standardized RT-PCR analysis of fusion gene transcripts from chromosome aberrations in acute leukemia for detection of minimal residual disease. Report of the BIOMED-1 Concerted Action: investigation of minimal residual disease in acute leukemia. Leukemia 13(12): 1901-1928.

16. Hirt C, Dölken G (2000) Quantitative detection of t(14;18)+ cells in patients with follicular lymphoma before and after autologous bone marrow transplantation. Bone Marrow Transplant 25: 419-426.

ISSN: 2574-1241

DOI: 10.26717/BJSTR.2021.39.006318

Pier Paolo Piccaluga. Biomed J Sci & Tech Res