European Review for Medical and Pharmacological Sciences 2015; 19: 4801-4810

Quantitative PCR detection of t(11;14) bcl-1/JH in mantle cell lymphoma patients: comparison of peripheral blood and bone marrow aspirate samples

S. DI MARTINO[1,2], O. CATAPANO[2], S.R. SIESTO[2], M. DI PAOLO[3], S. PUGLIESE[3], C.D. MORELLI[4], F. FIORICA[5], E. VARRIALE[6], R. DI FRANCIA[2-4], A. ABBADESSA[7]

[1]Pathology Unit, A.O. Sant'Anna e San Sebastiano, Caserta, Italy
[2]Italian Association of Pharmacogenomics and Molecular Diagnostics, Naples, Italy
[3]CETAC Research Center, Caserta, Italy
[4]GORI, Gruppo Oncologi Ricercatori Italiani, Pordenone, Italy
[5]Department of Radiation Oncology, Universitary Hospital "S. Anna", Ferrara, Italy
[6]UOS di Oncologia, Ospedale Fatebenefratelli, Naples, Italy
[7]Department of medical and surgical oncology, Haematology/Oncology Unit, A. O. Sant'Anna e San Sebastiano, Caserta, Italy

Abstract. – OBJECTIVE: Mantle cell lymphoma (MCL) is a non-Hodgkin lymphoma (NHL) featured by participation of the lymph nodes, spleen, blood and bone marrow with a short remission period to standard therapies and a median overall survival of 4-5 years.

PATIENTS AND METHODS: In this study, we compare the levels of bcl-1/JH fusion products detected by q-PCR in the concurrent peripheral blood (PB) and bone marrow (BM) aspirate samples from 7 patients with MCL.

RESULTS: In patients with moderate to high levels of bcl-1/JH copies, the results of q-PCR analysis of PB and BM aspirate samples correlate well. In patients with high levels of bcl-1/JH copies, instead, PB levels are a good indication of tumor burden. Finally, in patients with low levels of bcl-1/JH copies, the t(11;14) may be detected by identification of neoplastic cells.

CONCLUSIONS: Our data suggest that PB can be reliably used in place of BM aspirate both for detection of translocation status during minimal residual disease monitoring and for a possible molecular relapse, especially in those patients who have moderate to high levels of bcl-1/JH copies. If these results will be confirmed on a wider number of MCL patients, future study will be required to address the issue.

Key Words:
Disease monitoring, Molecular diagnostics, BCL1/JH, Quantitative PCR, Mantle cell leukaemia, Minimal residual disease.

Abbreviations

NHL = non-Hodgkin lymphoma; MCL = mantle cell lymphoma; iMCL = indolent form of MCL; cMCL = classical form of MCL; MBL = monoclonal B-cell lymphocytosis; IGH locus = immunoglobulin heavy chain locus; MTC = major translocation cluster region; MRD = minimal residual disease; PB = peripheral blood; BM = bone marrow; FCL = follicular cell lymphomas; IGHV = Immunoglobulin heavy chain variable region genes; JH consensus = Joining consensus region of immunoglobulin heavy chain.

Introduction

Mantle cell lymphoma (MCL) is relatively uncommon neoplasm, comprising 3-10% of non-Hodgkin lymphoma (NHL). It has a marked male predominance (male:female ratio = 5:1) and mainly affects middleaged to elderly individuals (median age about 60)[1,2]. MCL typically presents in advanced stage with lymphadenopathy, hepatosplenomegaly and bone-marrow involvement, but peripheral blood involvement is also present in about 25% of cases. MCL combines the worst features of indolent and aggressive lymphomas, in being incurable by currently available chemotherapy but clinically aggressive, with a median survival of only 3-5 years[1-4]. Re-

Corresponding Author: Salvo Di Martino, Ph.D; e-mail: salvo-dimartino@libero.it

cently however, an indolent form of MCL (iMCL) was described which differs from the classical form of MCL (cMCL) in having a benign clinical course with survival of more than 7 to 10 years, sometimes not even requiring chemotherapy for long periods. This iMCL subgroup often displays non-nodal leukemic disease with predominantly hypermutated Immunoglobulin Heavy Chain Variable Region genes (IGHV), noncomplex karyotypes and weak SOX11 expression[5-7]. MCL with an indolent clinical evolution may therefore represent a distinctive clinical and biological subtype of the disease. It has even been proposed that at least some iMCL cases may represent the MCL counterpart to the CLL-like monoclonal B-cell lymphocytosis (MBL)[7]. Recently, very low levels of long-lived monoclonal B-cells with a t(11;14) were detected in up 20 to 7% of healthy individuals, which may thus carry MCL-like MBL clones[8].

The t(11;14)(q13;q32) is regarded as the primary genetic event in the pathogenesis and the hallmark of MCL[9]. Its detection is crucial for the recognition of MCL cases underlying the development of neoplastic disease and for defining individual variations in response to specific drugs[10]. By analyzing the breakpoint regions it has been deduced that this translocation occurs at the pre-B-cell stage of differentiation in the bone marrow initiated by the recombination of the immunoglobulin heavy chain locus (IGH locus)[11]. The translocation juxtaposes the CCND1 locus (BCL-1) at 11q13 with the joining region (J) of immunoglobulin heavy chain genes (IGH locus) at 14q32, causing overexpression of the cyclin D1 protein at both mRNA and protein levels[12,13].

Only translocations involving the major translocation cluster region (MTC) of the BCL-1 gene can be revealed by a routine PCR analysis. Thus 30% to 40% of MCL cases can be shown to carry the t(11;14) translocation by PCR methods. Very recently, the quantitative evaluation of cells carrying the t(11;14) translocation, following conventional and high dose therapy, was shown to represent a powerful predictor of long-term remission in MCL patients. Therefore, the possibility of measuring molecular minimal residual disease (MRD) after appropriate therapy provides a powerful tool to define subgroups of MCL patients with a significantly different prognosis[14].

Molecular diagnosis of MCL is usually made either through a qualitative PCR (able to detect 30-40% of cases) or, indirectly, through detection of Cyclin D1 mRNA overexpression by REAL-time reverse transcriptase PCR. In this latter instance, Cyclin D1 overexpression needs to be referred to a B cell marker (e.g. CD19 or CD20) and cannot be exploited for direct MRD evaluation since it does not represent a direct measurement of neoplastic clone size[15,16]. Alternative methods for direct MRD evaluation such as IgH clone-specific PCR are cumbersome and time consuming[17].

The sensitivity of detection of t(11;14) rearrangement by any methodology, including q-PCR, also varies according to the extent of involvement by MCL in the tested sample.While most MCL patients have tumor cells present in bone marrow (BM) and peripheral blood (PB), the degree of lymphomatous involvement at these sites can often be low. Furthermore, BM aspirate samples frequently under-represent the extent of involvement because MCL cells have a tendency to be located in a paratrabecular location that are often not aspirated.

When PCR detection of t(11;14) rearrangements is used to monitor tumor burden, relapse or minimal residual disease in MCL patients, the choice of samples and the avoidance of false-positive and false-negative results becomes critical[17,18]. It is not known any study to address the question that PB can be used in place of BM aspirate to monitor minimal residual disease (MRD) in MCL patients and that either PB or BM aspirate testing give a rough approximation of the degree of BM involvement. While in NHL Follicular Cell Lymphomas (FCL) with the rearrangement t(14;18) was previously addressed: one study has shown that analysis of PB specimens is less predictive of relapse than analysis of BM aspirate specimens[19]. By contrast, other studies have demonstrated that PB and BM aspirate samples usually yield similar results[20].

Importantly, studies with transgenic mice suggest that cyclin D1 deregulation, although important for MCL initiation, may not be responsible for the complete cell transformation, and that secondary genetic alterations are required[21]. Comparison with other malignant lymphoid neoplasms has revealed that MCL, in particular the blastoid variant, is among those with the highest level of genomic instability[22]. Recurrent genomic aberrations observed in MCL[23] include deletions at 1p, 8p, 9p, 9q, 11q, 17p and gains at 3q, 8q, 15q, 18q. Although the target genes of most genomic aberrations have not been identified, a small group of candidate genes has been characterized, including ATM, BCL2, CDK4, CDKN2A, MYC, and TP53. Notably, most of these

target genes are involved in cell cycle regulation and cellular response to DNA damage, as discussed below[24].

As cyclin D1 is not generally expressed by normal B cells or other lymphomas, its positive expression has come to represent a highly specific marker for MCL in clinical practice[12]. It should be mentioned, however, that the t(11;14)(q13;q32) has been detected in other types of hematological malignancies such as multiple myeloma[1]. Furthermore, gene expression profiling studies have identified a subset of MCL cases that is cyclin D1 negative but cyclin D2 or cyclin D3 positive[25,26]. Translocations and resulting protein overexpression involving these alternative cyclin isoforms may be detected by FISH and RT-PCR respectively but immunostaining is not specific[27]. Genotyping is crucial to the identification of genetic markers underlying development of neoplastic diseases and individual variations in response to specific drugs. Cost and time-effective technologies able to accurately identify genetic polymorphisms will markedly affect routine diagnostics processes and future therapeutic developments[28].

Here, we compare the levels of bcl-1/JH MTC fusion products detected by q-PCR in concurrent PB and BM aspirate samples from 7 patients with MCL, all known to have bcl-1/JH rearrangement involving MTC. For patients with moderate to high levels of bcl-1/JH MTC copies, the results of q-PCR analysis of PB and BM aspirate samples correlates well, and thus PB can be used instead of BM aspirate material for this purpose. Most of the patients with high levels of bcl-1/JH MTC fusion products also had histologic evidence of BM involvement by MCL and, thus, PB levels are an indication of tumor burden. However, for patients with low levels of bcl-1/JH MTC fusion products, most of whom do not have histologic evidence of BM involvement by MCL, in this case the t(11;14) may be detected by identification of neoplastic cells and, thus, confirmation by analysis of the primary tumor is suggested.

Patients and Methods

Study Group

Quantitative PCR analysis for the t(11;14) involving the MTC is routinely performed on PB and/or BM aspirate samples of all MCL patients at time of presentation to CETAC research center institution. In this study, we focused on 7 MCL consecutive patients accessioned between 2003 to 2007 who had bcl-1/JH MTC fusion products and who had data from paired PB and BM aspirate samples. The primary diagnosis of MCL was established by excisional biopsy of lymph nodes or other tissues in 5/7 cases. Using the World Health Organization classification system for histological grading, 2 cases were grade 1, 2 cases were grade 2, and 3 were grade 3. The extent of MCL involvement in BM was established by examination of hematoxylin-eosin-stained sections of the decalcified, formalin-fixed trephine biopsy specimens with a semiquantitative assessment of the percentage of marrow cellularity comprised of tumor cells. This evaluation was effected by determination of Cyclin D1 expression levels by immunohistochemical methods.

DNA Isolation

High molecular weight genomic DNA was isolated from PB, BM and LN samples by conventional phenol-extraction method. After spectrophotometrically determining concentration at 260/280 nm (SmartSpec 3000, BioRad, Hercules, CA, USA), DNA samples were stored at 4°C.

DNAs for control studies

Lymphoblastoid B-cell line JVM2 with a chromosomal translocation t(11;14)(q13;q32) was used as positive control for PCR studies. It was diluted with DNA sample from White Blood Cells (WBC) of an healthy donor t(11;14)-negative at various percentages.

Rigorous precaution was taken to prevent cross contamination of sample and all experiments included negative control from all stage of the reaction.

Conventional PCR

500 ng of DNA sample was amplified in two steps (first Round and nested-PCR) by specific forward primers for t(11;14) that cover a Major Translocation Cluster (MTC) of Bcl-1 gene and reverse primers that annealing on Joining consensus region of Immunoglobulin heavy chain (JH consensus) gene located on chromosome 14[29].

A reaction mix containing: 10 X buffer (100 mM Tris-HCl pH 8.3, 0.5 mM KCl), 4 mM $MgCl_2$, 0.4 mM dNTPs, 200 nM of Primers *MTC forward* and *JH consensus reverse*, 2.5 U Taq Gold (Applied Biosystem, Foster City, CA, USA) distilled water for 50 ml of total volume[30].

PCR conditions for the first round were 10 minutes at 95°C followed by 35 cycles of 30 sec-

onds at 94°C (denaturation), 30 seconds at 58°C (annealing), 1.5 minutes at 72°C (extension) and final extension of 5 minutes at 72°C.

1 microliter of first round amplicon was diluted 1/10 to set up Nested amplification for a total of additional 30 cycles. This second round Nested amplification was performed by the same conditions of first round amplification adopting primers *MTC nested* e *JH consensus nested* [30].

In both cases ten microliters of amplified products were run on a 1.5% agarose ethidium bromide-stained gel.

Real time PCR

The ABI/Prism 7700 sequence detector (Applied Biosystem, Foster City, CA, USA) platform was used throughout this study for detection of t(11;14) by SYBR Green I method following by manufacturer's protocol of Quant Kit (Dia-Chem srl, Naples, Italy). Briefly, 250 nanograms of DNA sample and standards were amplified in duplicate in a total volume of 25 uL. Sample was heated for 2 minutes at 50°C (for UNG digest), 2 minutes at 95°C and 45 cycles of 30 seconds at 95°C, 30 seconds at 61°C, 70 seconds at 72°C and 10 seconds at 80°C for fluorescent acquisition, in order to eliminate eventually non-specific signal derived from primers dimers (our observation).

In parallel albumin reference reaction, (500 ng of DNA sample) was performed in duplicate in the same previously described conditions.

As SYBR green intercalates non-specifically with any double-stranded DNA generated during PCR, the *Temperature melting (Tm)* curve analysis was performed following amplification to confirm the identity of amplified products by its specific *Tm* profile. The *Tm* curve analysis included one cycle of denaturation at 94°C for 1 min, followed by 60°C for 10 seconds and a ramp to 94°C at a rate of 0.1°C/10 seconds with continuous fluorescence measurement.

A software for results interpretation and absolute quantification of *bcl-1*/*JH* MTC positive cells in analyzed samples is included in the kit.

Statistical Analysis

Results from two replicates of each sample are presented as a mean value of *bcl-1*/*JH* MTC rearranged product levels normalized to the mean value of amplification of the albumin gene. Because of the expected variance of PCR efficiency over different target concentrations, log *bcl-1*/*JH* ratios were also computed to obtain a mean log difference between PB and BM aspirate samples.

We calculated correlation coefficients using a pairwise comparison of *bcl-1*/*JH* MTC fusion product levels in PB and BM aspirate samples. We similarly calculated correlation coefficients for *bcl-1*/*JH* MTC fusion product levels in BM aspirate specimens with histologic extent of lymphomatous involvement in the BM biopsy specimen. R-squared values were taken graphically and used to calculate correlation coefficients (r).

Results

Interpretation of Results

The cycle number at which the reporter dye emission intensities rises above background noise is called the threshold cycle (Ct). The Ct is directly proportional to the copy number of the target template at the beginning of the reaction. The use of standard curves (Figure 1) of known amounts of both endogenous albumin reference control and *bcl-1*/*JH* MTC rearranged copies (JVM-2 DNA), allows the calculation of the ratio of specific BCL1/IgH gene copies to reference DNA (Albumin) in each sample. Given that 500 ng of DNA correspond about to 90,000 cells, the results are expressed as absolute number of *bcl-1*/*JH* MTC positive cells over 90,000 analyzed cells[31-33].

Assuming equal efficiency of amplification for MTC and albumin DNA targets, this ratio approximates the number of cells bearing a MTC rearrangement compared to the overall number of cells analyzed. Thus, there is a threshold of approximately 10 MTC-bearing cells in 100,000 cells that defines a sensitivity lower limit below which the t(11,14) products are detected by Real Time-PCR; in those samples whose Real Time PCR could be doubtful but qualitative Nested PCR was positive, neoplastic cells were considered at level <10 *bcl-1*/*JH* MTC + cells over 100,000 analyzed cells. In this circumstance, at diagnosis, the avoidance of false-positive and false-negative results becomes critical, therefore concurrent analysis of the primary tumor to confirm the true translocation status is likely required for definitive interpretation of the significance of a low/borderline PB or BM aspirate MTC positive result[30].

Quantitative evaluation of t(11;14) Copies at Diagnosis

The PB and BM normalized copies values at diagnosis were highly correlated in most patients regardless of time or not there was histologic evi-

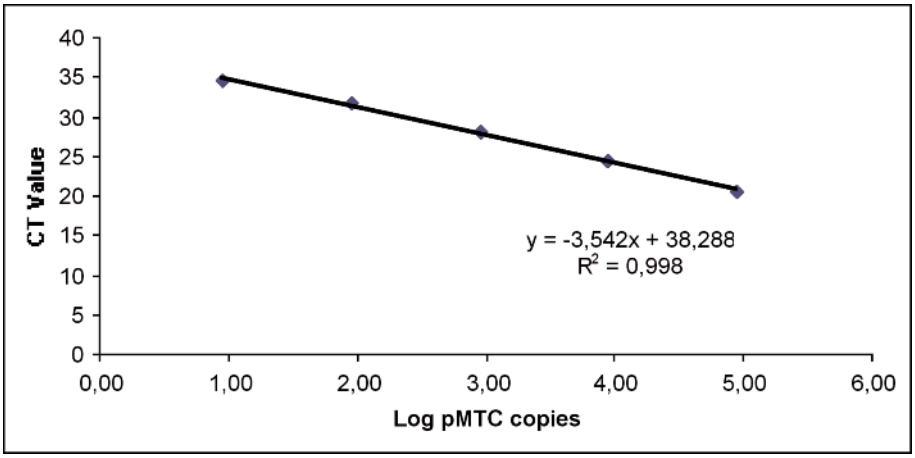

Figure 1. Standard curve: The logarithm of 10-fold serial dilution of pMTC (to 90000 copies down 9), was plotted as function of CT values. The lower detection limits (reproducibility 70%) of the method were 4.5 copies of pMTC. To perform parallel Albumin reference gene, each magnitude of pMTC was diluted into DNA from whole blood of healthy donor to a final concentration of 500 ng.

dence of MCL in the concurrent BM biopsy specimen. Following a Figure 2A, a less significant correlation coefficient was noted in both BM aspirate and PB samples (r = 0.169 and r= 0.07 respectively). The range of normalized *bcl-1/JH* MTC copies in BM was 106-92261 and in PB 172-130000. The normalized level of *bcl-1/JH* MTC was higher in BM aspirate than in PB in 4/7 of cases and was higher in PB than in BM aspirate samples in the remaining patients (Table I).

Among the 3 of 7 (57%) patients who had histologic evidence of MCL in the concurrent BM biopsy specimens, the median of *bcl-1/JH* MTC was 34640 copies in BM aspirate and 28158 copies in PB samples (Table II). As shown in Figure 2A, 1 of 7 (pat#2) MCL patients with histologically involved BM biopsy specimen had *bcl-1/JH* MTC levels in PB and BM aspirate samples that were within one log difference. However, approximately 1 MCL patients (pat#6) had low levels of *bcl-1/JH* MTC products detected in PB and BM aspirate, down 180 copies or less. Finally, the pat#5 had no evidences in BM, even so he had the high-test level of MTC.

Quantitative evaluation of t(11;14) copies after therapy

In contrast, there was a more significant correlation, at response to therapy, of the normalized *bcl-1/JH* MTC detected in concurrent BM aspirate samples (r = 0.84) and PB samples (r= 0.81) as shown in Figure 2B. In this case the range of normalized *bcl-1/JH* MTC copies in BM was < 10-2105 and in PB < 10-583 (Table I). The normalized level of *bcl-1/JH* MTC was higher in

Table I. Comparision between extent of Mantle cell lymphoma present in bone marrow biopsy and level of normalized bcl-1/JH MTC product in BM aspirate and PB at diagnosis and after therapy.

Patient	T(11;14) at diagnosis (copies normalized)		BM involvement	T(11,14) after therapy (copies normalized)		
N#	BM	PB	(%cyclin D1)	BM	PB	Tissues
1	57766	50000	Yes (40)	2105	583	
2	58176	2196	Yes (<10)	545	53	
3	4374	3677	Yes (<10)	143	< 10	
4	5611	1305	No	< 10	< 10	
5	92261	85000	No	< 10	< 10	
6	106	172	Yes (<10)	11	<10	
7	18230	56761	Yes (<10)	< 10	<10	

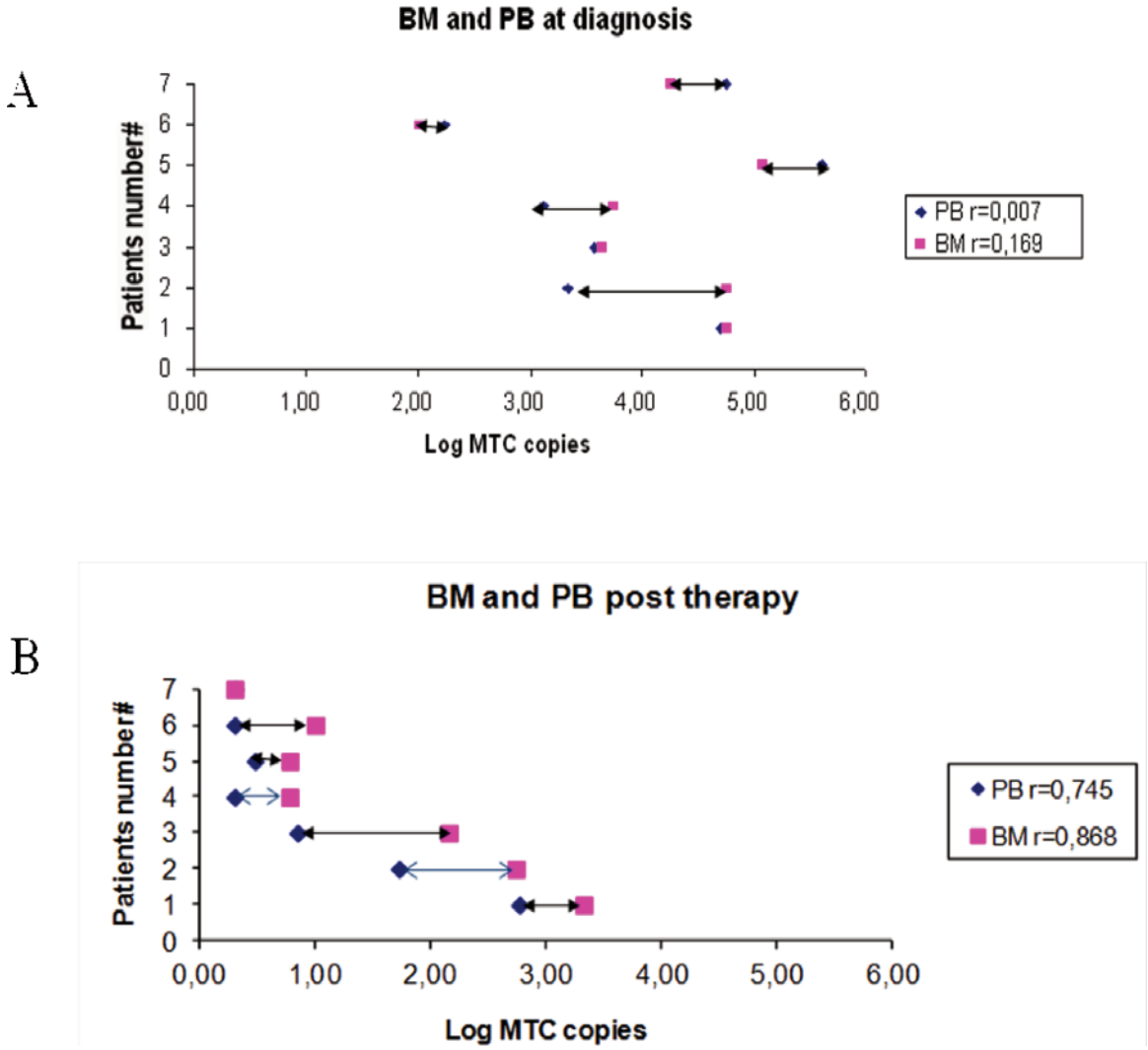

Figure 2. Correlation between normalized bcl-1/JH MTC levels in paired bone marrow and peripheral blood samples. ***A,*** Levels in patients in both BM and PB at diagnosis. ***B,*** Levels in patients in both BM and PB after therapy.

Table II. Comparison of bcl-1/JH MTC copies detected in PB and BM samples at diagnosis and after therapy.

Category	# of cases	BM at diagnosis	BM after therapy	PB at diagnosis	PB after therapy
Mantle cell lymphoma with involved BM	3	34640	734	28158	161
Mantle cell lymphoma with negative BM	4	32659	11	46059	<10
Avarange total	7	33789	402	35833	93
p			0.0001	0.0021	

BM aspirate than in PB in 7/7 of cases; however, in concurrent PB sample, t(11;14) was always detectable.

Among the 5 of 7 (71.4%) MCL patients who had no histological evidence of MCL in the concurrent BM biopsy specimens, the median of *bcl-*

1/JH MTC was 8.01% in BM aspirate and 0.83% in PB samples. As shown in figure 2B, 2 of 7 MCL patients (pat#2 and pat#3) with histologically involved BM biopsy specimens at diagnosis, had *bcl-1/JH* MTC levels in PB and BM aspirate samples that were within one log difference; patient #3, instead, had a lower involvement of BM biopsy after therapy.

In the 5th of 7 patients with the lowest *bcl-1/JH* copies (<10), conventional Nested PCR of the primary tumor revealed *bcl-1/JH* rearranged products with a size identical to that detected in the PB and BM aspirate samples. These results demonstrate that the low level of *bcl-1/JH* fusion products detected in PB and BM aspirate samples of these 5 patients was derived from the neoplastic clone (data not shown).

Quantitative evaluation of t(11;14) copies during follow up

Describe the parallel performance of PB and BM in all 7 cases with Figure 3 as example of the pat# 2: this is to prove that PB can be safely used for monitoring of minimal residual disease and therefore for a possible molecular relapse

Discussion

This study was designed to address whether levels of *bcl-1/JH* MTC fusion products detected by q-PCR in PB and BM samples obtained from MCL patients were similar, thereby obviating the need for BM examination solely for this purpose. We included 7 consecutive MCL patients with MTC *bcl-1/JH* fusion products who had q-PCR analysis performed on paired PB and BM aspirate specimens. The high degree of correlation between PB and BM aspirate samples shown here suggests that PB can be reliably used in place of BM aspirate for determination of translocation status in minimal residual disease monitoring, especially in those patients who have moderate to high levels of *bcl-1/JH* copies.

The correlation between *bcl-1/JH* copies detected in BM aspirate specimens and the extent of MCL involving the concurrent BM biopsy specimens also suggests that q-PCR may be used as an alternate method of determining the presence and general extent of BM lymphomatous involvement. Although in one case (pat #2) there was trend toward correlation, *bcl-1/JH* levels in PB were less useful for predicting the extent of BM involvement.

At our institution, patients with MCL commonly are first diagnosed by tissue biopsy. Elsewhere, fresh tissue or paraffin blocks of the primary tumor are often not available for molecular testing. In those cases, in terms of patient comfort and expense, PB is clearly the preferred specimen type over BM aspirate for determination of translocation status. We believe this study demonstrates that real-time q-PCR combined with "Amplicon temperature melting point analysis" can reliably allow analysis of *bcl1/JH* MTC molecular profile in PB samples in lieu of BM aspiration. This approach is highly sensitive when compared with non-quantitative PCR[34].

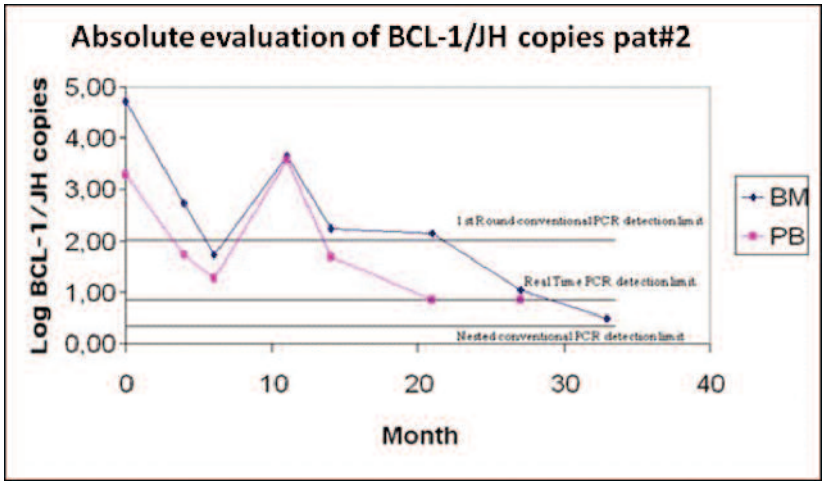

Figure 3. Monitoring follow up by detection of t(11,14) on paired BM and PB.

Furthermore, the good correlation (Figure 2B) between the results obtained from PB and BM aspirate specimens after therapy at all levels of disease (ranging from very high to very low levels of *bcl-1*/*JH* copies), suggests PB analysis is likely to be as informative as BM aspirate analysis for monitoring tumor burden in MCL patients over time.

Anyway, for newly diagnosed cases of MCL with low *bcl-1*/*JH* levels in PB or BM aspirate samples, confirmation of the *bcl-1*/*JH* amplicon size by testing of the primary tumor is justified. The data in this study also suggest that high levels of *bcl-1*/*JH* fusion products detected in PB or BM aspirate samples can be used as a substitute for testing the primary neoplasm. However, as already stated, relatively low levels of bcl-1/JH detected in PB and BM aspirate samples, necessitating the original biopsy specimen, if available, for t(11;14) analysis.

It has to be taken in mind that, as often happens, new analytical principles, innovative methods with extraordinary sensitivity and specificity features, preanalytical suitable procedures and standardized protocols for sample collection and analysis, are needed for the next future[35]. A deeper understanding of the biology of t(11;14) gene rearrengment can derive firstly from an advance in the methodological and technological support to the study of these molecules and secondarily to find medical expertise to interpret genomic results[36].

Conclusions

We suggest that PB can be used in place of BM aspirate to test for the presence of *bcl-1*/JH MTC and to monitor minimal residual disease in MCL patients. Either PB or BM aspirate testing yield a rough approximation of the degree of BM involvement.

Furthermore, future studies will be required to address the issue.

Acknowledgements

The authors are grateful to the GORI "Gruppo Oncologico Ricercatori Italiani" for the use of its bibliography resources.

Conflict of Interest

The Authors declare that they have no conflict of interests.

References

1) Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, Thiele J, Vardiman JW. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Fourth Edition ed. IARC WHO Classification of Tumours 2008, World Health Organization.
2) Jares P, Campo E. Advances in the understanding of mantle cell lymphoma. Br J Haematol 2008; 142: 149-165.
3) Herrmann A, Hoster E, Zwingers T, Brittinger G, Engelhard M, Meusers P, Reiser M, Forstpointner R, Metzner B, Peter N, Wormann B, Trumper Lorenz, Pfreundschuh M, Einsele H, Hiddemann W, Unterhalt M, Dreyling M. Improvement of overall survival in advanced stage mantle cell lymphoma. J Clin Oncol 2009; 27: 511-518.
4) Geisler CH, Kolstad A, Laurell A, Andersen NS, Pedersen LB, Jerkeman M, Eriksson M, Nordström M, Kimby E, Boesen AM, Kuittinen O, Lauritzsen GF, Nilsson-Ehle H, Ralfkiaer E, Akerman M, Ehinger M, Sundström C, Langholm R, Delabie J, Karjalainen-Lindsberg ML, Brown P, Elonen E. Long-term progression-free survival of mantle cell lymphoma after intensive front-line immunochemotherapy with in vivo-purged stem cell rescue: a nonrandomized phase 2 multicenter study by the Nordic Lymphoma Group. Blood 2008; 112: 2687-2693.
5) Fernàndez V, Salamero O, Espinet B, Solé F, Royo C, Navarro A, Camacho F, Beà S, Hartmann E, Amador V, Hernández L, Agostinelli C, Sargent RL, Rozman M, Aymerich M, Colomer D, Villamor N, Swerdlow SH, Pileri SA, Bosch F, Piris MA, Montserrat E, Ott G, Rosenwald A, López-Guillermo A, Jares P, Serrano S, Campo E. Genomic and gene expression profiling defines indolent forms of mantle cell lymphoma. Cancer Res 2010; 70:1408-1418.
6) Geisler CH, Kolstad A, Laurell A, Räty R, Jerkeman M, Eriksson M, Nordström M, Kimby E, Boesen AM, Nilsson-Ehle H, Kuittinen O, Lauritzsen GF, Ralfkiaer E, Ehinger M, Sundström C, Delabie J, Karjalainen-Lindsberg ML, Brown P, Elonen E. The Mantle Cell Lymphoma International Prognostic Index (MIPI) is superior to the International Prognostic Index (IPI) in predicting survival following intensive first-line immunochemotherapy and autologous stem cell transplantation (ASCT). Blood 2010; 115: 1530-1533.
7) Ondrejka SL, Lai R, Smith SD, Hsi ED. Indolent mantle cell leukemia: clinicopathologic variant characterized by isolated lymphocytosis, interstitial bone marrow involvement, kappa light chain restriction, and good prognosis. Haematologica 2011; 96: 1121-1127.
8) Lecluse Y, Lebailly P, Roulland S, Gac AC, Nadel B, Gauduchon P. t(11;14)-positive clones can persist over a long period of time in the peripheral blood of healthy individuals. Leukemia 2009; 23: 1190-1193.
9) Campo E, Raffeld E, Jaffe ES. Mantle-cell lymphoma. Semin Hematol 1999; 36: 115-127.

10) Di Francia R, Frigeri F, Berretta M, Cecchin E, Orlando C, Pinto A, Pinzani P. Decision criteria for rational selection of homogeneous genotyping platforms for pharmacogenomics testing in clinical diagnostics. Clin Chem Lab Med 2010; 48: 447-459.

11) Welzel N, Le T, Marculescu R, Mitterbauer G, Chott A, Pott C, Kneba M, Du MQ, Kusec R, Drach J, Raderer M, Mannhalter C, Lechner K, Nadel B, Jaeger U. Templated nucleotide addition and immunoglobulin JH-gene utilization in t(11;14) junctions: implications for the mechanism of translocation and the origin of mantle cell lymphoma. Cancer Res 2001; 61: 1629-1636.

12) Bosch F, Jares P, Campo E, Lopez-Guillermo A, Piris MA, Villamor N, Tassies D, Jaffe ES, Montserrat E, Rozman C. PRAD-1/cyclin D1 gene overexpression in chronic lymphoproliferative disorders: a highly specific marker of mantle cell lymphoma. Blood 1994; 84: 2726-2732.

13) De Boer Carla J, Schuuring Ed, Dreef Enno, Peters Gordon, Bartek Jiri, Kluin PM, van Krieken Johan HJM. Cyclin D1 protein analysis in the diagnosis of mantle cell lymphoma. Blood 1995; 86: 2715-2723.

14) Pott C, Schrader C, Gesk S, Harder L, Tiemann M, Raff T, Bruggemann M, Ritgen M, Gahn B, Unterhalt M, Dreyling M, Hiddemann W, Siebert R, Dreger P, Kneba M. Quantitative assessment of molecular remission after high-dose therapy with autologous stem cell transplantation predicts long-term remission in mantle cell lymphoma. Blood 2006; 107: 2271-2278.

15) Howe JG, Crouch J, Cooper D, Smith BR. Real-time quantitative reverse transcription-PCR for cyclin D1 mRNA in blood, marrow, and tissue specimens for diagnosis of mantle cell lymphoma. Clin Chem 2004; 50: 80-87.

16) Thomazy VA, Luthra R, Uthman MO, Davies PJ, Medeiros LJ. Determination of cyclin D1 and CD20 mRNA levels by real-time quantitative RT-PCR from archival tissue sections of mantle cell lymphoma and other non-Hodgkin's lymphomas. J Mol Diagn 2002; 4:201-208.

17) Andersen NS, Donovan JW, Zuckerman A, Pedersen L, Geisler C, and Gribben JG. Real-time polymerase chain reaction estimation of bone marrow tumor burden using clonal immunoglobulin heavy chain gene and bcl-1/Jh rearrangements in mantle cell lymphoma. Exp Hematol 2002; 30: 703-710.

18) Olsson K, Gerard CJ, Zehnder J, Jones C, Ramanathan R, Reading C, Hanania EG. Real-time t(14;18) and t(11;14) PCR assays provide sensitive and quantitative assessments of minimal residual disease (MRD). Leukemia 1999; 13: 1833-1842.

19) Gribben JG, Neuberg D, Freedman AS, Gimmi CD, Pesek KW, Freedman AS, Nadler LM. Detection of residual lymphoma cells by polymerase chain reaction in peripheral blood is significantly less predictive for relapse than detection in bone marrow. Blood 1994; 83: 3800-3807.

20) Bowman A, Jones D, Medeiros LJ, Luthra R. Quantitative PCR detection of t(14;18)bcl-2/JH fusion sequences in follicular lymphoma patients. Comparison of peripheral blood and bone marrow aspirates. J Mol Diagn 2004; 4: 396-400.

21) Gladden AB, Woolery R, Aggarwal P, Wasik MA, Diehl JA. Expression of constitutively nuclear cyclin D1 in murine lymphocytes induces B-cell lymphoma. Oncogene 2006; 25: 998-1007.

22) Salaverria I, Perez-Galan P, Colomer D, Campo E. Mantle cell lymphoma: from pathology and molecular pathogenesis to new therapeutic perspectives. Haematologica 2006; 91: 11-16.

23) Obrador-Hevia A, Fernández de Mattos S, Villalonga P, Rodríguez a J. Molecular biology of mantle cell lymphoma: from profiling studies to new therapeutic strategies. Blood Rev 2009; 23: 205-216.

24) Fernàndez V, Hartmann E, Ott G, Campo E, Rosenwald A. Pathogenesis of mantle-cell lymphoma: all oncogenic roads lead to dysregulation of cell cycle and DNA damage response pathways. J Clin Oncol 2005; 23: 6364-6369.

25) Rosenwald A, Wright G, Wiestner A, Chan WC, Connors JM, Campo E, Gascoyne RD, Grogan TM, Muller-Hermelink HK, Smeland EB, Chiorazzi M, Giltnane JM, Hurt EM, Zhao H, Averett L, Henrickson S, Yang L, Powell J, Wilson WH, Jaffe ES, Simon R, Klausner RD, Montserrat E, Bosch F, Greiner TC, Weisenburger DD, Sanger WG, Dave BJ, Lynch JC, Vose J, Armitage JO, Fisher RI, Miller TP, LeBlanc M, Ott G, Kvaloy S, Holte H, Delabie J, Staudt LM. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell 2003; 3: 185-197.

26) Fu K, Weisenburger DD, Greiner TC, Dave S, Wright G, Rosenwald A, Chiorazzi M, Iqbal J, Gesk S, Siebert R, De Jong D, Jaffe ES, Wilson WH., Delabie J, Ott G, Dave BJ, Sanger WG, Smith LM, Rimsza L, Braziel RM, Muller-Hermelink HK, Campo E, Gascoyne RD, Staudt LM, Chan WC. Cyclin D1-negative mantle cell lymphoma: a clinicopathologic study based on gene expression profiling. Blood 2005. 106: 4315-4321.

27) Quintanilla-Martinez L, Slotta-Huspenina J, Koch I, Klier M, His ED, de Leval L, Klapper W, Gesk S, Siebert R, Fend F. Differential diagnosis of cyclin D2+ mantle cell lymphoma based on fluorescence in situ hybridization and quantitative real-time-PCR. Haematologica 2009; 94: 1595-1598.

28) De Monaco A, D'Orta A, Fierro C, Di Paolo M, Cilenti L, Di Francia R. Rational selection of PCR-based platforms for pharmacogenomic testing. WCRJ 2014; 1: e391.

29) Tsujimoto Y, Jaffe E, Cossman J, Gorham J, Nowell PC, Croce CM. Clustering of breakpoints on chromosome 11 in human B-cell neoplasms with the t(11;14) chromosome translocation. Nature 1985; 315:340-343.

30) Andersen NS, Donovan JW, Zuckerman A, Pedersen L, Geisler C, Gribben JG. Real-time polymerase chain reaction estimation of bone marrow tumor

burden using clonal immunoglobulin heavy chain gene and bcl-1/Jh rearrangements in mantle cell lymphoma. Exp Hematol 2002; 30: 703-710.

31) Dolken L, Schuller F, Dolken G. Quantitative Detection of t(14;18) positive cells by Real Time Quantitative PCR using fluorogenic Probes. Biotechniques 1998; 25: 1058-1064.

32) Debled M, Hostein I, Astier-Gin T, Eghbali H, Bonichon F, Soubeyran I, Richaud P, Hœrni B, Soubeyran P. Competitive PCR to quantify tumour cells in peripheral blood of patients with t(14;18)-bearing follicular Non-Hodgkin's Lymphoma: an exploratory study in 8 patients. Int J Cancer 1999; 84: 558-561.

33) Mejerink JP, Smetsers JM, Raemaekers J, Bogman T, De Witte T and Mensink EJ. Quantification of follicular non-Hodgkin's lymphoma cells carrying t(14;18) by competitive polymerase chain reaction. Br J Haematol 1993; 84: 250-256.

34) Olsson K, Gerard CJ, Zehnder J, Jones C, Ramanathan R, Reading C, Hanania EG. Real-time t(14;18) and t(11;14) PCR assays provide sensitive and quantitative assessments of minimal residual disease (MRD). Leukemia 1999; 13: 1833-1842.

35) Berretta M, Di Francia R, Tirelli U. Editorial – The new oncologic challenges in the 3RD millennium. WCRJ 2014; 1: e133.

36) Di Francia R, Valente D, Pugliese S, Del Buono A, Berretta M. What health professions in oncology needs to know about pharmacogenomics? WCRJ 2014; 1: e90.