Fluorescent In Situ Hybridization Monitoring and Effect of Detected Early Responses in the Outcome of Patients With Chronic Phase Chronic Myeloid Leukemia: A Report From a Latin American Country

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Abstract

Our aim was to assess the routine use of fluorescent in situ hybridization in the monitoring of chronic phase chronic myeloid leukemia. We retrospectively analyzed data from 63 patients. The monitoring adherence assessment revealed better compliance rates compared with previous reports. Excellent correlation between fluorescent in situ hybridization and chromosome banding analysis was found. Achieving a complete cytogenetic response, assessed by fluorescent in situ hybridization, was an independent prognostic factor in the outcomes of patients.

Introduction: The cytogenetic hallmark of chronic myeloid leukemia (CML) is the Philadelphia chromosome. Monitoring the response in patients receiving therapy is a standard of care. The present study was conducted to assess the monitoring adherence and reliableness of fluorescent in situ hybridization (FISH) as a monitoring tool and the effect of a complete cytogenetic response (CCyR) assessed by FISH on the prognosis of patients in a chronic phase (CP)-CML cohort. **Materials and Methods:** We retrospectively analyzed the data from 63 newly diagnosed CP-CML patients treated with imatinib mesylate at a dose of 400 mg/day as frontline therapy. The clinical data and cytogenetic test results at diagnosis and during monitoring were collected. The cytogenetic monitoring adherence assessment rates were measured. A correlation between chromosome banding analysis (CBA) and FISH was performed. The CCyR assessed by FISH was defined as $< 1\%$ *BCR-ABL1*$^{+}$ nuclei. The Kaplan-Meier method was used for overall survival analysis and time-to-event estimates. **Results:** The cytogenetic monitoring assessment adherence was 50.8% at 3 months, 93.5% at 6 months, 96.7% at 12 months, and 88.6% at 18 months. The Pearson correlation coefficient showed a significantly positive association ($r = 0.84$; $P < .001$) between CBA and FISH. The median follow-up duration after imatinib mesylate initiation was 60 months. A CCyR was achieved in 90.4% of patients within the first 18 months of therapy. At 3 months, 31 patients underwent a FISH evaluation, and 13 (41.9%) had achieved a CCyR. The patients who did not achieve a CCyR at 3 months had a significantly inferior probability of 5-year failure-free survival (38% vs. 94%; $P = .001$) and progression-free survival (80% vs. 100%; $P = .043$) compared with those with a CCyR. **Conclusion:** We found improved monitoring adherence compared with the previous reports of Latin American populations. In countries with a high incidence of failure for CBA and a lack of real-time polymerase chain reaction standardization, FISH is a sensitive monitoring tool. In our cohort, patients not achieving an early CCyR, as tested by FISH, were a poor prognosis subgroup with worse rates of failure-free survival and progression-free survival.

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Introduction

Chronic myeloid leukemia (CML) is a clonal hematopoietic disease, the hallmark of which is the Philadelphia (Ph) chromosome and its molecular counterpart, the *BCR-ABL* fusion gene.[1,2] The oncogenic fusion codes for a constitutively active tyrosine kinase protein, resulting in an uncontrolled cellular division and inhibition of apoptosis.[3-5]

Upfront therapy with imatinib mesylate (IM) in patients with chronic phase (CP)-CML has shown an overall survival (OS) rate of 86% and a complete cytogenetic response (CCyR) rate of 82%.[6-8] Despite these excellent results, some patients fail to respond and have poor long-term outcomes.[9]

The achievement of an early CCyR remains a major determinant of outcome in CML, regardless of whether a major molecular response has been achieved.[10,11] Patients not achieving a cytogenetic or molecular response at 3 months have had worse event-free survival (EFS) and failure-free survival (FFS) rates.[12,13]

Monitoring the response in patients receiving tyrosine kinase inhibitor (TKI) therapy is considered the standard of care. The European LeukemiaNet's most recent consensus has recommended using quantitative real-time polymerase chain reaction (RT-PCR) for molecular testing and/or chromosome banding analysis (CBA) in $\geq$ 20 bone marrow (BM) cell metaphases, if cytogenetic techniques are used.[14]

Data from centers with resources to perform ideal monitoring have varied greatly from the data from centers in which the availability of drugs and monitoring tools is limited because of sociocultural status and financial standing.[15-19] A recent study performed in 16 countries of Latin America reported low rates of monitoring: 31% at 3 months, 54% at 6 months, and 9% at 12 months.[20]

In our country, in addition to the known limited availability of RT-PCR, a high incidence of failure for the CBA technique has been reported (73% to 94%). In contrast, the detection rate reported for the FISH technique has been 83% to 100%.[21-23] Previous studies have successfully reported data suggesting that FISH and RT-PCR are reliable methods with which to monitor the response, with strong correlations to the CBA results.[24-26]

The aim of the present study was to assess the monitoring adherence and the routine use of FISH in the monitoring of the initial therapy for CP-CML, when "high-quality" CBA is not available and RT-PCR–based molecular tests are unavailable because of financial and technical reasons. Additionally, we report the patterns of the early cytogenetic response and their effect on OS, EFS, FFS, and progression-free survival (PFS) in patients with CP-CML receiving IM therapy.

Materials and Methods

Patients

From January 2002 to April 2013, we retrospectively collected the demographic information, clinical data, and outcomes of patients. We included patients aged $\geq$ 18 years who had been diagnosed with CP-CML and treated with IM 400 mg daily as frontline therapy with $\geq$ 3 months of follow-up since IM initiation at our institution (Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán) in Mexico City.

IM was provided at no cost to our patients by Novartis Mexico under the Glivec International Patients Assistance Program of the Max Foundation. All patients in the IM group provided written informed consent as a mandatory requirement of the Glivec International Patients Assistance Program.

Definitions and Monitoring Response

CP-CML was defined as the presence in the peripheral blood of $<$ 15% blasts, $<$ 20% basophils, $<$ 30% blasts and promyelocytes, platelets $> 100 \times 10^9$/L, and no extramedullary blastic disease. CCyR was defined as 0% Ph^+ metaphases by CBA or $<$ 1% *BCR-ABL1*$^+$ nuclei by FISH analysis.[14,27]

The cytogenetic response was assessed in optimal samples, defined as CBA of BM cells using the G-banding technique, with $\geq$ 20 metaphases analyzed and/or by FISH dual color test for *BCR/ABL* in 200 BM interphase cell nuclei.[14,24,25] Correlation between CBA and FISH was performed between the tests, which were both optimal and obtained from the same sample.

The OS, EFS, FFS, and PFS were measured from the date of IM initiation to the date of the first event of interest or death during IM therapy. EFS referred to survival without the loss of a complete hematologic response, loss of CCyR, or failure to achieve CCyR after 18 months of therapy, progression to accelerated or blast phase, death from any cause with or without therapy, or treatment cessation for toxicity. FFS was defined as survival without any of the events previously described, with the exception of treatment cessation for toxicity. PFS implied survival without progression to the accelerated or blast phase of the disease.

Statistical Analysis

Continuous variables are described in terms of the median and range. Categorical variables are described as frequencies and proportions. Fisher's exact test was used to compare the differences between the numerical values, and the Student t test was used for categorical variables. Correlation analysis was performed using the Pearson correlation test. The Kaplan-Meier method was used to construct OS curves, and differences were analyzed using the log-rank test. A P value of $\leq$ .05 was considered statistically significant. We used SPSS, version 21, software (IBM Corp, Armonk, NY) to perform the data analysis.

Results

Study Group

A total of 73 patients with newly diagnosed CP-CML were found in our database, and 63 patients fulfilled the inclusion criteria. The patient baseline characteristics are summarized in Table 1.

Monitoring

The incidence of cytogenetic evaluation during the disease monitoring in our group, with either CBA and/or FISH, was 50.8% at 3 months, 93.5% at 6 months, 96.7% at 12 months, and 88.6% at 18 months. The total number of BM samples sent for analysis was 235 for CBA and 258 for FISH. Optimal results were obtained for 52.8% of the CBA and 98.0% of the FISH tests (Table 2).

The Pearson correlation coefficient test showed a significantly positive association ($r = 0.84$; $P < .001$) between the percentage of Ph^+ cells by CBA and the percentage of positive signals for