MOLECULAR DIAGNOSIS OF ACUTE promyelocytic leukemia in 30 minutes by single-step reverse transcription loop mediated amplification reaction (RT-LAMP).


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INTRODUCTION

A rapid detection of PML-RARα translocation is crucial for effective management of patients affected by Acute Promyelocytic Leukemia (APL). This life-threatening disease, if diagnosed promptly, benefits from treatment with retinooids and arsenic trioxide that have revolutionized therapy over the last decade. The molecular detection of the three PML-RARα transcripts (bcr1, bcr2, bcr3) is actually based on conventional RT-PCR, a long and laborious multi-step method often not suitable for clinical needs. We have developed two ultra-rapid RT-LAMP (Reverse Transcription Loop-mediated Isothermal Amplification) assays to overcome the limitations of traditional RT-PCR in order to obtain a diagnosis in real time within 30 minutes.

METHODS

Two multiplex fluorescent RT-LAMP assays have been developed to detect and distinguish the three fusions transcripts (bcr1, bcr2 and bcr3) of the PML-RARα translocation.

RESULTS

RT-LAMP is highly sensitive

The level of sensitivity was established on mutated RNA from positive patients diluted into negative cell line RNA (HL-60)

RT-LAMP is highly specific

The assays specificity was established on negative PML-RARα RNA extracted from 8 cell lines.

CLINICAL VALIDATION

The PML-RARα RT-LAMP assays were validated on RNA obtained from 34 clinical samples previously diagnosed at Ospedali Riuniti di Bergamo by using conventional RT-PCR (biomed).

CONCLUSIONS

The PML-RARα RT-LAMP assays represent an ultra-rapid, specific, sensitive and cheap method for molecular confirmation of APL. The single-step format monitored in real-time simplifies the entire reaction set-up and ensures reliability.

This 30 minutes reaction can significantly improve the outcome of APL patients allowing to start the therapy timely.