29th ECCMID Amsterdam, Netherlands 13 – 16 April 2019

The congress of ESCMID

P2181 Validation of reference genes for quantification of microRNAs by RT-qPCR in blood samples of patients with candidaemia

Silvio Ragozzino*[1], Jorge Luis Torres[1], María Vaquero-Herrero[1], María Ángeles Pérez[2], Edgar Bernardo[3], Guadalupe Sabio[3], Inmaculada García-García[1], Hugo Guillermo Ternavasio De La Vega[1,2], Miguel Marcos[1,2,4]

[1] Hospital Universitario de Salamanca, Spain, [2] Instituto de Investigación Biomédica de Salamanca, Spain, [3] Centro Nacional de Investigaciones Cardiovasculares-CNIC, Madrid, Spain, [4] Universidad de Salamanca, Spain

Background: Reverse Transcription – quantitative Polymerase Chain Reaction (RT-qPCR) is the gold standard procedure to study the expression pattern of specific microRNAs in different tissues and/or experimental conditions. Normalization of RT-qPCR data is a critical step for the reliability of the assay, because this process controls for technical variations. The use of an internal reference gene is the most accurate strategy for normalization. A single universal reference gene does not exist and the suitability of each candidate should be tested for each study. The best reference gene is the least variable among the samples, regardless of experimental conditions. The aim of our study is to validate candidate reference genes in blood samples of patients with candidemia and controls without sepsis.

Materials/methods: Blood samples were collected at the University Hospital of Salamanca (Spain) from patients with candidemia and controls with a similar baseline condition, but without sepsis. A panel of small RNAs, including miR-103, miR-16, U6 and SNORD48, were selected as candidate reference genes, either based on literature reviews or manufacturer's recommendation. RNA expression was quantified by RT-qPCR using the Applied Biosystems® StepOnePlus™ real-time PCR machine. The stability of each candidate gene was tested using GeNorm and NormFinder statistical algorithms, included in the GenEX software and the Excel template of BestKeeper algorithm.

Results: The expression of the 4 abovementioned small RNAs was examined in 10 cases and 10 controls. The results of stability analysis are shown in Figure 1. Low values indicate more stable expression. The most stable genes are at the left of each graphic.

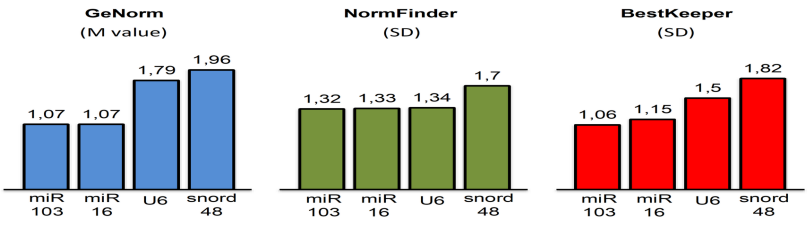

Figure 1. Stability analysis of the candidate reference genes.

Conclusions: Our results show that miR-103 is the ideal reference gene for the normalization of RT-qPCR quantification of microRNAs in blood samples of patients with candidemia.