Validation of appropriate reference genes for the normalization of real-time quantitative RT-PCR data obtained from platelets of post-myocardial infarction patients

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Introduction
Reference genes (RGs) used for the quantification of mRNA expression could vary with the experimental and disease conditions and their validation is a crucial requirement. Previous reports on platelet mRNA expression used conventional RGs, but no studies have been reported on checking the validity of these RGs. In other words, the expression of RGs has not been thoroughly investigated in platelet. The aim of our study was the establishment of RGs in platelets from healthy persons and in patients with the history of myocardial infarction.

To ensure experimental transparency, accuracy, and repeatability, we followed the MIQE guidelines (minimal information for publication of real-time qPCR experiments).

Materials and methods
12 mL blood samples were collected in ACD tubes from 21 patients who suffered myocardial infarction. We also included seven healthy individuals into the study as control. The tubes were stored at ambient temperature, and were transported within 4 hrs (also at ambient temperature) from the clinical ward to the analytical laboratory.

Primer pairs were chosen to span an exon-intron boundary to exclude amplification of genomic DNA.

WBC contamination
Contaminating leukocytes were completely removed by three consequent centrifugation as judged by PCR analysis.

Total RNA extraction
Assessment of leukocyte depleted-platelet (LDP) RNA purity
To monitor the effectiveness of leukocyte depletion, we used a sensitive RT-PCR assay. RNA from platelet preparations tested positive for a platelet-specific mRNA (vWF) and negative for granulocyte-specific mRNA (CD15 and lymphocyte-specific mRNA (HLA-DQβ). Ethidium bromide-stained agarose gel containing Reverse transcription polymerase chain reaction-amplified products of platelet mRNA was positive for vWF and negative for CD15 and HLA-DQβ after 40 cycles of polymerase chain reaction (Figure 1).

Conclusions
We recommend ACTB and HDGF as stable RGs most suitable for gene expression studies of human platelets after myocardial infarction. We propose the use of GNAS, OAZ1 and GAPDH average as RGs for the accurate normalization of qRT-PCR performed on normal platelets.

Our results clearly demonstrated that the expression levels of RGs change in certain conditions. Thus, the selection of RGs is important for platelet gene expression studies. The use of these genes as RGs may further enhance the robustness of qRT-PCR in this model system.