"Novel molecular biomarkers for detection of autologous blood transfusion in sport: fetal hemoglobin and microRNAs"

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PROJECT REVIEW

No method of direct detection of autologous blood transfusion (ABT) is currently available in sport. The implementation of the Athlete's Biological Passport has increased the search for new biomarkers or new techniques as 'omics' technologies to detect ABT. Otherwise both the phases of blood withdrawal and blood reinfusion might be accompanied by changes of different parameters sensitive to the oxygen availability with generation of integrated molecular profiles.

We hypothesized that among the potential sensitive parameters, two biomarkers might show changes of interest during ABT: microRNA, small non coding RNAs that regulate gene expression and fetal haemoglobin (HbF), the main oxygen transport protein in the human fetus and still present at low concentration in red blood cells of healthy adults. The objective of the project is to test this hypothesis during three experimental phases by means of 6 tasks: i) the determination of possible changes of HbF and miRNA pathways following blood withdrawal and re-infusion and the identification of proteins and miRNAs to be candidate as molecular markers of ABT (Phase-1-TASK 1); ii) the determination of the reproducibility of HbF and selected microRNA patterns with respect to age, sex and training status. (Phase-2-TASK 2); iii) the determination of the effects on these molecular markers of the exposure to acute exercise and altitude (Phase-2-TASKS 3-4); iv) the development of protocols, assays and platforms suitable for applications in the field of anti-doping (Phase-3-TASK 5-6). When the hypothesis was confirmed, the information of the microRNA profile and of HbF levels should be reported in the Athlete's Biological Passport to allow the detection of ABT in presence of deep changes, even after combined analysis, of the biomarkers.

RESULTS and CONCLUSIONS

The lack of a method for directly detecting ABT stimulates the search for new biomarkers and the use of new technologies. This project aimed to identify novel molecular markers useful in the anti-doping field to detect ABT. The hypothesis was that both the phases of ABT, blood withdrawal and reinfusion, might be accompanied by changes in different parameters sensitive to oxygen availability with the generation of integrated molecular profiles. The study focused on changes induced by ABT in: i) HbF, the primary oxygen transport protein in the human fetus and still present at low concentrations in RBC of healthy adults and never tested for anti-doping purposes, and ii) microRNA (miRNAs), small non-coding RNAs that regulate gene expression and are related to erythroid differentiation, HbF production and transcriptional regulation of γ-globin genes.

Twenty-four healthy trained male subjects were enrolled and randomized into Transfusion (T) and a Control (C) groups. The T subjects underwent nine seriated blood samples before and after the procedures of withdrawal, and their blood was refrigerated (4°±2°C) or cryopreserved (-80°C). They also received reinfusions. Blood samples were obtained for analysis from the C subjects at the same time points. For the entire population, we analyzed traditional hematological parameters, physical performance assessed by a treadmill test, HbF and other Hgb forms. Microarray analysis of a set HbF-related miRNAs was also performed for 6 subjects of the T group and for a pool of C group blood samples at selected time points.

For the T group, a moderate change in traditional hematological parameters, particularly following blood withdrawal, and increased performance following reinfusion were observed.

HbF showed a marked increase in the T group following withdrawal and a decrease after blood reinfusion. Moreover, HbA1c, another form of Hb that can be robustly measured and whose values correlated with HbF values, was found to be stable in the C group. We noted a continuously decreasing trend in HbA1c both after blood withdrawal and reinfusion in the T group. This unusual consistent trend in the T group resulted in a significantly lower level of HbA1c for the parameter compared to baseline values, with abnormally low values in 42% of the transfused subjects. HbF-related miRNA analysis performed in a subgroup of T subjects revealed a pool of 12 miRNAs associated with HbF and regulating transcriptional repressors that were differently expressed in the two phases of the intervention in 5 out of 6 subjects and stable in the pool of control subjects. Notably, among the 5 T subjects with overexpressed miRNAs, 3 individuals exposed an HbA1c value below the cut-off value and, one subject has an HbA1c value roughly equal to the cut-off level.

In conclusion, this study supports the hypothesis that ABT evokes changes of potential interest in the anti-doping field in sensors of relative hypoxia-hyperoxia. In particular, HbA1c, a biomarker largely used in metabolic diseases and never before tested for anti-doping purposes, showed an interesting pattern following ABT. Similarly, miRNA expression was found to be modified following ABT with person-to-person variability. However, the selective changes in T-group individuals supports the concept that miRNAs analysis might be useful for the detection of ABT. This framework of integrated parameters, which enabled us to recognize a relevant group of transfused subjects, requires further studies.

Publications

- Gasparello J et al., Altered erythroid-related miRNA levels as a possible novel biomarker for detection of autologous blood transfusion misuse in sport. Transfusion, (2019) May 30. doi: 10.1111/trf.15383.