

“Verification of a stable blood storage small RNA biomarker signature in subjects after autologous blood doping”

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Project Overview

The detection of illegal applied autologous blood transfusion is an everlasting and unsolved problem in the human doping scene. Since no external drugs are applied, no substance residues can be detected in the body. But there might be traceable physiological changes in the transfused blood cells, introduced by sampling or blood/erythrocyte storage. In such a case the detection of the physiological response of the illegal manipulation will be a promising approach.

In animal husbandry the problem of applied growth promoting substances is also present. We could show that illegal given drugs change the transcriptome level of RNA biomarkers in various tissues. In the case of autologous blood doping, blood cells are directly affected by sampling or storage and thus blood would be a promising matrix for the detection of such transcriptional changes. In a previous study we could show, that storage of erythrocyte concentrates in stabilization buffer has a tremendous and stable effect on the miRNA profile.

This study was designed to investigate whether those changes are also visible in subjects after autologous blood transfusion and if the storage conditions, e.g. the storage buffer, induces the same changes in the recipient's blood miRNA profile. Another point of investigation will be to monitor physiological changes in the blood cells that occur after receiving erythrocyte concentrates on small RNA level. A holistic approach is planned to detect physiological changes on small RNA level. We want to analyze all kinds of small RNAs (miRNA, piRNA, etc.) in different blood fractions (whole blood or plasma) via small RNA sequencing. This technology allows a holistic detection of all small RNAs in the biological sample. The clinical trial with healthy subjects that receive autologous erythrocyte concentrates is already approved. Finally, a transcriptional biomarker signature for field analysis via RT-qPCR will be developed.

Results and Conclusions:

The World Anti-Doping Code was established to ensure equality and the athletes' health by listing methods and substances whose usages are considered as rule violations. Whereas the abuse of many performance enhancing applications mentioned therein is already detectable, the definite unveiling of autologous blood doping (ABD), for example, is still unfeasible. Based on forward-looking results in biomarker research in the field of

microRNAs (miRNAs), the present study aimed at revealing ABD-dependent changes of miRNA signatures in blood samples after ABD.

Therefore, an ethically approved human study was conducted based on a total of 30 healthy and sportive males, who were equally distributed to three groups with different extents of ABD and several sampling time points. Hematological markers were determined by standard clinical laboratory measures, showing indeed, highly significant physiological changes in response to blood donation as well as erythrocyte concentrate re-transfusion. Especially clinical parameters related to erythrocyte and iron metabolism were clearly affected, while coagulation and urine markers remained largely ABD-unaffected. In addition, transcriptomic analyses were performed by small RNA sequencing allowing for high-throughput microRNA (miRNA) screening without the need for a prior knowledge. In the following, ABD-dependent changes in the miRNA profile were identified by bioinformatic-driven data analyses. This generated a set of a minimum of 29 miRNAs that could be used as biomarker signature to discriminate doped and non-doped conditions at a high prediction level. Further, sequencing results were selectively validated by real-time reverse transcription quantitative polymerase chain reaction demonstrating exceedingly high and significant correlation.

Next steps will be focusing on the confirmation of the miRNA biomarker signature by applying it to the complete study dataset on the individual level, since inter- and intra-individual variations are still permanent challenges in unequivocal ABD detection. Nevertheless, the present study already contributed enormously to the overall understanding of ABD-dependent changes on the blood level. Due to its high abundance of data as well as comprehensive and well-constructed study design, this study could also act as impeccable starting position for future studies on ABD detection.

Publications/presentations

- "Outlook: The WADA blood doping project" by Veronika Mussack, Oral presentation at the TUM colloquium of animal physiology and immunology; 19th December 2016; Freising, Germany
- "Detection of autologous blood doping of the miRNA level in a human study" by Veronika Mussack; Poster presentation at the 3rd HEZ PhD Symposium; 25th April 2017; Freising, Germany
- "Update: The WADA blood doping project" by Veronika Mussack, Oral presentation at the TUM colloquium of animal physiology and immunology; 19th June 2017; Freising, Germany
- "Update: The WADA blood doping project" by Veronika Mussack, Oral presentation at the TUM colloquium of animal physiology and immunology; 22nd January 2018; Freising, Germany
- "WADA blood doping project: Basics, interim findings, and future perspectives" by Veronika Mussack; Oral presentation at the LMU colloquium of transfusion medicine; 19th February 2019; Munich, Germany

- “MIQE-Compliant Validation of MicroRNA Biomarker Signatures Established by Small RNA Sequencing” by Veronika Mussack, Stefanie Hermann, Dominik Buschmann, Benedikt Kirchner, Michael W. Pfaffl; Springer Nature – Methods Molecular Biology; to be published in October 2019