PROJECT REVIEW

"Detection of autologous blood transfusion by analysis of erythrocyte density fractions and flow cytometry"

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Although autologous blood transfusions are a prohibited method, there is at present no unequivocal test and it seems from recent scandals that the technique remains a significant problem in various sports. During the past months and years, several research attempts to develop a valid detection method have been performed but so far have failed to identify a definite, unequivocal test. Whereas a method for detection of homologous blood transfusion was established in 2002, the most contemporary (indirect) attempt to detect autologous transfusions is the Athlete Biological Passport.

In this one-year project, our group focuses on the detection of autologous blood transfusion by a detailed analysis of red blood cell density fractions which could be altered in the time course after transfusion. In this context, the so-called 'neocytolysis' as a presumed selective destruction of young red blood cells (neocytes) was described as a process to possibly down-regulate hemoglobin mass when it is "excessive" or maladaptive for the environment and was seen as potentially important in blood doping settings. Several researchers assume that this regulation of red cells can be expressed when measuring their main density fractions.

In a pilot study, as an unexpected, but compelling and promising finding, our group was able to show that the transfusion of autologous blood leads to a distinct shift in the red cell density fractions with an increase of red cells of a high density. Flow cytometry with measurement of red blood cell surface markers shall be used to further evaluate these shifts and to allow identification of a certain set of markers being specific for autologous transfusion. This project will consist of a cross-sectional and longitudinal part where multiple transfusions and a control group shall be included to test for reproducibility and specificity of the methods.

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Results and Conclusion

The most important result of this project was that the transfusion of autologous blood, predominantly with long-term storage for 7 weeks, lead to distinct changes of the RBC population shortly after transfusion. These effects were not detectable in full blood but in RBC samples from a high density band established by self-forming Percoll gradients based on several parameters. However, the time window for detection of these changes seems to be short, possibly being limited to a maximum of 24 up to 48 / 72 hours depending on the tested parameter. Systemic reactions to autologous transfusions were supported by the analysis of classical markers such as serum ferritin and hematology measurements showing significant changes which support the high value of serum ferritin as an important marker of autologous transfusion. However, we were unable to demonstrate any significant effect of autologous transfusion on the RBC surface marker profile in full blood or one of the three RBC subpopulations.

In summary, we believe that the methods will be difficult to implement in the routine of any anti-doping laboratory. Because of the elaborate preparations and short detection window (perhaps only valid for long-stored blood), this project unfortunately may not reveal a direct test for autologous blood doping at this stage. Nevertheless, the results are very interesting from a physiological point of view and will be discussed in the next paragraphs. In the last section, we will further comment on possible applications for on-going and future research in the field.