***"Detection of Autologous Blood Transfusions by flow cytofluorimetry: a multiparametric approach"***

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## PROJECT REVIEW

Blood doping is banned by the World Anti-Doping Agency in all sports due to its effects on sport performance, especially in endurance disciplines. WADA accredited laboratories have developed testing methods for the detection of blood doping by eryhtropoietins, synthetic hemoglobins, RSR13, and homologous blood transfusions, while no direct, internationally recognized method is yet available for the detection of autologous blood transfusions. We propose a flow cytofluorimetric approach based on the recognitions of markers of storage in red blood cells. More specifically, markers of apoptosis (namely, phosphatidylserine) and markers to detect reduction of antigen expression on red blood cells membrane (primarily among them CD55 and CD59 proteins) have already been evaluated by our laboratory as potential diagnostic parameters to detect the infusion of previously stored blood, with very promising results. Preliminary data obtained on several different blood samples, tested at different times after collection and in different storage conditions, showed that the selected parameters are significantly modified by red blood cells storage. We are planning to implement the number of markers considered for this study, possibly broadening the panel of diagnostic markers to be monitored to effectively detect the recourse to autologous blood transfusions. Once the most suitable diagnostic markers will be identified and selected, the effectiveness of the approach will be verified on subjects undergoing preoperative autologous donation in the framework of pre and post- surgery practices.

We strongly believe that the proposed approach could effectively complement the analysis presently under evaluation for the detection of autologous blood transfusions, representing a significant advancement towards the development of a robust and reliable direct method to detect autologous blood transfusion.

## Results and Conclusions

One of the current challenges for the Antidoping Laboratories worldwide is the detection of Autologous Blood Transfusion (ABT). At present, ABT can be detected only by indirect methods, which require the longitudinal evaluation of the stability of selected hematological parameters, as in the hematological module of the athlete biological passport.
This project aimed to study and to explore a multi---parametric strategy, based on flow cytofluorimetry, targeting specific morphological/biochemical changes of red blood cells, as well as the alteration of other hematological parameters (e.g. the increase of

circulating microparticles), consequent to the storage period of the withdrawn blood prior to the reinfusion in the receiver person. This approach is in fact a “direct” detection strategy, since it recognizes specific changes on the “exogenous” (this meaning the transfused) red blood cells (RBCs).
We have conducted a series of experiments on human whole blood samples that were focused on the identification of diagnostic signs of red blood cells aging also matching all the following conditions: (i) to be easily detected by flow cytofluorimetry; (ii) to be stable after addition of the stored sample to fresh blood; and (iii) to show a sufficiently pronounced variation, allowing their identification also in mixed blood samples generated after a transfusion practice.
From the data we obtained two main conclusions can be drawn:

i) Flow cytofluorimetry is able to identify multiple indicators of erythrocytes aging that are generated after a storage time in the fridge and blood banks condition.
ii) The signals of RBC aging that have been detected in our experimental conditions (I.e., by considering a period of storage up to 40 days) are the following:
a. reduction of the expression of surface proteins on RBC;
b. moderate increase in the concentration of glycated HB (HbA1c);
c. reduction of RBC main size, that generates a population of smaller and more dense erythrocytes;
d. the consequent formation of a population of microparticles, that is a direct consequence of red blood cells microvesiculation process.

Unluckily, it was not possible to verify in vivo the observation recorded ex vivo, as well as to evaluate the potential effectiveness of “markers of reinfusion”, due to the lack of samples collected from auto-
--transfused subjects. Experiments in this direction are still in progress, thanks to newly activated, ongoing cooperation with other research groups and clinical laboratories.
In spite of the above limitation, the data we collected at this stage are very promising in the development of a universal, direct method for detecting both autologous and homologous blood transfusions: based on our results, a detection strategy based on the counting of the number of microvesicles and the counting of red blood cells of the more dense fraction (smaller in size) seem to present a higher diagnostic value than that based on the variation over time of the expression of specific proteins on the red blood cell membrane.
Finally, it has to be stressed out that our proposed approach needs a solid standardization: indeed, the same counting, when performed on different flow cytofluorimetry instruments, that use different detectors settings, can lead to different measurements, inevitably resulting in an increase of the inter-laboratory variability of the results.