PROJECT REVIEW

"Investigation of indirect markers of autologous blood transfusions in peripheral blood samples"

M. Ashenden (Science and Industry Against Blood Doping, Australia)

The long term goal of this research is to develop a test or tests to detect the presence of autologous transfused red cells in peripheral blood samples. Autologous transfusion is the reinfusion of the donor's blood that has been withdrawn and stored for an indefinite period, with the intent to increase total red cell mass and thereby endurance performance. There is unequivocal evidence that autotransfusion has been used, and is currently used, by elite endurance athletes. Prior research has found that autotransfusion alters red cell membrane structure, haematological parameters, and antigen expression. It is self-evident that autotransfusion also alters total red cell mass (which represents the goal of this practice). Our pilot research has also found that the expression levels of multiple genes remains altered for several weeks after reinfusion of autologous blood. Each of these parameters can be measured using appropriate technology, consequently they offer potential avenues for the detection of autologous transfusion for use by antidoping agencies. We hypothesise that a combination of these methods, supplemented with additional biologic information, will yield a diagnostic with superior sensitivity and specificity.

We will conduct autologous transfusion trials at the Copenhagen Muscle Research Centre (CMRC) in Denmark which has successfully performed such trials in the past. All samples and measurements required for each candidate parameter will be collected simultaneously, which will enable us to retrospectively scrutinise the sensitivity and specificity of various permutations of test data. We will evaluate cumulative sensitivities when the different approaches are applied to a single blood sample, and thereby determine whether one, or a combination of several, methodologies have potential to be applied in an antidoping setting.

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Results and Conclusions

In the absence of a test to detect autologous blood transfusion, this project was an exploration and comparison of several different avenues of research. The initial 2-year project was extended to permit investigation of promising early findings that autologous transfusion altered gene expression levels.

Our follow up studies have demonstrated that the gene signal was most evident 7 days after
reinfusion of three bags of blood, remained strong at 14 days and persisted through 28 days. There was an indication that a change does occur following transfusion with only one bag of blood, but this was of much smaller magnitude.

We found that haematological parameters were relatively insensitive to the reinfusion of blood, but were disturbed to a greater extent in the days after blood had been withdrawn. A novel marker was found to have greater sensitivity than conventional variables such as haemoglobin concentration or OFF-score.

An in-depth evaluation of total haemoglobin mass estimation via the CO rebreathing method
showed that this variable was the only one able to detect transfusion within the first few days after blood had been reinfused. However from one week onwards its sensitivity was no better than the OFF-score model.

Following initial promising results exploring red cell lesions as a means to reveal transfused
cells, basic problems in the development of that assay led us to conclude that a test based on this premise was most unlik