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APPLICATION OF FLOW CYTOMETRY FOR THE DETECTION OF THE ABUSE OF BLOOD TRANSFUSIONS IN SPORT DOPING: STATE OF THE ART AND NEW PERSPECTIVES

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Background and Aims

In doping control, anti-doping laboratories introduced flow cytometry techniques and instrumentation for the detection of blood transfusion (BT) abuse among cheating athletes. The method for detecting Homologous Blood Transfusion (HBT) abuse is based on flow cytofluorimetric analysis of phenotypic mismatches between minority blood group antigens on surface of donor and recipient erythrocytes. Since minor blood groups mismatch approach obviously cannot be applied when autologous blood transfusion (ABT) is used, several alternative strategies are currently being tested with the aim of identifying possible markers that are able to reveal an ABT. One of the most promising strategy concerns the analysis of the morphological and biochemical changes that erythrocytes undergo during storage in the blood bags conditions before being reinfused for transfusion purposes.

The aim of this work is to highlight how flow cytometry is used in the wide context of blood doping abuse detection. The strategies applied over the years in improving the sensitivity and the reliability of the results of the HBT method and the recent advances in the identification of ABT abuse are highlighted.

Methods

Erythrocytes (RBC) surface markers are detected by immunohematology techniques. Primary antibodies for the target of interest are used after titration in order to identify the best workflow concentration. Staining of RBC with fluorochrome-bound secondary antibodies are used in all those cases where the primary antibody linked to a detector fluorochrome is not commercially available. Minor blood group antigens currently being tested are big-C, small-c, big-E, small-e, Jka, Jkb, Fya, Fyb, big-S, small-s and big-K. More surface antigens are screened to assess the modification undergoing on RBC during storage in blood bags conditions: Glycophorin-A, CD47, Band3, CD55, CD59. Characterization of erythrocyte-derived microparticles (EMPs) is obtained by both morphological gating and immunological staining. Counting of EMPs is achieved by both absolute counting and relative percentage with respect of mature erytrhocytes.

Results and Discussion

A panel of 8-12 blood group antigens allows achieving an adequate global sensitivity to detect HBT abuse even many days after its execution. Accurate gating strategy together with high-brightness fluorochromes allow the clear separation of the donor and recipient erythrocyte cells populations. As for ABT, the counting of EMPs is emerging as the most effective strategy to identify the autologous transfusion as it is more sensitive than the monitoring of the decrease in expression of surface antigens in RBC subjected to storage.

Conclusion

Flow cytometry has become a basic technique in doping control for all aspects related to the detection of doping practices with the use of blood cells and at present the only technique suggesting the possibility of developing a direct method of identifying ABT abuse in sport doping.