Contemporary issues in the fight against blood doping in sport

One Olympic cycle has passed since international media and scientific scrutiny focused upon breakthroughs in the fight to deter the widespread abuse of recombinant human erythropoietin (rHuEPO) in sport. The Sydney 2000 Olympic Games was a watershed in that, for the first time, blood samples were collected from athletes and abnormal fluctuations in hematological parameters associated with rHuEPO use could be used as evidence to sanction the athlete for doping. Post-Sydney, the World Anti-Doping Agency (WADA) reviewed the scientific basis of both the blood and urine methodologies used in Sydney, and concluded that the presence of rHuEPO in the urine was sufficient evidence in itself to impose a sanction.[1] This decision relegated abnormal hematologic parameters to providing a screening tool to identify suspect athletes for urinalysis. However in the lead up to the 2004 Athens Olympics, the blood matrix has regained prominence, and is widely acknowledged by contemporary researchers as fulfilling a key role in future antidoping strategies. This article will review recent research, outline key challenges, and inform readers of likely future directions in blood doping research.

As forecast in a previous article,[2] and evinced by current press coverage, contemporary endurance athletes have focused their illegal attempts to enhance performance via blood doping on three areas: rHuEPO, blood substitutes and blood transfusions. Stemming the abuse of rHuEPO remains the major challenge to antidoping authorities, and this is reflected by the priority WADA gives to research in this field. Despite concerted efforts to improve upon the sensitivity of the electrophoretic technique, four years after inception the urine test remains virtually unchallenged in its efficacy. This stands as testimony to both the elegance and sophistication of the original methodology,[3] and the complexity of successfully discriminating between the endogenous and exogenous hormone.

Considering the rapid clearance of rHuEPO from circulation, it is unlikely there will be a major improvement of the current 3-4 day reach-back offered by detection methodologies reliant upon the pharmacologic approach. Frustratingly, it seems that athletes have learnt to manipulate rHuEPO regimens to successfully dodge current out-of-competition testing programs. This demands a more innovative approach, centered around sophisticated targeting of suspect athletes (awareness of their competition and training schedule will reveal likely windows for rHuEPO treatment). Such targeting must be supported by improved knowledge of athlete whereabouts (as promised by the WADA's Athlete Passport program) and greater dexterity of out-of-competition testing programs.

Research efforts to detect the presence of hemoglobin-based oxygen carriers (HBOC) or blood substitutes have conjointly received concerted funding and industry support. This blueprint has yielded commensurate results. Blood substitutes are not excreted by the kidney in sufficient quantities to warrant a urine-based test, mandating the collection and analysis of blood samples. Therefore international research has focused on three avenues to detect the presence of HBOC in blood: electrophoresis,[4] high performance liquid chromatography,[5] and mass spectrometry.[6] Pharmaceutical companies have given unequivocal support to these endeavors, providing methodology (Baxter), clinical trial support (Biopure), and/or access to products prior to their commercial release (Apex, Northfield, Sangart). This successful partnering between antidoping researchers and pharmaceutical companies has fast-tracked research to the point that authorities are currently addressing logistic concerns prior to the introduction of a sanctionable, blood-based test capable of detecting the entire gamut of HBOC products.

Perhaps the most disturbing, and from a medical viewpoint unconscionable, development in contemporary doping has been the re-appearance of homologous transfusion in sport. Fear of being caught for rHuEPO use, and awareness of sport's inability to test for homologous transfusion, has driven rogue athletes to expose themselves to the profound health risks posed by transfusion. In anticipation of this trend, the Royal Prince Alfred Hospital in Sydney embarked on research quantifying mixed red blood cell populations via the flow cytometric technique used to detect feto-maternal hemorrhage. The group has since demonstrated this method is clearly capable of detecting the small (<5%) populations of donor red blood cells that would result from a homologous transfusion of just one unit of blood.[7] Evidence of a mixed population in any of the 12 red cell antigens that are screened provides irrefutable evidence of prior transfusion. Screening for an additional, undisclosed antigen negates the possibility for a cynical athlete to find a perfectly matched donor. Authorities are expediting final preparations for the implementation of this blood-based test. Due to the unmistakable presence of donor cells for many weeks post-transfusion, and the corresponding fear of

being caught, it can be safely presumed that the implementation of the test will halt the alarming trend for athletes to transfuse homologous blood. Research is currently underway to explore whether the same technique can be utilized for the detection of autologous transfusion.

A paradigm shift in recent times has been how to best leverage information gleaned from the tell-tale fluctuations in haematological parameters caused by blood doping. The initial exuberance surrounding the ON model used at the Sydney 2000 Olympics[8] subsided as it was acknowledged that rogue athletes could remain below threshold limits by titrating their rHuEPO dosage. This was supplanted by enthusiasm toward the concept of a Hematologic Passport, whose longitudinal evaluation of individual athletes promised to magnify evidence of erythropoietic disturbances. The potential of this concept was recently illustrated in pilot research.[9] However, it is currently unclear what effect the unexpectedly large fluctuations in some key parameters will have on the legal surety of this approach, and by extrapolation its eventual deterrent effect.

Current energies are dedicated to the implementation of an OFF model, which combines hemoglobin concentration and reticulocyte percentage into an algorithm that can highlight athletes who have doped for several weeks after stopping rHuEPO injections. The attractiveness of this approach, stems principally from its ability to fortify the Achilles heel of the urine test (the inability to detect rHuEPO in the urine for more than 3-4 days after the last injection). An important recent breakthrough has been the capacity to compare reticulocyte results obtained on different platforms.[10] This negates the absence of a universal calibration material for reticulocytes, enabling a direct comparison of results collected over time on different instruments. The new approach complements not only the Hematologic Passport concept, but also facilitates the universal introduction of blood screening. In particular, it is now tenable for federations to supplement the existing hemoglobin limit with a threshold OFF score, beyond which athletes would be similarly ineligible to compete. In 2004 the International Cycling Union (UCI) will become the first federation to exclude from competition those athletes with abnormal OFF model scores.

A key issue confronting antidoping authorities today is how to combat the cynical use of water-based products that are infused minutes before an athlete must report for a blood screening. Freshly bleeding intravenous puncture sites are frequently encountered at competition venues; hemodilution masks hematologic evidence of prior blood doping. Although manipulating hematologic parameters is an illegal practice under the World Anti Doping Code, use of intravenous fluids has been tolerated as they are ostensibly required to assist rehydration and nutrition. However establishing an intravenous puncture for such purposes is not only against the spirit of sport, but is devoid of sound medical practice. Except in the case of genuine medical emergencies (severe dehydration), decisive action must be taken to eradicate this unseemly practice that is callously eroding the efficacy of blood screens.

One area of research whose anticipated application is seemingly immune to any type of masking is the quantification of gene expression profiles associated with blood doping. Changes in virtually all biological systems are accompanied by an alteration in the abundance of certain gene transcripts. Therefore knowledge of changes in gene expression profiles associated with blood doping may prove a decisive tool to reveal the use of otherwise invisible doping products. Unpublished preliminary research results emanating from Skuld Tech (University of Montpellier) have revealed a myriad of gene activity changes during and after rHuEPO treatment — the daunting intellectual challenge to sift through and seize upon those changes with sufficient discriminatory power to definitively identify rHuEPO use is currently underway.

In the midst of the various technical, legal and scientific challenges faced by antidoping authorities, a recent ethical challenge has surfaced from an unexpected quarter — whether or not to ban the use of simulated altitude facilities. Athletes have long utilized natural altitude exposure in the belief that it will confer a hematologic benefit similar to the use of rHuEPO. Against this backdrop, contemporary research is yielding an increasingly persuasive case that the physiologic response to altitude is multi-faceted, and that attributing the performance benefit to increased red cell production is overly simplistic and greatly exaggerated. Anti-ban supporters contrast the 1-2% performance improvement associated with hypoxic exposure against the 7-8% improvement routinely found with rHuEPO doping, and highlight the financial burden imposed on athletes who must relocate across international borders to enjoy access to natural altitude. Pro-ban advocates point to the artificial stimulation of biological processes as damning evidence that simulated altitude is against the spirit of sport. One of the issues that must be weighed by officialdom is whether banning simulated altitude facilities would necessarily warrant banning other edifices which utilize artificial environmental conditions to stimulate a physiologic response, such as the climate chambers used to acclimatize athletes to hot/humid conditions.

Four years on from the announcement of a blood-urine test capable of detecting rHuEPO use, progress to rid sport of the blood doping scourge has been deceptive. There have been quantum leaps forward in parallel areas, such as the increasing presence of the international coordinating agency WADA, and the advent of the World Anti Doping Code, the import of which cannot be overstated. During this time science, long the poorly-funded laggard in antidoping realms, has enjoyed considerable support and appears set to reward this investment with handsome dividends, such as the tests that will permanently close the door on several avenues of blood doping. However a solution to the initial challenge, seemingly answered four years ago by the test for rHuEPO, remains frustratingly elusive, and this thoroughfare will demand concerted attention in the future.

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Blood doping research in Haematologica

This issue of Haematologica contains an editorial and papers on issues surrounding blood doping.[1-3] More detailed information on this topic may be found in the Science and Industry Against Blood doping (SIAB) research consortium website (*http://www.siab.ws*).[4] In addition the reader might be interested in other papers on the same topic that have appeared in the journal in recent years.[5-20]

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Diagnosis and treatment of essential thrombocythemia

This issue of Haematologica reports a study on the value of bone marrow biopsy in the diagnosis of essential thrombocythemia.[1] The journal has recently reported practice guidelines for the therapy of this chronic myeloproliferative disorder.[2] Additional papers on essential thrombocythemia have been published[3-11] in the last few years. All Haematologica articles can be accessed for free online.

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