Doping en el deporte: la hematología-hemoterapia en alerta roja

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Blood doping

Blood doping is an illicit practice aimed at increasing blood oxigen carrying capacity and athlete's aerobic performance. Various techniques have been introduced in the last decades, including homologous[1] and autologous red blood cell transfusion[2], recombinant human erythropoietin (r-HuEpo) or related erythropoietic stimulants[3], rHuEpo-enhanced autologous transfusion[4], and hemoglobin-based oxygen carriers (blood substitutes)[3,5-7]. These illicit practices are mainly associated with endurance disciplines (e.g. cyclism, athletics and cross-country ski), but they can confer significant advantage in recovery after intermittent efforts[3]. Therefore other disciplines have to be considered at risk of blood doping[8].

The detection of these unacceptable practices is, for several technical reasons, problematic. The introduction of arbitrary limits in critical hematologic parameters to evaluate the elegibility to compete is a poorly specific and sensitive strategy[9]. The high biological variance increases the risk of false positive cases, while a pharmacological expansion of plasma volume could lead to falsely classify as negative dishonest athletes[8].

Australian researches elaborated a model including indices of accelared eythropoiesis[10] and defined algorithms able to detect the rHu-Epo during the administration phase (ON-model) and during the wash-out phase (OFF-model)[11,12].

An alternative approach could be based on the collection and recording of regular sequential tests of a single athlete in out-of-competitions periods in order to create an individual profile, the "hematologic passport", providing reference ranges and variation for a correct evaluation of the investigations carried out during competition. The inclusion within these tests, in addition to hemoglobin level and hematocrit, of additional erythropoietic indices could provide a strict control on values added to the profile[3,8]. Indeed, we showed that, using proper sequential determinations of hematologic variables, subject-specific reference ranges can be defined for hemoglobin and hematocrit[13]. Recent data support the notion that longitudinal monitoring of athletes' blood profiles will help detect blood doping[14]. This information could be used to instigate target-testing of suspicious athletes, or even warrant the exclusion from competition of athletes with aberrant variations in key hematologic values. Thus, the hematologic passport should be used within a global strategy to deter blood doping.

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TRENDS IN BLOOD DOPING IN SPORT

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Extended periods of strenuous physical exercise require the provision and replenishment of the fuels consumed by exercising muscle. To meet this physiological challenge, the body must drastically increase muscle blood flow to provide nutrients, and to ensure sufficient oxygen is available to 'burn' these fuels. The point at which the capacity to transfer oxygen from the lungs to exercising muscle is exceeded generally determines the highest level of sustained exercise that can be performed.

For many decades athletes and their coaches have recognised that this (genetically- and training-determined) maximal level of exercise could be exceeded if additional red blood cells were made available to transport oxygen to the muscles. Extra red blood cells, or more precisely the additional haemoglobin contained in these cells, translate into more oxygen being available to the exercising muscle, which in turn enables the athlete to perform at a higher exercise intensity.

The 1968 Mexico City Olympic Games, which took place at an altitude of 2,200 metres, marks one of the first occasions when the importance of oxygen supply to exercising muscle was brought to the attention of exercise physiologists. Previous publications in the 1940s and 1950s had demonstrated the impact that red cell transfusion could have on altitude acclimatization, and these results were rediscovered and subsequently built upon in the lead up to the Olympics. However as a post-Games report showed[1], there was still an imperfect understanding of precisely how altitude influenced exercise capacity (primarily due to the mistaken belief that performance should only have been limited when athletes were performing at maximal levels, which is seldom the case).

However athletes and coaches were apparently much swifter to extrapolate from the obvious performance decrements caused by altitude. Reports subsequently confirmed that one Finnish athlete had admitted to using blood transfusion at the 1972 Olympic Games in Montreal, and clouds of doubt hung over one of their most famous Olympians Lasse Viren:

> When asked about the charged of blood doping, Lasse Viren played dumb.
>
> "I don't know what it is," he said. "I've never heard of it."
>
> "It's supposed to raise the haemoglobin," he was told.
>
> "How do you raise the haemoglobin?" he said. "I don't know. Do you know?"
>
> "That's a non-denial denial, a Watergate denial".
>
> *Dave Anderson, New York Times*[2]

The use of blood transfusion apparently peaked around 1984, which was marked by numerous high profile cases of blood doping. Francesco Moser admitted to using blood transfusion in his successful attempt to break the 1-hour World Cycling Record, an entire national team were caught transfusing at the world cross-country running championships, and the US track cycling team infamously admitted to using both autologous and homologous blood transfusion in the lead up to and at the Los Angeles Olympic Games[3].

In 1985 the gene for erythropoietin was cloned, and four years later Amgen introduced a recombinant form of the protein to market under the trade name Epogen. It seems athletes readily adopted this convenient alternative to blood transfusion as their modus operandi. Unfortunately this heralded an era of numerous unexplained deaths in the cycling fraternity as EPO treatment was abused to extremes – one famous cyclist became known as "Mr 60%" in reference to the haematocrit he achieved via EPO doping.

EPO abuse went completely unfettered until 1997 when some sport federations introduced haematological limits for parameters such as haemoglobin concentration and haematocrit. This initiative seems to have had some effect on EPO doping – today it is very rare to find Hct values in excess of 50% in those sports that police this threshold. However several publications that gathered and compared historical data from different years suggest athletes have merely titrated their regimens in order to remain below the limit, rather than stop using EPO altogether[4,5].

A much-heralded breakthrough came in 2000 when French researchers developed a urine-based test that was able to discriminate between synthetic EPO and the endogenous hormone found in circulation[6]. The test relied upon a nuance of recombinant hormone production wherein the glycosylation of the proteins is exceedingly difficult to control during the protein synthesis stage. Proteins produced in mammalian cell lines display different glycosylation patterns to endogenous EPO, and thus possess a different negative charge. When an electric field is applied to a gel in which a urine sample is contaminated with synthetic EPO, the isoforms with different electric charge locate to regions that are different to those of endogenous EPO, so that a comparison with appropriate positive and negative controls enables authorities to confidently detect the presence of banned synthetic EPO.

Considerable publicity accompanied the release of data from the 1999 Tour de France which showed that at least six of the urine samples provided by the eventual champion Lance Armstrong contained synthetic EPO. Although the science behind these results remains uncontested, no sanction could be imposed due to a rule technicality.

The electrophoresis test for synthetic EPO remains the mainstay of antidoping laboratories today. There is considerable concern that biosimilar EPOs, which are being produced in an attempt to avoid patent infringements but capitalise on the lucrative anaemia drug market, may be produced in a manner that yields different glycosylation profiles to contemporary EPOs. Were the glycosylation profiles of these new EPOs indistinguishable from endogenous EPO this would be a confronting problem for antidoping authorities.

Similar concerns have been raised about genetic doping. Many press reports suggest that this technology will mark the end of an effective antidoping effort – based on the premise that gene doping will be undetectable. There appears to be ample opportunity to subvert genomic research for blood doping purposes. Due to favourable laboratory characteristics numerous models have been successfully developed which enable genetically modified cells to produce EPO in vivo. However research published in 2004 demonstrated that, contrary to public opinion, genetic doping with EPO was found to be detectable using the electrophoretic test[7].

Also in 2004, the Athens Olympic Games marked the introduction of a test for homologous blood transfusion. Researchers in Australia modified a flow cytometry test previously used to detect feto-maternal haemorrhage in order to apply the concept in the sporting domain[8]. The basis of the test is to screen whole blood samples for the presence of mixed populations of minor red cell antigens. Although a blood sample collected at these Olympics from cyclist Tyler Hamilton (who placed first in the road race) showed a mixed cell population for multiple antigens, no sanction could be imposed as the mandatory 'B' sample had been frozen and rendered useless for analytical confirmation. As red cells persist in circulation, the same athlete was tested several weeks later and sanctioned accordingly (despite protestations from the athlete that the mixed cell population was in fact due to either a vanishing twin in utero, severe bruising or insufficient research publications from the test authors). Tyler Hamilton's name was later implicated in Operation Puerto where seized documents revealed the use of multiple blood transfusions during 2004.

A less publicised, but nevertheless important breakthrough, came at the Athens Olympics with the introduction of a test for haemoglobin-based oxygen carriers, or 'artificial blood'. Rumours that these blood substitutes had surfaced in sport had persisted for some time, perhaps most explicitly in the admissions of Jesús Manzano who collapsed during the seventh stage of the 2003 Tour de France from what he claimed were the side-effects of using a veterinary version of artificial blood. In contrast to endogenous hormones such as EPO, blood substitutes are foreign molecules which make their detection relatively straightforward.

In a commendable demonstration of altruistic cooperation between the pharmaceutical industry and antidoping authorities, an arrangement was brokered wherein each of the six pharmaceutical companies who were in various stages of development of their product agreed to provide access to proprietary knowledge of their compounds. This enabled authorities to engineer an appropriate test methodology that was universal for all known products. This cooperation peaked at the Athens Olympics when each company also provided the antidoping laboratory with a sample of their product to enable positive identification of doping substances.

Today there is a growing realisation that athletes have resorted to autologous blood transfusion in order to escape detection. This has been famously revealed in the Operation Puerto case where more than 200 bags of blood were seized in Madrid. Many high profile cyclists have been implicated, including 1997 Tour de France champion Jan Ulrich and 2006 Giro d'Italia champion Ivan Basso.

No test currently exists to detect the presence of autotransfused blood cells. Research is currently underway investigating the persistence of membrane damage in frozen/thawed red cells that may eventually yield a diagnostic of suitable sensitivity and specificity. Other avenues being explored include measurement of total haemoglobin mass, and even changes in the profile of genes switched on and off after blood reinfusion or phlebotomy. Although haematological parameters such as haemoglobin and percent reticulocytes are known to change post-transfusion, and thus could be used as an indirect marker of autologous transfusion, they lack the desired level of specificity due primarily to the large inter-individual variability of these parameters.

The difficulties inherent with these inter-individual variances of haematological parameters are not new to antidoping. Although first proposed many years ago[9], only recently has well-deserved attention focussed on the concept of establishing a 'haematological passport' for athletes. Such a passport would establish the athlete's true baseline values, and thus eradicate inter-individual variability. An especially appealing characteristic is that longitudinal fluctuations of haematological variables (such as haemo-

globin, percent reticulocytes, transferrin receptor, and ferritin) might have the capacity to reveal blood manipulation irregardless of the type of blood doping used. Currently there is an ongoing effort to harmonise the parameters, and criteria for classifying a blood sample as 'abnormal', so that the passport concept can be adopted internationally across all sport. It seems the initial approach will rely on exclusion from competition, rather than sanctioning, of athletes who possess abnormal blood profiles.

Unquestionably a new kind of investigation – based upon non-analytical evidence – has dawned upon sport. The sanctions recently imposed on Austrian athletes found in possession of transfusion equipment at the 2006 Winter Olympic Games in Turin, and then the Puerto affair in Madrid, have underlined how effective, and indeed necessary, law enforcement intervention has become to deter (currently undetectable) practices such as autologous transfusion.

Blood doping has a long and multifaceted history in elite sport. Traditionally athletes resort to whatever means are available to increase circulating red cells, provided that they cannot be detected by authorities. Over time this tendency has seen the rise and fall of homologous transfusion, EPO injections, the use of blood substitutes and finally autologous blood transfusion. As the enormous performance advantage bestowed by blood doping seems an irresistible incentive for athletes to cheat, controlling this plaque upon international sport seems to rest upon the successful development of both detection methodologies and deterrence strategies.

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