Short Communication

Erythropoietin Abuse in Sport

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INTRODUCTION

In a sporting context 'doping' refers to the use by athletes of banned substances for the purpose of enhancing physical performance. The word 'dope' originated in the African continent and referred to a primitive alcoholic drink that was used as a stimulant in ceremonial dances. The term 'blood doping' was introduced in the 1970s to describe the use of blood transfusion (homologous or autologous) to increase the red cell mass artificially and so enhance both maximal oxygen uptake and performance in endurance sports, particularly in international cycling and cross-country skiing. Since the late 1980s blood doping is no longer only achieved by autologous transfusion but by the administration of recombinant human erythropoietin (rHuEpo) and more recently still the use of Haemoglobin-Based Oxygen Carriers (HBOCs) and other oxygen transport molecules. The International Olympic Committee and other major sporting bodies officially prohibit the use of rHuEpo. The International Ski Federation (FIS) was the first organisation to introduce haemoglobin (Hgb) limits to allow participation. The idea was to limit both the degree of health risk (hyperviscosity, thrombosis and hypertension) and the degree of performance enhancement. This was followed by the introduction of haematocrit (Hct) limits by the International Cycling Union (UCI) and the International Biathlon Union (IBU). An editorial review of strategies for the prevention and detection of doping with rHuEpo was published in 2000[1].

Since 2000 it has been possible to identify recombinant EPO. The electrophoretic mobility technique provides a direct measurement of urine levels of rHuEpo, and is based on the principle that the rHuEpo molecule is less negatively charged than the endogenous EPO molecule. Isoelectric focusing has emerged recently as a method for the direct analysis of rHuEpo in urine[2, 3]. However, the concept of indirectly detecting rHuEpo or other EPO mimetic substances through their effect on other blood characteristics is promising. Some sports have already imposed upper thresholds on Hgb concentration and Hct. The disadvantages of using thresholds are many including natural inter-individual variation, effect of posture and ease of manipulation through interventions such as, for example, infusion of saline. Suitable indirect markers include the concentration and physical properties of erythrocytes and reticulocytes (Ret) and the serum concentration of soluble transferrin receptor (sTfr) either singly or in combination. Algorithms that combine scores from multiple blood parameters are demonstrably effective in highlighting long term continuous rHuEpo administration and have been used to deter its use by athletes[4-7].

CURRENT PRACTICAL ACTIVITIES

Since 1996, the Laboratoire Suisse d'Analyse du Dopage (Swiss Laboratory for Analysis of Doping, Institut Universitaire de Medicine Légale, Lausanne, Switzerland) has been highly involved in blood analysis in sport. Indeed, during the Tour de Suisse 1996, and mandated by the officials of the UCI personnel from the laboratory obtained blood specimens for analysis for the first time in cycling. The aim was to determine whether or not it was possible to conduct blood specimen collection during a major cycling event. Specimens were collected and brought to the laboratory for determination of the Hct level and the Hgb concentration. Other parameters related to erythropoiesis were also measured, notably the plasma EPO, the sTfr concentration and the ferritin concentration. This first attempt resulted in two major findings: first, it was feasible to collect blood specimens quickly (approximately 2 minutes per cyclist) and secondly, the use of indirect blood parameters to detect rHuEpo abuse was not as good as expected. Indeed, at that time, rumours were circulating that quite a few athletes were taking rHuEpo, and even though, very few of them had out of range indirect blood parameters (serum EPO and sTfr concentrations and Hct levels)[8] .

Following this first trial, the UCI in 1997 decided to monitor athletes taking part in cycling events routinely by measuring Hct levels. The protocol involved collect-

ing blood specimens within two hours of the start of the competition and performing the analysis immediately so that athletes with abnormal values could be excluded from the competition. In order to have the results available with the minimum of delay and to avoid any inter-technological variations, it was decided to perform the analysis at the site of competition. The protocol was not primarily designed as part of an anti-doping programme to detect rHuEpo abuse but as a test to detect dangerously elevated blood viscosity. In the early 1990s there was considerable speculation that doping with rHuEpo was involved in the death of professional cyclists[9]. The artificially increased Hct compounded by dehydration during strenuous exercise predisposed these individuals to thromboembolic complications[10,11]. EPO enhances both endothelial activation and platelet reactivity in humans substantially increasing the risk of thromboembolic complications particularly in persons with a genetic predisposition to thrombophilia.

PARAMETERS AND POLICY SELECTED FOR SCREENING

For more than ten years, scientists have been looking for an ideal blood parameter or algorithm that combines multiple blood parameters to demonstrate the abuse of rHuEpo. Unfortunately this is possible only in the case of continuous long-term rHuEpo treatment[12]. The discovery of the direct detection of rHuEpo in urine in 2000 entirely changed the philosophy of blood testing to combat its abuse. Blood testing was designed to be a very specific anti-doping test[13] but with poor sensitivity; then it was redesigned to be very sensitive and less specific[14]. Indeed the aim of the blood test was then to screen out the most suspicious athletes who were then submitted to a urinary anti-doping test. This procedure was necessary because the urinary test is expensive and time consuming.

The most relevant parameters from our experience and from the Australian models were the Hct or the Hgb and the Ret count[14,15]. Both parameters are inexpensive to measure and most laboratories are used to performing such analyses. Ret count (%) was preferred to the absolute count because it is less influenced by plasma volume changes[16,17].

SELECTION OF INSTRUMENTATION

Since 1997, some seven European laboratories have been interested in performing the blood tests. At that time it was decided to use the Coulter AcT-8 (Beckman Coulter, CA, USA) performing the analyses at the competition site. At that time this analyser fulfilled all the analytical requirements and was light, compact, robust and reliable. The need to add the Ret count (%) which arose in 2001 altered these requirements. It was decided to use the Sysmex R-500 (Sysmex Corporation, Kobe, Japan) together with the AcT-8. The R-500 was the only entirely automated reticulocyte analyser small enough to be transported from one competition site to another. This instrument combination made it possible to analyse the necessary parameters for the screening test but was not without problems. The solution of using the AcT-8 for determination of the Hct and the Hgb concentration and the R-500 for the Ret count (%) satisfied the requirement to combat rHuEpo doping but was inconvenient for laboratory personnel since all specimens had to be analysed twice, once on each analyser. This was time-consuming and fairly expensive to run requiring two technologists to optimise the return of results. Each analyser required its own reagents, spare parts, and maintenance contracts. The costs of transporting the two analysers by air from one race to another were prohibitive. It was clear that this could only be a temporary solution until such times as a suitable analyser performing all the relevant analyses became available.

In the year 2002, Sysmex launched a new analyser, the XT-2000*i*, on the European market. This analyser combined aperture impedance and optical technologies in addition to differential lysis and fluorescence capabilities and generated 30 haematology parameters. These included all standard blood count parameters for red cells, white cells and platelets and incorporated a spectrum of reticulocyte parameters (absolute and proportional counts, immature reticulocyte fraction, high, medium and low fluorescence ratios)[18]. Following a brief evaluation, it was decided that the XT-2000*i* could be used in our programme of health checks organised by the UCI for the measurement of the Hgb concentration, the Hct and the Ret count (%). Not only were the results excellent, but above all, the analyser was faster (approximately 80 specimens per hour instead of 45). The analyser weighed less than the AcT-8 and R-500 together, thus reducing transportation costs, and only a single set of reagents was required. Finally the XT-2000*i* possesses user-friendly software capable of providing follow-up for patients and controls. Some interesting parameters, e.g. RET-Y, RBC-Y, can easily be displayed on the service screen of the instrument and provide information on real time bone marrow activity and the presence of functional iron deficiency.

SELECTION OF DECISION POINTS / PRECISION AT DECISION POINTS

At the time the 50 % Hct cut off limit was introduced, it corresponded approximately to the mean Hct value of a normal population plus two standard deviations. This limit was much too high to identify those athletes abusing rHuEpo, because in the meantime, they were injecting themselves with isotonic saline, physiogel and albumin prior to the blood controls. Our experience with regular rHuEpo injections on healthy volunteers showed us that 47 % was a reasonable cut off limit to select the most suspicious athletes following long-term hormonal treatment. In order to detect the same athletes right at the beginning of rHuEpo treatment, a Ret count (%) cut off limit was established at 2.4 %, because it was well above the mean Ret count (%), and especially it covered the period prior to the increase in the Hct level[15]. We therefore recommended that the federations introduce blood

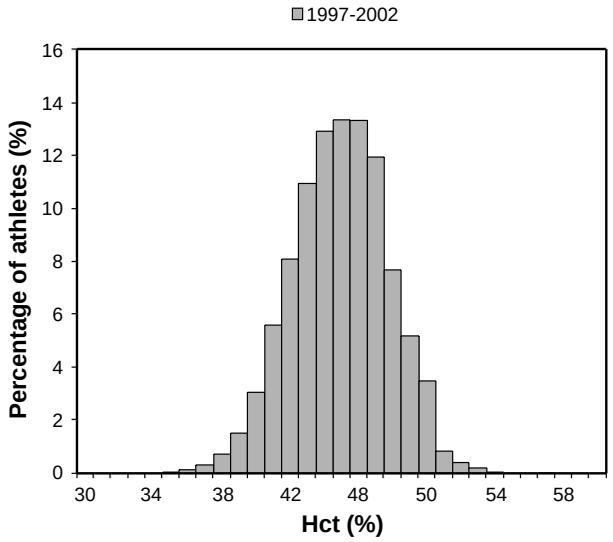

Fig. 1 *Haematocrit distribution of all cyclists tested by the UCI from 1997 until 2002 (n=9789, mean=44.5, SD=2.8)* [8)]

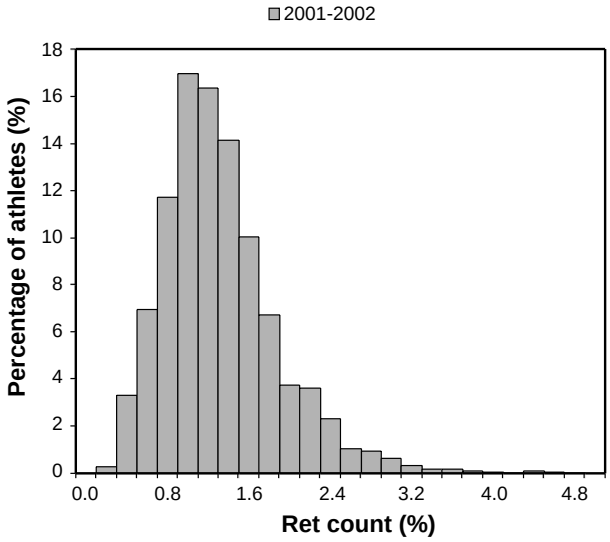

Fig. 2 *Reticulocyte distribution of all cyclists tested by the UCI from 2001 until 2002 (n=3524, mean=1.2, SD=0.6)*

screening tests to identify the most suspicious athletes, i.e., those individuals having either a Hct (%) close to 47 % (mean Hct % +/- 1 SD) ***(Fig. 1)*** or a Ret count (%) close to 2.4 % (mean Ret (%) +/- 2 SD) ***(Fig. 2)*** or other abnormalities of the haematological profile (biological follow up).

Indeed, it has been demonstrated that a single blood analysis cannot necessarily identify an athlete abusing rHuEpo. On the other hand, follow up of the Hct/Hgb and the Ret count (%) levels can give a much better indication when drug manipulation has occurred. This policy has notably enabled some federations to identify athletes abusing bone marrow stimulators such as rHuEPO or Aranesp® (Amgen Inc., CA, USA) .

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