



A global strategy for prevention and detection of blood doping with erythropoietin and related drugs

The definition blood doping was introduced by the media in the 1970s to describe the use of blood transfusion to increase red cell mass artificially, and in turn to enhance both maximal oxygen uptake and performance in endurance sports. Since the late 1980s blood doping is no longer achieved by autologous transfusion but instead through administration of recombinant human erythropoietin (rHuEpo).

Magnitude of blood doping and prospects for the next years

Clearly we cannot adopt the principles of evidence-based medicine in examining this issue, simply because there is no conventional data source. Most of the available information derives from articles in newspapers and, more recently, from police investigations. According to data collected by CONI (*Comitato Olimpico Nazionale Italiano*), [*Italian National Olympic Committee*],¹ blood doping with rHuEpo is particularly common in professional cycling and cross-country skiing. This is largely expected since these are typical endurance sports. Nevertheless, several observations by CONI indicate that the abuse of rHuEpo is extending to other disciplines. It is believed, in fact, that blood doping can help reduce physiologic strain during exercise and accelerate recovery after training. It should also be noted that blood doping is no longer a problem restricted to career athletes, since it now involves also amateurs and young athletes.

Prospects for the next years are discouraging. In fact, the major pharmaceutical companies are currently developing long-acting, modified Epo molecules. One weekly injection of a long-acting stimulator of erythropoiesis would be the ideal procedure for dishonest athletes.

Medical risks of blood doping with rHuEpo and vital importance of this drug for thousands of patients

There is speculation that blood doping with rHuEpo may be involved in the death of professional cyclists from the Netherlands in the early 1990s.² At that time, rHuEpo abuse was largely uncontrolled and Hct values in excess of 60% were presumably achieved. These polycythemic conditions compounded by dehydration during exercise readily predisposed athletes to thromboembolic complications. Nowadays rHuEpo abuse is undoubtedly more finely tuned. However, the medical risks associated with blood doping are still considerable.

Erythropoietin markedly enhances endothelial acti-

vation and platelet reactivity in humans,³ and these may substantially increase the risk of thromboembolic complications especially in individuals with a genetic predisposition to thrombophilia.⁴ Although a minority of athletes abusing rHuEpo will eventually develop a thromboembolic disease, the unlucky ones might die because of this, or experience serious handicaps for the rest of their life. Administration of rHuEpo also involves an increase in the systolic blood pressure during submaximal exercise.⁵ A large portion of the professional cyclists whose data have been examined in recent investigations by Italian magistrates show a degree of iron overload comparable to that of patients with genetic hemochromatosis, with ferritin levels often in excess of 1,000 ng/mL. These individuals were clearly given intravenous iron together with rHuEpo. Although intravenous iron is primarily taken up by the reticuloendothelial cells, it is later redistributed to parenchymal cells. Therefore, this type of iron overload will eventually produce organ damage comparable to that occurring in genetic hemochromatosis, including the risk of developing hepatic carcinoma. Finally, preliminary observations¹ suggest that the abuse of rHuEpo might involve a risk of post-treatment blunted endogenous erythropoietin production, including severe anemia. In particular, these individuals would be unable to develop an adequate erythropoietic response to stress conditions. More generally, we still do not know the effects of long-term treatment with hematopoietic growth factors, but observations in animals suggest that there may be a risk of development of myeloproliferative disorders.

Over 500,000 patients throughout the world are now receiving rHuEpo for the treatment of anemia of renal failure and deriving great benefit from such treatment in terms of both quality of life and prolongation of survival. Interestingly, the potential adverse effects on normal hemostasis are useful in renal patients to prevent hemorrhagic complications.³ In the last years, in addition, rHuEpo has been approved for other indications,⁶ including prevention of anemia in surgical patients or in patients undergoing platinum-based chemotherapy, treatment of anemia of prematurity, of anemia induced by zidovudine therapy in HIV-infected patients, and of anemia induced by chemotherapy for non-myeloid malignancies. One of the many adverse effects of rHuEpo abuse is that a vitally important drug – that can prolong survival of thousands of patients – is nowadays reported by the media as a doping drug. It must be clearly stated that, although blood doping is very common, the large majority of rHuEpo preparations are used for patients who benefit from them. Misinformation may be no less harmful than doping itself. It must also be clearly stated, however, that pharmaceutical companies, just because of the vital importance of rHuEpo for so many patients, should be more active in adopting measures to discourage erythropoietin abuse in sport.

What is currently being done to prevent blood doping

The use of rHuEpo is officially prohibited by the *International Olympic Committee* (IOC) and other major sporting organizations. In 1989, the IOC Medical Commission introduced the new doping class of peptide hormones and analogues, which includes rHuEpo, human chorionic gonadotrophin and related compounds, adrenocorticotrophic hormone, human growth hormone, and all the releasing factors of these hormones.

However, there is no reliable method for routinely detecting doping with rHuEpo at present since the recombinant molecule cannot be readily differentiated from the endogenous hormone. Even if reliable tests could be developed quickly, rHuEpo will be replaced by modified molecules in the next years, so that current efforts might become useless.

To dissuade the abuse of rHuEpo, some sports have imposed upper limits on hematocrit and hemoglobin [50% and 17 g/dL, respectively, in males, *International Cycling Union* (UCI)] or hemoglobin [18.5 g/dL in males, *International Ski Federation* (FIS)]. This strategy has many pitfalls, which have been extensively discussed elsewhere and include: large natural variation between individuals, postural effects on hematocrit, risk of false positivity and ease of manipulation through interventions such as saline infusion. In particular the upper limit of 18.5 g/dL adopted by the FIS is difficult to understand: in a prospective study that we are conducting on elite soccer players in Italy, no Hb level greater than 17 g/dL has so far been observed.

In my opinion, the adoption of upper limits might paradoxically generate more blood doping. In fact, it induces cleverly manipulated uses of rHuEpo with the aim of approaching the target hematocrit or hemoglobin without exceeding it.¹ This is the only explanation I can provide for the elevated – although below 50% – hematocrit values frequently found in some professional endurance sport athletes. One should consider, in fact, that hematocrit levels greater than 47% are found in only 1-2 out of 100 elite soccer players (see below *The hematologic passport*). Marginally elevated levels are even more suspicious if the same individuals show considerable lower values during non-competition periods: the reverse, in fact, should be expected. A not negligible adverse effect of adopting upper limits is that they generate aberrant beliefs in athletes: doping is no longer taking rHuEpo but instead having hematocrit levels greater than the upper limit. In other words, abusing rHuEpo and having hematocrit values below 50% is felt by some athletes as a fully normal behavior.

Indirect methods for detecting erythropoietin abuse in athletes

In the last few years, a number of studies have investigated indirect methods to detect of rHuEpo abuse by means of parameters indicative of accelerated erythropoiesis. In this issue of *Haematologica*, Parisotto and coworkers⁷ describe a novel method for the detection of rHuEpo abuse in athletes utilizing markers of altered erythropoiesis.⁸ A number of parameters proved to be closely related to rHuEpo administration: Hct, reticulocyte Hct, percentage of macrocytes,

soluble transferrin receptor and serum erythropoietin. It should be noted that these parameters can be determined automatically in a few minutes, so that this approach could be adopted to test athletes before a race. The above parameters were then utilized to develop mathematical models aimed at discriminating between athletes given rHuEpo and those given placebo. Interestingly the ON-model repeatedly identified 94-100% of rHuEpo group members during the final 2 weeks of the rHuEpo administration phase (one false positive from a possible 189), while the OFF-model repeatedly identified 67-72% of recent users with no false positives.

The many sophists who have a seat in sports organizations state that the use of indirect methods for detecting rHuEpo abuse is not formally correct. I invite them to reflect on the following points: a) rHuEpo is officially prohibited; b) rHuEpo cannot be analytically detected; c) indirect methods are not allowed. If point c) is true, the only conclusion is that point a) is no longer true in reality. As hematologists we have defined criteria and pathways for differential diagnosis of erythrocytosis.⁹ Based on these algorithms we routinely make a diagnosis of polycythemia vera (PV), in spite of the fact that we still lack a marker of this myeloproliferative disorder.^{10,11} Diagnosing PV involves a lot of medical responsibility¹² but an indirect diagnostic method is nevertheless accepted by the scientific community. Why should we not be allowed to diagnose *erythrocytosis due to previous administration of rHuEpo or related drugs* using a rational approach?

The indirect method reported in this issue of *Haematologica* can be further improved and the Australian researchers are currently conducting several studies in Australia and worldwide. Clearly, the first objective is to abolish false positives as far as is possible. The refined approach, however, should be part of global strategy for preventing and detecting blood doping. Such a strategy must include definition of the individual hematologic profile, the so-called hematologic passport.

The hematologic passport

CONI and FIGC (*Federazione Italiana Giuoco Calcio*) [*Italian Soccer Federation*] have recently launched a campaign called *lo non rischio la salute!* [*I take care of my health!*]. This involves regular hematologic investigations (2-3 times a year) including blood counts, reticulocyte count, serum ferritin and soluble transferrin receptor measurements. These determinations must be performed by laboratories participating in quality control programs to keep analytical errors as low as possible. Sequential evaluation of the above parameters allows definition of the individual hematological profile.

A top-level Italian soccer team has undergone such sequential studies over the last two years. Hematocrit values ranged from 37.2% to 47.3% (with less than 2% of values being $\geq 47\%$), and hemoglobin levels ranged from 12.8 to 16.5 g/dL. Interestingly, mild-field players showed the lowest hematocrit values (37-40%) and hemoglobin (between 13 and 14 g/dL), confirming that the so-called sports anemia is a spurious anemia, caused by an expanded plasma volume that dilutes red blood cells as a beneficial adaptation to aerobic exer-

cise. Considering blood cell counts associated with normal values for reticulocytes, serum ferritin and soluble transferrin receptor, the within-subject biological coefficients of variation ranged from 1.2% to 4.7% for hematocrit, and from 1.6% to 5.0% for hemoglobin. This study, which will be reported elsewhere, indicates that it is possible to define the individual hematologic profile: a physiologic individual range for hematocrit and hemoglobin can be defined as the mean value \pm 10% of the mean. We can discuss for years about the best size of variation (5%, 7.5%, 10%): a major factor of variability here is produced by between-laboratory differences, and the figure of 10% represents a better guarantee for the athlete at present.

The CONI campaign *I take care of my health!* not only involves regular hematologic evaluations performed by athletes themselves, but also random controls decided by the central authority. Increases in hematocrit and hemoglobin $>$ 10% of the mean value are considered potentially harmful to the athlete's health. For instance, if the mean hematocrit value (calculated on previous sequential determinations) is 42% and the present value is 47%, the increase is equal to 11.9% and therefore non-physiologic. Any athlete showing one of the following: a) increases in both hematocrit and hemoglobin $>$ 10%; b) an increase in hematocrit or in hemoglobin $>$ 10% plus an abnormal reticulocyte count, or serum ferritin, or soluble transferrin receptor levels, is stopped to prevent damage to his/her health. He or she will start to compete again once hematologic values return within normal ranges.

We are fully aware that the approach of the CONI campaign *I take care of my health!* is not an anti-doping procedure, for several reasons, but mainly because this campaign has been designed primarily to take care of the athletes' health. For instance, the inclusion of serum ferritin within the laboratory battery has already allowed us to identify several individuals with genetic hemochromatosis.

Conclusions

As physicians, one of our major duties is to prevent diseases, and we have sworn this with the Hippocratic Oath. Since blood doping exposes athletes to several medical risks, we must be against blood doping, and more generally against any form of doping. Blood doping is not an abstract, intellectual challenge on how to circumvent sports regulations licitly, but a betrayal of the Hippocratic Oath for the physicians who are involved in it. Sport is intended to improve people's health, doping worsens it.

As hematologists, over the next years we could face problems related to blood doping with increasing frequency: atypical cases of iron overload, erythrocytosis of unknown origin, unexplained anemias, atypical thromboembolic complications, and so on.

Those of us who are involved in sports medicine have a hard task trying to prevent and detect blood doping. There is no question that hematologists must play a central role in this task force. To reach the objective, a global strategy will be required. Although indirect methods for the detection of rHuEpo abuse will be

refined, this approach will likely be insufficient by itself. A careful definition of one individual's hematologic profile should form the basis for a more successful application of any indirect method. Introducing into a powerful predictive model a comparison with basal hematologic data will improve the method's sensitivity and reduce the risk of false positivity. Should ongoing studies in French laboratories provide us with a test that readily differentiates between exogenous and endogenous Epo, also this test could become part of a global strategy to prevent and detect blood doping.

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Professor Mario Cazzola is a member of the FIGC [Italian Soccer Federation] Scientific Committee. In the last two years he has been collaborating with CONI in the developing the campaign *I take care of my health!* and is currently following this campaign and conducting studies to improve it further. On behalf of the FIGC, Professor Mario Cazzola has collaborated with major Italian soccer teams by analyzing the hematologic data of their players in order to define the concept of the individual hematologic profile.

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