Original article

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Haematological and biochemical parameters from all professional cyclists during the Tour de Suisse 1999

Summary

Since 1997, the International Cycling Union (ICU) and the Institut Universitaire de Médecine Légale de Lausanne (IUML) have undertaken a medical program to prevent professional cyclists from abusing of recombinant erythropoietin (rhEPO). This program is based on the measurement of the haematocrit only. During several races organised by the ICU, blood samples are randomly collected from the sportsmen in the morning before breakfast and exercise. Then, the haematocrit is analysed online and all values exceeding 50% imply that the cyclists have to stop racing for a minimum period of two weeks. Cyclists with a physiological haematocrit above 50% can have a certificate delivered by the ICU. During the 1999 Tour de Suisse (TDS) in Lausanne, all cyclists (n = 146) had to give a blood sample before the start of a single stage. The haematocrits and other parameters related to the erythropoiesis, the iron metabolism and the liver function were measured. The parameters observed during this major race («Hors Classe»), just a fortnight before the Tour de France were the following: 5 cyclists had a haematocrit exceeding 50%, but one of them had a certificate. A narrow haematocrit distribution was observed in some teams and more than 50% of the cyclists had ferritin levels above 300 ng/ml. Most of the ferritin levels were also related to the teams. 5 men had an EPO level above the reference interval established by the manufacturer.The main potential secondary marker mentioned in the literature indicating an abuse of rhEPO, the sTFR (soluble transferrin receptor), was not out of range. On the other hand, the reticulocyte indices (absolute count, percentage, IRF) showed a large inter and intra team variability. These high or low values could be normal, due to some pathologies or some manipulations of the erythropoiesis thanks to high altitude training, hypobaric chamber sessions or rhEPO treatments. Without a proper medical follow up or at least a second blood test taken a few days later, it is difficult or even impossible to determine whether someone has manipulated his haematopoietic activity.

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Introduction

Cycling is an endurance sport, where oxygen transport plays an essential role. The possibility of increasing the haematocrit for a better oxygenation of the tissues and organs thanks to the use of auto blood transfusions or rhEPO injections provides noticeable advantages on the performances. Such artificial means have been prohibited by the International Olympic Committee (IOC). Unfortunately, at this time, there is no existing direct way of detecting an abuse of rhEPO [1,2], a drug widely used in endurance sports such as cycling (see Tour de France 1998).

In 1997, on the first day of the race Paris–Nice, the ICU decided to introduce for health and security reasons, a randomised blood check up to prevent sportsmen from having a too high haematocrit level [3]. The measurements were under the responsibility of our institute and that morning, two cyclists exceeded the limit introduced by the ICU. The cyclists had to stop riding for a minimum period of two weeks. Then, after a check up of their haematological parameters, they got their license back, because their haematocrit was below 50%. From then onwards, 4210 blood samples were analysed during different cyclist events and just above 1% of the runners were not allowed to take part to the departure. Since 1999, an important decision was taken by the ICU; the cyclists were not allowed to destroy their blood sample after the haematocrit analysis. Instead, the samples were brought back to the laboratory for further analyses such as ferritin, reticulocytes, total proteins and other relevant parameters. All these investigations could be undertaken only after ICU approval.

The reticulocyte count/percentage is thought to be a possible secondary marker of a manipulation of the erythropoiesis [4, 5, 6, 7]. The upper values can be eventually due to some diseases [8], an abuse of rhEPO or a high altitude training [9, 10, 11]. On the other hand, low ones can come from some diseases (red cell aplasia or disorders of maturation) or from a down regulation after an interruption of a treatment with rhEPO. For these reasons, the ICU has established for the year 2000 medical follow up, two cut off limits. For the time being no value out of range will cause any sanctions, but in case of a percentage of reticulocytes lower than 0.4% or higher than 2.4%, the cyclists will have to undertake complementary analyses in order to understand the origin of the unusual value.

The measurement of EPO in plasma is interesting and necessary to establish a normal range for each person [12]. In this respect, some people have high values, because they «suffer» from a hyper-secretion of EPO [13]. Others have low concentrations, because their EPO receptors are sensitive enough and are able to give a proper answer [14]. The analyses and specially the interpretation of hormones such as EPO have to be done on blood samples collected in the morning before breakfast for a better standardisation (specially to avoid any circadian rhythm effect [3]). Once the pre-analytic conditions have been established, it is necessary to take into consideration the history of the athlete; has he been in the

mountains at moderate or high altitude [15], has he recently taken an aircraft [16], has he done not long ago, a strenuous effort in anaerobic conditions or has he spent a few hours under hypobaric conditions [17, 18]? All these parameters can/could modify the concentration of EPO measured in plasma.

Regarding to the literature [19, 20] the follow up of the sTFR (soluble transferrin receptor) is one of the most valuable indirect ways to determine a modification of the erythropoiesis due to an intake of rhEPO. Unfortunately, this parameter is highly dependent on the iron availability [21] and can also be modified by the same factors as mentioned for EPO.

The ICU has been concerned by the abuse of iron intake for a couple of years. The medical follow up established for the year 2000 mentions that cyclists must stop taking iron in case of values exceeding 300 ng/ml. Whenever a cyclist has twice consecutively more than 500 ng/ml of ferritin, he must go and see a specialist to be sure that he does not suffer from a hereditary hemochromatosis.

We described here some of the haematological and biochemical parameters found on all cyclists taking part in the Tour de Suisse 1999. The reference intervals were those given by the literature, the manufacturer of the immunological tests, or the ICU's medical follow up for the year 2000. Our objective was to put forward a relation between these parameters in order to find out whether some cyclists were manipulating their haematocrit thanks to rhEPO injections.

Subjects and Methods

All cyclists taking part in the Tour de Suisse (TDS) 1999 were tested on the third day of the race in Lausanne after a transition stage (profile of the stage given in *Figure 1*). According to the

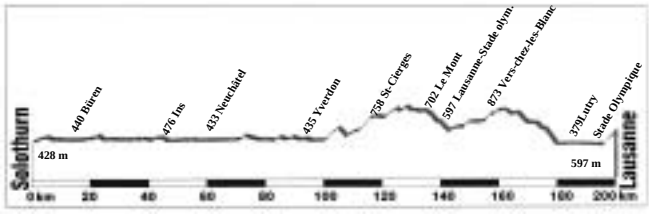

Figure 1: Profile of the race on the second day of the Tour de Suisse 1999, one day before the blood test. No real difficulties such as high or moderate altitude mountains, bad weather conditions (maximum temperature 24 °C, around 520 minutes of sun and no precipitation) were present on that day and professional sportsmen should not be exhausted and dehydrated on the day of the blood testing.

ICU's rules in 1999, the sportsmen were woken up 15 minutes before the blood testing. The riders were on an empty stomach (even if a cyclist had already eaten something, it does not seem to modify the haematocrit level [22]) and had to be in a seated position; a tourniquet was settled and 4 ml of EDTA blood (Sarstedt) was collected by a physician within less than 45 seconds. Then, the blood samples were put into a cool box (around 4 °C) and were transported to a hotel where the analyses were undertaken. Before analyses, all the samples were rolled for a good homogenisation and temperature stabilisation during a minimum period of 15 minutes. The analyses were performed with a Coulter Counter of the A^c•T Serie analyser (Beckman Coulter Diagnostics Division, Nyon, Switzerland). It was previously calibrated as mentioned in the maintenance guide (Guide de maintenance, PN 4237347). All blood samples were measured twice. If one or both values was below 50%, the rider could take part in the race. If both values were above 50%, the cyclist or a representative of his team could come and assist to three other measurements. Then, if all values were above 50% and the average was above 51% (1% of tolerance due to technical «hazard») the cyclist did not get his license back and could not take part in the race.

Once the haematocrit analyses were done and the results validated, the blood samples were transported to the laboratory for further investigations. The samples were rolled again for 15 minutes, and the measurement of the reticulocytes including the IRF (Immature Reticulocyte Fraction), the red and white blood cell populations were run with a Cell-Dyn 4000 (Abbott Diagnostics Division, Baar, Switzerland). Then the tubes were centrifuged and the plasma was aliquoted into three tubes. Some parameters could not be measured due to analytical problems or sample shortage. One tube was used directly for the determination of some chemical and biochemical parameters. They were measured by standard methods at the main hospital in Lausanne, the Centre Hospitalier Universitaire Vaudois (CHUV). The following parameters were obtained from Roche Diagnostics (Rotkreuz, Switzerland) and run on Hitachi 717 and Hitachi 917 according to the manufacturer's instructions. Albumine BCG (bromo-cresol-green), bilirubine direct (method Jendrassik), total bilirubine (methode DPD, dichloro-2,5-benzene diazonium), creatinine (method Jaffe kinetic), ferritin (Tina-quant, immunoturbidimetry), cholesterol CHOP-PAP, proteins (Biuret), urea (UV kinetic). Both parameters, ALAT and ASAT were obtained from BioMérieux (Lyon, France) (Enzyline standardized 50 IFCC/SFBC, BioMérieux, France) and CRP came from Dako (Glostrup, France). The other two aliquots were put into a deep-freeze (–20 °C) to prevent a possible degradation of the sTFR and EPO. Both parameters were analysed all at the same time, a couple of months later, with fully automated chemiluminescence assays. EPO's test was obtained from DPC (Bühlmann Laboratories, Schönenbuch, Switzerland) and was run on an Immulite and sTFR's test came from Nichols Institute Diagnostics (Allschwil, Switzerland) and was run on an Advantage.

Results

Haematocrit

The haematocrit measurements done in Lausanne during the TDS 1999 were close to expected values in a healthy male population *(Fig. 2)*. The average was 46% (n = 146, SD ± 2.4) with a minimum

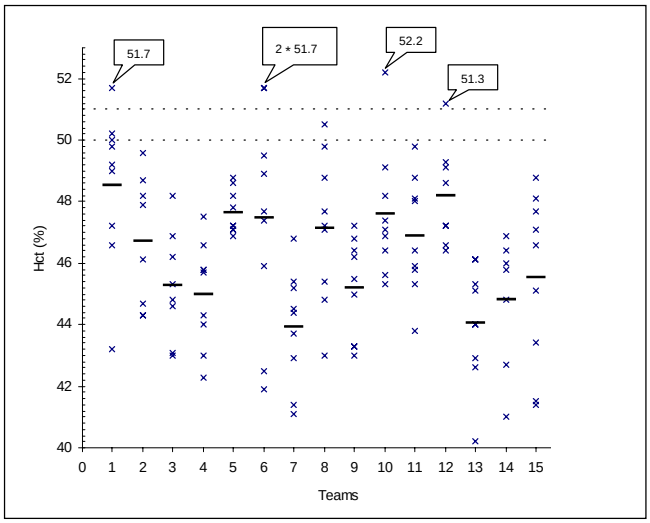

Figure 2: Haematocrit values of all cyclists taking part in the TDS on June 17th 1999 in Lausanne. The blood samples were collected before breakfast and exercise and were measured with a Coulter Counter (A^c•T Serie analyser). The cut off limit established by the ICU is 50%, but all men having a haematocrit between both horizontal lines could take part to the race, because 1% was deducted to the measured value due to technical hazard.

value of 40.2% and a maximum value of 52.2%. Five men belonging to four different teams (team 1, 2 x 6, 10 and 12) were above 50% (+ 1% due to technical «hazard»), the cut off limit established by the ICU. One cyclist had a haematocrit certificate (team 1, normal haematocrit above 50%) and could then take part in the race; the four others did not get their license back and had to stop riding for a minimum period of two weeks.

Reticulocytes

The average reticulocyte percentage was 1.3% (n = 146, SD ± 0.6) *(Fig. 3)*. 8 values were above the upper limit established by the

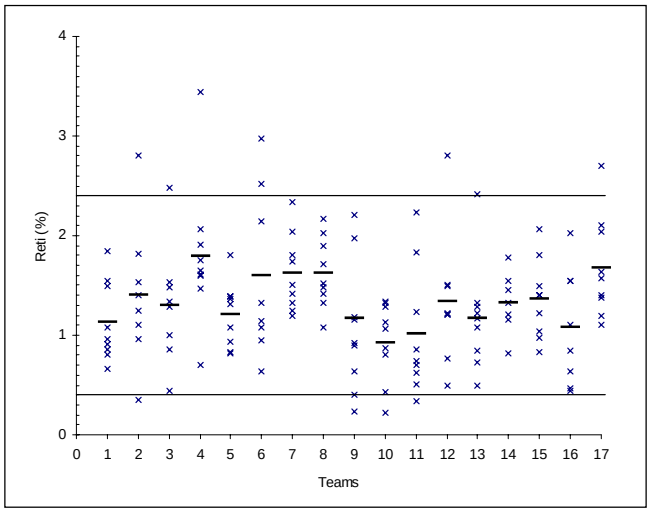

Figure 3: The reticulocytes were measured with a Cell-Dyn 4000 (Abbott) at the laboratory (IUML) within less than eight hours, once the blood was collected. Both horizontal lines are the cut off limits of 0.4 and 2.4% established by the ICU for the year 2000 medical follow up.

ICU, and 4 were below the lower limit of 0.4% (team 2, 9, 10 and 11). 3 cyclists had values very close to 2.4% (team 3, 6 and 13) and 5 were above 2.6% (team 2, 4, 6, 12 and 17). One was even above 3.4% (team 4).

EPO

The normal range for EPO was given by the manufacturer, respectively 1.6 and 34 mIU/ml. The normal range was determined in serum, but the blood collecting organised by the ICU was done with EDTA tubes. A correlation between serum and EDTA values is documented in the manual guide, but due to the small total number of samples (22 samples), we did our own with 43 samples (data not shown). The strong correlation allowed us to correct the EDTA values into serum values.

The average of EPO was 14.3 mIU/ml (n = 136, SD ± 9.7) *(Fig. 4)*. 5 men (team 7, 10, 2 x 15, 17) were above the upper limit (34 mIU/ml) but none were under the lower limit (1.6 mIU/ml).

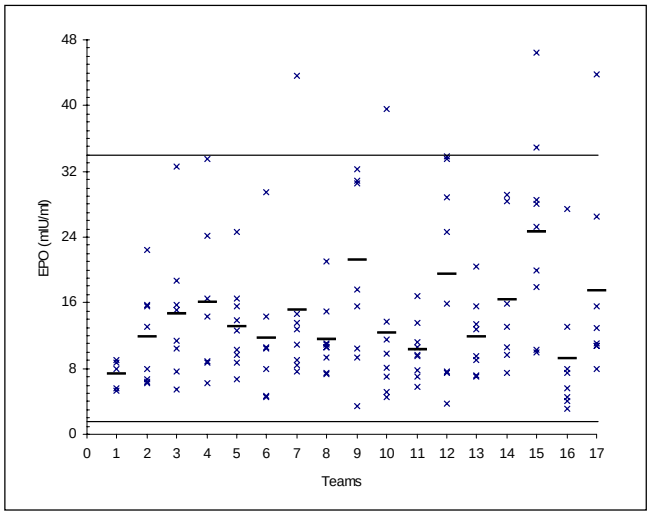

Figure 4: EPO was measured with a chemiluminescence test from DPC (immulite) on unfrozen EDTA samples. Both horizontal lines are the 95 percentile reference intervals for healthy individuals.

sTFR

The normal range for the sTFR depends on the method used and hardly no standardisation of the methods are available, because very few laboratories have purified the protein and know its exact weight (according to the manufacturers, the conversion factor from nmol/l into mg/l is 3.4). So for the time being, the normal range established by the manufacturer and the literature will be used [23].

A correlation between EDTA and serum values was done in our laboratory thanks to 47 samples (data not shown) and allowed us to correct the values of the TDS.

The average of sTFR was 19.1 nmol/l (n = 113, SD ± 5.2) with a maximum of 35.7 nmol/l (team 6) and a minimum of 9.6 nmol/l (team 9) *(Fig. 5)*. 7 cyclists were just above the upper limit

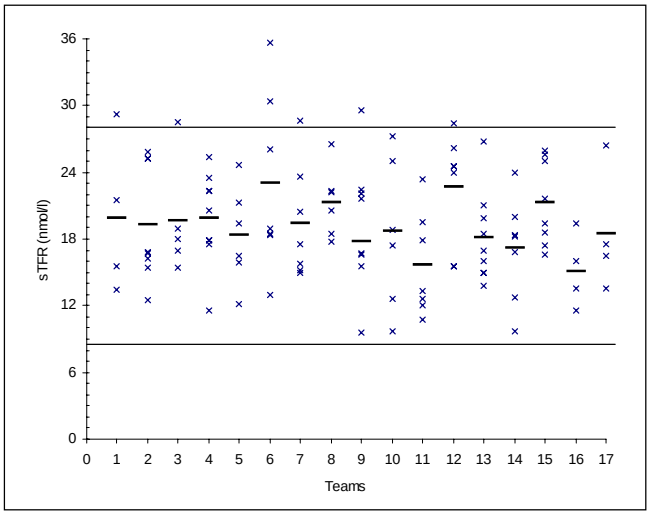

Figure 5: sTFR was measured with a chemiluminescence test from Nichols (Advantage) on unfrozen EDTA samples. Both horizontal lines are the 95% reference intervals established by the manufacturer for non-African Americans living at low altitude.

(28.1 nmol/l for non-African Americans living at low altitude), but none were below the lower limit (8.8 nmol/l for non-African Americans living at low altitude).

Ferritin

7 sportsmen had values above 1000 ng/ml *(Fig. 6)*. The average for ferritin was 406.4 ng/ml (n = 145, SD ± 250.2) with a maximum of

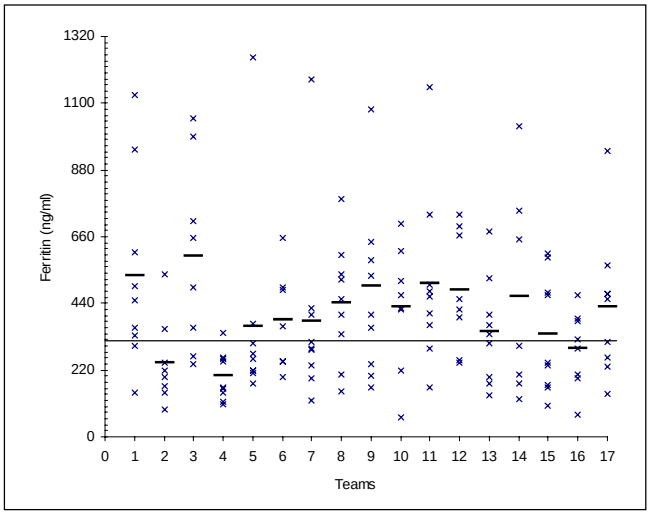

Figure 6: Ferritin was measured with a immunoturbidimetry test from Roche on EDTA samples the day after the blood collecting. The horizontal line is the cut off limit of 300 ng/ml established by the ICU for the year 2000 medical follow up.

1252 ng/ml and a minimum of 63 ng/ml. 57.9% of the cyclists were above 300 ng/ml (one of the cut off limits established by the ICU indicating that the cyclist has to stop taking iron).

Discussion

The medical program concerning the measurement of the haematocrit before cycling races is critical, but for the time being, it is still the unique way to prevent sportsmen from abusing of rhEPO. At the beginning of this program established by the ICU, many doctors and sportsmen tried to stop these blood analyses. Their arguments were mainly based on the arbitrary cut off limit of 50% (normal distribution of the haematocrit in healthy men with a 95% confidence interval: 39–55% [24]) except for men owning a certificate delivered by the ICU, the possibility for some cyclists to increase their haematocrit up to 50%, or even the establishment of the ideal haematocrit level. Indeed, above 50%, the blood viscosity is such, that it counterbalances the benefits of an increase in the number of red blood cells [25]. Some sportsmen also disagreed with the measurement of the haematocrit, because it is not an anti doping test. Indeed, high values do not necessarily indicate an abuse of rhEPO, but could come from a high altitude training or a hypobaric session and these training practices a not banned by the ICU or the IOC (International Olympic Committee). Nowadays, other sport federations (International Skating Union, Modern Pentathlon) agree with this health and security program and want/have to introduce(d) such measurements.

Knowing the limits of the haematocrit measurements, the ICU decided to improve its fight against doping by doing not only urine analyses but also other complementary analyses on blood samples collected during the races. Some of them concern the verification of a possible manipulation of the haematocrit via a hemodilution or injections of plasma expanders in order to increase the plasma volume [26, 27]. Others are done in order to look for possible secondary markers highly expressed after an intake of rhEPO. These parameters must be sufficiently specific so that it is possible to discriminate people under rhEPO from healthy men.

Haematocrit measurements necessitates EDTA tubes and this matrix can be problematical for other complementary analyses. Indeed, most of the hormones, peptides and enzymes have to be measured in serum or heparinazed tubes. Some parameters cannot be analysed on EDTA tubes, or little is known on the normal range. So, a hard work has to be done in the laboratories to standardise the methods and analytical procedures. Perhaps in a close future, during the blood testing, a second tube with another matrix (serum, heparinate) will be necessary for complementary analyses.

The establishment of cut off limits for some parameters such as hormones, peptides must take into consideration the hydration state (plasma volume) of the sportsmen. Unfortunately, for the time being, standardisation is not well documented by the manufacturers of the diagnostic kits or by scientific publications.

Looking at the haematocrit distribution, there were some noticeable differences. The cyclists belonging to the team 5 had an unusual haematocrit distribution; all values were contained between 46.9 and 48.8% (mean 47.7%) whereas the other teams had a much wider distribution. This could come from a good control of the haematocrit levels thanks to a proper hydration after the race. In some cases, some cyclists could have possibly injected themselves some physiological water (NaCl 0.9%) in order to reequilibrate their body and stabilise their too high haematocrit a couple of percentages below the cut off limit of 50%.

One cyclist had a high reticulocyte percentage (> 3.4%) and from our knowledge and from the literature, very few people have such odd reticulocyte percentages without suffering from some kind of disease (haemolytic anaemia, iron deficiency, megaloblastic anaemia, red cell aplasia,…). The understanding of odd values are essential and the advise of a specialist and a medical follow up would be necessary to find out the origin of these abnormal values. The possibility of measuring the IRF (Immature Reticulocyte Fraction), the youngest of reticulocytes, could provide a more sensitive tool to detect a recent stimulation of the erythropoiesis, but this parameter did not give significant results (data not shown).

In 1991, Berglund and Ekblom [28] did not notice any changes within the reticulocyte percentage, before and after a rhEPO treatment, whereas others did so [29]. Taking into consideration the known results, it could not be excluded that after a certain while, the reticulocyte percentage goes back to normal even under a treatment with rhEPO. In this case, if a sportsman has a continuous treatment with a low dosage of this hormone, a normal percentage of reticulocytes could be found after a while. When looking at *table 1*, no relation could be established between the haematocrit level and the reticulocyte percentage. Some men had a high haematocrit with a low reticulocyte percentage whereas others had a high haematocrit with a reticulocyte percentage above normal.

The few EPO values above the normal range *(table 1)* could come from a slight hypoxia caused by the use of a hypobaric chamber or a strenuous effort in anaerobic conditions. The intake of rhEPO also increases its concentration in plasma, but the values observed were much too close to the normal range in order to be able to say that there was an exogenous intake of the hormone.

Team	Hct (%)	Reti (%)	Ferritin (ng/ml)	EPO (mIU/ml)	sTFR (nmol/l)
1	51.7	0.97	361	9.0	21.5
1	49.8	0.67	1126	NA	29.2
2	44.7	0.35	355	6.5	12.5
2	44.3	2.80	218	8.0	25.2
3	44.8	2.48	1051	15.2	28.6
4	45.8	3.44	160	NA	22.3
6	41.9	2.52	248	29.5	19.0
6	51.7	1.14	250	8.0	26.1
6	42.5	0.24	240	30.5	29.5
6	47.4	0.63	483	10.6	30.4
6	48.9	2.97	196	10.5	35.7
6	51.7	1.08	NA	NA	NA
7	42.9	1.81	312	43.7	15.2
7	41.4	1.42	1178	14.6	28.7
10	46.4	0.43	515	39.6	18.8
10	52.2	1.13	63	5.1	27.2
10	48.2	0.22	704	8.2	25.0
11	45.9	0.34	478	5.7	NA
12	51.3	0.77	244	7.5	26.2
12	49.3	2.81	735	33.4	28.4
13	44.0	2.41	678	20.4	26.8
15	41.4	1.40	165	46.5	18.6
15	45.1	2.07	171	34.9	25.5
17	45.9	2.70	455	43.8	26.4

Table 1: Comparison of some parameters involved in the erythropoiesis. At least one of them (Hct, Reti, sTFR or EPO) was out of range except for ferritin. Some parameters could not be analysed due to analytical problems or sample shortage (—).

The cyclists who had a concentration of EPO above the upper limit established by the manufacturer had a tendency of having fairly low haematocrits, and the lower concentrations of EPO were more or less related to high haematocrit levels. But no proper relation could be established between EPO and the haematocrit levels.

High ferritin values have already been measured in 1996 during the Tour de Suisse (data not published). At that time the cyclists were warned that too much iron intake (specially IV injections) could cause irreversible liver damage [30]. Three years later, very little has changed. The ferritin analyses were done together with the C-reactive protein (CRP), so that high ferritin levels could not only be associated to an inflammatory disease. We noticed that ferritin levels were more or less related to some cycling teams. Indeed, some doctors seemed to prescribe either intravenous or oral iron treatments [31, 32]. The iron overload due to regular parenteral administrations could be discovered by measuring the ferritin. The cyclists belonging to the teams 4 and 5 did not seem to exaggerate with iron intake, because their values were below

430 ng/ml, except for one man (team 5, ferritin = 1252 ng/ml, CRP = 0). This man might have just changed teams and could come from a team where periodic intravenous injections took place.

None of the cyclists had sTFR values well above normal indicating a possible abuse of rhEPO, but most of them had values close to the upper limit established by the manufacturer. The cyclists could not suffer from a lack of iron as illustrated by their ferritin levels *(table 1).* The slight functional iron deficiency expressed by the observed sTFR values just below the upper limit could not be explained.

Regarding the white blood cell count and white blood cell population, very few people were out of range and the analysis of the CRP, except for one cyclist (CRP = 43), confirmed that no cyclist was suffering from a sever inflammation *(table 2).* Some of

Parameter	Mean	Standard deviation	Units	Min-Max	Sample size	Laboratory Range
WBC	7.6	1.8	G/l	4.1–13.7	146	4–10
NEU %	49.9	7.8	%	31.9–76.7	146	40–7
LYM %	39.1	7.0	%	21.2–54.7	146	25–40
MONO %	8.7	2.0	%	1.8–14.9	146	2–8
EOS %	2.0	1.3	%	0.3–9.6	146	1–5
BASO %	0.3	0.2	%	0–0.7	146	0–1
ALAT	30.7	13.4	U/l	14–143	144	11–60
Albumin	47.1	1.9	g/l	42–52	144	33–53
Bilirubin (direct)	*	*	mmol/l	<10–10	144	0–10
Bilirubin (total)	**	**	mmol/l	<10–35	144	0–21
ASAT	30.4	8.8	U/l	16–82	144	14–50
Cholesterol	4.4	0.8	mmol/l	2.8–7.9	144	3.1–5.0
CRP	0.8	4.0	mg/l	0–43	144	<10
Creatinin	87.3	8.2	nmol/ml	69–122	144	44–106
γ-GT	16.1	14.1	U/l	8–165	144	11–62
Total proteins	67.7	3.2	g/l	57–77	144	61–82
Urea	7.0	1.2	mmol/l	4.3–10.1	144	2.9–7.7

* 143 men had concentrations of direct bilirubin < 10 μmol/l and one man had 10 μmol/l

** 73 men had concentrations of total bilirubin < 10 μmol/l and 69 men had concentrations of total bilirubin contained between 10 and 35 μmol/l (mean 13.9, SD ± 5.2)

Table 2: Some parameters giving an idea of the health state of the body of runners during a major cycling tour.

the parameters (albumin, total proteins) were measured in order to check whether some cyclists had roughly manipulated their haematocrit via a hemodilution (perfusion of physiological water) done just before the blood collecting. Looking at these values, no evidence of dilution of the blood could be discovered. The measurement of creatinine was done to detect important kidney problems, but the cyclists did not seem to suffer from renal dysfunction. Otherwise, the cholesterol values for some of the cyclists were just above normal range. One cyclist had hepatic enzymes (ASAT, ALAT, γ-GT) well above normal, so a proper medical follow up will facilitate to discover such abnormalities. In this particular case, the cyclist should go see a specialist for further investigations.

Conclusions

Regarding a unique value of haematocrit, reticulocyte, EPO and sTFR it is impossible to discriminate the cyclists taking part in the Tour de Suisse 1999 who manipulated their haematocrit from the others. A regular medical follow up is compulsory to establish a normal range for each person concerning the haeematocrit and these specific parameters. Very soon, it may be necessary to compare the values obtained during the medical follow up and those obtained during the races. These in and out competition blood analyses could provide useful information concerning a manipulation of the haematopoiesis. This step is not yet on the agenda, but such an approach could facilitate the work of the scientists or at least make life more difficult for the sportsmen who want to take rhEPO.

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Bibliography

1 *Wide L., Bengtsson C., Berglund B., Ekblom B.:* Detection in blood and urine of recombinant erythropoietin administrated to healthy men. Medicine & Science in Sports & Exercise, 27(11): 1569–76, 1995.

2 *Wide L., Bengtsson C.:* Molecular charge heterogeneity of human serum erythropoietin. British Journal of Haematology, 76(1): 121–7, 1990.

3 Communiqué de presse de l'Union Cycliste Internationale effectué le 28 janvier 1997 à Lausanne concernant la réunion du 27 janvier 1997.

4 *Breymann C., Bauer C., Major A., Zimmermann R., Gautschi K., Huch, A., Huch R.:* Optimal timing of repeated rh-erythropoietin administration improves its effectiveness in stimulating erythropoiesis in healthy volunteers. British Journal of Haematology, 92(2): 295–301, 1996.

5 *Casoni I., Ricci G., Ballarin E., Borsetto C., Grazzi G., Guglielmini C., Manfredini F., Mazzoni G., Patracchini M., De Paoli Vitali E., et al.:* Hematological indices of erythropoietin administration in athletes. International Journal of Sports Medicine, 14(6): 307–11, 1993.

6 *Major A., Bauer C., Breymann C., Huch A., Huch R.:* rh-erythropoietin stimulates immature reticulocyte release in man. British Journal of Haematology, 87(3): 605–8, 1994.

7 *Brugnara C., Colella G.M., Cremins J., Langley R.C. Jr., Schneider, T.J., Rutherford, C.J., Goldberg, M.A.:* Effects of subcutaneous recombinant human erythropoietin in normal subjects: development of decreased reticulocyte hemoglobin content and iron-deficient erythropoiesis. Journal of Laboratory & Clinical Medicine, 123(5): 660–7, 1994.

8 *Begui Y., Clemons G.K., Pootrakul P., Fillet G.:* Quantitative assessment of erythropoiesis and functional classification of anemia based on measurements of serum transferrin receptor and erythropoietin. Blood, 81(4): 1067–76.

9 *Boning D., Maassen N., Jochum F., Steinacker J., Halder A., Thomas A., Schmidt W., Noe G., Kubanek B.:* After-effects of a high altitude expedition on blood. International Journal of Sports Medicine, 18(3): 179–85, 1997.

10 *Klausen T., Mohr T., Ghisler U., Nielsen O.J.:* Maximal oxygen uptake and erythropoietic responses after training at moderate altitude. European Journal of Applied Physiology & Occupational Physiology, 62(5): 376–9, 1991.

11 *Brau D., Andersen I., Villiger B.:* Reticulozytenzahl als Verlaufsparameter im Höhentraining. Schweizerische Zeitschrift für «Sportmedizin und Sporttraumatologie», 47(3): 149, 1998.

12 *Breidbach A., Schänzer W.:* Individual reference ranges for serum erythropoietin (sEPO) – A possible approach to detect misuse. In Schänzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.): Proceeding of the Manfred Donike Workshop, 16th Cologne Workshop on Dope Analysis, 15th to 20th March 1999, Sport und Buch Strauss: 301–310, 1998.

13 *Manglani M.V., DeGroff C.G., Dukes P.P., Ettinger L.J.:* Congenital erythrocytosis with elevated erythropoietin level: an incorrectly set «erythrostat»?. Journal of Pediatric Hematology/Oncology, 20(6): 560–2, 1998.

14 *Messinezy M., Westwood N.B., Woodcock S.P., Strong R.M., Pearson T.C.:* Low serum erythropoietin – a strong diagnostic criterion of primary polycythaemia even at normal haemoglobin levels. Clinical & Laboratory Haematology, 17(3): 217–20, 1995.

15 *Chapman R.F., Stray-Gundersen J., Levine B.D.:* Individual variation in response to altitude training. Journal of Applied Physiology, 85(4): 1448–56, 1998.

16 *Bialas B., Breidbach A., Schänzer W.:* Individual reference ranges for erythropoietin (sEPO) – Part II: Influence of long flight distance Flying. In Schänzer, W., Geyer, H., Gotzmann, A., Mareck-Engelke, U. (eds.): Proceeding of the Manfred Donike Workshop, 17th Cologne

Workshop on Dope Analysis, 14th to 19th March 1999, Sport und Buch Strauss: 301–9, 1999.
17 *Bauer C.:* The oxygen sensor that controls EPO production: facts and fancies. Journal of Perinatal Medicine, 23(1–2): 7–12, 1995.
18 *Fengels I., Habicht S., Braun D., Accola C., Villiger B.:* Kein Einfluss eines simulierten Höhentrainings (Spezialhaube zur O_2-Partialdruckminderung) auf ausgewählte hämatologische Parameter. Schweizerische Zeitschrift für «Sportmedizin und Sporttraumatologie», 47(3): 154, 1998.
19 *Gareau R., Audran M., Baynes R.D., Flowers C.H., Duvallet, A., Senecal, L., Brisson, G.R.:* Erythropoietin abuse in athletes. Nature, 380(6570): 113, 1996.
20 *Audran M., Gareau R., Matecki S., Durand F., Chenard C., Sicart M.T., Marion B., Bressolle F.:* Effects of erythropoietin administration in training athletes and possible indirect detection in doping control. Medicine & Science in Sports & Exercise, 31(5): 639–45, 1999.
21 *Beguin Y., Loo M., R'Zik S., Sautois B., Lejeune F., Rorive G., Fillet G.:* Early prediction of response to recombinant human erythropoietin in patients with the anemia of renal failure by serum transferrin receptor and fibrinogen. Blood, 82(7): 2010–6, 1993.
22 *Leppänen E., Dugué B.:* When to collect blood specimens: mid-morning vs fasting samples. Clinical Chemistry, 44(12): 2537–42, 1998.
23 *Allen J., Backstrom K.R., Cooper J.A., Cooper M.C., Detwiler T.C., Essex D.W., Fritz R.P., Mean, R.T. Jr., Meier P.B., Pearlman S.R., Roitman-Johnson B., Seligman P.A.:* Measurement of soluble transferrin receptor in serum of healthy adults. Clinical Chemistry, 44(1): 35–9, 1998.
24 *Lentner C. (eds.):* Geigy scientific tables, Volume 3, Physiological chemistry, Composition of the blood, Hematology, Somatometric Data. Ciba Geigy, West Caldwell, 1984.
25 *Stone H.O., Thompson H.K. Jr., Schmidt-Nielsen K.:* Influence of erythrocytes on blood viscosity. American Journal of Physiology, 214(4): 913–8, 1968.
26 *Dill D.B., Costill D.L.:* Calculation of percentage changes of blood volume plasma, and red cells in dehydration. Journal of Applied Physiology, 37(2): 247–8, 1974.
27 *Oscai L.B., Williams B.T., Hertig B.A.:* Effect of exercise on blood volume. Journal of Applied Physiology, 24(5): 622–4, 1968.
28 *Berglund B., Ekblom B.:* Effect of recombinant human erythropoietin treatment on blood pressure and some haematological parameters in healthy men. Journal of Internal Medicine, 229(2): 125–30, 1991.
29 *Major A., Mathez-Loic F., Rohling R., Gautschi K., Brugnara C.:* The effect of intravenous iron on the reticulocyte response to recombinant human erythropoietin. British Journal of Haematology, 98(2): 292–4, 1997.
30 *Halliday J.W., Searle J.:* Hepatic iron deposition in human disease and animal models. Biometals, 9(2): 205–9, 1996.
31 *Macdougall I.C.:* Strategies for iron supplementation: oral versus intravenous. Kidney International – Supplement, 69: S61–6, 1999.
32 *Fishbane S., Frei G.L., Maesaka J.:* Reduction in recombinant human erythropoietin doses by the use of chronic intravenous iron supplementation. American Journal of Kidney Diseases, 26(1): 41–6, 1995.