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RECENT ADVANCES IN DOPING ANALYSIS (8)

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Secondary markers of erythroid activity: any use for doping diagnosis in professional cyclists?

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We report the results obtained from blood samples collected from all cyclists taking part in the Tour de Suisse 1999. The models involving markers of altered erythropoiesis seem to suffer a serious limitation, because they do not take into consideration the high ferritin levels found in most of the cyclists. This work reflects information available after the oral presentation during the 18th Cologne Workshop in February 2000.

The discovery of recombinant human erythropoietin (rhEPO) ampoules in the main body of runners during the Tour de France 1998 made it clear that presumably many sportsmen were abusing this hormone. Since 1992, several laboratories have tried to find a direct or indirect way of detecting a recent administration of rhEPO in urine or in blood. The direct way of detecting rhEPO in urine has the advantage of isolating the drug itself [1] but has the disadvantage of detecting only a recent injection (around 48 to 72 hours). The indirect way [2] can detect a manipulation of the erythropoiesis such as an intake of rhEPO or mimetic peptide [3] during a much longer period.

The IOC (International Olympic Committee) Medical Commission has recently decided to perform simultaneously the direct and indirect test during the Olympic Games in Sydney. Both tests will have to be positive before taking any sanction against an athlete.

These last years, some publications have mentioned possible secondary markers to detect rhEPO abuse [4] . The models showed significant differences in the reticulocyte (Reti) count, the haematocrit (Hct) percentage, the soluble transferrin receptor (sTFR) and erythropoietin (EPO) concentration between groups under rhEPO treatment and placebo.

In the light of these findings, we have reanalysed the data of all cyclists (n=146) taking part in the Tour de Suisse 1999 who had been subject to a blood sampling with a haematocrit control on the third day of the race in Lausanne. Results are shown in Table 1 and in Figure 1.

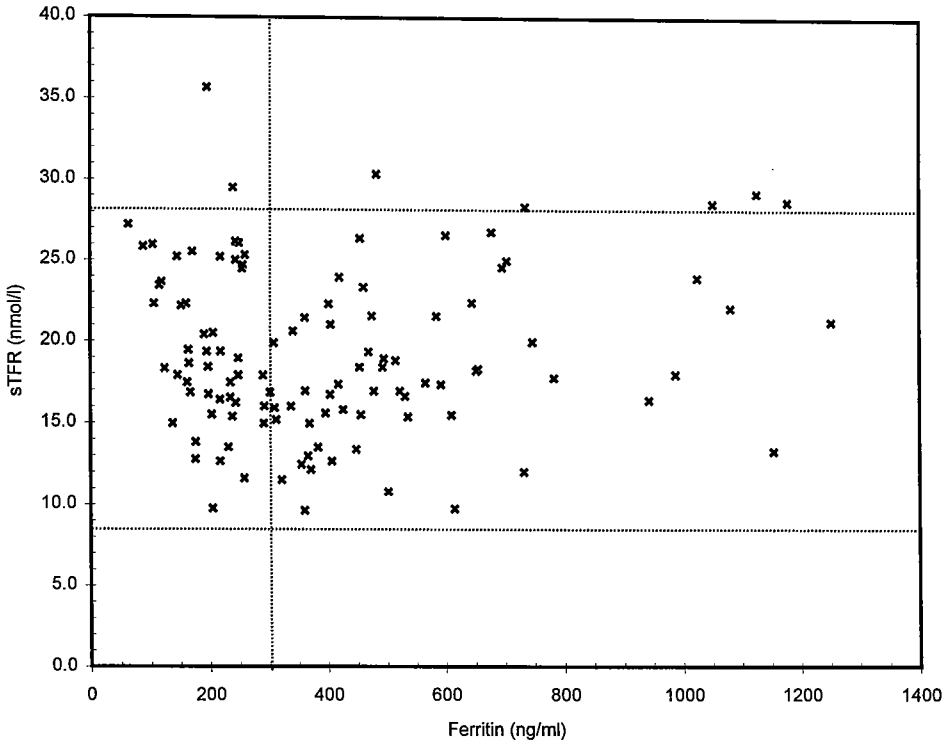

Figure 1. Ferritin and sTFR for all cyclists taking part in the Tour de Suisse 1999. The vertical line shows the cut off limit established by the UCI's year 2000 medical follow up for ferritin (ferritin = 300 ng/ml). Both horizontal lines are the 95 % reference intervals established by the manufacturer of the assay used for non-African Americans living at low altitude (sTFR = 8.8 – 28.1 nmol/l).

Five of the men with a haematocrit above 50 % had to stop racing for a minimum period of two weeks. One rider had a certificate stipulating that his haematocrit was physiologically above 50 % (certificate delivered by the International Cycling Union (UCI)). Figure 1 shows that more than half of the main body of runners had high ferritin values; of the 6 runners with excessively high ferritin values (around 1000 ng/ml), 3 also had out-of-range sTFR values. Taking haematocrit, reticulocytes, EPO and sTFR together, we can observe a total of 25 out-

of-range values which came from 24 different runners; i.e. only one runner had two out-of-range values at the same time (Reti and EPO).

Table 1. Possible secondary markers of rhEPO abuse in professional cyclists (n=146)

Parameter (unit)	Mean	Standard deviation	Number with out-of-range value*
Haematocrit (%)	46.0	2.4	5
Reticulocytes (%)	1.3	0.6	7
EPO (mU/ml)	14.3	9.7	6
sTFR (nmol/l)	19.1	5.2	7
Ferritin (ng/ml)	406.4	250.2	84

* as defined by UCI's year 2000 medical follow up for haematocrit (> 50 %), reticulocyte count (< 0.4 or > 2.4 %), ferritin concentration (> 300 ng/ml) and as indicated by the manufacturers for EPO (< 1.6 or > 34 mU/ml) and sTFR (< 8.8 or > 28.1 nmol/l).

From this non-clustering of secondary markers of erythroid activity in identifiable cyclists of the main body of runners of the Tour de Suisse, two major hypotheses can be deduced. Most of the cyclists were not taking any rhEPO shortly before and/or during the Tour de Suisse 1999. Given the overall impression of our results with, for example, the high absolute level and the narrow distribution of the haematocrit values, we do not consider this explanation to be very likely. The alternative hypothesis could be that the existing models would not be appropriate to professional sportsmen such as cyclists. Indeed the known models hardly take into consideration that sportsmen, and specially cyclists, have iron stores well above normal as illustrated in our results. Elevated iron stores especially due to regular intravenous iron injections should change drastically the expression of such markers of altered erythropoiesis and should for example down-regulate the expression of the sTFR [5], one of the most discriminant parameters supposed to increase in case of an abuse of rhEPO.

The amount, the way of administration and the length of the rhEPO treatment combined with elevated iron intake could easily modify the pattern of secondary markers. In such a case, the actual models proposed in the literature would lack specificity, because habits such as huge iron consumption for most of the cyclists have not been taken into consideration.

1. Lasne F, de Ceaurriz J. Recombinant erythropoietin in urine. *Nature* 2000; 405: 635.
2. Birkeland KA, Stray-Gundersen J, Hemmersbach P, Hallen J, Haug E, Bahr R. Effect of rhEPO administration on serum levels of sTFR and cycling performance. *Medicine & Science in Sports & Exercise 2000*; 32(7):1238-43.
3. Macdougall IC, Gray SJ, Elston O, Breen C, Jenkins B, Browne J, Egrie J. Pharmacokinetics of novel erythropoiesis stimulating protein compared with epoetin alfa in dialysis patients. *Journal of the American Society of Nephrology* 1999; 10(11): 2392-5.
4. Parisotto R, Gore CJ, Emslie KR, Ashenden MJ, Brugnara C, Howe C, Martin DT, Trout GJ, Hahn AG. A novel method utilising markers of altered erythropoiesis for the detection of recombinant human erythropoietin abuse in athletes. *Haematologica* 2000; 85: 564-72.
5. Hentze MW, Kuhn LC. Molecular control of vertebrate iron metabolism: mRNA-based regulatory circuits operated by iron, nitric oxide, and oxidative stress. *Proceedings of the National Academy of Sciences of the United States of America* 1996; 93(16): 8175-82.