The Blood profile of Claudia Pechstein

Background

Since the initiation of the ISU blood profiling program in February 2000, a total of 95 blood samples (20 out-of-competition and 75 in-competition collections) were collected from Claudia Pechstein. In a recently defined period starting from October 20th 2007 until today, a total of 24 blood samples (6 out-of-competition and 18 in-competition) are included. Most likely, a complete blood count was determined although only selected variables are published. In an anti-doping context, the relevant variables are presented (haemoglobin concentration and the number of reticulocytes in percent). The remaining variables are considered to constitute additional information. Variables from October 2007 are shown in Table 1 and Figure 1.

Explanation of given and calculated parameters

[Hb] and [Hb] CV

Hemoglobin is found in the red blood cells, where it binds oxygen. It is measured in grams pr. deciliter blood (g/dL). The individual fluctuations in haemoglobin concentration ([Hb]) in athletes have been determined to be < 5% calculated as the coefficient of variation in percent (CV%) (Malcovati et *al.*, 2003):

$$c_v = \frac{\sigma}{\mu} * 100$$

where σ is the standard deviation and μ is the mean

When artificial erythropoietin (EPO) is administered, the [Hb] increases because the bone marrow is stimulated to produce more red cells and hemoglobin. The [Hb] is increased weeks after artificial EPO has been administered. If blood is transfused, the [Hb] increases in a proportional manner to the amount of blood infused. There are contradictory reports on how long [Hb] is increased after infusion of different amounts of blood. In a recent study, [Hb] was only elevated compared to

baseline values for 3 days after infusion of on average 1.3 l of centrifuged blood (Damsgaard et *al.*, 2006)

[Hb] is decreased markedly, when blood is withdrawn from the body. In one study, [Hb] remained significantly lower compared to baseline values for two weeks following withdrawal of blood (Damsgaard et *al.*, 2006*)*.

Dilution of the blood by means of isotonic saline or plasma expander infusions is possible and would mask an underlying increase in haemoglobin concentration. Thus, a regularly, tight controlled hemodilution would theoretically be able to keep the haemoglobin concentration low at all times.

Reticulocytes

The percentage of reticulcoytes in the blood represents the relative amount of immature red blood cells in the red blood cell pool (immature + mature red blood cells). The normal range of reticulocytes is 0.2-2%.

Initially, when artificial EPO is administered, the reticulocytes rises to a high levels (>2%) and stays elevated for weeks (Morkeberg et *al.*, 2007). After EPO administration is terminated the reticulocytes decreases to levels lower than baseline levels returning to baseline levels after several weeks.

When on average 1.3 l of blood is lost from the body, reticulocytes rises to high levels (>2%) and stay elevated for at least two weeks (Damsgaard et *al.*, 2006). When less blood is lost from the body, only small or undetectable increases in reticulocytes are anticipated to show.

When blood is infused increasing [Hb] to supra-physiological high values a negative feed back signal to the bone marrow is initiated reducing the stimulation of the bone marrow. The result is – as with post EPO administration - a depressed level of reticulocytes compared to baseline values returning to baseline levels within two to three weeks (Damsgaard et *al.*, 2006, Morkeberg J et *al.*, 2007)

OFF-score

The OFF-score is used to detect artificial EPO administration after the administration has ceased. The score combines the [Hb] and reticulocytes into a score, which is calculated from the following formula: OFF-model score = [Hb] – 60 · √% reticulocytes, where [Hb] is in g/L. In the weeks after artificial EPO administration has ceased the [Hb] is still elevated above normal as previously mentioned. A supra-physiological [Hb] 'tells' the body to decrease the normal production of blood cells, resulting in a decrease in the immature population. An increased [Hb] in tandem with a low % reticulocytes will give a high OFF-score (see formula above). Different cut-offs have calculated in order to determine the levels, where different number of false positive cases appear. 1 out of 10 non-doped athletes will have an OFF-score above 92.2, while the remaining 9 will have scores below. The 1 in 100 and 1 in 1000 cut-offs are 104.4 and 113.5, respectively (Gore et *al.*, 2003).

Hb z-score

The Hb z-score is a score, where the [Hb] from all previous samples from the athlete is compared with his latest sample. It represents the number of standard deviations the last [Hb] is below or above the mean in the whole sample population (all previous tests). Because all athletes have different [Hb], which is a result of the individual genes, and which is fairly stable over time, it is practical to compare the athletes data over time, since large fluctuations are un-physiological and therefore will indicate doping abuse (Sharpe et *al.*, 2006). The Hb z-score is calculated from the following formula:

$$\text{Hb z-score} = (Hb_{current} - Hb_{mean}) / \sqrt{(\sigma^2 (1 + 1 / n))}$$

- $Hb_{current}$ is the [Hb] of the current sample.
- Hb_{mean} is the mean [Hb] score of all samples taken prior to the current sample.
- σ^2 is the variance in [Hb] in the reference group.
- n is the number of samples taken prior to the current sample.

Also here different statistical calculations are used to determine a false positive cut-off rate.

1 in 100:	≥ 2.33
1 in 1000:	≥ 3.09

If these limits are exceeded the variations in the [Hb] are considered 'un-physiological'.

OFF z-score

The OFF z-score is a score, where the OFF-score from all previous samples from the athlete is compared with his latest sample. The same rationale as for the [Hb] lies behind this score. The OFF z-score is calculated from the following formula:

$$\text{OFF z-score} = (\text{OFF}_{\text{current}} - \text{OFF}_{\text{mean}}) / \sqrt{(\sigma^2 (1 + 1 / n))}$$

- $\text{OFF}_{\text{current}}$ is the OFF-model score of the current sample.
- OFF_{mean} is the mean OFF-model score of all samples taken prior to the current sample.
- σ^2 is the variance in the reference group.
- n is the number of samples taken prior to the current sample.
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The same cut-offs for the Hb z-score apply to the OFF z-score.

Upper and Lower Limits in Two International Sport Federations

Upper and lower limits of blood and urine variables within the international cycling federation (UCI) and the international skiing federation (FIS) causing different sanctions or follow-up analyses unless a congenital or medical condition explains the exceeding.

UCI:

- Hemoglobin ≥17 g/dL (males)

- Reticulocytes ≤ 0.2% or ≥2%
- OFF-score ≥133
- "Positive Bayesian Model" (a model that in essence calculate the same likelihood of having un-physiological parameters in a profile as the z-score does)

FIS

- Hemoglobin ≥17 g/dL (males)
- Reticulocytes ≤ 0.2% or ≥2%
- OFF-score ≥113.5
- OFF z-score ≥3.09 or ≤ -3.09
- Hb z-score > 3.09 or ≤ -3.09

EPO urine results

When the WADA accredited laboratories are screening for artificial EPO administration (rEPO) they measure the intensity of the isoform bands by means of an isoelectric gelelectrophoresis. The intensity is given as a percentage of both basic and acidic bands. The percentage of EPO isoforms present in the Basic Area (BAP) in the urine is an indication of the use of the most widespread rEPOs (epoetin alfa and beta) whereas bands in the acidic area indicates use of darbepoetin alfa (ARANESP). According to the new WADA EPO technical document (TD2009EPO) various results leads to an adverse analytical finding i.e. BAP >82%. Thus, reporting the EPO screening results and compare these with the blood findings would indeed strengthen the evidence "pro et contra" an illegal administration of rEPO. A "negative" EPO urine test gives no information on whether the rider has actually used rEPO. As explained above a urine EPO screening result is always determined thus reporting a sample as "negative" may hide important information as to "how negative" the sample actually was. A "suspicious" EPO results reported by the WADA accredited laboratory in tandem with a time corresponding suspicious blood profile would support a possible rEPO administration.

Blood Results

A total of 94 blood results provided by the lawyer of Claudia Pechstein representing the ISU blood collections since 2000 of which 23 blood samples since 2007 has been chosen to constitute the basis of this evaluation. From a scientific point of view, it is not relevant to exclude any collected results. On the contrary, any results that can contribute to clarify physiological patterns are important and indispensible in a clinical setting as long as the results are treated with the necessary scientific cautions. Thus, the blood readings collected prior to October 2007 will be used to confirm or refute the factors explaining the blood profile under the caution of the factors that may have influenced those readings.

The mean of the variables and calculated CV are as follows:

Haemoglobin (range) = 14.4 g/dL (12.1-15.0)
Reticulocytes (range) = 2.3% (1.0-3.4)
CV (haemoglobin) = 2.8 %

The haemoglobin concentration in a non-trained background population is set at 9.7- 16.1 g/dl with a mean 12.7 g/dl. Thus, the normal range of such a population is large and includes values above 16 g/dl. In comparison, world-class cross-country female skiers have an average haemoglobin concentration between 13.5-15.2 g/dl (Morkeberg et *al.*, 2009). The haemoglobin results of the athlete indicate a "normal" mean haemoglobin value compared to elite athletes and slightly elevated compared to a non-trained healthy background population with no readings exceeding the UCI and FIS upper haemoglobin limits. The variations in % from the lowest to the highest measured haemoglobin and are 11.7%. Scientific reports suggest that the absolute variations of haematocrit (which by most scientists is consistent with haemoglobin in g/dl *3) 15% or lower are considered normal (Thirup P, 2003). Malcovati et *al.* suggested when taking previous readings into consideration that the physiological variation (CV) in athletes would be less than 5% and if exceeding 10%, then physiological abnormalities may play a role. The CV in this case is 2.8%. The Hb z-score ranges from -0.95 – 1.37 and is thus not suspicious at any time (a z-score >2.33 or <-2.33 is considered suspicious) (Sharpe et *al.*, 2006)

The reticulocytes show a roller coaster pattern between 1.3% and 3.8%. The mean is 2.3% and thus beyond the upper limit constituting follow-up analyses by some international sport federations (UCI

and FIS). Elevated reticulocytes are in an anti-doping context found with the use of exogenous EPO or after withdrawal of considerable amounts of blood (~1.3 l) as described previously. Thus, still in an anti-doping context, the athlete must repeatedly through nine years have received significant supra-physiological units of exogenous EPO or performed extensive blood withdrawals in order to achieve the reticulocytes of the magnitude presented in the ISU data. It should be noted that a theoretically, extensive blood withdrawal causing the some in-competition measured significantly elevated reticulocytes would have caused serious damage to the performance. For the record, it is the opposite behaviour – infusion of blood just prior to competitions - which have been found in cheating athletes.

Staying at altitude is not likely to explain the repeatedly, extensively elevated reticulocytes (Lundby et *al.*, 2005)

If reticulocytes are considered congenital elevated, however, it is not likely that exogenous EPO nor transfusion of up to 1.3 l of blood would depress the reticulocytes to <1% since the athletes' baseline is elevated as previously stated by one expert. The response in reticulocytes to exogenous EPO, blood transfusions or a combination of these will in post treatment period be depressed but in a parallel deferred manner to higher levels than normally reported.

The at times vastly increased and depressed reticulocytes in the ISU data does not clearly correlate with what is physiological expected. Normally, significantly high reticulocytes are a response to an induced stimulation of the bone marrow causing a significant, delayed haemoglobin increase. In a parallel manner and as a consequence of a negative feed-back system elevated haemoglobin causes depressed reticulocytes compared to baseline levels. Why haemoglobin seems unaffected by the elevated and depressed reticulocytes remains a mystery sought explained by other experts. However, in an anti-doping context the only reasonable explanation would be that the athlete artificially decreases the haemoglobin values by dilution of the blood. This is in a clinical setting performed to stabilise blood pressure (not to dilute the blood although the outcome of decreased haemoglobin levels are the same) carefully performed by infusing iv isotonic saline, albumin, plasma or plasma expanders one by one or in combination. To keep haemoglobin levels stable with a CV = 2.8% and never too high for nine consecutive years, the athlete would have had to meticulously regulate and control the haemoglobin value with *iv* infusions of various plasma

products of which at least one can be tested for (plasma expanders) on a regular basis. Keeping body fluids within their designated compartments in the body so that haemoglobin is stable is not possible in a clinical setting with 24 hours attention and all possible medicaments available. Theoretically, excessive use of any of the plasma expanders which is highly common in clinical settings would bring haemoglobin to very low levels which can only be masked by acute blood transfusions. Thus blood for acute transfusions in order to titrate the haemoglobin values within very narrow limits must have been available for nine years.

The OFF-score which is a cross sectional sign of a previous terminated exogenous EPO administration and the [Hb] and OFF z-scores which are kinds of "real-time" tell-tales of either exogenous EPO administration or blood transfusions are neither since 2007 nor back from 2000 at any stage exceeding the respective upper and lower limits set by scientific papers and implemented by some International Federation. To a large extend, this demonstrates that haemoglobin and reticulocytes in this athlete are solid, robust and stable with variations to be considered physiological.

EPO urine tests

I've been informed that in excess of 220 urine tests (the ratio of EPO urine tests is not indicated) have been collected by ISU, German NADO, IOC etc. since 2000. Looking through the few that have been presented to me with dates of collections and whether the test was collected in-competition or out-of-competition, I have noted that the EPO urine test have not been reported with its corresponding BAPs. I also note that no EPO urine test have been reported "suspicious". There is one EPO urine test reported with non- detectable bands. In FIS more than >15% of all athletes has had such a result. At a seminar this month in Vancouver, Francoise Lasne of the WADA accredited laboratory in Paris reported that the number of non-detectable bands is vastly reduced if the urine sample is kept cold and analysed quickly after collection. Thus, non-detectable bands are not suspicious but can to a large extend be explained by the handling of the sample. Non-detectable bands are to my knowledge not mentioned in the WADA EPO technical document (TD2009EPO).

If rEPO administration is suspected, reticulocytes >2% on 17 occasions in unannounced in and out-of-competition tests would according to the scientific papers on rEPO administration have caused a

number of suspicious if not positive EPO (depending on which rEPO is used and the prevailing EPO technical document) results. Reticulocytes >3% has only been found in subjects when rEPO administration have been at its highest intensity with a window of detection impossible to miss. So, either the different anti-doping bodies responsible for the EPO test have not reported the suspicious or positive nature of the tests or rEPO has not been found and thus can be excluded as the cause of the high reticulocytes.

The only other explanation of the high reticulocytes is blood withdrawal for a possible later infusion (autologous blood transfusion). However, it is unlikely to believe that any athlete or doping advisor would ever urge an athlete to withdraw in the vicinity of 1.3 l of blood which is necessary to cause a rise in reticulocytes of >3% in the days to hours prior to a competition.

One FIS female athlete

As the anti-doping program manager in FIS, I evaluate blood and urine profiles on a daily basis. As opposed to ISU, FIS receives the EPO urine screening results and compare these with the blood profiles. Administration of rEPO as have been demonstrated may be detected in two tissues and since the window of detection in the urine is only very short for most rEPOs a large variation in the EPO urine profile in tandem with a rEPO suspicious blood profile may create the necessary amount of evidence needed to sanction according to the new WADA EPO technical document (TD2009EPO).

In this context, we have at least one female athlete with a blood profile very similar to Claudia Pechstein. This athlete is not related to Claudia Pechstein (family, coaches, medical advisors etc.). Haemoglobin ranges from 11.9 g/dl-14.4 g/dl and reticulocytes from 0.9%-3.6% (Figure 2). The FIS EPO urine screening results since 2008 have shown BAPs from 9%-42% including occasions when reticuloytes were >2%.

Again, blood withdrawal explaining the high reticulocytes seems highly unlikely since these have also been found in-competition.

Facts

Potential sanctionable facts:

1. Haemoglobin > 16.0 g/dl (never)
2. OFF-score > 113.5 (never)
3. Hb and OFF z-scores >3.09 or <-3.09 (never)
4. EPO test (never)

Suspicious facts:

1. OFF-score > 104.4 (never)
2. Hb z-score >2.33 or <-2.33 (never)
3. **OFF-z-score >2.33 or <-2.33 (on 5 occasions since October 2007)**
4. Haemoglobin ~16 g/dl (since October 2007; never)
5. **Reticulocytes > 2% (multiple)**
6. Reticulocytes < 0.2% (never)
7. **Occasionally simultaneously for this athlete "high" haemoglobin and reticulocyte values**
8. EPO suspicious test (never)

Conclusion

In a consecutive period of nine years - reduced to two years due to instrumental variations - haemoglobin values have been stable with a baseline similar to the mean of other female endurance athletes. In contrast, reticulocytes has shown a highly fluctuating pattern with values up to 3.5% very rarely seen in humans unless a (congenital) medical condition or doping activities in research subjects have been present.

Reaching 3.5% in reticulocytes in healthy humans takes either withdrawal of approximately 1.3 l of blood or high doses of rEPO administrations. Considering the detrimental effect on performance by drawing 25% (1.3 l) of the blood volume on multiple occasions in-season combined with a needed extensive infusion program consisting of saline, albumin or other plasma expanders to avoid suspicious variations in haemoglobin after blood reinfusion, blood withdrawal and reinfusion seems unlikely.

Considering rHuEPO to be the hormone in question then multiple EPO screening results must have shown very suspicious patterns in the band distributions (BAPs) on several occasions. None of the reported EPO results have been declared suspicious or positive.

Applying known blood algorithms and upper and lower limits to the blood results, none of them but the “stand-alone” reticulocytes indicates any known doping behaviour.

At least one blood profile in a female athlete from a different sport has an identical pattern where administration of rEPO has been excluded.

In conclusion, the presented blood profile does not mimic the blood profiles found in the majority of female endurance athletes. In an anti-doping context, the fluctuations in reticulocytes cannot be explained by a well-known and sensible doping behaviour in order to enhance performance. Although, the blood profile like any other anti-doping profile cannot exclude the use of forbidden substances and/or methods at some point during the nine years, it has not been established - beyond any reasonable doubt – that the use of forbidden substances or methods has taken place.

Copenhagen, 13 October 2009

Rasmus Damsgaard, MD, PhD

CV

I am a medical doctor since 1994 currently specialising in anaesthesiology at the University Hospital of Copenhagen, Rigshospitalet. I have a PhD in growth and maturation in children in competitive sports focusing on the endocrinology aspects. From 2000-2004, I was secretary general of the Danish Anti-Doping Agency. Since 2006, I have been the anti-doping program manager in the international skiing federation (FIS). I am member of the anti-doping "Review Boards" in the international Tennis and Cricket federations. As a FIS representative I joined a WADA formed blood profile group in 2006 which in a new context is still active. Parallel to the practical implementations of anti-doping activities in international skiing, I am involved in research co-authoring several anti-doping papers focusing on blood profiles and blood manipulations.

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Table 1

The table shows the results if the mean, maximum and minimum of the ISU reported measurements of haemoglobin haematocrit and reticulocytes from October 2007. In addition, the calculated OFF-score, OFF and Hb z-scores are expressed

Statistics	Haemoglobin	Haematocrit	Reticulocytes	OFF-score	Hb-zscore	OFF-zscore
Mean values	14.4	40.6	2.3	53.2	-0.02	-0.05
Max values	15.3	44	3.8	77.8	1.37	2.76
Min values	13.7	37	1.3	25.1	-0.95	-2.84

Figure 1

The Figure gives a visual impression of the small variations in hemoglobin (green bars) and the overall elevated reticulocytes (red line) in **Claudia Pechstein**. Only measurements since December 2007 are included.

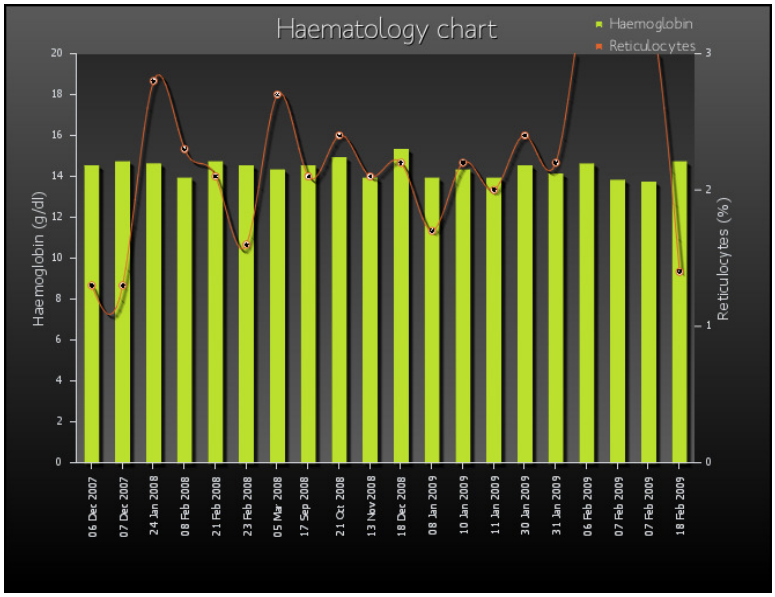

Figure 2

The Figure gives a visual impression of the small variations in hemoglobin (green bars) and the overall elevated reticulocytes (red line) in one **female FIS athlete**. Measurements since February 2005 are included