lymphoma. As far as we know, this phenomenon has not been previously reported, and the significance of this observation remains elusive. In addition, in our series *MGMT* hypermethylation does not seem to play a significant role in the response to chemotherapy in CTCL patients. The possibility that *MGMT* hypermethylation may be a negative prognostic factor identifying a specific subset of lymphomas cannot be completely ruled out.

In conclusion, our results demonstrate that hypermethylation in $p15^{INK4b}$ and $p16^{INK4a}$ promoter genes seems to play a role in the pathogenesis of cutaneous T-cell lymphoma. These phenomena occur independently as tumor-specific events and probably are not-dependent on the stage of the disease. *MGMT* promoter hypermethylation occurs in a significant percentage of T-cell cutaneous lymphomas.

Fernando Gallardo,° Manel Esteller,# Ramon M. Pujol,*° Carlota Costa,*° Teresa Estrach,°@ Octavio Servitje°^*

**Department of Dermatology, Hospital del Mar, IMAS, Barcelona; °Cutaneous Lymphoma Network of Catalonia; #Cancer Epigenetics Laboratory, Spanish National Cancer Centre (CNIO), Madrid; @Department of Dermatology, Hospital Clínic, Barcelona; ^Department of Dermatology, Hospital de Bellvitge, Barcelona, Spain*

Funding: this study was partially supported by the grant FIS 00/225 from the Spanish Ministries of Health and Science and Technology.

Key words: methylation status, MGMT, primary cutaneous T-cell lymphoma.

Correspondence: Fernando Gallardo, Department of Dermatology, Hospital del Mar, IMAS, Passeig Maritim 25-29, 08003, Barcelona, Spain. Phone: international +34.93.2483380. Fax: international +34.93.2483328. E- mail: fgallardo@imas.imim.es

References

1. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 2003; 349: 2042-54.
2. Drexler HG. Review of alterations of the cyclin-dependent kinase inhibitor INK4 family genes p15, p16, p18 and p19 in human leukemia-lymphoma cells. Leukemia 1998;12:845-59.
3. Pinyol M, Cobo F, Bea S, Jares P, Nayach I, Fernandez PL, et al. p16(INK4a) gene inactivation by deletions, mutations, and hypermethylation is associated with transformed and aggressive variants of non-Hodgkin's lymphomas. Blood 1998; 91: 2977-84.
4. Villuendas R, Sanchez-Beato M, Martinez JC, Saez AI, Martinez-Delgado B, Garcia JF, et al. Loss of p16/INK4A protein expression in non-Hodgkin's lymphomas is a frequent finding associated with tumor progression. Am J Pathol 1998;153:887-97.
5. Esteller M, Hamilton SR, Burger PC, Baylin SB, Herman JG. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation is a common event in primary human neoplasia. Cancer Res 1999;59:793-7.
6. Dolan ME, McRae BL, Ferries-Rowe E, Belanich M, van Seventer GA, Guitart J, et al. O6-alkylguanine-DNA alkyltransferase in cutaneous T-cell lymphoma: implications for treatment with alkylating agents. Clin Cancer Res 1999;5:2059-64.
7. Navas IC, Ortiz-Romero PL, Villuendas R, Martinez P, Garcia C, Gomez E, et al. P16 gene alterations are frequent in lesions of mycosis fungoides. Am J Pathol 2000;156:1565-72.
8. Navas IC, Algara P, Mateo M, Martinez P, Garcia C, Rodriguez JL, et al. p16 (INK4a) is selectively silenced in the tumoral progression of mycosis fungoides. Lab Invest 2002;82:123-32.
9. Scarisbrick JJ, Woolford AJ, Calonje E, Photiou A, Ferreira S, Orchard G, et al. Frequent abnormalities of the p15 and p16 genes in mycosis fungoides and Sézary syndrome. J Invest Dermatol 2002;118:493-9.

Blood Doping

Longitudinal variation of hemoglobin and reticulocytes in elite rowers

Longitudinal monitoring of athlete's hematologic parameters holds considerable promise as a strategy to detect and thereby deter illicit blood doping. This study documents temporal changes of hemoglobin concentration (Hb) and reticulocyte counts in elite rowers. The 'within subject' variation in rowers was comparable to that of athletes from other sports. Reticulocyte results were dependent on the type of instrument used.

haematologica 2003; 89:1403-1404
(*http://www.haematologica.org/2004/11/1403*)

The concept of utilizing a *Hematologic passport* as a tool to highlight athletes who have used illegal blood doping to enhance performance is gaining credibility. Faced with a limited budget, anti-doping authorities must balance the frequency of testing with the scope of hematologic evaluation. Two cornerstones of this approach will be Hb concentration and reticulocyte counts, which can both be measured on *portable* analyzers relocated to the event venue. Thus samples collected at the time of competition for routine blood screens could also provide a cost-effective and convenient source of Passport data.

A crucial element of the Passport approach is to define in advance normal fluctuations in blood parameters, to enable authorities to discriminate between *expected* and *suspect* changes. It is also necessary to recognize whether biological variability is sport-specific or whether such changes are *universal* across different sports. The first aim of this research was to report the variation of Hb and reticulocytes in rowers, and then to contrast these with data reported previously for athletes from another discipline. A second aim was to verify whether it was tenable to compare hematologic results obtained on different instruments.

Blood samples were obtained from members of the French National Rowing squad (n=83 males, n=31 females). Between two and eight (average 4.8 males and 3.7 females) EDTA blood samples were collected from each rower between July 2001 and March 2004, and were measured on either an ADVIA 120 Hematology Analyzer or a Sysmex Roche XE2100 (instruments were calibrated according to manufacturers' specifications). To provide heightened resolution reticulocytes are reported on a square root scale.[1] These results were contrasted with previously reported data comprising longitudinal evaluations from a subset of n=288 male professional football players measured on average 2.9 times between May 1999 and July 2002.[2] Analysis of variance was used to partition the total variation present in each data set into three components: the variation *within subjects*, the variation *between days* (within instruments) and the variation *between subjects*.

Table 1 gives estimates of the variances. In each case the estimate allows for the other two components of variation. All of the *between subject* and *between days* variances are significantly greater than zero (F-test, $p \leq 0.001$). The estimates show that the *within subject* variance for both Hb and reticulocytes is comparable among (male) football players and rowers. For Hb the *between subject* variances for males are comparable (estimates are at least double those of the *within subject* estimates, although lower for females). In

©Ferrata Storti Foundation

Table 1. Components of variation of hemoglobin concentration and reticulocytes in two cohorts of athletes.

	Rowers		Footballers
Males (n=83)	*Females (n=31)*	*Males (n=288)*	
Hb (g/L)[2]			
Between subjects	45.1	18.3	49.7
Between days	4.4	5.9	5.4
Within subjects	18.9	13.5	17.3
Retics (√%)[2]			
Between subjects	0.0127	0.0139	0.0135
Between days	0.0031	0.0043	0.0036
Within subjects	0.0083	0.0103	0.0085

Analysis of variance partitioned total variation into 'Between subjects', 'Between days' (within instruments) and 'Within subjects' from hematologic data collected longitudinally over a period of 33 months (rowers) or 38 months (football players).

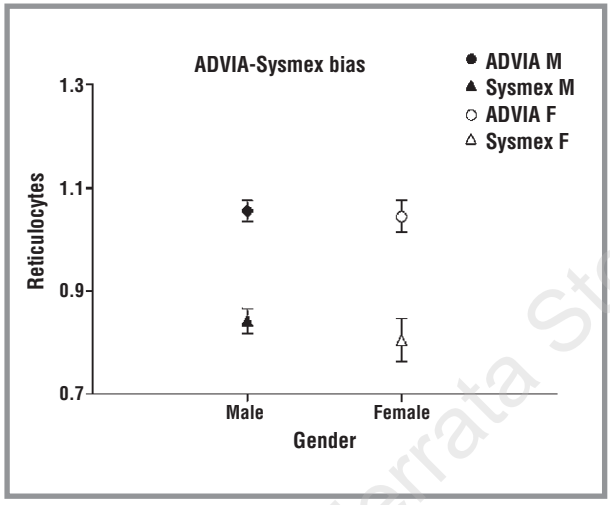

Figure 1. Square root transformed reticulocyte values (√%; mean ± SE, standard error of the mean was used to facilitate comparison of differences between instruments) of elite rowers derived from either an Advia 120 Hematology analyzer or a Sysmex Roche XE2100. Subgroups of a cohort of n=83 males and n=31 females were measured on an ADVIA (n=66M and 26F) and one month later on a Sysmex (n=58M and 18F).

comparison, the variance components for both males (n=739) and females (n=413) competing in various sports[3] who were measured over a 21-day period, was of the same magnitude reported here for males (the *between subject*, *between days* and *within subject* variances being 56.5, 4.6 and 16.1 for males, 48.9, 6.0 and 18.9 for females, respectively). Reticulocyte variance *within subject*s was approximately 0.75 times that of *between subject* variance, regardless of sport or gender (similar results were found for males and females from mixed sports).[3] Bias between instruments for Hb, after allowing for differences *between subjects*, was statistically significant but not important, being within expected tolerance limits (ADVIA values were 3.9 g/L higher than Sysmex ones, p=0.004). As shown in Figure 1 reticulocyte values derived from the ADVIA were consistently higher than when measured on the Sysmex platform (p<0.001). These quantitative data illustrate that for Hb the major component of variance is attributable to *between subject* variation, which supports the intuitive belief that comparing an athlete's current values with their own longitudinal data rather than population-derived thresholds will provide greater resolution when searching for signs of blood doping. The similarity of *within subject* variation apparent across different sports augurs well for the universality of this approach. Although of secondary importance to the Passport concept (which is contingent upon consistent *within subject* variance), there was no apparent explanation for why *between subject* variation in the cohort of female rowers was markedly lower than for other groups, and this deserves closer scrutiny.

Our results also emphasize the need to quantify, and adjust for, instrument bias for reticulocyte assays. Instrument bias can be quantified by using the mean value from a cohort of athletes as a *de facto* calibration agent, and bias negated by using a paper adjustment to standardize values.[1] The ADVIA-Sysmex bias in this study is consistent with that found in previous research,[1] and underlines the need to document the type of instrument used when measuring reticulocytes.

Michael J. Ashenden, Alain Lacoste,° Emmanuel Orhant,# Michel Audran,@ Ken Sharpe^*

**Science and Industry Against Blood doping (SIAB) research consortium, Gold Coast, Australia; °Fédération Internationale des Sociétés d'Aviron (FISA), Lausanne, Switzerland; #Fédération Française des Sociétés d'Aviron (FFSA), Nogent/Marne, France; @Biophysical Laboratory, Faculty of Pharmacy, University of Montpellier I, France; ^Department of Mathematics and Statistics, The University of Melbourne, Australia*

Funding: Data from the rowers were collected in a program funded by the French Ministry of Sport, data from the footballers were collected during a study facilitated by the Italian Ministry of Health, and data from the mixed group of athletes were collected during research funded by the Australian Government and the International Olympic Committee. This study received support from the World Anti-Doping Agency (WADA). We are grateful for the encouragement provided by the International Rowing Federation (FISA) to pursue this research.

Key words: doping, longitudinal variation.

Correspondence: Michael Ashenden, Science and Industry Against Blood doping (SIAB) research consortium, PO Box 572, Gold Coast, QLD 4217, Australia. E-mail: heyasho@hotmail.com

References

1. Ashenden MJ, Sharpe K, Damsgaard R, Jarvis L. Standardisation of reticulocyte values in an antidoping context. Am J Clin Pathol 2004;121:816-25.
2. Malcovati L, Pascutto C, Cazzola M. Hematologic passport for athletes competing in endurance sports: a feasibility study. Haematologica 2003;88:570-81.
3. Sharpe K, Hopkins W, Emslie K, Howe C, Trout G, Kazlauskas R, et al. Development of reference ranges in elite athletes for markers of altered erythropoiesis. Haematologica 2002;87:1248-57.

©Ferrata Storti Foundation