PROJECT REVIEW

"Alternative steroid profiling part II"

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The project aims to contribute to the development of the steroidal subunit of the Athlete's Biological Passport (ABP) which is hitherto solely validated on a few markers, such as the T/E ratio.

Recently, novel biomarkers were found that increase significantly the time detection window after administration of small doses of T, DHT and DHEA using the Adaptive Model of the ABP. These biomarkers consist of steroid ratios including minor metabolites sensitive to steroid administration. More data on intra-individual variation of the involved minor metabolites are nevertheless necessary to validate the proposed biomarkers. Therefore a large-scale investigation of long-term within-subject behaviour of an extended steroid profile will be conducted.

Data obtained on a larger cohort with comprehensive steroid profiling methods will allow the development of a multi-parametric marker of steroid doping that comprises the whole steroid profile. This model statistically classifies abnormal steroid profiles by outputting a single score. Longitudinal evaluation of this 'Abnormal Steroid Profile Score' (cfr. Abnormal Blood Profile Score in the Blood Passport) monitors any alteration in the steroid profile regardless to its cause. The goals are twofold. Firstly, when applied at the individual level, this model will allow the general screening of doping with endogenous steroids, food supplements and substances manipulating the steroid profile, such as after ethanol consumption. Secondly, and in contrary to blood doping and doping with growth hormone wherein markers having a detection time long enough to estimate the prevalence of doping already exist, this score might provide accurate estimates of the prevalence of steroid doping in elite sports when applied at the population level.

Moreover, the influence of genetic polymorphism on the new steroid profile parameters and an Abnormal Steroid Profile Score will be studied in order to increase the sensitivity of the model.

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Results and Conclusion

A combination of the Support Vector Machine (SVM) algorithm with a comprehensive approach of steroid profiling resulted in a steroidomic model that enables to differentiate normal steroid profiles from abnormal ones. Theoretically, the SVM tool plots all monitored steroids in a multi-dimensional hyperspace which makes the use of steroid ratios redundant to obtain a strategy with optimal detection sensitivity. Hence, the whole set of steroid profile values can be evaluated at once. In our model, however, the degree of abnormality was quantified by an Abnormal Steroid Profile Score (ASPS) for which values greater than 0.79 could be considered as deviating from normal.

Since the introduction of the Athlete Biological Passport, the results of steroid profiling tests can be systematically stored in a central database enabling the estimation of the individual reference ranges. From such databases, longitudinal steroid profiling data can be made readily available to elaborate longitudinal strategies, thereby omitting a large contribution of the inter-individual variance. Similarly, the raw SVM model was improved by standardizing the training set using individual mean and standard deviation obtained with the adaptive model. The combination of the adaptive model and the SVM enhances the general performance accuracy of the raw SVM model from 62% to 84%, disregarding the kind of endogenous steroid administered. The diagnostic sensitivity of the resulting ASPS was 55% in a post-administration period of 7 days. Altered steroid profiles can be found until 5 days after ingesting a small single doses of T or DHEA or after topical application of T or DHT in therapeutically recommended doses. This drastic increase in sensitivity can be explained by the ability of the model to sensitively distinguish a prolonged recovery state of the steroid metabolism which is restoring the homeostasis of steroid profile to known basal levels.

Since the model was trained on data obtained after T, DHT and DHEA administration, the model risked to be overfitted i.e. a specific detection tool for these steroids. This problem was addressed by leave-one-subject-out cross-validation and testing of the model on another volunteer, with another dose of DHEA and with other steroids. Testing of the excretion data from a 100mg dose of DHEA, 50mg Adion and 7-keto-DHEA ingested by another volunteer showed a clear response of the ASPSs. This indicates the polyvalent nature of the SVM model to detect any small disturbance of the steroid profile. Moreover, the high sensitivity of 97% obtained for this new test set illustrates the potential of the ASPS as a powerful biomarker for the general detection of misuse with

endogenous steroids. Although, this single model shows excellent sensitivity for a wide range of administered steroids, it cannot specify which cause resulted in an aberrant steroid profile. For this information, specific metabolites should be evaluated separately.
Despite the excellent preliminary results on low dose administration studies conducted on a limited study population - including subjects with atypical T/E's that challenge the classification -, the applicability of this strategy will require further work and large scale validation procedure. In order to implement the ASPS in routine testing as a sensitive marker for of any misuse with endogenous steroids, the model should be tested on larger cohorts of data and external influences on the steroid profile that can alter the ASPS should be scrutinized in the future.

In conclusion, a new strategy was developed that returns a single value ASPS as a denotation of the degree of abnormality of a steroid profile containing 24 steroid metabolites. With this strategy, the alteration of the steroid profile, caused by a variety of endogenous steroids, can be detected very sensitively. The longitudinal SVM model was shown to be a general model which can result in long detection of small doses of oral and topical steroid formulations up to 5 days. The overall model performance was very good, particularly when coupled with the longitudinal results from the adaptive Bayesian model. The combination of computer aided techniques as the Bayesian adaptive model and SVM algorithm provide a valuable steroidomic strategy for the long term detection of misuse with endogenous steroids in complement with current steroid profiling methods.