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Trends in the Detection of Erythropoietin Receptor Agonists (ERAs) in Anti-Doping: An Analysis of Recent Adverse Analytical Findings (AAFs)

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ABSTRACT

Anti-doping efforts aim to reduce the prevalence of doping through a combination of education, deterrence, and detection. Detection of doping practices, for example through testing and/or investigations, aims both to catch committed dopers and deter potential dopers. To date, little empirical evidence is available examining the ability of detection strategies to deter athletes from doping. Here, trends in adverse analytical findings (AAFs) for EPO or other EPO-Receptor Agonists (ERAs) were examined over an 8-year period in order to assess the impact of ERA testing and detection on athlete behavior. It was observed that the majority (62.8%) of ERA AAFs occur on samples collected on the day of a competition. Evidence is also presented that the largest fraction of ERA AAFs occurs on the first sample ever taken from an athlete (43.2%), and that the ERA AAF rates decline steadily as athletes continue to be tested. These findings provide evidence of a deterrent effect of testing on ERA use in sport.

1 | Introduction

Effective detection of doping aims to encourage clean sport by catching those who are committed to doping practices and by deterring potential dopers from choosing to dope in the first place. Deterrence is predicated on the ability of anti-doping measures to change or disrupt the behavior of a group of athletes, diverting their choice to dope because of, for example, knowledge of significant consequences. However, behavioral changes can also be observed in those who still choose to dope, where their doping practices are disrupted by anti-doping measures and they must therefore adapt their doping strategies accordingly.

Like most pharmacological responses, doping effectiveness is not a binary response where one either dopes and has the maximal effect or does not dope and has no impact. Instead, the effectiveness of doping regimes lies on a continuum of potential impacts on performance, ranging from zero to highly impactful. Factors, which may reduce the effectiveness of a particular doping strategy include reduced dosage, reduced duration of use, or increased time between doping and competitions. While so-called adverse analytical findings (AAFs), which identify the presence of a prohibited substance or its metabolites or markers in a sample, are an important outcome for anti-doping, adaptations in doping strategies which result in less effective doping practices are also a desirable outcome of detection strategies. Importantly, the very desirable outcome of deterring a potential cheating athlete from doping at all is not easily captured in anti-doping statistics.

While a true measure of deterrence would require knowledge of doping prevalence over time, which is currently not available, changes in athlete behavior in response to anti-doping measures can be observed from anti-doping data. For example, monitoring blood variables in elite cyclists indicated a marked change in extreme values both following

the introduction of the erythropoietin (EPO) test in cycling in 2001–2002 and again following introduction of the Athlete Biological Passport in 2008 [1]. Here we examined trends in AAFs for EPO and other EPO-receptor agonists (ERAs) over an 8-year period in order to assess the impact of ERA detection on athlete behavior.

2 | Methods

A dataset of all testing records between January 1, 2016 and December 31, 2023 was extracted from the Anti-Doping Administration & Management System (ADAMS) platform of the World Anti-Doping Agency (WADA). This period was chosen as data entry into ADAMS related to sample collection sessions became mandatory in 2016, providing a more complete dataset. Only the test type ("In Competition" (INC) and "Out of Competition" (OOC)), year and time of sample collection, ordinal number of the test, sport category (see Table S1), performance of ERA analysis and its results, gender, and anonymized athlete ID were extracted into an anonymized dataset, in accordance with the WADA International Standard for the Protection of Privacy and Personal Information (ISPPPI) [2]. A "sample collection session" refers to a single testing event and can include the collection of one or several sample types (ex. urine, serum, whole blood). Test ordinal number is defined as the sample collection session chronological order during the observed period. A sample collection session is considered as an ERA AAF if at least one of the samples collected during the session fulfils the criteria to declare an AAF [3], whether it be a urine or blood sample. The time of the sample collection session is defined as the time when the first sample of this session was taken. The "AAF rate" is defined as the ratio between the number of ERA AAF sample collection sessions and the total number of sample collection sessions analysed for ERAs calculated within a same dimension.

Data processing and analysis were performed with R software version 4.2.

3 | Results

ERAs can be detected in blood or urine, however because the detection method is time consuming and costly, it is not systematically applied to all samples as part of the standard test menu in WADA-accredited Laboratories. Instead, ERA analysis must be ordered specifically by the Anti-Doping Organizations (ADOs) for a given sample. In order to study trends in ERA detection and potential impacts on athlete behavior, a dataset spanning 8 years from 2016 to the end of 2023 was considered. During this period, a total of 1,679,868 unique sample collection sessions were conducted, of which 390,197 (23.2%) included samples analyzed for ERAs resulting in 522 ERA AAFs, for an AAF rate of 0.13% (see Table 1). Of these AAFs, 80.3% were for EPO, 10.2% were for darbepoetins (dEPO), and 9.5% were for methoxy polyethylene glycol-epoetin beta (CERA).

Of these 522 ERA AAFs, 79.9% originated from male and 19.1% from female sample collection sessions, with ERA AAF rates of 0.16% and 0.08%, respectively (See Table 1). Most ERA AAFs (83.9%) were from athletes competing in cardio-vascular endurance sports, with the remaining AAFs coming from muscular endurance sports (6.7%), power and strength sports (3.3%), combat sports (1.5%), and ball and team sports (1%). While the majority of the ERA AAFs comes from cardio-vascular endurance sports, these sports represent less than 50% of the ERA analyses performed, resulting in a 0.23% AAF rate. As a counter point, the ball-and-teams sports category is underrepresented in terms of ERA AAFs (1.0%), while having an ERA analysis share reaching 19.5%, for an ERA positivity rate of only 0.01%. Most of the AAFs (97%) were from summer sports, with only 3% being from winter sports. Moreover, the ERA AAF rate of summer cardio-vascular endurance sports is much greater than their winter counterparts, showing values of 0.26% and 0.03%, respectively. The relative rate of ERA AAF is highest among older athletes, reaching respectively 0.37% and 0.96% for INC and OOC sample collection sessions in those over 40 years of age (see Figures S1 and S2).

When considering the distribution of ERA AAFs occurring in samples collected INC versus those collected OOC, it was observed that 62.8% of all ERA AAFs occur in INC samples, while these INC samples account for only 40.6% of all the ERAs analyses performed (Figure 1). Overall, INC sample collection sessions show an ERA AAF rate of 0.21% compared to 0.08% for OOC sample collection sessions (Table 1). This trend was generally stable over the years examined (Figure 1B).

The time-of-day of the sample collection for ERA AAFs was also examined. The greatest number of ERA AAFs was observed during the afternoon (12 pm–6 pm) for INC samples (185) and during the morning (6 am–12 pm) for OOC samples (76). The rate of positivity by time-of-day was calculated by normalizing the number of AAFs by the number of total tests carried out at each period of the day. Using this approach, the rate of positivity across the day for OOC samples was generally stable (see Figure 2), while the rate of positivity was greater in the morning compared to later in the day (6 pm–12 am) for INC samples.

In order to study the potential impact of testing history on the likelihood of obtaining an ERA AAF, the ordinal position of a positive sample relative to all other sample collection sessions for a given athlete across the 2016–2023 period was considered. In this case, all collections were included, regardless of whether an ERA analysis was carried out or not, noting that the athlete would not be aware of whether an ERA analysis would be carried out on any of their samples. It was observed that 43.1% of all ERA AAFs occurred on the first sample ever collected for an athlete, decreasing to 14.1% on the second test, and continuing to decrease for each successive sample collection (see Figure 3A and Table 1). In order to examine the rate of ERA AAFs for each ordinal test number, the number of ERA AAFs was normalized by the total ERA tests carried out on all athletes for each test ordinal number (see Figure 3B). Using this approach a steady decrease in rate of positivity can be observed as athletes are tested more frequently. When separating ERA AAFs occurring INC from those happening OOC, this decreasing trend was more pronounced in those AAFs reported for INC session samples. Finally, the rate of ERA positivity for OOC samples was examined across the test

TABLE 1 | Frequency and relative frequency table of ERA analyses and ERA AAFs.

		ERA Analysis % (% Relative Share)	ERA AAFs (% Relative Share)	ERA AAF Rate (%)
Overall		**23.2%**	**522**	**0.13%**
Gender	M	23.2% (66.1%)	417 (79.9%)	0.16%
	F	23.4% (33.9%)	105 (19.1%)	0.08%
Sport (Top 5)	Endurance	51.4% (49.5%)	438 (83.9%)	0.23%
	Ball and team	13.7% (19.5%)	5 (1.0%)	0.01%
	Power and strength	13.3% (10.3%)	17 (3.3%)	0.04%
	Muscular endurance	27.8% (10.1%)	35 (6.7%)	0.09%
	Combat	15.0% (6.4%)	8 (1.5%)	0.03%
Age categories	Below 15	9.1% (0.9%)	0 (0.0%)	0.00%
	15–19	15.8% (17.9%)	52 (10.0%)	0.11%
	20–24	22.6% (32.7%)	108 (20.7%)	0.09%
	25–29	26.1% (28.4%)	106 (20.3%)	0.09%
	30–34	27.8% (13.8%)	112 (21.5%)	0.18%
	35–39	28.4% (4.3%)	75 (14.4%)	0.37%
	40 and above	2.9% (2.0%)	68 (13.1%)	0.70%
Test ordinal number	1	14.2% (18.9%)	225 (43.1%)	0.31%
	2	17.9% (10.0%)	78 (14.9%)	0.20%
	3	20.4% (7.2%)	40 (7.7%)	0.14%
	4	22.3% (5.7%)	32 (6.1%)	0.15%
	5	23.8% (4.7%)	21 (4.0%)	0.12%
	6	25.1% (4.0%)	17 (3.3%)	0.11%
	7	26.1% (3.5%)	13 (2.5%)	0.10%
	8	27.4% (3.1%)	9 (1.7%)	0.07%
	9	28.3% (2.8%)	8 (1.5%)	0.07%
	10	29.1% (2.5%)	6 (1.1%)	0.06%
	11 to 50	35.4% (32.4%)	70 (13.4%)	0.06%
	More than 50	48.8% (5.3%)	3 (0.6%)	0.01%
Test type	INC	19% (40.6%)	328 (62.8%)	0.21%
	OO1C	28% (59.4%)	194 (37.2%)	0.08%
Time of day	Morning	31% (32.1%)	141 (27.0%)	0.11%
	Afternoon	21% (32.4%)	229 (43.9%)	0.18%
	Evening	20% (33.3%)	140 (26.8%)	0.11%
	Night	23% (2.2%)	12 (2.3%)	0.14%

"ERA Analysis %" indicates the percentage of sample collection sessions with at least one sample analyzed for ERAs. The "% Relative Share" in parentheses indicates the relative share of ERA analyses in terms of ERA analyses of the variable within a given dimension. "ERA AAFs" shows the number of AAFs per variable and the relative share in terms of percentage of ERA AAFs within a given dimension (in parentheses). "ERA AAF Rate" is the percentage of ERA analyses returning an AAF. The sample collection session is considered as an ERA AAF if at least one of the samples collected during the session fulfils the criteria to declare an AAF. Age categories are based on the age of the athlete at the time of the sample collection session. Time of day is defined as Morning (6 am to 12 pm), Afternoon (12 pm to 6 pm), Evening (6 pm to 12 am) and Night (12 am to 6 am).

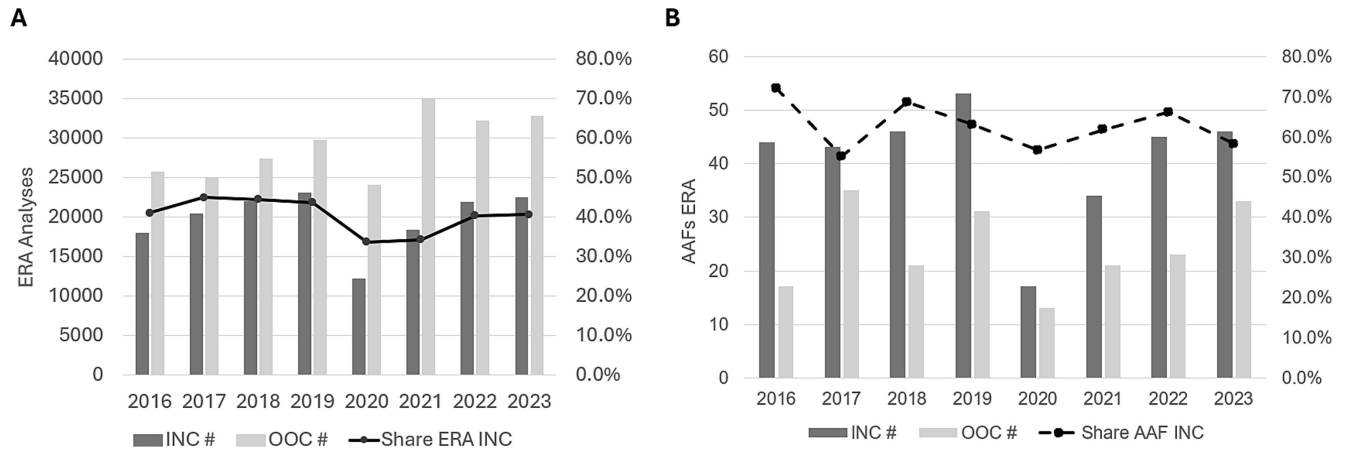

FIGURE 1 | (A) Number of INC and OOC samples with an ERA analysis per year (dark gray: INC #, light gray: OOC #). The solid line indicates the share of INC samples among the ERA analyses performed each year. (B) Number of INC and OOC samples with an ERA AAF per year (dark gray: INC, light gray: OOC). The dashed line indicates the share of INC samples among the AAF of each year.

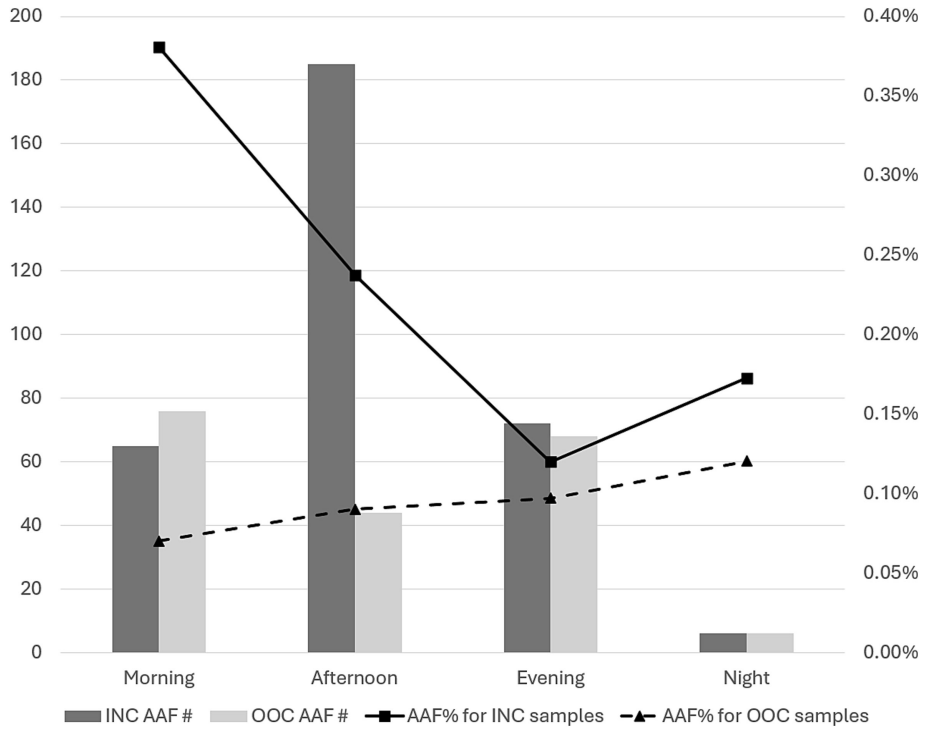

FIGURE 2 | INC and OOC distributions of observed frequencies of ERA AAFs across periods of the day: Morning (6 am–12 pm), Afternoon (12 pm to 6 pm), Evening (6 pm to 12 am), and Night (12 am to 6 am). The relative AAF frequency per ERA analysis is represented with a solid line for INC samples and a dashed line for OOC samples. Note that among the 12 AAFs collected during the night, for eight of them the athletes were notified in the evening and the sample collection sessions then continued into the night, while the rest have a sample collection time between 5 and 6 am.

ordinal number of each OOC sample collection session only (see Figure 3C). The largest decrease in AAF rate is observed between the sample position 1 and 2, decreasing from 0.21% to 0.09%.

Finally, we examined the impact of targeting ERA analysis using the hematological module of the ABP. The hematological module allows the longitudinal profiling of blood variables over time, which can then be used to target suspicious samples for ERA analysis. Our findings indicate that the proportion of ERA tests returning a positive finding is greater for athletes with at least one hematological passport sample compared to those without, with ERA positivity rates of 0.2% and 0.08%, respectively. The difference is even more pronounced among endurance athletes, where the ERA positivity rate is 0.62% for those with a hematological passport, compared to 0.1% for those without. This finding suggests that the ABP positively impacts the likelihood of uncovering ERA doping.

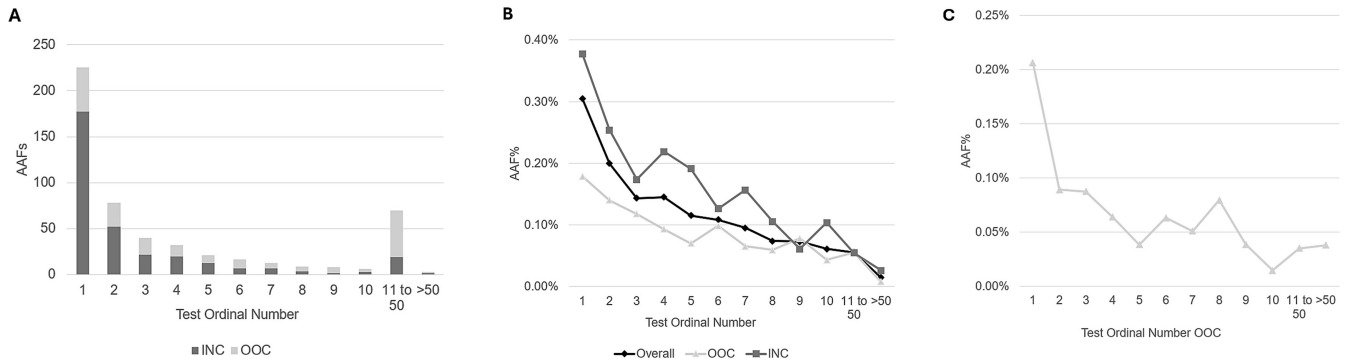

FIGURE 3 | (A) Distribution of the number of ERA analyses returning an AAFs per test ordinal number. (B) %AAF per test ordinal number for all sample types ("Overall"), vs. INC vs. OOC tests. Test ordinal number is defined as the sample collection session chronological order during the observed period (C) %AAF OOC samples per test ordinal number OOC. Test ordinal number OOC is defined as the chronological order of samples collected OOC only.

4 | Discussion

Deterrence theory argues that effective deterrence requires a perception of the risk of punishment [4]. In anti-doping, the risk of a sanction is strongly tied to the risk of being tested. While the ability to investigate has undoubtedly bolstered anti-doping efforts, few sanctions are currently levied without the support of analytical data originating from sample collections. Thus, testing remains the most likely means to catch doping athletes, which, coupled with the fact that athletes are explicitly aware that they have been tested due to their participation in the sample collection process, supports the potential for the act of being tested as having a likely potential for deterrence.

In general, athletes are first tested INC, as it is generally of lower cost to the ADOs to carry out such type of testing, and as an athlete's level increases, they are then included in a registered testing pool to be subjected to OOC testing. Because OOC testing is based on a whereabouts system, whereby athletes provide their location for testing purposes, athletes are also explicitly aware that they could be subjected to OOC testing. In order to test positive during a competition, an athlete must either have mismanaged their doping regime, felt that the risk of being tested was very low, or be uneducated about anti-doping in general and the potential consequences of testing positive. Comparing the number of INC AAFs occurring on the first test ever for an athlete to those occurring on the second test already shows a significant decrease, and by the third test the rate of INC positivity decreases by almost half (Figure 3B). Interestingly, we observed a similar decrease when looking at the positivity rate for OOC samples in function of OOC sample ordinal number with the rate of positivity decreasing by half between the first and the second OOC sample (Figure 3C). The findings suggest that the decrease in ERA positivity rate correlates inversely with the theoretical perceived risk, where the lowest perceived risk would be for an athlete never tested, followed by athletes only tested INC, and the greatest perceived risk being for athletes providing whereabouts and being tested OOC. Such a trend can be observed in overall AAF rates in specific populations, where the greatest number of AAFs are found INC in recreational athletes and the lowest number of AAFs is found in the most tested athlete population providing whereabouts [5].

Even with the theoretical identification of these aforementioned three athlete groups, another degree of heterogeneity in the potentially increasing levels of deterrence could be expected based on the quality of testing by their respective ADOs. Indeed, while some athlete perception surveys indicate that the majority of some athlete populations believe that testing is an effective deterrent to doping [6, 7], other athlete populations have indicated a lower perceived likelihood of being caught when tests are infrequent and predictable [8].

The present analysis assumes similar test "quality" for athletes regardless of their testing history, however it is likely that athletes tested more frequently are subjected to more intelligent testing, including OOC testing, than athletes tested less frequently. Thus, it is possible that the decrease in the rate of positivity is even greater than the one shown by the numbers due to the increase in intelligent testing as athletes are tested more frequently.

The current analysis also assumes a constant sensitivity of the ERA detection method over the period in question (2016–2023). However, several modifications to the analytical method were implemented across this period that could have improved the sensitivity of ERA detection, most notably with the introduction of sarcosyl-polyacrylamide gel electrophoresis (SAR-PAGE) coupled with updated specifications for method characteristics. Although it is challenging to assess the impact of a modest increase in sensitivity on the current conclusions—since the observed ERA AAF rate is due to a combination of true doping prevalence, testing, and method sensitivity—it could be argued that it would increase the rate of ERA positivity on later test ordinal positions across the period examined. This is because the likelihood of a sample being analyzed with the new method increases with its ordinal position, meaning that the average analysis date is, in general, more recent for samples with higher indices.

A decrease in the rate of ERA positivity could be explained by several factors other than by deterrence. It could be speculated that a decrease in AAFs is the result of the use of lower doses, altered timing of administration or doping with other substances. There could be the impact of a "survivorship bias",

whereby the positive findings reduce the ability to detect AAFs at later test ordinal positions (however new athletes are expected to enter the pool at a constant rate). Indeed, without a true measure of doping prevalence, the interpretation of the data presented here is subject to speculation. However, given the difficulties in obtaining reliable estimates of doping prevalence over time, such alternative strategies to obtain objective data to examine the impacts of anti-doping efforts can prove beneficial.

Another weakness of the current analysis is with regards to the truncation of testing history prior to 2016. Undoubtedly, a number of athletes included in the current dataset were actually tested prior to 2016 but were counted as having their testing history beginning in 2016. It is likely that the individuals with a first test being OOC in our dataset were in fact already tested prior to the period covered in this dataset. If this is the case, we may be underestimating the positivity rate for this first test position as some of the samples classified in this test ordinal number are not the athlete's first test. Concordantly, the observed AAF rate in 2016 (INC and OOC) is lower than the one observed for all the other years except 2021 (0.28% for 2016 vs. 0.31% in 2021), which aligns with the expected effect of truncating testing history on the AAF rate. Therefore, it is likely that we may be underestimating the decrease in AAF rate between the first and subsequent test ordinal positions.

The current findings also suggest a positive impact of the ABP on ERA targeting, which supports the observation of a 2–3 fold increase in ERA positivity that occurred upon implementation of the ABP across different sports in 2008–2009 [9]. A more controlled study would be needed to more accurately quantify the impact of the ABP, including an analysis of passport status and the timing of the ERA positive test compared to the first blood sample collected for the purpose of the hematological module of the ABP. However, the impact of the ABP in improving targeting for ERA analysis is balanced against the potential deterrent effect of increased testing, as suggested here, where it could be argued that increased ABP blood testing would eventually reduce the likelihood of obtaining an ERA positive, which is nevertheless also a key target of anti-doping programs. One could hypothesize that there is a tipping point at which the ABP shifts from the benefit of improved ERA targeting on early tests towards deterrence on later tests during an athlete's career. The same could be said for testing in general and would argue against simply using rates of AAFs as a surrogate for success in anti-doping.

The present findings raise several perspectives, including whether it would be more effective to allocate a greater number of ERA tests to the first tests in an athlete's testing history and whether testing more athletes earlier in their careers would improve deterrence. It is also unknown what frequency of testing is required to maintain an established deterrent effect. It may also be useful to compare rates of ERA positivity with methods for assessing blood doping prevalence [10] in order to better understand the relationship between the two metrics. Finally, the analysis approach taken here could also be applied to other substances, such as steroid AAFs, and may provide complementary information to that obtained from doping prevalence estimates or from annual testing statistics.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are subject to contractual and/or privacy restrictions.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section.