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Special Issue

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Biomarkers of Recent Cannabis Use in Blood, Oral Fluid and Breath

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Abstract

Proving driving under the influence of cannabis (DUIC) is difficult. Establishing a biomarker of recent use to supplement behavioral observations may be a useful alternative strategy. We determined whether cannabinoid concentrations in blood, oral fluid (OF) or breath could identify use within the past 3 h—likely the period of the greatest impairment. In a randomized trial, 191 frequent (≥4/week) and occasional (<4/week) cannabis users smoked one cannabis (placebo [0.02%], or 5.9% or 13.4% Δ^9-tetrahydrocannabinol [THC]) cigarette *ad libitum*. Blood, OF and breath samples were collected prior to and up to 6 h after smoking. Samples were analyzed for 10 cannabinoids in OF, 8 in blood and THC in breath. Frequent users had more residual THC in blood and were more likely to be categorized as 'recently used' prior to smoking; this did not occur in OF. *Per se* limits ranging from undetectable to 5 ng/mL THC in blood offered limited usefulness as biomarkers of recent use. Cannabinol (CBN, cutoff = 1 ng/mL) in blood offered 100% specificity but only 31.4% sensitivity, resulting in 100% positive predictive value (PPV) and 94.0% negative predictive value (NPV) at 4.3% prevalence; however, CBN may vary by cannabis chemovar. A 10 ng/mL THC cutoff in OF exhibited the overall highest performance to detect its use within 3 h (99.7% specificity, 82.4% sensitivity, 92.5% PPV and 99.2% NPV) but was still detectable in 23.2% of participants ~4.4 h post-smoking, limiting specificity at later time points. OF THC may be a helpful indicator of recent cannabis intake, but this does not equate to impairment. Behavioral assessment of impairment is still required to determine DUIC. This study only involved cannabis inhalation, and additional research evaluating alternative routes of ingestion (i.e., oral) is needed.

Introduction

Higher blood Δ^9-tetrahydrocannabinol (THC) concentrations are associated with increased crash risk (1–7), but defining cutoffs for safe driving limits is difficult. This is because blood THC concentration varies by smoking topography, frequency of use and route of ingestion (8–10). Previous studies demonstrated weak relationships between THC blood concentration and driving performance (7, 11), with impaired psychomotor function more pronounced in occasional than frequent users (12). Peak blood THC concentrations occur during smoking, but drop rapidly, while subjective 'high' per-

sists for several hours and varies significantly between users (13). Furthermore, THC remains detectable in the blood of chronic frequent cannabis users longer than occasional users (14, 15). Instead of trying to find a biomarker that indicates impairment, a more practical approach may be to identify a biomarker that indicates recent use. This can then be combined with officer observations to identify DUIC (16).

Despite growing cannabis popularity, not all states have established *per se* laws or legally allowable blood detection limits for DUIC. Current *per se* limits range from zero tolerance to 5 ng/mL (17). To date, few states have any restriction on THC metabolite concentrations. When using sensitive measurement techniques with lower limits of quantification (LLOQs) of 0.25–0.5 ng/mL, THC and certain metabolites can be detected in blood for weeks to months after use and do not necessarily indicate impairment (14). Therefore, current *per se* limits may lead to false accusations of DUIC.

The goal of this study was to investigate biomarkers as indicators of recent cannabis use. Recent use was defined as smoking within the last 3 h because the greatest impairment is observed within that time frame, although some aspects of impairment may persist beyond that period (7, 11, 18, 19). Blood, OF and breath were collected immediately before and at various time points after smoking. Sensitivity and specificity of up to 10 different compounds in each fluid were determined and predictive values based on previously published prevalence data were estimated.

Methods

Recruitment and requirements of study participants

This placebo-controlled, double-blinded randomized study was conducted under guidelines outlined in the Declaration of Helsinki and approved by the University of California, San Diego Human Research Protections Program (Institutional Review Board no. 160641). Volunteers 21–55 years old with a valid driver's license who self-reported cannabis use at least four times in the past month were recruited and pre-screened. Demographic information can be found in the study of Hoffman et al. (20). Participants were classified as 'frequent' or 'occasional' users based on self-reported cannabis use of $\geq$4/week or <4/week, respectively. Participants were compensated and medically evaluated prior to and during their visit for safety. They were asked to refrain from cannabis use for at least 2 days prior to participation and their OF was screened for recent use with a Dräger Drug Test 5000. Anyone ($n = 7$) with OF THC concentration $\geq$5 ng/mL or who were positive for any other drugs on the Dräger OF test were excluded. After exclusion criteria were applied, 191 participants were included and randomly assigned to receive a cigarette containing placebo (0.02%), or 5.9% or 13.4% THC (Supplementary Figure S1). Use patterns between the placebo (54% occasional users), and 5.9% (50%) and 13.4% (50%) THC content groups were not significantly different. In a negative pressure room, participants smoked a 700 mg cigarette *ad libitum* within 10 min, with a minimum of four puffs. Blood, OF and breath were collected prior to smoking. After smoking, 4 additional OF and breath and 8 blood collections were completed at time points up to ~6 h from the start of smoking. Participants ate and drank water between collections, although not within 10 min of OF collection.

OF was collected with the Quantisal™ device (Alere, Inc., San Diego, CA, USA). The absorptive pad was placed under the tongue until the indicator turned blue (indicating 1 mL OF collected) or until 10 min passed. The pad was placed in 3 mL extraction/stabilization buffer in the supplied tube, capped and stored at room temperature for 4–24 h. The pad was removed and decanted, and the remaining buffer was transferred into Nunc 3-mL cryovials (Wheaton, Millville, NJ, USA) and stored at 4°C for up to 2 months (21). Venous blood was collected from the arm in gray-top vacutainer tubes containing sodium fluoride and potassium oxalate, and 2 mL blood was transferred to Nunc vials and stored at −20°C for up to 3 months (22). Aerosol breath was collected for ~3 min via exhalation into a SensAbues® device (AB, Sweden). Mouthpieces were discarded and the device containing the collection pad was capped and stored at 20°C for up to 6 months (23). Samples were analyzed by liquid chromatography–tandem mass spectrometry by previously described methods (24, 25). LLOQs were as follows: in OF, 0.4 ng/mL THC, Δ^9-tetrahydrocannabinol-glucuronide (THC-gluc), 11-hydroxy-Δ^9-tetrahydrocannabinol (11-OH-THC), cannabidiol (CBD), Δ^9-tetrahydrocannabivarin (THCV) and CBN, and 1.0 ng/mL 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol-glucuronide (THCCOOH-gluc), 11-nor-9-carboxy-Δ^9-tetrahydrocannabinol (THCCOOH), cannabigerol (CBG) and Δ^9-tetrahydrocannabinolic acid A (THCA-A); in blood, 0.5 ng/mL THC, CBD, THCV and CBN, 1.0 ng/mL CBG, THCCOOH and 11-OH-THC, and 2.0 ng/mL THCCOOH-gluc; and in breath, 80 pg/pad THC.

Statistical analysis

Data were analyzed with R (v3.6.0) in RStudio (Boston, MA); visualizations were generated by ggplot2 (v3.3.2) (26). *t*-tests compared compound concentrations between frequent and occasional users at the pre-smoking time point. The *P*-values were adjusted for number of comparisons using Bonferroni correction.

Time post-smoking was divided into time windows. All samples collected between pre-smoking to 3 h ($n = 908$ for blood, $n = 601$ for OF and $n = 588$ for breath) were included in all analyses for recent use. Participants had a mean (standard deviation) of 4.8 (0.9) blood, 3.1 (0.4) OF and 3.1 (0.3) breath samples within that time frame.

Receiver operator characteristic (ROC) curves were generated by plotting the percentage sensitivity versus (100 − specificity) using various time windows pre- and post-smoking. A Youden's *J* statistic ('Youden's index') was calculated (J = sensitivity + specificity − 1) to determine optimal cutoffs; it weighed sensitivity and specificity contribution equally (27). A perfect test provides a Youden's index of one whereas a test yielding no useful information a Youden's index of zero. For example, a test with 80% sensitivity and 80% specificity would yield a Youden's index of 0.6.

Sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) were calculated for each biomarker of recent use (within 3 hours). Participants receiving 5.9% and 13.4% THC were grouped together because cannabis users are known to 'self-titrate' or smoke until the desired effects are achieved (28). A participant receiving placebo whose analyte concentration was below the specified cutoff was considered a true negative. A participant receiving either the 5.9% or 13.4% THC and whose analyte concentration was greater than or equal to the cutoff was considered a true positive. A participant receiving the placebo whose analyte concentration was greater than or equal to the cutoff was considered a false positive. A participant receiving active THC whose analyte concentration was below a cutoff was a false negative. At the pre-smoking time point, any participant whose analyte concentration was below the specified cutoff was considered a true negative whereas any participant whose analyte concentration was greater than or equal to the specified cutoff was considered a false positive, regardless of group assignment. The prevalence for PPV and NPV calculations was the percentage of weekend nighttime drivers

who tested positive for THC in various jurisdictions in California (29). This ranged from 4.3% in Fresno to 18.3% in Eureka with an average prevalence of 8.5% (29).

The relationships of THC across matrices and between THC and CBN within a single matrix were determined by linear regression, reporting the effect size estimated by the coefficient (β_1), *y*-intercept (β_0) and correlation coefficient (*r*) for each. Relationships were confirmed with a Spearman correlation (data not shown). A heatmap calculating the Pearson correlation (*r*) between the various compounds within the same matrix was generated for blood and OF.

Results

Detection of cannabinoids in blood, OF and breath

Prior to smoking, frequent users had higher blood concentrations of THC, 11-OH-THC, THCCOOH and THCCOOH-gluc compared to occasional users (Figure 1). A notable outlier in the pre-smoking time point was a frequent user who had the highest concentrations of THC (16.2 ng/mL), 11-OH-THC (9.3 ng/mL), THCCOOH (133 ng/mL) and THCCOOH-gluc (571 ng/mL) in the study. CBN, CBD, CBG and THCV in blood, all compounds except THC in OF, and THC in breath are not displayed in Figure 1 because <10 participants had detectable concentrations prior to smoking. No significant difference in median THC concentrations in OF was observed between frequent and occasional users at the pre-smoking time point.

The median and range cannabinoid concentrations detected in blood, OF and breath are listed in Supplementary File 1; any compound not detected in any participants in a particular matrix was excluded from this file. THC, THCCOOH and THCCOOH-gluc were detected in blood in the majority of samples post-smoking whereas THCV and CBD were rarely detected.

In OF, CBN, CBD, THC, CBG, THCV and THCA-A were detected after smoking whereas the metabolites THCCOOH, THC-gluc and THCCOOH-gluc were not at LLOQs of 1.0, 0.4 and 1.0, respectively. 11-OH-THC was only detected in seven OF samples, all at low concentrations.

THC in breath was measurable in 99.2% of participants within 40 min post-smoking either 5.9% or 13.4% THC and 25.4% in those who received placebo, which contained 0.02% THC. After smoking (41–90 min), THC was detected in 37% of participants who received active THC, and only one participant in the placebo group.

Biomarker potential for determining recent use

Cutoffs for recent use were optimized utilizing ROC curves (Supplementary Figure S2) and Youden's *J* statistic (Supplementary Table SI) with data from pre-smoking to 3 h post-smoking. In blood, 1.6 ng/mL THC yielded the highest Youden's index of 0.54. Although CBN exhibited a lower Youden's index (0.48) with a cutoff of 0.5 ng/mL, it offered 98.2% specificity.

A 3.8 ng/mL THC cutoff in OF exhibited the highest overall sensitivity and specificity with a 0.87 Youden's index. In OF, only five (2.6%) participants had concentrations ≥3.8 ng/mL pre-smoking. CBN had the next highest Youden's index (0.76) at 0.6 ng/mL cutoff. CBN was detectable in three participants at ≥0.6 ng/mL prior to smoking.

Breath was an excellent biomarker of use within the first 40 min post-smoking. After this time, most participants had undetectable concentrations and the utility of THC in breath as a biomarker was diminished.

Considering the potential delay between smoking and a traffic stop fluid collection, we calculated the ideal Youden's index for the last data point of each participant within 3 h after smoking, representing ~1.5–3 h post-smoking (Supplementary Table SII). The highest Youden's index (0.90) was a 0.9 ng/mL THC OF cutoff.

Performance of THC cutoffs in blood and OF

The Department of Health and Human Services recommended a 4 ng/mL THC cutoff in OF for workplace drug testing (30). For recent use, alternative cutoffs may be necessary. The sensitivity and specificity of these cutoffs and current *per se* limits were determined (Figure 2). In blood, false positives were observed in the pre-smoking time window resulting in decreased specificity. In OF, the specificity at the pre-smoking time point was 100% for all cutoffs ≥5 ng/mL, but this was an artifact of study design. Participants with ≥5 ng/mL THC in OF were excluded from the study to eliminate confounding factors from self-administered cannabis prior to study initiation. With lower OF cutoffs, false positives were noted in the pre-smoking time point, but at a lower rate than in blood. The specificity of each cutoff for OF dropped significantly 0–30 min after smoking due to the presence of 0.02% THC in the placebo (Supplementary Figure S1). Except for this dip, the specificity was superior to that of blood.

Alternative biomarker correlations within and between matrices

We explored correlations between and within matrices in various time windows to identify biomarkers that could serve as a 'proxy'

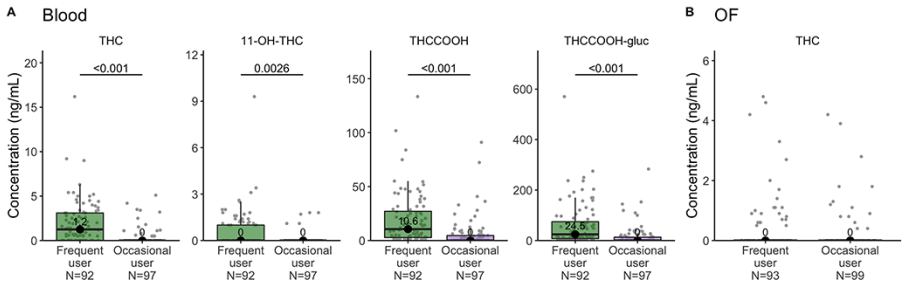

Figure 1. Cannabinoid blood and OF concentrations prior to smoking cannabis. Box and whisker plots represent the median and 1.5× the interquartile range of concentrations. (A) Median concentrations of THC, 11-OH-THC, THCCOOH and THCCOOH-gluc in blood and (B) THC in OF in frequent (green) and occasional (purple) users prior to smoking cannabis. Median concentrations are written for each compound and 0 indicates ≤ LLOQ. The number of users (*N*) included in each group is listed.

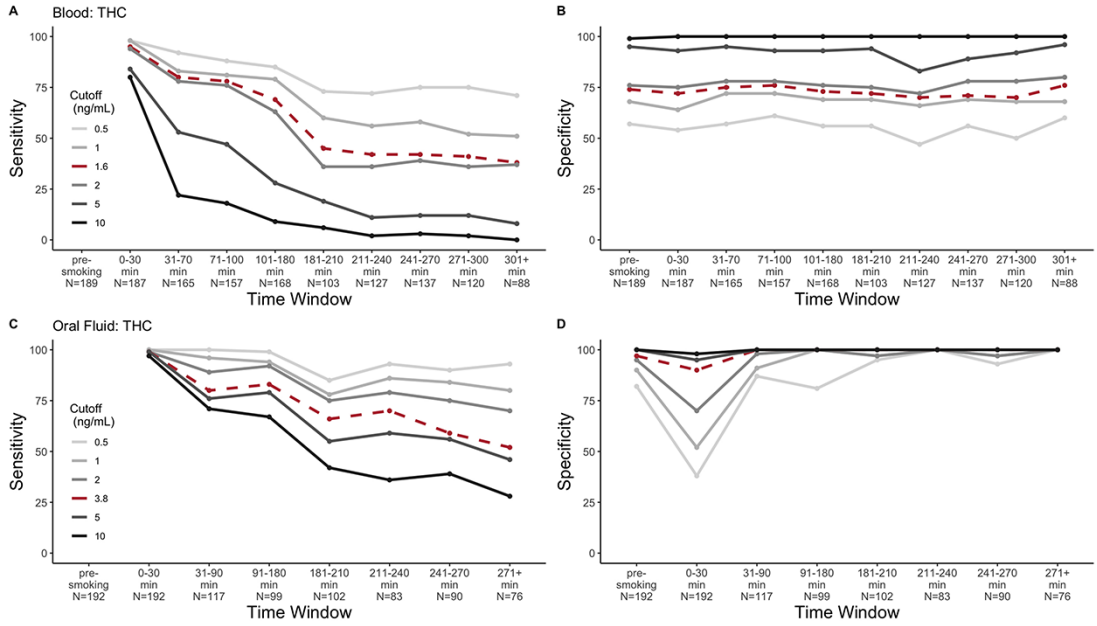

Figure 2. Sensitivity and specificity of select cutoffs for THC in blood and OF. Graph of percentage (A) sensitivity and (B) specificity over time using different blood THC cutoffs to determine recent use. The cutoff (1.6 ng/mL) with the highest Youden's index, maximizing both sensitivity and specificity, is represented by the dashed red line. Graphs of percentage (C) sensitivity and (D) specificity over time using different OF THC cutoffs to determine recent use. The cutoff (3.8 ng/mL) with the highest Youden's index is represented by the dashed red line. The vertical dotted line indicates the end of the 0–3 h time window. The number of users (*N*) included in each time window is listed.

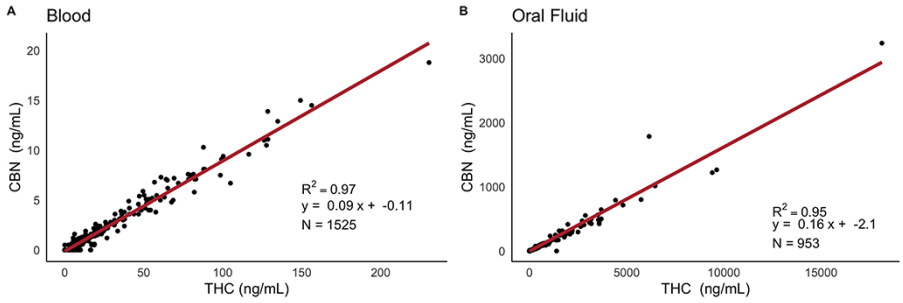

Figure 3. Within matrix correlation of THC and CBN. Linear regression correlation between THC and CBN concentrations (ng/mL) in (A) blood and (B) OF for all participants. The linear regression equation and coefficient of determination (R^2) is listed. The number of users (*N*) included in each analysis is listed.

for blood THC concentrations. While there is a significant correlation ($p < 0.001$) between concentration of THC in blood and OF, the correlation is modest ($r = 0.41$) and it is not possible to accurately predict THC WB concentrations from OF or breath specimens. (Supplementary Figures S3–S6). However, CBN and THC exhibited a strong correlation in both blood and OF (Figure 3, Supplementary Figure S7). Therefore, we explored the biomarker potential of CBN.

Performance of CBN cutoffs in blood and OF

The specificity of CBN in blood as a recent use biomarker remained excellent throughout the study, but the sensitivity dropped quickly over time (Figure 4). CBN in OF offered better sensitivity than in blood and had excellent specificity at the pre-smoking time point with all cutoffs used. A drop in specificity was noted 0–30 min post-smoking due to the small amount of CBN present in the placebo (Supplementary Figure S1).

To highlight the optimal cutoff concentrations, the sensitivity and specificity for THC and CBN in blood and OF at select concentrations within 3 h of smoking were plotted (Figure 5). THC (3.8 ng/mL cutoff) and CBN (0.6 ng/mL cutoff) in OF offered superior overall sensitivity and specificity compared to the same biomarkers in blood.

PPV and NPV of CBN and THC as biomarkers of recent use

PPV and NPV of THC and CBN in blood and OF at select concentrations were calculated (Table I). The frequency of driving after 'recent' cannabis use is unknown and likely lower than the average prevalence of THC-positive drivers (29); therefore, we focused on 'low prevalence' data. THC in blood at 0–5 ng/mL cutoffs exhibited poor performance. A zero tolerance limit had 56.9% specificity with 8.6% PPV. A 5 ng/mL cutoff offered 94.2% specificity and 97.7% NPV, but only 51.7% sensitivity.

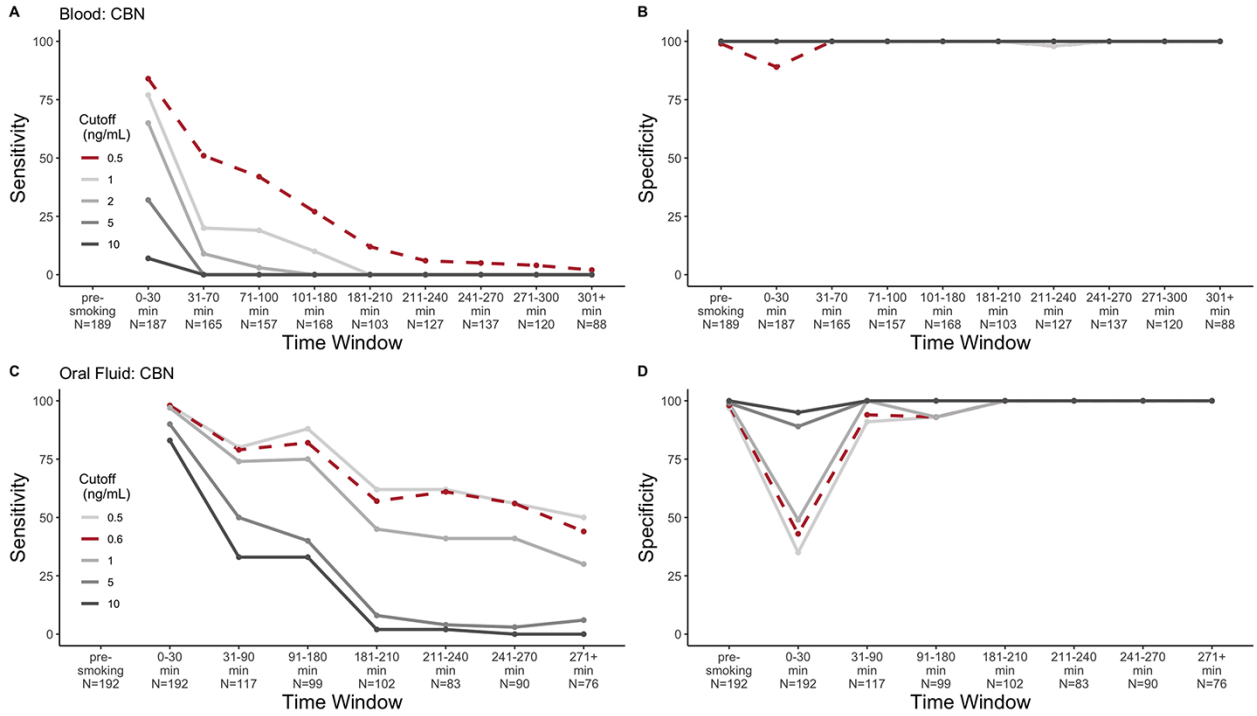

Figure 4. Sensitivity and specificity of select cutoff concentrations for CBN in blood and OF. Graph of percentage (A) sensitivity and (B) specificity over time with different blood CBN cutoffs to determine recent use. The cutoff (0.5 ng/mL) with the highest Youden's index in blood, maximizing both sensitivity and specificity, is represented by the dashed red line. Graph of percentage (C) sensitivity and (D) specificity over time with different OF cutoffs to determine recent use. The cutoff (0.6 ng/mL) with the highest Youden's index is represented by the dashed red line. The vertical dotted line indicates the end of the 0–3 h window. The number of users (*N*) included in each time window is listed.

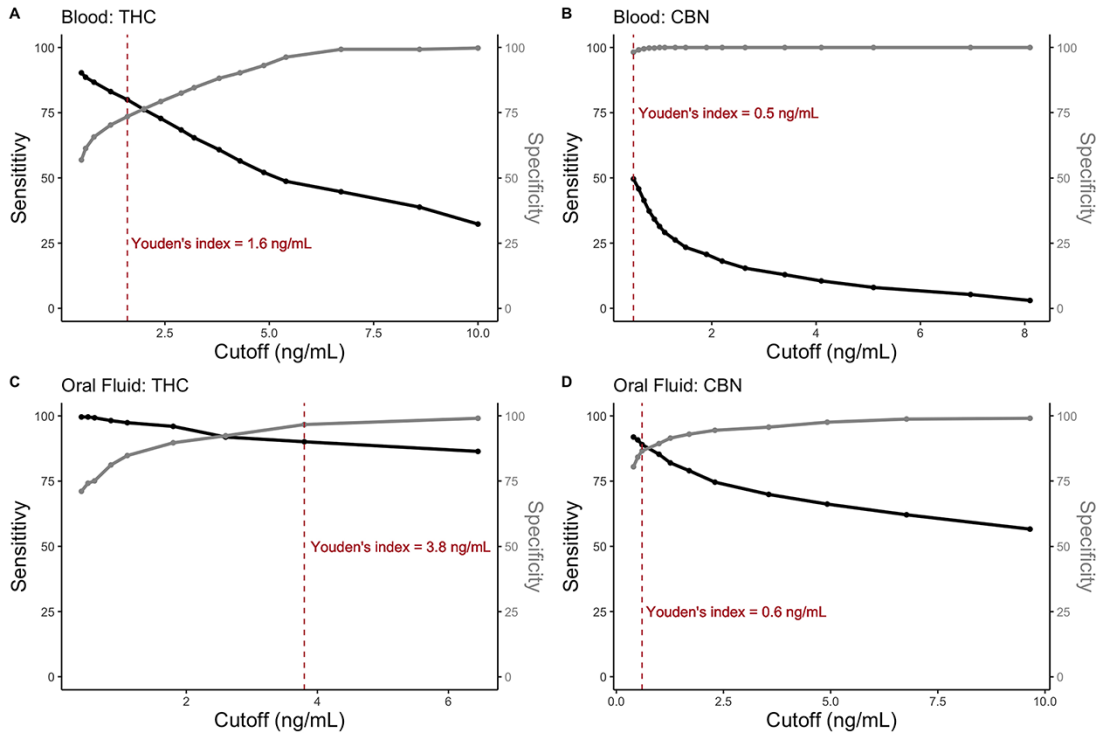

Figure 5. Sensitivity and specificity of THC and CBN in blood and OF within 3 h of smoking cannabis. Percentage sensitivity (left *y*-axis; black) and percentage specificity (right *y*-axis; gray) are plotted against cutoff concentrations of (A) THC and (B) CBN in blood as well as (C) THC and (D) CBN in OF. The vertical red dashed line indicates the Youden's index.

Table I. PPV and NPV of CBN and THC as Biomarkers of Recent Use (Within 3 h Post-Smoking) in Blood and OF

					Low prevalence 4.3%		Average prevalence 8.5%		High prevalence 18.3%	
Biomarker	Matrix	Cutoffs (ng/mL)	% Sensitivity	% Specificity	PPV	NPV	PPV	NPV	PPV	NPV
THC	Blood	0.5	90.3	56.9	8.6	99.2	16.3	98.4	31.9	96.3
THC	Blood	1	84.6	68.4	10.7	99.0	19.9	98.0	37.5	95.2
THC	Blood	1.6	80	73.5	11.9	98.8	21.9	97.5	40.3	94.3
THC	Blood	2	76.2	76.5	12.7	98.6	23.1	97.2	42.1	93.5
THC	Blood	5	51.7	94.2	28.6	97.7	45.3	95.5	66.6	89.7
THC	Blood	10	32.3	99.8	87.9	97.0	93.8	94.1	97.3	86.8
THC	OF	0.5	99.6	74.2	14.8	100	26.4	99.9	46.4	99.9
THC	OF	1	97.4	83.6	21.1	99.9	35.6	99.7	57.1	99.3
THC	OF	2	94.5	91.2	32.5	99.7	49.9	99.4	70.6	98.7
THC	OF	3.8	90.1	96.7	55.1	99.5	71.7	99.1	85.9	97.8
THC	OF	5	87.9	99.1	81.4	99.5	90.1	98.9	95.6	97.3
THC	OF	10	82.4	99.7	92.5	99.2	96.2	98.4	98.4	96.2
CBN	Blood	0.5	49.6	98.2	55.3	97.7	71.9	95.4	86.1	89.7
CBN	Blood	1	31.4	100	100	97.0	100	94.0	100	86.7
CBN	Blood	2	19.8	100	100	96.5	100	93.1	100	84.8
CBN	Blood	5	8.4	100	100	96.0	100	92.2	100	83.0
CBN	Blood	10	1.9	100	100	95.8	100	91.6	100	82.0
CBN	OF	0.5	90.8	84.2	20.5	99.5	34.8	99.0	56.3	97.6
CBN	OF	0.6	89	86.6	23.0	99.4	38.2	98.8	59.8	97.2
CBN	OF	1	85.3	89.4	26.6	99.3	42.8	98.5	64.3	96.4
CBN	OF	5	66.2	97.6	55.3	98.5	71.9	96.9	86.1	92.8
CBN	OF	10	56.6	99.1	73.9	98.1	85.4	96.1	93.4	91.1

Table II. Percentage of All Participants (Placebo, 5.9% THC and 13.4% THC Groups) above Specific Cutoffs in Blood and OF at Study Completion

Matrix	Compound	Cutoff	% Above cutoff
Blood	THC	0.5	54.0
Blood	THC	1.0	41.6
Blood	THC	1.6	34.2
Blood	THC	2.0	30.4
Blood	THC	5.0	6.2
Blood	THC	10.0	0.6
OF	THC	0.5	59.5
OF	THC	1.0	54.7
OF	THC	2.0	50.0
OF	THC	3.8	39.5
OF	THC	5.0	35.8
OF	THC	10.0	23.2
Blood	CBN	0.5	0.6
Blood	CBN	1.0	0.0
Blood	CBN	2.0	0.0
Blood	CBN	5.0	0.0
Blood	CBN	10.0	0.0
OF	CBN	0.5	35.8
OF	CBN	0.6	31.6
OF	CBN	1.0	23.7
OF	CBN	5.0	2.6
OF	CBN	10.0	0.5

At all cutoffs examined, THC in OF offered a NPV >99% with 4.3% prevalence. The overall best performing cutoff was 10 ng/mL with 82.4% sensitivity, 99.7% specificity, 92.5% PPV and 99.2% NPV. The Youden's index cutoff (3.8 ng/mL) had better overall sensitivity and specificity, but at the expense of 55.1% PPV. As prevalence increased, the PPV increased while the NPV decreased.

CBN in blood offered 100% specificity and PPV at all concentrations ≥1 ng/mL. Even though this was at the cost of low sensitivity, the NPV was ≥95.8% with 4.3% prevalence. The highest performing OF CBN cutoff was 10 ng/mL with 98.1% NPV and 73.6% PPV.

Performance of biomarkers beyond 3 h post-smoking

To assess biomarkers' performance beyond 3 h, we determined the percentage of participants (placebo, 5.9% THC and 13.4% THC groups) above the recommended cutoffs at the end of our study, including only data points beyond 3 h, representing a mean of 5.1 h post-smoking in blood and 4.4 h in OF (Table II). With a zero tolerance cutoff of THC in blood, 54.0% were still categorized as 'recently used'. In OF, 39.5% and 23.2% of participants had concentrations above 3.8 ng/mL and 10 ng/mL THC, respectively. CBN was detectable in 0.6% of participants in blood at study completion.

Discussion

This study determined the potential of cannabinoids as biomarkers of recent cannabis use. It was the first to examine cannabinoids as biomarkers of recent use with a large sample size and high THC (13.4%) content cannabis. This approaches THC content in currently marketed cannabis (31). Participants receiving 5.9% and 13.4% THC were grouped together because cannabis users are known to self-titrate to achieve the desired effects (28). Hoffman et al. (20) found that the number of puffs did not differ between the 5.9% and 13.4% groups, but participants who received 5.9% THC had higher concentrations of most cannabinoids in blood than those

who received 13.4% THC. Dose delivery, such as depth of inhalation and breath holds, may account for this difference (32). Subjects take smaller inhalation volumes and shorter puffs with higher-potency cannabis (9). Therefore, the concentration of THC in cannabis is not the primary determinant of the resulting THC concentrations in blood. Due to self-titration, previous studies using lower potency cannabis ranging from 2.9 to 6.7% (33, 34) likely accurately reflect the physiological and cognitive responses to currently marketed cannabis.

Previous studies estimated time since cannabis use with mathematical models and blood/plasma cannabinoid concentrations (35–37). These were good predictors after both single and multiple doses of cannabis. However, controlled dosing was used and models failed in chronic frequent smokers during sustained abstinence (37). Predicting time since dosing based on a single blood sample is difficult without knowing the baseline concentrations. We sought to identify a marker that could predict recent use without multiple sample collections or mathematical modeling.

Smoking cannabis reduces driving performance (7), but no data unequivocally demonstrate that state-specific cutoffs equate to impairment (29, 38). As recent use markers, *per se* cutoffs were limiting as several participants had detectable concentrations prior to smoking, including an outlier with 16.2 ng/mL THC. At study completion, this participant's THC concentration returned to the pre-smoking concentration (16.5 ng/mL), suggesting chronic past use, but cannabis ingestion shortly before study participation cannot be ruled out. An additional limitation to blood biomarkers is the ∼1.5 h delay between a traffic stop and blood draw. In that time, blood THC concentrations drop by as much as 90% (38). If blood is the preferred matrix, CBN offered superior overall performance with 100% specificity (cutoff ≥1 ng/mL) for detection of use within 3 h. CBN concentration in blood drops faster than THC and is less likely to be detected at times >3 h (past the time of maximal impairment). A drawback is that CBN concentration varies with both the cannabis preparation and age of cannabis, because CBN is a primary degradation product of THC (39, 40).

Prior to smoking, frequent users had higher concentrations of THC in blood than occasional users, likely due to residual drug from past self-administration. This makes it difficult to find a uniform national approach to DUIC and THC blood concentrations in a court of law (41). No statistical difference in pre-smoking OF THC concentration existed between frequent and occasional users. OF may eliminate the bias of frequent past use seen in blood.

OF can easily be collected roadside with currently marketed collection devices (42–44). A concentration of 10 ng/mL THC in OF offered the overall highest performance for a biomarker of use within 3 h post-smoking. The specificity was <100% because 0.02% THC was present in the placebo and detectable in OF. In a real-world application, the specificity would likely be higher. PPV and NPV remained >90% at all prevalence values examined and would likely perform well within 3 h after smoking. However, 23.2% of participants still had concentrations of THC ≥10 ng/mL at an average of 5 h after smoking. While the exact time window of impairment is unknown and varies in the literature (45), THC will likely remain detectable in OF after maximal impairment ends, limiting its long-term specificity.

Several cannabinoids were not considered for biomarkers of recent use. In blood, 11-OH-THC, THCCOOH and THCCOOH-gluc are detected for days to several weeks after smoking cessation in chronic users (14) and were rarely detected in OF. CBD is a poor biomarker because its concentration varies significantly between cannabis preparations and is offered in formulations without the presence of THC, the psychoactive component of cannabis (46). CBG, THCV, THC-gluc and THCA-A were infrequently detected and/or offered limited specificity in either blood or OF. THC in breath quickly dissipated after smoking and would be an excellent marker of use within 40 min, but has limited potential thereafter.

This study has several limitations. Subjects were dosed in a single 10-min period. Only inhalation as a route of administration and a single source of 5.9% and 13.4% THC cannabis was evaluated. Route and chemovar vary according to user preference, and peak THC concentrations occur significantly later after oral administration (8,47). Cannabis chemovar affects the concentration of certain phytocannabinoids such as CBN and CBD, thereby limiting their potential as biomarker proxies for THC. Participants were asked to refrain from smoking for 2 days prior to the study and were excluded if their OF THC concentrations exceeded 5 ng/mL; this may not accurately reflect cannabis consumption of the average user. Additionally, dry mouth is a common side effect of cannabis use. About half the OF samples collected immediately post-smoking had less volume than suggested by the manufacturer of the Quantisal™ device. A Kruskal–Wallis test demonstrated no statistically significant difference in OF THC concentration between those with sufficient and insufficient volumes, so all OF samples were in the analyses. The prevalence used in our calculations did not distinguish between recent and past use. Not all participants completed the targeted goal of nine blood collections. Several participants only donated the first few blood draws but were still included in the analysis, resulting in more samples at earlier time points. Breath samples were extracted from SensAbues® devices, and the possibility of OF contamination cannot be entirely ruled out.

A biomarker of recent cannabis use would fulfill the critical need to determine who recently smoked cannabis and could corroborate or contradict police officer observations. Recent use should not be confused with driving impairment that requires documented physiological and behavioral changes that adversely affect driving performance. THC concentrations in OF may offer a means to determine recent use in an easy-to-collect matrix. Although we show promising data for CBN in OF as a biomarker of recent use, this does not replace the need for behavioral assessment of impairment. Additional research with alternative routes of cannabinoid administration and varying dosing conditions are needed.

Supplementary data

Supplementary data is available at *Journal of Analytical Toxicology* online.

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Data Availability

The data underlying this article are available upon contacting the Center for Medicinal Cannabis Research at cmcr@ucsd.edu.

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