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RESEARCH ARTICLE

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Detectability of cannabinoids in the serum samples of cannabis users: Indicators of recent cannabis use? A follow-up study

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Abstract

Forensic toxicologists are frequently required to predict the time of last cannabis consumption. Several studies suggested the utility of minor cannabinoids as indicators of recent cannabis use. Because several factors influence blood cannabinoid concentrations, the interpretation of serum cannabinoid concentrations remains challenging. To assess the informative value of serum cannabinoid levels in cannabis users (in total $N = 117$ patients, including 56 patients who stated an exact time of last cannabis use within 24 h before blood sampling), the detectability of cannabinoids, namely, delta-9-tetrahydrocannabinol (delta-9-THC), 11-hydroxy-delta-9-THC, 11-nor-9-carboxy-delta-9-THC, cannabichromene (CBC), cannabidiol (CBD), cannabinol (CBN), cannabidivarin, tetrahydrocannabivarin, cannabigerol (CBG), cannabicyclol, delta-8-THC, tetrahydrocannabinolic acid A, cannabichromenic acid, cannabidiolic acid (CBDA), cannabigerolic acid, cannabicyclolic acid (CBLA), 11-nor-9-carboxy-THCV (THCVCOOH), and 11-nor-CBN-9-COOH, was investigated. Excluding CBDA and CBLA, all investigated cannabinoids were detected in at least one analyzed sample. The interval between cannabis consumption and sample collection (reported by the patients) was not correlated with cannabinoid concentrations. Minor cannabinoids tended to be more easily detected in samples obtained shortly after consumption. However, some samples tested positive for minor cannabinoids despite an interval of several hours or even days between consumption and sampling (according to patients' statements). For instance, CBC, CBG, THCVCOOH, CBD, and CBN in certain cases could be detected more than 24 h after the last consumption of cannabis. Thus, findings of minor cannabinoids should always be interpreted with caution.

KEYWORDS
cannabinoids, LC–MS/MS, serum

1 | INTRODUCTION

According to current scientific knowledge, cannabis-related impairment is especially expected within the first 2 h after consumption, whereas individual performance deficits may persist for up to 8 or even 24 h after consumption.[1] Because of the temporal occurrence of cannabis-induced impairment, forensic toxicologists are frequently required to determine the time of consumption to evaluate the degree of influence at the time of a legally relevant event (e.g., driving a car under the influence of cannabis).

Usually, delta-9-tetrahydrocannabinol (delta-9-THC) and its main metabolites 11-hydroxy-delta-9-THC (11-OH-THC) and 11-nor-9-carboxy-delta-9-THC (THCCOOH) are determined in the routine forensic toxicological analysis of blood samples (including serum or plasma). Because of the limited detection times of delta-9-THC and 11-OH-THC in serum or plasma,[2,3] their concentrations provide an initial rough estimation of the time of consumption (at least in occasional cannabis users).

Based on the kinetics of delta-9-THC and its main metabolites, Huestis et al. developed mathematical models (considering the delta-9-THC and THCCOOH concentrations) for estimating the last time of cannabis consumption.[4,5] However, delta-9-THC is distributed into tissues, such as adipose tissue.[6–10] After frequent cannabis consumption, delta-9-THC can accumulate in adipose tissue, in which it can be retained.[6,10] The subsequent release of delta-9-THC from adipose tissue (resulting in a long terminal half-life of delta-9-THC in blood[6]) can complicate the interpretation of blood concentrations of delta-9-THC and its metabolites. Toennes et al. stated that the blood concentrations of delta-9-THC and its main metabolites in a chronic cannabis user in the late elimination phase can be similar to those of an occasional user recently exposed to cannabis products.[2] Other study results uncovered enhanced delta-9-THC concentrations in heavy cannabis users even more than 24 h after the discontinuation of cannabis use.[11] Bergamaschi et al. revealed that delta-9-THC can be detected in blood up to 30 days after chronic daily cannabis use despite abstinence.[12]

Another approach to estimating the last time of cannabis consumption is the use of minor cannabinoids as markers of recent consumption. Several studies on cannabinoid pharmacokinetics suggested the utility of minor cannabinoids or the phase II metabolite delta-9-THC-glucuronide as indicators of acute intake. In particular, the minor cannabinoids cannabidiol (CBD), cannabinol (CBN), cannabigerol (CBG), tetrahydrocannabivarin (THCV), and 11-nor-9-carboxy-THCV (THCVCOOH) were described as markers of recent cannabis use.[13–15] However, it was concluded that the absence of detection of these cannabinoids cannot preclude the possibility of recent consumption.

In a previous investigation, we determined the detection rates of minor cannabinoids/cannabinoid metabolites in plasma samples with varying delta-9-THC concentrations. Based on the assumption that a high plasma delta-9-THC concentration is indicative of a short period between consumption and blood sampling, several minor cannabinoids were preferentially detected in samples taken from subjects with short intervals between use and sampling (or correspondingly higher delta-9-THC concentrations).[16]

In the present study, we analyzed 117 serum samples from individuals (psychiatric patients) with self-reported cannabis consumption via liquid chromatography coupled with triple-quadrupole tandem mass spectrometry (LC–MS/MS) to assess the detectability of cannabinoids/cannabinoid metabolites that might be useful for evaluating recent cannabis exposure. The analysis results were evaluated in consideration of the last time of consumption and consumption frequencies reported by the patients.

2 | MATERIAL AND METHODS

2.1 | Serum samples of cannabis users

Clinical serum samples were collected from psychiatric patients at the "Freud" ward of the Clinic and Polyclinic for Psychiatry and Psychotherapy, University Hospital Bonn and from psychiatric patients at the Clinic for Psychiatry and Psychotherapy, University Medical Center Mainz. The "Freud" ward of University Hospital Bonn is a protected ward focusing on addiction medicine for qualified detoxification treatments. Patients treated in the "Freud" ward usually exhibit abuse or dependence on opiates or other illegal substances. Ward 6 (not protected) of the Department of Psychiatry and Psychotherapy of University Medical Center Mainz specializes in the treatment of patients with various types of addiction. However, at both clinics, admission and treatment are voluntary on the part of patients.

During admission in both wards, patients are questioned about their substance abuse. As part of the medical anamnesis, the patients were asked about their drug use behavior within the last 12 months. The substances, the regularity of substance use, and the respective type of use were asked and documented for each patient in a form. The frequency of use was divided into the following categories: habitual (>3×/week), regular (1–3×/week), occasional (>1/month but <1×/week), and infrequent (<1×/month). In addition, the patients were asked about the respective last consumption of the substances mentioned. The last time of use (date and time) and the type of use were documented, if precisely remembered. Furthermore, the time of blood collection was noted in the form.

Blood samples were collected in serum tubes and stored at 4°C. Blood serum was obtained by centrifuging blood samples. Sodium fluoride was added to the collected serum samples (final concentration, approximately 0.25% [w/w]). Prior to analyses, serum samples were stored at −20°C.

The conducted study was approved by the regional ethics committee of the University of Bonn (number: 252/18). A positive ethics committee vote was also obtained from the State Medical Association of Rhineland-Palatinate (number: 2019-14254).

The cohort comprised 117 serum samples (see Table S3) obtained from patients (of the above-mentioned clinics in Bonn and Mainz) reporting prior cannabis use. Sixty-seven patients (57%) reported

inhalative consumption, whereas 50 patients (43%) did not specify the mode of use (or no type of use was documented).

2.2 | Chemicals and reagents

(−)-Delta-8-THC, (−)-delta-9-THC, (−)-delta-9-THC-D3 (THC-D3), cannabichromene (CBC), cannabichromenic acid (CBCA), (±)-cannabicyclol (CBL), cannabicyclolic acid (CBLA), CBD, CBD-D3, cannabidiolic acid (CBDA), CBN, CBN-D3, CBG, cannabidivarin (CBDV), THCV, cannabigerolic acid (CBGA), (±)-11-OH-THC, (±)-11-OH-THC-D3, (−)-THCCOOH, and (±)-THCCOOH-D9 were obtained from Cerilliant (Round Rock, TX, USA), Delta-9-tetrahydrocannabinolic acid A (THCA) was obtained from Sigma-Aldrich (St. Louis, MO, USA). 11-Nor-CBN-9-COOH (CBNCOOH) and THCVCOOH were obtained from ElSohly Laboratories (Oxford, MS, USA).

n-Hexane, ethyl acetate, and acetonitrile for gas chromatography, high-performance liquid chromatography (HPLC), or LC–MS, as well as glacial acetic acid, were purchased from Merck (Darmstadt, Germany). Ammonium acetate and hydrochloric acid were purchased from Sigma-Aldrich, and methanol (for LC–MS) was purchased from Honeywell Riedel-de-Haën (Seelze, Germany).

2.3 | Methods

2.3.1 | LC–MS/MS analysis

Analyses regarding the detection of cannabinoids were performed via LC–MS/MS. The LC–MS/MS apparatus consisted of an LC-20 series HPLC system (binary pump, degasser, column oven, and autosampler; Shimadzu, Duisburg, Germany) coupled to an API 4000 QTrap mass spectrometer (Sciex, Darmstadt, Germany). The analysis was performed using negative electrospray ionization and the multiple reaction monitoring mode. The following settings were utilized: collision gas, nitrogen; collision gas, high; curtain gas, 25 psi; ion source gas 1, 40 psi; ion source gas 2, 60 psi; ion spray voltage, −4500 V; and temperature, 475°C. The used mass transitions and corresponding mass spectrometric adjustments are presented in Table S1.

Chromatographic separation was achieved using a NUCLEODUR® C18 Isis (5 μm, 4.6 × 150 mm) column (Macherey-Nagel, Düren, Germany) and gradient elution (total flow, 0.8 ml/min). Eluents A and B consisted of 5 mM ammonium acetate in deionized water (adjusted to pH 5.7 with 0.1 M acetic acid) and acetonitrile/methanol (1:9, v/v, adjusted to pH 5.7 with 0.1 M acetic acid), respectively. The following gradient program was used: starting at 75% B, hold for 2 min, linear to 90% B at 8 min, hold for 5 min, back to 75% B at 14 min, and equilibration for 2 min. The injection volume was 30 μl.

The described method enabled the quantification of delta-9-THC, 11-OH-THC, THCCOOH, CBC, CBD, CBDV, CBG, CBN, THCV, delta-8-THC, THCA, CBDA, CBGA, CBLA, THCVCOOH, and CBNCOOH and provided qualitative proof of CBL and CBCA. CBL and CBCA cannot be quantified because there are no specific mass transitions and chromatographic and mass spectrometric interferences caused by delta-8-THC (in case of CBL) or CBLA (in case of CBCA).

Hydrolysis of the phase II metabolite THCCOOH-glucuronide due to sample treatment (see Extraction procedure section) was examined but could not be observed. Acidification during liquid–liquid extraction results in a pH value of approximately 4. Ether compounds (e.g., ether-linked glucuronides) generally exhibit higher chemical stability, so their hydrolysis requires certain conditions. Preanalytical acidic hydrolysis of ether glucuronides is usually carried out using concentrated acids and elevated temperatures.[17] Therefore, it is assumed that only free forms of cannabinoids or phase I metabolites are determined by means of the herein applied method.

A chromatogram of an extracted plasma sample previously spiked with an analyte mixture is presented in Figure 1.

The method was validated for plasma analysis according to forensic guidelines[18,19] regarding the selectivity, analytical limits, linearity, accuracy, recovery, matrix effects, and stability of the processed samples. Validation results are summarized in Table S2.

Extraction procedure

An aliquot (600 μl) of serum was spiked with 50 μl of a mixture of deuterated internal standards (containing 200 ng/ml each of THC-D3, CBN-D3, CBD-D3, and 11-OH-THC-D3 and 400 ng/ml of THCCOOH-D9). The first liquid–liquid extraction step was conducted using 1 ml of *n*-hexane/ethyl acetate (80:20, v/v). After vortexing (3 min) and centrifugation (10 min at 9447 × *g*), the organic supernatant was transferred to a separate vial. The sample residue was acidified with 40 μl of 1 M hydrochloric acid. After vortexing, 1 ml of *n*-hexane/ethyl acetate (80:20, v/v) was used for the second liquid–liquid extraction step (vortexing and centrifugation as described before). The supernatants were combined and evaporated on a rotary evaporator at room temperature. After complete evaporation of the solvents, reconstitution of the analytes and internal standards was performed using 50 μl of a mobile phase mixture (eluent A/eluent B, 10:90, v/v).

2.3.2 | Data analysis

Data analysis was performed using Microsoft Office Excel 2010 (Microsoft Corp., Redmond, WA, USA) and IBM SPSS Statistics 25 (IBM, Armonk, NY, USA).

3 | RESULTS

3.1 | Detectability of (minor) cannabinoids/cannabinoid metabolites

One hundred seventeen patient serum samples were analyzed. All cannabinoids excluding CBDA and CBLA were detected in at least

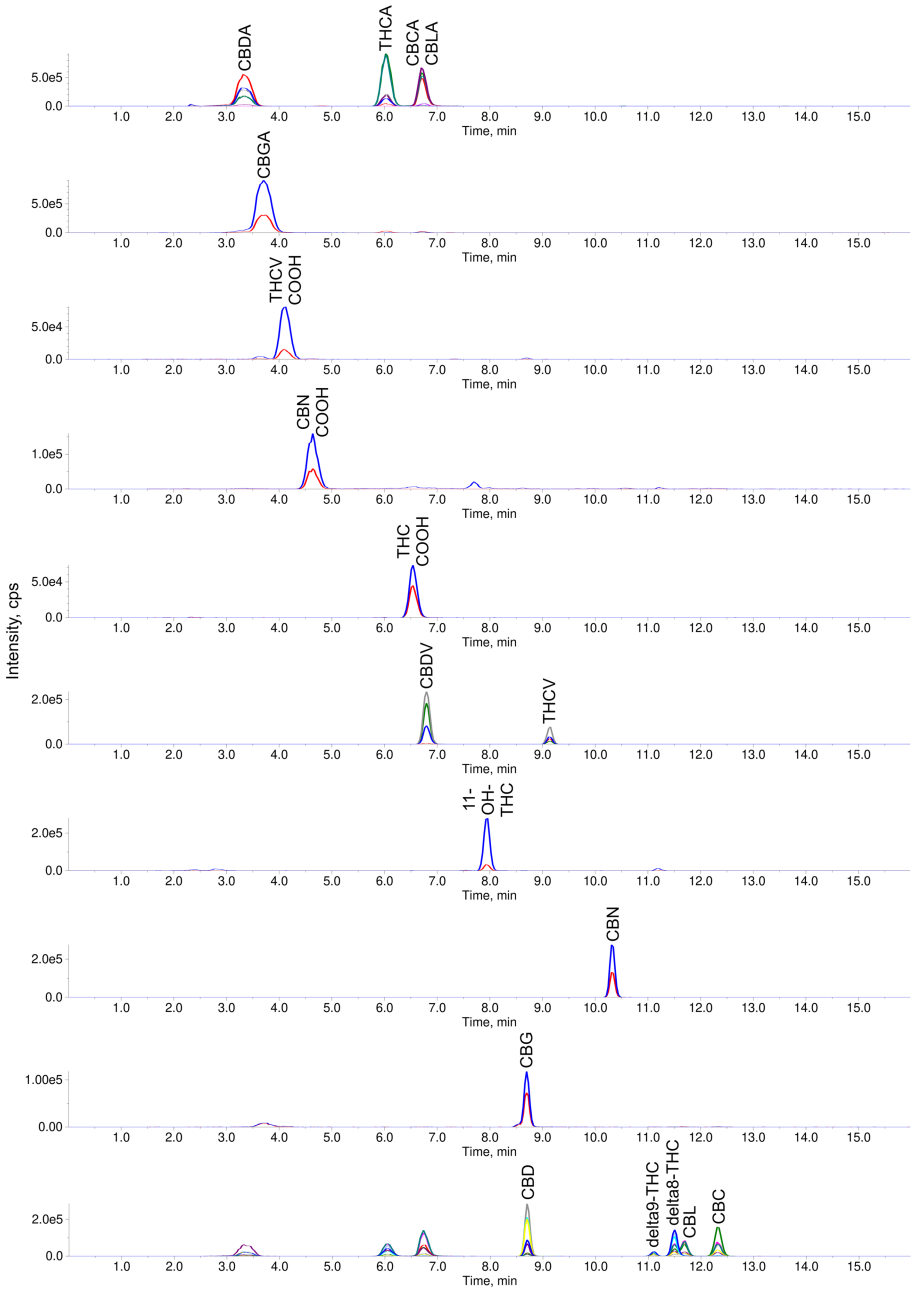

FIGURE 1 Chromatogram of an extracted plasma sample previously spiked with an analyte mixture (plasma cannabinoid concentration, 20 ng/ml, excluding THCCOOH [40 ng/ml]) [Colour figure can be viewed at wileyonlinelibrary.com]

one sample. The serum concentrations of minor cannabinoids/cannabinoid metabolites were generally low. Patients' statements on their last cannabis use, the frequency of cannabis use, the type of consumption, and detailed results of the conducted analyses are presented in Table S3. The frequency of use was divided into the following categories: habitual (>3×/week), regular (1–3×/week), occasional (>1/month but <1×/week), and infrequent (<1×/month).

Fifty-six patients (44 [79%] with stating habitual consumption, 35 [62.5%] with reporting inhalative use, and 21 [37.5%] with not specifying the type of use [or no type of use was documented]) provided exact and credible information about a last consumption of cannabis within the last 24 h before blood collection (stated time intervals: average, 13.3 h; median, 15.5 h; minimum, 0.6 h; maximum, 24.0 h). The results of serum analysis were used to evaluate possible correlations between the detectability or concentration of a (minor) cannabinoid (metabolite) and the interval between consumption and blood collection.

In addition to delta-9-THC and its main metabolites 11-OH-THC and THCCOOH, CBC, THCA, CBG, and THCVCOOH were the most frequently detected cannabinoids in the investigated serum samples ($N = 56$ [patients providing exact information about a last consumption of cannabis within the last 24 h before blood collection]). The relationships of serum cannabinoid concentrations (for cannabinoid-positive samples) with the time between last cannabis consumption and blood sampling (according to patients' statements) or the respective serum delta-9-THC concentrations are presented in Figures 2–7, and the corresponding Pearson's correlation coefficients are presented in Table 1.

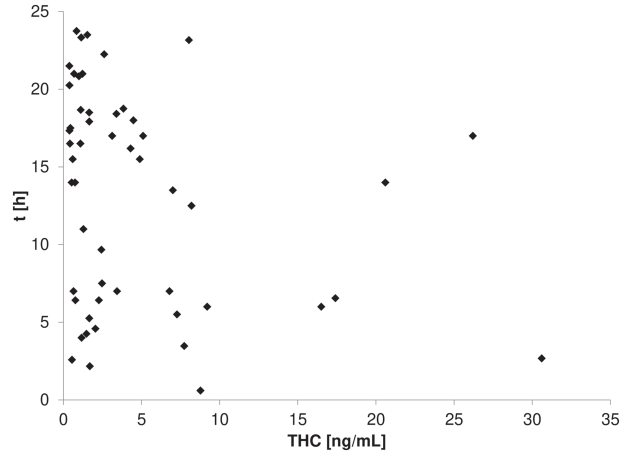

FIGURE 2 The correlation (♦) of serum delta-9-THC concentration with the interval between the last consumption of cannabis and blood sampling (according to patients' statements) ($N = 52$)

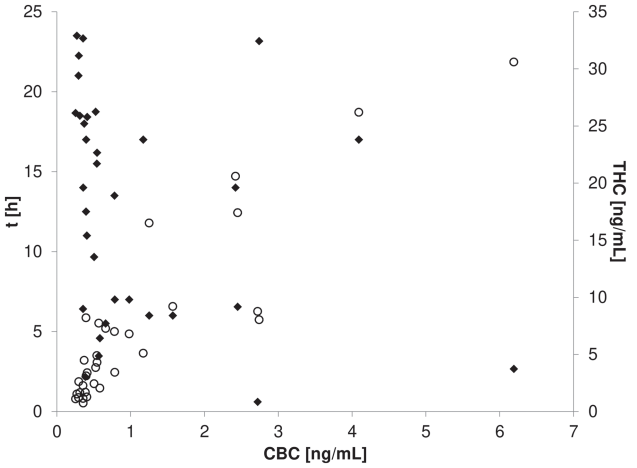

FIGURE 4 Correlations of serum CBC concentrations with the time between the last use of cannabis and blood sampling (according to patients' statements) (♦) and with the corresponding serum delta-9-THC concentrations (○) ($N = 33$)

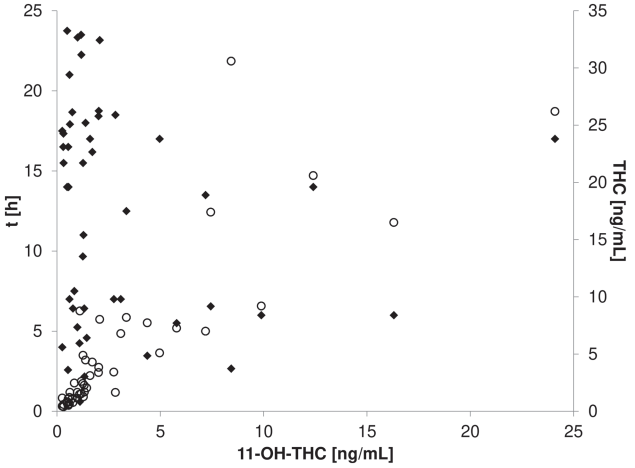

FIGURE 3 Correlations of serum 11-OH-THC concentrations with the time between the last use of cannabis and blood sampling (according to patients' statements) (♦) and with the corresponding serum delta-9-THC concentrations (○) ($N = 48$)

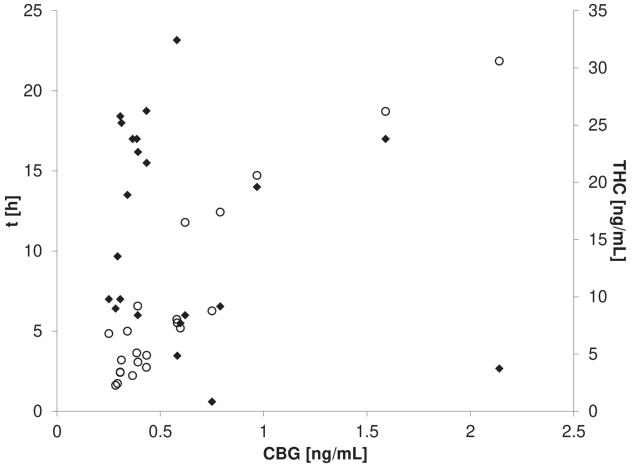

FIGURE 5 Correlations of serum CBG concentrations with the time between the last use of cannabis and blood sampling (according to patients' statements) (♦) and with the corresponding serum delta-9-THC concentrations (○) ($N = 22$)

Within the cohort of 56 serum samples from patients providing exact information on the last use of cannabis within 24 h before sample collection, CBNCOOH (five positive samples), delta-8-THC (two positive samples), THCV (one positive sample), CBDV (one positive sample), CBL (one positive sample), CBDA (zero positive samples), and CBLA (zero positive samples) were rarely detected. Thus, these cannabinoids were not considered for further evaluation.

To evaluate delta-9-THC, 11-OH-THC, CBC, CBCA, CBD, CBG, CBGA, CBN, THCA, and THCVCOOH, samples were divided into two groups according to positivity for each certain cannabinoid. The corresponding times between the last use of cannabis and blood sampling (according to patients' statements) were statistically evaluated for every group. The time (median, minimum, maximum, and average) between the last consumption of cannabis and blood sampling for every group is presented in Table 2.

To assess possible discrepancies of the elapsed times between cannabis use and blood sampling for positive and negative samples, box plots of the elapsed times are presented in Figures 8 and 9.

Considering the entire cohort ($N = 117$), it appears that minor cannabinoids/cannabinoid metabolites can be detected more than 24 h after cannabis consumption. There were 17 patients (eight of them stating habitual cannabis consumption) stating an exact time of last cannabis consumption in a range of 26.5–72.8 h (median, 39.0 h; average, 41.6 h) before blood sampling. Among these samples, the cannabinoids shown in Table 3 were detectable with the indicated frequencies.

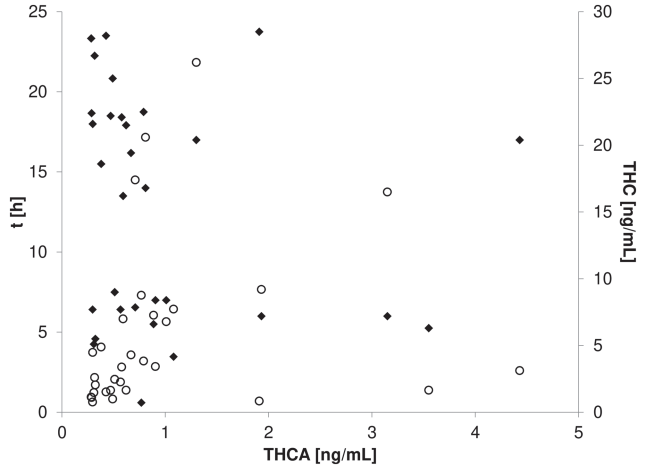

FIGURE 6 Correlations of serum THCA concentrations with the time between the last use of cannabis and blood sampling (according to patients' statements) (♦) and with the corresponding serum delta-9-THC concentrations (○) ($N = 31$)

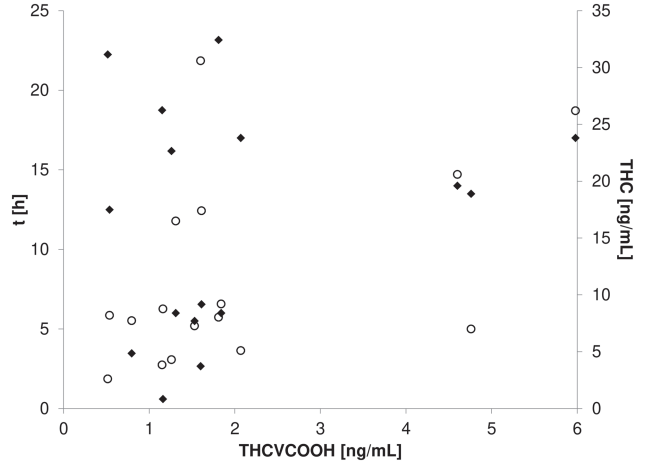

FIGURE 7 Correlations of serum THCVCOOH concentrations with the time between the last use of cannabis and blood sampling (according to patients' statements) (♦) and with the corresponding serum delta-9-THC concentrations (○) ($N = 16$)

TABLE 1 Pearson's correlation coefficients of the correlations presented in Figures 2–7

Cannabinoid	Correlation with the interval between use and sampling	Correlation with the delta-9-THC concentration
Delta-9-THC	−0.26	-
11-OH-THC	−0.12	0.84
CBC	−0.28	0.90
CBG	−0.22	0.93
THCA	−0.15	0.18
THCVCOOH	0.21	0.47

Considering the detection frequencies presented in Table 3, CBC appears to be detectable over longer periods after cannabis consumption (at least in some cases). Four of six samples being positive for CBC were collected from patients stating habitual cannabis consumption whereas one patient stated an occasional use and one other patient did not specify the frequency of consumption. CBN, on the contrary, was merely detectable in a single case of a habitual user stating a last time of cannabis use 29.0 h before blood sampling.

Exemplary cases with the detection of minor cannabinoids/cannabinoid metabolites despite intervals between consumption and blood sampling exceeding 24 h (according to patients' statements) are listed in Table 4.

3.2 | THCCOOH concentrations in habitual cannabis users

Seventeen patients claimed habitual cannabis consumption and recent consumption within less than 10 h before blood sampling. Eighteen patients claimed habitual cannabis consumption and last cannabis use between 10 and 20 h before blood sampling, and 18 patients claimed habitual cannabis consumption and last cannabis use more than 20 h before blood sampling. THCCOOH concentrations were evaluated for the aforementioned groups of habitual cannabis users. The median, minimum, maximum, and mean serum THCCOOH concentrations for each group are presented in Table 5.

The distribution of serum THCCOOH concentrations within the different groups is presented as box plots in Figure 10.

4 | DISCUSSION

4.1 | Detectability of (minor) cannabinoids/cannabinoid metabolites

Several factors influence serum cannabinoid concentrations after cannabis exposure, including, but not limited to, the ingested amount of the cannabis product, the cannabinoid contents of the consumed product (inter alia depending on the cannabis strain, growing conditions, age of the products, or their storage conditions), the type of consumption (e.g., inhalative [inter alia including number and duration of puffs and inhalation volume] or oral uptake), the degree of previous decarboxylation of cannabinoid precursor acids, the time of consumption, the frequency of consumption, interindividual differences in pharmacokinetics (absorption, distribution, metabolism [possibly also affected by other ingested substances], and excretion), and the stability of cannabinoids in the sample material.[2,3,6,12,20–24]

According to the European Drug Report, the potencies of herbal cannabis and cannabis resin have increased since 2008.[25] Several studies examining cannabinoid contents in seized materials[26–31] revealed variations in composition among the cannabis products. Elzinga et al. reported that the chemical composition of cannabis can vary

TABLE 2 Number of serum samples positive and negative for cannabinoids and the corresponding interval (median, minimum, maximum, and mean) between the last use of cannabis and blood sampling (according to patients' statements)

Cannabinoid	Result	N	Median (h)	Min (h)	Max (h)	Mean (h)
Delta-9-THC	Positive	52	14.8	0.6	23.8	13.1
	Negative	4	17.5	7.0	24.0	16.5
11-OH-THC	Positive	48	14.0	0.6	23.8	12.4
	Negative	8	20.5	7.0	24.0	18.7
CBC	Positive	33	14.0	0.6	23.5	12.8
	Negative	23	16.5	2.6	24.0	14.1
CBCA	Positive	13	6.6	2.7	23.3	10.3
	Negative	43	16.5	0.6	24.0	14.2
CBD	Positive	12	8.3	0.6	18.0	9.3
	Negative	44	16.5	2.6	24.0	14.4
CBG	Positive	22	11.6	0.6	23.2	11.3
	Negative	34	16.5	2.2	24.0	14.6
CBGA	Positive	12	11.2	3.5	23.8	11.9
	Negative	44	16.0	0.6	24.0	13.7
CBN	Positive	10	6.8	0.6	18.0	9.1
	Negative	46	16.5	2.2	24.0	14.3
THCA	Positive	31	14.0	0.6	23.8	12.7
	Negative	25	16.5	2.2	24.0	14.1
THCVCOOH	Positive	16	13.0	0.6	23.2	11.6
	Negative	40	16.5	2.2	24.0	14.0

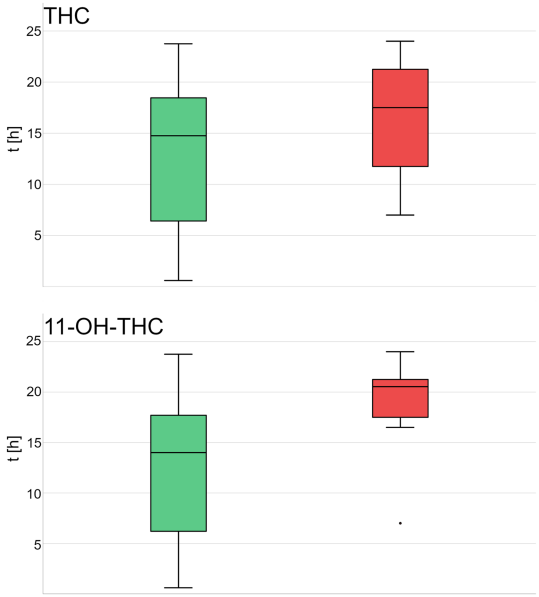

FIGURE 8 Comparison of the elapsed times between cannabis use (according to patients' statements) and blood sampling for samples positive (green, left box plot) and negative (red, right box plot) for delta-9-THC (top) or 11-OH-THC (bottom) [Colour figure can be viewed at wileyonlinelibrary.com]

within a strain, probably resulting from differences in the cultivation conditions.[20]

The degree of conversion of precursor acids (which are predominantly present in the plant material) to the corresponding cannabinoids was indicated to depend on the reaction temperature (e.g., during baking hash cookies or smoking a marijuana cigarette).[23] Accordingly, depending on the reaction temperature, different proportions of precursor acids present in the plant material are decarboxylated. Consequently, the resulting precursor acid residues and amounts of cannabinoids released by decarboxylation vary. Interestingly, in Case 46 of this study, comparatively high concentrations of THCA and CBGA were detected (additional qualitative detection of CBCA), although the concentrations of delta-9-THC and its main metabolites were not excessively high. According to a study by Raikos et al., high concentrations of THCA (exceeding the corresponding delta-9-THC concentrations) might be indicative of the oral intake of THCA (e.g., unheated or slightly heated marijuana).[32] According to conclusions drawn by Nadulski et al.,[33] oral ingestion of delta-9-THC is indicated by an 11-OH-THC/delta-9-THC ratio > 1, whereas the concentration of delta-9-THC was more than threefold higher than that of 11-OH-THC in Case 46 in the present analysis. Although oral consumption of delta-9-THC (e.g., consumption of slightly heated hash cookies) cannot be assumed in this case, the measured concentrations of THCA and CBGA as well as the qualitative detection of CBCA suggest the (additional) ingestion of cannabis material that was not heated or only heated slightly.

In a previous study,[16] we evaluated the detection rates of (minor) cannabinoids/cannabinoid metabolites among plasma samples previously tested positive for delta-9-THC or its metabolites. The dependency of cannabinoid concentration or detectability on the plasma delta-9-THC concentration or on the probable time of consumption

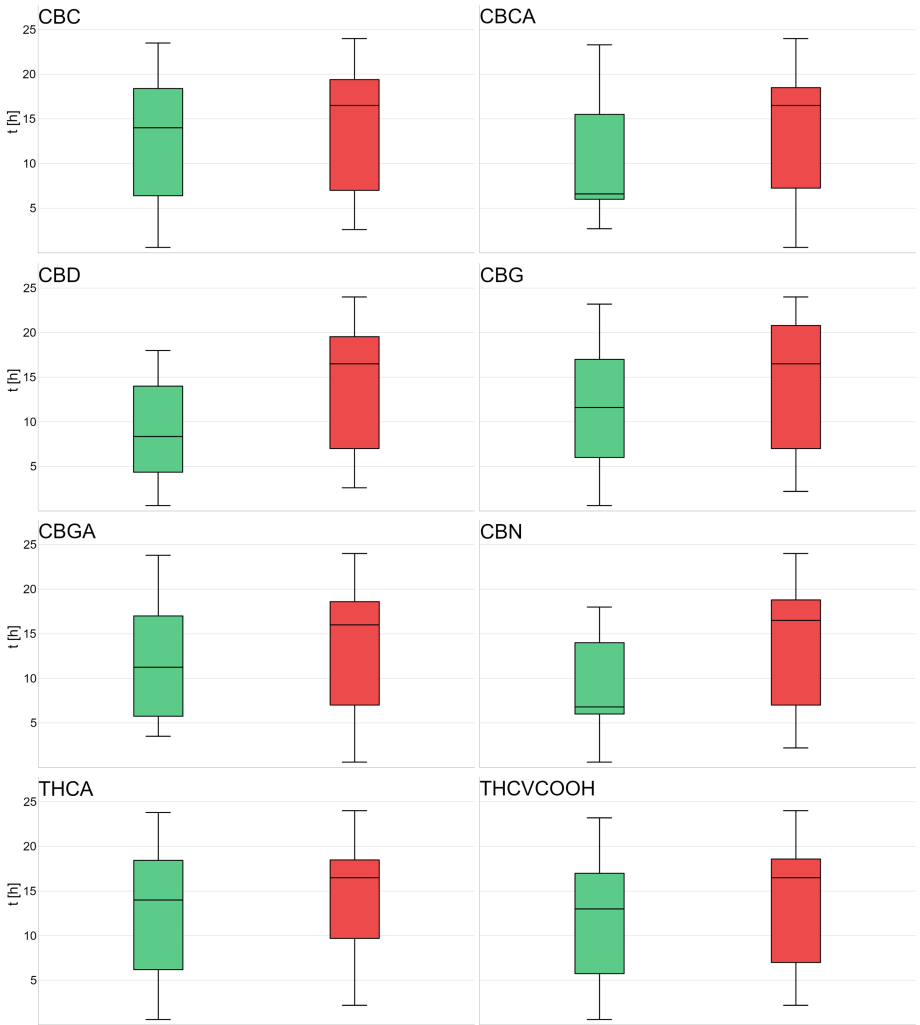

FIGURE 9 Comparison of the elapsed times between cannabis use (according to patients' statements) and blood sampling in samples positive (green, left box plot) and negative (red, right box plot) for various cannabinoids [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 3 Frequency of cannabinoid detection among 17 serum samples of patients stating an exact time of last cannabis consumption in a range of 26.5–72.8 h before blood sampling

Analyte	Frequency of detection
Delta-9-THC	13 (76.5%)
11-OH-THC	12 (70.6%)
THCCOOH	17 (100%)
CBC	6 (35.3%)
CBG	4 (23.5%)
THCA	3 (17.6%)
THCVCOOH	3 (17.6%)
CBD	3 (17.6%)
CBCA	2 (11.8%)
CBGA	1 (5.9%)
CBN	1 (5.9%)

(estimated by a mathematical model of Huestis et al.[5]) was examined. Detection incidences of some cannabinoids increased with increasing delta-9-THC concentration. Assuming recent cannabis consumption in cases of elevated delta-9-THC plasma concentrations (also consistent with the results of model I described by Huestis et al.[5]), these results imply that minor cannabinoids can be considered as additional markers for recent cannabis use.

In general, it was described that the mathematical models of Huestis et al.[5] are reliable using both models (models I and II) in combination but only for rather short time intervals (<8 h). There is considerable uncertainty for longer periods of time and for habitual users.[34] In the case of chronic users, the increased inaccuracy results particularly from residual delta-9-THC (and THCCOOH) concentrations[35] that may be detectable over long periods of time despite abstinence.[11,12] Thus, it was already mentioned in our previous study[16] that in order to further evaluate the marker suitability of minor cannabinoids, it is necessary to examine samples of patients with known (or self-reported) consumption history.

In the present study, the evaluation of the detection of (minor) cannabinoids/cannabinoid metabolites was not carried out by comparison with the delta-9-THC concentrations or the time of consumption estimated from it but on the basis of the patients' statements (often habitual consumers) on their consumption behavior. No distinct relationship was observed between the cannabinoid concentrations

TABLE 4 Exemplary cases with the detection of minor cannabinoids/cannabinoid metabolites despite intervals of more than 24 h between consumption and blood sampling

Case	Stated interval (h)	Stated frequency of consumption	Detected cannabinoids
8	29.0	Habitual	Delta-9-THC, 11-OH-THC, THCCOOH, CBC, CBG, THCVCOOH, CBD, and CBN
20	39.0	Habitual	Delta-9-THC, 11-OH-THC, THCCOOH, CBC, THCA, CBG, THCVCOOH, and CBD
21	27.7	Habitual	Delta-9-THC, 11-OH-THC, THCCOOH, CBC, CBG, THCVCOOH, and CBCA
26	67.5	Habitual	Delta-9-THC, 11-OH-THC, THCCOOH, CBC, and CBG
43	38.3	Not specified	Delta-9-THC, 11-OH-THC, THCCOOH, CBC, and CBD
46	39.5	Not specified	Delta-9-THC, 11-OH-THC, THCCOOH, THCA, CBGA, and CBCA
57	41.7	Not specified	Delta-9-THC, 11-OH-THC, THCCOOH, and THCA
61	188.3	Habitual	Delta-9-THC, THCCOOH, CBC, and THCA
63	41.3	Occasional	Delta-9-THC, 11-OH-THC, THCCOOH, and CBC

TABLE 5 Serum THCCOOH concentrations of habitual cannabis users with varying intervals between the last use of cannabis and blood sampling

Elapsed time (h)	N	Median (ng/ml)	Min (ng/ml)	Max (ng/ml)	Mean (ng/ml)
<10	17	40.6	8.7	>120 (approximately 229)	59.4
10–20	18	46.3	<1.0 (approximately 0.8)	>120 (approximately 648)	113
>20	18	15.5	2.1	>120 (approximately 160)	31.8

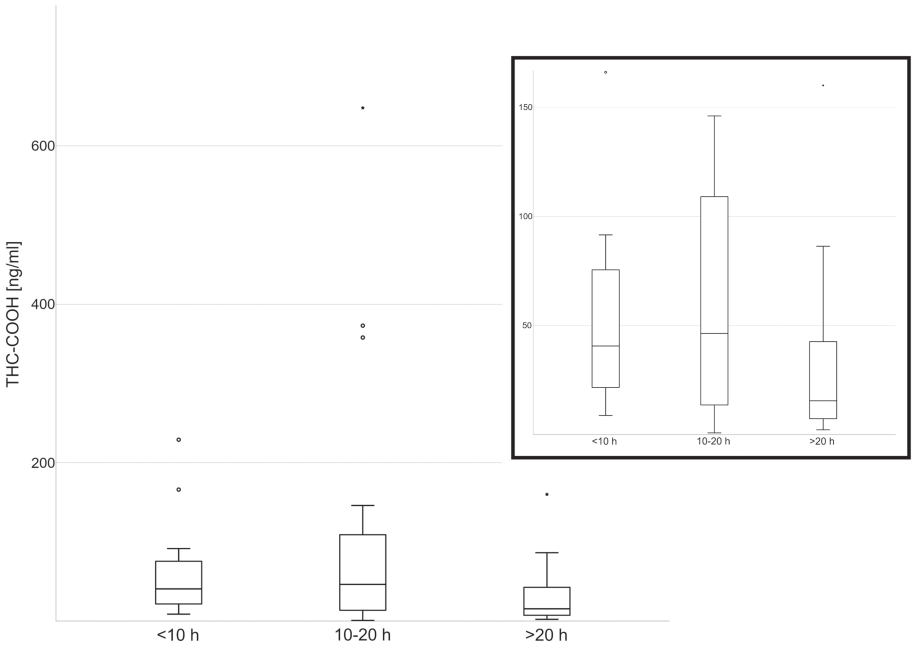

FIGURE 10 Serum THCCOOH concentrations in habitual cannabis users with varying intervals between the last use of cannabis and blood sampling (according to patients' statements)

(including delta-9-THC concentrations) and the interval between cannabis consumption and blood sampling (according to patients' statements). In general, cannabinoid concentrations are elevated with increasing delta-9-THC concentrations (see Figures 3-7 and Table 1). Assuming the accuracy of patient memory, these results imply that the interval between the last consumption of cannabis and blood sampling is not the most decisive factor regarding the resulting serum cannabinoid concentrations. Varying cannabinoid concentrations in consumed cannabis products, the frequency of consumption (particularly close to hospitalization/blood sampling), and other influencing factors are assumed to considerably affect the detectable concentrations of cannabinoids. Based on the present findings, the last time of

use cannot be estimated precisely using serum cannabinoid concentrations in general.

Within the cohort of 56 serum samples from patients providing exact and credible information on cannabis consumption within 24 h before blood sampling, CBNCOOH, delta-8-THC, THCV, CBDV, CBL, CBDA, and CBLA levels were rarely detected. Thus, these cannabinoids were not considered for further evaluation. These findings suggest that these cannabinoids are unsuitable for assessing the time of last cannabis use.

According to the data presented in Table 2 and in Figures 8 and 9, the mean or median interval between use and blood sampling generally appears shorter for cannabinoid-positive serum samples. This result implies the possible utility of (minor) cannabinoids/cannabinoid metabolites as markers of recent cannabis consumption. However, there was strong overlap between the reported intervals for cannabinoid-positive and cannabinoid-negative samples. Thus, differentiation between recent use and consumption several hours before blood sampling is not possible solely based on the detection of (minor) cannabinoids/cannabinoid metabolites.

Considering patients who provided exact information about the last time of consumption within the last 24 h ($N = 56$; 44 of them [79%] with stating habitual consumption; 35 of them [62.5%] with reporting inhalative use; and 21 of them [37.5%] with not specifying the type of use [or no type of use was documented]), delta-9-THC and 11-OH-THC were detected in the sera of patients reporting consumption between 0.6 and 23.8 h before blood sampling. In agreement with the results of previously published studies,[11,12] the obtained data revealed that delta-9-THC and its metabolite remain detectable for hours or possibly even days after the last consumption (particularly in the case of habitual users). The absence of delta-9-THC and 11-OH-THC in serum samples was found exclusively for patients reporting consumption times of at least 7 h before blood sampling. However, the comparatively low numbers of negative samples must be considered (delta-9-THC, 4; 11-OH-THC, 8). All delta-9-THC-negative samples were also negative for 11-OH-THC. Delta-9-THC concentrations in samples lacking 11-OH-THC ranged from approximately 0.3 to 0.9 ng/ml, which were relatively low. In summary, the results indicated that the detection of delta-9-THC and 11-OH-THC cannot immediately prove recent cannabis use. Meanwhile, the absence of delta-9-THC and 11-OH-THC detection suggests cannabis use (at least) several hours before sample collection or the uptake of only a small amount of delta-9-THC.

CBC, CBCA, CBD, CBG, CBGA, CBN, THCA, and THCVCOOH displayed broad intervals for both positive and negative serum samples (among patients providing exact information regarding the last time of consumption within the last 24 h). Serum samples positive for CBCA, CBD, or CBN correspond to median intervals between consumption and blood sampling of 6.6, 8.3, and 6.8 h, respectively. These intervals were shorter than those for other cannabinoids, indicating the possible utility of these cannabinoids as markers of recent cannabis consumption. Regarding CBCA-positive samples, patients reported elapsed times between cannabis use and blood sampling of up to 23.3 h. Conversely, CBCA-negative serum samples were also obtained from patients reporting consumption shortly before sampling (minimum, 0.6 h). CBCA is a cannabinoid precursor acid. As already discussed, the (residual) content of cannabinoid precursor acids in cannabis products (or in cannabis smoke or vapor) and therefore the detectability of precursor acids in biological specimens depend inter alia on the heat treatment of the consumed products.[23,36] The detection of CBCA in subjects who used cannabis several hours before sampling or the lack of detection in cases of recent consumption could readily be explained by the varying degree of heating of the consumed cannabis material. Similar results were also observed for CBGA. Because of their variable detectability, the utility of cannabinoid precursor acids for estimating the time of cannabis use appears questionable.

CBD and CBN were both detectable in serum samples from patients who reported cannabis use within 18 h before blood collection (among patients providing exact information on the last time of consumption within the last 24 h). Both CBD and CBN were previously identified as potential markers for recent cannabis consumption, but their absence in blood cannot exclude recent use.[13–15] However, according to the data of the present study, the detection of most recent-use cannabis markers such as CBD and CBN (or other discussed markers such as CBG or THCVCOOH) is also possible for up to several hours after the most recent consumption of cannabis (assuming the correctness of the patients' information; see also Table 2). Thus, when interpreting findings of minor cannabinoids, the assumption of recent use should not only be based on minor cannabinoid detection. In the case of CBD, the (additional) consumption of CBD joints or other CBD containing products leading to considerable blood CBD concentrations[37] should also be considered as a potential reason for its detection. For example, a single patient reported also using CBD. In this case (Case 10), CBD was found at a comparatively higher concentration of 3.8 ng/ml.

Considering the entire cohort, particularly cases evaluated in Table 3 or presented in Table 4, it appears that minor cannabinoids/cannabinoid metabolites can be detected more than 24 h after cannabis consumption. The THCV metabolite THCVCOOH has already been described as an unsuitable marker for recent cannabis use due to its prolonged detectability.[13]

Some cannabinoids are chemically similar to THC. It is conceivable that other cannabinoids with similar lipophilicity also exhibit similar distribution behavior as THC. For example, CBN is only slightly more polar than THC, displaying an *n*-octanol/water partition coefficient of 1.7×10^6 (delta-9-THC octanol–water coefficient, 9.44×10^6).[38] CBC is expected to have similar lipophilicity as THC.[39] Because of the comparable lipophilicity of other cannabinoids, similar tissue distributions or accumulations and the subsequent release of the corresponding cannabinoids into the bloodstream appear conceivable. Consequently, the window for detecting minor cannabinoids after regular cannabis consumption may be prolonged. In chronic cannabis users in particular, the exclusive occurrence of these minor cannabinoids only shortly after consumption would not be expected, and their suitability as markers for recent cannabis use cannot be generally confirmed.

4.2 | THCCOOH concentrations in habitual cannabis users

The delta-9-THC metabolite THCCOOH can be detected in the blood of chronic cannabis smokers for several days despite abstinence.[12] Because of the long plasma half-life of THCCOOH (approximately 6 days[40]) and its resulting accumulation in the blood of chronic cannabis users, blood THCCOOH concentrations are used to estimate the frequency of cannabis consumption.[41] According to Daldrup et al., serum THCCOOH concentrations ≥ 75 ng/ml are indicative of regular cannabis use (if the blood sample is obtained no more than 8 days after the discontinuation of cannabis use).[42] Concentrations in the range of 5 ng/ml ≤ THCCOOH < 75 ng/ml are indicative of substantial consumption (with suspicion of regular consumption).[43] Without considering the time until blood sampling (up to 8 days), a THCCOOH concentration ≥ 150 ng/ml is indicative of regular use.[43]

The plasma THCCOOH concentration after smoking was reported to peak no more than 4 h after consumption.[3] Following oral or oromucosal administration, the plasma THCCOOH concentration was expected to peak approximately 5 h (up to 7.5 h) after intake.[44] In the present study, 53 patients self-reported habitual cannabis use (>3×/week) and a precise last time of use. The samples were divided into three groups depending on the stated time of last use (<10, 10–20, and >20 h). As presented in Table 5 and Figure 10, the median THCCOOH concentration in serum samples of habitual cannabis users with stated consumption less than 10 h before sample collection was comparable with the concentration for habitual cannabis users with last consumption 10–20 h before sampling. Because of the long plasma half-life of THCCOOH, this is an unsurprising finding. The median serum THCCOOH concentration in habitual cannabis users with last consumption more than 20 h before sampling (average, 38 h; median, 25 h; maximum, approximately 8 days) was comparatively lower. Nevertheless, there was overlap of THCCOOH concentrations among the three groups as presented in Figure 10. Every group included patients with both low and high serum THCCOOH concentrations. Thus, in the case of habitual cannabis users, a distinct differentiation of serum THCCOOH concentrations depending on the last time of use cannot be made.

In total, six and 14 patients (11 and 26% of the considered cases [$N = 53$], respectively) exhibited THCCOOH concentrations of at least 150 and 75 ng/ml (indicating regular use[43]), respectively. Thirty-five patients (66%) had THCCOOH concentrations in the range of 5 ng/ml ≤ THCCOOH < 75 ng/ml (indicating substantial consumption and suspicious for regular consumption[43]). Only four habitual cannabis users had THCCOOH concentrations < 5 ng/ml. The corresponding intervals between consumption and blood sampling stated by the patients were 17.3, 18.5, 26.5, and 40.0 h, respectively. In summary, considering the described decision limits,[43] most patients reporting habitual cannabis use (>3×/week) and precise information about the most recent use of cannabis within 8 days before blood sampling could be classified as regular or substantial users (with suspicion of regular consumption) according to the measured THCCOOH concentrations.

4.3 | Strengths and limitations of the study

4.3.1 | Study collective

Many data on the temporal detectability of cannabinoids in blood were obtained during pharmacokinetic studies. These studies usually involve a single administration or consumption of certain cannabinoid doses and a defined mode of administration. The results of the present study, however, considering the variability of the circumstances of each case (e.g., frequency of use, last use, and different cannabinoid doses), are rather reflective of daily routine cases. Thus, data from studies such as the present one are equally important for the interpretation of cannabinoid findings as data obtained in a controlled manner.

However, the study collective had some limitations that should be considered in the future when using the data for the comprehensive interpretation of cannabinoid levels in serum samples. The samples were obtained from psychiatric patients (of two different wards in university clinics) who often abused other intoxicating substances in addition to cannabis and possibly exhibited conditions such as liver damage. These conditions can significantly influence serum concentrations of (minor) cannabinoids/cannabinoid metabolites. Therefore, the study collective of the present study may not fully represent the community of cannabis users.

The study results were evaluated in consideration of the timing and frequency of use reported by the patients. These statements were assumed to be true without restriction. However, because in both clinics admission and treatment were voluntary on the part of the patients and no disadvantages had to be feared when stating drug consumption in the past, the (comprehensive) statements on drug consumption made by the patients are to be considered as credible to a large extent.

4.3.2 | Data evaluation

The analyzed cohort predominantly comprised samples from patients who consumed cannabis on a regular basis (according to their own statements). In some cases, patients reported several instances of use in the recent past that could not be considered in the evaluation, as only the last instance of use was considered. In addition, no distinction was made between patients who consumed cannabis several times in the recent past and those with only one instance of use. Because of the availability of only one sample per patient, the possibility of residual cannabinoids in blood at the time of the last consumption (or blood sampling) resulting from previous consumption cannot be excluded. Furthermore, the cannabis product consumed or the dose (particularly the amounts of cannabinoids) was not known and thus could not be considered for data evaluation.

4.3.3 | Method of analysis

The detectability of a substance always depends on the sensitivity of the used analytical method. A number of minor

cannabinoid/cannabinoid metabolite findings of the present study were in the range of the lower limit of quantification indicating that minor cannabinoid/cannabinoid metabolite concentrations in serum of cannabis users are often comparatively low. When using detection rates of minor cannabinoids/cannabinoid metabolites for comparing purposes, the respective analytical limits of the method used for the determination of the detection rates should always be considered.

Some limitations of the herein applied method may have had an impact on the results. The validation data (see Table S2) did not meet the acceptance criteria of the considered validation guideline[18,19] for all analytes and parameters. In case of CBC, CBDV, delta-8-THC, THCA, CBDA, CBGA, and CBNCOOH, for example, a higher imprecision is assumed when determining concentrations of these analytes (relative standard deviation > 15%).

For 11-OH-THC (and its deuterated standard 11-OH-THC-D3), THCA, and CBLA, high standard deviations for recovery were observed during method validation. While in case of 11-OH-THC these variations might be compensated using 11-OH-THC-D3 for quantification, the mentioned variations can affect the quantification of THCA und CBLA. Moreover, the sensitivity of the method can vary depending on recovery achieved during extraction.

Additionally, an ion suppression was observed for delta-9-THC (and THC-D3), CBC, CBN (and CBN-D3), and delta-8-THC, all eluting comparatively late. Because this ion suppression was determined consistent among different sera (standard deviation < 25%), ion suppression is not expected to affect quantitation of these analytes. In general, however, ion suppression influences the sensitivity.

4.3.4 | Phase II metabolites

As in our previous study, phase II metabolites (particularly delta-9-THC-glucuronide) were not evaluated because they could not be detected using the LC–MS/MS method employed in this study. Based on the results of controlled studies, delta-9-THC-glucuronide has been suggested as a marker of recent cannabis consumption.[13–15] The utility of delta-9-THC-glucuronide as a marker of recent exposure among typical cannabis users should further be investigated.

5 | CONCLUSION

We investigated the detectability of several cannabinoids in serum samples from psychiatrically treated cannabis users. The presented results imply that the detection of minor cannabinoids can help to confirm recent cannabis use. It has often been emphasized that a lack of detection of minor cannabinoids cannot eliminate the possibility of recent use. Conversely, some patients presented with detectable minor cannabinoid levels despite a long period between use and blood sampling. In this respect, when interpreting findings of minor cannabinoids, the assumption of recent use should not be based only on minor cannabinoid detection. The circumstances of the individual case should always be considered (e.g., information on the quantity or dose and frequency of use, if the information is available). The analysis of other cannabinoids (next to delta-9-THC and its main metabolites), the data of the present study, and further published data allow a comprehensive evaluation of cannabinoid concentrations in serum samples, particularly in frequent consumers.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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