Research Paper

Use of levamisole-adulterated cocaine is associated with increased load of white matter lesions

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Background: Cocaine use has been associated with vascular pathologies, including cerebral white matter hyperintensities. Street cocaine is most often adulterated with levamisole, an anthelminthic drug that may also be associated with vascular toxicity. However, whether levamisole exposure from cocaine consumption further accelerates the development of white matter lesions remains unknown. **Methods:** We investigated the association of cocaine and levamisole exposure with white matter hyperintensities in 35 chronic cocaine users and 34 healthy controls. We measured cocaine and levamisole concentrations in hair samples, which reflected exposure up to 6 months previously. We assessed the number and total surface area of the white matter hyperintensities using structural MRI (FLAIR sequence). Using generalized linear models, we analyzed the contributions of cocaine and levamisole to the number and area of white matter hyperintensities, accounting for several confounding factors. **Results:** Analysis using generalized linear models revealed that cocaine users had more white matter hyperintensities in terms of total surface area, but not in terms of number. Further generalized linear models that included cocaine and levamisole hair concentrations (instead of group) as predictors indicated that levamisole exposure was strongly associated with more and larger white matter hyperintensities, suggesting that the elevated white matter hyperintensities in cocaine users were driven mainly by levamisole exposure. Finally, white matter hyperintensities in levamisole-exposed cocaine users were located primarily in the periventricular and juxtacortical white matter. **Limitations:** The sample size was moderate, and blood pressure was not systematically assessed. **Conclusion:** As an adulterant of cocaine, levamisole appears to increase the risk of white matter injury.

Introduction

Cocaine is the second most consumed illicit drug in Europe and North America, after cannabis. In 2018, cocaine use was estimated to be 1.2% in the European Union (3.9 million people) and 2.3% in the United States (5.5 million people).[1,2] Cocaine is a potent sympathetic nervous system stimulant with local anesthetic properties and, importantly, euphorigenic effects that emerge via strong modulation of the brain's reward system, accounting for its strong addictive potential.[3] By inducing endothelin-1 release and inhibiting nitrous oxide release, cocaine acts as a potent vasoconstrictor.[4] It can also cause cardiovascular disease, including ischemic and hemorrhagic stroke.[5] Cocaine causes strokes via multiple pathways, including direct (i.e., constriction of brain arterioles) and indirect (e.g., chronic hypertension, arrhythmias, induction of plaque growth and alterations of platelet aggregation) effects.[6] In contrast to clinically apparent stroke, white matter hyperintensities of the brain are more subtle, chronic ischemic lesions caused by constriction and damage of subcortical small vessels.[7] Cocaine consumption has also been associated with white matter pathology in studies analyzing white matter hyperintensities[8] or white matter integrity based on diffusivity.[9] A high load of white matter hyperintensities is associated with neurocognitive disturbances and functional decline in elderly patients.[10] With sustained drug consumption, chronic cocaine users also experience neuropsychiatric symptoms,[11,12] including neurocognitive sequelae.[13,14] Grey matter atrophy also occurs in cocaine users, particularly in laterofrontal regions of the brain.[15,16] These structural adaptations are correlated with cognitive changes but seem to be partially reversible with longer periods of abstinence.[15]

Nevertheless, cocaine is not usually consumed in a pure form; it is often laced with levamisole, a substance currently used to treat parasitic worm diseases in animals. Levamisole was once also approved for use in humans, but it was retracted from the market because of severe adverse effects, including rheumatic diseases and vasculitis.[17–19] Levamisole was proposed to have stimulant properties itself, amplifying or prolonging the effects of cocaine.[20,21] According to a recent

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report by the United States Drug Enforcement Agency, most seized cocaine bricks (87%) contained levamisole.[22] Levamisole-adulterated cocaine use has been repeatedly associated with increased risk for neutropenia and agranulocytosis, vasculitis, retiform purpura and other forms of skin necrosis, and arthralgia.[17,23] Moreover, potential toxic effects in the central nervous system have been reported in animal models, showing levamisole-induced white matter pathology such as disseminated perivascular cuffing with mononuclear cells throughout the brain.[24] However, the dose-dependent effect of levamisole exposure on neurocognitive function and the thinning of distinct cortical areas in human cocaine users has been demonstrated only recently.[25] Although the mode of action of levamisole has yet to be fully elucidated, interaction with nicotinic acetylcholine receptors, stimulation of monoamine transmission and modulation of immune responses have been implicated.[17] Based on existing knowledge of levamisole's action on vessel function, it is likely to contribute to white matter damage in the human brain as well. In fact, multifocal leukoencephalopathy has been shown in people who received levamisole to treat ascariasis, recurrent aphthous ulcers, malignant melanoma and adenocarcinoma of the colon (in combination with 5-fluorouracil),[26–29] but also in case reports of cocaine users who used levamisole-adulterated cocaine.[30,31] However, systematic case–control studies to disentangle the effects of cocaine and levamisole on white matter in human users are currently lacking.

In the present study, we aimed to reanalyze a previously published data set with respect to the long-term neurotoxic effects of levamisole-adulterated cocaine on white matter structures.[25] In a previous study, we showed that cocaine users with a high exposure to levamisole displayed reduced cortical thickness, specifically in the middle frontal gyrus, compared to controls and to cocaine users with low levamisole exposure.[25] In the present study, we also analyzed fluid attenuated inversion recovery (FLAIR) images, which were not reported in the previous study. We hypothesized that although cocaine exposure in itself would be associated with more white matter hyperintensities, sustained exposure to levamisole as an adulterant would further exacerbate the white matter lesions. For objective quantification of cocaine and levamisole exposure, we used toxicological hair analyses, which provided cumulative measures of cocaine use and levamisole exposure over previous months (1 cm of hair is equal to approximately 1 month of time).[32,33] In volunteer samples of cocaine users, cocaine hair concentrations were correlated specifically with self-reported cumulative cocaine dose parameters, cognitive dysfunction and changes in cortical thickness.[14,34]

Methods

Participants

We recruited 78 people (37 chronic cocaine users and 41 healthy cocaine-naive controls) for a previous imaging study investigating the effect of levamisole exposure on cortical thickness in cocaine users.[25] (For further recruitment details, see Appendix 1, Supplementary Methods S1, available at jpn.ca/200057-a1) Healthy controls were matched to cocaine users in terms of age, sex, education level and body mass index.

As an inclusion criterion, cocaine users had to report cocaine as their primary drug of use, at a consumption level of more than 0.5 g per month and an abstinence duration of less than 6 months to ensure regular and recent use (confirmed by hair analysis, see below). General exclusion criteria were as follows: any history of a neurologic disorder or head injury (including migraine), any clinically significant medical disease (including hypertension and other cardiovascular diseases), or current use of any prescription drugs that affected the central nervous system. The presence of major medical disorders was assessed by self-report. The included images had no neuroradiological signs of head injury (such as hemorrhages, infarcts or typical larger chronic lesions corresponding to encephalomalacia) as detected by a trained neurologist–neuroradiologist. Specific exclusion criteria for cocaine users were the regular use of opioids (confirmed by hair analysis), a polytoxic drug use pattern according to DSM-IV (confirmed by hair analysis) and the presence of DSM-IV Axis I adult psychiatric disorders (except for cocaine, cannabis, nicotine or alcohol abuse or dependence; attention-deficit/hyperactivity disorder; or a previous depressive episode). Specific exclusion criteria for controls were a current or previous DSM-IV Axis I psychiatric disorder (except for nicotine dependence) and illegal drug use of more than 15 lifetime occasions or during the past 6 months (confirmed by hair analysis), except for occasional cannabis use (regular users who smoked cannabis on more than 2 occasions per week and participants who had a positive result on urine drug screening were excluded).

Before the testing session, participants were asked to abstain from illegal substances for at least 72 hours and not to consume alcohol for 24 hours. Urine samples were collected to verify self-reports. When available, we cut 6 cm hair samples from the occiput, enabling us to objectively estimate drug use over the previous 6 months. Hair samples were analyzed using liquid chromatography–tandem mass spectrometry with a robust multianalyte routine method (Appendix 1, Supplementary Methods S2).[35] Hair samples were used as follows: to confirm regular cocaine use (participants were included only if cocaine hair concentration was at least 0.5 ng/mg, an accepted threshold for chronic cocaine use[36]); to confirm that cocaine was the primary illicit drug used; and to provide cocaine and levamisole concentrations, both serving as main predictors in our statistical models. We excluded 2 cocaine users because 1 did not provide a hair sample and the other showed a clear pattern of polydrug use on hair analysis. Five controls were excluded, 2 because of positive results on drug urine tests (1 for opioids, 1 for cannabis) and 3 because hair tests revealed traces of illegal drugs (2 for cocaine, 1 for cocaine and MDMA [ecstasy]). Two further controls reported a history of migraine with regular intake of antimigraine medication. These exclusions led to a final sample of 35 cocaine users and 34 healthy controls.

The ethics committee of the Canton of Zurich approved the study (KEK-Nr.: E-14/2009 and 2014–0006), which was in

accordance with the guidelines of the Declaration of Helsinki. All participants gave written informed consent and were compensated for their participation.

Procedure

We acquired MRI scans and collected demographic information and drug-use parameters on the same day. A trained psychologist conducted a structured clinical interview to determine the presence of DSM-IV Axis I disorders (Mini International Psychiatric Interview or Structured Clinical Interview for DSM-IV, Axis I disorders).[37] We estimated intelligence quotient (IQ) using a standard German vocabulary test.[38] We assessed self-reported drug use with the structured Interview for Psychotropic Drug Consumption, capturing the quantity, duration and frequency of present and past consumption for a large number of psychotropic substances.[39] Based on former and current cocaine intake periods across the previous 6 months and the lifespan, we estimated respective cumulative doses of cocaine (average cocaine grams per week in the period × total number of weeks of cocaine use in the period).

Structural MRI acquisition and image processing

All participants were scanned using a 3 T Achieva whole-body scanner (Philips) equipped with a 32-channel receiver head coil. We collected a FLAIR sequence with the following parameters: repetition time 4800 ms; echo time 275.1 ms; inversion time 1.650 ms; field of view 250 × 250 mm; 180 slices (thickness 0.9766 mm); and voxel size 1.0000 × 0.9766 × 0.9766 mm^3. The FLAIR images were analyzed for white matter hyperintensities by 2 independent reviewers (a trained neuroradiologist–neurologist and a resident in neurology) who were fully blinded to patient characteristics, using the semiautomated global thresholding tool implemented in the open-source image-processing program ImageJ (version 1.52n; National Institutes of Health). With this tool, the optimal upper and lower signal thresholds to best discriminate a target signal (i.e., white matter hyperintensities) are applied to all pixels in a given image. Both independent raters used this tool to better discriminate and document the classification of white matter hyperintensities. First, white matter hyperintensities were judged visually by extent and location and assigned a Fazekas classification score.[40] All white matter hyperintensities corresponded to a Fazekas score of 1 or 2. Then, white matter hyperintensities were grouped according to their location — juxtaventricular, periventricular, deep or juxtacortical — because certain pathologies may result in different distributions of white matter hyperintensities.[41] Then, we summed the total surface area of white matter hyperintensities per slice for the periventricular, deep and juxtacortical hyperintensities. The total surface area was proportional to a volume measure (10 mm^2 total surface area x 0.9766 mm slice thickness equals a volume of 9.766 mm^3). We excluded juxtaventricular hyperintensities because they are considered to be of a purely nonischemic etiology.[7,41] We also counted the number of white matter hyperintensities for each participant. The interrater reliability calculated using Spearman ρ was excellent, both for total surface area ($r = 0.93$, $p < 0.001$) and for number ($r = 0.94$, $p < 0.001$). For further statistical analysis, the measures for total surface area and number of white matter hyperintensities provided by both reviewers were averaged.

Statistical analysis

We analyzed demographic and drug-use data using Pearson χ^2 tests and Student independent t tests, where appropriate. The number and total surface area of white matter hyperintensities were clearly not normally distributed (Shapiro–Wilk $W < 0.001$). Although the number of white matter hyperintensities was best explained by a negative binomial distribution, total surface area was best explained by a lognormal function (as tested by the car and MASS packages in R [R Core Team]). Accordingly, we used a negative binomial regression within a generalized linear model (GLM), with a log function to analyze the number of white matter hyperintensities (because the number of white matter hyperintensities was averaged and the model accepted only integers, we multiplied data by 10 before analysis) and a lognormal function to analyze total surface area.[42] We used SPSS 25 (IBM) for the GLM analyses.

In a first step, we analyzed group effects on the number of white matter hyperintensities and total surface area with the respective GLM including the fixed factors group (cocaine users v. controls) and sex (female v. male). We also included age, years of education and weekly alcohol intake as further covariates, because they are important factors associated with the risk of white matter lesions.[43,44] Finally, we included intracranial volume (ICV) as a further covariate, because it could affect the probability of detecting white matter hyperintensities and their volume and area.[45]

In a second step, we analyzed the dose effects of levamisole and cocaine hair concentrations on the number of white matter hyperintensities and total surface area. We included log-transformed hair concentrations of levamisole and total cocaine (cocaine + benzoylecgonine + norcocaine) as covariates in the respective GLM to evaluate their potential effects on white matter hyperintensities.[25] We again included age, years of education, ICV and weekly alcohol intake as further covariates. To plot the marginal mean effects of significant model variables, we used the ggeffects package implemented in R. For correlation analyses between cocaine use and levamisole parameters and white matter hyperintensities data, we used Spearman rank correlations. All statistical tests were carried out at a significance level of $p < 0.05$.

Because no previous studies had investigated the effect of levamisole-adulterated cocaine on white matter hyperintensities, a plausible a priori power analysis was not possible. However, the mean effect of higher versus lower levamisole exposure on whole brain cortical thickness shown previously in this sample was $d = 0.56$.[25] This effect was likely underestimated, because we compared low- and high-exposure cocaine users without a baseline of no levamisole exposure. A compromise power analysis using G*Power 3.1.9[46] — which considered this previous effect size (equivalent to f^2 =

0.0784) and the 69 included participants in a linear regression model with 7 predictors (2-tailed) — revealed a power of 83% for the detection of a significant single regression coefficient, and this was acceptable.

Results

Demographic characteristics

Included participants were mostly right-handed (92.8%), and we found no group difference in handedness (Fisher exact test, $p = 0.356$).

The 2 groups did not differ with respect to education, sex distribution, smoking status, body mass index or ICV (Table 1). As expected, cocaine users showed lower verbal IQ scores and more intense alcohol, nicotine and illicit drug intake than healthy controls (Appendix 1, Table S1). Among the cocaine users, 31.4% fulfilled the criteria for cocaine abuse and 57.1% for cocaine dependence according to DSM-IV. Cocaine was used mostly nasally (94.3% of cocaine users); only a few (5.7%) reported smoking it (free base). No injection users were included.

Group effects

The measures of white matter hyperintensities among cocaine users and controls are shown in Table 2. In GLMs (assuming a negative binomial function for the number of white matter hyperintensities and a lognormal function for total surface area with group and sex as factors and age, education, ICV and weekly alcohol intake as covariates), we found a significant group effect for total surface area but not for number (Table 3). Age was a significant covariate of strong effect size in both models. We found significant differences in ICV in the GLM for total surface area but not for number; we found the opposite for education (although there was a statistical trend for total surface area). Sex and weekly alcohol intake did not have a significant effect in any model. Thus, cocaine users showed significantly more white matter hyperintensities in terms of total surface area but not in terms of number. We also confirmed the well-known age effect on the number and area of white matter hyperintensities, and an effect of education.[47] As we assumed, ICV also had a significant effect on the area of the white matter hyperintensities.

Table 1: Participant demographic characteristics and drug use

Characteristic	Healthy controls ($n = 34$)*	Cocaine users ($n = 35$)*	Value†	p value
Age, yr	31.8 ± 7.8	33.0 ± 7.5	$t_{67} = -0.62$	0.54
Female/male	12/22	8/27	$\chi^2_1 = 1.3$	0.26
Education, yr	10.5 ± 1.5	10.4 ± 1.5	$t_{67} = 0.40$	0.69
Body mass index, kg/m^2	23.7 ± 3.5	23.2 ± 2.8	$t_{67} = 0.62$	0.54
Verbal IQ	108.3 ± 11.4	102.3 ± 9.9	$t_{67} = 2.33$	0.023
Intracranial volume, cm^3	1621.7 ± 144.7	1582.8 ± 150.1	$t_{67} = 1.09$	0.28
Nicotine use				
Cigarettes per day, *n*‡	31.2 ± 42.9	58.3 ± 61.2	$t_{67} = -2.1$	0.037
Duration of use, yr	7.9 ± 7.6	12.3 ± 9.1	$t_{67} = -2.1$	0.038
Smoking, yes/no	24/10	29/6	$\chi^2_1 = 1.5$	0.23
Alcohol use				
Pure ethanol, g/w‡	67.8 ± 74.8	207.7 ± 179.1	$t_{67} = -4.2$	< 0.001
Duration of use, yr	12.1 ± 6.9	13.6 ± 8.2	$t_{67} = -0.81$	0.42
Cocaine use				
Amount, g/w‡	0.00 ± 0.00	1.5 ± 1.8	$t_{67} = -4.9$	< 0.001
Duration of use, yr	0.06 ± 0.34	9.6 ± 6.4	$t_{67} = -8.7$	< 0.001
Maximum dose, g/d	—	2.2 ± 1.3	—	—
Cumulative dose, last 6 mo, g	—	40.1 ± 48.1	—	—
Cumulative dose, lifetime, g	0.04 ± 0.21	1244.9 ± 1415.6	$t_{67} = -5.1$	< 0.001
Last consumption, d	—	9.3 ± 7.9	—	—
Urine toxicology negative/positive§	34/0	17/18	$\chi^2_1 = 21.9$	< 0.001
Hair concentrations				
Cocaine, ng/mg	0.00 ± 0.02	20.5 ± 24.4	$t_{67} = -4.9$	< 0.001
Benzoylecgonine, ng/mg	0.00 ± 0.01	7.3 ± 3.3	$t_{67} = -4.6$	< 0.001
Norcocaine, ng/mg	0.00 ± 0.00	0.54 ± 0.64	$t_{67} = -4.9$	< 0.001
$\text{Cocaine}_{\text{total}}$, ng/mg¶	0.01 ± 0.03	28.3 ± 33.5	$t_{67} = -4.9$	< 0.001
Levamisole, ng/mg	0.00 ± 0.00	5.8 ± 9.7	$t_{67} = -3.5$	< 0.001
Levamisole:$\text{cocaine}_{\text{total}}$ ratio	—	0.24 ± 0.21	—	—

*Values are mean ± standard deviation unless otherwise indicated.
†Independent *t* test for dimensional data; χ^2 test for frequency data.
‡Average use over the last 6 months.
§Cut-off values for cocaine = 150 ng/mL.
¶$\text{Cocaine}_{\text{total}}$ = cocaine + benzoylecgonine + norcocaine.

Table 2: White matter hyperintensity markers

Marker	Healthy controls (n = 34)*	Cocaine users (n = 35)*
Number*	3.81 ± 6.08 (0–24.5)	6.01 ± 12.7 (0–50.5)
Total surface area, mm^2†	58.4 ± 102.2 (0–452.2)	98.4 ± 223.9 (0–841.8)
Periventricular surface area, mm^2	9.31 ± 20.9 (0–91.8)	21.8 ± 59.3 (0–258.8)
Deep surface area, mm^2	23.1 ± 55.8 (0–257.8)	32.8 ± 75.4 (0–271.5)
Juxtacortical surface area, mm^2	6.69 ± 15.3 (0–62.5)	10.52 ± 31.1 (0–169.0)

*Values are mean ± standard deviation (range).
†Average across both reviewers.

Table 3: Group effects on white matter hyperintensity number and total surface area

	White matter hyperintensities					
	Number*			Total surface area†		
Effect	Wald χ^2_1	p value	Exp(B)	Wald χ^2_1	p value	Exp(B)
Group (reference cocaine users)	0.127	0.72	1.121	5.526	0.019	3.164
Sex (reference female)	1.079	0.30	1.692	0.042	0.84	0.673
Age, yr	33.38	< 0.001	1.116	30.12	< 0.001	1.154
Intracranial volume, cm^3	2.678	0.10	1.002	12.66	< 0.001	1.004
Education, yr	5.236	0.022	1.227	3.210	0.07	1.242
Weekly alcohol intake, pure ethanol g/w	0.429	0.51	0.999	1.978	0.16	0.999
Omnibus test (n = 69)	$LQ_6 = 52.16$, $p < 0.001$			$LQ_6 = 50.78$, $p < 0.001$		

LQ = likelihood quotient.
*Negative binomial generalized linear model with log-link function.
†Lognormal generalized linear model.

Levamisole and cocaine dose effects

Figure 1 shows examples of FLAIR images with white matter hyperintensities in participants of different ages and with low or high levamisole consumption. We assessed the dose effects of levamisole and cocaine using analog GLMs (Table 4), replacing the fixed factor group with levamisole and total cocaine hair concentrations. The estimated marginal means for the significant effects are shown in Figure 2 (number of white matter hyperintensities) and Figure 3 (total surface area of white matter hyperintensities). Both levamisole and cocaine had significant effects on total surface area and number; however, levamisole showed much stronger effect sizes. Again, age had significant effects on both independent variables, with strong effect sizes. In these models, ICV had a significant effect only on the number of white matter hyperintensities, and weekly alcohol intake had a significant effect only on total surface area. Sex and education had no significant effects.

Inspection of exp(B) (Table 4) and of the plotted estimated marginal means (Figures 2 and 3) revealed that higher levamisole concentrations and higher age were clearly associated with more and larger white matter hyperintensities. As well, increased ICV was associated with a higher number of white matter hyperintensities (Figure 2). Surprisingly, in these models higher cocaine hair concentrations were associated with fewer and smaller white matter hyperintensities (Figures 2 and 3, respectively), although the effect was much smaller than that of levamisole. Higher weekly alcohol intake was also associated with smaller white matter hyperintensities (Figure 3). Importantly, when levamisole hair concentration was deleted from the models, the cocaine effect was reversed: higher cocaine hair concentrations became associated with increased total surface area (p = 0.028; exp[B] 1.263). The reversed direction of the effect was also present for the number of white matter hyperintensities, although the effect was smaller and nonsignificant (p = 0.57, exp[B] 1.043). Alcohol use per week was no longer significant after the exclusion of levamisole as a predictor (total surface area p = 0.22, exp[B] 0.999; number of white matter hyperintensities p = 0.45, exp[B] 0.999). In contrast, the exclusion of hair cocaine concentration from the models did not change the direction of the levamisole effects on total surface area (p = 0.006, exp[B] 1.343) and the number of white matter hyperintensities (p = 0.13, exp[B] 1.143), although the levamisole effect was weakened specifically with respect to the number of white matter hyperintensities. Importantly, model fit indices and explained total variances were always better with the full models than with the reduced models (in which cocaine or levamisole concentrations were left out). Consequently, the counterintuitive reduction of the risk of white matter hyperintensities with increased cocaine exposure was likely an artifact induced by the high collinearity of cocaine and levamisole hair concentrations (Appendix 1, Table S2).

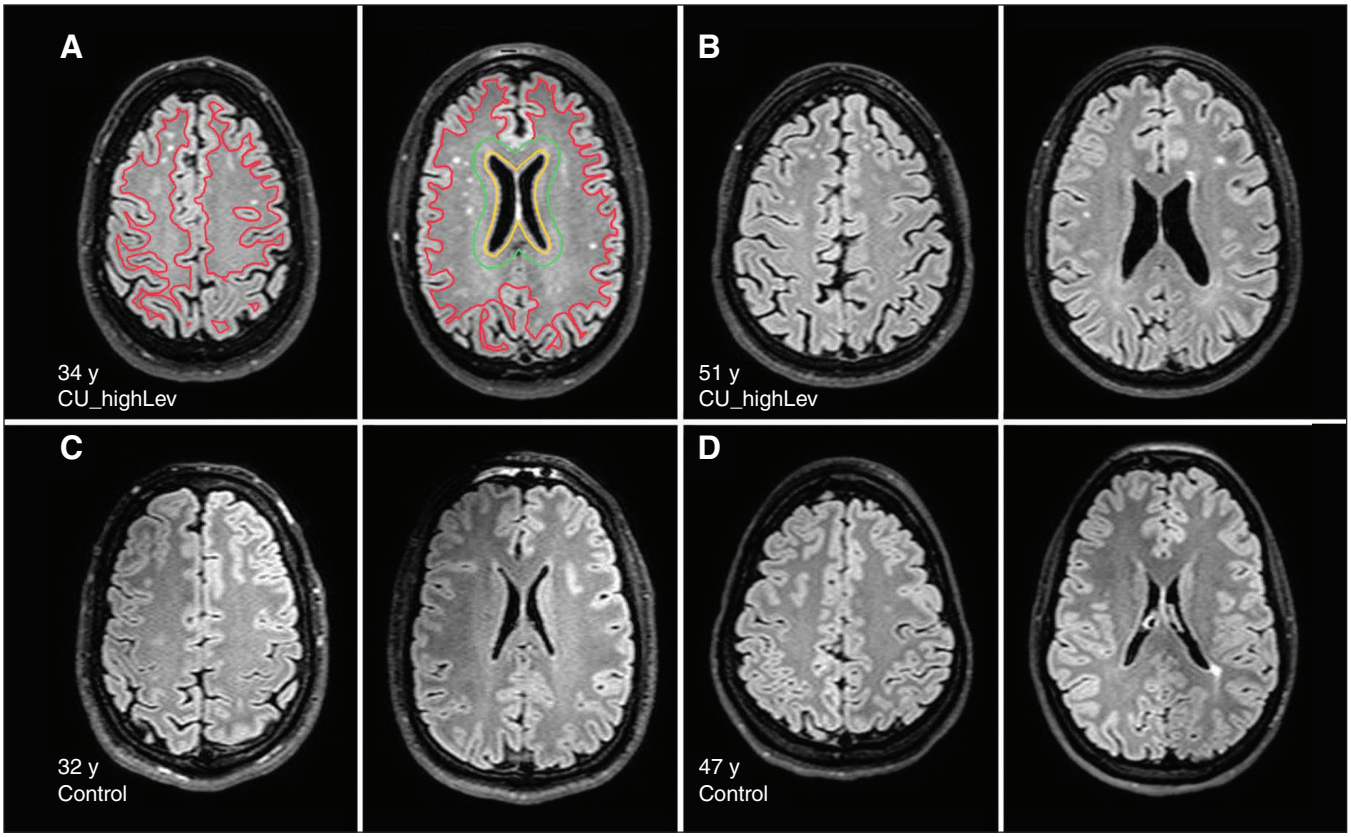

Fig. 1: White matter hyperintensities at different ages in controls and cocaine users with high levamisole exposure (CU_highLev). Representative FLAIR images (2 slices each) of (A) a younger cocaine user and (B) an older cocaine user, both with high levamisole hair concentrations; FLAIR images of (C) a younger control participant and (D) an older control participant. In A, different white matter hyperintensity locations are visualized according to Kim and colleagues[41]: superficial cortex to red line, juxtacortical; between red and green lines, deep; between green and yellow lines, periventricular; between yellow line and ventricles, juxtaventricular. FLAIR = fluid attenuated inversion recovery.

Table 4: Levamisole and cocaine dose effects on white matter hyperintensity number and total surface area

	White matter hyperintensities					
	Number*			Total surface area†		
Effect	Wald χ^2_1	*p* value	Exp(B)	Wald χ^2_1	*p* value	Exp(B)
Sex (reference female)	2.577	0.11	2.156	0.730	0.39	2.460
Age, yr	22.21	< 0.001	1.092	13.849	< 0.001	1.132
Intracranial volume, cm^3	4.021	0.045	1.002	2.893	0.09	1.003
Education, yr	0.500	0.48	1.070	2.801	0.09	1.289
Weekly alcohol intake, pure ethanol g/w	0.106	0.75	1.000	7.468	0.006	0.995
$Cocaine_{total}$ hair concentration, log	19.10	< 0.001	0.384	12.90	< 0.001	0.068
Levamisole hair concentration, log	21.06	< 0.001	3.441	14.23	< 0.001	27.89
Omnibus test (*n* = 69)	$LQ_7 = 68.61$, $p < 0.001$			$LQ_7 = 68.65$, $p < 0.001$		

LQ = likelihood quotient.
*Negative binomial generalized linear model with log-link function.
†Lognormal generalized linear model.

Interactions

Within the GLMs, we investigated potential interactions between levamisole and cocaine hair concentrations, and between both substances and age, but neither of these interactions was significant (data not shown).

Location of white matter hyperintensities

By examining the distribution of white matter hyperintensities across different locations, we observed that (over all participants) 53.6% were located in the deep or juxtacortical white matter, 29.9% in the periventricular white matter and

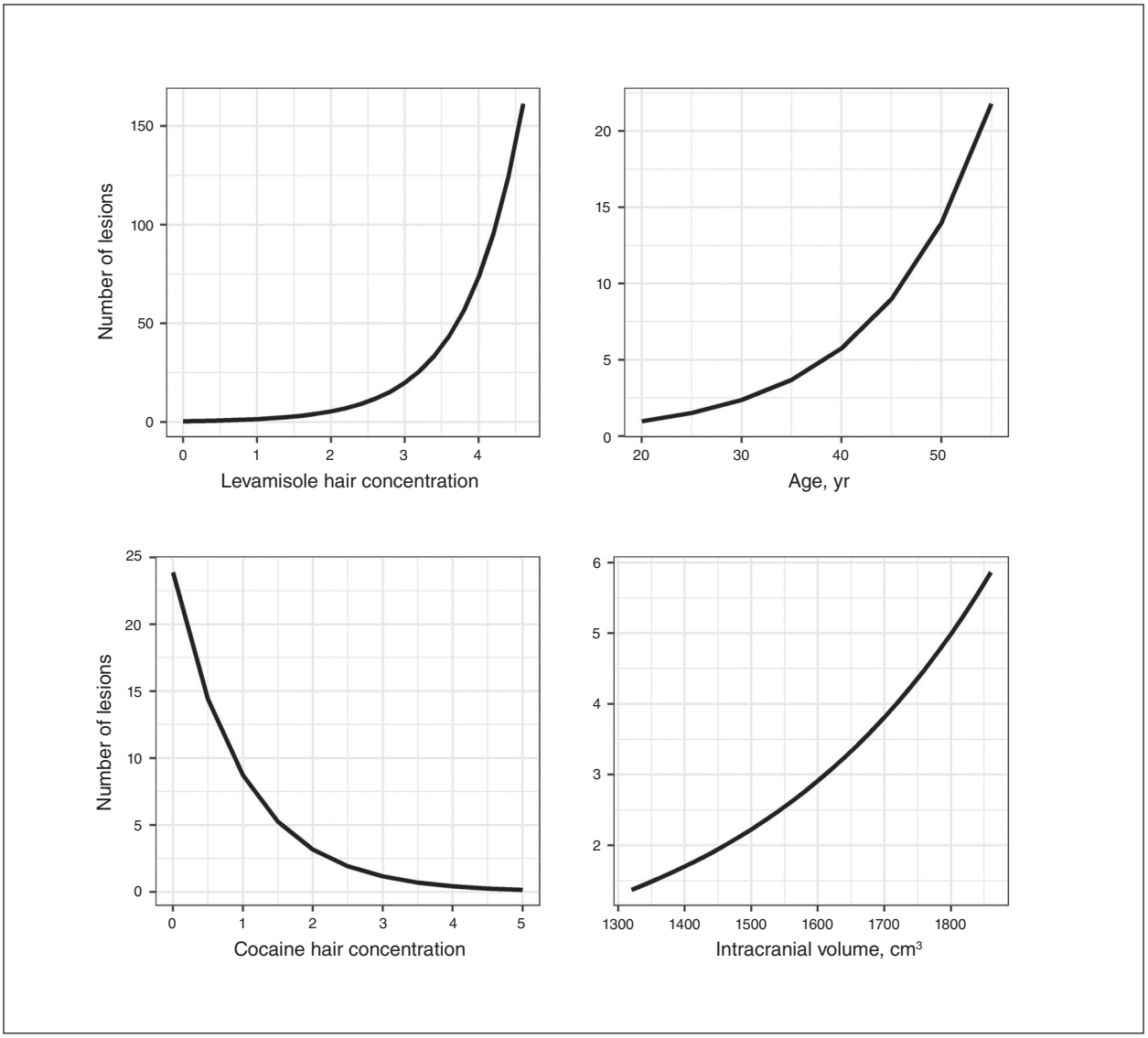

Fig. 2: White matter hyperintensities were more frequent in cocaine users with increased levamisole exposure. The estimated marginal means of the significant effects (negative binomial generalized linear model with log-link function) showed that elevated levamisole hair concentration (upper left), higher age (upper right) and larger intracranial volume (lower right) were associated with more white matter hyperintensities. In this model that included healthy controls, lower cocaine concentration was associated with more white matter hyperintensities (lower left); however, note the scale of the *y*-axis, suggesting a weak effect.

16.5% in the juxtaventricular white matter. Cocaine users did not differ from controls in terms of percentages in each of these regions (Fisher exact test; deep $p = 0.26$; periventricular $p = 0.26$; juxtaventricular $p = 0.45$). In a GLM with the factor group of surface areas in the different locations (Table 2), we detected significant group effects in the periventricular ($p = 0.008$, exp[B] for cocaine users 4.79) and juxtacortical areas ($p = 0.021$, exp[B] for cocaine users 4.05), but not the deep area ($p = 0.29$, exp[B] for cocaine users 1.895). The pattern of the covariates was largely similar to the effects of total surface area. When we replaced group with cocaine and levamisole hair concentrations, the GLM again revealed significant effects for the periventricular (levamisole $p < 0.001$, exp[B] 20.95; cocaine $p < 0.001$, exp[B] 0.102) and juxtacortical areas (levamisole $p = 0.005$, exp[B] 22.74; cocaine $p = 0.005$, exp[B] 0.110), but not the deep area (levamisole $p = 0.99$, exp[B] 1.01; cocaine $p = 0.76$, exp[B] 0.83). These results indicate that levamisole exposure is mainly associated with increased white matter hyperintensities in the periventricular and juxtacortical areas.

Correlations between white matter hyperintensities and drug use

In cocaine users ($n = 35$), the number and area of white matter hyperintensities were significantly correlated with the maximum reported cocaine dose during a single day

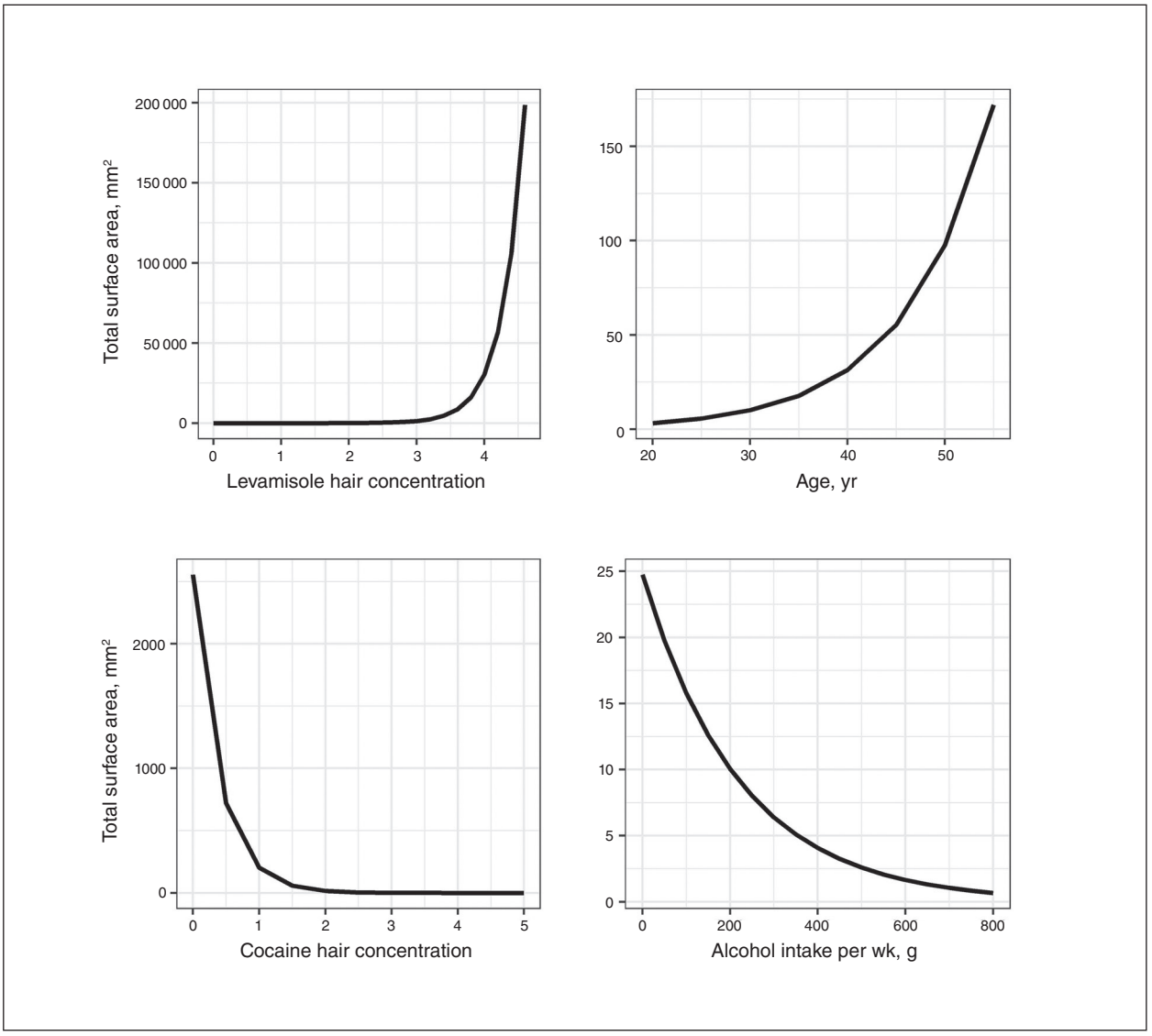

Fig. 3: White matter hyperintensities were larger in cocaine users with increased levamisole exposure. The estimated marginal means of the significant effects (lognormal generalized linear model) showed that elevated levamisole hair concentration (upper left) and higher age (upper right) were associated with higher total surface area of the white matter hyperintensities. In this model that included healthy controls, lower cocaine concentration (lower left) and lower weekly alcohol intake (lower right) were associated with larger white matter hyperintensities; however, note the scale of the *y*-axis, suggesting weak effects.

(number $r = 0.40$, $p = 0.029$; area $r = 0.37$, $p = 0.047$) and the duration of cocaine use in years (number $r = 0.36$, $p = 0.032$; area $r = 0.36$, $p = 0.037$); however, these significant correlations disappeared when the number and area of white matter hyperintensities were adjusted for age and ICV.

Correlations between parameters of objective and subjective cocaine use

In cocaine users, total cocaine hair concentrations were significantly correlated with cocaine grams per week, cumulative cocaine dose from the last 6 months (g), cumulative cocaine lifetime dose (g), last cocaine consumption (days) and levamisole hair concentrations (Appendix 1, Table S2), indicating that higher self-reported cocaine use and a shorter abstinence period were associated with higher cocaine hair concentrations. As well, levamisole hair concentrations were significantly correlated with cocaine grams per week and both cumulative cocaine dose measures (Appendix 1, Table S2), demonstrating that higher self-reported cocaine use was associated with higher levamisole hair concentrations.

Discussion

The present investigation was the first case–control study to systematically analyze the effects of cocaine and levamisole

exposure on the white matter structure of chronic cocaine users. We demonstrated that cocaine users with predominantly nasal cocaine use had more white matter hyperintensities in terms of total surface area, but not in terms of number. Extended analyses revealed that higher levamisole exposure during the previous months (confirmed by toxicological hair analyses) increased the risk for white matter hyperintensities, both in number and total surface area. Remarkably, the levamisole effect on white matter hyperintensities was most prominent in the periventricular white matter regions; this finding was well in line with an earlier study showing that levamisole can induce periventricular white matter abnormalities in dogs,[24] and it was in accordance with case reports showing multifocal leukoencephalopathy, including periventricular white matter hyperintensities, in cocaine users with confirmed[30] and suspected[31] intake of levamisole-contaminated cocaine. Therefore, white matter hyperintensities with periventricular preponderance are likely induced not only by short-term levamisole medical treatment,[26–29] but also by unintended chronic levamisole exposure. Periventricular white matter regions are supplied by terminal branches of small vessels and particularly vulnerable to ischemic injury and small vessel dysfunction.[48] White matter hyperintensities in periventricular regions are associated with cognitive decline in stroke patients[49] and elderly adults without clinically apparent stroke.[50] However, multifocal leukoencephalopathy in patients who received levamisole as a medication (e.g., for ascariasis) had MRI findings suggestive of acute demyelinating encephalomyelitis, and demyelination has been verified on biopsy in at least 2 studies.[26,28] Thus, it remains to be ascertained whether white matter hyperintensities in cocaine users reflect acute demyelinating encephalomyelitis or ischemic changes, or both. Of note, the burden of white matter hyperintensities was not sufficient to justify a diagnosis of a toxic leukoencephalopathy in any of the cocaine users, as seen in levamisole-medicated patients. Finally, the calculated effect sizes suggest that the levamisole-induced burden of white matter hyperintensities might be more severe than the cocaine-induced fraction. This, in turn, may indicate that street cocaine with a larger proportion of levamisole adulteration could result in more long-term harmful sequelae, including neurocognitive and neuropsychiatric symptoms.

Although the initial GLM indicated larger white matter hyperintensities among the cocaine users, further GLM including cocaine and levamisole hair concentrations (instead of the group factor) unexpectedly showed a decreased risk for white matter hyperintensities with increasing cocaine dose. However, when levamisole was excluded from the model, higher cocaine hair concentrations were again related to more white matter hyperintensities in terms of surface area (but not number), indicating that in the full model the predictor levamisole clearly better explained the variance in white matter hyperintensities than cocaine, resulting in a statistical artifact of an inverse risk relation regarding cocaine. We concluded that levamisole exposure likely had a stronger effect on white matter hyperintensities than cocaine in our sample. This result stands somewhat in contrast to a previous study showing a pronounced burden of white matter hyperintensities in cocaine users from Boston,[8] who were likely less exposed to levamisole, given that levamisole appeared as a cocaine adulterant around 2003 in the United States,[51] but the levamisole contamination of cocaine began at a low level and has increased in concentration and frequency in more recent years.[23] However, this previous sample was an average of 10 years older and used cocaine 6 years longer than the present sample, and contained only cocaine-dependent individuals. In contrast, in our study about 40% of the cocaine users were regular but not dependent users. Thus, cocaine effects on white matter hyperintensities might be more pronounced with longer and more severe use. As well, a recent study in an urban sample of young stroke patients showed that, along with white matter hyperintensities, cocaine use was associated with an increased risk for cerebral microbleeds.[52] However, this study did not control for levamisole exposure; the white matter hyperintensities burden, and possibly also the microbleeds in this sample, might be partially explained by levamisole exposure rather than by cocaine consumption alone. The neuropathological underpinnings of MRI-detected white matter hyperintensities are mostly chronic ischemic lesions of the white matter with consecutive dilatation of perivascular spaces.[53] In elderly people, a high burden of white matter hyperintensities is strongly correlated with a rapid global decline in cognitive function over time.[10,54] It is undisputed that large and confluent areas of white matter hyperintensities resemble accelerated brain aging and negatively affect cognitive health. However, white matter hyperintensities are rare in healthy people younger than age 40.[55]

In our sample of cocaine users, the main route of administration was nasal, so it remains unclear whether other routes, such as smoking or injecting levamisole-contaminated cocaine, might reveal different results. Future studies are needed to investigate the effects of levamisole on white matter lesions in crack cocaine or injection users.

Although chronic cocaine abuse is known to elicit alterations of brain structure and function,[11,12] we have shown here that additional injury to the brain's white matter stems from the adulteration of cocaine with levamisole. Mechanisms of levamisole-induced generation of white matter hyperintensities may include synergistic effects to those of cocaine; elevation of postsynaptic noradrenaline levels may contribute to orchestrated sympathomimetic vasoconstrictive actions of both substances.[56] In addition, more recent reports have demonstrated that levamisole-induced vasculopathy has a marked immunological component.[57] Another potential mechanism of chronic subcortical ischemia could be destruction of the blood–brain barrier as a consequence of cocaine use, which could be further amplified by levamisole.[58,59]

Limitations

Our study had some limitations that should be kept in mind. First, the study had a moderate sample size. However, detailed assessment of drug use and consumption history; objective quantification of the levels of cocaine, levamisole and other drugs in hair samples; and strict exclusion of neuropsychiatric comorbidities or severe polysubstance use were particular strengths of our study. Second, although we aimed

to recruit people who used only cocaine, we detected a certain amount of co-use of other substances (specifically moderate cannabis and MDMA [ecstasy] use; Appendix 1, Table S1) in self-reports and in hair samples. Thus, we cannot completely rule out the possibility that the effects we observed could have been partially explained by concomitant drug use or interaction effects of levamisole or cocaine with other substances. Third, we did not systematically assess blood pressure during the study visits, although its association with white matter hyperintensities has been well established.[60] However, we excluded all self-reported and treatment-relevant cardiovascular disease indications, and none of our participants was taking any antihypertensive medication.

Conclusion

Our finding of accelerated brain aging through progressive white matter injury in cocaine users exposed to high doses of the adulterant levamisole is particularly worrisome, because this substance is increasingly found in street cocaine.[23] Consequently, levamisole-exposed cocaine users may be at risk of more severe and protracted neurocognitive consequences earlier in their lives. The reduction of levamisole in street cocaine should be a major aim of current drug policy-making. Systematic public drug-checking for those concerned would be a feasible prevention approach.[61] Finally, the hazardous effects of levamisole need to be recognized by the public, given that avoiding levamisole-contaminated cocaine likely prevents white matter disease and related symptoms.

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Contributors statement: S. Hirsiger, S. Wegener, E. Seifritz and B. Quednow designed the study. F. Conrad, S. Hirsiger, M. Baumgartner and P. Stämpfli acquired the data, which S. Winklhofer, E. Seifritz, S. Wegener and B. Quednow analyzed. F. Conrad, E. Seifritz, S. Wegener and B. Quednow wrote the article, which S. Hirsiger, S. Winklhofer, M. Baumgartner, P. Stämpfli, E. Seifritz and B. Quednow reviewed. All authors approved the final version to be published and can certify that no other individuals not listed as authors have made substantial contributions to the paper.

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