Study title:

Ketamine in treatment resistant major depression (TRD) -
A placebo controlled, double blind, randomized trial of efficacy, safety and response prediction by resting state fMRI

Test substance:	Ketamin
Eudra-CT number:	2010-023414-31
BfArM-submission-number:	4038024
Short appellation:	KETREST

Final report (abstract)

V1.1 / Date: 27.02.2020; Ergänzung zu V01 vom 27.04.2017

Sponsor of the clinical trial:

Otto-von-Guericke University Magdeburg, Medical Faculty
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Study initiation – Study completion
10.08.2012 – 09.05.2016

Signatures

The signing authors agree on the content of this final report by their signatures. The clinical trial reported here was conducted in accordance with the principles of the Declaration of Helsinki, Good Clinical Practice (GCP) and applicable laws.

Die unterzeichnenden Autoren stimmen den Inhalten des vorliegenden Abschlussberichtes durch ihre Unterschriften zu. Die hier berichtete, klinische Prüfung wurde nach den Grundsätzen der Deklaration von Helsinki, der Guten Klinischen Praxis (GCP) sowie den geltenden Gesetzen durchgeführt.

Sponsor

Prof. Dr. med. H.-J. Rothkötter

28.02.2020
Magdeburg, date

Project managerin

Frau Dr. rer. nat. Antje Wiede

27.02.2020
Magdeburg, date

Title of study	Ketamine in treatment resistant major depression (TRD) -A placebo controlled, double blind, randomized trial of efficacy, safety and response prediction by resting state fMRI
Type of project **Phase:**	A placebo-controlled, double-blind, monocentrically, randomized, non-commercial clinical trial to predict the efficacy and safety according to AMG II
Sponsor **Representative**	Prof. Dr. med. Hermann-Josef Rothkötter Medical Faculty, Otto-von-Guericke University Leipziger Str. 44, 39120 Magdeburg Tel.: 0391/67-15750 Email: hermann-josef.rothkötter@med.ovgu.de Dr. rer. nat. Antje Wiede KKS Magdeburg Medical Faculty, Otto-von-Guericke University Leipziger Str. 44, 39120 Magdeburg Tel.: 0391/67-15307 Email: antje.wiede@med.ovgu.de
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Registration: **Publication of the study (reference)**	DRKS: DRKS00003527 WHO UTN: U1111-1127-4851 Li, M., Demenescu, L. R., Colic, L., Metzger, C. D., Heinze, H. J., Steiner, J., ... & Walter, M. (2016). Temporal Dynamics of Antidepressant Ketamine Effects On Glutamine Cycling Follow Regional Fingerprints of Ampa and Nmda Receptor Densities. Neuropsychopharmacology.

Study period	10.08.2012 – 09.05.2016
Study objectives	To assess the efficacy of a single dose ketamine infusion in treatment resistant depression against 0.9 % saline infusion and to assess effects of glutamate, glutamine and GABA concentrations as measured by MRS. Secondly resting state fMRI (RS-fMRI) and MRS measures pretreatment is assessed for predictive potential on decrease of depressive symptoms after verum infusion. To understand the underlying mechanism, we include a healthy control population, where we want to 1. Investigate the general effects of a single ketamine administration on resting-state functional connectivity using fMRI at ultra high resolution 2. Investigate the correlations between glutamatergic measures and resting state functional connectivity at baseline and following ketamine administration 3. Investigate the time-course of sequential changes in amino acid neurotransmitters following ketamine administration
Primary outcomes	Primary efficacy endpoint : Improvement on HAMD scores, 24 hours after ketamine infusion.
Secundary outcomes	Secondary Endpoint 1: Predictive value of resting state regional homogeneity (ReHo), glutamine concentration and functional connectivity (FC) of pgACC for treatment response to ketamine in patients. Secondary Endpoint 2: Greater changes of glutamine concentration after ketamine infusion acutely and after 24 hours compared to placebo.
Study design	Placebo controlled, double blind, randomized clinical trial with four parallel treatment arms, to evaluate efficacy and effects of a single infusion of ketamine on brain metabolites concentrations in healthy controls and patients. The experimental design is considered as double-blind. The procedures will be followed in order to prevent the investigator and subjects from coming into contact with the study materials thereby compromising the blinding of the study. The study materials will be administered by a designated person other than the investigator. Additionally, both the person in charge of drug administration and the subject will be instructed not to discuss the study materials and treatment schedule with the investigator. Subjects will be randomized either to ketamine or placebo. Subjects will be imaged three times: once at baseline, before being administered ketamine, the second scan will be performed at the time of initial separation of antidepressants

	effects from dissociative-like symptoms (i.e., at 60 minutes following ketamine administration), and the third scan will be performed 1 day after the infusion. - *treatment arm 1*: TRD patients (n=20) receiving ketamine i.v. infusion - *treatment arm 2*: TRD patients (n=20) receiving placebo i.v. infusion - *treatment arm 3*: healthy controls (n=40) receiving ketamine i.v. infusion - *treatment arm 4*: healthy controls (n=40) receiving placebo i.v. Infusion
Trial test medication / treatment strategy	Name: Ketamine Hydrochloride Form: Solution Dose or Concentration: 500 mg/10ml Dosage: Single Route of Administration: Infusion Frequency of Administration: Single Duration of Treatment: Single treatment
Name of Finished Product	Ketamin-ratiopharm® 500 mg/10ml injection solution
Batch-No (expiry date)	L42872R (11/2014) M08443 (05/2015) M38959 (11/2015) N14676 (06/2016)
Treatment / intervention	All treatment interventions will be done in TRD patients meeting the inclusion criteria, and in a selected group of healthy controls. Both immediate and sustained effects of ketamine infusions at subanaesthetic doses will be explored and compared during acute and sustained investigation of RS-fMRI paralleled by GABA and Glu/Gln MRS. **Treatment:** Both patients and healthy subjects in the verum group will receive a single intravenous ketamine hydrochloride injection (0.5mg/kg body weight over 40 minutes). Diagnostic intervention: Resting state fMRI and MRS before and after administration will be assessed on a 7T clinical MR scanner. **Control treatment:** Both the patients and healthy subject allocated to the control group will receive placebo injections of 0.9 % saline (0.5mg/kg body weight over 40 minutes) in the same fashion as the verum group. Ketamine and placebo applications are given double blind with subjects pseudorandomly assigned to either placebo or verum group to minimize confounding effects of age or gender due to small sample size of the pilot study. Administration will be done outside the scanner. RS-fMRI will be compared before infusion, 60 minutes afterinfusion, and 24 hours after infusion.
Comparator treatment / comparative condition	Name: Isotone NaCl (Berlin Chemie) Form: Solution Dose or Concentration: 0.9%

	Dosage: Singe Route of Administration: Infusion Frequency of Administration: Single Duration of Treatment: Single treatment
Total number of patients	Planned number of cases: 120 (40 patients/ 80 healthy subjects) Finally screened study participants: 192 (including 91 patients) Included study participants: 91 (including 10 patients) Randomized study participants: 94 (including 10 patients) Drop-outs / Lost to FU: 3/1
Study population	TRD subjects can be either drug-free or on a standard antidepressant treatment for at least 6 weeks without changes in dosage in the 2 previous weeks before the baseline assessment. The inclusion and exclusion criteria for enrolling subjects in this study are described in the following sections. Healthy subjects are requested to be off any prescribed medication and will be recruited via public announcements.
Inclusion Criteria	TRD subjects must satisfy the following criteria before entering the study: • Male or female subjects between 18 and 55 years of age, inclusive. • Subjects must fulfill ICD-10 for recurrent Major Depression (F 33) without psychotic features, based on clinical assessment and confirmed by structured diagnostic interview. • Subjects must have an initial score of at least 16 on the 21-item HAMD at screen and at baseline of study phase I. • Current history of lack of response to two adequate antidepressant trials (may be from the same chemical class) operationally defined using the Antidepressant Treatment History Form (ATHF), see Appendix. • Current major depressive episode of at least 4 weeks duration. Both healthy controls and TRD subjects must fulfill the following criteria: • Women of childbearing potential must have a negative pregnancy test on the day of each scan, prior to participating in MRI imaging part of the study. • Healthy or medically stable on the basis of physical examination, medical history, vital signs and 12 lead ECG performed at screening. • Healthy or medically stable on the basis of clinical laboratory tests performed at screening. • Healthy or medically stable on the basis of physical

	examination, vital signs (including orthostasis test) or 12 lead ECG (including QTcB <470 ms) at Screening. Minor deviations in ECG, which are not considered to be of clinical significance to the investigator are acceptable. • Willing to adhere to the prohibitions and restrictions specified in this protocol. • Subjects must have signed an informed consent document indicating that they understand the purpose of and procedures required for the study and are willing to participate in the study.
Exclusion Criteria	*Potential TRD subjects who meet any of the following criteria will be excluded from participating in the study:* • Current or past history of psychotic features or a diagnosis of Schizophrenia or any other psychotic disorder as defined in the ICD-10. *Potential healthy control subjects who meet any of the following criteria will be excluded from participating in the study:* • Current or past history of psychotic features or a diagnosis of any psychiatric disorder as defined in the ICD-10. *Both TRD subjects and healthy control subjects who meet any of the following criteria will be excluded from participating in the study:* • Subjects with a history of drug or alcohol dependency or abuse within the preceding 6 months. • Female subjects who are either pregnant or nursing. • Serious, unstable illnesses including hepatic, renal, gastroenterologic, respiratory, cardiovascular (including ischemic heart disease), endocrinologic, neurologic, immunologic, or hematologic disease. • Subjects with uncorrected hypothyroidism or hyperthyroidism. • Subjects with one or more seizures without a clear and resolved etiology. • Presence of metallic (ferromagnetic) implants (heart pacemaker, aneurysm clips) or tattoos. • Subjects with any medical illness likely to alter brain morphology and/or physiology (uncontrolled hypertension, diabetes) will be excluded. • Treatment with a reversible MAOI within 4 weeks prior to baseline assessment. • Treatment with clozapine or ECT within 3 months prior to baseline assessment.

	• Judged clinically to be at serious suicidal risk. • Drinks, on average, more than 8 cups of tea/coffee/cocoa/cola per day. • Use of energy drinks within 3 days before the baseline assessment and during the duration of the study. • Clinically significant acute illness within 7 days prior to study drug administration. • Clinically significant history of drug and/or food allergies. • Donation of 1 or more units (approximately 450 mL) of blood or acute loss of an equivalent amount of blood within 90 days prior to study drug administration. • Have received an experimental drug or used an experimental medical device within 90 days before the planned start of treatment.
Depiction of demographics and baseline characteristics	Demographics: Age, gender, HAMD scores, relative concentrations of glutamine Assessment schedule: Psychological scores are assessed on the day of infusion and 1 day post infusion. On the day of infusion, HAMD scales are assessed 1 hr before infusion and 120 minutes after infusion. Additional scores include Hamilton Anxiety Rating Scale (HAM-A), Becks Depression Inventory (BDI), Young mania rating scale (YMRS) and the positive symptoms score on the brief psychiatric rating scale (BPRS). The scale for subject assessment will be found in section 12, Attachments - Appendices. Assessment of safety: Monitoring of adverse effects includes perceptual disturbance confusion and psychotic experience, changes in blood pressure and heart rate as well as hematological and biochemical values, dizziness, gastrointestinal distress, increased thirst, headache and constipation and will be systematically assessed at the day of infusion.
Depiction of efficacy	*Primary efficacy Endpoint*: Improvement on HAMD scores, 24 hours after ketamine infusion. *Secondary Endpoint 1:* Predictive value of resting state regional homogeneity (ReHo), glutamine concentration and functional connectivity (FC) of pgACC for treatment response to ketamine in patients. *Secondary Endpoint 2:*

	Greater changes of glutamine concentration after ketamine infusion acutely and after 24 hours compared to placebo. *Efficacy Measurements:* - Linear mixed model, Cohen's d for ketamine/placebo difference - Cohen's d for responders, for RS-fMRI differences, two sampled t-tests will assess differences in pgACC ReHo and whole brain functional connectivity (RSFC). - Prediction of ketamine related responses will be tested using multivariate discriminant function analysis of pgACC ReHo and pgACC RSFC's. - Exploratory analysis of multimodal response prediction will make use of a support vector machine based approach (SVC, Craddock et al. 2009) - Linear dependence of changes in HAMD on pre-infusion resting state fMRI will be tested using Spearmans Rho.
Depiction of safety	Overall safety: monitoring of adverse effects includes perceptual disturbance confusion and psychotic experience, changes in blood pressure and heart rate as well as hematological and biochemical values, dizziness, gastrointestinal distress, increased thirst, headache and constipation and will be systematically assessed at the day of infusion. Monitoring of arterial blood pressure will be performed at the beginning and at 30 minutes intervals for 2 hours post infusion. Patients will further be seen by an ophthalmologist before treatment to exclude potential risk factors to ketamine such as older perforating eye trauma or glaucoma. All patients will further be neurologically screened for pathologies seen in a 32 channel EEG which would indicate increased risks for seizures.
Statistical methods:	**Principal Statistical Methods :** The primary efficacy endpoints will be tested in a linear mixed model and Cohen's d for ketamine/placebo difference on HAMD scores (two-tailed $p<0.05$). The secondary endpoints will be tested by Cohen's d for measuring the RS-fMRI differences. Paired t-tests will assess changes of regional homogeneity (ReHo) and the whole brain resting state functional connectivity (RSFC) in pgACC region. Prediction of ketamine related responses will be tested using multivariate discriminant function analysis of pgACC ReHo and pgACC RSFC's. The primary analysis is done in the per-protocol population. Description of the primary efficacy analysis and population: Objective 1: Efficacy of ketamine

	Superiority of ketamine on placebo will be tested for reduction of HAMD values between T0 and T1 in both conditions in the per protocol population. A linear mixed model and Cohen's d for ketamine/placebo difference will be performed with an alpha = 0.05. Pearson Chi-square Test for two independent samples of TRD patients and controls (40/80) will be performed with an alpha= 0.05. Objective 2: Effects on metabolite concentration MRS Spectra will be accepted for a CRLB of 20 or lower. Changes will be assessed in a treatment (pre-post) * substance (glutamine, glutamate and GABA) ANOVA. We will specifically test the hypothesis of a significant increase of glutamine acutely after infusion and after 24 hours.

SUMMARY:

RESULTS OF EFFICACY ANALYSES:

The inclusion of healthy controls providing a full data set was completed (n = 80). Due to three drop-outs and one lost to follow-up, the recruitment was prolonged. In total, 84 subjects were randomized and 81 received treatment. The patient recruitment was topped after 33 months due to insufficient chances to reach the inclusion sample. The problem in finding patients was mainly due to poor acceptance and existence of MRI counter indications for those in principle eligible. Therefor the following primary and secondary outcomes could not be tested: improvement on HAMD scores 24 hours after ketamine infusion (primary), predictive value of resting state regional homogeneity (ReHo), glutamine concentration and functional connectivity (FC) of pgACC for treatment response to ketamine in patients (secondary).
We did however test the following outcome on the healthy sample: greater changes of glutamine concentration after ketamine infusion acutely and after 24 hours compared to placebo.

We further investigated the clinical safety parameters for the full sample of HC.

The two treatment groups in the full sample of HC did not differ in demographic properties.

Table 1: Demographic data; there were no differences between the groups. Data is represented in mean± S.D.

	Verum (N= 40)	**Placebo (N= 40)**	**Mann- Whitney U-test or Pearson χ^2 interaction test**
Age	25.95± 5.65	25.83± 4.98	U= 794.0, p= 0.95
Sex	16 females	17 females	χ^2= 0.05, df= 1, p= 0.82
BMI	23.81± 3.0	23.76± 2.94	U= 793.5, p= 0.95
Smoking yes	11	15	χ^2= 0.91, df= 1, p= 0.34
Alcohol usage [1]	23	27	χ^2= 0.85, df= 1, p= 0.36
Drugs yes [2]	13	15	χ^2= 0.22, df= 1, p= 0.64
Contraceptive pill [3]	10	8	χ^2= 0.79, df= 1, p= 0.37
Days baseline [4]	5± 2	5± 3	U= 776.5, p= 0.80
Days follow – up [4]	14± 1	14± 2	U= 704.5, p= 0.44
Delta days	19± 2	19± 3	U= 693.5, p= 0.39

[1] Frequency of 1-3 drinks per week; [2] Ever consumed drugs; [3] Only for women; [4] Days baseline denotes days between baseline blood collection and infusion, and follow–up days between infusion and follow–up blood collection

To elucidate ketamine's mechanism of action, we tested whether the clinical time course of the improvement is mirrored by the change of glutamine/glutamate ratio and if such effects show a regional and temporal specificity in two distinct subdivisions of ACC with different AMPA/N-methyl-D-aspartate receptor profiles. We measured glutamate and glutamine in the pregenual ACC (pgACC) and the anterior midcingulate cortex (aMCC) at 1 and 24 h post infusion. The initial sample size of 40 HC was increased to 58 successful inclusions due to higher drop outs because of insufficient data quality. The recruitment was continued for the total number of 80 to provide sufficient cases for MRS-fMRI correlations. The secondary outcome was tested for the test sample of 58 and reported in a publication. The result was then tested for consistency in the total sample:

After exclusion due to spectra quality criteria and abnormal absolute Cramér–Rao Lower Bound (CRLB), 12 subjects (28.0±8.1 years, 5 female) in the ketamine group and 14 subjects (27.5±6.6 years, 5 female) in the placebo group out of 58 in total were included in the primary analysis. Ketamine and placebo groups did not differ significantly in age ($t(21.3)=-0.198$, $p=0.844$) or sex ($\chi2(1,n=26)=0$, $p=0.755$). A significant interaction of time, region, and treatment was found ($F(2,96) = 5.483$, $p =0.005$) on the glutamine/glutamate ratio. Significant main effects was found for time ($F(2,96) =6.307$, $p = 0.002$) and region ($F(1,24) =48.072$, $p =0.001$), but not for treatment ($F(1,24) =0.077$, $p =0.784$).

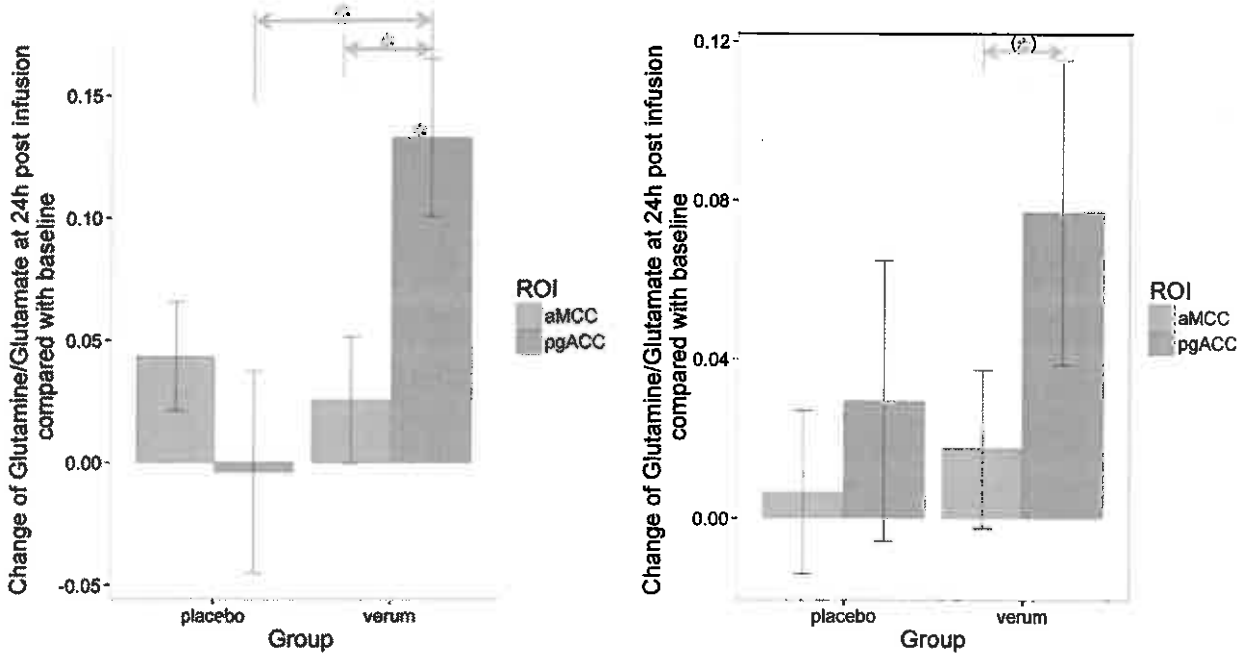

Figure 1: Results of regional comparison from the primary analysis. The change of glutamine/glutamate ratio at 24 h after intravenous ketamine infusion over baseline in the two anterior cingulate cortex subregions (aMCC, anterior middle cingulate cortex, in red; pgACC, pregenual anterior cingulate cortex, in blue) in placebo (n=14, saline) and verum groups (n=12, ketamine) of the test sample (left), and in placebo (n=18) and verum groups (n=16) of the total sample (right), respectively. Asterisks denote statistically significant (*p<0.01, (*)p<0.1) changes in glutamine/glutamine relative to baseline levels. Error bars denote one SEM.

Posthoc analyses in the test sample revealed that the glutamine/glutamate ratio increased significantly in the ketamine group, compared with placebo, specifically in the pgACC after 24 h. In

the pgACC, the change in the glutamine/glutamate ratio at 24 h post ketamine infusion compared with baseline was significant ($t(11) = 4.136$, $p = 0.001$), which is also significantly larger than the change observed at 24 h in the placebo group ($t(23.3) = 2.618$, $p = 0.015$).
Furthermore, in the test sample the metabolite levels at 1 h after infusion in pgACC in the ketamine group was not significant different from the ones at baseline ($t(11) = 1.482$, $p = 0.166$), as well as the difference of changes between ketamine and placebo at 1 h ($t(22.1) = -0.487$, $p = 0.630$), compared with their baseline level. In contrast, the changes between the ketamine and the placebo group in the aMCC were not significant at 1 or 24 h post infusion (1 h: $t(15.9) = 0.456$, $p = 0.654$; 24 h: $t(22.7) = -0.523$, $p = 0.605$). The changes in the glutamine/glutamate ratio in aMCC from baseline in ketamine group (1 h: $t(11) = 1.237$, $p = 0.241$; 24 h, $t(11) = 0.978$, $p = 0.349$) was not significant.
In the total sample of 80 subjects, 16 subjects of the ketamine group and 18 subjects of the placebo group went into analysis after exclusion due to previous quality criteria. In this sample, acquired for fMRI / MRS correlations, results of increased glutamine/glutamate ratio 24 h post ketamine infusion compared to baseline and to aMCC at 24 h were replicated on a trend level ($p<0.1$).

Our results support a significant temporal and regional response in glutamine/glutamate ratios to a single subanesthetic dose of ketamine, which mirrors the time course of the antidepressant response and reversal of the molecular deficits in patients and which may be associated with the histoarchitectonical receptor fingerprints of the ACC subregions.

RESULTS OF SAFETY ANALYSES:

Safety assessment comprised psychotomimetic side effects, blood pressure and heart rate on the infusion day, as well as hematological and biochemical values in serum in the follow-up measurement.

Psychotomimetic Side Effects
Dissociative symptoms were assessed via the Clinician Administered Dissociative States Scale (CADSS) in all 80 healthy subjects. Dissociative symptoms were increased only in the ketamine group post-infusion. While all subjects reported no dissociative symptoms before drug intake, only the ketamine group showed increased CADSS scores of >5 after treatment. Chi square test revealed a significant difference between the two groups ($p < .001$).

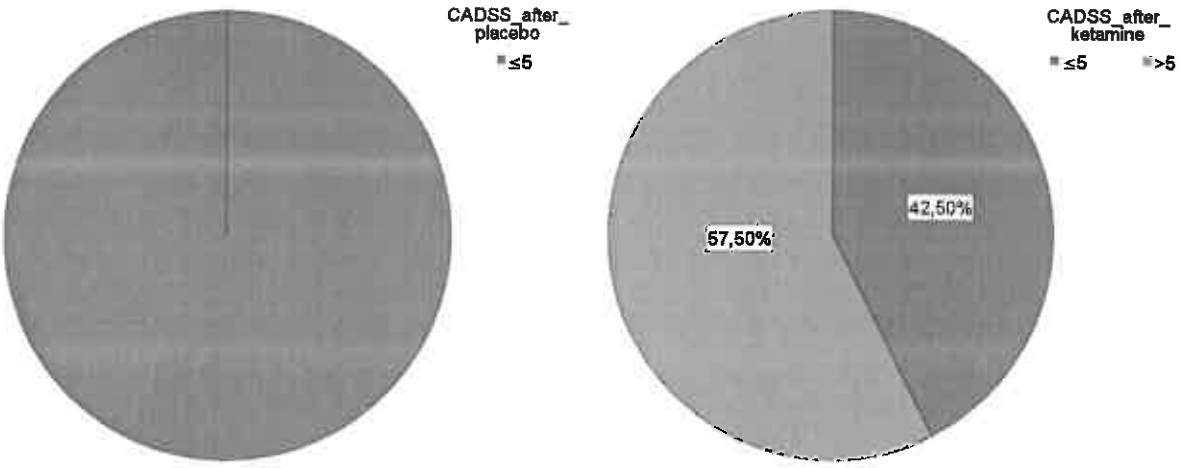

Figure 2: CADSS scores post-infusion grouped in ≤ 5 (blue) and > 5 (green) plotted for the placebo (left) and ketamine (right) group.

Blood Pressure and Heart Rate

Blood pressure and heart rate were monitored during and following administration of ketamine or placebo in 78 healthy participants (mean age 26.04 ±5.562 years). The influences of baseline blood pressure/heart rate and gender on blood pressure and heart rate changes were investigated. Systolic (RR_{Sys}) and diastolic (RR_{Dia}) blood pressure as well as heart rate (HR) increased significantly upon ketamine administration, but without reaching hypertensive levels. Significant time by group interactions were observed with respect to RR_{Sys}, RR_{Dia} and HR when comparing the baseline with the 40 minutes post-infusion (TP40) values (RR_{Sys} ($F_{1,76}$=80.312, p<0.0005, part. η^2=0.514), RR_{Dia} ($F_{1,76}$=75.878, p<0.0005, part. η^2=0.500), HR ($F_{1,75}$=16,989, p<0.0005, part. η^2=0,185)).

Sub-anesthetic ketamine increased both blood pressure and heart rate without causing hypertensive events.

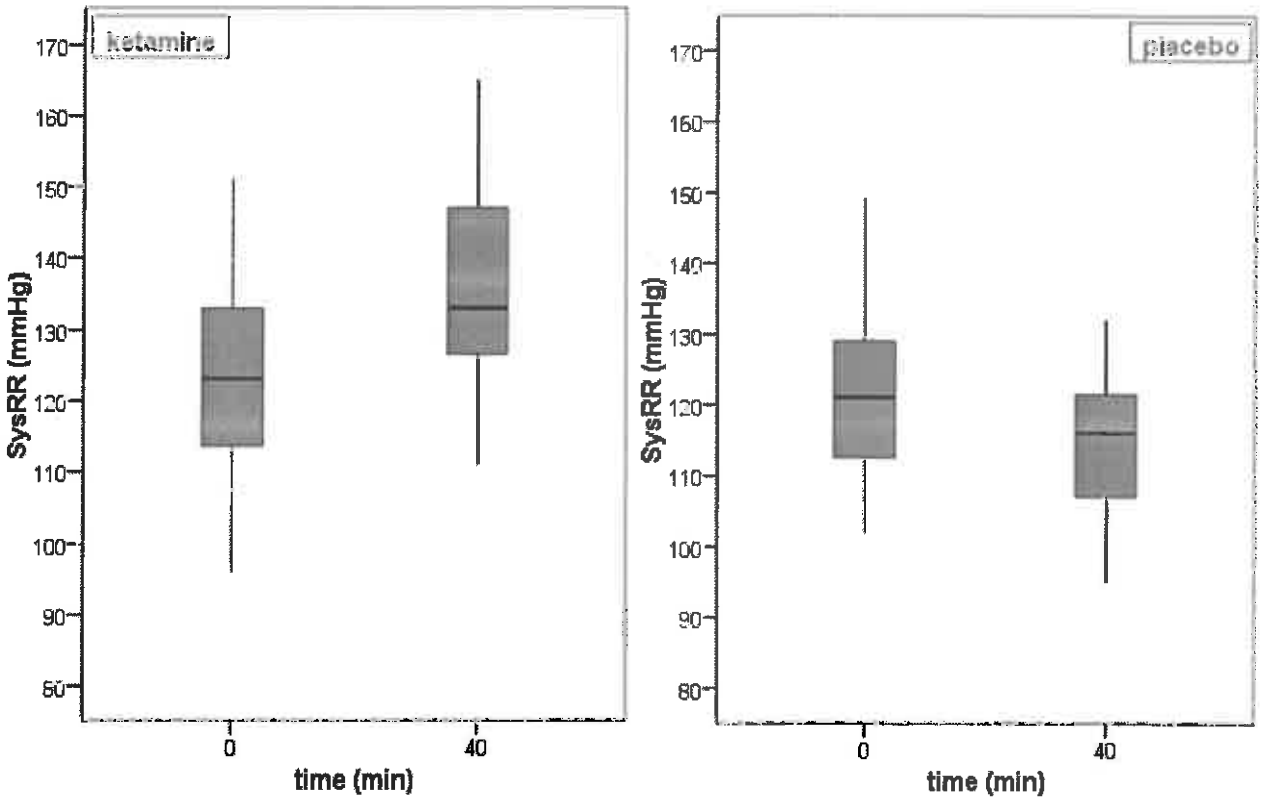

Figure 3: Systolic blood pressure at TP0 and TP40, separately for the ketamine and placebo groups. Systolic blood pressure increased in the ketamine group (p<0.0005), and decreased in the placebo group (p<0.0005). The difference between the groups was significant on direct comparison (p<0.0005).

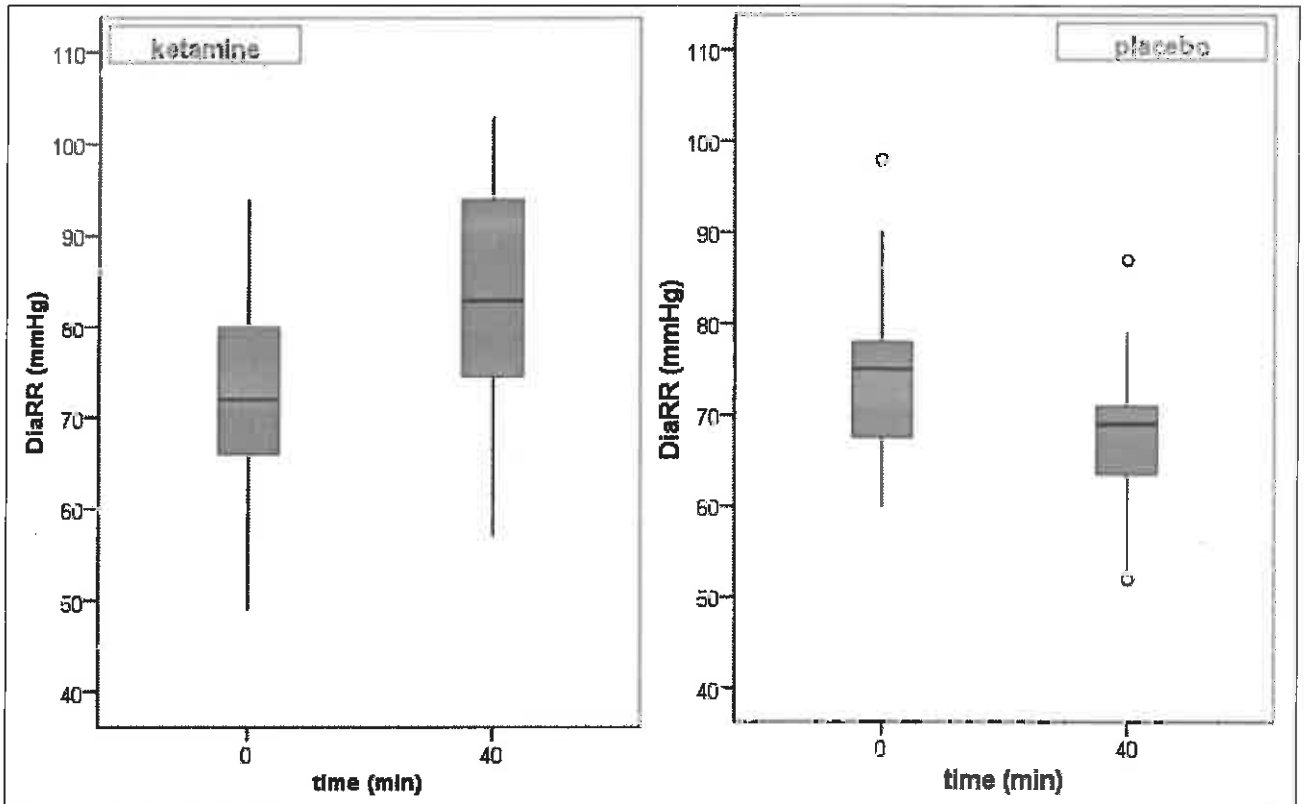

Figure 4: Diastolic blood pressure at TP0 and TP40 separately for the ketamine and placebo groups. The ketamine group showed an increase ($p<0.0005$), and the placebo group a decrease ($p<0.0005$). The difference between the groups was significant on direct comparison ($p<0.0005$).

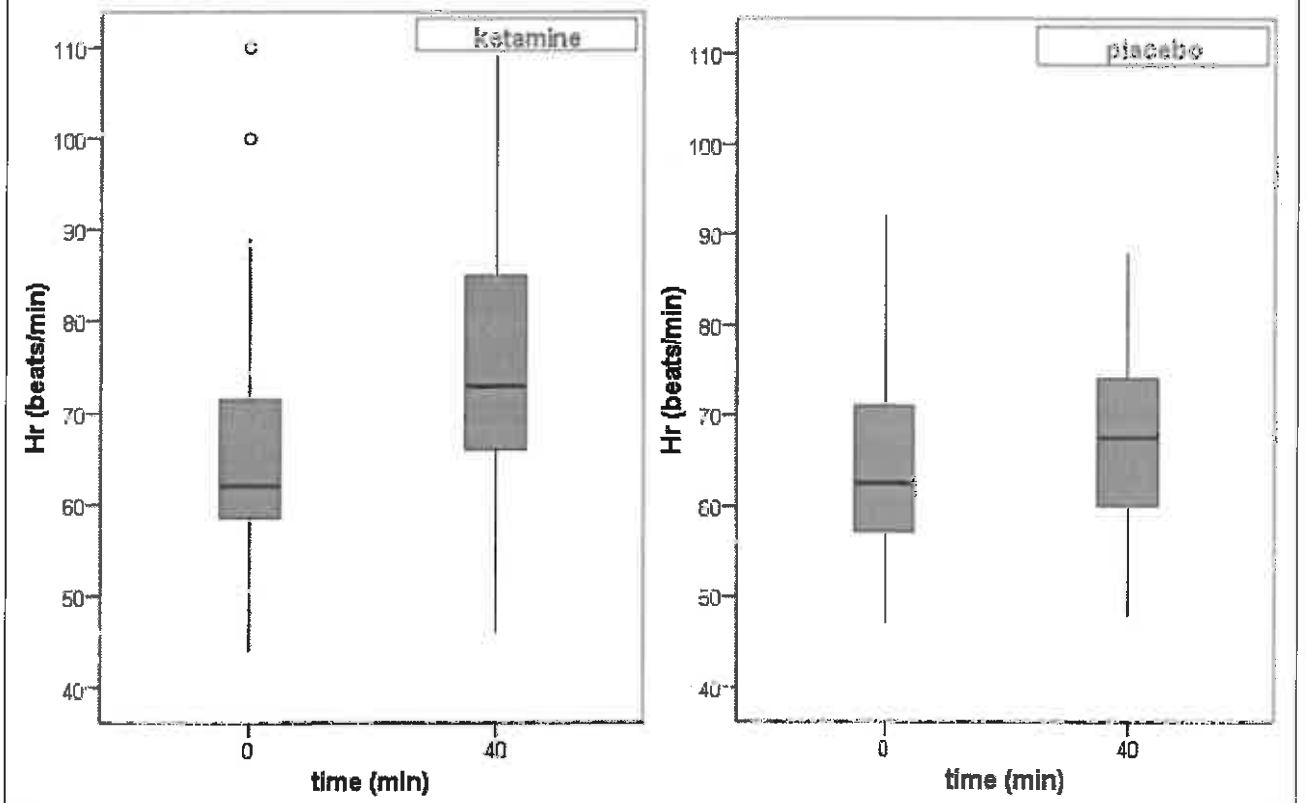

Figure 5: Heart rate at TP5 and TP40 separately for the ketamine and placebo groups. The ketamine group showed an increase ($p<0.0005$), while the placebo group showed no change in the heart rate ($p=0.794$).

Hematological and Biochemical Values

Furthermore, we investigated potential sustained effects of ketamine administration on

hematological and biochemical values in serum. To assess the group effect, repeated measure analyses of co–variance taking account of age, BMI and sex as covariates (rmANCOVA) were conducted for the following blood parameters: levels of sodium, potassium, calcium, hemoglobin and number of erythrocytes, lymphocytes and thrombocytes. RmANCOVA revealed a significant time by treatment effect on thrombocyte levels, driven by an increase in the ketamine group (paired t-test, t= -3.51, df= 38, p= 0.001).

Table 4: A) Results of repeated measures analysis of co–variance for thrombocytes. Results for interaction time by group are indicated with statistical threshold set at $p < 0.05$. B) Results of repeated measures analysis of co–variance for thrombocyte number, men and women separately. Data are represented in mean± S.D.

A		Verum	Placebo	rmANCOVA – time* group
Thrombocytes (Gpt/L)[1]	Pre	246± 54	244± 55	**$F_{1, 74}$= 13.54, p= 0.001, η^2= 0.155**
	Post	266± 59	238± 52	
B		Verum	Placebo	rmANCOVA – time* group
Thrombocytes (Gpt/L) *men*	Pre	237± 53	227± 46	**$F_{1, 43}$= 9.20, p= 0.004, η^2= 0.176**
	Post	261± 62	224± 46	
Thrombocytes (Gpt/L) *women*	Pre	260± 55	268± 58	**$F_{1, 28}$= 4.23, p= 0.049, η^2= 0.131**
	Post	273± 55	257± 55	

[1] Gpt/L denotes gigaparticles (10^9) per liter; [2] Tpt/L denotes teraparticles (10^{12}) per liter; [3] mmol/ L denotes millimol (10^{-3}) per liter;

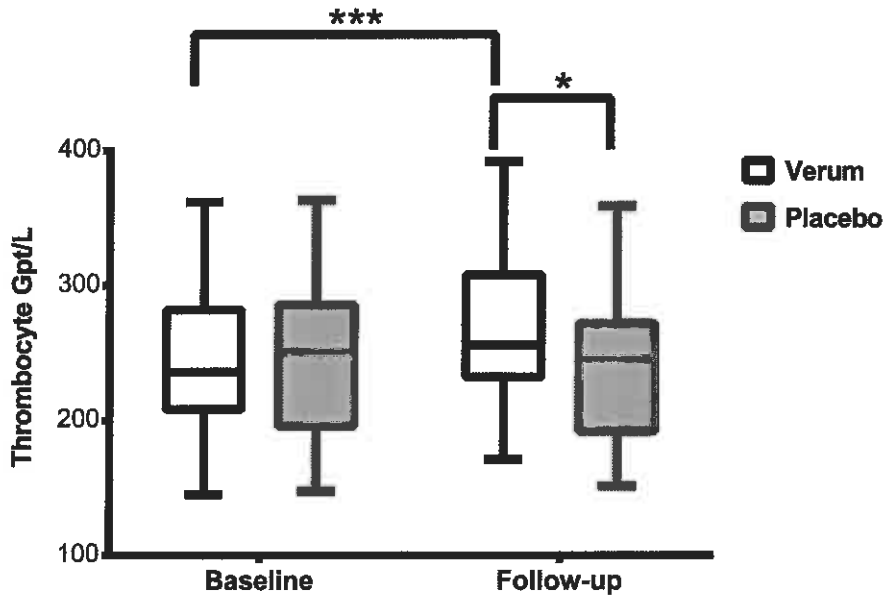

Figure 6: Results of rmANOVA for the thrombocyte count ($F_{1, 74}$= 13.54, p= 0.001, η= 0.155) (box-plot, mean± 95%CI). *Post – hoc* test show group difference for the follow–up (Student's t-test, t= 2.20, df= 77, p= 0.03*) and within subject difference between baseline and follow–up in the ketamine group (paired t-test t= -3.51, df= 38, p= 0.001***). There was no difference between groups (Student's t-test, t= 0.070, df= 78, p= 0.94) at baseline, and no difference between baseline and follow–up in the placebo group (paired t-test, t= 1.47, df= 39, p= 0.15).

The effect of treatment was confirmed by logistic regression (χ^2= 26.16, df= 7, p< 0.001), with a significant effect for the relative increase in thrombocyte numbers (B= -12.55, Wald's χ^2= 15.62, df= 1, p< 0.001). Additional rmANCOVA's of other coagulation parameters did not reveal

significant interactions. An increase in the frequency of subjects exceeding the maximum of reference range of thrombocyte number was also observed in the ketamine group. In the follow–up, 3 subjects in the verum group were above reference range value with none in the placebo, as compared to 2 in the whole sample at baseline.

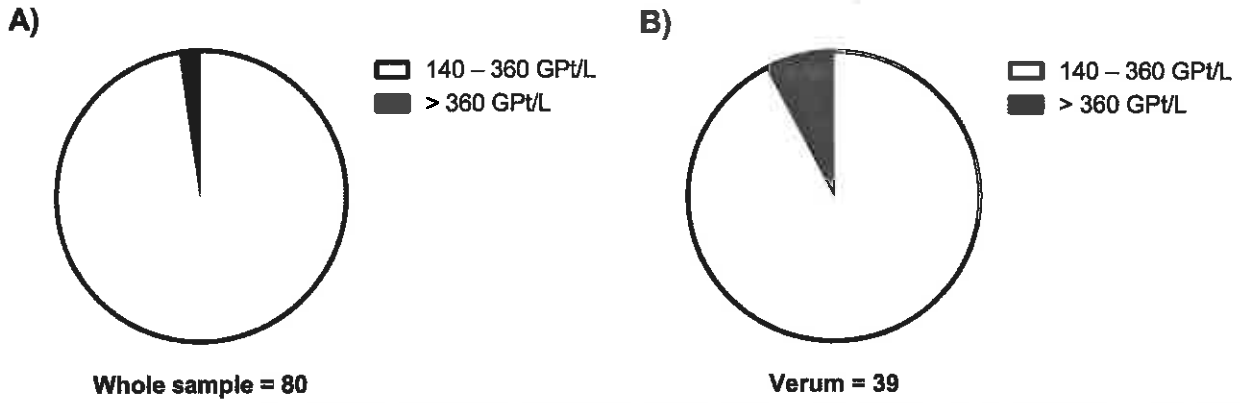

Figure 7: Frequency of subjects exceeding reference range (140 – 360 GPt/L) A) at baseline in the whole sample, 2.5% (2 subjects out of 80), and B) at follow–up in the verum group, 7.7% (3 out of 39)

Moreover, the relative increase in the ketamine group was stable across sexes and not predicted by age, BMI, smoking, alcohol or drug use including contraception. Our results describe aftereffects of sub anesthetic ketamine administration on blood coagulation parameters, which should be considered especially when targeting psychiatric populations with relevant clinical comorbidities.

Substantial Amendments:

1. Amendment V2/2 vom 08.08.2012, Protokolländerungen zu V1 26.01.2012

1) Page 2 (Synopsis, Title of Study) there was an inconsistency between the protocol title and the synopsis title, this has been adjusted

2) Page 24 (4.2 Time and Event Schedule); Page 25 (4.3.1 Study Visits Description); Page 30 (5.4.2. Study Unblinding) For the benefit of patient safety, a pregnancy test based on the blood samples obtained is carried out during the screening visit instead of the planned pregnancy test using a urine sample (urine sample is therefore not required). A possible pregnancy can be detected earlier in the blood sample. Day 2 is also defined as FU and at the same time the end of the study at 10 to 20 days after baseline (infusion). As described above, the pregnancy test is also carried out on this day using a blood sample.

3) Page 27 (5.1.3.2 Subject Randomization Number) In its algorithm, the stratification software takes into account not only age and severity (HAMD, patients) but also the gender of the test subjects.

4) Page 34 (7.3. Procedure for Reporting a Serious Adverse Event) Recipient of the SAE form and any relevant medical information is "Koordinierungszentrum Klinische Studien Halle" and not the sponsor. This has been adjusted.

2. Amendment V3/3 vom 12.11.2013 zu V2/2

1. Additional determining the BDNF gene expression and BDNF blood level
2. Post-Infusion-EEG

In the more recent literature, there are still indications that brain activity measured by EEG changed after the administration of ketamine. So far, the EEG was carried out only in the context of screening to exclude a contraindication, but not after administration of ketamine. Therefore, an EEG measurement was added at visit 3. This means an additional expenditure of time of 30 minutes for the patients or

subjects.

3. Time Management Screenings

   The time interval between screening and baseline is increased to 14 days. Examinations of which the results can fluctuate significantly depending on time and health, will continue to be carried out a maximum of 7 days before the baseline visit.

4. Increase number of healthy volunteers

   It turned out that the data could not be collected with sufficient quality in some individual subjects using the current method. Therefore, the sample size should be increased from 40 to 80 in the study.

CONCLUSIONS:

In conclusion, in healthy subjects we found that ketamine specifically increases glutamine/glutamate ratios in the pgACC, a region previously identified to display a deficit in these ratios in subjects with depression. This translated into a change of the balance between regions: the glutamine/glutamate ratio in the pgACC relative to the levels in the aMCC was significantly increased only 24 h after the ketamine infusion. This finding largely confirms our hypothesis of a regional specificity of ketamine effects on glutamatergic levels. In an earlier study, Rowland et al (2005) reported evidence for acute glutamine (but not glutamate) changes following ketamine infusion. Rowland's finding was specific to the loading dose of ketamine rather than the maintenance dose afterwards. The results of this study most closely match previous observations in a temporally similar study by Iltis and colleagues (2009) who administered the NMDA antagonist phencyclidine to rats. They found a significant increase in glutamine/glutamate ratio compared with the baseline after phencyclidine infusion. Our findings describe novel neurochemical correlates of a subanesthetic ketamine infusion, which appear as late as 24 h after treatment. Scheidegger et al (2012) report the reduction of functional connectivity as a similar mechanism underlying ketamine's antidepressant efficacy, which may counteract the repeatedly observed hyperconnectivity within the default mode network in depressed patients. Consequences for clinical translation need to be considered with caution, given that we only healthy subjects could be recruited in sufficient numbers. Future studies are needed to broaden the time windows of observations especially the acute infusion stages.

Dissociative symptoms were generally increased only in the ketamine group post-infusion. Arterial blood pressure and heart rate increased after ketamine infusion. Elevated arterial blood pressure can lead to neuronal, cardiac, ophthalmological and renal diseases or at least to uncomfortable adverse effects (Chobanian et al, 2003). While ketamine-related elevation in blood pressure and heart rate in our participants was within a moderate range, with no outliers reaching concerning levels, our participant group comprised healthy young individuals. The impact is potentially greater, however, when treating patients with initially high blood pressure. Based on the hypothesis that the sympathetic nervous system plays an important role in blood pressure regulation during ketamine administration, we were able to identify factors predictive of the degree or of the time course of blood pressure change, e.g. the baseline systolic blood pressure and gender. Moreover, we identified the number of thrombocytes as a specific parameter affected by ketamine, an effect stable across sexes and not predicted by a range of demographic factors. Our findings thus suggest that even if we did not observe serious increase or complications in any subject receiving ketamine, clinical significance of this effect still needs to be considered. On the other hand, our safety lab indicated that all other blood parameters were not affected by the ketamine infusion in the follow–up time point.

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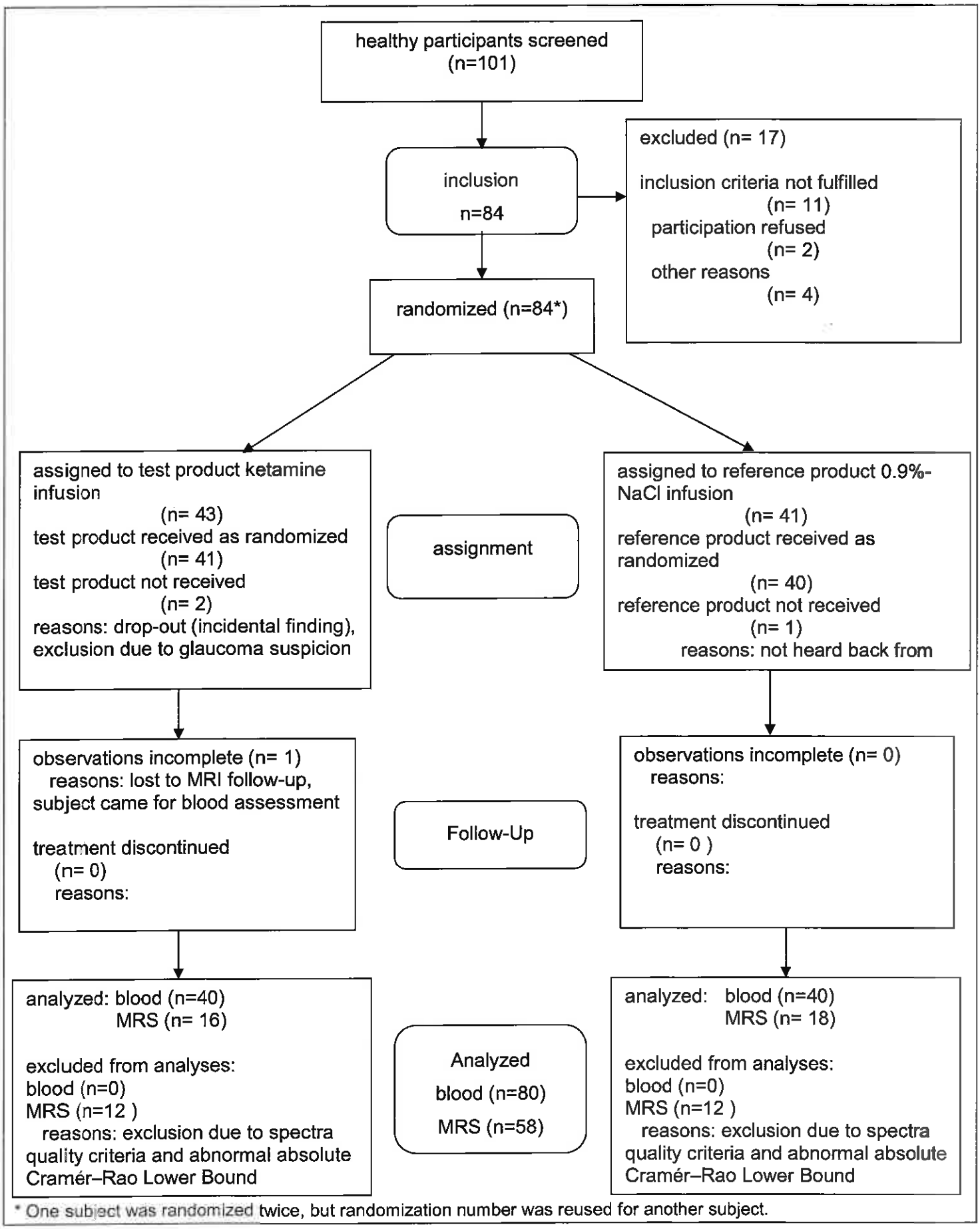
CONSORT Flow Diagramm



* One subject was randomized twice, but randomization number was reused for another subject.

CONSORT Flow Diagramm