UNIVERSITÀ DEGLI STUDI DI TRIESTE

UNIVERSITÀ DEGLI STUDI DI TRIESTE

XXXIV CICLO DEL DOTTORATO DI RICERCA IN SCIENZE DELLA RIPRODUZIONE E DELLO SVILUPPO

PHARMACOGENETIC, PHARMACOKINETIC AND EPIGENETIC PROFILES

IN CHILDREN WHO EXPERIENCE VOMITING OR RECOVERY AGITATION

DURING SEDATION WITH KETAMINE

Settore scientifico-disciplinare: **PEDIATRIA GENERALE E SPECIALISTICA**

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ANNO ACCADEMICO 2020/2021

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1. Abstract

Background: Ketamine is one of the sedatives most used in North America for procedural sedation outside the operating room in children. Vomiting and recovery agitation are the adverse events most frequently reported after ketamine administration in children. The aim of this study was to identify genetic variants associated with the development of vomiting or recovery agitation after intravenous ketamine administration.

Material and methods: This was a single-center prospective pharmacogenetic study performed at the tertiary level children's hospital Institute for Maternal and Child Health IRCCS Burlo Garofolo of Trieste, Italy. Children, between 1 and 17yrs of age, performing procedural sedation with intravenous ketamine as the sole sedative agent were enrolled. Pharmacogenetic, pharmacokinetic and epigenetic analysis were predisposed. The genotyping was performed through Illumina Omni 2.5+ exome array. The search for biomarkers was performed through the analysis of the variants of 10 candidate genes, codifying for proteins responsible for the metabolism and pharmacodynamics of ketamine. A target sequencing genome-wide analysis was carried out to identify the presence of genetic variants associated with the development of adverse events but not related to the candidate genes. The plasma concentration of ketamine and norketamine were determined and pharmacokinetic parameters were determined. A pharmacogenomic approach provided neural-derived exosomal miRNA profiles for patients. The miRNA contained in neural-derived exosomes were quantified and related to the occurrence of ketamine adverse events, through a next-generation sequencing technique.

Results: From September 2019 to October 2021, 106 patients were enrolled in the study. Among them, 87 patients were analyzed. The median age was 8yrs (5-11). 47 (55%) were females, 32 (37%) were affected by chronic diseases. Arthrocentesis and bone fracture reduction were the procedures most frequently performed. A median dose of 1.7mg/kg of intravenous ketamine was employed to perform the procedures.

Thirty-one patients (35%) experienced vomiting or recovery agitation and were compared to the 56 patients (65%) without these events.

Polymorphisms on GRIN2A, GRIN2B, and CYP2A6 were associated with the development of vomiting or recovery agitation. A stronger association was found with polymorphisms related to the pharmacodynamic of ketamine.

Conclusion: The development of vomiting and recovery agitation after ketamine administration seems associated with specific genetic variants. These findings should be confirmed in a greater population.

Keywords: children, ketamine, pharmacogenetic, recovery agitation, vomiting.

2. Introduction

The need for procedural sedation and analgesia in children has significantly raised in the last decades along with the increasing awareness of the detrimental consequences of untreated pain and fear in pediatric patients (1,2).

Undertreated pain could affect the child's long-term mental health, resulting in detrimental psychological effects, exaggerated responses to future painful clinical procedures, and negative lasting memories (3).

The best management is based on a careful patient assessment and effective use of pharmacological and non-pharmacological techniques (1).

At the Institute for Maternal and Child Health IRCCS Burlo Garofolo of Trieste, a solid experience in the management of sedation outside the operating room both by anesthesiologists and specifically-trained pediatricians, has been built in the last two decades (4-16). We aim to continue our research to improve the standard of care for patients in this setting.

Ketamine is the sedative most commonly used in North America for painful procedures in children at the emergency department (17,18). Compared to the other sedatives and combination of sedatives commonly employed including propofol, benzodiazepines,

barbiturates, and opioids, ketamine provides the higher safety profile for procedural sedation and analgesia, in the pediatric emergency setting (17).

Due to its unique pharmacological properties, ketamine allows pain control and sedation without loss of protective airways reflexes or respiratory depression, thus leading to a very low rate of respiratory adverse events during procedures. Moreover, it maintains cardiovascular stability (19). On the other hand, ketamine, like every drug, can cause adverse events. The most frequently reported adverse events after the administration of ketamine are vomiting and recovery agitation (20). The rate of vomiting varies between studies, but in the most extensive available meta-analysis regarding 8,282 children undergoing procedural sedation outside the operating room, its prevalence was 8.4% (20). Recovery agitation is the second most frequently reported adverse event after ketamine administration, with a prevalence in the above-mentioned meta-analysis of 7.6%. Recovery agitation is defined as any abnormal behavioral response including any combination of agitation, crying, hallucinations, or nightmares after sedation.

Vomiting and recovery agitation are not severe adverse events, but they can be perceived as very unpleasant for patients and families and they may play a considerable role in the perception of the quality of sedation, especially in children who need repeated procedures. Our hypothesis is that the development of vomiting and recovery agitation after ketamine administration may be favored by the presence of some specific genetic profiles of patients. To the best of our knowledge, only very limited evidence is available about the role of pharmacogenetics and pharmacokinetics in the occurrence of ketamine adverse events, especially in children, with only preliminary data from a few papers addressing this issue (22).

Therefore, the aim of this study is to investigate if there are some specific genetic and epigenetic profiles related to the development of adverse events after ketamine sedation in children.

The possibility of quick identification of patients at risk of developing vomiting or recovery agitation after ketamine administration in emergency settings and pediatric wards could improve the quality of clinical practice especially for children needing repeated procedures including children with leukemia, solid tumors, juvenile idiopathic arthritis, and osteogenesis imperfecta.

2.1 Ketamine pharmacology

Ketamine, 2-(o-chlorophenyl)-2-(methylamino) cyclohexanone, is a dissociative agent, member of the arylcyclohexylamine class (Fig 1) (23). It was designed in the mid-1960s as a replacement for phencyclidine, in order to obtain a dissociative agent with a lower incidence of hallucinogenic side effects.

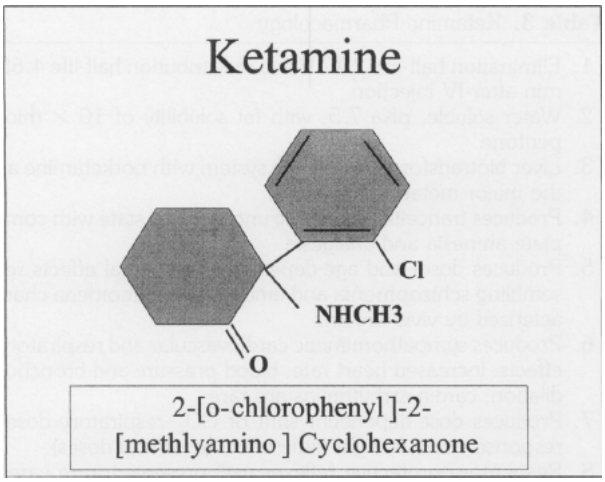

Figure 1. Chemical structure of ketamine

Ketamine induces a dissociation between the thalamo-cortical and limbic systems, inducing a cataleptic-like state in which the patient is dissociated from the surrounding environment.

The limbic system is involved in the regulation of emotions and serves as a processing center receiving sensory input and information from the sensory association cortex. It invests the sensory experience with emotional significance and controls the hypothalamus and brainstem that coordinate the visceral motor responses associated with these emotions. Moreover, the limbic system is involved in the development of short and long-term memory. The sensory association areas of the cortex, components of the limbic system are directly depressed by ketamine. Consequently, higher central nervous system centers are unable to receive or process sensory information and its emotional significance. The result of ketamine administration is sedation, analgesia, amnesia, suppression of fear, and anxiety (23).

Ketamine is a lipid-soluble molecule that rapidly crosses the blood-brain barrier.

It is a noncompetitive N-methyl-D-Aspartate (NMDA) receptor antagonist that blocks glutamate excitatory effects (Fig 2) (24).

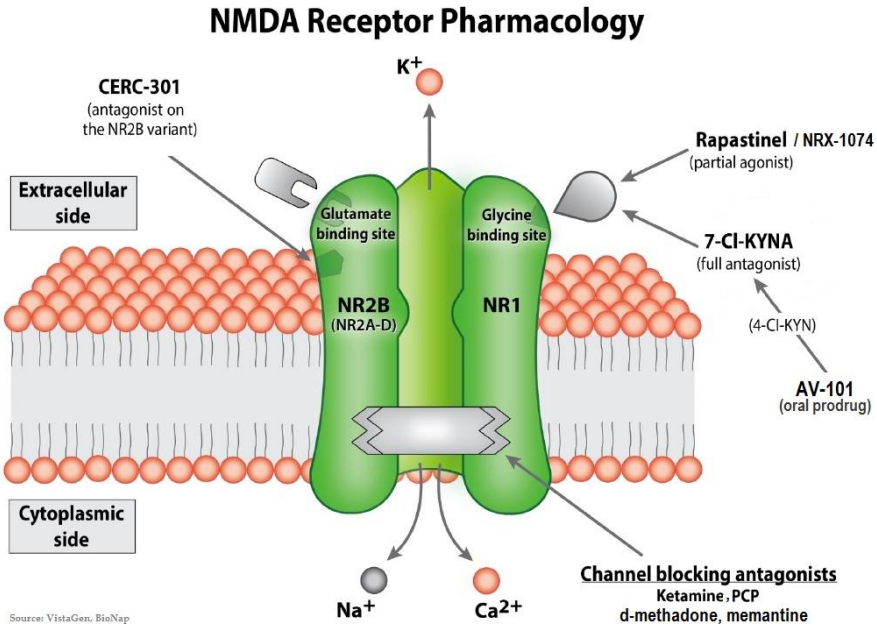

Figure 2. NMDA receptor pharmacology.

The NMDA receptor is a cation-gated channel receptor permeable primarily to calcium and to a lesser extent to sodium and potassium. Most of the NMDA receptors are constituted by two NR1 and two NR2 subunits. NMDA receptor binds to excitatory amino acids including glutamate, glycine, and aspartate. The binding results in the opening of the ion channel and depolarization of the neuron. NMDA receptors are involved in sensory input at the spinal, thalamic, limbic, and cortical levels.

Ketamine is not selective for specific NMDA receptor subtypes, because it produces a trapping type of open channel block, in which the drug binds a site that is deep within the ion channel, occludes the flow of ions through the open channel, preventing depolarization, and can remain in the channel when the channel closes (25). The reduction of synaptic excitation mediated via NMDA receptors contributes to the anesthetic/analgesic properties of ketamine. However, ketamine's molecular mechanism is not limited to the NMDA receptor, and several studies indicate interactions with a series of receptor systems, including agonism at the opioid, AMPA, cholinergic, dopaminergic receptors (25). It acts as an agonist at alfa and beta-adrenergic receptors, and as an antagonist at muscarinic receptors of the central nervous system. It blocks the reuptake of catecholamines and is and an agonist at opioid sigma receptor. Ketamine also acts as a weak agonist at opioid mu-receptors (23). These interactions lead to the development of sympathomimetic

cardiovascular and respiratory effects, including increase heart rate, blood pressure, and bronchodilation. Respiratory protective reflexes are preserved, with rare cases of apnea and respiratory obstruction reported. Ketamine produces increased salivary and tracheobronchial secretions. It produces random head and extremity movements, with skeletal muscle hypertonicity and rigidity. In most cases, the severity of these phenomena has not been great enough to interfere with the procedure being performed (23).

From a pharmacokinetic point of view, ketamine is readily distributed (Vd=160-550L/70kg; serum alpha phase half-life about 2-11 minutes, with relatively low plasmatic protein binding (10-30%) (26).

Ketamine is water soluble with a pKa of 7.5, which allows nonirritating, intravenous, intramuscular, oral, intranasal, and rectal administration (23).

Metabolism of ketamine is mainly hepatic, by the cytochrome p450 enzyme system, via N-dealkylation to norketamine and with hydroxylation of the cyclohexone ring and dehydration of the hydroxylated metabolites to other metabolites. Norketamine is 33% as potent as the parent compound. Enzymes involved in phase-I biotransformation are CYP2B6, CYP2C9, CYP3A4, CYP3A5, and CYP2A6 (Fig 3).

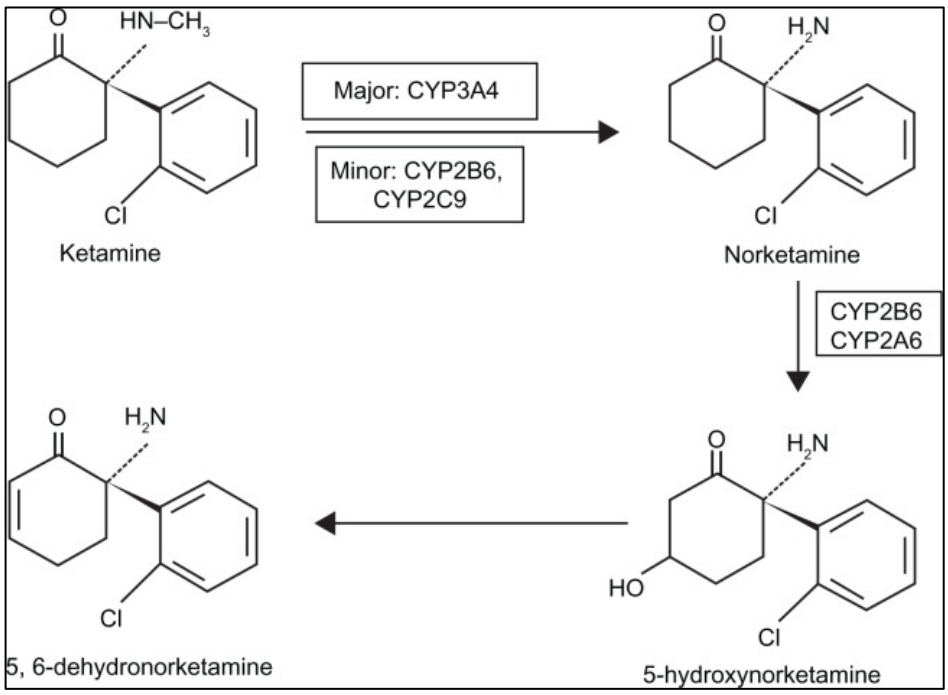

Figure 3. Ketamine metabolism

Serum beta-phase elimination half-life is of 2-4 hours. Excretion of metabolites occurs mainly through urine (urine 91%, feces 3%).

Ketamine molecule has a chiral structure, consisting of two optical enantiomers, with S-ketamine two to four times more potent as an anesthetic and analgesic than the racemate and R-isomer, respectively (27). Commercially available racemic ketamine preparations contain equal concentrations of the 2 enantiomers.

2.2 Clinical use of ketamine for sedation outside the operating room

Ketamine provides dissociative sedation, inducing a trance-like cataleptic state characterized by profound analgesia and amnesia, with the maintenance of protective airway reflexes, spontaneous respirations, and cardiopulmonary stability (28).

It acts dissociating the central nervous system from outside stimuli, like pain, sight, and sound. Its clinical effect varies with dosage. After the intravenous administration, low doses (0-1-0.3mg/kg) induce analgesia, but not sedation. Higher doses (1-2mg/kg) induce dissociation with no response to outside stimuli. Once the dissociative threshold is reached, the administration of additional ketamine does not increase or deep sedation. The only need for titration is to maintain the dissociative state over time (28).

Evidence shows the safety and efficacy of dissociative sedation for various painful procedures in children, including orthopedic reduction, laceration repair, abscess drainage, foreign body removal, lumbar puncture, bone marrow aspirate, arthrocentesis, and tissue biopsies (17,28).

According to a widely used clinical practice (28), absolute contraindications to its use are: age under three months and patients with schizophrenia. Relative contraindications to its use outside the operating room include: age between 3 and 12 months because of concerns about a potentially higher risk of airway complications; laryngeal stimulation during procedures for an increased risk of laryngospasm; upper respiratory infections and asthma exacerbation for an increased risk of respiratory adverse events as laryngospasm; anatomy abnormalities for an increased risk of adverse events during procedures; cardiac disease as coronary artery disease, congestive heart failure or hypertension, for the sympathomimetic properties of the drug; increased intracranial pressure; increased intraocular pressure; porphyria and thyroid disease.

There isn’t a recommended specific fasting duration before dissociative sedation.

The dissociated state could be usually reached with 1.5-2mg/kg via the intravenous route and with 4-5mg/kg through the intramuscular route. To prolong sedation further doses of 0.5mg/kg can be administered intravenously. A repeated half-dose or full dose via the

intramuscular route can be administered if the first dose is not sufficient to dissociate the patient.

Usually, the intravenous bolus of ketamine is administered in 60 seconds, to avoid the development of a transient respiratory depression, as historically reported. However, recent reports do not show an increase of respiratory events after a very quick administration, in 5 seconds, of doses of 0.7-0.8mg/kg (29).

Ketamine can be used alone, without the routine administration of other drugs, including anticholinergics, benzodiazepines, and antiemetics (28).

It can be administered together with other sedatives like propofol (30), dexmedetomidine (31), fentanyl, or midazolam (17). In the emergency setting, a recent study showed that the rate of adverse events was lower with the administration of ketamine alone, compared to the administration of ketamine combination with other sedatives (17).

2.3 Ketamine adverse events

A prospective multicenter observational cohort study conducted in six pediatric emergency departments in Canada, that collected the adverse events of 6295 sedations in children showed that the use of ketamine, as the only sedative drug, was associated with the lowest rate of adverse events, with the need for medical intervention during the procedure in 0.9% of cases and serious adverse events in 0.4% of cases (17).

Another report regarding 22,645 sedations with ketamine, alone or combined with other sedatives, in different settings outside the operating room, showed a rate of adverse events of 7.3% and of severe adverse events of 1.7% (18).

Both reports proved the high safety profile of the use of ketamine for sedation outside the operating room in children.

In general, adverse events of ketamine are not dose-related across the standard dosing range (19,20,28).

Respiratory adverse events with ketamine are rare events. A large meta-analysis observed airway and respiratory complications in 3.9% of children including transient apnea in 0.8% and laryngospasm in 0.3% (19). When present, respiratory depression presents at the time of peak drug levels in the central nervous system (1-2 min after intravenous administration or 4-5min after intramuscular administration). The intramuscular and intravenous routes of administration share a similar risk of airway and respiratory adverse events. This very

low rate of respiratory adverse events makes ketamine so attractive for sedation in the emergency setting.

The adverse events most frequently reported after ketamine administration are vomiting and recovery agitation.

2.3.1 Vomiting

The rate of vomiting varies among studies. A large meta-analysis (8282 patients) reported a prevalence of vomiting of 8.4%. The prevalence of vomiting was related to patients' age with a peak among adolescents (Fig 4). The intramuscular route of administration was associated with a higher rate of vomiting when compared to the intravenous one. Moreover, unusually higher doses of intravenous ketamine (>2.5mg/kg) were associated with an increased occurrence of vomiting (Fig 5) (20).

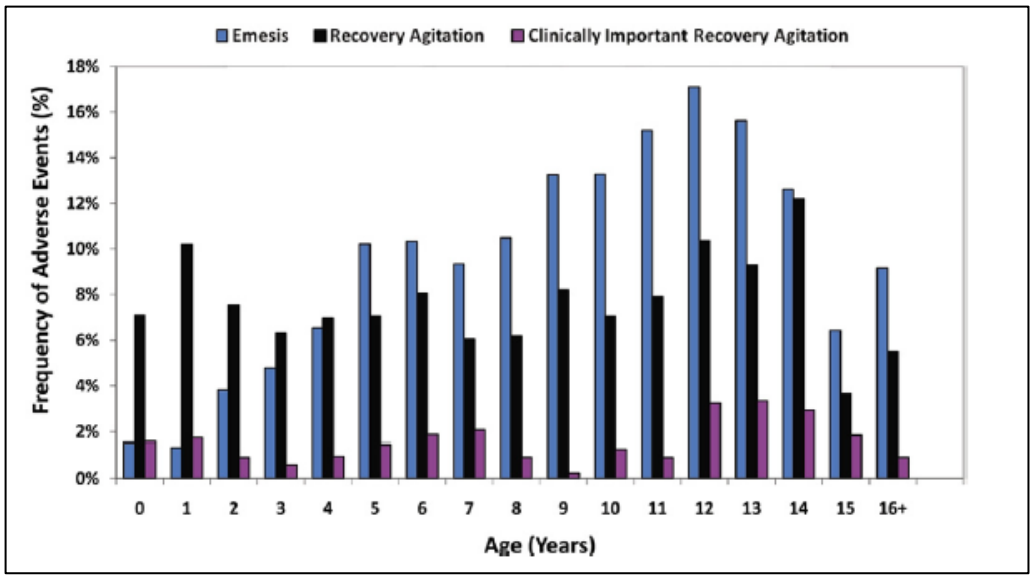

Figure 4. Frequency and distribution of vomiting and recovery agitation by age. Modified by reference 20.

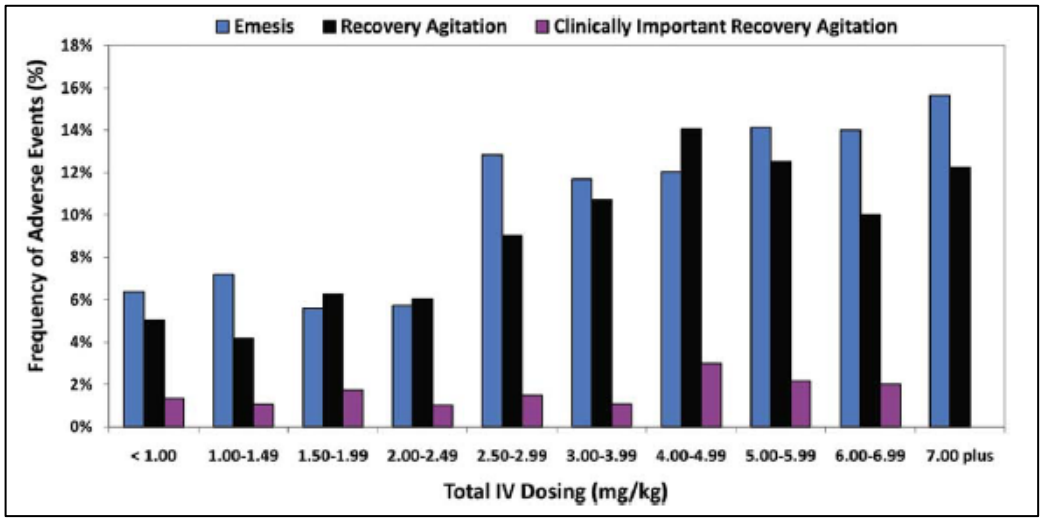

Figure 5. Frequency and distribution of vomiting and recovery agitation by total intravenous dosing. Modified by reference 20.

Usually, vomiting occurs late during the recovery phase from sedation, when the patient is already alert. Sometimes it occurs after discharge. Rarely, near 1% of cases, it occurs during sedation, when the patient is still dissociated. Nevertheless, no cases of aspiration during ketamine sedation outside the operating room are reported.

Routine premedication with antiemetics (ondansetron) is not recommended, even if a randomized controlled trial showed a significantly decreased rate of vomiting by 8%, but with a relatively high number need to treat (NNT 13) (32). The number need to treat lowers to 9 in early adolescent patients, who have a higher risk of vomiting.

2.3.2 Recovery agitation

Recovery agitation is defined as any abnormal behavioral response including any combination of agitation, crying, hallucinations, or nightmares after sedation.

Recovery agitation is the second most frequently reported adverse event after ketamine administration, with a prevalence in the above-mentioned meta-analysis of 7.6% (20). Rarely, it is severe (1.4% of cases) (20,28).

Recovery agitation is not related to the age of patients, the dose of ketamine. Adolescents don't have a clear higher risk of developing this phenomenon (20).

The intramuscular and intravenous route of administration share a similar risk of clinically important recovery agitation.

Recovery agitation usually is transient with spontaneous resolution, but in severe cases, the use of benzodiazepines can rapidly diminish such reaction. On the contrary, a routinely preventive use of benzodiazepines is not associated with a clear reduction of this phenomenon in children (28).

2.4 Study background and hypothesis

As presented, vomiting and recovery agitation are not severe adverse events, but they occur frequently after ketamine administration. They can be perceived as unpleasant by patients and families and may have a negative influence on the perception of the quality of sedation, especially in children who need repeated procedures.

Despite clinical data suggesting which are the patients at higher risk of developing such adverse events, we are not still able to preliminarily identify the patients who will develop these events.

The possibility of quick identification of patients at risk could suggest the choice of different pharmacological regimens, improving the quality of our practice.

Our hypothesis is that some specific genetic profiles may be associated with the development of vomiting or recovery agitation in children. To the best of our knowledge, only very limited data are available in children about the role of pharmacogenetics in the occurrence of ketamine adverse events (22).

Moreover, we would like to identify if these patients can be characterized by some epigenetic biomarker.

Recently, an innovative source of clinically useful biomarkers has been identified in circulating extracellular vesicles, named exosomes. Exosomes are nanoscale particles comprising a lipid barrier membrane and a variable cargo of DNA, RNA, and protein. Accumulating evidence shows that extracellular vesicles can cross the blood-brain barrier in both directions, and neuron-derived extracellular vesicles can be consistently collected from plasma. Micro-RNAs are single-strand non-coding RNA that play a role in post-transcriptional gene regulation. The micro-RNA (miRNA) contained in these vesicles can be used as innovative epigenetic biomarkers for the identification of patients at risk for ketamine adverse events. The combination of comprehensive genetic analysis and epigenetic assessment could provide innovative insights on the molecular mechanism of action of ketamine.

3. Material and methods

3.1 Study objectives

This exploratory study aimed to identify any biomarker useful to predict the likelihood of occurrence of vomiting and recovery agitation after ketamine administration. A comprehensive set of potential pharmacological predictors, including pharmacogenomic and pharmacokinetic determinants, were considered.

The primary objective of the study was to identify genetic variants associated with the development of vomiting or recovery agitation in children sedated with intravenous ketamine.
The study was carried-out analyzing genetic variants derived from candidate genes involved in the metabolism and pharmacodynamic of ketamine, comparing children with and without adverse events.

Secondarily we aimed to compare the pharmacokinetic and epigenetic profiles of children who developed vomiting and recovery agitation with children who didn't, in order to investigate if some miRNA profile, derived from neuron-derived exosomes, may be used as a biomarker of the development of adverse events.

3.2 Study design and settings

A prospective pharmacogenetic study was conducted at the tertiary level, university teaching, children's hospital, Institute for Maternal and Child Health IRCCS Burlo Garofolo of Trieste, Italy. Pharmacogenetic, pharmacokinetic and epigenetic analyses were performed.
Children were recruited in three different hospital settings: pediatric emergency department, pediatric ward, pediatric rheumatological, nephrological, and oncological day hospital.

3.3 Population

We organized the consecutive enrolment of children performing sedation outside the operating room with intravenous ketamine as the only sedative drug, considering as cases, patients who develop vomiting and/or recovery agitation and, as controls, patients who do not develop these side effects.
We planned to enroll 50 patients with vomiting and recovery agitation and to compare them with the population of patients without these adverse events.
Eligible patients were children and adolescents, from 1 to 17yrs of age, needing analgesia and sedation for painful procedures and receiving intravenous ketamine as the sole sedative agent.
The procedural sedation was performed in accordance with the protocols and standard of care of the Institute. The choice of using ketamine as the sole sedative agent was made by the physician in charge for the procedural sedation (who could be both a trained pediatrician or an anesthesiologist) according to standard protocols, type of procedure and patients' characteristics.
Patients were enrolled before the procedural sedation. Children and their parents were able to revoke their consent to participate in the study at every moment.

3.4 Inclusion criteria

Inclusion criteria were:

- Patients between 1 and 17yrs of age needing sedation and analgesia for painful procedures, such as fracture reduction, suture laceration, burn medication, arthrocentesis, bone marrow aspiration, lumbar puncture.
- Use of intravenous ketamine as the sole sedative agent for the procedure.

3.5 Exclusion criteria

Exclusion criteria were:

- Patients who were needed to be administered with other sedatives during the procedure

- Patients who were administered ondansetron or any other antiemetic before the procedure.
- Patients with intellectual disability or autism spectrum disorder, because recovery agitation could be more difficult to be objectivated in these subjects.
- Patients who received ketamine via other routes than the intravenous route.
- Patients whose parents or legal guardians were not able to provide written informed consent to the study.

3.6 Intervention

The parents or legal guardians of eligible patients were approached and informed about the aim and scope of this study. After obtaining written informed consent from parents or legal guardians, eligible children and adolescents were enrolled in the study.

The procedural sedation was undertaken regardless of this study and was carried out following the international guidelines and the standard protocols for procedural sedation outside the operating room.

In brief, children undergoing sedation were initially examined by a trained pediatrician or an anesthesiologist and certified fit for sedation. Peripheral vascular access was obtained, if not already present before sedation.

Intravenous ketamine was administered with an initial bolus (usually 1.5mg/kg) in 1-2 minutes, to achieve the dissociated state. The diagnostic or therapeutic procedure started once achieved dissociation. Further boluses of intravenous ketamine were administered to achieve a Ramsay score of 4 or to maintain dissociation during the procedure, if necessary.

The monitoring of the patients during sedation included a continuous recording of oxygen saturation, heart rate, and capnography. Monitoring was continued until patients were awake with a minimum Aldrete score of 9 points and they were able to tolerate clear fluids (33).

Children who needed a sedative drug other than ketamine during the procedure were excluded from the analyses.

The sedating physician recorded the occurrence of side effects during the procedure and after the procedure on a predisposed sedation chart. The latter was also be fulfilled with the patient data, including age, sex, weight, type of ongoing painful procedure, ASA score, ketamine dose administered and time of administration, need for further ketamine boluses during the procedure.

Adverse events were recorded in a dedicated form by the sedating physician and included:

- oxygen desaturation (defined as saturation <92% for more than 20 seconds)
- upper airway obstruction
- urgent airway intervention (tracheal intubation, positive ventilatory pressure, positioning of respiratory support devices such as Mayo cannula, laryngeal mask or nasopharyngeal trumpet, aspiration)
- unscheduled recovery or pediatric intensive care admission
- apnoea (defined as a respiratory pause of at least 15 seconds)
- laryngospasm
- vomiting
- recovery agitation, defined as any abnormal behavioral response such as any combination of agitation, crying, hallucinations or nightmares after sedation.
- hemodynamic changes, defined as a change of heart rate or systolic blood pressure higher than 30% of the baseline.
- cardiac arrest
- death

The occurrence of vomiting was recorded at the moment of discharge from the hospital or within 6 hours from the administration of ketamine. The occurrence of recovery agitation was evaluated by the physician responsible for the sedation following the definition provided in the study protocol.

Clinical data were stored on a specific electronic database to ensure the reliability and the internal coherence of all the data.

Sampling for the pharmacogenetic and epigenetic analyses were performed before ketamine administration collecting 3 mL of blood into K-EDTA containing tubes, from the established vascular access. Samples were stored at a 4°-degree temperature and then centrifugated in order to separate plasma from buffy coat within 24 hours from collection. Samples were eventually stored at -80° at the Institute until the analysis was carried out.

The pharmacokinetic analysis was limited to a subgroup of 15 subjects developing adverse events, applying a limited sampling strategy in accordance with the work by Sherwin et al. (34). One mL of blood was obtained in K-EDTA-containing tubes, before the procedure and at 1, 6, 15, and 120 minutes after ketamine administration. Plasma was separated immediately by centrifugation of blood samples (3000 g for 10 minutes at 4 °C), then split

into two polypropylene tubes and stored as two independent aliquots at -80 °C in 2 different freezers in order to avoid the loss of samples in the case of freezer failure.

3.7 Samples analysis

3.7.1 Pharmacogenetic analysis

The samples were processed at the Institute for the extraction of the nucleic acids. The genotyping was performed through Illumina Omni 2.5+ exome array, in collaboration with the University of Trieste. The search for biomarkers was performed through the analysis of the variants of 10 candidate genes, codifying for proteins responsible for the metabolism (CYP2B6, CYP2C9, CYP3A4, CYP3A5, CYP2A6) and pharmacodynamics of ketamine (NMDA receptor subunits: GRIN1 e GRIN2 A-D), including 1300 genetic variants, selected based on the available literature. The independent variable associated with the occurrence of ketamine side effects was the presence of functional variants of candidate genes.

Moreover, a target sequencing genome-wide analysis with Illumina Global Screening Array was carried-out to identify the presence of genetic variants associated with the development of adverse events but not related to the candidate genes.

As mentioned, DNA was extracted from a peripheral blood sample, obtained immediately before the start of the procedural sedation.

3.7.2 Pharmacokinetic analysis

The plasma concentration of ketamine and its primary metabolite, norketamine, was determined, by using a new LC-MS/MS method specifically developed and validated according to European Medicine Agency guidelines. A non-compartmental analysis was applied to determine the pharmacokinetic parameters. The parameters that were taken into account were Ke (elimination rate constant), $T_{1/2}$ (plasma half-life in the terminal phase), Vd (volume of distribution), Cl_p (plasma clearance defined as Ke x Vd), and $AUC_{0\text{-last}}$ (area under the concentration-time curve from time 0 to the last detectable sample, defined as drug dose/Cl_p). They were calculated through the R software version 4.0.1. For comparisons, the plasmatic concentrations of ketamine and norketamine at each time point, were normalized as weight adjusted doses. The samples, collected at the already mentioned time points, consisted in an optimal sampling schedule for the estimation of the pharmacokinetic parameters in children receiving ketamine (34).

3.7.3 Epigenetic analysis

The sequencing of the miRNA, derived from plasmatic neuron-derived exosomes, was extracted in collaboration with the University of Trieste and the Department of Neurosciences, University of California, San Diego, La Jolla, CA, United States. The miRNA contained in the neural-derived exosomes were quantified and related to the occurrence of ketamine adverse events, through a next-generation sequencing technique. For the statistical analysis, the independent variable, associated with the occurrence of side effects, was the level of expression of each miRNA. An analysis of the pathways related to the differently expressed levels of miRNA in patients with and without adverse events was performed, with the aim of interpreting the different biological processes.

An innovative pharmacogenomic approach provided neural-derived exosomal miRNA profiles for each patient. Plasma extracellular vesicles enriched for neuronal origin were isolated as described in several papers (35-36).

Total RNA was extracted from the exosome pellet, and small RNA fragments were sequenced using the Illumina platform. RNA sequencing was performed for circulating exosomes obtained from the plasma of 50 patients with adverse events and 50 patients without. Selected miRNAs with significant differences in expression were validated by quantitative reverse transcription-polymerase chain reaction (qRT-PCR), using TaqMan miRNA assays.

3.8 Statistical analysis

3.8.1 Pharmacogenetic analysis

The study aimed to enrol 50 patients with vomiting and recovery agitation and to compare them to almost 150-200 patients. Through the analysis of this minimum number of patients, we hypothesized to be able to find out genetic variants, related to the occurrence of vomiting and/or recovery agitation. All the enrolled patients were tested for the presence of pharmacogenetic biomarkers. Expecting the presence of 100 variants for each of the 10 candidate genes (CYP2B6, CYP2C9, CYP3A4, CYP3A5, CYP2A6 for the pharmacokinetics NMDA receptor subunits GRIN1 e GRIN2A-D for the

pharmacodynamics) the score for statistical significance for multiple tests was 0.05/1000 = 5x10-5.

The pharmacogenomic analysis was performed through Illumina Global Screening Array. The genome-wide association between the genetic variants and the occurrence of adverse events was performed through the Plink software. The chi-square test was used to compare the two groups in search of any pharmacogenetic variant useful to predict the occurrence of ketamine adverse events. The p-value was corrected for multiple testing, using the False Discovery Rate (FDR). When analysing results from genome wide studies, often thousands of hypothesis tests are conducted simultaneously, one for each single nucleotide polymorphism. Use of the traditional Bonferroni method to correct for multiple comparisons is too conservative, since guarding against the occurrence of false positives will lead to many missed findings. In order to be able to identify as many significant comparisons as possible while still maintaining a low false positive rate, the FDR are utilized, according to the experience of Benjamini Y et al, published on the Journal of the Royal Statistical Society in 1995.

3.8.2 Pharmacokinetic analysis

Patients enrolled undergo the pharmacokinetic study, until the collection of data from 15 patients with vomiting or recovery agitation. This number has been calculated considering as the guiding parameter, a clearance value of 87,9 l h-1 70 kg-1, with a standard deviation of 11,6 (34): hence a sample of 15 patients allow to evaluate the clearance with an accuracy of 13% (37). This sample size also allows the detection of any significant difference in the average clearance, in the children with and without side effects. The comparison of the pharmacokinetic profiles of patients with and without adverse events was performed through the Welch's t test, calculated through Graphpad Prism 8.

3.8.3 Epigenetic analysis

We planned to perform epigenetic analysis in 50 patients with vomiting or recovery agitation and in 50 other subjects, randomly selected among enrolled patients, who did not experience these adverse events, matching them in accordance to age and sex. This sample size allows the detection of miRNA with a strong association with the occurrence of adverse events (d = 0.8), with a power of 80% and a significant level of 0.002. The edgeR

software is used for the identification of the different levels of expression of the miRNA. The miRNA displaying a significantly different level of expression between patients with and without adverse events will be validated through the real-time PCR.

3.9 Feasibility and expertise

The Institute for Maternal and Child Health "Burlo Garofolo" of Trieste has developed a Pediatric Sedation Unit since 2001, fostering a specific activity of collaborative research on the field documented by several papers on the topic.

The pharmacokinetic studies were performed in collaboration with the prof De Corti laboratory, of the University of Trieste. All the pharmacogenetic analyses were conducted by prof. A.P. d'Adamo from the University of Trieste.

The genetic unity, led by Prof. d'Adamo, has comprehensive experience in genetic association studies. The laboratories are equipped with platforms for High-throughput Genotyping, and Next Generation Sequencing, and part of the staff is composed of bioinformatics and biostatisticians.

The study was conducted according to the Good Clinical Practice standards.

No additional risk is foreseen for patients: all children enrolled have been previously considered as in need of sedation for a painful procedure according to the Institute-specific protocols exclusively on clinical ground. The study enrollment was proposed only to patients needing sedation with intravenous ketamine by means of intravenous access, thus the enrollment in the study only added to patients the burden of a limited amount of additional blood sampling, maximum 7 ml.

Selection of patients and monitoring during sedation were performed according to standard protocols and practices already in place.

Since sedation practices already require high specific standards of training and privileges for evaluation of patients, sedation management, monitoring skills, no additional training activity was scheduled for the purpose of the study from this perspective.

4. Results

The recruitment of patients in this study is still ongoing. Therefore, here we present the results available until October 2021.

From September 2019 to October 2021, 106 patients were enrolled in the study. The pharmacogenetic analyses have been performed and were available in the first 95 of them. Among these 95 patients, 8 patients (8%) were excluded because they didn't fulfill the inclusion and exclusion criteria of the study (Fig 6). In particular, two patients were excluded because they received premedication with midazolam per os; one patient underwent the procedure with intravenous ketamine in combination with propofol; one patient underwent the procedure with intravenous ketamine in combination with sevoflurane; one patient performed the procedure with intramuscular ketamine; one patient received premedication with sublingual ondansetron; demographical and clinical features of two patients were not reported.

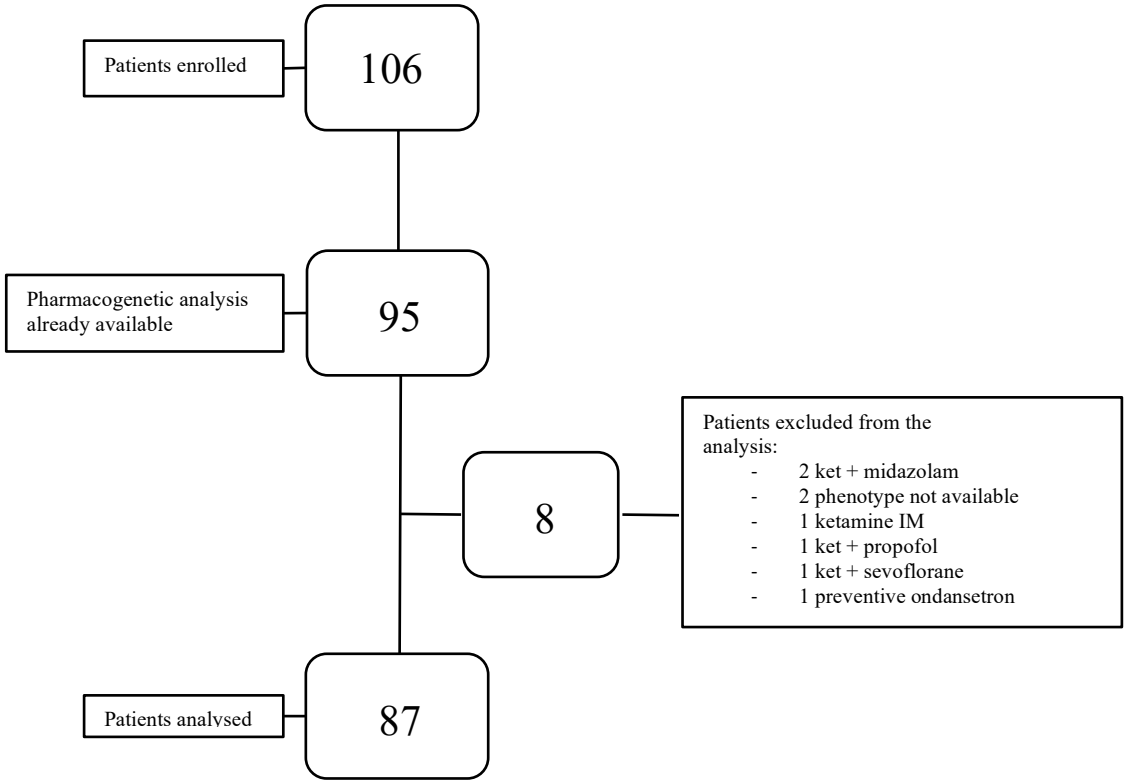

Figure 6. Study flow diagram

Finally, 87 enrolled patients were analyzed.

The table 1 shows the main baseline features of the analyzed patients enrolled in the study.

Analyzed patients	
Patients, n (%)	87 (92%)
Female sex, n (%)	47 (55%)
Age yrs, mean (SD); range; median (IQR)	8 +/- 4.3; 1-17; 8 (5-11)
Weight kg, mean (SD); median (IQR)	36 +/- 19.9; 30 (17.8-47.5)
Affected by chronic diseases: - Yes - No	 32 (37%) 55 (63%)
Chronic diseases: - Juvenile idiopathic Arthritis - Chronic glomerulonephritis - Autoimmune Hepatitis - Psoriasis	 28 (88%) 2 (6%) 1 (3%) 1 (3%)
Taking chronic medications: - Monoclonal drugs - NSAIDs - Oral steriods - Methotrexate - Mycophenolate	13 (15%) 6 (7%) 3 (3%) 2 (2%) 1 (1%) 1 (1%)

Table 1. Baseline features of analyzed patients.

Among the 87 patients analyzed, 47 (55%) were females. The mean age was 8yrs. Thirty-two patients (37%) were affected by chronic diseases, mainly Juvenile Idiopathic Arthritis (88%). Thirteen patients (15%) were taking chronic medication, mainly monoclonal drugs (7%).

The Table 2 shows the type of procedure who underwent the 86 analyzed patients.

Type of procedure	
Procedures, n (%)	87 (100%)
Arthrocentesis, n (%)	32 (37%)
Bone fractures reduction, n (%)	26 (30%)
Bone marrow aspirate, n (%)	5 (6%)
Dental extraction, n (%)	5 (6%)
Renal, Hepatic, Skin Biopsies, n (%)	5 (6%)
Foreign body removal, n (%)	3 (3%)
Wound suture, n (%)	1 (1%)
Dislocation reduction, n (%)	1 (1%)
Kirschner wires removal, n (%)	1 (1%)
Nasal brushing, n (%)	1 (1%)
Angioma removal, n (%)	1 (1%)
Eye evaluation, n (%)	1 (1%)
Trans-tympanic drainage, n (%)	1 (1%)
Electromyography, n (%)	1 (1%)
Others, n (%)	3 (3%)

Table 2. Type of procedures performed with intravenous ketamine.

Dissociative sedation with intravenous ketamine was employed to perform a wide range of painful procedures. The procedures more commonly performed were arthrocentesis (37%) and bone fracture reduction (30%). Intravenous ketamine was used for other procedures typically performed at the emergency department including dental extraction, foreign body removal, wound suture, and dislocation reduction. A notable number of biopsies and bone marrow aspirates were also performed.

The table 3 summarizes the use of ketamine during procedures.

Ketamine use during procedures	
Patients, n (%)	87 (100%)
Patients who performed the procedure with a single bolus, n (%)	60 (69%)
Patients who needed more boluses, n (%): - Not adequately sedated after the first bolus - Awakening during the procedure	27 (31%) 13 (48%) 14 (52%)
First bolus dose, mean (SD); range; median (IQR)	1.4mg/kg +/- 0.48; 0.6-3.3mg/kg; 1.3mg/kg (1-1.75)
Second bolus dose, mean (SD); range; median (IQR)	0.7mg/kg +/- 0.51; 0.2-1.5mg/kg; 0.7mg/kg (0.5-1)
Mean and median (IQR) first bolus dose in patients who needed a single bolus	1.6mg/kg; 1.5mg/kg (1.07-2)
Mean and median (IQR) first bolus dose in patients who needed more boluses	1.2mg/kg; 1.2mg/kg (1-1.35)

Table 3. Ketamine use during procedures.

The majority of patients (69%) underwent the procedure with a single bolus of intravenous ketamine. Among the patients who needed more boluses, 48% didn't reach dissociation after the first bolus, 52% didn't maintain the dissociation during the procedure. Ketamine was administered with a mean first bolus dose of 1.4mg/kg. Patients who underwent the procedure with a single bolus received a median initial dose slightly higher (1.5mg/kg) than patients who needed more boluses (1.2mg/kg).

Among the 87 patients analyzed, 31 patients experienced vomiting or recovery agitation (35%) and formed the study group. On the other hand, two patients experienced headache, 1 patient excessive drooling and transient apnea, 1 patient a skin rash. No patients experienced laryngospasm. For the purpose of the study, patients experiencing adverse events different from vomiting and recovery agitation were considered in the same group of patients without any adverse events, namely in the control group.

Among the 31 patients of the study group, 16 patients (18%) experienced vomiting, 12 patients (14%) recovery agitation, and 3 patients (3%) vomiting and recovery agitation. Table 4 summarizes the main clinical features of patients with vomiting or recovery agitation compared to patients without these adverse events.

	Study group		**Control group**
	Vomiting	**Recovery agitation**	**No adverse events or other events**
Patients, n (%)	19 (22%)	15 (17%)	56 (64%)
Mean age, yrs (SD)	9.7 +/- 4.2	8.5+/- 4.0	7.7 +/- 4.9
Median age, yrs (IQR)	10 (7-12.5)	9 (6-11.5)	8 (3.7-11)
Mean weight, kg (SD)	35.7 +/- 16.0	36.0 +/- 21.1	33.1 +/-19.8
Median weight, (IQR)	30 (23.5-48.5)	38 (17.4-47)	30 (16.8-45)
Female sex, n (%)	13 (68%)	8 (53%)	24 (43%)
Affected by chronic disease, n (%)	7 (37%)	7 (37%)	16 (29%)
Single bolus, n (%)	12 (63%)	8 (53%)	43 (77%)
Mean ketamine dose administered during procedure (SD)	1.8mg/kg +/- 0.5mg/kg	1.8mg/kg +/- 0.6mg/kg	1.6mg/kg +/- 0.5mg/kg
Median ketamine dose administered during procedure (IQR)	1.9mg/kg (1.55-2)	1.7mg/kg (1.35-2)	1.6mg/kg (1.17-2)
Lower dose at which the event occurred	1mg/kg	1mg/kg	-
Higher dose without vomiting or recovery agitation	-	-	2.8mg/kg

Table 4. Main clinical features of patients with vomiting, recovery agitation compared to the control group.

Patients with vomiting and recovery agitation were similar to patients without these adverse events regarding age, weight and mean ketamine dose received, according to the Welch's t test. Among groups, no statistically significant differences were noticed regarding the prevalence of females, the presence of chronic diseases and the number of boluses of ketamine received during the procedure, according to a Fisher exact test. Nevertheless, we observed that patients without vomiting and recovery agitation underwent the procedure more frequently with a single bolus of ketamine.

We noticed that vomiting and recovery agitation occurred even for doses of ketamine of 1mg/kg and that no adverse events were recorded in patients who were administered higher doses of ketamine, even 2.8mg/kg. Remarkably, 19 patients (34%) without adverse events received a dose of ketamine higher than the mean dose of ketamine received by patients with vomiting or recovery agitation.

Moreover, we observed that despite the similar mean dose of ketamine received (1.8mg/kg in patients with vomiting and recovery agitation and 1.6mg/kg in patients without adverse events), patients without adverse events carried out the procedures more commonly with a single bolus of ketamine.

These clinical observations seem to confirm the hypothesis that factors different from the simple dose of ketamine received, may influence the development of vomiting or recovery agitation.

4.1 Pharmacogenetic analysis

As explained in the method section, the pharmacogenetic analysis was focused on 10 candidate genes, namely CYP2B6, CYP2C9, CYP3A4, CYP3A5, CYP2A6, GRIN1, GRIN2A-D. The first five are involved in the metabolism of ketamine and the second five in the pharmacodynamics of ketamine, coding for the subunits of NMDA receptor.

These analyses investigated approximately 1300 genetic variants. We decided to present here only the variants more associated with the development of vomiting and recovery agitation.

We present in the following table, the polymorphisms with a higher statistical significance related to the analyses of the above-mentioned cytochromes. Results for the CYP2C9 are not presented because they didn't show any relevant association.

CYP3A5 (chrom 7)	**polymorphism**	**p value**	**Odd Ratio (OR)**	**number of permutations**
	rs200579169	0.1116	1.83	326
	GSA-rs28365067	0.1561	1.96	220
CYP3A4 (chrom 7)	**polymorphism**	**p value**	**OR**	**number of permutations**
	7:99428736	0.01794	5.89	2257
	rs35599367	0.1016	2.56	363
	7:99381766	0.1412	2.22	254
	GSA-rs45537741	0.1392	3.79	254
CYP2B6 (chrom 19)	**polymorphism**	**p value**	**OR**	**number of permutations**
	rs2279345	0.04239	0.45	919
	rs2279344	0.08721	0.49	429
	rs2099361	0.08824	0.49	424
CYP2A6 (chrom 19)	**polymorphism**	**p value**	**OR**	**number of permutations**
	GSA-rs28399442	**0.006592**	**4.97**	**6143**
	GSA-rs28399443	0.04774	5.89	816
	rs28399442.1	0.04774	5.89	816
	rs8192726	0.05501	0.2	708

Table 5. Polymorphisms present in the cytochromes studied associated with the development of vomiting or recovery agitation.

The analyses of the genes involved in the metabolism of ketamine showed one polymorphism more associated with phenotypes of patients who experienced vomiting or recovery agitation: **GSA-rs28399442** on the gene CYP2A6 on chromosome 19 (p =0.006592; OR 4.97) (Figure 7).

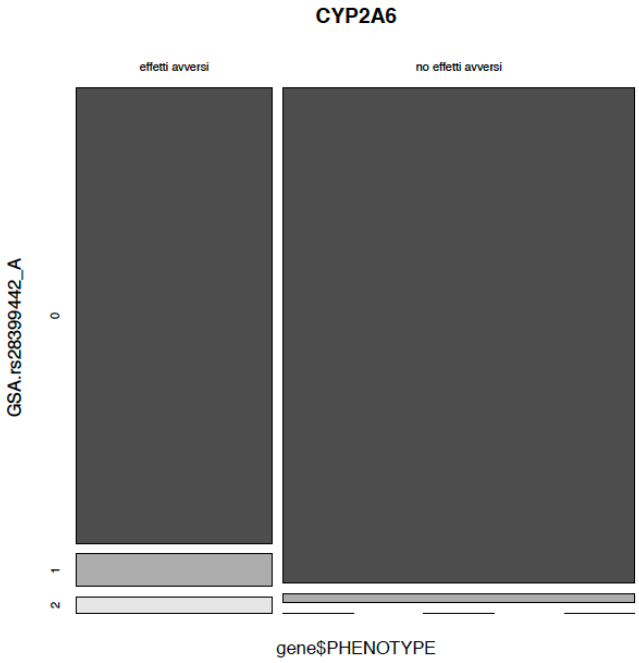

Figure 7 legend		
Haplotypes GSA-rs28399442_A	Patients with vomiting or recovery agitation	Controls
0	28	55
1	2	1
2	1	0

Figure 7. Frequency of haplotypes for allele **GSA-rs28399442_A** on the gene CYP2A6 in patients with and without vomiting or recovery agitation.

The table 6 shows the results of the pharmacogenetic analysis focused on the gene coding for the subunits of the NMDA receptor.

GRIN2B (chrom 12)	**polymorphism**	**p value**	**Odd Ratio (OR)**	**number of permutations**
	rs77384356	**0.0104**	**4.41**	**3943**
	GSA-rs114543590	0.01774	4.03	2254
	rs2300252	0.0187	2.33	2165
	GSA-rs17220663	0.02813	3.74	1403
GRIN2A (chrom 16)	**polymorphism**	**p value**	**OR**	**number of permutations**
	GSA-rs71379063	**0.003857**	**7.16**	**10629**
	rs11648559	0.012	3.12	3374
	rs117572631	0.02166	4.97	1846
	rs8058295	0.02651	2.1	1508
GRIN2D (chrom 19)	**polymorphism**	**p value**	**OR**	**number of permutations**
	GSA-rs10424366	0.06331	2.56	615

Table 6. Polymorphisms present in the genes coding for the subunit of the NMDA receptor associated with the development of vomiting or recovery agitation.

This analysis shows the presence of polymorphisms more associated with the development of vomiting or recovery agitation on the gene GRIN2B and GRIN2A.
The polymorphism **GSA-rs71379063** on the GRIN2A gene is the one more associated with the development of vomiting and recovery agitation (p 0.003857; OR 7.16) among the genetic variants analyzed focused on the candidate genes (Figure 8).
The second polymorphism more associated with the development of vomiting and recovery agitation was **rs77384356** on the GRIN2B (p=0.0104; OR 4.41) (Figure 9).
Results for the GRIN1 and GRIN2C are not presented because they didn't show any relevant association.

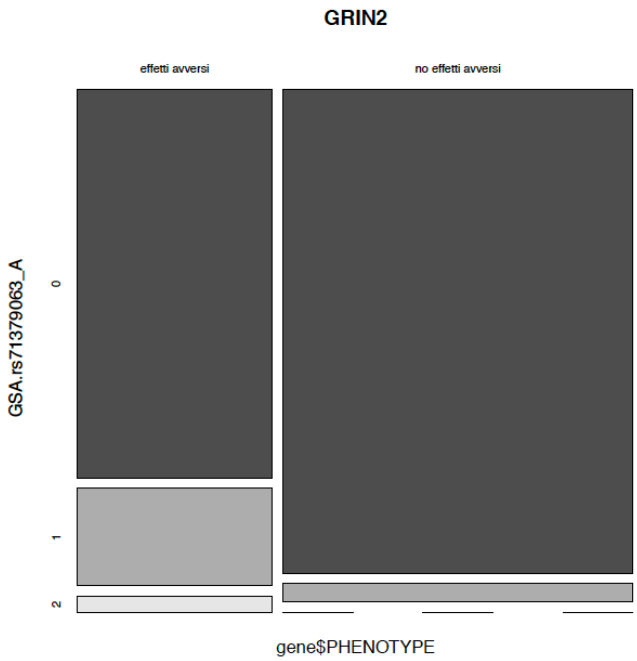

Figure 8 legend		
Haplotypes GSA-rs71379063_A	Patients with vomiting or recovery agitation	Controls
0	24	54
1	6	2
2	1	0

Figure 8. Frequency of haplotypes for the allele GSA-rs71379063_A on the gene GRIN2A in patients with and without vomiting or recovery agitation.

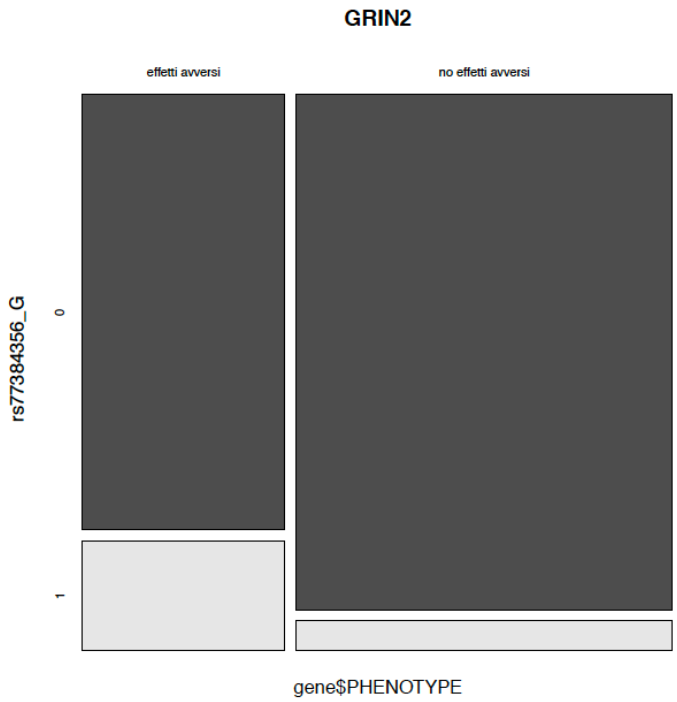

Figure 9 legend		
Haplotypes rs77384356_G	Patients with vomiting or recovery agitation	Controls
0	24	53
1	6	3
2	0	1

Figure 9. Frequency of haplotypes for the allele rs77384356_G on the gene GRIN2B in patients with and without vomiting or recovery agitation.

To extend our pharmacogenetic analysis beyond the candidate genes, we performed a genome-wide approach. Through the Plink software, 645027 polymorphisms on the entire genome were analyzed.

Notably, a series of polymorphisms, mainly on chromosome 5, were associated to the development of vomiting or recovery agitation in the study population. In the table 7 we have enlisted the polymorphisms more associated with the development of these adverse events.

Chromosomes	Polymorphisms	p value
5	GSA-rs27578	0,0000010
5	Rs2662255	0,0000010
5	Rs66548630	0,0000075
7	Rs10252494	0,0000075
5	Rs368168553	0,0000080
5	GSA-rs79558474	0,0000080

Table 7. Polymorphisms more statistically associated with the development of vomiting or recovery agitation identified through the genome-wide analysis.

The figure 10 offers a graphical view of the results of the genome-wide analysis. In this plot higher spots indicate the polymorphisms more associated with the development of vomiting or recovery agitation.

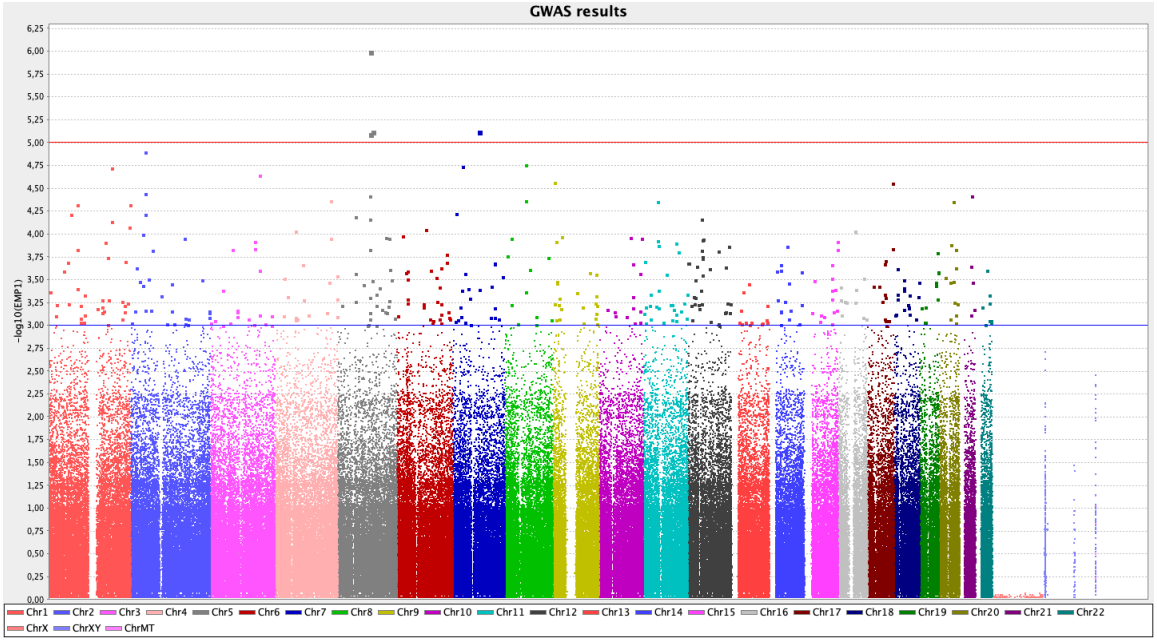

Figure 10. Graphical view of the results of the genome-wide analysis.

The polymorphisms more associated with the development of vomiting or recovery agitation were not related to candidate genes. According to the available gene libraries

these polymorphisms were not related to genes already known to have a relationship with ketamine.

4.2 Pharmacokinetic analysis

We were not able to collect the blood samples for the pharmacokinetic analysis in all the enrolled patients. This was related to the setting in which the procedures were performed and to the personnel involved in the procedure. In consideration of that, we decided to privilege the pharmacogenetic analysis in many cases, collecting just an initial blood sample before the procedure.

Plasma samples at different time points (T1= 1 minute, T6= 6 minutes, T15= 15 minutes, T120= 120 minutes) of 27 patients were collected in order to quantify the plasma concentration of ketamine and its metabolite norketamine.
Among the time points chosen we decided to exclude the T1 point because it could be assumed that ketamine was not yet distributed and because there was variability among operators in the duration of the administration of the initial bolus of ketamine.
Among the 27 patients in which we collected the samples, 11 patients were excluded from the analysis because they required more boluses of ketamine during their procedure and 2 patients were excluded because not enough samples were collected to calculate the pharmacokinetic parameters.

Finally, we compared the pharmacokinetic parameters of 14 patients, 5 with adverse events (3 with vomiting, 1 with recovery agitation, 1 with vomiting, and recovery agitation) and 9 without these adverse events.

Figure 11 shows that the 14 patients were similar for age, weight, and dose of ketamine received: Welch's t-test not significant.

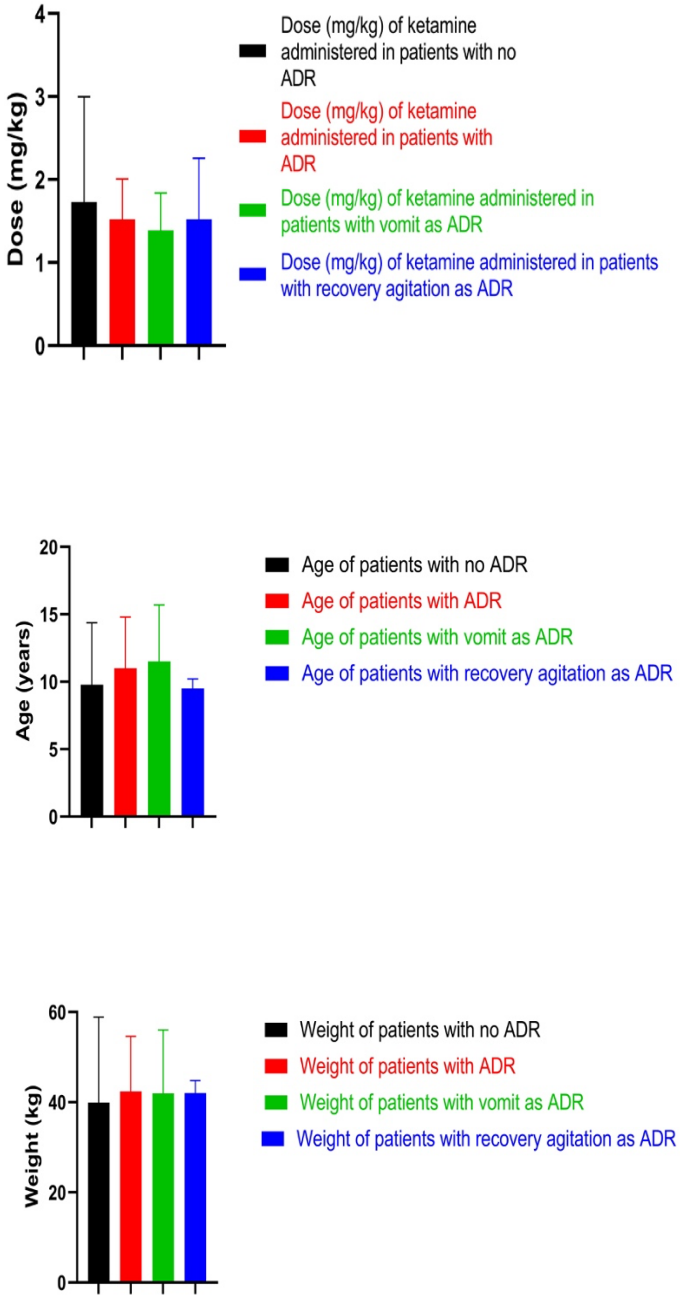

Figure 11. Comparison between patients who presented vomiting or recovery agitation and who didn't on the basis of the dose pro kilogram of ketamine received, of the age and of the weight.

The results of the pharmacokinetic parameters were as follows:

Ke (elimitation rate constant)

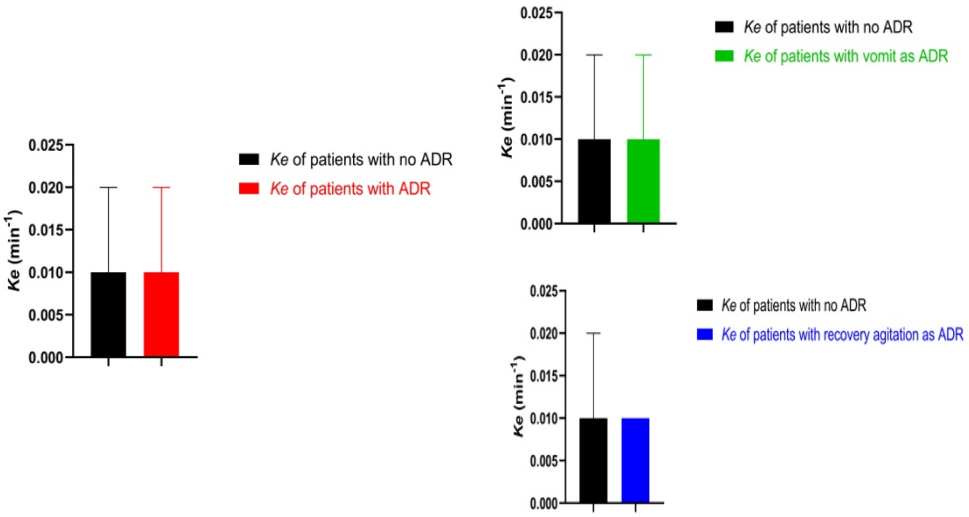

Figure 12. Comparison between the elimination rate constants (Ke) of patients with vomiting or recovery agitation and patients without. ADR (adverse events).

No statistically significant differences (Welch's t test) were observed regarding Ke in patients with or without vomiting or recovery agitation.

$T_{1/2}$ (half-life time)

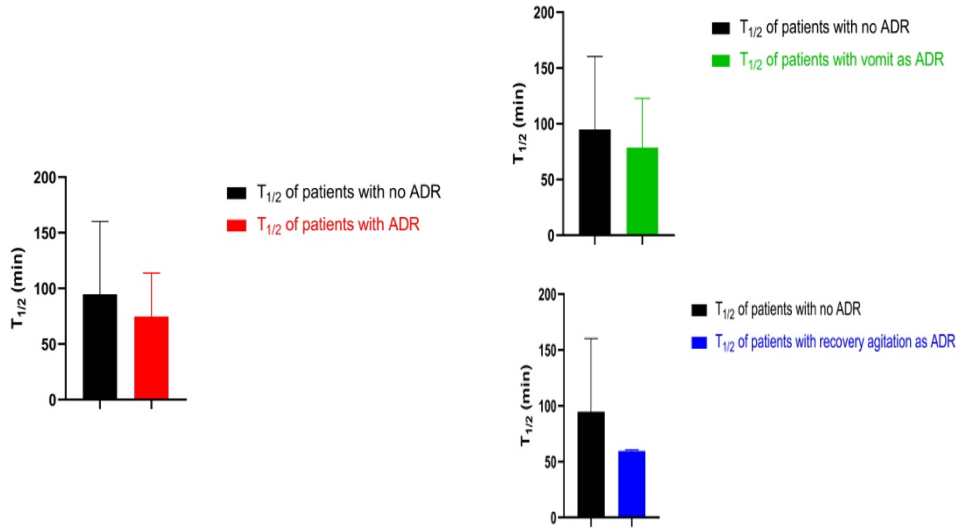

Figure 13. Comparison between the half-life times ($T_{1/2}$) of patients with and without vomiting and recovery agitation. ADR (adverse events).

No statistically significant differences (Welch's t test) were observed regarding $T_{1/2}$ in patients with or without vomiting or recovery agitation.

Vd (volume of distribution)

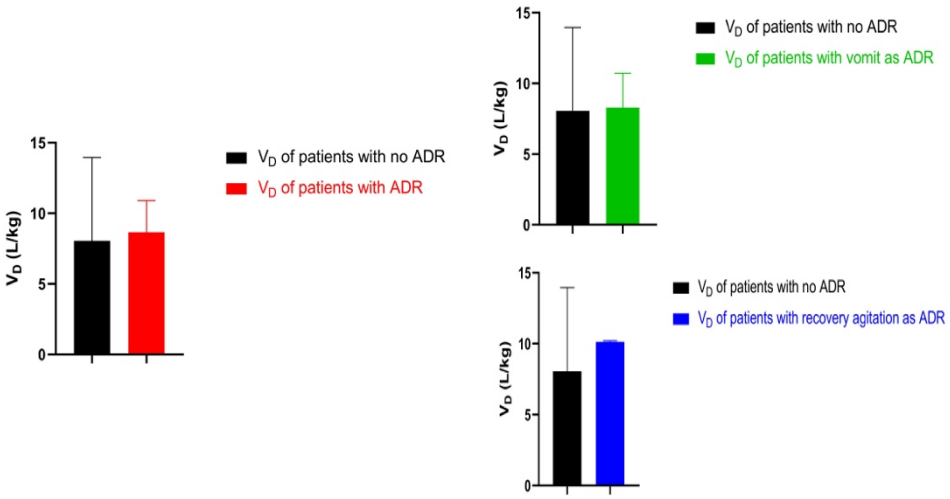

Figure 14. Comparison between the volumes of distribution (Vd) of patients with and without vomiting and recovery agitation. ADR (adverse events).

No statistically significant differences (Welch's t test) were observed regarding Vd in patients with or without vomiting or recovery agitation.

Cl (Clearance)

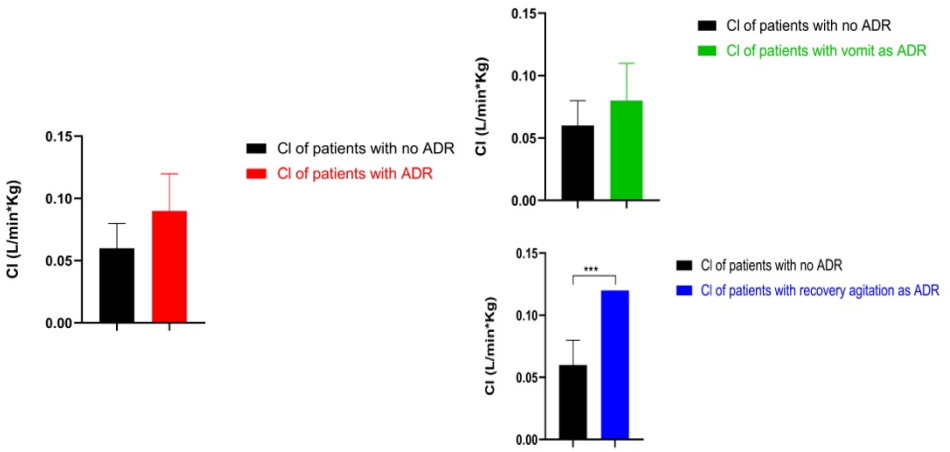

Figure 15. Comparison between the clearance values (Cl) of patients with and without vomiting or recovery agitation. ADR (adverse events).

No statistically significant differences (Welch's t test) were observed regarding Cl between patients without adverse events and patients with vomiting. A statistically significant difference, was found between patients without adverse events and patients with recovery agitation, Welch's t test *** p<0,001.

AUC (area under the curve)

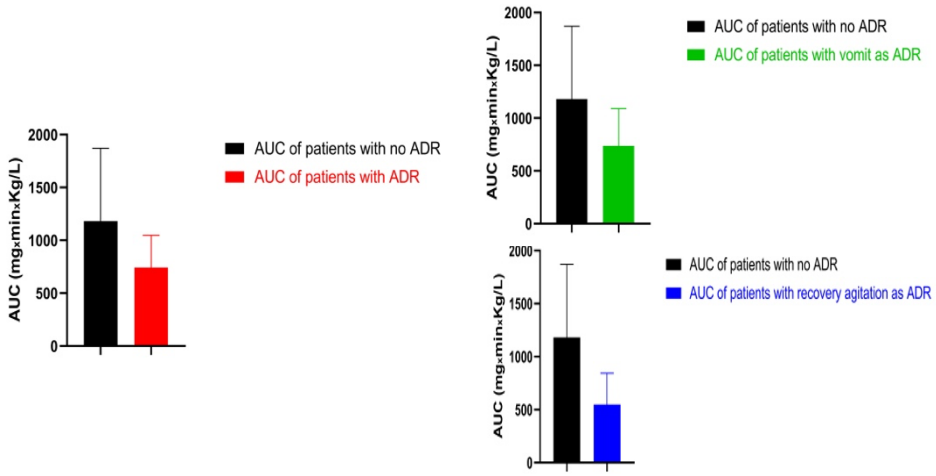

Figure 16. Comparison between the area under the curve values (AUC) of patients with and without vomiting or recovery agitation.

No statistically significant differences (Welch's t test) were observed regarding AUC in patients with or without vomiting or recovery agitation.

In general, the pharmacokinetic parameters didn't show any significant difference between patients without adverse events and patients with vomiting. On the contrary, the plasma clearance of ketamine was different between patients without adverse events and patients with recovery agitation. This difference seemed related more to a different distribution of the drug than to a different elimination.

Weight adjusted ketamine plasma concentration

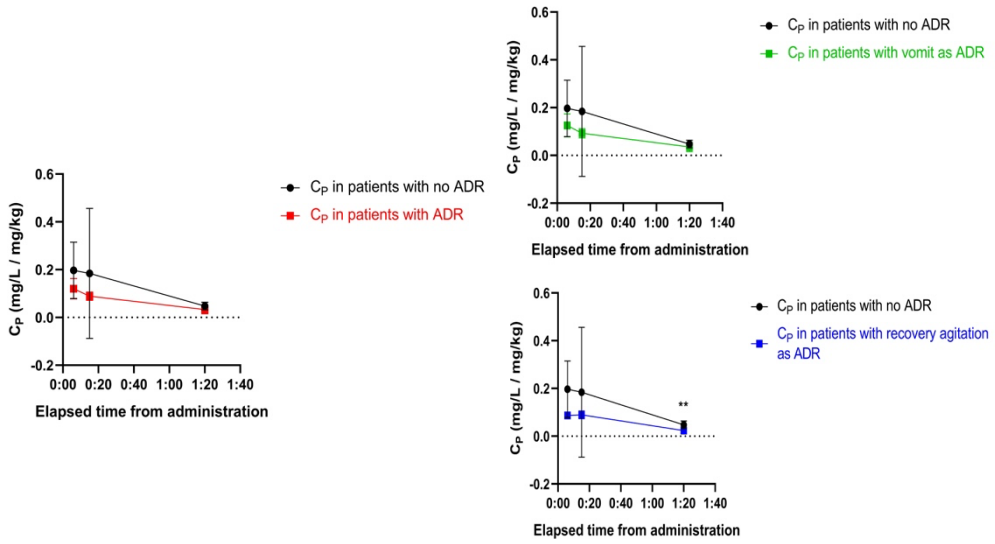

Figure 17. Concentration-time graphs showing the plasmatic concentration of ketamine (C_P) normalized on the dose pro kilogram in the elapsed time from administration in patients with and without vomiting or recovery agitation. These values were used for the comparison between the plasma concentrations at the 3 different time points.

Plasma concentration levels of ketamine at T120 were significantly different between patients without adverse events and patients with recovery agitation, Welch's t-test (** $p<0.01$). On the contrary no differences were observed between patient without adverse events and patients with vomiting.

The different plasma concentration of ketamine found in patients with recovery agitation, compared to patients without adverse events was consistent with the higher clearance of ketamine observed in these patients.

Weight adjusted norketamine plasma concentration

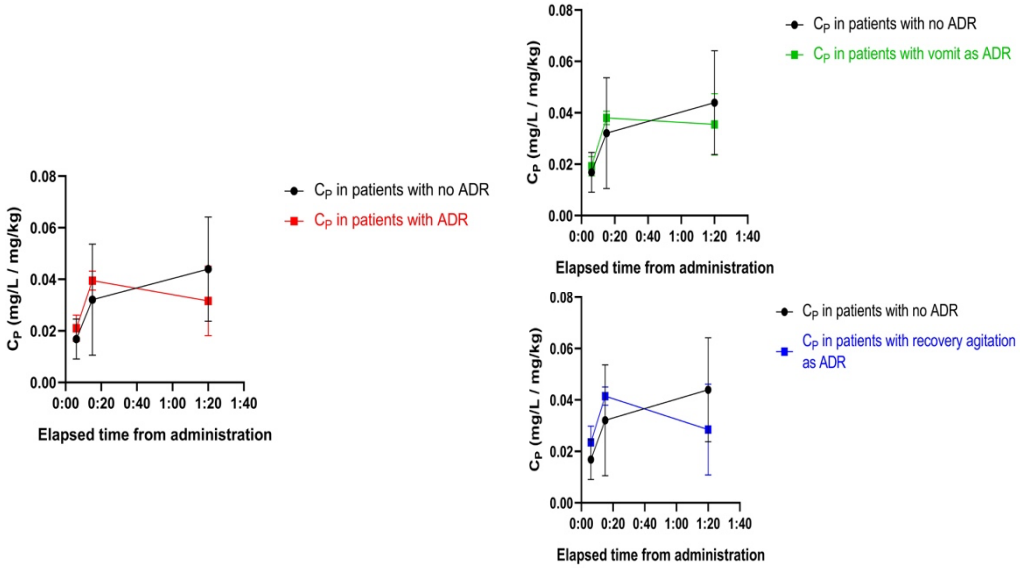

Figure 18. Concentration-time graphs showing the plasmatic concentration of norketamine (C_P) normalized on the dose pro kilogram in the elapsed time from administration in patients with and without vomiting or recovery agitation.

No statistically significant differences (Welch's t test) were noted regarding plasma concentration levels of norketamine at any time point, between patients with and without vomiting or recovery agitation.

4.3 Epigenetic analysis

We planned to perform epigenetic analysis in 50 patients with vomiting or recovery agitation and in 50 patients match for age and sex, who did not experience these adverse events. Considering the fact that the analysis will be performed in a laboratory in the USA, in order to limit costs, we planned to send the samples once we had reached the number of 50 patients with vomiting or recovery agitation.

Therefore, at the moment, we don't have any results to present regarding the epigenetic analysis.

5. Discussion

Ketamine is one of the sedatives most used in the US and Canada, for sedation outside the operating room in pediatric emergency settings. Its use is well consolidated, with strong evidence of safety and efficacy, published in the last two decades (17,18-20,28,38,39). On the other hand, in Italy, its use in this setting is still limited. A recent survey among 32 emergency departments in Italy, providing care to 1.4 million children each year, showed that ketamine was never used in 6 (19%) centers, used only by anesthesiologists on call in 20 (62%) centers and used autonomously by emergency physicians in 6 (19%) centers (40). Despite its wide use in North America, data regarding the association between patients' genetic variants and the development of adverse events are extremely limited.

We found a pilot study, performed on adult patients who underwent sedation with ketamine for minor surgery (22). The aim of the study was to investigate the relationship between two polymorphisms of the CYP2B6 gene and two polymorphisms of the GRIN2B gene and the development of recovery agitation in those patients. The study didn't show any significant association between the polymorphisms and the presence of the adverse event, but its results were greatly weakened by the very limited sample size (47 patients) and by the choice to enroll patients who use ketamine in combination with midazolam.

In our population, despite the limited size, we found some clinical aspects that, in our opinion, may suggest a relationship between an individual predisposition and the development of adverse events. As underlined in the results section, vomiting and recovery agitation were present in patients who received relatively low doses of ketamine and on the contrary, not present in a considerable number of patients who received high doses of ketamine. Moreover, despite a similar mean dose of ketamine needed to perform the procedures among patients with and without vomiting and recovery agitation, patients without these adverse events underwent the procedure more commonly with a single bolus of ketamine, even though this difference wasn't statically significant. These clinical observations may also suggest a possible individual predisposition, mediated by pharmacodynamic variants, influencing the efficiency of the drug.

Literature shows that the causes of adverse drug reactions are multi-factorial. Genetic predisposition has been estimated to cause about 50% of severe adverse drug reactions and strongly influence the drug response variability (41).

Pharmacological effects are commonly related to pharmacokinetic such as drug absorption, distribution, metabolism, and elimination; and pharmacodynamic effects, related to drug molecular targets in the body. Gene mutations and polymorphisms may result in structural and functional differences in proteins for metabolizing enzymes and drug receptors leading to the development of adverse events. Understanding the genetic bases of variability in drug pharmacokinetics and pharmacodynamics may have considerable implications for the prevention of adverse events. In this sense, a priori knowledge of which medication and dosage are most suitable for an individual patient based on his/her genetic background may maximize the potential therapeutic effects and minimize adverse events (42).

Pharmacogenetic and pharmacogenomic studies, performed also in our Institute, are exploring the link between genetic predisposition and drug efficacy and toxicity in many pediatric fields (43-46).

As mentioned, drug toxicity may be linked both to its metabolism and pharmacodynamics. Opioids' hypersensitivity linked to the liver metabolism of the drug, is a well-known event. It had a direct repercussion on pediatric everyday clinical practice, abolishing the use of codeine and recently leading to a warning for the use of tramadol (47,48).

As mentioned, evidence about ketamine is more limited.

In vitro studies have found that ketamine is metabolized primarily by the CYP2B6 and CYP3A4 and to a lesser extent by CYP2C9 and other isoforms (49,50). An in vivo study showed that polymorphisms on the CYP2B6 were associated with higher median steady-state plasma levels of ketamine and lower plasma clearance of ketamine (51).

Our study has found that the contribution of cytochrome isoforms on the development of adverse events after intravenous ketamine administration is limited. We didn't find any significant association with variants on CYP2B6 and CYP3A4. In our population, the polymorphism more associated with the presence of vomiting and recovery agitation was GSA-rs28399442 on CYP2A6 ($p=0.006$), which is not one of the cytochromes most involved in ketamine metabolism.

On the other hand, in this study, we found a significant association between the development of vomiting and recovery agitation and the polymorphism GSA-rs71379063 on the GRIN2A gene ($p=0.0038$), suggesting a major relationship between the pharmacodynamic of ketamine and the development of adverse events. Although the exact pathophysiological mechanism is still unknown, we can speculate that polymorphisms of the GRIN2A gene may lead to the production of NMDAr protein variants more prone to lead to the development of vomiting and recovery agitation after ketamine administration.

The NMDA receptor has multiple functions in synaptic transmission and neuronal plasticity. The GRIN2 subunit has a binding site for glutamate, which is the main excitatory neurotransmitter in the brain and spinal cord. Genetic variations of NMDA receptor have been found associated with various central nervous system diseases in children and adults. GRIN2 mutations have been described in patients with speech disorders and language delay in various epileptic syndromes (52). Genetic variants of the GRIN2A and other subunits have been reported in patients affected by autism and schizophrenia (53,54).
Specifically, regarding ketamine, an animal study showed that variations of the GRIN2A subunits reduced the hypnotic effect of ketamine (55).
In this study we found two other polymorphisms associated, but with minor power, with the development of adverse events in GRIN2A and GRIN2B genes, rs11648559 (p=0.012) and rs77384356 (p=0.010), respectively.
Literature shows that also polymorphisms in the GRIN2B gene were associated with the development of schizophrenia (56,57). If we considered recovery agitation as a brief psychiatric effect due to ketamine exposition, it is not surprising that GRIN2A and GRIN2B genetic variants may play a role in its development as they do in schizophrenia.
No data are available in the literature about the possible role of GRIN2A and GRIN2B genetic variants and the occurrence of vomiting.
Notably, the agnostic pharmacogenetic approach through a genome-wide analysis showed us a series of polymorphisms associated with the development of vomiting or recovery agitation, not related to candidate genes or to genes with an already known correlation with ketamine. Moreover, these polymorphisms seemed located in genome zones with regulatory functions. It is possible that the development of vomiting or recovery agitation depends more on the regulation of genes than on specific gene variants. Nevertheless, every interpretation should be taken with great caution, considering the limited size of our population.
This study has several limitations. First of all, the recruitment of patients is still ongoing and we were able to present only partial results. In pharmacogenetic studies, the sample size is critical to strengthen the results. Despite our population being the largest available, compared to the literature, focused on this specific topic, our results must be confirmed with greater sample size. Initially, we planned to design a multicenter study, we had identified three other Italian pediatric centers who initially agreed to participate, but then none of these centers was able to start the enrollment of patients. As shown (40), ketamine, as the only sedative for sedation outside the operating room, is still little used in Italy.

Sedation outside the operating room also in emergency settings is frequently managed only by anesthesiologists, so that several other choices are preferred. Moreover, in some centers, ketamine is used always in combination with midazolam, and the combination of ketamine with other sedatives was an exclusion criterion for the enrollment in this study.

COVID-19 pandemic had an indirect influence also on this study, limiting the enrolment of patients. The social distancing measures put in place to limit the spread of SARS-CoV-2 completely changed the rate and type of patients accessing the pediatric emergency department at our Institute and led to a transient delay for elective procedures. During the initial strict lockdown, from March to April 2020, we experienced a dramatic reduction of the visits at our emergency department, with a 73% reduction of accessed in general and a 76% reduction of accessing for injuries, compared to the same period of the year before (58). In the same way, the mitigated social distancing measures, that are still in place, led to a reduction of 52% of visits in general, with a reduction of 42% of the accesses for injuries, during the last winter season, compared to the previous one (59).

Compared to previous studies with experienced high rates of vomiting and recovery agitation (20). In this study, adverse events were reported by the physician responsible for the sedation. Even though well instructed, many physicians were involved. So that, we can't exclude that an interindividual variability could have influenced the number of the reported adverse events. This could be true mainly for recovery agitation. In fact, this condition has a wide and interpretable definition: "any abnormal behavioral response such as any combination of agitation, crying, hallucinations or nightmares after sedation". Milder cases could have been missed or on the contrary mild changes in the patients' behavior could have been recorded as adverse events. On the other hand, the presence or absence of vomiting is quite objective and easy to collect. Usually, after sedation with ketamine, patients remained observed for a mean of two hours, sometimes even more. We are well aware that vomiting can present even later than two-three hours after sedation with ketamine. Nevertheless, we decided not to organize a follow-up of the patients after discharge. Considering the initial multicenter design of the study, we planned to perform an enrollment as much as easy and adherent to common practice as possible. Anyway, in our population, we had high rates of predischarge vomiting (22%) compared to the available North American metanalysis (8%) (20), but lower than other European and North American reports in which ketamine was employed intramuscularly (60,61). In our population, we enrolled patients affected by chronic diseases, and who underwent a broad spectrum of procedures, so we can't exclude that some factors related to the baseline

conditions of patients or to the type and length of procedures may have influenced the rate of vomiting in our population.

Another limitation of this study is that we decided to consider together patients with vomiting and recovery agitation. In our population, most of the patients had only one of the two adverse events, with only 3% of patients experiencing both vomiting and recovery agitation. We focused on the most frequently reported adverse events of ketamine in general, but we can't exclude that the development of vomiting and the development of recovery agitation, may be triggered or favored by different genetic variants. Moreover, the results of the pharmacokinetic analysis suggest a different pharmacokinetic profile between patients with and without recovery agitation, but not between patients with and without vomiting. Nevertheless, considering the sample size of our population we decided not to perform a pharmacogenetic subgroup analysis exploring separately patients with vomiting and patients with recovery agitation.

Finally, we were not able to present the results related to some of the study's secondary outcomes, so we can't already provide the full view of the analyses that we planned when we designed this research project.

In conclusion, this work presents the preliminary results of an ongoing pharmacogenetic study that aims to investigate if some genetic profiles are more associated with the development of vomiting or recovery agitation after intravenous ketamine administration in children. We found polymorphisms on GRIN2A, GRIN2B, and CYP2A6 genes associated with the development of these events. The available results suggest that the development of vomiting or recovery agitation seems more associated with genetic variants related to the pharmacodynamic of ketamine. These findings should be confirmed in a larger population of patients.

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Acknowledgments

Questa tesi e il mio dottorato di ricerca non sarebbero stati possibili senza il prof Barbi, ideatore e anima di questo progetto.
Grazie ai molti specializzandi di pediatria che nel corso della rotazione nel servizio di sedazione hanno arruolamento pazienti per questo studio e messo da parte tappi rigorosamente viola.
Un grazie particolare a Gabriele, Martina, Pio e Marianna, che tanto mi hanno aiutato nel corso di questi tre anni e senza i quali nulla sarebbe stato possibile.

Grazie di cuore a tutti voi!