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A clinical trial of S-ketamine analgesia in third molar surgery

Effects, safety and influence on inflammatory biomarkers

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Abstract

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Background and aims: Third molar surgery is a common surgical procedure and a well-established pain model in research, which is generally performed under local anaesthesia. Post-operative pain management is often solved with a combination of paracetamol and ibuprofen. In this thesis, our goal was to find a better alternative for pain management in the early post-operative period. The overall aim was to study if preoperative IV S-ketamine (0.125mg/kg or 0.25mg/kg) can offer a more effective and safer transition from local anaesthesia to orally administered analgesia, to investigate whether surgery or S-ketamine have an impact on inflammatory biomarkers in plasma, and to examine differences in protein expression between saliva and plasma.

Patients and methods: 168 patients participated in the study. Physiological variables, questionnaires, pain diaries, and self-reported side effects were all registered in the study. Biomarkers in plasma and saliva were analysed using a multiplex panel (Olink).

Results: There was a globally significant lower pain score in the group with high dose S-ketamine, and a longer time to first rescue medication. There were minor, clinically insignificant changes of SpO2 and pulse rate in the S-ketamine 0.25 mg/kg group. Surgery significantly affected certain biomarkers (IL6, CST5, OSM, TGF-alpha, IL10, FGF-19, Flt3L, white blood cell count, neutrophil blood cell count, cortisol, and high sensitivity c-reactive protein). S-ketamine had no impact on the expression of biomarkers. Although major differences were observed in biomarker expression between saliva and plasma, the correlations were ultimately limited and weak.

Conclusion: A single dose of S-ketamine 0.25 mg/kg IV reduces postoperative pain for up to 24h with a longer time to first rescue medication, compared to S -ketamine 0.125 mg/kg and placebo. Single dose S-ketamine 0.25 mg/kg IV gives a minor reduction of SpO_2 and a minor increase of pulse rate at the end of infusion of S-ketamine. These changes are clinically insignificant. Third molar surgery causes an immune response within 2 hours expressed in plasma; infusion of S-ketamine 0.25 or 0.125 mg/kg does not cause any immune reaction. The immune response in saliva vs plasma differs and are not interchangeable.

Keywords: Pain, Postoperative pain, S-ketamine, Third molar surgery, Efficacy, Safety, Biomarkers, Inflammatory biomarkers, Plasma, Saliva.

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One of the painful things about our time is that those who feel certainty are stupid,
and those with any imagination and understanding
are filled with doubt and indecision.
Bertrand Russell, 1872-1970

Let doubt prevail.
Stephen Fry, 1957-

List of Papers

This thesis is based on the following papers, which are referred to in the text by their Roman numerals.

I. Eriksson LB, Gordh T, Karlsten R, Lo Martire R, Thor A, Tegelberg A. Intravenous S-ketamine's analgesic efficacy in third molar surgery. A randomized placebo-controlled double-blind clinical trial. Br J Pain. 2024;18(2):197-208.

II. Eriksson LB, Gordh T, Karlsten R, Thor A, Tegelberg A. Patient safety of adjunct pre-operative intravenous S-ketamine for pain relief in third molar surgery - a randomised, placebo-controlled, double-blind trial. Br J Pain. 2024:20494637241262509.

III. Eriksson LB, Larsson A, Tegelberg Å, Thor A, Freyhult E, Kultima K, Gordh T. Effects of surgical trauma and S-ketamine on inflammatory biomarkers in a third molar surgery pain model. (Submitted)

IV. Eriksson LB, Larsson A, Eriksson M, Tegelberg Å, Thor A, Gordh T. Methodological comparison between salivary and plasma cytokines in third molar surgery patients. (Submitted)

Contents

Abbreviations

AE	Adverse Event
Alb	Albumin
ASA	American Society of Anaesthesiologists classification
BBB	Blood-brain barrier
BMI	Body mass index
BP	Blood pressure
CCGF	Cytokines, chemokines, and growth factors
CLCF1	Cardiotrophin-like cytokine factor 1
CNS	Central Nervous System
CNTF	Ciliary neurotrophic factor
CO	Cardiac output
CPSP	Chronic Postsurgical Pain
CPT	Cold-pressor tolerance
Crea	Creatinine
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CST5	Salivary cystatin D
CT1	Creatine transporter-1
DBP	Diastolic Blood Pressure
DHNK	Dehydroxynorketamine
DRG	Dorsal root ganglion
ECMO	Extracorporeal Membrane Oxygenation
ECT	Electroconvulsive therapy
EDTA	Ethylenediaminetetraacetic acid
EVF	Erytrocyte volume fraction
EudraCT	European Union drug regulating authorities clinical trials database
FasL	Fas Ligand
FGF-21	Fibroblast growth factor-21
FLACC	Face, Legs, Activity, Cry, Consolability
Flt3L	FMS-related tyrosine kinase 3 ligand
GABA	Gamma-aminobutyric acid
GCP	Good clinical practice
GDPR	General data protection regulation
HADS	Hospital Anxiety and Depression Scale
HNK	Hydroxynorketamine

hsCRP	High-sensitivity C-reactive protein
IASP	International Association for the Study of Pain
ICU	Intensive Care Unit
IFN-gamma	Interferon-gamma
iGluRs	Ionotropic glutamate receptors
IV	Intravenous
JIA	Juvenile idiopathic arthritis
kD	Kilo Dalton
LHb	Lateral Habenula
LIF	Leukemia inhibitory factor
LOD	Limit of detection
MCID	Minimal clinically important difference
MDD	Major Depression Disorder
MEC	Minimal efficacy criteria
MSAE	Medical Serious Adverse Events
NMDA	N-methyl-D-aspartic acid
NMDAR	N-methyl-D-aspartic acid receptor
NNH	Number needed to harm
NNT	Number needed to treat
NPX	Normalized protein expression
NS	Not significant
NSAID	Non-Steroidal Anti-Inflammatory Drugs
NRS	Numeric Rating Scale
OFA	Opioid-free anaesthesia
OSM	Oncostatin M
PCA	Patient Controlled Analgesia
PCR	Polymerase chain reaction
PDGF	Platelet derived growth factor
PEA	Proximity extension assay
PI	Pain Intensity
Plt	Platelet count
PNS	Peripheral Nervous System
PONV	Postoperative Nausea and Vomiting
PPT	Pressure pain tolerance
PRO	Patient Reported Outcome
RCT	Randomized controlled trial
S	Significant
SBP	Systolic Blood Pressure
SD	Standard deviation
SPID	Sum of Pain Intensity Difference
SNRI	Selective Serotonin-Noradrenalin-Reuptake Inhibitor
SSRI	Selective Serotonin Reuptake Inhibitor
SV	Stroke volume
TGF-alpha	Transforming growth factor-alpha

TNF-alpha	Tumour necrosis factor alpha
TOTPAR	Total pain relief
VAS	Visual Analogue Scale
VEGFA	Vascular endothelial growth factor A
VRS	Verbal Rating Scale
WBC	White blood cell count

Introduction

The International Association for the Study of Pain (IASP) defines pain as "An unpleasant sensory and emotional experience associated with, or resembling that associated with, actual or potential tissue damage". Six additional key notes expand this definition (1):

- Pain is always a personal experience that is influenced to varying degrees by biological, psychological, and social factors.
- Pain and nociception are different phenomena. Pain cannot be inferred solely from activity in sensory neurons.
- Through their life experiences, individuals learn the concept of pain.
- A person's report of an experience as pain should be respected.
- Although pain usually serves an adaptive role, it may have adverse effects on function and social and psychological well-being.
- Verbal description is only one of several behaviours to express pain; inability to communicate does not negate the possibility that a human or a nonhuman animal experiences pain.

By this definition pain cannot be measured or categorized by an external bystander only, but with the help of the patient´s history. According to these key points, the study of pain is in many ways more complex than other medical or surgical phenomena. To process different aspects of pain one can find help in the bio-psycho-social model, first presented by Engel in 1977 (2). This model is especially well suited for theorizing about the core of chronic pain and why both acute and chronic pain is perceived differently in different individuals. Nevertheless, pain, whether short or long-term, is a significant part of every human´s life. Pain needs to be scientifically studied to understand the underlying pain generating mechanisms and be able to ease and treat pain when possible. A variety of pain models are used in the study of pain. One such well-established model for acute pain is lower third molar surgery (or wisdom tooth surgery) (3-6). Some of the advantages with this model are the large number of subjects, as it is a common procedure (7); the procedure is fairly standardized; and the fact that these patients are typically relatively young, relatively healthy and often free from ongoing pharmacological treatments - all factors contributing to reduced variability. In addition, this procedure involves both soft- and hard- tissue and is therefore often associated with tem-

porary local morbidity, such as signs of local inflammation, trismus, and postoperative pain of light to severe intensity. Besides that, impacted lower third molars are a common clinical complaint, due to local inflammation and pain that has to be surgically addressed. The definition of an impacted tooth is when other teeth, overlying bone, or soft tissue interfere with the eruption of a tooth into its normal position and occlusion; it may also be considered impacted when a third molar is not fully erupted by the approximate age of 20, which is the expected age of eruption (8). The third molar is a commonly impacted tooth with an incidence of 18-32% (8).

In Sweden, 20000-25000 third molars are surgically removed annually (9). Most of these procedures are performed under local anaesthesia. A minority of patients receive sedation, most often through administration of a benzodiazepine, such as midazolam or diazepam. Most patients experience postoperative pain of moderate to severe intensity. The pain intensity is highest during the day of surgery (10, 11). This pain usually peaks at five hours postoperatively (12), but then gradually fades out over several days or up to a couple of weeks (13-15). This acute pain responds well, in a majority of the cases, to a combination of ibuprofen and paracetamol (16). The time when the pain peaks coincides, unfortunately, with the wearing off of the local anaesthetic effect. This study aims to optimize early postoperative pain management and especially the transition from local anaesthesia to orally administrated analgesic (Figure 1). In patients where the combination of ibuprofen and paracetamol is not a sufficient strategy for pain management, more powerful supplementary analgesia may be necessary (17). Traditionally opioids have been used for this purpose, and are usually effective, but opioids are associated with some unwanted effects (18, 19). This may be problematic especially in day case surgery, when the patient is supposed to leave the hospital on the day of surgery and manage without professional care or supervision. In a previous single-blind RCT study, we explored whether the weak opioid tramadol could fit this purpose, but at the dose studied, 1 mg/kg iv, was not sufficient (20). Thus, we decided to search for other pharmacological solutions to our clinical inquiry.

The trend in day case surgery is to choose opioid free analgesia (21-24). One opioid free alternative is ketamine, which was developed and described by Domino et al., in 1965 (25-27). Ketamine was initially developed for general anaesthesia but has since been found to act as an analgesic when used in lower doses (17). Ketamine for analgesia in oral and maxillofacial surgery is only described in small studies with divergent methods, doses, and routes of administration (28-32). Ketamine in its original composition is a racemic mixture of mirror enantiomers, S-ketamine, and R-ketamine. Later the S-form has been commercially available as S-ketamine or esketamine. S-ketamine has

some advantages in relation to racemic ketamine, such as higher relative potency and fewer side effects (33-35). To our knowledge, there is no previous publication on S-ketamine as an analgesia in Oral and Maxillofacial Surgery.

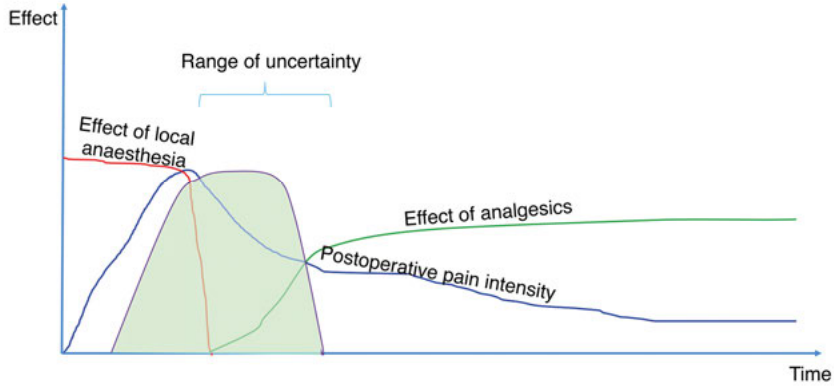


Figure 1. A schematic depiction of the illustration of the clinical and scientific challenge.

A brief history of dentistry

The very first known evidence of an impacted third molar is in the lower jaw of the so called Magdalenian girl, who lived during the Upper Palaeolithic age 18000-10000 BC, and was found in a cave in Cap Blanc, in the Dordogne region of France (8, 36). A 13000-14000 year old finding from northern Italy revealed that a cavity in an infected tooth had been carved out and filled with bitumen (37). The first evidence of dental fillings with beeswax was found in Slovenia and dates back to 6500 years ago (37). Archaeological findings from the neolithic era in Pakistan from 7500-9000 years ago provide evidence of in vivo drilling in the occlusal surface of maxillary second molars (38); Mehrgarh in Baluchistan is sited along the main route connecting the Indus valley and Afghanistan, which was at that time populated by hunters-gatherers who were in the process of converting to the cultivation of cotton, wheat, barley, and cattle breeding (38). The tooth drilling is believed to have been carried out using flint, bone, shells or turquoise (7, 38). The first evidence of a dental surgical process to relieve pain of an abscess is a mandible from c2500 BC, with drilled holes near the roots of the first molar (37). The first tooth extraction was probably performed by Hippocrates (c.460-c.370 BC) using an instrument called *plumbeum odontogagon* (7). Aristotle (384-322 BC) developed the practice and used a forceps-like instrument to more easily move or luxate the tooth (7). The oldest known extraction forceps were found in Greece

and date back to the 5th century BC (39). It is believed that the first person to propose to detach the gingiva from the bone was Aulus Cornelius Celsus (c.25 BC-c.50AD) (7). Celsus also described a narcotic potion to treat postoperative pain (7). In addition, Celsus treated jaw fractures and drained dental abscesses with incision (40). The Middle Ages did not include any further developments in the field. During the late 1700s, however, an Englishman, Walter Harris expressed the opinion that incisions should be used for difficult extractions (7). At the end of the 1700s, techniques to extract third molars also gained attention (7). A method for removing third molars took form after dental surgeons who had developed their approaches began sharing their ideas (7). This development is thought to have taken place in Germany and became a standard practice in Europe and North America during the 1800s (7). In 1903, the American National Dental Association published a manual for the extraction of third molars (7), and in 1918, Charles Edmund Kells (1856-1928) described a more humane approach to the removal of third molars in a paper, in *Dental Cosmos* (7). George B. Winter (1878-1940), a professor at Washington University School of Dentistry, published *Principles of exodontia as applied to the impacted mandibular third molar* in 1926 (7). The first written record of using facial bandages to manage mandible fractures represents the origin of oral and maxillofacial surgery, which dates back to ancient Egypt (2700 BC), and is known as the Edwin Smith Papyrus (40, 41). This document contains 48 cases describing the treatment of mandibular fractures by a military surgeon, who used egg and honey soaked bandages (40). Much later, Hippocrates treated jaw fractures with interdental wires rather than facial bandages (37, 41). Hippocrates also described the repositioning of a luxated jaw by applying pressure on the external oblique ridge, which is similar to the technique used today (41). The first textbook on oral surgery was written in 1778 by Anselme Jourdain, a French surgeon (40). The American Society of Exodontists established in 1918, became the American Association of Oral and Maxillofacial Surgeons (AAOMS) in 1978, after three previous changes of the name (41). Facial traumas from the trench warfare during World War I (WWI) significantly influenced the scope of the oral and maxillofacial surgery as a speciality (41). The influx of facial traumas also required more than frontline responders, and so many nations developed maxillofacial injury units (41). The first to form such a unit in the British Army was Sir Harold Gillies (41). Gillies introduced several methods to the practice of reconstructive surgery, most notably the tube pedicle flap to close large soft tissue defects (41). Gillies also described the method for repositioning a fractured zygomatic arch - a method still in use (41). It is thus perhaps unsurprising that Hippocrates stated, “War is the only proper school for a surgeon” (40).

Dentistry´s historical contribution to anaesthesiology

Before the introduction of ether, there had been several unsuccessful attempts to develop general anaesthetics by using herbal substances such as opium (41). Nitrous oxide (N_2O) was first synthesised by Joseph Priestly in 1772 (42). Humprey Davy later observed the chemical properties of the gas and published his findings in a book "*Researches, chemical and philosophical; chiefly concerning nitrous oxide: or dephlogisticated nitrous air and its respiration*" 1800 (42). A milestone in the history of medicine was the discovery of anaesthesia by Horace Wells, a dentist, in 1844 (42). Wells had one of his own molars extracted under N_2O anaesthesia to prove his discovery (42). Stanislav Sigismund Klikovich (1853-1910), who was born in Poland and studied medicine in St Petersburg, graduated in 1876 with a diploma in medicine and surgery and went on to study the use of nitrous oxide (43). His studies of nitrous oxide were what led to him being awarded an MD on 23 April 1881 (43). His findings led to the introduction of the concept of using a mixture of oxygen and nitrous oxide to provide analgesia without loss of consciousness, and thus avoiding the risk of hypoxia (43).

William Morton, a dentist, was the first to publicly demonstrate the use of dietyl ether for a dental extraction, at Massachusetts General hospital, in 1846 (41, 44). A few weeks later, the ether method was once again successfully used during the surgical removal of a benign tumour of the neck (41).

Pain

Acute vs chronic pain

Acute pain has an obvious evolutional value to warn the organism to avoid harmful conditions (45). The pain sensation or rather the nociception enables an organism to localise and swiftly withdraw from a painful and potentially harmful stimulus (45). Chronic pain is pain that lasts for more than 3 months, and in some cases chronic pain even exceeds the time it takes for the healing of acute tissue damage. Chronic pain is often described as a disease of its own, though the function is more obscure, and the treatment regimens and prognosis differ distinctly between acute and chronic pain. A non-negligible cause of chronic pain is surgery, resulting in chronical postsurgical pain (CPSP). Some procedures include more risk than others. For example, thoracic and breast surgeries have a 20-50% risk of CPSP, while amputation has an 80% risk (46-48).

To measure and compare pain

Due to its nature, pain intensity cannot be measured in an objective, observational manner (1). Nevertheless, it is of great value in research to be able to

compare pain in some way. One of the most common ways of measuring and comparing pain intensity is obtained via self-reported pain, expressed on some scale of continuous, interval or nominal type, such as the visual analogues scale (VAS) (49), numeric rating scale (NRS), or verbal rating scale (VRS) (50). To measure indirect or surrogate factors is also common in pain research; common examples include consumption of analgesics (type and amount), frequency of sick leave, catastrophizing, physical activity or fear/avoidance of physical activity, and experiences of well-being. Other outcome measures can be used such as, number needed to treat (NNT), total pain relief (TOTPAR), number needed to harm (NNH), summed pain intensity difference (SPID), or minimal efficacy criteria (MEC) (50-52). Every measurement of pain experience must consider other factors such as dementia or cognitive impairment (53). The initiative on methods, measurements, and pain assessment in clinical trials (IMMPACT) recommend six core domains (53, 54):

1. Pain
2. Physical functioning
3. Emotional functioning
4. Patient rating of improvement and satisfaction with treatment
5. Other symptoms and adverse events during treatment
6. Patient's disposition and characteristics data.

Physiology and nociception

The physiology of pain is inherently complex, since various events are detected in the periphery by different types of nociceptors involved in somatic functions (55). The nociceptors also localise the event while sending the information to more central parts of the nervous system through afferent pathways; the perception of pain sensation occurs as a strict psychological sensation in the brain (56). The nervous system has its own mechanisms to regulate sensitivity to pain at different levels (56), which also makes it possible to influence the system through various drugs, medicines, and chemicals. Lastly, the pain system is sensitive to various psychological factors such as anxiety and depression. Certain parts of the brain are involved in both pain processing and mood regulation, such as prefrontal cortex (PFC), anterior cingulate cortex (ACC), hippocampus, thalamus, and amygdala (57, 58). These areas are physically connected and show altered activity in both pain and depression (57). Furthermore, there seems to be psychological and inflammatory connections between pain and depression (59-61) (Figure 2 & 3).

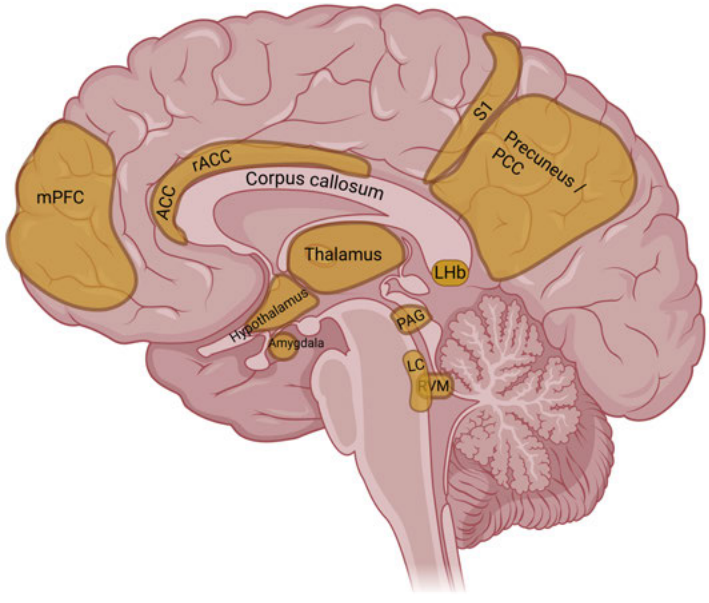


Figure 2. Regions of the brain associated with perception and modulation of pain signals. Medial aspect. Created with BioRender.com.

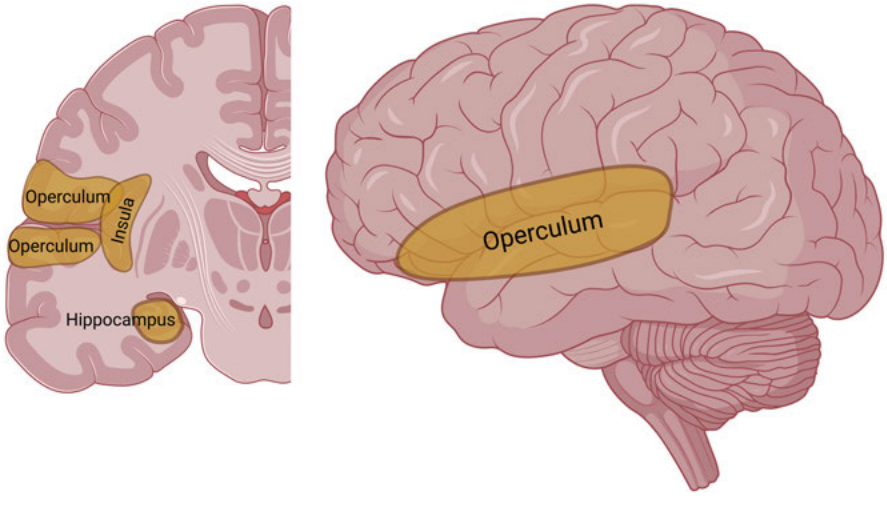


Figure 3. Regions of the brain associated with perception and modulation of pain signals. Frontal and lateral aspect. Created with BioRender.com.

Nociception is the physiological process that enables the detection and localisation of noxious stimuli (62, 63). Pain is thus the conscious perception. These two are often parts of a common process, but they can also exist independently of one another.

The dental pulp is an example of a peripheral end organ. The pulp is innervated predominantly by unmyelinated C-fibres (80-90%), thin myelinated A-delta fibres, and a small part of thickly myelinated A-beta fibres (64, 65).

The endogenous pain inhibitory system is located at several places in CNS, but mainly at the rostral ventromedial medulla (RVM), locus coeruleus (LC), rostral anterior cingulate cortex (rACC), spinal dorsal horn, amygdala and midbrain periacueductal grey (PAG). These are all parts of the endogenous descending inhibitory pathway. These effects are mediated by restoring connectivity in prelimbic-prefrontal cortex or supressing activity in the ACC (66).

Nociceptors transmit pain signals via the dorsal root ganglion (DRG) to the dorsal root of the spinal cord and then upward to the somatosensory cortex where the pain sensation is formed (67). The organisation of the cranial nerves, such as the trigeminal nerve, is different. The trigeminal nerve afferent impulses, from the facial and dural sensitivity, reaches the trigeminal division to the trigeminal ganglion, nuclei, thalamus and somatosensory cortex (68). The conscious pain experience is believed to be a result of cooperation between operculoinsular complex (OIC), ACC, prefrontal cortex (PFC), amygdala, and S1(56) (Figure 2 & 3).

Experimental pain research

In the research of specific physiological processes, it might suit the aim better to use an experimental pain model than a clinical one. In other words, these methods are provided to give a more complete exposition of possible models. Most of these experimental methods focus on inducing cutaneous pain. These include electrical (transcutaneous or intra cutaneous), thermal/heat (radiant or contact thermode), thermal/cold (ice water or contact thermode), mechanical (pin prick or pressure or impact stimuli), and chemical (capsaicin or mustard oil) forms of inducing cutaneous pain (69). Intramuscular infusion of hypertonic saline can also generate experimental pain (70). The experimental pain models give the researcher control over the stimuli that can be reproduced from patient to patient and from time to time. Nevertheless, the pain experience and the patient-reported pain intensity is still personal and subjective.

Clinical pain research

To study different aspects of pain and pain management, including pharmacological trials, the use of established pain models is common. A few models are more common than others. Namely, joint replacement, dental extraction, bunionectomy, and soft tissue surgery (71). When choosing a model for clinical pain research, Singla et al. have published a list of seven desirable qualities for such a model (71):

- Produce a homogeneous and predictable pattern of pain in a diverse population
- Allow a range of experimental manipulations that reliably alter the postoperative pain experience in order to meet the needs of the experiment
- Closely match the clinical environment in which the agent will ultimately be administered
- Include volunteers whose demographics are a representative sample of the target clinical population
- Produce pain via a well-understood physiological process
- Be common enough so that recruitment can be achieved in a reasonable time frame
- Allow state-of-the-art care to be provided in a manner that minimizes analgesic cofounders.

Placebo vs Active comparator

To evaluate the effect of an intervention it is necessary to work with a comparison. Depending on the phenomenon being studied, different comparisons can be suitable or ethically varied. In cases where a new pharmacological treatment is studied and when there already exists an effective treatment, the new drug is supposed to be compared to the best available treatment or drug (72). An active comparator is a pre-existing treatment to which the new treatment is compared. In a clinical trial placebos are control treatments with a similar appearance as the study treatment, but without its specific activity (73). When a new analgesic compound with unknown effects and side effects is studied, the use of two active comparators is preferable, one opioid and one NSAID. This is to provide two different comparators with a different AE profile (50).

A placebo is an inactive or inert comparator and can be used when there is no effective comparator (72) - placebo (lat. *I will please*) (74). In 1955, Beecher wrote, “It does not matter in the least what the placebo is made of or how much is used so long as it is not detected as a placebo by the subject or the observer” (75). He also wrote that “From work on postoperative pain… it appears that placebos can relieve pain arising from physiological cause” (75).

Beecher also stated that “Many ‘effective’ drugs have power only a little greater than that of a placebo” (75).

In a Cochrane review, it was concluded that placebo intervention in general did not have any important clinical effects, but in certain settings such as pain and nausea, placebo interventions can affect the patient-reported outcome (76). The effect of placebo on acute pain often resembles the response to analgesic, including a similar peak effect after 1-2 hours (74).

Third molar surgery - a model for pain research

Regarding third molar surgery “…there is a common misperception that the postoperative pain course is mild, and as such the experimental model is appropriate only for low-potency oral analgesics” (5, 71). Third molar surgery is a common model that has been used for more than 50 years, and is typically used for studying the effects of various investigational drugs intended for pain treatment (3, 51, 71, 77, 78). Third molar surgery causes moderate to severe pain (52), and the surgical protocol can be adjusted so that the postoperative pain becomes intense enough to be able to evaluate strong intravenous analgesics (71). Modification can be achieved by choosing to remove a number of third molars at the same time, by anatomical position, and by the degree of impaction (71). The enrolment rate for third-molar-surgery-model studies is typically faster than the same rate for joint replacement, bunionectomy, or soft tissue surgery studies, among others (71).

Third molar surgery

Third molar surgery as it is known and practiced today was developed during the 1800s and the first quarter of the 1900s (7). The reasons for removing a third molar in a Swedish context are often local pathology or recurring complaints such as pain or swelling due to local infection/inflammation. In the United Kingdom (UK), National Institute for Health and Care Excellence (NICE) guidelines introduced in 2000 describe the clinical indications for the removal of third molar teeth (79).

The NICE guidelines consists of the following points (79):

- Unrestorable caries
- Non-treatable pulpal and/or periapical pathology
- Cellulitis, abscess and osteomyelitis
- Internal/external resorption of the tooth or adjacent teeth
- Fracture of tooth
- Disease of follicle including cyst/tumour
- Tooth/teeth impending surgery of reconstructive jaw surgery and when a tooth is involved in or within the field of tumour resection.

Both dental practitioners and oral surgeons are now more prone to base their decision on whether to remove mandibular third molars, on clinical factors (80). In the past, prophylactic removal of third molars was more common (80).

Perioperative pain management

By perioperative pain, one usually means pain resulting from surgical or other procedures. However, it can also involve strategies to manage pre-existing pain conditions such as pain caused by an acute injury or chronic pain related or unrelated to the current procedure.

Perioperative sedation

The use of sedation is common and widespread among most medical specialities, either as a standalone solution to enable a patient to undergo surgical or medical treatment, or for examination. It may also be a part of a more complex pharmacological regimen that includes analgesics (see above) or other agents.

Perioperative sedation can furthermore be used as preparation before an imminent anaesthesia.

Ketamine

History

CI-581 was first synthesized by Calvin Stevens at Parke-Davis and Co. in 1962 (81). and the first human test took place on August 3, 1964. Male inmate volunteers at the Jackson Prison in the State of Michigan were given CI-51 intravenously (26, 27). CI-51 later become ketamine, a drug with analgesic and anaesthetic properties. Ketamine is primarily a general anaesthetic agent but is also widely used for analgesic purposes (17). Ketalar® (Pfizer AB) was approved for the Swedish market in March 1973 (82). Ketamine is described as a unique drug, though it combines hypnotic, analgesic, and amnestic properties in the same drug (83).

Chemistry

Racemic mix

Ketamine is a 2-(2-chlorophenyl)-2-(methylamino) cyclohexanone with a molecular weight of 274.4 (48, 81); $C_{13}H_{16}ClNO$ is the chemical formula (81). Ketamine is a racemic mixture of the two enantiomers, S(+) ketamine and R(-) ketamine (48, 84). The difference is one asymmetric carbon atom that leads to two different enantiomers (48, 85). The analgesic effect is related to the relative strength of affinity when binding to the NMDA receptor at the phencyclidine (PCP) site. Both S(+) ketamine and R(-) ketamine reduced pain in an experimental ischemic pain model; however, S(+) ketamine was found to be more effective (85).

S-enantiomer

When comparing the racemic mix to the s-enantiomer of ketamine, S-ketamine was found to give a significant increase in systolic and diastolic blood pressure for the first 15 minutes (86). The s-form of ketamine (Ketanest-S®, Pfizer AB) was approved in Sweden, in May 2000. The more potent S(+) enantiomer gave a more rapid recovery compared to the racemic ketamine, when psychomotor skills were evaluated (84). S-ketamine is known to have shorter sedation time and fewer adverse events than racemic ketamine (17, 86). Due

to its superior potency, the S(+) enantiomer was found to be more clinically advantageous, compared to the racemic ketamine (84). The analgesic effect is approximately four times more potent in S(+) ketamine than R(-) ketamine (81, 85).

R-enantiomer

A study of five volunteers who on separate occasions received anaesthetic doses of racemic ketamine, S(+) ketamine, and R(-) ketamine, revealed that R(-) ketamine had less impact on electroencephalographic (EEG) registrations (84). R(-) ketamine gave less slowing overall. No large slow-wave complexes were produced by the racemic ketamine or the S(+) ketamine (84). R-ketamine has more of an antidepressant effect at a lower dose than S-ketamine (87). R-ketamine produces less sedation, dissociative effects, and abuse potential than S-ketamine (88). R-ketamine has a milder side-effect profile than S-ketamine (88).

Pharmacokinetics

Both isomers, R(-) and S(+)-ketamine, undergo extensive metabolism with active metabolites (89) (Table 1). Ketamine has three main metabolites - norketamine, hydroxynorketamine (HNK), dehydroxynorketamine (DHNK) - and is metabolized in the liver by cytochrome enzymes (CYP) (48, 66, 87). Norketamine is the dominant metabolite, and it is active (48). CYP3A, CYP2B6, and CYP2C9 are all involved in the ketamine metabolism (48, 83). The metabolites of ketamine are excreted in urine and bile (48, 90). S-ketamine is demethylated 20% faster than R-ketamine and 10% faster than racemic ketamine (89). The faster demethylation of S-ketamine is probably related to S-ketamine's superior affinity to the CYP3A4 system (89, 91). In a recent study on pigs, 30 new ketamine metabolites have been identified, which were found to be distributed unevenly across the brain due to regional differences (92). These findings provide a foundation for further studies of ketamine metabolism and its neurological effects. Interaction with the cytochrome P450 system can influence the metabolism of ketamine. Inhibition of CYP3A mediated metabolism caused by grapefruit juice or clarithromycin gives increased levels of S-ketamine (48). On the other hand, the CYP3A mediated metabolism can be induced by rifampicin or St John´s wort, leading to a reduced plasma concentration of S-ketamine (48).

Table 1. Comparison of pharmacokinetic properties for ketamine enantiomers and racemic mixture.

	Pharmacokinetic variables of ketamine enantiomers (mean±SE)		
	Racemic	S(+) ketamine	R(-) ketamine
T½ γ [min]	196±73	146±33	137±30
NMDA-receptor affinity		++++	+
Opioid-receptor potency (μ, Ƙ, δ)		++(+)	+
Analgesic potency	++	++++	+
Protein binding [%]	50%	50%	50%
VSS [L/kg]	2.18±0.81	2.7±0.68	1.34±0.71
CL [ml/kg/min]	14.8±1.7	26.3±3.5	13.8±1.3

T½γ: terminal half-life, V_{ss}: volume of distribution at steady state, CL: elimination clearance (27, 89, 93, 94).

Pharmacodynamics

Ketamine is a non-competitive antagonist of the NMDA-receptors (N-methyl-D-aspartic acid receptor) (48, 95). In addition to blocking NMDA receptors, ketamine seems to partly exert its effect via the opioid and monoaminergic systems (66). “Dissociative anaesthesia” is the characteristic effect of ketamine that is not hypnotic or sedative but amnesic, unconscious, and analgesic (81). Neuronal and extra-neuronal catecholamine reuptake is especially inhibited by S-ketamine, causing a hyper adrenergic state that raises the concentration of circulating norepinephrine (48). Ketamine binds to the phencyclidine site of the NMDA-receptor and thereby prevents calcium ions flowing through the canal (48, 66, 90) (Figure 4). In contrast to many anaesthetics, ketamine maintains or increases heartrate and cardiac output, and even increases blood pressure due to its sympathetic stimulation (48). In end-stage heart disease, there is a risk that ketamine decreases the myocardial contractility through its decreasing effect on beta-stimulation (48). Ketamine leads to bronchodilation through an inhibitory effect on muscarinic receptors, while maintaining protective reflexes (pharynx and larynx) and causing no respiratory depression (48, 66, 81, 83, 96). The R(-) ketamine is more potent in relaxing airway smooth muscle contraction induced by acetylcholine (89, 97). This is in line with clinical experience that racemic ketamine more effectively counteracts bronchoconstriction and severe asthmatic attacks, compared to s-ketamine (89). Ketamine maintains the functional residual capacity (FRC), thus decreasing the risk of perioperative hypoxia (98).

The NMDA-receptor

Glutamate mediates excitatory synaptic transmission in the CNS (99). The ionotropic glutamate receptors (iGluRs) are activated when glutamate binds to it (99). The iGluRs are composed of four subunits around a central ion channel in the membrane of nerve cells in the central nervous system. There are four classes of iGluRs: one of them is the NMDA-receptor (NMDAR), and the others are AMPAR, kainite receptors, and delta (δ). There are several subtypes of the NMDAR with a variety of properties (99, 100). When activated, it opens the ion channel for an influx of Ca^{2+} ions (100). NMDA-receptors are mainly located in the CNS. Peripherally located NMDA-receptors are believed to contribute to pathological pain and itch (101). Ketamine´s anaesthetic and analgesic effects are induced by its affinity to the NMDA-receptor (Table 2), but ketamine also targets other receptors with a lower affinity, for example GABA, serotonin, dopamine, sigma, cholinergic, voltage-gated sodium, and opioid (87).

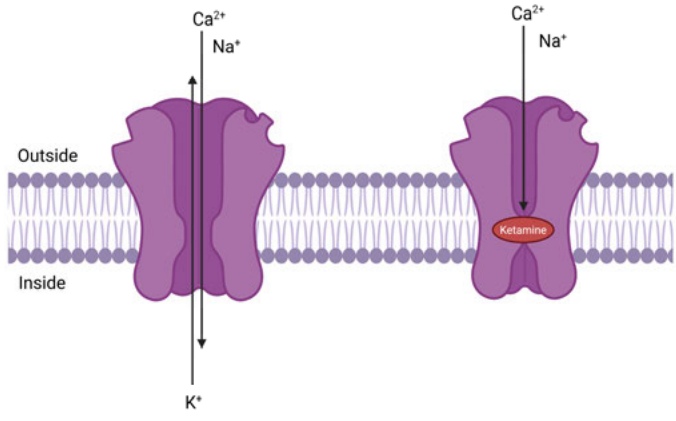


Figure 4. Ketamine´s antagonistic action on the NMDA-receptor blocking the ion channel. Created with BioRender.com.

Table 2. The effect of different substances on the NMDA-receptor (95).

NMDA receptor antagonists	Mechanism
Ketamine S-ketamine R-ketamine Dextromethorphan Dextromethadone/Esmethadone Methadone Nitrous oxide Lanicemine Riluzole Memantine	Uncompetitive antagonism
NMDA receptor agonists	**Mechanism**
Sarcosine	Agonist
D-cycloserine	Partial agonist

Anaesthesia properties

Ketamine is listed by the World Health Organization (WHO) as an essential drug for anaesthesia due to its ability to provide analgesia and anaesthesia without cardiorespiratory depression (48, 89).

Analgesia properties

In sub-anaesthetic doses ketamine can be used to manage pain during procedures such as suturing, burn-wound care, reposition of dislocated joints, and repositioning of fractures (83). Even in conscious patients, ketamine can produce a dreamlike state with hallucinations as an unpleasant side effect. However, benzodiazepines such as diazepam, or the more effective midazolam or propofol, can reduce that risk (26). Benzodiazepines such as midazolam reduce the psychotomimetic adverse effects of S-ketamine and R-ketamine (17). Ketamine has been used for treatment of neuropathic pain (102) and cancer pain (17).

Clinical use

The dissociative anaesthesia

Ketamine anaesthesia is different from anaesthesia induced by other injectable agents, such as opioids, propofol, pentothal, or gases for inhalation, such as N_2O, isoflurane, or sevoflurane. Ketamine anaesthesia induces a dissociative, dreamlike state, where body movements may occur and the patient may have open eyes despite being deeply anaesthetised (103). To induce anaesthesia

using S-ketamine intravenously, a dose of 0.5-2.5 mg/kg is needed. A similar dose 0.5-2.5 mg/kg/h is required to maintain the anaesthesia using S-ketamine. When given intramuscularly, (IM) 4.0-8.0mg/kg S-ketamine is required to induce anaesthesia (89). Other authors have stated that the recommended dose to induce anaesthesia is 0.5 to 1 mg/kg IV or 2 to 4 mg/kg intramuscular IM (48, 104).

Prehospital and battlefield anaesthesia and analgesia

Due to its strong analgesic effect along with its minor impact on airway and respiration, ketamine is especially suited for trauma, prehospital care, and in circulatory unstable patients (105-107). The preservation of protective airway reflexes and absence of respiratory depression despite full dissociation add to its positive properties (48, 96). Ketamine´s sympathomimetic effects lead to tachycardia, an increase in blood pressure, and preserved cardiac output (48, 108-110). From experimental laboratory data Watso et al. stated that morphine reduces tolerance to haemorrhage compared to ketamine or fentanyl and therefore morphine should not be used for an individual who is haemorrhaging in a prehospital context (107). In a prehospital or battlefield setting, a single drug regimen is preferred to adding a benzodiazepine. The simplicity of application, moreover, reduces the risk of adverse events (AE) related to polypharmacy. Ultimately, it is safer to give more ketamine instead of adding another drug, if the analgesic effect is insufficient (111, 112). A 2020 systematic review on the subject of ketamine use on the battlefield concluded that "all studies tend to strengthen the belief in the efficacy and safety of ketamine when given at 50-mg to 100-mg intravenous for prehospital analgesia in combat casualties" (113). Others have reached similar conclusions (112).

Vascular catastrophes

By maintaining blood pressure and heartrate, ketamine counteracts the negative physiological consequences of bleeding, unlike other anaesthetic drugs, which usually increase the negative consequences of bleeding (48, 83, 107). Ketamine raises the arterial blood pressure and cardiac output, and decreases the risk of perioperative hypoxia, by maintaining the functional residual capacity (FRC) (98). In a study on rats (Sprague-Dawley, 11-13 weeks), it was found that ketamine preserves but fentanyl impairs both respiration and the peripheral vasoconstriction response due to haemorrhage (106). The study also states that the findings supported the use of ketamine in prehospital settings, in contrast with fentanyl (106). Ketamine bolus is associated with higher cardiac output (CO) and stroke volume (SV) compared to fentanyl bolus in septic shock patients (114).

Post operative pain management and analgesia

Inadequate management of postoperative pain increases the risk for postoperative complications such as chronic postsurgical pain (17, 47, 115) The mechanism of ketamine´s effect on chronic pain is unclear. Perioperative ketamine IV reduces opioid consumption by 8 mg equivalents for 24 hours (17). In a large systematic review from 2018, perioperative ketamine was compared to placebo or basic analgesic regimen without ketamine, in a non-stratified study population (17). The systematic review (n=2449) revealed that the participants receiving ketamine consumed 12.6 mg less of morphine equivalent during the first 48 hours (17). During the first 24 hours (n=5004) pain intensity at rest measured on VAS was 5 mm less in ketamine than in the control group. During movement, the corresponding difference was 6 mm (n=1806) (17). Ketamine has shown short-term positive effects on migraine, neuropathic pain, ischemic pain, fibromyalgia, temporomandibular, and whiplash pain (48, 90, 116). In a study of patients undergoing transthoracic lung and heart surgery, the patients received postoperative analgesia using intravenous patient-controlled analgesia (IV-PCA) (117). The morphine only group (MO) were given 1.5 mg morphine bolus while the morphine + ketamine group (MK) had 1mg morphine + 5mg ketamine bolus, both with seven minutes lockout time (117). Similar findings are reported in a study on major abdominal surgery where the addition of ketamine in the postoperative pain management regime gave a decrease in morphine consumption, resulted in fewer side effects of morphine such as PONV, and the low dose ketamine was not associated with any psychomimetic effects (118). Ketamine´s analgesic effect as well as adverse events are dose-related, but the optimal dose or route of administration is yet unknown (17). Patients going through major lumbar fusion surgery were randomized to infusion 0.12 mg/kg/h, 0.6 mg/kg/h, or placebo. The authors of this study concluded that intraoperative S-ketamine did not reduce postoperative oxycodone consumption (119). A set of American consensus guidelines states that the common subanesthetic dose of S-ketamine used in clinical practice is IV 0.3-0.5 mg/kg bolus with or without an infusion (120). For analgesia, 0.125-0.25mg/kg is a “helpful” range (81). In a patient-controlled analgesia (PCA) study, combining oxycodone and S-ketamine at a ratio of 1:0.75, it showed a reduced cumulative opioid consumption over 24 hours compared to groups with lower relative S-ketamine dose (121).

Chronic pain

S-ketamine has been examined in Complex Regional Pain Syndrome type1 (CRPS-1) patients, in combination with an acute experimental thermic pain (122). In doses too low to influence the acute pain, S-ketamine still showed an effect on chronic pain, suggesting that desensitization of NMDA-receptors in the spinal cord or increased inhibitory sensory control in the brain may be

involved (122). In the case of repetitive stimulation of spinal C-fibers, NMDA-receptors will enhance the spontaneous activity of the fibers and receptive fields (48). This can be blocked by ketamine and thereby may prevent acute pain from becoming chronic (48).

Depression

In 2000, the first RCT was published which showed that ketamine can play a part in the treatment of major depression (123). Ketamine has shown to be a rapid antidepressant and may be useful in selected cases in the treatment of therapy resistant depressions (48, 83). In a non-inferiority study in 2023, the authors showed that ketamine is not inferior to ECT in treating therapy-resistant major depression without psychosis (124). A population with treatment resistant depression received S-ketamine low-dose infusion, on six occasions, with the result that the plasma-concentration of interleukin-6, cortisol, and tumour necrosis factor-alpha decreased, and the depression improved (125). Recent studies indicate that the ketamine effect on the lateral habenula (LHb) is an important mechanism in the antidepressant properties (126). LHb regulates reward behaviours and negative reward signals (126). Ketamine´s rapid antidepressant effects are thought to depend on its ability to block the NMDAR-dependant bursting activity in LHb (127) (Figure 2 & 3).

Recreational and abusive use

Ketamine vials produced and distributed for veterinary use were and still are exploited for recreational and abusive use. On the illegal drug market ketamine is found as K, Ket, Kit Kat, Kizzo, Special K, Super Acid, Vitamin K, Monkey Mix, Monkey Business, K-land, or K-hole (26, 128) (Figure 5). Street ketamine abuse may cause cystitis and contracted bladder (83). The damage appears to be caused by inflammation and nerve hyperplasia induced by the metabolites of ketamine, which then causes damage to the urothelium, covering the walls inside the bladder (129). In severe cases, renal damage occurs which may or may not be reversible, and could mean that patients in these cases need dialysis (83). A frequent side effect among ketamine abusers is upper gastrointestinal symptoms, most commonly epigastric pain (approximately 70% among abusers) (83, 130). Less common are urological and hepatic toxicity related to pain management but finally become the effects of long-term and recreational use (48, 131).

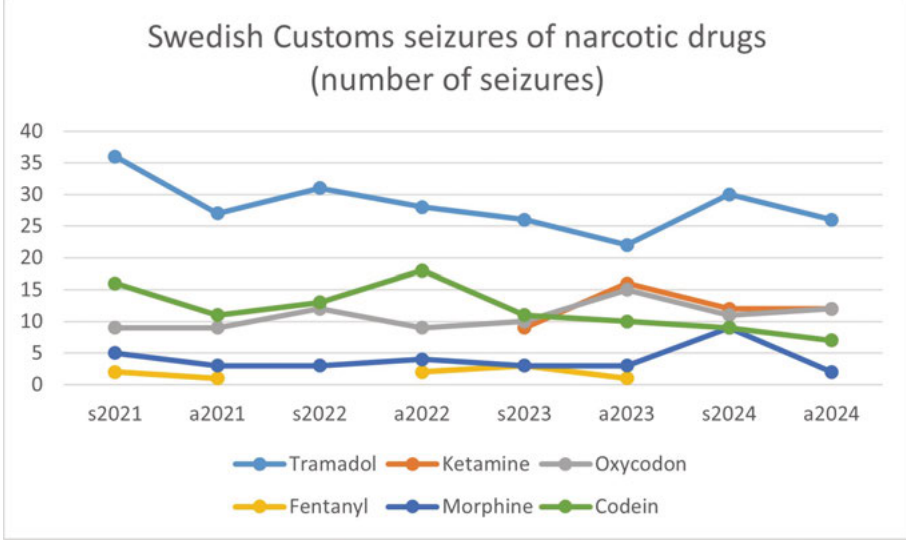


Figure 5. Public statistics from the Swedish Customs Service, showing some of the most common analgesics sized, and the number of seizures regardless of size (132).

Saliva – some physiological aspects

The purpose of saliva might not be obvious to everyone but to those who suffer from a lack or total absence of saliva, its purpose and importance is clear. The three most important functions of saliva are lubrication, protection, and digestion (133). Saliva has several other important specific functions, such as protecting mucous membranes and teeth from attack by microorganisms, initiating the digestion of food, as well as moistening and lubricating to enable swallowing. The salivary glands are exocrine glands that participate in the digestive system (134). Saliva also buffers organic acids formed by microorganisms in the biofilm and thus protects the enamel of the teeth from demineralisation (135). There are three buffer systems in saliva: the dominating one is the bicarbonate buffer; the phosphate and protein buffer systems are also present in saliva (136). The buffer capacity is dependent on salivary flow rate. The bicarbonate buffer has its peak capacity in stimulated saliva, while the phosphate buffer dominates in unstimulated saliva (137). Saliva is a mixture of secretion from the paired major salivary glands, sublingual glands, submental glands, and parotid glands, which together produce approximately 90% of the total volume of saliva (134, 138). There are also several minor salivary glands on the tongue and palate and in the labial and buccal mucosa. The minor glands produce a majority of the proteins found in saliva, 70%, though they contribute to only a small part of the volume. The average daily flow of whole saliva is 1-1.5L (139). The normal flow rate for unstimulated saliva is 0.3-0.5 ml/min; the flow rate of stimulated saliva is 1.0 -2.0ml/min. Salivary pH is normally slightly acidic, approximately 6-7. The pH is associated with a flow range from a low flow of pH 5.3 to a peak flow of pH 7.8. Saliva is not an ultra-filtrated plasma. When it is formed in the acini, saliva is isotonic, but it becomes hypotonic when moving through the ducts. The salivary glands are innervated by both parasympathetic and sympathetic nerves (139). The parasympathetic stimulation gives a more watery saliva while the sympathetic stimulation produces saliva that contains more proteins from the acinar cells. The salivary flow has a circadian (daily) variation with its lowest flow rate during sleep and peaks during high stimulation episodes, typically during the waking period (139). Secretion from the submandibular glands is a mixture of serous and mucous content; the sublingual glands are mainly mucous with a mucin-rich saliva, while the parotid glands are serous, producing a watery sa-

liva when stimulated. Parotid saliva contains enzymes such as lipase and amylase. The saliva/salivary fluid is composed almost entirely of water, but approximately 1% of the saliva includes electrolytes: sodium, potassium, magnesium, bicarbonate, phosphate, and calcium (133, 138, 140). Furthermore, saliva contains glucose, and nitrogenous: ammonia and urea. Several proteins are also found in saliva, for example immunoglobulins (IgA, IgG, IgM), antimicrobial factors, polypeptides, oligopeptides, glycoproteins, as well as traces of albumin (138, 140). Cytokines in saliva have different origins (141). They may be expressed by immune cells in the mouth or by the salivary glands. They may come from lymphoid cells in the oral mucosa or from the general circulation via serum that passes into the oral fluid. Cytokines found in saliva secreted from cells in the oral cavity are thought to be a part of the local immune processes (141).

The evidence supporting significant associations between cytokines measured in plasma and saliva is limited (141).

Biomarkers

The inflammatory part of pain

Inflammation is one of several factors that causes pain and influences the experience of it (46, 142-145). A significant number of publications on the topic pain and inflammation have been published during the last one hundred and thirty years, with a noticeable increase in the last thirty years. A 19th century publication stated that the pain in tooth ache is caused by an acute inflammation either in the pulp of the tooth or in the periosteum in the dental alveolus (146). In a study from 2019, saliva was analysed for inflammatory biomarkers, interleukin-1-beta (IL-1beta), interleukin-6 (IL-6), cortisol, and C-reactive protein (CRP); regarding periapical or pulpal inflammation, the authors of this study found that patients with acute inflammatory dental pain had increased levels of IL-1beta, IL-6, and cortisol, compared to pain-free controls (147). Inflammatory mediators such pro-inflammatory cytokines, chemokines, and prostaglandin, induce pain (46). Their induction of pain takes place via activation of nociceptors, the primary neurons that detect noxious stimuli (46, 148). A nociceptor is a part of an afferent neuron, with its cell body located in the dorsal root ganglion (DRG) or the trigeminal ganglia (46). Painful events are associated with the activation of glia cells. The glia cells are of three kinds, microglia, astrocytes, and oligodendrocytes in the CNS (149) and satellite glia cells in the trigeminal ganglia and the dorsal root (149, 150). Chronic pain and painful syndromes are associated with various kinds of glia activation. Also, acute pain conditions trigger glia activation and the acute use of opioid treatment activates peripheral glia (150). Chronic pain is thought to cause a dysregulation of glia activity in CNS and the peripheral nervous system (PNS) (150). As a result of noxious stimulation, microglia, the immune cells of the CNS, are activated, which changes the local environment by releasing cytokines, and which leads to a chain of further responses (150, 151). One such response is the alteration of permeability of the blood-brain barrier (BBB) (151, 152). That in turn enhances the passage of cytokines and other inflammatory proteins from the periphery into the CNS as well as the other way around (153, 154). In summary, there is an increase in the neuroimmune communication (153). Glia activation is documented in chronic pain patients (155, 156). In animal models, glia activation is also found to induce increased pain sensitivity and central sensitization (157). Through various animal models it is known that common orofacial pain states result in glia activation (149). Orofacial pain

involves several influences on the nervous system, such as peripheral sensitization of afferent neurons, central sensitization of the spinal cord and brain, loss of inhibitory transmission increase in the descending facilitation of pain signalling, and a change from acute to chronic pain (149).

Biomarkers of inflammation

Blood cells and acute phase proteins

In everyday clinical life a few common analyses are repeatedly used to diagnose and monitor inflammatory conditions. The type of analysis chosen usually depends on severity, speciality, and the specific diagnoses of each case. The first publication on the subject of white blood cells dates back to 1843, and it concluded that the "white globules" were altered in disease (158). White blood cell count (WBC) is the number of lymphocytes, monocytes, neutrophils, eosinophils, basophils, and immature cells per one micro litre of blood (159). The neutrophils (NEUT) are the predominant WBC in teenagers and adults (160).

Neutrophils were first time described in 1865 (161). Regarding the first line of defence, neutrophils capture and destroy invading microorganisms (162). Neutrophils respond to multiple signals by producing cytokines and other biomarkers to regulate inflammation and play a central part in the immune defence from bacterial infections (160, 162). Reactive neutrophilia are seen in inflammation, bacterial infection, stress, and trauma (162).

C-reactive protein (CRP) is an acute phase reactant protein primarily induced by IL-6, which is synthesized in the liver, and was first described in 1930 (163, 164). Foreign pathogens and damaged cells are recognized and cleared by the CRP (163). The CRP concentration can increase more than x1000 in severe inflammatory states (165). High sensitivity C-reactive protein (hsCRP) make it possible to detect CRP-values < 10 mg/L (166). Cortisol was first described in 1937-1938 by separate researchers (167). This acute phase response contains proteins from the liver, including CRP, $\alpha_2$macroglobulin, and fibrinogen (142, 168, 169). The CRP concentration rises 4-12 hours postoperatively and peaks at 24-72 hours (169).

Cytokines Chemokines and Growth Factors (CCGF)

To gain a better understanding of the inflammatory part of pain, smaller, more specific components had to be identified, studied, and compared. Several researchers have studied these components previously, but have mainly focused

on chronical pain states, for example diabetic neuropathy (170), cluster headaches (171), chronic fatigue syndrome (172), peripheral neuropathic pain (173), chronic widespread pain (174, 175), neuropathy after traumatic nerve injury (176), severely impaired chronic pain patients (177), fibromyalgia (178), trigeminal neuralgia (179), and postoperative inflammation after knee surgery (180). The cytokine group includes interleukins and interferons (142). Leukocytes, endothelia cells, and fibroblasts produce cytokines as an early response to tissue damage in order to direct the inflammatory response to the site of infection or tissue damage (142, 181). Among the inflammatory biomarkers, there are infinite associations and interactions in their dynamic responses to external and internal threats. Nonetheless, the biomarkers are usually sorted into families according to their function. The pro-inflammatory cytokines are as follows: IL-1-beta, IL-6, IL-8, IL-12, IFN-gamma, and TNF-alpha (182). IL-1 receptor antagonist, IL-6, IL-10, IL-11, and IL-13 are all anti-inflammatory cytokines (182). Cytokines can act distally or locally depending on whether they are distributed through endocrine, paracrine, or autocrine mechanisms (183). Depending on the cell they are secreted from, cytokines divide into lymphokines, monokines, and interleukins (184). Cytokines are usually produced by macrophages and T helper cells (184). Chemokines are cytokines with chemotactic activities (184, 185). Even chemokines are subdivided into four families: CXC, CC, C3XC, and XC (186). Cytokines are arranged the five following families: IL-1, IL-6, TNF, TGF, and INF (182). The IL-6 family consists of IL-6, IL-11, LIF, CNTF, CLCF1, OSM, and CT-1, and has recently been expanded to include three further members: IL-27, IL-35, and IL-39 (187). The family of cystatins are cysteine peptidase inhibitors including salivary cystatin D (CST5), S-type, and cystatin C (188). CST5 was first found in saliva as a protease inhibitor with the capacity to regulate genes (189). The inflammatory cascade is modulated via CST5, which influences the production of cytokines (189, 190). FGF-19, FGF-21, and FGF-23 are all members of one of seven subfamilies to FGF. FGF-21 is broadly associated with metabolic functions and its complexity (191, 192). Increased levels of FGF-21 in humans is connected to coronary heart disease, obesity, metabolic syndrome, type 2 diabetes, and chronic kidney disease (193). Every traumatic tissue damage causes various inflammatory and immune reactions. The severity and extent of the tissue damage correlates in some extent to the reaction (142). Though surgery causes tissue damage at the cellular level, it also triggers an immune response in relation to the surgical trauma. After major surgery IL-1, IL-6, and TNF-alpha are the main biomarkers released. IL-1 and TNF-alpha are released from activated monocytes and macrophages in the damaged tissue (142, 168, 181). This event stimulates the release of IL-6, which triggers an acute phase response (142, 181). Within 30-60 minutes from the start of surgery, the level of IL-6 increases, and the concentration becomes significant after 2-4 h. After major surgery the cytokine level peaks after approximately 24 h and the concentration stays elevated

for 48-72 h postoperatively (142). The production of cytokines mirrors the extent of the tissue damage, so low minor surgery such as laparoscopic surgery triggers lower titres of IL-6 (142), which is most likely similar for third molar surgery. The IL-6 level is a marker associated with the severity of the tissue damage (169). IL-6 is involved in the neuronal reaction to nerve injury and in the development of pathological pain. Not only is IL-6 associated with the activation of astrocytes and microglia but also to the neuropathic pain behaviour following peripheral nerve injury (184).

As members of the same family OSM and IL-6 share the ability to influence the differentiation of a variety of cell types (194). OSM is involved in the early parts of wound healing, liver regeneration, and bone remodelling (195). Inflammatory diseases such as inflammatory bowel disease, arthritis, skin disease, asthma, pulmonary fibrosis, COVID-19 and cardiovascular disease are all associated with OSM (195). OSM is also significantly involved in different cancer types including brain, skin, lung, testicular, breast, cervical, ovarian, colon, and gastrointestinal (195). TNF-alpha is connected to inflammatory disorders like rheumatoid arthritis and osteoarthritis and by inducing collagenase, TNF-alpha can reduce the extracellular matrix (ECM) (196). Flt3L is a growth factor associated with immune and hematopoietic cell production (197). IL-8 is a pro-inflammatory chemokine that attracts and activates neutrophils (198). VEGFA promotes angiogenesis and is involved in the formation of new blood vessels (199, 200). CDCP-1 is associated with some solid tumour cells and cancers with poor prognosis (201). IL-1 alpha is expressed in the epithelial cells of liver, kidney, skin, and gastrointestinal tract. Neutrophil infiltration is the result of chemokine secretion from IL-1 alpha (202). TNFSF14 is a glycoprotein with a central role in the acute immune response and the body's capacity to combat a virus (203). CCL28 belongs to the chemokine family and shows a broad spectrum of capabilities in fighting fungi, gram-positive, and gram-negative bacteria (204). EN-RAGE facilitates the activation of the inflammatory monocyte. EN-RAGE is found to be increased in chronic inflammatory disorders such as chronic kidney disease, type 2 diabetes mellitus, inflammatory bowel disease, coronary artery disease, and atherosclerosis (205). CASP-8 is involved in programmed cell death and apoptosis, which can be initiated by TNF that in turn activates CASP-8 (205, 206). CD8A/CD8 T-cell is related to the antiviral response of the immune system (207). The signalling by CD244 is associated with the hepatitis B and C virus and tuberculosis in humans (208). AXIN1is thought to be a tumour suppressor (209). SCF is mainly related to cell proliferation, survival, and cancer (210). The pro-inflammatory protein, MCP-4, is overexpressed in several malignant tumours and is thought to be important in the progression of tumours and metastasis (211). Both breast- and cervical- cancer progression are promoted by CCL-19 and lung-, colorectal-, gastric-, and ovarian- cancer are suppressed

(212). IL-12 is important for the antiviral immune response (213). CCL-23 is related to the outcome of acquired brain damage and stroke (186).

To measure in serum, plasma, or saliva

CCGF can be analysed in almost any body fluid. In addition to serum plasma and saliva, urine and CSF have been used in several studies. The research question to answer is of course the main factor when choosing body fluid to investigate. Secondarily, convenience and availability might be considered. Integrity and ethical considerations must be taken into account. For example, if a saliva sample can answer your research question, it is difficult or even unethical to justify a lumbar puncture to collect CSF.

There are some similarities but also differences regarding serum, plasma, and saliva to be aware of when choosing body fluid to analyse; this also applies when interpreting and comparing analysis results.

Plasma is blood without the blood cells but with the coagulation factors remaining. When collecting blood samples for plasma analysis, a tube prepared with some anti-coagulant, for example EDTA, is required (214, 215).

When the blood is prevented from coagulating, the plasma and the blood cells can be separated through centrifugation. Serum is blood that has coagulated for 30-60 minutes, and the clot is then removed (216). Serum is blood without blood cells and without coagulation factors. The coagulation is an extensive and complex process that affects the properties of the serum and separates its properties from those of plasma. In some studies, saliva has shown similarities with plasma when considering the expression of CCGF, but the differences, when comparing the expression of CCGF in plasma vs saliva, are more significant.

Analysis of inflammatory biomarkers for better understanding of pain pathophysiology

Because of the definition of pain as an experience, there is currently no possibility to detect or quantify pain solely using objective methods. Most likely, there will never be a specific pain biomarker. Nevertheless, biomarkers can be used to detect and quantify biological processes associated with pain, such as inflammation.

Aims

The aim of the thesis was to optimize early, postoperative pain management in the junction between local anaesthesia and postoperative analgesia in third molar surgery. In addition, the aim was to gain a better understanding of the inflammatory mechanisms involved in surgical trauma, the S-ketamine effect, and the third molar surgery pain model.

Paper I

The aim of this study was to investigate if preoperative single dose intravenous (IV) S-ketamine has a role in day-case surgery of third molars, in relation to pain experience and the need for complimentary analgesics.

Paper II

The aim of this study was to analyse patient safety of S-ketamine analgesia in third molar surgery, with special reference to oxygen saturation of the blood (SpO_2). The secondary aim was to study alterations in respiratory rate, blood pressure, pulse and/or adverse events - all following the administration of two different doses of S-ketamine, 0.125 mg/kg or 0.25 mg/kg, compared to placebo (saline) in midazolam sedation.

Paper III

The aim of this study was to identify important biomarkers in the early postoperative inflammation in third molar surgery and to evaluate the systemic effect of third molar surgery and of s-ketamine.

Paper IV

The aim of this study was to compare inflammatory biomarker expression in saliva and plasma.

Methods

The study was designed as a randomized placebo-controlled double-blind trial. The inclusion process started in February 2017 and was completed in March 2022. The clinical part of the study suffered some delay due to the Covid-19 pandemic.

The study enrolled healthy individuals according to the American Society of Anaesthesiologists classification, I or II (ASA) (217-219) (Table 3). Potential participants aged between 18 and 44, with a body weight between 50 and 100 kg were invited to participate in the study. The participants were selected from patients, who had been referred to the Oral and Maxillofacial Surgery clinic, at Falun County Hospital (Sweden), for surgical removal of an impacted lower third molar.

Table 3. ASA classification system.

ASA Physical Status Classification System (Last Amended December 13, 2020)	
ASA PS Classification	Definition
ASA I	A normal healthy patient
ASA II	A patient with mild systemic disease
ASA III	A patient with severe systemic disease
ASA IV	A patient with severe systemic disease that is a constant threat to life
ASA V	A moribund patient who is not expected to survive without the operation
ASA VI	A declared brain-dead patient whose organs are being removed for donor purposes

The exclusion criteria included the following conditions: potential participants were excluded from the study if they were receiving ongoing regular medication with analgesics, hypnotics, thyroid hormones, psychoactive drugs, or MAO inhibitors. Potential participants were excluded from the study if they were diagnosed with or were currently undergoing, hypertension, heart failure, psychosis, epilepsy, hyperthyroidism, myasthenia gravis, glaucoma, verified sleep apnea, diabetes, porphyria, pregnancy, breast-feeding, blood transmitted infections (such as HIV and hepatitis B and C), or if they had a known

hypersensitivity to midazolam, ketamine, ibuprofen, or local anesthetics. Potential participants were excluded from the study if informed oral and written consent was not attained or if the person was unable to understand information in spoken or written Swedish. A flow-chart of the study design is presented in Figure 7 (Figure 7).

The subjects were randomized into three groups, comparing two doses of S-ketamine, 0.25 mg/kg or 0.125 mg/kg, or a placebo (saline). In a consecutive series, patients were offered to participate in the study until a total number of 168 participants was reached. The number of subjects was decided based on a power analysis.

We estimated this to be a necessary sample size to detect a clinically meaningful mean difference of 15 mm in the VAS-score (0-100 mm) between high-dose ketamine and placebo at four hours post-surgery (220, 221). From our previous observations, we expected a standard deviation of 26 mm, which for 80% power, a two-sided 5% significance level, and allowing for 15% attrition, corresponded to 56 patients per intervention group.

A total of 168 subjects were randomized at a 1:1:1 allocation ratio to high-dose ketamine, low-dose ketamine, or placebo, using stratified block randomization with a fixed block size of six within sex. In practice, the randomization sequence was generated through manual card shuffling, whereby two piles of six cards each were sequentially dealt and re-shuffled until the assignment was completed. The randomization sequence was stored at the local hospital's pharmacy under the supervision of independent pharmacists, who also dispensed the study's pharmaceuticals, which had an identical appearance to maintain concealment. Patients were allocated according to eligibility criteria assessment, informed consent, and following the completion of baseline measurements. All parties remained unaware of allocation until completion of the statistical analyses (Table 6).

A list of serial numbers and randomized groups affiliation was produced, which was accessible for the pharmacy's manufacturing staff and closed to all staff performing the clinical part of the trial. The clinical trial leader informed the pharmacy which serial numbers were in turn for treatment and the pharmacy prepared filled syringes with the investigational drug in accordance with the randomized group affiliation. Preparation took place in the pharmacy's manufacturing unit. The syringe containing the test drug was marked with a code/serial number, shelf life/expiration date, the time of manufacture, the patient´s name, id-number and EudraCT number. The prepared investigation drug was delivered to the clinic no more than 24 hours in advance.

A peripheral venous catheter (BD Venflon™1.3mm Ø) was placed in a superficial vein of the subject's forearm or on the back of the hand. The peripheral venous catheter was used for drug administration and for blood sampling. Blood samples were collected in EDTA tubes (BD Vacutainer®) on two occasions, immediately preoperatively and two hours after the start of surgery. Saliva was only collected immediately preoperatively. The blood samples were prepared by centrifugation at 2400g for 5 minutes, and the plasma portions were stored in aliquots at -80°C. Both plasma and saliva were prepared and put in a freezer within one hour - time affects the majority of proteins measured in plasma and therefore there is a risk of causing pre-analytic errors, especially when centrifugation is delayed by more than one hour (214, 215).

All participants were sedated with midazolam intravenously (Table 4) to a defined end point: Ptosis and / or dysarthria. The oral and maxillofacial surgeon (LBE) and an independent observer assessed sedation depth at the endpoint according to the Observer's Assessment of Alertness / Sedation Scale (OAA / S scale) (222-224). The rating was from 1 (deep sleep) to 5 (fully awake). Four variables were evaluated: responsiveness (1 to 5), speech (2 to 5), facial expression (3 to 5), and eyes (3 to 5). The lowest of all values from the four variables was noted. The scale is regarded as valid and reliable in relation to VAS, the University of Michigan sedation scale (UMSS), and the electroencephalogram bispectral index analysis (EMG-BI) (222-224).

Table 4. Midazolam – pharmacological properties.

	Pharmacological properties				
	Midazolam IV	Midazolam IM	Midazolam PO	Midazolam PR	Midazolam IN
T½ γ [h]	1.5-2.5				
Receptor	$GABA_A$	$GABA_A$	$GABA_A$	$GABA_A$	$GABA_A$
Protein binding [%]	94				
V_{SS} [L/kg]	0.7-1.2				
CL [ml/kg/min]	5.63				
Bioavailability [%]	100	90	44	50	50
Onset with in [min]	2	2	15	10-15	10
Active metabolite	1-OH-M				
-metabolite T½ [h]	1				

Intravenous=IV, Intramuscular=IM, Oral=PO, Rectal=PR, Nasal=IN (94, 225-228).

In the literature, we found a broad spectrum of doses, 0.075mg/kg to 1mg/kg as bolus, but there appears to be no consensus as to which S-ketamine dose should be used in postoperative analgesia (17). We thus used two different doses to enable comparisons. We looked for doses likely to give analgesic effect but not cause general anaesthesia. One American consensus guidelines states that the common subanesthetic dose of ketamine used in clinical practice is IV 0.3-0.5 mg/kg bolus with or without an infusion (120). The recommended dose to induce anaesthesia is 0.5 to 1 mg/kg IV or 2 to 4 mg/kg intramuscular (IM) (48, 104). We chose to give the ketamine prior to surgical incision to enable a standardized protocol. An injection at the end of or after surgery would have been less coordinated with the midazolam effect.

The drugs were administered to the three groups as follows:

1. Placebo group: Sodium Chloride solution (9 mg / ml) 0.2 ml/kg slow intravenous injection (2 ml/min).
2. Low-Dose Ketamine group: S-Ketamine (0.125 mg/kg body weight). (0.625 mg / ml x 0.2 ml/kg) slow intravenous injection (2 ml/min).
3. High-Dose Ketamine group: S-Ketamine (0.25 mg/kg body weight). (1.25 mg/ml x 0.2 ml / kg) slow intravenous injection (2 ml/min).

The test drug was injected using a syringe pump (Alaris CC, Cardinal Health, Rolle, Switzerland) at a rate of 120 ml / h (2 ml/min) with volume accuracy ± 2%. One infusion set (Alaris Medical System Ref MFX2299E Impromediform GmbH, Lüdenscheld, Germany) was coupled between the syringe and a three-way connection, which in turn was connected to a peripheral venous catheter (Venflon® 18G 1.3x32 mm). The test drug was administered as a preoperative single dose, immediately after the sedation. Total injection time varied between 5 to 10 minutes depending on the patient's body weight of 50 to 100kg.

The surgical procedure was performed under local anesthesia (LA) - Xylocaine adrenaline 20 mg/ml, 4 ampoules of 1.8ml - supplemented if necessary to achieve intraoperative pain relief. The LA was applied as an inferior alveolar nerve block in combination with buccal infiltration. The affected lower third molar was removed according to an established surgical procedure, which started with a mucoperiosteal incision at a 45° angle from the distal buccal part of the tooth and a small buccal flap was mobilized. After bone removal around the root, the tooth was luxated and, if necessary, sectioned at the root furcation before the parts were removed. The wound was inspected for hemostasis, irrigated with saline and the incision closed with a resorbable (Vicryl®) suture (3).

The patients were continuously monitored with pulse oximetry, and a non-invasive blood pressure measurement was taken every fifth minute using an automatic patient monitor (Dash 5000, General Electric Company, Nellcor algorithm). The respiratory rate was registered manually for one minute on four occasions before and during the surgery. The antidote for benzodiazepines (flumazenil) was on hand. Supplemental oxygen, in case of oxygen desaturation, assisted breathing support, as well as suction catheters for cleaning the upper airways were available, tableside. After the surgery, all patients stayed for recovery time, and before being discharged, the patients were given oral and written postoperative information. Every participant was given a box with 30 rescue pills of Ibuprofen (Ibumetin® Orifarm Generics AB Stockholm, Sweden) in doses of 400mg, to be taken up to four times a day, and administered as needed by the patient.

Primary outcome

The primary outcome of the study was VAS Δ = VAS pain 4 hours - VAS pain preoperatively [mm] (220, 221, 229). The secondary outcome variables were time to first rescue medication [minutes], number of rescue medications/pills used in the first 24 hours, use of complementary analgesics, and safety aspects measured, such as capillary saturation [SpO_2], blood pressure, pulse, and side effects.

The patients rated their spontaneous pain intensity on eight occasions during the first 24 hours (immediately before and after surgery - 2, 3, 4, 6, 8, 24 hours after end of surgery) using a 100 mm scale, VAS. The endpoints of the scale were marked "no pain" and "worst pain imaginable" (50, 54). Pain during function such as chewing or swallowing was not measured. Analgesics consumption was described as the time to first rescue medication and total analgesic consumption for the first 24 hours, as well as the use of any other analgesics than those provided in the study (54). The self-reported pain intensity was indicated by a short line or a cross (X) on the 100 mm long line of the VAS. Using a steel ruler LBE meticulously measured the VAS-value. Markings to the left of 0 were given the value 0 and markings to the right of 100 were given the value 100. When rough crosses and markings were found, the center of the marking was used. If the marking was a line crossing the VAS-line obliquely, the center of the crossing was used for the measuring.

Proximity Extension Assay (PEA)

The PEA was performed using the Proseek Multiplex Inflammation kit (Olink Bioscience, Uppsala, Sweden) (230). In summary, one µL of saliva or plasma was combined with 3 µL of incubation mix, containing two probes (antibodies conjugated with unique DNA oligonucleotides). The mixture was incubated at 8 ◦C overnight. Following incubation, 96 µL of extension mix including the PEA enzyme and PCR reagents, was added. The samples were then incubated at room temperature for five minutes before undergoing seventeen cycles of DNA amplification in a thermal cycler. A 96.96 Dynamic Array IFC (Fluidigm, South San Francisco, CA, USA) was prepared and primed according to the manufacturer's instructions. In a separate plate, 2.8 µL of the sample mixture was combined with 7.2 µL of detection mix, and 5 µL of this solution was loaded into the right side of the primed 96.96 Dynamic Array IFC. Unique primer pairs for each cytokine were loaded into the left side of the array. The protein expression analysis was then performed using the Fluidigm Biomark reader, following the Proseek protocol. Each Proseek kit quantified ninety-two biomarkers.

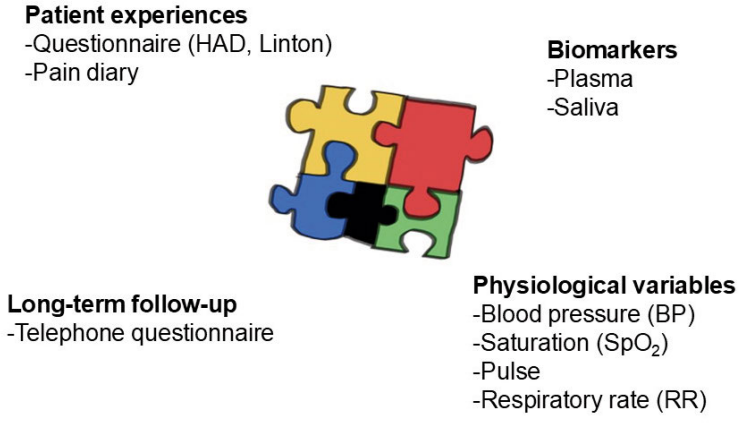


Figure 6. Schematic overview of the data sources featured.

Statistics and data management

Statistics – Paper I

The basis of the power calculation was the pain by VAS 3 hours after surgery (20). Based on the power analysis, subjects were allotted to one of the three groups. Data were analysed according to the intention-to-treat principle (ITT), where participants are coded to their allocation status. In the primary analysis, the VAS Δ-score at 4 hours post-surgery was compared with the Mann–Whitney U test in a hierarchical procedure of predetermined order, to retain the nominal 5% significance level1: high-dose ketamine and placebo and level2: low-dose ketamine and placebo (R v4.2.2). Four secondary analyses were conducted. First, the VAS-score was compared between the intervention groups per time point in a linear mixed model for repeated measures (nlme v3.1–160 in R v4.2.2). The model included time as a factor, the interaction between time and intervention groups for all time points except baseline, and the stratification factor 'sex' as fixed effects. To account for dependency within the patient, it included a patient-specific random intercept and an autoregressive correlation structure for the repeated measures. Based on this model, the marginal means per time point and intervention group as well as globally across time points were estimated (emmeans v1.8.2 in R v4.2.2). Second, the time to first rescue medication was computed with the Kaplan–Meier estimator and compared between high-dose ketamine and placebo using the log-rank test (survival v3.4.0 in R v4.2.2). Finally, the total number of pain medication pills were compared between high-dose ketamine and placebo using the Mann–Whitney U test (R v4.2.2).

Statistics – Paper II

The comparison was conducted in a hierarchal procedure of predetermined order: (1) high-dose S-ketamine versus placebo and (2) low-dose S-ketamine versus placebo. The physiological variables, SpO_2, respiratory rate, pulse rate, systolic blood pressure, and diastolic blood pressure, were compared between groups using the independent nonparametric method, Mann–Whitney U test. Fisher's exact test was used to compare the groups regarding self-reported side-effects, such as any occurrence of nausea and vomiting on the day of surgery, nausea and vomiting the day after surgery, nightmares on the day of surgery, and hallucinations on the day of surgery. For all our analyses, we used the software Jamovi, version 2.3.21.

Statistics – Paper III

Protein NPX values were compared between baseline and after surgery using paired t-tests. Blood status values were compared between baseline and after

surgery using a Wilcoxon signed rank test. Multiple testing correction was performed using Benjamini-Hochberg's false discovery rate method (FDR), and FDR 10% was used as a significance threshold.

The delta values between the Ketamine groups, high vs placebo, low vs placebo, and high vs low, were compared using Welch's t-test. Again, correction for multiple tests was performed using Benjamini-Hochberg's FDR method.

Statistics – Paper IV

All the output data from the OLINK set-up was utilized, analysing both saliva and plasma. 71 proteins in plasma, 63 proteins in saliva, and 58 proteins in both saliva and plasma out of 92 analysed showed >80% samples with NPX values above the limit of detection (LOD). LOD was defined as three standard deviations (SD) above the average of the negative controls. Spearman´s rank correlation coefficient was calculated using Statistica (StatSoft (Version 14), Tulsa, OK, USA) to examine the correlation of expression of various cytokines as NPX in saliva vs plasma (231).

Spearman rank correlation (r_s) was calculated; r_s=+1 is a perfect positive correlation and r_s=-1 is a perfect negative correlation. We interpreted r_s<±0.3 as a weak correlation (232, 233). For some descriptive parts of data, Jamovi (ver 2.3.28) was used.

	Paper			
	I	II	III	IV
Sample size	168	168	165	165
Primary aim	S-ketamine efficacy	S-ketamine safety	Biomarker expression	Biomarker association
Primary outcome	Δ VAS 4h	Saturation at end of analgesia	Surgical trauma effect on inflammatory biomarkers	Association between inflammatory biomarkers in saliva vs plasma
Secondary outcomes	Global VAS first 24h Time to rescue medication Number of rescue medication	Saturation Respiratory rate SBP DBP Pulse Nightmares/Hallucinations PONV on operation day PONV day after operation AE	S-Ketamine effect on inflammatory biomarkers	
Contrast	S-Ketamine vs placebo	S-Ketamine vs placebo	S-Ketamine vs placebo	Baseline
Analysis/Method	• Mann-Whitney U test • Linear mixed model • Kaplan-Meier estimator	• Mann-Whitney U test • Fisher´s exact test	• Welch´s t-test • Wilcoxon signed rank • Benjamini-Hochberg´s false discovery rate method	• Spearman rank correlation
Software	R v4.2.2 nlme v3.1–160 in R v4.2.2 emmeans v1.8.2 in R v4.2.2 survival v3.4.0 in R v4.2.2 R v4.2.2	Jamovi, version 2.3.21	R v4.2.2	Statistica (StatSoft Version 14) Jamovi, version 2.3.21

List of analysed inflammatory biomarkers

Cytokines, chemokines, and growth factors (CCGF) analysed in plasma and saliva, using the Proseek Multiplex Inflammation kit (Olink Bioscience, Uppsala, Sweden).

Table 5. Overview analysed CCGF.

Gene	Protein name	UniProt ID
CD6	T-cell differentiation antigen CD6	P30203
KITLG	Kit ligand	P21583
IL-18	Interleukin-18	Q14116
SLAMF1	Signaling lymphocytic activation molecule	Q13291
TGFA	Protransforming growth factor alpha	P01135
CCL13	C-C motif chemokine 13	Q99616
CCL11	Eotaxin	P51671
TNFSF14	Tumour necrosis factor ligand superfamily member 14	O43557
FGF-23	Fibroblast growth factor 23	Q9GZV9
IL-10RA	Interleukin-10 receptor subunit alpha	Q13651
CCL4	C-C motif chemokine 4	P13236
FGF5	Fibroblast growth factor 5	P12034
LIFR	Leukemia inhibitory factor receptor	P42702
FGF-21	Fibroblast growth factor 21	Q9NSA1
CCL19	C-C motif chemokine 19	Q99731
IL-15RA	Interleukin-15 receptor subunit alpha	Q13261
IL-10RB	Interleukin-10 receptor subunit beta	Q08334
IL-22RA1	Interleukin-22 receptor subunit alpha-1	Q8N6P7
IL-18R1	Interleukin-18 receptor 1	Q13478
CD274	Programmed cell death 1 ligand 1	Q9NZQ7
NGF	Beta-nerve growth factor	P01138
CXCL5	C-X-C motif chemokine 5	P42830
MMP1	Interstitial collagenase	P03956
TNFSF11	Tumour necrosis factor ligand superfamily member 11	O14788
TSLP	Thymic stromal lymphopoietin	Q969D9
IL-2	Interleukin-2	P60568
VEGFA	Vascular endothelial growth factor A	P15692
CCL7	C-C motif chemokine 7	P80098
GDNF	Glial cell line-derived neurotrophic factor	P39905

CDCP1	CUB domain-containing protein 1	Q9H5V8
CD244	Natural killer cell receptor 2B4	Q9BZW8
IL-7	Interleukin-7	P13232
TNFRSF11B	Tumour necrosis factor receptor superfamily member 11B	O00300
TGFB1	Transforming growth factor beta-1 proprotein	P01137
PLAU	Urokinase-type plasminogen activator	P00749
IL-6	Interleukin-6	P05231
CXCL1	Growth-regulated alpha protein	P09341
IL-17C	Interleukin-17C	Q9P0M4
IL-17A	Interleukin-17A	Q16552
CXCL11	C-X-C motif chemokine 11	O14625
AXIN1	Axin-1	O15169
TNFSF10	Tumour necrosis factor ligand superfamily member 10	P50591
IL-20RA	Interleukin-20 receptor subunit alpha	Q9UHF4
CXCL9	C-X-C motif chemokine 9	Q07325
CST5	Cystatin-D	P28325
IL-2RB	Interleukin-2 receptor subunit beta	P14784
IL-1A	Interleukin-1 alpha	P01583
OSM	Oncostatin-M	P13725
CCL2	C-C motif chemokine 2	P13500
CXCL8	Interleukin-8	P10145
DNER	Delta and Notch-like epidermal growth factor-related receptor	Q8NFT8
CCL28	C-C motif chemokine 28	Q9NRJ3
SIRT2	NAD-dependent protein deacetylase sirtuin-2	Q8IXJ6
IL-20	Interleukin-20	Q9NYY1
EIF4EBP1	Eukaryotic translation initiation factor 4E-binding protein 1	Q13541
CXCL10	C-X-C motif chemokine 10	P02778
CXCL6	C-X-C motif chemokine 6	P80162
FLT3LG	Fms-related tyrosine kinase 3 ligand	P49771
S100A12	Protein S100-A12	P80511
CCL3	C-C motif chemokine 3	P10147
CCL23	C-C motif chemokine 23	P55773
TNF	Tumour necrosis factor	P01375
IL-10	Interleukin-10	P22301
MMP10	Stromelysin-2	P09238

ARTN	Artemin	Q5T4W7
IL-13	Interleukin-13	P35225
IL-24	Interleukin-24	Q13007
IL-12B	Interleukin-12 subunit beta	P29460
CD5	T-cell surface glycoprotein CD5	P06127
HGF	Hepatocyte growth factor	P14210
CD40	Tumour necrosis factor receptor superfamily member 5	P25942
IFNG	Interferon gamma	P01579
CSF1	Macrophage colony-stimulating factor 1	P09603
LTA	Lymphotoxin-alpha	P01374
ADA	Adenosine deaminase	P00813
IL-5	Interleukin-5	P05113
STAMBP	STAM-binding protein	O95630
SULT1A1	Sulfotransferase 1A1	P50225
CCL20	C-C motif chemokine 20	P78556
TNFSF12	Tumour necrosis factor ligand superfamily member 12	O43508
IL-33	Interleukin-33	O95760
NTF3	Neurotrophin-3	P20783
CX3CL1	Fractalkine	P78423
CCL25	C-C motif chemokine 25	O15444
CASP8	Caspase-8	Q14790
CCL8	C-C motif chemokine 8	P80075
NRTN	Neurturin	Q99748
LIF	Leukemia inhibitory factor	P15018
IL-4	Interleukin-4	P05112
FGF19	Fibroblast growth factor 19	O95750
TNFRSF9	Tumour necrosis factor receptor superfamily member 9	Q07011
CD8A	T-cell surface glycoprotein CD8 alpha chain	P01732

Ethical considerations

A difference from conventional clinical routine treatment was the use of venous blood sampling, which implied a further invasive step. However, in relation to local anesthesia and surgery, this was considered negligible or of minor importance.

Further, we obtained verbal and written informed consent from each patient prior to the initiation of any study procedures. In all clinical and administrative aspects, Good Clinical Practice (GCP), the General Data Protection Regulation (GDPR), and the Helsinki Declaration were followed.

S-Ketamine is a registered drug and is thus already well documented. This study is a phase IV clinical trial and has therefore gone through a rigorous application process in order to obtain permission from MPA for clinical drug testing on humans. As part of this process, a general safety assessment was carried out, but a plan was also put in place to detail how any adverse events (AE), serious adverse events (SAE), or suspected unexpected serious adverse reactions (SUSAR) would be handled. Grounds for the detection of these events were spontaneous reports, from observations including laboratory reports, and when needed, active questioning. Furthermore, the principal investigator (PI) and the sponsors' responsibilities were described. Possible actions within the trial could be to break the blinding code or to stop the trial. Any necessary medical attention was to be provided in order to ensure the ultimate protection of the participants' health. As part of the trial´s specific standard operating procedures (SOP), contact information to related authorities was available in case of any AE, SAE, or SUSAR occurred.

The trial leader (LBE) had already acquired clinical experience of the drug in clinical use; although these previous treatments have not been recorded and studied systematically, no obvious side effects were noted.

Nevertheless, there are several known side effects to ketamine use: dreams, nightmares, vertigo, restlessness, blurred vision, palpitations, increased blood pressure, irregular or slow pulse, decreased blood pressure, effects on breathing, nausea, vomiting, and increased salivation. Others, such as increased body movements (e.g. muscle twitching that may resemble seizures), increased eye movements, double vision, increased intraocular pressure, skin rashes, pain and/or redness at the injection site, allergic reactions, hallucinations, depression, anxiety and confusion have also been reported (98, 234).

An independent, external person performed monitoring at four occasions during the study period: the first visit took place before inclusion, the second and

third visits during ongoing inclusion, and the last visit took place soon after the inclusion was completed.

Legal permits and registrations

Swedish Ethical Review Authority (EPM) Dnr 2015/378 revised and approved the study protocol. Swedish Medical Products Agency (MPA) has granted us permission for this study regarding clinical drug testing on humans (Dnr 5.1-2016-48439). The study was registered in the European Union Drug Regulating Authorities Clinical Trials Database (EudraCT) with number: 2014-004235-39. We have also registered the study on ClinicalTrials.gov, ID: NCT04459377.

Permission to set up a sample collection at the local biobank has been obtained (sample ID 873-2014-004-235-39).

Baseline Characteristics

Table 6. Demographic and clinical characteristics of the study participants at baseline.

	Group		
	Placebo Saline solution	Low dose S-ketamine 0.125 mg/kg	High dose S-ketamine 0.25 mg/kg
No.	53	55	60
Sex			
Female	36 (68%)	38 (69%)	41 (68%)
Male	17 (32%)	17 (31%)	19 (32%)
Age, mean (SD), year	30 (6.7)	29.5 (6.7)	28 (6.7)
Weight, mean (SD), kg	74.8 (13.5)	74.9 (14.6)	72.8 (13.0)
Height, mean (SD), cm	171.3 (8.4)	171.6 (8.7)	171.6 (8.8)
BMI, mean (SD)	25.4 (4.1)	25.5 (8.7)	24.7 (4.1)
Circulation			
SBP, mean (SD), mmHg	116.6 (13.0)	115.9 (13.1)	117 (13.1)
DBP, mean (SD), mmHg	69.4 (8.5)	68.6 (9.2)	68.8 (8.1)
Pulse, mean (SD)	70.1 (68.8	68.6 (12.3)	69.8 (13.3)
Respiration			
Respiration rate, mean (SD)	16.5 (3.1)	16.3 (3.4)	16.4 (3.4)
Pulse oximetry SpO_2, mean (SD), %	98.8 (1.4)	98.9 (1.1)	98.8 (1.1)
Duration of surgery, minutes			
Median	15	16	14
IQR	9	9.5	12
Min	5	7	6
Max	70	35	48
Clinical degree of retention			
Erupted	2	3	1
Partially covered by mucosa	46	50	53
Entirely covered by mucosa	5	2	6
Entirely covered by bone	0	0	0
Radiological position			
Vertical 	10	8	13
Mesioangular 	18	10	14
Horizontal 	14	18	13
Distoangular 	11	19	20

Systolic blood pressure=SBP, Diastolic blood pressure=DBP.

Results

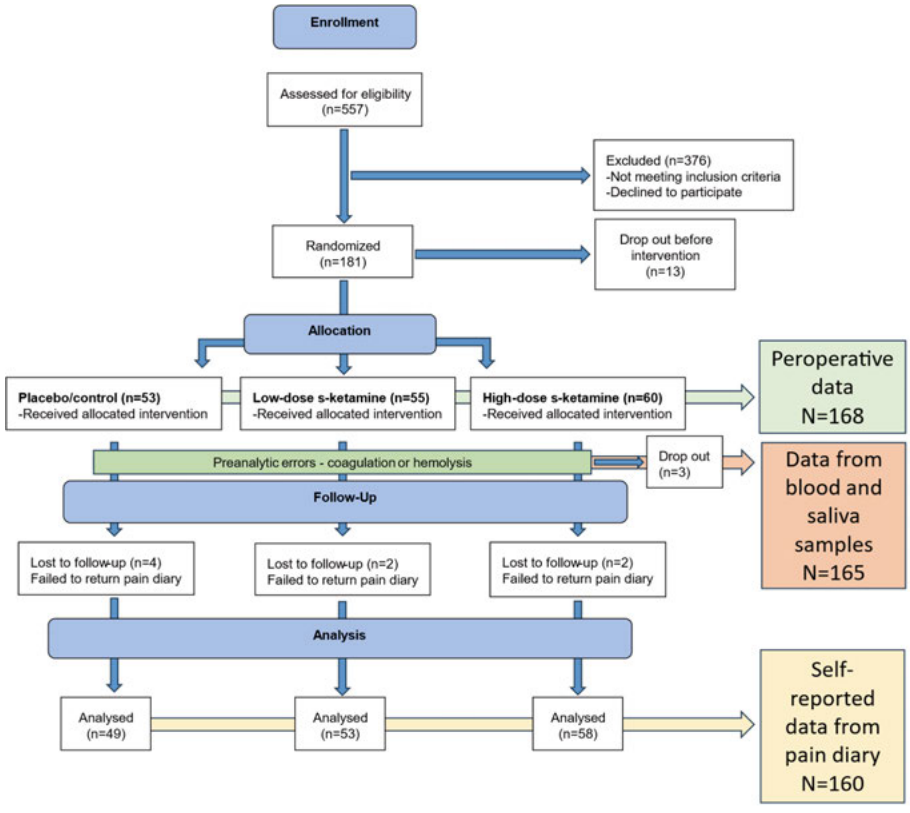


Figure 7. CONSORT flowchart.

Paper I

Median duration of surgery was 15 minutes with no significant difference between the groups.

The primary outcome, VAS at 4 hours postoperatively, showed no significant difference between the placebo and high-dose ketamine group. There was a significant difference in mean VAS between the groups for the period 2-24 hours, with a lower VAS-score in the high-dose S-ketamine group (Figure 8).

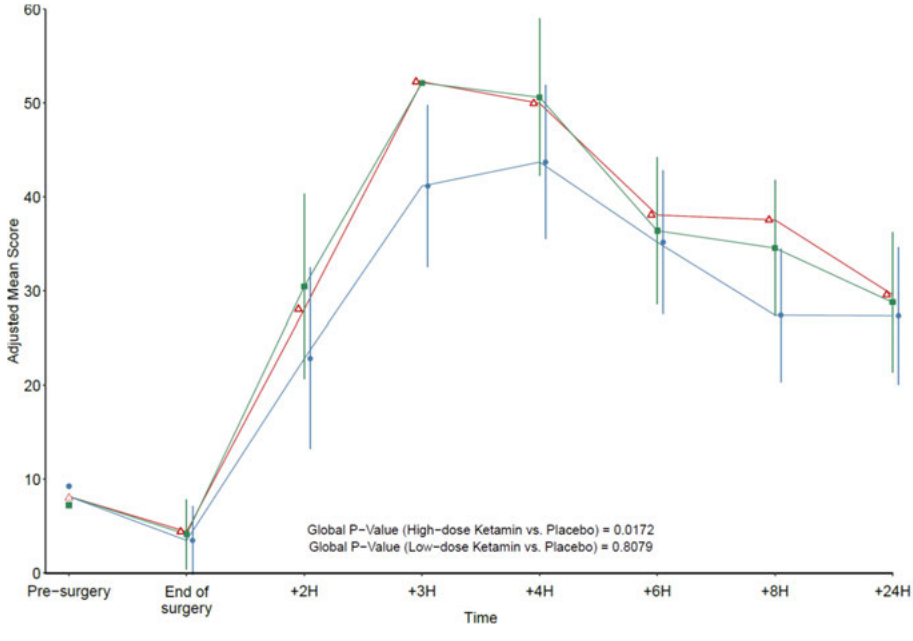


Figure 8. Global pain intensity VAS first 24 hours. Blue circle: high-dose S-ketamine 0.25mg/kg; green square: low-dose S-ketamine 0.125mg/kg; red triangle: placebo

The median time to first rescue medication was 12 minutes longer in the high dose S-ketamine group. The total amount of rescue pills during the first 24 hours did not differ between the groups. The use of additional analgesics was similar between the groups.

Paper II

For the safety aspects of this study, oxygen saturation (SpO_2) after finished administration of the investigational drug was the main outcome measured. There was a significant difference between the placebo and the high-dose group at that point (p=0.021), with a decrease of saturation in the high-dose group (Table 7). No other significant differences between groups regarding saturation or respiratory rate were noted.

The lowest saturation and the number of registrations of SpO_2<90% did not show any difference between groups. Oxygen supplementation was given in around 40% of the cases with no differences between the intervention groups.

Table 7. Effects on respiratory parameters during the perioperative period.

Parameters and groups	Baseline	End of sedation	End of analgesia (Primary end-point)	End of surgery
Saturation SpO_2				
Placebo[1]	98.8(1.4)	98.0(1.7)	97.9(1.7)	99.3(1.0)
Low-dose[2]	98.9(1.1)	98.0(1.7)	98.0(1.7)	99.3(1.1)
High-dose[3]	98.8(1.1)	97.8(1.5)	97.1(1.9)	99.2(1.1)
p (1 vs 3)			0.021	
p (1 vs 2)			0.793	
Respiratory rate				
Placebo[1]	16.5(3.1)	17.9(2.7)	17.3(2.5)	18.1(2.9)
Low-dose[2]	16.3(3.4)	17.0(2.6)	16.8(2.6)	17.4(2.4)
High-dose[3]	16.4(3.4)	17.6(2.6)	16.7(2.7)	17.5(2.5)
p (1 vs 3)			0.308	

Placebo=saline, Low-dose=0.125mg S-ketamine/kg, High-dose=0.25 S-ketamine/kg. Mean(std). Respiratory rate=breath/minute.
P-value based on Mann-Whitney U test.

At the end of the investigational drug administration, the pulse rate differed significantly between the placebo and the high-dose group (p=0.007) with an increased rate in the high-dose group. No other differences between the groups regarding pulse rate or blood pressure were observed (Table 8).

Table 8. Effects on hemodynamic parameters during the perioperative period.

Parameters and groups	Baseline	End of sedation	End of analgesia (Primary end-point)	End of surgery
Pulse rate[a]				
Placebo[1]	70.1(9.9)	71.7(9.2)	70.3(10.4)	86.3(11.7)
Low-dose[2]	68.6(12.3)	71.6(10.4)	73.2(11.8)	83.7(14.0)
High-dose[3]	69.8(13.3)	70.6(10.2)	75.9(10.8)	88.2(12.6)
p (1 vs 3)			0.007	
p (1 vs 2)			0.155	
Systolic BP[b]				
Placebo[1]	116.6(13.0)	106.0(11.1)	103.8(11.2)	117.2(14.2)
Low-dose[2]	115.9(13.1)	106.0(11.3)	107.0(14.3)	118.5(14.7)
High-dose[3]	117.0(13.1)	107.4(10.4)	108.3(13.7)	120.4(15.9)
p (1 vs 3)			0.122	
Diastolic BP[b]				
Placebo[1]	69.4(8.5)	63.2(7.3)	62.0(7.2)	63.5(8.8)
Low-dose[2]	68.6(9.2)	62.7(7.9)	63.1(10.1)	65.1(8.8)
High-dose[3]	68.8(8.1)	63.7(7.6)	65.2(9.5)	66.3(9.9)
p (1 vs 3)			0.074	

Placebo=saline, Low-dose=0.125mg S-ketamine/kg, High-dose=0.25 S-ketamine/kg. [a]Frequency= bpm. [b]mmHg. Mean(std). P-value based on Mann-Whitney U test.

Nausea and vomiting on the day of surgery was found to be more frequent in the high-dose group (p=0.006), (Table 9). No other differences were found between all groups.

Table 9. Frequency of known side effects of ketamine.

	Groups Placebo[1]	Low-Dose S-ketamine[2]	High-dose S-ketamine[3]	Fisher´s exact test p-value
No	49(29.1)	51(30.3)	56(33.3)	
Missing	4(2.3)	4(2.3)	4(2.3)	
Nausea and vomiting day of surgery	1(0.6)	7(4.5)	12(7.7)	0.006 (1 vs 3) 0.060 (1 vs 2)
Nausea and vomiting day after surgery	2(1.3)	2(1.3)	6(3.9)	0.351
Nightmares day of surgery	1(0.6)	1(0.6)	4(2.6)	0.372
Hallucinations day of surgery	0(0)	1(0.6)	1(0.6)	1.000

Placebo=saline solution, Low-Dose=0.125mg/kg, High-Dose=0.250mg/kg. Number (%).

The subjects had the opportunity to describe any other complaints in a free text section. Sixty-two out of 168 subjects used that opportunity. Local problems at the site of surgery as well as pain that was not sufficiently relieved by the rescue pills were the most common complaints.

Paper III

Several inflammatory biomarkers in plasma showed significant changes when compared preoperatively with two hours after the start of the operation. IL-6, CST5, FGF-21, TGF-alpha, OSM, IL-10, WBC, and NEUT increased while Flt3L, FGF-19 Cortisol, and hsCRP decreased (Table 10 & 11). The administration of S-ketamine 0.125mg/kg and 0.25mg/kg did not significantly influence any measured inflammatory biomarkers in plasma within two hours.

Table 10. Effects of the surgical trauma on biomarkers. Wilcoxon signed rank test comparing B to A in the **placebo** group. A=direct prior to start of surgery. B=2 hours after start of surgery.

Name	Unit	n	Median A	Median B	B-A (median (IQR))	p-value	Σ	s/ns
Corti-sol	nmol/L	52	348.05	169.65	-152.90 (-265.38 - 80.00)	1.1 × 10−8	↓	s
NEUT	$x10^9$/L	51	3.00	4.10	0.70 (0.30 - 1.75)	7.8 × 10−8	↑	s
WBC	$x10^9$/L	51	5.20	6.40	0.60 (-0.15 - 1.50)	9.2 × 10−5	↑	s
hsCRP	mg/L	51	0.70	0.70	-0.10 (-0.10 - 0.00)	1.0 × 10−4	↓	s

All changes are significant at 95% CI. Σ=direction of change.

Table 11. Effects of surgical trauma on biomarkers.

N=52							
Assay	Mean A	Mean B	Ratio (B/A)	Σ	p-value	q-value	s/ns
IL-6	1.86	4.46	6.05 (4.81 - 7.61)	↑	3.0×10^{-21}	2.8×10^{-19}	s
Flt3L	8.40	8.04	0.78 (0.73 - 0.83)	↓	2.0×10^{-10}	9.3×10^{-9}	s
CST5	6.44	6.98	1.45 (1.31 - 1.60)	↑	7.7×10^{-10}	2.4×10^{-8}	s
FGF-21	3.71	4.15	1.36 (1.20 - 1.53)	↑	5.5×10^{-6}	1.3×10^{-4}	s
FGF-19	7.46	7.02	0.74 (0.65 - 0.84)	↓	3.1×10^{-5}	5.8×10^{-4}	s
TGF-alpha	2.10	2.28	1.13 (1.06 - 1.21)	↑	1.9×10^{-4}	2.8×10^{-3}	s
OSM	3.25	3.72	1.39 (1.18 - 1.65)	↑	2.2×10^{-4}	2.9×10^{-3}	s
IL-10	2.09	2.34	1.19 (1.08 - 1.30)	↑	4.9×10^{-4}	5.7×10^{-3}	s
TRANCE	4.60	4.43	0.89 (0.83 - 0.97)	↓	5.9×10^{-3}	6.0×10^{-2}	ns

Paired t-test comparing B vs A for patients within the **placebo** group. The columns show: Assay, mean A - mean NPX among A, mean B - mean NPX among B; ratio - the ratio B/A (with 95% CI); % change; Σ=direction of change ↑increase ↓decrease; p-value - the p-value; q-value - adjusted p-value using Benjamini-Hochberg's FDR method; significant/not significant. Top proteins are shown (q-value < 0.10), sorting according to p-value. A=direct prior to start of surgery. B=2 hours after start of surgery. N=52.

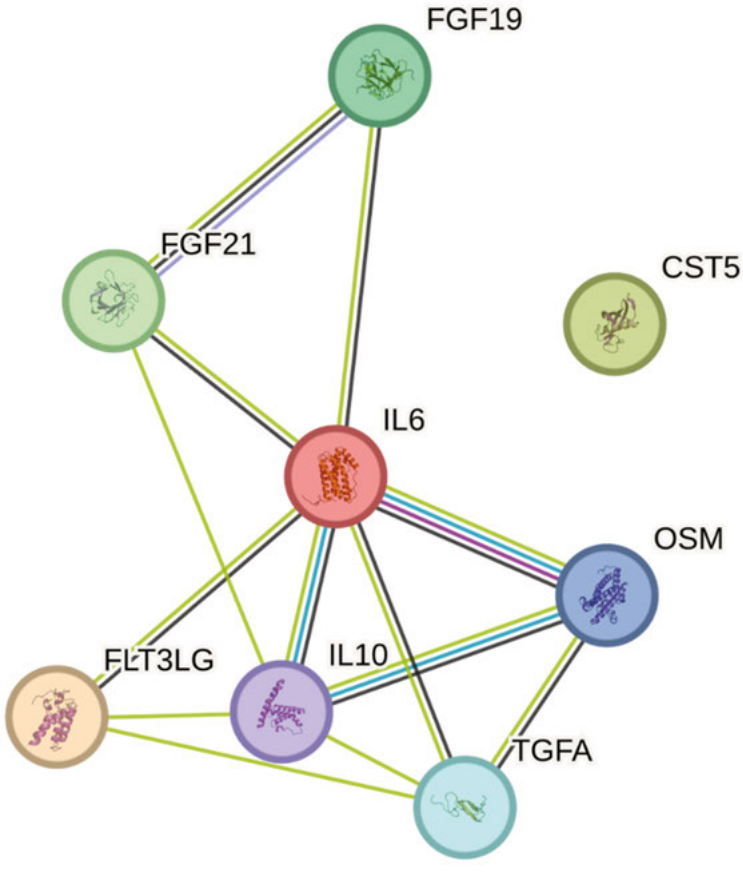


Figure 9. Protein interactions, 8 nodes (proteins), 13 edges, expected number of edges 3, protein-protein-interaction (PPI) enrichment p-value: 1.28 x 10-5. The edge width represents the strength of the interaction (From the web page of STRING database, String Consortium 2025: SIB – Swiss Institute of Bioinformatics, CPR – Novo Nordisk Foundation Centre Protein Research, EMBL – European Molecular Biology Laboratory).(235-237).

Paper IV

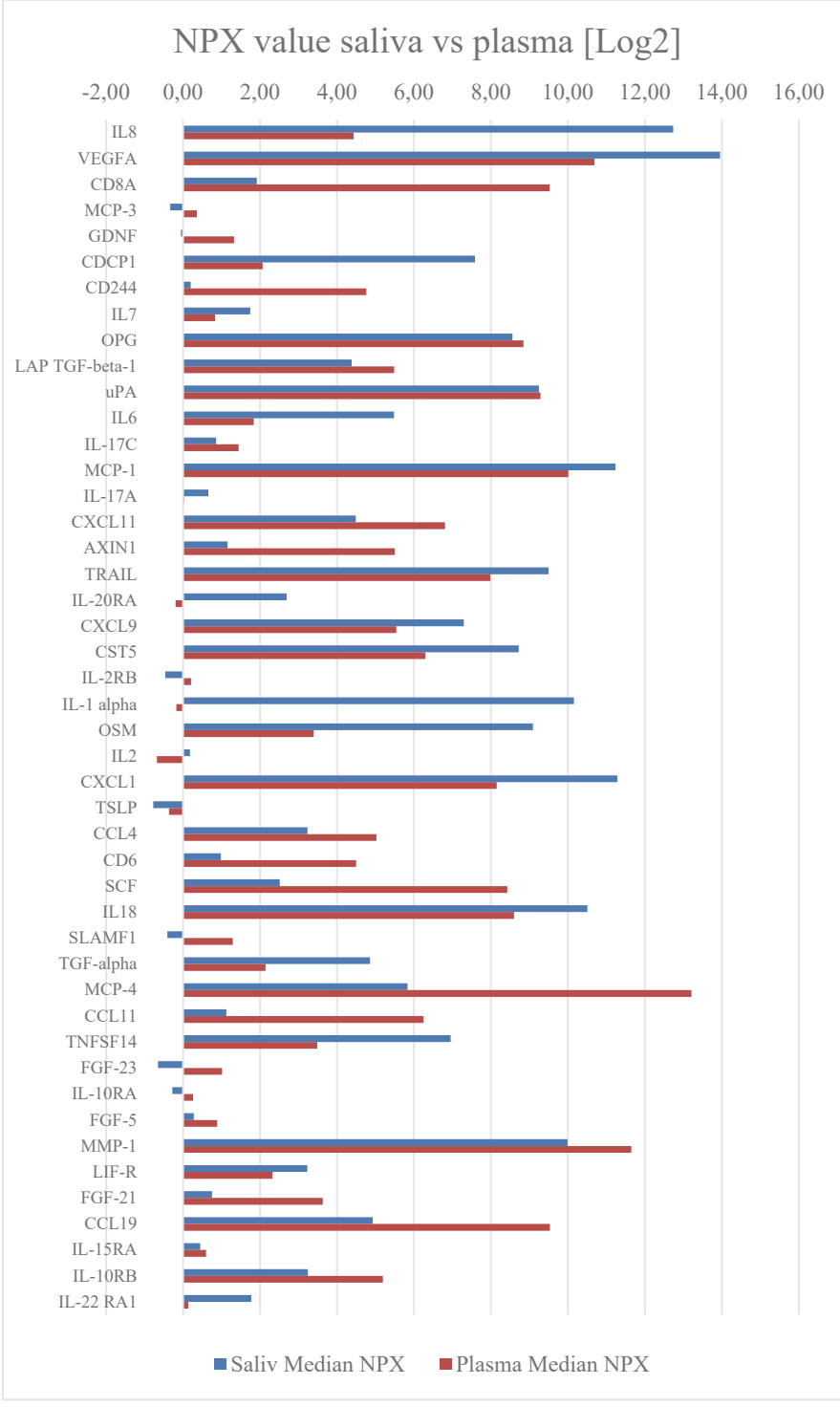
The inflammatory biomarkers IL-6 and CST5 showed a correlation between their expression in saliva and plasma. The correlation showed statistical significance even though the correlation was weak (Table 12). A few CCGF were more clearly expressed in saliva than in plasma and vice versa. This exploratory study cannot explain any similarities or differences between the biomarker's expression in these two biofluids (Figure 10). The question is whether measurements in saliva are useful as a surrogate for plasma. Further research is needed to clarify this relationship.

Table 12. An earlier study (Eriksson et al., submitted 2025) showed eight cytokines in plasma that significantly increased or decreased, pre- versus post- operatively, due to third molar surgical trauma; these were tested for correlations, against the same cytokines in saliva (s/ns = significant/not significant).

Saliva-cytokine	Plasma cytokine	Uniprot	Spearman(r_s)	p-value	s/ns
IL6	IL6	P05231	0.153554	0.048937	s
CST5	CST5	P28325	-0.254204	0.000985	s
OSM	OSM	P13725	0.076021	0.331802	ns
TGF-alpha	TGF-alpha	P01135	0.104004	0.183712	ns
FGF-21	FGF-21	Q9NSA1	0.079302	0.311299	ns
IL10	IL10	P22301	0.078783	0.315976	ns
Flt3L	Flt3L	P49771	0.105294	0.178308	ns
FGF-19	FGF-19	O95750	0.023853	0.761040	ns

Table 13. Thirty-six significant correlations between inflammatory biomarkers in saliva and plasma.

Saliva-cytokine	Plasma-cytokine	Spearman(r_s)	p-value	s/ns
IL6	IL6	0.153554	0.048937	S
CST5	IL-17A	0.153931	0.048378	S
CST5	CXCL11	0.171429	0.027692	S
CST5	CXCL9	0.155734	0.045778	S
CST5	CST5	-0.254204	0.000985	S
OSM	IL6	0.166711	0.032341	S
FGF-21	GDNF	-0.186047	0.016731	S
FGF-21	CXCL9	0.163984	0.035321	S
IL10	AXIN1	0.154051	0.048201	S
FGF-19	IL-17A	-0.156349	0.044918	S
FGF-19	TRAIL	0.198042	0.010777	S
FGF-19	CST5	0.190500	0.014250	S
CST5	CCL11	0.186838	0.016264	S
FGF-21	OSM	-0.160254	0.039767	S
Flt3L	IL18	0.190564	0.014216	S
FGF-19	IL18	0.170855	0.028225	S
FGF-19	IL-15RA	0.191099	0.013942	S
FGF-19	IL-18R1	0.193329	0.012846	S
IL6	IL-24	0.190666	0.014164	S
FGF-21	ARTN	-0.158114	0.042524	S
FGF-21	CXCL10	0.212342	0.006179	S
Flt3L	CD5	0.156090	0.045279	S
FGF-19	DNER	0.195825	0.011711	S
IL6	CCL20	0.201867	0.009318	S
CST5	MCP-2	0.185895	0.016822	S
CST5	CCL20	0.199685	0.010127	S
OSM	MCP-2	-0.171520	0.027608	S
OSM	TNFB	-0.184818	0.017478	S
TGF-alpha	CCL25	0.177012	0.022938	S
TGF-alpha	TNFRSF9	0.206492	0.007791	S
FGF-21	IFN-gamma	0.161259	0.038525	S
IL10	FGF-19	-0.207023	0.007630	S
IL10	TNFB	-0.212160	0.006224	S
Flt3L	TNFRSF9	0.159100	0.041235	S
FGF-19	TNFRSF9	0.164184	0.035094	S
FGF-19	ST1A1	0.163751	0.035585	S


NPX value saliva vs plasma [Log2]
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0,00
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
IL8
VEGFA
CD8A
MCP-3
GDNF
CDCP1
CD244
IL7
OPG
LAP TGF-beta-1
uPA
IL6
IL-17C
MCP-1
IL-17A
CXCL11
AXIN1
TRAIL
IL-20RA
CXCL9
CST5
IL-2RB
IL-1 alpha
OSM
IL2
CXCL1
TSLP
CCL4
CD6
SCF
IL18
SLAMF1
TGF-alpha
MCP-4
CCL11
TNFSF14
FGF-23
IL-10RA
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CCL19
IL-15RA
IL-10RB
IL-22 RA1
Saliv Median NPX
Plasma Median NPX

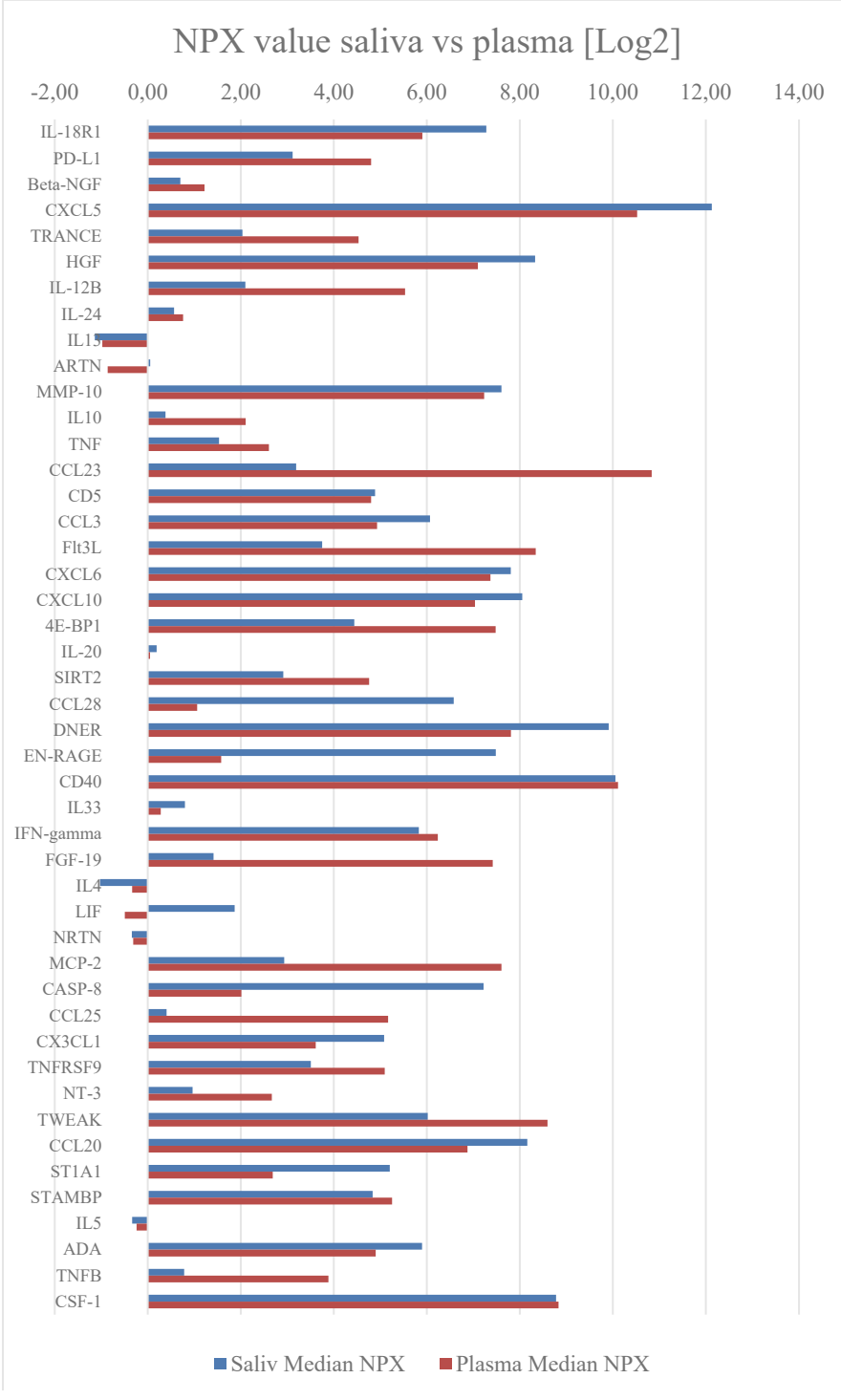

Figure 10. Relative expression of inflammatory biomarkers in saliva and plasma.

Supplementary results

Table 14. Perioperative midazolam [MZ] dose aspects at different times related to group.

	Midazolam dose			
Group	MZ dose at sedation endpoint [mg]	MZ dose at end of surgery [mg]	Total MZ dose / minute operation time [mg/min]	Total MZ dose / kg bodyweight [mg/kg]
Placebo				
Median	7.50	12.5	0.94	0.10
Max	22.5	70.0	12.5	0.28
Min	2.5	7.5	0.26	0.04
S-Ketamine 0.125 mg/kg				
Median	7.50	12.5	0.79	0.09
Max	20.0	30.0	3.2	0.32
Min	2.5	3.75	0.29	0.02
S-Ketamine 0.250 mg/kg				
Median	7.50	13.8	0.89	0.11
Max	27.5	37.5	3.75	0.27
Min	2.5	5	0.34	0.03

Table 15. Duration of surgery in relation to radiological position - a possible indicator of surgical complexity.

	Duration of surgery [minutes]					
Radiological position	N	Missing	Median	IQR	Minimum	Maximum
Vertical	31	0	12	7.5	5	28
Mesioangular	42	0	16	6.75	8	31
Horizontal	45	0	19	11	8	70
Distoangular	50	0	13	12.5	6	48

Discussion

Methodological strengths and weaknesses

The most obvious strength with this study's method is that it is a randomized controlled trial. Furthermore, it is placebo-controlled and double blind. It is also stratified by sex. The study population is homogenous in reference to age (18 to 44 years). The study population is a sample of the population and includes patients with no or few health problems, as well as no or few prescribed medicines. In summary, these circumstances minimize the occurrence of bias and other confounders that could adversely affect the outcome and therefore the reliability of the study. The same surgeon (LBE) performed all operations, which increases the likelihood that every patient was treated the same way. This might also be seen as a weakness: the method is admittedly effective when performed by a single surgeon, but it is unclear whether the same method would work in the hands of several surgeons. However, studies always involve compromise due to factors such as time and feasibility. The goal was to mimic the standard clinical setting as much as possible and minimise the effects of the scientific part of the study on the participants. It would have been an advantage to having multiple samples of saliva and plasma from different occasions. And yet, if the subjects had to be admitted to the ward for a more thorough blood sample regime during a longer period of time, then we would have deviated from the standard clinical procedure. Another potential weakness is that this is a single centre study, yet we found the study sufficient in scope for an individual student´s PhD project. A multicentre study would have required the enrolment a much larger population and would have required significantly more administrative work to coordinate. Given the framework of a PhD thesis our compromises are deemed to be reasonable. Another potential weakness may be the relatively long period for enrolment, which was partially caused by the SARS Covid-19 pandemic. It is not entirely straightforward to decide whether to use a placebo or an active comparator in this study, since the intervention occurs at a time when no medication is typically administered in routine care. Nevertheless, in this study, participants received either S-ketamine (in two different doses) or a placebo. The active comparator might have been ibuprofen, which is the standard postoperative pain management regime, but in this study, ibuprofen was given as a rescue medication.

Methodological considerations

To identify and quantify protein-based biomarkers in different body fluids a number of methods can be used. Immunoassays as: Enzyme-Linked Immunosorbent Assay (ELISA) (238), Western blotting (239), and Immunoprecipitation. Mass spectrometry methods (240) such as Liquid Chromatography - tandem mass spectrometry and Surface Enhanced Raman Spectroscopy (SERS) or other methods: UV absorbance, Protein microassay can be employed. In this study proximity extension assay (PEA) a multiplex immunoassay process provided by OLINK (230) were chosen mostly based on the fact that quite a lot of recent pain research focused on biomarkers had used the same method (170, 174, 176, 179, 241-246). This circumstance of course increased the ability to more easily compare findings with those of other researchers. An advantage with the method is the small amount sample, only 1μL (saliva or plasma), needed in combination with the large number of protein analysis it delivers. The method is widely used in neighbouring fields such cardiovascular research (237, 247, 248) and cancer research (249, 250), and while other biomarkers are often analysed, the same technique is used.

Skewed sex representation

A weakness of the study is the imbalance between sexes regarding the number of enrolled participants, since females represent a two thirds majority. A worldwide prevalence study pointed out a slightly higher likelihood of third molar impaction among women compared to men, with an odds ratio = 1.173 (251). This explains a minor part of the difference but is far from being conclusive. One might speculate that women as a group are more likely to seek healthcare and more likely to accept offered treatment. The same kind of skewed sex distribution has been found in a previous study (20). It is possible to consider that women are generally more open to participate in scientific studies as an unselfish act, but this interpretation has, in part, been challenged by other studies (252, 253).

Pain and how to measure it – a never-ending story

The first study showed positive results on the pain reducing effect measured by self-reported VAS in the high-dose group (S-ketamine 0.25 mg/kg) and not in the low-dose (0.125 mg/kg) or placebo groups. This may imply that the population was too small to detect minor differences. It may also signal that a higher dose is needed to achieve the desired analgesic effect.

To measure VAS and calculating changes in VAS is just a mathematical exercise, so the core question is what constitutes a clinically important difference? What is acceptable pain control? Myles et al. states that 10mm is an important change and VAS 33mm or less indicates acceptable pain control (254). Another author states that a 30% reduction of pain intensity measured on VAS is a clinically important difference (255). And yet, use of the VAS can be questioned. When compared to NRS and VRS, the VAS tool is found to be more sensitive to treatment effects than the other two. On the other hand, VAS is often found to be more complicated to comprehend for older patients and those who are cognitively challenged; it is unfamiliar to most patients according to Cooper et al. (50). I would argue that VAS is, however, much more familiar to the average person than NRS or VRS, which are often completely unknown. Nevertheless, whichever scale is chosen, patients must be instructed on how to use it correctly. Cooper et al. further state that "until there is more definitive evidence of a superior rating scale, we recommend that an NRS measure of Pain Intensity or Pain Relief be included in all acute pain trials involving adults" (50). Yet, the choice of pain assessment scale used in other comparable studies must also be considered.

VAS was chosen in this study to evaluate pain intensity despite its shortcomings and was considered to be the best instrument for the task. If time from intervention to evaluation had been significantly longer than a few hours, a quality-of-life questionnaire would have been more relevant, even though this instrument is better placed to measure how the pain experience is interpreted by the patient.

The patients in the high-dose group (S-ketamine 0.25 mg/kg) had a slightly longer time (12 min) from end of surgery to first request for rescue medication, compared to the low-dose and placebo-groups. In the literature there are indications that this timeframe can be longer (17).

Safety

Oxygen desaturation as an effect of the pharmacological regime is a safety hazard to the patient. In the second study, patients given the highest dose of S-ketamine had a slightly lower SpO_2 at the end of infusion of S-ketamine compared to the low-dose and placebo groups. Even though the difference was statistically significant it was small enough to be found clinically insignificant. The study did not include an opioid comparator. Nevertheless, it can be assumed that switching S-ketamine for an opioid would have caused even more desaturation. Ketamine has been used to reduce the risk of unwanted effects on respiration (256). A similar situation was found in relation to pulse-rate, with the high-dose S-ketamine group compared to placebo, where a significant

higher pulse-rate was observed in the high-dose group. Even in this comparison the difference displayed is statistically yet not clinically significant. Previous research tells us that S-ketamine has little or no influence on vital parameters such as pulse, respiratory rate, blood pressure, and SpO_2, compared to other equipotent analgesics or anaesthetics (48, 89, 96, 105-107, 114, 257).

On the day of surgery, the high-dose S-ketamine group reported more PONV than the other groups. This is not in line with previous publications and should therefore be interpreted with caution. There is even evidence that suggests that ketamine reduces the risk of PONV compared to placebo (17).

The frequency of oxygen supplementation was described in paper II. This was more frequent in both the intervention groups than in the placebo group. S-ketamine's pharmacological properties cannot explain this finding. Indeed, the opposite is what would normally be expected. There were no strictly defined criteria about when to give supplementary oxygen. The instruction was based on clinical experience on when it is appropriate to give extra oxygen. Obviously, every patent whose saturation (SpO_2) dropped below 90%, and even some with a substantial decrease in saturation (including above 90%), received supplementary oxygen in accordance with the clinical situation.

In a large study from USA, 42954 patients with the COVID-19 infection required mechanical ventilation. In a retrospective cohort study design, the use of early ketamine sedation was related to clinical outcomes (258). Only 1423 (3.3%) received ketamine sedation according so the study definition, which was initiated within 2 days of intubation and continued for more than 1 day. The authors concluded that early ketamine may have an association with higher hospital mortality, ICU stay and days on ventilation days (258). However, the higher use of extracorporeal membrane oxygenation (ECMO) in the ketamine group was 7.8 %, compared to 4.7% in the matched group, which may indicate that these patients were more severely ill than first thought. The use of ketamine was merely an attempt to stabilize the cardiovascular parameters rather than the cause of an inferior outcome.

Findings in plasma and saliva samples

Paper III showed significant changes in routine blood samples as well as in CCGF within two hours of the surgical trauma, following third molar surgery. The fast and diverse response indicates a high grade of sensitivity in the defence system; it also indicates that the third molar surgery as a pain model is relevant. S-ketamine infusion 0.25 or 0.125 mg/kg did not cause significant alterations in routine blood samples or in CCGF.

Not least in an odontological context, saliva is easily available for sampling and analysing. Paper IV therefore aimed to compare saliva and plasma to see to what extent saliva could provide the same protein information as plasma. Even though the findings show some similarities, there are significant differences, which led to the conclusion that saliva cannot replace plasma when monitoring certain biomarkers. There are differences between unstimulated saliva and stimulated saliva. The content of saliva varies with the flow rate. The flow rate of saliva has a circadian variation (139). These aspects may well influence our findings when saliva samples are analysed.

S-ketamine pros and cons

Ketamine is known to have anti-inflammatory properties (83). The indication that S-ketamine reduces the titre of IL-6 in a dose dependent manor is not new, but we failed to reach significance in our material. Through its anti-inflammatory effect, S-ketamine might influence the analgesic efficacy of S-ketamine that is unrelated to or at least beyond the effect exerted via NMDA-receptors. In vitro, ketamine reduces interleukin-6 (IL-6), IL-8, and tumour necrosis factor-alpha (TNF-alpha) in human whole blood (48, 259). S-ketamine reducing IL-6, cortisol, and TNF-alpha are also shown to have an effect in the treatment of depression (125). Similar effects have been shown in vivo in rats (260, 261). Several studies have shown that ketamine suppresses the production of pro-inflammatory cytokines (259-262). Ketamine has been reported to have both immunomodulatory and anti-inflammatory properties. The pro-inflammatory cytokines IL-1-beta, IL-6, and TNF-alpha are decreased by ketamine (260, 261, 263).

S-ketamine has in different studies, including one of ours (Paper I), been shown to reduce pain experience or pain intensity during a longer period, despite the T½ of S-ketamine (17). The explanation most likely are related to differences in the milieu inside versus outside the blood-brain-barrier. The T½ relates to the concentration in periphery blood or plasma whilst the long-term effect relates to things like concentration and receptor affinity in the CNS, and more specifically in the brain. These factors are not fully known and difficult to investigate in humans.

A recent publication has revealed several new metabolites of ketamine (92). The exact action of every one of these are of course yet unknown. It is reasonable to expect, however, that at least some of these metabolites will play an active role in ketamine´s properties.

S-ketamine can be used in an abusive context, though there is a minimal risk for oral and maxillofacial surgery in general, and third molar surgery in particular, that this would lead to an addiction. S-ketamine is never prescribed to the patient for use outside the clinic. The exposure to S-ketamine via third molar surgery is limited to only one or a few occasions, and under strict clinical conditions.

There are indications that ketamine has a valid place in the treatment of certain chronic pain states. The fact that ketamine has been found non inferior to ECT in treating major depressions (124) might relate to its strength in treating pain. Chronic pain patients, for example, also often suffer from depression. The lateral habenula (LHb) in the brain is a key area for the regulation of depression, pain, and cognition (264). Patients suffering from neuropathic pain frequently suffer from impaired cognitive function and affective behaviour (264). Neuropathic pain patients are more likely to suffer from affective disorders such as depression (264, 265). Blocking the activity of the LHb relieves negative emotions related to pain as well as nociceptive hypersensitivity (67, 264). There is epidemiological evidence that depression has a lot of co-occurrent disorders, such as nicotine dependence, substance use, anxiety disorders, insomnia, and chronic pain (266). LHb plays a central role in pain-associated negative behaviours: depression- and anxiety-like behaviours, aversion or anti-award, aggression, defensive behaviour, and substance use disorder (67).

S-ketamine most certainly has a place in third molar surgery. Most likely, S-ketamine will be useful in cases where a significant but short-acting form of pain control is preferred; in cases where opioids ought to be avoided; in cases that involve pre-existing pain conditions; and/or in cases where there is a known risk of developing chronic pain. Elderly people may also benefit from S-ketamine as an alternative to opioids. The current study model would likely benefit from a slight increase in the dose/kg bodyweight ratio.

In some cases, the goal is not to eliminate the use of opioids but to optimize the pain control; a combination of opioid and ketamine can then be the best solution. Positive experimental effects on preventing hyperalgesia and wind-up like pain sensations has been shown by Schulte et al., when combining ketamine and morphine (267).

In a double-blind comparison of remifentanil (1µg/kg) vs ketamine (0.7mg/kg) for induction of anaesthesia in 75 preschool children in day case adenoidectomy, Tarkkila et al. found BP and heart rate better attenuated in the remifentanil group than the ketamine group (268). They concluded that remifentanil provides a hemodynamically more stable induction in comparison to ketamine or placebo. Further, they did not find any advantage with ketamine concerning opioid-sparing effects (268).

This illustrates that every population and every type of surgical procedure has its own optimal matching analgesics or anaesthetics. In most clinical cases, opioids are more prone to interfere with hemodynamic and respiratory variables, compared to ketamine. The choice of drug, as well as the dose and the size of the study population will influence the outcome. Remifentanil are associated with decreased heart rate (269), decreased blood pressure (270), and decreased cardiac output (270, 271).

Conclusion

Preoperative S-ketamine 0.25 mg/kg gave a global statistically significant reduction of spontaneous pain by VAS during the first 24 h postoperatively.

Preoperative S-ketamine 0.125 and 0.25 mg/kg gave no significant pain reduction at 4 h postoperative.

The time from end of surgery to first rescue medication was slightly longer in the 0.25 mg/kg group compared to both low-dose S-ketamine and placebo groups.

It was safe to use adjunct, preventive intravenous S-ketamine 0.25 mg/kg body weight, for pain relief in midazolam-sedated patients receiving third-molar surgery.

There were no serious adverse events or symptoms of overdose nor any clinically relevant effects on circulatory or respiratory parameters.

Third molar surgery influenced several inflammatory biomarkers in plasma by increasing IL-6, CST5, FGF-21, TGF-alpha, OSM, and IL-10 and decreasing Flt3L and FGF-19.

Third molar surgery influenced certain blood cells within 2 hours. WBC and NEUT were increased by third molar surgery.

S-ketamine IV in analgesia dose did not influence the early inflammatory response measured by inflammatory biomarkers.

Several cytokines in saliva are weakly but still significantly correlated to cytokine expression in plasma.

Saliva and plasma are not interchangeable when monitoring inflammatory biomarkers.

Clinical implications

S-ketamine IV can be considered in third molar surgery, especially in cases with IV midazolam sedation. To gain the maximum benefit of S-ketamine use, patients with preexisting pain states or in cases with expected extraordinary postoperative pain intensity should be prioritized. In cases where opioids should be avoided or minimized, S-ketamine may also be an advantageous choice. Sub-anaesthetic doses are not associated with any severe side effects. Nightmares and hallucinations are rare, and the influence on cardiopulmonary functions is negligible, and lacks clinical relevance. All of this can be reassuring for patients who will undergo this treatment. Extrapolations, however, should always be made with caution. Yet, it is still reasonable to assume that IV S-ketamine also has a place in other dentoalveolar surgery such as removal of smaller cysts and tumours, as well as implant surgery. The tissue trauma of third molar surgery causes early changes in inflammatory biomarkers in blood and plasma. This must be considered when assessing possible postoperative infections/inflammations, especially when deciding on the need for other treatments such as antibiotics. It is also important when evaluating postoperative blood test results in general. All new knowledge deepens the understanding of third molar surgery as a pain model.

Future perspectives

Starting with the findings of this thesis, there are several interesting research questions to answer or attempt to answer. There is room for further pharmacological questions, such as, what happens to side effects vs pain relief if the sub-anaesthetic dose of S-ketamine is increased even further? Now we know which biomarkers are the most prominent in plasma and blood two hours after the start of surgery, but which biomarkers dominate after thirty minutes, a day, or a week? What connection do these biomarkers have to the patient´s reported pain intensity? Can the patient´s mental status, such as the presence of depression, anxiety, and/or dental fear, be reflected in the biomarkers and/or self-reported pain intensity? Artificial intelligence (AI) will inevitably have a major impact in the scientific world for the foreseeable future. Whether these impacts truly benefit humanity is something future generations will have to evaluate. From what is obvious today, there is great potential in AI to interpret large amounts of data, such as biomarkers, to find significant patterns that can help us to better understand biological processes.

Today´s patients who are more demanding and the profession handling an increasing number of available methods, requires more individualized pain management. More medicines and combinations of medicines must be scientifically tested for every type of surgical procedure and every type of patient in a broader range than ever before, to be able to offer the best possible pain management. Beyond sex, age, and bodyweight, studies will need to involve genetic information about various kinds of vulnerability. Both somatic and psychological properties need to be addressed when designing the optimal pain management for tomorrow and further into the future.

The era of genetically designed pain management is just beginning but will most likely have a central role in the future management of pain, especially in chronic pain and various complex pain syndromes where the treatment options we have today are too few, too blunt, or too inaccurate. I believe that we can look forward to pain treatments that will target specific cells and receptors, as well as limited areas or functions in the central and peripheral nervous systems.

There are a lot of exciting new projects to explore and develop. One interesting area includes neuropathic pain – depression – ketamine, where the lateral habenula region of the brain is a common denominator. Here are several possible approaches: pharmacological, radiological with fMRI, biochemical with cytokines in CSF, psychiatric one at a time or in combinations.

Inflammation seems to be associated with an increasing number of diseases and pathological conditions. By expanding our knowledge about the mechanisms of immune reactions and inflammations, this holds the key to many future treatments, including the treatment of chronic pain states.

The field of inflammatory biomarkers is as vast as the field of pain research, and the combination opens endless possibilities to find new pieces to fit into the eternal puzzle of science.

Sammanfattning på svenska (Summary in Swedish)

Att kirurgiskt avlägsna visdomständer i underkäken är ett av de vanligaste kirurgiska ingreppen i världen. Visdomstandskirurgi är också en väletablerad modell för att studera smärtafysiologiska processer och vid utveckling av smärtstillande läkemedel. Vanligtvis avlägsnas visdomständer i lokalanestesi. Tandvårdsrädsla är relativt vanligt inför ingreppet. Vanligast är att de tandvårdsrädda patienterna erbjuds sedering (lugnande medicinering) med bensodiazepin företrädesvis midazolam.

Visdomstandskirurgin ger en postoperativ smärta. Denna smärta är värst ca 5 timmar efter operationsstart, vilket i stort sett sammanfaller med när lokalbedövningen försvinner. Denna smärta kvarstår vanligen under flera dagar och i enstaka fall i veckor innan smärtfrihet inträder. När lokalbedövningen slutar verka behöver patienten annan effektiv smärtlindring. Det vanligaste är en kombination av läkemedlen: paracetamol och ibuprofen i tablettform, som har visat sig vara mer effektivt än enbart ett av preparaten för sig. Ibland är denna smärtlindring otillräcklig.

I denna avhandling valde vi att prova S-ketamin för detta ändamål. Ketamin utvecklades i USA och registrerades på den svenska marknaden i början 1970-talet som ett narkosläkemedel. Ketamin har genom åren använts inte enbart som narkosmedel utan också för smärtlindring/analgesi då i lägre doser.

I våra studier har personer som remitterats till käkkirurgiska kliniken vid Falu lasarett för kirurgiskt avlägsnande av en eller flera underkäksvisdomständer erbjudits deltagande i studien om de uppfyllt inklusionskriterierna. De personer som tackade ja fick vid undersökningstillfället fylla i enkäter med frågor om smärta, depression & ångest. Vid besök två (operationstillfället) randomiserades deltagarna till en av tre grupper 1) midazolam + placebo, 2) midazolam + S-ketamin 0.125 mg/kg kroppsvikt eller 3) midazolam + S-ketmin 0.25 mg/kg kroppsvikt. De fick före operationsstart lämna saliv- och blodprover. Blodprovstagningen upprepades två timmar efter operationsstart. Alla patienter fick intravenös sedering med midazolam och i två av de tre grupperna fick patienterna också S-ketamin intravenöst. Under operationen övervakades och protokollfördes läkemedelsdoser, tidpunkter för operationens start och slut

samt syrgasmättnad (SpO_2), andningsfrekvens, blodtryck, puls och sederingsgrad. Efter operationen fick patienterna med sig en smärtdagbok för att fylla i smärtintensitet (VAS), tidpunkt och antalet använda värktabletter som behövts som extra smärtlindrande hjälp och eventuella biverkningar. Totalt inkluderades 168 patienter i studierna.

Delarbete 1 avsåg att besvara frågan om S-ketamin tillförde något ytterligare smärtlindring till den aktuella gruppen och om det läkemedlet påverkade tiden till att extra smärtlindring behövdes samt om den totala analgetikakonsumtionen påverkades. Vi fann att de som fick den högre dosen av S-ketamin hade över 24 timmar totalt sett lägre smärta (VAS), men smärtan vid den primära utvärderingen 4 timmar postoperativt skiljde inte mellan grupperna. Tiden till extra smärtlindrings-hjälp var något längre i gruppen med högsta S-ketamindosen, men skillnaden var så liten att den är försumbar.

Delarbete 2 avsåg att besvara frågan om det är patientsäkert att ge S-ketamin till aktuell patientgrupp vid aktuellt ingrepp. Det primära utfallsmåttet för säkerhet var SpO_2 och i andra hand förändringar i andningsfrekvens, puls, blodtryck eller biverkningar. Vi fann att SpO_2 uppvisade en signifikant skillnad mellan gruppen som fick högst dos S-ketamin jämfört med placebogruppen med en lätt sänkning av SpO_2 i högdos gruppen, ingen ytterligare skillnad hittades avseende SpO_2 eller andningsfrekvens. Vid samma tidpunkt noterades högre pulsfrekvens för gruppen som fick högst S-ketamin dos jämfört med placebogruppen. Det fanns inga ytterligare skillnader avseende pulsfrekvens eller blodtryck. Illamående och kräkningar under operationsdagen var vanligare hos de som fick högst dos S-ketamin. Inga skillnader sågs mellan grupperna avseende hallucinationer, mardrömmar eller illamående och kräkningar dagen efter operationen.

Delarbete 3 avsåg att kartlägga i vilken utsträckning hur det kirurgiska traumat vid visdomstandskirurgi och tillförseln av prövningsläkemedel (S-ketamin) påverkar uttrycket av inflammatoriska biomarkörer (CCGF), såsom cytokiner, chemokiner och tillväxtfaktorer. Blodprover togs såväl preoperativt som postoperativt, en del var rutinprover som analyserades direkt (exempelvis; CRP, hsCRP, leukocyter, neutrofila granulocyter samt kortisol). En del av proverna centrifugerades och plasman frystes in för senare analys (-80°C). När alla patienter var inkluderade analyserades de frysta plasmaproverna med en specifik analysmetod från företaget OLINK som gav 92 svar per prov, analyssvaren visade relativ mängd av de inflammationsrelaterade proteinerna, CCGF. Vid jämförelser pre- och postoperativt fann vi att det kirurgiska traumat påverkar flera inflammatoriska biomarkörer i plasma; motsvarande effekt åstadkoms inte av prövningsläkemedlet.

Delarbete 4 avsåg att utröna om det finns samband mellan CCGF i saliv och i plasma. Studien jämförde salivprover med plasmaprover tagna vid samma tillfälle preoperativt och analyserades med OLINK:s inflammationspanel. Det fanns ett fåtal cytokiner som korrelerade mellan saliv och plasma, därutöver noterades några CCGF som hade ett påtagligt tydligare uttryck i saliv jämfört med plasma. Dessa fynd kan ligga till grund för fortsatta studier som kan påvisa mekanismerna till såväl likheter som skillnader.

Avhandlingens slutsatser och kliniska tillämpning vid denna form av kirurgi är:

- S-ketamin kan användas för att förbättra smärtlindringen de första 24 timmarna efter operationen.
- S-ketamin kan användas i stället för opioider i de fall där man förväntar sig kraftigare postoperativ smärta.
- S-ketamin kan övervägas till patienter med pågående smärta.
- S-ketamin kan användas i sub-anestetiska doser med bibehållande av hjärt- och lungfunktion och med liten risk för allvarliga bieffekter.
- Mardrömmar och hallucinationer är ovanliga med givna sub-anestetiska doser. Detta kan vara fördelaktigt för patienter att veta vid behandling med S-ketamin.
- Vävnadstraumat orsakat av visdomstandskirurgi åstadkommer tidiga reaktioner hos inflammatoriska biomarkörer i blod och plasma. Detta bör beaktas när man utvärderar möjlig postoperativ infektion/inflammation.
- S-ketamin i sub-anestetiska doser verkar inte påverka uttrycket av inflammatoriska biomarkörer i plasma.
- Några av de inflammatoriska biomarkörerna i saliv och plasma (IL-6 och CST5) är svagt korrelerade till varandra.
- Saliv och plasma är inte inbördes utbytbara/likvärdiga när man mäter förekomst av inflammatoriska biomarkörer.

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