

# THE BIOCHEMICAL IMPACT OF SURGERY AND ANESTHESIA



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# THE BIOCHEMICAL IMPACT OF SURGERY AND ANESTHESIA

## DE BIOCHEMISCHE GEVOLGEN VAN CHIRURGIE EN ANESTHESIE

### **Proefschrift**

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# CHAPTER 1

## Introduction

The impact of surgical injury and anesthesia on stress related biochemical intermediates in human plasma



General anesthesia has been considered by some medical historians as one of the most important contributions to modern medicine second to perhaps the concept of antiseptic medicine and hygiene. The first historical mention of a deep unnatural sleep so that surgery can take place can be found in the old testament, "So the LORD God caused the man to fall into a deep sleep; and while he was sleeping, he took one of the man's ribs and then closed up the place with flesh." However, when inhalational anesthesia was administered for the first time in 1846, it was discovered that there were potential dangerous side effects that could lead to death and potentially complicate surgical outcome. It was also postulated that it could "poison" the blood in unforeseen ways. Today, medical practitioners and patients know that anesthesia provides the required level of comfort and protection necessary to carry out invasive medical procedures.

Prior to the age of anesthesia, most surgery was limited to amputations and cauterizing wounds with hot oil and irons. The speed of the surgery was essential and was associated with lower mortality. Faster surgeons provided better results. The foremost surgeon of his age, London surgeon Sir Robert Liston was known for his ability to amputate a leg within 30 seconds. Only one out of ten of his patients died, while slower surgeons had mortality rates of one in four. Perhaps, faster surgery allowed for a less distressing surgical experience and therefore ultimately improved survival <sup>1</sup>.

Since the first days of anesthesia it has been immediately recognized that anesthesia can reduce pain and discomfort during surgery, however, the discussion about whether or not anesthesia dangerously alters a patient's physiological homeostasis is not new. Prior to 1800 surgeons considered pain and stress as a necessary part of surgery. It was considered an essential and crucial stimulant that prevented shock and hemorrhage. In fact, many surgeons attributed death from surgery to a patient's lack of manliness and cowardice.

Anesthesia has subsequently been accepted by the medical community as a necessary medical intervention required during surgery. A great deal of research has focused on the physiological effects of anesthesia, however, surprisingly little is known about the biochemical effects of anesthesia and surgery as a whole. The focus of our research is to study the effects of anesthesia on metabolic and inflammatory pathways during surgery. It is also our intention to look at how severity of surgery influences these pathways.

Craniotomy under general anesthesia and awake function-controlled craniotomy allow for a unique clinical setting where measurements can be made quantifying the biochemical effects of anesthesia during one type of surgical



procedure where the intended variable is general anesthesia. Cytokine profiles will be analyzed in order to scrutinize the effect anesthesia might have on inflammation.

We will also use this opportunity to measure amino acids and determine whether or not the effects of anesthesia influence plasma amino acids levels. It is already known that varying amino acid levels can increase sensitization to pain, neuromodulate and influence nitric oxide levels<sup>2-4</sup>. Glutamine, for example, is an important excitatory neurotransmitter that could be influenced by the hypnotic effects of anesthesia while tryptophan is the direct precursor of serotonin, a well-known neurotransmitter<sup>2,5</sup>.

The second phase of our research intends to find out how the severity of surgery influences inflammatory and associated metabolic pathways. There is a particular emphasis on how severity of surgery influences nitric oxide regulation, the molecule at the center of inflammatory, hemodynamic and clotting mechanisms; a molecule of immense importance, known to only exist for milliseconds at a time<sup>6,7</sup>. In order to attain our goal, we will measure citrulline, ornithine and arginine, key amino acid intermediates involved during pre en post nitric oxide pathways<sup>8</sup>. Ornithine is an amino acid we will pay extra attention to when comparing different types of invasive surgery because it is essential for collagen synthesis and wound repair<sup>9</sup>.

It is well recognized that nitric oxide is a regulatory molecule at the core of the inflammatory cascade<sup>10</sup>. In order to better unravel how severity of surgery affects this inflammatory cascade we will measure the well-studied cytokine IL-6, while at the same time the lesser-known inflammatory mediators kynurenine and neopterin. The relationship between these mediators and that of the amino acid tryptophan is also of interest because it is not only an essential precursor of serotonin, but also that of kynurenine<sup>5</sup>. In addition, tryptophan degradation is correlated to neopterin formation<sup>11</sup>. Not only is neopterin associated with a pro inflammatory state, but it also serves as an estimate of the oxidative stress caused by an activated immune system and has thus been shown to predict the development of septic complications in surgical patients<sup>12</sup>.

While comparing surgery groups of different severity, it is our intention to apply, as far as it is ethically possible, similar anesthesia regimens. Thus, the level of severity will be the intended variable in these chapters. However, due to the tendency that more invasive surgery involves longer operating times, we will have the opportunity to evaluate whether the expected rise of the well established IL-6 cytokine is correlated to the amount of propofol used. It is our intention to analyze the relationship between propofol and IL-6 because there

is conflicting information about what exactly the immunological impact of propofol is <sup>13-15</sup>.

By looking at two operations of differing severity and two similar operations where general anesthesia was the variable in question, we will be able to better understand how anesthesia and surgical severity modulates inflammation, it's amino acid intermediates and the free radical nitric oxide. We hope that this research will help us to better understand the enormously complex mechanisms responsible for various clinical outcomes after surgery and anesthesia.

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# CHAPTER 2

Inflammatory profile of awake function-controlled craniotomy and craniotomy under general anesthesia



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## **Running title**

Cytokine profile during craniotomy

# ABSTRACT

## Background

Surgical stress triggers an inflammatory response and releases mediators into human plasma such as interleukins (IL). Awake craniotomy and craniotomy performed under general anesthesia may be associated with different levels of stress. Our aim was to investigate whether those procedures cause different inflammatory responses.

## Methods

Twenty patients undergoing craniotomy under general anesthesia and 20 patients undergoing awake function-controlled craniotomy were included in this prospective, observational, two-armed study. Circulating levels of IL-6, IL-8, and IL-10 were determined pre-, peri-, and postoperatively in both patient groups. VAS scores for pain, anxiety and stress were taken at four moments pre- and postoperatively to evaluate physical pain and mental duress.

## Results

Plasma IL-6 level significantly increased with time similarly in both groups. No significant plasma IL-8 and IL-10 change was observed in both experimental groups. The VAS pain score was significantly lower in the awake group compared to the anesthesia group at 12 hours postoperative. Postoperative anxiety and stress declined similarly in both groups.

## Conclusion

This study suggests that awake function-controlled craniotomy does not cause a significantly different inflammatory response than craniotomy performed under general anesthesia. It is also likely that function-controlled craniotomy does not cause a greater emotional challenge than tumor resection under general anesthesia.

## KEYWORDS

Awake function-controlled anesthesia, general anesthesia, pain, plasma IL-6, plasma IL-8, plasma IL-10, stress.

## INTRODUCTION

General anesthesia using endotracheal intubation is the standard procedure during brain tumor resection. Vital parameters are monitored and intubation provides a safe airway; drugs ensure analgesia and suppress vegetative reactions. Immobilization is relatively simple, even for patients in an atypical position. However, the use of general anesthesia precludes intra-operative monitoring of higher brain functions, and lesions made to the central nervous system being detected when reversibility of damage control might still be possible. Therefore, awake function-controlled neurosurgery may be beneficial in that respect. During awake craniotomy, the cerebral cortex of the patient is electrically stimulated. This allows the surgeon to properly identify and spare functionally relevant areas of the brain. Awake craniotomy has been shown to be a well-tolerated procedure with minimal side effects. Nevertheless, it is considered to be more challenging for the patient. By allowing for maximal tumor excision while keeping healthy tissue intact, awake craniotomy has the potential for better patient outcomes <sup>1</sup>. In such a procedure, the need to provide sufficient analgesia and sedation without interfering with electrophysiological monitoring is essential <sup>2</sup>.

Before, during and after craniotomy all patients are confronted with anxiety, stress, and pain. These factors can all negatively influence the perioperative experience. Patients undergoing craniotomy using general anesthesia, however, have to endure additional physical stress factors like intubation, longer hospital stays and mechanical ventilation <sup>3</sup>.

Patient perspectives regarding awake brain surgery have been investigated and adequate preoperative consultation has been found to be essential for patient confidence. In addition, scalp incisions and fixation of pin-holding sites were regarded as major sources of pain and discomfort. Still, the benefits far outweigh those of general anesthesia because awake craniotomy patients report less pain, anxiety and fear <sup>4,5</sup>. Even though there are drawbacks, the majority of patients tolerate awake craniotomy very well.

No study has attempted to compare the inflammatory impact of awake craniotomy versus general anesthesia procedures. Pathological inflammatory states can have far ranging clinical effects and negatively influence a patient's neurological outcome <sup>6-8</sup>. Recent research has demonstrated that cytokine levels can be correlated to the degree of brain tissue manipulation <sup>9</sup>. Plasma cytokine levels could reflect stress-related biochemical pathways after surgery <sup>10-12</sup>.



Cytokines orchestrate the complex network of cellular interaction that regulate both cell mediated and humoral immunity, as well as the acute phase response<sup>13</sup>. Cytokines are glycopeptide signaling molecules that act at extremely low concentrations, mediating key immune responses. Several cytokines are released during periods of stress, including interleukin-6 (IL-6), IL-8, and IL-10<sup>14</sup>. IL-6 is a pro-inflammatory cytokine secreted by T-cells, macrophages, and other cells. IL-6 is involved in both the immune response to trauma and the acute phase response; its targets being T- and B-cells. IL-8 is a chemokine produced mainly by macrophages and epithelial cells and functions to attract neutrophils towards inflammation sites. These proinflammatory cytokines play a key role in the physiological response to trauma and surgery, whereas IL-10 is an anti-inflammatory cytokine produced by Th2-cells that cause a reduction in pro-inflammatory cytokine synthesis<sup>15</sup>.

Our aim was to investigate whether awake function-controlled craniotomy causes a significantly different inflammatory response than craniotomy performed under general anesthesia. We thought both procedures would create similar inflammatory profiles despite differing anesthesia techniques used. In order to test our hypothesis, plasma levels of IL-6, IL-8, and IL-10 were measured during the pre-, peri-, and postoperative period in both patient groups. We also noted corresponding subjective outcome parameters for pain, anxiety and stress to investigate whether performing an awake procedure causes more physical pain and mental duress.

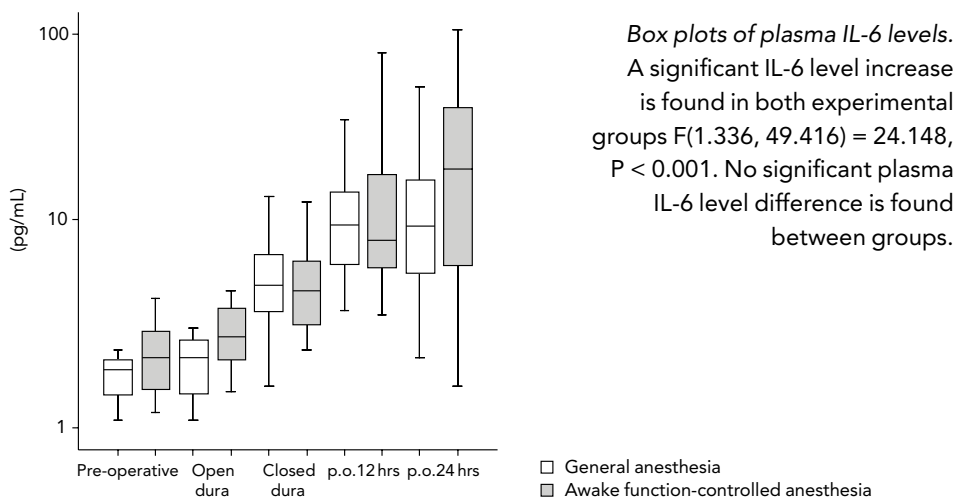
## **PATIENTS AND METHODS**

### **Study design and inclusion criteria**

This was a prospective, single centre, two-armed observational study with 40 patients (20 men and 20 women). The protocol was approved by the Medical Ethics Committee of the Erasmus Medical Center, Rotterdam. All procedures were performed in accordance with the Helsinki declaration. Written informed consent was obtained from all patients.

Plasma cytokine determinations were performed blinded, but randomization was limited. The decision to perform either function-controlled awake craniotomy or craniotomy under general anesthesia was determined by the neurosurgeon who based his decision on the intra-cerebral location of the tumor. The type or size (WHO classification of brain tumors) had no influence on whether or not awake craniotomy was chosen. By proxy patients were allo-

**FIGURE 1: Plasma IL-6 levels throughout surgery**



cated to the general anesthesia group unless the location of the tumor warranted the benefits of an awake procedure. Patients with tumors close to functional relevant areas like the motor cortex or areas related to speech require the awake monitoring made possible by the awake craniotomy procedure. By allocating these patients to the awake craniotomy group maximal tumor resection is made possible with a minimal risk of functional neurological damage.

Eligible patients were >18 years of age and were undergoing craniotomy for an intracerebral tumor. Patients were excluded if they were 1) ASA-classification IV-V, 2) did not provide written informed consent, 3) had a tumor location other than intra-cerebral, 4) had surgery beginning later than 11:00 a.m., 5) had a disease of the endocrine system or 6) were taking drugs that alter endocrine metabolism (like thyroxine). Non-co-operative or non-compliant patients could be withdrawn from the study, as could patients who developed serious adverse effects.

### Anesthesia procedure

Patients in both groups received 1.5 mg lorazepam on the evening before the surgery. All patients were on a regimen of dexamethasone 4 x 4 mg/day with the first dose given at least one day prior to surgery; regular personal

drug regimens were continued during the study. In the awake function-controlled group, 7.5 mg piritramide and 25 mg promethazine was given 30 minutes prior to induction. In the general anesthesia group, premedication consisted of 50 mg promethazine. In both groups propofol was administered for sedation and remifentanyl for analgesia. The general anesthesia group received an additional 0.25 mg fentanyl before intubation and placement of the Mayfield clamp. Cis-atracurium was used for muscle relaxation prior to intubation. In order to provide adequate pain control during awake craniotomy patients were infiltrated with bupivacaine 0.375% with adrenaline 1:200000 at the site of scalp incision. Postoperatively all patients were offered 4 times one gram paracetamol per day, and if required, supplemental morphine.

### **Outcome measures**

Patient characteristics, medications used during and after surgery, fluid balance, and duration of surgery were documented. Pain, anxiety and stress were measured at 12 and 24 hours pre and postoperatively, using visual analogue scale (VAS) scores (0 = none, 10 = extreme).

EDTA blood samples (7 ml) for cytokine level determinations were collected preoperatively, during the opening and closing of the dura, and 12 and 24 hours postoperatively. Plasma was isolated by centrifugation at 2650 gmax for 10 min at 20 °C; samples were stored at -80°C until assay.

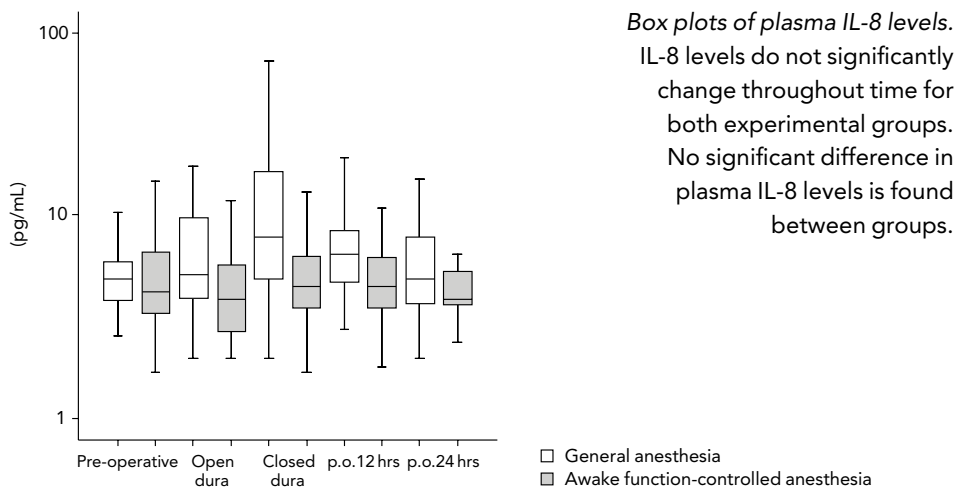
Enzyme immunoassays for the quantitative determination of human IL-6, IL-8, and IL-10 were performed with a sandwich ELISA (Pelikine Compact™ and additional Pelikine Toolset™, Sanquin, Amsterdam, The Netherlands) as described previously<sup>16</sup>. Data were calculated as pg/ml plasma and presented in figures 1-3 as (log) pg/ml.

### **Statistical analysis**

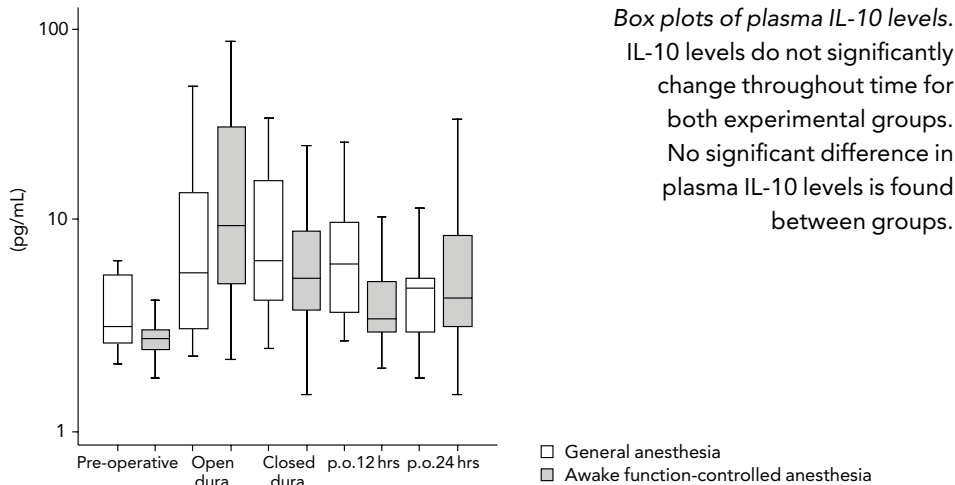
Data were analyzed using SPSS for Windows, version 16.0.1. The independent sample t-test was used to compare means for patient demographics (excluding ASA classification) and perioperative characteristics. The Pearson Chi-square test was used to evaluate differences in ASA classification. All data were reported as the mean (SD), counts, or median (25%-75%).

Sample size was calculated using the O'Brien-Shieh Algorithm for the MANOVA repeated measures test. Assuming a medium effect, an effect size of 0.6 was used and a power of 0.8. There were two experimental groups and 5 repetitions. The required a priori sample size computed by this method was 39.

**FIGURE 2: Plasma IL-8 levels throughout surgery**



**FIGURE 3: Plasma IL-10 levels throughout surgery**



For the VAS scores and cytokine data the MANOVA test was used. Differences in VAS score or cytokine values between the experimental groups across all time points and interaction between experimental groups and time were analyzed using multivariate repeated measures. Experimental group and time were the independent variables. When Mauchly's Test of Sphericity was significant, the Greenhouse-Geisser test of within subjects effects was used. When a significant difference was found between experimental groups a one-way ANOVA test with post-hoc multiple comparisons (Bonferroni correction) was used to analyze the relationship between the cytokines or VAS scores from the first preoperative measurement until 24 hours postoperative. The same Bonferroni correction was employed to analyze differences between experimental groups and time.

A P-value < 0.05 was considered statistically significant.

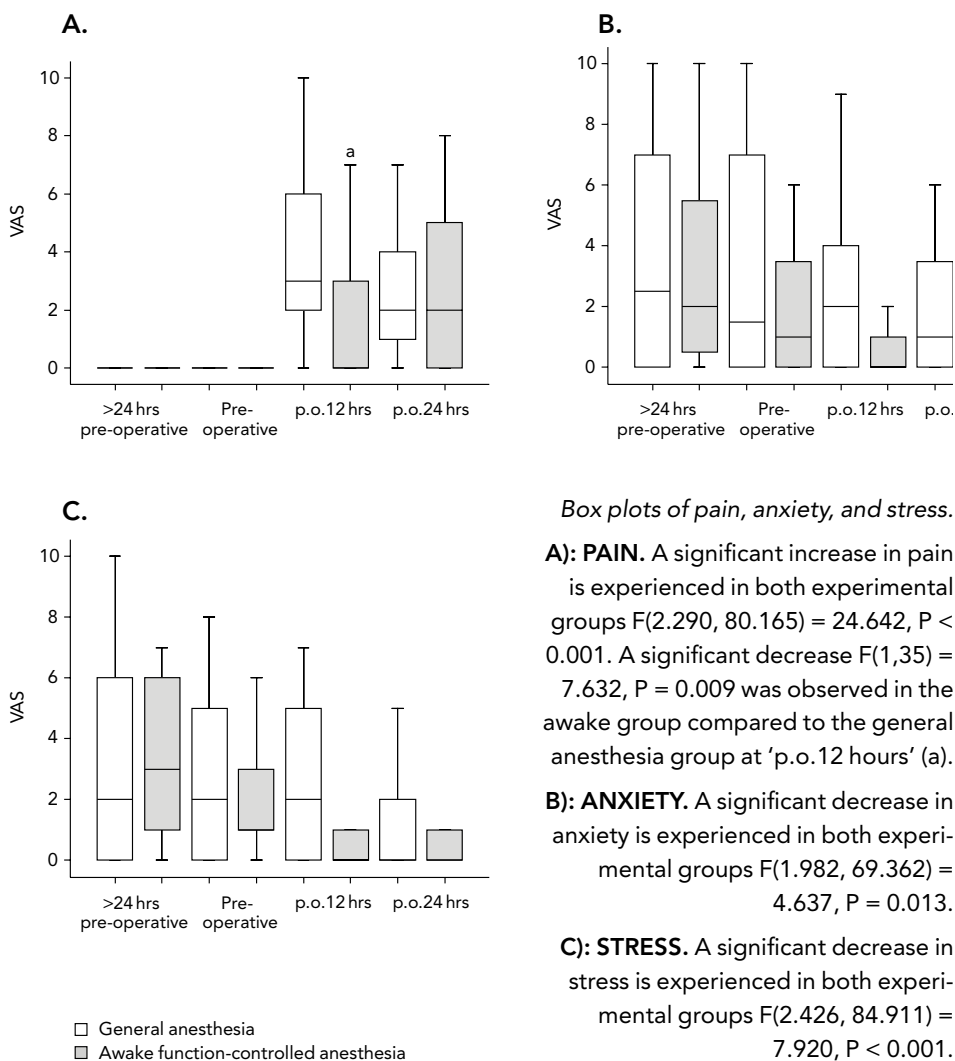
## RESULTS

Forty patients were included in the study. The awake function-controlled and general anesthesia groups contained 20 patients each, stratified for gender (10 males and 10 females). No significant intergroup differences were observed for age, height, weight, ASA classification, or Hb concentration (table 1).

Perioperative characteristics are described in table 2. As expected, the total amount of propofol administered throughout the operation was significantly less in the awake group than in the general anesthesia group. The general anesthesia group also received more crystalloids during the operation. The total amount of remifentanil used in the general anesthesia group was  $8.4 \pm 5.4$  mg. No more than 200 $\mu$ g of remifentanil was given to the awake craniotomy group.

Plasma concentrations of IL-6, IL-8, and IL-10 during all time points are displayed in figures 1 through 3. IL-6 level significantly increased with time in both experimental groups (main effect of time:  $F(1,49) = 24.1$ ,  $P < 0.001$ , observed power = 1.00). However, there was no between groups differences (group-time interaction:  $F(1,37) = 1.3$ ,  $P=0.3$ , observed power = 0.20). Furthermore, IL-8 levels did not significantly change with time in both experimental groups (main effect of time:  $F(1, 48) = 2.2$ ,  $P=0.1$ , observed power = 0.35) and no significant IL-8 between groups differences (group-time interaction:  $F(1, 37) = 2.8$ ,  $P=0.1$ , observed power = 0.37). The same applied for IL-10 levels, there

**FIGURE 4: Perioperative variations in pain, anxiety and stress**



was no significant change with time in both experimental groups (main effect of time:  $F(1, 39) = 2.6, P=0.1$ , observed power = 0.36) and no significant between groups differences (group-time interaction:  $F(1,37) = 0.6, P=0.4$ , observed power = 0.12).

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**TABLE 1: Patient demographics**

	General anesthesia	Function-controlled
Age (years)	48 ± 15.4	44 ± 13.2
Gender (m/f)	10/10	10/10
Height (cm)	174 ± 11.3	176 ± 9.6
Weight (kg)	74 ± 16.5	81 ± 14.7
ASA classification 1/2/3 (number of patients)	9/10/1	5/15/0
Hb concentration (mmol/l)	9.3 ± 1	9.0 ± 0.6

*Data presented as mean ± SD.*

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**TABLE 2: Perioperative characteristics**

	General anesthesia	Function-controlled
Propofol during operation (mg)	3277 ± 1632	673 ± 313 <sup>a</sup>
Operation time (min)	327 ± 104	275 ± 56
Blood loss during operation (ml)	400 (300 – 500)	450 (300 – 600)
Colloids during operation (ml)	500 (500 – 500)	500 (0 – 500)
Colloids after operation (l)	0.05 ± 0.2	0.1 ± 0.4
Crystalloids during operation (l)	3.7 ± 2.0	1.6 ± 0.7 <sup>a</sup>
Crystalloids after operation (l)	2.0 ± 1.0	2.0 ± 0.9
Urine during operation (ml)	1620 (1043 – 2050)	1042 (480 – 1483) <sup>b</sup>
Urine after operation (ml)	1759 ± 836	1668 ± 620
Remifentanyl	8.4 ± 5.4mg	200µg*
Postoperative paracetamol (mg)	2100 ± 1483	1900 ± 1477
Postoperative morphine (mg)	1.60 ± 4.72	2.00 ± 5.48

*Function controlled versus general anesthesia significantly decreased:*

*a. P < 0.001 and b. P = 0.004.*

*Data presented as mean ± SD and median (25%-75%).*

*\*Maximum total amount of boluses given.*

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There was no between groups significant differences in the amount of post-operative morphine and paracetamol used. The mean subcutaneous post-operative morphine administered in the general anesthesia group was 1.60 ( $\pm$  4.72mg), while the mean given to the awake group was 2.00 ( $\pm$  5.48mg). The mean postoperative paracetamol administered to the general anesthesia group was 2100 ( $\pm$  1483mg), while the mean given to the awake group was 1900 ( $\pm$  1477mg).

Pain increased significantly with time in both experimental groups (main effect of time:  $F(2, 80) = 24.6, P < 0.001$ ). However, a significant between groups difference ( $F(1,35) = 7.6, P = 0.009$ ) was noted with the awake group having less pain at the 12 hour postoperative time point.

Anxiety significantly decreased with time in both experimental groups (main effect of time:  $F(2, 69) = 4.6, P = 0.013$ ) and there was a significant stress decrease with time in both experimental groups (main effect of time:  $F(2, 85) = 7.9, P < 0.001$ ).

## DISCUSSION

We believe we are the first to compare the cytokine profiles of awake and general anesthesia craniotomy groups. Cytokine release is also a known physical reaction to tissue damage. The influence of surgery on cytokine plasma levels have been addressed during several studies. There is a great amount of evidence linking IL-6 to the degree of surgical trauma<sup>17-22</sup>. In addition, there are also studies that establish a clear relationship between dynamic IL-6 changes and cortisol plasma levels during the perioperative period<sup>11,23</sup>. The non-significant differences in IL-6 levels between groups found during this experiment suggest from an immunological perspective that both procedures are likely to be similarly stressful for the body. However, the low and medium observed powers of our negative findings requires a larger patient group to provide more certainty.

It is interesting to note the significant plasma IL-6 increase despite the exact dexamethasone 4 x 4 mg/day regime given to both experimental groups. Another study investigating the effects of dexamethasone produced different results. Morariu et al. found that after receiving dexamethasone (1 mg/kg) before anesthesia induction, plasma levels of both IL-6 and IL-8 were significantly reduced, while levels of IL-10 increased perioperatively<sup>24</sup>.



Our finding that there was a significant plasma IL-6 increase throughout time for both experimental groups and a significant increase in reported pain can be partially explained by the expected increase in pain after tissue damage. It is noteworthy that an increasing pain trend matches the increasing IL-6 tendency observed. The important role interleukin-6 plays in nociception and the pathophysiology of pain during a variety of different conditions might explain this trend<sup>25</sup>. A study done with rat models observed that higher IL-6 concentrations was linked to more intense hyperalgesia<sup>26</sup>.

Recently, plasma IL-8 has been measured as a key mediator for neuro-inflammation in patients with severe traumatic brain injuries<sup>27</sup>. Central venous plasma IL-8 levels were significantly lower in survivors than in non-survivors. In our study, the insignificant in-between and within-subject plasma IL-8 change in both experimental groups was unexpected. Due to IL-8's presence in neutrophils, microglia, astrocytes, and endothelial cells of the brain<sup>28-31</sup> we expected damaged brain tissue to cause an increased release of IL-8 over time from these sources. However, the studies involving traumatic brain injury patients contain a different patient population than ours and different confounders. The additional hypoxia and ischemia experienced in these severely injured traumatic brain injury patients can be attributed to shock and resulting hypoperfusion and might account for increased plasma IL-8 levels<sup>32</sup>.

Awake craniotomy is considered a stressful procedure. It seems logical that being awake while a neurosurgeon removes pathological brain tissue would lead to a more intense emotional response than undergoing the same procedure under general anesthesia. However, perhaps good psychological support and active coping mechanisms may actually make awake craniotomy less stressful for the patient. This might be due to the awake group having decreased feelings of dependency and loss of control than those in the general anesthesia group.

Our results show that patients undergoing awake function controlled craniotomy experience less 12 hour postoperative pain than their general anesthesia counterparts. The intensive preoperative consultation patients received might have influenced results due to the subjective nature of the VAS scoring system<sup>33</sup>. It could be argued that perioperative medication may also have influenced VAS score results. Patients who underwent awake function controlled craniotomy received 25 mg of promethazine and 7.5 mg of piritramide 30 minutes before surgery. In comparison, general anesthesia patients received 50 mg of promethazine and two boluses of fentanyl, one prior to induction and another prior to placement of the Mayfield clamp. Piritramide and fenta-

nyl are both opiates with additive sedative and euphoric properties. They are also accepted drugs for surgical procedures like craniotomy<sup>34</sup>. Additionally, the seven and six hour half life of piritramide and fentanyl makes them unlikely to affect the first postoperative VAS score measurement taken at 12 hours postoperative<sup>35,36</sup>. We think the local anesthesia provided by lidocaine infiltration at the site of scalp incision was the primary reason why VAS scores were significantly lower in the awake group.

The differing nature of awake craniotomy and general anesthesia techniques requires a larger amount opiates to be given to the general anesthesia group. There is some evidence that opiates can modulate the immune system<sup>37-39</sup>. However, our results reveal similar pro and anti-inflammatory profiles for both groups with no significant difference having been found between groups. It is still important to consider that the larger opiate amount given to the general anesthesia group could have altered its immunological profile. However, the aim of this study is to compare the inflammatory profile of two different anesthesia techniques. General anesthesia cannot be performed without a greater amount of opiates being used by the anesthesiologist.

The smaller amount of propofol administered to the awake group is due to the reduced need for sedation during the awake craniotomy procedure. On the other hand, the larger amount of crystalloids given to the general anesthesia group can be explained by the need to counteract the vasodepressive properties of propofol (table 2).

A limitation of our study is that for ethical reasons allocation of patients to one group or another could not be randomized. This restriction could bias our results. However keeping the previously mentioned limitations in mind, the plasma levels of pro and anti-inflammatory cytokines measured during this study suggests that awake function-controlled craniotomy does not cause a significantly different inflammatory response than craniotomy performed under general anesthesia. Furthermore, the non-significant difference in subjective outcome parameters for pain (with exception 12 hours postoperative), anxiety and stress insinuates that both procedures are equally mentally challenging. Therefore, it is likely that function-controlled craniotomy does not cause a greater inflammatory insult or emotional challenge than patients undergoing tumor resection using general anesthesia.

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# CHAPTER 3

Awake craniotomy induces fewer changes in the plasma amino acid profile than craniotomy under general anesthesia



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## **Running title**

Amino acid profile during craniotomy

The authors report no conflicts of interest.



## ABSTRACT

In this prospective, observational, two armed study we compared the plasma amino acid profiles of patients undergoing awake craniotomy to those undergoing craniotomy under general anesthesia. Both experimental groups were also compared to a healthy, age and sex matched reference group not undergoing surgery. It is our intention to investigate whether plasma amino acid levels provide information about physical and emotional stress, as well as pain during awake craniotomy versus craniotomy under general anesthesia. Both experimental groups received pre-, peri- and postoperative dexamethasone. The plasma levels of 20 amino acids were determined pre-, peri-, and postoperatively in all groups and were correlated with subjective markers for pain, stress and anxiety. In both craniotomy groups preoperative levels of tryptophan and valine were significantly decreased whereas glutamate, alanine and arginine were significantly increased relative to the reference group. Throughout time tryptophan levels were significantly lower in the general anesthesia group versus the awake craniotomy group. The general anesthesia group had a significantly higher phenylalanine/tyrosine ratio, which may suggest higher oxidative stress, than the awake group throughout time. Between experimental groups a significant increase in large neutral amino acids was found postoperatively in awake craniotomy patients, pain was also less and recovery was faster. A significant difference in mean hospitalization time was also found, with awake craniotomy patients leaving after  $4.53 \pm 2.12$  days and general anesthesia patients after  $6.17 \pm 1.62$  days;  $P = .012$ . This study demonstrates that awake craniotomy is likely to be physically and emotionally less stressful than general anesthesia and that amino acid profiling holds promise for monitoring postoperative pain and recovery.

## KEYWORDS

Awake craniotomy, general anesthesia, plasma amino acids, craniotomy, pain, stress, hospital discharge, tryptophan, phe/tyr ratio, BH4.

## INTRODUCTION

General anesthesia using endotracheal intubation is the standard procedure during brain tumor resection; however, it does limit intra-operative monitoring of functional lesions made to the central nervous system. The anesthetic drugs used suppress neuronal activity making it impossible to monitor certain higher cortical brain functions unless the patient regains consciousness during the operation. Examples of higher cortical brain functions that can only be checked during awake craniotomy are speech, sensibility, and complex motor functions like drawing. Therefore, awake craniotomy is the ideal anesthetic approach for when function controlled neurosurgery is necessary. During this procedure the cerebral cortex of the awake patient is electrically stimulated identifying and thus sparing functionally relevant areas of the brain. Details of our technique have been described previously<sup>1</sup>.

Public perception is that awake craniotomy is physically and emotionally more stressful than brain tumor resection under general anesthesia. However, with adequate local anesthesia and proper preoperative consultation patients undergoing awake craniotomy report less pain, discomfort and fear<sup>2,3</sup>. When proper steps are taken the majority of patients tolerate awake craniotomy very well. In comparison, patients undergoing craniotomy using general anesthesia have to endure more physical stress factors like intubation, longer hospital stays, and artificial ventilation<sup>4</sup>.

Biochemical factors relating to stress and anxiety, the perception of pain and the rate of postoperative recovery in neurosurgical patients have not been investigated. Cortisol levels have traditionally been used to indicate physical stress<sup>5</sup>; however, our standard operating procedure mandates the administration of dexamethasone, which influences cortisol levels. It was therefore decided to investigate whether or not plasma amino acids have potential to be used as biomarkers for pain and physical stress.

A number of amino acids play an important role in pain pathways. The neuropeptide bradykinin is known to increase sensitization of pain via the N-methyl-D-aspartate (NMDA)-receptor in the central nervous system, which in turn, is stimulated by the amino acid glutamate<sup>6</sup>. The amino acid glutamine also plays a role in this pathway because it is the precursor for glutamate<sup>7,8</sup>. Glutamine, on the other hand, may inhibit the generation of the amino acid arginine, a precursor for nitric oxide (NO) and citrulline<sup>9</sup>. Interestingly, the amino acid ratio of citrulline/arginine is used and accepted as an index of NO synthesis<sup>7</sup>. It is known that NO is a potent vasodilator. Less NO production causes

vasoconstriction resulting in diminished tissue blood perfusion and increased pain intensity<sup>10</sup>.

Glycine is an amino acid that acts as a co-agonist with glutamate on the NMDA receptor<sup>11</sup>. Both amino acids are thought to be mainly responsible for neuropathic pain and mood disorders<sup>12,13</sup>.

Taurine is an amino acid known to play a significant role in neuromodulation<sup>14</sup>. Animal studies have shown that physical stress is associated with a sharp rise in plasma taurine levels<sup>15</sup>. It has also been demonstrated that taurine diminishes neuropathic nociception<sup>16</sup>.

Current data about the effects of physical stress on large neutral amino acids (LNAAs), i.e. valine, leucine, isoleucine, tryptophan (trp), tyrosine (tyr), and phenylalanine (phe) are somewhat contradictory. A study performed with rats found that although rested rats had decreased plasma levels of valine and tryptophan, tyrosine levels increased<sup>15</sup>. Yet, patients undergoing cardiac surgery using general anesthesia had decreased levels of valine, leucine, isoleucine and tyrosine, while tryptophan and phenylalanine levels increased<sup>17</sup>.

This is the first study to compare absolute plasma values of amino acids over time during surgery between patient groups who received general anesthesia and patients who underwent an awake craniotomy procedure. Our aim was to determine whether or not the changes in plasma amino acid levels can be correlated to the type of anesthesia administered.

We also compared these plasma amino acid values to an age and sex matched reference group that did not undergo surgery. Furthermore, plasma amino acids were correlated with quality of life factors such as stress, anxiety and pain.

When we compare the general anesthesia and awake craniotomy groups, we hypothesize that awake craniotomy patients will have fewer changes in their amino acid profiles while having a faster recovery and resulting shorter hospitalization time.

## **PATIENTS, MATERIALS AND METHODS**

### **Study set-up and inclusion criteria**

This study was a prospective, single centre, two-armed observational study with 40 patients, stratified for gender. Gender stratification is necessary because there are known inter-sex differences in amino acid and hormone profiles<sup>18</sup>. The protocol was approved by the Medical Ethics Committee of the

Erasmus Medical Centre, Rotterdam. All procedures were performed in accordance with the Helsinki declaration. Written informed consent was obtained from all patients.

The patients were not randomized because allocation to an awake craniotomy procedure or a general anesthesia group had to do with location of the tumour. The type or size (WHO classification of brain tumours) had no influence on whether or not awake craniotomy was chosen. By proxy patients were allocated to the general anesthesia group unless the location of the tumor warranted an awake procedure. Patients with tumors close to functional relevant areas like the motor cortex or areas related to speech require the awake monitoring made possible by the awake craniotomy procedure. By allocating these patients to the awake craniotomy group maximal tumor resection is made possible with a minimal risk of functional neurological damage<sup>19</sup>.

Gender stratification was achieved by including consecutive patients to all groups until the maximum for a certain group (e.g. women, awake) was achieved. Once a maximum number of patients for a particular group was attained, only patients belonging to one of the other still open groups (e.g. man, general anesthesia) were included in the study.

Inclusion criteria were 1) undergoing craniotomy for a cerebral neoplasm situated in close proximity to an eloquent area, 2) age >18 years, 3) ASA-classification I-III, and 4) written informed consent. Exclusion criteria were 1) ASA-classification IV-V, 2) informed written consent missing, 3) tumour other than intra-cerebral, 4) surgery beginning later than 11:00 a.m. 5) endocrine problems, or 5) taking drugs that influence endocrine metabolism. Operations starting after 11:00 a.m. were excluded because Erikson et al. found that essential amino acids are affected by the circadian rhythm<sup>20</sup>. Patients had the right to withdraw from the study at any time. Patients who developed serious adverse effects were to be withdrawn from the study. Examples of serious adverse effects include prolonged unconsciousness, severe bleeding requiring a blood transfusion or any other event likely to strongly interfere with our protocol.

A healthy age- and sex-matched reference group was used to compare results obtained from the experimental groups. Blood plasma donors in this reference group donated blood after having had a light breakfast low in fat and protein.

## **Food intake**

All patients were hospitalised the day before surgery. They were allowed to eat and drink until midnight. Afterwards, only apple juice or tea with sugar

were permitted until 0:600 a.m. on the morning of surgery. Anesthesia was induced between 8:00 and 8:15 a.m. After surgery, all patients were transferred to the post-anesthesia care unit (PACU) and monitored for 14 hours. During this time morphine was available and if necessary titrated intravenously until acceptable pain levels were achieved. While in the PACU patients were told that food could be requested and delivered at any time during their stay.

### **Anesthesia procedure**

Patients in both groups received 1.5 mg lorazepam on the evening before surgery. Otherwise, all patients were on a regimen of dexamethasone 4 x 4 mg/day while regular personal drug regimens were continued. In the awake craniotomy group, 7.5 mg piritramide and 25 mg promethazine was given one-half an hour prior to induction. Piritramide was used to reduce pain perception during skull infiltration with 40ml bupivacaine 0.375% + adrenaline 1:300.000. Benzodiazepines were not an option due to the paradoxical reactions that are sometimes associated with its use. In addition, benzodiazepines would reduce the responsiveness of propofol, making it less effective for sedation. In the group undergoing general anesthesia, the premedication consisted of 50 mg promethazine. In both groups, propofol was used for sedation and remifentanyl for analgesia. In the general anesthesia group, cis-atracurium was used for muscle relaxation.

### **Postoperative pain control**

After surgery patients were transferred to the PACU where they were monitored and primarily treated with paracetamol for pain. If pain control was not adequate morphine was administered until adequate pain control was achieved. Postoperative pain medication administered was documented.

### **General Outcome Measures**

Patient demographics (table1) as well as perioperative characteristics were noted (table 2). Quality of life was measured using the visual analogue scale (VAS) for stress, pain and anxiety preoperatively and at 12 and 24 hours post-operative (table 3). Although there are overlapping elements relating to the concepts of stress and anxiety, VAS scores for each was obtained separately.

### **Blood sampling**

EDTA blood samples (7 ml) for amino acid level determinations were collected preoperatively (t1), during opening(t2) and closing of the dura (t3), and

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**TABLE 1: Patient demographics**

	General anesthesia	Awake
Age (years)	48 ± 15.4	44 ± 13.2
Gender (m/f)	10/10	10/10
Length (cm)	174 ± 11.3	176 ± 9.6
Weight (kg)	74 ± 16.5	81 ± 14.7
ASA classification 1/2/3 (number of patients)	9/10/1	5/15/0
Hb concentration (mmol/l)	9.3 ± 1	9.0 ± 0.6

*Data are mean ± SD except for gender and ASA classification.  
Hb, hemoglobin.*

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**TABLE 2: Perioperative characteristics**

	General anesthesia	Awake
Propofol during operation (mg)	3277 ± 1632	673 ± 313 <sup>a</sup>
Operation time (min)	327 ± 104	275 ± 56
Blood loss during operation (l)	0.8 ± 1.7	0.4 ± 0.2
Colloids during operation (l)	0.6 ± 0.5	0.4 ± 0.3
Colloids postoperative (l)	0.05 ± 0.2	0.1 ± 0.4
Crystalloids during operation (l)	3.7 ± 2.0	1.6 ± 0.7 <sup>a</sup>
Crystalloids postoperative (l)	2.0 ± 1.0	2.0 ± 0.9
Urine during operation (ml)	1857 ± 1583	1007 ± 469 <sup>b</sup>
Urine postoperative (ml)	1759 ± 836	1668 ± 620

*Data are mean ± SD. Awake vs. general anesthesia: a P < 0.001; b P < 0.03.*

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12(t4) and 24(t5) hours postoperative. Plasma was isolated by centrifugation at 2650 g<sub>max</sub> for 20 min at 20°C; samples were stored at -80°C until assay.

## Plasma amino acid level determination

Blinded plasma amino acid determinations were performed. Each plasma sample was deproteinised with 5-sulphosalicylic acid (6%, w/v) containing norvaline and homoserine as internal standards. Amino acids were assayed by high-performance liquid chromatography (HPLC) using automated precolumn derivatization with o-phthaldialdehyde and fluorescence detection<sup>21</sup>. The amino acids measured were: the essential amino acids including the LNAAs tryptophan, valine, leucine, isoleucine, tyrosine, and phenylalanine; as well as lysine, histidine, threonine, and methionine; and the non-essential amino acids (NEAAs) glutamate, glutamine, glycine, serine, taurine, asparagine, alanine, ornithine, arginine, and citrulline. The trp/LNAA ratio was calculated by dividing 100 times the plasma concentration of trp by the sum of all other LNAAs. The phe/Tyr ratio was calculated to estimate the functional availability of the cofactor tetrahydrobiopterin (BH<sub>4</sub>). Hydroxylation of phe to tyr is highly dependent on this cofactor.

The limits of detection depended on the amino acid because of the different fluorescence responses and differing peak shapes of the derivatives. Typical values were 54 fmol for glutamate and 167 fmol for serine. Concentrations of amino acids as low as 0.5 µmol/L in plasma can be measured accurately with our method. The inter-assay coefficient of variation was for all amino acids below 4%.

## Statistical analysis

Data were analysed using SPSS for Windows, version 12.0.1. The Kolmogorov-Smirnov test was used to analyze whether or not amino acids values were normally distributed. All amino acid values except glutamic acid measured at time points 1, 3, 5 and taurine 4 were normally distributed.

For the non-normal distributed values we still decided to use MANOVA's test. Although Manova's test requires that each dependent variable entered into the analysis be normally distributed it was still used because the Monte Carlo experiments have shown that for sample sizes of 3 or 5 it is still possible to analyze leptokurtic, rectangular, J-shaped, moderately and markedly skewed distributions. These experiments demonstrated that the empirically determined rejection region of the F-distribution would be no larger than  $\alpha = 0.08$  when the usual 5% rejection is used<sup>22</sup>. The results are therefore presented as mean  $\pm$  the standard deviation (SD).

Differences in plasma amino acid levels between experimental groups across all time points (5 moments of time) and interaction between experi-

**TABLE 3: Pre- and postoperative stress, anxiety, and pain as determined by visual analogue score**

	PREOPERATIVE		12H POSTOPERATIVE		24H POSTOPERATIVE	
	General anesthesia	Awake	General anesthesia	Awake	General anesthesia	Awake
<b>Stress (0-10)</b>	3.15 ± 3.2	2.4 ± 2.1	2.3 ± 2.7	0.90 ± 1.9	1.4 ± 2.1	0.90 ± 1.9
<b>Anxiety (0-10)</b>	3.5 ± 3.6	1.6 ± 2.1 <sup>b</sup>	2.1 ± 2.7	0.80 ± 2.0 <sup>b</sup>	1.8 ± 2.0	1.1 ± 1.8
<b>Pain (0-10)</b>	0.1 ± 0.3	0.3 ± 0.7	3.8 ± 2.8	1.5 ± 2.4 <sup>a</sup>	2.6 ± 2.3	2.7 ± 2.6

Data are mean ± SD. Awake vs. general anesthesia: a  $P < 0.01$ ; b  $P < 0.05$ .

mental group and time were analyzed using multivariate repeated measures. Experimental group and time were the independent variables.

When a significant difference was found between experimental groups a one-way ANOVA test with post-hoc multiple comparisons (Bonferroni correction) was used to analyze the relationship between plasma amino acids and time from the moment of plasma donation until 24 hours after donation. The same statistical method was employed to analyze differences between preoperative and 24 hour postoperative plasma amino acid levels in the two experimental groups.

Differences relating to patient demographics (excluding ASA classification), perioperative characteristics and preoperative plasma amino acid levels between experimental groups were tested using the t-test for independent samples. This same test was used to compare the mean amount of postoperative analgesia given as well as the mean number of days until discharge for both experimental groups. The Pearson Chi-square test was used to evaluate differences in ASA classification.

Correlations between patient characteristics, amino acids and the quality of life measures were evaluated using Pearson's correlation test. Sample size was calculated using the MANOVA repeated measures test. An effect size of 0.6 was used and a power of 0.8. There were two experimental groups and 5 repetitions. The required a priori sample size computed by this method was 39. For all statistics the  $\alpha$  was set at the traditional 0.05 level.



## RESULTS

### Demographics

A total of forty patients were included in the study with 20 being allocated to both groups. The two groups of 20 were stratified for gender (10 males and 10 females). No inter-group differences were observed for age, length, weight, ASA classification or hemoglobin concentration (table 1).

### Perioperative Characteristics

Perioperative characteristics are shown in table 2. The total amount of propofol administered throughout the operation was significantly less in the awake craniotomy group. Total operating time in the awake craniotomy group was also less. The general anesthesia group had more blood loss, higher urine output and as a result received significantly more crystalloids during the operation. The average total amount of remifentanyl used in the general anesthesia group was 8.4mg while an average total bolus of 200mcg was given to the awake group.

### Quality of Life Indicators

The awake craniotomy group reported significantly less pre and postoperative VAS scores for anxiety than the general anesthesia group. The awake craniotomy group also disclosed having less pain postoperatively (table 3).

### Recovery, food intake and hospitalization

Both patient groups were offered food postoperatively during recovery in the PACU. All awake craniotomy patients requested and received their first meals within twelve hours of surgery. As a result, the awake craniotomy patients had blood taken (t3) after their first meal. All the patients in the general anesthesia group, however, requested and had their first meal after the 12 hour postoperative blood sample (t3) was taken. Consequently, the general anesthesia group had blood taken before their first meal.

From the moment of arrival in the intensive care until 24 hours postoperative all patients were offered 4 times one gram paracetamol, and if required, nurses titrated I.V. morphine until acceptable pain levels were achieved. There was no significant difference found between both groups in postoperative morphine and paracetamol use. The mean postoperative morphine administered in the general anesthesia group was  $1.60 \pm 4.72\text{mg}$ , while the mean given to the awake group was  $2.00 \pm 5.48\text{mg}$ ;  $P = 0.806$ . The mean postopera-

tive paracetamol administered to the general anesthesia group was  $2100 \pm 1483\text{mg}$ , while the mean given to the awake group was  $1900 \pm 1477\text{mg}$ ;  $P = 0.668$ .

A significant difference in mean hospitalization time was also found. Awake craniotomy patients left the hospital after an average of  $4.53 \pm 2.12$  days. Patients in the general anesthesia group left on average after  $6.17 \pm 1.62$  days;  $P = .012$ .

### Preoperative plasma amino acid levels

We detected no differences in the preoperative plasma amino acid levels between craniotomy groups (table 4 and 5); therefore, anesthesia procedure

**TABLE 4: Pre- and postoperative plasma levels of essential amino acids**

Amino acid ( $\mu\text{mol/L}$ )	CRANIOTOMY PATIENTS				REFERENCE
	General anesthesia		Awake		
	Preop	Postop 24h	Preop	Postop 24h	
Tryptophan <sup>a</sup>	$30 \pm 8^*$	$43 \pm 12^*$	$36 \pm 9^*$	$48 \pm 11$	$50 \pm 8$
Valine <sup>a,b</sup>	$236 \pm 76^*$	$262 \pm 54$	$232 \pm 54^*$	$269 \pm 67$	$285 \pm 58$
Leucine <sup>a,b</sup>	$132 \pm 37$	$164 \pm 50$	$134 \pm 28$	$168 \pm 44$	$147 \pm 31$
Isoleucine <sup>a,b</sup>	$69 \pm 21$	$91 \pm 26$	$67 \pm 14^*$	$85 \pm 23$	$82 \pm 23$
Tyrosine <sup>a</sup>	$58 \pm 12^*$	$80 \pm 25$	$61 \pm 10$	$81 \pm 17$	$70 \pm 16$
Phenylalanine <sup>a</sup>	$62 \pm 10$	$79 \pm 17^*$	$63 \pm 10$	$76 \pm 13^*$	$62 \pm 10$
Lysine <sup>c</sup>	$201 \pm 29$	$216 \pm 63^*$	$214 \pm 54^*$	$220 \pm 59^*$	$185 \pm 35$
Histidine <sup>c</sup>	$81 \pm 11$	$80 \pm 18$	$85 \pm 16$	$78 \pm 15$	$84 \pm 11$
Threonine	$117 \pm 29^*$	$139 \pm 43$	$124 \pm 32$	$140 \pm 50$	$144 \pm 33$
Methionine	$29 \pm 5$	$38 \pm 12$	$32 \pm 7$	$38 \pm 12$	$31 \pm 6$

Data are mean  $\pm$  SD. a. LNAAs: large neutral amino acids; b. BCAAs: branched chain amino acids; c. BAAs: basic amino acids.

No significant differences between craniotomy groups, pre- and postoperative.

\*  $P < 0.05$  vs. reference group.

assignment did not affect preoperative amino acid levels. However, significant preoperative gender differences were observed for glutamate (males,  $104 \pm 47 \mu\text{mol/L}$ ; females,  $73 \pm 44 \mu\text{mol/L}$ ;  $P = 0.040$ ) and tryptophan levels (males,  $36 \pm 8.1 \mu\text{mol/L}$ ; females,  $30 \pm 9.1 \mu\text{mol/L}$ ;  $P = 0.037$ ).

There are significant differences in the preoperative and postoperative levels of a number of amino acids when comparing the experimental groups with the reference group (tables 4 and 5). Preoperative fasting could have influenced these levels. However, when comparing the plasma amino acid levels of experimental groups to the reference group at the 24-hour postoperative time point when fasting is not a problem, it was found that the general anesthesia group still had decreased levels of tryptophan and ornithine. In addition, postoperative levels of lysine, arginine, glutamate and alanine were still increased in both experimental groups.

At t5 citrulline and phenylalanine are worth noting. Citrulline is decreased in both experimental groups in comparison to the reference group, while the inverse was true for phenylalanine.

### **Time related effects of anesthesia and surgery**

We analysed the time-related effects of anesthesia and surgery on the plasma levels of amino acids. Figure 1 shows the plasma levels of tryptophan and the other essential LNAAs. During anesthesia/surgery, both experimental groups demonstrate a similar decline in plasma tryptophan levels. However, worth noting is that tryptophan levels were significantly lower in the general anesthesia group when compared to the awake craniotomy group both during anesthesia/surgery and 12 hours postoperatively.

Figure 1 also shows a plot of the phenylalanine/tyrosine ratio. The ratios before and at the start of surgery are remarkably elevated for both groups when compared to our healthy non-surgical reference group. The ratio then peaks at the end of surgery. However, postoperatively both experimental groups experience a considerable drop to levels similar to the healthy reference group. It is worth noting that throughout time, the general anesthesia group has a significantly higher ratio than the awake group ( $P=.016$ ).

The rest of the LNAAs show a general trend with a major increase occurring postoperatively, except, a faster increase with significantly higher LNAA levels is noted in the awake craniotomy patients. This reflects the faster recovery awake craniotomy patients experience during their first 12 hours in the PACU.

The levels of NMDA receptor related NEAAs glutamate, glutamine, and glycine are presented in figure 2. Glutamate and glutamine exhibited time

**TABLE 5: Pre- and postoperative plasma levels of non-essential amino acids**

Amino acid ( $\mu\text{mol/L}$ )	CRANIOTOMY PATIENTS				REFERENCE
	General anesthesia		Awake		
	Preop	Postop 24h	Preop	Postop 24h	
Glutamate <sup>d</sup>	99 $\pm$ 67*	85 $\pm$ 63*	84 $\pm$ 29*	66 $\pm$ 24*	47 $\pm$ 20
Glutamine <sup>d</sup>	587 $\pm$ 131	531 $\pm$ 135	616 $\pm$ 126	520 $\pm$ 108	559 $\pm$ 85
Glycine <sup>d</sup>	224 $\pm$ 50	220 $\pm$ 68	226 $\pm$ 72	216 $\pm$ 86	216 $\pm$ 40
Serine	104 $\pm$ 22	108 $\pm$ 23	110 $\pm$ 20	112 $\pm$ 30	111 $\pm$ 20
Taurine	50 $\pm$ 12	46 $\pm$ 30	53 $\pm$ 20*	38 $\pm$ 9	42 $\pm$ 8
Asparagine	51 $\pm$ 9*	59 $\pm$ 14	51 $\pm$ 11	57 $\pm$ 18	58 $\pm$ 11
Alanine	503 $\pm$ 171*	487 $\pm$ 184*	480 $\pm$ 167*	439 $\pm$ 189	396 $\pm$ 85
Ornithine <sup>c</sup>	59 $\pm$ 9*	66 $\pm$ 25*	61 $\pm$ 14*	60 $\pm$ 16*	86 $\pm$ 23
Arginine <sup>c</sup>	109 $\pm$ 24*	93 $\pm$ 36*	108 $\pm$ 18*	90 $\pm$ 22*	71 $\pm$ 22
Citrulline	33 $\pm$ 9	23 $\pm$ 7*	30 $\pm$ 7	22 $\pm$ 6*	35 $\pm$ 8

Data are mean  $\pm$  SD. c. BAAs: basic amino acids; d. NMDA: N-methyl-D-aspartate -related amino acids.

No significant differences between craniotomy groups, pre- and postoperative.

\*  $P < 0.05$  vs. reference group.

dependent level changes, although no group differences were found. On the contrary, glycine showed no significant time dependent or group differences.

Plasma levels of endothelium related NEAAs arginine and citrulline demonstrated a time-dependent decline but there were no intergroup differences (figure 3).

### **Correlations between patient characteristics, amino acid levels, and quality of life**

Preoperative stress and anxiety were correlated to each other ( $r=0.77$ ,  $P<0.001$ ). However, none of the quality of life factors like stress, anxiety, and

pain related to amino acid levels. Additionally, levels of citrulline ( $r = 0.40$ ,  $P = 0.011$ ), serine ( $r = -0.53$ ,  $P = 0.001$ ), and methionine ( $r = -0.38$ ,  $P < 0.001$ ) were found to be related to patient age.

## DISCUSSION

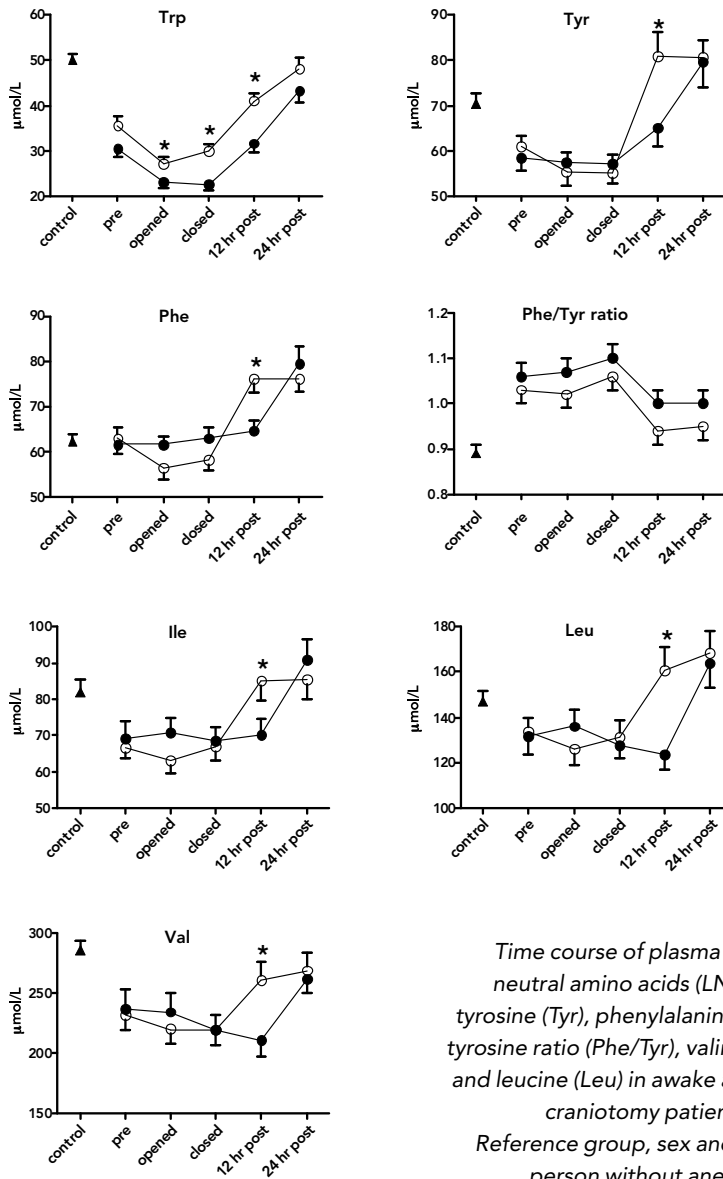
### Pain and amino acids

As is expected after surgery, pain is significantly increased 12 hours postoperatively in both groups. However, this increase was significantly greater in the general anesthesia group. Anxiety and stress, however, declined similarly for both groups (table 3).

The subjective nature of pain could explain why the awake craniotomy group reports feeling less postoperative pain than the general anesthesia group. Administration of postoperative pain medication cannot be a factor because in both groups no significant difference in the amount of pain medication given was found. Intensive preoperative consultation might have helped reduce fear in the awake group by giving patients the opportunity to know what to expect during and after the operation<sup>23</sup>. It is also possible that postoperative pain might have been lessened by the 7.5mg of piritramide given to the awake craniotomy group, although the 7 hour half life of piritramide and relatively low dosage makes this less likely<sup>24</sup>. A synergistic effect with remifentanyl given during surgery is also not very likely considering the remifentanyl half life of 3 minutes<sup>25</sup>. In retrospect, although selectively giving piritramide to the awake craniotomy group in order to make scalp infiltration more bearable might be a confounder, we think the confounding influence is limited considering that around the same time a much more than equivalent dose of opioids is given for the purpose of anesthesia induction in the general anesthesia group.

In both craniotomy patient groups, plasma glutamine levels decreased 24 hours postoperatively (figure 2). Despite the administration of analgesics, patients reported mild pain. Reduced levels of plasma glutamine have also been found in burn patients, for whom immunological function and wound healing are the most prominent issues, in addition to pain<sup>26</sup>. Preoperative levels of plasma glutamate were significantly elevated in both patient groups as compared to our healthy reference group (figure 2). Pain perception was not determined at that time, but patients with brain tumours generally have little or no preoperative pain. Preoperative stress and anxiety were mild to moderate in the general anesthesia groups and were lower in the awake group

**FIGURE 1**



○ awake  
 ● general  
 ▲ healthy reference group

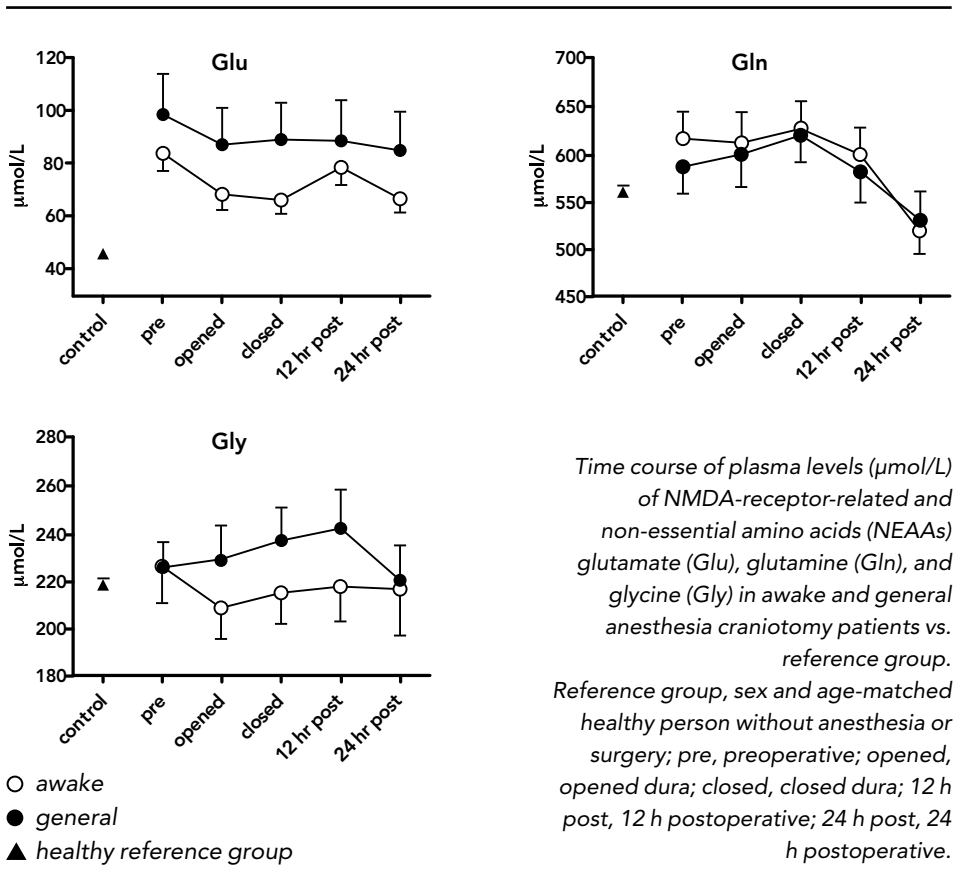
Time course of plasma levels ( $\mu\text{mol/L}$ ) of large neutral amino acids (LNAA) tryptophan (Trp), tyrosine (Tyr), phenylalanine (Phe), phenylalanine/tyrosine ratio (Phe/Tyr), valine (Val), isoleucine (Ile), and leucine (Leu) in awake and general anesthesia craniotomy patients vs. reference group. Reference group, sex and age-matched healthy person without anesthesia or surgery; pre, preoperative; opened, opened dura; closed, closed dura; 12 h post, 12 h postoperative; 24 h post, 24 h postoperative. \*  $P < 0.05$  awake craniotomy vs. general anesthesia

(table 3). Although not significant, plasma glutamate was 15% lower in the awake craniotomy group, suggesting that physical stress and anxiety might influence levels of the NMDA-receptor-related glutamate. Lastly, glutamate levels in the general anesthesia group are noteworthy because it has previously been reported that propofol causes a reversible increase in plasma glutamate<sup>27</sup>. We were not able to confirm this trend.

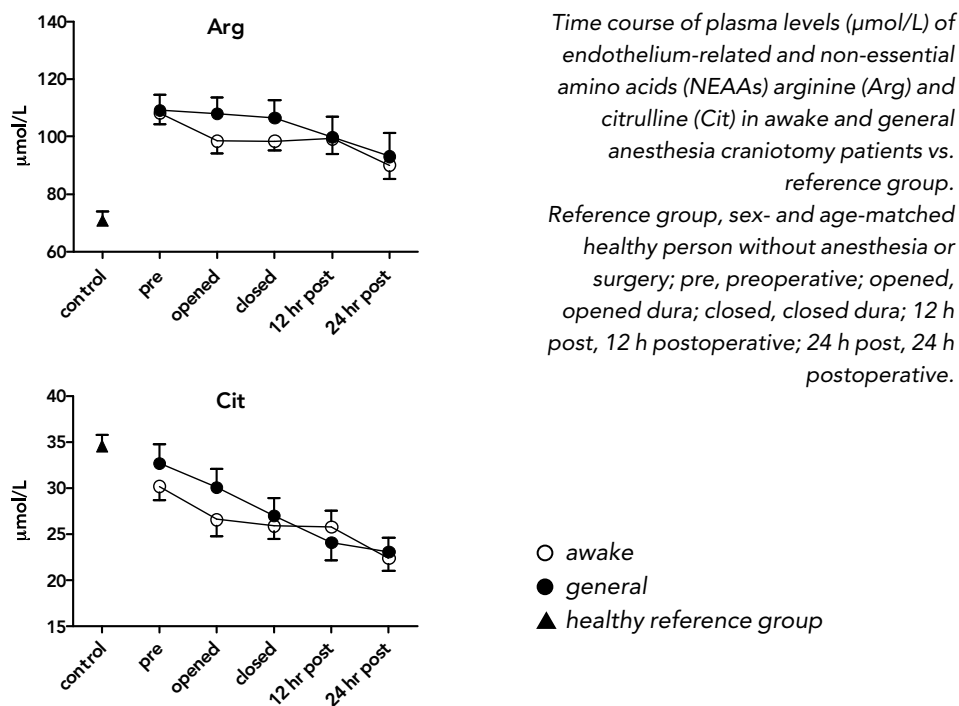
### Gender, age and amino acids

As table 1 shows, observed differences between groups cannot be accounted for by gender, however, glutamate and tryptophan were an exception. They were found to be greater in males than in females.

**FIGURE 2**



**FIGURE 3**



Age was positively correlated with preoperative levels of citrulline, suggesting that more NO formed in older individuals. Nonetheless, serine and methionine were negatively correlated with age. These findings agree with data from aging studies<sup>28,29,18</sup> and suggest altered uptake and or production with advancing age. However, the levels of these amino acids did not undergo significant changes during surgery or anesthesia.

### Effects of premedication, fasting, stress, and anxiety

Dexamethasone, a selective glucocorticoid-receptor antagonist, has been found to increase plasma levels of glutamate, glutamine, and alanine<sup>30</sup>. On the contrary; both tryptophan and tyrosine are diminished after administration of dexamethasone<sup>31</sup>. In this study when comparing to the reference group, preoperative levels of glutamate and alanine are indeed increased, although glutamine showed no significant changes.



Preoperative fasting probably affects plasma amino acid levels. A limitation of our study is that our reference group was not fasted. This influenced the level of essential amino acid levels as illustrated in figure one. Preoperatively, levels of essential amino acids in both experimental groups were in all cases, with exception of phenylalanine, lower than in our reference group.

In rats, fasting plus physical stress reduces the plasma levels of the NEAAs alanine and arginine while increasing glutamate and glutamine<sup>32</sup>. However in our patients, plasma levels of alanine, arginine, and glutamate were increased.

### **Time related effects of surgery and anesthesia**

It is known that surgery can cause a decline in fasting plasma levels of alanine, arginine, glutamate, glutamine and glycine relative to fasting control groups not undergoing surgery<sup>33</sup>. In another study involving patients undergoing thoracic surgery, perioperative plasma levels of tryptophan, glutamine, glycine, and arginine declined rapidly, whereas the levels of valine, leucine, and phenylalanine were slightly or not affected<sup>34</sup>. A study with patients experiencing abdominal aortic aneurysm surgery had glutamine levels decline and remain below preoperative levels for at least seven days<sup>35</sup>.

Results from this study reveal that only levels of tryptophan are lower during surgery. An additional anesthesia dependent effect was demonstrated by tryptophan levels being significantly lower in the general anesthesia group. This observation agrees with those of Nunn et al<sup>36</sup> who found a 15% reduction in plasma tryptophan after short-term routine surgery (mean duration 88 min).

Recent research has revealed a remarkable stress mechanism likely to explain the levels tryptophan and phe /tyr ratios found in figure one. Stress induces the enzyme indole amine dioxygenase responsible for metabolizing the amino acid tryptophan via the kynurenine pathway<sup>37</sup>. This causes a decrease of available tryptophan in blood plasma. Our results confirm this with the general anesthesia group having a more significant reduction in tryptophan than the awake craniotomy group. Furthermore, it is known that oxidative stress causes a decrease in the co-factor BH4. This cofactor is necessary for the production of serotonin, dopamine, NO and the conversion of phenylalanine (phe) into tyrosine (tyr)<sup>38</sup>. Therefore, the phe/tyr ratio serves as a reflection of the co-factor BH4 concentration.

The phe/tyr ratio shown in figure 1 nicely symbolizes expectations before, during and after surgery. In comparison with our healthy reference group the ratio for both experimental groups is increased preoperatively. At the end of surgery the ratios peak, a time when the body has experienced the maximum

amount of physical stress. Twelve hours postoperatively, levels are drastically reduced being lower than preoperative levels and close to levels found in our healthy reference group. Throughout time, the general anesthesia group has significantly higher ratios than the awake group suggesting that this group experienced higher levels of oxidative stress.

### **Nitric Oxide**

The results show a marked preoperative increase in arginine levels in the two experimental groups with a decrease after surgery but still being increased 24 hours postoperatively relative to our reference group. Considering that citrulline is a marker of NO synthesis, the continuous decline of this amino acid could indicate diminished postoperative c-GMP-dependent vasodilatation. If so, this would result in diminished tissue blood distribution and the spread of pain.<sup>10</sup>

## **CONCLUSION**

Preoperative plasma levels of all LNAAs, with the exception of phenylalanine, were decreased in craniotomy patients when compared to levels in our reference group. On the contrary, the NEAAs glutamate, alanine and arginine were markedly increased prior to surgery. Only tryptophan, the precursor of serotonin, decreased significantly during general anesthesia and surgery.

The phe/tyr ratio needs additional study in order to establish whether it can be used as a molecular marker for emotional and/or physical stress. Furthermore, patients undergoing awake craniotomy showed rapid postoperative improvement, as displayed by a faster and significant increase in plasma LNAA levels and shorter hospital discharge times. This fits with our clinical impression that these patients experience less perioperative physical stress than patients undergoing general anesthesia.

This study indicates that amino acid profiling holds promise as an extra physiological tool that could potentially help monitor postoperative recovery. Therefore its value for monitoring surgery-induced stress and pain should be investigated further. In future studies, levels of kynurenine will also be determined along with tryptophan to unravel more direct changes in the activity of indoleamine dioxygenase during surgical stress.

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# CHAPTER 4

Effect of major and minor surgery on plasma levels of arginine, citrulline, nitric oxide and ornithine in humans



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## **Type of study conducted**

Prospective, single centre, two-armed observational study.

## **Declarations of authors**

This project is funded by department research budget.  
The authors declare that they have no conflict of interest.

## **Short running title**

Plasma amino acid levels during surgery

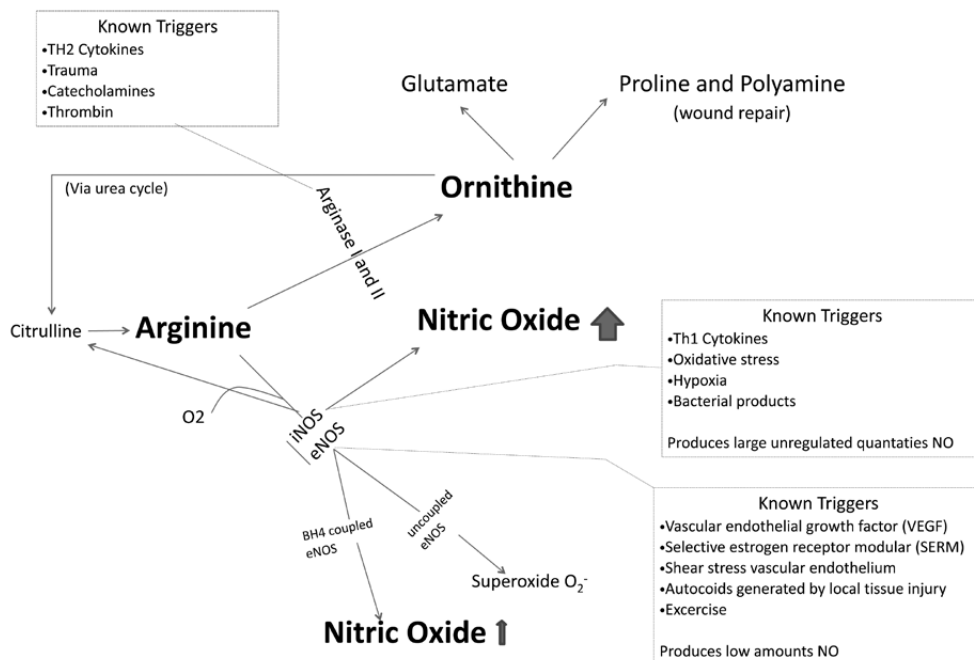


## INTRODUCTION

Nitric Oxide (NO) is an enormously clinically relevant molecule having been the subject of intense medical research because of the important role it plays during critical illness and in maintaining healthy homeostasis. This versatile molecule is difficult to study because this single mediator is responsible for both pathological and physiological actions *in vivo*. NO levels have been implicated in maintaining vascular tone, inhibiting platelet aggregation and monocyte adhesion<sup>1-3</sup>. Levels of NO also seem to regulate immune status with high levels associated with compensatory anti-inflammatory response (CARS) and systemic inflammatory response syndrome (SIRS)<sup>4-6</sup>. The exclusive substrate precursor of NO is the amino acid arginine<sup>7</sup>. It is known as a semi-essential or conditionally essential amino acid. In adults, endogenous synthesis from citrulline is sufficient making it possible to classify arginine as non-essential, however, in growing children or acute moments like trauma endogenous synthesis is not sufficient making it essential during these circumstances<sup>8</sup>. The enzyme arginase transforms arginine into ornithine<sup>9</sup>. There are two isoforms of arginase: arginase I is mainly found in the lungs, kidney and macrophages, while "mitochondrial arginase II" is limited to the liver and leukocytes<sup>10,11</sup>. Ornithine is essential for collagen synthesis and wound repair because it is the precursor for polyamine and proline synthesis<sup>10</sup>. Ornithine is also converted to citrulline in the urea cycle, and citrulline in turn may be converted to arginine<sup>12</sup>. In addition, citrulline is synthesized from arginine by nitric oxide synthase (NOS)<sup>13</sup>. Therefore, a cyclic relationship exists between these three amino acids (figure 1)<sup>14</sup>.

It is well known that surgical trauma may have a profound effect on the metabolism of NO. Rat studies investigating the role of trauma and NO have demonstrated that trauma-hemorrhage and fluid resuscitation influence NO metabolism. In one study rats were rapidly bled to induce hemorrhagic shock. These rats were found to have significantly higher NO levels than sham operated animals where soft tissue trauma was inflicted without enough blood loss to induce hemorrhagic shock. This experiment documented that blood loss followed by untimely and insufficient fluid resuscitation causes increased NO synthesis<sup>15</sup>. Other studies confirm that hemorrhagic shock induces iNOS<sup>16-19</sup>. On the other hand, another study involving human subjects measured plasma NO in trauma patients admitted to the emergency room and reported reduced NO levels compared to controls<sup>20</sup>. It is possible that trauma victims received adequate and timely pre-hospital hemodynamic support preventing

**FIGURE 1: The relationship between nitric oxide and its amino acid intermediates**



the advent of hemorrhagic shock like in the previously mentioned rodent experiments. Similar results have been found in other rodent studies where tissue trauma, but not hemorrhagic shock were intended experimental variables<sup>21</sup>.

Our aim is to get more insight into the biochemical relationship between arginine, citrulline, ornithine and NO during surgery by measuring their plasma levels at five strategic time points. We decided to perform this study in two types of surgery with different levels of invasiveness, i.e. major surgery (abdominal hysterectomy) and minor surgery (vulvectomy), to measure the effect of invasiveness on their plasma levels. Our hypothesis is that major abdominal surgery has a more profound effect on these levels than minor surgery.

## METHODS

### Study set-up and inclusion criteria

This study was a prospective, single centre, two-armed observational study with 28 female patients. The protocol was approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam (MEC-2008-134). All procedures were performed in accordance with the Helsinki declaration. Informed consent was obtained from all patients.

Inclusion criteria were 1) scheduled for vulvectomy or open abdominal hysterectomy, 2) expected surgery duration greater than 0.5 h, 3) postoperative hospitalization lasting more than 4 days, 4) age greater than 18 years, 5) ASA (American Society of Anesthesiologists) classification I-III, 6) oral informed consent. Exclusion criteria were 1) ASA-classification IV-V, 2) patients unable to speak Dutch, 3) and patients not able to consent. Patients had the right to withdraw from the study at any time. Patients who developed serious adverse side effects were to be withdrawn from the study.

### Anesthesia procedure

All patients received 1.0 mg tablet lorazepam and 100 mg celecoxib (selective COX-2 inhibitor) approximately one hour before surgery. Personal drug regimens were continued during the study. The observational nature of this study allowed the staff anesthesiologist to place an epidural catheter if the anesthesiologist felt it was indicated for adequate postoperative pain control. All patients received total intravenous anesthesia, using propofol for sedation and sufentanil for analgesia. Cisatracurium provided muscle relaxation for patients being intubated. Prior to the first incision all patients received antibiotics (1 g cefazoline and 500 mg metronidazol). For all patients the minimum postoperative pain control regimen included 4000 mg paracetamol and 200 mg celecoxib per 24 hours. While in the recovery room, morphine was titrated until sufficient pain control was achieved. The daily regimen of paracetamol and celecoxib was continued until patients no longer experienced pain with a VAS greater than four. Patients with an epidural catheter had it removed when the anesthesiologist determined that it was no longer indicated for adequate pain control.

### Outcome measures

Patient demographics, medications used during and after surgery, and duration of surgery were documented. EDTA blood samples (4ml) were collected

for determination of amino acids and NO at 24 hours preoperative, right after IV placement preceding induction, ten minutes before the operation was expected to end, and at 24 and 96 hours postoperative. Plasma was isolated by centrifugation at 2650 gmax for 20 minutes at 20 °C; samples were stored at -80 °C until assay.

### Biochemical determinations

Amino acids were determined as previously described<sup>22</sup>. NO was determined by measuring plasma nitrite + nitrate by the Griess reaction after conversion of nitrate to nitrite using a commercially available assay kit (R&D Systems, Abingdon, UK). Figures were made with Sigma Plot, version 9.0. The results are presented as mean ± the standard error of the mean (SEM).

### Statistical Analysis

Data was analyzed using SPSS for windows, version 16.01. The independent sample t-test was used to compare means for patient demographics (excluding ASA classification) and perioperative characteristics. The Pearson Chi-square test was used to evaluate differences in ASA classification. The Fisher exact test was used to analyze difference in the type of pain control techniques used between groups (NSAID only, NSAID + Opiates, NSAID + Opiates + Epidural). All data are reported as the mean ± SD. The amino acid data was analyzed using MANOVA. Differences in amino acid values between the experimental groups across all time points and interaction between experimental groups and time were analyzed using multivariate repeated measures. Experimental group and time were the independent variables. When

**TABLE 1: Patient demographics**

	Vulvectomy	Laparotomy
Age (years)	62 ± 12	44 ± 9*
Height (cm)	166 ± 5	167 ± 6
Weight (kg)	71 ± 10	71 ± 7

Data are mean ± SD except for ASA classification.

\*Significant difference between vulvectomy and laparotomy groups, P<0.001.

Mauchly's Test of Sphericity was significant, the Greenhouse-Geisser test was used. When a significant difference was found between experimental groups a one-way ANOVA test with post-hoc multiple comparisons (Bonferroni correction) was used to analyze the relationship between the level of the amino acid from the first preoperative measurement until 96 hours postoperative. The same Bonferroni correction was employed to analyze differences between experimental groups and time. Pairwise comparisons were used to analyze significant differences between longitudinal time points. A P-value < 0.05 was considered statistically significant.

## RESULTS

### Demographics

Twenty eight female patients were included in the study; no patients were withdrawn from the study (table 1). The vulvectomy group contained 15 patients, while the laparotomy group contained 13 patients. Significant inter-group differences were found for age and ASA classification (P<0.05). Laparotomy patients were younger and had significantly lower ASA scores.

**TABLE 2: Perioperative characteristics**

	Vulvectomy	Laparotomy
Propofol during operation (mg)	1146 ± 828	1983 ± 105*
Operation time (min)	126 ± 50	188 ± 46*
Blood loss during operation (ml)	134 ± 271	959 ± 335 <sup>a</sup>
Colloids during operation (ml)	170 ± 242	540 ± 335*
Crystalloids during operation (ml)	1182 ± 627	1917 ± 655*
Sufentanil during operation (µg)	33 ± 15	37 ± 14
Cisatracurium during operation (mg)	20 ± 25	68 ± 18*

Data are mean ± SD.

\* Significant difference between vulvectomy and laparotomy groups, P<0.05.

<sup>a</sup> Significant difference between groups, P<0.001.

**TABLE 3: Postoperative characteristics**

	Vulvectomy		Laparotomy	
	24 h post-operative	96 h post-operative	24 h post-operative	96 h post-operative
Paracetamol (mg)	3769 ± 832	4000 ± 0	3833 ± 577	4000 ± 0
Celecoxib (mg)	200 ± 0	186 ± 38	200 ± 0	233 ± 82
Morphine (mg)	6 ± 2	-	12 ± 1	-

*Data are mean ± SD. Celecoxib (selective COX-2 inhibitor)*

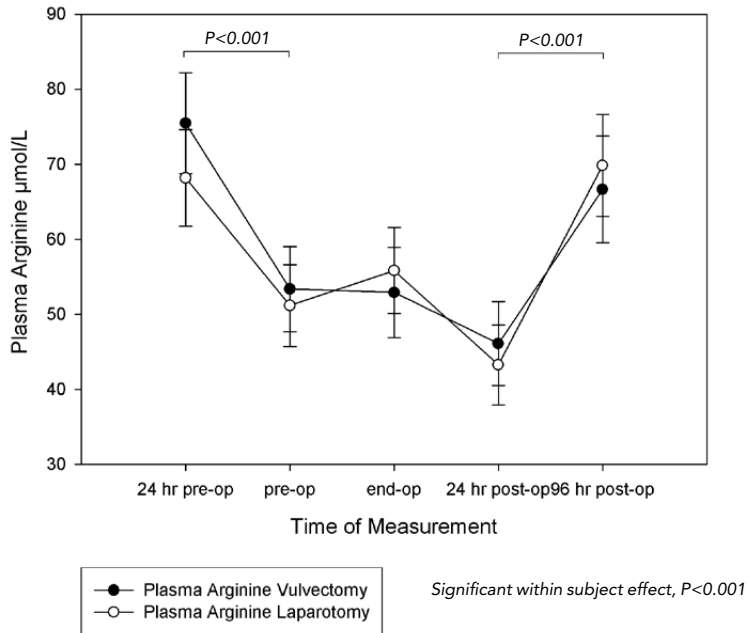
### Perioperative characteristics

Differences in perioperative characteristics were found (table 2). The abdominal hysterectomy group had a significantly longer operating time ( $P=0.002$ ), more blood loss ( $P<0.001$ ) and correspondingly more fluid replacement in the form of colloids ( $P=0.002$ ) and crystalloids ( $P=0.005$ ). In addition, significantly more propofol was used ( $P=0.026$ ), however, there was no significant difference in the amount of sufentanil used between groups. One of the patients in the laparotomy group received a blood transfusion with 285ml of erythrocytes and one patient in this group used a beta-blocker. There was no significant difference in the amount of postoperative morphine, paracetamol or celecoxib given to both groups (table 3). However, 9 patients from the laparotomy group were given preoperative epidural catheters while only 3 patients were given one in the vulvectomy group. All epidural catheters were removed by 24 hours postoperative because pain control was found to be adequate.

### Plasma Arginine levels (figure 2)

Baseline levels of plasma arginine 24 hours before surgery did not significantly differ between groups ( $P=0.478$ ). Throughout time, no significant difference between experimental groups was found ( $P=0.856$ ). However, arginine levels changed significantly over time in both groups (within group effect,  $P<0.001$ ). Pairwise comparisons revealed a significant decrease during the first 24 hours between the first and second time points ( $P<0.001$ ). No significant

**FIGURE 2: Plasma Arginine levels**



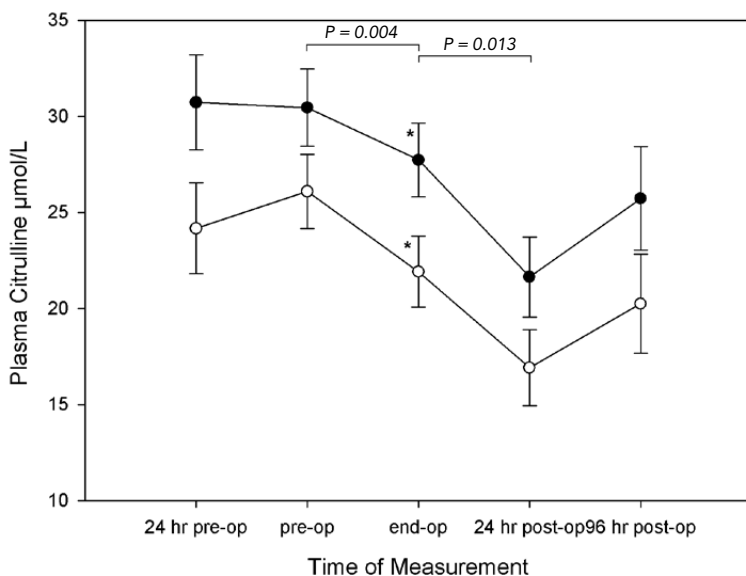
The effect of vulvectomy versus laparotomy on perioperative levels of plasma arginine. Values are the mean and SEM. There was a significant within subject effect ( $P<0.001$ ). Horizontal brackets represent significant pairwise changes.

change in plasma arginine levels occurred during surgery until 24 hours postoperative. Between 24 and 96 hours postoperative arginine levels returned towards the baseline.

### Plasma Citrulline levels (figure 3)

Baseline levels of plasma citrulline were not significantly different between both groups at the 24 hour preoperative time point. However, throughout time plasma levels of citrulline were lower in the laparotomy group than in the vulvectomy group (between group effect,  $P=0.030$ ). This difference was significant at the end of operation (time point 3,  $P=0.043$ ). There was also a significant within group effect ( $P<0.001$ ). Pairwise comparisons revealed a significant citrulline decrease between preoperative and end of operation time

**FIGURE 3: Plasma Citrulline levels**



● Plasma Citrulline Vulvectomy  
○ Plasma Citrulline Laparotomy

\*Significant intergroup difference at time point 3,  $P = 0.043$   
Significant within subject effect,  $P < 0.001$   
Significant between subject effect,  $P = 0.030$

The effect of vulvectomy versus laparotomy on perioperative levels of plasma citrulline. Values are the mean and SEM. A significant intergroup difference was found with a lower level of plasma citrulline measured throughout time for the laparotomy group ( $P = 0.030$ ). There was a significant within subject effect ( $P < 0.001$ ). Horizontal brackets represent significant pairwise changes.

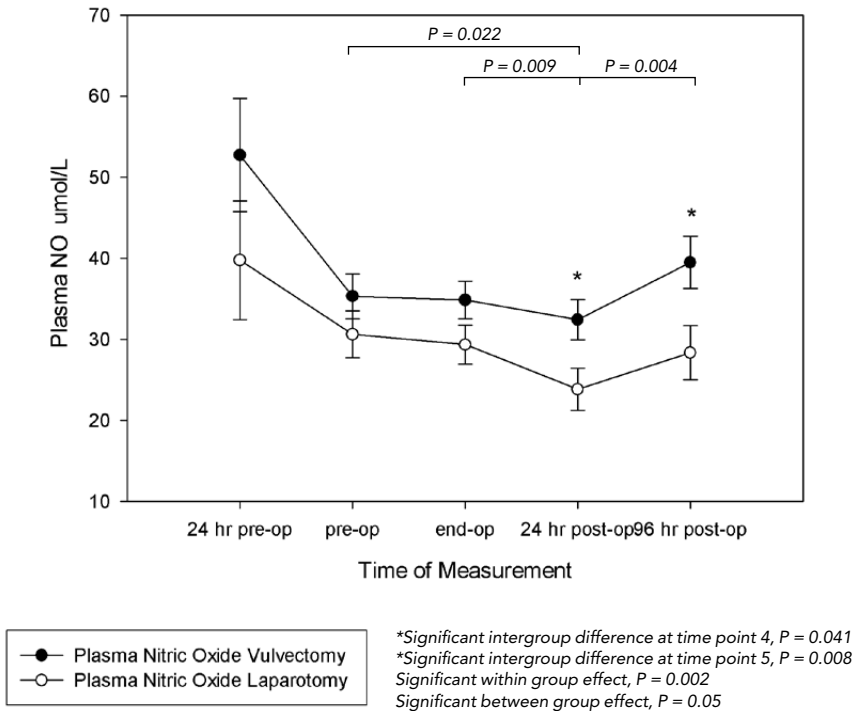
points ( $P = 0.004$ ). A further significant drop occurred from the end of the operation until 24 hours postoperative ( $P = 0.013$ ).

### Plasma NO metabolites (figure 4)

Baseline levels did not significantly differ between groups. There was a significant change in plasma NO metabolite levels over time for both groups (within group effect,  $P = 0.002$ ) as well as a significant difference between experimental groups ( $P = 0.05$ ). Plasma NO metabolite levels were not significantly different from each other until 24 hours postoperative. At 24 hours



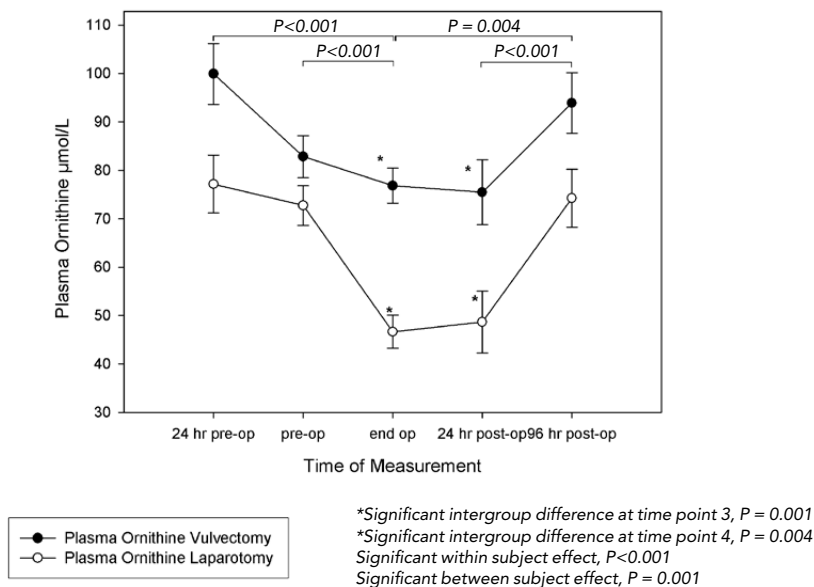
**FIGURE 4: Plasma Nitric Oxide levels**



The effect of vulvectomy versus laparotomy on perioperative levels of plasma nitric oxide. Values are the mean and SEM. There was a significant within group change in plasma NO levels over time for both groups ( $P = 0.002$ ), as well as a significant between group difference ( $P = 0.05$ ). Horizontal brackets represent significant pairwise changes.

postoperative, the laparotomy group had significantly lower levels of plasma NO than the vulvectomy group (time point 4,  $P=0.041$ ). This difference continued until 96 hours postoperative where there still was a significant difference between groups (time point 5,  $P=0.008$ ). Pairwise comparisons revealed a significant decrease occurring between preoperative and 24 hour postoperative time points ( $P=0.022$ ). There was also a significant decrease in plasma NO metabolites occurring between the end of the operation and 24 hours postoperative ( $P=0.009$ ). Lastly, NO metabolite levels showed an increase from 24 to 96 hours postoperative ( $P=0.004$ ), but without returning to baseline levels.

**FIGURE 5: Plasma Ornithine levels**



The effect of vulvectomy versus laparotomy on perioperative levels of plasma ornithine. Values are the mean and SEM. Both a significant within subject effect ( $P < 0.001$ ) and a significant difference in plasma ornithine levels between experimental groups ( $P = 0.001$ ) were found. Horizontal brackets represent significant pairwise changes.

### Plasma Ornithine levels (figure 5)

Baseline levels of plasma ornithine did not significantly differ between both groups. A significant change occurred over time for both groups (within group effect,  $P < 0.001$ ) as well as a significant difference between experimental groups ( $P = 0.001$ ). Plasma ornithine levels were not significantly different between groups during the first two measurements (24 hours preoperative and preoperative). However, at the end of surgery the laparotomy group showed a highly significant decrease compared to the vulvectomy group ( $P = 0.001$ ). Plasma ornithine remained significantly lower until 24 hours postoperative ( $P = 0.004$ ). Pairwise comparisons revealed a significant decrease during the operation from baseline values measured preoperatively and values obtained at the end of surgery ( $P < 0.001$ ). Between 24 hours and 96 postoperative plasma ornithine levels returned towards the baseline.

## DISCUSSION

We found that major surgery has a more profound effect on plasma levels of ornithine and NO than minor surgery. We believe we are the first to compare two patient groups in two well defined surgical interventions with different severity in a controlled clinical setting where all three amino acids related to NO metabolism are measured at the same time. Arginine, citrulline, and ornithine are key amino acids involved in NO metabolism<sup>23,14</sup>. By comparing these two patient groups and their respective amino acid profiles it is possible to shed light on how surgical invasiveness affects NO metabolism.

Previous studies involving human subjects measured plasma NO in trauma patients admitted to the emergency room and reported reduced NO levels compared to controls<sup>20</sup>. Similar results have been found in other, rodent studies where tissue trauma was the intended variable<sup>21</sup>. Arginine is the sole substrate for NO production and its metabolism is complex<sup>14</sup>. When fasting, 85 percent of arginine is made from protein turnover while the rest is made from de novo synthesis from citrulline via the intestinal-renal axis of arginine synthesis<sup>13,12</sup>. Levels of dietary arginine intake do not seem to influence endogenous production. Instead, arginine levels are maintained by controlling arginine metabolism<sup>24</sup>. Changes in the activity of Arginase II are thought to be the main method in which the body is able to regulate arginine levels. Rat studies using arginase knock out mice provide convincing evidence because they have much higher levels of plasma arginine compared to control animals<sup>25</sup>. Arginase is able to increase arginine catabolism and therefore decreases the available endogenously made arginine substrate for NO synthesis. Studies using cultured macrophages show that arginase can regulate the production of NO by competing with NO synthases for arginine availability<sup>20</sup>.

The significant decrease in arginine plasma levels we found during the first twenty four hours in both groups might be explained by preoperative stress induced catecholamine synthesis. Catecholamines are known to stimulate arginase activity both in vitro and in vivo. Experiments performed in animals clearly show that the catecholamine induced increase in arginase activity could be annulled with catecholamine inhibitors<sup>11</sup>. Previous reports also indicate that arginine levels drop approximately 50% during trauma<sup>20,26,27</sup>. A recent study showed that in human trauma patients arginase activity exhibited a significant increase on day 1 after intensive care admission, which is in line with the nadir of plasma arginine we observed 24 hours postoperative<sup>28</sup>. It was also demonstrated in this study that in a murine model of laparotomy the decrease

in early systemic arginine is very likely explained by the release of circulating arginase, and that systemic arginine depletion in their model is limited to a few hours due to the limited arginase release. The 50% drop in our study occurs before the first incision, possibly indicating that preoperative anxiety and associated increase in catecholamines and resultant arginase activation could explain this drop<sup>11</sup>. Plasma dilution caused by IV fluids is not likely to be an explanation due to the absence of a drop in citrulline levels during this same time period. As previously described, arginine levels are not dependent on dietary intake, therefore the 6 hour fasting period before the operations cannot sufficiently explain this drop<sup>24</sup>. The non-significant change in plasma citrulline levels during the first twenty four hours provides further evidence that fasting is not the explanation for this significant drop. The observation that no further arginine level decrease occurs from preoperative until 24 hours postoperative when general anesthesia and postoperative morphine is administered further supports this argument.

Our ornithine results are completely different than those of arginine. Unlike our arginine results, significant intergroup differences start occurring from the end of the operation until 24 hours postoperative. Using our arginine and ornithine data, one can deduce that heavier surgical trauma stimulates the laparotomy group to consume significantly more ornithine than the vulvectomy group. The equivalent arginine level drop in both groups leads us to believe that arginase has equally been activated and that equal amounts of ornithine are produced. However, the significant decrease of plasma ornithine in the more invasive laparotomy group suggests that more of this substrate is being used to produce the proline and polyamines required for wound healing<sup>29-31</sup>.

Our results on the NO metabolites reveal a significant drop from the start of surgery until 24 hours after surgery when levels start regaining ground. It is noteworthy that significantly less NO metabolites are present after surgery in the laparotomy group until our last measurement taken 96 hours later. It seems that the heavier trauma experienced by the more invasive laparotomy group results in lower NO metabolite levels after surgery. These results are in line with various rat and human studies<sup>1,20,32</sup>. When considering the role NO plays in maintaining vascular tonus, it seems logical that NO is lower in the surgical group requiring vasoconstriction in order to preserve physiological hemodynamic parameters. Too much NO production would have resulted in circulatory collapse, while too little NO would have allowed an overzealous vasoconstrictive response possibly resulting in ischemic injury.

The lower levels of plasma ornithine and NO metabolites measured during surgery in the laparotomy group are very likely attributed to invasiveness experienced since ASA scores and age were higher in the vulvectomy group. If these parameters would have been the same in both groups we would expect that the differences in ornithine and NO metabolite levels might have even been greater. It must be stressed, that there are differences in age and ASA-score between the groups. It was not our intention to compare two more or less identical groups. Due to the fact that the higher age and the higher ASA-score are associated with the minor surgery group while most changes occur in the younger and healthier major surgery group, we can conclude that the observed effects are caused by the procedure and not by comorbidity.

There has been discussion on whether citrulline can be used as a reference for NO synthesis. It is thought that because citrulline is a byproduct of NO synthesis that its level may reflect NO synthase activity<sup>13,23,33</sup>. Our results might support this hypothesis. Plasma citrulline levels were significantly different at the end of the operation with the vulvectomy group having more plasma citrulline than the laparotomy group, however, before the operation these curves do not run parallel (figure 3). Perhaps this symbolizes the increased NOS activity responsible for the significantly increased NO metabolite levels measured in the vulvectomy group at the 24 and 96 hour time points (figure 4).

## Limitations

The definition of major vs. minor surgery seems to be a somewhat arbitrary one, defined by many by the length of the operation and amount of blood loss to be expected. However, one factor that supports this intuition is that open abdominal surgery is considered to have an intermediate perioperative cardiovascular risk while gynecological surgery is considered to have a low risk factor<sup>34</sup>.

The significantly less amount of propofol given to the vulvectomy group can be partly explained by the shorter operating time. However, women in this group were older and had higher ASA scores explaining why less propofol was used. In addition, the greater amount of colloids and crystalloids given to the laparotomy group is related to the longer operative time and increased amount of blood loss in this group of patients. Furthermore, these perioperative characteristics provide additional evidence that abdominal hysterectomy is a heavier, more traumatic operation in comparison to the vulvectomy procedure.

Epidural anesthesia could possibly have influenced our NO results. The decreased vascular tonus resulting from epidural anesthesia<sup>35</sup> could have some

influence on NO metabolite levels because of its central role in modulating vascular tonus<sup>1</sup>. Very little or no data are available that compares plasma NO levels of patients undergoing surgery with or without epidural anesthesia. We were only able to find one relevant study, however, they investigated the effects of combined spinal-epidural analgesia (CSEA) instead of epidural anesthesia alone<sup>36</sup>. These researchers compared plasma concentrations of NO metabolites nitrate and nitrite during labor in two patient groups with or without CSEA. At baseline they found no significant difference between patient groups. However, they found significantly higher NO levels during full cervical dilation and at two hours post-delivery in the group using CSEA. These authors claim to be the first to publish data comparing NO metabolites with combined spinal-epidural analgesia. Their results possibly suggest that our abdominal laparotomy group might have had even lower levels of NO metabolites from the preoperative until 24 hour postoperative time points if epidural anesthesia was not used during this time frame, although labor remains an entirely different pain mechanism than surgery.

Epidural anesthesia may also blunt catecholamine release and this may have resulted in lower arginase activity and consequently relatively higher arginine levels in our hysterectomy patients compared to the vulvectomy patients who had no epidural anesthesia. However, we neither can confirm nor reject this possibility, because we did not measure catecholamines in our study, but if catecholamine release would have been blunted by the epidurals, then arginine levels in our hysterectomy patients possibly would have been lower in this group compared to the vulvectomy group.

## CONCLUSION

We found a significant decrease in the plasma levels of citrulline, ornithine and NO metabolites during surgery, and that major surgery has a more profound effect on plasma levels of ornithine and NO metabolites than minor surgery. It may be that the lower postoperative ornithine levels in the laparotomy group are caused by a higher need in this group for ornithine in the process of wound healing compared to the minor surgery group. It should, however, be stressed that, although a decrease in amino acid levels is an important observation, this will not reveal the exact cause.

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# CHAPTER 5

The tryptophan kynurenine pathway, neopterin and IL-6 during vulvectomy and abdominal hysterectomy



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## ABSTRACT

Surgery has wide ranging immunomodulatory properties of which the mechanism is poorly understood. In order to investigate how different types of surgery influence inflammation, we designed a longitudinal observational study investigating two inflammatory profiles of two separate patient groups undergoing gynaecological operations of differing severity. In addition to measuring the well known inflammatory markers neopterin and IL-6, we also determined the kynurenine/tryptophan ratio.

This study was a prospective, single center, two-armed observational study involving 28 female patients. Plasma levels of tryptophan, kynurenine, neopterin and IL-6 were determined from samples taken at: 24hrs preoperative, prior to induction, ten minutes before the operation was expected to end, and at 24 and 96 hours postoperative in patients undergoing abdominal hysterectomy and vulvectomy.

There were 15 and 13 patients included in the vulvectomy and abdominal hysterectomy groups, respectively. In this study we show that anesthesia and surgery significantly increases the enzyme activity of indoleamine 2, 3 dioxygenase (IDO) as measured by the kynurenine/tryptophan ratio ( $P=0.003$ ), while maintaining stable neopterin levels. However, abdominal hysterectomy causes a considerable IL-6 increase ( $P<0.001$ ).

Surgery and associated anesthesia cause a significant tryptophan level decrease while significantly increasing IDO activity. Both types of surgery produce nearly identical neopterin time curve relationships, with no significant change occurring in either group. However, even though neopterin is unaffected by the severity of surgery, IL-6 responded to surgical invasiveness by revealing a significant increase during abdominal hysterectomy.

## KEYWORDS

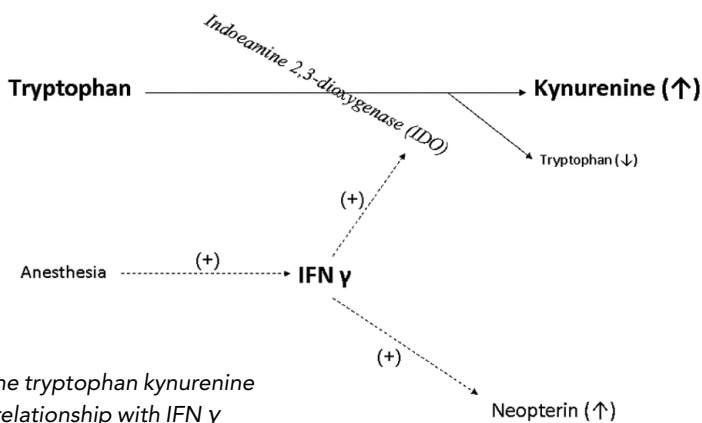
Kynurenine, tryptophan, Indoleamine 2,3-dioxygenase, neopterin.

## BACKGROUND

Surgery has wide ranging immunomodulatory properties of which the mechanism is poorly understood<sup>6,26,37</sup>. In order to better understand the effect of surgery and anesthesia on inflammation, we designed a longitudinal observational study investigating two inflammatory profiles of two separate patient groups undergoing surgery of differing severity while undergoing general anesthesia. In addition to measuring the well known inflammatory markers neopterin and IL-6, we also determined kynurenine and tryptophan.

Tryptophan is an essential precursor for serotonin and kynurenine (KYN)<sup>54</sup>. The metabolism of tryptophan to kynurenine is facilitated by the enzyme Indoleamine 2,3 dioxygenase (IDO)<sup>15</sup>. Tryptophan is a vital amino acid for growth. When present in limited amounts it inhibits viral, bacterial and parasitic development. Not only are microorganisms dependent on tryptophan, but T-cell production is also limited by decreased levels<sup>7,28,29</sup>. Kynurenine, the direct product of tryptophan catabolism has several important physiological and immunosuppressive properties. It regulates immune function by suppressing T-cells and natural killer cells<sup>12,42</sup>. Moreover, recent research has found that it contributes to arterial vessel wall relaxation and causes hypotension in a dose dependent manner in systemically inflamed mice<sup>48</sup>.

**FIGURE 1: Tryptophan Kynurenine pathway**



An overview of the tryptophan kynurenine pathway and its relationship with IFN  $\gamma$  as well as neopterin. (+) denotes that it activates and or stimulates production.

The tryptophan kynurenine pathway is regulated by the rate limiting enzyme IDO<sup>15,19</sup> (figure 1). IDO is made in the vascular endothelial cells and is activated via autocrine and paracrine mechanisms by means of interferon  $\gamma$  (IFN  $\gamma$ ) released by dendritic and T-cells<sup>16,17</sup>. The amount of kynurenine produced relates to its activity<sup>19,36</sup>. In addition, IDO contributes to the regulation of blood pressure because it controls the production of kynurenine. This has been demonstrated by the ability of IDO inhibitors to restore normal systolic blood pressure in septic mice<sup>48</sup>.

Tryptophan degradation is correlated to neopterin formation; both are stimulated by IFN  $\gamma$ <sup>14,27,50,52,54</sup>. Increased neopterin levels are associated with a pro-inflammatory state mediated by cellular immune system activation<sup>13,33,46</sup>. It is an endogenous regulator of cytotoxic effects by activated macrophages and a potent enhancer of peroxynitrite<sup>51</sup>. It is a stable molecule eliminated only by the kidney. Higher levels are associated with higher levels of reactive oxygen species and thus serve as an estimate of the oxidative stress caused by the immune system<sup>21,30</sup>. Levels of neopterin have been shown to predict the development of septic complications in trauma and post surgical patients. There is a direct correlation between increased neopterin levels and non-survivors<sup>41</sup>.

IL-6 is an acute phase pro-inflammatory cytokine capable of being released by macrophages, endothelial cells and T-cells minutes after injury. It is a good marker for acute immune system activation and is accepted as a sensitive early marker of tissue damage with peak serum levels being proportional to the amount of surgical trauma<sup>3,9,10,23</sup>. Known to be made within minutes after injury, it is also broken down in a relatively short amount of time<sup>40,55</sup>.

Quantifying the above mentioned biochemical substances in blood may give us more insight into perioperative immunomodulatory processes. Significantly different inflammatory profiles can have wide clinical implications. We hypothesize that more invasive surgery when compared to minor surgery causes significantly more increases in all inflammatory biomarkers measured.

## METHODS

### Study set-up and inclusion criteria

This study was a prospective, single centre, two-armed observational study with 28 female patients. The protocol was approved by the Medical Ethics Committee of the Erasmus Medical Centre, Rotterdam (MEC-2008-134). All

procedures were performed in accordance with the Helsinki declaration. Informed consent was obtained from all patients.

Inclusion criteria were 1) scheduled for vulvectomy or abdominal hysterectomy, 2) expected surgery duration greater than 0.5 h, 3) postoperative hospitalization lasting more than 4 days, 4) age greater than 18 years, 5) ASA (American Society of Anesthesiologists) classification I-III. Exclusion criteria were 1) ASA-classification IV-V, 2) patients unable to speak Dutch, 3) and patients not able to consent. Patients had the right to withdraw from the study at any time. Patients who developed serious adverse side effects were to be withdrawn from the study.

### **Anesthesia procedure**

All patients received 1.0 mg tablet lorazepam and 100 mg celecoxib (selective COX-2 inhibitor) approximately one hour before surgery. Personal drug regimens were continued during the study. The observational nature of this study allowed the staff anesthesiologist to place an epidural catheter if the anesthesiologist felt it was indicated for adequate postoperative pain control. All patients received total intravenous anesthesia, using propofol for sedation and sufentanil for analgesia. Cisatracurium provided muscle relaxation for patients being intubated. Prior to the first incision all patients received antibiotics (1 g cefazoline and 500 mg metronidazol).

For all patients the minimum postoperative pain control regimen included 4000 mg paracetamol and 200 mg celecoxib per 24 hours. While in the recovery room, morphine was titrated until sufficient pain control was achieved. The daily regimen of paracetamol and celecoxib was continued until patients no longer experienced pain with a VAS greater than four. Patients with an epidural catheter had it removed when the anesthesiologist determined that it was no longer indicated for adequate pain control.

### **Outcome measures**

Patient demographics, medications used during and after surgery, and duration of surgery were documented. EDTA blood samples (4ml) were collected for determination of neopterin, kynurenine, and tryptophan at 24 hours preoperative, right after IV placement prior to induction, ten minutes before anesthesia was expected to end, and at 24 and 96 hours postoperative. Plasma was isolated by centrifugation at 2650 g<sub>max</sub> for 20 minutes at 20 °C; samples were stored at -80 °C until assay. At the same time points blood was collected for preparation of serum used for the measurement of IL-6.



Concentrations of kynurenine and tryptophan were determined via their natural fluorescence using an isocratic, reversed-phase HPLC system (Agilent) and an FP-2020 fluorescence detector (Jasco) as described previously<sup>47</sup>. The analytical column consisted of a 250 x 2.1 mm i.d. column packed with 5 µm particles of GraceSmart RP-18 (Grace Davison Discovery Sciences), which was protected by a guard cartridge column (4.0 x 2.0 mm i.d.) containing Phenomenex C18 material. An HP ChemStation (Hewlett Packard) was used for data collection and handling. The kynurenine/tryptophan ratio was calculated to estimate the activity of the enzyme indoleamine 2,3 dioxygenase (IDO)<sup>53</sup>.

Total neopterin was measured after acid oxidation. Plasma (0.4 ml) was oxidized in 0.1 ml 1 M trichloroacetic acid and 0.05 ml iodine solution (0.5% I<sub>2</sub>, 1% KI in 0.2 M trichloroacetic acid). After standing for 60 min under reduced light, excess iodine was reduced by the addition of 20 µl of 1% ascorbic acid solution and the mixture was centrifuged at 12 000 x g for 15 min at 4 °C. The supernatant (0.4 ml) was transferred to an amber glass vial and 10 µl was injected directly onto the analytical column using an HPLC system with an auto sampler and a fluorescence detector as described previously<sup>45</sup>.

Enzyme immunoassays for the quantitative determination of human IL-6 were performed with a sandwich ELISA (Pelikine Compact™ and additional Pelikine Toolset™, Sanquin, Amsterdam, The Netherlands) as described previously<sup>18</sup>. Data were calculated as pg/ml. IL-6 figures were made with Sigma Plot, version 9.0. The results are presented as mean ± the standard error of the mean (SEM).

## Statistical Analysis

Data was analyzed using SPSS for windows, version 16.01. The independent sample t-test was used to compare means for patient demographics (excluding ASA classification) and perioperative characteristics. The Pearson Chi-square test was used to evaluate differences in ASA classification. The Fisher exact test was used to analyze difference in the type of pain control techniques used between groups (NSAID only, NSAID + Opiates, NSAID + Opiates + Epidural). All data are reported as the mean ± SD.

Our biochemical data were analyzed using MANOVA. Differences in values measured between the experimental groups across all time points and interaction between experimental groups and time were analyzed using multivariate repeated measures. Experimental group and time were the independent variables. When Mauchly's Test of Sphericity was significant, the Greenhouse-Geisser test was used.

When a significant difference was found between experimental groups a one-way ANOVA test with post-hoc multiple comparisons (Bonferroni correction) was used to analyze the relationship between the level of the amino acid from the first preoperative measurement until 96 hours postoperative. The same Bonferroni correction was employed to analyze differences between experimental groups and time. Pairwise comparisons were used to analyze significant differences between longitudinal time points. P-value < 0.05 was considered statistically significant.

**TABLE 1: Patient demographics**

	Vulvectomy	Laparotomy
Age (years)	62 ± 12	44 ± 9*
Height (cm)	166 ± 5	167 ± 6
Weight (kg)	71 ± 10	71 ± 7

Data are mean ± SD.

\*Significant difference between groups, P<0.001.

**TABLE 2: Perioperative characteristics**

	Vulvectomy	Laparotomy
Propofol during operation (mg)	1146 ± 828	1983 ± 105*
Operation time (min)	126 ± 50	188 ± 46*
Blood loss during operation (ml)	134 ± 271	959 ± 335 <sup>a</sup>
Colloids during operation (ml)	170 ± 242	540 ± 335*
Crystalloids during operation (ml)	1182 ± 627	1917 ± 655*
Sufentanil during operation (µg)	33 ± 15	37 ± 14
Cisatracurium during operation (mg)	20 ± 25	68 ± 18*

Data are mean ± SD.

\* Significant difference between groups, P<0.05.

<sup>a</sup> Significant difference between groups, P<0.001.

## RESULTS

### Demographics

Twenty eight female patients were included in the study; no patients were withdrawn from the study (table 1). The vulvectomy group contained 15 patients, while the laparotomy group contained 13 patients. Significant intergroup differences were found for age and ASA classification ( $P < 0.05$ ). Laparotomy patients were younger and had significantly lower ASA scores.

### Perioperative characteristics

Differences in perioperative characteristics were found. The abdominal hysterectomy group had a significantly longer operating time ( $P = 0.002$ ), more blood loss ( $P < 0.001$ ) and correspondingly more fluid replacement in the form of colloids ( $P = 0.002$ ) and crystalloids ( $P = 0.005$ ). In addition, significantly more propofol was used ( $P = 0.026$ ), however, there was no significant difference in the amount of sufentanil used between groups. One of the patients in the laparotomy group received a blood transfusion with 285ml of erythrocytes.

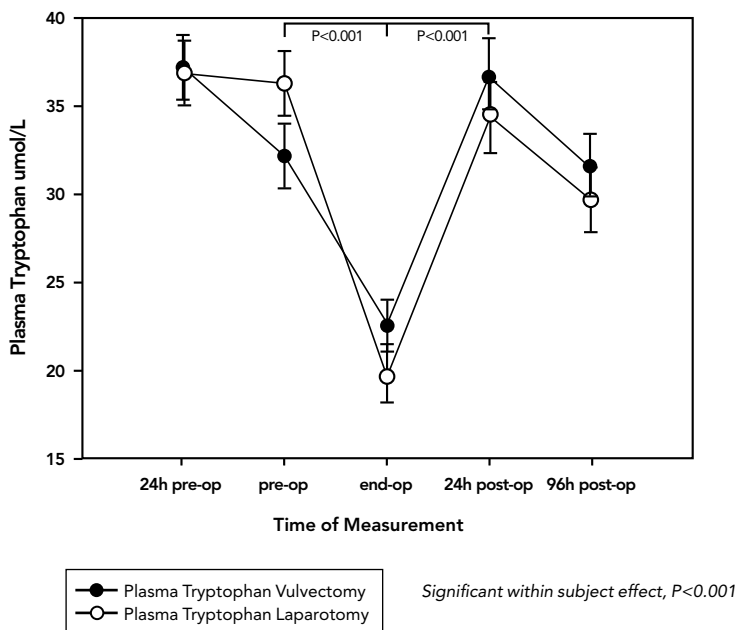
There was no significant difference in the amount of postoperative morphine, paracetamol or celecoxib given to both groups (table 2). However, 9 patients from the laparotomy group were given preoperative epidural catheters while only 3 patients were given one in the vulvectomy group. All epidural catheters were removed by 24 hours post-operative because pain control was found to be adequate.

**TABLE 3: Postoperative characteristics**

	Vulvectomy		Laparotomy	
	24 h post-operative	96 h post-operative	24 h post-operative	96 h post-operative
Paracetamol (mg)	3769 ± 832	4000 ± 0	3833 ± 577	4000 ± 0
Celecoxib (mg)	200 ± 0	186 ± 38	200 ± 0	233 ± 82
Morphine (mg)	6 ± 2	-	12 ± 1	-

*Data are mean ± SD. Celecoxib (selective COX-2 inhibitor).*

**FIGURE 2: Plasma Tryptophan levels**



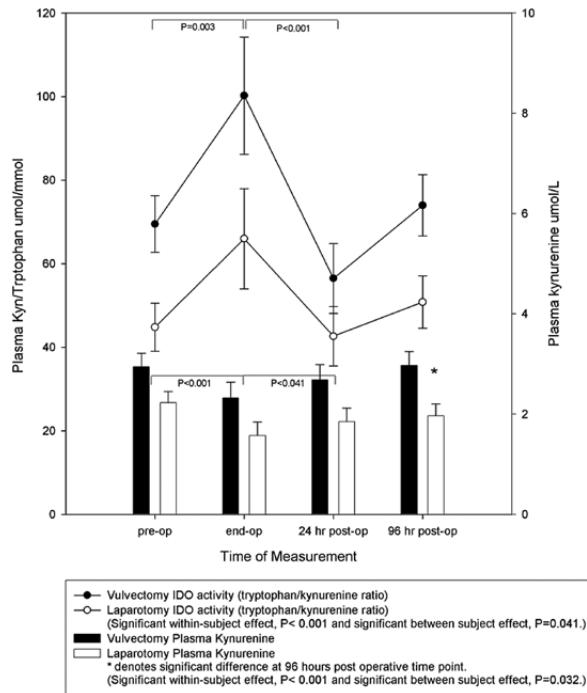
The effect of anesthesia in patients undergoing vulvectomy and major abdominal surgery on perioperative levels of plasma tryptophan. Values are the mean and SEM. There was a significant within subject effect ( $P < 0.001$ ). There was a significant decrease prior to induction and 30 minutes prior to the end of operation (bracket  $P < 0.001$ ). A significant increase occurred 30 minutes prior to the end of operation and 24 hours postoperative (bracket  $P < 0.001$ ).

### Plasma Tryptophan levels (figure 2)

Baseline levels of plasma tryptophan 24 hours before surgery did not significantly differ between groups. Throughout time, no significant difference between experimental groups was found. There was however a significant within subject effect over time ( $P < 0.001$ ).

Pairwise comparisons reveal a significant decrease between plasma tryptophan measured prior to induction and thirty minutes prior to the end of the operation ( $P < 0.001$ ). Following this significant decrease there was a significant increase between plasma tryptophan measured thirty minutes prior to the end of the operation and plasma measured 24 hours postoperative ( $P < 0.001$ ).

**FIGURE 3: Plasma Kynurenine levels and Indoleamine activity**



**Line graph:** The effect of anesthesia in patients undergoing vulvectomy and major abdominal surgery on perioperative IDO activity as defined by the ratio of tryptophan/kynurenine. Values are the mean and SEM. There was a significant within subject effect ( $P<0.001$ ). In addition, a significant difference between experimental groups was found ( $P=0.041$ ). There was a significant increase in plasma IDO activity measured prior to induction and thirty minutes prior to the end of the operation (bracket  $P=0.003$ ). A significant decrease occurred between IDO activity measured prior to the end of the operation and levels measured at 24 hours postoperative (bracket  $P<0.001$ ).

**Bar graph:** The effect of anesthesia in patients undergoing vulvectomy and major abdominal surgery on perioperative levels of KYN. Values are the mean and SEM. There was a significant within subject effect ( $P<0.001$ ) as well as a significant difference between experimental groups ( $P=0.032$ ). At 96 hours postoperative, the laparotomy group had significantly lower levels of plasma KYN than the vulvectomy group ( $P=0.028$ ). There was a significant plasma KYN level decrease during measurements made prior to induction and the end of operation (bracket  $P<0.001$ ). A significant increase occurred between points measured prior to the end of operation and 24 hours postoperative (bracket  $P<0.041$ ).

### **Plasma IDO activity, defined by the ratio of tryptophan/kynurenine (figure 3)**

Baseline levels of plasma IDO activity were not significantly different between both groups. There was a significant within subject effect over time ( $P<0.001$ ). In addition, a significant difference between experimental groups was found ( $P=0.041$ ).

Pairwise comparisons reveal a significant increase in plasma IDO activity measured prior to induction and thirty minutes prior to the end of the operation ( $P=0.003$ ). Following this significant increase there was a significant decrease between plasma IDO activity measured thirty minutes prior to the end of the operation and plasma measured 24 hours postoperative ( $P<0.001$ ).

### **Plasma Kynurenine (figure 3)**

Baseline levels of plasma kynurenine were not significantly different between both groups. There was a significant change in plasma kynurenine levels over time for both groups ( $P<0.001$ ) as well as a significant difference between experimental groups ( $P=0.032$ ). At 96 hours postoperative, the vulvectomy group had significantly higher levels of plasma kynurenine than the laparotomy group ( $P=0.028$ ).

Pairwise comparisons reveal a significant decrease in plasma kynurenine measured prior to induction and thirty minutes prior to the end of the operation ( $P<0.001$ ). Following this significant decrease there was a significant increase between thirty minutes prior to the end of the operation and plasma measured 24 hours postoperative ( $P<0.041$ ).

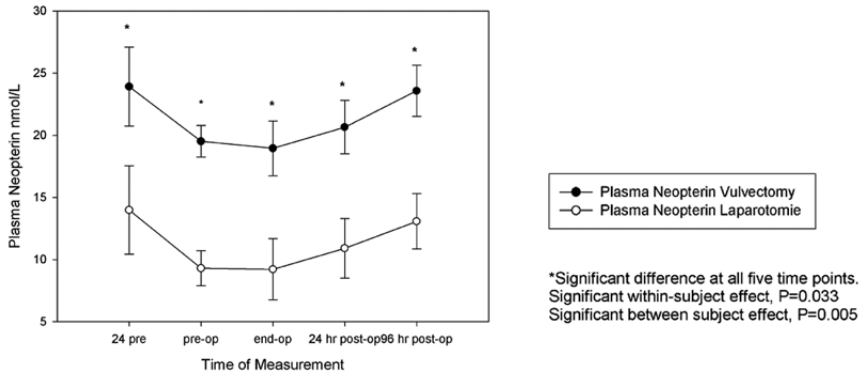
### **Plasma Neopterin levels (figure 4)**

Baseline levels of plasma neopterin significantly differed between both groups ( $P=0.002$ ); the vulvectomy group having much higher levels than the laparotomy group. A significant plasma neopterin level change occurred over time for both groups ( $P<0.033$ ). There was also a significant difference between experimental groups ( $P=0.005$ ).

### **Plasma IL-6 levels (figure 5)**

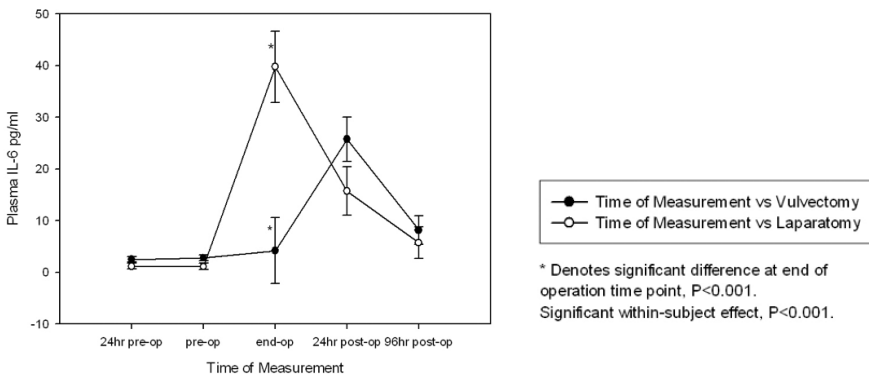
Baseline levels of plasma IL-6 were not significantly different. A significant plasma IL-6 level change over time occurred for both groups ( $P<0.001$ ). There was no significant difference between groups. However, the major abdominal surgery group had produced significantly more IL-6 than the vulvectomy group 30 minutes prior to the end of the operation ( $P<0.001$ ).

**FIGURE 4: Plasma Neopterin levels**



The effect of anesthesia in patients undergoing vulvectomy and major abdominal surgery on perioperative levels of plasma Neopterin. Values are the mean and SEM. There was a significant within subject effect ( $P<0.033$ ) and a significant difference between groups ( $P=0.005$ ).

**FIGURE 5: Plasma IL-6 levels**



The effect of anesthesia in patients undergoing vulvectomy and major abdominal surgery on perioperative levels of plasma IL-6. There was a significant within subject effect ( $P<0.001$ ). The major abdominal surgery group had produced significantly more IL-6 than the vulvectomy group 30 minutes prior to the end of the operation ( $P<0.001$ ).

## DISCUSSION

In this study we show that surgery and associated anesthesia significantly increase IDO activity, while maintaining stable neopterin levels. However, abdominal hysterectomy causes a major IL-6 increase. Via this longitudinal observational study, we believe we are the first to compare how two well defined surgical interventions of differing severity affect the tryptophan kynurenine pathway and the two inflammatory mediators neopterin and IL-6. It must be stressed, that there are differences in age and ASA-score between the groups. It was not our intention to compare two more or less identical groups. Due to the fact that the higher age and the higher ASA-score are associated with the vulvectomy group while most changes occur in the younger and healthier abdominal hysterectomy group, we can conclude that the observed effects are caused by the procedure and not by comorbidity.

Our tryptophan results show that in both groups stable baseline levels significantly decrease after induction with a significant rebound occurring during the 24 hour period after surgery. This data suggest that tryptophan is being rapidly consumed after induction. Anesthesia has been shown to cause IFN gamma release which in turn activates the enzyme IDO<sup>4,5,49</sup>. Our results show that there is significant activation ( $P=0.003$ ) of the IDO enzyme as suggested by the tryptophan/kynurenine ratio measured in both groups. During the 24 hours following surgery, however, a significant deactivation of IDO occurs ( $P<0.001$ ), although 96 hours after surgery IDO activity returns to preoperative levels. The significant kynurenine increase seen between the end of operation and 24 hours postoperative might provide an explanation for the corresponding drop in IDO activity. Allowing for a negative feedback mechanism capable of maintaining KYN homeostasis, KYN suppresses the activation of T cells and NK cells which are responsible for IFN gamma secretion, one of the known factors responsible for activating IDO.

Neopterin is primarily produced by human monocytes and macrophages. Increased neopterin concentrations are indicative of cellular immune activation<sup>52</sup>. We found significantly higher levels of neopterin at all five time points in the vulvectomy group. The cancer necessitating vulvectomy may explain these higher levels because cancer has been correlated with higher neopterin levels<sup>30,39</sup>. It is clearly evident that the time curve relationship is nearly identical for both groups. Remarkably, anesthesia and surgery did not significantly dampen or increase neopterin levels. Instead, there seems to be a neopterin level drop during the 24 hour period prior to induction with a slower recovery



to baseline level occurring during the 96 hour time period after surgery.

Not surprisingly, patients awaiting surgery suffer high levels of anxiety<sup>22</sup>. There is good evidence that in healthy individuals, acute psychological stress causes increased levels of catecholamines and cortisol, which in turn modulate the immune system<sup>1,25,31,34</sup>. We speculate that increased stress and associated increased cortisol synthesis prior to surgery caused a neopterin level decrease in the 24 hours prior to anesthesia. Opioid based anesthesia, however, is known to suppress cortisol synthesis and this may explain why neopterin levels do not continue to drop after induction of anesthesia<sup>5,20</sup>. Another explanation may be that the decrease in neopterin is counteracted by an increased release of neopterin induced by IFN  $\gamma$ .

Unlike our neopterin results, there are barely measurable IL-6 levels during the 24 hours prior to surgery. As would be expected from an acute phase pro-inflammatory cytokine known to be a sensitive early marker of tissue damage, there was a sharp increase resulting in significantly more IL-6 being made in the major abdominal surgery group at the end of the operation compared to the vulvectomy group. It is noteworthy that at 96 hours postoperative IL-6 levels almost return to baseline levels in both groups. The high prevalence of mast cells in the abdominal cavity might explain why an earlier and overwhelmingly more significant amount of IL-6 is released during the abdominal operation. A recent study provides convincing evidence that intestinal handling during open gynecological surgery causes mast cell activation and associated release of IL-6 and other inflammatory mediators<sup>43</sup>. When comparing with neopterin results it is reasonable to conclude that the large IL-6 peak found in the major abdominal surgery group is due to the more severe surgical trauma and intestinal handling experienced by this group.

The course of the tryptophan kynurenine pathway measured in both groups suggests that activated IDO might buffer overzealous cellular immune system activation by allowing for the restoration of KYN levels<sup>5</sup>. The trend seen in our neopterin results supports this idea. While our IL-6 results confirm that major abdominal surgery causes a more acute activation of the cellular immune system when compared to the less invasive vulvectomy group, the flare up seen in the major abdominal surgery group is short lived. This is confirmed by all of the data in this study. Between the end of the operation and 24 hours after surgery, the IL-6 peak quickly recedes to the same level seen in the less invasive (vulvectomy) group, while a significant kynurenine increase is seen. During this time frame, neopterin is still depressed when compared to baseline and 96 hour postoperative levels.

In addition to its ability to suppress natural killer and T cells, kynurenine also has a vasodilating effect that might explain our results. It is likely that the vasodilating properties of propofol help depress kynurenine levels after induction. A recent study using animal models has found that kynurenine plays a similarly important role in determining arterial wall tonus as nitric oxide does<sup>48</sup>. The two mediators act redundantly to influence vascular tone<sup>8,32,44</sup>.

Although this study is primarily intended to study the effects of differing degrees of surgery on inflammatory markers, this study also provides data that can be interpreted to show that anesthesia does not seem to be strictly immunosuppressive, but might have a buffering effect. It seems that adequate anesthesia preserves proper immune function while at the same time suppressing pathological immune activation in operations like major abdominal surgery which are more prone to a systemic inflammatory response syndrome (SIRS). Further research is necessary in order to investigate how opiates, hypnotic agents and the epidural technique specifically contribute to these effects. Most evidence suggests that opiates have immuno-modulating properties, while propofol allows for proper function of the immune system<sup>2,11,24,35,38</sup>. It is noteworthy that in this study there was significantly more propofol used in the group expressing a significant IL-6 increase. Taking this into account, it is likely that propofol is not responsible for immunosuppression.

## Limitations

The significantly less amount of propofol given to the vulvectomy group can be partly explained by the shorter operating time. However, women in this group were older and had higher ASA scores also explaining why less propofol was used. In addition, the greater amount of colloids and crystalloids given to the laparotomy group is related to the longer operating time and increased amount of blood loss in this group of patients. Nevertheless, the results shown in our figures make it clear that dilutional phenomena are not responsible for trends seen. Likewise, these perioperative characteristics provide additional evidence that abdominal hysterectomy is a heavier, more traumatic operation in comparison to the vulvectomy procedure. The more severe nature of abdominal hysterectomy also explains why anesthesiologists used significantly more invasive analgesia techniques during the 24 hour postoperative period as seen in table 4. Generally, a heavier regimen was chosen for heavier surgery. According to our results this seems to have an appropriate effect because IL-6 only showed one moment where levels were different between groups at 24 hours postoperative.

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**TABLE 4: Analgesia technique applied 24 hours postoperative**

	Only NSAIDs	NSAIDs and Intra-venous Morphine	NSAIDs, Intravenous Morphine and Epidural Anesthesia
Vulvectomy	8	4	3
Laparotomy *	1	3	9

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*\*Laparotomy group had significantly more invasive pain control,  $P < 0.05$ .*

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Furthermore, we did not consider or note the menopausal status of women when conducting this study, however, if the menopausal status had a confounding effect it would make our results even stronger: From the literature we know, that after menopause, there is an increase in pro-inflammatory serummarkers (IL-6 and TNF-alpha). When taking age into account, our vulvectomy group would have had more women who were post-menopausal than in the abdominal hysterectomy group. However, we found the higher IL-6-levels in the abdominal hysterectomy, the group with the younger patients. This supports the claim that the effect of the procedure is stronger than changes due to aging.

One might also discuss, whether it is prudent, to use COX-2-inhibitors as analgetics in a study on inflammation processes. We decided to do so, because it reflects our clinical standard practice and it would be considered an ethical problem by our institutional Medica Ethics Committee to change our pain control procedures just to create better study conditions. There is growing evidence that the suppression of inflammatory processes by COX-2-antagonists has more than only analgetic effects: depression and the immune response in cancer patients are two of the most interesting fields. We know that COX-2-inhibition can lead to down-regulation of IDO1 and decreased kynurenine levels. However, in our study the dosages administered were not that extremely different and nevertheless the group with the higher total dose of COX-2-inhibitor had the more pronounced changes in the immunological profile. Despite the use of a COX-2-inhibitor we found increased IDO-activity in both groups.

Finally, one might discuss the possible impact of the fact, that more patients in the abdominal hysterectomy used an epidural catheter. However, we know that an epidural catheter is not able to block the stress response to major surgery. If the epidural anesthesia would have had an anti-inflammatory effect, the differences between the groups might have even been bigger than observed in our study.

While taking all these factors into consideration, we are aware of the fact, that our groups are not totally similar. However, when there was a difference between patient demographics or in the anesthetic regimen used, they should have led to smaller differences than what were actually observed, which must be interpreted as extra evidence to support our theory.

## **CONCLUSION**

We conclude that surgery and associated anesthesia cause a significant decrease in tryptophan levels, while significantly increasing IDO activity. Both types of surgery produce nearly identical neopterin time curve relationships, with no significant change occurring in either group. However, even though neopterin is unaffected by the severity of surgery, IL-6 responded to surgical invasiveness by revealing a significant increase during major abdominal surgery.

## CHAPTER 5 • REFERENCES

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# CHAPTER 6

Surgery duration and not propofol influences  
IL-6 synthesis in patients undergoing surgery



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# ABSTRACT

## Background

Proper immune function during surgery is important in order to preserve healthy immunological reactions following surgery. There has been concern that anesthesia might play a role in causing pathological deactivation of the immune system. It is difficult for many studies to identify which elements of surgery are responsible for a perioperative dampened immune status. This observational study was intended to investigate whether or not propofol has any immunomodulatory effects in patients undergoing two distinct operations of different severity and duration.

## Methods

This study was a prospective, single center, two-armed observational study. EDTA blood samples (4ml) were collected for determination of IL-6: 24hrs preoperative, prior to induction, at the end of the operation, and at 24 and 96 hours postoperative in patients undergoing abdominal hysterectomy and vulvectomy.

## Results

Total plasma IL-6 measured at the end of the abdominal hysterectomy operation was significantly correlated to the amount of propofol given ( $r = 0.622$ ,  $P = 0.031$ ). No correlation was found in the vulvectomy group ( $r = -0.032$ ,  $P = 0.913$ ). Post surgery plasma IL-6 levels were significantly correlated to surgery duration in both surgery groups ( $r = 0.798$ ,  $P = 0.002$  and  $r = 0.568$ ,  $P = 0.034$ ).

## Conclusion

This study demonstrates that propofol does not diminish plasma levels of IL-6. Surgery duration positively correlates to the amount of IL-6 produced in both patient groups undergoing surgery. Our findings emphasize that there is a clinical benefit to reduce the duration of surgery as much as possible.

## KEYWORDS

Propofol, plasma IL-6, and surgery duration.

## INTRODUCTION

The possible immunomodulatory effects of anesthesia has received a lot of attention in recent literature<sup>1</sup>. Proper immune function during surgery is important in order to preserve healthy immunological reactions following surgery. There has been concern that anesthesia might play a role in causing pathological deactivation of the immune system, making the patient more susceptible to cancer metastasis, infection or sepsis after surgery<sup>2</sup>. On the other hand, it has been thought that the potentially dampening effects of anesthesia could be used to reduce the chance of a systemic inflammatory response syndrome from occurring during high risk surgery<sup>3-5</sup>.

There are wide ranging theories that describe mechanisms responsible for these actions. However, it is difficult for many studies to identify which elements of surgery are responsible for a dampened immune status during and after an operation. There is little evidence to support the concept of clinically relevant immune modulation that can be solely attributed to propofol use during surgery<sup>6</sup>. Tissue damage and manipulation resulting from surgery, the individual anesthetic drugs used and the endocrine stress response can all be responsible for these actions<sup>7-11</sup>.

Interleukin-6 (IL-6) is a cytokine that gives more insight into the reported anti-inflammatory actions of propofol. IL-6 is an acute phase pro-inflammatory marker that is released within minutes after tissue injury<sup>3,5</sup>. It is an accepted early indicator of inflammation with peak plasma levels being proportional to the amount of surgical trauma<sup>7-14</sup>. This pro-inflammatory cytokine stimulates the hepatic acute phase protein synthesis reaction that is required for a systemic inflammatory reaction. High levels of IL-6 have been linked to impaired cardiac function and correlated to postoperative complications and negative outcomes for trauma patients<sup>15,16</sup>.

It is our intention to investigate how propofol affects the release of IL-6 in the bloodstream during two different types of surgery of differing severity and duration.

## MATERIALS AND METHODS

### Patients

This study was a consecutive, single center, two-armed observational study with 28 female patients. The protocol was approved by the Medical Ethics

Committee of the Erasmus Medical Centre, Rotterdam (MEC-2008-134). All procedures were performed in accordance with the Helsinki declaration. Informed consent was obtained from all patients.

Inclusion criteria were 1) scheduled for vulvectomy or abdominal hysterectomy, 2) expected surgery duration greater than 0.5 h, 3) age higher than 18 years, 4) ASA (American Society of Anesthesiologists) classification I-III, 5) informed consent. Exclusion criteria were 1) ASA-classification IV-V, 2) patients unable to speak Dutch, 3) and patients not able to consent. Patients had the right to withdraw from the study at any time. Patients who developed serious adverse side effects were to be withdrawn from the study.

### **Anesthesia procedure**

All patients received a 1.0 mg tablet lorazepam and 100 mg celecoxib (selective COX-2 inhibitor) approximately one hour before surgery. Personal drug regimens were continued during the study. The observational nature of this study allowed the staff anesthesiologist to place an epidural catheter if the anesthesiologist felt it was indicated for adequate postoperative analgesia. All patients received total intravenous anesthesia, using propofol for sedation and sufentanil for analgesia. Cisatracurium provided muscle relaxation for optimal intubation conditions. Prior to the first incision all patients received antibiotics (1 g cefazoline and 500 mg metronidazol).

### **Postoperative pain management**

For all patients the minimum postoperative pain control regimen included 4000 mg paracetamol and 200 mg celecoxib per 24 hours. While in the recovery room, morphine was titrated until sufficient pain control was achieved. i.e. a score of less than 4 on the visual analog scale. The daily regimen of paracetamol and celecoxib was continued until patients no longer experienced pain with a VAS greater than four. Patients with an epidural catheter had it removed when the anesthesiologist determined that it was no longer indicated for adequate pain control.

### **Outcome measures**

Patient demographics, medications used during and after surgery, and duration of surgery was documented. EDTA blood samples (4ml) were collected at 24 hours preoperative, right after IV placement prior to induction, at the end of the operation, and at 24 and 96 hours postoperative.

Enzyme immunoassays for the quantitative determination of human IL-6 were performed with a sandwich ELISA (Pelikine Compact™ and additional Pelikine Toolset™, Sanquin, Amsterdam, The Netherlands) as described previously<sup>17</sup>. Data were calculated as pg/ml.

### **Statistical Analysis**

Data were analyzed using SPSS for windows, version 16.01. The independent sample t-test was used to compare means for patient demographics (excluding ASA classification) and perioperative characteristics. The Pearson Chi-square test was used to evaluate differences in ASA classification. The Fisher exact test was used to analyze difference in the type of pain control techniques used between groups (NSAID only, NSAID + Opiates, NSAID + Opiates + Epidural). The correlations between peak plasma IL-6 levels and all perioperative characteristics (see table 2) were assessed in both patient groups using the Spearman rank test or the Pearson correlation test in case of normal distributions. Calculations were performed using GraphPad Software (version Prism 5, San Diego, USA). All data are reported as the mean ± SD.

## **RESULTS**

### **Demographics (table 1):**

Twenty-eight female patients were included in the study; no patients were withdrawn from the study. The vulvectomy group contained 15 patients, while the abdominal hysterectomy group contained 13 patients. All patients were female and there was no significant difference in weight. Significant intergroup differences were found for age and ASA classification ( $P < 0.05$ ). Abdominal hysterectomy patients were younger and had significantly lower ASA scores.

### **Perioperative Characteristics (table 2):**

There was no significant difference in the amount of sufentanil used between groups. The abdominal hysterectomy group had a significantly longer operating time ( $P = 0.002$ ) and significantly more propofol was used in this group ( $P = 0.026$ ) compared to the vulvectomy group. One of the patients received a blood transfusion with 285ml of erythrocytes. The abdominal hysterectomy group used significantly more crystalloids, colloids and more cisatracurium.



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**TABLE 1: Patient demographics**

	Vulvectomy	Abdominal Hysterectomy
Age (years)	62 ± 12	44 ± 9*
Height (cm)	166 ± 5	167 ± 6
Weight (kg)	71 ± 10	71 ± 7

Data are mean ± SD except

\*Significant difference between groups,  $P < 0.001$ .

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**TABLE 2: Perioperative characteristics**

	Vulvectomy	Abdominal Hysterectomy
Propofol during operation (mg)	1146 ± 828	1983 ± 105*
Operation time (min)	126 ± 50	188 ± 46*
Blood loss during operation (ml)	134 ± 271	959 ± 335 <sup>a</sup>
Colloids during operation (ml)	170 ± 242	540 ± 335*
Crystalloids during operation (ml)	1182 ± 627	1917 ± 655*
Sufentanil during operation (µg)	33 ± 15	37 ± 14
Cisatracurium during operation (mg)	20 ± 25	68 ± 18*

Data are mean ± SD.

\* Significant difference between groups,  $P < 0.05$ .

<sup>a</sup> Significant difference between groups,  $P < 0.001$ .

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There was no significant difference in the amount of postoperative morphine, paracetamol or celecoxib given to both groups (table 3). However, 9 patients from the abdominal hysterectomy group were given preoperative epidural catheters for postoperative analgesia while only 3 patients were given one in the vulvectomy group. All epidural catheters were removed by 24 hours postoperative because pain control was found to be adequate.

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**TABLE 3: Postoperative characteristics**

	Vulvectomy		Abdominal Hysterectomy	
	24 h post-operative	96 h post-operative	24 h post-operative	96 h post-operative
Paracetamol (mg)	3769 ± 832	4000 ± 0	3833 ± 577	4000 ± 0
Celecoxib (mg)	200 ± 0	186 ± 38	200 ± 0	233 ± 82
Morphine (mg)	6 ± 2	-	12 ± 1	-

*Data are mean ± SD. Celecoxib (selective COX-2 inhibitor).*

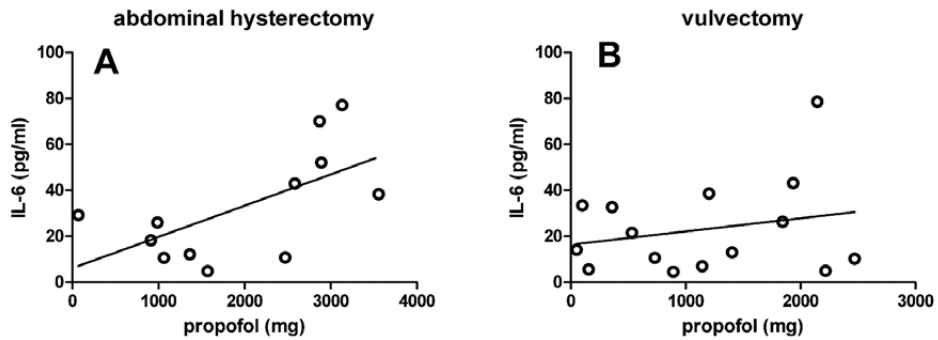
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### Plasma IL-6 levels

Baseline levels of plasma IL-6 in the abdominal hysterectomy group ( $1.1 \pm 0.8$  pg/ml) were not different from the levels in the vulvectomy group ( $1.7 \pm 0.8$  pg/ml). However, the abdominal hysterectomy group produced significantly more IL-6 than the vulvectomy group at the end of the operation ( $P < 0.001$ ). In addition, the abdominal hysterectomy group had peak levels of IL-6 at the end of the operation while the vulvectomy group experienced peak levels 24 hours after surgery.

Total plasma IL-6 measured at the end of the abdominal hysterectomy operation was significantly correlated to the amount propofol given ( $r = 0.622$ ,  $P = 0.031$ ; fig. 1A). No correlation was found in the vulvectomy group ( $r = -0.032$ ,  $P = 0.913$ ; fig. 1B). The results were found to be the same when the amount of propofol was designated as mg/kg body weight. However, the post surgery plasma IL-6 levels were significantly correlated to surgery duration both in the abdominal hysterectomy and in the vulvectomy group ( $r = 0.798$ ,  $P = 0.002$  and  $r = 0.568$ ,  $P = 0.034$ ; figs. 2 A and B). In both patient groups, there were no correlations between plasma IL-6 levels post surgery on the one hand, and blood loss and colloids, crystalloids and cisatracurium given during surgery on the other hand.

**FIGURE 1**



Effect of propofol on the plasma levels of IL-6 in the abdominal hysterectomy group at the end of surgery (A) and in the vulvectomy group 24 hours after surgery (B). There was a positive correlation between propofol given and IL-6 levels in the abdominal hysterectomy group only ( $r = 0.622$ ,  $P = 0.031$ ).

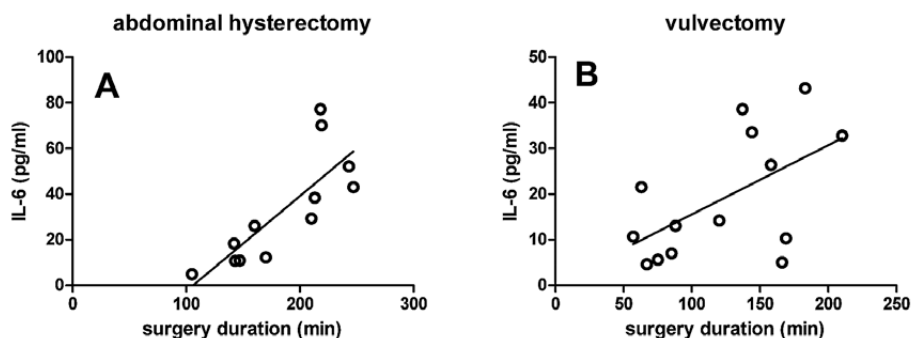
## DISCUSSION

This study demonstrates that propofol does not diminish plasma levels of IL-6. In addition, we found that the amount of IL-6 released was highly correlated to surgery duration.

There is a great deal of information about the immunological impact of anesthetic drugs<sup>18</sup>. Often times it is unclear whether opiates or hypnotic drugs contribute to immunomodulation. There is evidence that opiates can influence the immune system, although results are inconsistent<sup>19-21</sup>. Furthermore, the mechanism by which opiates influence the immune system are still unclear although it is apparent that immune cells have surface receptors for opiate drugs. These receptors are thought to influence cAMP pathways, which in turn are considered to influence IL-6 secretion<sup>22-24</sup>.

Hypnotic drugs have wide ranging cytokine effects *in vitro*, but data are inconclusive<sup>19,20,25</sup>. Propofol has been found to inhibit IL-6 production by lipopolysaccharide stimulated mononuclear cells *in vitro*<sup>25</sup>. *In vivo* data collected from rat studies revealed that propofol has anti-inflammatory properties and is able to dampen IL-6 increases<sup>26</sup>. A clinical study comparing anesthesia regimens found that anesthesia using propofol and alfentanil reduced the re-

**FIGURE 2**



Effect of surgery duration on the plasma levels of IL-6 in the abdominal hysterectomy group at the end of surgery (A) and in the vulvectomy group 24 hours after surgery (B). The correlations between surgery duration and IL-6 levels were significant in both patient groups.

lease of IL-6 when compared to anesthesia maintained with isoflurane and nitrous oxide, although it was not clear whether propofol or alfentanil was responsible. However, another study suggested that propofol/fentanyl anesthesia promoted a pro-inflammatory state<sup>27</sup>. When considering these studies, it is clear that different results are realized when propofol is used in different *in vivo* or *in vitro* experimental settings. Furthermore, most clinical studies available were unable to isolate the individual effect of propofol from various confounders such as varying anesthesia regimens. In most cases, the confounders were the relative effects of individual anesthetic drugs required to maintain anesthesia.

One focus of this study was to isolate the effect of propofol from the potentially confounding effects of opiates. This was made possible by investigating the effect of propofol on the release of IL-6 during two distinctive operations in which the use of sufentanil was not significantly different. The experimental setup of this study is unique because it compares the relationship between IL-6 and two variables in two well-defined surgical settings of different severity. The two variables are the amount of propofol administered and the length of the operation. These comparisons are made for an abdominal surgical procedure expected to generate an intense IL-6 response, with another surgical

procedure less prone to the same level of acute phase inflammatory reaction<sup>12,28</sup>.

Our results provide some interesting points. It is noteworthy that propofol was not immunosuppressive in the vulvectomy group, an operation expected to have less activation of the acute phase reaction. In addition, the amount of propofol used in the abdominal hysterectomy group, associated with the strongest acute phase reaction, also seemed to be positively correlated to IL-6. However, our study showed that this positive correlation was due to the longer operating time of this group. In both patient groups, increased IL-6 was correlated to surgery duration. When comparing the results of the abdominal hysterectomy group to our vulvectomy results, which show no correlation in the amount of propofol used and IL-6 measured, one can conclude that propofol does not dampen or heighten plasma IL-6 levels. Rather, it is clear that surgery duration is the most important factor influencing IL-6 synthesis.

Gilliland has previously been able to demonstrate that IL-6 response during abdominal surgery does not seem to be influenced by isoflurane and propofol, while Cruisank has shown that IL-6 responds to the severity of surgery with length of surgery or severity playing a role<sup>12,29</sup>. Our results seem to provide good evidence that the primary determinant that causes IL-6 levels to rise is surgery duration instead of the amount of propofol given or the type of surgery performed.

## Limitations

Epidural anesthesia is not expected to dampen the immune system; in fact it is expected to keep it intact. However, there is evidence that epidurals do not alter IL-6 profiles, thus it should not provide a confounding effect<sup>2,14,30-33</sup>. The ASA values are higher in the vulvectomy group due to the increased age at which vulva carcinoma presents. However, this did not influence IL-6 levels at baseline.

In conclusion, propofol does not inhibit IL-6 synthesis, however, longer operating times are paired with increased IL-6 production. In addition, our findings emphasize that there is a clinical benefit to reduce the duration of surgery as much as possible.

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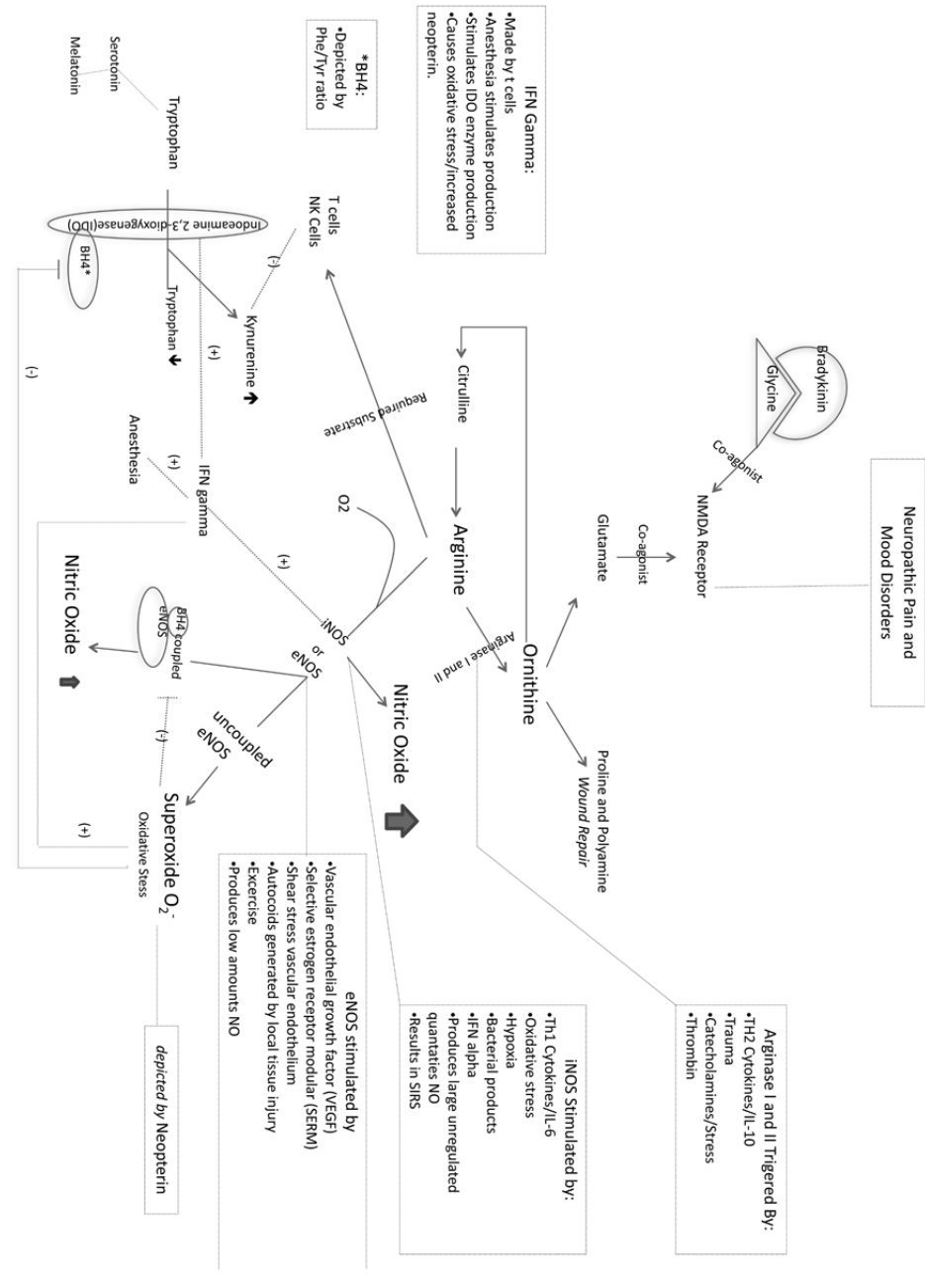


# CHAPTER 7

Discussion



**FIGURE 1: A summary of the biochemical impact of surgery and anesthesia**



Our first case series clearly demonstrated that cytokine concentrations significantly increased over time in both anesthesia and non-anesthesia (awake) groups. However, it is important to note that there was no significant difference between experimental groups when general anesthesia was the only clinically relevant variable being applied to patients.

Cytokine release is a known reaction to tissue damage. The non-significant difference between groups suggests from an immunological perspective that craniotomy performed during an awake procedure and one under general anesthesia have similar immunological effects. Not only did this trend apply for both pro-inflammatory cytokines IL-6 and IL-8, but also for the anti-inflammatory cytokine IL-10.

What makes these results more substantial is that there were cytokine responses throughout time for both experimental groups during surgery, but levels were almost similar in both groups and never significantly different from each other. Therefore, one can conclude that surgery is the main determinant when it comes to cytokine responses during surgery and not general anesthesia.

These results shed new light to the discussion about the potential immunosuppressive effects of general anesthesia on patients with cancer disorders. Most anesthesia care providers realize that it is important to keep the immune system intact in a way that provides cancer cell immune surveillance thought to be vital to prevent and inhibit tumor metastasis.

It is logical that our general anesthesia craniotomy group utilized significantly more propofol and opiate drugs than the awake group. What is clinically relevant however is that this did not significantly alter the immunological profile of both pro and anti-inflammatory cytokines. In addition when graphing the results it is clear that the trends over time seen were very similar in both groups.

IL-6 and IL-8 were the pro-inflammatory cytokines measured. IL-6 is known to protect cancer cells from apoptosis<sup>1,2</sup>, while IL-8 is thought to be a regulatory factor within the tumor environment playing an important role during angiogenesis, the proliferation and migration of cancer cells. Also, IL-8 has been shown to cause chemotherapeutic resistance in cancer cells<sup>3</sup>.

IL-10 is an anti-inflammatory cytokine and is known to inhibit the action of NK cells. It is produced by mastocytes and TH2 regulatory t-cells<sup>4</sup>. It also inhibits pro-inflammatory cytokines and COX activation<sup>5</sup>. Our results show that neither general anesthesia nor being awake during a well orchestrated awake craniotomy did not cause a significant increase over the course of 5 time points

and no difference at any point in time between the two surgical groups. This suggests that there was no dangerous immunosuppression caused by general anesthesia that could have been detrimental to the cancer patient in question. Furthermore, when taking the opportunity to look at the effect propofol might have on IL-6 during abdominal surgery, we were able to discover that propofol does not reduce plasma levels of IL-6, making this anesthetic a good choice during cancer surgery.

Pro-inflammatory cytokines have been associated with postoperative cancer recurrence and metastasis<sup>6</sup>. The balance between T1 helper cell (TH1) and T2 helper cells (TH2), also known as the Th1/Th2 balance, and the activity of Natural Killer cells (NK) are primary players during perioperative immunosuppression and cancer metastasis. NK cell function is necessary for anticancer immunity, while a TH2 dominant status causes immunosuppression that is detrimental for the cancer patient<sup>7,8</sup>.

It has been previously established that perioperative stress can significantly suppress anti-tumor immunity<sup>8</sup>. When the sympathetic nervous system is activated it causes neuroendocrine activation via the hypothalamic pituitary adrenal axis causing catecholamine release via noradrenaline and adrenaline. These catecholamines attach to Tcells, NKcells, and macrophages causing an internal cyclic AMP rise, inhibiting NK cells, instructing T cells and macrophages to produce pro TH2 cytokines ultimately causing a detrimental Th2 shift placing the cancer patient in immunologically detrimental state<sup>9,10</sup>. The balance between Th2 and Th1 dominance is partially determined by arginase and nitric oxide synthase by competing for the amino acid arginine, and will be subsequently discussed.

Keeping this in mind, our first case series clearly shows that effective and safe stress reduction can be attained via two methods, general anesthesia and an awake procedure. Indeed, it seems that mind and body are truly interconnected. However, as described during in our paper, the awake procedure must be carried under the condition that time and effort is invested prior to the operation to mentally prepare the patient for the procedure. In addition, the awake procedure is only possible if an optimal relationship exists between the operating team and patient.

When comparing amino acid profiles between craniotomy performed under general anesthesia or as an awake procedure, we were able to discover that general anesthesia causes tryptophan levels to decrease significantly more than in the awake group. It is possible that anesthesia allows for more tryptophan to be utilized by neuronal cells leading to elevated levels of neuro-

transmitters like serotonin and melatonin<sup>11</sup>. It has been previously been reported based on findings using biological models that anesthetics are capable of changing amino acid availability by influencing amino acid permeases<sup>12</sup>.

After matching our tryptophan results to measurements made during our second case series, where surgical severity was the variable being examined, one notices that there is a strikingly similar and significant drop in available tryptophan once surgery and anesthesia is initiated. We will return to this finding when discussing the tryptophan kynurenine pathway later during this general discussion.

While returning to our craniotomy findings, it is noteworthy that both experimental groups demonstrated increased postoperative levels of the excitatory amino acid and NMDA co-agonist glutamate, its precursor glutamine, and the other NMDA receptor co-agonist glycine when compared to healthy controls. Although postoperative pain was well managed, it seems reasonable that these amino acids could be elevated as a logical consequence of the relationship these amino acids have to the N-methyl-D-aspartate (NMDA) receptor. Furthermore, it is remarkable that for both groups the excitatory amino acid glutamate was significantly elevated both pre and postoperatively when compared to our healthy control group. When looking at intergroup differences, our awake group had 15% less plasma glutamate than the awake craniotomy group. This could suggest that physical stress and anxiety might influence levels of glutamate considering our awake group reported significantly less preoperative and postoperative VAS scores for anxiety than the general anesthesia group. The enzyme arginase is likely to be the mechanism responsible, it is triggered by catecholamines and stress<sup>13</sup>. All NMDA related amino acids reach their lowest point 24 hours postoperatively when they almost return to normal levels.

The NMDA receptor is an inotropic glutamate receptor found on the dendrites of post synaptic cells responding to the glutamate ligand allowing for the flow of K<sup>+</sup>, Ca<sup>2+</sup>, and Na<sup>+</sup> creating an excitatory postsynaptic current. It is also the NMDA receptor that is the predominant molecular mechanism known for controlling synaptic plasticity<sup>14</sup>.

Synaptic plasticity allows synapses to become more or less sensitive over time. This is the key mechanism making learning and memory possible<sup>15</sup>. More relevant to the perioperative process, is the role synaptic plasticity plays in establishing neuropathic pain syndromes. Synaptic plasticity allows for improved cation conduction allowing for the potentiation of a synapse<sup>16</sup>. Pain hypersensitivity and hyperalgesia is what results when this mechanism is not

corrected by negative feedback systems<sup>17</sup>. Although appropriate negative feedback mechanisms are present to prevent pain sensitization, an alarming number of elective surgery patients fail to do so after tissue or nerve injury developing extremely morbid neuropathic pain conditions. For example, it has been reported that for inguinal hernia and thoracic surgery that anywhere between 11.5–47% of the patients develop chronic pain conditions<sup>18,19</sup>.

Given that both surgical patient groups have elevated NMDA related amino acids postoperatively, it is worth contemplating whether or not it would be wise to use a NMDA receptor antagonists like ketamine or gabapentin and pregabalin in order to minimize the risk of developing a neuropathic pain condition when these drugs are not contra indicated<sup>18</sup>.

Pain, inflammation, tumor cytotoxicity, neural transmission, hemodynamics, sexual function, fertilization, clotting, and metabolism are all bound and controlled by a simple molecule known to exist for only a split second at a time, nitric oxide. This molecule is very influential, the physiological mechanisms elucidating the functions and workings of nitric oxide in health and disease resulted in various researchers winning the Nobel Prize in 1998.

Among the other biological activities already mentioned, levels of nitric oxide seem to regulate immune status with high levels associated with an anti-inflammatory response and in pathological cases, the opposite effect, the morbid systemic inflammatory response syndrome<sup>20</sup>. Studies of the enzyme responsible for making nitric oxide, nitric oxide synthase, have led to the conclusion that treatment of animals with cytokines increases the production of NO while also demonstrating that arginine is the exclusive precursor of nitric oxide<sup>21,22</sup>. As far as we know, we are the first to demonstrate that these mechanisms can be observed in human plasma as well.

Although nitric oxide synthase is responsible for producing nitric oxide, arginase is also an enzyme capable of regulating NO production because it directly competes with NOS for arginine availability in order to make ornithine<sup>23</sup>. Remarkably, arginase is an enzyme known to be stimulated by catecholamines<sup>13</sup>. We found a 50% drop in arginine availability in the 24 hours before the first surgical incision was performed in our vulvectomy and laparotomy groups indicating that preoperative anxiety and associated increase of catecholamines causes arginase activation resulting in depleted amounts of available arginine. Similar results have been reported in studies involving rats<sup>24</sup>.

Our main finding is that more invasive surgery has a more profound effect on plasma levels of ornithine and nitric oxide. We are the first to compare two patient groups in two well defined surgical interventions with different severity

in a controlled clinical setting where all 3 amino acids related to NO metabolism are measured at the same time. As illustrated in our figure, arginine, citrulline and ornithine are the key amino acids involved in NO metabolism.

Ornithine is essential for collagen synthesis and wound repair because it is the precursor for polyamine and proline synthesis. Unlike in our arginine results, significant intergroup differences start occurring from the end of the operation until 24 hours postoperatively. Using this data, one can deduce that heavier surgical trauma stimulates the laparotomy group to consume significantly more ornithine than the vulvectomy group. The more significant decrease of plasma ornithine levels in the more invasive surgery group suggest that more ornithine is being used to produce the proline and polyamines required for wound healing.

Rat studies have previously shown that surgical trauma profoundly affects nitric oxide metabolism, while an observational study has shown that trauma patients admitted to the emergency room have decreased levels when compared to controls<sup>23</sup>. Our results reveal a significant drop from the start of surgery until 24 hours after surgery and stays decreased in the severe group until our last measurements taken 96 hours later. We can conclude that the more significant drop in nitric oxide levels experienced by the more invasive laparotomy group is a physiological response to heavy surgery. When considering the role NO plays in maintaining vascular tonus, it seems logical that NO levels are lower in the group requiring vasoconstriction to preserve physiological hemodynamic parameters. Too much NO created during pathological conditions would have resulted in circulatory collapse like that seen in SIRS, while too little would create ischemic conditions causing injury and leaving tissue susceptible to infection.

When looking at the levels of tryptophan measured while surgical severity was the variable being examined, one notices that there is a strikingly similar and significant drop in available tryptophan once surgery and anesthesia is initiated as seen in the craniotomy data.

The tryptophan kynurenine pathway is a notable stress mechanism likely to explain why available tryptophan markedly decreases after starting surgery in all our patient groups. Stress and pro-inflammatory cytokines activate the enzyme indole amine dioxygenase (IDO) metabolizing tryptophan into kynurenine. In addition, not only does surgical stress cause the activation of IDO, anesthesia has been shown to cause levels of interferon gamma (IFN) gamma to increase, which in turn is also known to be an activator of the IDO enzyme<sup>25-27</sup>. We were able to confirm that there was indeed significant activation of the

IDO enzyme by using the tryptophan/kynurenine ratio as an indicator of IDO enzyme activity. Furthermore, by measuring neopterin levels, we were able to quantify the amount of oxidative stress being experienced by the body<sup>28,29</sup>. We also know that too much oxidative stress causes inhibition of the IDO cofactor tetrahydrobiopterin (BH4) effectively<sup>30</sup>, inhibiting the production of kynurenine, and allowing for uncontrolled immune activation via T cells and natural killer cells making the possibility of an uncontrolled systemic inflammatory response syndrome (SIRS) reaction a possibility. Considering that the neopterin levels measured revealed that there was no clinically relevant increased amounts of oxidative stress, the IDO BH4 complex was free to convert tryptophan into kynurenine allowing for a healthy negative feedback loop. In our study, kynurenine, neopterin and IDO activity returned to preoperative levels within 96 hours postoperative, while IL-6 levels almost return to preoperative levels. Taking all these mediators together, we were able to document that surgery under general anesthesia allowed for a proper immune response considering that IL-6 was able to peak for a short time frame while a simultaneously activated IDO enzyme was able to buffer any potential overzealous immune system activation by restoring kynurenine to physiological levels.

Recent research published in *Nature Immunology* describes IDO as an enzyme that catalyzes the degradation of tryptophan, which limits its availability as an essential amino acid required for T cell growth, thereby making it essentially a protective negative regulator of immune responses. In addition it has been found that IDO acts via phosphatase SHP-1 to inhibit the kinase IRAK1 responsible for the up regulation of the inflammatory cytokine IL-6<sup>31</sup>. Our *in vivo* results seem to mirror this theory.

Further emphasizing the importance of a proper functioning kynurenine, tryptophan metabolic pathway and associated inflammatory intermediates is the finding that increased kynurenine/tryptophan ratios in ICU patients has been associated with fewer days alive and less days free from delirium and coma free days. However, these findings were found in seriously sick ICU patients, suggesting that the increased oxidative stress produced more of the neurotoxic 3-hydroxykynurenine instead of the neuroprotective kynurenic acid. It seems that oxidative stress decides which form predominates. Considering that our patients had normal levels of oxidative stress as measured by neopterin, it is likely that our patients benefited from kynurenic acid<sup>32</sup>.

In summary, we have found that surgery and its duration is the main determinant that causes cytokine fluctuations, not anesthesia. This implies that, if done properly, anesthesia is safe for immunologically vulnerable patients. We



have also found that a craniotomy done during an expertly performed awake procedure provides the same level of comfort and stress reduction, as does general anesthesia. This was not only confirmed by biochemical measurements, but also the reported perception of our patients.

It was also discovered that anesthesia causes plasma tryptophan levels to decrease, why this is the case is not entirely clear. This observation gives us a small hint about how anesthesia works on a biochemical level. More research could unravel this mystery and perhaps provide for new therapeutic options.

Our research also provides biochemical evidence that it is worthwhile to consider NMDA receptor antagonists during routine surgery especially when combining this data with recent publications about unacceptably high numbers of patients developing neuropathic pain syndromes after routine elective surgery. Future research should focus on which NMDA receptor drugs are most effective at reducing the incidence of neuropathic pain after surgery.

Wound healing might benefit from our observation that the amino acid ornithine is more significantly consumed during more invasive surgery. This has profound implications; it would be very remarkable if ornithine supplementation can safely improve the quality and speed of wound healing.

We can also safely state that adequate anesthesia during surgery allows for a proper and normal physiological immune response. An immune response that was not paired with excessive oxidative stress or explosive pathological nitric oxide levels associated with morbid pathology.

As mentioned during our introduction, before anesthesia was invented, the medical community was rightfully concerned about whether or not reducing stress via anesthesia would be detrimental to patients. In those times concepts like immunosuppression were not commonplace like today. The forefathers of modern medicine were rightfully concerned about whether human physiology would be pathologically disturbed by anesthesia. Patients and medical practitioners today could not imagine modern medicine without anesthesia and it has been deemed relatively safe when practiced in experienced hands. However, we hope that our research will provide more insight into the biochemical workings of anesthesia and give some reassurance that biochemically, anesthesia can be deemed safe, and certainly necessary when looking at risk benefit ratios of various medical interventions.

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# SUMMARY



Anesthesia has been accepted by the medical community as an obligatory medical intervention required during surgery. A great deal of research has focused on the physiological effects of anesthesia, however, surprisingly little is known about the biochemical effects of anesthesia and surgery as a whole. The focus of our research is to study the effects of anesthesia on metabolic and inflammatory pathways during surgery. It is also our intention to look at how severity of surgery influences these pathways.

Craniotomy under general anesthesia and awake function-controlled craniotomy allow for a unique clinical setting where measurements can be made quantifying the biochemical effects of anesthesia during one type of surgical procedure where the intended variable is general anesthesia. Cytokine profiles were analyzed in order to scrutinize the effect anesthesia might have on inflammation. Amino acids were also measured in order to look at metabolic effects.

We are able to conclude that awake craniotomy (surgery performed without anesthesia) does not cause a significantly different inflammatory response than craniotomy performed under general anesthesia.

Our amino acid results indicate that craniotomy performed under general anesthesia causes more oxidative stress when comparing to patients undergoing the procedure without general anesthesia. We also found that pain was less in awake patients and that they were able to recover more quickly and leave the hospital significantly earlier. We were also able to confirm that awake craniotomy does not cause a greater emotional challenge than tumor resection done under general anesthesia. It is safe to summarize that at the very least, an awake craniotomy provides the same level of comfort and stress reduction, as does general anesthesia when performed in experienced hands.

Although patients from both groups undergoing craniotomy were adequately treated for perioperative pain, we were able to show that there were increased levels of pain related amino acids in both surgical groups when comparing to a control group that was not operated on.

The second phase of our research aimed to find out how the severity of surgery influences inflammatory and associated amino acid pathways while intending to maintain similar anesthesia mechanisms. We were particularly interested in how surgical severity influences nitric oxide, a molecule known to be at the center of almost every important physiological process in the body. By also simultaneously measuring citrulline, ornithine and arginine we were able to have a better look and pre and post nitric oxide pathways. Ornithine's status as being essential for wound repair makes this amino acid even more

remarkable. Also of interest was the excitatory amino acid and neurotransmitter glutamine and glutamate because of the prominent role it plays during pain perception via the NMDA receptor. Tryptophan was also a noteworthy amino acid worth observing because it is the direct precursor of serotonin, one of the main neurotransmitters found between synapses.

We discovered that more severe surgery causes a decrease in nitric oxide levels and that surgery is the main determinant that causes a cytokine response and not general anesthesia. We also found that severity of surgery causes an increase in NMDA pain receptor related amino acids and that heavier surgery causes an increased consumption of ornithine, an amino acid considered essential for wound healing. Furthermore, anesthesia and not surgery causes plasma tryptophan levels to decrease, possibly giving us a clue as to how anesthesia works on a biochemical level.

On the whole, adequate anesthesia during various types of surgery allows for a proper immune response without uncontrolled or excessive oxidative stress or overzealous immune system activation. We were also able to confirm that propofol does not cause immunosuppression. Our results suggest that the type of surgery, its invasiveness and operating time are the main determinants influencing various biochemical pathways. General anesthesia and well performed regional anesthesia as performed during awake craniotomy obviously provides comfort, but does not seem to impact biochemical pathways like surgery does.





# SAMENVATTING



Anesthesie is geaccepteerd door de medische wereld als een essentiële medische interventie die noodzakelijk is tijdens chirurgie. Veel onderzoek is al besteed aan de fysiologische effecten van anesthesie, maar er is weinig bekend over de biochemische effecten van chirurgie en anesthesie als geheel. Het doel van ons onderzoek is om de effecten van anesthesie te onderzoeken op metabolische- en ontstekingsparameters tijdens chirurgie. Het is tevens onze intentie om te kijken hoe de omvang van de chirurgie hier invloed op uitoefent.

Wanneer je een craniotomie operatie (onder algehele anesthesiologie) en een wakkere craniotomie operatie met elkaar vergelijkt, maak je het mogelijk om een unieke klinische situatie te creëren, waarbij de biochemische effecten van anesthesie gemeten en ook onderscheiden kunnen worden van chirurgische effecten. Cytokine-profielen werden geanalyseerd om te kunnen onderscheiden hoe anesthesie inflammatie beïnvloedt. Aminosuren werden geanalyseerd om metabolische effecten te bekijken.

Het was mogelijk om te concluderen dat wakkere craniotomie (chirurgie zonder algehele anesthesie) geen significant verschillende ontstekingsreactie veroorzaakte dan craniotomie gedaan onder algehele anesthesie.

Onze resultaten met betrekking tot de aminosuren laten zien dat een craniotomie onder algehele anesthesie meer oxidatieve stress veroorzaakt wanneer je deze patiënten vergelijkt met patiënten die de procedure ondergaan zonder algehele anesthesie. Het was ook duidelijk dat pijn minder was bij de wakkere patiënten en dat ze sneller herstelden en het ziekenhuis eerder konden verlaten. Tevens hebben wij kunnen aantonen dat wakkere craniotomie niet gepaard gaat met toegenomen emotionele consequenties wanneer je deze groep vergelijkt met de procedure onder algehele anesthesiologie. Het gaat gepaard met dezelfde comfort- en stressreductie als bij algehele anesthesiologie op voorwaarde dat het anesthesiebeleid door een ervaren professional wordt uitgevoerd.

Hoewel al onze chirurgische patiënten adequate postoperatieve pijnstilling hebben ontvangen, hebben wij wel kunnen aantonen dat er toegenomen spiegels van aan pijn gerelateerde aminosuren aanwezig waren in beide chirurgische groepen wanneer je dit vergelijkt met een gezonde controle groep die geen operatie heeft ondergaan.

Het tweede deel van ons onderzoek is bedoeld om te kijken hoe de intensiteit van een chirurgische ingreep ontstekings- en aminozuurprofielen beïnvloedt, terwijl een vergelijkbaar anesthesiologisch beleid gevoerd wordt. Wij hadden een bijzondere interesse in hoe dit nitric oxide (NO) zou beïnvloeden,

een molecuul dat een sleutelrol speelt tijdens bijna alle belangrijke fysiologische processen in ons lichaam. Door ook tegelijkertijd citrulline, ornithine en arginine te meten was het ook mogelijk om pre en post nitric oxide pathways beter te begrijpen. Ornithine was zeer interessant omdat het een essentieel aminozuur is voor een normale wondgenezing. Glutamine en glutamaat hadden onze interesse omdat deze aminozuren een sleutelrol spelen tijdens pijnperceptie via de NMDA receptor. Ook tryptofaan was de moeite waard om te meten omdat het een voorloper is van serotonine, een van de belangrijkste neurotransmitters in onze synapsen.

We hebben kunnen ontdekken dat intensievere chirurgie NO spiegels doet afnemen en dat chirurgie de primaire determinant is die de cytokinereactie veroorzaakt en niet algehele anesthesiologie. Wij hebben ook kunnen vaststellen dat de ernst van chirurgie een grotere toename veroorzaakt van NDMA gerelateerde aminozuren en dat zwaardere chirurgie een toegenomen consumptie van ornithine veroorzaakt, het aminozuur dat essentieel is voor de wondgenezing. Verder doet anesthesie en niet chirurgie tryptofaanspiegels dalen wat mogelijk een aanwijzing geeft in hoe anesthesiologie werkt op biochemisch niveau.

Het lijkt erop dat adequate anesthesiologie, tijdens verschillende soorten chirurgie, het mogelijk maakt om een normale en effectieve afweerreactie op te roepen zonder een ongecontroleerde of overmatige afweer en oxidatieve stress teweeg te brengen. Wij hebben ook kunnen bevestigen dat propofol geen immunosuppressie veroorzaakt. Onze resultaten suggereren dat de ernst van de chirurgie en het type en de duur van een operatie het meeste invloed hebben op biochemische reacties in het lichaam. Algehele anesthesie en een goed uitgevoerde regionale anesthesie, zoals tijdens een wakkere craniotomie, bieden uiteraard comfort aan een patiënt, maar lijken geen dempende werking te hebben op de biochemische reacties die chirurgische incisies doen oproepen in het lichaam.



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# **CURRICULUM VITAE**



## **Jaap Willem Hol**

**Geboren 22 november, 1977 te San Salvador**  
**Nederlandse nationaliteit**

## **DIPLOMA'S**

### **Erasmus Medisch Centrum – Registratiecommissie Geneeskundig Specialisten**

Medisch Specialist - Anesthesioloog  
*Juli 2009 tot juli 2014*

### **Erasmus Universiteit**

Doctorandus in de Geneeskunde en arts examen  
Rotterdam, Nederland  
*September 2002 tot en met juli 2008*

### **Duke University**

Bachelor of Science  
Plan Cell Biologie (Major) en Scheikunde (Minor)  
Durham North Carolina, Verenigde Staten van Amerika  
*Augustus 1996 tot en met september 2000*

### **Foothill Highschool**

Tustin, California, Verenigde Staten van Amerika  
*September 1994 tot en met juni 1996*

## **WERKERVARING**

### **Onze Lieve Vrouwe Gasthuis**

Anesthesioloog – Fellow Pijnbestrijding  
*Juli 2014 tot heden*

### **Erasmus Medisch Centrum**

Arts in opleiding tot Anesthesioloog  
*Juli 2008 tot juli 2014*

### **Genzyme**

Research and Development Medewerker Lysozomal Storage Disorders  
Naarden Bussum, Nederland  
*Februari 2002 tot en met juli 2002*

### **Medix Emergency Ambulance Service**

Emergency Medical Technician  
Mission Viejo, California  
*September 2000 tot en met november 2002*

### **United Business Media**

Entry Level Sales CMP Media's Technology Group  
Los Angeles, California  
*Januari 2001 tot en met mei 2001*

### **City of Newport Beach California Fire Department**

Professional Ocean Lifeguard and Emergency Medical Technician (EMT)  
Supervisor (*zomer 1999, 2000, 2001*)  
*Maart 1995 tot en met november 2001*

## **ACTIVITEITEN NAAST DE OPLEIDING TOT ANESTHESIOLOOG**

### **Voorzitter CASA (Commissie Arts in Opleiding tot Specialist Anesthesioloog) Nederlandse Vereniging Anesthesiologie (NVA)**

MSRC visitaties en de belangenbehartiging van AIOS anesthesiologie op  
nationaal niveau.  
*Oktober 2011 tot april 2014*

### **Assistenten Vertegenwoordiger AIOS Anesthesiologie Erasmus MC**

*Februari 2011 tot heden*

## WETENSCHAPPELIJKE PUBLICATIES

Effect of major and minor surgery on plasma levels of arginine, citrulline, nitric oxide and ornithine in humans. *Annals of Surgery*.

*Hol JW, van Lier F, Valk M, Klimek M, Stolker RJ, Fekkes D.*

Awake craniotomy induces fewer changes in the plasma amino acid profile than craniotomy under general anesthesia. *Journal of Neurological Anesthesia*.

*Hol JW, Klimek M, Heide-Mulder M, Stronks D, Vincent AJ, Klein J, Zijlstra FJ, and Fekkes D.*

Inflammatory profile of awake function-controlled craniotomy and craniotomy under general anesthesia. *Mediators Inflamm*.

*Klimek M, Hol JW, Wens S, Heijmans-Antonissen C, Niehof S, Vincent AJ, Klein J, Zijlstra FJ.*

Giant Brunner's hamartomas of the duodenum and obstructive jaundice, an overview of the literature and suspicion of malignancy in a case. *Digestive Surgery*.

*Hol JW, Stuijbergen WNHM, Teeppen JLJM, van Laarhoven CJHM.*

Epidural Analgesia Is Associated with Improved Health Outcomes of Surgical Patients with Chronic Obstructive Pulmonary Disease. *Anesthesiology*.

*van Lier F, van der Geest PJ, Hoeks SE, van Gestel YR, Hol JW, Sin DD, Stolker RJ, Poldermans D.*

Risk modification for postoperative pulmonary embolism: Timing of postoperative prophylaxis. *Thrombosis Research*.

*van Lier F, van der Geest PJ, Hol JW, Leebeek FW, Hoeks SE.*

Modafinil reduces patient – reported tiredness after sedation/analgesia but does not improve patient psychomotor skills. *Acta Anaesthesiologica Scandinavica*.

*Galvin E, Boesjes H, Hol JW, Ubben JF, Klein J, Verbrugge SJ.*

## **VRIJWILLIGERSWERK EN NEVENFUNCTIES**

### **Operatiekamer centrum locatie H gebouw, Erasmus MC, Rotterdam**

Studenten Teamleider

*3 jaar*

### **Duke Emergency Medical Service**

President (1999 en 2000)

Automated External Defibrillator (AED) programma geïmplementeerd.

Emergency Medical Technician (EMT) trainingsprogramma opgezet.

Donatie Ambulance gerealiseerd.

Coördinatie van 25 vrijwillige EMT's die een populatie van 30,000 mensen dienden op de Duke University campus.

*Lid van september 1996 tot en met mei 2000*

### **American Red Cross**

Board of Directors Central North Carolina Chapter

*September 1997 tot en met mei 1999*

### **Duke University Journal of Science and Technology Vertices**

Science Writer

*September 1999 tot en met mei 2000*

## **HOBBY'S**

SKADI Algemene Rotterdamse Studenten Roeivereniging tijdens geneeskunde opleiding.

Zwemmen en waterpolo.

Zeilen. Nederlands vaarbewijs en 13 jaar kajuit zeilervaring.

Golfsurfing.

Scuba diving. Padi en Naii gecertificeerd. Ben lid geweest van het body recovery team Newport Beach Fire Department.

## ONTVANGEN OORKONDEN

Irvine Company (California) spectrum leadership scholarship

Hitachi LTD community safety scholarship

American Volunteers Association "Circle of Life" award for service

Elks Lodge student leadership award

California State Federation scholarship

Boys State California nomination

## **Stellingen bij het proefschrift *The Biochemical Impact of Surgery and Anesthesia*, door Jaap Willem Hol**

1. Surgery is the main determinant that causes a cytokine response and not general anesthesia. *(This thesis)*
2. An awake craniotomy provides the same level of comfort and stress reduction as does general anesthesia. *(This thesis)*
3. Surgery causes an increase in NMDA receptor related amino acids requiring appropriate pharmacological intervention to prevent surgery related neuropathic pain syndromes. *(This thesis)*
4. Heavier surgical trauma causes increased consumption of ornithine, the amino acid used to produce proline and polyamine required for wound healing. *(This thesis)*
5. Heavy surgery causes nitric oxide to decrease more significantly when compared to less invasive surgery. *(This thesis)*
6. Propofol is not immunosuppressive.
7. Having an open mind and possessing keen observational skills are common traits shared by history's most pioneering scientists who have made revolutionary discoveries by reporting accidental findings or unintended experimental outcomes.
8. The discovery of and application of anesthesia and pain relief is one of the most important contributions to medicine and mankind.
9. Just because there is no evidence does not mean that it is not true.
10. Every single individual should be encouraged to stick their heads "boven het maaiveld".
11. The ones who are crazy enough to think that they can change the world, are the ones who do. *(Steve Jobs)*

