# The effect of intra-abdominal local anaesthetics following major gyneacological surgery

## Clinical and experimental studies

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*Till Julia och Astrid*

# Abstract

**Background:** Local anaesthetics (LA), in addition to inhibition of pain signalling, also have anti-inflammatory properties. In vitro studies have demonstrated anti-proliferative and cytotoxic effect of LAs on cancer cells when administered in therapeutic concentrations.

Intraperitoneal administrated LA is shown to reduce pain, improve surgical recovery and to blunt the postsurgical inflammatory response. Retrospective studies have indicated beneficial oncological outcome of regional anaesthesia on cancer recurrence when used in cancer surgery. Abdominal hysterectomy causes moderate to severe pain, and assessing new tools for pain treatment is crucial. The postoperative period of extensive surgery for advanced ovarian cancer is associated with high morbidity. When the patients have recovered from cancer surgery, chemotherapy can be initiated. New therapeutic approaches to enhanced recovery with reduced postoperative pain and inflammation is of great interest.

**Methods and aim:** The thesis aimed to evaluate the efficacy of intra-abdominal local anaesthetics on pain, inflammatory response, serum concentration of LA and patient recovery after gynaecological surgery (study I, II and III). The aim of study IV was to determine the effects of LA on ovarian cancer cells in vitro. The clinical studies were prospective, double blind, randomized and placebo-controlled. In study I, women scheduled for abdominal hysterectomy, were randomised to local infiltration analgesia (Group LIA) or placebo (group C). Rescue analgesic consumption and opioid related side effects were analysed. In study II and III, women undergoing cytoreductive surgery for advanced ovarian cancer were randomised to receive either intraperitoneal ropivacaine (Group IPLA) or saline (Group Control) peroperatively. Inflammatory markers in serum, LA concentrations (study II), and objective measures of patient comfort, postoperative complications, pain, home readiness and time to initiation of chemotherapy (study III) were analysed.

In study IV proliferation and migration in two ovarian cancer cell lines, exposed to LA in concentrations corresponding to doses used in study II and III, were analysed. Analysis of cancer stem cells (CSC) phenotypes were performed.

**Results:** The median supplemental requirements of morphine during 0–24 hours after abdominal hysterectomy was significantly lower in group LIA compared to group C (18 mg vs. 27 mg, $p = 0.028$) and the median time to first analgesic injection was significantly longer in group LIA (40 min vs. 20 min, $p = 0.005$) (Study I).

Perioperative intraperitoneal LA resulted in significantly decreased serum cortisol levels. Serum concentrations of ropivacaine were well below toxic concentrations (study II). Time to initiation of chemotherapy was significantly shorter in group IPLA (Median 21, IQR 19-29 vs. 29 days, IQR 21-40, $p = 0.021$). No differences in standardised recovery endpoints were found between the groups (Study III) .

The laboratory study showed a significantly reduced cell number and an inhibited cell migration. Cell size were significantly increased and CSC phenotype analysis showed a reduction in all cells by up to 50% (Study IV).

**Discussion:** Local infiltration analgesia results in a significantly lower rescue morphine consumption following abdominal hysterectomy.

Intraperitoneal local anestetics can be administered in ovarian cancer cytoreductive surgery safely, without achieving toxic doses. Although IPLA do not provide further anti-inflammatory effects, the stress response is briefly blunted and there might be positive effects such as earlier start of chemotherapy. LA reduce the ability of cancer cells to metastasise.

Intra-abdominal LA offers a potential to have beneficial effects on pain, recovery and circulating tumour cells after gynaecological surgery.

**Keywords:** Local anaesthetics, postoperative pain, hysterectomy, inflammation, ropivacaine, toxicity, recovery, ovarian cancer, ovarian cancer cells.

# Populärvetenskaplig sammanfattning på svenska

Syftet med avhandlingsarbetet var att undersöka effekten av lokalbedövningsmedel (LA) givet in i bukhålan, så kallad intraperitoneal lokalanestesi (IPLA), vid gynekologisk kirurgi. Vårt mål var att finna en tilläggsbehandling som optimerar måendet hos kvinnor som opererar bort livmodern, samt hos kvinnor som genomgår omfattande kirurgi på grund av äggstockscancer.

Den bakomliggande teorin är att om man blockerar smärtimpulserna med LA direkt vid platsen för vävnadskadan, minskar den postoperativa smärtan och det kirurgiska stressvaret.

Lokalbedövningsmedel har beskrivits vara inflammationshämmande och även ha en direkt hämmande effekt på cancerceller. IPLA har i andra studier visats förbättra återhämtningen efter stor bukkirurgi. Ytterligare andra studier har beskrivit att valet av narkosmedel och bedövningsmetod kan påverka prognosen i cancersjukdomen. Äggstockscancer har en hög dödlighet och en hög risk för komplikationer efter operationen. När patienten har återhämtat sig efter kirurgin kan cellgiftbehandling påbörjas. Om man kan ge en behandling som är smärtlindrande, som påverkar det inflammatoriska svaret och möjligen även återhämtningen, kan det ha positiva effekter för patientens prognos.

Två kliniska studier med patienter som genomgick kirurgi utfördes. Studierna delades upp i tre delarbeten. Delarbete I undersökte effekten på postoperativ smärta efter att livmodern opererats bort. Delabete II studerade det inflammatoriska svaret och halten av lokalbedövningsmedel i blodet vid tillägg av IPLA i samband med äggstockscancerkirurgi. Delarbete III undersökte smärta, tarmfunktion, rörlighet, mående, komplikationer och tiden till start av cellgifter efter kirurgi mot äggstockscancer. Det avslutande delarbete IV utfördes i laboratoriemiljö där framodlade äggstockscancerceller exponerades för lokalbedövningsmedel i de doser som patienterna behandlats med i delarbete II och III.

I delarbete I undersökte vi 60 kvinnor som lottades till att antingen standardiserat få aktiv behandling alternativt koksalt infiltrerat i vävnaden som skadats i operationsområdet.

Vi kunde visa att morfinbehovet reducerades med 30%, smärtskattningen var lägre under de första timmarna och det tog längre tid innan patienterna behövde den första dosen med smärtlindring i den gruppen som fått aktiv behandling. Vi drar slutsatsen

att man kan erbjuda den testade metoden till de kvinnor som inte kan, eller inte vill, ha ryggbedövning eller morfin som smärtbehandling efter livmoderoperation.

I den andra kliniska studien inkluderades 40 kvinnor som lottades till att antingen få IPLA eller koksalt in i bukhålan under 72 timmar vid operation av avancerad och metastaserad äggstockscancer.

Vi fann att IPLA ledde till lägre cortison-nivåer och att ingen patient uppnådde toxiska nivåer av lokalbedövningsmedel i blod. Vi kunde även visa att IPLA-gruppen påbörjade cellgiftsbehandling 8 dagar tidigare. Vår slutsats är att IPLA är en säker metod som till viss del hämmar det kirurgiska stressvaret och som vidare kan korta ned tidsintervallet till start av cellgifter. Resultaten behöver upprepas i en större studie.

Eftersom LA har visat sig ha direkt effekt på cancerceller utförde vi i det avslutande fjärde delabetet, ett experimentellt försök där ovarialcancerceller i laboratoriemiljö behandlades med det läkemedel, i de doser, som våra patienter fått vid ovarialcancerkirugin. Försöket visade på ett minskat antal ovarialcancerceller, minskad förmåga hos cellerna att röra sig samt ett förändrat utseende.

Sammanfattningsvis har vi visat att IPLA kan ha positiva effekter vid behandling av gynekologisk cancer och kan vara en adjuvant smärtlindringsmetod efter livmoderkirurgi.

# List of Papers

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

I. Hayden J, Oras J, Karlsson O, Olausson K, Thörn SE, Gupta A.
*Post-operative pain relief using local infiltration analgesia during open abdominal hysterectomy: a randomized, double-blind study.*
Acta Anaesthesiol Scand. 2017;61(5):539-548.
DOI: https://doi.org/10.1111/aas.12833

II. Hayden J, Gupta A, Thörn SE, Thulin P, Block L, Oras J.
*Does intraperitoneal ropivacaine reduce postoperative inflammation? A prospective, double-blind, placebo-controlled pilot study.*
Acta Anaesthesiol Scand. 2019;63(8):1048-1054.
DOI: https://doi.org/10.1111/aas.13410

III. Hayden J, Oras J, Block L, Thörn S-E, Palmqvist C, Salehi S, Nordstrom J, Gupta A.
*Intraperitoneal ropivacaine reduces time interval to initiation of chemotherapy after surgery for advanced ovarian cancer. A randomized controlled double-blind pilot study.*
BJA 2020;124(5):563-570
DOI: https://doi.org/10.1016/j.bja.2020.01.026

IV. Hayden J, Oras J, Block L, Thörn S-E, Gupta A, Oredsson S.
*Ovarian Cancer Cell Inhibiting Effects of Local Anaesthetics.*
Manuscript.

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# Abbreviations

| | |
|---|---|
| AMP | Adenosine monophosphate |
| ASA | American Society of Anesthesiologists |
| BPI-SF | Brief pain inventory-short form |
| CC | Cardio collaps |
| CDC | Clavien-Dindo classification |
| CI | Confidence interval |
| CNS | Central nervous system |
| CRP | C-reactive protein |
| CSC | Cancer stem cells |
| CT | Computed tomography |
| DNA | Deoxyribonucleic acid |
| ECG | Electrocardiogram |
| EOC | Epithelial ovarian cancer |
| FIGO | Fédération Internationale de Gynécologie et d'Obstétrique |
| FLACC | Face Legs Arms Cry Consolability Scale |
| GI | Gastro intestinal |
| IARC | International Agency for Research on Cancer |
| IL | Interleukin |
| IV | Intravenous |
| IPLA | Intraperitoneal local anaesthetic |
| IQR | Interquartile range |
| LA | Local anaesthetics |
| LAK | Lymphocyte activated killer |
| LAST | Local anaesthetic systemic toxicity |
| LIA | Local infiltration analgesia |
| MTT | 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide |
| NK | Natural killer |
| NRS | Numeric rating scales |
| NSAID | Nonsteroidal anti-inflammatory drugs |
| PACU | Post-anaesthesia care unit |
| PCA | Patient-controlled analgesia |
| PSPP | Persistent post-surgical pain |
| QoR | Quality of Recovery |
| RCT | Randomized controlled trial |
| RIFLE | Risk, Injury, Failure, Loss, End-stage |
| RIOT | Return to intended oncologic therapy |
| SD | Standard deviation |

| | |
|---|---|
| SSR | Surgical stress response |
| TAP | Transversus abdominis plane |
| TEA | Thoracic epidural analgesics |
| TTC | Time to chemotherapy |
| VAS | Visual analogue scales |
| VGSC | Voltage gated sodium channels |
| WHO | World Health Organisation |

# 1. Introduction

### 1.1 Epidemiology of uterus and ovarian tumours

Hysterectomy is the surgical removal of the uterus. The indications for hysterectomy include: endometriosis, abnormal uterine bleeding, pelvic pain, gynaecologic cancer, including cancer prophylaxis and transgender male affirmation.[1] Depending on tumour characteristics, sometimes even the adnexa, including the ovaries, the fallopian tubes and the cervix may also be removed. Approximately 7000 women undergo hysterectomy each year in Sweden, 4000 for a benign indication. The surgical approach is either open abdominal or vaginal surgery, or laparoscopic/robot assisted techniques, depending on indication or patient/surgeon preference. There is an increasing trend towards minimal invasive techniques and today 40% of the women are operated via open abdominal hysterectomy today, compared to five years ago when 60% were operated with this technique.

Ovarian cancer is the most common cause of gynaecologic cancer-related death among females.[2] Nine out of ten ovarian cancers evolve from the epithelium of the ovaries or the fallopian tubes. Ovarian, fallopian tube and primary peritoneal cancer are all included under the same definition of epithelial ovarian cancer (EOC). EOC is staged according to the extent of tumour spread, described after surgery, using the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) staging system (Table 1). Advanced FIGO stage III and IV is tumour found outside of the pelvis, with metastasis to the lung, spleen or liver in stage IV. The five-year relative survival rate for all stages is 44 percent, with approximately 28 percent in higher stages.[3] At the time of diagnosis, 75–93% of the women are in advanced stage (III or IV) due to few and non-specific symptoms in early stages. The mainstay treatment regimen for EOC is surgery with the aim to resect all macroscopic tumour bulks, followed by adjuvant chemotherapy to eradicate residual cancer cells when the patients have recovered from surgery. In some patients, neo-adjuvant therapy is started before surgery to reduce tumour size. The single most important independent prognostic factor for long-term survival is the extent of cyto-reduction.[4, 5] The time to reccurrance of tumour is better described by surgery performed to lack of visual residual tumour than tumour staging.[6, 7] The median time to recurrence after complete radical surgery (microscopic residuals) with additional adjuvant chemotherapy is about 16 month, compared to 8 month after suboptimal debulking surgery (more than 10 mm residual tumour). The overall 5-year survival increases from 25% after suboptimal resection to 75% after complete resection. Corresponding figures for progression-free survival are from 10% to 50%.[7] The surgical trauma after cytoreductive surgery is extensive, and postoperative morbidity high, with a moderate to severe inflammatory response which may delay administration of adjuvant chemotherapy.[8] The interval from surgery to initiation of chemotherapy has an impact on survival, where 28 days is described as a cut off in

one study[9], whereas 42 days was described in another study using a different cohort of patients.[10] It is, however, commonly accepted that initiating chemotherapy as early as possible is favourable and the Swedish oncology association recommends < 21 days as ideal. In Sweden, approximately 350–400 women are operated each year for advanced ovarian cancer, and the intent is to centralize surgery of these patients to high-volume and surgical-proficiency tertiary referral centres. Time to chemotherapy (TTC) is individually planned in each case according to a joint decision by the clinicians and the oncologists when the patient is deemed to have recovered from surgery.

| Stage | Findings |
|---|---|
| I | Growth limited to the ovaries |
| II | Tumor involves one or both ovaries with pelvic extension |
| III | Peritoneal metastasis outside the pelvis and/or retroperitoneal or inguinal lymph node metastasis |
| IIIa | Micropscopic peritoneal metastasis beyond plevis |
| IIIb | Macropscopic peritoneal metastasis beyond plevis 2 cm or less in greatest dimension |
| IIIc | Peritoneal metastasis beyond plevis more than 2 cm in greatest dimension and/or retroperitoneal or inguinal lymph nodes |
| IV | Distant metastasis including liver parenchyma or malignant pleural effusion, whichmust be cytologically positive. |
| V | Death of the patient |

**Table 1.** FIGO staging classification of ovarian cancer

### 1.2 Inflammation in health and disease

Inflammation is the protective response to harmful stimuli involving the immune system, cells locally within the injured tissue, as well as the vascular system. The purpose is to destroy, dilute and transport the injurious material to restore tissue function or interrupt infectious agents if this is the underlying cause of the tissue injury. The immune system is divided into two types of immune responses: The innate and the acquired. The innate system reacts immediately to a harmful stimuli and does not require an immunologic memory. It includes phagocytic cells (neutrophils in blood and tissues, monocytes in blood and macrophages in tissues) that ingest and destroy antigens. It also involves granulocytes, mast cells and natural killer (NK) cells. NK-cells are lymphocytes that contain cytotoxic granules that are released and lead to lysis of tumour cells and virus-infected cells. NK cells secrete cytokines, which enhances the immune response. Recent studies have demonstrated an ability of the NK-cells to formulate an immunological memory, raising the possibility of NK cells as a potential cancer therapy target.[11, 12] The acquired immunity remembers previous exposure of an antigen and includes T and B cells responsible for cytotoxicity, and cytokine and

anti-body production. Cytokines are polypeptides or glycoproteins synthesized locally at the site of injury or by systemic immune cells to direct the proper immune response. They act via binding to cellular receptors to regulate immune cell activity. Following injury a cascade of pro-inflammatory cytokines is activated followed closely by anti-inflammatory cytokines resulting in a self-limiting balance. An over exaggerated pro-inflammatory situation renders a risk of systemic and hemodynamic instability leading to organ failure. On the other hand, excessive anti-inflammatory response can manifest as immuno-compromise. Pro-inflammatory cytokines consists of interleukin (IL)-1, IL-2, IL-6, IL-8, IL-12, IL-18, TNF-α and IFN-γ. Major anti-inflammatory cytokines include IL-4, IL-6, IL-10, IL-11, and IL-13. Following an injection of endotoxin to healthy volunteers, Lin *et al.* found that TNF-α and IL-1 are inducers of the cascade and together with IL-6, amongst the earliest cytokines with a short half-life of three to 6 hours.[13] However, during prolonged and ongoing tissue damage, as during surgery, cytokines persist and present with a similar initial pattern that is maintained for more than 48 hours postoperatively (Figure 1). The concentrations of cytokines in plasma are proportional to the extent of the surgery and the magnitude of tissue damage.[13] There is a wide inter-individual variability of expression between different cytokines.[14]

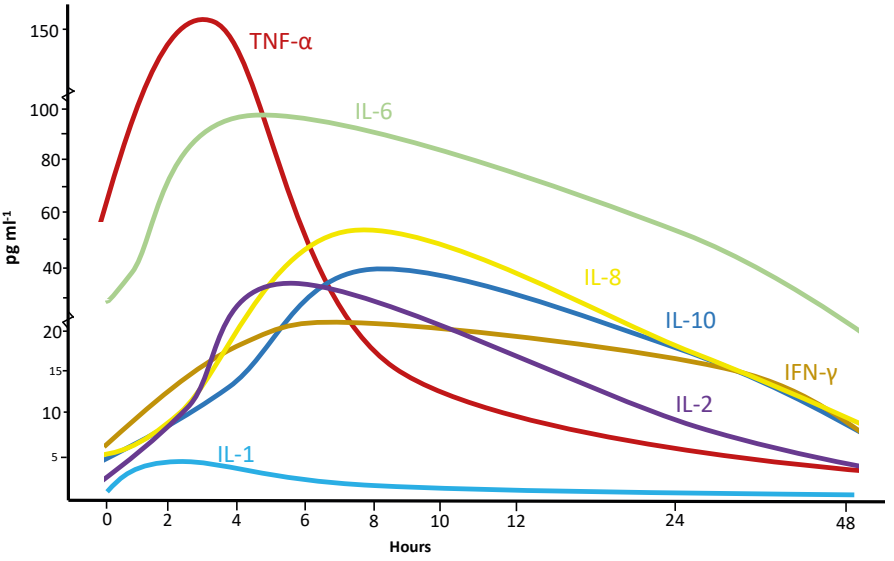


**Figure 1.** Diagrammatic presentation of changes in cytokine concentration during the perioperative period is shown. (Adapted from [15–21])

Disorders of the immune system can result in inflammatory diseases as well as cancer.[22] Cancers may arise from sites of chronic inflammation and tumour cells are shown to use inflammatory mediators for invasion, migration and metastasis.[23, 24] In chronic inflammation the normal self-limiting process is lost with a failure to resolve

the inflammatory response. A plausible hypothesis is that repeated interference on proliferating cells by phagocytic cells induces DNA damage resulting in permanent genomic alterations with mutations. Examples of chronic inflammatory conditions associated with cancer is inflammatory bowel disease leading to colon cancer, oesophagitis to oesophageal cancer and cystitis to bladder carcinoma. Yet, attraction of inflammatory cells to the tumour environment may also represent a suppression of the tumour growth by the host. The pattern of cytokines in cancer patients has been shown to determine prognosis.[25, 26] Anti-inflammatory therapeutic approaches to cancer development has recently been of great interest and reducing inflammation may therefore have prognostic significance.

### 1.3 Surgery and the stress response

In order to prevent ongoing tissue damage during the physical trauma of surgery, hormonal, metabolic and inflammatory changes are induced. This is called the surgical stress response (SSR), necessary to activate the repair process and restore normal function[15] (Figure 2). The overall metabolic goal is increased catabolism to provide energy as well as retaining fluid volume and cardiovascular haemostasis. The SSR is initiated in the following way. At the site of injury, afferent impulses follows sensory nerve roots through the dorsal horn of the spinal cord and up to the medulla to activate the hypothalamic-pituitary-adrenal axis with secondary effect on target organs. Adrenocorticotropic hormone from the pituitary stimulates cortisol release from the adrenal gland, while anti-diuretic hormone causes fluid retention by the kidneys. Cortisol plays several metabolic roles including promoting lipolysis, protein breakdown and gluconeogenesis. The release of cortisol also causes inhibition of glucose uptake by cells resulting in increased blood glucose. Furthermore, SSR causes anti-inflammatory effects by inhibiting macrophages, leucocytes and the synthesis of inflammatory mediators. Surgery also results in α-adrenergic inhibition on β-cells in the pancreas leading to a diminished secretion of insulin and a failure to match the hyperglycaemia. The cellular sensitivity to insulin is altered, resulting in insulin resistance.

The tissue damage from trauma and the resulting SSR induces cytokine release from activated leukocytes, endothelial cells and fibroblast mediating an pro-inflammatory response, followed by immuno-compromise. IL-1 and TNF-alpha are released which stimulates a cascade of cytokines, in particular IL-6. These three cytokines are the major mediators of the acute phase response.[27, 28] Plasma concentration of cytokines during and after surgery has been shown to correlate directly with the magnitude of surgery, and with associated postoperative complications.[14, 29–33]

The impaired immunity after surgery is caused by a reduction in T-helper cells type 1 producing pro-inflammatory cytokines and a shift to immunosuppression. Additionally glucocorticoids are stimulants of T-helper cells type 2, producing anti-inflammatory cytokines which may contribute further to the immunosuppression.

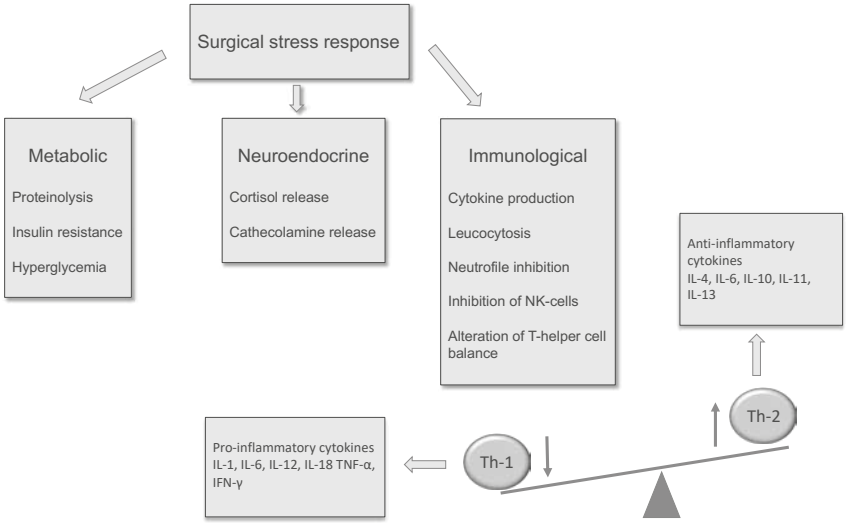


**Figure 2.** Schematic diagram of the surgical stress response

Although the stress response is evolutionarily advantageous to allow survival after injury, the response to surgery may be unnecessary because of resulting immunosuppression. If the SSR is exaggerated or unbalanced, the physiological changes may be excessive and prolonged, and may contribute to an increased risk of postoperative complications. Perioperative immunosuppression in cancer patients implies a risk of cancer growth and metastasis, and therefore it may be desirable to diminish the surgical stress response. Additionally, surgery itself might enhance cancer cell dissemination due to tumour manipulation.[34–37] Some studies have shown a decreased anti-tumour activity of Natural killer (NK) cells and lymphocyte activated killer (LAK) cells immediately after surgery, which may contribute to tumorigenesis perioperatively.[38, 39] The concentration of macrophages as well as the anti-inflammatory cytokines known to potentiate tumour growth, are lowest at day three after surgery.[23, 40] Hence a peak in immunosuppression is said to occur between 48 to 72 hours postoperatively.[37] Additionally, *in vitro* studies have observed altered biological properties of cancer cells after surgery with enhanced proliferation and reduced apoptosis that might lead to improved tumorigenesis.[34, 39]

The perioperative period offers a short timeframe in the progression of tumour and metastasis, but nevertheless several studies have described this period to be critical for prognosis.[41, 42] The neuroendocrine response described above may act directly on the cancer cells facilitating motility, proliferation, survival and the release of angiogenic factors.[31, 42, 43] There is strong evidence that inflammation itself is tumorigenic and several studies have proposed a relationship between surgery, trauma, inflammation and tumour growth.[23, 24, 44]

### 1.4 Cancer, surgery, metastases and chemotherapy

The world health organisation (WHO) has defined cancer as "rapid creation of abnormal cells that grow beyond their usual boundaries, and which can then invade adjoining parts of the body and spread to other organs, the latter process is referred to as metastasizing".[45] Cancer is a leading cause of sickness and death globally, estimated by the International Agency for Research on Cancer (IARC), to 17.0 million new cancer cases and 9.5 million cancer deaths worldwide in 2018.[46] The treatment for cancer consists of several alternatives depending on the specific type of tumour. Surgery, radiation, chemotherapy, bone marrow transplant, immunotherapy, hormonal therapy, targeted drug therapy, cryo-ablation or radiofrequency ablation are used solely or combined to cure or reduce the tumour burden or inhibit the progression. Cancer development and progression is hypothesized to derive from cancer cells of many different phenotypes. All have different potentials to proliferate extensively, and recent data suggests that a small proportion of certain phenotypes within the population of cancer cell have a hierarchical advantage of efficiently initiating tumours, named cancer stem cells (CSC). The population of cells are similar to normal stem cells with capability of differentiation and self-renewal.[47, 48] Sub-populations of CSC within the bulk of cancer cells is found in recurrent tumours, in metastatic tumours and tumours resistant to chemotherapy.[49–51]

### 1.5 Anaesthesia and cancer

A growing interest in the choice of anaesthesia and analgesia during cancer surgery is emerging. *In vitro* studies, animal studies and mostly retrospective clinical trials have suggested an association between perioperative anaesthetic technique and drugs, and the long-term outcome following cancer surgery. This has been hypothesised to be via modulation of the immune response, the surgical stress response as well as through direct effect on cancer cells.

#### 1.5.1 Regional anaesthesia and cancer

Several retrospective studies have reported that the use of perioperative regional anaesthesia (paravertebral block or epidural block) during cancer surgery extend time to cancer recurrence. De Oliviera *et al.* described an increased relapse-free survival after surgery of ovarian cancer when using intraoperative epidural compared to postoperative use, or general anaesthesia alone.[52] In their retrospective study on 182 patient, the intraoperative epidural group had a mean (95% CI) time to recurrence of 73 (56–91) months, compared to 33 and 38 month in the other two groups. Similar results have been presented after surgery of breast cancer, colon cancer, prostate cancer and melanoma.[53–55] A meta-analyses of studies, including several different cancers, found a correlation between improved overall survival and perioperative epidural analgesia.[56] A possible explanation is reduction in inflammation by blocking the SSR and suppression of lymphocyte activity as well as cytokine release.[57–59] However, contradictory results have been reported and therefore the evidence is inconclusive.[60] A large prospective, randomised trial published recently assigned 2100 patients, scheduled

for mastectomy for breast cancer received paravertebral block with propofol sedation or general anaesthesia with sevoflurane and opioid analgesia. The authors found that regional anaesthesia-analgesia did not reduce recurrence of breast cancer.[61]

### 1.5.2 Inhalation anaesthetics and cancer

Volatile anaesthetic agents (i.e., isoflurane, sevoflurane, desflurane, halothane) are known to have proinflammatory effects.[62] Sevoflurane is shown to alter the release of cytokines by NK and NK-like cells *in vitro*.[63] A meta-analysis of five retrospective and one RCT including over 7800 cancer patients found that the use of total intravenous analgesia compared to volatile anaesthesia was associated with improved recurrence-free survival.[64] Exposing ovarian cancer cells to volatile agents in clinically relevant concentrations have shown increased cell proliferation and migration and increased expression of genes related to metastasis.[65, 66] However, as with regional anaesthetics, other studies have reported conflicting results.[67] In contrast, the choice of propofol over inhalation agents has increased recently since a variety of antitumor effects of propofol have been demonstrated.[68] For example, while volatile agents have been shown to supresses NK-cell activity leading to increased metastasis, propofol does not.[69]

### 1.5.3 Opiates and cancer

Opiates promote migration and proliferation of cancer cells *in vitro*. They also have immune modulating effects on lymphocyte proliferation, phagocytic activity, NK-cell activity and cytokine-production.[70, 71] Healthy volunteers have been shown to have suppressed NK-cell function after opioid infusion.[72] One proposed explanation is the over-expression of μ-receptors on certain cancer cells which stimulates cell proliferation upon activation.[73] Consequently opioids have been shown to promote metastasis and tumour growth and perioperative or long-term treatment with opiates may have clinical implications in cancer patients.[74] Once again, the evidence is conflicting and clinical data are limited.[75]

### 1.5.4 Local anaesthetics and cancer

Local anaesthetics (LA) may influence tumour growth by several pathways described in the literature. Multifactorial hypothesis have been presented where epigenic effects, mechanisms involved in voltage gated sodium channel block and inhibition of cancer cell adhesion through src-signaling. The anti-inflammatory properties of LAs have been hypothesised to result in cytotoxicity, inhibited cell proliferation and migration.

#### Epigenics

LA may interact with the tumour genome thereby altering cell differentiation. Demethylation of DNA caused by LA in clinical doses can affect transcriptional programs related to tumorigenesis.[76, 77] Lidocaine in 10μM doses has been shown to induce apoptosis due to demethylation of DNA in breast cancer cell lines.[78]

### Voltage gated sodium channels

Local anaesthetics may influence tumour growth by blocking voltage gated sodium channels (VGSC). VGSC are highly expressed in primary cancer cells and in metastatic cells including ovarian cancer, colon cancer, melanoma, lung cancer, prostate and breast cancer.[79] The level of expression correlates with their potential for invasiveness.[80] Lidocaine binding to VGSC on colon cancer cells inhibits the metastatic potential of the cell.[81] Baptista *et al.* found that clinical doses of ropivacaine inhibit expression of VGSC on colon cancer cells and at the same time causes a decreased ability of the cells to migrate.[82]

### Src-signaling

One hypothesis of the inhibition of cancer cell migration by LA is via inhibition of the src-signalling pathway. Src is an oncogene encoding of a tyrosine kinase. The tyrosine kinase is responsible for phosphorylation of proteins involved in cellular function. Src-mediated phosphorylation is necessary for cell adhesion, for migration and for cancer cells to metastasise. Peigler *et al*. demonstrated a dose dependent decreased src-activation by ropivacaine in lung cancer cells.[83] They thereby proposed a possible theory that ropivacaine could inhibit migration and exert antimetastatic effects independent of VGSC-blockade. Enhanced Src activation has been observed in cancer development and inflammation.

### Inflammation

There is increasing evidence that inflammation and cancer are linked.[23] Local anaesthetics have anti-inflammatory properties through several mechanisms. They act directly on immune cells and on mediators of inflammation.[84, 85] LAs have been shown to inhibit leukocyte adhesion, phagocytosis, T-cell proliferation and cytokine secretion.[86–89]

## 1.6 Local anaesthetics

The first anaesthetic to be discovered was cocaine, being introduced in Europe in the 1800s. Subsequently all local anaesthetics have been developed synthetically. The most widely used cocaine synthetic derivate, lidocaine was developed in Sweden in the 1940s. Local anaesthetics bind reversibly to sodium channels in the nerve cell membrane and thereby prohibit the sodium influx through these channels. No depolarization occurs and hence the subsequent propagation of impulse is blocked, producing anaesthesia.

Potency, time of onset, duration of action and time of regression of local anaesthetics is of great interest in the anaesthetic setting. High lipid solubility enhances potency by diffusion through nerve sheets and membranes. While potency correlates with lipid solubility, the onset of action is determined by if the LA exist in ionized or non-ionized forms. Since it is the non-ionized base that is able to diffuse through membranes and bind to sodium channels, the more closely the equilibrium pH for a given anaesthetic

is to the pH of the tissue, the more rapid is the onset of action. Once inside the cell the base reacts with a hydrogen ion and it is the ionized form that binds to the voltage gates sodium channel. (Figure 3). The duration of action of LAs is due to the amount of protein binding while circulating in the bloodstream. High affinity to protein causes longer blockade. Lidocaine has a moderate duration of action, while bupivacaine and ropivacaine are long-acting amides. The potency is bupivacaine > levobupivacaine > ropivacaine.[90]

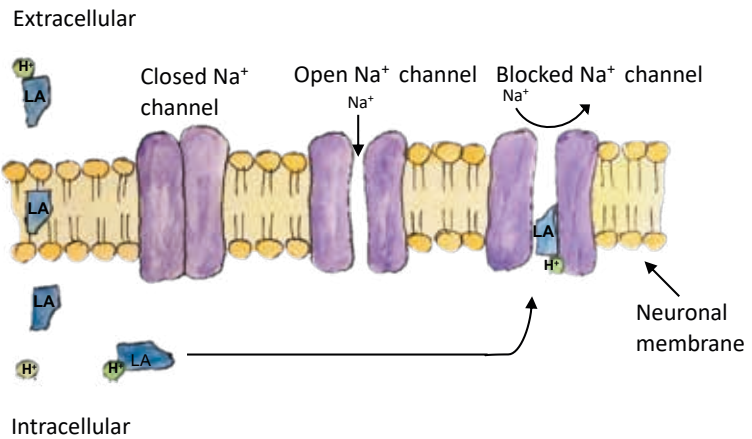


**Figure 3.** The neural cell membrane lipid bilayer with the Na+ Channel. Schematic illustration of LAs mechanism of action.

### 1.6.1 Mechanism of action

Binding of LA to the voltage-gated sodium channel inside the cell, results in a decreased chance of depolarization and succeeding action potential. This causes increased threshold for excitation in the nerve and blocks generation and the conduction of nerve impulses. A nerve impulse is a sudden reversal of the electric charge across the membrane of a resting neuron, distributed along the axon. Myelin sheets insulate the axon with continuous interruptions at short areas of non-myelinated nerve segments, the nodes of Ranvier. Electrolytes can easily pass the membrane at these segments and as a result sodium rushes through the membrane creating a movement of ions already inside the cell. This signal reaches the next node and although the transmission of action potential appears to jump along the non-myelinated areas it is rather a conduction of the signal inside the axon. This phenomenon is called saltatory conduction. LA exerts its anaesthetic effect on the nodes of Ranvier in myelinated neurons. The progression on anaesthesia of LA is thus related to morphological parameters of the cell such as the fibre-, and the axon diameter, the thickness of the myelin sheet and the internodal length.[91]

### 1.6.2 Local anaesthetic toxicity

Adverse events of LA with systemic toxicity can be life-threatening. The incidence of local anaesthetic systemic toxicity (LAST) is estimated to be 0.03%.[92] In most cases, systemic toxicity results from accidental intravasal injection. CNS toxicity is the most common feature (68%–77%).[93] Early manifestations of LA toxicity include auditory or visual disturbance, perioral paraesthesia, dizziness, confusion, agitation, reduced level of consciousness and eventually seizures. In one third of all cases, LAST is initiated by CNS symptoms that evolve into a cardiovascular event. Isolated cardiovascular symptoms are seen in 20% of the cases.[94] The symptoms commonly include hypotension, dysrhythmias and cardiac arrest. The risk factors for developing LAST depend on drug of choice, dose, patient characteristics and the technique.

Different LAs differ in their tendency to cause cardiovascular or CNS symptoms. LAs associated with less cardiotoxic tendency allows for a larger safe margin because of the earlier presentation of CNS signs. The dose that cause cardiac collapse (CC) in relation to the dose causing severe CNS symptoms, the CC/CNS ratio, can be used to compare cardiotoxicity of local anaesthetics. The lower the CC/CNS ratio, the more cardiotoxic is the LA. The CC/CNS ratio for bupivacaine is 2, versus 7 for lidocaine and 3.8 for ropivacine.[95, 96] Most LAs have vasodilatory properties leading to rapid absorption, while ropivacaine and levobupivacaine have vasoactive properties that may potentially prolong the effective duration of action.

The amount of LA molecules available in plasma are, in addition to the mass of drug disposition, dependent on the vascular supply and the rate of systemic absorption at the injections site. It is the unbound fraction that is available for pharmacological effects and hence for toxicity. Distribution and metabolism of LA is determined by patient characteristics such as perfusion, cardiac output, liver function, age and inflammatory state. Amino amides are highly bound to α1-acid glycoprotein, an acute phase protein that increases during trauma, surgery or inflammatory states. Children have reduced plasma concentrations of α1-acid glycoprotein while elderly, exhibit a reduced clearance of LA, rendering an increased free fraction of LAs in both groups.

The risk of LAST additionally differs between block techniques. Peripheral nerve blocks in lower and upper limbs have low risk of LAST while paravertebral, neuroaxial blocks and continuous infusions accounts for higher.[97] This is probably reflected by the vascularity of the injected tissue. Studies evaluating fascial plane blocks and local infiltration techniques using large volume of LA have demonstrated that the unbound concentration remains well below toxic threshold. The risk of LAST appears to be higher using continuous catheters than single shot injections reflecting an accumulation.[98] However, significant inter-individual variation is described and LAST has been reported even following single injections as during intravenous regional anaesthesia using tourniquet, which is associated with a significant risk LAST with symptoms, even at low doses.[99]

Intravenous lidocaine is used in pain treatment and following cardiac dysrhythmias, with doses up to 3 mg·kg–1 as a bolus and 5 mg·kg–1·hour–1 as an infusion. Cardiovascular signs arise in 4%–15% of these patients with an onset of mild neurological symptoms followed by cardiovascular compromise in a narrow range of plasma concentration.[100]

Plasma concentration of local anaesthetics infused during epidural anaesthesia in up to 120 minutes is shown to reach levels 0.096 μg ml–1 and when administrated intraperitoneally, peak concentration was found to be 0.008 μg ml–1.[21, 101]

The treatment of LA toxicity is immediate maintenance of airway and oxygenation, cardiovascular support, and immediate administration of 20% intravenous lipid emulsion therapy.

| Agent | Maximum bolus dose (mg) | Maximum dose (mg $kg^{-1}$) | Maximum dose 24 hours (mg) | Onset (min) | Duration (min) |
|---|---|---|---|---|---|
| Bupivacaine | 150 | 2 | 400 | 5-10 | 200 + |
| Lidocaine | 400 | 4 | 1200 | <2 | 30-60 |
| Ropivacaine | 300 | 3 | 800 | 5-15 | 200 + |
| Mepivacaine | 400 | 5 | 1000 | 3-5 | 45-90 |

**Table 2.** Suggested dose recommendations for commonly used local anaesthetic agents, onset and duration. Data from Miller RD (Ed.). Miller's Anesthesia, eighth ed. Philadelphia: Elsevier; 2015:2718 and from manufactures https://www.fass.se/LIF/startpage?userType=0. Recommended doses differ slightly between countries and literatures.

#### 1.6.2.1 Total and free concentration

Approximately 90–94% of LA in plasma is bound to alpha-1-acid glycoprotein, an acute-phase reactant that increases after trauma and surgery. It is the unbound fraction that is available for pharmacological effects and hence for toxicity. Free concentration of bupivacaine > 0.3 (0.1–0.5) μg mL-1 has been shown to cause LAST in humans while for ropivacaine free concentration should be < 0.6 (0.3–0.9) μg mL-1.[102]

#### 1.6.2.2 CNS and cardiovascular effects

The mechanism by which LA exerts it toxic effects is due to the blockade of inhibitory and excitatory neuronal pathways causing CNS depression as well as excitatory clinical features. The blockade of voltage-gated sodium channels in the heart disrupts normal conduction leading to decreased action potential propagation and prolonged PQ and QT-intervals. LAs pursue several additional effects on the cardiac function, by blocking calcium-channels and reduce intracellular calcium causing impaired contractility and by inhibiting mitochondrial phosphorylation of AMP.[92]

### 1.6.3 Ropivacaine

Ropivacaine was developed with an altered molecular structure after evidence of bupivacaine-related severe toxicity, so as to achieve a less toxic, more potent and longer acting LA.[103]

Ropivacaine is 94% protein bound to α1-acid glycoprotein. It is metabolized in the liver, mainly by aromatic hydroxylation. All metabolites have anaesthetic effect but with considerably less potency and duration. The metabolites are excreted in the urine. The mean ± SD terminal half-life of ropivacaine is 1.8 ± 0.7 h after intravascular administration and 4.2 ± 1.0 h after epidural administration.[104] In human volunteers given intravenous ropivacaine, the maximum tolerated free arterial plasma concentrations until mild CNS symptoms were noted was 0.6 (0.3–0.9) μg mL-1 and threshold for total concentration was 4.3 (3.4–5.3) μg mL-1.[102] This study evaluated the incidence of CNS symptoms and echocardiographic changes after acute tolerance of 10 mg min-1 ropivacaine. Both venous as well as arterial concentrations were presented in this study and the literature sometimes refers to the venous concentration as limit for toxicity. However as indicated by the authors, venous blood returning from various tissues with disparity in perfusion is not representative of the plasma concentration directly after rapid systemic administration.

### 1.7 Postoperative pain

The innervation of the tissues in the pelvic viscera is complex involving somatic as well as visceral affarent nerve fibres predominantly from the thoraco-lumbo-sacral region (Figure 4). Therefore, nerve blocks cannot produce complete analgesia even under direct vision due to the complex innervation of the uterus and adenexa but can reduce pain intensity. However, spinal or low thoraco-lumbar epidural block usually ensures complete analgesia in these segments.

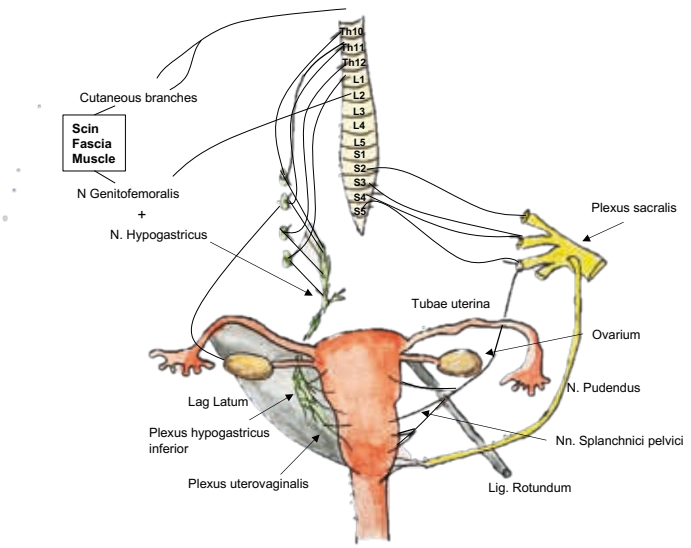


**Figure 4.**
Sensory innervation of tissues traumatised during hysterectomy

The incidence of acute postoperative pain after abdominal surgery is reported to be > 80% with moderate to severe pain in 75% of the cases.[105] Adequate pain management is unfortunately seen in only half of the patients undergoing surgery, and inadequate post-operative pain control may lead to increased morbidity, poor recovery, diminished quality of life and risk of persistant postoperative pain.[106] Pain is the symptom of tissue damage caused by disease, injury or surgery. There are two aspect of pain; the sensory discriminative, and the affective, aversive behavioural that is accompanied by anxiety and uncomfort. A large prospective cohort study evaluating postoperative pain on the first postoperative day, in 179 surgical procedures demonstrated orthopaedic/trauma procedures on the extremities and major abdominal surgery to be the most painful, ranking hysterectomy as number 1.[107]

Postoperative pain following open abdominal hysterectomy is moderate to severe during the first 24 h and rescue analgesic consumption is medium-high.[108] Pain stimulating mediators such as prostaglandins, serotonin, bradykinin, histamine and substance P, are released by the damage cells, from circulating blood cells or are synthesised by activated enzymes following tissue damage caused by surgical incisions. These chemicals activate primary afferent nociceptors (nociceptive pain). Pain signals are transmitted from the periphery in to the central nervous system by primary afferent neurons of thin myelinated A-delta fibres and unmyelinated C fibres. The afferent nociceptor terminates in the dorsal horn of the spinal cord and releases transmitters that activate second order pain transmission cells.

The axons cross over to the opposite side of the spinal cord and are transmitted upwards via the spinothalamic and spinoreticular tracts. Hence there are two major ascending pathways to the brain stem and the thalamus. The spino-thalamic pathway is primarily responsible for sharp pain arising from peripheral nociception while in contrast the spino-reticular pathway responds to visceral pain (Figure 6). Autonomic nerves are also involved in the transfer of pain signals. Sensory signals from the viscera runs along sympathetic and parasympathetic nerves.

### 1.7.1 Types of pain

Pain originating in the skin, muscles or bones is called nociceptive somatic, while pain signals from visceral organs is called nociceptic visceral. Direct damage to peripheral or central nerves also causes pain and is called neuropathic pain. Usually a combination of these pain mechanisms are involved following surgery. Following open abdominal hysterectomy, somatic pain arises from musculoskeletal structures and abdominal wall and is often referred to as sharp, or aching. Visceral pain is diffuse, localisation is often difficult, and the pain is described as squeezing, deep or sickening. While some sensory afferents innervating the viscera run via paravertebral ganglia through the sympathetic innervation and terminating in the spinal cord, other afferents innervating the same organs follow the vagal nerve terminating in the brainstem. This implies a possible explanation for pain sensations during neuraxial blockade (spinal/epidural) despite an adequate distribution.[109, 110]

Damage to the nerve system during surgery can result in persistent (also called chronic) postoperative pain. Chronic postoperative pain is defined as pain in the operated region lasting more than 3 months after surgery. The incidence is reported between 10 to 30% in surgical patients.

Several factors may result in chronic pain syndromes including: poorly treated severe pain in the postoperative period, pain prior to surgery, the surgical approach, psychological factors and age.[111] The patho-physiology behind persisting pain is multifactorial and complex. After injury, inflammatory mediators are released causing a sensitization of the surrounding tissue enabling the nerves to trigger impulses at lower thresholds. Simultaneously, when afferents persistently transmits signal to the nerves in the dorsal horn, they enhance their response. Subsequently the sensory cortex in the brain is remapped and this pathological circle of events results in chronification of pain.

### 1.7.2 Measurement

Pain treatment has to be evaluated, repeatedly, to obtain optimal postoperative pain relief after surgery (ref Chou). A number of validated pain assessment tools are available. Quantification of pain can be done by using numeric rating scales (NRS) where patients use numbers to quantify the intensity of pain, 0 = no pain and 10 = the worst pain imaginable. The visual analogue scales (VAS) is an alternative method using a horizontal line where the patient indicates a point corresponding to a number on the opposite side. In children, the self-assessed Faces Pain Scale or the observational FLACC (Face Legs Arms Cry Consolability Scale) is widely used. Pain intensity only takes into account one dimension of pain. Additional measurements of quality, or character of the pain and its impact on life contributes to more diverse information. Although several questionnaires for assessment of pain character are available they are not routinely used, and therefore most studies define pain intensity using only quantitative scales, which is a major limitation. Another problem in comparative studies is the variability of pain assessment methods using different scales (NRS, VAS), intervals (mild, moderate, severe) and nature (at rest or on activity).

### 1.7.3 Management techniques

Efficient postoperative pain management is imperative in order to promote early mobilisation and improve patient satisfaction. Poor pain control in the postoperative period is associated with increased morbidity and may sometimes evolve into persistent chronic pain.[112, 113] A multimodal approach to treatment of postoperative pain is preferable to reduce the need for opioids and improve pain scores.[114] A combination of analgesics with different mechanisms of action, acting at different sites (peripheral and central) will enhance the analgesia and reduce the opioid-related side effects. Nonsteroidal anti-inflammatory

Drugs (NSAID), local anaesthetics, paracetamol, alpha2-agonists and ketamine are examples of drugs included in this multi-modal pain management strategy. The cor-

nerstone in pain management is opioids. However, it is well known that they may cause nausea, sedation, respiratory failure, ileus and/or urinary retention. The use of patient-controlled analgesia (PCA) using opioids intravenously administered by a pump controlled by the patient provides better autonomy and control, rendering lower dosage of rescue opioid analgesia.[115]

Advanced regional analgesia techniques should be integrated in the multimodal regimen when feasible. Intrathecal analgesia is a commonly used analgesic technique either solely or in combination with general anaesthesia during abdominal hysterectomy. Local anaesthetics with or without the adjunct of opioids or alpha2-agonists are placed intrathecally, below the level of conus medullary. Intrathecal LA generates total block of motor and sensory afferent fibres distal to the level of injection. Severe adverse events are rare following spinal anaesthesia, less than 0.1%, but some might be permanent.[116] Major complications include nerve injuries, severe hypotension, epidural hematoma, meningitis and cardiac arrest.[117] Thus the technique is associated with several contraindications.

Another available neuraxial technique is epidural block where local anaesthetic drug is injected into the epidural space through a catheter enabling blocking of impulse transmission below the level of injection site.

The epidural technique may be administered at the lower thoracic level during abdominal hysterectomy and low to mid-thoracic level during ovarian cancer surgery and due to minimal neuromuscular block of the lower extremity it is preferably used in postoperative pain management. Epidural anaesthesia has been shown to reduce the surgical stress response and to have comprehensive positive effects on postoperative recovery.[106, 118, 119] Systematic reviews have implied that regional anaesthesia is protective against persistent postsurgical pain.[120]

Peripheral nerve blocks, such as the transversus abdominis plane (TAP) block may be used during abdominal surgery and may be efficacious but require expertise in the use of ultrasound devices to identify the nerves. They also require bilateral (two quadrant) block for abdominal hysterectomy and four quadrant block for ovarian cancer surgery, which is not always practicable and often not very successful. Additionally, they provide short-term analgesia unless catheters are placed in two (or four) quadrants and local anaesthetics injected intermittently. Although they may be effective, they are time consuming, operator dependent and may be nurse-intensive (and therefore costly) which is why they are not used commonly following abdominal cancer surgery. Local infiltration analgesia (LIA) using large volumes of local anaesthetics combined with non-steroidal anti-inflammatory drugs and adrenaline, given as a single bolus using infiltrative techniques locally at the site of surgical trauma by the surgeon has also been used successfully for pain management following orthopaedic surgery.[121] Peripherally acting drugs injected at the site of tissue trauma may have an important

role in providing anaesthesia and analgesia locally but may provide short-duration analgesia from a few hours to a day but tide over the period of maximum pain intensity. Intraperitoneal administration of LA has been used to block pain impulses generated locally at the most peripheral nerve endings within the peritoneal cavity. Continuous intraperitoneal infusion has been reported to reduce morphine requirement by 30–40%, and leads to less postoperative nausea.[122] Instillation and infusion of intraperitoneal ropivacaine after abdominal surgery is shown to improve surgical recovery and to blunt the postsurgical inflammatory response.[21]

Finally, some authors have used intravenous lidocaine for pain management and shown that it shortens the length of hospital stay, increases gastrointestinal motility and inhibits the inflammatory response.[123] Administering analgesics before the painful stimulus (pre-emptive analgesia) is hypothesised to prevent or reduce the subsequent pain signal, but is debateable.[124]

#### 1.7.4 Advantages/disadvantages of opiates vs. non-opiates

Opiate-sparing techniques using local anaesthetics in some way are increasingly popular because of the known disadvantages of opiates as well as opiate-induced hyperalgesia, risk of misuse and abuse and the development of opiate tolerance. However, all methods having advantages and disadvantages. The use of epidural analgesia is opiate-sparing and significantly decreases morbidity but is associated with pruritus, hypotension, and urinary retention.[106, 125] Intrathecal anaesthesia and analgesia may cause nausea and vomiting, transient neurological symptoms, urinary retention and post-dural-puncture headache. The use of NSAID may cause gastro-duodenal ulcer, exacerbate asthma, platelet dysfunction with increased postoperative bleeding while paracetamol is known for its hepatoxicity when used in large doses. Opiates are recommended to be a rescue medication for acute postoperative pain and remains an effective instrument for treatment of moderate and severe pain.

#### 1.7.5 Monitoring postoperative recovery and health status

Besides pain, there are many other aspects of postoperative health status. Validated standardised assessment of patient comfort and recovery of life quality are important when comparing perioperative outcomes. The Standardised Endpoints in Perioperative Medicine defined by Myles categorize patient comfort into five objective measures and are increasingly used in the surgical setting.[126] Pain, Nausea and/or vomiting, Time to GI-recovery, Time to mobilization, Quality of Recovery and Sleep are summarised. Myles *et al.* also developed and validated the Patient-perceived quality of recovery score (QoR-40).[127] QoR-40 is designed to measure the patient's health status after surgery and anaesthesia.[128] The QoR-40, measured pre- and post-operatively, contains 40 items measuring five dimensions of recovery: physical comfort (12 items), emotional state (9 items), physical independence (5 items), psychological support (7 items), and pain (7 items). The QoR-40 has a minimum possible score of 40 (extremely poor quality of recovery) to 200 (excellent quality of recovery). A recently introduced measurement

of the quality of surgical therapy for cancer is Return to Intended Oncologic Therapy (RIOT) as a quality indicator.[129] Cancer surgery is associated with high morbidity which sometimes prevent the patient from initiating postoperative chemotherapy. An inability to complete the intended oncologic therapy may impact outcome.[128]

# 2. Aims of this thesis

The following were the main aims of this thesis:

1. Can local infiltration analgesia provide better postoperative pain management compared to placebo after open abdominal hysterectomy? (study I),
2. Does intraperitoneal local anaesthetic (IPLA) injection perioperatively lead to lower postoperative inflammation in patients undergoing ovarian cancer surgery? (study II)
3. Are total and free plasma concentration of ropivacaine following intraperitoneal and epidural injection in high doses over 72 h reach levels considered to be toxic? (study II)
4. What is the effect of perioperative IPLA on postoperative recovery, home discharge and start of adjuvant chemotherapy following cytoreductive surgery for ovarian cancer? (study III)
5. Does ropivacaine or lidocaine inhibit ovarian cancer cell proliferation in clinically relevant concentrations *in vitro?* (Study IV)

# 3. Patients and Methods

### 3.1 Approvals and registration

Studies I–III were approved by the Regional Ethics Committee in Gothenburg, Sweden, registration number 125–16 (study I) and 1043-13 (studies II–III). Studies I and II–III were registered in an international database ClinicalTrials.gov (reg. no: NCT01782781 and NCT02256228, respectively) prior to patient recruitment. Written, informed consent was obtained from all patients prior to inclusion. No changes were made in the protocol after study commencement. The studies I–III adhere to the CONSORT guidelines and to the Declaration of Helsinki.

The collected blood samples were registered prior to study initiation in the corporate biobank for research and clinical trials at Sahlgrenska University hospital in accordance with the regulations covering the healthcare sector. Registration: reg 890 SaB Sahlgrenska biobank.

### 3.2 Patients and setting

Study I

The patients included women > 18 years old, scheduled for open abdominal hysterectomy for a benign lesion of the uterus. Exclusion criteria were physical status of ASA > III, language difficulties or comprehension problems, analgesic medication with opioids for more than 3 month prior to surgery, signs of renal failure with serum creatinine > 90μmol/L, simultaneous participation in another clinical study and known intolerance to local anaesthetics or non-steroidal anti-inflammatory drugs (NSAIDs). This study was performed at the Eastern University Hospital, Gothenburg.

Study II and III

Eligible patient were all women planned to undergo extensive abdominal debulking surgery for advanced ovarian cancer, FIGO stage III-IV. Exclusion criteria were: neoadjuvant chemotherapy prior to surgery, ASA ≥ 4, language difficulties or comprehension problems, chronic analgesic medication with opioids for > 3 months, known intolerance to local anaesthetics and an absolute contraindications to the insertion of an epidural catheter. Patients in whom extensive surgery was not performed due to extensive spread of the tumour or a less malignant macroscopic feature than FIGO III were excluded by the surgeon after laparotomy. These studies were multicentre and included Sahlgrenska University Hospital, Gothenburg and Karolinska University Hospital, Stockholm.

### 3.3 Randomisation and blinding

In studies I–III, computer-generated randomised numbers were concealed in identical, opaque, sealed envelopes and stored in a locked room. Simple randomisation in

a 1:1 ratio was used. The study drugs were prepared in an adjacent room by a nurse not involved in the study or in the care for the patient. The patients and the health care professionals involved in patient care were fully blinded during the complete trial.

Study I

Patients were randomised to receive either local infiltration analgesia using a combination of ropivacaine, ketorolac and adrenaline (Group LIA) or 0.9% saline (Group C).

Study II and III

Patients were randomly allocated to receive either intraperitoneal ropivacaine per- and postoperatively (Group IPLA) or 0.9% saline (Group Control).

### 3.4 Anaesthesia, surgical technique and intervention

Patients in both study groups in each trial were treated identically in all respects with the exception of study drug. Premedication, induction and maintenance of anaesthesia, as well as monitoring during the surgical procedures were standardized in all patients and followed local hospital clinical practice.

Study I

Hysterectomy was performed in a standardised way using a Pfannenstiel, Joel-Cohen or midline incision, depending on surgeon's preference. Before closure of the wound the surgeon administered the study drug around the proximal vagina, the sacro-uterine ligament and the round ligament bilaterally and finally in the fascia and subcutis. The procedure were standardised and an instruction video were recorded before commencement of the study. All patients had a patient controlled analgesia (PCA) pump intravenously and could self-inject morphine 1 mg as needed during 0–48 h when perceived pain intensity was moderate to severe or NRS > 3.

Study II and III

Epidural anaesthesia and analgesia was used in all patients for perioperative pain management. Surgery was conducted by a conventional laparotomy through a midline incision with the attempt to maximally resect all visible and palpable disease. In addition to standard removal of the ovaries, uterus, omentum, and lymph nodes, extended surgical techniques used to debulk advanced disease included bowel resection, splenectomy, partial liver resection, peritoneal and/or diaphragmatic stripping when indicated. At the commencement of the surgical procedure, in the midst, and before closure of the peritoneum, study drug was administered. At the end of the surgery a multi-port catheter was inserted lateral to the incision through the abdominal wall and connected to an infusion pump (Figure 5). During the following 72 hours, a bolus injection of the study drug was injected every other hour by an automatic pump connected to the intraperitoneal catheter via a bacterial filter. Additionally, all patients had a PCA pump with morphine 1 mg/ml intravenously for postoperative pain management, as needed.

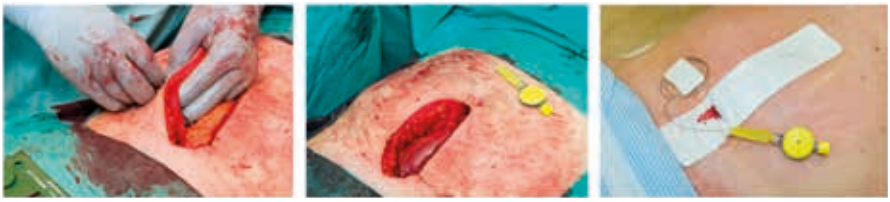

**Figure 5.** Placement of intraperitoneal catheter.

### 3.5 Postoperative assessment

#### 3.5.1 Clinical parameters

##### 3.5.1.1 Pain

Postoperative pain was registered as rescue analgesic consumption using patient controlled analgesia (PCA) pump. Pain intensity was measured on a numeric rating scale (NRS) where 0 = no pain and 10 = worst imaginable pain self-assessed at rest and during coughing.

Study I

All patients registered pain intensity before surgery and after 3 months using a standardised questionnaire, the brief pain inventory-short form (BPI-SF) to evaluate persistent post-surgical pain.

Study III

In addition to intravenous opioid consumption using PCA and NRS analyses, the quantity of drugs given via epidural route were also recorded.

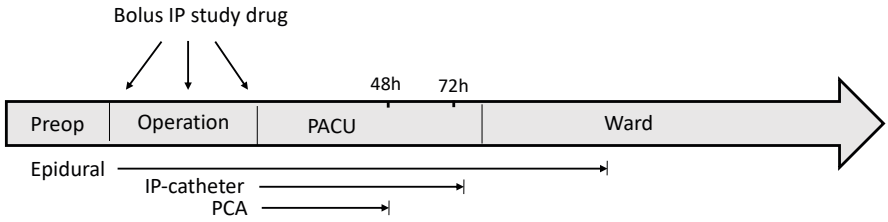


**Figure 6.** Flow chart of intervention in paper II and III. PACU: post-anaesthesia care unit. PCA: patient controlled analgesia. Patients received epidural preoperatively which was activated during surgery and continued for approximately 4 to 5 days. Study drug were administered intraperitoneally at three time points peroperatively, and for 72 hours postoperatively. PCA with opioids was used during 48 hours.

#### 3.5.1.2 Recovery

Study I

Postoperative opiate related side effects were recorded. Nausea and/or vomiting (PONV) and pruritus, were recorded using a categorical scoring system (0 = none, 1 = mild, 2 = moderate, 3 = severe). The Ramsey scale was used to evaluate sedation while respiratory rate < 10/min and saturation < 92% were chosen as evidence of respiratory depression.

Study III

Functional recovery assessing time to stand, walk and return of bowel function were recorded. Patient-perceived quality of recovery was assessed using a Swedish version of a validated quality of recovery score (QoR-40). Objective measures of patient comfort in the perioperative period were summarised in predefined measures according to The Standardised Endpoints in Perioperative Medicine clinical trials defined by Myles (Pain, Nausea and/or vomiting, Time to GI-recovery, Time to mobilization, Quality of Recovery and Sleep).

#### 3.5.1.3 Postoperative complications

In *study* I, surgical complications were noted postoperatively. In study III, the following predefined postoperative complications were recorded: deep vein thrombosis (clinical) or pulmonary embolus (confirmed by CT scan), respiratory failure (prolonged ventilator management), evidence of pneumonia or new pleural fluid accumulation (Chest X-ray), prolonged paralytic ileus or bowel obstruction, evidence of local or systemic infection (laboratory parameters), anastomosis leakage (CT scan ), abdominal abscess (CT scan), acute myocardial infarction (rise of troponin), atrial fibrillation or other major arrhythmia (ECG), renal failure (RIFLE criteria) and urinary infection (urine culture). For surgical quality assessment the Clavien-Dindo classification with 5 grades for postoperative complications was used (Table 3).

| Grade | Definition |
|---|---|
| I | Any deviation from the normal postoperative course without the need for pharmacological treatment or surgical, endoscopic and radiological interventions |
| II | Requiring pharmacological treatment. Blood transfusion and parenteral nutrition included |
| III | Requiring surgical, endoscopic or radiological intervention |
| IIIa | Intervention without general anaesthesia |
| IIIb | Intervention with general anaesthesia |
| IV | Life-threatening complication |
| IVa | Dysfuction of an organ system |
| IVb | Multiorgan dysfunction |
| V | Death of the patient |

**Table 3.** Clavien-Dindo definition of surgical complications.

#### 3.5.1.4 Home discharge, readmission, start of chemotherapy

In *study I*, home discharge was recorded in the case report form and readmission was recorded using patient record follow-up.

In *study III,* home readiness was evaluated every other day starting from day 4. Criteria for home readiness are shown in Figure 7. Home discharge was recorded in the case report form. Time to initiation of chemotherapy and RIOT were recorded using patient medical records. Initiation of chemotherapy was defined as the time between the end of surgery and the date of the first cycle of chemotherapy.

| | Yes | No |
|---|---|---|
| 1. Mild pain, NRS <3 | | |
| 2. The patients nutrion is adequate without nausea or vomiting | | |
| 3. GI-function restored | | |
| 4. In case of ostomy, the patient manage the nursing | | |
| 5. No weight gain the last two days | | |
| 6. Renal function ok | | |
| 7. No signs of infection, locally or systemically | | |
| 8. Adequate mobility without dyspnoea | | |
| 9. There are signs of surgical complications | | |

Home readiness when 1-8 = yes and 9 = no.

**Figure 7.** Criteria for home readiness.

### 3.5.2 Laboratory measurements

#### 3.5.2.1 Cytokines

In *study II*, serum concentration of cytokines was measured preoperatively, and at 6, 24 and 48 hours after incision. The following inflammatory parameters were analysed in combination: IL-1β, IL-2, IL-6, IL-8, IL-10, IL-15, IFN-γ, and TNF-α. The samples were analysed at the Department of Clinical Immunology, Sahlgrenska University Hospital, Gothenburg, Sweden. Arterial blood was collected in collecting tubes and left at room temperature for 60 minutes to allow the cells to settle and the liquid portion of the blood was removed followed by subsequent centrifugation at 2260 g at 4oC for 10 minutes. 500 μL aliquots of the supernatant were stored in cryo-tubes at -80oC until study completion and analysis. Cytokines were quantified from serum using a Procarat-Plex multiplex cytokine assay (ThermoFisher Scientific), a Bio-Plex 200 assay reader and using the Bio-Plex Manager™ software (Bio-Rad Laboratories AB, Sundbyberg, Sweden). The analysis was performed according to the manufacturer's instructions.

#### 3.5.2.2 Local anaesthetics

In *study II*, arterial blood was collected in 6 mL Na-Heparin collecting tubes at 6, 24, 48 and 72 hours after induction of anesthesia. Samples were centrifuged at 2260g at 4oC within 30 minutes after sampling. The samples were stored at -80°C until analysis. Quantification of total and unbound plasma ropivacaine was performed at Therapeutic Drug Monitoring Laboratory, Department of Clinical Pharmacology at Karolinska University Hospital, Stockholm. The method was developed and validated in-house and utilised liquid chromatography coupled to single mass spectrometry.

#### 3.5.2.3 Stress markers

Cortisol and insulin were analysed at the Sahlgrenska University Hospital laboratory (*study III*). The blood samples were collected preoperatively and 6, 24 and 48 hours after incision. Blood-glucose was analysed at induction, and after 6, 24 and 48 hours after incision using the departmental blood gas analyser. C-reactive proteins (CRP) were analysed at 72 hour postoperatively at the Sahlgrenska University Hospital laboratory.

#### 3.5.2.4 Cell multiplication, migration

In *study IV*, the human ovarian carcinoma cell lines SW626 and SKOV-3 were purchased from the American Tissue Culture Collection (Manassass, VI, USA). Culture and analyses were performed at the Department of Biology at Lund University, Sweden. The cells were exposed to ropivacaine and lidocaine in concentrations of 1, 10, 100 and 1000 μM. Ropivacaine at concentrations of 1000 μM corresponds to clinical doses after intraperitoneal administration whereas 1 and 10 μM corresponds to total plasma concentration after epidural anaesthesia. Lidocaine 10 and 100 μM is within the range of i.v. administration for analgesia.

Cell number were determined by using a phase contrast microscope, and via MTT assay (for assessing cell metabolic activity) to assess dose response relationships. The MTT assay offers indirect information about cell number, and studies dose response relationship. MTT is a salt widely used to determine cell viability in different assays. In the electron transport chain of the mitochondria MTT is reduced. MTT added to drug incubated cells is assumed to be taken up, and reduced in proportion to the cell number. The percentage of MTT reduction was calculated using the following equation: MTT reduction (%) = (absorbance treatment group / absorbance control group) × 100. Living ovarian cancer cells were also observed and counted manually, before and after treatment with ropivacaine and lidocaine.

The wound healing assay was used to study cell migration. A scratch on a cell monolayer is done and cell movement into the scratch is captured taking images at regular intervals by the microscope. The scratch produces a cell-free area where the cells migrate towards each other to close the gap, creating a semi-quantitatively approach to measure migration.

Physical characteristics of the cell population were analysed using flow cytometry technique. Light scatter through the cells were measured by two optical detectors. One detector measures forward scatter, where the intensity is proportional to cell size. The other detector measures at a 90o angle to the laser and is called side scatter, providing information about granularity of a cell.

Analysis of cancer stem cells phenotypes were done with Aldehyde dehydrogenase assay and CD44+CD24+ assays. Aldehyde dehydrogenase (ALDH) is a family of enzymes found throughout the human body that catalyse the conversion of various aldehydes. The proteins are highly expressed in cancer stem cells (CSCs). ALDH assay expression is used as a marker for CSCs. The method quantifies the ALDH enzymatic activity. The surface markers CD44 and CD24 are highly expressed on cancer cells possessing stem cell characteristics. Antibodies targeting the CD44 and CD24 surface were incubated with the live cells and analysed using a flow cytometry method.

### 3.6 Statistical analyses

Normal distribution was checked for all continuous variables using Shaprio-Wilks test and inspection of histogram. Mean and standard deviation was used for normally distributed variables while non-normally distributed variables are presented as median and interquartile range. Students T-test was used to compare means of normally distributed variables and Mann-Whitney U-test was used to compare medians in non-normally distributed variables.

Multiple comparisons were adjusted with Bonferroni correction when appropriate. Fisher's exact tests was used to compare binary variables. Association over time between cytokine levels and LA concentrations were tested with a lineal mixed model and whether the two variables co-vary was tested with Spearman rank test. All statistical calculations were accomplished using IBM SPSS Statistics 22.0. A p-value $<0.05$ was considered to be significant.

# 4. Results

Study I

In all, 60 patients were included and randomized in this study during the period January 2013 and October 2014 (Figure 8). Median age for all patients was 46 years and no differences were seen between the groups in demographic data or perioperative variables.

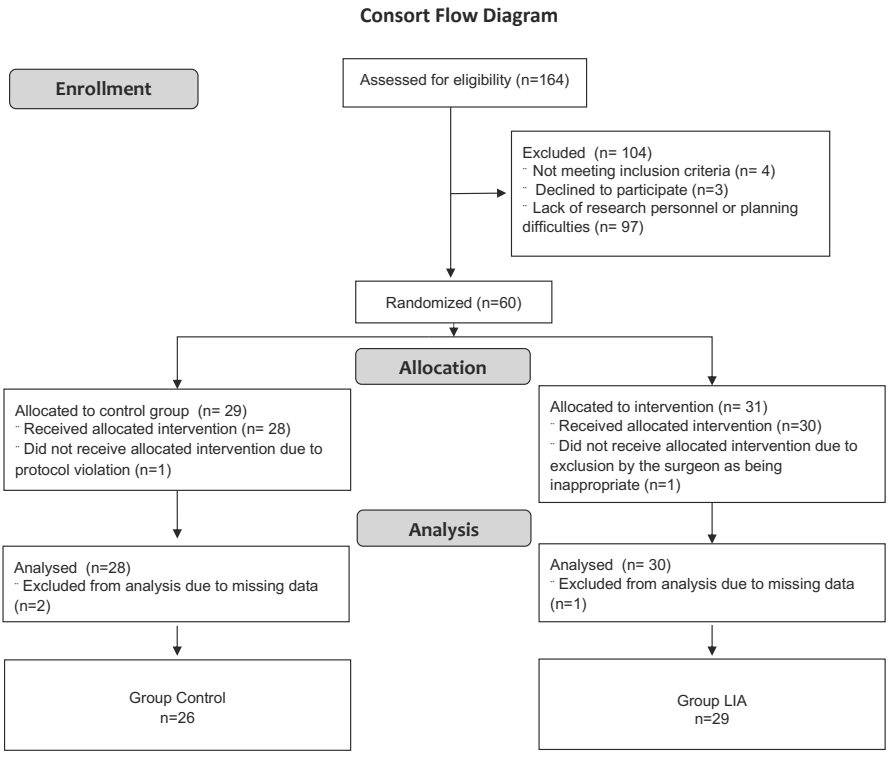


**Figure 8.** Consort flow diagram study I.

The median rescue morphine consumption during 0–24 hours after extubation was significantly lower in group LIA compared to group C (18mg vs. 27mg, p = 0.028). (Table 4)

| | LIA (n=29) | Control (n=26) | p-value |
|---|---|---|---|
| Total morphine consumption first 24h, mg | 18 (5 - 25) | 27 (15 - 43) | 0.028 |
| Time to first morphine given, min | 40 (20 - 60) | 20 (13 - 30) | 0.005 |
| Total morphine consumption 24h-48h, mg | 0 (0 - 7) | 4 (0 - 8) | 0.214 |
| Opiate related side-effects | | | |
| Nausea and/or vomiting, N (%) | 11 (38) | 15 (57) | 0.292 |
| Pruritus, N (%) | 5 (17) | 10 (38) | 0.141 |
| Dizziness, N (%) | 16 (55) | 20 (77) | 0.274 |
| Sedation, N (%) | 22 (85) | 19 (73) | 1 |
| Respiratory depression, N (%) | 0 (0) | 0 (0) | 1 |
| PACU stay, min | 225 (190 - 300) | 230 (170 - 270) | 0.533 |
| Time to hospital discharge, hours | 48 (31 - 52) | 51 (47 - 53) | 0.155 |
| Surgical complications, N (%) | 1 (4) | 3 (12) | 0.335 |

**Table 4.** Opioid consumption and postoperative variables.

Time to first analgesic injection was significantly longer in group LIA (40 min vs. 20min, p = 0.005) and pain intensity was significantly lower on arrival at PACU and one hour after extubation. (Figure 9). The following important surgical complications were noted: vaginal hematoma (n = 1), re-operation due to bleeding (n = 1) and wound infection (n = 2). The overall incidence of surgical complications was 7% (4/55) with no significant difference between the groups. The response rate for the brief pain inventory questionnaire was 71%. The incidence of persistent post-surgical pain was 23% (9/39) in the cohort, with no significant difference between the groups.

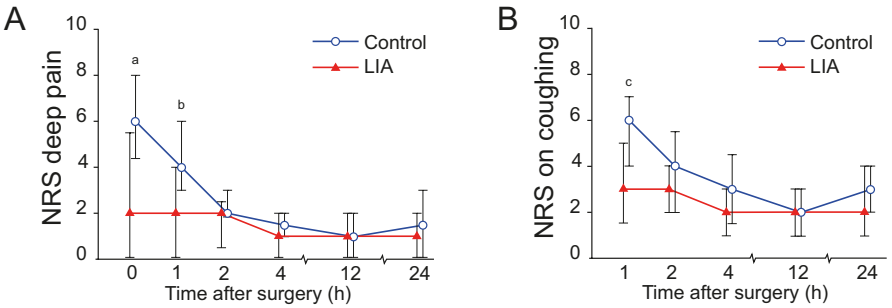


**Figure 9.** NRS at incision postoperatively. (A) Numeric rating scale for deep pain at the incision site, presented as median and IQR. aP-value < 0.001, bP-value = 0.001(B) NRS for pain on coughing at incision site, presented as median and IQR. cP-value < 0.001

Study II and III

Forty patients were recruited, 20 patients in each group, between November 2014 and September 2017 (Figure 10). There were no differences between the groups in demographics or perioperative characteristics. In all, 13 (65%) patients in the IPLA-group and 14 patients (70%) in the placebo group were macroscopically resected with no gross residual disease.

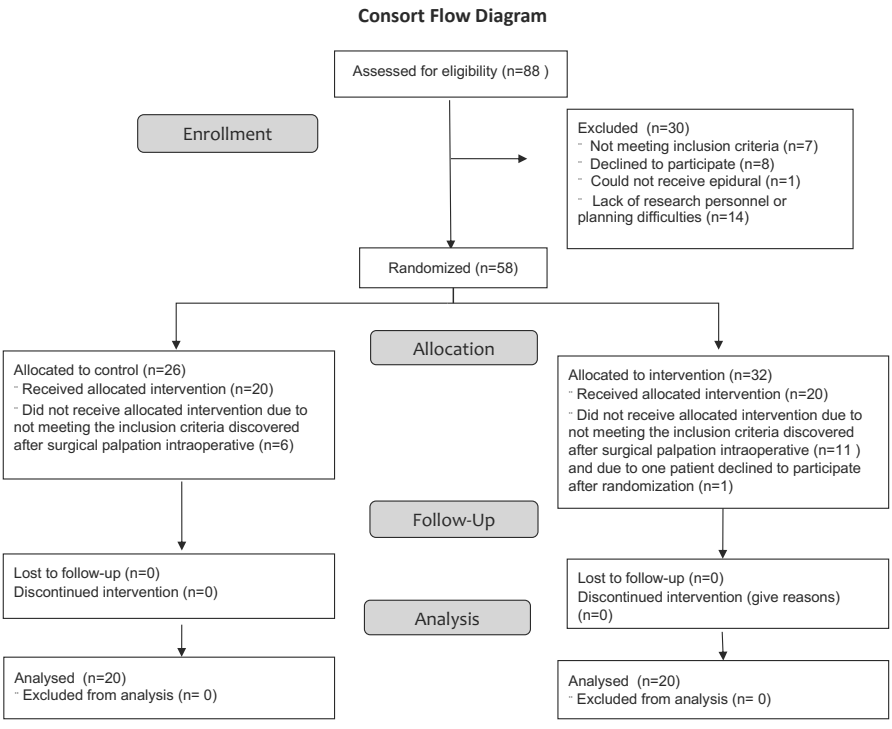


**Figure 10.** Consort diagram study II and III.

Four cytokines reached detectable levels, IL-2, IL-6, IL-8 and IL-10. Perioperative serum concentrations of cytokines are shown in Figure 11. The only cytokine concentration that was significantly higher in the IPLA group compared to the control group at baseline and at 6 hours was IL-2. Serum cortisol was significantly lower in the IPLA group at 6 hours, median 103 nmol/l (53–250) vs. 440 nmol/l (115–885) in the IPLA and placebo groups respectively (p=0.023). No differences were found in insulin, glucose or CRP levels between the groups.

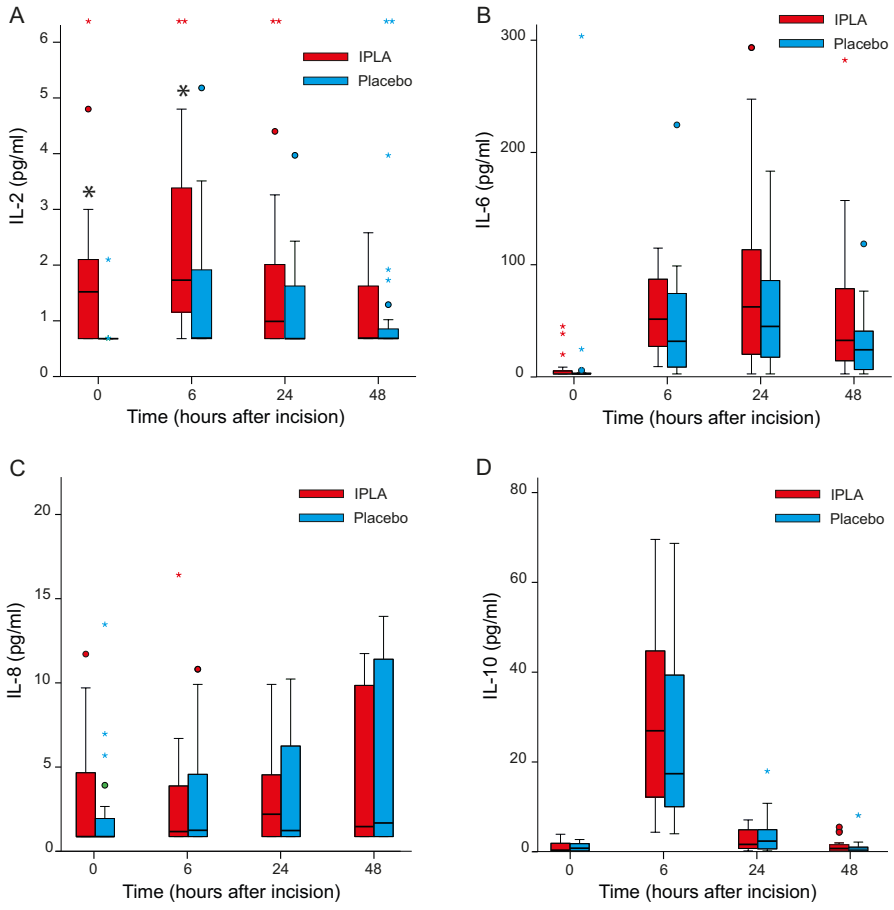


**Figure 11.** Serum levels of Cytokines. * p-value < 0.05

The total dose of ropivacaine administered via epidural catheter route did not differ between groups, median (IQR) 368 mg (292-408) vs. 384 mg (292–412) in the IPLA and placebo groups respectively (p=0.693). In IPLA group, an additionally 520 mg ropivacaine was given intraperitoneally. Thus, the IPLA group received a median (IQR) total of 888 mg (812–928) ropivacaine which was significantly higher vs the placebo group. The free and total serum concentrations of ropivacaine at 6, 24 and 48 hours are shown in Figure 12. No patient had LA concentrations above known central nervous system toxic concentrations in humans. One patient in the control group had a total serum concentration higher than the potential toxic levels of ropivacaine (5.7 mg/L) at 48 hours. However, the free concentration was only 0.034 mg/L in this patient. No adverse events occurred.

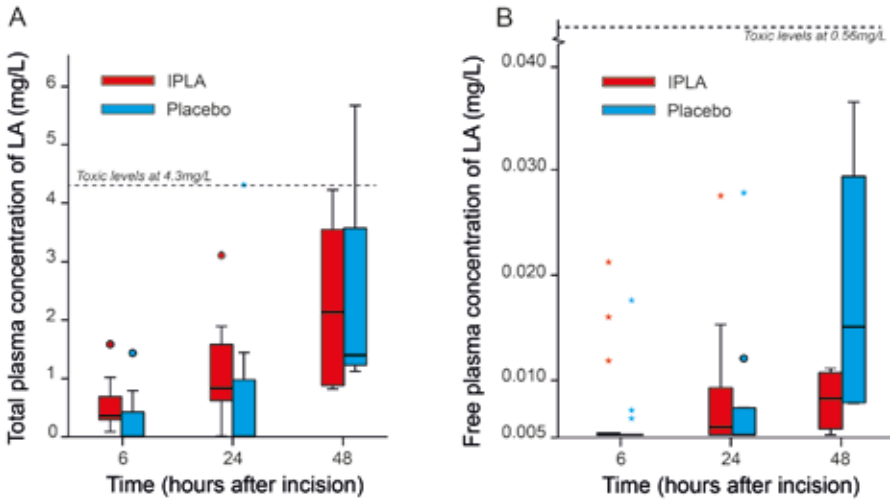


**Figure 12.** Total and free concentration of ropivacaine.

Postoperative pain, recovery, complications and time to chemotherapy are shown in table 5. Median time to initiation of chemotherapy was significantly shorter in the IPLA group compared to placebo, 21 days vs. 29 in the control group (Figure 13). The global score of QoR-40 declined in the postoperative period, but with no statistical differences between the groups (Figure 14).

| | IPLA (n=20) | Control (n=20)) | p-value |
|---|---|---|---|
| Morphine requirements at 24h, mg (IQR) | 9 (2-29) | 9 (1.5-17) | 0.505 |
| Morphine requirements at 48h, mg (IQR) | 21 (5.5-48) | 10 (5.5-42) | 0.432 |
| Dose epidural ropivacaine 48h, mg | 368 (292-408) | 384 (292-412) | 0.693 |
| Total dose ropivacaine (epidural+IP) 48 h (mg) | 888 (812-928) | 384 (292-412) | <0.001 |
| Drain losses/ascites 24 h (ml) | 700 (570-1100) | 540 (415-832) | 0.243 |
| | (n=14) | (n=16) | |
| Functional recovery | | | |
| Time to stand (hours) | 17 (14-24) | 19 (16-24) | 0.433 |
| Time to walk (hours) | 48 (44-68) | 57 (43-73) | 0.449 |
| Time to return of bowel function (days) | 7 (5-9) | 7 (5.5-8.5) | 0.985 |
| Time to readiness for discharge (days) | 14 (10-15) | 11.5 (9-14) | 0.225 |
| Complications Clavien-Dindo score | | | 0.548 |
| Grade I, n (%) | 9 (45) | 7 (35) | |
| Grade II, n (%) | 5 (25) | 7 (35) | |
| Grade IIIa, n (%) | 4 (20) | 3 (15) | |
| Grade IIIb, n (%) | 2 (10) | 1 (5) | |
| Time to initiation of chemotherapy, days, median (IQR) | 21 (20-29) | 29 (21-40) | 0.021 |
| | (n=19) | (n=18) | |
| RIOT, n (%) | 19 (95) | 18 (90) | 0.795 |

**Table 5.** Postoperative pain, recovery, complications and time to chemotherapy.
Results are presented as median and interquartile range (IQR).
RIOT = Return to Intended Oncologic Treatment.

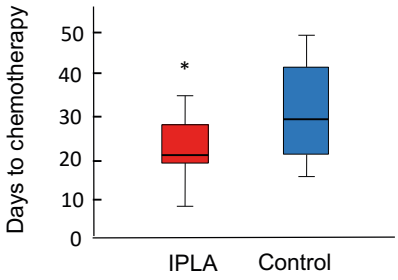


**Figure 13.** Time to initiation of chemotherapy. * p-value < 0.05

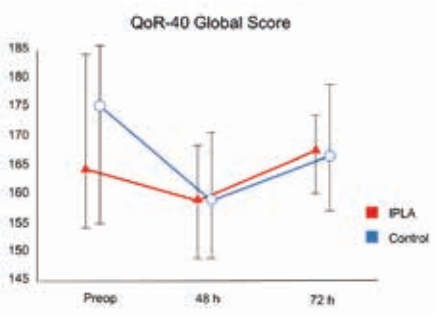


**Figure 14.** QoR-40 Global score preoperatively, after 48 and 72 hours, presented as median and IQR.

Study IV

Ropivacaine and lidocaine in 1000 μM but not 100 μM concentration significantly reduced cell number in SW626 cell line ($p < 0.05$). However, no effect was seen in the SKOV-3 in relation to control (Figure 15).

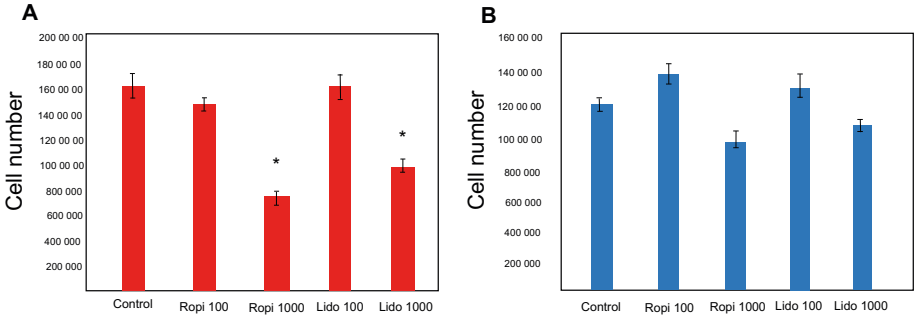


**Figure 15.** Effect on cell number after treating the ovarian cancer cell lines SW626 (A) and SKOV-3 (B) with lidocaine or ropivacaine for 72 hours. Ropi, ropivacaine. Lido, lidocaine. * p-value < 0.05

MTT reduction (% of control) assumed to be proportional to total cell number after treatment with ropivacaine or lidocaine was increased only after treatment with lidocaine at concentrations of 1000 µM (Figure 16).

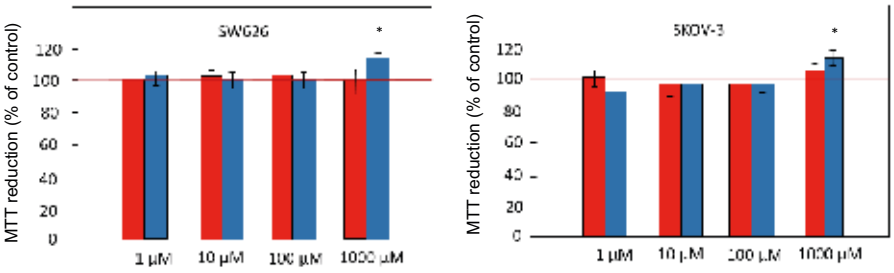


**Figure 16 .** MTT reduction (% of control) assumed to be proportional to cell number after treatment with ropivacaine or lidocaine. Horizontal line shows MTT reduction in the control group. The bars show the mean and SEM of 3–4 independent experiments. * p-value < 0.05

Cell migration was significantly diminished by up to 50% in SW626 cell lines after 72 hours of incubation with 1000 µM ropivacaine and lidocaine, but not after incubation with 100 µM. Compared with control, the SKOV-3 cell line showed significant inhibition of cell migration when exposed to 1000 µM ropivacaine for 72 hours but the opposite effect was seen when treated with lidocaine (Figure 17). Microscopic photo of wound closure in SW626 is shown in Figure 18.

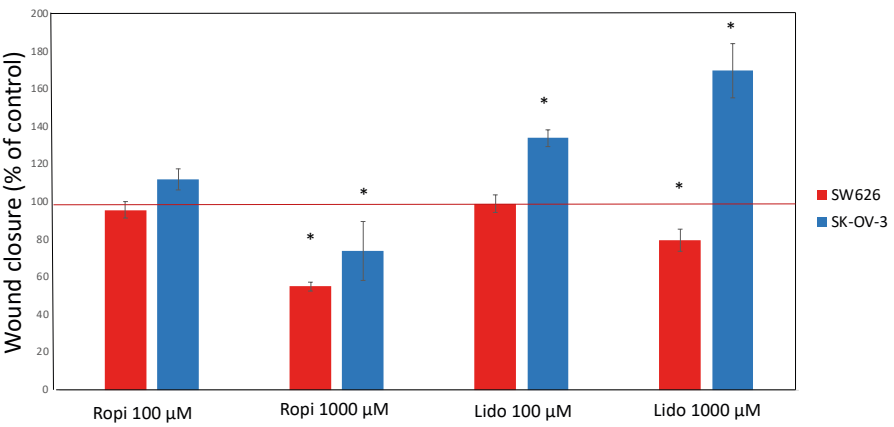


**Figure 17.** Effect on cell migration after treating the ovarian cancer cell lines SW626 (red bars) and SKOV-3 (blue bars) with lidocaine or ropivacaine after 72 hours, except for lidocaine in SKOV-3 group (48 hours). Ropi, ropivacaine. Lido, lidocaine. * p-value < 0.05

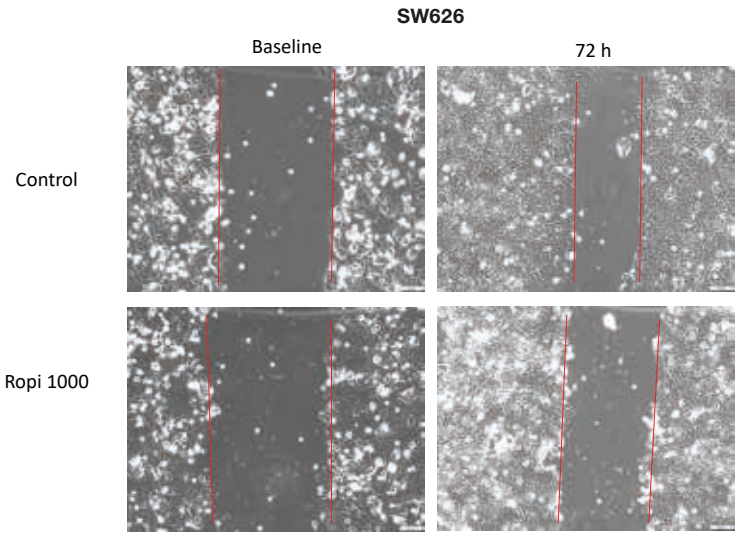


**Figure 18.** Ropivacaine suppressed cell migration (wound closure) in SW626 cells.

Phase contrast microscopy showed morphologic changes at 100 μM and 1000 μM concentrations, resulting in larger and more granulated cells (Figure 19). This finding was repeated using the flow cytometric analysis of light scatter that showed significantly increased cell size and granulation of the ovarian cancer cell lines treated with LA. Ovarian cancer cells with cancer stem cell properties were significantly decreased in cell number by up to 50% after treatment using both LAs (Figure 20).

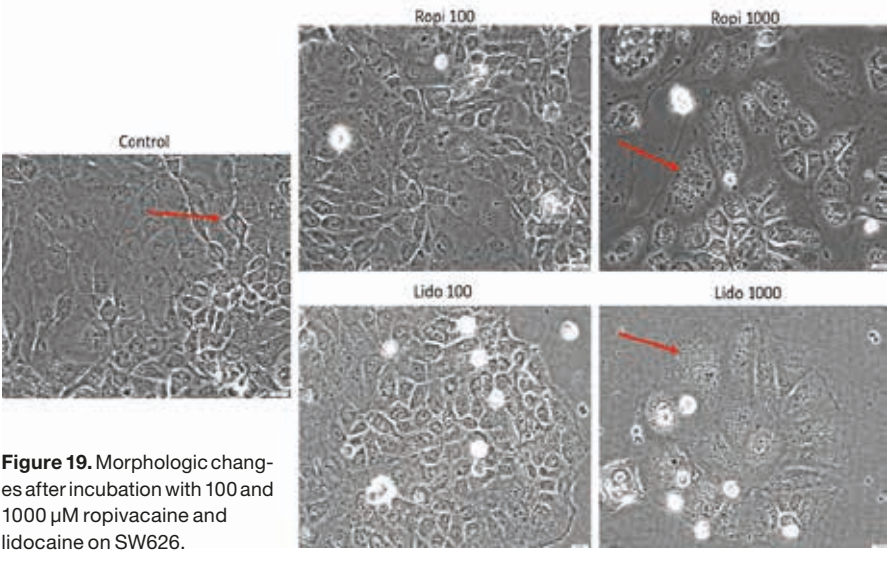


**Figure 19.** Morphologic changes after incubation with 100 and 1000 μM ropivacaine and lidocaine on SW626.

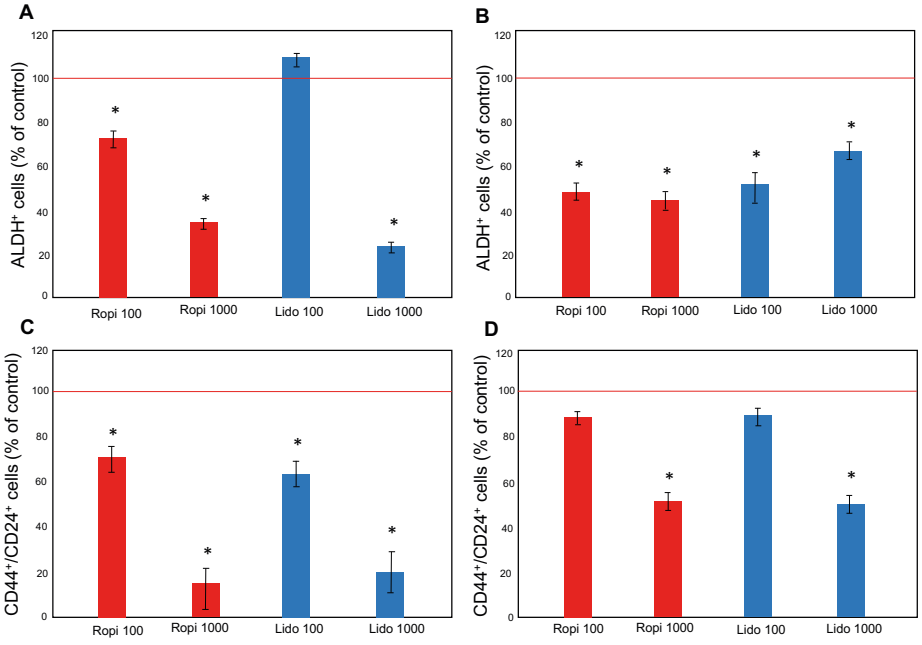


**Figure 20.** Treatment with lidocaine and ropivacaine for 72 hours on the ALDH+ (A and B) and the CD44+CD24- (C and D) population in the ovarian cancer cell lines SW626 (A and C) and SKOV-3 (B and D). * p-value < 0.05

# 5. Discussion

### Postoperative pain and rescue analgesia

In study I, we found that the systematic injection of local anaesthetics in combination with ketorolac and adrenaline results in a significantly lower rescue morphine consumption during the first 24 hours, lower pain intensity in the early hours after surgery and a longer time to first analgesic consumption after abdominal hysterectomy. However, we could not show that the reduction in opioid use in the LIA group lead to a significant reduction in the incidence of opiate-related side effects or earlier home discharge, which is likely because the study was not powered to detect these differences. Local anaesthetics infiltrated in the abdominal cavity or infused intraperitoneally as a single bolus or following continuous infusion have showed a reduction in opioid consumption and lower pain score.[130–132] Wound infiltration of superficial layers, subcutaneously and/or in muscle layers of the abdomen have, however, not demonstrated satisfactory analgesia, indicating the need for blocking of all sensory affarents in the surgical field.[133] The efficacy of high-volume multimodal wound infiltration has been extensively studied after orthopaedic surgery with no, or few and minor side effects.[134] This technique combined with inserting of a catheter into the operating field following orthopedic surgery or indeed even wound catheters has not shown increased risk of infection, and the use of ketorolac has not resulted in a higher incidence of hematoma formation locally.[134] However, systemically administered ketorolac has been associated with increased risk of postoperative bleeding in some studies.[135] A meta-analysis by Gobble *et al.* reviewing twenty-seven double-blind, randomized, controlled studies including 2314 patients did not find a significantly increased risk of bleeding when using ketorolac compared with controls. This is in agreement with our own findings where only one patient suffered from postoperative bleeding (1.7%) and one from vaginal vault hematoma (1.7% ) after abdominal hysterectomy. The incidence of postoperative haemorrhage, regardless of the surgical approach (laparoscopic, vaginal, or abdominal), varies between 1–5%, and is up to 16% for vault hematoma.[136] Therefore, it is unlikely that the use of ketorolac in the local infiltration analgesia in our study lead to an increased risk of postoperative bleeding.

Intraperitoneal local anaesthetics (IPLA) have been shown to inhibit visceral pain following abdominal surgery leading to a chemical affarentectomy.[137] However, in our small pilot study, we did not find any analgesic benefit of supplemental IPLA to epidural analgesia after extensive abdominal surgery, neither in patient reported pain-intensity or morphine consumption. Kahokher *et al.* have reported a significantly reduced opioid consumption and lowered NRS on pain at rest and movement using IPLA for 3 days after colectomy over and above that could be associated with the use of epidural analgesia.[21] These contrasting result may be explained by the fact that extensive ovarian cancer surgery in all four abdominal quadrants may not lead to complete

inhibition of all visceral affarents or that a greater volume of LA infused continuously postoperatively is needed than was used in our present study. It is also possible that sub-diaphragmatic analgesia following peritonectomy may not be achieved using an intraperitoneal catheter placed in the mid-abdominal region. The vagus nerve carries afferent fibres from visceral structures in the thoraco-abdominal viscera, directly to the brain (bypassing the spinal cord).[110, 138] Viscera is predominantely innervated by the vagus nerve. Thoracic epidural analgesics (TEA) block the somatic afferent nerve fibres entering the dorsal column and hence part of the visceral affarent stimuli that follow vagal innervation are not feasible for inhibition by TEA. Therefore, it is conceivable that IPLA may supplement the analgesic effects of TEA. No studies have previously explored the possibility of combining IPLA and TEA during cytoreductive surgery for ovarian cancer. It is possible that TEA provided adequate postoperative pain relief in our study, and therefore the added beneficial effects of IPLA may not have been detectable in our study.

In summary, our findings suggest that LIA is a good adjunct to a multi-faceted analgesic strategy for abdominal hysterectomy while IPLA as supplemental pain management to epidural after cytoreductive surgery for ovarian cancer offers no additional benefit for pain relief under the limitations of this pilot study.

### Postoperative Inflammation and the stress response

Epidural anaesthesia has been shown to diminish the inflammatory response after abdominal surgery. By blocking afferent nerve fibres from peripheral nerves to the central nervous system, the initiation of the hormonal and metabolic responses to stress and trauma are altered.[139] In a prospective study by Beilin *et al.* patients undergoing abdominal surgery were randomised to receive PCA, epidural analgesia or intermittent morphine intravenously.[59] Patients in the epidural group had reduced lymphocyte proliferation and an inhibited cytokine response during the postoperative period. In a clinical study by Kuo *et al.*, assessing inflammation, pain and bowel function after colectomy using epidural and i.v lidocaine, both i.v LA and epidural analgesia were better than control. The decline in cytokine-levels were most profound in the epidural analgesia group.[118] Intravenously administered LA have been shown to reduce inflammation.[140] Therefore, it remains unclear whether LA have beneficial effects via systemic absorption (from the epidural space) or by reduced stress response by blocking affarent nerve transmission. In our study, systemic uptake of epidurally administrated ropivacaine was demonstrated in the plasma even in the control group (without intraperitoneal local anaesthetics), indicating a plausible systemic effect of LA, since no significant difference was found in most cytokines, between the groups. We did not include a group without epidural analgesia in our pilot study to assess the anti-inflammatory effect of IPLA alone, which may have been unethical in view of the magnitude of surgery. Reduced cytokine expression using IPLA have been reported by Kahokher *et al.* who evaluated IL-6, IL-8; IL-10 and TNF-α in 60 patients undergoing colectomy and found significantly decreased concentrations in the study group at 8 and

20 hours postoperatively.[21] One important caveat is that there is a large intra-individual variations in the expression of cytokines, and sampling time postoperatively is crucial. Matte *et al.* profiled 120 cytokines in ascites in 10 women with advanced ovarian cancer and found a wide variability of expression between cytokine levels and patients.[14] Immunological mediators (and cytokines) are elevated in almost all stages of ovarian cancer, and are an important prognostic factor.[141] One possible explanation for the lack of differences between the groups in our study is the elevated cytokines preoperatively in the cohort, causing small differences between groups difficult to detect. With the exception of the significant differences in IL-2 and cortisol at 6 hours, we did not find any significant differences in any of the other inflammatory markers between those patients receiving IPLA or placebo in this small pilot study. IL-2 levels were actually higher at baseline in the IPLA-group. This difference must have been by chance since no treatment was given at this time-point. It is likely that this could also expain the higher levels at 6h. The lower cortisol concentration after 6 hours in the IPLA group followed by a rise that was greater than in the placebo group at 24 hours suggests that IPLA might delay the normal stress-induced rise in cortisol, but does not abolish it. Taken together, our findings indicate that epidural and intraperitoneal LA combined do not further add to the anti-inflammatory effect of epidural alone. However, IPLA blunts the stress response, briefly.

### Local anaesthetic toxicity

Current recommendation regarding maximum doses of LA are based on small exploratory studies, case reports or extrapolated from animal studies. A review by Rosenberg *et al.* suggests that because of the lack of randomised clinical trials, clinically safe LA doses should not be presented as milligram doses or mg/kg, but instead be prescribed according to patient-realted factors.[142] In a recent review by Gitman *et al.*, 47 separate cases of LAST were described in 35 peer-reviewed articles.[93] High concentrations, or high volumes of LA, and the use of continuous peripheral catheters and intravenous lidocaine infusions were associated with a higher risk of LA toxicity. The absorption of LA administered intraperitoneally has been previously evaluated by Perniola *et al.* administrating 10 ml $h^{-1}$ infusion of 1.75 mg $ml^{-1}$ levobupivacaine intraabdominally postoperatively for 48 h after hysterectomy, with no adverse events and LA concentration was well below known toxic values in humans.[130] In *study I*, we administered 300mg of ropivacaine via local inflitration analgesia intraabdominally without side effects and therefore consider it to be safe. Some authors have used cumulative doses of 1340 mg ropivacaine after combined intraperitoneal and epidural analgesia, without toxic plasma concentration, but since the maximum recommended safe dose of ropivacaine during 24 h is recommended to be about 800 mg, we measured ropivacaine concentration in *study II* and found it to be far below toxic concentrations, and without side effects.[21] Both total and free serum ropivacaine concentrations remained well below known toxic concentrations despite administration of up to 928 mg ropivacaine in one patient. We conclude that ropivacaine is safe when administered intraperitoneally

together with epidural analgesia in the doses used in *study II*, without risking toxic concentration.

### Postoperative recovery and complications

Clavien-Dindo classification (CDC) is an international system for rating postoperative complications in the surgical population.[143] The incidence of postoperative major complications (CDC > 2) after cytoreductive surgery for advanced ovarian cancer is 22–30%, medical and surgical complications included.[8] Chi *et al.* investigated 141 patients after extensive abdominal surgery for ovarian cancer, stage III or more and found that CDC grade 3–5 complications occurred in 22% of the patients.[8] The most common complications were pleural effusion requiring drainage (39%) followed by intra-abdominal infection, pancreatic leak and bleeding. The complications rate in our study (III) is similar to previously reported, 25% major complications in ovarian cancer surgery and 1.7% after hysterectomy. The Swedish national gynaecologic register reported an average of 3.1% major complications after hysterectomy for benign indication in 2018. The overall complication rate after hystererctomy in the Swedish national gynaecologic register was 25% compared to 7% in our small cohort. This implies that there are no major adverse effects using the new methodologies evaluated in this thesis. Furthermore, the incidence of complications was similar between the groups receiving IPLA or LIA, or not.

Assessment of patient-perceived quality of recovery in terms of mobilisation, pain, GI-function and sleep were performed with a qualitative intent using standardised questionnaires in study III. The two groups did not differ in either the postoperative global score or in the five dimensions of recovery at any time point, with the exception of pain where the control group had a discretely higher pain score (3 vs 2, p=0.053). The global QoR-40 score, however, significantly declined at 48 hours and improved at 72 hours as predicted due to the extensive surgery. There are no studies evaluating QoR-40 after extensive ovarian cancer. Gornall *et al.* summarized the postoperative global score in a meta-analysis in 2013, reviewing 17 studies and 3621 patients where the mean (95% CI) global score postoperatively in patients with complications was 159 (153–166) compared to 170 (163–179) in patients without complications (p =0.002).[127]

In our study, the pre- and postoperative value is comparable to high versus low quality of recovery implying that the severity of discomfort is likely to be from the cancer itself. The ability to measure clinical impairment of health status after surgery is an important tool to assess outcome. However, in our study, we did not find any difference between the groups. Persistent post-surgical pain (PPSP) is frequently reported after abdominal hysterectomy. The estimated frequency is 5–32%.[112] Poorly managed postoperative pain is known to be a risk factor for PPSP. However, this is a predictable factor in less than 20% of patients with a risk of developing persistent pain.[144] We followed up the intensity of pre-surgical pain after 3 month following abdominal hysterectomy using the Brief Pain Inventory questionnaire and 20% of our patients suffered from pain in

the surgical area. Despite significant difference in NRS-scores in the PACU between the groups, we did not find a difference in PPSP at 3 months. This further supports the multifactorial mechanisms behind PPSP, and the need for additional pain treatment strategies.

### Initiation of adjuvant chemotherapy

In study III, we found that IPLA significantly reduced median time to initiation of chemotherapy from 29 to 21 days. Several studies have reported a positive prognostic correlation between survival and the interval to chemotherapy but the optimal time is not defined. In most clinical trials the patients receive their first cycle within 6–8 weeks. Hofsetter *et al*. showed a significant correlation with long-term outcomes following ovarian cancer when the first cycle of chemotherapy was < 28 days, while Mahner *et al.* found a slightly improved survival when initiation was <19 days.[9, 145] From our study, we cannot conclude that a difference of 8 days between the groups altered the long-term outcome, it is nevertheless relevant since IPLA offers an earlier start of chemotherapy without side effects. This needs to be further studied in larger studies with long-term outcome. Unfortunately, there is no uniform criteria on the decision as to when adjuvant chemotherapy may or may not be started. For this reason, the Swedish national guidelines for epithelial ovarian cancer does not provide any other recommendation than to state that "adjuvant chemotherapy for advanced ovarian cancer should start as soon as it is possible, based on the general status of the patient and postoperative recovery, especially in patients who have undergone microscopic radical surgery". The decision is ultimately made by the treating medical oncologist and is a subjective decision, depending on the patient´s general postoperative status. The reasons for the underlying decision to initiate chemotherapy were not monitored in our study, which is a limitation. The decision to start chemotherapy may be affected by several causes due to structural differences among hospitals for administrative or logistic reasons, for example referral to rehabilitation institutions. These differences are present in the two Swedish centres that participated in the study and may account for underlying biases. However, the study was completely blinded to the oncologists and the surgeons involved in the decision to initiate chemotherapy. Even though the optimal time to chemotherapy is uncertain, evidence suggests not to delay initiation since the favourable effect on prognosis is important.

### Effects of Local Anaesthetics on Ovarian Cancer Cells

The pharmacokinetics of ropivacaine during epidural analgesia for 120 hours were evaluated by Wiedemann *et al.* in 12 patients undergoing total knee arthroplasty. Ropivacaine 15 mg $h^{-1}$ was administered for 1–5 days, with an absolute mean dose of ropivacaine of 1786 mg. The highest individual free plasma concentration was 0.16 mg $L^{-1}$ and mean peak total concentrations was 4.1 mg $L^{-1}$, yielding a molarity of 6–22 µM in plasma.[146] Intravenous administration of lidocaine in anti-arrhythmic doses reaches similar plasma-concentrations of 10–25 µM. Our own study showed a free and total plasma concentration of 0.03 and 8 µM respectively in the blood after IPLA

and epidural infusion with ropivacaine. The local concentration at the site of injection is approximately equivalent to the administered concentration, 2 mg $ml^{-1}$ or 7.2 mM. The ovarian cancer cells in study IV were exposed to 1, 10, 100 and 1000 μM. 1 and 10 μM only in the initial dose response MTT reduction assay evaluating cell viability at different concentrations. As the cells were mostly affected at 1000 μM, it is unlikely that lower concentrations would have had further impact.

### Influence of local anaesthetics on cancer cells in vitro

The results from study IV indicates that amide LA may provide beneficial antimetastatic effects. Cell number was significantly reduced by 1000 μM lidocaine and ropivacaine in SW626 cells and a trend toward the same in SKOV-3. Migration was significantly inhibited after 1000 μM ropivacaine in both cell lines. Lidocaine, however, showed divisive effects with inhibition in SW626 but enhanced migration in SKOV-3. Lower concentrations did not have any impact on cancer cell number or migration. Our findings are in line with Martinssons *et al.* and Siekman *et al.* who showed reduced cell proliferation in colon cancer cell lines at clinical concentrations of ropivacaine.[147, 148] Martinsson *et al.* found a less anti-proliferative effect of lidocaine than of ropivacaine, while Siekman *et al.* found no effect of lidocaine. Similar contrasting results have been reported by others. Boselli *et al.* found that lidocaine, but not ropivacaine, increased apoptosis in Jurkat leukemia cells.[149] This indicates possible differences between drugs as well as cancer cell lines.

Cell viability indirectly estimated by mitochondrial activity in the MTT assay was significantly inhibited in both cell lines after 1000 μM lidocaine in our study. This finding has no clinical implication because of the doses are much higher than those used in clinical practice. The indirect measurement of viability may be an improper methodology if the alterations caused by LA in the cells are due to increased mitohocondrial activity. An altered morphology in both cell lines was observed at 100 μM and 1000 μM when exposure time was more than 48 hours. The mechanism for this observation remains unanswered. The cell size increases, with enhanced granulation, while the cell number decreases. One can ponder if activation of intrinsic properties with increased cell size and granulation might be a result of alterations in intracellular metabolism and organelle expression. Ropivacaine has been shown to alter energy metabolism in cells by inhibiting Adenosine triphosphate synthesis and by a direct inhibitory effect on mitochondrial enzyme complexes.[78] Observed inhibition of cell migration has been reported by several authors.[82, 83, 148] Piegler *et al.* showed inhibitory effects on lung cancer cells after ropivacaine and lidocaine exposure at clinical concentrations, presenting a possible molecular mechanism where cell signalling was disrupted.[83]

Cells with tumour-initiating properties within the cancer cell bulk were analysed using well-studied surface biomarkers for ovarian cancer stem cells.[150] The range of cells with CSC phenotypes was in line to previously reported data (4.9–9.4%). In the present study, we found that exposure of CSC phenotypes to ropivacaine in clinical

doses resulted in a 50% decline in this cell population. For ovarian cancer, CSCs are believed to be resistant to chemotherapy.[51] In concentrations that are used clinically, our study (IV) indicates that the ability of cells to grow and multiply can be inhibited, which may delay cancer recurrence. We could demonstrate reduction of cell viability and proliferation at doses that can be achieved by intraperitoneal administration of LA during several days following abdominal surgery.

# 6. Conclusions

Systematic injection of local anaesthetics after open abdominal hysterectomy is an efficient adjuvant to conventional pain management. Local anaesthetics administered in the abdominal cavity during and/or after gynaecologic surgery are safe, with no apparent side effects.

Intraperitoneal administered LA in combination with epidural analgesia do not further add to the anti-inflammatory effects compared to epidural alone, except for a brief inhibition of the surgical stress response. Advanced ovarian cancer treated with supplemental perioperative intraperitoneal ropivacaine seems to result in earlier start of postoperative adjuvant chemotherapy. However, this needs to be validated in a larger randomized trial.

Clinically equivalent doses of LA exposed to ovarian cancer cells *in vitro* result in mitigated cell numbers, inhibited cell migration and altered phenotype.

We have shown that the perioperative administration of intra-abdominal local anaesthetics may have beneficial effects on pain, recovery and circulating tumour cells after gynaecological surgery.

# 7. Methodological considerations

The major advantage of the two clinical studies was their randomized, double blind design. Study II–III were the first randomized controlled trial evaluating the effect of IPLA in extensive ovarian cancer surgery. Despite the RCT design, we set the study to be a phase II pilot trial to test a relatively new method in oncological surgery, before embarking on a larger prospective clinical study. Since there is no previously published study on IPLA in late-stage ovarian cancer, we used a convenient sample size based on the number of patients that could be potentially recruited over 1–2 years in this initial trial. The inclusion criteria were stringent and only late-stage epithelial ovarian cancer patients were recruited in a small country from two hospitals.

Our hypothesis was that ropivacaine, being anti-inflammatory, might reduce pain and inflammation and thereby promote early mobilization, quicker return of function and earlier start of chemotherapy. Considering that the data was extensive, we decided to publish a pre-clinical study with inflammation, pharmacokinetics and toxicology of ropivacaie and later a clinical study looking at more explorative outcome data. Therefore, study III can be considered as a secondary analysis of clinical parameters evaluating the effect of perioperative intraperitoneal local anaesthetics following late-stage ovarian cancer surgery.

We chose ropivacaine because it is the safest long-acting local anaesthetic available. The recommended maximum dose is 800 mg per day, and to avoid toxicity a free concentration level below 0.56 mg/L is tolerated. In our study, median free concentrations of ropivacaine were much lower, and no patient had any signs or symptoms of LA toxicity.

A limitation of study III is that initiation of chemotherapy was not monitored according to predefined criteria at specific time intervals. However, since the Swedish Association of Obstetrics and Gynaecology do not, to date, recommend pre-defined criteria, these would have been arbitrarily chosen.

# 8. Future perspectives

Local infiltration analgesia will most probably be further refined and evaluated in different surgical settings. New strategies and techniques to improve pain management and to reduce opioid use will remain a future and continuing quest.

Even though we found significant effect on RIOT, the results are not conclusive and have to be interpreted with caution until a larger trial confirms (or rejects) our initial findings.

Despite the findings on ovarian cell lines, little is known about the underlying mechanisms, or the relationship between laboratory results and *in vivo* outcome. It will be of interest to conduct studies focused on cellular mechanism by which LA inhibit cancer cell proliferation.

Our *in-vitro* experiments were conducted on two ovarian cancer cell lines. To verify the results, two other ovarian cancer cell lines, examined with identically set-up would be relevant.

Since cancer stem cells have been hypothesized to be resistant to chemotherapy, a follow-up on women included in our clinical study on IPLA following surgery for ovarian cancer would be of great interest to explore the recurrence rate and long-term survival.

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# 11. Appendix (Paper I–IV)