RECOVER - 2

Low impact laparoscopy *(low pressure pneumoperitoneum and deep neuromuscular blockade)* versus standard laparoscopy during robot assisted radical prostatectomy to improve the quality of recovery and immune homeostasis; a randomized controlled study.

NCT number: NCT04250883

Versie 1.6, 14-05-2020

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SUMMARY

Rationale: Intra-abdominal pressure (IAP) needed to create sufficient workspace during laparoscopic surgery affects the surrounding organs with ischemia-reperfusion injury and a systemic immune response. This effect is related to postoperative recovery, pain scores, opioid consumption, bowel function recovery, morbidity and possibly mortality. In clinical practice standard pressures of 12-16mmHg are applied instead of the lowest possible IAP, but accumulating evidence shows lower pressure pneumoperitoneum (PNP) (6-8mmHg) to be non-compromising for sufficient workspace, when combined with deep neuromuscular blockade (NMB) in a vast majority of patients. Therefore, low impact laparoscopy, meaning low pressure PNP facilitated by deep NMB, could be a valuable addition to Enhanced Recovery After Surgery (ERAS) Protocols.

Increased IAP can cause peritoneal mesothelial injury either directly or by compression of small vessels including capillaries, leading to a variable degree of ischemia reperfusion injury. The compromised perfusion of the parietal peritoneum can be visualized and quantified by a fluorescent marker such as Indocyanine green (ICG), as shown in a previous pilot study. Hypoxic injury of intra-abdominal organs and/or tissues may cause the release of Danger Associated Molecular Patterns (DAMPs). It is known that after trauma and sepsis, the release of DAMPs is associated with immunoparalysis and a higher susceptibility to infectious complications. The use of low pressure PNP may reduce hypoxic injury and the release of DAMPs and thereby contributing to a better preservation of innate immune function which may help to reduce the risk of infectious complications.

Objective:

Primary objectives:

- To establish the relationship between the use of deep neuromuscular blockade (NMB) with low pressure pneumoperitoneum (PNP) and the quality of recovery after RARP.
- To establish the relationship between the use of deep neuromuscular blockade (NMB) with low pressure pneumoperitoneum (PNP) and innate immune function after RARP.

Secondary objectives:

- To study whether low pressure PNP and/or deep NMB affects the perfusion of the parietal peritoneal layer during RARP.

Study design: A mono-center, randomized controlled clinical trial.

Study population: Adult patients undergoing elective RARP without extended lymph node dissection.

Intervention: The participants will be randomly assigned in a 1:1 fashion to:

A. Experimental group: low impact laparoscopy (low pressure (8 mmHg) and deep NMB (PTC 1-2))

B. Control group: standard laparoscopy (standard pressure (14 mmHg) and moderate NMB (TOF 1-2))

At the start of surgery, after intra-abdominal insufflation, ICG injection will take place to quantify *parietal peritoneum* perfusion, and a parietal peritoneal biopsy will be taken. At the end of surgery, a second parietal peritoneum biopsy will be taken.

Main study parameters/endpoints:

Primary endpoints

- Quality of recovery score (QoR-40) at postoperative day 1.
- IL-6 and IL-10 response upon whole blood LPS stimulation at postoperative day 7.
- Perfusion index of the parietal peritoneum as calculated from the slope of ICG fluorescence intensity, and time to maximal intensity in seconds. (extracted from video registration).

Secondary endpoints

- QoR-40 score (day 1 and day 10-12), SF-36 score (1 day before, 10-12 days and 3 months after surgery), McGill pain questionnaire (3 months after surgery).
- Pain scores, analgesia use, PONV, time to reach discharge criteria, length of hospital stay, surgical conditions and postoperative complications scored by Clavien Dindo classification (table 2 (1)).

Nature and extent of the burden and risks associated with participation:

The use of a deep NMB enables the safe use of low-pressure PNP. If despite a deep NMB visibility is compromised, pressure will be increased to minimize surgery related risks. A deep NMB is achieved by higher doses of rocuronium which are within normal therapeutic range used in clinical practice, and is safe to use. Depth of NMB will be monitored throughout the whole surgery. At the end of surgery, the effects of rocuronium will be antagonized by sugammadex to avoid residual paralysis. Indocyanine green is a registered product routinely used to quantify tissue perfusion during this surgery. The total dose during surgery is below the advised maximum dose of 5 mg/kg. Any possible risk factors or interactions as mentioned in the Summary of Product Characteristics are covered by exclusion criteria in order to fully eliminate risk of participation. Regarding the peritoneal biopsies; peritoneal tissue is directly visible and easily accessible during laparoscopic surgery and biopsies are obtained in a standardized manner with hemostasis when needed under direct vision. Therefore, no additional complications are expected. Blood samples will be combined with routine laboratory assessment where possible. Previous studies have shown low-pressure PNP is associated with reduced postoperative pain scores, reduced opioid consumption and improved bowel function. This may lead to enhanced recovery. The burden for participants is mainly related to the evaluation of the endpoints during the early postoperative phase. Assessment of pain scores, nausea, complications and discharge criteria are part of the normal treatment. Questionnaires will take approximately 10-15 minutes per time-point.

1 INTRODUCTION AND RATIONALE

Release of DAMPs in surgical patients

By induction of a pneumoperitoneum with carbon dioxide (CO2) a surgical field is created for laparoscopic surgery. A sufficient surgical field is important because of the intra-operative complications related to a limited workspace, although the side-effects of an increased IAP are just as important, but not directly present. The pressure to maintain a sufficient workspace affects the surrounding organs and vasculature. Standard PNP (usually 12-14 mmHg) causes a temporarily decreased perfusion leading to ischemia-reperfusion injury with oxidative stress and release of Danger Associated Molecular Patterns (DAMPs) (2–7). DAMPs elicit an immune response of innate immune cells, which results in a systemic inflammatory response syndrome (SIRS) followed by a compensatory anti-inflammatory reaction (8). This response causes sensitization of nociceptors enhancing postoperative pain, the anti-inflammatory reaction is related to fatigue and leaves more susceptibility for infections (9–11). Maca et al. shows the relation between DAMPs and the degree of surgical trauma, and predicted morbidity and mortality after elective major abdominal surgery (12). Fragidiakis et al described a strong correlation between immune status and recovery from surgery. Signaling responses downstream in monocytes were particularly strong correlates of recovery outcomes including pain and fatigue (13).

Upon (surgical) trauma DAMPS e.g. nuclear DNA, Heat Shock Protein-70, mitochondrial DNA, are released and bind to receptors on immune cells such as macrophages, which induces an anti-inflammatory response predominantly characterized by IL-10 release. In turn this leads to immune suppression, reflected by decreased monocytic HLA-DR expression as well as reduces production of TNF-alpha and IL-6 and increased production of IL-10 upon ex vivo stimulation with LPS (figure 1 (14)).

Alternatively, DAMPs can exert direct immunosuppressive effects, such as HSP10-induced LPS-tolerance in monocytes. All these events take place in a very early phase after surgery.

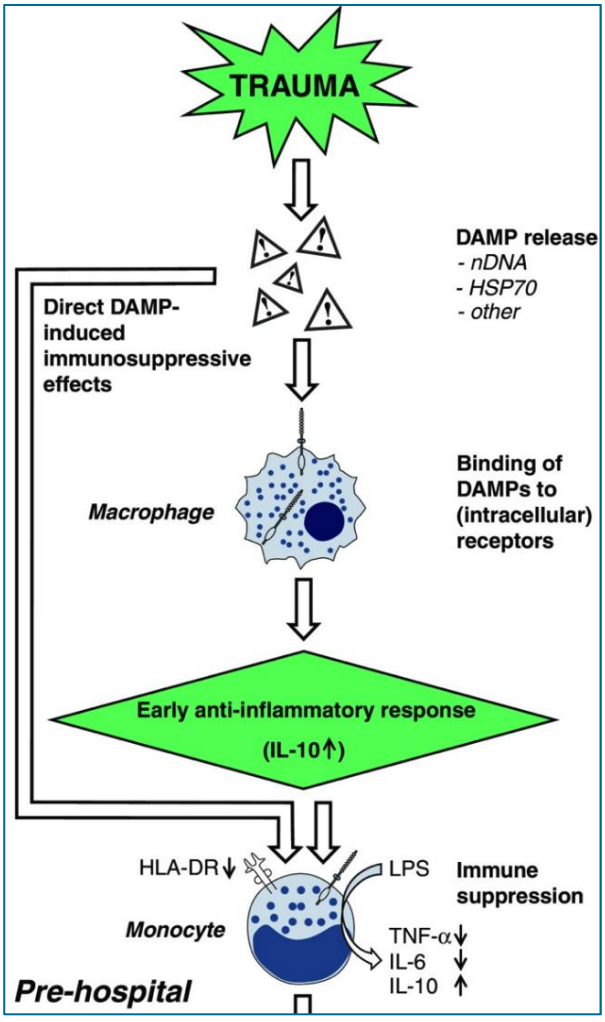


Figure 1. Adapted from Timmermans *et al*. (14)

Low pressure PNP and immune homeostasis after surgery

Accumulating evidence shows the safety and advantages of low-pressure PNP (6-8mmHg); clinically relevant reduction in postoperative pain (15,16), reduced cumulative opioid consumption (17) and improved bowel function (16,18). Furthermore, it preserves innate immune homeostasis, reflected by reduced increase in pro-inflammatory mediators IL-6, IL-10 and TNF-alfa (19–22). And a reduced decrease in monocytic HLA-DR expression (19). Therefore, it is of clinical importance to elucidate the relation between the degree of PNP with its peritoneal and organ perfusion and preservation of innate immune function, to enhance postoperative recovery.

A limited number of studies investigated the immune response after low pressure laparoscopy. It was found that low pressure pneumoperitoneum was associated with a reduced increase in serum levels of pro-inflammatory cytokines like IL-6 and TNF-alpha (19–21) and also with lower levels of anti-inflammatory IL-10 (22). Moreover, Schietroma *et al.* also described a better preservation of monocyte function as reflected by higher levels of HLA-DR expression on monocytes (19). Altogether this provides indirect evidence that lower intra-abdominal pressures may contribute to recovery by a reduced stress response and subsequently a better preservation of innate immune function as compared to laparoscopy with higher intra-abdominal pressures. The interim-analysis of the RECOVER PLUS study showed that patients undergoing laparoscopic colorectal surgery showed a reduced *ex vivo* TNF-α and IL-6 response upon whole blood stimulation at postoperative day 1 which reflects immune suppression due to surgical trauma (Figure 2) (23).

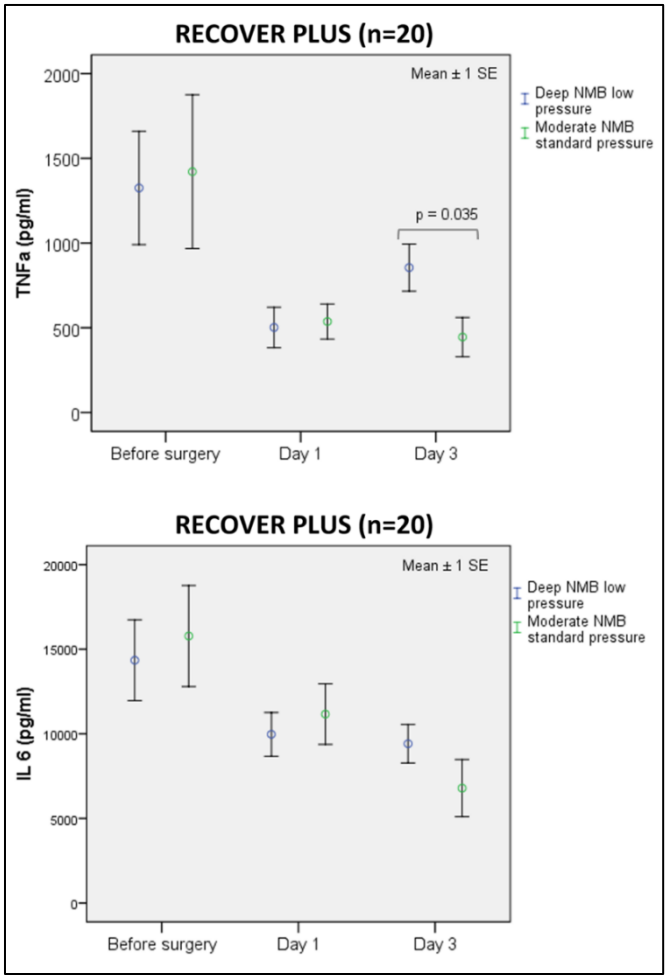


Figure 2. Preliminary/interim results from the RECOVER PLUS study. After ex vivo stimulation of whole blood, the release of TNF-α and IL-6 is significantly reduced at POD 1. In patients allocated to low pressure PNP with deep NMB, TNF-α release is almost restored as compared to baseline. This indicates that low pressure with deep NMB is associated with better preservation of immune function after laparoscopic colorectal surgery. (23)

Patients allocated to low pressure PNP with deep NMB the TNF-α response was almost restored to baseline at postoperative day 3, whereas patients allocated to standard pressure with moderate NMB showed ongoing immune suppression.

To ensure the safety of low-pressure PNP, deep NMB facilitates a sufficient surgical field. Several studies show a significant reduction in IAP with deep NMB compared to moderate NMB, without compromising surgical conditions in patients undergoing laparoscopic surgery (18,24). Current evidence indicates a reduction of 3-4mmHg of IAP when deep NMB is used, with a sufficient and safe surgical field measured and quantified by Leiden-surgical-rating-scale (L-SRS) (25).

Low pressure PNP improves intra-abdominal perfusion

Our group performed the PERFUSION study in which patients undergoing robot assisted laparoscopic colorectal surgery were allocated to an intra-abdominal pressure of 8, 12, or 16 mmHg. Subsequently fluorescence of the parietal peritoneum was recorded after injection of a standardized dose of indocyanine green (26). The time to maximum fluorescent intensity was significantly lower in patients allocated to 8 mmHg. Also, the maximal fluorescent intensity was significantly lower at 8 mmHg as compared to 12 and 16 mmHg (Figure 3). These data indicate that perfusion of intra-abdominal organs and/or tissues is better below 12 mmHg.

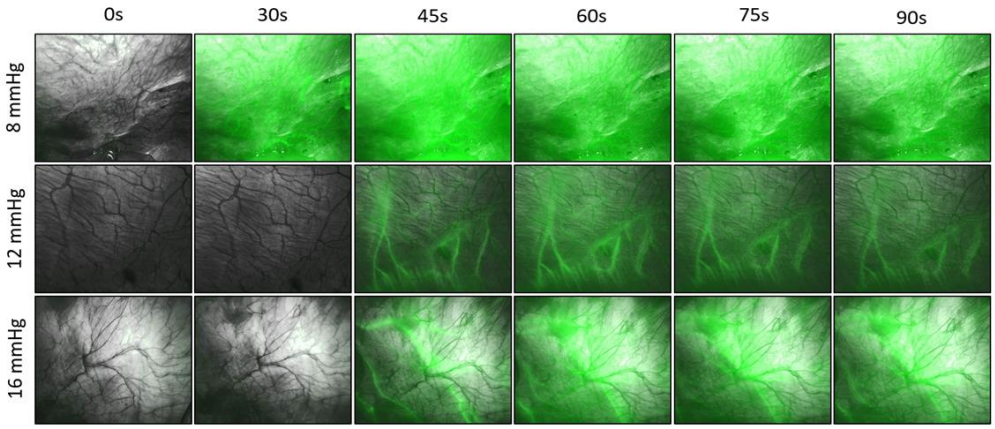


Figure 3. Recordings of the parietal peritoneum after injection of a bolus of indocyanine green. The images are typical examples of three patients representing the mean time to maximum fluorescent intensity at 8, 12 and 16 mmHg. (26)

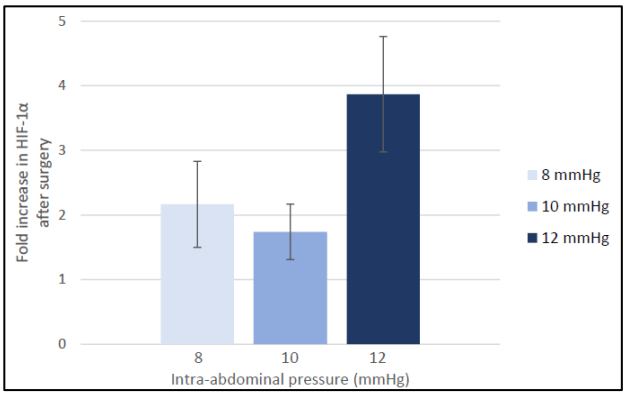


Figure 4. Preliminary/interim results from the RECOVER PLUS study. The increase in expression of HIF-1ain peritoneal biopsy after laparoscopic colorectal surgery compared to the intra-abdominal pressure used. Values are presented as means with standard error of the mean. 8 mmHg (n=4), 10 mmHg (n=3), 12 mmHg (n=12). (26)

Biopsies of the parietal peritoneum were taken after trocar introduction and at the end of surgery. Patients who underwent surgery with a pneumoperitoneum pressure of 8 or 10 mmHg showed lower expression of Hypoxia Induced Factor-1a as compared to those with an intra-abdominal pressure of 12 mmHg. These data support the hypothesis that the use of intra-abdominal pressures below 12 mmHg leads to an improved perfusion of intra-abdominal organs and tissues and thereby reducing hypoxia and ischemic injury.

The aim of this study is to assess the mechanisms and impact of low pressure on:

- perfusion of the parietal peritoneum,
- tissue markers of ischemia and inflammation in the parietal peritoneum,
- serum cytokines and DAMP´s,
- ex vivo innate immune function,
- clinical outcomes i.e. pain scores, quality of recovery and (infectious) complications.

The study is designed to reveal the precise mechanism underlying the clinical benefits of the use of low-pressure PNP facilitated by deep NMB.

2 OBJECTIVES

Primary objectives:

- To establish the relationship between the use of deep neuromuscular blockade (NMB) with low pressure pneumoperitoneum (PNP) and the quality of recovery after RARP.
- To establish the relationship between the use of deep neuromuscular blockade (NMB) with low pressure pneumoperitoneum (PNP) and immune function after RARP.

Secondary objectives:

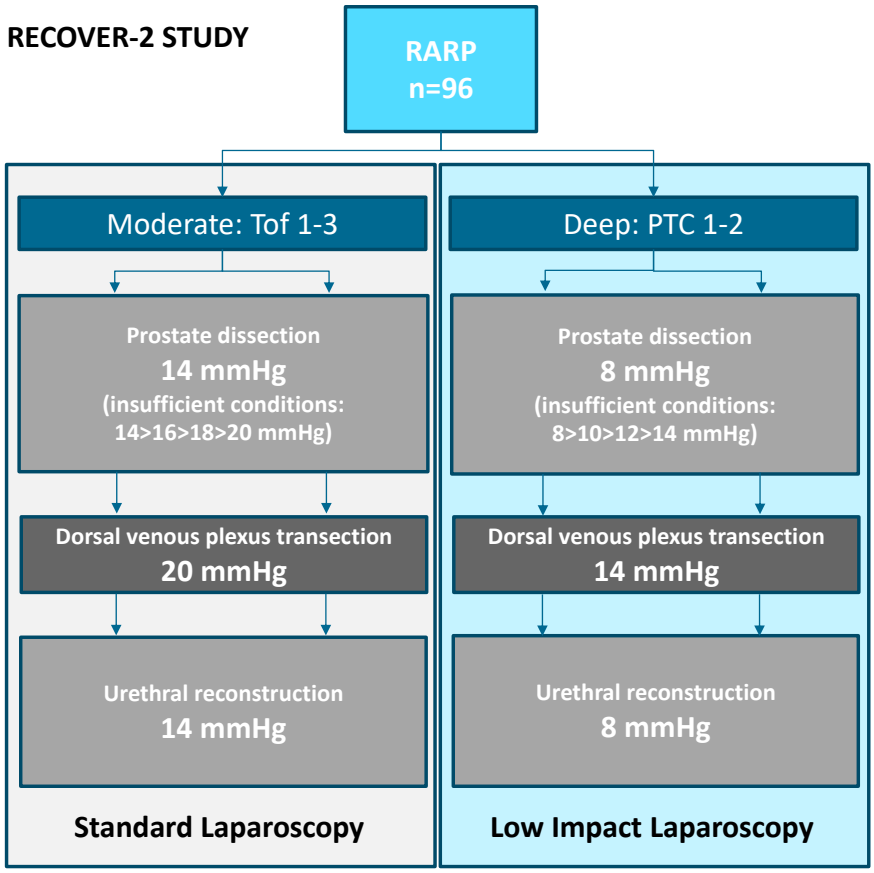
- To study whether low pressure PNP and/or deep NMB affects the perfusion of the parietal peritoneal layer during RARP.

3 STUDY DESIGN

A mono-center blinded randomized controlled clinical trial at the Canisius Wilhelmina Hospital in Nijmegen, where approximately 300 robot assisted radical prostatectomy (RARP) procedures are performed annually. We expect that the screening and inclusion period (recruitment period) will be 15 months.

Treatments to be compared

- Experimental group: *Low impact laparoscopy;* low pressure PNP (8 mmHg) and deep NMB and reversal of NMB with sugammadex 4mg/kg.



- Control group: *Standard laparoscopy;* standard pressure PNP (14 mmHg) and moderate NMB and spontaneous recovery of neuromuscular function (only if one or more additional boluses of rocuronium are required, it is allowed to reverse with sugammadex 2mg/kg)

All study endpoints will be arbitrated by a blinded researcher. Also the perfusion index is extracted from video registration with MATLAB (R2017a version 9.2.0) by a researcher blinded to the level of pressure and NMB depth.

4 STUDY POPULATION

4.1 Population

Adult patient undergoing elective robot assisted radical prostatectomy

4.2 Inclusion criteria

To participate in this trial, a subject must meet all of the following criteria:

- Age ≥ 18 years
- Undergoing elective robot assisted radical prostatectomy (RARP)
- Obtained informed consent

4.3 Exclusion criteria

A potential subject will be excluded from participation when meeting any of the following criteria:

- Laparoscopic radical prostatectomy without robot assistance
- Insufficient control of the Dutch language to read the patient information and to fill out de questionnaires
- (Chronic) use of immunosuppressive medication
- Chronic use of NSAID's and/or opiods or psychotropic drugs
- Use of NSAID's shorter than 5 days before surgery
- Severe liverdisease (Child-Pugh B and C) or chronic kidney disease (stage 4 and 5)
- Neuromuscular disease
- Hyperthyroidism or thyroid adenomas
- Deficiency of vitamin K dependent clotting factors or coagulopathy
- Planned diagnostics or treatment with radioactive iodine < 1 week after surgery
- Indication for rapid sequence induction
- BMI >35kg/m^2
- Known of suspected hypersensitivity to ICG, sodium iodide, iodine, rocuronium or sugammadex
- Use of medication interfering with ICG absorption as listed in the summary of product characteristics (SPC); anticonvulsants, bisulphite compounds, haloperidol, heroin, meperidine, metamizol, methadone, morphium, nitrofurantoin, opium alkaloids, phenobarbital, phenylbutazone, cyclopropane, probencid.

4.4 Sample size calculation

The main study includes 48 patients per study arm (low impact and standard laparoscopy). With this number of patients we will have sufficient power for the primary and secondary analyses:

1) Quality of recovery at POD 1 as reflected by the QoR-40 questionnaire. Although Myles et al. reported a minimal clinically relevant difference (MCID) of 6.3 for the QoR-40 questionnaire, consensus about the MCID is lacking. As 6.3 is a relatively small difference on a scale ranging from 40-200, we feel that a MCID of 10 points is more appropriate. A sample size of 48 patients per group is needed to provide 90% power to detect a 10 point difference in the QoR-40 at POD 1 (alpha 5%) with an estimated standard deviation of 15.
2) Innate immune function as reflected by the *ex vivo* release of IL-6 and IL-10 upon LPS stimulated leukocytes at POD 7. A sample size of 45 patients per group is needed to provide 90% power to detect a 3.000 pg/mL difference in IL-6 release (alpha 2.5%) with an estimated standard deviation of 4.000 pg/mL at POD 7. A sample size of 42 patients per group is needed to provide 90% power to detect a 50 pg/mL difference in IL-10 release (alpha 2.5%) with an estimated standard deviation of 65 pg/mL. As two cytokines are measured, a Bonferroni correction was used (alpha 2.5%).

5 TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

The head practitioner will ask the patient for permission to let the researcher contact and inform him. After informed consent is given the participants will be randomly assigned in a 1:1 fashion to:

- Experimental group: low impact laparoscopy (low pressure (8 mmHg) and deep NMB (PTC 1-2))
- Control group: standard laparoscopy (standard pressure (14 mmHg) and moderate NMB (TOF 1-2))

At the start of surgery, after intra-abdominal insufflation, ICG injection will take place with peritoneal perfusion, and a parietal peritoneal biopsy will be taken. At the end of surgery a second parietal peritoneum biopsy will be taken.

Perioperative ERAS protocol

A standardized perioperative ERAS protocol is used for this study. Adherence of ERAS key elements (27) as presented by the ERAS society will be scored.

Anesthesia protocol and titration of intra-abdominal pressure

Standardized anesthesia protocol:

- Premedication according to local protocol
- No epidural anesthesia: if conversion to open surgery is necessary, epidural anesthesia can be given postoperatively according to the preference of the attending anesthesiologist.
    - General anesthesia will be achieved with Total Intravenous Anesthesia (TIVA), consisting of propofol bolus 1-3 mg/kg and maintenance aimed at a bispectral index score between 45 and 55, remifentanil 0,25-2mcg/kg/min, lidocaine 1% 1-1,5mg/kg bolus followed by 0,5-3mg/kg/h and esketamine bolus 0,25-1mg/kg.
    - Dexamethason is avoided to prevent influencing the immune response
    - Neuromuscular monitoring is initiated, after the set-up and calibration procedures, with rocuronium (intubation dose 0,6mg/kg). Tracheal intubation is performed 2 minutes after administration in all groups.
    - Postoperative pain management will be paracetamol and oxycodone or patient controlled analgesia (PCA) with morphine.
- After introduction of the camera trocar, insufflation of carbon dioxide is titrated to an IAP of 8mmHg in the experimental group, and 14 mmHg in the control group. After placement of the last trocar the injection of ICG and video registration of peritoneum will take place, and a peritoneal biopsy will be taken. There after surgery will take place, for dissection of the venous plexus, IAP is titrated to 14 mmHg in the experimental group and 20mmHg in the controle group. A second parietal peritoneum biopsy will be taken at the end of surgery. If surgical conditions remain compromised despite adequate pressure and neuromuscular block, the surgeon can decide to convert to an open- or hand-assisted procedure.
- Neuromuscular monitoring is initiated after set-up and calibration procedures.
    - The control group with standard laparoscopy will be monitored with TOF count every 5 minutes in standardized fashion. After intubation, a bolus or continuous infusion of rocuronium is administered as normally done in clinical practice, titrating towards TOF count 1-2. When TOF count is 0, infusion of rocuronium will be stopped until neuromuscular function is recovered to maximal TOF 2.
    - The experimental group with low impact laparoscopy will be monitored with PTC every 5 minutes. After intubation, continuous infusion of rocuronium (0,3-0,4mg/kg) is started and titrated towards PTC 1-2. When PTC is 0, continuous infusion of rocuronium will be decreased, not stopped, until a PTC of 1-2 is reached.
- During surgery patients are ventilated by pressure-regulated volume-controlled ventilation through an endotracheal tube with a mixture of oxygen in air, PEEP of 5cmH_2O and tidal volumes between 6-8ml/kg. Minute ventilation is adjusted to main end-tidal carbon dioxide between 31-43mmHg by changing respiratory rate. Oxygenation is adjusted to main saturation between 97-100% by changing the mixture of oxygen in air.
- According to local protocol a nasogastric tube will be placed for gastric decompression.
- Minimal infusion of fluids and losses will be replaced.
- Core temperature is measured continuously, aiming at 36-37 C.
- The use of drains is avoided

- After skin closure, the NMB is reversed using sugammadex 4mg/kg in the experimental group, and 2mg/kg in the control group unless the TOF ratio is >0,9. If neuromuscular function has recovered spontaneously in the control group, no sugammadex will be administered.
- Extubation will be performed when TOF ratio is stable at >0,9 for more than 2 minutes and the patient is fully awake.

5.2 Use of co-intervention (if applicable)

Not applicable

5.3 Escape medication (if applicable)

Not applicable

6 NON-INVESTIGATIONAL PRODUCT

Not applicable

7 INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

a. Rocuronium 10 mg/ml solution for infusion. Rocuronium bromide is indicated as an adjunct to general anaesthesia to facilitate tracheal intubation during routine sequence induction and to provide skeletal muscle relaxation during surgery.
b. Sugammadex (Bridion®) 100 mg/mL solution for injection. Bridion reverses neuromuscular blockade induced by rocuronium or vecuronium in adults, by selective binding with the relaxant agent.

7.2 Summary of findings from non-clinical studies

a. Rocuronium: preclinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity, toxicity to reproduction and development. Carcinogenicity studies have not been performed with rocuronium bromide (28).
b. Sugammadex: preclinical data reveal no special hazard for humans based on conventional studies of safety pharmacology, repeated dose toxicity, genotoxicity potential, and toxicity to reproduction, local tolerance or compatibility with blood. Sugammadex is rapidly cleared in preclinical species, although residual sugammadex was observed in bone and teeth of juvenile rats. Preclinical studies in young adult and mature rats demonstrate that sugammadex does not adversely affect tooth colour or bone quality, bone structure, or bone metabolism. Sugammadex has no effects on fracture repair and remodelling of bone (29).

7.3 Summary of findings from clinical studies

a. Rocuronium: the standard intubation dose for geriatric patients and patients with hepatic and/or biliary tract disease and/or renal failure during routine anaesthesia is 0.6mg/kg rocuronium bromide. Regardless of the anaesthetic technique used, the recommended infusion rate is 0.3-0.4 mg/kg/h. For children and adolescents the recommended intubation dose during routine anaesthesia and maintenance dose are similar to those in adults. When used in overweight or obese patients (defined as patients with a body weight of 30% or more above ideal body weight) doses should be reduced taking into account ideal body weight. The following conditions may influence the pharmacokinetics and/or pharmacodynamics of Rocuronium: hepatic and/or biliary tract disease and renal failure, prolonged circulation time, neuromuscular

disease, hypothermia, obesity, burns, hypokalaemia, hypermagnesaemia, hypocalcaemia, hypoproteinaemia, dehydration, acidosis, hypercapnia and cachexia (28).

b. Sugammadex: the use of sugammadex in patients with severe renal impairment (including patients requiring dialysis (CrC1 < 30 mL/min)) is not recommended. Studies in patients with severe renal impairment do not provide sufficient safety information to support the use of sugammadex in these patients. For mild and moderate renal impairment (creatinine clearance ≥ 30 and < 80 mL/min): the dose recommendations are equal to adults without renal impairment. In obese patients, the dose of sugammadex should be based on actual body weight. For mild to moderate hepatic impairment no dose adjustments is required, as sugammadex is mainly excreted renally (29).

7.4 Summary of known and potential risks and benefits

a. Rocuronium: the most commonly occurring adverse drug reactions include injection site pain/reaction, changes in vital signs and prolonged neuromuscular block. The most frequently reported serious adverse drug reactions during post-marketing surveillance is 'anaphylactic and anaphylactoid reactions' and associated symptoms (28).

b. Sugammadex: sugammadex is administered concomitantly with neuromuscular blocking agents and anaesthetics in surgical patients. The causality of adverse events is therefore difficult to assess. The most commonly reported adverse reactions in surgical patients were cough, airway complication of anesthesia, anaesthetic complications, procedural hypotension and procedural complication (common ((≥ 1/100 to < 1/10).

System organ class	Frequencies	Adverse reactions (preferred terms)
Immune system disorders	Uncommon	Drug hypersensitivity reactions
Respiratory, thoracic and mediastinal disorders	Common	Cough
Injury, poisoning and procedural complications	Common	Airway complication of anaesthesia Anaesthetic complication Procedural hypotension Procedural complication

The following adverse reactions were reported in placebo controlled trials where subjects received anaesthesia and/or neuromuscular blocking agents (1,078 subject exposures to sugammadex versus 544 to placebo) (29):

[Very common (≥ 1/10), common (≥ 1/100 to <1/10), uncommon (≥ 1/1.000 to < 1/100), rare (≥ 1/10.000 to <1/1.000), very rare (<1/10.000)]

Fusidic acid and flucloxacillin can cause displacement of sugammadex and should not be administered 6 hours after administration of sugammadex. Sugammadex can also encapsulate hormonal contraceptives, which may leave them less effective (30). Therefore, sugammadex use should be considered as one missed dose of hormonal contraceptive (but this issue is not applicable in this study)

7.5 Description and justification of route of administration and dosage

a. Rocuronium: rocuronium bromide is administered intravenously (i.v.) either as a bolus injection or as a continuous infusion. The standard intubating dose during routine anaesthesia is 0.6 mg rocuronium bromide per kg body weight, which results in adequate intubation conditions within 60 seconds in nearly all patients. If rocuronium bromide is administered by continuous infusion, it is recommended to give a loading dose of 0.6 mg rocuronium bromide per kg body weight and, when the neuromuscular block starts to recover, to start administration by infusion. In adults under intravenous anaesthesia, the infusion rate required to maintain the neuromuscular block ranges from 0.3 - 0.6 mg/kg/h. Under inhalational anaesthesia the infusion rate ranges from 0.3 - 0.4 mg/kg/h (28).
b. Sugammadex: sugammadex should be administered intravenously as a single bolus injection. A dose of 4 mg/kg sugammadex is recommended if recovery has reached at least 1-2 post-tetanic counts (PTC) following rocuronium or vecuronium induced blockade. A dose of 2 mg/kg sugammadex is recommended, if spontaneous recovery has occurred up to at least the reappearance of T2 following rocuronium or vecuronium induced blockade (29).

7.6 Dosages, dosage modifications and method of administration

a. Rocuronium: in both groups, a bolus of 0.6 mg/kg rocuronium is administered intravenously before tracheal intubation. After tracheal intubation, in group A (low pressure with deep NMB), a bolus of 0.6 mg/kg rocuronium is administered and continuous infusion of rocuronium (0.4 mg/kg) is started and titrated towards PTC 1-2. In group B (normal pressure with moderate NMB), a bolus or continuous infusion of rocuronium is administered, titrating towards a TOF count of 1-2. Dosage in both groups is within normal therapeutical range used in clinical practice (28).
b. Sugammadex: if TOF-ratio is <0.9 the NMB is reversed with an intravenous bolus injection of 2 (moderate NMB) or 4 (deep NMB) mg/kg. If TOF-ratio > 0.9 the patient can be extubated safely (29).

7.7 Preparation and labelling of non-investigational medicinal product

Sugammadex will be provided by MSD. Both Rocuronium and Sugammadex will be prepared and labelled at the Radboudumc pharmacy. The clinical trials unit of the Radboudumc pharmacy will distribute batches of Rocuronium and Sugammadex to the pharmacy of the Canisius Wilhelmina hospital by courier.

7.8 Drug accountability

Administration of stock, preparation and labelling, distribution of batches and returns will be coordinated and logged by the clinical trials unit of the Radboudumc pharmacy. Locally, in the Canisius Wilhelmina hospital, drug registration will be logged on patient level (e.g. batch and ampoule number for each patient).

8 METHODS

8.1 Main study endpoints

- Quality of recovery score (QoR-40) at postoperative day 1.
- IL-6 response upon whole blood LPS stimulation at postoperative day 10-12
- Perfusion index of the parietal peritoneum as calculated from the slope of ICG fluorescence intensity, and time to maximal intensity in seconds. (extracted from video registration).

8.2 Other study parameters

General

- Baseline parameters as age, gender, length, weight, body mass index, comorbidity, indication for and type of surgery.
- Intraoperative parameters routinely measured (at administration of ICG): blood pressure, temperature and level of NMB (train of four response).

Questionnaires

- Quality of recovery score (QoR-40) at 1 day before surgery and day 10-12 after surgery.
- SF-36 general health questionnaire (Dutch version) 1 day before surgery, day 10-12 and 3 months after surgery.
- McGill pain Questionnair 3 months after surgery

Pain scores

- Pain at rest and movement (NRS 0-10) at 1, 8, 24, 72 hours after surgery
- Is pain acceptable (yes/no) at 1, 8, 24, 72 hours after surgery
- Referred shoulder pain (yes/no) at 1, 8, 24, 72 hours after surgery

PONV

- PONV (yes/no) at 1, 8, 24 and 72 hours after surgery

Medication use

- Cumulative opiate use
- Cumulative use of other analgesics and anti-emetics

Clinical parameters

- Time to reach discharge criteria*
- Length of hospital stay
- Surgical conditions: Leiden Surgical Rating Scale to quantify the quality of the surgical field during the pneumoperitoneum phase (after introduction of the Hasson trocar, after introduction of the third trocar and then every 15 minutes)
- Postoperative complications scored by Clavien Dindo classification (table 2 (1))

Immune function

- Peritoneal mesothelial hypoxia as reflected by peritoneal HIF1-alfa mRNA expression
- Histological peritoneal mesothelial cell injury.
- Plasma levels of DAMPs, cytokines and ex vivo immune function at baseline, 24 hours after surgery and 10-12 days after surgery.

*discharge criteria are: adequate pain control with oral medication, passage of flatus or defecation, intake of solid food tolerated, patient is mobilized and independent and patient accepts discharge. Discharge criteria will be evaluated daily. If the donor for social reasons wants to stay longer (e.g. long distance from partner of child who are still hospitalized) the 'virtual' discharge date is listed. A physician who is independent and blinded (ward physician) is responsible for the actual discharge date.

8.3 Study procedures

Randomization:

Computer-generated randomization (supported by our statistician) will be used with stratification for center. To ensure a balanced distribution, we will use block randomization.

Blinding and treatment allocation:

Blinding of the surgeons to the level of pressure and NMB is ensured by facing monitoring equipment away or covering these under sterile drapes. The level IAP will be set and adjusted by the anesthesiologist who is not blinded to the allocation of treatment. Surgical conditions will be assessed after introduction of the trocars, every 15 minutes or when the surgeon indicates inadequate surgical conditions. When SRS is ≤3 (table 1), pressure is increased with 2mmHg to max 14mmHg in group A and B or 16mmHg in group C and D. The anesthesiologist (not blinded) or his/her assistant titrates the dose of rocuronium to the desired level of NMB. The nerve stimulator and computer are placed behind the sterile drapes away from the surgeons. The research physician alongside the anesthetic staff will not be blinded in order to ensure depth of the NMB is continuously adequately monitored. To minimize the risk of observer bias and/or unblinding of the entire team, the following measures will be taken: Surgical adverse events will be registered directly after surgery by the blinded, primary surgeon. Surgeons, scrub nurses, postoperative care nurses, and ward physicians are blinded. Postoperative clinical outcomes (e.g. QoR-40 questionnaires) are collected by a blinded researcher.

Perioperative procedures:

The peri-operative flowchart is shown in figure 6.

During insufflations of the abdomen, the insufflator will be set at an intra-abdominal pressure of 8 or 14 mmHg. The usual dose of indocyanine green (0.2mg/kg) as regularly used will be injected intravenously. The camera trocar is aimed at the lateral abdominal wall and video is recorded for 3 minutes.

An overview of measurements and questionnaires for the primary and secondary endpoints, are given in table 3. Assessment of pain, PONV, discharge criteria and postoperative complications are part of normal medical treatment. Extra for this study, patients are asked to complete the QoR-40, SF-36 and McGill questionnaire. For the secondary endpoints on immune function, blood samples (a standard 6ml lithium/heparine,10 ml EDTA tube and 2,5ml

Paxgene RNA tube) will be combined with routine laboratory assessment as much as possible. Otherwise, blood will be drawn by vena puncture. Peritoneal biopsy will be performed during laparoscopy when the patient is under anesthesia and peritoneal tissue is directly visible and easily accessible. A biopsy will be taken as previously described and performed by Schaefer et al. (31) and Williams et al. (32) at the start and end of surgery. Briefly, a small part of the parietal peritoneum (0.5-1 x 0.5-1 cm, 2-4mm deep) tissue sample is excised. Half of the sample is fixed and used for histology the other half is used to determine HIF1α mRNA expression.

Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons or with one of the following criteria:

- Before operation (for example postponement or adjustment of operation)
- During surgery (for example conversion to open procedure)
- After surgery (for example non response to questionnaire)

In case of withdrawal the patient will not be followed. Patients withdrawing will be replaced, with a maximum of 10 patients.

Premature termination of the study

Not applicable

9 SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AE's, SAE's and SUSAR's

9.2.1 Adverse events (AE's)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product, trial procedure and/or the experimental intervention. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAE's)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- or any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events. The product summaries for rocuronium and sugammadex will be used to evaluate whether the adverse event or reaction is expected or unexpected. The following adverse events are considered to be inevitable in surgery and will not be recorded: blood loss <500 ml, surgical site infection and wound dehiscence. However, if any of these complications is considered an serious adverse event, they will be recorded.

9.2.3 Suspected Unexpected serious adverse reactions (SUSAR's)

Not applicable

9.3 Annual safety report

Not applicable

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist.
SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB)

The need for a DSMB is assessed taking the EMEA guidelines on data monitoring committees into consideration. We believe that an interim evaluation does not increase patient safety, because safety of the procedure has already been proven. Study conduct and progress will be monitored according a monitor plan. Thus, a DSMB is not beneficial considering the proposed study design and will therefore not be established.

10 STATISTICAL ANALYSIS

The principal investigator (PI) has final responsibility regarding the data; a web-based encrypted data management system will be used to minimize errors, to ensure traceability and privacy of the subjects. An independent statistician will provide assistance for data-analysis. The data will be unblended after completion of the follow-up period and identification of protocol violations.

10.1 Primary study parameter(s)

- Quality of recovery score (QoR-40) at postoperative day 1.
- For QOR-40 score, factorial ANCOVA will be used to compare groups and to adjust for co-variates *i.e.* age and gender. P-values <0.05 will be considered statistically significant.
- IL-6 response upon whole blood LPS stimulation at postoperative day 7.
- For mononuclear cell responsiveness, ANCOVA will be used to compare groups and to adjust for co-variates *i.e.* age and gender. As the primary endpoint includes changes in both IL-6 and IL-10 release, Bonferroni correction for multiple comparisons will be used. P-values < 0.025 will be considered statistically significant.
- Perfusion index in sigmoid as calculated from the slope of ICG fluorescence intensity, and time to maximal intensity in seconds. (extracted from video registration).
- Factorial ANCOVA will be used to compare groups and adjust for covariates i.e. age and gender. P-values <0.05 will be considered statistically significant.

10.2 Secondary study parameter(s)

Study parameters/endpoints	Presentation of data
QOR-40 (primary endpoint), SF-36, McGill pain questionnaire	Quantative
Pain scores	Quantative
PONV	Quantative
Medication use	Quantative
Length of hospital stay (days)	Quantative
Time to reach discharge criteria	Quantative
Surgical conditions	Quantative
Mesothelial hypoxia (HIF1-alfa mRNA expression)	Quantative
Histological peritoneal mesothelial cell injury and plasma levels of DAMPs and cytokines	Quantative
Ex vivo immune function (primary endpoint 2)	Quantative

10.3 Interim analyse

Not applicable

11 ETHICAL CONSIDERATIONS

11.1 Regulation statement

This study will be conducted according to the principles of the Declaration of Helsinki (59th version, Seoul, October 2008) and other Dutch guidelines, regulations and Acts. Subjects will be coded by a numeric code (a unique study number will be assigned) in order to create an anonymous dataset. Investigators have access to this code and will store the subject identification code list at a separate location from the dataset. Data will be securely stored in the database of the department of surgery of the Radboud university medical center accessible to the investigators, in accordance with the Dutch Personal Data Protection Act. Videos are recorded in the operating room on CD-ROM, video files are imported from the disc into the database of the department of surgery of the Radboudumc by the researcher and saved anonymously under the study number. Only the investigators have access to the subject identification code list saved separately from the data.

11.2 Recruitment and consent

At Canisius Wilhelmina Hospital, 350 Robot-assisted radical prostatectomy procedures are performed annually. All eligible patients will be screened. With an inclusion rate of approximately 2 patients per week, we expect that the screening (and inclusion period) will be 12-15 months. We intend to include 96 patients at the Canisius Wilhelmina Hospital. Informed consent is obtained at least one week after providing written information about the study. During this 7-day interval, an independent physician can be consulted for questions. Informed consent will be obtained before or on the day of hospital admission. Then, a unique study number will be assigned.

11.3 Benefits and risks assessment

As explained in the rationale, the use of deep NMB enables safe use of low-pressure PNP. If visibility is compromised at low pressure, pressure will be increased to ensure no additional risks related to the surgery. A deep NMB is achieved by higher doses of rocuronium that are within normal therapeutical range used in clinical practice, and can safely be used. Depth of NMB will be monitored throughout the whole surgery. At the end of surgery, the effects of rocuronium are antagonized by sugammadex to ensure no extended effects. Randomized controlled trails have shown sugammadex can be safely administered. Regarding the biopsies, peritoneal tissue is directly visible and easily accessible during laparoscopic colorectal surgery. Biopsies are obtained in a standardized manner previously used in other studies (33,34) who report no complications. After biopsy, hemostasis will be established under direct vision and therefore no additional complications are to be expected. Blood samples will be combined with routine laboratory assessment as much as possible. Previous studies have shown low-pressure PNP is associated with reduced postoperative pain scores, reduced opioid consumption and improved bowel function. This may lead to enhanced recovery. The burden for participants is mainly related to the evaluation of the endpoints during the early postoperative phase. Assessment of pain scores, nausea, complications and discharge criteria are part of the normal treatment. Questionnaires will take approximately 10-15 minutes per

time-point. Regarding the use of indocyanine green, the risks are negligible, as addressed above.

11.4 Compensation for injury

The Radboudumc has an insurance in accordance with the legal requirements in the Netherlands (Article 7 of the WMO and the Measure regarding Compulsory Insurance for Clinical Research in Humans of 23th June 2003). This insurance provides cover for damage to research subjects though injury or death caused by the study.

1. € 650.000,-- (i.e. sixhundred and fifty thousand Euro) for death or injury for each subject who participates in the Research
2. € 5.000.000,-- (i.e. fivemillion Euro) for death or injury for all subjects who participate in the Research
3. € 7.500.000,-- (i.e. sevenmillion fivehundredthousand Euro) for the total damage incurred by the organisation for all damage disclosed by scientific research for the Sponsor as 'verrichter' in the meaning of said Act in each year of insurance coverage

The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

12 ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

Subjects will be coded by a numeric code in order to create and anonymous dataset. A Castor database will be developed and used for data management. Investigators have access to this code and will store the subject identification code list at a separate location from the dataset. Data will be securely stored in the database of the department of surgery of the Radboud university medical center accessible to the investigators, in accordance with the Dutch Personal Data Protection Act.

12.2 Monitoring and quality assurance

In our opinion, this study adds a low chance of mild damage and consequently adds a negligible risk according to the risk classification of the "Nederlandse Federatie van Universitair Medische Centra" (NFU). Monitoring will be conducted in accordance with negligible risk monitoring guidelines of the NFU, which will be reported in a monitorplan.

	Mild damage	Intermediate damage	Sever damage
Low chance	Negligible risk	Negligible risk	Intermediate risk
Intermediate chance	Negligible risk	Intermediate risk	High risk
High chance	Intermediate risk	High risk	High risk

Risk classification in relation to the chance and severity of damage (34)

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be notified to the METC that gave a favourable opinion. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last patient's last visit.
The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination. Within one year after the end of

the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

The study will be registered at ClinicalTrials.gov. Results will be published in a peer reviewed international journal.

13 STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

Not applicable

13.2 Synthesis

As explained in the rationale, the use of deep NMB enables safe use of low-pressure PNP. If visibility is compromised at low pressure, pressure will be increased to ensure no additional risks related to the surgery. A deep NMB is achieved by higher doses of rocuronium that are within normal therapeutical range used in clinical practice, and can safely be used. Depth of NMB will be monitored throughout the whole surgery. At the end of surgery, the effects of rocuronium are antagonized by sugammadex to ensure no extended effects. Randomized controlled trials have shown sugammadex can be safely administered. Regarding the biopsies, peritoneal tissue is directly visible and easily accessible during laparoscopic surgery in the pelvis. After biopsy, hemostasis will be established under direct vision and therefore no additional complications are to be expected. Previous studies have shown low-pressure PNP is associated with reduced postoperative pain scores, reduced opioid consumption and improved bowel function. The burden for participants is mainly related to the evaluation of the endpoints during the early postoperative phase. Assessment of the pain scores and the QOR-40 questionnaire will take approximately 10-15 minutes per time-point. Blood samples will be combined with routine laboratory assessment as much as possible, this is standard before- and 24 hours after surgery. Blood samples taken during surgery (while under anesthesia) and 10-12 days after surgery will be drawn by venapuncture if necessary.

Figure 6: Perioperative flowchart

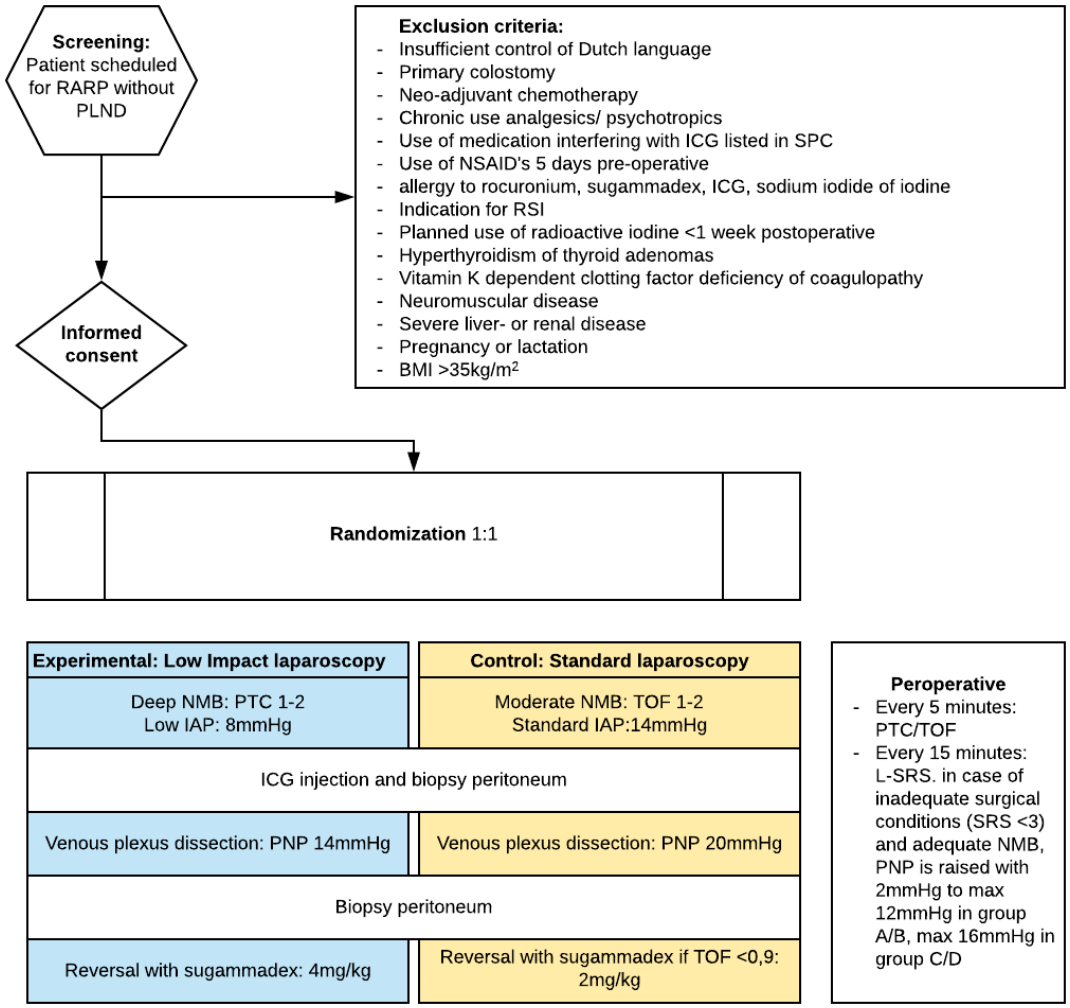


Table 1: Leiden Surgical-Rating-Scale (L-SRS) (25)

Scale	***Description***	
1	Extremely poor conditions	The surgeon is unable to work because of coughing or the inability to obtain a visible laparoscopic field because of inadequate muscle relaxation.
2	Poor conditions	There is a visible laparoscopic field, but the surgeon is severely hampered by inadequate muscle relaxation with continuous muscle contractions, movements or both with the hazard of tissue damage.
3	Acceptable conditions	There is a wide visible laparoscopic field but muscle contractions, movements or both occur regularly causing some interference with the surgeon's work.
4	Good conditions	There is a wide laparoscopic field with sporadic muscle contractions, movements or both.
5	Optimal conditions	There is a wide visible laparoscopic working field without any movement or contractions.

Table 2: Clavien Dindo classification(1)

	Definitions	Modes of therapy
I	Any deviation from the normal postoperative course	No pharmacological of surgical treatment, endoscopic of radiological interventions were required. Acceptable therapeutic regimens are drugs such as anti-emetics, antipyretics, analgesics, diuretics and electrolytes and physiotherapy. Wound infections of small abscess requiring incision at bedside is within this category.
II	Normal course altered	Pharmacological management other than in grad 1. Blood transfusions and total parenteral nutrition are also included.
III	Complications that require intervention of various degrees	Sub-classified into: - Grade IIIa – Complications that require an intervention performed under local anesthesia - Grade IIIb – Interventions that require general or epidural anesthesia
IV	Complications threatening life of patients (incl. CNS complication), requiring ICU support	Sub-classified into: - Grade IVa - Single organ dysfunction (incl. dialysis) - Grade IVb – Multi-organ dysfunction
V	Death of a patient	

Table 3: Overview of study endpoints

	QOR-40 (primary endpoint in main study)	Anti-coagulated blood (*ex vivo* leukocyte responses, DAMP and cytokines)	Indocyanine green fluorescence of parietal peritoneum	Peritoneal tissue biopsy (histology and HIF1alpha mRNA)	Pain scores, PONV, Analgesia use	Complications, Discharge criteria, Length of hospital days	SF-36 QoL	McGill Pain Questionnair
-1 day	X	X					X	
After trocar introduction			X	X				
At end of IAP				X				
1hr					X			
8 hr					X			
24 hr	X	X			X	X		
48 hr					X	X		
10-12 days	X	X					X	
3 months						X	X	X

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