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Inflammatory Response After Laparoscopic Versus Open Resection of Colorectal Liver Metastases
Data From the Oslo-CoMet Trial

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Abstract: Laparoscopic and open liver resection have not been compared in randomized trials. The aim of the current study was to compare the inflammatory response after laparoscopic and open resection of colorectal liver metastases (CLM) in a randomized controlled trial.

This was a predefined exploratory substudy within the Oslo CoMet-study. Forty-five patients with CLM were randomized to laparoscopic (n = 23) or open (n = 22) resection. Ethylenediaminetetraacetic acid-plasma samples were collected preoperatively and at defined time points during and after surgery and snap frozen at $-80\,^\circ C$. A total of 25 markers were examined using luminex and enzyme-linked immunosorbent assay techniques: high-mobility box group 1(HMGB-1), cell-free DNA (cfDNA), cytokines, and terminal C5b-9 complement complex complement activation.

Eight inflammatory markers increased significantly from baseline: HMGB-1, cfDNA, interleukin (IL)-6, C-reactive protein, macrophage inflammatory protein -1β, monocyte chemotactic protein -1, IL-10, and terminal C5b-9 complement complex. Peak levels were reached at the end of or shortly after surgery. Five markers, HMGB-1, cfDNA, IL-6, C-reactive protein, and macrophage inflammatory protein -1β, showed significantly higher levels in the open surgery group compared with the laparoscopic surgery group.

Laparoscopic resection of CLM reduced the inflammatory response compared with open resection. The lower level of HMGB-1 is interesting because of the known association with oncogenesis.

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Abbreviation: ASA = American Society of Anesthesiology classification, AUC = area under the curve, bFGF = basic fibroblast growth factor, cfDNA = cell-free DNA, CLM = colorectal liver metastases, CONSORT = Consolidated Standards of Reporting Trials, CRP = C-reactive protein, DAMP = damage-associated molecular patterns, EDTA = ethylenediaminetetraacetic acid, ELISA = enzyme-linked immunosorbent assay, ERAS = enhanced recovery after surgery, G-CSF = granulocyte colony-stimulating factor, GM-CMF = granulocyte macrophage colony-stimulating factor, HMGB-1 = high-motility group box 1, IFN = interferon, IL1-ra = interleukin-1 receptor antagonist, IP-10 = interferon-inducible protein, MCP-1 = monocyte chemotactic protein, MIP = macrophage inflammatory protein, PDGF-BB = platelet-derived growth factor-BB, RANTES = regulated upon activation T cell expressed and secreted, RCT = randomized controlled trial, TCC = terminal C5b-9 complement complex, TNF = tumor necrosis factor, tregs = regulatory T cells, VEGF = vascular endothelial growth factor, Y_{max} = maximum value across all time points.

INTRODUCTION

Laparoscopic liver surgery is replacing the open technique in many centers worldwide. Comparative studies, however, are limited because of their retrospective and nonrandomized nature. To date, no randomized controlled trial (RCT) has been conducted. The 2nd International Consensus Conference on Laparoscopic Liver Resection concluded that higher quality studies are needed to determine the role of laparoscopic liver surgery in relation to open surgery.[1]

Any surgical procedure is a controlled tissue injury. Traumatic tissue injury leads to release of intracellular substances, which act as endogenous triggers of the immune system, often referred to as alarmins or damage-associated molecular patterns (DAMP).[2,3] Alarmins such as high-mobility group box 1 (HMGB-1) and circulating cell-free DNA (cfDNA) are associated to tissue trauma-induced inflammatory responses.[4] Recent studies also strongly, however, associate HMGB-1 and cfDNA with cancer development and progression.[5–7]

A reduced inflammatory response has been considered an advantage of laparoscopy compared with open surgery. A reduced inflammatory response has been found following minimally invasive colorectal surgery,[8] cholecystectomy,[9]

appendectomy,[10] perforated ulcer repair,[11] and lung resection,[12] when compared with open procedures. Decreased interleukin 6 (IL-6) after laparoscopic surgery was the most consistent finding in these studies. A reduced inflammatory response has also been associated with better-preserved immune competence in the postoperative period, which may influence both surgical complications and tumor metastasis formation.[8,12] Results from previous studies have indicated a survival benefit of the laparoscopic method in patients with colorectal liver metastases (CLM).[13] A reduced inflammatory response has been discussed as one possible explanation for this.

Animal trials have shown decreased levels of IL-6 after laparoscopic liver resection,[14] but the inflammatory response following laparoscopic liver resection has not been described in humans. The aim of the current study was thus to compare the inflammatory response following laparoscopic and open liver resection for CLM.

MATERIALS AND METHODS

Study Overview

This study was a predefined exploratory substudy of the randomized Oslo CoMet-study.[15] The end point was inflammatory response after open compared with laparoscopic liver resection, assessed pre-, per- and postoperatively. The first 45 patients recruited to the Oslo CoMet-study were included. Inclusion and operations took place between February and October 2012 Figure 2. Because of the cost of obtaining and analyzing the samples, it was not possible to perform this study on the entire Oslo CoMet-study population. No power analysis was performed as this study was piggy backed onto the main trial. The decision of including 45 patients was thus based on previous experience and economical limitations. The study was approved by the Regional Committee for Health and Research Ethics (2011/1285/REK Sør-Øst B), by the Data Protection Official for Research at Oslo University Hospital, and the study was registered in Clinicaltrials.gov (NCT01516710, January 19, 2012). The Consolidated Standards of Reporting Trials checklist was used.

The Oslo CoMet-study includes all resections of less than 3 consecutive liver segments for colorectal metastases except resections where reconstruction of vessels or bile ducts is necessary and resections that need to be combined with ablation. All surgery was performed at Oslo University Hospital, Rikshospitalet, Oslo, Norway by 8 consultant hepato-pancreato-biliary surgeons.

Patient Management

The patients underwent computer-generated randomization to open (n = 22) or laparoscopic (n = 23) resection of CLM after giving informed, written consent. Patients were informed about the study and the surgical procedure at the outpatient clinic, and about the operation method on the day before surgery. One patient in each group was operated but did not undergo liver resection. The patient in the open group was deemed inoperable after laparotomy, when small metastases were found in all liver segments. The patient in the laparoscopic group had a metastasis that vanished completely after chemotherapy, and the tumor could not be found, even after application of a hand port for contrast-enhanced ultrasound examination of the liver. In addition, one patient randomized to laparoscopy was excluded from the study after randomization, as his tumor was re-evaluated to be benign on the day of surgery. He did not undergo surgery and is still cancer free.

Patients followed an enhanced recovery after surgery (ERAS) protocol when possible. In cases where patients could not follow an enhanced recovery after surgery protocol, as in the case of prolonged intensive care treatment, the protocol was initiated as soon as possible. Surgical technique was at the discretion of the operating surgeon. For open surgery, an L-shaped, subcostal or midline incision was used. For laparoscopy, three 12 mm trocars were used as a standard, with the addition of trocars or hand port when necessary. For both open and laparoscopic resections, liver parenchyma was transected with electrosurgical instruments, mainly LigaSure® (Covidien, Mansfield, MA), Thunderbeat® (Olympus, Tokyo, Japan), or Caiman® (B.Braun, Melsungen, Germany) sometimes assisted by ultrasonic aspirators, mainly SonoSurg aspirator® (Olympus, Tokyo, Japan) and Söring aspirator® (Söring, Quickborn, Germany). Endoscopic staplers, Endo-GIA® (Covidien, Mansfield, MA) and Endopath® (Ethicon, Bridgewater, NJ) were used for dividing large vessels and sometimes for parenchyma division. The safe surgery checklist was used.[16]

Collection of Samples

Whole blood samples were drawn from a central venous line after general anesthesia was established (T1), before liver transection (T2), 10 minutes into liver transection (T3), at end of surgery (T4), 2 (T5), 6 (T6), and 24 hours after surgery (T7). In total, 260 of the planned 308 samples (84.4%) were eligible for statistical analysis. Five samples were not drawn according to protocol. Two samples from 1 patient in the laparoscopy group were not drawn because of personal error. The patient who underwent laparotomy but was inoperable had no sample drawn at T3. The patient who underwent laparoscopy but had a vanished lesion had no samples drawn at T2 and T3. The remaining missing data were sample exclusions as reported by the analysis instruments.

Blood samples were drawn into vacutainer tubes containing Ethylenediaminetetraacetic acid (EDTA) and immediately put on ice. Samples were then centrifuged at 4 °C, $1400 \times g$ for 15 minutes, and EDTA-plasma was aliquoted in triplicate to 1 mL Nunc® CryoTubes® (ThermoFischer Scientific, Waltham, MA), and immediately frozen at −80 °C. Laboratory analyses were performed in one batch at the Department of Immunology at Oslo University Hospital, Rikshospitalet during March and April 2014.

Markers of Perioperative Immune Activation

High-mobility box group 1 was analyzed by standard enzyme-linked immunosorbent assay (ELISA) technique according to instructions from the manufacturer (HMGB-1 ELISA Kit II, Shino-Test Corporation, Kanagawa, Japan).

Cell-free DNA was analyzed in EDTA-plasma samples using the PicoGreen-based cfDNA-Quant kit from Trillium diagnostics (Brewer, ME). All samples were diluted 1:5 in PBS and analyzed according to the manufacturer's instructions. The fluorescent signal was measured with a Victor3TM 1420 multilabel counter (Perkin Elmer, Boston, MA) using wavelengths for excitation and emission at 485 nm and 530 nm, respectively. Obtained values were related to a standard of human placental DNA and results were given as ng/mL.

Cytokines, chemokines, and growth factors were analyzed in plasma samples using a multiplex cytokine assay (Bio-Plex Human Cytokine 27-Plex Panel; Bio-Rad Laboratories Inc., Hercules, CA) containing the following analytes: IL-1β, IL-1 receptor antagonist (IL1-ra), IL-2, IL-4, IL-5, IL-6, IL-7, IL-8,

IL-9, IL-10, IL-12 (p70), IL-13, IL-15, IL-17, eotaxin, basic fibroblast growth factor, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, interferon-γ, interferon-inducible protein 10, monocyte chemotactic protein, macrophage inflammatory protein (MIP)-1α, MIP-1β, platelet-derived growth factor-BB (PDGF-BB), regulated upon activation T cell expressed and secreted (RANTES), tumor necrosis factor-α, and vascular endothelial growth factor. The samples were analyzed on a Multiplex Analyser (Bio-Rad Laboratories) according to instructions from the manufacturer. Macrophage inflammatory protein 1-α, IL-1β, IL-12, and IL-15 could not be detected in levels above 20 pg/L, and were therefore excluded from further analysis. Platelet-derived growth factor and regulated upon activation T cell expressed and secreted were excluded for methodological reasons, as platelets spontaneously release these after sampling unless special platelet preservation precautions are taken.

The terminal C5b-9 complement complex (TCC) concentration was measured by an ELISA based on the mouse antihuman TCC antibody (clone aE11) reacting with a neoepitope exposed in C9 when incorporated into C5b-9. The assay has been described in detail previously.[17]

Total protein was measured with a colorimetric assay, and C-reactive protein (CRP) with a particle-enhanced turbidimetric immunoassay on the Modular p800® (Roche, Basel, Switzerland) at the Department of Medical Biochemistry, Oslo University Hospital.

To compensate for hemodilution during surgery, total protein concentration was measured in all samples, and every analysis result was corrected against baseline protein concentration.

Statistical Analysis

All 44 patients who underwent surgery, and thus had blood samples drawn, were included in the statistical analysis.

The statistical analyses were performed with SPSS® for Mac v. 22 (IBM, Armonk, NY). For all 25 analytes, 2 measures were calculated: the area under the curve (AUC), and the maximum value across all time points (Y_{max}). The differences between the means for the open and laparoscopic groups were estimated with linear regression. All regression models included an adjustment for the baseline value.

When analyzing repeated measurements of biologic substances, more than one statistical measure can be appropriate. The "Y_{max}" analysis provides information on the peak value of each analyzed substance, whereas the AUC provides information on the total production of the substance during the study period. In our experience, AUC and Y_{max} supplement each other and should therefore both be reported.

In this exploratory study we performed 25 tests on the same set of samples. We discussed whether multiple testing corrections should be applied, for instance the Bonferroni test. The analyses we performed, however, are biologically correlated, as they are measures of inflammation. When multiple outcomes that represent the same underlying mechanism are examined, a Bonferroni correction would unacceptably increase the risk of Type 2 error, thus failing to detect an effect that is present.[18] Therefore, no multiple testing correction has been applied on these results.

RESULTS

Patient Characteristics

Patient characteristics such as age, sex, body mass index, American Society of Anesthesiology classification, Basingstoke Predictive Index,[19] and tumor size were similar in the laparoscopic and open surgery group (Table 1).

Inflammatory Response in the Laparoscopic Versus the Open Group

Of the 25 inflammatory markers examined, 8 showed a significant increase from baseline to maximum levels: HMGB-1, cfDNA, IL-6, CRP, MIP-1β, MCP-1, IL-10, and TCC (Tables 2 and 3). Five of these, HMGB-1, cfDNA, IL-6, CRP, and MIP-1β, showed significantly ($P < 0.05$) lower levels in the laparoscopic surgery group compared with the open surgery group either by maximum level (Table 2), AUC (Table 3) or both (Fig. 1, where 4 markers are shown). Three markers did not show a significant difference between the groups (MCP-1, IL-10, and TCC). For the other 17 markers the surgery did not induce any significant increase from baseline to maximum, indicating no impact of surgery.

DISCUSSION

In the current study, we found significantly lower levels of several inflammatory markers in patients randomized to laparoscopic resection of CLM compared with patients randomized to open resection. This is the first RCT to study the impact of laparoscopic liver resection on the immune system in humans, and thus the first attempt to find immunologic differences between the techniques that could explain a possible survival benefit of laparoscopy. Randomization and a consistent use of an enhanced recovery pathway minimized bias in this study.

The low number of patients is a limitation to this study; however, statistically significant differences could be seen despite this. Another possible limitation is that 2 different methods were used for comparing the groups. In our experience, both methods, however, should be reported to completely describe the inflammatory response.

Levels of HMGB-1 were lower in the laparoscopic group than the open group. High-mobility group box 1 initiates inflammation; therefore, this finding strengthens the existing evidence that laparoscopic surgery diminishes the inflammatory response. High-mobility group box 1 also, however, plays a role in cancer development.[20–23] Recently, HMGB-1 was found to stimulate tumor cell proliferation through an alteration of tumor metabolism. In the same study, inhibition of HMGB-1 or its receptors reduced tumor growth.[24] Thus, in this trial

TABLE 1. Preoperative Patient Characteristics

	Laparoscopic (n = 22)	Open (n = 22)
ASA-classification, mean (SD)	2.2 (0.7)	2.0 (0.6)
BPI, preoperatively, mean (SD)	6.0 (3.5)	5.9 (3.0)
Age, mean (SD)	66.6 (10.2)	64.4 (11.6)
Body mass index, mean (SD)	26.4 (4.7)	24.9 (3.5)
Tumor diameter, mean (SD)	34.2 (20.4)	41.7 (33.6)

ASA = American Society of Anesthesiology, BPI = Basingstoke Predictive Index, SD = standard deviation.

TABLE 2. Difference in Time Independent Maximum Levels (Y_{max}) From Open Group to Laparoscopic Group

	Difference in Time-Independent Maximum Level (from OLR to LLR)	95% CI	*P* Value
HMGB-1 (ng/mL)	−4.6	(−8.4, −0.8)	0.018
cfDNA (ng/mL)	−291.4	(−585.9, 3.0)	0.052
IL-6 (pg/mL)	−43.9	(−87.4, −0.5)	0.047
CRP (mg/L)	−49.4	(−94.3, −4.4)	0.032
MIP-1β (pg/mL)	−57.8	(−115.6, 0.02)	0.050
MCP-1 (pg/mL)	−19.8	(−44, 5)	0.118
TCC (AU/mL)	−0.3	(−1.6, 1.1)	0.710
IL-10 (pg/mL)	−13.8	(−46, 18)	0.390

AU = arbitrary units, cf-DNA = cell-free DNA, CI = confidence interval, CRP = C-reactive protein, HMGB-1 = high-mobility group box 1, IL-10 = interleukin 10, IL-6 = interleukin 6, LLR = laparoscopic laparoscopic liver resection group, MCP-1 = monocyte chemotactic protein, MIP-1β = macrophage inflammatory protein 1β, OLR = open open liver resection group, TCC = terminal complement complex, Y_{max} = time-independent maximum level.

TABLE 3. Difference Between Open Liver Resection Group and Laparoscopic Liver Resection, Measured by Area Under the Curve

	Difference in AUC From OLR to LLR	95% CI	*P* Value
HMGB-1 (ng*min/mL)	−1756	(−3584, 73)	0.059
cfDNA (ng*min/mL)	−270160	(−499140, −41180)	0.022
IL-6 (pg*min/mL)	−38215	(−74767, −1663)	0.041
CRP (mg*min/L)	–	–	–
MIP-1β (pg*min/mL)	−27041	(−51905, −2178)	0.034
MCP-1 (pg*min/mL)	−14635	(−30768, 1499)	0.074
TCC (AU*min/mL)	164	(−704, 1034)	0.70
IL-10 (pg*min/mL)	7101	(−13893, 28096)	0.68

AU = arbitrary units, AUC = area under the curve, cf-DNA = cell-free DNA, CI = confidence interval, CRP = C-reactive protein, HMGB-1 = high-mobility group box 1, IL-10 = interleukin 10, IL-6 = interleukin 6, LLR = laparoscopic liver resection group, MCP-1 = monocyte chemotactic protein, MIP-1β = macrophage inflammatory protein 1β, OLR = open liver resection group, TCC = terminal complement complex.
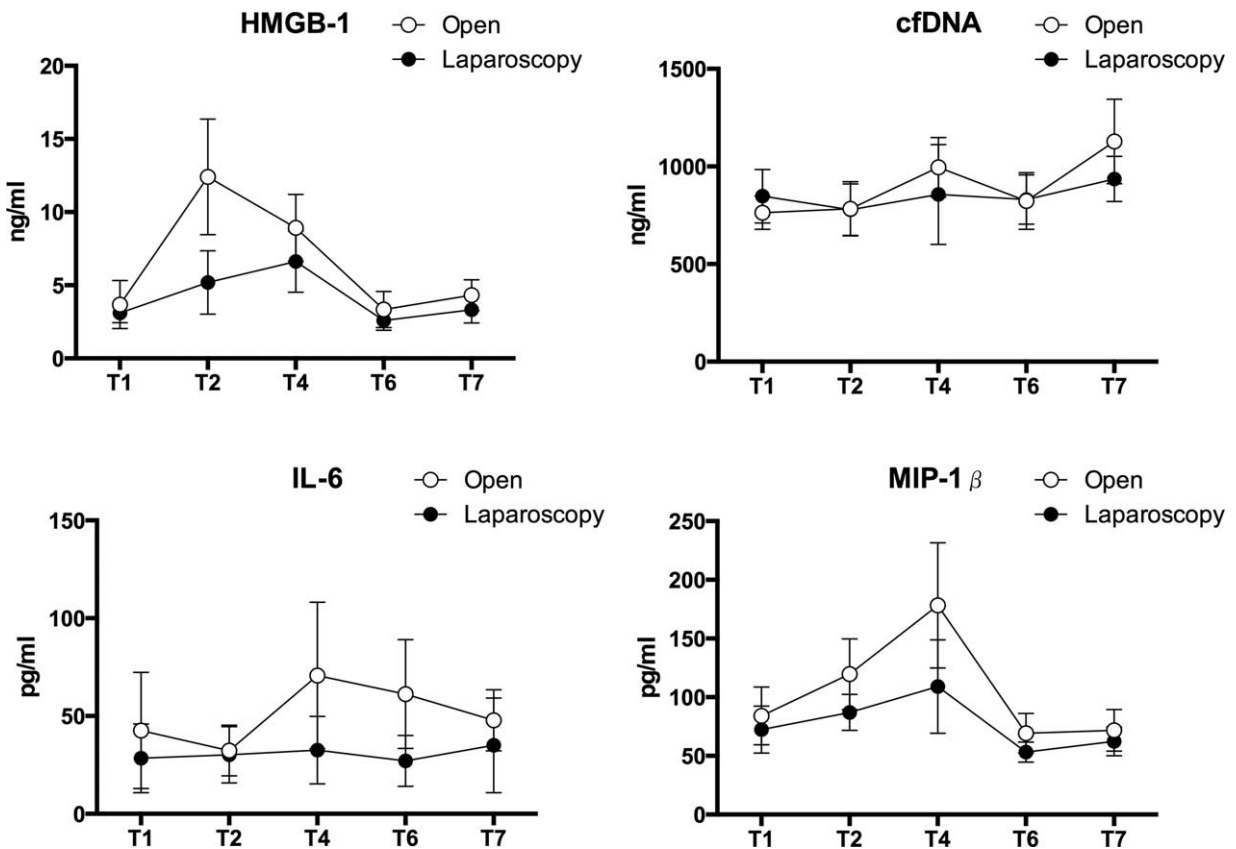*Represents multiplication.

FIGURE 1. Levels of high-mobility box group 1, cell free DNA , interleukin 6, and macrophage inflammatory protein 1β at T1, T2, T4, T6, and T7 (n = 22) in each group, graph shows mean values with 95% confidence interval.
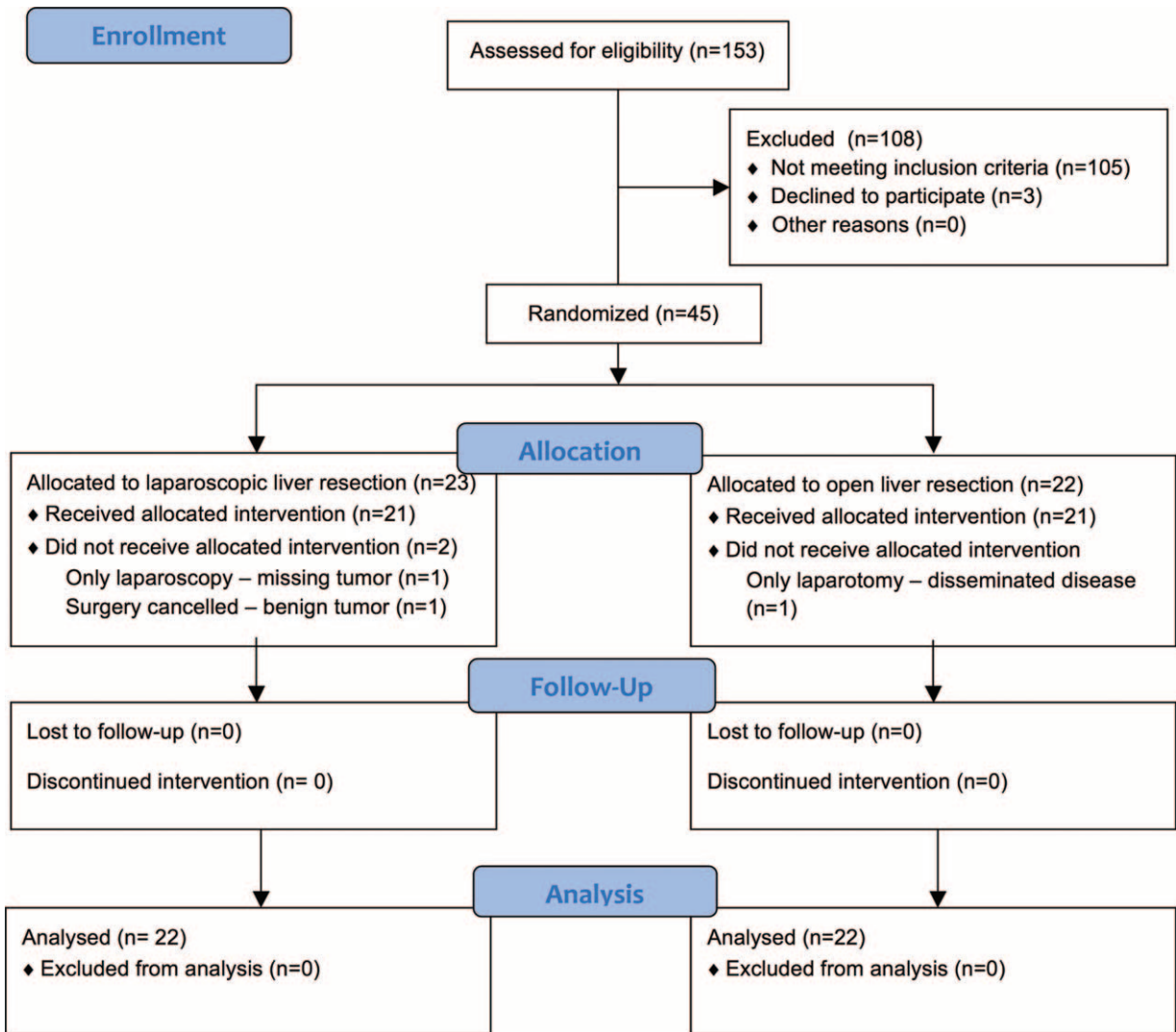
FIGURE 2. Consolidated Standards of Reporting Trials flow diagram.

laparoscopy seemed to reduce not both inflammation, and levels of a potentially oncogenic protein.

Levels of cfDNA turned out to be significantly lower in the laparoscopic group. Cell-free DNAs are fragments of extracellular DNA detectable in plasma, and are shown to initiate an inflammatory response together with HMGB-1 and other damage-associated molecular patterns. Cell-free DNA is a marker of tissue damage, but concentrations also increase in cancer patients. Therefore, cfDNA has been proposed as a biomarker for the diagnosis, monitoring, and prognosis in cancer.[7]

Levels of IL-6 were lower in the laparoscopic group compared with open. This is consistent with previous comparisons of open and laparoscopic surgery. Interleukin-6, an acute phase mediator involved in B-cell stimulation, antibody production, and further release of acute phase proteins including CRP, is considered an indicator of the extent of surgical trauma, and might serve as a predictor for complications. Two previous studies comparing open and minimally invasive surgery found a correlation between increased IL-6 postoperatively and a reduced cellular immunity in the following days.[8,12]

Levels of CRP and MIP-1β were also lower in the laparoscopic group compared with the open group. C-reactive protein enhances the inflammatory response by complement activation and other effects. C-reactive protein is widely used as a marker of inflammatory response, and is also used as a prognostic marker in hepatocellular carcinoma[25] and colorectal cancer.[26] Macrophage inflammatory protein-1β attracts monocytes, natural killer cells, and regulatory T cells (Tregs) to the site of inflammation. Inhibiting the MIP-1β-dependent attraction of Tregs to a tumor site is associated with delayed tumor growth in mice,[27] and has been suggested as a target for treatment of systemic colorectal cancer in humans.[28]

Terminal C5b-9 complement complex was just moderately and similarly increased in both open and laparoscopic groups. Terminal C5b-9 complement complex reflects complement activation as the final downstream activation product. Previous studies from our group have shown significantly increased TCC levels after cardiopulmonary bypass, but not after thoracotomy or open abdominal aortic aneurysm repair.[29] This is consistent with the current findings. Thus, none of the techniques seem to

activate the complement system to a degree that is measurable in the systemic circulation.

CONCLUSIONS

In this randomized study we found a reduced inflammatory response after laparoscopic liver resection compared with open, represented by decreased levels of HMGB-1, cfDNA, IL-6, CRP, and MIP-1β. In a previous study, we found that patients undergoing laparoscopic liver resection have a better survival than predicted.[13] Our current results indicate 2 possible explanations to this: first, a reduced inflammatory response followed by improved postoperative immune competence; second, a reduced release of potentially oncogenic proteins as HMGB-1. Randomized controlled trials with long-term follow-up are needed to verify this.

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