Minimally invasive esophagectomy for cancer; beyond techniques

Maas, K.W.

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PART III

Impact of minimally invasive esophagectomy

Chapter 8

Immunological changes after minimally invasive or conventional esophageal resection for cancer: a randomized trial

Maas KW
Biere SSAY
van Hoogstraten IMW
van der Peet DL
Cuesta MA

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ABSTRACT

Background

This study was performed as a substudy analysis of a randomized trial comparing conventional open esophagectomy (OE) by thoracotomy and laparotomy with minimally invasive esophagectomy (MIE) by thoracoscopy and laparoscopy. This additional analysis focuses on the immunological changes and surgical stress response in these two randomized groups of a single center.

Methods

Patients with a resectable esophageal cancer were randomized to OE (n=13) or MIE (n=14). All patients received neoadjuvant chemoradiotherapy. The immunological response was measured by means of leukocyte counts, HLA-DR expression on monocytes; the acute-phase response by means of C-reactive protein (CRP), interleukin-6 (IL-6) and interleukin-8 (IL-8), and stress response by cortisol, growth hormone and prolactin. All parameters were determined at baseline (pre-operatively) and 24, 72, 96 and 168 hours post-operatively.

Results

Significant differences between the two groups were seen in favor of the MIE group with regard to leukocyte counts, IL-8 and prolactin at 168 hours (1 week) post-operatively. For HLA-DR expression, IL-6 and CRP-levels there were no significant differences between the two groups, although there was a clear rise in levels upon operation in both groups.

Conclusion

In this substudy of a randomized trial comparing the minimally invasive and conventional esophagectomy for cancer, a significantly better preserved leukocyte counts and IL-8 levels were observed as compared to the open group. Both findings can be related to fewer respiratory infections found post-operatively in the MIE group. Moreover, significant differences in the prolactin levels at 168 hours post operation imply that the stress response is better preserved in the MIE group. These findings indicate that less surgical trauma could lead to better preserved acute-phase and stress responses and fewer clinical manifestations of respiratory infections.

INTRODUCTION

Previous studies have shown that trauma, surgery and anesthesia induce a state of immuno-suppression.[1,2] A downward regulation of the immune response and host defense against tumor cells may occur in the peri-operative period. As a consequence, patients are more susceptible to infections and sepsis.[1] The immunological response to surgery has been increasingly studied since the introduction of minimally invasive techniques. Laparoscopic surgery reduces the magnitude of operative trauma. If alterations of the systemic immune response are proportional to the extent of injury, then the response to minimally invasive techniques will be reduced when compared with open surgery. A key factor in post-operative morbidity is the surgical stress response with ensuing increased demand on the patient's reserves and the immunological competence. Increased demands on organ functions are thought to be mediated by trauma-induced endocrine and metabolic changes. The stress response has been studied by monitoring levels of hormones such as cortisol, prolactin and growth hormones, whereas the acute phase response has been measured by means of various cytokine and acute-phase response protein levels. Of these, interleukin-6 (IL-6), interleukin-8 (IL-8) and C-reactive protein (CRP) are among the most frequently studied after surgical trauma.[3-9] Each of the two cytokines represent a different component of the inflammatory response: IL-6 is a mediator of the acute phase, inducing synthesis of proteins by the liver, whereas IL-8 has chemoattractant activity and is able to activate and degranulate neutrophils.[10]

Immune status, inflammatory response and stress response have been analyzed in different studies comparing conventional open surgery to minimally invasive surgery favoring the minimally invasive approach.[3-5]

Currently, for patients with advanced esophageal cancer the best curative treatment is surgical resection. This extensive surgical procedure involves a thoracotomy and laparotomy with gastric tube reconstruction and an esophagogastric anastomosis. A high morbidity rate, mostly due to respiratory complications, and a long intensive care unit and hospital stay are consequences of this procedure

In recent years, many surgical techniques have been developed in order to reduce morbidity rates after esophageal resection. Minimally invasive approaches are likely to be favorable over conventional open procedures, as observed in previous studies.[11] This study was performed as a substudy of a randomized trial (TIME trial: traditionally invasive versus minimally invasive esophagectomy) which compares conventional open esophagectomy by thoracotomy and laparotomy (OE) with minimally invasive esophagectomy (MIE) by thoracoscopy and laparoscopy.[12] Different parameters - stress response hormone levels, acute-phase response protein levels and immunological status - were studied during different postoperative stages in both groups.

METHODS

Study design

Data were collected of patients who had been included in a randomized, multicenter trial comparing conventional esophagectomy with minimally invasive esophagectomy (TIME-trial, NTR TC 2452). Details of the trial design have been published elsewhere.[12,13] From 2009 to 2010, 27 consecutive patients who participated in the TIME-trial in the VU University Medical Center were included into this substudy.

Patient selection

Patients were eligible for this trial if they had a histologically proven squamous cell carcinoma, adenocarcinoma or undifferentiated carcinoma of the intrathoracic esophagus and gastro-esophageal junction tumors which were considered surgically resectable (T1-3, N0-1, M0). Moreover, the eligible patients must have had a Eastern Cooperative Oncology Group (ECOG) performance status of 0, 1 or 2. All patients in this trial received neoadjuvant chemoradiotherapy, as defined in the CROSS protocol.[14]

Surgical technique

The open surgical technique (OE) involves a right thoracotomy with lung blockade and laparotomy with either a cervical or thoracic anastomosis. The minimally invasive procedure (MIE) involves a right thoracoscopy in prone position with a single lumen tube and laparoscopy with either a cervical or thoracic anastomosis.

Endpoints

A choice was made of the most standardised markers to study the endpoints. Endpoints were stress response measurements of cortisol, prolactin and growth hormone levels, immune status as manifested by the preservation of HLA-DR on monocytes, white blood cell count, and acute inflammatory response measured by means of IL-6, IL-8 and CRP level determinations. All measurements were performed pre-operatively and on the 1st, 3rd, 4th and 7th postoperative day.

Material and methods

Peripheral blood and serum (BD Vacutainer Systems, Plymouth, UK) were collected pre-operatively (baseline), 24, 72, 96 and 168 hours after surgery. Serum IL-6 and IL-8 samples were obtained by centrifugation for 10 min at 3,000 rpm at 4°C. All samples were stored in aliquots at -80°C until tested.

Immunological response

White blood cell count and phenotype were determined in fresh heparinized venous blood (within 2 hours after obtainment). Phenotyping was performed by using CD14-PE and HLA-DR-FITC moAbs (Becton Dickinson), lysis of erythrocytes, and fixation with paraformaldehyde. Evaluation of monocyte HLA-DR expression was performed by FACS analysis (FACS Calibur, Becton Dickinson, San Jose, CA, USA) quantified by using calibration beads (Quantum TM 26, Flow Cytometry Standards Corp, Bangs Laboratories Inc, Fishers, IN) and expressed as ratio of the mean fluorescence intensity post/presurgically.

Acute Inflammatory response

Concentrations of IL-6 and IL-8 were measured using commercially available enzyme-linked immunosorbent assay kits (PeliKine compact ELISA kit, Sanquin, Amsterdam, The Netherlands). CRP was measured in blood serum by immunoturbidimetric testing, using the BM/Hitachi 705 (Boehringer, Mannheim, Germany).

Stress response

Cortisol and growth hormone concentrations were measured by competitive immunoassay (Bayer Diagnostics, Mijdrecht, The Netherlands). Prolactin was measured by immunometric assays (DPC, Los Angeles, USA).

Statistical analysis

Statistical analysis was performed using the SPSS software package 15 (SPSS Inc., Chicago, IL, USA). The results for the two different groups were compared by means of Mann-Whitney U-test. Categorical parameters were analyzed with Fisher's exact test. Significance was set at $p<0.05$.

RESULTS

A total of 27 patients were included in this substudy of the TIME trial, 14 patients in the minimally invasive group and 13 patients in the open group.

Clinical characteristics

Demographic parameters, surgical data and pathological tumor indices are shown in table 1. No significant differences were observed in gender, age, tumor location and operation time. There was significantly less blood loss in the MIE group (450 vs 275 ml, p<0.05). General outcome and post-operative morbidity are depicted in table 2.

Immunological response

At baseline, white blood cell count was comparable in both groups (table 3). At 24, 72 and 96 hours after surgery there were no significant differences between the open and minimally invasive group. However, at 168 hours (1 week) after surgery there was a significant difference in white blood cell count due to an increase in the OE group as opposed to a sustained decrease in the MIE group (Figure 1). The change in leukocyte count after one week is also significantly different between the groups (p 0.011).
The expression of HLA-DR on monocytes showed a decrease of 60 percent or more in both groups at all measured moments (P<0.05). This was lowest for the open group at 72 hours after surgery (23.8%), although no statistical significance between the groups was reached (figure 2).

Acute inflammatory response

IL-6, IL-8 and CRP levels were comparable at baseline (table 3). In the post-operative IL-6 and CRP levels there were no significant differences between the groups. However, CRP levels peaked at 72 hours after surgery for both groups (Figure 3) and both IL-6 and IL-8 levels showed an increase at 24 hrs compared to baseline measurements (Figure 4 and Figure 5). Remarkably, at 168 hours after surgery the IL-8 level in the OE group was significantly higher than that in the MIE group (P=0.047) (Figure 5).

Stress response

Cortisol, prolactin and growth hormone at baseline in both groups were comparable (table 3). Cortisol levels upon surgery were elevated at all time measurements for both groups as growth hormone peaked at 24 hours for the OE group, but showed no significant differences between the groups (data not shown). Prolactin levels in the OE group, however, fluctuated in time, being significantly elevated at 168 hours (1 week) after surgery compared to the MIE group (Figure 6).

Table 1. Patient characteristics

	Open esophagectomy (n = 13)	MIE (n = 14)	P
Age (years)	62 (52-74)	65 (56-75)	ns
Gender			
Male	12	10	ns
Female	1	4	ns
BMI (kg/m2)	23 (21-33)	24 (16-33)	ns
Carcinoma type			
Adenocarcinoma	11	13	ns
Squamous cell carcinoma	2	1	
No. of lymph nodes retrieved	19 (7-40)	19 (3-30)	ns
Operation time (minutes)	266 (228-337)	309 (247-430)	ns
Blood loss (ml)	450 (100-1200)	275 (200-950)	0.045

MIE, Minimally Invasive Esophagectomy. Continous variables are expressed as median (range). Ns, not significant. BMI, Body Mass Index.

Table 2. General outcome and Morbidity

	Open esophagectomy (n = 13)	MIE (n = 14)	P
Total ICU stay (days)	1 (1-16)	1 (1-15)	ns
Hospital stay (days)†	18 (9-33)	15 (7-56)	ns
Mortality	0	1	ns
Pneumonia	7	3	ns
Anastomotic leakage	1	3	ns
Empyema not related to anastomotic leakage	1	0	ns
Pulmonary embolism	0	1	ns
Vocal cord palsy	2	1	ns

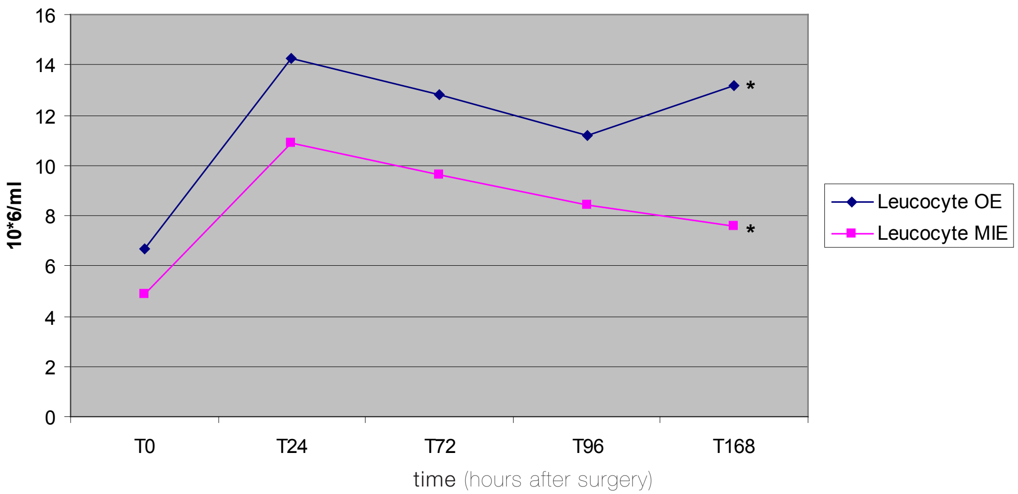
MIE, Minimally Invasive Esophagectomy. ICU, Intensive Care Unit. Continous variables are expressed as median (range). Ns, not significant. †, the patient who died in the hospital was included for calculation of the hospital stay.

Table 3. Postoperative immune, inflammatory and stress response

	Pre-op (T0)	24 hr (T24)	72 hr (T72)	96 hr (T96)	168 hr (T168)
Leucocytes					
OE	6.4 (4.2, 7.6)	14.3 (8.8, 17.2)	13.3 (7.1, 15.1)	11.6 (6.7, 14.6)	13.2 (10.8, 14.4)
MIE	4.9 (4.1, 5.5)	10.9 (8.2, 15.4)	9.4 (7.0, 12.2)	8.4 (6.4, 9.3)	7.5 (6.7, 8.4)
IL-6					
OE	6.0 (6.0, 8.8)	118.7 (68.5, 429.9)	30.6 (27.1, 68.7)	3.21 (12.5, 65.8)	23.3 (17.5, 51.9)
MIE	6.0 (6.0,6.0)	116.2 (43.0, 315.0)	29.9 (22.6, 99.6)	27.0 (14.7, 65.3)	11.8 (8.0, 33.1)
IL-8					
OE	11.2 (8.6, 19.9)	27.5 (17.1, 92.8)	18.6 (18.0, 31.6)	18.1 (10.8, 30.0)	33.7 (19.1, 67.2)
MIE	9.9 (7.8, 14.7)	32.2 (16.4, 41.7)	19.8 (12.9, 22.7)	14.5 (13.2, 22.9)	16.5 (12.6, 30.0)
CRP					
OE	5 (2, 23)	109 (75, 150)	183 (152, 286)	131 (116, 221)	114 (79, 171)
MIE	2 (2, 4)	65 (49, 113)	189 (144, 258)	125 (83, 243)	72 (55, 126)
Cortisol					
OE	365 (312, 603)	757 (604, 1197)	464 (353, 715)	700 (561, 843)	539 (474, 677)
MIE	329 (206, 455)	714 (605, 903)	600 (438, 675)	765 (563, 844)	692 (521, 798)
Prolactin					
OE	0.19 (0.11, 0.78)	0.18 (0.13, 0.35)	0.24 (0.15, 0.47)	0.18 (0.13, 0.35)	0.31 (0.18, 0.51)
MIE	0.12, 0.93)	0.18 (0.07, 0.24)	0.15 (0.11, 0.20)	0.18 (0.11, 0.31)	0.18 (0.13, 0.31)
GH					
OE	1.1 (0.6, 3.2)	8.0 (4.0, 12.0)	5.8 (2.1, 7.6)	2.2 (1.5, 5.2)	2.1 (1.0, 8.5)
MIE	0.8 (0.5, 3.9)	3.0 (1.8, 5.4)	2.3 (0.9, 4.8)	5.4 (2.6, 6.5)	1.5 (0.9, 3.5)

Results are expressed as Median (Q1, Q3)
OE, open esophagectomy; MIE, Minimally Invasive Esophagectomy
Leucocytes in 106/ml
IL-6 and IL-8 in pg/ml
CRP in mg/ml
Cortisol in nmol/l
Prolactin and Growth hormone (GH) in ng/ml

Figure 1. Concentration of Leucocytes in blood

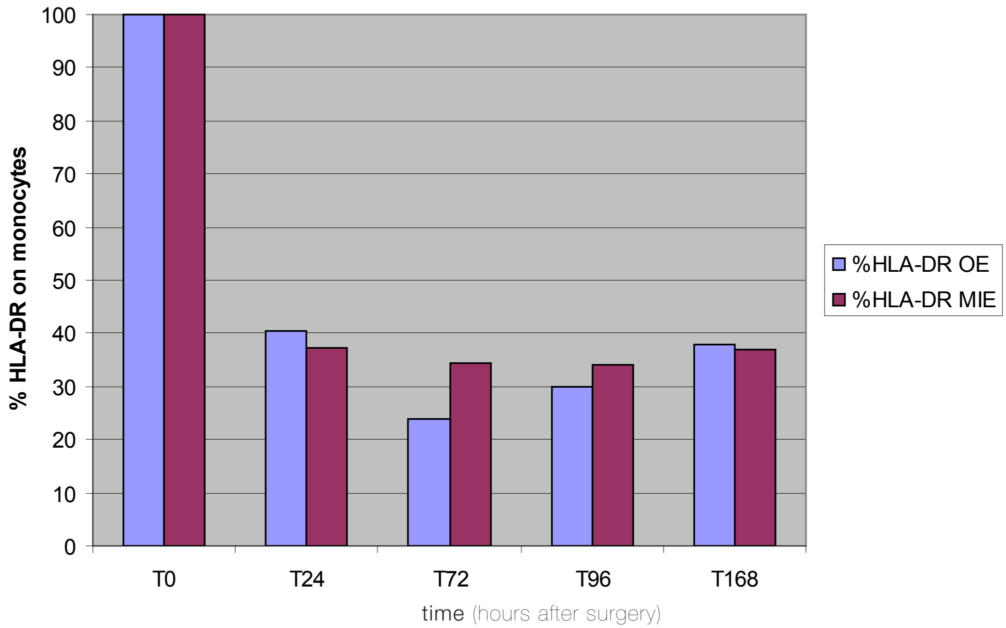


* significant difference (p 0.004)

Leucocyte OE = Concentration of Leucocytes in blood in the open esophagectomy group

Leucocyte MIE = Concentration of Leucocytes in blood in the minimally invasive esophagectomy group

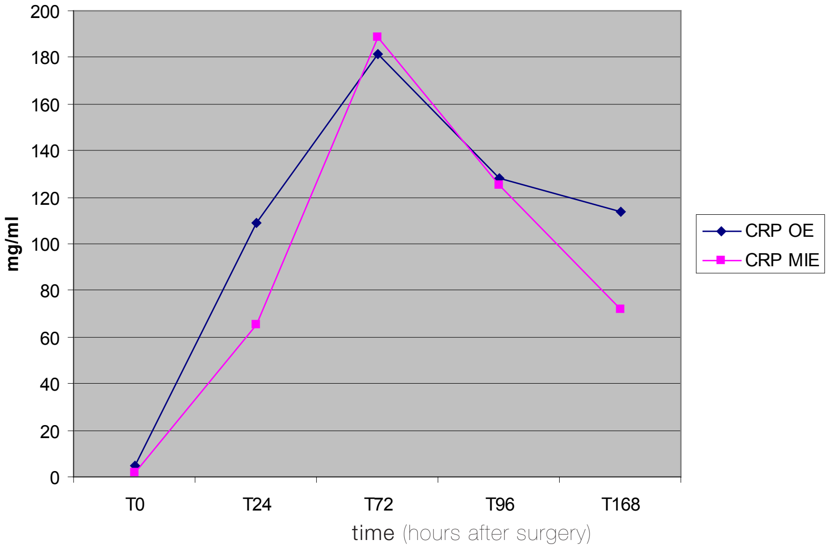
Figure 2. preservation of HLA-DR on monocytes



%HLA-DR OE = Percentage of HLA-DR on monocytes in blood in the open esophagectomy group

%HLA-DR MIE = Percentage of HLA-DR on monocytes in blood in the minimally invasive esophagectomy group

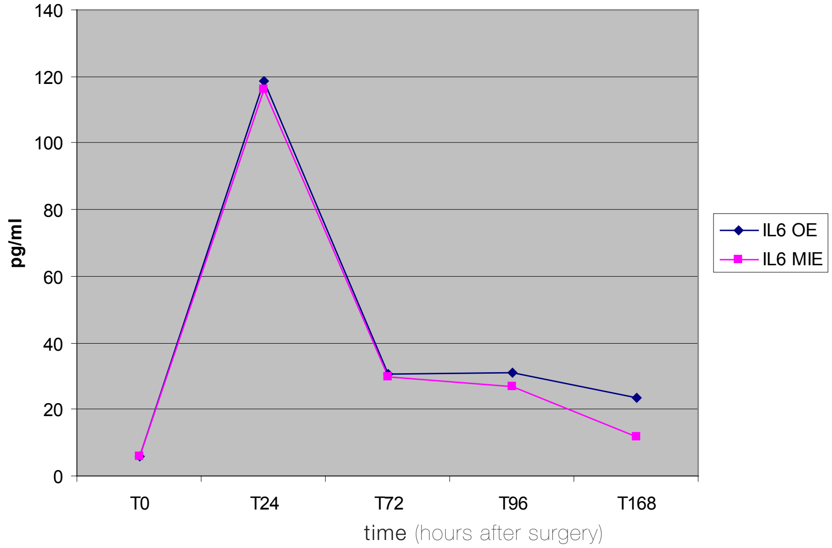
Figure 3. Concentration of C-Reactive Protein in blood



CRP OE = C-Reactive Protein concentration in blood in the open esophagectomy group

CRP MIE = C-reactive Protein concentration in blood in the minimally invasive esophagectomy group

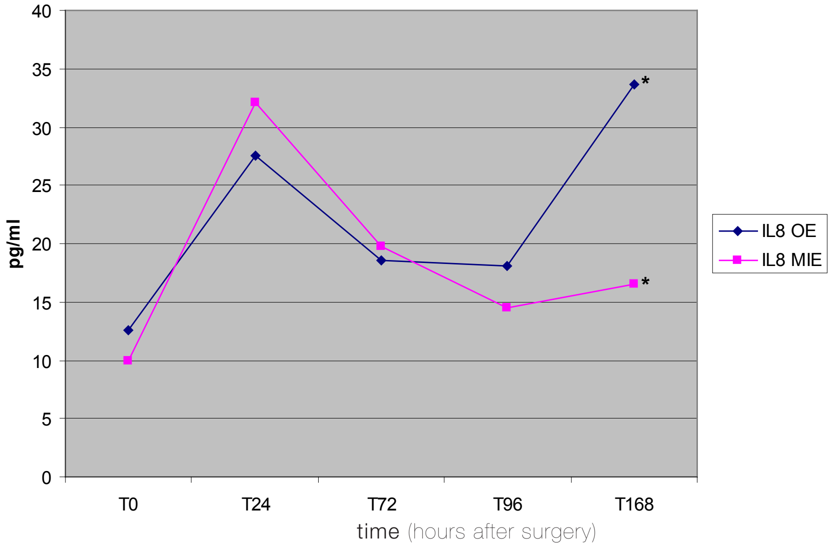
Figure 4. Concentration of Interleukin 6 in blood



IL 6 OE = Interleukin 6 concentration in blood in the open esophagectomy group

IL 6 MIE = Interlekin 6 concentration in blood in the minimally invasive esophagectomy group

Figure 5. Concentration of Interleukin 8 in blood

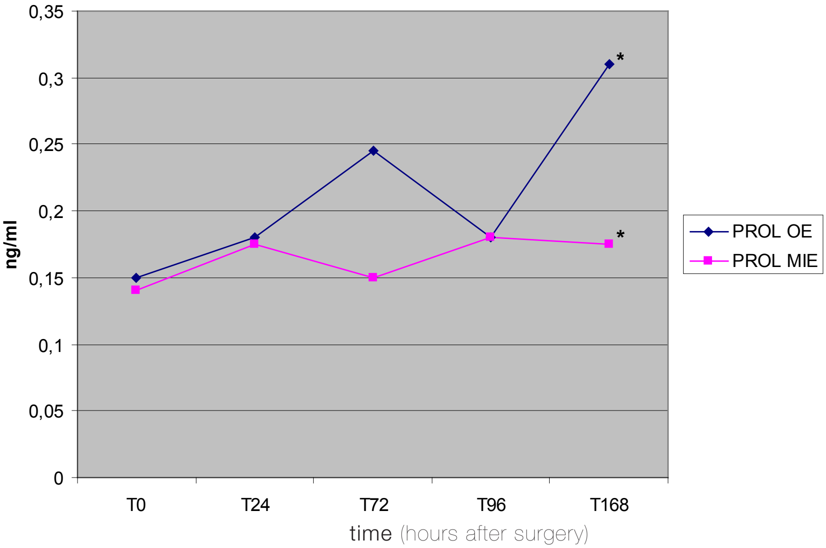


* significant difference (p 0.047)

IL8 OE = Interleukin 8 concentration in blood in the open esophagectomy group

IL 8 MIE = Interleukin 8 concentration in blood in the minimally invasive esophagectomy group

Figure 6. Concentration of Prolactin in blood



* significanct difference (p 0.49)

PROL OE = Prolactin concentration in blood in the open esophagectomy group

PROL MIE = Prolactin concentration in blood in the minimally invasive esophagectomy group

DISCUSSION

Immunological reaction after surgery may contribute to infectious complications, sepsis and tumor growth.[1,2] Minimally invasive surgery has shown to preserve immunological function better than conventional open surgery in different surgical procedures, including cholecystectomy, Nissen fundoplication and laparoscopic colorectal surgery.[3-5] In esophageal surgery for cancer, Scheepers et al. described the immunological consequences of laparoscopic versus open transhiatal resection for malignancies of the esophagus.[8] This non-randomized study compared 6 patients in the laparoscopic group with 11 patients in the open group. They found an increase of all markers with significantly higher levels of IL-6 for the open group, suggesting that the surgical trauma in the minimally invasive group is less extensive. This increase of IL-6, expression of the extent of trauma and predictor for postoperative complications is also seen in this study. A peak is observed at 24 hours after surgery, as in other studies.[3,6,8] However, no significant differences were found in IL-6 and CRP levels between the OE group and MIE group. Remarkably, leukocyte count was significantly higher in the OE group at seven days, whereas the expression of HLA-DR on monocytes was decreased postoperatively, but without a significant difference between the groups. A possible explanation is that the extent of trauma was so predominant in both groups of transthoracic esophagectomy that differences, measured by IL-6 and CRP, were masked. However, it must be acknowledged that this study includes a limited number of patients. Additional differences would probably might be seen with larger number of patients.

For IL-8 and white blood cell counts a significant increase in the OE group one week after surgery was found. In white blood cell count not only the difference at one week is significant, also the change in leukocyte count is significantly different in both groups, in favour of the minimally invasive group. IL-8 is thought to play an important role in the development of pneumonia.[7,10,15,16] Fujimori et al. investigated the role of IL-8 in interstitial pneumonia and found that an increased level is associated with fibrosis and injury of the lung.[15] Yamada et al. evaluated the serum IL-6 and IL-8 in patients undergoing conventional thoracic surgery.[7] They found a significant increase of IL-8 levels until the seventh post-operative day in patients developing postoperative pulmonary infections. The authors suggest that this increase of IL-8 may reflect the severe surgical stress due to reperfusion of ischemic lung tissue as a result of one-lung ventilation during thoracotomy. In our study we also found increased IL-8 levels in the early postoperative period for both groups, with a significant difference at seven days postoperatively in favour of the MIE group. This could explain the trend of fewer respiratory infections observed in the MIE group in comparison to the OE group (7 in the OE group and 3 in MIE group). The trend here observed was confirmed actually in the main study, the TIME trial, recently published.[12] Other studies comparing open to minimally invasive techniques in cholecystectomy, Nissen fundoplication and colorectal surgery have shown better immunological outcome in favour of the minimally invasive techniques. [3-5] However, an important difference between esophagectomy and the other interventions, for example cholecystectomy, is the enormous difference in amount of surgical trauma. Probably is more difficult to demonstrate significant differences in immunosuppression in interventions with huge wound surface like the esophagectomy. For example expression of HLA-DR was decreased atv all times for both groups in our study, whereas after Nissen fundoplication and even after colorectal surgery the expression of HLA-DR on monocytes returned to pre-operative levels within a week[4,5].

Since the increase in IL-8 levels has been identified both during and after surgery, the possibility has risen to block this increase intra-operatively in order to minimize respiratory infections after esophagectomy.[7,9] Kawahara et al. introduced the administration of a neutrophil elastase inhibitor in patients undergoing esophageal resection by thoracoscopy.[9] They showed that this inhibitor seems to partially suppress the postoperative increase in IL-8 levels and shorten the duration of systemic inflammatory response in a randomized trial. As a result, acute lung injury and respiratory infections could be prevented.

The increase of prolactin, cortisol and growth hormone levels seems to be less intense and of shorter duration after laparoscopic surgery in gyneacologic pelvic surgery.[17, 18] An increase in open surgery might not be due to surgery solely, but also to the administration of anesthetic drugs such as morfine.[19] All patients in our study received epidural anesthesia for at least 3 days. At 1 week after surgery a significant difference in prolactin levels between the two groups was observed. This might be attributable to the use of analgesics. At one week after surgery, the use of morphine was increased in the open group (11 versus 5 patients).

In conclusion, in this substudy of a randomized trial comparing the minimally invasive with the conventional esophagectomy for cancer, a significantly better preserved leukocyte count and IL-8 level were observed in the MIE group as compared to the open group. Though the differences are small, both values might be related to fewer respiratory infections found postoperatively in the MIE group. Moreover, stress response was also better preserved in the MIE group, as expressed by the slightly different prolactin values at 1 week postoperatively. These findings indicate that less surgical trauma could lead to better preserved acute-phase response and fewer respiratory infections. Overall, further studies will be needed to subscribe our findings.

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