Chapter 7

C-reactive protein and Interleukin-6 in perianastomotic exudate in diagnosis of major complications after major abdominal surgery

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Chapter 7

ABSTRACT

Early detection of major complications is key in decreasing the morbidity and mortality after major abdominal surgery. Here we assess C-reactive protein (CRP) and Interleukin-6 (IL-6) levels in perianastomotic drain fluid and serum as early predictors of major complications.

CRP and IL-6 were measured in both serum and perianastomotic drain fluid in 28 patients undergoing oesophagectomy or pancreaticoduodenectomy. Nine patients had a major complication.

Serum CRP levels were higher on POD3 in patients with major complications (276 mg/L versus 133 mg/L). Values for CRP and IL-6 in drain fluid were similar in uncomplicated and complicated cases (p > 0.05).

CRP in serum was found to be an important marker for early detection of major complications. CRP and IL-6 in perianastomotic fluid were not found to be significant markers for major complications. Technical difficulties found around postoperative drains are addressed. The search for optimal markers in assessing major complications continues.
INTRODUCTION

Major complications after major abdominal surgery (MAS) are reported in up to 20% of patients. These complications require invasive treatment and are associated with increased morbidity and mortality. Median time to diagnosis of major complications is currently 7 to 8 days, which is interesting in light of today’s fast track care, were patients are discharged as early as five days postoperatively. During the early postoperative phase it can be difficult to differentiate septic complications from the normal postoperative inflammatory response. In the case of major complications, early detection and intervention are crucial in improving outcomes.

Several studies have assessed the use of C-reactive protein (CRP) as a marker for postoperative complications and/or anastomotic leak. CRP is an acute-phase protein synthesized almost exclusively in the liver. Its release is stimulated by pro-inflammatory cytokines such as Interleukine-6 (IL-6).

Others studies have focused on the process of intraperitoneal physiology and the changes that occur in CRP and IL-6 in the peritoneal compartment in normal healing process and in the case of septic complications. Peritoneal drain fluid could supply important information in the early diagnosis of anastomotic complications. The aim of this study is to determine whether CRP and/or IL-6 in perianastomotic drain fluid can be used as a screening tool for anastomotic complications after MAS.

METHODS

A prospective cohort study was performed in order to assess the levels of CRP and IL-6 in serum and perianastomotic drain fluid as a marker for anastomotic complications after MAS. In the Department of Surgery of the VUmc, drains are positioned routinely after oesophageal and pancreatic resections and only on indication after rectal surgery. Hence, patients undergoing oesophagectomy or pancreaticoduodenectomy for cancer were included. The study was performed according to the principles of the Declaration of Helsinki. The medical ethics board approved the protocol. Participating patients were required to give informed consent prior to inclusion.

Oesophagectomy

Oesophagectomy was performed for oesophageal cancer after neoadjuvant chemoradiotherapy according to the CROSS-scheme and consisted of minimally invasive oesophageal resection by thoracoscopy or transhiatal with gastric tube formation. Anastomoses were performed cervical or intrathoracic. The drains were placed in the thorax and in the neck.
Pancreaticoduodenectomy

Pancreaticoduodenectomy was performed for cancer of the pancreatic head, duodenal papilla or duodenum. The type of procedure consisted of pancreaticoduodenectomy according to Whipple, or a pylorus preserving pancreaticoduodenectomy (PPPD). Drains were positioned routinely near the anastomosis and checked for pancreatic enzymes routinely as a marker of anastomotic leakage.

Data collection

Data was collected prospectively. Upon inclusion data regarding baseline characteristics were collected; age, gender, BMI, co-morbidities and American College of Anaesthesiologists (ASA) classification. Operative data were collected with regard to type of surgery, duration of surgery, TNM-classification, radicality and number of resected lymph nodes. Postoperatively drain fluid was collected routinely on postoperative day (POD) one and postoperative day three. POD1 and POD3 were deemed appropriate; day one to portray the postoperative systemic inflammatory response, whereas previous studies have shown CRP levels on postoperative day three to be a predictive marker for major postoperative complications. The drain fluid reservoir was emptied every morning, respecting the rules of sterility. At the same time blood samples were collected. Patients were followed up to 30 days postoperatively or until discharge. Complications were graded according to the Clavien-Dindo classification.

Interleukin 6 and C-reactive protein

CRP samples were analyzed with the Roche Modular P800 chemistry analyzer® (Roche Diagnostics, Mannheim, Germany ®). IL-6 samples were collected and centrifuged. The supernatant was stored at -80 degrees Celsius. IL-6 was measured with ELISA, using the Meso Scale Discovery human IL-6 assay (Meso Scale Discovery, Gaithersburg, MD ®).

Definitions

Complications were graded according to the Clavien-Dindo classification, which grades complications according to the necessitated treatment. The classification was modified into minor and major complications. Major complications consisted of grade III to V, encompassing all complications that require invasive treatment (i.e. percutaneous drainage or reoperation), ICU admission and even leading up to death. Mortality was defined as in-hospital mortality and 30-day mortality.

Statistics

Continuous variables were described as means and standard deviations or medians or medians and interquartile ranges as appropriate for normal and non-normal distributions respectively. Univariate analysis occurred via Student’s T-test or Mann-Whitney-U
test as appropriate. Wilcoxon rank sum test was used for paired samples with non-normal distributions. Frequencies were depicted as percentage and univariate analysis was performed with chi-square tests or McNemar analysis.

RESULTS

In the period from April 15th 2014 to August 19th 2014, 31 patients underwent oesophagectomy or pancreaticoduodenectomy for cancer in the VU Medical Centre. Three patients declined to participate. Twenty-eight patients signed informed consent and participated in the study. Baseline characteristics and outcome parameters of the patients are depicted in Table 1. In 13 of the 28 patients drain follow-up was not complete due to insufficient drain production in eight patients, the drain fell out in two patients and in three patients the drain was removed early. Major complications occurred in five

Table 1: Univariate analysis of baseline characteristics of the total population: patients that completed drain follow-up compared to patients that did not. I) GJ = gastrojejunostomy; HJ = hepaticojejunostomy. II) grade 3 or higher according to the Clavien-Dindo classification score.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Drain follow-up complete (n=15)</th>
<th>Drain removed earlier (n=13)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male gender, n (%)</td>
<td>7 (46,7)</td>
<td>8 (61,5)</td>
<td>0,476</td>
</tr>
<tr>
<td>Age, y, mean ± SD</td>
<td>70,1 ± 10,0</td>
<td>64,5 ± 11,5</td>
<td>0,181</td>
</tr>
<tr>
<td>ASA class</td>
<td></td>
<td></td>
<td>0,398</td>
</tr>
<tr>
<td>I , n(%)</td>
<td>1 (6,7)</td>
<td>0 (0,0)</td>
<td></td>
</tr>
<tr>
<td>II , n(%)</td>
<td>6 (40,0)</td>
<td>8 (61,5)</td>
<td></td>
</tr>
<tr>
<td>III , n(%)</td>
<td>8 (53,3)</td>
<td>5 (38,5)</td>
<td></td>
</tr>
<tr>
<td>BMI, kg/m2, mean ± SD</td>
<td>25,0 ± 3,7</td>
<td>24,9 ± 4,1</td>
<td>0,915</td>
</tr>
<tr>
<td>Type of procedure</td>
<td></td>
<td></td>
<td>0,397</td>
</tr>
<tr>
<td>Oesophagectomy, n (%)</td>
<td>6 (40,0)</td>
<td>5 (38,5)</td>
<td></td>
</tr>
<tr>
<td>Pancreaticoduodenectomy, n (%)</td>
<td>9 (60)</td>
<td>6 (46,2)</td>
<td></td>
</tr>
<tr>
<td>Palliative GJ/HJI, n (%)</td>
<td>0 (0,0)</td>
<td>2 (15,4)</td>
<td></td>
</tr>
<tr>
<td>Duration of surgery (minutes) mean ± SD</td>
<td>279,9 ± 86,3</td>
<td>274,8 ± 66,9</td>
<td>0,864</td>
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<tr>
<td>Neoadjuvant therapy, n (%)</td>
<td>6 (40,0)</td>
<td>5 (41,7)</td>
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<tr>
<td>Radical resection, n (%)</td>
<td>12 (80,0)</td>
<td>10 (90,9)</td>
<td>0,614</td>
</tr>
<tr>
<td>T-stage</td>
<td></td>
<td></td>
<td>0,164</td>
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<tr>
<td>T0, n (%)</td>
<td>5 (35,7)</td>
<td>1 (12,5)</td>
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</tr>
<tr>
<td>T1, n (%)</td>
<td>0 (0,0)</td>
<td>2 (25,0)</td>
<td></td>
</tr>
<tr>
<td>T3, n (%)</td>
<td>8 (57,1)</td>
<td>5 (62,5)</td>
<td></td>
</tr>
<tr>
<td>T4, n (%)</td>
<td>1 (7,1)</td>
<td>0 (0,0)</td>
<td></td>
</tr>
</tbody>
</table>
patients in the group that completed follow up and in three in whom the drain was insufficient or early removed.

**Postoperative complications**

Nine patients (32.1%) had major complications. Treatment for major complications and mortality rates are depicted in Table 1. Two patients died, one patient after oesophageal resection because of sepsis associated with anastomotic leakage and another one following a Whipple procedure, complicated by a fistula between the gastroduodenal artery and gastrointestinal tract. Fluid collections were treated by percutaneous drainage in four patients (two after oesophageal resection and two after pancreatioduodenectomy). In two patients after oesophageal resection the postoperative course was complicated by pneumonia with respiratory insufficiency. One patient after pancreaticoduodenectomy was reoperated with clinical and radiological symptoms of anastomotic leakage. Three patients had a minor complication, consisting of paralytic ileus requiring total parenteral nutrition in two patients and pneumonia treated with antibiotics in one patient.

**Postoperative drain production**

Median CRP and IL-6 levels in serum and in drain fluid on POD1 and POD3 are depicted in table 2. Differences in serum and perianastomotic fluid levels between complicated and uncomplicated groups are depicted in table 3.
The median serum CRP on POD1 was 87 mg/L in patients with major complications and 116 mg/L in patients without complications. The median serum CRP level on POD3 was 276 mg/L in patients with major complications as opposed to 133 mg/L in patients without major complications.

CRP levels in perianastomotic drain fluid on POD1 were 11 mg/L in patients with major complications, and 11,5 mg/L in uncomplicated patients. On POD3, the values were again similar, 47 mg/L in patients who presented with major complications and 50 mg/L in those without.

Serum IL-6 levels on POD 1 were median 86 pg/L in patients with major complications versus 67 pg/L in uncomplicated patients. The median serum IL-6 on POD3 was 23 pg/L in patients with major complications as opposed to 17 pg/L in uncomplicated patients. Median levels IL-6 in perianastomotic drain fluid on POD 1 were 21449 pg/L in patients with major complications versus 53021 pg/L in patients without major complications. The median drain fluid IL-6 levels on POD3 were 13127 pg/L in patients with major complications as opposed to 11844 pg/L in uncomplicated patients.
DISCUSSION

In search of early predictors of major complications after major abdominal surgery, a prospective cohort study was gathered in order to determine whether the measurement of CRP and/or IL-6 in serum and in perianastomotic drain fluid could be used as a non-invasive screening tool to assess patients who were at risk for developing anastomotic complications.

Serum C-reactive protein levels were higher on POD3 in case of major complications, as also previously described in several large cohorts \(^2,6\). Differences in levels of CRP in drain fluid showed no difference and a great variance between major complicated and uncomplicated groups according to Clavien-Dindo indicating that measurement of CRP in drain fluid was not able to differentiate between patients with major complications and those without.

IL-6 levels in both serum and drain fluid showed an early peak on POD1 and a decrease towards POD3. This is in line with the knowledge that IL-6 is produced locally as a result of surgical trauma and CRP is synthesized almost exclusively in the liver in response to the rise of IL-6 and other cytokines \(^5\). No differences were observed between IL-6 in serum and perianastomotic drain fluid levels in patients with major complications compared to those without, as described previously \(^11\). The higher perianastomotic drain fluid levels of IL-6 compared to the serum IL-6 values can be attributed to compartmentalization of cytokines with high production of IL-6 caused by discontinuity of vascular and lymphatic vessels by surgery \(^12\).

Literature concerning measurement of acute phase proteins in intra-peritoneal drain fluid after colorectal surgery has showed promising results, although policy about removal of drains and sufficiency of drain production has been unclear \(^7,13\). IL-6 levels in drain fluid strongly exceeded serum levels; it is proposed that surgical trauma causes local production of IL-6 whilst discontinuity of vascular and lymphatic structures due to surgery further maintains the local levels of IL-6 \(^14\). With great variance in reported levels of cytokines, significance of the measurement of these levels in a clinical setting remains unclear \(^15,16\). Moreover, with the great variance in reported levels of cytokines the significance of the measurement of these in a clinical setting remains unclear \(^15,16\). Furthermore the drain policy is disputable because of no utility, insufficient production or misplacement.

Several important limitations of this study should be emphasized, for one being a small size pilot study. Only oesophagectomies and pancreaticoduodenectomies were included since these patients receive routine placement of perianastomotic drains. In colorectal surgery drains are only left on indication by the surgeon. Another drawback of the study is that not in all patients the drain fluid analysis could be completed, probably like in other studies, adding a difficulty for this kind of studies.
In conclusion, CRP levels in serum were increased in patients with major complications. C reactive protein and IL-6 levels in perianastomotic drain fluid provided no differences between patients with major complications, and those without. The search for optimal markers and devices left in perianastomotic areas to detect major anastomosis complications continues.
REFERENCES


