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Original article

Interleukin-6 Levels to Identify Patients at Risk of Early Postoperative Complications after Open Radical Cystectomy: A Clinical Study

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Abstract

Background and Objective: Molecular data indicate that immune mechanisms are affected by surgical trauma. We performed a study to compare lymphocyte Th1/Th2 cytokine production profile in open cystectomy patients who developed early postoperative infectious and cardiac complications and patients whose postoperative course was uneventful. ***Methods:*** Perioperative and laboratory data of 54 patients who underwent elective open radical cystectomy with ileal conduit were assessed. Interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, IL-13, interferon - gamma (INF)-γ and tumor necrosis factor - alpha (TNF)-α concentrations were analysed at two points: before surgery and on postoperative day one (POD-1). Cardiac troponin levels on POD-1 were also analysed. Cytokine levels were determined using cytometric bead array and flow cytometry. Complications assessed as one composite outcome were pneumonia, surgical site infection and myocardial injury. Cytokine levels at two study points in patients with and without complications, as well as in patients with and without perioperative blood transfusion were compared. ***Results:*** Data from 54 cases (30% women) were analysed. Of eight assessed cytokines only IL-6 and IFN- γ levels differed at two study points. Seven patients developed infection and myocardial injury was diagnosed in six cases. Postoperative IL-6 levels increased postoperatively and were significantly higher in patients who developed complications (413.2 vs 250.2pg/ml, p=0.01), as well as in those transfused perioperatively (328.3 vs 160.2pg/ml, p=0.005). ***Conclusions:*** Open cystectomy results in major rise in proinflammatory IL-6, with higher levels in patients who develop postoperative complications and those requiring blood transfusion.

Keywords: ***open cystectomy, postoperative complications, cytokines, postoperative immune suppression, transfusion***

Introduction

Bladder cancer is a common malignancy, with its prevalence increasing with age. Open radical cystectomy with pelvic lymphadenectomy and ileal urine conduit is a well-recognized definite treatment option for patients with invasive bladder cancer that improves survival by cessation of local invasion and avoidance of distant metastases. At the same time, it is one of the most demanding surgical procedures, with 3-month mortality of up to 15% in elderly [1]. Significant complications after surgery markedly influence the length of hospital stay. Depending on the type of surgery, as much as 30 - 70% of patients suffer from delayed recovery due to unexpected complications [2]. Vast majority of postoperative morbidity and mortality is due to surgical site infection, pulmonary and cardiac complications. Advanced biochemical assays made it clear that most of the immune system is involved in perioperative response [3]. It is now understood that features of both proinflammatory and anti-inflammatory activation can be observed [4].

Ongoing struggle to identify possible biomarkers capable of predicting postoperative course suggest that specific immunological factors may be of substantial value. Postoperative cytokine profiles were studied mostly in abdominal and lung surgery, with results pointing towards proinflammatory factors as potential targets for future research [5-8].

Myocardial injury related to non-cardiac procedures (Myocardial Injury After Noncardiac Syrgery, MINS) is a major complication with potential to increase the postoperative morbidity [9]. It is reported that more than 10% of patients undergoing surgical procedures may present with raised cardiac troponin (cTn) levels

postoperatively, indicative of some form of myocardial damage and linked to increase in 30-day mortality [10]. As most of these patients show no clinical symptoms typical of cardiac ischemia, perioperative biochemical monitoring is currently indicated for intermediate and high-risk surgery in patients at risk or with known cardiovascular disease [11,12].

Open radical cystectomy is one of the most demanding urological procedures. Due to extensive tissue damage and long operative times, its perioperative pathophysiology may be considered representative for major, long - lasting abdominal surgeries. Intraoperative management is challenging, as the course of operation is likely to require blood transfusion and inotropic support. Postoperative infectious complications are serious adverse events, of which the most common are surgical site infections (SSI) and infections of respiratory system, with marked impact on morbidity, length of hospital stay (LOS) and mortality.

The aim of this study was to assess changes in type 1 helper T lymphocytes (Th1)- and type 2 helper T lymphocytes (Th2) - dependent cytokine concentration during the early postoperative period after open radical cystectomy and to identify the possible relation of cytokine production and development of early infectious complications and myocardial injury. As blood transfusion is known to affect immune status, differences in cytokine concentrations in patients who were given blood transfusion perioperatively and in those who were not were also assessed.

Materials and Methods

This study is a retrospective observational study, based on laboratory findings and retrospective data analysis. It was conducted in Department of Anaesthesiology and Intensive Care, Orlowski Clinical Hospital, Centre of Postgraduate Medical Education in Warsaw, Poland. Ethics Committee approval for the study was granted by the Ethics Committee of Centre of Postgraduate Medical Education, Warsaw, Poland on the 21st of February 2018, 28/PB-A/2018).

All analyzed blood samples were collected between 2013 and 2017, as a part of previous prospective clinical study (Ethics Committee of Centre of Postgraduate Medical Education, Warsaw, Poland, 13th of February 2013, 9/PB/2013), which was conducted in Department of Anaesthesiology and Intensive Care and Department of Urology, Orlowski Clinical Hospital, Centre of Postgraduate Medical Education in Warsaw, Poland.

Eligible patients were screened and enrolled between October 2013 and May 2017. The main inclusion criterion was radical open cystectomy due to advanced bladder cancer. Patients unable to understand and sign the informed consent form, with perioperative risk assessment graded as American Anesthesiology Association grade 4 or more and patients with absolute contraindications to epidural analgesia were considered not eligible for the study.

Perioperative regime was standardized, with epidural catheter placed in low thoracic or high lumbar portion of the epidural space prior to induction of general anesthesia with propofol and fentanyl, muscle relaxation with rocuronium or cis-atracurium and endotracheal intubation. Perioperative antibiotic prophylaxis with ceftriaxone, amikacin and metronidazole was used and continued for 2 days postoperatively. Anesthesia was maintained with sevoflurane in a mixture of oxygen and air, with fraction of inspired oxygen at 0,4 – 0,6 and a goal of oxygen saturation above 95%. Epidural analgesia was maintained with intermittent (2 hourly) doses of 10ml of 0.25% bupivacaine with epinephrine. Pressure – controlled ventilation was used routinely with end – expiratory pressure set to 5cm H_2O. The fluid therapy was based on crystalloids with colloids reserved for blood loss replacement (up to 1000ml of colloids per procedure). Estimated blood loss of more than 800ml was an indication to start blood transfusion with regime 1:1 (equal number of units of packed red cells (PRC) and plasma). Early low-dose norepinephrine infusion for maintenance of blood pressure was used as required and if used, it was continued until the end of surgery. Procedure time of more than 6 hours, blood loss requiring intraoperative transfusion of more than 2 units of PRC, and inability to cease norepinephrine infusion by the end of surgery were the indications for intensive care admission postoperatively. Epidural infusion of 0.125% bupivacaine with epinephrine was initiated after the procedure and continued with the rate adjusted to achieve adequate pain control until the next day. Hemoglobin levels were tested postoperatively and allogeneic blood transfusion given when anemia was diagnosed (routinely <8mg%). Routine postoperative blood tests were performed, including cTn I on postoperative day one (POD-1).

Two samples of venous blood were collected from each participating patient for the study: immediately before induction of anesthesia and early on POD-1 (approximately 24 hours after beginning of surgery). Blood was first drawn into 4ml vials with clot activator, then centrifuged, serum acquired was stored in the temperature -70°C until cytokine assessment. For cytokine measurements, BioLegend cytometric bead array kit was used (LEGENDplex Th1/Th2 Panel, BioLegend, San Diego, USA). Cytokines measured using this panel are: interleukin (IL)-2, IL-4, IL-5, IL-6, IL-10, IL-13, interferon-gamma (INF-γ) and tumor necrosis factor-alpha (TNF-α). The flow cytometer used for bead array was BD FACSCanto II.

As per the design of the current study, all the predefined infectious complications and myocardial injury in postoperative period were considered as one composite outcome, since perioperative immune disturbances may play a role in their development. Two types of infections diagnosed before discharge were considered: SSI and pneumonia. Wound infections diagnosed after discharge (during follow-up visits within 90 days from the surgery) and requiring readmission were also recorded and included in the analysis as SSI.

Surgical wounds were evaluated and their condition recorded in medical notes daily by nursing staff until discharge, signs of infection prompting commencement of systemic antibiotics. Data on episodes of SSI that occurred before discharge were acquired from medical records, as well as episodes of re-admissions due to SSI – related complications: wound hematoma, wound dehiscence, seroma, wound necrosis. Both clinical signs of chest infection and radiological features of pneumonia were required for diagnosis of pneumonia. All patients had cardiac troponin I (cTn I) levels measured as part of the routine laboratory tests on the first postoperative day, with the level above 99th percentile of the upper reference limit indicative of myocardial injury. Patients with either myocardial injury alone or subsequent diagnosis of myocardial infarction in postoperative period were included in analysis as patients with myocardial injury complication. Data on infectious complications, blood transfusion and cTn I levels were acquired from medical records and hospital electronic database.

Statistical analysis

The data were examined for normal distribution. Continuous variables of baseline characteristics and surgical data were assessed with t-test for independent variables and Fisher's exact test for categorical variables. Serum cytokine concentrations in two assessment points, as well as their change between these time points were compared in patients with and without complications and

transfused and not transfused patients with Mann-Whitney U test. Receiver – operator curve and statistic were employed to assess the efficacy of POD-1 IL-6 in predicting the occurrence of complications. MedCalc statistical software was used for data analysis.

Results

Complete data were available in 54 cystectomy cases. Demographic and intraoperative characteristic is presented in Table 1.

Table 1: Patient characteristics. The data are given as mean and standard deviation or number of cases and percentage in parentheses. BMI – body mass index. COPD - chronic obstructive pulmonary disease.

	Total N=54	Complications n=11	No Complications n=43	p	Transfusion n=32	No Transfusion n=22	p
Age (years)	70.26 (9.04)	73.72(9.65)	69.37(8.93)	0.16	69.34(8.49)	71.59(10.11)	0.38
Weight (kg)	75.8 (17.48)	78.09(19.00)	75.20(19.11)	0.65	79.25(19.56)	70.77(17.23)	0.10
Height (cm)	167.8 (9.26)	164.27(7.36)	168.69(10.05)	0.17	167.68(8.47)	167.95(11.4)	0.92
BMI	26.78 (5.20)	29.02(7.31)	26.2(5.16)	0.14	28.16(6.49)	24.77(3.58)	0.03
Women	16 (30)	2(18)	14(32)	0.47	8(25)	8(36)	0.38
Men	38 (70)	9(82)	29(68)	0.47	24(75)	14(64)	0.38
Diabetes	6 (11)	2(18)	3(7)	0.24	5(16)	1(4)	0.38
Hypertension	22 (40)	5(45)	17(39)	0.74	13(41)	9(41)	1.0
COPD	3 (5)	0	3(5)	1	0	3(13)	0.06
Procedure duration (min)	398.14 (80)	399(55)	397(85)	0.94	418(76)	369(78)	0.02
Intravenous fluids (ml)	3615.74 (1091.04)	4090(1044)	3494(1080)	0.06	3890(1029)	3215(1075)	0.02
Blood transfusion	32 (59)	9 (82)	23(53)	0.16	32(100)	0(0)	N/A

Most of the patients were men and the average age of the study population was 70 years. Large percentage of intraoperative transfusions was noted: more than half of our cases required blood products transfusion due to a significant blood loss causing hemodynamic alterations. In 11 patients' postoperative complications were identified as per study protocol. Seven patients have developed infectious complications. Six patients had raised postoperative cTn I levels, indicative of myocardial injury, whereas two patients presented both types of complications (infection and raised cTn I). Apart from IL-6 and INF-γ, serum concentrations of the other assessed cytokines in two study points (pre- and postoperative) were not different in the study group. Hence, the further analysis focused on IL-6 and INF-γ only. When patients with and without complications were compared, their preoperative IL-6 and INF-γ levels were not different, whereas postoperative IL-6 has differed significantly (413.23 vs 250.23, p=0.01, Table 2).

Table 2: Interleukin - 6 and interferon gamma analysis preoperatively (pre-OP) and on the first postoperative day (POD-1) in complication and no complication group. The data are presented as median and interquartile range. Exact p value is given.

	Complication (n=11)	No complications (n=43)	p
IL-6 pre-OP (pg/ml)	30.60 (0-62)	9.16 (0-12)	0.68
IL-6 POD-1 (pg/ml)	413.23 (183-547)	250.23 (110-338)	0.01
INF-γ pre-OP (pg/ml)	289.5 (37-424)	193.73 (94-286)	0.47
INF-γ POD-1 (pg/ml)	197.4 (37-139)	149.0 (37-195)	0.82

To investigate a possible link between particular postoperative IL-6 levels and development of early infectious complications and MINS, the area under the curve (AUC) of Receiver-Operator Curve (ROC) analysis was performed using IL-6 concentrations on the first day after surgery as the test assessed and complication as an outcome. AUC of the ROC analysis for IL-6 on POD-1 as predictor of postoperative complications was 0.67 (0.95% CI: 0.53 - 0.79), which indicate marked, but rather poor prognostic value of the test assessed. The Youden index indicating the highest sum of sensitivity and specificity was 0.33 for IL-6 concentration above 302.98 pg/ml (63.64 sensitivity, 69.77 specificity) (Figure 1).

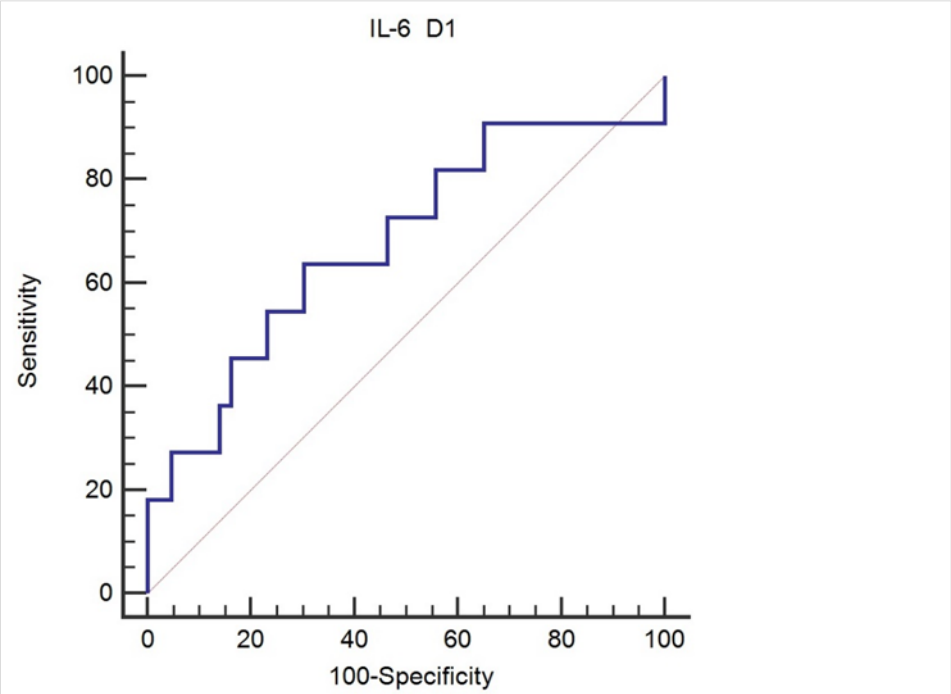

Figure 1: Receiver Operating Characteristics (ROC) for prediction of postoperative complications based on the serum levels of IL-6 at POD-1.

To analyze the possible influence of intraoperative blood transfusion on postoperative cytokine concentration, it was also assessed preoperatively and on the first postoperative day in a group of patients who received blood transfusion perioperatively and in those who have not. Interleukin-6 plasma concentration before surgery and on day 1 increased in both transfused and not-transfused patients. However, a significant difference was noted in the first postoperative day levels between the groups: patients who were transfused showed higher IL-6 concentrations when compared to patients who were not treated with blood transfusions (Table 3). The difference was also significant when changes in IL-6 levels between baseline and POD-1 were compared (p=0.005).

Table 3: Interleukin – 6 and interferon gamma analysis preoperatively (pre-OP) and on the first postoperative day (POD-1) in transfusion and no transfusion group. The data are presented as median and interquartile range. Exact p value is given.

	Transfusion (n=32)	**No transfusion (n=22)**	**p**
IL-6 pre-OP (pg/ml)	2.85 (0-22,5)	1.17 (0-9)	0.3181
IL-6 POD-1 (pg/ml)	328.33 (182-457)	160.20 (109-226)	**0.0058**
IL-6 change (pg/ml)	301.27 (182-428)	153.16 (99-217)	**0.0058**
INF-γ pre-OP (pg/ml)	147.64 (46-286)	151.74 (122-331)	0.44
INF-γ POD-1 (pg/ml)	94.90 (37-168)	49.47 (37-195)	0.5

The findings regarding IL-6 were not noted for other assessed cytokines. The only significant difference between preoperative and postoperative plasma concentration of other cytokines was found for INF-γ in not-transfused group (151.74pg/ml vs 49.47pg/ml, p=0.01), whereas in transfused patients decrease in INF-γ concentration was not significant (147.64 vs 94.90pg/ml, p=0.15). Postoperative INF-γ concentrations in transfused and not transfused patients were not different (Table 3). Similarly, no difference was found between transfused and not transfused groups when both preoperative and postoperative concentrations of IL-10, IL-2, IL-4, IL-5, IL-13 and TNF-α were considered (data not shown).

Discussion

Limiting the burden of postoperative complications is one of the key tasks of modern perioperative medicine, due to an increasing number of surgical procedures being performed each year worldwide. The aim of this study was to evaluate the possible relation of early postoperative complications and inflammatory cytokines levels on the first postoperative day. The results point to major changes in IL-6 production perioperatively, which were not shown for other cytokines (IL-10, IL-2, IL-4, IL-5, IL-13, INF-γ and TNF-α). The significance of the role of IL-6 is underlined by the difference in IL-6 levels in patients who developed predefined complications and in those who had not. Additionally, levels of POD-1 IL-6 were compared in patients who received blood transfusion and those who did not. The results of this comparison suggest the presence of some possible major disturbances caused by blood products transfusion in patient immunity postoperatively. Analysis of possible predictive value of IL-6 in terms of developing infectious complications and myocardial injury showed that POD-1 level of this cytokine had some marked, although minor value, and the concentration of 302pg/ml showed the best value in this regard.

The above results of postoperative cytokine profile are in line with the results of previous reports [5,6,7], although to our best knowledge this is the first report regarding a single type of surgery of this extent. All patients experienced major tissue trauma as a result of the same surgical oncological intervention, thus the study group may be considered relatively homogenous.

Disadvantageous postoperative course may stem from major immune system changes. Trauma – related immune changes are characterized by activation of innate inflammatory response and relative attenuation of adaptive response, mostly lymphocyte T -

dependent pathways [3]. Limiting this phenomenon could potentially reverse undesirable course of immune response and improve surgical outcomes. Interleukin-6 levels are greatly elevated immediately following major surgery and trauma [13], which can last for a number of days [7]. It is understood that IL-6 function in this scenario is not limited to stimulating immune defenses, but may have a role in limiting the activity of antigen – presenting cells like monocytes through a decrease in INF-γ production [13]. In vitro studies have shown that exogenous INF-γ may decrease immune impairment caused by trauma, whereas causative role of IL-6 is still discussed [14]. The magnitude of increase of IL-6 levels in our study was large, which reflects the complexity and duration of the procedure. Boersema et al. have shown significant rise of IL-6 levels following gastrointestinal surgery in a group of similar size, with relatively higher levels in patients with anastomotic leakage and infectious complications. Predictive value of indices derived from IL-6 levels measured on POD-1 and POD-3 was also similar, indicating poor ability to predict the occurrence of early postoperative complications. Nevertheless, as both number of infectious complications and transfusions are not available, comparison with our results is difficult [7]. Postoperative cytokine profile was also assessed in thoracic surgery by Kaufmann et al. Patients with postoperative complications have shown higher levels of serum IL-6 when compared to patients with uncomplicated postoperative course and a number of complications were analysed as one composite outcome. Although predictive value of POD-1 serum IL-6 was similar to the one in our study, nearly three times lower cut-off value proved to have the best sensitivity/specificity index for predicting postoperative complications. The difference may stem from defined types of complications and the fact that patients requiring blood transfusions were excluded from analysis. Our results partially confirm data from the study by Serpa Neto et al., where in the trial investigating intraoperative positive end-expiratory pressure (PEEP) on pulmonary complications higher levels of serum IL-6, IL-8 and TNF-α on POD-1 and POD-5 were noted in patients who developed postoperative pulmonary complications. When serious complications were considered, the difference was significant for IL-6 only and - similarly - predictive value of IL-6 was judged to be poor [5]. No change in POD-1 TNF-α levels were shown in our patients when compared to baseline.

Although it is well understood that blood products cause immune disturbances, literature data on inflammatory cytokine changes following blood transfusion are limited. Higher increase of IL-6 concentration noted on POD-1 in our study in patients with perioperative blood transfusion confirms the impact of this intervention on transfusion - related immunomodulation (TRIM). It appears that perioperative transfusion of allogeneic blood results in considerable and lasting release of inflammatory cytokines [15-17]. These observations have not been confirmed in studies regarding spinal and abdominal surgery, where a relation between transfusion and postoperative levels of IL-6, as well as other essential inflammatory cytokines was not present [18,19]. Major variation in type and duration of surgery could be a reason behind different (lower) increase in cytokines levels and produce different results. In the study by Theodoraki et al., analysis was restricted to 20 patients allocated either too restrictive or liberal transfusion policy, hence the difference between the groups could be linked to volume of transfused RBC [19].

There are obvious limitations of this study. First, the study group was relatively small, resulting in a limited number of predefined complications. Hence, regression analysis to investigate the causation between cytokines levels and complications could not be performed. Second limitation is timing of cytokine sampling. Only one postoperative measurement gives no insight into the dynamics of cytokine production. However, POD-1 for surgical patients is the time when risk stratification is crucial.

Conclusions

This study indicate that postoperative IL-6 levels rise considerably after major abdominal surgery, significantly more than in previous reports based on less extensive surgical interventions. In addition, increase is remarkably higher in patients with major complications and those subjects who required blood transfusion. This accounts for a need to further investigate the perioperative immune response with the aim to find the way to predict and reduce the rate of postoperative morbidity and mortality.

Ethics approval and consent to participate

Ethics Committee approval for the study was granted by the Ethics Committee of Centre of Postgraduate Medical Education, Warsaw, Poland on the 21st of February 2018, 28/PB-A/2018).

List of abbreviations

IL: Interleukin
INF-γ: Interferon gamma
TNF-α: Tumor necrosis factor – alpha
POD-1: Postoperative day one
cTn: cardiac troponin
SSI: surgical site infection
LOS: length of hospital stay
Th1: type 1 helper T lymphocytes
Th2: type 2 helper T lymphocytes
PRC: packed red cells
BMI: body mass index
COPD: chronic obstructive pulmonary disease
AUC: area under the curve
ROC: Receiver Operator Curve

Data Availability

All data regarding this manuscript is available from corresponding author on reasonable request.

Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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Authors' contributions

The individual contributions of authors to the manuscript should be specified in this section.

BH initiated the study, acquired samples and clinical data, and wrote the manuscript. MM-M contributed to initiation and design of the study and writing of the manuscript. MJ, PG and DE performed cytokine analysis and were contributors to data processing and reporting. MP contributed to study design, data collection and processing and manuscript preparation. All authors read and approved the final manuscript."

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