INTRODUCTION

Abdominal aortic aneurysm (AAA) is a disease of inflammatory origin with estimated 5% prevalence in the population >65 years old. Although in the last 20 years the mortality has been reduced to 12% in open elective AAA repair, it is still higher than in other surgical procedures. There are several factors responsible for the high risk of postoperative fatal complications. Concomitant diseases, as well as neutrophil activation and accumulation in the course of ischemia-reperfusion injury seem to be the crucial variables that make the operation different from others and more unpredictable during the postoperative period. Neutrophils (NU) appear to be predominant leukocytes that contribute to mediating ischemia-reperfusion injury. The role of chemotactic cytokines termed chemokines in NU activation and migration to the inflammatory site has been shown in experimental models and clinical situations.

Growth-related oncogene α (GROα, CXC ligand 1) belongs to CXC chemokine subfamily, being characterized by the separation the first 2
The response of GROα is mediated through CXC receptors that are expressed on neutrophils, monocytes/macrophages, T-lymphocytes, natural killer cells, mast cells, neurons and endothelial cells. The ability of GROα to suppress NUs apoptosis and stimulate its own production by these cells has been reported in in vitro studies and can result in amplification of leukocyte inflammatory response. Glynn et al. suggested that abnormal NU apoptosis simulated by GROα contributes to inflammatory response in acute respiratory distress syndrome (ARDS) and multiple organ dysfunction syndrome (MODS), potential severe complications of AAA repair. The increased level of this chemokine has been also found in bronchoalveolar lavage (BAL) fluid from patients with ARDS, bacterial pneumonia, post-lung transplantation ischemic-reperfusion injury, and sepsis, and in urine of uroseptic patients. A role of GROα in AAA patients undergoing surgery and its relation to clinical ischemia-reperfusion and the development of postoperative complications have not been studied yet.

The aim of this study was to evaluate serum GROα levels during elective AAA repair and the relationship between their changes and ischemia-reperfusion and the postoperative course.

PATIENTS AND METHODS Seventeen men hospitalized in the Department of Vascular Surgery and Transplantology of Medical University in Białystok with diagnosis of infrarenal AAA scheduled for open elective surgery were included in the study. Mean age of these patients was 65 years (44–77 years). Clinical characteristics of the study group are presented in Table 1. The exclusion criteria were a positive history of malignant, rheumatic or immunologic diseases. The Medical University of Białystok Ethical Committee on research on humans and animals has approved this study. All patients gave their written consent for participation in the study.

The control group comprised of 11 healthy men recruited from the hospital personnel, with no medical history of any inflammatory disease, cancer and nicotineism, with abdominal ultrasonography done during the last 6 months with no evidence for AAA. Mean age of the control group was 61 years (55–72 years).

All patients were qualified by an anesthesiologist the day before surgery and perioperative risk was estimated according to the American Society of Anesthesiologist (ASA) score and Goldman Cardiac Risk. The operation was performed under general anesthesia with fentanyl, etomidate and cis-atracurium for induction, maintained with sevoflurane, oxygen/air mixture and fentanyl. Additionally continuous epidural anesthesia was provided unless contraindications were present. Antibiotic prophylaxis consisted of 1 g cefazoline on induction, followed by 2 further postoperative doses. Patients underwent elective AAA repair with bifurcated graft placement with identical stents Uni-Graft KD.V supplied by B/Braun (Aesculap AG & CO KG, Germany).

Peripheral venous blood samples were taken before surgery in the Operating Room before anesthesia induction (Preop), just before aortic unclamping (Pre-Xoff), 90 min after unclamping (90min-Xoff), and 24 h after surgery. Three-ml samples of blood were taken with minimal stasis from the antecubital vein to tubes for serum and centrifugated for 10 min at 3000 rpm. The serum was removed to 2-ml micro tubes and stored at a temperature of −70°C till measurement. The level of GROα was measured 2 times in each sample with an enzyme-linked immunosorbent assay (ELISA) (R&D Systems, Abingdon, UK), according to the manufacturer’s instructions. Other tests like blood cell count, electrolyte, creatinine, bilirubin, albumin and total protein levels, aminotransferase activity, and coagulation tests were measured by standard laboratory methods before and after surgery. Based on clinical observations and laboratory tests the clinical assessment of patients was performed on the 1st and 2nd day after surgery, according to Acute Physiology and Chronic Health Evaluation II and III scores (APACHE II and APACHE III), the Sequential Organ Failure Assessment (SOFA) and the Multiple Organ Disfunction (MOD) score.

Statistical analysis was performed using the STATISTICA 5.0 for Windows. Since the data were not normally distributed, the results were presented as median and range, and for comparison within and between the groups non-parametric Wilcoxon and Mann-Whitney U tests were used. To analyze correlations the Spearman rank correlation coefficient was calculated. A p <0.05 was considered as statistically significant.

RESULTS Nine patients made an uncomplicated recovery. Eight patients developed complications, local complications like minor wound infection and thrombosis of the left prosthetic arm were observed in 2 patients. Among the remaining 6 patients 2 developed pneumonia, and the cases of disseminated intravascular coagulation, intraabdominal bleeding, an acute cardiac ischemia and decompensated chronic heart failure...


**TABLE 1 Clinical characteristics of the study group**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>AAA patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of patients</td>
<td>17</td>
</tr>
<tr>
<td>age (years)</td>
<td>65 (44–76)</td>
</tr>
<tr>
<td>ASA score</td>
<td>3 (2–4)</td>
</tr>
<tr>
<td>Goldmann score</td>
<td>8 (3–29)</td>
</tr>
<tr>
<td>duration of surgery (min)</td>
<td>230 (180–310)</td>
</tr>
<tr>
<td>duration of clamping (min)</td>
<td>66 (45–85)</td>
</tr>
<tr>
<td>duration of hospital stay (days)</td>
<td>13 (10–25)</td>
</tr>
<tr>
<td>concomitant diseases (%)</td>
<td></td>
</tr>
<tr>
<td>atherosclerotic vascular disease</td>
<td>94</td>
</tr>
<tr>
<td>hypertension</td>
<td>60</td>
</tr>
<tr>
<td>coronary heart disease</td>
<td>50</td>
</tr>
<tr>
<td>chronic heart failure</td>
<td>12</td>
</tr>
<tr>
<td>diabetes</td>
<td>18</td>
</tr>
<tr>
<td>nicotinism</td>
<td>65</td>
</tr>
<tr>
<td>aneurysm diameter (mm)</td>
<td>45 (35–70)</td>
</tr>
</tbody>
</table>

Values are shown as medians and ranges.

Abbreviations: AAA – abdominal aortic aneurysm, ASA – American Society of Anesthesiologists

with MODS were observed. Complications were defined based on specialist consultations.

**Serum GROα levels** The median serum GROα levels were similar in controls and AAA patients before surgery (86 pg/ml vs. 79 pg/ml, p >0.05) (**FIGURE 1**). During surgery the GROα level showed nearly the same median value of 76 pg/ml at Pre-Xoff, with a tendency to a decrease to 61 pg/ml 90 min after cross-clamp release, followed by an increase to 100 pg/ml 24 h after surgical procedure. The differences were not statistically significant.

**The level of GROα in serum of patients with complicated and uncomplicated postoperative courses** In an attempt to examine the role of GROα in the development of complications, chemokine levels in patients with systemic complications and with uncomplicated courses were analyzed (**FIGURE 2**). The preoperative median GROα level was slightly lower in the group with complications compared to those without complications (70 pg/ml vs. 80 pg/ml). The GROα level was almost unchanged during AAA repair and was from 70 pg/ml to 85 pg/ml at Pre-Xoff and 76 pg/ml at 90min-Xoff, with a higher postoperative value of 133 pg/ml in serum of 6 patients who developed systemic complications. In 11 patients with an uncomplicated course a tendency to a decrease in GROα levels from 80 pg/ml to 63 pg/ml at Pre-Xoff and to 58 pg/ml at 90min-Xoff has been observed, followed by an increase to 90 pg/ml 24 h after surgery (p = 0.05 vs. 90min-Xoff). There were no statistically significant differences in GROα levels between patients with complicated and uncomplicated courses, or patients and healthy controls.

**Correlation calculations** The relationships between serum level of GROα and preoperative, operative and postoperative patient data which influence morbidity and mortality were analyzed. Significant positive correlations are presented in **TABLE 2**. No statistically significant relationship between chemokine level and age, APACHE II and APACHE III score and aneurysm diameter was found.

**DISCUSSION** Local chemokine expression is the first step in the development of inflammation after ischemia-reperfusion. The GROα is a potent chemotactic molecule for neutrophils and expression of this chemokine has been found in several inflammatory models and diseases, vital implicating its role in the pathogenesis of inflammation.

Detailed analysis of data obtained during the present study showed slightly lower preoperative GROα levels in patients with complicated courses comparing to those with uncomplicated courses. Differences in the preoperative GROα level, however insignificant, might be associated with some kind of NU dysfunction as the effect of local aortic inflammation and degeneration.

Preoperative chemokine levels can also be influenced by the degree of atherosclerosis and coexistence of diabetes, that are directly associated with vessel inflammation and were diagnosed in the study group with AAA.

During AAA repair, cross clamping of the aorta is associated with ischemia of distal tissues. GROα levels were measured just at the end of ischemia lasting 45–85 min (median 66 min), after 90 min of reperfusion and 24 hours after surgery. The analysis showed a tendency to a decrease in serum GROα levels after 90 min of reperfusion in patients with uneventful outcomes contrary to the group with complications. The lack of statistically significant differences between GROα serum levels may be explained by mostly local not systemic chemokine activation and a limited number of studied patients. The dominance of local GROα was underscored by Mathiak et al. as they found the high chemokine level in BAL fluid, but not in serum from septic patients. Similarly, Olzyna et al. observed elevated GROα levels in urine, but not in serum from patients...
TABLE 2  Correlations between the patient data and growth-related oncogene α levels (pg/ml) in serum of patients undergoing abdominal aortic aneurysm surgery

<table>
<thead>
<tr>
<th>Patient data</th>
<th>serum GROα (pg/ml) level</th>
</tr>
</thead>
<tbody>
<tr>
<td>ASA score</td>
<td>R = 0.258, p = 0.033</td>
</tr>
<tr>
<td>duration of clamping (min)</td>
<td>R = 0.322, p = 0.0074</td>
</tr>
<tr>
<td>duration of surgery (min)</td>
<td>R = 0.317, p = 0.0084</td>
</tr>
<tr>
<td>MODs 2nd day after surgery</td>
<td>R = 0.417, p = 0.0006</td>
</tr>
<tr>
<td>SOFA 2nd day after surgery</td>
<td>R = 0.352, p = 0.0043</td>
</tr>
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Spearman rank correlation coefficients (R) and p values are presented. Abbreviations: ASA – American Society of Anesthesiologist, MODs – multiple organ dysfunction score, SOFA – sequential organ failure assessment

FIGURE 2  Serum levels of GROα (pg/ml) before operation (Preop), before unclamping of the aorta (PreXoff), 90 min after unclamping (90minXoff), and 24 h after surgery in the group of patients with systemic complications (n = 6) (solid boxes) and in patients with the uncomplicated course (n = 11) (open boxes) undergoing AAA surgery. Dotted line presents the median GROα level in the control group. Values are presented as median (line, number), 25th and 75th percentile (box) and range.

with urosepsis. Because the current study was the first to investigate GROα serum levels in such clinical conditions, it was planned as preliminary and thus included the limited number of patients, which reduced the chance of getting statistically significant differences in the studied parameter. Taking into account studies by Belperio et al. and Sievert et al. that showed that NU activation with increased activity of CXC chemokines and its interaction with receptor enhance ischemic-reperfusion injury during organ transplantation, changes observed during AAA repair seemed to be worth discussing. As it has been shown that the removal of leukocytes using leukocyte-depleting filters decreases organ injury during ischemia and reperfusion, we assumed that the lower GROα level during AAA repair could represent the protective mechanism against systemic inflammatory response. Munford et al. suggested that such a reaction may prevent organ injury and systemic complications in response to ischemia and reperfusion during AAA repair.

Unlike the uncomplicated group, the analysis of patients with postoperative complications showed a tendency to the higher GROα serum level during surgery, with an increase by about 50% at 24 hours after procedure. High risk of perioperative complications assessed with the ASA score, and the long time of aorta clamping and surgery were associated with the higher chemokine serum level during follow-up. High GROα levels during the perioperative period also correlated with higher degrees of organ dysfunction according to the MOD score and SOFA score, calculated on the 2nd day after surgery. We failed to find other clinical studies on such relationships. There are however data confirming the association between the GROα level and inflammatory events, ischemia-reperfusion or operative injury. Experimental studies have provided strong evidence for higher expression of mouse GROα in infiltrating inflammatory cells during ischemia and myocardial or renal reperfusion. Simultaneously, there was a relationship between the high chemokine level and reperfusion organ injury. Treatment with neutralizing antibodies to mouse GROα was associated with inhibited NU infiltration, restored renal function and reduced mortality. Authors suggested that the use of strategies directed at blocking neutrophil chemokine receptors should be introduced to reduce tissue damage during ischemia and solid organ transplantation. Similarly, Souza et al. showed that repertaxin, an inhibitor of rat CXCR2 function, inhibits inflammatory response that follows intestinal ischemia-reperfusion, suggesting that drugs known to block CXCR2 receptor may be effective in the prevention of reperfusion injury in relevant clinical situations.

Features of lung and liver injury and an increase in lung and serum GROα levels with the maximum value at 2 hours after reperfusion were also observed in the experimental mouse model of visceral ischemia. The presence of bacteria and/or LPS in blood might also induce systemic GROα production and secretion during AAA repair. Cumming et al. observed increased GROα levels in plasma from septic patients and decreased CXCR2 expression on circulating polymorphonuclear NU. The possible mechanisms of down-regulation of CXCR2 expression on NUs from patients with severe sepsis may involve the activity of other cytokines like TNF-α, hypoxic conditions and LPS. The suppression of chemotactic response to the CXC chemokines which bind with high affinity to only CXCR2 may be included in strategy to limit NU migration, inflammation and organ dysfunction in septic patients. Shokuh et al. showed that GROα levels were significantly higher in patients with severe acute pancreatitis than in subjects with a mild form of the disease and healthy controls. The median peak level of chemokine within the first 48 hours of admission was higher in patients who died than in survivors. Elevated serum levels of GROα were also found in patients with liver cirrhosis and these levels were higher in patients with spontaneous bacterial peritonitis than in non-infected cirrhotic patients. The highest serum levels of GROα were detected in those patients who died, but the differences were not statistically significant.

The tendency to elevated serum GROα levels observed in AAA patients with the complicated postoperative course in comparison to the uncomplicated group is in agreement with observations made by other authors and may contribute
to continuation of the studies that can lead to development of new therapeutic models. There is a new approach in clinical studies to use CXCR2 as a trigger to lessen tissue neutrophil accumulation to lung in patients with post-lung transplantation injury.11 We believe that the modulation of enhanced production and secretion of GROα during surgery through its receptor CXCR2 will be a new important step in protecting ischemia-reperfusion organ injury in the postoperative period, which however requires further experimental and clinical studies. This paper is a continuation of our previous research that assessed a profile of interleukin-1226 and measured the serum levels of regulated-on-activation normal T cell expressed and secreted with monocyte chemotactic protein-125 in patients undergoing reconstructive surgery.

In conclusion, AAA repair is associated with insignificant changes in serum GROα levels, with their decrease after ischemia and 90 min of reperfusion. A higher level of GROα may result from longer ischemia and/or surgery. Decreased chemokine levels after ischemia and reperfusion may suggest an uncomplicated post-operative course, which however should be evaluated in further studies in such clinical settings. On the contrary, lack of these changes with a tendency to the high GROα level during surgery may be associated with high risk of postoperative organ dysfunction in patients undergoing AAA repair.

Acknowledgments The study was supported by a research grant from the Medical University in Białystok, Poland (No 3-14678L).

REFERENCES

ARTYKUŁ ORYGINALNY

Stężenie chemokiny GROα w surowicy podczas operacji tętniaka aorty brzusznej

Badanie wstępne

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SŁOWA KLUCZOWE
chemokiny, niedokrwienie-reperfuzja, tętniak aorty brzusznej

STRESZCZENIE

WPROWADZENIE Głównymi czynnikami dużej śmiertelności chorych poddawanych operacji tętniaka aorty brzusznej (aortic abdominal aneurysm – AAA) są niedokrwienie i reperfuzja, prowadzące do uszkodzenia naprawów oraz zespołu dysfunkcji wielonarządowej (multiple organ dysfunction syndrome – MODS). Badania doświadczalne wykazały udział chemokiny GROα (growth-related oncogene α) w rozwoju niedokrwienia i reperfuzji.

CELE Celem badania była ocena stężenia GROα w surowicy podczas planowej operacji AAA oraz związku obserwowanych zmian z niedokrwieniem, reperfuzją i przebiegiem pooperacyjnym.

PACJENCI I METODY Krew obwodową do badań pobierano u 17 chorych przed operacją (Preop), przed zaciśnięciem zacisku z aorty, 90 minut po jego zaciśnięciu (90 min-Xoff) i 24 godziny po operacji oraz u 11 osób z grupy kontrolnej. Stężenie GROα oznaczano metodą immunoenzymatyczną.

WYNIKI Podczas operacji AAA obserwowano nieistotne zmniejszenie stężenia GROα z 79 pg/ml w Preop do 61 pg/ml w 90 min-Xoff, które zwiększyło się do 100 pg/ml 24 godziny po operacji. W przypadkach powikłanych stężenie GROα podczas operacji wykazywało trend wzrostowy, a największy poziom – 133 pg/ml – obserwowano 24 godziny po operacji. Stwierdzono istotną dodatnią korelację między GROα i czasem trwania operacji (r = 0,317), czasem zaciśnięcia aorty (r = 0,322) i wskaźnikiem MODS (r = 0,417).

WNIOSKI Operacja AAA jest związana z nieistotnymi statystycznie zmianami stężenia GROα w surowicy. Zmniejszenie stężenia chemokiny po niedokrwieniu i reperfuzji może wskazywać na prawdo podobieństwo niepowikłanego przebiegu pooperacyjnego. Tendencja do dużego stężenia chemokiny może być związana z ryzykiem pooperacyjnej dysfunkcji narządowej u chorych poddawanych operacji AAA.