Original Article

STANDARDIZATION OF PROCALCITONIN, C-REACTIVE PROTEIN AND LACTATE LEVELS IN PIGS SUBMITTED TO LAPAROTOMY

PADRONIZAÇÃO DOS NÍVEIS DE PROCALCITONINA, PROTEÍNA-C REATIVA E LACTATO EM SUÍNOS SUBMETIDOS À LAPAROTOMIA

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ABSTRACT: This study was undertaken to determine the procalcitonin, C-reactive protein and lactate levels in pigs anesthetized and submitted to laparotomy during 5 hours. A total of 05 landrace pigs with 3 months of age and weighing 26 kg were used. After a midline xiphopubic laparotomy, the procalcitonin, C-reactive protein (CRP) and lactate levels were measured at intervals of 60 minutes for 5 hours with samples taken from the jugular vein. The procalcitonin value remained below 0.05 µg. The CRP values were between 6.7 and 8.3 and lactate levels remained between 2.9 and 3.7 for the confidence interval of 95%. We conclude thtat in pigs submitted to laparotomy for 5 hours, the procalcitonin levels did not exceed 0.05 ng / mL. The C-reactive protein and lactate levels were between 6.7 and 8.3 mg / L and 2.9 and 3.7 mg / d, respectively. These data allowed reducing the number of animals.

KEYWORDS: Pigs. Inflammation mediators. Reference standards.

INTRODUCTION

Animal models allow investigations that otherwise would be impossible to be studied in human beings. Pigs have been widely used in medical research to the understanding of various diseases and pathological processes (ROUSSI et al., 1996).

Surgery is often the proposal of new surgical procedures that can be simulated in animals. Studies may evaluate not only the feasibility of the procedure itself, but also its pathophysiologic consequences and therapeutic effectiveness (FAGUNDES e TAHA, 2004).

Several studies have been conducted using procalcitonin, C-reactive protein (CRP) and lactate in an attempt to establish early diagnosis, degree of severity of injuries and also prognostics in several pathologies (ANDRIOLO et al., 2004).

Procalcitonin and CRP are acute phase proteins, produced from inflammatory and infectious stimuli (ANDRIOLO et al., 2004).

Under normal conditions, this protein is present in very low concentrations in the circulation, remaining inside the cells as the main calcitonin-precursor peptide, but it may rise in severe infections, serious burns, multiple traumas, in thoracic surgery, cardiovascular and colorectal postoperative period, and in response to endotoxin and inflammatory cytokines (ANDRIOLO et al., 2004).

In the study by Meisner et al. (1998) in a population of 130 patients in the postoperative period, high procalcitonin levels in major surgeries were found. However, the correlation with inflammation processes after surgery and the prognosis have not yet been well established.

In a literature review by Uzzan et al. (2006) in order to quantify the accuracy of procalcitonin for diagnosis of sepsis in adult patients in intensive care units after trauma surgery in comparison with C-reactive protein describe that procalcitonin may be high in the non-infectious systemic inflammatory response syndrome and immediately after surgeries or trauma.

The C-reactive protein is produced in the liver in response to stimulation by inflammatory cytokines (interleukin-1, interleukin-6 and tumor necrosis factor). It is a positive-behavior acute phase protein, i.e., its concentration rises markedly shortly after the occurrence of an injury. There is an increase both in infectious and non-infectious processes and its serum levels depends on the intensity of the inflammatory response (UZZAN et al., 2006).

The lactate level is used in a number of diseases. Surgical trauma causes changes in the hemodynamics, metabolism and immune response

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of patients in the postoperative period. Like most physiological responses, the response to injury is a dynamic process that follows specific paths defined by clinical and scientific observation (CHOILEAIN e REDMOND, 2006).

The activation of the cascade of cytokines in response to surgical trauma consists of a complex biochemical network with diverse effects on the local wound (CHOILEAIN e REDMOND, 2006).

This study aims to: define the procalcitonin, C-reactive protein and lactate levels in pigs anesthetized and submitted to laparotomy for 5 hours and provide researchers with an established model for future researches on these mediators that need to conduct laparotomy in experimental pathologies of the abdominal cavity, thus reducing the number of animals used.

MATERIAL AND METHODS

Five landrace pigs with 3 months of age and weighing 26 kg were used, given by the vivarium of the University Center of Espírito Santo - UNESC. The animals were submitted to complete blood count, biochemistry, vaccination and worming prior to the execution of the experiment. This study was approved by the Ethics Committee of the Meridional Hospital (Vitoria, Espírito Santo, Brazil) through law 03-29/2009.

The experiment was conducted at the Laboratory of Experimental Surgery at the University Center of Espírito Santo (UNESC), supported by the Tommasi Clinical Analysis Laboratory and the Animal Center of the University Center of Espírito Santo - UNESC.

After anesthetic induction with thiopental, the animals were placed in supine position on the operating table receiving saline solution of NaCl at 0.9% through the auricular vein for maintenance of anesthesia by continuous infusion of ketamine, xylazine and Midazolam.

Dissection and cannulation of the jugular vein were performed for collection of samples and a xiphopubic laparotomy was performed according to standard technique.

The procalcitonin, C-reactive protein and lactate levels were measured at intervals of 60 minutes (T0, T1, T2, T3, T4, T5) for 5 hours with samples taken from the jugular vein.

At the end of the experiment, animals were euthanized as recommended by Resolution 714 of June 20, 2002 the Federal Council of Veterinary Medicine through anesthetic overdose of thiopental 5% IV (20 ml), followed by infusion of potassium chloride (3 ml) up to the detection of cardiorespiratory arrest.

The dead pigs were kept frozen in a freezer and discarded through the medical waste collection service in the city of Colatina, Espírito Santo, Brazil.

For the lactate and C-reactive protein analysis, the Vitros 250 device (JOHNSON AND JOHNSON CLINICAL DIAGNOSTICS, USA) was used by the method of dry chemistry with colorimetry.

The procalcitonin analysis was performed with the Mini-Vidas device (BIOMERIEUX BRASIL S.A.) using the ELFA methodology (Enzyme Linked Fluorescent Assay), which carries an enzyme immunoassay and finalizes the analysis using immunofluorescence.

The data were submitted to inferential statistical analysis to determine the confidence interval of 95% for the average population for variables lactate, procalcitonin and C-reactive protein. Statistical analyses were made using the BioEstat 4.0 (AYRES et al., 2005) computer program according to procedures recommended by Vieira, 2000 (VIEIRA, 2000).

RESULTS

The procalcitonin evaluations did not reveal any increase during the times. All remained with value below 0.05 µg, minimum value detected by the equipment.

The C-reactive (CRP) protein values were between 6.7085 and 8.2915 for the confidence interval of 95% (Figure 1).

Lactate levels remained between 2.8594 and 3.7406 for the confidence interval of 95% (Figure 2).

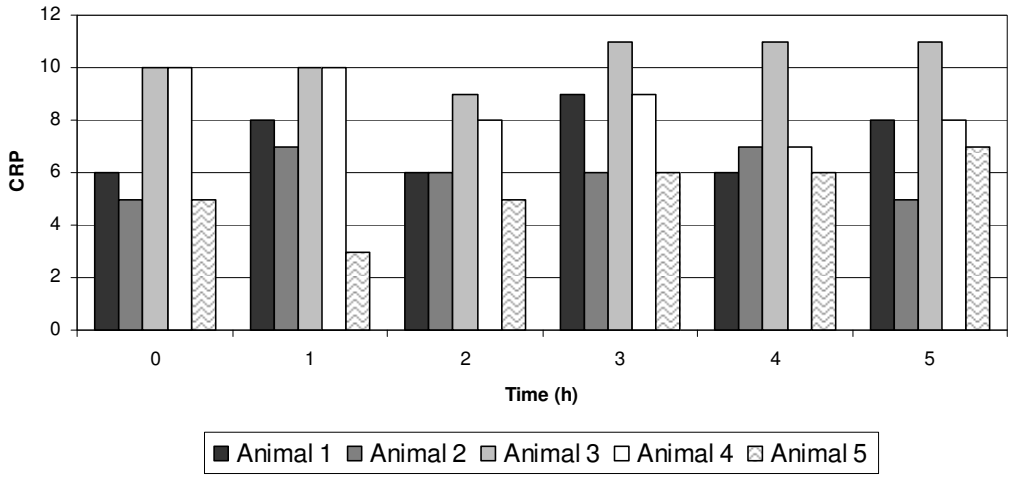

Figure 1. C-reactive (CRP) protein values during five hours of laparotomy.

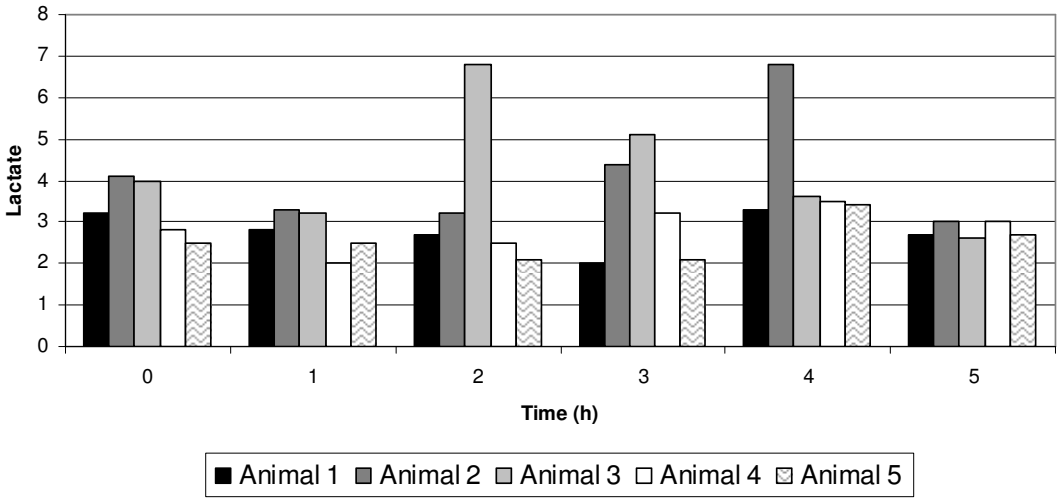

Figure 2. Lactate values during five hours of laparotomy.

DISCUSSION

Several diseases can be generated in animal models aiming at a more detailed study on diagnosis, treatment and prognosis.

Experimental models of abdominal pathologies are widely used by researchers.

Pimenta et al. (2007) used the clamping of the abdominal aorta to generate ischemia/reperfusion lesion in order to prove the production of interleukin 6 and C-reactive protein. Three groups of 8 Wistar rats were used.

The control group was submitted to laparotomy, the ischemia group was submitted to laparotomy followed by 2 h of aortic clamping and the ischemia/reperfusion group was submitted to 2 hours of ischemia and 2 hours of reperfusion. The C-reactive protein levels did not show statistical changes in any of the three groups.

This result indicates that laparotomy does not affect the C-reactive protein levels; however, the procedure time was only two hours, unlike our experiment, which kept the animal in laparotomy for 5 hours. Buunen et al. (2004) reported that the C-reactive protein undergoes increases of up to 1000 times after conventional surgery.

Kurimoto et al. (2008) used an experimental pig model of mesenteric ischemia to assess the lactate concentration in blood and in the superior mesenteric vein. These authors found blood lactate values in the control group of 9.4 mg / dl, higher than those found in our study, which were between 2.9 and 3.7 mg / dl. This difference in values may be related to a greater manipulation degree that the animals were submitted in the study of Kurimoto.

After laparotomy, the animals had the femoral vein, femoral artery and hepatic vein cannulated for collection of samples. In our study, the pigs were submitted to laparotomy only with a small access to the jugular vein to collect blood samples.

Ayten et al. (2005) evaluated the predictive value of procalcitonin for the diagnosis of intestinal strangulation in New Zealand rabbits collecting venous blood samples through the auricular vein. Thirty animals were used and divided into three groups. The control group was submitted to laparotomy only. In the second group, a simple obstruction was created and the third group was submitted to bowel strangulation.

The results showed increases in PCT only in the third group, starting after 30 minutes and with results statistically significant after 120 minutes.

Solomon et al. (2002) conducted a randomized study comparing the inflammatory stress among patients submitted to abdominal rectopexy in patients with rectal prolapse through laparoscopy and laparotomy.

The researchers assessed cortisol, interleukin-6, C-reactive protein and erythrocyte sedimentation rate in preoperative times and in 4, 24 and 48 hours of postoperative times. The C-reactive protein values proved to be higher in laparotomy at the time of 24 hours (7.7 mg / L) than in the laparoscopy group (4.4 mg / L). At time of 48 hours, there was also a higher value in laparotomy compared to laparoscopy - 11.2 *versus* 7.0 mg / L, indicating a more intense inflammatory process in the open abdominal surgery.

Also interested in knowing the inflammatory stress level between laparoscopy and laparotomy, Uzunkoy et al. (2000) performed preperitoneal hernia repair using both techniques and measured the glucose, cortisol, C-reactive protein, creatinine phosphokinase, transferrin, fibrinogen and albumin concentrations at 2, 24 and 48 hours. The C-reactive protein level for the laparoscopic procedure was on average 6.1 mg / L, while the level after laparotomy was 8.3 mg / L. Grande et al. (2002) also described the C-reactive protein values between groups of open cholecystectomy and laparoscopy. The values between groups were 8.4 mg / L in the open laparotomy group and 1.30 mg / L in laparoscopy.

The values of these two latter studies in humans have been corroborated by our experimental study in pigs, indicating that the values found in these animals can be extrapolated to humans.

Zegin et al. (2002) studied the inflammatory response levels after surgery using 30 patients submitted to surgery for morbid obesity to check the C-reactive protein, haptoglobin, albumin, transferring and interleukin-6 levels, among others. Patients were divided into 2 groups for the placement of adjustable gastric band. One group was through laparotomy and another through laparoscopy. Samples were collected at 6, 12, 24 and 48 hours after surgery and compared using the nonparametric Kruskal Wallis test.

The C-reactive protein values in both groups were extremely high. In humans, the reference value is 0-5 mg / L and the group submitted to laparoscopy showed 160 mg / L and the laparotomy group 254 mg / L, indicating that open surgery was more aggressive than the closed type.

These extremely high values compared to our study may indicate that the presence of the gastric band induces an inflammatory stress process in the body.

Wade et al. (1989) established that lactate could be used as a survival predictor in controlled hemorrhage in pigs. The group of animals that survived have maintained the lactate levels on average 43.9 mg / dl and the animals that died had lactate 106.5 mg / dl. In conclusion, the lactate level of 125 mg / dl had successful predictive of 81%.

Machado et al. (2007), studied the correlation between increases in serum lactate levels and complications in the immediate postoperative period of orthotopic liver transplantation. They found that the serum lactate was the only parameter significantly different at the first evaluation after transplantation among patients who had a favorable course and patients who developed severe complications. This suggests that high lactate levels at the time of acceptance in the intensive care unit should be alert to the increased risk of postoperative complications. They also conclude that neither aminotransferases nor total bilirubin proved to be of prognostic use in this context.

Kouame et al. (2005) evaluated the predictive importance of procalcitonin in children with appendicitis. The normal values considered were <0.05 µg and from 33 patients submitted to the surgical procedure and detected with appendicitis, only 4 had levels above this standard, thus indicating that procalcitonin does not have good predictive value for appendicitis in children.

Rau et al. (1997) evaluated procalcitonin as predictive parameter of acute pancreatitis with infected necrosis in adults and concluded that it is an excellent marker for monitoring of cases after debridement.

These experimental studies indicate that C-reactive protein, procalcitonin and lactate have been studied for many decades in an attempt of finding

patterns in their levels indicating postoperative complications, severity of disease and predictive values at the bedside.

Pigs are the closest animals to humans in terms of anatomy and physiology, particularly in hemodynamics. Thus, this study demonstrates the levels of these markers in order to collaborate with researches in this area, defining the standard of these tests in animals submitted to midline laparotomy, which is the most widely used access to the creation of several experimental models of diseases in abdominal organs. Knowing the behavior of procalcitonin, C-reactive protein and lactate in laparotomy provides subsides to researchers about the injury and inflammatory response related to this procedure, so that misinterpretations on these levels in future researches will no longer occur.

In our experimental surgery laboratory, we have a research line in inflammatory and ischemic chemical mediators, and this work is part of an analysis that will be performed in models of mesenteric ischemia, liver and kidney transplantation and pancreatic resections, as well as bariatric surgeries to reduce weight.

CONCLUSIONS

Some studies indicate that acute inflammatory phase proteins and lactate may be useful as indicators of various pathological processes in abdominal organs.

This study shows that pigs submitted to laparotomy for 5 hours do not show changes in the procalcitonin values; thus, future experimental studies using this acute phase protein can adopt any value above 0.05 ng / mL as reference.

Regarding the C-reactive protein, values between 6.7 and 8.3 mg / L were found in this model. Also, studies using pigs as experimental models that adopt the midline laparotomy may consider these values as reference.

The values found in this study for lactate were between 2.9 and 3.7 mg / dL. Further researches using pig models to simulate pathologies that require laparotomy may adopt these values as reference.

Thus, the number of animals can be reduced, reducing the impact to society and the financial cost of such studies.

This work is the basis of a research line developed in our institution on ischemic and inflammatory mediators in experimental surgery.

RESUMO: Este estudo objetivou definir os níveis de procalcitonina, proteína-c reativa (PCR) e lactato em porcos anestesiados e submetidos à laparotomia mediana por 5 horas fornecendo aos pesquisadores um modelo estabelecido para futuras pesquisas sobre estes mediadores em que se necessite realizar laparotomia em patologias experimentais da cavidade abdominal, reduzindo assim o número de animais utilizados. Após a indução anestésica com tiopental, os animais foram posicionados em decúbito dorsal e realizada a dissecção e canulação da veia jugular para as coletas das amostras. Os níveis de procalcitonina, proteína-c reativa e lactatemia foram mensurados em intervalos de 60 minutos por 5 horas com amostras colhidas da veia jugular. As avaliações da procalcitonina não revelaram aumento durante nenhum dos tempos. Todas se mantiveram com valor abaixo de 0,05 µg. Os valores do PCR ficaram entre 6,7085 e 8,2915 e os valores de lactato permaneceram entre 2,8594 e 3,7406 para o intervalo de confiança de 95%. Conclui-se que suínos submetidos à laparotomia mediana por 5 horas não apresentam alterações nos valores de procalcitonina, podendo então, futuras pesquisas que se utilizarem desta proteína de fase aguda adotar o valor acima de 0,05 ng/mL como referência. Em relação à proteína-c reativa, encontramos valores entre 6,7 e 8,3 mg/L e o lactato entre 2,9 e 3,7 mg/dL. Futuras pesquisas que utilizarem suínos, como modelo experimental de patologias adotando a laparotomia mediana, poderão considerar estes valores como referência. Desta forma, podemos diminuir o número de animais, reduzindo o impacto perante a sociedade e o valor financeiro destes estudos.

PALAVRAS CHAVE: Suínos. Mediadores inflamatórios. Valores de referência.

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