Evaluation of blood biomarkers in rats submitted to nanotextured and polyurethane foam-coated silicone implants

Avaliação de biomarcadores sanguíneos em ratas submetidas à colocação de implantes de silicone nanotexturizados e revestidos pela espuma de poliuretano

Introduction: In recent decades, there has been a great evolution in breast implants’ lining surface, which has resulted in decreased complications. In the postoperative period, the inflammation is constant and can be evaluated by the blood count, as it is a fast, inexpensive, and highly available examination. The present study evaluates blood biomarkers in rats submitted to the placement of nanotextured silicone implants and implants coated with polyurethane foam. Methods: 60 Wistar rats were used divided into two groups for nanotextured mini-implants and others mini-implants coated with polyurethane foam, subdivided into subgroups according to the animals’ euthanasia in the 30, 60, and 90 days. At the time of euthanasia, blood samples were obtained by cardiac puncture and the blood count was analyzed. Results: Hemoglobin, hematocrit, mean corpuscular hemoglobin, leukocytes, neutrophils, lymphocytes, and platelets had very similar results in all subgroups evaluated (30, 60, and 90 days). However, when the different subgroups were compared within each group, statistically significant results were obtained in the mean corpuscular hemoglobin (nanotextured p=0.032 and polyurethane p=0.007) and leukocytes (nanotextured p=0.038 and polyurethane p=0.034). Changes in postoperative blood biomarkers were hypochromic anemia, normal leukocyte count, neutrophilia, lymphopenia, and thrombocytopenia. Conclusion: After the placement of mini-silicone implants, the rats of both groups evolved with hypochromic anemia, normal leukocyte count at the expense of neutrophilia and lymphopenia, and thrombocytopenia. Keywords: Biomarkers; Blood cell count; Rats; Breast implant; Mammoplasty.
Evaluation of biomarkers in rats submitted to implants

One way to evaluate the inflammatory response is the blood count, which is divided into red and white sets, and platelets. Its advantages are to be a quick, inexpensive and high availability exam.

Through this examination, the biological response to the implant can be evaluated by toxicity and rejection (number of red blood cells) and by the response to cellular stress (neutrophil/lymphocyte/NLR ratio), correlated with mortality in response to device implantation or infections.

As an example, we can mention a rare complication, toxic shock syndrome, after breast implant surgery. This disease has as characteristic the lack of local signs such as edema or erythema but presents fever and a marked increase in white blood cell count, suggesting severe infection. Therefore, early diagnosis is usually difficult, and appropriate treatment initiation can be delayed without the knowledge of this characteristic.

INTRODUCTION

Breast implant mammoplasty is one of the most performed plastic surgeries worldwide1. Thus, breast implants have been the target of research, mainly focusing on their coating’s surface, which can be divided into smooth and textured, the latter being subdivided into micro and textured macro2-5.

Thus, in recent decades, there has been a great evolution in breast implants’ lining surface, resulting in the prevention of mammoplasties complications, such as capsular contracture6,7, infection7, and, more recently, large cell anaplastic lymphoma (BIA-ALCL).8

BIA-ALCL has been the subject of studies in recent years, as there has been an increase in its incidence, especially in patients undergoing mammoplasties with implants6,8,9. In the pathophysiology of this disease, it is known that neoplasia is directly related to the host’s inflammatory response to the implant6,9.
In the inflammatory response, neutrophils are one of the first immunological cells to be recruited to go to the injured site and secrete various inflammatory cytokines that contribute to the beginning of this response. However, the biological response of neutrophils to implanted devices remains uncertain.

OBJECTIVES

The present study aims to evaluate at the laboratory through blood biomarkers the rats submitted to the placement of mini-implants of silicone nanotextured and mini-implants coated with polyurethane foam.

METHODS

The research was carried out in the Universidade Estadual de Ponta Grossa (UEPG) experimental surgery vivarium after being approved by the Ethics Committee on the Use of Animals (CEUA) of UEPG. CEUA Case - 041/2018. EUPG Protocol: 16450/2018. All procedures strictly followed existing animal research regulations.

The study design was a primary study (randomized clinical trial), interventional, experimental in animals (rats), prospective, analytical, controlled, randomized, double-blind and unicentric.

A total of 60 albino rats (Rattus norvegicus albinus, Rodentia Mammalia) weighing between 190 to 250 grams and 30 to 60 days of life had free access to water and diet specific to the species, with room temperature and circadian cycles of 12 hours.

They were randomly divided into two groups of 30 animals for each type of mini-silicone implant (nanotextured and polyurethane foam) and subdivided into three subgroups, according to the euthanasia time of the animals (30, 60, and 90 days).

In the nanotextured group, n = 30, mini-implants with a nanotextured surface (Silimed®, Rio de Janeiro, Brazil) were placed, and in the polyurethane group, n = 30, mini-implants with polyurethane foam coating were placed (Silimed®).

The implanted materials had the same layers of a human breast silicone implant, discoid shape, with 22 +/- 1 mm (mm) in diameter and 9 +/- 1 mm in height in mini-implants with a nanotextured surface, and with 24 +/- 1 mm in diameter and 11 +/- 1 mm high in polyurethane foam-coated mini-implants. Height was defined as the point of greatest implant projection on the vertical axis (Figure 1).

Concerning pores on the surface of mini-implants, those with nanotextured surface had the following dimensions: diameter 0.3 to 8.7 micrometers (300 to 8700 nanometers); average roughness (Ar) 4.12 micrometers (4120 nanometers); and depth 3.08 to 10.74 micrometers. The mini-implants coated by polyurethane foam had the following dimensions: diameter 120 to 320 micrometers; average roughness (Ar) 1500 micrometers; and pore depth 480 to 1200 micrometers.

After group distribution, the rats were randomly removed from the cages and anesthetized by intraperitoneal injection, composed of an association of ketamine hydrochloride 1% (Dopalen®, Hertape, Belo Horizonte, Brazil) at a dose of 40mg/kg and xylazine hydrochloride 2% (Dopasen®, Hertape) at a dose of 8mg/kg according to the anesthesia and analgesia guide of laboratory animals - UNIFESP/CEUA (2017)14.

The effectiveness of anesthesia was evaluated by the absence of movement, corneal-eyelid reflex, and motor reaction after gripping with tweezers of one of the hind legs’ adipose cushions, in addition to a good ventilatory pattern.

With the rats positioned in ventral decubitus, trichotomy was performed in the dorsal region, with subsequent antisepsis and sterile surgical field placement.

The incision’s delimitation was performed regarding a subcostal horizontal line, following the posteroinferior costal edge, which met with the middle sagittal line. With a scalpel cable no. 3, coupled with a blade no. 15, a horizontal incision was made, with an extension of 20 mm at the intersection of these reference lines.

The store was made for the mini-implants in a retromuscular plane (below the Panniculus carnosus), and, later, the mini-implant was introduced vertically and positioned horizontally according to the group (nanotextured or polyurethane). The suture of the skin was intradermal with mononylon 5-0 (Ethicon®) with buried knots. There was no removal of the stitches in
Evaluation of biomarkers in rats submitted to implants

the postoperative period, and the surgical wound was kept exposed (Figure 2).

Postoperative analgesia was with a single intramuscular application of sodium dipyrone (20mg/kg) in the posterior limb’s lateral region. No postoperative dressings or stitches were performed.

Euthanasia occurred according to the subgroups of 30, 60 and 90 days by applying four times the therapeutic dose of Dopalen® and Dopasen’s subsequent cervical dislocation. There was no death, infection of the surgical site, and no implants’ extrusion, so no rats were excluded.

Evaluation methodology

Blood samples with 4mL were obtained on the day of euthanasia, according to each subgroup, by intracardiac puncture with a 10mL syringe performed by the veterinarian (Video 1), and were arranged in tubes with anticoagulant Ethylenediamine tetraacetic acid (EDTA) and homogenized for 1 minute.

After that, the tubes were immediately placed on the veterinary hematological analyzer Max Cell 200 – KT 6200 VET® (Green Medical Instrument, Tokyo, Japan) previously calibrated for blood count reading, in which blood separation was performed in red and white series, and platelets.

The use of this equipment is a way of not committing cell counting errors that frequently occur through a blood smear. After that, the results were analyzed 15,16.

Statistical evaluation

The results were described by median, minimum and maximum values. For comparing the groups (nanotextured and polyurethane), the Mann-Whitney non-parametric test was used in each subgroup (30, 60 and 90 days). The differences between the subgroups for each group were made using the Kruskal-Wallis non-parametric test. P<0.05 values indicated statistical significance. The data were analyzed with the computer program Stata/SE v.14.1. StataCorpLP, USA.

RESULTS

Red series

Hypochromic anemia was found in the animals evaluated on the changes in the red series in the postoperative period.

Hemoglobin and hematocrit

In all groups and subgroups evaluated, the results were very similar and without statistical significance (Tables 1 and 2, Figures 3 and 4).

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p* |
|-----------|------------------------------|------------------------------|----|
| 30d       | 13 (3.9-13.7)                | 13 (12-13.6)                 | 0.762 |
| 60d       | 12.5 (7.2-13.9)              | 12.2 (11.5-37.1)             | 0.720 |
| 90d       | 13 (12.3-14.7)               | 13.4 (10.5-13.9)             | 0.631 |

p** (30 x 60 x 90d) 0.210 0.165

Table 1. Hemoglobin analysis in the nanotextured and polyurethane groups over time

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p* |
|-----------|------------------------------|------------------------------|----|
| 30d       | 48.4 (16.3-50)               | 48.3 (44-50.6)               | 0.829 |
| 60d       | 46.7 (0.7-51.2)              | 47.5 (45.2-51.1)             | 0.780 |
| 90d       | 46.3 (44.2-51.3)             | 48.5 (37.2-49.9)             | 0.631 |

p** (30 x 60 x 90d) 0.594 0.632

Table 2. Analysis of hematocrit in the nanotextured and polyurethane groups over time

Mean corpuscular hemoglobin

When the different subgroups were compared among themselves, statistically significant results were obtained (Table 3 and Figure 5).
Leukocytes

When comparing the different subgroups among each other, within each group, results with statistical significance were obtained (Table 4 and Figure 6).

Table 4. Analysis of leukocytes in the nanotextured and polyurethane groups over time.

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p*  |
|------------|-------------------------------|--------------------------------|-----|
| 30d        | 8.1 (3.3-12.6)                | 10.5 (3.8-17.6)                | 0.122 |
| 60d        | 8.3 (2-11.9)                  | 7.9 (1.1-11.7)                 | 0.905 |
| 90d        | 11.9 (4.4-16.3)               | 12 (6-30.6)                    | 0.631 |
| p** (30 x 60 x 90d) | 0.038*** | 0.034*** |     |

Source: The authors. *Mann-Whitney nonparametric test, p<0.05; **Kruskal non-parametric test -Wallis, p<0.05; ***Nanotextured: 30d x 60d: p=0.590; 30d x 90d: p=0.052; 60d x 90d: p=0.013 Polyurethane: 30d x 60d: p=0.060; 30d x 90d: p=0.416; 60d x 90d: p=0.010.

White series

Normal leukocytes count at the expense of neutrophilia and lymphopenia was evidenced in the changes in the white series postoperatively.
Neutrophils and lymphocytes

In all groups and subgroups evaluated, the results were similar and without statistical significance (Tables 5 and 6, Figures 7 and 8).

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p* |
|-----------|-------------------------------|-------------------------------|----|
| 30d       | 35.1 (25.5-44.1)              | 37.8 (28.4-46.3)              | 0.408 |
| 60d       | 39 (25.9-43.8)                | 35.1 (22.2-43.9)              | 0.211 |
| 90d       | 40.4 (28.7-49.1)              | 41 (23.4-60.2)                | 0.631 |

$p** (30 \times 60 \times 90d)$ 0.589 0.148

Table 5. Analysis of neutrophils in % in the nanotextured and polyurethane groups over time. Source: The authors.*Mann-Whitney nonparametric test, p<0.05; **Kruskal nonparametric test-Wallis, p<0.05.

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p* |
|-----------|-------------------------------|-------------------------------|----|
| 30d       | 53.6 (44.5-70.8)              | 51.6 (43.6-62.5)              | 0.360 |
| 60d       | 49.6 (44-68.1)                | 55.9 (45.7-72.6)              | 0.079 |
| 90d       | 48 (41.4-60.2)                | 47.2 (23.5-63.8)              | 0.481 |

$p** (30 \times 60 \times 90d)$ 0.372 0.075

Table 6. Analysis of lymphocytes in % in the nanotextured and polyurethane groups over time. Source: The authors.*Mann-Whitney nonparametric test, p<0.05; **Kruskal nonparametric test-Wallis, p<0.05.

| Subgroups | Nanotextured median (min-max) | Polyurethane median (min-max) | p* |
|-----------|-------------------------------|-------------------------------|----|
| 30d       | 114 (37-667)                  | 211 (42-739)                  | 0.573 |
| 60d       | 146 (110-759)                 | 292 (134-645)                 | 0.489 |
| 90d       | 301.5 (137-662)               | 203.5 (98-839)                | 0.247 |

$p** (30 \times 60 \times 90d)$ 0.265 0.535

Table 7. Analysis of platelets in the nanotextured and polyurethane groups over time. Source: The authors.*Mann-Whitney nonparametric test, p<0.05; **Kruskal nonparametric test-Wallis, p<0.05.

Platelets

Platelets presented with thrombocytopenia, and the results were very similar between the two groups, in the various subgroups evaluated, and without statistical significance (Table 7 and Figure 9).

DISCUSSION

Choice of animal model

Based on Zarini et al. (2004), it was considered that the life expectancy for a laboratory rat is approximately 2.5 years. Correlating with human life, three months of
this animal correspond to 90 months (approximately eight years) in a human being, based on an average life expectancy of 75 years. Due to this, we chose to divide into subgroups of 30, 60 and 90 days to give greater veracity to a late postoperative period.

The rat (*Rattus novergicus Albinus*, Rodentia Mammalia) chosen by the authors is the animal most used in healing studies around silicone implants due to the easy reproducibility of results, resistance to surgical interventions and high availability in bioteries17,18.

**Red series**

According to reference values in Wistar rats 19, hemoglobin, hematocrit and mean corpuscular hemoglobin were analyzed. Concerning hemoglobin, there was a total decrease and, on the other hand, hematocrit increased, regardless of the type of mini-implant.

This configured a possible postoperative hypochromic anemia and may suggest iron deficiency, blood loss, inflammation, and postoperative infection. As a consequence, anemia can increase postoperative morbidity and mortality20.

Partially agreeing with Ersoy et al. (2015) 21, who implanted intracardiac device in humans, demonstrated that hemoglobin and hematocrit decreased significantly in the postoperative period. They attributed this gastrointestinal bleeding and hemolysis of red cells secondary to high shear tension, which the implanted device could cause.

Disagreeing with Zarini et al. (2004) 15, who placed hydrogel implants in female mice, the authors mentioned above found no alteration in the red series, indicating no postoperative systemic involvement in the red series due to the implant.

Disagrees with Csendes et al. (2014) 9, who underwent gastrectomy in humans, did not show a change in hemoglobin's reference values before and after surgery. However, concerning hematocrit, they obtained a decrease in this parameter, which contradicts our study. We had increased hematocrit values, even if not absolutely, in all subgroups, regardless of the type of mini-implant.

**White series**

When analyzing leukocytes in the present study, a large amplitude was evidenced between the minimum and maximum values; however, the median remained within the reference value. When the subgroups were compared longitudinally, there was a gradual increase in leukocytes, indicating a higher production of these cells.

Neutrophilia accompanied by lymphopenia was also evidenced. In this case, lymphopenia is considered relative because there is an increase in neutrophils, unbalancing the leukocyte equation, which is a marker of inflammation11.

Neutrophilia is immediate in the postoperative period because interleukin-1 is formed by stimulation of the inflamed and traumatized areas, resulting in the neutrophil reserve’s release to the periphery13.

In the present study, neutrophilia was persistent, probably due to the perpetuation of the implant’s inflammatory reaction, especially in the polyurethane group, which showed relatively higher values.

It is partially agreed with Csendes et al. (2014) 9, which in their study found an increase in leukocytes at the expense of neutrophilia in the postoperative period; however, according to these authors, the values returned to the reference on the fifth day, suggesting a physiological inflammatory process due to surgical stress, unlike the present study in which the values remained high until 90 days9.

We disagree with Zarini et al. (2004) 15, who described a mild leukocytosis accompanied by neutrophilia attributed to the foreign body reaction15; on the contrary, in the present study, normal leukocyte values were found due to a balance between neutrophilia and lymphopenia.

Regarding the leukocyte equation, Hashimoto et al., in 201814 reported that the neutrophil/lymphocyte ratio (NLR) might be a method of stratification of mortality risk, with the characteristics of being noninvasive, inexpensive and readily available for patients with implants.

The advantage of using both neutrophils and lymphocytes can be explained because NLR combines two different immunological pathways. The first pathway, composed of neutrophils, is involved in rapid response, while the second pathway contemplates lymphocytes in the long-term adaptive response of the immune system, which is a synonym for physiological stress11.

**Platelets**

Regarding platelet changes, thrombocytopenia was observed, suggesting splenic sequestration or decreased production, destruction or accelerated consumption of platelets20.

It corroborates with Zarini et al. (2004) 15, who in their study demonstrated early and transient thrombocytopenia as a consequence of hemostatic activation due to microhemorrhage at the implant site.

It partially disagrees with Zhang et al. (2015)22, who evaluated the alteration in platelets after stent implantation in humans in their study. These authors found patients with decreased platelet count (mild, moderate and large amounts) and reported patients with increased platelet count, but these values returned to normality after six months.
The same authors attributed the alteration in platelets due to the minimization of surgical stress and the accelerated consumption of these cells to stent-related hypercoagulability. This study also demonstrated that the change in platelet count was positively correlated with the change of leukocytes and not with the number of stents. 

**CONCLUSION**

After placement of mini silicone implants, the rats of both groups evolved with hypochromic anemia, normal leukocyte count at the expense of neutrophilia and lymphopenia, and thrombocytopenia.

**REFERENCES**

1. Puskas JE, Luebbers MT. Breast implants: the good, the bad and the ugly. Can nanotechnology improve implants?. Wiley Interdiscip Rev Nanomed Nanobiotechnol. 2012 Mar/Abr;4(2):153-68.
2. Gomes RS, Garcia EB, Gomes HFC, Ferreira LM. New technologies and innovations in breast surgery. Rev Bras Cir Plast. 2019 Jan;34(1):150-6.
3. Brown T, Harvie F, Stewart S. A different perspective on breast implant surface texturization and anaplastic large cell lymphoma (ALCL). Aesthet Surg J. 2019 Jan;39(1):56-63.
4. Calobrace MB, Schwartz MR, Zeidler KR, Pittman TA, Cohen R, Stevens WG. Long-term safety of textured and smooth breast implants. Aesthet Surg J. 2017 Dec;37(8):38-48.
5. Shauly O, Gould DJ, Patel KM. Microtexture and the cell/biomaterial interface: a systematic review and meta-analysis of capsular contracture and prosthetic breast implants. Aesthet Surg J. 2019 May;39(5):603-14.
6. Prantl L, Püppel N, Horvat N, Heine N, Eisenmann-Klein M. Serologic and histologic findings in patients with capsular contracture after breast augmentation with smooth silicone gel implants: Is serum hyaluronan a potential predictor?. Aesthetic Plast Surg. 2005 Nov/Dec;29(6):510-8.
7. Ajdic D, Zoghi Y, Gerth D, Panthaki ZJ, Thaller S. The relationship of bacterial biofilms and capsular contracture in breast implants. Aesthet Surg J. 2016 Mar;36(3):297-309.
8. Real DSS, Resendes BS. Linfoma anaplásico de grandes células relacionado ao implante mamário: revisão sistemática da literatura. Rev Bras Cir Plast. 2019 Out/Dez;34(4):531-8.
9. Csendes A, Muñoz A, Burgos AM. Evolução do hemograma e proteína C-reactiva em pacientes com câncer gástrico operados por gastrectomia total. Arq Bras Cir Dig. 2014;27(4):234-6.
10. Ulum MF, Nasution AK, Yusop AH, Arafat A, Kadir MRA, Juniantito V, et al. Evidences of in vivo bioactivity of Fe-bioceramic composites for temporary bone implants. J Biomed Mater Res Pt B: Appl Biomater. 2011 Nov;103(7):1354-65.
11. Hashimoto N, Arimoto T, Narumi T, Iwayama T, Kutsuzawa D, Ishigaki D, et al. The neutrophil-to-lymphocyte ratio predicts all-cause mortality in patients with implantable cardioverter defibrillators. Pacing Clin Electrophysiol. 2017 Feb;40(2):135-44.
12. Hisanaka K, Kadota H, Fukushima S, Inatomi Y, Shimamoto R, Kamizono K, et al. Toxic shock syndrome caused by staphylococcal infection after breast implant surgery: a case report and literature review. Ann Plast Surg. 2018 Sep;80(3):559-62.
13. Jhunjhunwala S, Silva SA, Tang K, Alvarez D, Webber MJ, Tang BC, et al. Neutrophil responses to sterile implant materials. PLoS One. 2015 Sep;10(9):e0137550.
14. Universidade Federal de São Paulo (UNIFESP). Comissão de Ética no Uso de Animais (CEUA). Guia de anestesia e analgesia de animais de laboratório, São Paulo (SP): UNIFESP/CEUA; 2017.
15. Zarinia E, Supino R, Pratesi G, Laccabue D, Tortoreto M, Scanziani E, et al. Biocompatibility and tissue interactions of a new filler material for medical use. Plast Reconstr Surg. 2004 Set;114(4):534-42.
16. Acharya V, Kumar P. Identification and red blood cell automated counting from blood smear images using computer-aided system. Med Biol Eng Comput. 2018 Mar;56(3):483-9.
17. Silva EN, Ribas-Filho JM, Czeczko NG, Pachnicki JPA, Montemor Netto MB, Lópera HP, el al. Histological evaluation of capsules formed by silicone implants coated with polyurethane foam and with a textured surface in rats. Acta Cir Bras. 2016 Dec;31(12):774-82.
18. Silva EN, Ribas-Filho JM, Tabushi FI, Silva MAR Siqueira EBD, Noronha L, et al. Smooth muscle alpha actin immunoeexpression (alpha-Sma) and CD-117 antibody (C-Kit) in capsules formed by polyurethane foam-coated silicone implants and with textured surface: a study on rats. Aesthetic Plast Surg. 2019 Feb;43(1):233-42.
19. Melo MGD, Dória GAA, Serafini MR, Araújo AAS. Valores de referência hematológicos e bioquímicos de ratos (Rattus norvegicus linhagem Wistar) provenientes do biotério central da Universidade Federal de Sergipe. Sci Plena. 2012 Abr;8(4):e049903.
20. Cappellini MD, Musallam KM, Taher AT. Iron deficiency anaemia revisited. J Intern Med. 2020 Feb;287(2):153-70.
21. Ersoy O, Gultekin B, Ozkan M, Akkaya I, Umaroglu S, Sezgin AT, et al. Neutrophil counts and cytometry of blood smear images using computer-aided system. Exp Clin Transplant. 2015 Nov;13(Suppl 3):137-9.
22. Zhang Z, An L, Du D, Lai X, Wang F, Li Y. The change in platelet count in patients with acute coronary syndrome 6 months after coronary stent implantation. Blood Coagul Fibrinolysis. 2015 Set;26(6):661-4.