



# 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*

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## ■ ABSTRACT

**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.

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## ■ RESUMO

**Introdução:** Nas últimas décadas houve uma grande evolução na superfície de revestimento dos implantes mamários, o que resultou na diminuição das complicações. No pós-operatório a inflamação é uma constante e pode ser avaliada pelo hemograma, pois é um exame rápido, barato e com alta disponibilidade. O presente estudo tem como objetivo avaliar os biomarcadores sanguíneos em ratas submetidas à colocação de mini-implantes de silicone nanotexturizados e revestidos por espuma de poliuretano. **Métodos:** Foram utilizadas 60 ratas Wistar divididas em dois grupos para utilização de mini-implantes nanotexturizados e revestidos com espuma de poliuretano, subdivididos em subgrupos de acordo com a eutanásia dos animais nos 30, 60 e 90 dias. No momento da eutanásia, as amostras de sangue foram obtidas por punção cardíaca e foi analisado o hemograma.

**Resultados:** A hemoglobina, o hematócrito, a hemoglobina corpuscular média, os leucócitos, os neutrófilos, os linfócitos e as plaquetas tiveram os resultados muito semelhantes em todos os subgrupos avaliados (30, 60 e 90 dias). Entretanto, quando os diferentes subgrupos foram comparados entre si dentro de cada grupo, obteve-se resultados estatisticamente significantes na hemoglobina corpuscular média (nanotexturizado  $p=0,032$  e poliuretano  $p=0,007$ ) e nos leucócitos (nanotexturizado  $p=0,038$  e poliuretano  $p=0,034$ ). Sobre as alterações dos biomarcadores sanguíneos no pós-operatório encontrou-se anemia hipocrômica, contagem de leucócitos normais, neutrofilia, linfopenia e trombocitopenia. **Conclusão:** Após a colocação de mini-implantes de silicone, as ratas de ambos os grupos evoluíram com anemia hipocrômica, contagem de leucócitos normais às custas de neutrofilia e linfopenia, e trombocitopenia.

**Descritores:** Biomarcadores; Contagem de células sanguíneas; Ratos; Implante de mama; Mamoplastia.

## INTRODUCTION

Breast implant mammoplasty is one of the most performed plastic surgeries worldwide<sup>1</sup>. 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 macro<sup>2-5</sup>.

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 contracture<sup>5,6</sup>, infection<sup>7</sup>, and, more recently, large cell anaplastic lymphoma (BIA-ALCL)<sup>8</sup>.

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 implants<sup>3,4,6,8</sup>. In the pathophysiology of this disease, it is known that neoplasia is directly related to the host's inflammatory response to the implant<sup>6,8</sup>.

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<sup>9</sup>.

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)<sup>10</sup>, correlated with mortality in response to device implantation or infections<sup>11</sup>.

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<sup>12</sup>.

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<sup>13</sup>.

## 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<sup>®</sup>, Rio de Janeiro, Brazil) were placed, and in the polyurethane group, n = 30, mini-implants with polyurethane foam coating were placed (Silimed<sup>®</sup>).

The implanted materials had the same layers of a human breast silicone implant, discoid shape, with 22 +/- 1 mm (mm) in diameter and 9 +/- 1mm in height in mini-implants with a nanotextured surface, and with 24 +/- 1 mm in diameter and 11 +/- 1mm 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)

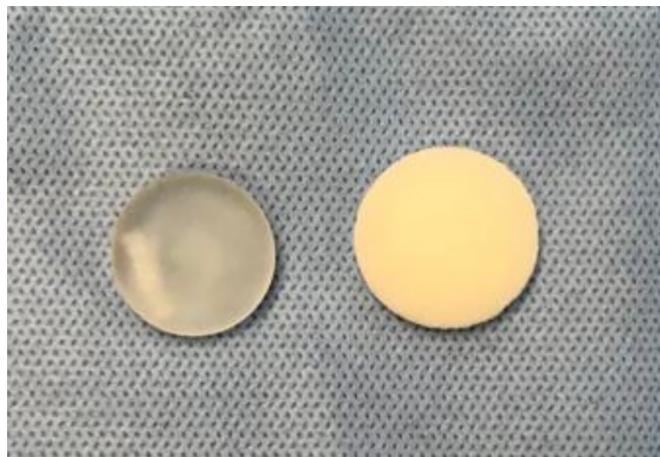


Figure 1. Mini silicone implants nanotextured and mini implants coated with polyurethane foam.

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<sup>®</sup>, Hertape, Belo Horizonte, Brazil) at a dose of 40mg/kg and xylazine hydrochloride 2% (Dopasen<sup>®</sup>, Hertape) at a dose of 8mg/kg according to the anesthesia and analgesia guide of laboratory animals - UNIFESP/CEUA (2017)<sup>14</sup>.

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 antiseptis 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<sup>®</sup>) with buried knots. There was no removal of the stitches in



Figure 2. Immediate post-op.

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® 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<sup>15,16</sup>.

### 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).

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

Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
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	

Source: The authors. \*Mann-Whitney nonparametric test,  $p < 0.05$ ; \*\*Kruskal-Wallis nonparametric test,  $p < 0.05$ .

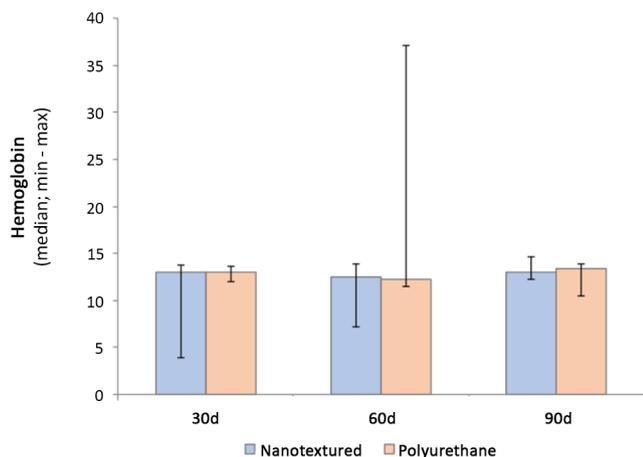
**Table 2.** Analysis of hematocrit in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
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**(30x60x90d)	0.594	0.632	

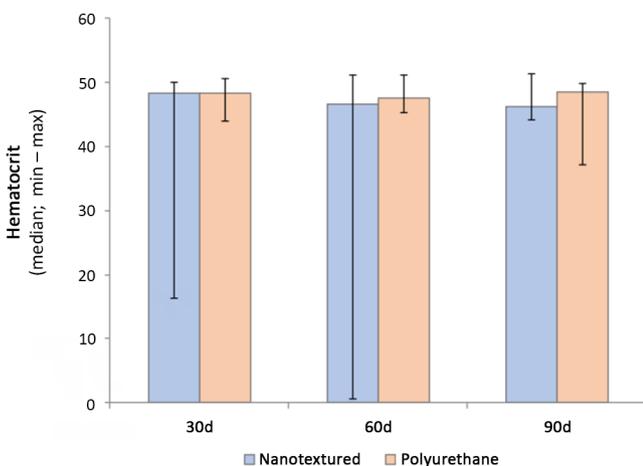
Source: The authors. \*Mann-Whitney nonparametric test,  $p < 0.05$ ; \*\*Kruskal-Wallis nonparametric test,  $p < 0.05$ .

### Mean corpuscular hemoglobin

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



**Figure 3.** Hemoglobin analysis in the nanotextured and polyurethane groups over time.



**Figure 4.** Analysis of hematocrit in the nanotextured and polyurethane groups over time.

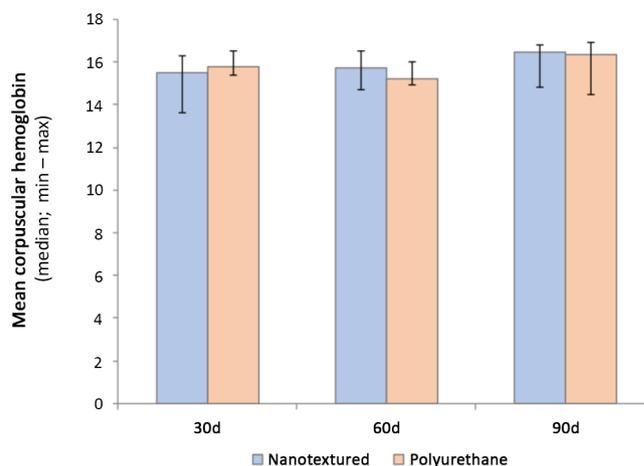
**Table 3.** Analysis of mean corpuscular hemoglobin in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
30d	15.5 (13.6-16.3)	15.8 (15.4-16.5)	0.515
60d	15.7 (14.7-16.5)	15.2 (14.9-16)	0.190
90d	16.5 (14.8-16.8)	16.4 (14.5-16.9)	0.631
p** (30 x 60 x 90d)	0.032***	0.007***	

Source: The authors. \*Mann-Whitney nonparametric test, p<0.05; \*\*Kruskal nonparametric test -Wallis, p<0.05; \*\*\*Nanotextured:30d x 60d: p=0.032; 30d x 90d: p=0.009; 60d x 90d: p=0.061; Polyurethane: 30d x 60d: p=0.120; 30d x 90d: p=0.039; 60d x 90d: p=0.001.

### White series

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



**Figure 5.** Analysis of mean corpuscular hemoglobin in the nanotextured and polyurethane groups over time.

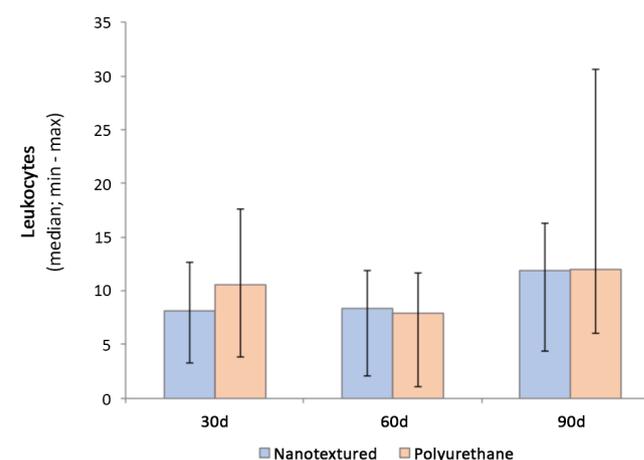
### Leukocytes

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	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
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.



**Figure 6.** Analysis of leukocytes in the nanotextured and polyurethane groups over time.

### 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).

**Table 5.** Analysis of neutrophils in % in the nanotextured and polyurethane groups over time

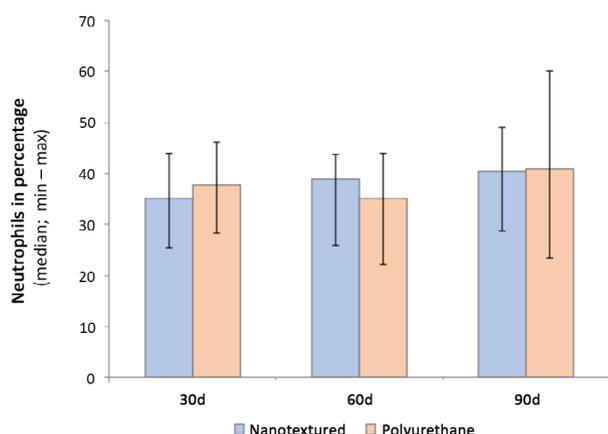
Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
30d	35.1 (25.5-44.1)	37.8 (28.4-46.3)	0.408
60d	39 (25.9-43.8)	35.1 (22.2-43.9)	0.211
90d	40.4 (28.7-49.1)	41 (23.4-60.2)	0.631
p** (30 x 60 x 90d)	0.589	0.148	

Source: The authors.\*Mann-Whitney nonparametric test, p<0.05; \*\*Kruskal nonparametric test-Wallis, p<0.05.

**Table 6.** Analysis of lymphocytes in % in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
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 (23.5-65.8)	0.481
p** (30 x 60 x 90d)	0.372	0.075	

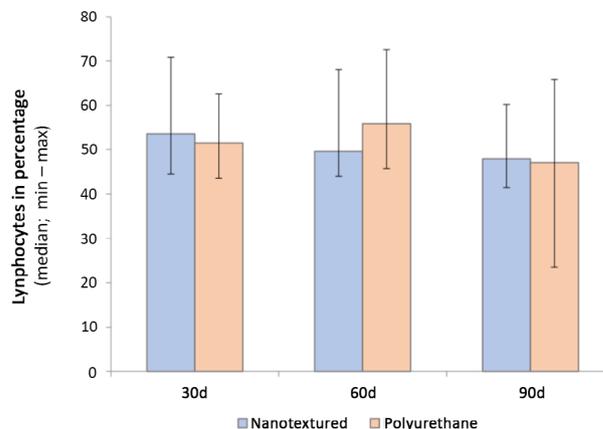
Source: The authors.\*Mann-Whitney nonparametric test, p<0.05; \*\*Kruskal nonparametric test-Wallis, p<0.05.



**Figura 7.** Análise dos neutrófilos em % nos grupos nanotexturizado e poliuretano ao longo do tempo.

### 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).

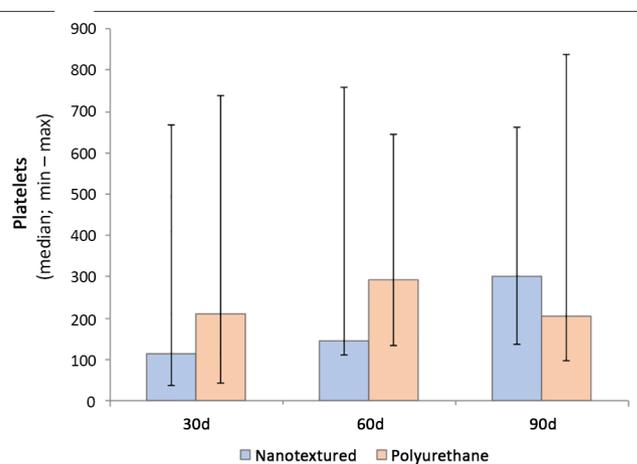


**Figure 8.** Analysis of lymphocytes in % in the nanotextured and polyurethane groups over time.

**Table 7.** Analysis of platelets in the nanotextured and polyurethane groups over time.

Subgroups	Groups		p*
	Nanotextured median (min-max)	polyurethane median (min-max)	
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 x 60 x 90d)	0.265	0.535	

Source: The authors.\*Mann-Whitney nonparametric test, p<0.05; \*\*Kruskal nonparametric test-Wallis, p<0.05.



**Figure 9.** Analysis of platelets in the nanotextured and polyurethane groups over time.

## DISCUSSION

### Choice of animal model

Based on Zarini et al. (2004)<sup>15</sup>, 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 norvegicus 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 bioterries<sup>17,18</sup>.

### Red series

According to reference values in Wistar rats<sup>19</sup>, 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 mortality<sup>20</sup>.

Partially agreeing with Ersoy et al. (2015)<sup>21</sup>, 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)<sup>15</sup>, 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)<sup>9</sup>, 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 inflammation<sup>11</sup>.

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 periphery<sup>13</sup>.

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)<sup>9</sup>, 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 days<sup>9</sup>.

We disagree with Zarini et al. (2004)<sup>15</sup>, who described a mild leukocytosis accompanied by neutrophilia attributed to the foreign body reaction<sup>15</sup>; 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 2018<sup>11</sup>, 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 stress<sup>11</sup>.

### Platelets

Regarding platelet changes, thrombocytopenia was observed, suggesting splenic sequestration or decreased production, destruction or accelerated consumption of platelets<sup>20</sup>.

It corroborates with Zarini et al. (2004)<sup>15</sup>, 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)<sup>22</sup>, 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<sup>22</sup>.

## 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.

## COLLABORATIONS

<b>ENS</b>	Analysis and/or data interpretation, Conception and design study, Conceptualization, Data Curation, Final manuscript approval, Formal Analysis, Investigation, Methodology, Project Administration, Resources, Supervision, Visualization, Writing - Original Draft Preparation, Writing - Review & Editing
<b>GHP</b>	Realization of operations and/or trials
<b>FMB</b>	Analysis and/or data interpretation
<b>AYK</b>	Data Curation
<b>LMF</b>	Conception and design study
<b>LCL</b>	Realization of operations and/or trials

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