

## Histological aspects of autologous transplantation of different fragments of the spleen in rats<sup>1</sup>

### Aspectos histológicos do transplante autólogo de diferentes fragmentos de baço em ratos

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#### ABSTRACT

**PURPOSE:** To evaluate macro and microscopically the evolution of autotransplants of fragments of spleen different fragments in the greater omentum, after eight weeks of observation.

**METHODS:** Twenty rats Wistar were used, males and adults, submitted to total splenectomy and divided in two groups. The group I - ten animals with implant of spleen fragment (25% weight of spleen) in the omentum; and group II - ten animals with implant of spleen fragment (30% weight of spleen) in the omentum. It was analyzed macro and microscopically the evolution of the implant.

**RESULTS:** It was observed adherences to the adjacent tissues and vascularization in all of the fragments transplanted. The group I and II presented white pulp with follicular formations and lymphoid tissue preserved, and the red pulp in cordon aspect. The group II presented white pulp more disorganized and red pulp hemorrhagic. The active macrophages were observed in the group I and II.

**CONCLUSION:** The splenic autotransplantation of the group I showed better regeneration.

**Key words:** Transplantation, Autologous. Spleen. Histology. Rats.

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#### RESUMO

**OBJETIVO:** Avaliar macro e microscopicamente a evolução do autotransplante de diferentes fragmentos de baço no omento maior, após oito semanas de observação.

**MÉTODOS:** Foram utilizados 20 ratos Wistar, machos e adultos, submetidos a esplenectomia total e distribuídos em dois grupos. O grupo I – dez animais com implante de fragmento com 25% do peso do baço no omento e o grupo II – dez animais com implante de fragmento com 30% do peso do baço no omento. Foram observados macro e microscopicamente a evolução dos implantes.

**RESULTADOS:** Foi observada no fragmento transplantado aderência aos tecidos adjacentes e vascularização preservada. Os grupos I e II apresentaram polpa branca e vascularização preservada, polpa branca com formação folicular e tecido linfóide preservado, e a polpa vermelha com aspecto cordonal. O grupo II apresentou polpa branca mais desorganizada e polpa vermelha hemorrágica. Os macrófagos ativos foram observados nos grupos I e II.

**CONCLUSÃO:** O autotransplante esplênico do grupo I mostrou melhor regeneração.

**Descritores:** Transplante Autólogo. Baço. Histologia. Ratos.

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## **Introduction**

The spleen is a lymphoid organ that plays important part in the organism defense, participating in filtration processes, phagocytosis and immunoglobulin production. Therefore, their main functions are: haematopoietic, immunological in the production of lymphocytes, plasmocytes and macrophages, phagocytosis also denominated of haemocatheresis and it is part of the “pool” outlying of blood storage<sup>1,2</sup>. Recent studies showed that the spleen has a role in the lipid metabolism and may influence atherosclerosis<sup>3</sup>. Histologically, the spleen is formed by a stroma and the parenquima being its cellular arrangement differentiated in white and red pulps. The white pulp acts, about 80% of the parenquima while the remaining 20% are the red pulp, which is constituted by the lymphoid splenic strings. The main cellular types in the splenic structure are: T and B lymphocytes, macrophages, plasmocytes, fibrocytes, reticulocytes and dendritic cells<sup>1,2</sup>.

Some authors recommend the autotransplantation in a homogenized tissue form, with the justification that is better to maintain the splenic filter function, because the architecture of the spleen is conserved. Besides, it is discussed if the viability is superior in the implants of smaller fragments for regenerating in less time than the larger implants<sup>4,5</sup>. As for the location of the implants, several places have been studied: great omentum, peritoneal cavity, splenic store, retroperitoneum, intraportal, abdominal muscle, armpits and subcutaneous of abdominal wall<sup>5,6</sup>. The greater omentum has several advantages, as the accentuated vascularization, allowing great sanguine contribution, that it is resulted essential for its appropriate revascularization, which avoids the migration of the implant, and its permanence in the portal territory. The capacity of implant growth in the abdominal muscles is smaller than in the omentum and peritoneum<sup>1,7</sup>.

There isn't consensus on the experimental study regarding the size of the implant. Researchers have used with implant 1mm, 2mm, 3 to 5mm and 1cm pieces larger than 1cm. In general the results of the searches revealed that regeneration was implants ranging from 1mm to fragments larger than 1cm thick. The fragments very thick undergoes degeneration occurs before the higher regeneration process, as to start the regeneration process areas have central necroses totally affected<sup>8</sup>.

For the spleen has a working state, it takes at least 25% to 30% of its normal tissue mass. Smaller fragments, even if they have specific functions are not sufficient to fight infections induced experimentally in animal organisms<sup>9,10</sup>.

The objectives of the present research were to evaluate macro and microscopically the evolution of the autotransplantation

of different fragments with 25% and 30% weight of the spleen in the greater omentum.

## **Methods**

Twenty Wistar rats (*Rattus Novergicus Albinus, Rodentia, Mammalia*), males, with about three months of age under room temperature and conditions of natural light, fed with ration for rodents were employed. The animals divided in two groups were submitted to total splenectomy and intra-abdominal spleen tissue autotransplantation, being the Group I with ten animals and implant fragments with 25% weight of the spleen in the omentum; Group II with ten animals and implant fragments with 30% weight of the spleen in the omentum. This research was approved by the Ethics Committee (Protocol n° 012/2011).

### *Autotransplantation technique*

The anesthetized (xylazine: 5mg/kg; ketamine: 90mg/kg and fentanyl: 0.03mg/kg) animals were submitted to laparotomy and to splenectomy. The spleen was put in solution of sodium chloride 0.9% for cleaning of the excess of blood and dry with aid of a gauze, being weighed then in a precision scale and measured with ruler in milimeters.

The organ was fragmented in three similar portions, and the fragments of the extremities left and right of the spleen were called, respectively, of “F1” and “F3”, while the fragment of the middle, called of “F2.” The fragments F1 and F3 were put in formalin 10% for histological studies as controls. The fragment F2 was autotransplanted. The fragment F2 had 25% and 30% weight of the spleen. The fragment was involved in the greater omentum, very close to the stomach great curvature, being formed a bag which was sutured with thread of 4-0 nylon. The procedures total duration time was approximately from 20 to 25 minutes in all the animals.

### *Sacrifice of the animals, removal of the fragments and histological studies*

The animals were observed and after eight weeks they were anesthetized again and submitted to laparotomy and to inspection of the peritoneal cavity to look for the splenic tissue implanted. The fragments were examined carefully in search of revascularizations and being dissected the adjacent tissues. They were removed and again weighed in a precision scale, and measured with ruler in millimeters.

The collected fragments were conserved in formalin 10% for histological studies in red-faced cuts using hematoxylin-eosin stain. Two criteria of histological evaluation were adopted: the qualitative analysis.

The qualitative analysis consisted of the evaluation of the fragments, with emphasis in the comparison with the normal splenic tissue being verified the capsule, the white pulp, the red pulp, the splenic tissue structure, and the vascularization.

#### Statistical analysis

To check normality of the data we used the statistical method Shapiro-Wilk. The existent of the significant difference between the size before and after the autotransplantation were used Student's test.

#### Results

All the animals had gain of weight in a homogeneous way after eight weeks of observation. There were no deaths. However, important reduction was registered in the weight of the splenic tissue implanted in the two groups.

The size of the implanted fragments also suffered reduction. The average size in the preoperative, in the groups I and II, respectively, was of 8.16mm and 9.00mm. After the implant, the average size was, respectively, 6.00mm and 5.75mm.

#### Macroscopic analysis of the grafts

The macroscopic analysis showed, after 8 weeks, that the implants of splenic tissues were visualized revascularization in all of the fragments of the groups (Figure 1).

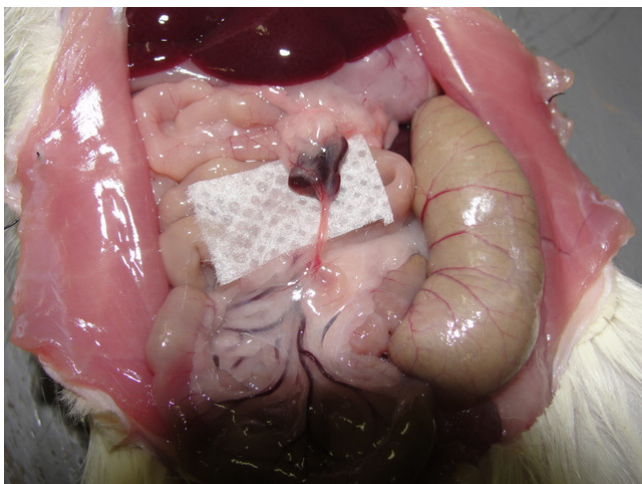


FIGURE 1 – Neovascularization of spleen fragment transplanted.

#### Histological qualitative analysis

1. Group I: splenic capsule thickness thinner, lymphoid tissue partially preserved white pulp with follicular formations and red pulp with cordon aspect (Figure 2).

2. Group II: thicker splenic capsule, lymphoid tissue partially preserved, more disorganized white pulp and red pulp with cordon aspect and hemorrhagic (Figure 3).

Many activated macrophages were found with repleted cytoplasm of hemosiderine granules (Figure 4).

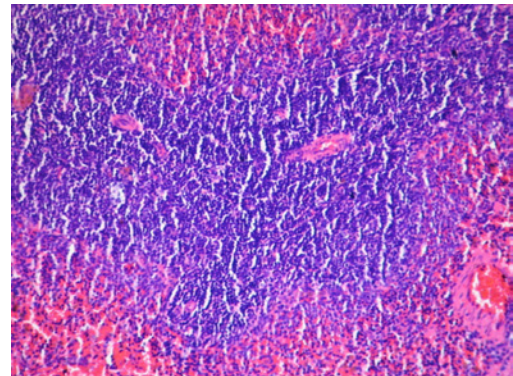


FIGURE 2 - With pulp of spleen fragment.

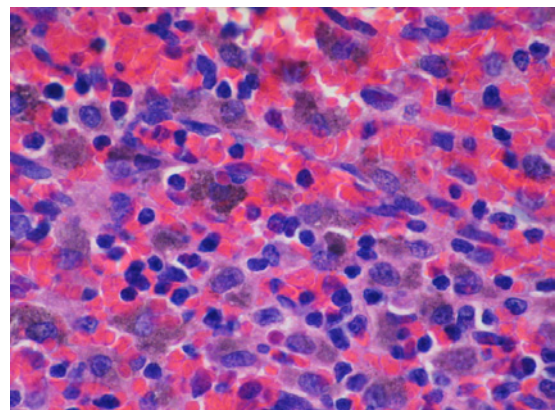


FIGURE 3 - Red pulp of spleen fragment.

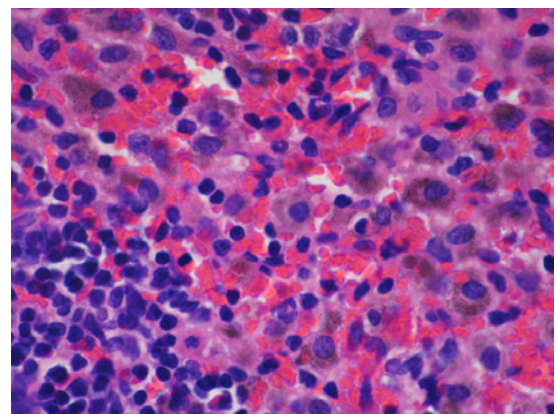


FIGURE 4 - Macrophages with cytoplasm of hemosiderine.

## Discussion

Wistar rats are pointed laboratory animals as appropriate for the investigation of the splenic vascularization and they present the spleen relatively larger than other species of mammals<sup>1</sup>. Torres *et al.*<sup>11</sup> related that it is an animal of easy manipulation, favoring the subtotal or total splenectomy, due to its anatomical location and still the postoperative to have low operational cost. Despite the morphology peculiar of each species, their histological aspects of the splenic tissue are similar to the humans<sup>1</sup>. Kovacs *et al.*<sup>12</sup>, studying autologous grafts of rats, concluded that the youngs are more capable to regenerate the splenic tissue and they emphasize that the development of the graft depends not only of the age of the recipient, but also of the local blood supply.

This study shows that after eight weeks of grafting of 25% and 30% in the spleen weight, a reduction of the initial weight. In principle the fragments, the 25% group showed a mean of 127.17mg, while the 30% group showed a mean of 171.5mg. Postoperatively the weight of fragments in the groups 25% and 30% respectively, were 59.67mg and 42.12mg. As Tavassoli *et al.*<sup>4</sup>, the results confirm that fragments greater than 100 mg not increased weight.

Christenson *et al.*<sup>13</sup> reported that the implants greater omentum, even at 24 weeks increased the doubling of the initial weight. This situation was not observed in the experiment, however, there was a reduction of the weight of the fragment in the initial eight weeks, as ascertained by Malago *et al.*<sup>8</sup> over 24 weeks of grafting.

Malago *et al.*<sup>8</sup> proposes to justify the weight reduction of the fragments using implants thick (14.53mm) which were subjected to a regeneration process later, as described by Torres *et al.*<sup>14</sup>. However this work, which used implants with sizes smaller than 10mm, still shows that fragments undergo a reduction in the weight, the fragments with an average of approximately 8.17mm (group 25%) and 9mm (group 30%) thick to the animals were implanted. Particularly, according Tavassoli *et al.*<sup>4</sup>, the histological evaluation of implants is very important, for the variations in weight depend on the microscopic events developed during the regeneration process.

Regarding the percentage weight loss of spleen tissue, the study was demonstrated loss of about 53.1% (group 25%) and 75.5% (30% group). However the differences between the groups, the statistically this weight loss was not significant.

Malago *et al.*<sup>8</sup> and Paredes *et al.*<sup>7</sup> showed loss of weight between 34.66% and 44.75% of the initial weight of the fragments, by grafting of more than three months. The results show weight

losses of 53.1% (25% group) and 75.5% (group 30%), higher than the studies cited. It is supposed that this difference is related to fragment size, the higher, more time is spent for the degeneration of the tissue.

According to Torres *et al.*<sup>14</sup> areas of central necrosis should be totally affected in order to initiate the regenerative process. As studies of Torres *et al.*<sup>14</sup> and Malago *et al.*<sup>8</sup> with, respectively, time of grafting of 28 days, 16 and 24 weeks, it was observed the presence of white pulp partially preserved.

Evidence of lymphocytes around the arterioles could be observed after 60 days of grafting, as evidenced by Sotelo *et al.*<sup>15</sup> when he collected fragments after 30 days of implantation.

Sotelo *et al.*<sup>15</sup>, Torres *et al.*<sup>14</sup> and Malago *et al.*<sup>8</sup> was examined cordon aspect of the red pulp, with congested sinusoids by erythrocytes, this could hemorrhage is connected to the implantation and subsequent tissue regeneration.

The hemosiderin deposits in activated macrophages were also found in the work reported by Paredes *et al.*<sup>7</sup>, Malago *et al.*<sup>8</sup>, Freitas *et al.*<sup>16</sup> and Giusti *et al.*<sup>17</sup>

According to the studies Tavassoli *et al.*<sup>7</sup> and Torres *et al.*<sup>14</sup> thick fragments require more time for regeneration, and suggested a correlation between the weight, size and regeneration.

The study showed weight loss fragment differently for the groups, although there was no statistical significance. The regenerative process was more clearly observed in the group 25%, which showed the percentage of reduction of the group weighing less than 30%. This report highlights the fact Malago *et al.*<sup>8</sup> proposes that the regenerative process delayed for thick implants, since the group 30% of the implanted fragments had a mean of 9mm.

The study suggests that the fragments implanted with 8.17mm regeneration develop faster because the pulps, white and red, resembling the normal splenic tissue. The group 30% had delayed the regenerative process, which is evidenced by histological features, when compared to 25%. In addition, the fragments confirm that the group 30% is also implanted to animals thick.

## Conclusion

Microscopic analysis revealed that both groups showed regeneration, however, there was a better regeneration in group 25% compared with the group 30%.

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