

## Phagocytic function of lower spleen pole and autogenous splenic implants in rats<sup>1</sup>

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### ABSTRACT

**PURPOSE:** To investigate whether there are differences between the phagocytic function of the remaining lower spleen pole after subtotal splenectomy and autogenous splenic implants.

**METHODS:** Thirty-six male Wistar rats, weighting  $364 \pm 60$ g were used. They were subjected to subtotal splenectomy preserving the lower spleen pole and to autogenous splenic implant in the greater omentum. Its viability was assessed microscopically. Phagocytic function was assessed by splenic uptake of the radioisotope-labeled colloid and by macrophages counting.

**RESULTS:** The viability of the autogenous splenic implant and of the lower spleen pole was found in 33 animals, with no difference between them. The weight of the implants was higher than the lower pole of animals from groups G1, G7, G30, G60 and G120. The implants phagocytic function by radioisotope uptake was higher than the lower pole in G7 and G120 groups and it did not differ from the other groups. The number of macrophages was higher in G1, G60, G90 and G120 and did not differ from the other groups.

**CONCLUSION:** Until the 16th week, the phagocytic function was more pronounced in autogenous splenic implants when compared with the lower spleen pole, but it became similar thereafter.

**Key words:** Spleen. Splenectomy. Transplantation, Autologous. Macrophages. Rats.

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## Introduction

For many years, it was believed that the spleen was not an important organ, and that its removal would cause no major repercussions. As a result, total splenectomy was performed indiscriminately, regardless the severity of the trauma or disease<sup>1</sup>. Nowadays, complications derived from splenectomy are well known and worrisome because of their seriousness. In order to reduce these complications, total splenectomy has been progressively replaced by procedures that preserve the splenic tissue<sup>2</sup>. The better knowledge of the spleen and its functions has influenced the use of less invasive procedures<sup>3,4</sup>.

There are situations in which it is not possible to preserve the spleen and then splenectomy becomes inevitable. In these circumstances, the autogenous splenic implant is able to keep, at least in part, splenic functions with small risk of complications. According to Holdsworth, Griffini and Tizzone<sup>5</sup> performed the first splenic implants in dogs. In 1896, Albrecht performed this surgery in humans in order to treat splenic traumas.

The spleen function of removing foreign substances, bacteria (mainly the capsulated ones) and senescent erythrocytes from the body was studied by scintigraphy and macrophages counting in the upper pole and in autogenous splenic implants in rats<sup>6</sup>. It should be considered that, especially in splenic trauma, keeping the upper spleen pole may be infeasible and that there must be an alternative treatment. Once concerned about this possibility, Paul *et al.*<sup>7</sup> performed a subtotal splenectomy preserving the lower spleen pole in dogs, which remained macroscopically feasible in 86.6% of the cases, even with the ligation of the main splenic vessels.<sup>7</sup> The lower pole viability was also observed in rats<sup>8-11</sup>.

Studies on the phagocytic function of the remaining lower pole from subtotal splenectomy as well as studies comparing this technique with autogenous splenic implants were not found in the literature. This gap motivated the current study.

## Methods

This study followed the Ethical Principles of Animal Experimentation and was approved by the Ethics Committee on Animal Experimentation of the Federal University of Minas Gerais, under N. 205/2012.

Prospective, experimental research, performed in 36 male Wistar rats, aged between 2 and 3 months with average weight of  $364 \pm 60$  grams. The animals were divided into seven groups of five animals, with the exception of the second group which consisted of six animals, according to the postoperative

follow-up time period, i.e.: one day (G1), seven days (G7) thirty days (G30), sixty days (G60) ninety days (90), one hundred and twenty days (120) and one hundred and fifty days (150).

The animals were placed in appropriate cages and were properly identified and kept in suitable conditions for the studied species. They received appropriate feed and water *ad libitum*.

The animals were weighed ((Filizola<sup>®</sup> electronic scale, Model MF-6 - 1g sensitivity) and anesthetized with ketamine hydrochloride (Vetaset<sup>®</sup>, Fort Dodge - Iowa, USA) at a dose of 50mg/kg of body weight, associated with xylazine hydrochloride (Kensol<sup>®</sup>, König - Avellaneda, Argentina) at an intraperitoneally delivered dose of 5 mg/kg. The surgical procedure consisted in: supraumbilical median laparotomy, mobilization of the spleen out of the abdominal cavity with ligation and division of the vessels that irrigated the upper and middle portion of the spleen along its border, with 5-0 mononylon sutures; subtotal splenectomy preserving the lower pole supplied by gastrosplenic ligament vessels, without suture in the bloody border. The part removed from the spleen was divided into five fragments for settling the autogenous splenic implants; the first upper fragment was submitted to control study, the other four fragments were sutured to the greater omentum by using 5-0 mononylon suture; hemostasis and laparotomy layered review.

The phagocytic functions of the remaining lower pole and autogenous splenic implants were verified by sodium phytate splenic uptake and by counting macrophages, which were performed after the animals were euthanized. The colloidal carbon was obtained from India ink, which consists of soot mixed with gum arabic and fragrance. Such ink was placed in a Becker bottle, and subsequently, in a drying oven at 100°C. The remaining pulp was diluted at the ratio of 50g/100ml water. The animals were anesthetized with ketamine and xylazine hydrochloride three hours before the removal of tissues for study purposes. A colloidal carbon solution was injected, by using a one milliliter syringe, at a dose of 1ml/kg in the rat's right jugular vein for counting macrophages. After two hours, a sodium phytate solution injection labeled with 110MBq (3mCi) of 99m technetium (99m Tc) was applied for splenic uptake. Three hours (3h) after the initial colloidal carbon injection, each animal was euthanized by means of intraperitoneal injection of pentobarbital (3% hipnol) at a dose of 120mg/kg of body weight. When no response was found to stimuli, 10% potassium chloride was injected in the 0.5 ml intracardiac dose. Then, re-laparotomy was performed in the same incision and the lower pole and autogenous splenic implants were removed for phagocytic function evaluation. The

intake of autogenous splenic implants and inferior pole was obtained by dose counter (Curimeter / Graduated Beacker), model CRC 7 (Radioisotope Calibrator by Carpintec), in which the intake calculation was made by counting the radioisotopes activity during samples reading. Then, the splenic tissues were processed for histological examination. Splenic fragments were embedded in paraffin, cut with a 3-micrometers-thick rotating microtome, and processed and fixed on microscope slides. The slide staining was performed with hematoxylin and eosin for morphological studies, and with light green for counting the macrophages that contained colloidal carbon pigment. This counting was performed in 10 microscopic fields with 400x magnification using the advanced free open source software, called Cellprofiler, developed for biomedical research<sup>12</sup>. The final number of macrophages was provided by the average of macrophages containing colloidal carbon found in these fields.

Statistical evaluation was initiated by data descriptive analysis in order to describe or summarize the study data. The comparison between the weights of autogenous splenic implants and lower poles, the technetium radioisotopes uptake and the macrophage number count in the different pre- and post-operative periods was performed by Mann-Whitney test<sup>13</sup>. In all tests, the 5% significance alpha level was used.

### Results

Subtotal splenectomy - maintaining the lower spleen pole - and autogenous splenic implants were feasible in all animals. There was one death in G60 after colloidal carbon injection. In the second laparotomy, the lower pole and the autogenous splenic implants were not found in an animal from G30 and in another one from G90. Several adhesions were found around the implants and the lower pole, making it sometimes difficult to remove these splenic remnants. By analyzing each group of animals, it was observed that the weight of autogenous splenic implants was greater than that of the lower pole in G1, G7, G30, G60 and G120 ( $p < 0.05$ ). However, there was no difference in the G90 and G150 groups ( $p > 0.05$ ).

The radioisotopes uptake was higher in the autogenous splenic implants than it was in the lower spleen pole in G7 and G120 ( $p < 0.05$ ); however, there was no difference in the other groups ( $p > 0.05$ ) (Table 1).

**TABLE 1** - Radioisotope uptake by autogenous splenic implants and lower spleen pole in separate groups (in mCi).

	N	Autogenous splenic implants uptake	Lower pole uptake	Value p*
		Median	Median	
G1	5	0.40	13.40	0.090
G7	6	5.18	0.68	<b>0.026</b>
G30	4	15.10	1.68	0.080
G60	4	26.05	6.72	0.080
G90	4	15.25	6.16	0.240
G120	5	11.40	6.50	<b>0.010</b>
G150	5	21.00	34.0	0.170

(\*) Mann-Whitney Test

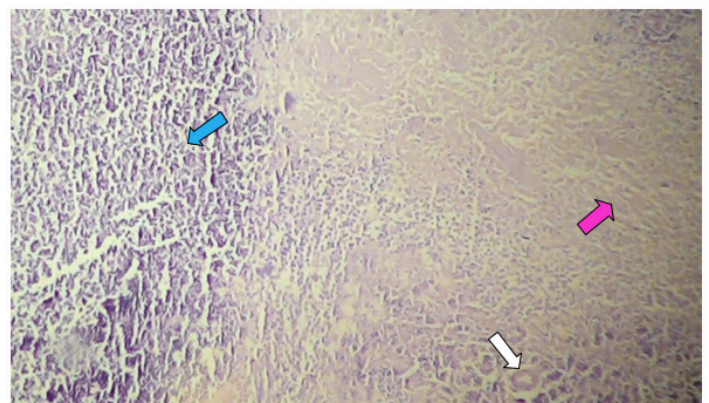
The number of macrophages was higher in the autogenous splenic implants than it was in the lower spleen pole in G1, G60, G90 and G120 ( $p < 0.05$ ). In the other groups, the difference was not significant ( $p > 0.05$ ) (Table 2).

**TABLE 2** - Number of macrophages from the autogenous splenic implants and lower spleen poles in separate groups (unit).

	N	Autogenous splenic implants	Lower pole	Value p*
		Median	Median	
G1	5	803.80	6.70	<b>0.0009</b>
G7	6	74.65	37.60	0.3367
G30	4	29.95	13.05	0.2482
G60	4	122.35	36.10	<b>0.0209</b>
G90	4	186.00	26.25	<b>0.0209</b>
G120	5	111.70	9.90	<b>0.0090</b>
G150	5	130.50	35.20	0.070

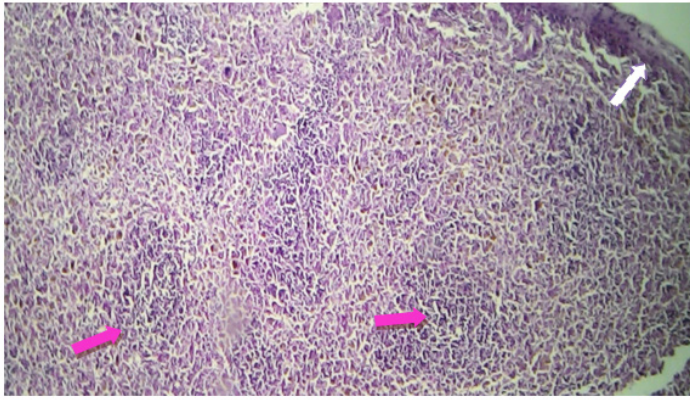
(\*) Mann-Whitney Test.

By histological evaluation, it was possible to see that animals from groups G7 and G30 showed almost the same characteristics. Figure 1 shows necrosis foci with severe fibrosis and vascular neoformation.



**FIGURE 1** - Photomicrograph of autogenous splenic implant histology showing extensive necrosis and fibrosis area in G7. 20X magnification. HE stain. Blue Arrow (area of fibrosis), pink arrow (necrotic areas) and white arrow (vascular neoformation).





**FIGURE 2** - Photomicrograph showing the autogenous splenic implant histology with normal morphology (G60). x10 magnification. HE stain.

In the G60, G90, G120 and G150 groups, the microscopic morphology of the autogenous splenic implant was considered as normal, since splenic tissue with splenic capsule (white arrow) and lymphoid follicles (pink arrow) are present (Figure 2).

### Discussion

In this study, an animal from G60 died shortly after the colloidal carbon injection. Its autopsy did not define the cause of death. It happened probably due to embolism once the animal showed respiratory failure followed by death right after the radioactive substance was administered. Lower spleen pole and autogenous splenic implants were not found in an animal from G30 and in another one from G90. A mass of about 4 cm, with fibrous tissue shell containing infectious-like material, was found in one of these animals, in the same location of the autogenous splenic implant and the lower pole. It is likely that these structures have undergone necrosis, followed by abscess. In another animal, the probable cause for the absence of structures was not found.

G7 had an extra animal because it was considered as the most controversial group regarding the phagocytic function recovery time in the autogenous splenic implants and lower pole. However, the results found for this animal did not differ from the ones found for the other animals.

Abnormalities were not found in the control fragment removed from the spleen. The histological architecture was considered as normal in all of them.

It is known that the spleen tissue exerts its function in several parts of the body in which it is implanted. In this study, we chose to implant them into the greater omentum, which has abundant vascularization and which venous drainage goes towards the portal vein. The splenic blood flows directly to the liver, as

well as it happens in normal conditions with the ectopic spleen venous drainage, which shows the best results according to the literature. Nevertheless, in the lack of omentum there is also an option for the mesentery or mesocolon, which drainage processes are also related to the portal system<sup>14,15</sup>.

Another aspect of great importance for the autogenous splenic implant success is the amount of spleen tissue needed to maintain splenic function. Studies have shown the need for at least 25% of normal splenic tissue in order to obtain the proper spleen function<sup>15,16</sup>. In the current study, the implants contained splenic mass exceeding 30% of rat's normal splenic mass.

In the first postoperative week of this study, there was no radioisotope uptake by the autogenous splenic implants (radioisotope uptake with a mean of 0.4 is considered insignificant). However, on the seventh postoperative day, the autogenous splenic implant showed evidences of phagocytic function which is characterized by both radioisotope uptake and the presence of colloidal carbon within their macrophages. The radioisotope uptake increased gradually until the date of the last group (G150) in both structures. This fact shows that the phagocytic function requires a period of time to fully recover. It is worth remembering that, in the current study, the splenic tissue mass from the autogenous splenic implant was significantly higher than the lower spleen pole mass. This may explain, in part, the best phagocytic function of these implants when compared with the lower pole, because the spleen function regeneration seems to be directly related to the implanted splenic mass<sup>15</sup>.

In the radioisotope uptake analysis performed in the separated groups, it was found that there was no difference between the autogenous splenic implants and the lower pole in G1, G30, G60, G90 and G150. In groups G7 and G120, however, the uptake by the autogenous splenic implant was higher, showing a significant difference. Importantly, the implants' weight was significantly higher than the lower pole one in these two groups.

In the phagocytic function analysis by counting macrophages, it was found that the counting of macrophages was higher in G1, G60, G90 and G120, in which there were also significant differences in the autogenous splenic implant weights when compared with the lower spleen pole weight. Perhaps, it can justify the abovementioned results. G150 showed difference neither in radioisotope uptake nor in the number of macrophages between the autogenous splenic implant and the lower spleen pole. There was also no difference between the autogenous splenic implant and lower pole weights. The lower pole seems to grow over time, and thus improve its function<sup>8</sup>.

After the initial period of necrosis, the autonomous

splenic implant is able to regenerate itself. The neovascularization originates from the arterial branches surrounding the implantation area. In the case of omental implant, it comes from gastro-omental arteries, with venous drainage towards the portal vein, similar to the spleen *in situ*, with centripetal orientation relative to the implant, starting a few days after this procedure<sup>17,18</sup>.

It is emphasized that results from the current study found that animals from G1, G7 and G30 showed necrotic areas and that the autogenous splenic implants morphological aspect was quite normal in the animals from G60, G90, G120 and G150, when compared with the *in situ* histological study of a spleen. This fact reinforces the autogenous splenic implant regeneration concept, which is in agreement with the results found in another study<sup>17</sup>, in which the histopathological analysis showed architecture typical of a normal spleen. There was morphological regeneration in all animals subjected to autogenous splenic implant.

Technical aspects related to the autogenous splenic implant have been reported and most authors consider that this procedure is not associated with major complications<sup>19,21</sup>. The immunocompetence degree that the autogenous splenic implant can provide to the host in response to bacteria have been investigated<sup>18,21</sup>. This procedure was performed in humans and the laboratory and scintigraphic tests found the recovery of some spleen functions. It has proved itself capable of removing colloidal and bacterial substances from rat's circulation<sup>6,22</sup>.

The autogenous splenic implant has not been widely performed in the treatment of traumatic splenic injuries. This practice should be encouraged and further research should be carried out aiming to develop less invasive techniques for treating spleen diseases, because when part of this organ is preserved, splenic function is maintained and asplenia adversities are prevented.

## Conclusion

Up to the 16th week, the phagocytic function was more pronounced in autogenous splenic implants when compared with the lower spleen pole, but it became similar after this period.

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