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The Influence of Spleen Conservation in Sepsis Experimentally Induced by Escherichiae Coli

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ABSTRACT
Many microorganisms, and particularly Streptococcus pneumoniae, are capable of causing fatal septicemias in splenectomized patients. E. coli is a very common microorganism, although postsplenectomy infection due to this bacteria has not yet been demonstrated experimentally.
The aim of the present study was to determine the influence of the complete or partial spleen on the appearance of sepsis induced by the intraperitoneal inoculation of E. coli.
A total of 125 rats were divided into groups of 25 each as follows: (I) Simulated surgery; (II) Total splenectomy; (III) Partial splenectomy (5%); (IV) Partial splenectomy (50%); (V) Partial splenectomy (75%).
Ninety days after the operation, E. coli was inoculated intraperitoneally. Ten minutes later 1 ml blood was extracted, and the procedure repeated 120 minutes after inoculation.
Integrity of the spleen may exert an influence on the presence of bacteriemia. However, once bacteria appear in circulating blood, their concentration is not affected by the amount of splenic tissue present.

Key words: Overwhelming postsplenectomy infection. Prophylaxis, postsplenectomy infection. Spleen conservation.

INTRODUCTION
Since 1952, when King and Schumaker (1) reported the possible presentation of fatal septicemias in splenectomized infants and children, attention has centered on the multiple aspects of this syndrome. It is well known that many microorganisms, and particularly Streptococcus pneumoniae, are capable of causing septicemia; the clearance of these microorganisms in blood may vary, depending on the total or partial preservation of the spleen when an animal is challenged experimentally with Neumococcus. Likewise, it has been proven that different immune properties disappear after splenectomy, but persist when using certain methods to preserve the organ.

The aim of the present study was to determine the influence of the complete or partial spleen on the appearance of sepsis induced by the intraperitoneal inoculation of Escherichiae coli commonly found in clinical practice and implied in postsplenectomy sepsis. An attempt was made to establish the possible utility of preserving the spleen as a prophylatic measure in this type of infection, thus contributing to a better understanding of postsplenectomy sepsis and its prevention.

MATERIAL AND METHODS
A total of 125 female Wistar rats (mean weight, 200 g) were used. The bacterial strain employed was encapsulated Escherichiae coli 20034 HUS, with proven pathogenicity and isolated from a patient hospitalized at the University Hospital in Seville (Spain). The inoculus administered consisted of E. coli 20034 HUS in 1 ml saline, obtaining a cloudiness of 1 on the McFarland scale - equivalent to 3×10^8 colony-forming units (CFUs). It was injected intraperitoneally 90 days after surgery.

One ml blood at a time was drawn from each rat by cardiac puncture, 10 and 120 minutes after inoculus injection.

We used liquid state soy-agar trypticase at 45 °C, posteriorly left to solidify to facilitate bacterial count on the surface and in depth. Blood-agar enriched medium was used for the isolation of all types of microorganisms, and Levine-selective medium favored the isolation of E. coli. Thioglycolate enriched liquid medium was used for the isolation of E. coli, including samples with a very low percentage of bacteria.

All animals were anesthetized with an intraperitoneal injection of ketamine (0.3 ml/100 g wgt.).

Animal	Culture at 10 min. (bacteria/ml)	Culture at 120 min. (bacteria/ml)
1	30	5840
2	0	+
3	1580	10
4	230	1010
5	70	0
6	80	0
7	0	80
8	0	0
9	0	+
10	0	0
11	30000	+
12	1460	8520
13	260	1240
14	0	+

+ (deaths)

Table I. Blood culture results made after 10 and 120 min. (Group I)

The same method was employed to obtain samples, giving the rats 0.1 ml/100 g wgt. on awakening within the two hours following inoculus administration.

The rats were divided into groups of 25 animals each as follows:

(a) Group I: Simulated surgery

A midline laparotomy was performed in this group, as in all animals operated on. The intestines and spleen were exteriorized, followed by closure of the cavity.

(b) Group II: Total splenectomy

After severing the splenorenal ligament, the spleen was luxated for optimum maneuverability. The short vessels and those forming the splenic pedicle were identified, isolated and ligated independently, performing a total splenectomy.

(c) Group III: Partial splenectomy (25%)

The short vessels were isolated, ligating the most peripheral vessel. Ligature of the remaining splenic parenchyma was carried out, followed by severing. No hemostatic agent was used.

(d) Group IV: Partial splenectomy (50%)

The short vessels were ligated and severed, leaving approximately 50% of the spleen without irrigation. Ligature was performed on the remaining spleen, removing the devascularized portion.

(e) Group V: Partial splenectomy (75%)

A technique similar to that used in the preceding group was carried out. In order to remove an additional 25% of the tissue, for a total 75% organ exeresis, the peripheral vessels of the pedicle were isolated and ligated, and the parenchyma was removed after ligature of the remaining organ tissue.

After anesthetizing each animal, 1 ml saline with Escherichiae coli (3 x 10^8 CFUs/ml) was inoculated intraperitoneally. Ten minutes later, 1 ml blood was extracted by percutaneous cardiac puncture. This same procedure was repeated 120 min after inoculation.

We deposited 0.1 ml blood in an empty, sterile petri dish, adding 15 ml of liquid soy-agar trypticase (SA) and mixing until completely homogeneous. After solidification, the medium was incubated at 35°C for 24 hours, followed by counting. A quantitative blood culture was achieved with this method, enabling the count of colonies growing on the surface and in depth throughout the medium.

A culture was also grown in the other media mentioned above. Density of the inoculus used was determined simultaneously, processing the blood samples in a similar fashion.

An analysis of variance (ANOVA) was applied to the results obtained for each parameter, to establish possible differences between the groups. The interrelation between pairs of results was studied applying Spearmann's range correlation tests, analyzing the level of significance for statistical differences. Results with a level of sigificance of 0.05 were considered to be of value (2).

RESULTS

Group I: Simulated surgery

Of the 20 animals used for the microbiological study, only 16 survived after 90 days. Two deaths occurred immediately after surgery, and two more in the late postoperative period, of unknown causes. Of these 16 animals, two died during anesthesia prior to the inoculation, while another four died after obtaining the first blood culture.

The blood culture results after 10 and 120 min. are given in Table I.

Group II: Total splenectomy

Of the 20 animals, 19 survived after 90 days, only one dying in the immediate postoperative period. The results obtained are shown in Table II.

Group III: Partial splenectomy (25%)

In this group of 20 animals, 16 survived after 90 days; three deaths occurred immediately after surgery and one due to unknown causes. Of the remaining 16, three died while obtaining samples, due to accidents during anesthesia, and another three after obtaining the first blood culture. The results are shown in Table III.

Animal	Culture at 10 min. (bacteria/ml)	Culture at 120 min. (bacteria/ml)
1	0	0
2	0	0
3	30	4200
4	10	780
5	3120	90
6	0	9680
7	60	28640
8	10	0
9	0	0
10	120	420
11	60	20160
12	0	0
13	70	4920
14	60	20
15	19520	540
16	20	1340
17	13560	280
18	130	20
19	800	2460

Table II. Blood culture results made after 10 and 120 min. (Group II)

Animal	Culture at 10 min. (bacteria/ml)	Culture at 120 min. (bacteria/ml)
1	740	0
2	0	0
3	23200	+
4	11600	19520
5	110	570
6	400	15040
7	360	410
8	0	10
9	120	+
10	4000	2800
11	11200	+
12	40	+
13	24000	70

+ (deaths)

Table III. Blood culture results made after 10 and 120 min (Group III)

Group IV: Partial splenectomy (50%)

Fifteen of the 20 animals operated on and used for the microbiological study survived after 90 days; of the five deaths recorded, three occurred in the immediate postoperative period and two later on due to unknown causes. Of the remaining 15, two died of accidents during ansthesia and three during the extraction of the first blood culture (only one being successful). The results are expressed in Table IV.

Animal	Culture at 10 min. (bacteria/ml)	Culture at 120 min. (bacteria/ml)
1	10	0
2	0	0
3	670	140
4	0	0
5	1800	0
6	45440	180
7	230	410
8	55000	250
9	0	0
10	0	10

Table IV. Blood culture results made after 10 and 120 min (Group IV)

Group V: Partial splenectomy (75%)

Fourteen of the 20 animals survived after 90 days. Four of the six deaths recorded occurred immediately after surgery and two later on due to unknown causes. Of the remaining 14, two died of accidents during anesthesia and three during the extraction of the first blood culture (only one being successful). The results are expressed in Table V.

Animal	Culture at 10 min. (bacteria/ml)	Culture at 120 min. (bacteria/ml)
1	30	50000
2	290	16480
3	10	10
4	1560	0
5	10	0
6	0	+
7	160	9680
8	290	780
9	740	0
10	0	0

+ (deaths)

Table V. Blood culture results made after 10 and 120 min. (Group V)

Groups	Culture at 10 min. (%)	Culture at 120 min. (%)
I	57.14	60.00
II	73.68	73.68
III	84.61	80.00
IV	60.00	50.00
V	80.00	55.55

Table VI. Percentages of positive cultures according to groups and parameters.

Groups	Level of significance
I	NS
II	NS
III	NS
IV	$p < 0.05$
V	NS

Table VII. Correlation of blood cultures at 10 min / blood cultures at 120 min

The percentages of positive culture according to groups and parameters are shown in Table VI. The results obtained have been compared by ANOVA, applying this test to each of the parameters and observing statistical differences between the different groups. The ANOVA was carried out for each, using the results of both blood cultures. No statistically significant differences were observed.

The results obtained on applying Spearmann's range correlation test are expressed in Table VII.

The corresponding ratios between the first and second blood cultures (bacterial clearance) were calculated to establish the presence of a decrease in the number of circulating microorganisms between the first 10 min. and after 120 min.

The different ratios between the two blood cultures are given in Table VIII. The first column lists those cases in which the first blood cultures showed a greater number of microorganisms than the second, i.e., those cases with greater or lesser clearance. The second column represents those cases in which the second blood culture displayed a greater number of microorganisms than the first, i.e., without any clearance.

As to mortality, during the observation period only two deaths occurred in Group I.

Groups	1ºB.C.>2ºB.C.	Both negative	1ºB.C.<2ºB.C.
I	36.36%	18.18%	45.45%
II	31.57%	21.05%	47.36%
III	30.00%	10.00%	60.00%
IV	50.00%	30.00%	20.00%
V	33.33%	22.22%	44.44%

Table VIII. Ratio of 1º/2º blood culture

DISCUSSION

In 1952, King and Schumacker (1) noted the possible incidence of fatal septicemia in splenectomized infants and children operated on in the first six months of life: they developed meningitis and generalized infection between six weeks and three years after splenectomy: three deaths were recorded. Current bibliography on this subject, including recent and multiple clinical and laboratory studies, shows an increase in the risk of fatal sepsis after splenectomy (3-6) that clearly depends on two factors: (a) patient age (7,8) and (b) indication for surgery (9).

In terms of age, pediatric patients are affected twice as often as adults (10); the incidence of severe sepsis ending in death is 58 times greater among children splenectomized for traumatism, than in the general population (5,11-13). The risk of septicemia in adults is difficult to establish, as each series consulted defines infection differently. The incidence of septicemia after splenectomy is approximately 4% among adults, about 1.5% being due to fatal sepis (3,10,14).

Zarrabi and Rosner (15) studied 48 adults with serious infections following splenectomy for traumatism; they concluded that there is also a potential risk of suffering this kind of sepsis in such patients.

According to the indication of surgery, children undergoing splenectomy due to a hemorrhagic disease presented greater risk than adults (5,16), with a greater rate of septicemia after surgery for reticulo-endothelial disease than for traumatism (17).

The frequency of microorganisms responsible for post-splenectomy sepsis varies according to different authors. Streptococcus pneumoniae causes the majority of cases, with up to 50% of cases documented. Other microorganisms involved are: (a) Bacteria: Neisseria meningitidis, Escherichiae coli, Haemophilus influenzae, Staphylococcus spp., Klebsiella spp., Mycobacterium tuberculosae, Pseudomona aeruginosa; (b) Viruses: Herpes zoster, Cytomegalovirus; (c) Protozoos (14).

Escherichiae coli is a very common microorganism responsible for multiple infections seen in daily clinical practice. Nevertheless, post-splenectomy infection due to this bacteria has not yet been demonstrated experimentally.

Having chosen E. coli for the present study, a strain of proven pathogenicity was used, employing a bacterial inoculus of sufficient density to provoke demonstrable bacteremias.

Studies by Greco and Alvarez (18) suggest that bacterial clearance from blood is the most important factor in the protective function of the spleen; microorganisms persist in the blood of splenectomized patients, permitting multiplication that in turn leads to sepsis and death.

The prevention of post-splenectomy septicemia included many different methods. Currently, there is a tendency to preserve the spleen, along with its vascular system in order to keep organ function intact.

Based on the above, we carried out this study to verify the influence of total or partial splenectomy on the induction of sepsis following E. coli inoculation.

The blood cultures made 10 min. after bacterial inoculation can be analyzed qualitatively and quantitatively. Qualitatively, the animals with an intact spleen presented a greater percentage of negative blood cultures (42.85%) than those subjected to total (26.31%) or partial 25% splenectomy (15.38%) and 75% splenectomy (20%). However, the group of animals with a 50% preservation of the spleen showed results very similar (40%) to those obtained in normal rats.

Quantitatively, statistical analysis showed no significant differences between the different groups.

Spleen integrity may exert a certain influence on the presence of bacteremia 10 min. after inoculation, resulting in a greater number of negative blood cultures in the control group than in the other series. However, once bacteria appear in circulating blood, concentration is not affected by the amount of spleen tissue present.

We observed that the incidence of negative blood cultures in animals with a complete spleen (42.85%) is greater in animals totally splenetomized (26.31%), this reflecting a defensive role of the organ against the intraperitoneal inoculation of E. coli.

Our results do not coincide with those reported by Cooney et al. (19) who, having obained blood cultures 10 min. after inoculation with Streptococcus pneumoniae, observed a lesser concentration of circulating bacteria in animals with partial splenectomy than in those totally splenectomized. The control group showed the lowest concentration of circulating bacteria. No such tendency was seen in our study with E. coli.

Our results also differ from those reported by Scher et al. (4) who, having used E. coli, observed bacteremia in both normal and splenectomized animals.

Two hours after bacterial inoculation there were clear differences between the blood cultures of the normal animals and those splenectomizd. The first group showed 40% negative blood cultures, while the second presented 26.31%. However, there was no correlation between the remaining spleen tissue and blood culture negativity in animals with partial spleen conservation. The 50% (50%) and 75% partial splenectomies (44.44%) paradoxically yielded better results than the 25% partial splenectomies (20%). Quantitatively there were no statistically significant differences between the cultures studied.

Differing from our results, Scher et al. (4) observed negative blood cultures in all normal animals, although the second blood cultures were carried out four hours after inoculation and not 120 min. after, as in our study.

The blood culture results after 120 min. confirm that complete spleen conservation protects the animal against E. coli, with lower bacteremia (60%) than when the animal has no spleen (73.68%). However, partial conservation of the spleen offers contradictory results.

When Spearmann's test is applied to the blood culture results, a significant correlation is only discovered for the group with a partial 50% splenectomy.

No disappearance or homogeneus decrease in circulating bacteria was seen in animals with an intact spleen, as the second blood culture presented a greater bacterial concentration than the first in 5 of the 11 studied. Similarly, there were no important differences between the remaining groups.

No mortality was recorded among animals totally splenectomized or in those with a partial 25, 50 or 75% splenectomy. Death only occurred in two rats with an intact spleen (40%). Our results differ from those reported by Cooney et al. (19) when using Streptococcus pneumoniae. These authors observed that all normal rats survived while all totally splenectomized rats died - 10% of the patially splenectomized animals surviving.

We accept that it is at least technically feasible to carry out certain procedures to preserve the spleen with a minimum guarantee of success.

This may be justified in the prevention of sepsis by Streptococcus pneumoniae. However, and considering the many reports published, this is not acceptable in the case of sepsis caused by E. coli, at least within the experimental context.

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