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Stereological study of tissue compartments of the human spleen

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Summary. Morphometric reports on animal and human spleen are very few and no studies have been carried out using stereological methods to investigate all of the tissue compartments of the human spleen. Eighteen samples of spleens, which were either surgically removed after traumatic injury or during treatment for early stage carcinoma or gastric ulcer, were investigated. The point-counting method was used to study the volume densities of the following tissue compartments: red pulp, perifollicular zone, white pulp (this tissue compartment was divided in two subcompartments: follicles and periarteriolar lymphatic sheath), marginal zone and connective tissue of trabeculas. The following stereological parameters of the follicles were investigated: the number of follicles per mm^2 of spleen section, numerical density, volume density, and the mean follicular diameter. The identity of spleen tissue compartments was verified using immunohistochemical staining for B- and T-lymphocytes. The volume densities of tissue compartments, as well as stereological parameters of lymphoid follicles, were similar in both groups of splenic samples, except for the volume densities of perifollicular zone and periarteriolar lymphatic sheath, where a statistically significant difference was registered.

Key words: Spleen, Stereology, Tissue compartments, Human

Introduction

The spleen is the largest peripheral lymphatic organ of the organism. Traditionally, it was considered as «the graveyard of erythrocytes», but more recently, it has been recognized that the spleen plays a significant role in protection and defence, which cannot be accomplished by other organs of the immune system. It has a delicate microarchitecture, with finely organized vascular network. Various compartments of the splenic tissue, i.e., red pulp, marginal zone and white pulp (with its subcompartments), are populated with different lymphoid and nonlymphoid cells, which have different functional roles (Pabst and Westermann, 1993). Considering this, it is surprising to find only a few histoquantitative studies on the tissue compartments of the spleen, which together with the data concerning spleen cells and vasculature, would enable more precise evaluation of pathological states that affect the spleen. Even more notable is a complete lack of stereological studies which would encompass all tissue compartments of the human spleen. Simple morphometric methods and approach were utilized in former studies: the individual subcompartments of splenic white pulp were not recognized, but in contrast it was studied as a single entity, whereby the stereological parameters of lymphoid follicles were completely disregarded (Watanabe, 1970; Stutte, 1974; Yamamoto, 1979; van Krieken et al., 1983, 1985; Barnard et al., 1990). Moreover, marginal zone was also considered as a part of white pulp which, considering its structure and function (Kraal, 1992), certainly is not true. Therefore, we felt that it would be advantageous to use the stereological approach, combined with immunohistochemical staining, to perform a complete study of all tissue compartments of the human spleen.

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Materials and methods

The blocks of splenic tissue were retrieved from the Department of Pathology, St. Luke's Hospital, Gwardamangia, Malta. Ten samples of spleens, surgically removed after traumatic injury, without signs of hemorrhage or immune reactivity (numerous plasmocytes or granulocytes within the red pulp), were selected. The age of patients was between 12 and 53 years (mean= 27 years). Eight patients, whose spleens were surgically extirpated during treatment for either early stage carcinoma (gastric or kidney) or gastric ulcer, were included in the study as a separate group. The age of these patients was between 53 and 83 years (mean=

71.3 years).

Preparation of tissue for microscopy

The slices of splenic tissue were fixed in 10% neutral buffered formalin. The tissue was processed and embedded in Paraplast. From each block several 6 μm-thick sections were sampled at 3 different levels of tissue 200 μm apart. Thereafter, the sections were either routinely stained with hematoxylin and eosin or used for immunocytochemical detection of T- and B-lymphocytes to verify the identity of splenic tissue compartments. Briefly, the standard three-step immunoperoxidase method (avidin-biotin) was performed, whereby 3,3'-diaminobenzidine tetrahydrochloride (DAB) was used for detection of peroxidase activity and monoclonal anti-CD45R (clone 4KB5; 1:60 diluted in PBS) and anti-CD45R0 (clone UCHL1; 1:100 dilution in PBS) antibodies (Dako, Denmark) were used for demonstration of B- and T-cells, respectively. The sections were counterstained with hematoxylin. Appropriate negative and positive controls were performed.

Spleen histomorphometry

Volume densities of the spleen tissue compartments

The point-counting method was used. The volume density of a tissue compartment i (v_{vi}) was calculated from the equation: $V_{vi}=P_i/P_t$, where P_i= number of points falling on the tissue compartment i, and P_t= total number of points counted on the reference tissue (Weibel, 1979). The following tissue compartments were separated: 1) red pulp; 2) perifollicular zone; 3) white pulp - this tissue compartment was divided in two subcompartments: 3a) follicles and 3b) periarteriolar lymphatic sheath (PALS); 4) marginal zone; and 5) connective tissue of trabeculas. All determinations were performed at a magnification of x100 using lattice with 36 points (B-36). Three sections per spleen were utilized for the analysis using the systematic field sampling technique and 20 fields were inspected on each section. The fields were randomly chosen, but did not involve the subcapsular area which consists predominantly of the red pulp.

Stereological parameters of the spleen follicles

The numerical density of the follicles per 1 mm^3 of spleen tissue (N_{vf}) was calculated from the following equation (Weibel, 1979):

$$N_{vf} = {}^{1}/_{\beta} \cdot K\sqrt{{}^{3}N_{Af}/V_{vf}}$$

where N_{Af}= number of follicles per mm^2 of spleen section; V_{vf}= volume density of the follicles, this was determined by the point-counting method on three spleen sections using a magnification of x 100 and lattice B 36; ß= 0.87 (shape factor of the follicles); K= 1.06 (factor for the size distribution of the follicles). The mean follicle diameter ($\bar{D}$) was calculated from the equation (Weibel, 1979):

$$\bar{D} = 2 \cdot \sqrt[3]{{}^{3}/_{4\pi} \cdot {}^{V_{vf}}/_{N_{vf}}}$$

which is based on the assumption that the follicles have a spherical shape.

The mean values for each scoring procedure were determined and standard deviation calculated. Student's t-test was employed for comparing of the means.

Results

In all examined samples, i.e., in both the traumatically injured spleens and spleens removed because of surgical reasons, all tissue compartments were easily distinguished (Figs. 1-3). B-lymphocytes were predominantly confined to lymphoid follicles (Fig. 2). T-lymphocytes were also present therein, especially in secondary follicles, between the germinal center and follicular mantle. However, their predominant location was the PALS (Fig. 3), which also contained a substantial number of B-lymphocytes (Fig. 2). Marginal zone was populated with both B- and T-lymphocytes (Figs. 2, 3).

In the traumatically injured spleens, the volume density of red pulp and perifollicular zone was 65.33% and 14.87%, respectively. In the spleens removed on surgical grounds, the volume density of red pulp was 71.11%. The volume density of perifollicular zone was 10.71%, which was significantly lower in comparison with the traumatically injured spleens ($p<0.05$). Jointly, red pulp and perifollicular zone composed 80.20% of the splenic volume in trauma group, whereas they comprised

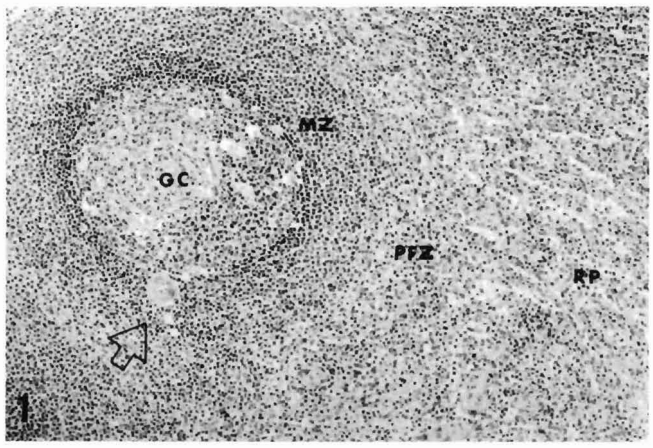

Fig. 1. Section of the human spleen. All tissue compartments are discernible. RP: red pulp; PFZ: perifollicular zone; MZ: marginal zone; GC: germinal center (with light and dark zones); arrow: periarteriolar lymphatic sheath. H&E. x 150

Table 1. Volume densities (%) of tissue compartments of the human spleen.

PARAMETER	V_{vrp}	V_{vpfz}	$V_{vrp} + V_{vpfz}$	V_{vmz}	$V_{vmz} + V_{vwp}$	V_{vwp}	V_{vfoll}	V_{vPALS}	V_{vct}
Trauma group	65.33	14.87	80.20	6.76	17.50	10.74	3.97	6.77	1.96
Surgery group	71.11	10.71*	81.82	5.72	14.36*	8.64*	3.25	5.39*	3.74

V_{vrp}: volume density of red pulp; V_{vpfz}: volume density of perifollicular zone; V_{vmz}: volume density of marginal zone; V_{vwp}: volume density of white pulp; V_{vfoll}: volume density of follicles; V_{vPALS}: volume density of periarteriolar lymphatic sheath; V_{vct}: volume density of connective tissue; *: statistically significant difference.

Table 2. Stereological parameters of splenic follicles (means±SD).

PARAMETER	TRAUMA	SURGERY
N_{Af} (mm^{-2})	0.69±0.1	0.75±0.2
N_{Vf}(mm^{-3})	3.75±0.8	4.19±0.3
$\bar{D}$(μm)	582.49±10.4	542.59±19.1
V_{Vf} (mm^3/mm^3)	0.03±0.001	0.03±0.002

N_{Af}: number of the follicles per mm^2 of section area; N_{Vf}: numerical density of the follicles; $\bar{D}$: mean follicular diameter; V_{vf}: volume density of the follicles.

81.82% of the splenic volume in surgery group. Marginal zone represented 6.76% of the splenic tissue and together with the white pulp it comprised 17.50% of the ruptured spleen volume. In the surgery group, marginal zone constituted 5.72% of the tissue and together with the white pulp it composed 14.36%, which was significantly different ($p<0.01$) from the values for the traumatically injured spleens. In the traumatically injured spleens the volume density of white pulp was 10.74%, with follicles and PALS comprising 3.97% and 6.77% respectively. In the surgery group the volume density of white pulp was 8.64%, which was significantly smaller than in the trauma group ($p<0.01$). Follicles were 3.25%, whereas PALS comprised 5.39% which was significantly different from values obtained for the spleens extirpated after traumatic injury ($p<0.05$) (Table 1). Various stereological parameters of the follicles (the number of follicles per mm^2, the numerical density of the follicles and the mean follicular diameter) of traumatically injured spleens and of the spleens removed because of surgical causes were not statistically different and are shown in Table 2.

Discussion

The histomorphometric data on the human spleen are very few and there is a complete lack of studies using the proper stereological approach to investigate all of the splenic tissue compartments. Our study, which corroborates earlier findings, adds new complementary data and fills this gap providing the complete data for all compartments of the human spleen (including all subcompartments of the white pulp), which may serve as a useful reference in future studies of this organ.

The values obtained for red pulp in both trauma and surgery groups corresponded very well to those obtained by Stutte (1974) (79%) and van Krieken et al. (1985) (79.6%), as well as by Yamamoto (1979) (69.4% -

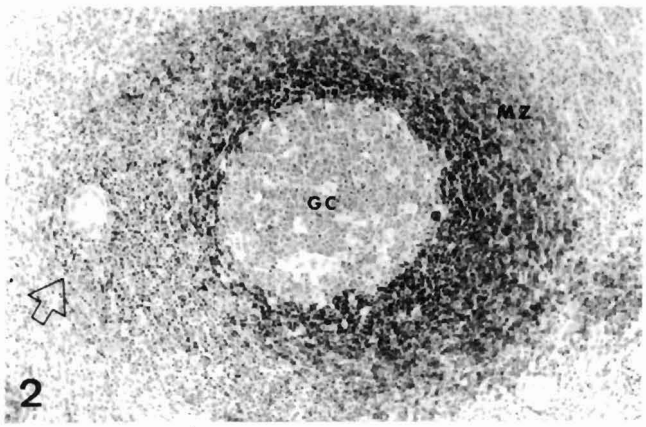

Fig. 2. Section of the human spleen. Mature B-lymphocytes are predominantly confined to the lymphoid follicle, but some are also positioned within periarteriolar lymphatic sheath. Marginal zone is populated with both B- and T-lymphocytes (compare with Figure 3). MZ: marginal zone; GC: germinal center; arrow: periarteriolar lymphatic sheath. Immunoperoxidase staining of B-lymphocytes (monoclonal anti-CD45R antibody). x 150

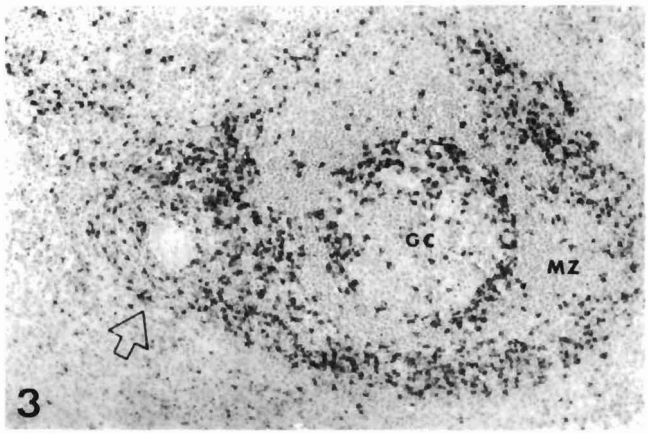

Fig. 3. Section of the human spleen. T-lymphocytes are predominantly located in the periarteriolar lymphatic sheath. However, they are also present in the secondary follicle, mostly between the germinal center and follicular mantle. Marginal zone is populated with both B- and T-lymphocytes (compare with Figure 2). MZ: marginal zone; GC: germinal center; arrow: periarteriolar lymphatic sheath. Immunoperoxidase staining of T-lymphocytes (monoclonal anti-CD45R0 antibody). x 150

without perifollicular zone) and van Krieken et al. (1985) (82.4%) in their respective groups. To compare the values obtained for the white pulp with those from the literature, we had to add the results for follicles, PALS and marginal zone together, because the individual subcompartments of white pulp were not recognized as such in earlier studies and the data on their respective volumes are not available. Moreover, marginal zone was also considered as a part of white pulp which, considering its structure and function (Kraal, 1992), certainly is not true. These pooled values also corresponded well to those obtained by Stutte (1974) and van Krieken et al. (1983) 16%, as well as those found by Watanabe (1970) and van Krieken et al. (1983) (12%), in their respective groups of patients. There are no data in the literature on the individual volumes of marginal zone and subcompartments of white pulp, i.e., of the follicles and PALS, nor on the stereological parameters of lymphoid follicles of the human spleen. So, the data presented in this study provide reference for the future work, especially for comparing with the pathologically changed spleens. The comparisons, thus have to be made with the existing data on the animal spleen. In the human spleen the marginal zone appeared to be the largest lymphoid compartment, similarly as in the rodent spleen, as demonstrated by histomorphometric (Pabst and Westermann, 1991; Milićević et al., 1994) and other methods (Kumararatne et al., 1982). In the human spleen the volume of PALS is larger than that of follicles, as is found in the rodent spleen (Pabst and Westermann, 1991; Milićević et al., 1994). The diameter of splenic follicles in the rat spleen (Milićević et al., 1994), is much smaller than that of follicles in the human spleen.

Comparing the results obtained for traumatically injured spleens with those obtained for spleens removed on surgical grounds, some notable differences, corresponding to those observed by van Krieken et al. (1983, 1985) and Barnard et al. (1990) were registered. Namely, the volumes of perifollicular zone and white pulp (actually, of follicles, PALS and marginal zone pooled together to enable the comparisons with the former results) were significantly larger in trauma group than those in surgery group. Our study shows that, in fact, there was no difference in the volume of marginal zone and lymphoid follicles. But, there was a significant difference between these two groups in the amount of PALS. So, when the values for PALS were pooled with those of follicles and then with those of marginal zone, this significance was carried over to the common values. Thus, we show that the reason for the difference is the amount of white pulp between trauma and surgery groups is the increased volume of PALS in the former. This is in accordance with the increased number of CD4-positive lymphocytes registered in the study of ruptured human spleens (Barnard et al., 1990). Van Krieken et al. (1983, 1985) reported that there was no influence of age on the difference observed between trauma and surgery groups and they concluded that the changes registered in trauma group could reflect the immune stimulation of the spleen, which could make this organ more susceptible to the traumatic injury. So, we believe that this issue deserves further attention. Currently, we are forming the age-matched groups of spleen samples from traumatically injured and surgery patients, so that this question can be addressed more precisely.

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