

## Liver-spleen axis: Intersection between immunity, infections and metabolism

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### Abstract

Spleen has been considered a neglected organ so far, even though is strictly linked to liver. The spleen plays an important role in the modulation of the immune system and in the maintenance of peripheral tolerance *via* the clearance of circulating apoptotic cells, the differentiation and activation of T and B cells and production of antibodies in the white pulp. Moreover, splenic macrophages are able to remove bacteria from the blood and protect from sepsis during systemic infections. We review the spleen function and its assessment in humans starting from the description of spleen diseases, ranging from the congenital asplenia to secondary hyposplenism. From the literature data it is clear that obesity in humans affects different compartments of immune system, even though there are still few data available on the implicated mechanisms. The intent is to enable clinicians to evaluate the newly recognized role of metabolic and endocrine functions of the spleen with special emphasis to obesity and nonalcoholic fatty liver disease in the context of the available literature.

Moreover, understanding the spleen function could be important to develop appropriate prevention strategies in order to counteract the *pandemia* of obesity. In this direction, we suggest spleen longitudinal diameter at ultrasonography, as simple, cheap and largely available tool, be used as new marker for assessing splenic function, in the context of the so-called liver-spleen axis.

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**Key words:** Spleen size; Obesity; Non-alcoholic fatty liver disease

**Core tip:** From the literature data it is clear that obesity in humans affects different compartments of immune system. The aim of this review is to let clinicians appreciate the new role of metabolic and endocrine functions of the spleen with special emphasis to obesity and nonalcoholic fatty liver disease in the context of the available literature. Moreover, understanding the spleen function could be important to develop appropriate prevention strategies in order to counteract the *pandemia* of obesity. In this direction, we suggest spleen longitudinal diameter at ultrasonography, as simple, cheap and largely available tool, be used as new marker for assessing the liver/spleen axis.

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### INTRODUCTION

In vertebrate evolution, spleen functions were performed by a spleen-like tissue scattered along the digestive tract, as seen in lamprey. Bony fishes and sharks are the first

vertebrates where it appears as individual organ<sup>[1]</sup>.

The spleen is a secondary peripheral lymphoid organ located in the abdominal cavity between the diaphragm and the fundus of the stomach of mammals. Its principal function was preserved during the evolution in all animal classes having that organ, while important differences can be observed histologically. For example, the red pulp is seen only from bony fishes upwards.

It is the largest lymphoid organ in the human body and it has a fundamental role as destruction of red blood cells and as actor in the immune response, filtering the blood from antigenic particles and from abnormal and aged cells. Table 1 summarizes the different functions of the spleen.

The spleen anatomical architecture is extremely sophisticated and little is still known about specific processes that are performed in its differentiation. Mesenchymal, hematopoietic and endothelial cells interact each other thanks to complex, organized and still undiscovered signals leading to the development of its complex micro-architecture<sup>[2-4]</sup>.

## WHAT EVIDENCE HAS SUGGESTED SPLEEN BE CONSIDERED A NEGLECTED ORGAN?

The congenital asplenia may occur with or without other clinically evident abnormalities. In the first case, with asplenia, other defects of organs of the thoracic and abdominal cavities can be found. One example is the heterotaxy syndrome, where there is a failure in the left-right axis specification<sup>[5]</sup>.

If the defect occurs before the ontogenesis of the spleen on the left side, it may not affect splenic development. The second type of congenital asplenia is less common<sup>[6-10]</sup> and includes subjects with no other obvious abnormalities that report recurrent infections from childhood. In those cases the diagnosis of asplenia often remains unravelled, due to the lack of necroscopy.

Studies in mice have highlighted that some genes are crucial for spleen development, such as *Tcf21*, *Bapx1*, *Pbx1*<sup>[11]</sup> and recently also *Tlx1*<sup>[12]</sup>. In this case it can be expected that asplenic animals suffer from additional several anomalies caused by the deficiency of specific genes. However, in the literature are not reported corresponding cases, probably because in humans and mice similar genes do not have overlapping functions, or these subjects die before or soon after birth and/or they were not extensively investigated.

A suitable example may be the Atrx syndrome, where the mutations of this gene result in athalassemia, myelodysplasia and mental retardation<sup>[13]</sup>. Individuals with this syndrome occasionally exhibit asplenia<sup>[14]</sup>, but the inactivation of Atrx similar gene in mice does not end up in asplenia<sup>[15]</sup>.

In individuals with congenital isolated asplenia are reported some mutations<sup>[16]</sup>, but the molecular mecha-

**Table 1 Function of the spleen**

Red pulp
Extramedullary hematopoiesis if required
Facilitating an environment wherein erythrocytes rid themselves of solid waste material
Blood filter for foreign material and damaged and senescent blood cells
Storage site for iron, erythrocytes, platelets, plasmablasts and plasma cells
Rapid release of antigen-specific antibodies into the circulation produced by red pulp plasma cells
Defense against bacteria using iron metabolism by its macrophages
White pulp
T cell zone (periarterial lymphatic sheath) and B cell zone (follicles)
Storage site for B and T lymphocytes
Development of B and T lymphocytes upon antigenic challenge
Release of immunoglobulins upon antigenic challenge by B lymphocytes
Production of immune mediators involved in clearance of bacteria such as complement, opsonins, properdin and tuftsin
Marginal zone
Phagocytosis of circulating microorganisms and immune complexes by MZ macrophages
Development of marginal zone B lymphocytes upon TI-2 antigenic challenge
Blood trafficking of B and T lymphocytes
Release of immunoglobulins upon antigenic challenge by splenic B lymphocytes

nisms and the etiology of spleen development are still unknown.

## ANATOMICAL AND HISTOLOGICAL COMPOSITION OF THE SPLEEN (ANIMAL VS HUMAN MODEL)

In the mice the spleen has a characteristic histological organization similar to a sponge, where the fibrous capsule form a reticular network with the trabeculae stemming from its internal side. The splenic artery enters the hilum of the spleen, divides itself into smaller branches and finally gives rise to “central arteriolar” of the white pulp and to the large sinusoids of the red pulp. The central arteriolar are surrounded by a sheath of small T lymphocytes, the so-called PeriArteriolar Lymphoid Sheath (PALS). They communicate with follicles, a highly organized accumulation of T and B lymphocytes. The red pulp, PALS and follicles are also surrounded by the marginal zone, filled with large memory B cells.

The human and mice spleen are not anatomically different. The fact that patients with autoimmune thrombocytopenia purpura and circulating antiplatelet antibodies improve after splenectomy<sup>[17]</sup>, supports the role of the red pulp of the spleen in the displacement of old and damaged platelets, aged erythrocytes and apoptotic cells in humans.

After apoptosis of aged erythrocytes, hemoglobin is digested and iron is released or stored by splenic macrophages. Thus, the spleen is fundamental in the recycling of iron<sup>[18]</sup>.

Interestingly, after abdominal surgery for trauma or neoplasia, the displacement of the spleen is often without immediate consequences. This is one of the reasons for which, until a recent past, the spleen was considered not a vital organ. Consequently, it was believed that the spleen could be removed without major consequences. Recently, Ozban *et al.*<sup>[19]</sup> have disproved this theory, because they have shown that exercise in splenectomized individuals can cause serious problems in form of decreasing splanchnic flow and increasing blood viscosity.

The spleen plays an important role in the modulation of the immune system and in the maintenance of peripheral tolerance *via* the clearance of circulating apoptotic cells, the differentiation and activation of T and B cells and production of antibodies in the white pulp<sup>[20,21]</sup>. Moreover, splenic macrophages are able to remove bacteria from the blood and protect from sepsis during systemic infections.

*Vice versa*, most important differences between mice and humans in the spleen organization and functionality are revealed in the immune response. The marginal sinus in mice and the perifollicular zone in humans are areas of particular activity. B cells in the marginal zone in mouse are highly reactive and specialized against pathogens invasion *via* a T-independent reaction<sup>[20]</sup>, however in humans, the same area contains memory B cells<sup>[22]</sup>. Several chemokines and adhesion molecules regulate the trafficking between the marginal zone and the white pulp.

## ROLE OF SPLEEN IN LIMITING BACTERIAL INFECTION

As seen before, congenital asplenic subjects have an increased risk of developing infective diseases and the lack of the spleen functionality causes more startling effects in the newborn.

Morris *et al.*<sup>[23]</sup> firstly described in 1919 that splenectomized patients are more susceptible to infections, especially caused by *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Neisseria meningitidis* (so called encapsulated bacteria)<sup>[23-25]</sup>. The risk of sepsis is 10- to 20-fold higher than non splenectomized population and, especially in young children, death can result<sup>[26]</sup>. Overwhelming post-splenectomy infection can occur some hours after the first signs of deterioration of health and can degenerate to multi-organ failure and death<sup>[27]</sup>. Most asplenic children die of infection during the neonatal period. In fact, among the causes of sudden and unexpected infant death, the congenital asplenia can also be included<sup>[28]</sup>.

Another condition is functional asplenia, when in patients with haematological or metabolic disorders the splenic tissue organization is altered and, for this reason, equally they develop the same type of infections. In patients with functional or anatomical asplenia is quite impossible to quantify the risk of developing infections and sepsis. Therefore, a method to conserve some splenic tissue during abdominal surgery with deracination of the spleen is to transplant small spleen fragments

into the well vascularised greater omentum. Clinical data have shown that this procedure has an important effect in increasing specific antibody responses after pneumococcal vaccination, as well as normalizing IgM levels<sup>[29,30]</sup>, and probably can also reduce the risk of opportunistic infections in immunodeficient subjects<sup>[31]</sup>. The increased susceptibility of hypo or asplenic individuals to encapsulated bacterial infections is mostly due to the lack of IgM memory B cells and to their not adherent reaction to polysaccharide vaccines. The absence of splenic macrophages with the reduced number of B cells in asplenic patients can result in the establishment of a favorable environment to the development of overwhelming bacterial infections.

## SPLEEN AND NATURAL ANTIBODIES

A particular subtype of B-cell population is involved in the immune deficiency and in the reduced response to polysaccharide antigens seen in the asplenic or splenectomized mice. B cells may be divided in two main subpopulations on the basis of life development (fetal or adult), surface markers and functions. Asplenic mice lack B-1a B cells<sup>[32]</sup>, a distinct population from the more conventional B-2 B cells that are involved in the adaptive immunity and collaborating with T cells<sup>[33]</sup>.

Functions of B-1a B cells are mainly three: (1) they can act in an T-cell independent mode during the immune response; (2) they produce natural antibodies and co-operate with the innate immune system to contrast bacterial and viral infections; and (3) in the intestinal mucosa they can differentiate into plasma cells producing IgA<sup>[34]</sup>.

In the specific immune response, produced antibodies have a very high affinity for a particular epitope and they can prevent re-infection from the single pathogen that previously has caused their own production. *Vice versa*, the pentameric IgM isotype produced by B-1a B cells binds various antigens with high avidity and low affinity and is therefore able to neutralize many different antigens. IgM antibodies are the so called “natural antibodies” and in recent years, it has been demonstrated that they may play a role in the protection against malignancy<sup>[35]</sup> and atherosclerosis<sup>[36]</sup>. Asplenic mice not only have a reduced number of B-1a B cells but they have a decreased concentration of serum IgM<sup>[32]</sup>.

B-1a B cells produce IgA immunoglobulins, and in the intestinal mucosa about half of the IgA plasma cells derives from B-1a B cells<sup>[37]</sup>. The homeostasis of the intestine is finely regulated by mucosal IgA. These immunoglobulins interact with antigens presented by the intestinal microbiota and by pathogens, preventing their overgrowth and subsequent invasion.

The precise reason why B-1a B cells are absent in asplenic mice and why their number rapidly declines after splenectomy is not yet defined. B-1a B cells are produced in the fetal liver, contrarily to B-2 B cells that derive from adult bone marrow. It is noteworthy to stress that the

first subtype cannot be replaced after adult bone marrow transplant. Moreover, Ig-positive precursors of B-1a B cells have been detected in the spleen, but it is unknown if these cells persist in the spleen during the adult life and derive from precursors situated in the fetal liver. According to a recent theory the spleen might be central to their generation or survival and therefore splenectomy would lead to the depletion of the B-1a population<sup>[32]</sup>.

## OTHER FUNCTIONS OF THE SPLEEN

An interesting hypothesis relates the spleen to the activity of gut-associated lymphoid tissue (GALT). The dysfunction of GALT is known to predispose to inflammatory bowel diseases (IBD), above all for its role in T cell activation and trafficking in the gut. Moreover, the frequency of IgM memory B cells is decreased in IBD subjects<sup>[38]</sup> establishing a relationship among GALT and spleen in humans.

The spleen also has important hematological functions. The spleen picks up from the circulation platelets that subsequently are stored or can be destroyed by lymphocytes. As storage organ the spleen stores about one third of the human body's platelets.

The thrombocytopaenia is a result of hypersplenism, because of the heightened functions of the spleen in sequestering and break-downing platelets. Conversely, after splenectomy mild thrombocytosis can be observed<sup>[39]</sup> because of the lack of sequestering and destruction of platelets by the spleen, and at the same time it can be observed a slight increase in platelet production in the bone marrow<sup>[40]</sup>. Erythrocytes are stored and removed from the blood circulation in this organ. After splenectomy, the presence in the blood of many substances released from circulating damaged erythrocytes with procoagulant activity can lead to the establishment of a procoagulant state and therefore to the occurrence of thromboembolic events (for example pulmonary embolism, deep vein and portal vein thrombosis).

Beyond haematopoietic stem cells, stem cells of other differentiation lines, such as stromal cells with osteogenic differentiation properties, seem to be present in the spleen, confirmed by *in vitro* studies<sup>[41]</sup>. It would be interesting to explore if in the spleen these osteogenic precursors may represent a monocyte/macrophage lineage common precursor cell population with the ability to differentiate along the osteoclast lineage<sup>[42]</sup>. Animal studies have also shown that splenocytes can differentiate into pancreatic islets and ductal epithelial cells when injected into diabetic non-obese diabetic mice, thereby splenocytes may be useful in the treatment of type 1 diabetes, thanks to their ability of restoring normal glycaemia<sup>[43,44]</sup>. Subsequently, Chong *et al.*<sup>[45]</sup> have questioned the origin of these stem cells.

## ASSESSMENT OF SPLEEN FUNCTION

Over the years, several methods have been developed to

study the activity of the spleen. Because of its ability in purifying the blood from old erythrocytes, the amount of altered red blood cells can be used as index of functionality of the spleen. The detection of Howell-Jolly bodies is one of these, although the sensitivity and specificity are questionable for the hyposplenism<sup>[46,47]</sup>. Other haematological parameters are finding membranes pits, large vacuoles situated near to the plasma membrane, or other cellular changes as acanthocytes, target cells, Heinz bodies (remnants hemoglobin), Pappenheimer bodies (iron granulocytes) and siderocytes<sup>[48]</sup>.

The count of B cells derived from the marginal zone, which have fundamental action in innate immunity, and in particular as defense against the invasion by encapsulated bacteria, is otherwise a possible method for evaluating the immunological activity of spleen<sup>[49]</sup>. B cells derived from the marginal zone and the memory cells producing IgM are in fact reduced in patients with diminished splenic function<sup>[38]</sup>.

All the tests described above could be used with ease in clinical practice, on the other hand they have proved not to be very sensitive and specific, or they are needed to be further studied and validated<sup>[46,50]</sup>.

To date, the radioisotope method is definitely the best way to quantify the filtering activity of the spleen. The (99m) Tc-labeled, heat-altered, autologous erythrocyte scintigraphy with multimodality single photon emission computed tomography (CT) - technology is considered the best approach to gauge all the facets of the splenic function<sup>[51]</sup>. However, this is a method that has the highest cost and it is difficult to perform.

## SPLEEN AS A NEW PLAYER

Nonalcoholic Fatty Liver Disease (NAFLD), the most common cause of liver steatosis, is associated with obesity, mainly visceral type, and insulin resistance. The liver inflammation (nonalcoholic steatohepatitis, the so called NASH) can progress from the simple hepatic steatosis or fatty liver (FL) lasting risk factors, as type 2 diabetes mellitus, major obesity and Metabolic Syndrome (MS). In its natural history, NASH can end up in perisinusoidal fibrosis and cirrhosis. Hepatocytes, during steatosis, are fat-laden and swollen, and in steatohepatitis the hydropic change (ballooning) causes further swelling and also sinusoidal distortion, as visualized by *in vivo* microscopy studies. This evenience leads to the reduction of intrasinusoidal volume and microvascular blood flow, as clearly described by Farrell *et al.*<sup>[52]</sup>. Sinusoidal endothelial cells, Kupffer cells and stellate cells are also involved in the pathological process in conjunction with the activation of the immune system. The microcirculation is skewed by inflammatory cells and platelets recruited in the liver. Animal models confirm these data and evidence that these pathological changes lead to a marked reduction of sinusoidal space (approximately 50% of control), and a decrease in the number of normally perfused sinusoids, according to a review, recently published<sup>[51]</sup>. The micro-

vascular damage is necessary for developing further liver injury and causing disease progression as in NASH. The lipid peroxidation of unsaturated fatty acids by reactive oxygen species is one of the main causes of the sensitivity of hepatic steatosis to ischemia-reperfusion injury. During the whole 24-h-period the most part of time is spent in postprandial state in humans. Therefore, the liver has a fundamental role in maintaining the correct energy state balancing the input, secretion, and oxidation of fatty acids. In abdominally obese men the oxidation of dietary fatty acids, hepatic desaturation and elongation of palmitic acid occur to a greater extent than in non-obese<sup>[53]</sup>.

This means, therefore, that donor's fatty livers are an obstacle to transplantation<sup>[52]</sup>. Between other hepatic cells, the dysfunction of Kupffer cells gives a major contribution to NASH progression. It is noteworthy that the reticular-endothelial system also plays a key role in the spleen and a good method for study Kupffer cell activity is the colloid scintigraphy. Duman *et al*<sup>[54]</sup> have followed 22 patients with biopsy-proven NASH who underwent colloid liver scintigraphy. Liver right/left lobe ratio was altered in all patients after intravenous injection of 185 MBq Tc tin colloid. The shift of colloid to the spleen and a prolonged blood pool clearance time was observed in 55% of patients with NASH.

Previously, Tsushima *et al*<sup>[55]</sup> aimed to determine if there was an association between spleen enlargement and NAFLD, measuring spleen volume at CT. It must be observed that the values were weighted according to the patient's demographic data, the Liver/Spleen (L/S) ratio of CT Hounsfield unit measurements, and liver function tests. L/S ratio was also used to perform the diagnosis. The authors evidenced an increased mean spleen volume ( $P < 0.0001$ ) between NAFLD and controls  $73.0 \pm 24.4 \text{ cm}^3$  (range, 21.1-106.1) in normal subjects and  $141.2 \pm 54.1 \text{ cm}^3$  (range, 44.1-267.3) in NAFLD subjects. Only the L/S ratio ( $P < 0.0001$ ) and age ( $P < 0.01$ ) were significantly correlated to spleen volume at multivariate linear regression analysis and at forward selection stepwise regression.

Basing on the evidence that obesity and insulin resistance are inflammatory chronic diseases and therefore are associated with systemic markers of inflammation, some scholars have attempted to find a non invasive diagnostic method for NASH to help clinicians to decide whether and when to perform liver biopsy.

Patients with histology proven-NAFLD (43 patients with NASH and 40 with FL), compared with healthy subjects, were evaluated with ultrasonographic exams, with particular interest to ultrasonographic spleen longitudinal diameter (SLD) and splenic artery resistive index, and laboratory measurements, as serum interleukin (IL)-6 and vascular endothelial growth factor (VEGF) concentrations. The NASH group demonstrated higher IL-6 blood levels, SLD values, and VEGF concentrations than controls. In this study was estimated that the SLD is more sensitive than IL-6 and VEGF in discriminating NASH from FL, and the optimal cut-off value for SLD is 116 mm (specificity 95% and sensitivity 88%). NASH and FL

subjects have a similar splenic artery resistive index, but it differs when compared with controls. On the other hand, normal values of SLD and IL-6 were associated with FL and normal values of IL-6 could confirm the absence of NASH<sup>[56]</sup>. Further confirmation of these findings comes from another study which highlighted that spleen enlargement may be a distinct feature of NASH, especially early-stage NASH<sup>[57]</sup>. Therefore, we suggest that SLD could be used as new marker for assessing splenic function, independently from its use in distinguishing the simple FL, also called benign, from NASH, the more severe form of NAFLD, benignity not always shared<sup>[58]</sup>.

In this study<sup>[59]</sup>, SLD and blood pressure were significantly correlated with insulin resistance, moreover measures of SLD were well predicted by body mass index values.

To let Authors duplicate this finding, SLD was measured by postero-lateral scanning. It was used the average value obtained by measuring the maximum length and the cranio-caudal diameter. All the indices were measured thrice.

A subsequent study showed that spleen enlargement was found at significant levels (38%) in obese female rats as determined by Cavalieri volume calculation, an unbiased stereological method<sup>[60]</sup>. These recent results clearly indicated that high fat diet caused splenomegaly *via* sinusoidal dilatation and intracellular or intercellular deposits<sup>[61]</sup>. Although these data are encouraging to find a non-invasive method for NAFLD diagnosis, liver biopsy remains the only reliable method to differentiate simple steatosis or FL from NASH in NAFLD subjects<sup>[58]</sup>. On this line, Kikuchi *et al*<sup>[62]</sup> evaluate the efficacy of non-invasive (99m) Tc-phytate scintigraphy in the diagnosis of NASH in humans and in a rat model. In the first study, patients with suspected NAFLD underwent liver biopsy and (99m) Tc-phytate scintigraphy. As region of interest, signal intensities of the liver and spleen were measured. Subsequently, they observed that the L/S uptake ratio at scintigraphy was significantly decreased in NASH subjects when compared to patients with FL. The L/S ratio was an independent predictor in distinguishing NASH from FL. More interestingly, the decrease of L/S ratio was found in all NASH stages, from its earliest stages (stages 1 and 0). In the second study, the authors induced NASH in rats feeding them with a Methionine- and Choline-Deficient (MCD) diet. In this case, the L/S uptake ratio was also significantly decreased after 8 wk of a MCD diet in comparison with control diet-fed rats. From these data, the authors concluded that non-invasive (99m) Tc-phytate scintigraphy is able to discriminate NASH from FL.

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## INFECTIONS TENDENCY IN OBESITY AND THE POSSIBLE LINK WITH THE SPLEEN

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The frequency of ischemic heart disease observed after traumatic splenectomy and the low cholesterol levels

Table 2 Main topics

Congenital asplenia in humans	
There are two types of congenital asplenia: with or without other clinically evident abnormalities	
Tcf21, Bapx1, Pbx1 and Tlx1 are crucial for spleen development	
The molecular mechanisms and the etiology of spleen development are still unknown	
How the anatomical and histological composition of the spleen can guarantee its function?	
The phagocytosis of old and damaged cells, particles and blood-borne microorganisms from local macrophages takes place in the red pulp	
The spleen is fundamental in the recycling of iron	
Exercise in splenectomized individuals can decrease splanchnic flow and increase blood viscosity	
Most important differences between mice and humans in the spleen organization and functionality are revealed in the immune response	
Role of spleen in limiting bacterial infection	
Splenectomized and asplenic patients are more susceptible to infections, especially caused by <i>Haemophilus influenzae</i>	
Subjects with functional asplenia develop the same type of infections	
The spleen and natural antibodies	
B cells may be divided in two main subpopulations on the basis of life development (fetal or adult), superficie markers and functions	
Spleen might be central to the generation or survival of the B-1a population and therefore splenectomy would lead to their depletion	
Other functions of the spleen	
There is a probable relationship among GALT and spleen in humans	
The spleen also has important hematological functions	
In the spleen were found stem cells with several differentiation properties: haematological, osteogenic and maybe pancreatic	
Assessment of spleen function	
Hematological and immunological parameters should be used in the assessment of spleen function	
The best approach to gauge all the facets of the splenic function is the radioisotope method	
Spleen as a new player	
There is an association between spleen enlargement and NAFLD	
SLD could be used as new marker for assessing splenic function	
Initial data have shown that SLD is more sensitive than IL-6 and VEGF in discriminating NASH from FL, and the optimal cut-off value for SLD is 116 mm	
Infections tendency in obesity and the possible link with the spleen	
Obese subjects have an increased risk to develop malignancies and infections	
The pathophysiological mechanisms by which cellular immune functions are affected by obesity are still under investigation but the spleen may have an important role	

GALT: Gut-associated lymphoid tissue; NAFLD: Nonalcoholic fatty liver disease; SLD: Spleen longitudinal diameter; VEGF: Vascular endothelial growth factor; IL-6: Interleukin-6; NASH: Nonalcoholic steatohepatitis.

found in patients with hypersplenism are observations that suggest a possible role for the spleen in lipid metabolism and in the etiology of atherosclerosis<sup>[63]</sup>. Previous studies showed that obese subjects, compared to non-obese, have an increased risk to develop cardiovascular disease, hypertension, cerebrovascular disease and type 2 diabetes mellitus. But, it is equally important that they have an impaired immune function, as demonstrated by the higher incidence of malignancies and infections. From the literature data it is clear that obesity in humans affects different compartments of immune system, even though there are still few data available on the implicated mechanisms. Elderly people (> 60 years of age) have

an increased risk of infection, showing their peripheral blood lymphocytes a decreased reactivity to mitogens and an impaired proliferative capacity<sup>[64,65]</sup>. The response of T lymphocytes to concanavalin A and response of B lymphocytes to pokeweed mitogen are decreased in obese subjects<sup>[66]</sup>. In addition to the T lymphocyte population, also natural killer cell activity is suppressed in obese men and women > 60 years of age, as mentioned in a report made by Moriguchi *et al*<sup>[66,67]</sup>. Moreover, the natural killer cells activity and percentage of body fat are negatively correlated in both elderly women<sup>[68]</sup>, and middle-aged men<sup>[69]</sup>. These data suggest that obesity is a risk factor for the progressive deteriorating of cellular immune functions. The pathophysiological mechanisms by which cellular immune functions are affected by obesity are still under investigation but the spleen may have an important role. In the splenic lymphocytes of obese mice, the expression of glucose transporter 1 (GLUT-1), analyzed by Western blot analysis, was lower compared to lean rats. The decreased expression of GLUT-1 in these rats is associated with a defective uptake of glucose into immune cells. It is probable that the decreased proliferation of splenic lymphocytes in obese rats is connected to the decreased expression of GLUT-1 and therefore to an impairment of glucose uptake<sup>[69]</sup>. An interesting report by Miyake *et al*<sup>[70]</sup> evaluates NAsFLD mice fed high-fat and high-calorie diet for 12 wk for assessing the extent of antigen-specific immunity response. NAFLD mice and control mice were immunized with hepatitis B vaccine containing hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (HBcAg) and, subsequently, antibody to HBsAg (anti-HBs) blood levels, HBsAg and HBcAg-specific cellular immune response and functions of whole spleen cells, T lymphocytes, B lymphocytes and spleen dendritic cells (DCs) of NAFLD and control mice were assessed *in vitro*. Interestingly, in NAFLD mice levels of anti-HBs and the proliferation activity of HBsAg and HBcAg-specific lymphocytes were significantly lower compared to controls. Higher levels of inflammatory cytokines were produced and T cells have showed an increased proliferation rate in spleen cells of NAFLD than lean mice. Concurrently, DCs processing and presenting antigen activities were significantly decreased in the spleen of NAFLD mice compared to controls. Moreover, the administration of saturated fatty acids caused impaired antigen processing and presenting capacity of murine DCs. These data emphasize that the modification of antigen-specific immunity in NAFLD mice depends on the action of different types of immunocytes, including DCs and lymphocytes, clarifying the role of the spleen in this specific pathological process.

## FUTURE PERSPECTIVES

This paper reports the studies on the use of simpler parameters in assessing the need for medical intervention with respect to healthy and non healthy overweight/obese individuals.

In a not too distant past, the spleen has been considered a neglected and expendable organ. In portal hypertension it was considered an ancillary organ<sup>[71-74]</sup> and it has some importance in infectious disease or as organ localization in lymphoproliferative diseases.

As described before, Table 2, now the spleen is deemed an important component of the immune system, crucial in immune response regulation<sup>[55,75,76]</sup>, and also it has a metabolic asset and it is involved in endocrine function with regard to NAFLD<sup>[51]</sup>.

It is suggested that adoption of a simpler tool to perform measurements could not only reduce the cost of medical care but also provide more reliable identification of patients in need of weight loss<sup>[77-80]</sup>.

Larger and well-implanted studies comprehending better characterized patients should be taken into account to ascertain the validity of this tool.

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