The role of Bruton's tyrosine kinase and the spleen in host defense against bacterial pathogens

Two essential gears in the immune clockwork

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7

Assessment of splenic function

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ABSTRACT

Hyposplenic patients are at risk for Overwhelming Post Splenectomy Infection (OPSI), which has a mortality of up to 70%. Therefore, preventive measures are warranted. However, patients with diminished splenic function are difficult to identify. In this review we discuss (i) immunological, (ii) haematological and (iii) scintigraphic parameters that can be used to measure splenic function.

(i) IgM memory B cells are a potential parameter to assess splenic function, however more studies are necessary for its validation. (ii) Detection of Howell Jolly bodies does not reflect splenic function accurately, whereas determining the percentage of pitted erythrocytes is a well evaluated method and seems a good first line investigation to assess splenic function. (iii) When assessing spleen function, ^{99m}Tc labelled heat-altered autologous erythrocyte scintigraphy with a multimodality Single Photon Emission Computed Tomography (SPECT)-CT technology is the best approach, as all facets of splenic function are evaluated. In conclusion, although scintigraphic methods are most reliable, they are not suitable for screening large populations. We therefore recommend using the percentage of pitted erythrocytes, albeit suboptimal, as a first line investigation and subsequently confirm abnormal readings by means of scintigraphy. More studies evaluating the value of potentially new markers are needed.

INTRODUCTION

The spleen is the largest lymphoid organ in the human body. Its rich and diverse population of immune cells and its ingenious anatomy that enables optimal surveillance and phagocytosis of circulating blood elements play an important role in the defence against pathogens. Table 1 summarizes the different aspects of splenic functions. After splenectomy, patients are at increased risk for Overwhelming Post Splenectomy Infection (OPSI), see Box [1-4].

Table 1: Functions of the spleen.

The red pulp
· Extramedullary hematopoiesis if necessary
· 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
· Defence against bacteria using the iron metabolism of its macrophages
The white pulp
T cell zone (periarterial lymphatic sheath) & 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 tufstin
Mariginal zone (MZ)
· 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 lymhocytes

Apart from patients with a status after splenectomy, there is a much larger group of patients with diminished splenic function. Many diseases are associated with a dysfunctional spleen (Table 2) and the degree of splenic dysfunction varies between patients [5]. For patients suspected to have a diminished functioning spleen, it is important to quantify their splenic function in order to assess the risk of developing OPSI. Subsequently, preventive measurements can be taken and, in case of infection, therapy can be started without delay. In this review we evaluate the available methods to measure splenic function.

Box: Overwhelming post-splenectomy infection.

After splenectomy, patients are at risk for overwhelming infection. This syndrome is called overwhelming post-splenectomy infection (OPSI) or post-splenectomy sepsis (PSS). Patients with functional asplenia are also at risk for this syndrome.

Symptoms: OPSI is characterized by a mild onset with flu-like symptoms such as low grade fever, chills, muscle aches and nausea. However, a subsequent fast deterioration may occur in hours rather than days leading to fulminant sepsis, disseminated intravascular coagulation and multi-organ failure[6].

Incidence: Incidence of OPSI is estimated to be low, 2-5 per 1000 asplenic patients per year[7]. The lifetime risk for developing OPSI is estimated to be 5%[8]. Although more than half of these infections occur within the first two years after splenectomy, the risk remains increased lifelong[1,9].

Mortality: Although incidence is low, mortality is high. Numbers in literature vary between 50 and 70%[1,3]. Notably, 68% of patients die in the first 24 hours, and 80% within 48 hours after onset[3,10].

Micro-organisms: Encapsulated bacteria are important causative organisms of OPSI. S. pneumoniae causes 70% of bacteraemic episodes after splenectomy[3]. Other pathogens, responsible for OPSI are H. influenzae, N. meningitidis, E. coli and Pseudomonas.

Guideline: To prevent OPSI several preventive measures should be taken, such as immunization against the encapsulated bacteria S. pneumoniae, H. influenzae B and N. meningitidis C. Furthermore, patients should use continuous prophylactic antibiotics during the first 2 years after splenectomy and have on-demand antibiotics to use in case of (suspected) infection [11-13]

APPROACHES TO MEASURE SPLENIC FUNCTION

Throughout the years, several methods have been developed to quantify the many different functions of the spleen. These methods are based on haematological, immunological and scintigraphic parameters.

Haematological parameters

Haematological methods reflect the capacity of the spleen to phagocytose deviant erythrocytes and to facilitate an environment wherein erythrocytes rid themselves of solid waste material [14,15]. In case of splenic dysfunction these capacities are impaired, which results in an increase of abnormal circulating red blood cells. Furthermore, large amounts of thrombocytes and leukocytes normally reside in the spleen. Circulating thrombocyte- and leukocyte counts can either be increased or decreased indicative of hyposplenism

in a patient with a dysfunctional spleen (for example thrombocytosis in asplenia and thrombopenia associated with splenomegaly)[5,16].

One of the first methods available to evaluate splenic function was the detection of erythrocytes containing Howell Jolly bodies, using a light microscope viewing a stained peripheral blood smear [17,18]. Howell Jolly bodies are basophilic DNA remains from the nucleus of the erythrocyte precursor cell. Normally, upon leaving the bone marrow, the erythrocyte precursor cell expels its nucleus. In some erythrocytes however, a small portion of DNA remains. Normally the spleen clears the erythrocyte of these nuclear remnants or removes the erythrocytes from the circulation, but when the spleen is absent or has a decreased function these Howell Jolly body containing erythrocytes remain in the circulation. A recently developed method uses flow cytometry to quantify the amount of erythrocytes containing Howell Jolly bodies [19].

Other abnormalities that can be seen on peripheral blood smears of patients with absent or diminished splenic function are acanthocytes (spur cells), target cells (condocytes: erythrocytes with a pattern of central staining, a ring of pallor and an outer ring of staining), haemoglobin remnants (Heinz bodies), siderocytes and iron granulocytes (Pappenheimer bodies) [5,16] .

In individuals with a dysfunctional or absent spleen the membrane of erythrocytes appears to contain so called “pits” when studied with interference phase microscopy [20]. With electron microscopy it was shown that these “pits” are in fact large vacuoles (about 300 nm in diameter) beneath or attached to the plasma membrane. These vacuoles have low optical density, due to contained waste material of the erythrocyte such as ferritine, haemoglobin and rest material of mitochondria and membranes [14,15,21]. In case of normal splenic function, pits are seen in 0-4% of the erythrocytes [20,22,23]. A pit count above 4% has been associated with hyposplenism, although asplenia or clinically relevant hyposplenism is most often associated with much higher values, ranging from 15 to 70% [22,24,25]. Casper *et al.* noted that in 5 patients with sickle cell disease who developed sepsis and/or meningitis, pit counts were higher than 15% and therefore the authors suggested this as a cut-off value for significant splenic dysfunction [23]. The same cut-off value was suggested by Corazza *et al.*, who noted that patients that underwent splenectomy had functional residual splenic tissue when pits counts were beneath 16% [26].

Another method to evaluate spleen function is counting erythrocytes containing argyrophilic inclusions, where normal values range from 0-3%. This method uses a silver stain and in comparison with a normal Wrights stain, the argyrophilic inclusions show to be Howell Jolly bodies, Pappenheimer bodies and other inclusions visible in patients with a decreased or absent splenic function [27].

Table 2: Causes of hyposplenism (Adapted from William B.M. *et al*, Table 1[5]).

Congenital disorders	*Haematologic/Neoplastic disorders*
· Congenital asplenia (isolated)	· Bone Marrow transplantation
· Ivemark's syndrome	· Graft versus host disease
· Stormorken's syndrome	· Acute leukemias
· APECED syndrome	· Chronic lymphocytic leukemia
· Fetal hydantion syndrome	· Non-Hodgkin's lymphoma
· Congenital cyanotic heart disease	· Essential thrombocythemia
· Normal and premature neonates	· Systemic mastocytosis
Sickle hemoglobinopathies	· Sezary syndrome
· SS / SC	· Pure red cell asplenia
· S/B-thalassemia	· Fanconi syndrome
· SE	· Advanced breast cancer
· SO-Arab	· Hemangiosarcoma of the spleen
· SD-Los Angeles	· Hemangioendothelioma of the spleen
Gastrointestinal diseases	· Malignant histiocytosis
· Celiac disease	*Sepsis/infectious diseases*
· Ulcerative Colitis	· Disseminated meningeococcemia
· Crohn's disease	· Acquired immunodeficiency syndrome
· Dermatitis herpetiformis	*Circulatory disorders*
· Tropical sprue	· Spenic artery thrombosis
· Whipple's disease	· Splenic vein thrombosis
· Idiopathic ulcerative enteritis	· Celiac artery thrombosis
· Intestinal Lymphangiectasie	*Miscellaneous*
Hepatic disorders	· Old age
· Alcoholic liver disease	· Alcoholism
· Chronic active hepatitis	· Sarcoidosis
· Liver cirrhosis and portal hypertension	· Amyloidosis
· Primary biliary cirrhosis	· Methyldopa administration
Autoimmune disorders	· Hypopituitarism
· Systemic lupus erythematosis	· Selective IgA deficiency
· Discoid lupus	· Primary pulmonary hypertension
· Antiphospholipid syndrome	· Splenic irradiation
· Vasculitis	· Thorotrast exposure
· Rheumatoid arthritis	· Total parenteral nutrition
· Glomerulonephritis	· high-dose corticosteroids
· Sjögren's syndrome	*Surgical Splenectomy*
· Mixed connective tissue disease	
· Graves'disease	
· Hashimoto's thyreoiditis	
· Multiple sclerosis	

Immunological parameters

The spleen contains a large amount of immune cells [28]. In comparison to the peripheral blood lymphocyte compartment, the spleen percentually contains more B-cells and less $CD4^+$ and $CD8^+$ T cells. The percentage $CD8^+$ T cells is higher in the spleen, leading to an inverse CD4/CD8 ratio. Both splenic $CD4^+$ and $CD8^+$ T cell populations show a higher number of activated cells and splenic $CD8^+$ T cells show a more differentiated cytotoxic $CD27^-CD45RA^+$ memory phenotype. Thus, the distribution of the different lymphocyte subsets is markedly different between spleen and peripheral blood, inferring an important and distinct role for the spleen in $CD4^+$ and $CD8^+$ T cell activation [29]. After splenectomy, some immunological functions of the spleen can be taken over by other organs such as liver, bone marrow and peripheral lymph nodes. Therefore these functions are not suitable as a reliable parameter for measuring spleen function. However, the spleen has a specific role in the defence against encapsulated bacteria [1-4]. This is mainly related to the marginal zone (MZ) containing marginal zone B cells (MZ B cells) and macrophages. Marginal zone macrophages are able to capture whole encapsulated bacteria from the circulation and subsequently initiate a humoral immune response [30]. MZ B cells are a distinct B cell lineage that, unlike other B cell lineages, develop and mutate Immunoglobin (Ig) receptors during the first years of life without being engaged in any immune response. Upon stimulation with thymus independent type 2 (TI-2) antigens expressed by encapsulated bacteria, the prediversified MZ B cells can rapidly proliferate and differentiate into antigen presenting cells or into IgM-, IgG-, and IgA- secreting plasma cells, circulating for several months. MZ B cells do not differentiate into memory cells and are therefore part of the (immediate) innate immunity against invading pathogens [31-33].

MZ B cells do not only reside in the MZ but are also present in the circulation and in other lymphoid tissue [34-36]. The spleen is however essential for the maintenance of the MZ B cell population, as appears from a decrease in MZ B cell counts after splenectomy. In contrast with one report [31], other studies have shown that young patients with congenital asplenia have a normal blood MZ B cell population whereas this circulating MZ B cell subset fails to expand in older asplenic individuals [32, 37]. Therefore, the amount of circulating MZ B cells may be an indication of immunological function of the spleen. The effect of diminished spleen function on the composition of naïve-, memory- and effector (antigen specific) T cells in the circulation is not yet known. Decreased numbers of circulating memory B cells have been described in patients with diminished splenic function [31, 32, 37] although this might be due to only a decrease in IgM memory B cells rather than other B memory cells [31, 37].

Some studies have described tuftsin as a potential marker for immunological spleen function, since production of this peptide is mainly depending on the spleen [38, 39]. Tuftsin is a tetrapeptide with protective bactericidal characteristics, since it has been shown to stimulate phagocytosis by neutrophils and macrophages [40]. Decreased serum levels of tuftsin are seen in splenectomised patients [38, 39], patients suffering from sickle cell disease [41] and celiac disease [42].

Scintigraphic parameters

Like haematological parameters, scintigraphic parameters use the capacity of the spleen to filter the blood of deviant cells and particles to measure its activity. The radiopharmaceutical most commonly used for this purpose is technetium-99m (^{99m}Tc) labelled heat-altered autologous erythrocytes, which has replaced the previously commonly used ^{99m}Tc labelled sulphur colloids [43-48]. ^{99m}Tc-labelled sulphur colloid scintigraphy has been used for visualisation of liver and spleen phagocytic function and was once a common study to evaluate for the presence or absence of neoplastic disease, cirrhosis or portal hypertension, being largely supplanted by other modalities like ultrasonography, (PET)-CT or MRI to date [49]. For the assessment of spleen function or presence of an accessory spleen, ^{99m}Tc-labelled heat-altered autologous erythrocyte scintigraphy is now recommended, because in contrast to sulphur colloid scintigraphy sensitivity is not hampered by the relatively high liver uptake [43-48, 50]. Sulphur colloids are captured by phagocytosis, whereas autologous heat-altered erythrocytes are sequestrated by the normal spleen [50, 51]. The normal spleen accumulates about 90% of injected autologous heat-altered erythrocytes, as compared to 10% of sulphur-colloids which are mainly phagocytosed by the liver [50]. After intravenous re-injection of these cells, splenic function can be determined by (i) measuring the clearance rate of the injected cells from the circulation, by analysing blood samples using a gamma well-counter, or (ii) by determining the splenic uptake either solely or by determining the spleen-to-liver uptake ratio using a gamma probe or -camera.

Besides quantitative information on splenic function, planar or dynamic scintigraphy enables visualisation of organ function. In addition to planar scintigraphy, modern multi-modality Single Photon Emission Computed Tomography (SPECT)-CT gamma camera's enable assessment of both function and anatomy (organ volume and structure) within a single investigation potentially introducing clinically useful parameters like organ specific functional volumes [52].

Alternatively, unaltered autologous or donor erythrocytes or platelets can be radio-labelled for assessment of pathological sequestration in the spleen in patients with low

peripheral cell counts such as idiopathic thrombocytopenic purpura or auto-immune anaemia [43, 50, 53-55]

DIFFERENT APPROACHES COMPARED

As there are many approaches to assess splenic function, the question arises what method is most reliable and what method is best for clinical use. Knowledge about correlation and functionality of the different available methods is required before a deliberate decision on which method to use can be made. In the next paragraphs we give an overview of studies comparing the haematological, immunological and scintigraphic parameters to measure splenic function.

Scintigraphic parameters compared

Although ^{99m}Tc-labelled heat-altered autologous erythrocytes as well as ^{99m}Tc-labelled sulphur colloids have been used in studies on splenic function, not much recent data can be found on their correlation when determining the amount of functional splenic tissue. When computing splenic volumes based on planar scintigraphy in two groups of celiac patients, splenic volumes derived from ^{99m}Tc-labelled sulphur colloid scintigraphy correlated well with those from ^{99m}Tc-labelled heat-altered erythrocyte scintigraphy [56]. Furthermore, there was a good correlation between volume of functional splenic tissue and splenic function measured using ^{99m}Tc-labelled heat-altered erythrocyte clearance rates from the circulation. Another publication by Smart *et al.* in patients with mainly Inflammatory Bowel Disease (IBD) showed a strong correlation between clearance of the cells from the circulation and functional spleen volume, with a large variation about the regression line leading to the conclusion that functional spleen size determination was not able to replace measurement of the rate of heat-altered erythrocyte clearance from the circulation in the assessment of hyposplenism [57]. However, these studies were performed in the pre-tomographic and ultrasonographic era using planar imaging for volume calculation, making it less reliable. Furthermore, only functioning spleen was visualized and eligible for volume calculation, implying a direct correlation between function and size [57, 58]

A more recent study by Gotthardt *et al.* showed that spleen-liver ratios as soon as 10 min after reinjection of ^{99m}Tc-labelled heat-altered erythrocytes reliably predict spleen function in IBD patients when compared to rate of clearance of the cells from the circulation. The

spleen-liver ratio measured with ^{99m}Tc-labelled sulphur colloids showed no correlation with the clearance of the ^{99m}Tc-labelled heat–altered erythrocytes [59].

Scintigraphic and haematological parameters compared

The correlation between haematological parameters and scintigraphic parameters has been studied more accurately. In patients with sickle cell disease (SCD), a correlation was found between the uptake of ^{99m}Tc-labelled sulphur colloid by functional splenic tissue and the percentage of pitted erythrocytes [23-25]. In a study by Pearson *et al.* amongst 64 children with homozygous SCD between 8 and 13 months of age it was found that sensitivity, specificity and predictive values were all between 90% and 98% when correlating uptake with a percentage of pitted erythrocytes less then 3,5% [24]. Another study by Lane *et al.* described patients with heterozygous SCD (HbSC), where it was demonstrated that pit counts of more than 20% were indicative for functional asplenia, whereas pit counts lower than 20% were associated with normal or near normal splenic function [25]. Furthermore, in a study of patients with celiac disease and dermatitis herpetiformis, a correlation was found between the percentage of pitted erythrocytes and the size of functioning splenic tissue, as measured by using ^{99m}Tc-labelled autologous heat-altered erythrocytes rather than sulphur colloids [22]. In this same group of patients, a significant correlation was found between the percentage of pitted erythrocytes and the clearance rate of ^{99m}Tc-labelled heat-altered erythrocytes. However, another study describing patients with megaloblastic anaemia and iron deficient anaemia, which are rare causes for hyposplenia, no correlation was found between the percentages of pitted erythrocytes and the blood clearance rate, splenic uptake values and splenic volumes [60]. An explanation for these results could not be given by the authors, however they state that erythrocyte pits may be heterogeneous in origin, composition, or removal kinetics and may be different in individuals that are hyposplenic for various reasons.

The presence of the Howell Jolly bodies has historically been associated with diminished splenic function. However, Howell Jolly bodies have been shown not to correlate with blood clearance of the ^{99m}Tc-labelled heat-altered erythrocytes [58, 59]. Similar results were obtained using ^{51}Chromium-labelled heat-altered erythrocytes [61]. The presence of Howell Jolly bodies did also not correlate with the spleen-liver activity ratio measured with either ^{99m}Tc-labelled heat-altered erythrocytes or ^{99m}Tc-labelled sulphur colloids [59].

Haematological parameters compared

Although there is discussion in literature, it was found that the percentage of erythrocytes containing Howell Jolly bodies correlated with the percentage of pitted erythrocytes [23, 62].

This correlation however, was only present at pit counts higher than 8% and when at least 10.000 erythrocytes were examined. Mild cases of hyposplenism could not be detected by determining percentages of erythrocytes with Howell Jolly bodies, since a pit count above 4% is indicative for hyposplenism. No notice was made of what percentage Howell Jolly Bodies indicates hyposplenism [62].

The argyrophilic inclusion positive erythrocyte count has a sensitivity of 88,9% and a specificity of 97,1% for splenic dysfunction when using the percentage of pitted erythrocytes as a golden standard [27].

Immunological and haematological parameters compared

Because the amount of circulating IgM memory B cells was first described in 2005 as a method to quantify splenic hypofunction, research on this subject is still limited. Two studies describe a correlation between the amount of circulating IgM memory B cells and the percentage of pitted erythrocytes in treated patients with celiac disease and IBD [37,63]. In one study, patients with IBD were divided into either having a decreased splenic function (>4% pitted erythrocytes) or having a normal splenic function (<4% pitted erythrocytes) and both were compared to a control group [37]. Patients with decreased splenic function were shown to have lower amounts of circulating memory B cells, mainly IgM memory B cells, as compared to healthy controls as well as individuals classified to have normal splenic function. Furthermore, IgM memory B cells were shown to be completely absent in the peripheral blood of splenectomised patients. As described above, serum tuftsin might be indicative for splenic function although not much research on the subject has been published. This potential marker was studied in 52 untreated patients with celiac disease [42]. In accordance with the study on IgM memory B cells, patients were divided into groups based on pit count. It was found that hyposplenic as well as eusplenic celiac patients had significantly lower tuftsin activity than healthy controls, but significantly higher than splenectomised patients. There was less tuftsin activity in hyposplenic patients than in eusplenic patients. Furthermore, a correlation was found between serum tuftsin activity and the percentage of pitted erythrocytes.

DISCUSSION

Knowledge about splenic function is important since patients with an absent spleen or decreased splenic function are at risk to develop severe infections with a high mortality rate. Quantification of spleen function could become an important tool for physicians in

their decision-making about the need for preventive measures. However, when assessing splenic function in a clinical setting, physicians should be aware of the multiple facets of spleen function (as described in Table 1) and thus the different possible approaches to determining splenic function.

In many diseases associated with splenic hypofunction such as sickle cell disease, celiac disease, IBD and Systemic Lupus Erythematosis, splenic function changes as the underlying disease activity alters [56, 57, 61, 64-67]. It has been suggested that these changes in splenic function are due to two components of splenic hypofunction in active disease; (i) impaired splenic function that may deteriorate during high disease activity but may improve with treatment, and (ii) splenic atrophy that may lead to irreversible loss of volume and therefore also irreversible loss of function. Illustrating this phenomenon, two patients are described in whom the size of the functional splenic tissue did not alter during relapse of the disease causing the hyposplenia, while the clearance rate of heat damaged autologous erythrocytes was prolonged [56, 57]. Furthermore, shifts in the splenic volume-function relation can also occur otherwise. For example, splenomegaly is frequently observed in hyposplenic heterozygote sickle-cell patients [68]. Also, hypersplenism with homogenous organ function, splenic infarction, splenomas (regenerating nodules) [69] or transition to autosplenectomy can shift the splenic volume-function relation [70].

Because functional splenic tissue can be temporarily impaired during increased disease activity, whereas splenic atrophy is permanent, it is important to be informed about function as well as the actual volume of the organ. To measure the activity of the functional compartment of the spleen, ^{99m}Tc-labelled heat-altered autologous erythrocyte scintigraphy with quantification of spleen uptake seems the most appropriate technique. This method is well evaluated, especially in comparison with other methods [59]. Clearance rates of ^{99m}Tc-labelled heat-altered autologous erythrocytes from the circulation should be considered carefully since this is not solely dependant on splenic sequestration as the liver also partially participates in this process. Although liver uptake of ^{99m}Tc-labelled heat-altered erythrocytes is low in controls, absolute liver uptake can vary considerably, potentially affecting secondary parameters like the spleen-liver ratio [52]. Consequently, as the spleen is not the unique sequestering organ, with variability of liver uptake that possibly increases when splenic function is diminishing, this phenomenon may affect the axioma that measured blood clearance of cells reflects pure spleen function. Therefore, assessment of pure splenic uptake in function of the administered dose might be a better strategy.

Performing ^{99m}Tc-labelled heat-altered erythrocyte scintigraphy on state-of-the-art SPECT-CT gamma cameras will enable combination of both function and anatomy (volume)

within a single investigation with the possibility of accounting for the exact organ volume and the volume of functional organ tissue within the organ.

The large amount of potential hyposplenic patients (Table 2) makes it almost impossible to evaluate splenic function by means of scintigraphy in every patient. Laborious preparation (cell isolation, denaturation and labelling), gamma (SPECT/CT) camera availability and even the radiation burden –albeit low- requires selection of patients eligible for this advanced technique. To screen a large group of potential hyposplenic patients, a more economical, simple and easily accessible method without radiation burden is needed. An alternative is counting the percentage of pitted erythrocytes, which is also well evaluated [22-25, 60]. It is quick, cheap and non invasive. However, interference phase microscopy needs to be available as well as trained personnel. It should also be considered that erythrocyte pits may be heterogeneous as to their origin, composition, or removal kinetics [60]. Percentages indicating hyposplenism may therefore be different in individuals who are hyposplenic for various reasons. Detection of Howell Jolly bodies does not seem to be a reliable method for evaluating splenic function, as correlation with other methods is poor [59]. However, measuring the percentage of Howell Jolly bodies via flow cytometry is a potentially more reliable parameter as large amounts of erythrocytes can be screened [19]. The percentage of argyrophilic inclusion positive erythrocytes is a parameter that is simple and seems reliable as well [27]. Measuring the percentages of both Howell Jolly bodies by flow cytometry as well as argyrophilic inclusion positive erythrocytes do not require special equipment. Both methods however require extensive validation. More studies evaluating the value of potentially new (immunological) markers are needed. Measuring the amount of IgM memory B cells seems a promising method giving the opportunity to measure the susceptibility to infection in a more direct way [31, 37, 63]. Until these new methods have been validated, quantification of the percentages of pitted erythrocytes seems most reliable to screen for potential hyposplenic patients. Abnormal readings can subsequently be confirmed by scintigraphy.

CONCLUSION AND RECOMMENDATIONS

Large studies comparing all available methods in various patient populations with splenic hypofunction are missing, and data on sensitivity and specificity are scarce. To measure splenic function accurately it is important to have knowledge about the volume and function of the active splenic tissue as well as the volume of the organ itself. Function in splenic tissue can temporarily be decreased due to increased disease activity while the

spleen might actually still be partially functioning and is not in state of atrophy. Assessment of spleen function using ^{99m}Tc labelled heat-altered autologous erythrocyte scintigraphy combined with a multimodality SPECT-CT approach seems best for this purpose as all facets of splenic function are evaluated. Measuring the clearance rates of ^{99m}Tc-labelled heat-altered autologous erythrocytes from the circulation should be considered carefully as a method to assess splenic function since this is not solely dependent on spleen activity. The population of hyposplenic patients is too large to screen by the use of scintigraphy as a first line investigation. Therefore a cheaper, simpler, more accessible method is necessary. At present, we recommend to use the percentage of pitted erythrocytes for this purpose, and refer patients with abnormal percentages for scintigraphy. Finally, more studies evaluating the value of potentially new (immunological) markers are needed.

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