

## Patients After Splenectomy: Old Risks and New Perspectives

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### Rezumat

#### *Pacienții post-splenectomie: complicații cunoscute și perspective noi*

Complicațiile care survin în urma splenectomiei pot fi împărțite în infecțioase și non-infecțioase. Legătura dintre splenectomie și aceste riscuri este doar parțial înțeleasă. Răspunsul imun al gazdei împotriva infecției este profund modificat post-splenectomie, iar acești indivizi sunt mai susceptibili să dezvolte sepsis și infecția are o evoluție fulminantă. Splenectomia este de asemenea un potențial factor de risc pentru numeroase complicații vasculare care rezultă în urma obstrucției parțiale sau totale ale unei artere sau vene. În plus, hipertensiunea pulmonară poate fi o complicație severă și uneori fatală a splenectomiei. Unii autori includ și neoplaziile, diabetul zaharat și pancreatita acută în categoria complicațiilor non-infecțioase post-splenectomie. Cea mai de temut complicație pentru pacienții splenectomizați rămâne sepsisul. Fiziopatologia sepsisului încă este controversată. Decesul în sepsis poate surveni fie în urma hiperinflamației, fie în urma imunosupresiei. Multiple dovezi experimentale au dovedit implicarea microRNA-urilor celulare și virale în sepsis. Considerăm faptul că microRNA-urile sunt de

asemenea asociate cu imunosupresia pacientului asplenic, ceea ce determină o creștere a riscului de sepsis fatal. Studiarea nivelului expresiei plasmatice a microRNA-urilor circulante la pacienții asplenic, ar putea conduce la o mai bună înțelegere a imunosupresiei post-splenectomie și la dezvoltarea unor noi metode diagnostice și terapeutice.

**Cuvinte cheie:** splenectomie, microRNA, sepsis, microRNA-uri virale, OPSI

### Abstract

The risks that arise after splenectomy can be divided in infectious and non-infectious. The link between splenectomy and these hazards remains partially unknown. Host defense against infection is altered after splenectomy and such individuals develop sepsis more easily and the infection has a fulminant course. Splenectomy is also a potential risk factor for several vascular complications that result from partial or total obstruction of an arterial or venous blood vessel. Furthermore, pulmonary hypertension can be a severe and sometimes fatal complication following splenectomy. Some authors also consider that malignancies, diabetes mellitus and acute pancreatitis are non-infectious complications after splenectomy. The most feared complication for splenectomized patients remains sepsis. The pathophysiology of sepsis is still controversial. Death in sepsis can occur due to either hyper-inflammation or “immune paralysis”. Multiple experimental evidences link cellular and viral microRNAs with sepsis. We presume that

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miRNAs are also associated with the immunosuppression of the asplenic patients which leads to the high risk of deadly sepsis. Studying the expression level of circulating miRNAs in asplenic patients could help us better understand the post-splenectomy immunosuppression and develop new diagnostic and therapeutic tools.

**Key words:** splenectomy, microRNA, sepsis, viral microRNAs, OPSI

## Introduction

The spleen is structured in two main areas, the red pulp and the white pulp which are separated by the marginal zone (1). The main function of the red pulp is to recycle iron from erythrocytes and to filter blood. The white pulp is a secondary lymphoid organ with key functions in immune response initiation and regulation. The cells that exert the functions of the white pulp are: macrophages, dendritic cells, subsets of B and T lymphocytes. These various immune cells of the white pulp trap antigens and generate an antigen specific response against invading pathogens (bacteria, viruses and fungi) (2).

There are several types of asplenia: surgical, functional and congenital. The most common type of asplenia is the surgical one. Most frequent causes for surgical asplenia are: trauma, hereditary spherocytosis, immune thrombocytopenic purpura, hypersplenism and sickle cell anemia (3).

In the United States, 25000 splenectomies are performed annually (4) and there are a total of 1 million asplenic patients (5). The benefits of performing a splenectomy are clear for each of the mentioned pathologies, but the potential risks that appear for the asplenic patient are challenging. In a recent paper, Schilling proposes that the best choice for patients with mild and moderate anemia suffering of hereditary spherocytosis is to avoid splenectomy (6). Hence, it is clear that for each patient undergoing splenectomy the benefits and risks must be carefully weighted. Numerous splenectomies are avoided because many physicians fear the complications of the asplenic patient (6).

The risks that arise after splenectomy can be divided in infectious and non-infectious. The link between splenectomy and these hazards remains partially unknown. In this paper we will review the main risks for asplenic patients and present a new approach that attempts to explain the mechanisms behind these risks. In the final section of our review we present future perspectives that can reduce the complication rate after splenectomy.

## Risks following splenectomy

### Infectious risks

Host defense against infection is altered after splenectomy and

such individuals develop sepsis more easily and the infection has a fulminant course (3). This phenomenon is called overwhelming post-splenectomy infection (OPSI). In the initial stage of OPSI patients usually experience abdominal pain, lethargy, weight loss, headache, nausea and vomiting. Meningitis and pneumonia are often associated in the more severe stages. The patient's clinical state can quickly deteriorate, leading to coma and death (7). There is a lifelong risk for developing OPSI with the highest incidence in the first two years post-splenectomy (8). Because of the high fatality rate (46%) associated with OPSI, asplenic individuals need to be vaccinated against encapsulated bacteria, which are most frequently responsible for the infection (i.e. *pneumococcus*, *meningococcus* and *Haemophilus influenzae type b*) (9,10). Some authors also recommend annual vaccination against influenza virus, because influenza predisposes to sepsis and pneumonia (11,12). Splenectomized patients are also more prone to develop infections caused by *Babesia*, *capnocytophaga* or *anaplasma phagocytophilum* (13-17). The problem is that for these kinds of infections there is no immunization available (6). *Capnocytophaga* is usually transmitted through dog bites; hence asplenic patients should be instructed to seek medical assistance quickly after such event (6,17). Also there is a higher prevalence of malarial parasite infection but it is uncertain whether such patients have also a higher mortality rate (6,18).

Asplenic children who present fever should promptly receive antibiotics in order to avoid the setting of sepsis, but the treatment compliance is often sub-optimal (19,20).

Although the spleen secretes opsonins (tuftsin, properdin, fibronectin) which intermediate the activation of the complement and phagocytosis and also contains lymphocytes, macrophages and antigen presenting cells, the mechanism through which the spleen is involved in the defense against infection is not currently understood (8).

### Non-infectious risks

#### Vascular complications

Splenectomy is a potential risk factor for several vascular complications that result from partial or total obstruction of an arterial or venous blood vessel. The pathophysiology behind these vascular events is complex, the activation of thrombocytes and hypercoagulability being the most incriminated mechanisms (21).

According to Schilling, splenectomized patients suffering from hereditary spherocytosis (HS) are seven times more likely to suffer an arterial event and three times more likely to undergo a venous event when comparing them to HS patients who had their spleen preserved (22).

#### Arterial events

Individuals suffering from HS with a preserved spleen have fewer adverse arterial events (i.e. stroke, myocardial infarction) than unaffected family members due to lower hemoglobin and cholesterol levels and higher bilirubin concentration (6,23).

Robinette et al found out that there is a greater risk of mortality (RR 1.85) from coronary disease in patients splenectomized for trauma (24). Even though mild chronic anemia seems to offer protection against atherosclerotic events, there is not enough data available regarding an improved survival (6).

### *Thromboembolic events*

Lin et al. reported a 1.97-fold increased risk of developing venous thromboembolism in patients who suffered splenic injury. However, there was no statistically significant difference between splenic injury patients who did or did not undergo splenectomy (25).

Cappellini et al. found out that 24 out of 83 patients with thalassemia intermedia suffered a venous thromboembolic complication, of whom 23 (96%) were asplenic (26).

Even though there is a remarkable thrombocytosis following splenectomy, the short-term risk for thromboembolism seems to be similar to the risk following other types of abdominal surgery (6).

### *Pulmonary hypertension*

Pulmonary hypertension (PH) can be a severe and sometimes fatal complication following splenectomy. Meera et al found out that patients suffering from thalassemia, myelofibrosis or hereditary spherocytosis who underwent splenectomy are at risk for developing PH (27). One of the mechanisms that could explain PH in splenectomized patients is that the abnormal red blood cells have a higher life span and they might activate the platelets which become entrapped in the pulmonary circulation, leading to an increased pressure (28). The conditions associated with PH after splenectomy are responsible for PH even in non-splenectomized patients, but the mean pulmonary artery pressure is higher in splenectomized individuals. Therefore, caution is needed when recommending splenectomy to patients suffering from PH (27).

Pulmonary hypertension also has a higher incidence in patients who underwent splenectomy after trauma leading to the conclusion that asplenic individuals have a higher risk for vascular events regardless of the preexistent abnormal hemolysis (6,29).

### *Splenectomy and cancer risk*

Several cancers have been correlated with immunodeficiency and it is known that the spleen plays a crucial role in the immune response hence, one might argue that splenectomy might be a risk factor for cancer, but the exact mechanism is still unknown (30,31).

Sun et al. found that asplenic patients have a high risk for developing cancer, especially head and neck cancer, digestive tract cancers and hematological malignancies (32). The risk for developing hematological malignancies is even greater in individuals who underwent splenectomy for non-traumatic reasons (33). Preexistent hematological conditions might be responsible for the increased risk found in these patients

(34,35). The higher incidence of gastric cancer might be explained by the fact that asplenic patients are more prone to develop *Helicobacter pylori* infections which is a known risk factor for gastric cancer (32,36).

While some studies suggest that splenectomy increases tumor growth, others advocate that splenectomy might inhibit tumor progression (37–39).

### *Other complications*

According to Wu et al, trauma patients who underwent splenectomy have a twofold risk for developing diabetes mellitus type 2 (DMT2) comparing with patients with a preserved spleen (40). Because various known factors for DMT2 including obesity, physical inactivity, family history were not taken into account, further studies should be performed in order to find out if the spleen has a pathogenic role in the development of DMT2.

Lai et al. reported that splenectomy could be a risk factor for acute pancreatitis (OR 3.57) (41). Even though the pancreas might be at risk for developing an infection after splenectomy which would lead to pancreatic inflammation, the pathogeny of acute pancreatitis is much more complex. More underlying factors such as preexistent conditions and diet and drinking habits should be considered.

Reviewing the literature, it seems that there is a general trend in finding interesting and suspicious correlations between splenectomy and any other possible disease. The only possible way to distinguish between true risks and false risks is by understanding the mechanisms behind the complications.

## **The mechanisms behind the complications**

The most feared complication for splenectomized patients remains sepsis. Styrt reports a risk for deadly sepsis after splenectomy of 0.29 cases per 100 patients among children and around 0.10 cases per 100 patients among adults (42). Bisharat et al report different numbers for surgical asplenia: the sepsis incidence is 3.2% and the mortality rate is 1.4%; the median follow up for these patients was 6.9 years (9).

The mechanisms that link splenectomy with fatal sepsis remain partially unknown.

Recently, new classes of blood-circulating molecules were demonstrated to play a role in the pathophysiology of sepsis (43). One of these potential new molecules is the microRNAs (miRNAs). MiRNAs are a subset of small noncoding RNAs that regulate gene expression. They are endogenous, 21-25 nucleotides RNAs that bind to the 3' untranslated region (3'UTR) of a target messenger RNA (mRNA) and cause post-translational repression or mRNA cleavage. In order to achieve this, miRNAs form the RNA-induced silencing complex (RISC) along with Dicer, Argonaute and other proteins (44). Several studies demonstrated that miRNAs not only play a key role in developing and normal functioning of the immune system (45) but are also associated with many diseases in which the immune function is impaired, including cancer (46). MiRNA levels of expression were observed to be modified in

many infectious (including viral, fungal, bacterial and parasitic) and inflammatory diseases (47). The pathophysiology of sepsis still remains controversial. Death in sepsis can occur due to either hyper-inflammation or “immune paralysis” (48). Multiple experimental evidences link miRNAs with sepsis.

Firstly, numerous research groups studied the levels of cellular and in-plasma circulating miRNAs, as potential biomarkers, for sepsis (49,50). Our group observed in plasma of septic patients, that miR-150 is significantly downregulated and that its value correlates with the aggressiveness of the disease. We proposed miR-150 as a new biomarker for sepsis (51). In a recent study, Tudor et al found 12 deregulated miRNAs in plasma of septic patients. The most intriguing fact was that 2 of these miRNAs are viral miRNAs (miR-K-12-10b and miR-K-12-12\*) belonging to the genome of Kaposi sarcoma-associated herpesvirus (KSHV). These two miRNAs are upregulated in sepsis (52). In a review article Benz et al presents all circulating miRNAs discovered and recommended as sepsis biomarkers (53). One important limit of these studies is that the biological role of these miRNAs is unclear.

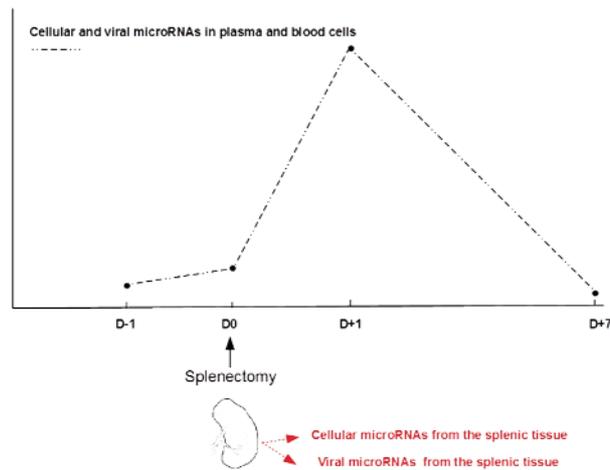
Secondly, Nahid et al demonstrate that miRNAs also interfere in the “endotoxin tolerance” phenomenon (54). The “immune paralysis” observed in sepsis is similar to the “endotoxin tolerance” phenomenon (55). By studying the “endotoxin tolerance” one can better understand sepsis (56). Recent research demonstrates that after LPS induced tolerance, the expression level of miR-146a, miR-132, miR-150 and miR-155 are modified. Thus, microRNAs play an important role in the pathophysiology of sepsis (57).

Thirdly, Quinn and his collaborators demonstrated that three miRNAs (miR-155, miR-21 and miR-146a) are fine tuners of the Toll-like receptor (TLR) signaling pathway. These three miRNAs control several mRNAs involved in the TLR4 intracellular pathway. The TLRs are the main receptors of the innate immune system and play a key role in the endotoxin tolerance phenomenon (58). Hence, microRNAs seem to be regulators of the immune mechanisms.

We presuppose that miRNAs are also associated with the immunosuppression of the asplenic patients which leads to the high risk of deadly sepsis.

Interestingly, Tudor et al observed that two KSHV miRNAs are up-regulated in the blood of septic and postoperative patients (52). It is well known that Kaposi Sarcoma (KS) is associated with immunosuppressive states, especially that of AIDS (59). The asplenic patient is an immunocompromised host. Jirillo et al compared the immunosuppression after splenectomy with that of HIV-infected patients, suggesting that asplenic patients present a quantitative and qualitative T cell dysfunction (60). Regarding the analogy between the cellular immunosuppression of the post-splenectomy infected patients who develop sepsis and HIV patients; we assume that KSHV miRNAs could also be deregulated in plasma of asplenic patients. The role of KSHV miRNAs is still debated. Tudor et al proposed that these miRNAs are agonist of TLR8 and are part of a positive feedback mechanism that induces an altered cytokine secretion (52).

We presume that microRNAs can be a missing link, which



**Figure 1.** We hypothesize that the level of cellular and viral microRNAs in plasma and blood cells rises after splenectomy (D0) and reaches a peak on day 1 (D+1). Most probably these microRNAs are specific for the splenic tissue.

explains the relationship between splenectomy and the high risk for deadly sepsis. Studying the expression level of circulating miRNAs in asplenic patients could help us better understand the post-splenectomy immunosuppression. We hypothesize that after performing a splenectomy the expression level of circulating viral and cellular miRNAs is elevated. This event could represent the association between asplenia and its complications (Fig. 1).

Furthermore, due to the complexity of carcinogenesis and its connection with the immune system a different approach, such as microRNA profiling might be suitable in order to understand the role of the spleen in the immunologic defense and oncogenesis.

Animal studies reveal that the lipid metabolism is altered after splenectomy, increasing the total cholesterol level, independent of hemolysis. These metabolic changes are one possible cause of the atherosclerotic complications after splenectomy (61,62). Similarly, a large amount of studies report that miRNAs are involved in the regulation of the lipid metabolism, leading to atherosclerosis and fat liver disease (63). One can speculate that shifts in miRNAs concentration after splenectomy are responsible for the development of atherosclerosis.

## Future perspectives

In the past decades, spleen preserving strategies became the preferred treatment options for hemodynamically stable patients with splenic injuries, various splenic tumors and hematological diseases (8,64–68). These approaches emerged in order to reduce the infectious and non-infectious complications after splenectomy. After arterial embolization the patients seem to have a good immunoglobulin response to pneumococci and *H. influenzae* (69,70), therefore vaccination being unnecessary in almost all of these patients. But the decrease of complications

remains highly debated. When comparing splenectomy with arterial embolization the complications rates differ between the institutions where the procedures are performed (71-73). In order to prove the benefits of splenic embolization over total splenectomy, Walusimbi et al compared the level of several immune cells and small proteins of the complement system. They found only a higher number of B-Lymphocytes and NK T-cells in splenectomized patients, these changes do not explain the altered immune function after splenectomy. In the final section of the article, they stress out that the used immune markers are not specific for evaluating the immune function of the spleen (64). Considering the role of miRNAs in the regulation of the immune system, we assume that these small non-coding molecules might be a suitable marker for assessing the immune function of asplenic patients.

Nevertheless, splenectomy remains the treatment of choice in hemodynamically unstable patients with splenic injury, leading to a decreased number of spleen-preserving procedures performed (74). For these patients the asplenia related complications remain high and new therapies and rapid diagnostic procedures are required in order to treat and avoid them.

Circulating miRNAs are a possible diagnostic tool, proposed for several diseases including sepsis (53) and most likely also for OPSI. The main problem regarding miRNA as a possible diagnostic method is the lack of specificity. In a review Haider et al report that many of the miRNAs considered specific for sepsis are diagnostic biomarkers for several different diseases. For example miR-146a, miR-223, miR-16 and miR-155, which were reported in several papers as biomarkers for sepsis (53), are also specific for more than 9 other diseases (75). It is clear that only one miRNA is not sufficient as a marker for a pathological condition; in most diseases an entire group of miRNAs is altered. This group of miRNAs can be seen as a circulating miRNA network, where the different miRNA molecules interact via the shared targets (mRNAs and other types of ncRNAs) (76-78). Thus, any diagnostic or therapeutic procedure should take into account the entire network of circulating miRNAs and not only isolated molecules. When trying to diagnose a disease one looks for a dysregulated network and the purpose of the therapy is to restore the integrity of the network (79).

The same applies for splenectomized patients. The first step is to detect the altered miRNA network (diagnostic step) and the second is to restore the network to resemble the one of a healthy individual (therapeutic step).

One possible limitation of this approach is that it is still unknown where the circulating miRNAs are produced and which is their tropism. Newer studies recommend exploring not just the circulating miRNAs, but also their carriers (80). Circulating miRNAs are present in blood in three different forms: associated with Argonaute complexes (81), connected to lipoproteins (82) and encapsulated in extracellular vesicles (EVs) (83). The most appealing form of blood circulating miRNAs, are those associated with EVs. On the basis of surface proteins of EVs it is possible to determine the cellular origin and the target of the miRNAs (80). Understanding the origin and tropism of the EVs makes it possible to engineer a

suitable drug delivery system containing miRNAs (84) that could restore the altered network.

## Conclusion

In conclusion, future extensive studies regarding the blood and spleen level of miRNAs (and other regulatory molecules) in patients treated for splenic pathologies are highly required. These studies could lead to a better understanding of the mechanism behind these complications and offer new therapies.

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