Splenic studies: prevention of pneumococcal disease on organisational, clinical and experimental level

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Summary

Infectious diseases are a major cause of morbidity and mortality worldwide. Especially people that are susceptible to infection, such as children, the elderly or immuno-compromised patients are at risk. Resistance of pathogens to antimicrobial therapy is increasing at an alarming rate, causing a major problem. This thesis aims to contribute to the prevention of severe (pneumococcal) infection in patients that are immunocompromised due to asplenia or diminished splenic function.

Chapter 1 is a general introduction and describes the normal immune response to invading pathogens, as well as the impaired immunological response to infection after splenectomy. Furthermore, the introduction elaborates on overwhelming intravascular infection after splenectomy (i.e. post-splenectomy sepsis; PSS), as well as recommended preventive strategies to prevent this syndrome.

The first part of this thesis describes studies we performed on an organisational level. In chapter 2, we report on the quality of care for patients after splenectomy in the Netherlands. Strikingly, whereas 80% of patients receive pneumococcal vaccination, only 30% receive all three recommended immunizations, and less than 25% of patients are adequately provided with prophylactic as well as stand-by antibiotics. In Chapter 3, analysis of 30% of all Dutch hospitals shows that admission to an academic hospital is associated with better adherence to best-practice than admission to a non-university teaching hospital or non-teaching hospital. Neither the presence of a hospital protocol, nor the size of the surgical staff was related to the better performance of academic hospitals; differences are therefore most likely due to practice organisation. All hospitals however need to improve care for asplenic patients. In chapter 4, we have investigated the experienced barriers among physicians in adhering to best-practice for splenectomised patients, as published by the British Committee for Standards in Haematology 1,2. Physicians, both general practitioners (GPs) and hospital specialists, reported that their knowledge about recommendations for asplenic patients is adequate and that they agree with the British guideline contents. However, responsibilities in the care for asplenic patients were reported to be unclear, and there was a lack of mutual trust between GPs and specialists. Caregivers suggest that the presence of a national guideline, preferably available online, would contribute to adherence to best-practice. Chapter 5 is the result of a national task force (WIHA; Werkgroep voor Infectiepreventie bij Hyposplenie en Asplenie) that has published recommendations for preventing infections in patients with hyposplenia and asplenia.

The second part of this thesis describes studies at clinical level. Chapter 6 is a literature review on accurate measurement of splenic function, and concludes that spleen scintigraphy with heat-altered, labeled autologous erythrocytes is best for this purpose. Chapter 7 is the result of a clinical study, where different diagnostic tests for determining splenic function were compared
between 3 groups: healthy volunteers, hyposplenic patients (due to sickle cell disease) and splenectomized patients. We demonstrate that diagnostic testing for hyposplenia is complicated, that the common method of determining Howell Jolly bodies should be used with caution, and that counting IgM memory B cells is a promising indicator for hyposplenic function.

In the third and last part, studies at experimental level are described. In chapter 8 we have investigated the role of the bacterial capsule of the pneumococcus during infections in asplenic mice. We have shown for the first time that S. pneumoniae mutants that don’t express a capsule (Cps-locus mutants), but still have other virulence factors, are not capable of causing morbidity and mortality during asplenia. Therefore, it is indeed the polysaccharide capsule that is responsible for the increased susceptibility after splenectomy.

Although TLR2 has been designated the major receptor for Gram-positive bacteria, this receptor has been shown not to play a major role in host defense against pneumococcal pneumonia. The results presented in Chapter 9 indicate that TLR2 does not have a significant role in host defense during S. pneumoniae pneumonia in the asplenic state either. Therefore, there are other components of the immune system than the spleen that can provide a sufficient backup mechanism for TLR2 deficiency in the defense against intrapulmonary infections with S. pneumoniae.
General discussion

In the first part of this thesis, we showed that the management of post-splenectomy patients in the Netherlands is inadequate. Although university hospitals offered higher adherence to the British guideline recommendations in the prevention of infections after splenectomy than other teaching and non-teaching hospitals, for all Dutch hospitals there is room for improvement of the quality of post-splenectomy patient care. Although we were expecting to find a better performance status by academic hospitals, we did not find an explanation for the difference: neither the presence of a (local) protocol nor the size of the surgical staff were associated with better compliance. Organisational factors that might contribute to better performance (such as the presence of a residency or fellowship program) need to be further elucidated.

Although pneumococcal vaccination rates after splenectomy in the Netherlands reported in this thesis are among the highest in the literature, hospitals could – and should- do much better at providing general practitioners with recommendations for post-splenectomy management. We have both identified and quantified physicians’ experienced barriers to comply with best practice recommendations. Better informed patients and better transmural collaboration and communication between general practitioners (GPs) and hospital based internists and surgeons, are likely to improve the quality of care of the asplenic patient population. It is important that all caregivers aim at 100% vaccination rates and all patients are discharged with antibiotics and clear instructions on how and when to use them. Since physicians reported that the lack of a national guideline might be the culprit in sustaining a persistent gap between best practice and actual clinical practice, we have urged the development of a Dutch guideline for this group of patients.

We hope that with current recommendations –which are now available for all care givers-, implementation of best-practice for asplenic patients will improve. Further studies will have to show whether our publication and implementation of these recommendations will result in higher vaccination rates, increased prophylactic and therapeutic treatment and more adequate education of patients.

Whereas asplenic patients are relatively easy to identify, diagnosing patients with diminished splenic function is complicated. Large studies comparing available methods for testing splenic function in various patient populations are missing, and data on sensitivity and specificity are scarce. In the second part of this thesis, we have reviewed the literature on how to measure splenic function accurately, and concluded that $^{99m}$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 used with caution as a method to assess splenic function, since this is not solely dependent on spleen activity. Unfortunately, the population of hyposplenic patients is too large to screen by use of scintigraphy. Therefore a cheaper, simpler, more accessible method is necessary. In the literature, counting the percentage
of pitted erythrocytes (PIT, erythrocyte membrane irregularity) in patients with potential hyposplenialia is suggested to be fit for this purpose \(^{11,12}\), and it is suggested to refer patients with abnormal PIT percentages for scintigraphy. However, evaluation of other or potentially new (immunological) markers is still needed. We aimed to find such a simple method for measuring splenic function, by investigating hyposplenic patients (due to sickle cell disease), and comparing them with healthy controls and asplenic patients due to total splenectomy after trauma. We confirmed that patients with homozygous sickle cell anemia (HbSS) are completely functionally asplenic. In patients with heterozygous sickle cell disease (HbSC), a reduced immunological function was seen. We have demonstrated that a diminished *functional* splenic volume correlates with both a reduced phagocytic function as represented by an increase in PIT percentages, a reduced anti-PS response upon pneumococcal vaccination, and reduced numbers of nonswitched memory B cells. Importantly, the absence of Howell Jolly bodies is not indicative of normal functioning splenic tissue. Whether these functional tests can also be correlated to the risk of overwhelming infection with encapsulated bacteria remains to be demonstrated. With current preliminary results based on small patients groups, implicating that further research in larger patient cohorts is needed, we suggest a diagnostic schedule as shown in figure 1.

*Suspected hyposplenia or asplenia?*

1. determine the percentage of \(\text{IgG}^+\text{CD27}^+\) B cells
   - <15\%: no hyposplenia
   - >15\%: no hyposplenia

2. determine *Howell Jolly bodies*
   - Not present
   - Present: hyposplenia proven

3. Determine splenic phagocytic capacity:
   - counting *pitted cells* or perform *spleen scintigraphy*
   - Pit count > 15\%: hyposplenia proven

Dynamic dual-head imaging up to 15 min p.i., followed by SPECT-CT; Functional splenic volumes < 0.3 %uptake/cm\(^3\): proven hyposplenia.

*Figure 1.* Suggested flow chart for diagnostic tests in determining splenic function.
Historically, asplenic patients are thought to be at risk for encapsulated bacteria, since splenectomy has repeatedly been shown to result in a strongly increased susceptibility to severe infections caused by *S. pneumoniae* \(^{13-15}\). We have demonstrated that pneumococcal strains that are lacking the capsule but still express other important virulence factors are rapidly cleared even in the absence of the spleen. As such, our data provide solid evidence that indeed the capsule has a prominent role in the enhanced vulnerability to pneumococcal disease in patients after splenectomy or with functional asplenia. Furthermore, the results presented in this thesis strongly argue against a significant role of TLR2 in host defense during *S. pneumoniae* pneumonia in the asplenic state. Therefore, in the immune competent host, there are other components of the immune system than the spleen that can provide a sufficient backup mechanism for TLR2 deficiency in the defense against intrapulmonary infections with *S. pneumoniae*.

Collectively, the studies described in this thesis show that care for patients without a spleen or with diminished splenic function is difficult, and importantly, that this care should be improved. Pitfalls probably are the few number of splenectomies performed each year, combined with a low incidence of PSS; thus physician exposure to the complications of asplenia is low. The studies described in this thesis are based on (hospital) performance in the Netherlands only, patient cohorts in the clinical study were small, and diagnosing hyposplenia or splenic rest function proved to be complicated. However, mortality associated with disease after splenectomy is alarmingly high, whereas at the same time its prevention is relatively uncomplicated.

We hope and expect that the national recommendations for managing splenectomized patients as given in this thesis will offer doctors a tool to provide their (functionally) asplenic patients with the high quality care they deserve.
References