Antibody Response to a T-Cell-Independent Antigen Is Preserved after Splenic Artery Embolization for Trauma


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Splenic artery embolization (SAE) is increasingly being used as a nonoperative management strategy for patients with blunt splenic injury following trauma. The aim of this study was to assess the splenic function of patients who were emboziled. A clinical study was performed, with splenic function assessed by examining the antibody response to polysaccharide antigens (pneumococcal 23-valent polysaccharide vaccine), B-cell subsets, and the presence of Howell-Jolly bodies (HJB). The data were compared to those obtained from splenectomized patients and healthy controls (HC) who had been included in a previously conducted study. A total of 30 patients were studied: 5 who had proximal SAE, 7 who had distal SAE, 8 who had a splenectomy, and 10 HC. The median vaccine-specific antibody response of the SAE patients (fold increase, 3.97) did not differ significantly from that of the HC (5.29; P = 0.90); however, the median response of the splenectomized patients (2.30) did differ (P = 0.003).

In 2 of the proximally emboziled patients and none of the distally emboziled patients, the ratio of the IgG antibody level postvacci nation compared to that prevaccination was < 2. There were no significant differences in the absolute numbers of lymphocytes or B-cell subsets between the SAE patients and the HC. HJB were not observed in the SAE patients. The splenic immune function of embolized patients was preserved, and therefore routine vaccination appears not to be indicated. Although the median antibody responses did not differ between the patients who underwent proximal SAE and those who underwent distal SAE, 2 of the 5 proximally emboziled patients had insufficient responses to vaccination, whereas none of the distally emboziled patients exhibited an insufficient response. Further research should be done to confirm this finding.

The spleen is one of the most commonly injured organs after blunt trauma (1, 2). It is involved in the antibody response against infection, most importantly against encapsulated bacteria such as Streptococcus pneumoniae, Haemophilus influenzae type B, and Neisseria meningitidis group C (3, 4). Other functions of the spleen include storing B and T lymphocytes, plasma cells, and iron and filtering the blood, including removing damaged or old erythrocyes.

Surgery (splenectomy) has long been the preferred treatment strategy for patients with traumatic injury to the spleen. After a splenectomy, patients have an increased risk of developing an overwhelming postsplenectomy infection (OPSI), which occurs after only 0.5% of all splenectomies in trauma patients but carries a mortality rate of around 50% to 70% (5). The risk of OPSI was one of the driving factors behind the evolution toward the use of more nonoperative treatment (NOM) strategies for splenic injury.

Splenic artery embolization (SAE) is a nonoperative treatment strategy that can be used as an adjunct to observation in cases with an arterial bleeding focus. Advantages of NOM over surgical treatment include the avoidance of surgery-associated complications and morbidity, the possibility of a nonoperative reattempt if rebleeding occurs following observation or SAE, shorter periods of hospitalization, and a possible concomitant reduction in costs (6, 7). In a recent study from our institution, it was shown that, when compared to splenic surgery, SAE was not associated with time loss, even in hemodynamically unstable patients (8).

Different techniques of SAE can be applied, depending on the number of bleeding sites, the location of the bleeding, and the urgency. In distal (or selective) embolization, coils or particles are inserted into the small arterial branch that supplies the segment in which the contrast extravasation, pseudoaneurysm, or abrupt termination (cutoff) is located. Consequently, infarction of only a small part of the parenchyma behind the coils occurs. In proximal (or central) embolization, the main splenic artery is embolized, thereby reducing arterial pressure and flow to the injured parenchyma of the whole organ (9). Different authors have argued that in proximal embolization, reconstitution of the blood supply is allowed through collateral vessels (e.g., short gastric arteries), which allows the spleen to heal (9, 10).

Several research groups have found that the immunocompetence of the spleen after SAE is preserved (11–14). However, different methods for assessing splenic function were applied in different studies, including quantifying immunoglobulins, anti-pneumococcal antibodies (to a mix of 14 or 23 serotypes), or lymphocyte subsets to assess the number of CD4+ T cells, including the CD4+ CD45RA− and CD4+ CD45RO+ subpopulations; assessing the presence of Howell-Jolly bodies; and performing complete blood count/blood chemistry analysis and ultrasound or computed tomography (CT) examinations. These differences make it difficult to compare the results. In addition, a gold standard for assessing splenic function does not exist. In their review of the literature, Skattum et al. concluded that existing studies on immune function after SAE do not provide enough evidence for any firm conclusions to be drawn about the preservation of splenic...
immunocompetence (15). In addition, only one study has compared the splenic function of patients treated with different types of embolization (proximal versus distal) in a subgroup analysis, and the groups in this study were small (4 versus 4, respectively) (14).

Therefore, it is currently unknown whether routine vaccination is indicated for embolized patients. This uncertainty is reflected in the low percentage (8.4%) of physicians providing routine vaccinations to patients who have been treated nonoperatively, as Shatz discovered (16).

The aim of this study was to assess the splenic function of patients who were embolized after blunt splenic injury. We also determined whether the splenic function of proximally embolized patients differed from that of distally embolized patients.

**MATERIALS AND METHODS**

**Patients.** A clinical study was performed. Adult patients (≥18 years old), embolized for splenic injury with a traumatic cause which had occurred in the past 6 months, were invited to participate in the study. This time point was chosen in order to reduce the chances of the immune function being affected by the trauma (14). Patients with limited mental capacity or who had language skills which meant that explanations and instructions could not be followed were excluded. Also excluded were patients with a splenic injury initially treated with observational management or splenic surgery, patients in whom a reintervention was performed after the initial SAE, patients who were treated with a combination of proximal and distal SAE, patients who had received a pneumococcal vaccine in the past 5 years or who had made a blood donation or had a blood loss of >400 ml in the past 3 months, patients who had participated in another medical study or studies, patients who had an allergic reaction to previously administered vaccines, and patients with an acute or chronic illness that possibly influenced their immunity or splenic function (i.e., hematological or immunological diseases). Since SAE is a relatively low-volume procedure in our medical center, a convenience sample was taken. The decision to perform proximal or distal SAE in our hospital is at the discretion of the interventional radiologist and is always adjusted to the individual patient. Generally, proximal SAE is performed in urgent cases (e.g., for a hemodynamically compromised patient or an initially stable patient with clinical deterioration), for multiple bleeding sites within the spleen, or for an anatomy that does not allow distal SAE. In all other cases, distal SAE is performed.

The SAE patients who were included were compared to previously studied surgically treated patients and to healthy controls (HC) (17). The serum samples of these patients and controls (who received the same vaccines and tests as did the SAE patients) had been stored, and their levels of antibodies against pneumococcal polysaccharides were reanalyzed, since a new method with new reference values had been introduced. Ethical approval was obtained by the Medical Ethical Committee of the Academic Medical Center (AMC, Amsterdam).

**Methods.** Patients were invited twice to the outpatient department of the Trauma Surgery Academic Medical Center. During the first visit, blood samples were drawn (a total of 24 ml per patient was collected; 1 clotted-blood tube and 4 EDTA tubes), and the pneumococcal 23-valent polysaccharide vaccine (PPV-23) (Pneumo-23; Sanofi Pasteur MSD) was administered. During the second visit, 14 days later, the same amount of blood was drawn. In addition, patients were asked to report their antibiotic use and the number of infectious episodes that had occurred after the accident compared to the same data before the accident. Patients who were incapable of coming to the hospital, for whatever reason, were visited at home. Vaccination after SAE is not part of the routine medical care in our hospital.

**Splenic function.** The immunological function of the spleen was assessed by measuring the antibody response to polysaccharide antigens (the ratio of the IgG antibody level postvaccination compared to that prevaccination). The pneumococcal polysaccharide vaccine induces a secondary T-cell-independent memory B-cell response. We administered the standard dose (0.5 ml) for adults intramuscularly in the deltoid muscle of the arm. The sera were stored at −20°C until use. Pre- and postvaccination serum samples were analyzed simultaneously by enzyme-linked immunosorbent assay (ELISA) (VaccZyme; The Binding Site Group Ltd., Birmingham, United Kingdom). The aggregate response to all 23 serotypes in the vaccine was measured. Antipneumococcal polysaccharide titers were reported in mg/liter. A ratio of <2 was considered an insufficient response to vaccination.

The absolute numbers and percentages of T (CD3, CD4, and CD8), B (CD19 and CD20), and natural killer (NK) lymphocytes were assessed using a fluorescence-activated cell-sorting (FACS) machine (BD Biosciences, Erembodegem, Belgium). Flow cytometric immunophenotyping of B cells was performed on isolated peripheral blood mononuclear cells (PBMCs), incubated with directly labeled fluorescent monoclonal antibodies (17). Three types of B cells were specified: naïve cells (IgD−CD27−), nonswitched memory B cells (IgD+CD27−), and switched memory B cells (IgD−CD27+). The numbers of nonswitched memory B cells (IgD−CD27+), in particular, have been shown to be decreased or absent in functional asplenia (17). Lymphocytes were analyzed only once (before vaccination), since previous work has shown that there were no differences in absolute cell counts or in percentages between pre- and postvaccination samples (17). HJB were made visible in the peripheral blood smear of fresh EDTA blood by Pappenheim (basophilic) staining (combination of May-Grunwald and Giemsa). HJB are nuclear remnants in erythrocytes. When the spleen is functioning normally, HJB-containing erythrocytes are removed quickly and efficiently from the blood circulation (18). A certified technician from the hematology laboratory assessed the presence of HJB by counting the ratio of HJB-containing erythrocytes per 1,000 red blood cells under the microscope and assigning them to the following categories: absent, 0 per 1,000 erythrocytes; +, 1 to 3 per 1,000 erythrocytes; ++, 3 to 7 per 1,000 erythrocytes; and ++++, >7 per 1,000 erythrocytes. HJB counting is regarded as a simple and reliable technique for identifying the presence of nuclear fragments in the peripheral blood (12). Technicians were blinded to the patients’ names and identification numbers.

**Statistical analysis.** The data were analyzed using the IBM SPSS software package, version 20 (IBM Corp., Armonk, NY). Categorical data are expressed as numbers (percentages), and continuous data are expressed as means with standard deviations (SD) or medians with the 25th and 75th percentiles. The Mann-Whitney U test was applied to test the fold increase in antipneumococcal polysaccharides and to compare the median lymphocyte counts. The independent t test was used to compare normally distributed continuous variables. A P value of <0.05 was considered statistically significant.

**RESULTS**

A total of 115 embolized patients were assessed for eligibility between January 2006 and May 2013. Pediatric patients, patients who had died (of their traumatic injuries), patients who were treated with a combination of proximal and distal embolization, and patients who had splenic surgery after SAE were excluded. After these exclusions, 29 patients were invited to participate, and 12 patients were included. Of the 17 patients who did not participate, 2 were unable to take part because they were resident in a foreign country, 1 had a comorbidity that might have affected immunity, 4 were not willing to participate, 9 were lost to follow-up (2 because their new addresses were unknown), and 1 was admitted to a clinic for treatment of his alcohol addiction. The characteristics of the included patients are listed in Table 1. The types of embolization were proximal (5 patients) and distal (7 patients).

Five patients treated with SAE received antibiotic treatment...
The splenic function of trauma patients treated with splenic artery embolization was preserved, which was supported by sufficient responses to vaccination with PPV-23, no differences in lymphocytes or B-cell subsets compared to those of healthy controls, and the absence of Howell-Jolly bodies. No statistically significant difference was found between proximally and distally embolized patients with regard to the immunological or phagocytic function of the spleen. These data are in agreement with the clinical course of our patients, who did not experience more infections after their injury.

One research group previously compared the splenic function of proximally embolized patients to that of distally embolized patients (14). In this comparison of 4 proximally and 4 distally embolized patients, no differences in CD4+ T cells were found. In the current study, we also found no significant difference in the median antibody responses to polysaccharide antigens between proximally and distally embolized patients (3.38 and 3.2, respectively). However, the results of antibody tests for the individual patients showed that 2 of the 5 proximally embolized patients had insufficient responses to vaccination, as expressed by a ratio of <2, whereas none of the distally embolized patients had an insufficient response. In one patient, the low ratio may be explained by a relatively high prevaccination titer, possibly caused by previous vaccination with a pneumococcal vaccine (≥6 years ago). Because of the high prevaccination titers, the antibody increase should have been much higher in order to achieve the same ratio. The insufficient response to vaccination in the other patient might reflect impaired splenic function.

Not only is the increase in antibodies (ratio) important, but absolute postvaccination titers also should be high enough for patients to be protected against infections with Streptococcus pneumoniae. Two of the proximally embolized patients had relatively low postvaccination titers (1 of these patients also had a ratio of <2), whereas all distally embolized patients had high postvaccination titers. Although there is no generally accepted standard regarding the required amount of antibodies to establish a protective effect, these findings suggest that there might be a difference in splenic function after proximal and distal SAE, a difference that may be related to the underlying pathophysiologic mechanism. In distal embolization, infarction of the parenchyma behind the coils occurs, but the majority of the parenchyma is left unchanged and retains its function. In proximal embolization, the main splenic artery is embolized. It is conceivable that, in some patients, blood flow is poorly reconstituted because of the slow development of collateral vessels or the complete occlusion of the splenic artery, leading to infarction, the loss of functional tissue of larger parts of the spleen, and, subsequently, impaired splenic function.

Several studies have addressed splenic function after embolization (11–14, 19). Although different in study design, control

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**TABLE 2** Response upon vaccination with PPV-23

<table>
<thead>
<tr>
<th>Response</th>
<th>Median pre- and postvaccination antipolysaccharide IgG titers (no. of patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAEa (12)</td>
<td>3.97 2.30 5.29</td>
</tr>
<tr>
<td>HCd (10)</td>
<td>0.90 0.003</td>
</tr>
</tbody>
</table>

a Ratios were calculated as the median of the ratio of all the individual patients (postvaccination divided by prevaccination titers).

b SAE, splenic artery embolization.

c The values of patients who underwent a splenectomy and the healthy controls were collected in a previously conducted study (17).

d HC, healthy controls.

e The ratios of the SAE and splenectomy groups were compared to those of the healthy controls with the Mann-Whitney U test. The value in bold type is considered statistically significant.
groups, splenic function tests, and the numbers of patients vaccinated, all but one study concluded that splenic immune function seems to be preserved and that routine immunization is not necessary. Our findings support this previous research but also raise new questions. More research with larger patient numbers is needed to make robust recommendations and to explore in greater depth the possible differences between proximally and distally embolized patients. A direct and specific test for investigating splenic function is still not available. It would be preferable if a test were developed to map the percentage of functional splenic tissue. Future research should include CT scanning to monitor the healing capacity of the spleen and ideally would include erythrocyte scintigraphy with multimodality single-photon emission CT technology.

Splenectomized patients had significantly lower rates of nonswitched memory B cells than did the healthy controls (17). In this study, we found that the percentage of nonswitched memory B cells for the proximally embolized patients was only slightly higher than that in the splenectomized patients. This is alarming because nonswitched memory B cells have been shown to protect patients from mortality caused by S. pneumoniae infection after splenectomy (20).

**Strengths and limitations.** One of the strengths of this study is that we compared SAE patients to healthy controls and splenectomized patients. In addition, we are among the first to compare the two embolization techniques, proximal and distal SAE. Some authors have measured only exposure-driven immunity (12, 14, 19). The disadvantage of this method is that if low antibody levels are found, they might be explained by low exposure or impaired splenic function. We measured both pre- and postvaccination antibody levels, which allowed us to objectify previous exposure to pneumococcal polysaccharides and to measure the immunologic function of the spleen (i.e., the increase in antibody levels).

Unfortunately, we included fewer patients than we intended. The fact that we were unable to observe a statistically significant difference between the patients treated with proximal SAE and those treated with distal SAE might be explained by low (statistical) power due to the small patient numbers. Since one of the aims of this study was to investigate whether differences exist between proximal and distal embolization, we nevertheless chose to conduct these statistical tests. Future research should be done to confirm and further explore our findings.

Another limitation is that, although the presence of HJB was found to be significantly associated with diminished functional splenic volume, the absence of HJB is not indicative of normally functioning splenic tissue, as assessed by scintigraphy (17). Lammers et al. (17) found that at a functional splenic volume of

![Image](http://cvi.asm.org/)

**FIG 1** Antibody response to polysaccharide antigens (ratio) for each patient. SAE, splenic artery embolization; HC, healthy controls. *, patients with a ratio of <2 (n = 2).

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**TABLE 3** Analysis of lymphocytes (absolute cell counts) and B-cell subsets

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Proximal SAE (n = 5)</th>
<th>Distal SAE (n = 7)</th>
<th>Splenectomy (n = 8)</th>
<th>Healthy controls (n = 10)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD3⁺ T cells</td>
<td>1.06</td>
<td>1.73</td>
<td>2.18</td>
<td>1.22</td>
<td>0.84⁴</td>
</tr>
<tr>
<td>CD8⁺ T cells</td>
<td>0.60</td>
<td>0.50</td>
<td>0.58</td>
<td>0.42</td>
<td>0.95⁵</td>
</tr>
<tr>
<td>CD4⁺ T cells</td>
<td>0.83</td>
<td>1.04</td>
<td>1.51</td>
<td>0.79</td>
<td>0.29⁶</td>
</tr>
<tr>
<td>CD4⁺ CD27⁺</td>
<td>0.34</td>
<td>0.23</td>
<td>0.50</td>
<td>0.23</td>
<td>0.55⁷</td>
</tr>
<tr>
<td>NK cells</td>
<td>0.34</td>
<td>0.23</td>
<td>0.50</td>
<td>0.23</td>
<td>0.55⁷</td>
</tr>
<tr>
<td>B cells</td>
<td>0.28</td>
<td>0.28</td>
<td>0.61</td>
<td>0.22</td>
<td>0.31⁸</td>
</tr>
<tr>
<td>IgD⁺ CD27⁻</td>
<td>75.50</td>
<td>70.60</td>
<td>77.55</td>
<td>65.87</td>
<td>0.22⁹</td>
</tr>
<tr>
<td>IgD⁺ CD27⁺</td>
<td>7.98</td>
<td>13.24</td>
<td>7.20</td>
<td>15.47</td>
<td>0.22⁹</td>
</tr>
<tr>
<td>IgD⁻ CD27⁺</td>
<td>12.30</td>
<td>13.71</td>
<td>12.83</td>
<td>15.82</td>
<td>0.28⁹</td>
</tr>
</tbody>
</table>

⁴ Median values are presented unless otherwise indicated. Absolute cell counts were measured in 10⁸ cells/liter.
⁵ All SAE (proximal and distal) patients versus healthy controls.
⁶ SAE versus splenectomized patients.
⁷ Significant result.
⁸ Proximal SAE versus distal SAE.
⁹ Mean values are shown.

*Measured in percentages of CD19⁺ cells. IgD⁺ CD27⁺, naive nonswitched B cells; IgD⁺ CD27⁺, nonswitched memory B cells; IgD⁻ CD27⁺, switched memory B cells.
that all embolized patients in our cohort had a remaining splenic volume above the defined threshold of 0.30% uptake/cm². In future research, it would be interesting to investigate what volume of the spleen is required for normal functioning. This would be particularly valuable for patients treated with distal embolization or those who have been treated surgically (e.g., partial splenectomy). If we learn to what extent (which branches of the splenic artery) embolization of the spleen can be performed or what part of the spleen can be surgically resected before splenic function is lost, we will be able provide tailor-made vaccinations.

Also, we measured IgG antibodies against all 23 different serotypes together. In some studies, antibody levels against specific serotypes of *S. pneumoniae* were measured separately (12, 13, 21). Theoretically, there is a possibility that responses to one or more specific serotypes differed between the different groups. Lastly, the opsonic capacity of the induced (pneumococcal) antibodies was not measured. This is a drawback since opsonic capacity has been shown to be associated with vaccine-induced immunoprotection (22, 23).

**Conclusion.** The splenic immune function of patients was preserved, and therefore routine vaccination appears to not be indicated. Although the median antibody responses did not differ between the patients who underwent proximal SAE and those who underwent distal SAE, 2 of the 5 proximally embolized patients had insufficient responses to vaccination, whereas none of the distally embolized patients had an insufficient response. Further research should be done to confirm this finding.

**ACKNOWLEDGMENT**

We have no conflicts of interest to declare.

**REFERENCES**


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