Antibody response to a T-independent antigen is preserved after splenic artery embolisation for trauma

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ABSTRACT

PURPOSE: Splenic Artery Embolisation (SAE) is increasingly being used as a non-operative management strategy for patients with blunt splenic injury following trauma. The aim of this study was to assess splenic function of patients who were embolised.

METHOD: A clinical study was performed. Splenic function was assessed by examining the antibody response to polysaccharide antigens (pneumococcal 23-valent polysaccharide vaccine), B-cell subsets and assessing the presence of Howell-Jolly Bodies (HJB). The data were compared to those obtained in splenectomised patients and healthy controls (HC) who had been included in a previously conducted study.

RESULTS: A total of 30 patients were studied: 5 with proximal SAE, 7 with distal SAE, 8 splenectomised patients and 10 HC. The median vaccine-specific antibody response of the SAE patients (fold increase 3.97) did not differ significantly from the HC (5.29; p value 0.90), the response of the splenectomised patients, however, did (2.30; p value 0.003). In 2 of the proximally embolised patients and none of the distally embolised patients the ratio was <2. There were no significant differences in lymphocytes or B-cell subsets between the SAE patients and the HC. HJB were not observed in the SAE patients.

CONCLUSIONS: The splenic immune function of embolised patients is preserved and routine vaccination appears not to be indicated. Although the median antibody response did not differ between proximal and distal SAE, 2 of the 5 proximally embolised patients had an insufficient response to vaccination, as opposed to none of the distally embolised patients. Further research should be done to confirm this finding.

Key words: splenic injury, trauma, immune function, vaccination
INTRODUCTION

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 the removal of damaged or old erythrocytes.

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 in only 0.5% of all splenectomies in trauma patients but carries a mortality rate of around 50-70% [5]. The risk of OPSI was one of the driving factors behind the evolution towards the use of more non-operative treatment (NOM) strategies for the treatment of splenic injury.

Splenic Artery Embolisation (SAE) is a non-operative treatment strategy that can be used as an adjunct to observation in case of an arterial bleeding focus. Advantages of NOM over surgical treatment include: avoidance of surgery-associated complications and morbidity, the possibility of a non-operative re-attempt 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 the author’s institution, it was shown that SAE was not associated with time loss compared to splenic surgery, even in haemodynamically unstable patients [8].

Different techniques of SAE can be applied, depending on the size of the bleeding, where the bleeding is located and the urgency. In ‘distal’ or ‘selective’ embolisation coils or particles are inserted into the small arterial branch that supplies the segment in which the contrast extravasation, pseudoaneurysm or abrupt termination (‘cut-off’) is located. Consequently, infarction of only a (small part of the) parenchyma behind the coils occurs. In proximal (also called ‘central’) embolisation, the main splenic artery is embolised, thereby reducing arterial pressure and flow to the injured parenchyma of the whole organ [9]. Different authors have argued that in proximal embolisation, 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 are applied in different studies (consisting of one or several of the following methods: quantification of immunoglobulins, anti-pneumococcal antibodies (to a mix of 14 or 23 serotypes) or lymphocyte subsets to assessing the amount of CD4+ T-cells including CD4+ CD45RA+ and CD8+ CD45RO+ subpopulations, assessing the presence of Howell-Jolly bodies, performing complete blood...
count/ blood chemistry, and ultrasound or CT-scan examination) and therefore it is 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. conclude 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, there has only been one study that compared splenic function of patients treated with different types of embolisation, proximal vs. distal, in a subgroup analysis and the groups in this study were small (4 versus 4) [14].

To date, therefore, it is unknown whether routine vaccination is indicated in embolised patients. This uncertainty is reflected in the low percentage (8.4%) of physicians providing routine vaccinations to patients treated non-operatively, as Shatz et al. discovered [16].

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

METHODS

Patients

A clinical study was performed. Adult (≥18 years) patients, embolised for splenic injury with a traumatic cause, which had occurred in the last 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. The following categories of patients were also excluded: patients with a splenic injury initially treated with observational management or splenic surgery, patients in whom a re-intervention was performed after 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 made a blood donation or suffered a blood loss of greater than 400 ml in the last 3 months, patients who had participated in (an)other medical study(ies), patients who had an allergic reaction to previously administered vaccines, and patients with an acute or chronic illness which could possibly influence their immunity or splenic function (i.e. haematological 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 case of urgency (e.g. haemodynamically compromised patient or an initially stable patient with clinical deterioration),...
multiple bleeding sites within the spleen or 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 patients after splenectomy, and healthy controls (HC) [17]. The serum samples of these patients and controls (who received the same vaccine and tests as the SAE patients) had been stored and were now re-analyzed for antibodies against pneumococcal polysaccharides 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 was collected; 1 clotted blood tube and 4 EDTA tubes) and the PPV-23 (Pneumo-23®, Sanofi Pasteur, MSD) vaccine was administered. During the second visit, 14 days later, the same (amount of) blood was drawn. In addition, patients were asked to report their antibiotics use and the number of infectious episodes that had occurred after the accident, compared to before the accident. Patient’s 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 IgG antibody levels post-vaccination compared to pre-vaccination). 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. Serum was stored at -20º C until use. Pre- and post vaccination serum samples were analysed simultaneously by ELISA (Vacczyme™, The Binding Site Group Ltd, Birmingham, UK). The aggregate response to all 23 serotypes in the vaccine was measured. Anti-pneumococcal polysaccharides titres were reported in mg/L. A ratio of less than two was considered an insufficient response to vaccination.

Absolute numbers and percentages of T (CD3, CD4 and CD8), B (CD19, CD20), and Natural Killer (NK)-lymphocytes were assessed using a fluorescence-activated cell sorting (FACS) machine (BD Biosciences, Erembodegem, Belgium). Flowcytometric immunophenotyping of B cells was performed on isolated peripheral blood mononuclear cells (PBMCs), incubated with directly-labelled fluorescent monoclonal antibodies [17].
Three types of B-cells were specified: naïve cells (IgD⁺CD27⁻), non-switched memory B-cells (IgD⁺CD27⁺) and switched memory B-cells (IgD⁻CD27⁺). The numbers of non-switched 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 had shown that there were no differences in absolute cell counts nor in percentages between pre- and post-vaccination samples [17]. HJB were made visible in the peripheral blood smear of fresh EDTA blood by a 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 Haematology laboratory assessed the presence of HJB by counting the ratio of HJB-containing erythrocytes per one thousand red cells under the microscope in the following categories: 0 per 1000 erythrocytes (absent), 1-3 per 1000 erythrocytes (+), 3-7 per 1000 erythrocytes (++) and > 7 per 1000 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 for patients’ names and identity numbers.

Statistical Analysis

Data were analysed using IBM statistics software package version 20 (Armonk, NY: IBM Corp., USA). Categorical data were expressed as number (percentage) and continuous data as mean with standard deviation (SD) or median with p25-p75. The Mann-Whitney U test was applied to test the fold-increase in anti-pneumococcal 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 embolised patients were assessed for eligibility between January 2006 and May 2013. The following patients were excluded: paediatric patients, patients who had died (of their traumatic injuries), patients who were treated with a combination of both proximal and distal embolisation, and the patients who had splenic surgery after SAE. After these exclusions, 29 patients were invited to participate of which 12 patients were included. Two patients could not take part because they were resident in a foreign country, one patient had comorbidity that might affect immunity, 4 were not willing to participate, 9 patients were lost to follow-up (2 because their new addresses were unknown), and one patient because he was admitted to a clinic for treatment of
his alcohol addiction. The characteristics of the included patients are depicted in Table 1. The type of embolisation included proximal in 5 patients and distal in 7 patients.

Five of the patients treated with SAE received antibiotic treatment after the accident, but except for 1 patient (who had repeated common colds), the infections seemed not related to splenic function (infection of the arm, recurring urinary infections, infections related to Coronary Artery Bypass Graft, because of asthma). None of the patients indicated that they had experienced more episodes of fever after the accident than before it.

**Immunological function**

The response to vaccination with pneumococcal polysaccharides for the different patient groups is presented in Table 2. The median ratio of the SAE patients did not differ from the HC (3.97 versus 5.29; p-value 0.90), the median ratio of the splenectomised patients, however, did (2.30 versus 5.29; p-value 0.003). The median ratio of the proximal SAE patient group (3.38) was comparable to the distal SAE group (3.21; p-value 0.29). Two patients with proximal SAE had a ratio <2, indicative of an insufficient response to vaccination (indicated by an asterisk in Figure 1). None of the distally embolised patients had a ratio <2.

Results of flowcytometric analysis of lymphocytes and B-cell subsets for the four groups are presented in Table 3. The total of CD3+ T-cells, CD4+ T cells, NK cells and B-cells was significantly higher in the splenectomised patients compared to the embolised patients (p-values 0.02 and 0.01, respectively). There were no significant differences between the embolised patients and the HC. B-cell subsets analysis (Table 3) showed that non-switched memory B-cells were significantly lower in the splenectomised patients compared to the healthy controls (7.20 vs. 15.47; p-value 0.03). There was a non-significant difference between proximal and distal SAE patients, where non-switched memory B-cell levels were 7.98 and 13.24 (p-value 0.14), respectively.

**Haematological or phagocytic function**

No HJB were visible in any of the embolised patients (0%). One of the HC (10%) had HJB in a frequency of 1-3 per 1000 erythrocytes. HJB were visible in 4 of the 8 splenectomised patients (50%). In 3 patients 1-3 HJB were detected per 1000 erythrocytes and in 1 patient 3-7 HJB per 1000 erythrocytes were observed.
The splenic function of trauma patients treated with splenic artery embolisation is preserved, which was supported by a sufficient response to vaccination with PPV-23, no difference in lymphocytes and B-cell subsets compared to healthy controls, and the absence of Howell Jolly Bodies. No statistically significant difference was found between proximally and distally embolised 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 embolised patients to that of distally embolised patients [14]. In this comparison of 4 proximally and 4 distally embolised patients, no differences in CD4+ T cells were found. In the current study, we also found no difference in the median antibody response to polysaccharide antigens between proximally or distally embolised patients (3.38 and 3.2, respectively).

However, the results of the antibody responses of individual patients show that 2 of the 5 proximally embolised patients had an insufficient response to vaccination, as expressed by a ratio < 2, as opposed to none of the distally embolised patients. In one patient the low ratio could be explained by a relatively high pre-vaccination titre, possibly caused by previous vaccination with a pneumococcal vaccine (6 years or longer ago). The 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. Absolute post-vaccination titres should be high enough for patients to be protected against infections with *Streptococcus pneumoniae*. Two of the proximally embolised patients had relatively low post-vaccination titres (1 of these patients also had a ratio <2) whereas all distally embolised patients had high post-vaccination titres. 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 which may be related to the underlying pathophysiologic mechanism. In distal embolisation, infarction of the parenchyma behind the coils occurs, but the majority of parenchyma is left unchanged and retains its function. In proximal embolisation, the main splenic artery is embolised. It is conceivable that, in some patients, the blood flow is poorly reconstituted because of the slow development of collaterals or the complete occlusion of the splenic artery, leading to infarction, the loss of functional tissue of larger parts of the spleen and subsequently to impaired splenic function.

Several studies have addressed splenic function after embolisation [11-14;19]. Although different in study design, control groups, splenic function tests, and the number of patients vaccinated, all but one study have concluded that splenic immune function seems to be preserved and 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 the possible differences between proximally and distally embolised patients at greater depth. A direct and specific test for investigating splenic function is still not available. It would be preferable if a test was developed to map the percentage of functional splenic tissue. Future research should include a CT-scan to monitor the healing capacity of the spleen and ideally an erythrocyte scintigraphy with multimodality single photon emission computed tomography-CT technology.

Splenectomised patients had significantly lower rates of non-switched memory B-cells compared to the healthy controls [17]. In this study we found that the percentage of non-switched memory B cells for the proximally embolised patients was only slightly higher than in the splenectomised patients. This is alarming because non-switched memory B-cells have shown to protect patients from mortality caused by S. Pneumoniae after splenectomy [20].

Strengths & Limitations

One of the strengths of this study is that we were able to compare SAE patients to both healthy controls and splenectomised patients. In addition, we are among the first to compare the two embolisation techniques: proximal and distal SAE. Some authors only measure exposure driven immunity [12;14;19]. The disadvantage of this method is that if low antibody levels are found, this could be explained by both low exposure or impaired splenic function. We measured both pre - and post-vaccination antibody levels which allowed us to objectify previous exposure to pneumococcal polysaccharides and to measure the immunological function of the spleen (i.e. the increase in antibody levels).

Unfortunately, we included fewer patients than we had indented. The fact that we could not observe a statistically significant difference between the patients treated with proximal SAE and those patients treated with distal SAE might be explained by low (statistical) power due to the small patient numbers. Since this study was designed to investigate whether differences exist between proximal and distal embolisation, we nevertheless chose to conduct these statistical tests. Future research should be done to confirm and explore our findings further (e.g. using larger patient groups, and including diagnostic strategies such as scintigraphy or CT-scanning to assess the percentage to which the spleen is infarcted after embolisation and to closely monitor the healing process of the spleen).

Another limitation is that, although the presence of HJB was found to be significantly associated with diminished functional splenic volume, the absence of HJB was found not to be indicative of normal functioning splenic
tissue as assessed by scintigraphy [17]. Lammers et al. found that above a functional splenic volume of 0.30 % uptake/cm³, no HJB were observed. This might suggest that all the embolised patients in our cohort had a splenic volume remaining which was above the defined threshold of 0.30. 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 embolisation or those who have been surgically treated. If we would learn to what extent (which branches of the splenic artery) embolisation of the spleen could be performed or what part of the spleen could be surgically resected before splenic function was lost, we could provide tailor-made vaccinations. Also, we measured IgG antibodies against all 23 different serotypes together. In some studies, antibody levels against specific serotypes of Streptococcus 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, 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
Splenic function of embolised patients is preserved and therefore routine vaccination appears not to be indicated. Although not significant, there were differences in the memory B-cell count and in vaccination responses between proximally and distally embolised patients: 2 of the 5 proximally embolised patients had an insufficient response to vaccination while none of the distally embolised patients exhibited an insufficient response. Further research should be done to confirm this finding.

Conflict of interest
None.

Conflict of interest
None.

Conflict of interest
None.
References


Table 1. Patient characteristics of the embolised patients

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SAE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>38 (22-56)</td>
</tr>
<tr>
<td>Male gender</td>
<td>8 (67%)</td>
</tr>
<tr>
<td>Grade of splenic injury</td>
<td>3 (2-4)</td>
</tr>
<tr>
<td>Injury Severity Score at time of trauma</td>
<td>24 (13-40)</td>
</tr>
<tr>
<td>Time since trauma (years)</td>
<td>5 (3-6)</td>
</tr>
<tr>
<td>Blunt trauma mechanism</td>
<td>12 (100%)</td>
</tr>
</tbody>
</table>

Data are expressed as number (percentage) or median (p25-p75). Abbreviations: SAE: splenic artery embolisation.

The characteristics of the healthy controls and the splenectomised patients are presented in a study completed by Lammers AJ et al. [17].
**Table 2.** Response upon vaccination with Pneumo-23® vaccine

<table>
<thead>
<tr>
<th></th>
<th>SAE n=12</th>
<th>Splenectomy ‡ n=8</th>
<th>HC ‡ n=10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre-vaccination (mg/L)</td>
<td>118.25</td>
<td>234.20</td>
<td>69.66</td>
</tr>
<tr>
<td>Post-vaccination (mg/L)</td>
<td>553.25</td>
<td>539.40</td>
<td>404.80</td>
</tr>
<tr>
<td>Ratio</td>
<td>3.97</td>
<td>2.30</td>
<td>5.29</td>
</tr>
<tr>
<td>P-value* (ratio compared to the HC)</td>
<td>0.90</td>
<td><strong>0.003</strong></td>
<td>-</td>
</tr>
</tbody>
</table>

Abbreviations: SAE: splenic artery embolisation, HC: healthy controls

Pre-and post vaccination anti-polysaccharide IgG titres are reported in median values. The ratio was calculated as the median of the ratio of all the individual patients (post-vaccination divided by pre-vaccination titres).

*: the ratios of both the SAE and splenectomy group were compared to the healthy controls with the Mann-Whitney U test. Emboldened values are considered statistically significant

‡: the values of patients who underwent a splenectomy and the healthy controls were collected in a previously conducted study [17].
### Table 3. Analysis of lymphocytes (absolute cell counts) and B-cell subsets

<table>
<thead>
<tr>
<th></th>
<th>Proximal SAE</th>
<th>Distal SAE</th>
<th>Splenectomy</th>
<th>Healthy Controls</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n=5</td>
<td>n=7</td>
<td>n=8</td>
<td>n=10</td>
<td></td>
</tr>
<tr>
<td>CD3+ T cells</td>
<td>1.06</td>
<td>1.73</td>
<td>2.18</td>
<td>1.22</td>
<td>1. 0.84</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. <strong>0.02</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. 0.47</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>1. 0.95</td>
</tr>
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<td></td>
<td></td>
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<td>2. 0.17</td>
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<td></td>
<td></td>
<td></td>
<td>3. 0.37</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>1. 0.29</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>2. <strong>0.01</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3. 0.44</td>
</tr>
<tr>
<td>NK cells</td>
<td>0.34</td>
<td>0.23</td>
<td>0.50</td>
<td>0.23</td>
<td>1. 0.55</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2. <strong>0.01</strong></td>
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<td></td>
<td></td>
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<td>3. 0.37</td>
</tr>
<tr>
<td>B cells</td>
<td>0.28</td>
<td>0.28</td>
<td>0.61</td>
<td>0.22</td>
<td>1. 0.31</td>
</tr>
<tr>
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<td>2. <strong>0.01</strong></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>3. 0.69</td>
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**B-cell subsets‡†‡**

<table>
<thead>
<tr>
<th></th>
<th>IgD+ CD27-</th>
<th>IgD+CD27+</th>
<th>IgD-CD27+</th>
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<tbody>
<tr>
<td></td>
<td>n=5</td>
<td>n=7</td>
<td>n=8</td>
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<tr>
<td>IgD+ CD27-</td>
<td>75.50</td>
<td>70.6</td>
<td>77.55</td>
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<tr>
<td>IgD+CD27+</td>
<td>7.98</td>
<td>13.24</td>
<td>7.20</td>
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<tr>
<td>IgD-CD27+</td>
<td>12.30</td>
<td>13.71</td>
<td>12.83</td>
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</table>

Median values unless otherwise reported. †: mean values.
IgD+ CD27-: naive non-switched B cells, IgD+CD27+: non-switched memory B cells, IgD-CD27+: switched memory B cells.

*1: All SAE patients vs. healthy controls; 2: SAE vs. splenectomised patients; 3: proximal SAE vs. distal SAE.

Absolute cell counts were measured in 10^6 cells/L. ‡: were measured in % of CD19+ cells.
Figure 1. Antibody response to polysaccharide antigens (ratios) for the individual patients

Abbreviations: SAE: splenic artery embolisation, HC: healthy controls.
*: patients with a ratio<2 (n=2)