

## The efficacy and immunogenicity of Pneumo-23 and ACT-HIB in patients undergoing splenectomy

Splenektomi yapılan hastalarda Pnemo-23 ve Act-HIB'nin etkinliği ve immünojenitesi

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### BACKGROUND

The objective of this prospective study is to validate the efficacy of *Streptococcus pneumoniae* and *Haemophilus influenzae* vaccines in splenectomized patients via the demonstration of seroconversion and uninterrupted ability for opsonization.

### METHODS

Thirty-two adult patients (18 males, 14 females; mean age 46.1 years; range 18 to 79 years) who underwent elective or urgent splenectomy for various benign and malignant hematological disorders, splenic trauma and splenic masses were reviewed. Pneumo-23 and Act-HIB were administered to all patients on routine basis. In order to demonstrate the ongoing opsonizing capacity of the immune system and the seroconversion of immunoglobulins after vaccination, antibody titers of IgG and IgM and plasma C3 and C4 levels were quantitatively measured.

### RESULTS

The operative morbidity was 9% and overall mortality was 16%, with no early postoperative death in this series. Five patients with various malignant disorders died due to dissemination of their primary tumor. None of the patients with benign hematological disorders or those with splenic trauma died during the mean follow-up of 427 days. Furthermore, death from overwhelming postsplenectomy infection was nil in our clinical survey. All of the patients including those with malignancy had normal IgG (mean: 1383.1 mg/dL) and IgM levels (mean: 80.9 mg/dL) during discharge and at the last follow-up. Among the patients with benign hematological disorders, splenic trauma and splenic masses necessitating splenectomy, C3 and C4 levels were entirely within normal limits with a mean of 108.8 mg/dL and 21.4 mg/dL, respectively.

### CONCLUSION

This preliminary study reveals adequate seroconversion of immunoglobulins in all patients and normal C3 and C4 levels in patients with benign hematological disorders and splenic trauma. Moreover, none of the patients in the latter group had *S. pneumoniae* or *H. influenzae* infection nor did they expire due to overwhelming sepsis during the follow-up period. Long-term follow-up is required to determine the continuation of this immunologic response and the necessity of repeated vaccination.

**Key Words:** Act-HIB; *H. influenzae*; pneumococcal vaccines; Pneumo 23; splenectomy; *S. pneumoniae*.

### AMAÇ

Bu prospektif çalışmada, splenektomili olgularda opsonizasyon yetisinin devamlılığını ve serum immünglobulin düzeylerinin dönüşümünü ortaya koyarak, *Streptococcus pneumoniae* ve *Haemophilus influenzae* tip B aşılarının etkinliği gösterildi.

### GEREÇ VE YÖNTEM

İyi ve kötü huylu hematolojik hastalığı olan, splenik travma veya splenik kitle nedeniyle elektif ya da acil splenektomi uygulanmış 32 erişkin hasta (18 erkek, 14 kadın; ort. yaş 46.1; dağılım 18-79) incelendi. Tüm hastalara rutin olarak Pneumo-23 ve Act-HIB aşıları uygulandı. Aşılama sonrası immün sistemde opsonizasyon kapasitesinin devamlılığı ve immünglobulin düzey dönüşümlerini ortaya koymak amacıyla, serum IgG, IgM, C3 ve C4 düzeyleri kantitatif olarak tayin edildi.

### BULGULAR

Serimizde cerrahi mortalite ve morbidite sırasıyla %16 ve %9 olup, erken ameliyat sonu ölüm gözlenmedi. Farklı malin hastalıklı 5 olgu primer tümörün sistemik yayılımı nedeniyle kaybedildi. Benin hematolojik hastalık ve splenik travma nedeniyle splenektomi uygulanan hiçbir olguda, 427 günlük ortalama izlem süresince ölüm saptanmadı. Ayrıca, serimizdeki hiçbir hastada splenektomi sonrası şiddetli enfeksiyona bağlı ölüm gelişmedi. Malin olgular dahil tüm hastaların çıkış ve son kontrol kan örneklerinde IgG ve IgM düzeyleri normal olup, ortalama değerler sırasıyla 1383.1 mg/dL ve 80.9 mg/dL idi. Splenik travma, splenik kitle ve benin hematolojik hastalık grubunda C3 ve C4 düzeyleri tümüyle normal sınırlarda olup, ortalama değerler sırasıyla 108.8 mg/dL ve 21.4 mg/dL idi.

### SONUÇ

Bu öncül çalışmada, aşılama sonrası splenektomiyi takiben tüm olgularda immünglobulin düzeylerinin, selim hematolojik hastalık ya da splenik travmalı olgularda ise ek olarak C3 ve C4 düzeylerinin normal sınırlarda korunduğu gösterildi. Bunun ötesinde, son grupta yer alan olguların hiçbirinde takip süresince sepsise bağlı ölüm ve *H. influenzae* veya *S. pneumoniae* enfeksiyonu gelişmedi. Bu immünolojik yanıtların devamlılığının ve rapel gereksiniminin ortaya konması için uzun dönem takipler gerekmektedir.

**Anahtar Sözcükler:** Act-HIB; *H. influenzae*; pnömokok aşısı; Pneumo 23; splenektomi; *S. pneumoniae*.

Splenectomy used in the surgical management of various hematological disorders, renders the patients susceptible to the development of overwhelming sepsis by certain encapsulated bacteria.<sup>[1-3]</sup> The encapsulated microorganisms most frequently implicated are *Streptococcus pneumoniae*, *Meningococcus* and *Haemophilus influenzae* type B but uncommon offending organisms such as *E. Coli*, *Pseudomonas sp.*, *Candida sp.* and *Herpes zoster* have also been reported.<sup>[3-6]</sup> This increased potential for sepsis is considered to be lifelong, despite vaccination in this group.<sup>[2,7,8]</sup> The high incidence of fulminant sepsis and the spectrum of the offending agents are distinct among patients splenectomized for benign or malignant hematological disorders and those who are vaccinated or not.<sup>[1]</sup>

Therefore, the objective of this prospective study was to validate the efficiency of *Streptococcus pneumoniae* and *Haemophilus influenzae* vaccines in patients with splenic trauma, malignant or benign hematological disorders, and intraabdominal solid tumors which necessitate urgent or elective splenectomy. Furthermore, the occurrence of seroconversion and antibody titers of IgG, IgM and C3, C4 have been quantitatively measured in order to demonstrate immunological responses and the capacity for opsonization in this group.

## PATIENTS AND METHODS

Thirty-two adult patients with various benign and malignant hematological disorders, splenic trauma and splenic masses who had undergone elective or urgent splenectomy for diagnostic and therapeutic indications were reviewed. There were 18 males and 14 females patients with a mean age of 46.1 years (range; 18-79 years). The clinical and demographic characteristics of the patients are shown in Table 1.

At presentation, all patients with clinical features of ITP underwent a diagnostic bone marrow aspirate. The first line treatment was medical for all patients with ITP and only those who failed to respond to steroids with or without IVIg within a period of 3 months were sent to surgery. The surgical approach was either through a left subcostal or midline incision. Routine preoperative vaccination was a standard policy for all patients scheduled for elective splenectomy. Those cases were vaccinated with a mean of eight days (range: 3 to 27 days)

before surgery. A 23-valent polysaccharide vaccine against *Streptococcus pneumoniae* and a vaccine against *Haemophilus influenzae* type B were administered to all patients for the immunization. All urgent cases were vaccinated on the day of surgery and received routine antibiotic with amoxicillin + clavulanic acid (625-1gm bid) or piperacillin + tazobactam (4.5 gm bid or tid) prophylaxis for two weeks to cover the gap between vaccination and seroconversion. In order to demonstrate the seroconversion of immunoglobulins after vaccination and to reveal the ongoing opsonizing capacity of the immune system, antibody titers of IgG and IgM and plasma C3 and C4 levels were quantitatively measured. Blood specimens were collected twice; first after the third post-operative week and then at the last follow-up. All patients received perioperative antibiotic prophylaxis. All infections were recorded. Follow-up ranged between 12 to 1693 days, with a mean of 427 days. In hospital death or death within thirty days of discharge were accepted as early postoperative death.

**Table 1.** The clinical characteristics of patients

Patient number	32
Males / Females	18 / 14
Age (range: 18-79 years)	46.1
<b>Splenectomy indications</b>	
<b>Solid tumors</b>	<b>6</b>
Pancreas carcinoma	4
Gastric carcinoma	1
Adrenal adenocarcinoma	1
<b>Hematologic diseases</b>	<b>13</b>
Non-Hodgkin lymphoma	3
Chronic myeloid leukemia	1
Immune thrombocytopenia	5
Autoimmune hemolytic anemia	2
Thalassemia intermedia	1
Hereditary spherocytosis	1
<b>Others</b>	<b>13</b>
Isolated hydatid cyst	3
Pancreatic pseudocyst	2
Splenic hemartoma	1
Splenic hemangioma	1
Traumatic rupture	5
Sarcoidosis	1

## RESULTS

There were no major complications such as subphrenic abscess, pancreatic injury including pancreatitis or pancreatic fistula. Postoperative hemorrhage to the left subphrenic space occurred in a female patient with chronic myeloid leukemia. The patient recovered after laparotomy. The patient with sarcoidosis developed a lower respiratory tract infection and the patient with penetrating abdominal trauma experienced wound infection during the postoperative period. The causative agents were *Enterobacteria spp.* and *Staphylococcus aureus*, respectively. One patient with chronic myeloid leukemia and two patients with ITP required platelet transfusion and it has been done intraoperatively after the splenic artery ligation. For all patients the hospital stay ranged between 5 to 55 days with a mean of 13.8 days. The overall morbidity was 9%. All of the patients with ITP recovered without any bleeding complication.

None of the patients died during early postoperative period. Five patients with malignancy died with a mean of 176 days following splenectomy. In all of them, the cause of death was the progression of the underlying malignancy. The overall mortality was 16%.

C3 and C4 level could not be analyzed in patients with malignancy. Among the patients with benign diseases and splenic trauma, postoperative C3 (normal: 79.0-152.0 mg/dL) and C4 (normal: 16.0-38.0 mg/dL) levels ranged between 82.7 to 182.0 mg/dL and 13.9 to 40.4 mg/dL, with a mean of 108.8 mg/dL and 21.4 mg/dL respectively. C3b and C4b measurements were not undertaken because of the insufficiency in the laboratory setting. However; C3 and C4 levels were almost within normal range in all subjects who underwent splenectomy due to benign disorders. All of the patients had normal IgG (normal: 751-1560 mg/dL) and IgM levels (normal: 46-304 mg/dL) ranging between 777.0 mg/dL to 2590.0 mg/dL and 29.6 mg/dL to 317.0 mg/dL with a mean of 1383.1 mg/dL and 80.9 mg/dL, respectively. Only two patients, one with sarcoidosis and the other with traumatic splenic rupture had subnormal levels of IgM (43.7 and 29.6 mg/dL, respectively) during follow-up but had no evidence or history of any serious infection.

## DISCUSSION

Along with the peripheral lymphoid tissues, the spleen is the major site of IgM production in the body. This unique property and the production of IgM against previously unrecognized antigens allows clearance of encapsulated microorganisms.<sup>[15]</sup> Removal of this antigens occur in the red pulp by the phagocytic activity of macrophages. The T-cell rich periarteriolar lymphoid sheaths in the white pulp are essentially composed of CD4+ (helper-inducer) T-cells possessing the same phenotype as those that reside in the paracortical region of the peripheral lymph node. On the other hand, the primary B-cell follicles in the white pulp differ from the follicles in the lymph nodes in their propagation of memory B-cells that express CD19, CD20 surface antigens. When exposed to an antigen, these memory B-cells interact with antigen-primed T-cells and proliferate into antibody secreting mature plasma cells.

The spleen was postulated to be the only source of opsonins and C3, C4, C3b and C4b are the major opsonins generated.<sup>[1]</sup> Bacteria coated by opsonins are optimally and rapidly destructed by phagocytic cells. Therefore, splenectomized patients are theoretically vulnerable to sepsis indefinitely because of the loss of opsonizing capacity and the lack of memory B-cell proliferation during antigen exposure. In fact, before and mid-1980s, when administration of vaccine was not being carried out yet on routine basis, postsplenectomy sepsis syndrome levels were as high as 15% after splenectomy with 20-80% ultimate deaths according to much published data.<sup>[11,12,14]</sup>

There was also no standard policy for vaccination after splenectomy during that era. Although some authors advocated daily prophylactic oral penicillin for many years after splenectomy in childhood, noncompliance with this regimen appeared as a major issue.

The overall complication and mortality rate of splenectomy remains higher particularly in patients with hematological malignancies.<sup>[6]</sup> The incidence of fulminant sepsis in adults splenectomized for ITP and for hereditary spherocytosis was within a range of 1.5-2.0% over 8 years of follow-up during the era when routine vaccination was not attempted. Laboratory evidence indicates that polyvalent vac-

cines are immunogenic in asplenic subjects, also Ruben FL. et al. clearly demonstrated adequate antibody response to meningococcal polysaccharide vaccine in asplenic subjects with nonlymphoid tumors or splenic trauma.<sup>[1]</sup>

We also found that IgG, IgM, C3 and C4 levels were entirely within normal limits in patients with benign hematological disorders or splenic trauma during a mean follow-up of 427 days. Similar observations have been reported in 135 patients who underwent splenectomy for hematological malignancies and received *Pneumococcal* and *Haemophilus influenzae* type B vaccines as a standard policy before elective splenectomy. The incidence and mortality of septic complications was 9% with the identification of a single sepsis case caused by *Haemophilus influenzae* (an encapsulated organism). However the profile of causal organisms was divergent and included *Staphylococcus aureus*, *E. coli*, *Pseudomonas*, *Enterobacteria*, and *Candida spp.* in patients in this series with sepsis related death.<sup>[6]</sup>

The phenomenon of De Novo ITP in splenectomized patients, which has been purported to arise from antiplatelet antibodies produced by remote lymphoid organs where B-cell reside, challenges the concept of the unique existence of CD19+ and CD20+ memory B-cells in follicles of splenic white pulp. Moreover, the normal C3 and C4 levels achieved in all patients with benign disorders in our study led to scepticism about the absolute loss of opsonizing capacity of the immune system following splenectomy. Clearly, detection of the opsonizing activity and seroconversion ability of the immune system together with the differences between vaccinated vs. unvaccinated splenectomized subjects requires quantitative measurements of IgG, IgM, C3, C4, C3b, C4b levels and flow-cytometric analysis of CD(19)+, CD(20)+ B-cells both in peripheral lymph nodes and in plasma to be made. These analyses are of further concern of the authors participating in this study and will soon be reported.

### CONCLUSION

Splenectomized patients with different etiologies display 7% sepsis risk in the next ten years with the highest frequency being within the first three years. The Advisory Committee on

Immunization Practice recommends the administration of pneumococcal vaccine (23-valent polysaccharide type) to all patients two weeks before elective splenectomy, in so far as its' possible.<sup>[10]</sup> Besides, addition of preventive measures other than vaccination, particularly the long-term antibiotic prophylaxis, still remains controversial currently.

The evaluation of our preliminary study revealed adequate seroconversion of immunoglobulins in all patients and normal C3 and C4 levels in patients with benign hematological disorders and splenic trauma. Furthermore, neither of the patients in the latter group had pneumococcal or *Haemophilus influenzae* infection nor deceased due to overwhelming sepsis during the follow-up. The efficacy of routine vaccination before splenectomy is clearly evident in this study.

The determination of the persistence of this immunologic response and the necessity of repeated vaccination requires long-term follow-up.

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