Response to vaccination in patients immunocompromised due to asplenia, allogeneic stem cell transplantation or chemotherapy

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Respons op vaccinatie in patiënten met een verminderde afweer ten gevolge van afwezigheid van de milt, allogene stamceltransplantatie of chemotherapie

(met een samenvatting in het Nederlands)

Proefschrift

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Voor pap en mam

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Chapter 1:

General introduction

IMMUNOLOGICAL BASIS OF THE RESPONSE TO VACCINATION

THE IMMUNE RESPONSE

The immune response to infectious agents consists of two general systems: innate (or natural) immunity and acquired (adaptive) immunity. The innate immune system involves a cellular component consisting of macrophages, natural killer cells, granulocytes and proteins including complement factors which act in concordance to kill micro-organisms by means of opsonisation, phagocytosis and lysis.[1,2] In case of infection, polymorphonuclear neutrophils (PMN's) are attracted to the site of infection through agents generated by micro-organisms or complement. At the site of infection, the PMN's adhere to the micro-organism (receptor-mediated) and consequently are able to engulf the micro-organism (phagocytosis). After phagocytosis, the PMN's release granules with microbicides and kill the micro-organism by means of toxic oxygen metabolites.
The innate immune system is a fast-acting response, but is less specific than the acquired immune system; no immunological memory is created.[3,4]

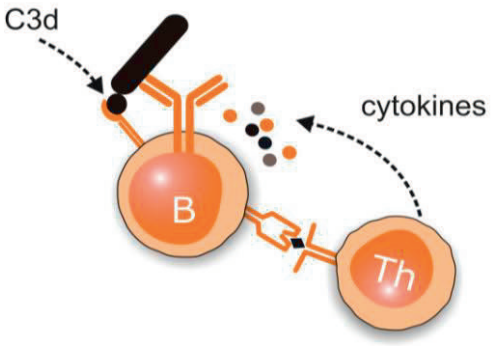
Innate immune responses are initiated by recognition of molecular patterns on micro-organisms (pathogen-associated molecular patterns; PAMP). Receptors for these molecules, the so-called pattern recognition receptors (PRR), are expressed on cellular surfaces or in the cytoplasm. An important group of PRR's consists of the Toll-like receptors (TLR). The TLR's were first discovered in the fruit-fly Drosophila melanogaster. The mammalian family of TLR's consists of 10 different receptors. Most TLR's are trans-membrane proteins but TLR's for viral DNA and RNA are endosomal. Stimulation of a TLR by binding of ligand leads to activation of signalling pathways, resulting in induction of inflammatory cytokines, co-stimulatory molecules and increased antigen-presenting capacity.[3] Next to cell-bound pattern recognition receptors, soluble pattern recognition molecules are also essential for the proper functioning of the innate immune system. An example of a soluble pattern recognition molecule is mannose-binding lectin (MBL). MBL recognises terminal mannose residues on the surface of many micro-organisms, including gram-negative and gram-positive bacteria, yeasts, parasites and several viruses. After binding to a pathogen, MBL subsequently activates downstream components of the complement system ultimately leading to activation of the membrane attack complex and lysis.[3,5]

The acquired immune system consists of lymphocytes and antigen-presenting cells (APC's) and develops during the first years of life. The response consists of a cellular and a humoral pathway, based on the involvement of respectively T-

lymphocytes and B-lymphocytes. In the cellular pathway, T-lymphocytes are activated through binding of their T-cell receptor to antigen bound and presented by an APC. Two important types of T-lymphocytes can be discerned: cytotoxic (or $CD8^+$) T-lymphocytes, for killing of viral infected cells and helper (or $CD4^+$) T-lymphocytes, that promote intracellular killing by macrophages and antibody production by B-lymphocytes.[1]

The humoral immune response to bacteria depends on recognition of the antigen by B-lymphocytes. These B-lymphocytes are stimulated by cytokines of T-helper lymphocytes and co-stimulation by complement fragments such as C3d takes place (Figure 1).[1,2]

Figure 1. Interaction of a B-lymphocyte with a T-helper lymphocyte in case of recognition of antigen by a B-lymphocyte



The activation of a B-lymphocyte leads to proliferation and differentiation of the B-lymphocyte, ultimately leading to the production of antibodies (immunoglobulin or Ig). The B-lymphocyte then is called a plasma cell. If the immune system for the first time encounters a specific micro-organism, the B-lymphocyte primarily produces IgM.
The IgM that is produced has low affinity. During B-lymphocyte proliferation in germinal centres, the cells undergo a process which is called somatic hypermutation: the mutation rate is higher than that in rapid proliferating non-lymphoid cells. As a result of the mutations which will also occur in immunoglobulin genes, B-cell receptors with higher affinity for the antigen in question may arise. After interaction with T-lymphocytes in the germinal centres, these B-lymphocytes form either memory cells or plasma cells, producing antibodies of high avidity.[6-8] Antibody avidity is defined as the antigen-binding capacity of polyclonal antibodies with different affinities.[6,9,10] After a second infection with the same antigen, these

memory B-lymphocytes are capable of rapid production of antibodies with higher affinity.

The humoral response as described above that needs the help of T-lymphocytes is called the T-cell dependent (TD) response. B-lymphocytes can produce antibodies against capsular polysaccharides of bacteria without the help of T-lymphocytes, the so-called T-cell independent (TI) response.[11,12] The absence of B-T cell interaction precludes the formation of memory B-lymphocytes. The magnitude of the antibody response can, however, be augmented by T-lymphocytes. Polysaccharides are therefore classified as TI-2 antigens, in order to distinguish them from lipopolysaccharides, which are truly independent of T-lymphocyte help (TI-1).

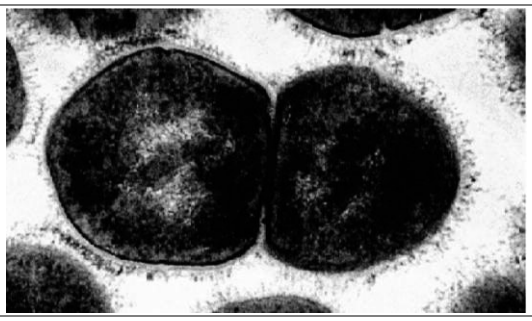
As described above, the predominant class of immunoglobulin produced after the first encounter with a micro-organism is IgM. IgG is produced during the primary immune response, but in smaller amounts and later on in the response. In case of repeated contact with the same micro-organism, the predominant isotype of Ig in terms of quantity and quality switches to IgG. Four subclasses of IgG exist: IgG1-IgG4. In general, after contact with a TD antigen (first as well as repeated contact), IgG1 is predominantly formed. In the response to TI antigens, both IgG2 and IgG1 are produced.

Innate and acquired immune systems co-operate in many aspects and one example of this form of interaction is the complement system. Complement components play an important role in the stimulation of the acquired immune system. The complement components can promote antigen uptake, processing and presentation by B-lymphocytes to antigen specific T-lymphocytes, can directly activate B-lymphocytes or facilitate the interaction of B-lymphocytes with follicular dendritic cells (FDC's), which are important antigen-presenting cells.[2]

Polymorphisms and/or deficiencies in both the innate and acquired immune system can lead to higher susceptibility to infections with for example the polysaccharide-encapsulated bacteria *Streptococcus pneumoniae* (Figure 2). Infections with these bacteria worldwide cause major morbidity and mortality in infants, the elderly and immunosuppressed individuals. The polysaccharide capsule is an important virulence factor, because it hinders the phagocytic uptake of bacteria by neutrophils and macrophages. This diminished uptake is at least in part caused by resistance to and degradation of complement components.[13,14] Opsonisation of the bacteria with complement and antibodies enables efficient phagocytosis and subsequent killing of the micro-organism by PMN's.[15] Anti-polysaccharide capsule antibodies can promote phagocytosis by binding of the Fc-part of the

immunoglobulin to the Fc-receptor (FcR) of an effector cell, for example a PMN.[16] Furthermore, the antibodies can act as a receptor for complement components.[17]

Figure 2. *Streptococcus pneumoniae*



The antibodies produced against the pneumococcus are mainly of the IgG2 subclass. To induce adequate opsonisation of the pneumococcus, IgG2 has to bind to its receptor, human Fcγ-RIIa, on the PMN. Two co-dominant allotypes of Fcγ-RIIa exist due to a single nucleotide polymorphism, resulting in either arginine (R) or histidine (H) at residue 131 of the protein. Both receptors have different binding capacities for IgG2. Fcγ-RIIa-H131 has high affinity for IgG2, whereas Fcγ-RIIa-R131 has low affinity.[18] Therefore, a homozygous Fcγ-RIIa-R/R131 genotype leads to less efficient phagocytosis, with higher incidence of pneumococcal bacteraemia and severe sepsis after community-acquired pneumoniae (CAP) caused by *S. pneumoniae*.[19] Moreover, the homozygous Fcγ-RIIa-R/R131 genotype was more frequent in CAP caused by *Haemophilus influenzae* type b (*Hib*) and was associated with severity of disease caused by *Neisseria meningitidis*, both polysaccharide-encapsulated bacteria.[19,20]
Next to genetic variation in Fcγ-RIIa, mutations in the gene coding for MBL and deficiencies in TLR-mediated cytokine production have also been described in association with susceptibility to pneumococcal disease.[21,22]

The B-lymphocyte mediated TI-2 response is dependent on marginal zone (MZ) B-lymphocytes that after the age of 18-24 months develop a protein on their surface, CD21. This CD21 binds complement C3d, which is bound and activated by bacterial capsular polysaccharides. Due to this binding, B-lymphocytes are activated that produce specific antibodies against these capsular polysaccharides.[2,23]
In children under the age of 24 months, expression levels of CD21 on B-lymphocytes are minimal and without an effective interaction between CD21 and C3d, B-lymphocytes are not triggered to induce an anti-polysaccharide response.

Young children are therefore vulnerable for infections with polysaccharide encapsulated bacteria.[23]

In patients with an absent or dysfunctional spleen, the TI-2 response is impaired due to the absence of the MZ macrophages and B-lymphocytes.[24] The TI-response of the splenic B-lymphocytes is particularly effective against bacterial capsular polysaccharides, therefore, asplenic patients are at increased risk of infections with encapsulated bacteria like *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Hib*.[25,26] In the marginal zone of the spleen, blood flow is low, allowing prolonged and intimate contact between antigens in the bloodstream and the lymphocytic system.[27] Prolonged contact between an antigen and phagocytes enables phagocytosis of poorly opsonised particles, like capsular polysaccharides. The spleen is therefore the most important site of clearance during the early phase of bacterial invasion. Thus, in asplenic patients the increases risk of infection with encapsulated bacteria can be explained by both the absence of antibody production by MZ B-lymphocytes and the decreased clearance of bacteria from the bloodstream by splenic macrophages.

VACCINES: PLAIN POLYSACCHARIDE VERSUS PROTEIN-CONJUGATED

The purpose of vaccination is to improve the immune system in such a way that subsequent exposure to corresponding micro-organisms does not cause disease. For most micro-organisms, vaccination induces both a cellular as well as a humoral immune response and immunological memory. For micro-organisms for which protective immunity involves an immune response against capsular polysaccharides, only the humoral branch of the acquired immune system contributes to protection, i.e. antibodies. The antibody response to polysaccharides is T-cell independent. T-cell independent antibody responses have a number of characteristics which distinguish them from classical T-cell dependent antibody responses to protein antigens (see Table 1). Two of these characteristics are of special importance when TI-2 antigens are used as vaccines. First, TI-2 antigens do not induce immunological memory. This means that if primary vaccination, for whatever reason, would not induce an antibody response, booster-vaccination would be of no use. Indeed, booster vaccinations are not recommended for polysaccharide vaccines. Because of the absence of memory induction, and because antibody levels decline over time, polysaccharide vaccines are repeated every 3 to 5 years (see below). A second restriction of polysaccharide vaccines is the fact that they are not effective in young children.[28,29]

Table 1. Characteristics of the humoral antibody response to T-cell dependent and T-cell independent antigens

Characteristics	**T-cell dependent**	**T-cell independent**
Type of antigen	proteins	polysaccharides
Involvement of T-lymphocytes	yes	no
Induction of memory B-lymphocytes	yes	no
Isotype switch antibodies	yes	restricted
Main antibody (sub)isotype	IgG1	IgM, IgG2
Onset of responsiveness in ontogeny	< 2 years	> 2 years

The results of vaccination in asplenic patients are variable. Some studies show limited rise in antibody titre after vaccination, while others show induction of protective antibody titres.[30-33] However, the absence of splenic tissue necessary for the initial response to polysaccharide antigens suggests that polysaccharide vaccines are not optimal for asplenic patients. Because of the limited use of polysaccharide vaccines, polysaccharides have been covalently coupled to protein carriers. This principle was first described by Avery and Goebel, but it took over 50 years until this discovery was put to practical use in the form of conjugate vaccines.[34] These so-called protein conjugated vaccines greatly improve the immunogenicity of the polysaccharide by inducing an immune response with the characteristics of TD antigens.[35-37] Starting in the previous decade, conjugate vaccines are incorporated into the Dutch National Vaccination Programme for children and infants. Conjugate vaccines are now also used or considered for patient groups who otherwise would not respond to polysaccharides, including asplenic patients.[28,38]

The polysaccharide conjugate vaccines have the ability to elicit higher avidity antibodies with increased functional activity than do polysaccharide vaccines.[9,39,40] The height of the avidity is dependent on the type of conjugate vaccine.[41,42] The affinity of the antibodies also depends on IgG isotype. Human IgG consists of 4 subclasses, IgG1-IgG4. In a TI-response, both IgG2 and IgG1 are produced. In a T-cell dependent response, primarily IgG1 is produced. IgG1 is of higher affinity than IgG2.[43,44] The ability to induce an IgG1 T-cell dependent (TD) response is among others dependent on age at vaccination, type of vaccine and type of adjuvant.[45-47]

In this thesis, both polysaccharide and conjugate vaccines are studied for *S. pneumoniae*. For *Hib*, one conjugate vaccine is used for indicated patient groups. The polysaccharide of *Hib* is covalently bound to the tetanus toxoid protein (Table 2).

Of the known meningococcal serogroups, 5 groups (A, B, C, Y and W135) are responsible for more than 95% of invasive disease.[39] Both polysaccharide and protein-conjugated vaccines are available, directed against different serotypes. For asplenia, the meningococcal C (MenC) conjugate vaccine is recommended. In this vaccine, the meningococcal polysaccharide is bound to diphtheria toxoid or tetanus toxoid. Vaccination against diphtheria, tetanus and polio (DTP) induces a T-cell dependent response, as does vaccination against the influenza virus.

Table 2. Vaccines used in this thesis. One dose consists of 0.5 mL.

Vaccine	**Polysaccharide**	**Conjugated protein**	**Manufacturer**
Pneumo®-23	*S. pneumoniae*[1]	-	Sanofi Pasteur SA
Prevnar®	*S. pneumoniae*[2]	Diphtheria CRM_{197}	Wyeth Pharmaceuticals
Act-HIB®	*Hib*[3]	Tetanus toxoid	Sanofi Pasteur SA
NeisVac-C®	MenC[4]	Tetanus toxoid	Baxter AG
Revaxis®	DTP	-	Aventis Pasteur SA
Influvac®	Influenza H and A	-	Solvay Biologicals BV
Vaxigrip®	Influenza H and A	-	Sanofi Pasteur SA

S. pneumoniae = *Streptococcus pneumoniae*
Hib = *Haemophilus influenzae* type b
MenC = *Neisseria meningitidis* serogroup C
DTP = diphtheria, tetanus, poliomyelitis
Influenza H and A = influenza haemagglutinin and neuraminidase
[1] one dose contains 575 µg polysaccharides (23 serotypes included in the vaccine, 25 µg polysaccharide per serotype)
[2] one dose contains 16 µg polysaccharides (2 µg of serotypes 4, 9V, 14, 18C, 19F and 23F and 4 µg of serotype 6B) and 20 µg protein
[3] one dose contains 10 µg polysaccharides and 20 µg protein
[4] one dose contains 10 µg polysaccharides and 10-20 µg protein

IMMUNE SYSTEM DEFICIENCIES AND VACCINE RECOMMENDATIONS

With the development of polysaccharide conjugate vaccines, which have turned polysaccharides into T-cell dependent antigens, it has become possible to vaccinate young children effectively against infections with encapsulated bacteria. Young children are at risk of these infections due to the incomplete development of the immune system. Therefore, in the Netherlands all children under the age of two years are vaccinated in the Dutch National Vaccination Programme against for example *S. pneumoniae*, *Hib*, MenC and the toxin of *Clostridium tetani*. Beside young children, vaccination is indicated for a number of specific patient groups. Recommendations for vaccination of these patients are given by the Health Council of the Netherlands and other (inter)national institutes.[48-50] These recommendations include the use of vaccines to prevent infections that are more frequent and/or severe in these patients groups than in the normal population and vaccines against infectious agents for which vaccination recommendations exist to protect the general population. An example of the latter is the DTP vaccine. Vaccines to prevent severe infections include pneumococcal, *Hib*, meningococcal and the influenza virus vaccine.
While the recommendations are given for each specific vaccine, there are differences but also overlap in patient groups. For an overview of the possible indications for immunisation, guidelines for two prevalent vaccines (pneumococcal and influenza virus) will be discussed below.

PNEUMOCOCCAL VACCINATION

In Table 3, the indications for pneumococcal vaccination as stated by the Health Council of the Netherlands are given. For only two patient groups, pneumococcal vaccination is strongly recommended: patients with anatomical or functional asplenia and patients with a scull trauma with leakage of liquor. For other categories of patients, for example patients with haematological malignancies or patients who underwent a stem cell transplantation or solid organ transplantation, it is advised to make an individual decision about the necessity of pneumococcal vaccination.

For *S. pneumoniae*, two different types of vaccines are available. The pneumococcal polysaccharide vaccine (PPV-23) contains capsular polysaccharides of 23 pneumococcal serotypes, responsible for 85-90% of the invasive pneumococcal infections among children and adults.[51] This polysaccharide vaccine is not effective in young children.[28,29] Beside, due to the

absence of generation of memory B-lymphocytes and the decline in antibody levels over time, re-immunisation is recommended every three to five years.[49,52] In 2006, a protein-polysaccharide conjugate vaccine (PCV-7) containing the capsular polysaccharides of seven pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) was introduced in the Dutch National Vaccination Programme for children and infants. These seven serotypes originally were chosen on their prevalence in otitis media in American infants and are responsible for 64% of the invasive pneumococcal infections in young children (< 2 years) in the Netherlands. Because PCV-7 only covers seven pneumococcal serotypes, for children with functional or anatomic asplenia an additional dose of PPV-23 at the age of two years is recommended.[53] In December 2009 a 13-valent pneumococcal conjugated vaccine received European authorisation, but is not incorporated in the National Vaccination Programme yet.

Table 3. Indications for pneumococcal vaccination stated by the Health Council of the Netherlands in 2003

Indication	**Patient group**
Strongly advised	Functional or anatomical asplenia
	Liquor leakage due to scull trauma
Individual base	Myeloma
	CLL
	Morbus Hodgkin
	Non-Hodgkin lymphoma
	HIV
	Stem cell transplantation
	Solid organ transplantation
	Auto-immune diseases
	Kidney disease (nefrotic syndrome in children, haemodialysis)
	Alcohol abuse
No indication	Hypo- and agammaglobulinaemia
	Solid tumours
	Diabetes mellitus
	Chronic pulmonary disease
	Chronic heart disease

CLL = chronic lymphatic leukaemia
HIV = human immunodeficiency virus

INFLUENZA VACCINATION

Every winter season, an estimated 1000-2000 persons die directly or indirectly due to an infection with the influenza virus in the Netherlands.[54] Especially patients ≥ 60 years are vulnerable for the influenza virus; they are offered yearly vaccination. Besides the elderly population, several patient groups are advised to be immunised with the influenza A and B virus vaccine (Table 4). In contrast to pneumococcal vaccination, for the influenza virus vaccine no distinction is made between 'strongly recommended' and 'decision on individual base'.

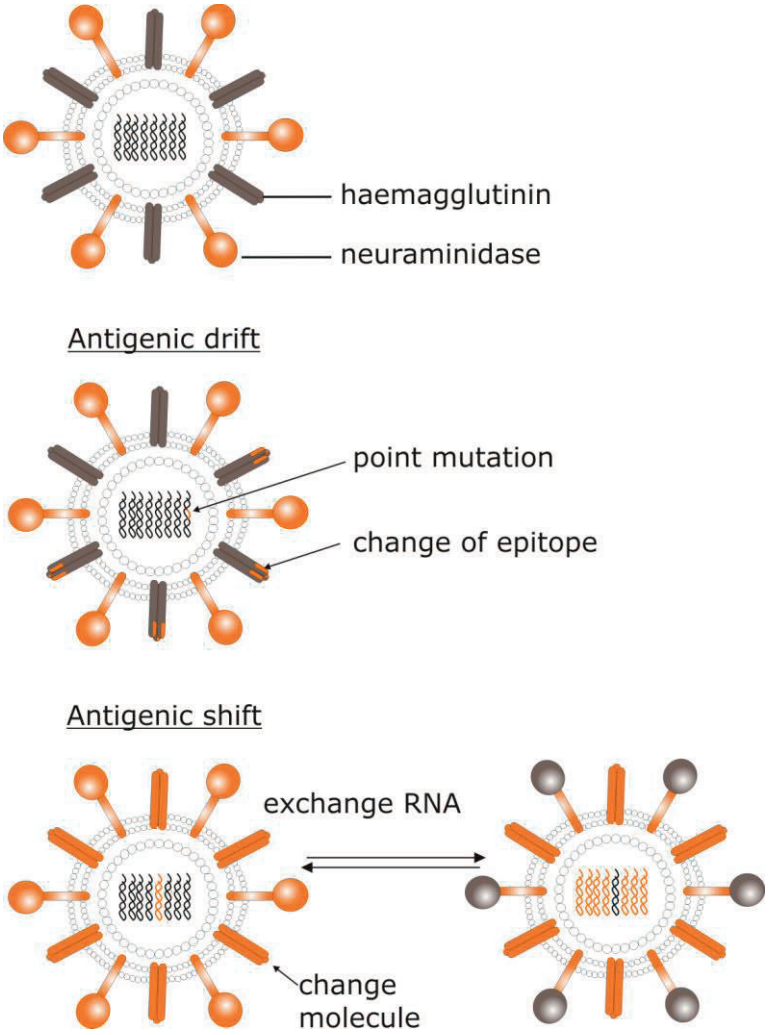
The influenza A and B viruses belong to the RNA-virus family of the *Orthomyxoviridae*. The viral RNA is covered by a lipid membrane with two 'spike' proteins: haemagglutinin (H) and neuraminidase (N) (Figure 3). The influenza A virus has several subtypes, based on the combination of different H and N proteins. Of the influenza B virus, no subtypes exist. Due to small cumulative changes in the H and N proteins, every 1-5 years a new subtype of virus develops (this is called antigenic drift). Because of these changes in the influenza virus, each year a new influenza virus vaccine is produced, based on the most prevalent influenza subtypes of the year before. More sporadically, one of the subtypes of the influenza A virus is replaced by another (avian) subtype. This is called antigenic shift and may lead to an influenza pandemia.

Table 4. Indications for influenza virus vaccination stated by the Health Council of the Netherlands in 2007

Age ≥ 60 years
Chronic heart disease (e.g. heart failure)
Pulmonary disease (e.g. COPD)
Diabetes mellitus
Immunosuppression due to
Recent stem cell transplantation
HIV
Leukaemia
Recent chemotherapy or radiation therapy
Children aged 6 months - 18 years with long-term use of salicylates
Mentally retarded, living in institutions

COPD= chronic obstructive pulmonary disease
HIV= human immunodeficiency virus

Figure 3. Schematic structure of the influenza virus with 'spike' proteins



From the recommendations for pneumococcal and influenza vaccination, it might be concluded that indicated patients groups for vaccination consist of two major groups:

1) patients with an impaired immune system and
2) patients with an underlying disease, in whom infection may result in progressive loss of function of the affected organ (lungs, heart).

Progressive loss of function of organs due to pneumococcal or influenza infections may occur in patients with heart failure or patients with a reduced lung function, such as patients with chronic obstructive pulmonary disease (COPD).
Deficiencies in the immune system can occur because the immune system has not completely been developed, for example in young children or, on the contrary, because of aging of the immune system. Besides age, various medical conditions can lead to a diminished activity of the immune system or increased susceptibility for infections. Underlying defects of the immune system in these patients groups are diverse and may consist of leukocyte dysfunction (e.g. lack of ability of phagocytic clearance in patients with diabetes mellitus) or absence or diminished function of B-lymphocytes or T-lymphocytes (e.g. absence of marginal zone B-lymphocytes in asplenia). Above mentioned patient groups will be discussed in more detail in the next paragraphs.

UNDERLYING DISEASE: CHRONIC OBSTRUCTIVE PULMONARY DISEASE AND HEART FAILURE

COPD encompasses a group of pulmonary diseases including chronic bronchitis and lung emphysema. The underlying mechanism of disease is a chronic inflammation of the lungs, mostly caused by smoking. Currently, more than 300.000 patients in the Netherlands suffer from COPD and COPD causes 4% of total mortality in the Netherlands.[55] In patients with COPD, the morbidity and mortality of acute exacerbations are high. Most acute exacerbations are caused by community-acquired respiratory infections like the influenza virus. Vaccination against the influenza virus reduces the number of acute exacerbations significantly and tends to lower all-cause mortality in COPD.[56,57] The role of the pneumococcal vaccine in reducing all-cause mortality and infection rates is less clear, and therefore is not recommended in the Netherlands.[49,56] Heart failure predisposes to influenza virus infections, although the underlying mechanisms are not yet fully clarified. In heart failure patients, B-lymphocyte function seems adequate when studying the response to vaccination.[58] However, the pattern of cytokine induction seems different with reduced activity of cytotoxic T-lymphocytes.[59] As for COPD patients, influenza virus vaccination is strongly recommended; the pneumococcal vaccine is not.

AGE: IMMUNOSENESCENCE

In elderly patients, the aging of the immune system (immunosenescence) leads to susceptibility to infectious diseases and a decreased efficacy of vaccination.[60,61] Changes in the humoral immune system lead to production of low-affinity antibodies that are less specific and consist mostly of IgM in stead of IgG.[62]

Decreased efficacy of vaccination is observed especially for T-cell dependent antigens.[63] It is thought that the involution of the thymus, with decline in the absolute numbers of B-lymphocytes and T-lymphocytes ($CD4^+$ as well as $CD8^+$) and a loss of the co-stimulatory molecule CD28 on $CD8^+$ cells are responsible for the decline in immune function.[60,64] In the elderly population, that is vulnerable for infection with the influenza virus, vaccination is strongly recommended.[48,61] In the Netherlands, vaccination of the elderly against *S. pneumoniae* is not yet recommended, in contrast to e.g. the United States of America and most European countries.[65]

DIABETES MELLITUS: LEUKOCYTE DYSFUNCTION

In patients with diabetes, infection rates are higher, probably due to defects in innate immunity (PMN's) rather than caused by deficiencies in B- or T-lymphocytes.[66] In diabetic patients, a global dysfunction of the PMN's takes place. All previously mentioned functions of attraction to the site of infection, adherence to micro-organisms, phagocytosis, and lysis of micro-organisms are diminished.[66-68] Besides the dysfunction of PMN's, in diabetes the hyperglycaemia and the occurrence of ketoacidosis makes the patient vulnerable to infections. In the Netherlands influenza vaccination is, therefore, recommended.[48] Pneumococcal vaccination is not yet indicated in the Netherlands for patients with DM.

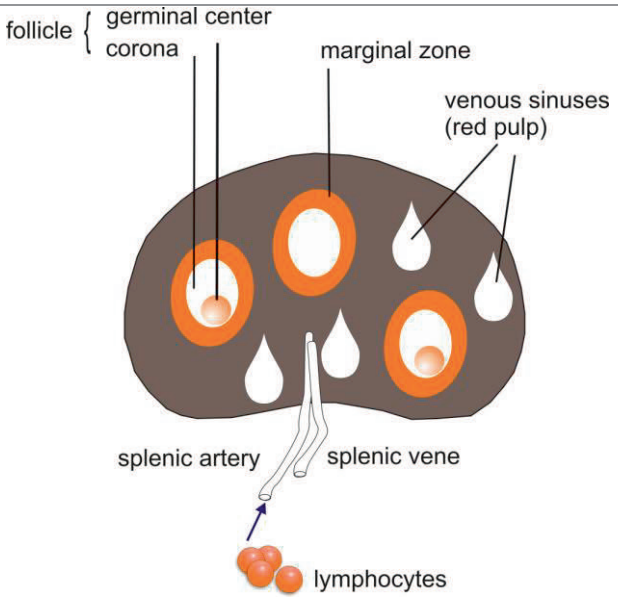
ASPLENIA: ABSENCE OF MARGINAL ZONE B-LYMPHOCYTES AND MACROPHAGES

The spleen is the largest secondary lymphoid organ of the human body which is historically organised in red and white pulp. The red pulp primarily consists of blood sinuses with macrophages, removing damaged erythrocytes and thrombocytes and enabling phagocytic clearance of bacteria from the circulation. The white pulp consists of T- and B-lymphocytes.[69] The B-lymphocytes are organised in two different compartments: the follicles and the marginal zone (Figure 4). The marginal zone is the splenic compartment where bacterial polysaccharides accumulate following immunisation.[23] The marginal zone B-lymphocytes express high levels of CD21 and this most probably is the B-lymphocyte population responding to polysaccharide antigens.

For these reasons, the spleen is an important organ for protection against encapsulated bacteria like *S. pneumoniae*, *Hib* and *N. meningitidis*.[70-72] These bacteria are filtered from the bloodstream by marginal zone macrophages and the marginal zone B-lymphocytes are able to produce antibodies against the capsular

polysaccharides.[24] The anti-capsular antibodies and the following activation of complement, facilitate phagocytosis and destruction of the bacteria.[38,69]

Figure 4. Schematic overview of the spleen



Lacking the functions of the spleen as described above, asplenic individuals are at increased risk of fulminant and life-threatening infections with these encapsulated bacteria.[25,26] The estimated life-time risk of such an overwhelming post-splenectomy infection (OPSI) in asplenic or hyposplenic patients is approximately 5% with a mortality of 50–80%.[73] The occurrence of these infections is dependent on the reason for asplenia (infections are seen more often after splenectomy for haematological malignancies than after trauma) and age at splenectomy (younger age predisposes for infections). Infections are mostly seen in the first three years after splenectomy.[74,75]

Strategies to prevent these infections are based on awareness of the patient about the increased infection risks, vaccination and antimicrobial prophylaxis.[75-79] Vaccines recommended for asplenic patients target the encapsulated bacteria as mentioned above: *S. pneumoniae*, *N. meningitidis* (in particular serogroup C) and *Hib*.[80,81] Moreover, the influenza virus vaccine is recommended every year, in order to prevent a bacterial super-infection after a viral upper airway infection caused by the influenza virus.[52]

CHEMOTHERAPY: TRANSIENT DEPLETION OF B- AND T-LYMPHOCYTES

Two factors influence the response to immunisation in patients with cancer: the disease itself may be directly immunosuppressive and the treatment for cancer can be immunosuppressive. Chemotherapy is used to destroy rapidly growing tumour cells. Other rapidly growing cells, including leukocytes, however are also adversely affected by these interventions. Therefore, cancer patients are more susceptible to infection because of their suppressed immune system, and they may not be capable of mounting an adequate response to vaccination.[82]
The myelosuppression that is induced by the chemotherapy is transient. The duration of myelosuppression varies between agents due to variable drug distribution of the chemotherapeutics and different pharmacokinetic patterns in patients. In general, acute myelosuppression starts 7-14 days after chemotherapy, continues from 10 to 14 days and will recover in 3-4 weeks post-chemotherapy administration.[83]

In patients with cancer treated with chemotherapy or immunosuppressive drugs, the immune response to natural viral infections is severely impaired, and this patient group can be considered a high-risk group at risk of particularly serious post-influenza complications.[84] Vaccination is therefore recommended, with some vaccines advised to all cancer patients, and other vaccines indicated for specific patient groups.[82,85] In the Netherlands, for patients treated with immunosuppressive agents like chemotherapy, the influenza virus vaccine is strongly recommended, whereas the pneumococcal vaccine has to be considered.[48,49,86]

In solid tumours, chemotherapy can be given for palliation in metastatic disease or as adjuvant therapy in potentially curable disease. In this latter group it is important to follow the chemotherapy schedule and minimally delay chemotherapy. Therefore, in addition to the prevention of infection, in the adjuvant setting influenza vaccination is given in order to prevent postponing of the chemotherapy due to infection. Information about the timing of vaccination during chemotherapy is scarce. In general it is advised to vaccinate patients before start of the chemotherapy, if possible.[83,86]

STEM CELL TRANSPLANTATION: MYELOSUPPRESSION

Autologous or allogeneic stem cell transplantation (SCT) is the therapy of choice for a number of haematological malignancies like non-Hodgkin lymphoma, chronic lymphatic leukaemia or multiple myeloma.[87-90] In recipients of an autologous SCT (autoSCT), pre-transplantation high dose chemo- or radiotherapy depresses the

immune system.[50] Because there is no immunological disparity between the graft and the host, post-transplantation immunosuppressive therapy is not given. Although the immune system regenerates more quickly after autologous than after allogeneic stem cell transplantation, complete recovery of immunological function can be impaired for a year or longer, making patients prone for infections.[91] Prolonged impairment of IFN-γ production for example might contribute to a higher incidence of viral infections.[92] Beside viral pathogens, infections with encapsulated bacteria are common and appropriate vaccination is indicated. An immunisation schedule with pneumococcal, DTP and *Hib* vaccines starting six months post-transplantation induces protective antibody concentrations.[93]

After an allogeneic stem cell transplantation, the immune system of the recipient is fully replaced by the immune system of the donor.[50] Pre-transplantation, high-dose chemo- or radiotherapy is given in order to reduce the tumour load and to eradicate all immune cells of the recipient.[87]
To prevent severe graft versus host disease immunosuppressive therapy is required, such as the T-lymphocyte activation blocker cyclosporine or the purine synthesis inhibitor mycophenolate mofetil (MMF). The chemotherapy given pre-transplantation, together with the underlying haematological disease and the immunosuppressive therapy, make these patients even more susceptible to bacterial and viral infections than autoSCT patients.[50,94-97] Defects in B-lymphocyte function can last for 12-24 months, involving defects in the response to T-cell independent antigens, including the capsular polysaccharides of *S. pneumoniae* and *Hib*, as well as T-cell dependent protein antigens.[98-100] Beside B-lymphocyte defects, T-lymphocyte counts are low in the first three months after transplantation and may take much longer to reconstitute functionally.[101]

To reduce the post-transplantation complications of a myeloablative conditioning regimen used in standard allogeneic transplantation, a less intense conditioning regimen was developed. This so-called non-myeloablative or reduced intensity allogeneic SCT (allo-RIST) is now used in older patients or in patients with significant morbidities.[87,102]
The reduced conditioning regimen leads to less severe neutropenia and the mucosal and dermal barriers are not as damaged as after myeloablative regimens. Moreover, the allo-RIST regimen does not eliminate all the T-lymphocytes of the recipient (so-called mixed chimerism). These T-lymphocytes remain functional and can prevent infectious complications.[90,95]

Loss of immunity is mostly studied in myeloablative regimens and scarce data are available of non-myeloablative regimens. The loss of immunity post-transplantation

depends on the degree of pre-transplant immunity of the patient, the vaccination status of the donor and the occurrence of chronic graft versus host disease (cGVHD).[50,101,103-107] The susceptibility to bacterial and viral infections has guided the vaccine recommendations in these patients. Pneumococcal, *Hib* and influenza virus vaccines are recommended, as well as diphtheria, tetanus toxoid and polio vaccines, starting at about six months after vaccination.[108,109] Meningococcal serogroup C vaccination has to be considered.[50,110,111]

This thesis focuses on the efficacy of vaccines against the encapsulated bacteria *S. pneumoniae*, *Hib* and *N. meningitidis*, against DTP and against the influenza virus in patient groups with an impaired immune system. The response to these vaccines is studied in detail in the next chapters in patients with asplenia, patients on chemotherapy and patients after allogeneic stem cell transplantation.

AIMS AND OUTLINE OF THIS THESIS

This thesis addresses the response to vaccination in three categories of immunocompromised patients. The first part concerns patients with asplenia. In previous studies, it was found that the vaccination status of asplenic patients was suboptimal. Therefore, an inventory was made of the awareness of asplenic patients about their infectious risks, the need for microbial prophylaxis and their vaccination status. This study was conducted in the Utrecht area of the Netherlands (**Chapter 2**). This is the first Dutch study to obtain information about the preventive measures and follow-up of asplenic patients. In case these patients were not vaccinated according to international standards, the asplenic patients received the recommended vaccines against *S. pneumoniae*, *Hib* and *N. meningitidis*. The response to pneumococcal and *Hib* vaccines is described in **Chapter 3**. The response to meningococcal serogroup C conjugate vaccine in terms of antibody concentration, subclass distribution, and avidity is studied in **Chapter 4**.

The second part of this thesis describes the response to vaccination in two different categories of patients with malignancies. In **Chapter 5**, the response to the influenza virus vaccination is studied in patients with breast cancer treated with FEC-containing chemotherapy in the adjuvant setting. There is little information about the optimal moment for vaccination during chemotherapy. In general, it is advised to vaccinate before start of chemotherapy. However, for season-bound vaccinations as the influenza vaccine, this is not always feasible. Furthermore it is known that vaccination at the day of the chemotherapy reduces the response on vaccination with 50%. We randomised patients for vaccination at day 4 or day 16 of the chemotherapy cycle and measured the increase in antibody titre after vaccination, in order to establish the best moment of vaccination during chemotherapy.
In Chapter 6, the response to pneumococcal, *Hib* and DTP (diphtheria, tetanus and polio) vaccination is studied after reduced intensity stem cell transplantation (allo-RIST). These patients are immunocompromised due to a combination of the underlying haematological malignancy, the pre-treatment chemotherapy and the post-transplantation immunosuppressive therapy. Almost all previous studies have been conducted in patients who underwent a myeloablative conditioning regimen. Our study in patients with a non-myeloablative (or reduced intensity) regimen is the first to analyse the effectiveness of vaccination (in terms of antibody concentration) in relation to recovery of the immune system in the allo-RIST patient.
In **Chapter 7**, the findings of this thesis are summarised and discussed and future perspectives are given.

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Chapter 2:

Vaccination coverage and awareness of infectious risks in patients with an absent or dysfunctional spleen in the Netherlands

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ABSTRACT

We evaluated the current practice to prevent infections in patients with an absent or dysfunctional spleen in a part of the Netherlands and measured serum antibody levels against *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (*Hib*).

This is an observational study of vaccination coverage by analysis of questionnaires and serum antibody levels, in primary care practices in the Utrecht area of the Netherlands, catchment area 750,000 inhabitants, period 2006–2007.

One hundred and thirty adult patients with an absent or dysfunctional spleen were included. Patients were asked whether they were informed about infectious risks and the need of timely use of antimicrobial prophylaxis. After determination of vaccine coverage against *S. pneumoniae*, *Hib* and *Neisseria meningitidis*, levels of serum antibodies against *S. pneumoniae* and *Hib* were measured.

Fifty-six patients (43%) have not received up-to-date information about the infectious risks associated with their condition; 65 patients (50%) are not aware of the need to contact a physician immediately in case of high fever; 37 patients (28%) are keeping antimicrobial prophylaxis at home. Pneumococcal vaccination has been administered within the last 5 years to 103 of 130 patients, antibody levels above the threshold of ≥ 0.35 µg/mL are found in 83 of the 101 patients (data lacking in 2 patients). Complete coverage against *S. pneumoniae* is only 64% (83/130). A minority of patients (respectively 32% and 27%) has been vaccinated against *Hib* and *N. meningitidis*.

In conclusion, vaccination coverage and education about infectious risks in patients with an absent or dysfunctional spleen can be improved markedly in the Netherlands.

INTRODUCTION

The spleen is an important site of antibody production and phagocytic clearance of bacteria. Asplenic individuals are at increased risk for fulminant and life-threatening infections, especially with encapsulated bacteria such as *Streptococcus pneumoniae, Haemophilus influenzae* type b (*Hib*) and *Neisseria meningitidis*. The estimated life-time risk of such an overwhelming infection in asplenic or hyposplenic patients is approximately 5% with a mortality of 50–80%.[1] Strategies to prevent these infections are based on education of the patient about the increased infection risks, vaccination and antimicrobial prophylaxis.[2-5] The aim of this study is to evaluate the current practice of preventive strategies in patients with an absent or dysfunctional spleen in a part of the Netherlands.

PATIENTS AND METHODS

This study, approved by the medical ethics committee of the St. Antonius Hospital Nieuwegein, was conducted in asplenic or hyposplenic patients of ≥ 18 years during the period 2006–2007 in the area of Utrecht in the centre of the Netherlands. Medical ethical considerations played an important role in the design and execution of the study, in particular in the recruitment procedure of the patients. Because of privacy legislation, patients had to be approached indirectly after permission of their general practitioner (GP). If the patient was informed by the GP about this study and had signed informed consent, the study team was justified to contact the patient.

Three strategies to identify patients were used. First, we asked 384 GP's within a 30 km distance around the hospital to search their registry for adult, splenectomised or functionally asplenic patients with emphasis on administrative codes (International Classification of Primary Care; ICPC) for trauma, sickle cell anaemia, idiopathic thrombocytopenic purpura (ITP), spherocytosis or Hodgkin's lymphoma. Secondly, splenectomised patients in the St. Antonius Hospital in Nieuwegein were identified from a nationwide database. The GP's of the patients found in this database were subsequently contacted. Thirdly, we had our own database of the Division of Haematology of the St. Antonius Hospital Nieuwegein, consisting of patients who had undergone a splenectomy because of Hodgkin's disease. The GP's of these patients also were asked to recruit these patients.

The patients who signed a written informed consent were asked to fill in a questionnaire about vaccination coverage, previous education and awareness of the need for antimicrobial prophylaxis. Blood samples were taken for measurements of serum antibodies against *S. pneumoniae* (serotypes 3, 4 and 9V)

and *Hib* by ELISA as described previously.[6-8] Briefly, microtitre plates coated with either pneumococcal polysaccharide serotype 3, 4 or 9V were incubated with serial dilutions of patient sera. Sera were pre-absorbed with cell wall polysaccharide (CPS; 50 µg/mL) and serotype 22F polysaccharide (2 µg/mL) overnight.[9,10] Antibody levels were determined with goat anti-human immunoglobulin conjugated to alkaline phosphatase, and developed with *p*-nitrophenylphosphate. The reference serum 89SF was kindly provided by Dr. CE Frash, Division of bacterial products, Center for biologics evaluation and research, Rockville, MD. An anti-pneumococcal antibody threshold for protection against invasive disease was defined as ≥ 0.35 µg/mL, in accordance with the recommendation by the World Health Organisation (WHO) for the pneumococcal conjugate vaccine in children.[11] Because it has been estimated that in adults higher antibody levels are required for protection against pneumococcal pneumonia, a second threshold was defined as ≥ 1.0 µg/mL. In this study, the threshold had to be reached for two out of three tested pneumococcal serotypes.[12]

For *Hib*, an anti-PRP antibody concentration of ≥ 1.0 µg/mL is considered to correlate with long-term protection.[13] For asplenic individuals, a higher threshold is recommended, although the exact level is not known. In this study therefore we also calculated the fraction of patients with antibody concentrations ≥ 2.5 µg/mL.

RESULTS

In the Netherlands, about 2000 patients are served by one GP. Our domain of 384 approached GP's therefore consists of 750,000 patients. Of the 384 GP's, 231 (60%) actively recruited patients in this study. Twenty-five (11%) GP's did not use a disease classification system and therefore were unable to identify asplenic or hyposplenic patients. The participating GP's identified 235 patients who fulfilled the study criteria. The search in the nationwide database yielded another 33 patients of which 22 patients could not be approached because the GP did not participate in this study. The haematology database revealed another 17 patients who were all recruited by their GP. A total of 263 asplenic patients were identified and approached by their GP, of whom 130 (49%) responded and were included. Reasons for not participating could not be obtained due to the design of the study. The characteristics of the 130 participants are summarised in Table 1.

Table 1. Clinical characteristics of the study population

Patients (n)		130
Male gender (n (%))		63 (48)
Mean age (years, range)		50.3 (21-85)
Mean time since Sx (years, range)		20.4 (0.1-53)
Reason for asplenia (n (%))		
Functional (n=3)		
	Rx for Hodgkin's disease	2
	β-thalassaemia	1
Splenectomy (n=127)		
	Trauma	38 (30)
	ITP	22 (17)
	Hodgkin's disease	20 (16)
	Spherocytosis	13 (10)
	Iatrogenic lesion	8 (6)
	Haemolytic anaemia	4 (3)
	Non-Hodgkin Lymphoma	3 (2)
	Splenomegaly	3 (2)
	Pancreas surgery	3 (2)
	Other reasons	13 (10)

Sx = splenectomy, Rx = radiotherapy, ITP = Idiopathic thrombocytopenic purpura

The group consisted of 63 men and 67 women with a mean age of 50 years. Mean time since splenectomy was 20.4 years, with a range of 0.1–53 years (Figure 1). The most frequent cause of splenectomy in this patient group was traumatic rupture of the spleen (30%).

Figure 1. Time since splenectomy

Information

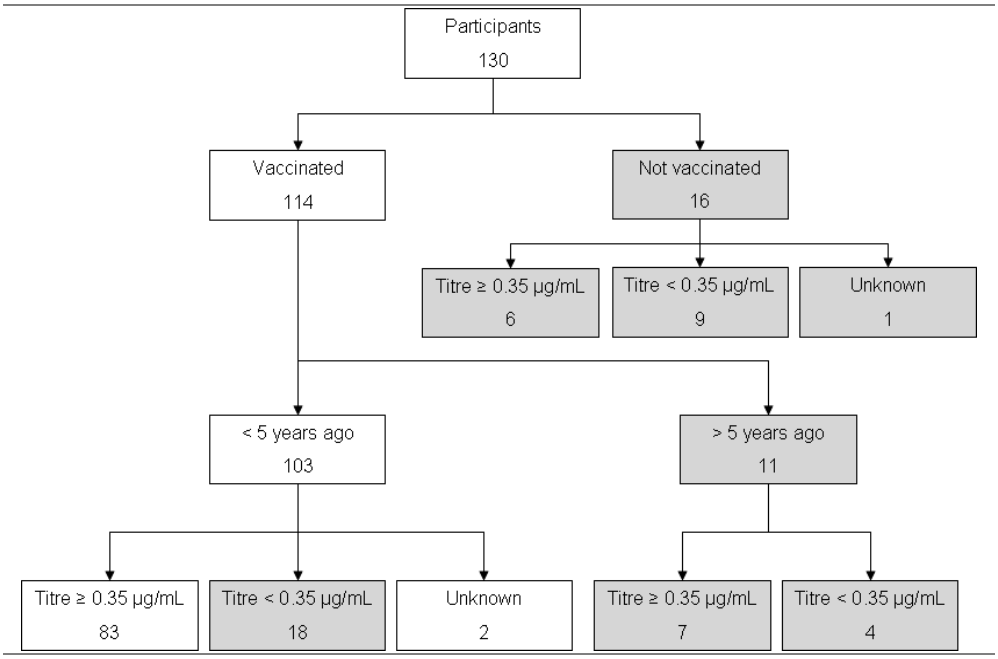
Fifty-six patients (43%) were not aware of the increased risk of infections and the need for preventive measures. The 74 patients who were informed about these risks, received the information from their GP (n=32, 43%), travel clinics (n=14, 19%), internists or surgeons at the time of splenectomy (n=11, 15%), relatives (n=10, 14%), the Internet (n=8, 11%) or newspapers (n=4,5%).

None of the patients had received written information on this subject. Sixty-five patients (50%) were not aware of the need to immediately contact a physician in case of high fever.

Vaccination

Of the 130 asplenic participants, 114 were vaccinated against *S. pneumoniae* and 16 patients (12%) never received a (pneumococcal) vaccination (Figure 2). According to recommendation of the British Committee for Standards in Haematology, 103 patients (79%) received this vaccination during the last 5 years; in 11 patients vaccination took place more than 5 years ago. Despite vaccination in the past 5 years, 18 patients did not reach the threshold of ≥ 0.35 µg/mL antibodies against *S. pneumoniae* (Figure 2).

Figure 2. Antibody levels against *S. pneumoniae* polysaccharides



"shade": patients who are not vaccinated according to international guidelines or who have antibody levels < 0.35 µg/mL for 2 out of 3 pneumococcal serotypes.

Of two patients, serum samples were not available. As can be seen from Figure 2, only 83 of 130 (64%) patients received vaccination against *S. pneumoniae* during the last 5 years and had antibody levels above the chosen threshold of ≥ 0.35 µg/mL. Forty-one of the 130 (32%) patients had antibody levels of ≥ 1.0 µg/mL for 2 out of 3 tested pneumococcal serotypes (Table 2).

Table 2. Anti-pneumococcal polysaccharide and anti-*Hib* polysaccharide antibody levels

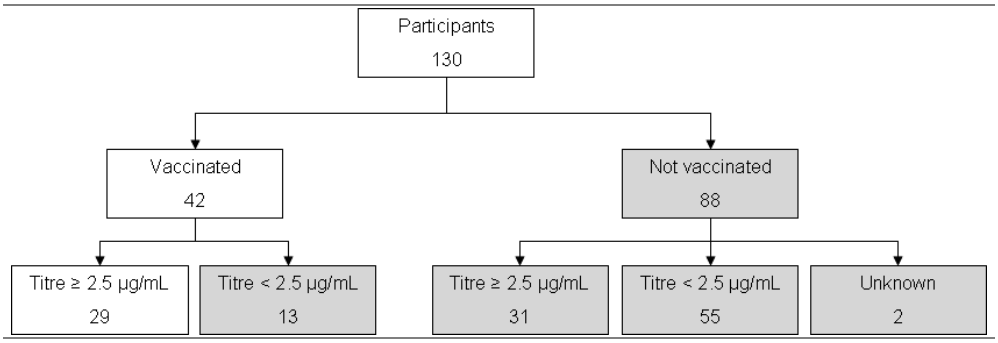
Antigen	**PPS 3**	**PPS 4**	**PPS9V**	***Hib***
GMC	0.17	1.32	0.78	3.02
% ≥ 0.35 µg/mL	25	85	76	
% ≥ 1.0 µg/mL	2	56	38	75
% ≥ 2.5 µg/mL				46

GMC= geometric mean concentration
PPS = pneumococcal polysaccharide
Hib = *Haemophilus influenzae* type b
The fraction of patients with antibodies ≥ 0.35 µg/mL for 2 out of 3 pneumococcal serotypes was 74%, ≥ 1.0 µg/mL was 32%.

The group of 18 patients with antibody concentrations < 0.35 µg/mL despite vaccination in the last 5 years did not differ from the patients with adequate

antibody levels with regard to reason for asplenia, age and time of asplenia (data not given).
For *Hib*, 88 patients (68%) were not vaccinated (Figure 3). Of the 42 patients that did receive a *Hib* vaccination, 13 individuals (31%) did not have levels of serum IgG antibodies ≥ 2.5 µg/mL (Figure 3).

Figure 3. Antibody levels against *H. influenzae* type b polysaccharide



"shade": patients who are not vaccinated according to international guidelines or who have antibody levels < 2.5 µg IgG/ml.

Of the studied population, the majority (95 patients, 73%) never received a meningococcal vaccination. Twenty-four of the 34 patients who were vaccinated against *N. meningitidis*, received this vaccination in the last 3 years. Only 28 (22%) patients received all three recommended vaccines (pneumococcal, *Hib* and meningococcal).

Antimicrobial prophylaxis

Thirty-seven (28%) patients keep (a prescription for) antibiotics at home in case of high fever and during holidays. Twenty-one (16%) patients had taken a pre-emptive course of antibiotics at least once.

DISCUSSION

In this study of 130 Dutch patients with an absent or dysfunctional spleen, vaccine coverage was inadequate in 21% (for *S. pneumoniae*), 68% (for *Hib*) and 73% (for *N. meningitidis*) of the patients. Despite international guidelines, 78% did not receive all three recommended vaccines.[14-17] A substantial part (43%) of the splenectomised patients was not informed (or did not recall having received this information) about infectious risks and the necessity of antimicrobial prophylaxis. Fifty percent of the patients were not instructed to immediately seek medical attention in case of febrile illness. In case of infection, antibiotics were not

immediately available for 72% of the patients. The design of our study may even overestimate the actual education and vaccination status of asplenic individuals in the general population. For reasons of privacy, we were only allowed to recruit patients indirectly, i.e. by their general practitioner. This might have resulted in a selection bias. It is possible that patients whose splenectomy is not recorded by their GP (or for whom adequate vaccination strategies probably are not implemented) are not approached for participation. Furthermore, half of the identified patients did not respond for reasons that could not be obtained. This might have lead to a representation bias, for patients that are not interested in joining this study might also not be aware of the risks associated with their asplenic state. On the contrary, it might be possible that patients not responding were already fully informed and therefore did not feel the need to participate.

Pneumococcal immunisation is the mainstay of preventive therapy for asplenic patients; it is recommended since 1977. A 23-valent polysaccharide vaccine (PPV-23) has been available since 1983.[18] Previous studies also show that many patients are not informed about infectious risks and antimicrobial prophylaxis, and that vaccination coverage is low.[19,20] Kinnersley *et al.* showed that in 1993 only 16% of the splenectomised patients received pneumococcal vaccination.[21] In 2000, Brigden *et al.* concluded that 68% of the hospitalised splenectomy patients were vaccinated with a pneumococcal vaccine.[22] Despite vaccination in the past 5 years, 17% (18/103) of the splenectomised patients in our study does not have antibody concentrations against *S. pneumoniae* above the recommended threshold. Hosea *et al.* found that splenectomised patients have lower baseline concentrations of type-specific antibody and, after stimulation with pneumococcal polysaccharides, respond to a lesser degree than do healthy volunteers.[23]

Besides the necessity of improving vaccine administration, the substantial number of patients not responding adequately to polysaccharide vaccines indicates the need for additional vaccination strategies. The use of a seven-valent pneumococcal conjugate vaccine (PCV-7) leads to a significant rise in pneumococcal antibodies in splenectomised patients.[24,25] This conjugated vaccine causes T-cell-dependent induction of antibodies with development of immunological memory.[24,25] Musher *et al.* showed that in two asplenic patients who failed to respond serologically and clinically to repeated doses of PPV-23, immunisation with PCV-7 resulted in high levels of IgG to all of the capsular polysaccharides included in the vaccine.[26] Introduction of a sequential pneumococcal vaccination strategy with PCV-7 and PPV-23 might therefore lead to protective antibody levels in a higher fraction of asplenic individuals. This vaccination schedule is currently being evaluated in our patient population.

CONCLUSIONS

Vaccination coverage and education about infectious risks in patients with an absent or dysfunctional spleen in (part of) the Netherlands is low. Strategies towards improvement of vaccination coverage have to be developed. We suggest a unique, voluntarily registration of splenectomised patients, from which patients are being informed on a regular base about infectious risks and the need for revaccination.[19,22] Due to limited efficacy of the pneumococcal polysaccharide vaccine in patients with an absent or dysfunctional spleen, the efficacy of alternative vaccination schedules (containing combinations of conjugated and polysaccharide vaccines) should be investigated.

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Chapter 3

Response to conjugate pneumococcal and *Haemophilus influenzae* type b vaccines in asplenic patients

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ABSTRACT

We determined the efficacy of conjugated *Haemophilus influenzae* (*Hib*) type b and pneumococcal vaccines by quantitative analysis of the antibody response in asplenic patients. To that end we vaccinated 92 patients with a conjugated *Hib* vaccine and 54 received two doses of conjugated pneumococcal vaccine (PCV-7), followed at six months by a plain polysaccharide pneumococcal vaccine (PPV-23). Antibody concentrations were measured before and three weeks after vaccination. After one dose of pneumococcal conjugate vaccine, 82% of the patients reached the antibody threshold of ≥ 1.0 µg/mL for 5 out of 7 tested vaccine serotypes. This percentage rose to 85% and 92% after the second PCV-7 and the PPV-23, respectively. For serotypes, included in the PPV-23 vaccine only, the majority of patients reached antibody concentrations above the chosen threshold after one dose of PPV-23. For *Hib*, 97% of the patients reached the threshold concentration of ≥ 1.0 µg/mL after one dose of vaccine. It can be concluded that the vast majority of asplenic patients had a sufficient response to conjugated vaccines against *S. pneumoniae* and *Hib.* We recommend to include conjugated and plain polysaccharide pneumococcal vaccines in the vaccination schedule for asplenic patients.

INTRODUCTION

Functional or anatomical asplenic patients are at increased risk of a severe overwhelming post-splenectomy infection (OPSI). The estimated life-time risk of OPSI in asplenic patients is approximately 5% with a mortality of 50-80%. Micro-organisms as *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (*Hib*) and *Neisseria meningitidis* are most often involved in OPSI.[1] Guidelines for the prevention and treatment of the infections in asplenic patients have been developed and can be divided into three categories: education, vaccination and antibiotic prophylaxis.[2-4]

In 1983, a pneumococcal polysaccharide vaccine (PPV-23) consisting of the capsular polysaccharides of the 23 most prevalent pneumococcal serotypes became available. PPV-23 has a coverage of 85-90% of the invasive pneumococcal infections among children and adults.[5] Although this polysaccharide vaccine induces an immune response, it does not result in generation of memory B-cells and long lived plasma cells. To maintain sufficiently high antibody levels, re-immunisation with PPV-23 every five years is therefore recommended in hyposplenic and asplenic patients.[3,6] The heptavalent pneumococcal polysaccharide protein conjugate vaccine (PCV-7) was introduced in the Dutch National Vaccination Programme for children and infants in 2006. The seven serotypes included in the conjugate vaccine are responsible for 64% of the invasive pneumococcal infections in young children (< 2 years) in the Netherlands. This conjugate vaccine leads to a T-cell dependent induction of antibodies and immunological memory. Because PCV-7 only covers seven pneumococcal serotypes, an additional dose of PPV-23 at the age of two years is recommended in children with functional or anatomic asplenia.[7]

In immunocompromised patients, the response to non-conjugated polysaccharide vaccines is suboptimal and antibody concentrations decline rapidly over time.[8,9] Giebink *et al.* showed a 24-32% decline in serum antibody concentration from the peak antibody level during the first year after vaccination.[10] By adding a conjugate vaccine to the pneumococcal vaccination schedule, a higher and sustained increase in antibody concentrations was obtained.[11-13]
Beside pneumococcal vaccination, vaccination against *Hib* and *N. meningitidis* is also recommended for asplenic patients.[2,14] We recently described that lack of adherence to international guidelines is common. Of the patients who were adequately vaccinated with PPV-23, 17% had anti-pneumococcal antibody concentrations below the chosen antibody threshold of 0.35 µg/mL.[15] Because the conjugate pneumococcal vaccine induces higher and sustained antibody

concentrations in asplenic patients and the considerable number of patients (17%) with low antibody concentrations after PPV-23, we included PCV-7 in our vaccination protocol. We prospectively studied the effect of conjugated pneumococcal and *Hib* vaccination in our cohort of asplenic patients.

PATIENTS AND METHODS

In 2006 and 2007, we performed a study to evaluate the vaccination coverage of adult asplenic patients in the Utrecht area of the Netherlands. The study was approved by the local institutional ethics committee and all patients gave written informed consent. In all patients, pre-vaccination antibody concentrations against *Hib* and *S. pneumoniae* were determined.[15] If vaccination was not performed according to international guidelines, patients were vaccinated with conjugated pneumococcal and *Hib* vaccines and serum samples were taken after vaccination to determine antibody concentrations. Catch-up vaccination with meningococcal type C (Men C) conjugate vaccines was also offered, the outcome of latter vaccination will be reported separately. The last serum samples were obtained in 2008.

Vaccination protocol

Patients are vaccinated according to a standard protocol in our hospital. The vaccination schedule and indications are:

- In patients previously not vaccinated against *S. pneumoniae* or, if the vaccination took place more than five years ago, two doses of conjugate pneumococcal vaccine (PCV-7) and one dose of polysaccharide pneumococcal vaccine (PPV-23) are given according to the schedule given in Table 1.
- In patients previously not vaccinated against *Hib*, one dose of conjugate *Hib* vaccine will be administered.

In case both pneumococcal and *Hib* vaccines are indicated, the *Hib* vaccine was given simultaneously with the conjugate pneumococcal vaccine.

Table 1. Vaccination schedule for asplenic patients

Time	**Day 0**	**+ 6 weeks**	**+ 6 months**
Vaccine	PCV-7, *Hib*	PCV-7, MenC	PPV-23

PCV-7 = 7-valent pneumococcal conjugate vaccine
Hib = *Haemophilus influenzae* type b conjugate vaccine
MenC = meningococcal group C conjugate vaccine
PPV-23 = 23-valent pneumococcal polysaccharide vaccine

One dose of the conjugated pneumococcal vaccine used (Prevnar®, Wyeth Pharmaceuticals, Philadelphia PA, USA) contains 2 µg of each of the

pneumococcal polysaccharide serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of 6B coupled to the CRM_{197} carrier protein. One dose of the plain polysaccharide pneumococcal vaccine (Pneumo®23, Sanofi Pasteur SA, Lyon, France) contains 25 µg of each of the following serotypes: 1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F and 33F. The *Hib* vaccine used is Act-HIB®, Sanofi Pasteur SA, Lyon, France. Each dose contains 10 µg of purified capsular polysaccharide of *Haemophilus influenzae* type b covalently bound to 20 µg of tetanus protein.

Antibody determinations

Serum samples were collected prior to vaccination and three weeks after each vaccination and stored at -20°C until use.

Antibody concentrations against *Hib* were determined by ELISA as described previously.[16-18] Briefly, microtitre plates coated with *Hib* polysaccharide were incubated with serial dilutions of patient sera. Next, plates were incubated with goat anti-human IgG conjugated to alkaline phosphatase, and developed with *p*-nitrophenylphosphate. For *Hib*, an antibody concentration of ≥ 1.0 µg/mL is considered to correlate with long-term protection.[19] For immunocompromised patients this threshold is often considered to be not protective. We therefore also calculated responsiveness at a threshold of ≥ 2.5 µg/mL.

Antibody levels to eight pneumococcal serotypes (PPS 3, 4, 6B, 9V, 14, 18C, 19F and 23F) and cell wall polysaccharide (CWPS) were determined by multiplex immunoassay (Luminex®). Patient sera were pre-absorbed with pneumococcal serotype 22F to remove CWPS2.[20,21] Two dilutions of patient sera (1:100 and 1:1,000) were incubated with the mixture of polysaccharide-coated beads. Goat anti-human IgG conjugated to phycoerythrin was added and the plate was read on a Luminex-100 system (Bioplex, Biorad). Sera from a selected number of patients were also analysed with the 14-plex Luminex Pneumococcal immunity panel (Luminex corporation, Austin TX). The reference serum 89SF was kindly provided by dr CE Frash, Division of bacterial products, Center for biologics evaluation and research, Rockville, MD. The chosen anti-pneumococcal antibody threshold for protection against invasive disease was ≥ 0.35 µg/mL, in accordance with World Health Organisation (WHO) recommendations.[22] As for *Hib*, in immunocompromised patients this threshold is generally considered not to be protective. Therefore, we also calculated the response rate using a higher threshold of ≥ 1.0 µg/mL.[23-25] A successful response to vaccination was defined as post-vaccination concentrations of ≥ 1.0 µg/mL for at least five out of seven pneumococcal serotypes included in the conjugate vaccine.[26]

All antibody concentrations were log-transformed and geometric mean concentrations (GMC) were calculated.

Statistical analysis

Comparisons of GMC before and after vaccination (paired samples) were performed using the paired samples T-test. For unrelated samples, the independent samples T-test and Chi-square were used. A *p*-value of ≤ 0.05 was considered statistically significant. For statistical analysis, the Statistical Package for the Social Sciences (SPSS) for Windows® software, version 15.0 (SPSS Inc., Chicago, IL, USA) was used.

RESULTS

We included 184 patients in the study on the vaccination coverage of asplenic patients in the Netherlands.[15] In short, only 32 of the 184 patients (17%) were vaccinated according to international guidelines; 152 patients were not vaccinated adequately. These 152 patients were offered a catch-up vaccination for pneumococci, *Hib* and meningococcal type C. After vaccination, 129 (85%) of the 152 patients returned for withdrawal of serum samples. This study is based on the analyses of 129 patients. Catch-up vaccination for pneumococci was indicated in 54 patients and *Hib* vaccination was indicated for 92 patients.
The patient group consisted of 65 men and 64 women. Mean age at vaccination was 52.6 years. Baseline characteristics of the patients are given in Table 2.

Table 2. Baseline characteristics of the total patient group and the patient groups receiving pneumococcal and *Hib* vaccines respectively

		Total	***S. pneu***	***Hib***
Patients (n)		129	54	92
Male gender[1]		65 (50)	28 (52)	50 (54)
Reason asplenia[1]	Trauma	39 (30)	16 (30)	31 (34)
	ITP	19 (15)	8 (15)	16 (17)
	Hodgkin's disease	17 (13)	10 (19)	10 (11)
	Spherocytosis	16 (12)	6 (11)	10 (11)
	Other reasons	36 (28)	13 (24)	24 (26)
	Functional asplenia	2 (2)	1 (2)	1 (1)
Age at Sx[2]		29.6 ± 19.4	27.8 ± 17.6	30.9 ± 20.5
Age at vaccination[2]		52.6 ± 13.2	51.5 ± 12.2	54.6 ± 13.6
Time to vaccination[2]		23.0 ± 14.8	23.9 ± 13.4	23.6 ± 14.8

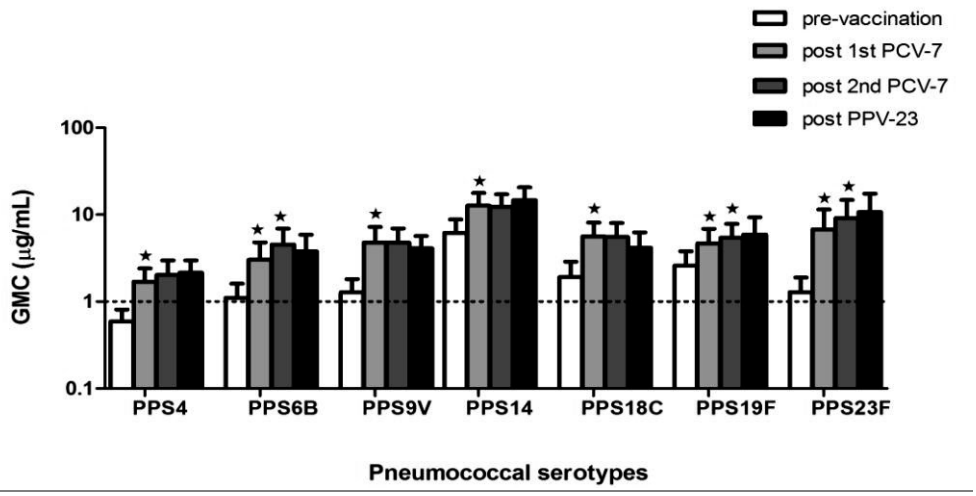
S. pneu = *Streptococcus pneumoniae*, *Hib* = *Haemophilus influenzae* type b, ITP= idiopathic thrombocytopenic purpura, Sx= splenectomy, Time to vaccination = time between splenectomy and vaccination.
[1] data given in numbers with percentages in parentheses
[2] data given in mean years ± standard deviation

All but two patients were asplenic because of splenectomy, the most prevalent reason being a trauma (30%). Two patients were considered to be functional asplenic, because of irradiation of the spleen for Hodgkin's disease and β-thalassaemia, respectively.

Of the fifty four patients that were vaccinated with pneumococcal vaccines, 41 (76%) were immunised against *S. pneumoniae* in the past. Previous vaccination had taken place once in 21 patients, whereas multiple immunisations before start of the study had taken place in 20 patients. The mean time since the last vaccination was 8.6 years (± SD 6.4 years). Only five patients (9%) received the first pneumococcal vaccine before splenectomy.

GMC's after the first pneumococcal conjugate vaccine rose significantly ($p<0.01$) for all vaccine serotypes (Figure 1). After the second conjugate vaccination, a further significant increase was found for antibodies against serotypes 6B, 19F and 23F. Serum samples after the PPV-23 vaccination were available for 48 of the 54 patients, six patients were lost to follow-up after the second vaccination. The pneumococcal polysaccharide vaccine did not lead to a further increase in antibody levels to the conjugate vaccine serotypes (Figure 1). No influence was seen of previous vaccination on the height of the antibody concentration before or after complete vaccination except for PPS14.

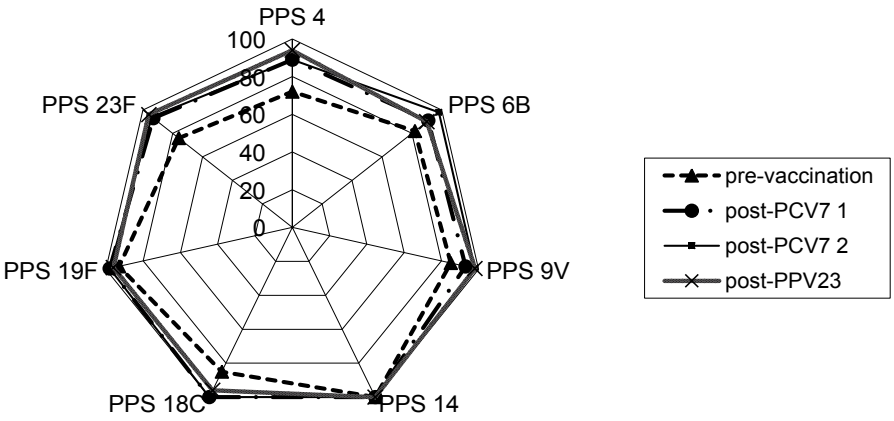
Figure 1. *S. pneumoniae*: serotype-specific IgG response (GMC)



PPS= pneumococcal polysaccharide, ★ = statistically significant increase of antibody concentration compared to pre-vaccination values ($p < 0.05$). For serotypes 6B, 19F, and 23F, a second dose of PCV-7 induced a significant further increase in GMC

In Figure 2, the percentage of patients that obtained pneumococcal antibodies above the chosen threshold of 0.35 µg/mL after the first and second (conjugate) vaccine and the polysaccharide pneumococcal vaccine are given.

Figure 2. Percentage of patients with pneumococcal serotype-specific antibody concentrations of ≥ 0.35 µg/mL after pneumococcal vaccination

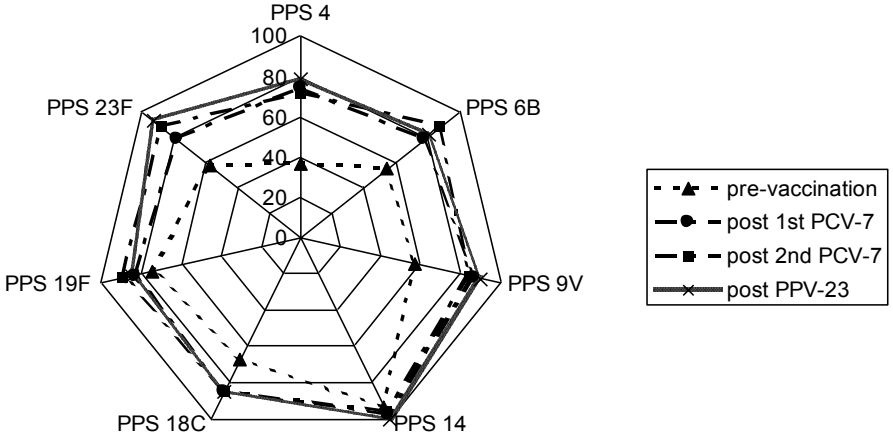


PPS= pneumococcal polysaccharide

In Figure 3, the percentage of patients that obtained pneumococcal antibodies above the chosen threshold of 1.0 µg/mL are given for every pneumococcal vaccination.

Before vaccination, 56% of the patients had antibody concentrations of ≥ 0.35 µg/mL against all seven pneumococcal serotypes that are included in the conjugate vaccine, whereas 76% of the patients reached this chosen threshold for five out of seven vaccine-serotypes. When applying a threshold of ≥ 1.0 µg/mL pre-vaccination, these percentages are 17% and 48% respectively.

Figure 3. Percentage of patients with pneumococcal serotype-specific antibody concentrations of ≥ 1.0 µg/mL after pneumococcal vaccination

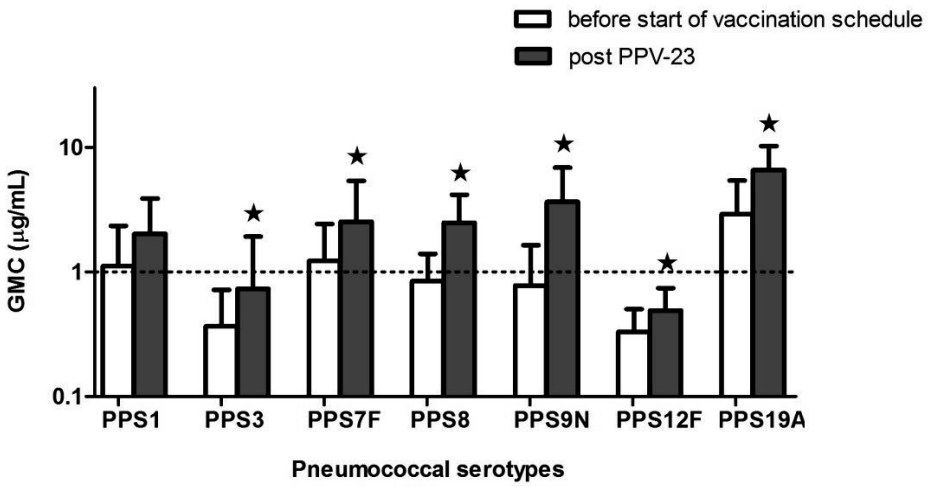


PPS= pneumococcal polysaccharide

After the first conjugate vaccination, 82% reached the chosen antibody threshold of ≥ 1.0 µg/mL for five or more of the seven pneumococcal serotypes included in the vaccine. After the second conjugate vaccine, this percentage rose to 85% and after the polysaccharide vaccine, 92% of the patients reached protective antibody concentrations for at least five out of seven pneumococcal serotypes.
Four patients did not reach protective antibody concentrations (≥ 1.0 µg/mL) for five or more conjugate vaccine serotypes. This group existed of three women and one man. One patient was splenectomised because of Hodgkin's disease, the other patients had other reasons for the splenectomy (pancreas surgery, splenic abscess, haemangioma). Mean age at vaccination was 57 years (± SD 8,8 years), mean age at splenectomy was 45 years (± SD 17 years).

To determine whether the pneumococcal polysaccharide vaccine induces a good response against serotypes only included in the PPV-23 vaccine, a second assay was done in a subset of patients to measure seven additional polysaccharides included in the PPV-23 vaccine. Of 16 patients, antibody concentrations were measured in sera before vaccination and after the pneumococcal polysaccharide vaccine (Figure 4).

Figure 4. GMC's before and after PPV-23 for 7 pneumococcal serotypes included in the PPV-23 vaccine



PPS= pneumococcal polysaccharide
★ = statistically significant increase after vaccination ($p < 0.05$) compared to pre-vaccination

In Table 3, the percentages of patients who reach the chosen threshold concentrations of ≥ 0.35 µg/mL and ≥ 1.0 µg/mL respectively are given for seven additional pneumococcal polysaccharides included in the PPV-23 vaccine. After vaccination, the threshold of ≥ 0.35 µg/mL was reached in 56-100% of the patients, dependent of the serotype. The threshold of ≥ 1.0 µg/mL was reached in the majority of patients for all tested serotypes, except PPS3 and PPS12F.

Table 3. Percentages of patients who reach the threshold concentrations after the pneumococcal polysaccharide vaccine (PPV-23)

Serotype	**IgG concentration ≥ 0.35 µg/mL**	**IgG concentration ≥ 1.0 µg/mL**
PPS1	94	69
PPS3	56	25
PPS7F	100	63
PPS8	100	88
PPS9N	100	88
PPS12F	56	31
PPS19A	100	100

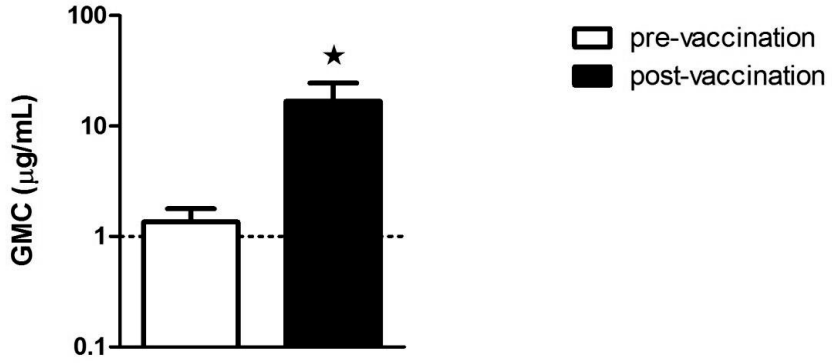
PPS = pneumococcal polysaccharide

In univariate analysis no influence was observed with regard to age at splenectomy, age at vaccination, previous vaccination or reason for splenectomy (trauma versus other causes).

Pneumococcal revaccination, while not leading to a further increase in antibody levels to the 7 conjugate vaccine serotypes, thus was able to induce ≥ 0.35 µg/mL antibody concentrations for the majority of non-conjugated pneumococcal polysaccharides.

Mean *Hib* antibody concentrations before and after conjugate *Hib* vaccination are shown in Figure 5. Of the 92 patients vaccinated for the first time with the conjugated *Hib* vaccine, 55 patients (60%) already had a anti-*Hib* antibody concentration ≥ 1.0 µg/mL before the vaccination. After vaccination, 89 patients (97%) reached this antibody concentration. When applying a post-vaccination antibody concentration threshold of ≥ 2.5 µg/mL, the vaccination was successful in 75 patients (82%). No association was found between the magnitude of the *Hib* antibody response and reason of splenectomy, sex, age at splenectomy, age at vaccination or time from splenectomy to vaccination.

Figure 5. *H. influenzae* type b: IgG response (GMC)



★ = statistically significant increase after vaccination ($p < 0.01$)

Three patients did not reach the chosen threshold of ≥ 1.0 µg/mL after one vaccination. The hypo-responders were two men and one woman, with a mean age at vaccination of 56 years. Reasons for splenectomy were pancreas surgery, thrombocytopenia and stomach cancer. Two out of three *Hib* concentrations pre-vaccination were below 1.0 µg/mL. The female patient was also a hypo-responder to the pneumococcal vaccination.

DISCUSSION

In this study the antibody response to pneumococcal and *Hib* vaccines is studied in a group of 129 asplenic patients. In this high-risk population for pneumococcal and *Hib* infections, the response to polysaccharide vaccines, expressed as increase in antibody concentration, is suboptimal.[27,28] In previous studies it was shown that the efficacy of vaccination with a polysaccharide pneumococcal vaccine in asplenic patients, in terms of the prevention of pneumococcal infections, was 77% (95% CI 14-95%).[29] Defining protective antibody levels is difficult, the protective level may be different for different serotypes and may vary with age. Therefore two different thresholds were chosen to evaluate the effect of vaccination.[27]

Musher *et al.* showed that in two asplenic patients who failed to respond serologically and clinically to repeated doses of PPV-23, immunisation with PCV-7 resulted in high levels of IgG to all of the capsular polysaccharides included in the vaccine.[30] In asplenic children (n=21, mean age 11.7-12.6 years) who were revaccinated with pneumococcal polysaccharide or conjugate pneumococcal vaccines, the antibody concentrations showed a greater increase in the conjugate group for serotypes 6B and 23F and the percentage of patients with a four-fold increase in antibody coccentration from baseline was greater than in the polysaccharide group.[31] Rose *et al.* showed that in children unresponsive to PPV-23, after two doses of PCV-7 and a booster dose of PPV-23, specific antibody concentrations rose to ≥ 1.0 µg/mL in 64-100% for every serotype included in PCV-7. For the PPV-23 specific serotype 5, after pneumococcal polysaccharide vaccine 43.7% of the patients did not reach the threshold of ≥ 1.0 µg/mL.[13] Our data indicate that a single dose of conjugate polysaccharide vaccine is sufficient to induce antibody levels ≥ 0.35 µg/mL, including serotypes 6B and 23F, independent of whether or not the patients were previously vaccinated with PPV-23.

In the current study it was seen that after the pneumococcal polysaccharide vaccine, in the majority of patients the chosen threshold of ≥ 0.35 µg/mL and ≥ 1.0 µg/mL was also reached for seven tested serotypes not covered by the conjugate vaccine. After immunisation with PPV-23, for 5 of the 7 tested non-conjugated pneumococcal serotypes included in this vaccine the majority of patients reached the chosen threshold of ≥ 1.0 µg/mL with a mean of 63%. The conjugated vaccine on the contrary led to antibody concentrations above the highest chosen threshold (≥ 1.0 µg/mL) in more than 89% for every serotype included in the conjugate vaccine after the first dose of vaccine, with 82% of the patients reaching this threshold for five out of seven vaccine-included serotypes.

In this patient group of splenectomised patients, an excellent response was obtained after one dose of conjugate vaccine. In the study of Stoehr *et al.* with patients splenectomised for hereditary spherocytosis similar results were obtained after a single dose of conjugated vaccine, whereas in studies with specific antibody deficiencies repeated vaccination was necessary to reach protective antibody concentrations.[32-34] Our results are therefore in accordance with previously conducted studies in splenectomised patients. The conjugate pneumococcal vaccine thus is an immunogenic vaccine in asplenic patients. A second dose of conjugate vaccine does not lead to a significant additional increase in antibody concentration; whether it does lead to an improvement of antibody function was not determined in this study. A single dose of conjugate vaccine therefore seems adequate and a change in protocol may be considered.

A number of clinical variables (age at splenectomy, reason of splenectomy, age at vaccination, previous vaccination) have previously been associated with an effect on the antibody response to pneumococcal polysaccharide vaccination.[9,32] We have analysed the impact of these variables on serotype specific antibody concentrations. Factors that had a statistically significant ($p<0.05$) influence on reaching the chosen threshold antibody concentration of ≥ 1.0 µg/mL, were: trauma as reason of asplenia (PPS19F), gender (PPS18C) and age at vaccination (PPS9V, PPS23F). For the lower threshold of ≥ 0.35 µg/mL, associations with other serotypes and other clinical characteristics were found. When correcting for multiple testing, significance of these findings were lost. It therefore can be concluded that factors previously implicated as influencing the magnitude of the antibody response were of very limited influence on increase in antibody concentrations against specific serotypes in this study. Our results are in line with the observations of Landgren *et al*.[35] Taken together, it can be concluded that clinical characteristics of splenectomised patients cannot predict responsiveness to pneumococcal vaccination.

For *Hib*, high antibody concentrations were reached in almost all patients included in this study. The response rate was 97% for the threshold of ≥ 1.0 µg/mL and 82% if a threshold of ≥ 2.5 µg/mL was chosen. Only three patients did not reach the threshold of ≥ 1.0 µg/mL for *Hib*. One dose of conjugated *Hib* vaccine therefore seems adequate for protection of asplenic patients. In contrast, a single dose of meningococcal type C conjugate vaccine appears to be insufficient in a significant number of asplenic patients (Meerveld-eggink et al.; manuscript in preparation).

CONCLUSIONS

Pneumococcal and *Hib* conjugate vaccines induce an adequate increase in antibody concentrations after vaccination. Higher response rates were reached after conjugated pneumococcal vaccines than after pneumococcal polysaccharide vaccination. Therefore, the pneumococcal conjugate vaccine should be incorporated in the guidelines for vaccination in asplenic patients.

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Chapter 4

Impaired antibody response to conjugated meningococcal serogroup C vaccine in asplenic patients

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ABSTRACT

We determined the quantity and quality of antibodies against the meningococcal serogroup C (MenC) conjugated vaccine in asplenic patients. In 116 asplenic patients, antibody concentrations (IgG) were measured against meningococcal serogroups A, C, W135 and Y before and after immunisation. Of MenC-specific IgG, both antibody avidity and subclasses of IgG1 and IgG2 were determined. Mean MenC IgG concentration rose from 0.16 µg/mL prior to vaccination to 3.69 µg/mL 3 weeks post-vaccination, with 67% of patients reaching the threshold of ≥ 2.0 µg/mL. Mean IgG concentration at 35 weeks post-vaccination was 3.10 µg/mL. IgG2 concentrations increased more than IgG1. Marginal avidity maturation was seen. Hypo-responders to the first MenC vaccine (IgG anti-MenC ≤ 2.0 µg/mL) were offered a booster dose. After revaccination, 59% reached the chosen IgG threshold. The IgG concentration rose from 0.29 to 1.12 µg/mL, with an increase in the IgG1/IgG2 ratio. Avidity indices remained below 33%.

In conclusion, in asplenic patients the quantity and quality of antibodies produced after one dose of conjugated MenC vaccination is lower than observed in previous studies in healthy adults. Booster vaccination does lead to a rise in IgG GMC's, but does not lead to higher avidity of antibodies.

INTRODUCTION

Functional or anatomical asplenic patients are at increased risk of a severe overwhelming post-splenectomy infection (OPSI) with encapsulated bacteria. After *Streptococcus pneumoniae* as the major cause, one of the other prevalent causative organisms is *Neisseria meningitidis*.[1-3] To reduce the incidence of these infections in asplenic patients, a vaccination schedule including vaccines against *N. meningitidis* is strongly recommended.[4]
Of the known meningococcal groups, 5 groups (A, B, C, Y and W135) are responsible for more than 95% of invasive disease.[5] In 2002, after an outbreak of MenC infection, a national vaccination campaign was performed in the Netherlands in which all persons 1-18 years of age were vaccinated against MenC. Since then, due to vaccination and herd immunity, the circulation of MenC is very low.

Data on the ability of asplenic individuals to establish an adequate antibody response to vaccination with polysaccharide vaccines are conflicting. The response depends among others on the reason for asplenia and on the vaccine that is used (polysaccharide versus conjugate).[6-9] Immunisation with meningococcal polysaccharide vaccines was not recommended for patients with asplenia due to the short duration of protection and the absence of protection against the most common serogroup B.[2,10] With the introduction of conjugated vaccines, inducing long-term immunity, vaccination of asplenic patients with meningococcal conjugated vaccines now is recommended.[10,11]

For determination of immunogenicity of a vaccine, increase in antibody concentration is a surrogate marker of infection protection. A more useful biomarker reflecting the generation of immunological memory may be the increase in antibody avidity after immunisation.[12-14]
Antibody avidity is defined as the overall antigen-binding capacity of polyclonal antibodies with different affinities.[12,13,15,16] Polysaccharide conjugate vaccines have the ability to elicit higher avidity antibodies with increased functional activity than do plain polysaccharide vaccines.[13] The magnitude of the avidity index is dependent on the type of conjugate vaccine.[17]

In this study, the efficacy of a MenC conjugated vaccine in terms of increase in antibody concentration after vaccination (quantity) and the avidity of the antibodies (quality) is determined in adult, asplenic patients.

PATIENTS AND METHODS

In 2006 and 2007, patients were included in a study to evaluate the vaccination coverage of adult asplenic patients in the Utrecht area of the Netherlands.[18] This study was approved by the local institutional ethics committee and all patients gave written informed consent. When applicable, patients were vaccinated with conjugated pneumococcal, *Hib* and meningococcal vaccines according to the institutions protocol and serum samples were taken pre- and post-vaccination to determine antibody concentrations. Results of the pneumococcal and *Hib* vaccines will be reported separately (Meerveld *et al.*, manuscript submitted).

Vaccination protocol

Patients were vaccinated against *N. meningitidis* when they were never vaccinated before, when vaccination status against *N.meningitidis* could not be retrieved or when vaccination with a polysaccharide meningococcal vaccine occurred more than three years ago. A first dose of conjugate meningococcal group C vaccine was given, consisting of MenC polysaccharide covalently coupled to tetanus toxoid (NeisVac-C®, Baxter AG, Vienna, Austria). Blood samples were drawn pre-immunisation, 3 weeks and 35 weeks post-immunisation. Serum samples were stored at -20°C until use. When antibody concentrations after the first vaccination remained below 2 µg IgG/mL, a second dose of conjugate MenC vaccine was advised according to previously published recommendations.[19]

Laboratory determinations

Meningococcal antibody concentrations were measured against serotypes A, C, W135 and Y by multiplex immunoassay (MIA) as described previously.[20-22] All antibody concentrations were log-transformed and geometric mean concentrations (GMC) were calculated. Seroconversion was defined as a ≥ fourfold increase in antibody concentration after vaccination. The chosen anti-meningococcal antibody threshold for correlation with long-term protection was ≥ 2.0 µg/mL.[23]

MenC-specific IgG1 and IgG2 subclasses were measured by a modification of the MIA as described above.[24] Previously assigned concentrations of meningococcal IgG1, and IgG2 antibodies were used in this assay.[25,26]

Avidity of MenC-specific IgG antibodies was assessed by using a modification of the MIA for determining MenC-specific IgG.[20,21] Serum samples with an IgG concentration of ≥ 0.25 µg/mL were diluted to an antibody concentration of 25 ng/mL in a buffer composed of 25% antibody depleted human serum (Valley Biomedical, USA). Ammonium thiocyanate (NH_4SCN; Sigma-Aldrich, St. Louis,

MO) was added to a final concentration of 0.5 M in order to dissociate low-avidity antigen-antibody binding.[27] After incubation of polysaccharide-conjugated-beads with serum, 0.5M NH_4SCN was added for 10 minutes exactly. Beads were washed, *R*-phycoerythrin(*R*PE)-conjugated goat anti-human IgG (gamma chain specific) (Jackson ImmunoResearch Laboratories Inc., West Grove, PA) was added and incubated for a further 30 minutes. After a final wash, the plate was read on a Luminex-100 system (Bio-plex; Bio-Rad Laboratories, Hercules, CA). Data are expressed as the avidity index (AI) which is the percentage of antibodies that remained bound to the MenC polysaccharide-conjugated beads after treatment with NH_4SCN and was calculated as follows: AI = (amount of IgG bound in the presence of 0.5 M NH_4SCN) / (amount of IgG bound in PBS) x 100. Sera were categorised into high, intermediate and low avidity, based on the AI: high, 100-67%; medium, 66-34%; and low, 0-33%.

Statistical analysis

Comparisons of GMC before and after vaccination (paired samples) and comparisons of antibody avidity before and after vaccination were performed using the paired samples T-test. Independent sample T-test and Chi-square were used when appropriate. A *p*-value of ≤ 0.05 was considered statistically significant. In case of subset analysis, in small groups the Wilcoxon signed rank test was used.
For statistical analysis, the Statistical Package for the Social Sciences (SPSS) for Windows® software, version 15.0 (SPSS Inc., Chicago, IL) was used.

RESULTS

We included 184 asplenic patients in this study. Previously, we have described the characteristics of the majority of this group.[18] In 117 patients a MenC conjugated vaccine was indicated. Samples after vaccination, of all but one patient, were available and these 116 sera were analysed in this study. Baseline characteristics of the patients are given in Table 1. All but one patient underwent a splenectomy, the prevalent reason being a trauma (30%). One patient was considered to be functional asplenic, because of irradiation of the spleen for Hodgkin's disease.

Seventeen (15%) patients had previously received a meningococcal vaccination, either MenA, -C, -W135 and -Y polysaccharide vaccine (n=6), MenA, -C polysaccharide vaccine (n=4) or an otherwise not-specified meningococcal vaccine (n=7). The median time since the last vaccination was 4 years (range 2-6 years).

Table 1. Baseline characteristics of asplenic patients vaccinated with MenC conjugate vaccine

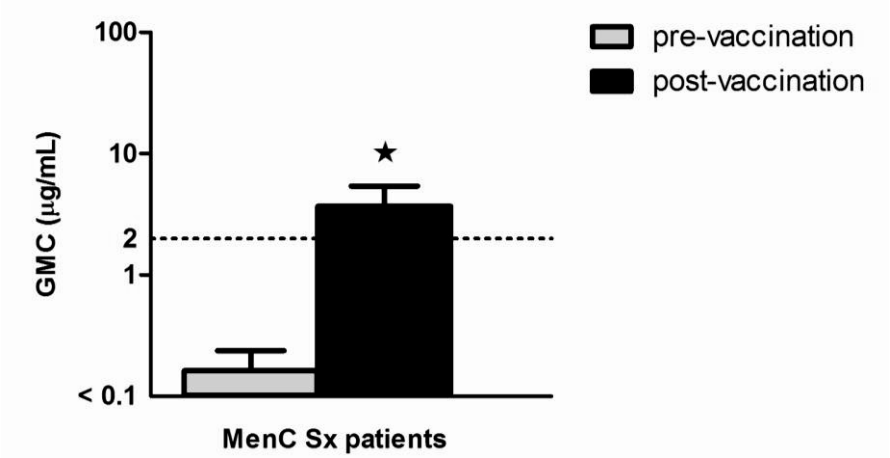
Patients (n)	116
Male gender	59 (51)
Reason for Sx	
Trauma	35 (30)
ITP	18 (16)
Hodgkin's disease	16 (14)
Spherocytosis	16 (14)
Other reasons	30 (26)
Functional asplenia	1 (1)
Age at Sx in years	28.3 (3-78)
Age at vaccination in years	52.5 (23-86)
Time from Sx to vaccination in years	24 (0-55)

ITP= idiopathic thrombocytopenic purpura
Sx= splenectomy
For gender and reason for asplenia, data are given in numbers of patients with percentages recorded in parentheses. For age at Sx, age at vaccination and time from Sx to vaccination, data are given in median number of years, with the range in parentheses

Meningococcal antibody concentrations

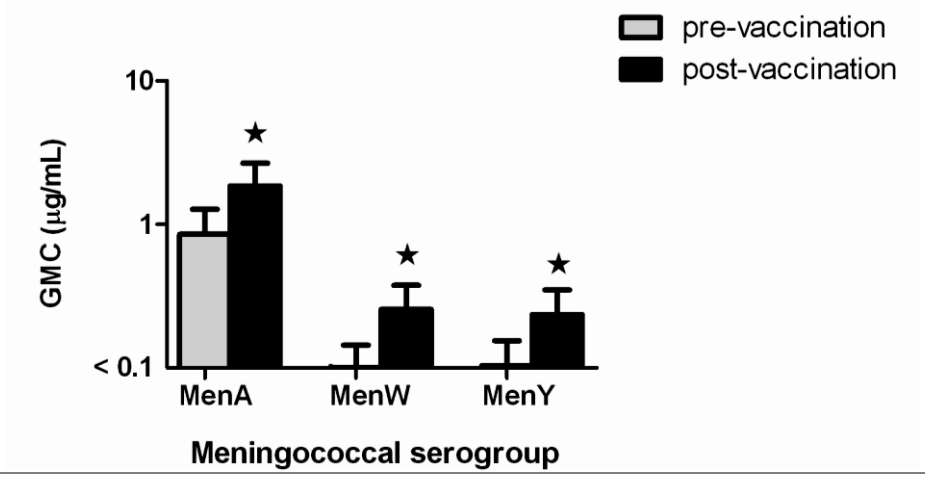
In asplenic patients, MenC geometric mean antibody concentrations (GMC) rose from 0.16 µg/mL prior to vaccination to 3.69 µg/mL three weeks post-vaccination (Figure 1a). Following immunisation with the MenC vaccine, the GMC's for serogroups A, W135 and Y also rose significantly (Figure 1b; post-vaccination concentrations 1.85, 0.26, and 0.24 µg/mL, respectively). For MenC, trauma patients had significantly higher post-vaccination antibody concentrations than the other asplenic patients ($p<0.05$). We observed no statistically significant influence of age at splenectomy, age at vaccination or time from splenectomy to vaccination on the antibody concentrations of MenA, -C, -W135 or -Y. Previous meningococcal vaccination had a statistically significant influence on the MenC GMC's pre- and post-vaccination ($p<0.05$). For MenC, GMC pre-vaccination in previously vaccinated patients was 1.6 µg/mL (95% CI 0.5-5.7) versus 0.1 µg/mL (95% CI 0.1-0.2) in patients not vaccinated before. For GMC post-vaccination, these data were 10.7 µg/mL (95% CI 4.8-24.0) and 3.1 µg/mL (95% CI 2.1-4.8), respectively. GMC's post-vaccination were 10% higher in patients receiving the MenC vaccine alone compared to patients receiving concomitant pneumococcal and/or *Hib* vaccines. This difference was not statistically significant.

Figure 1a. Geometric mean concentrations of MenC IgG before and after MenC conjugate vaccination in asplenic patients (n=116)



GMC = geometric mean concentration
Sx = splenectomy
★ statistically significant increase ($p < 0.05$) in antibody concentration post-vaccination compared to pre-vaccination

Figure 1b. Geometric mean concentrations of MenA, -W135 and -Y IgG before and after MenC vaccination (n=116)



GMC: geometric mean concentration
★ statistically significant increase ($p < 0.05$) in antibody concentration post-vaccination compared to pre-vaccination

Post-vaccination, 67% of the patients reached the threshold of 2.0 µg/mL for MenC (Table 2). Patients who were splenectomised because of trauma reached this threshold more often than other patients ($p<0.05$). Time since splenectomy did not influence seroconversion of MenC. A seroconversion post-vaccination was seen for MenA in 22% (n=25) of patients, and for MenW135 and MenY in 38% (n=44) and 34% (n=39), respectively.

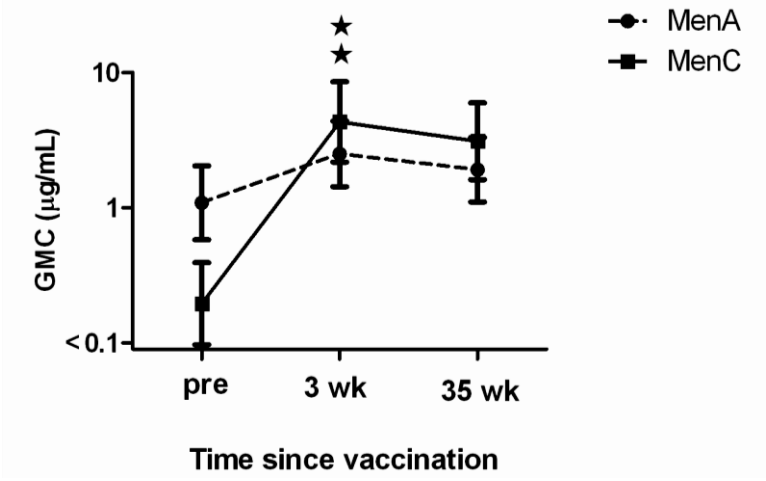
Table 2. *Neisseria meningitidis* serogroup C: response to vaccination

≥ 2.0 µg IgG/mL pre-vaccination: n (%)	14 (12)
≥ 2.0 µg IgG/mL post-vaccination: n (%)	78 (67)
Seroconversion: n (%)	93 (80)

Data are given in numbers of patients with percentages in parentheses
Seroconversion: a ≥ fourfold increase in antibody concentration after vaccination

In 38 patients, we were able to determine meningococcal antibody concentrations up to approximately eight months after the first conjugate MenC vaccine. Mean time since vaccination was 8.3 months (range 6,5 – 10,5). GMC's for both serogroup A and C are given in Figure 2. In this group of 38 patients, for MenC GMC pre-vaccination was 0.21 µg/mL and rose to 3.71 µg/mL post-vaccination ($p < 0.05$). After eight months, a decrease in GMC to 3.10 µg/mL was observed. This decrease was not significant compared to the GMC 3 weeks post-vaccination, nor was the decrease in GMC at eight months after vaccination for serogroups A, W135 and Y.

Figure 2. Geometric mean concentrations eight months after vaccination



GMC: geometric mean concentration in µg/mL with 95% confidence interval
★ statistically significant increase ($p < 0.05$) in antibody concentration post-vaccination compared to pre-vaccination, for both MenC and MenA

Thirty eight patients (33%) did not reach adequate antibody concentrations after one dose of conjugate vaccine. Characteristics of this group of hyporesponders are given in Table 3. These hyporesponders had an adequate response to *Hib* vaccination (98% reached the threshold) and pneumococcal conjugate vaccination (93,5% reached protective antibody concentrations of ≥ 1.0 µg/mL for 5 out of 7 vaccine-serotypes after one vaccination). Of the 38 patients, 58% (n=22) did reach seroconversion for serogroup C after the first vaccination. Because of their subnormal antibody response, a revaccination with the MenC conjugate vaccine was performed.

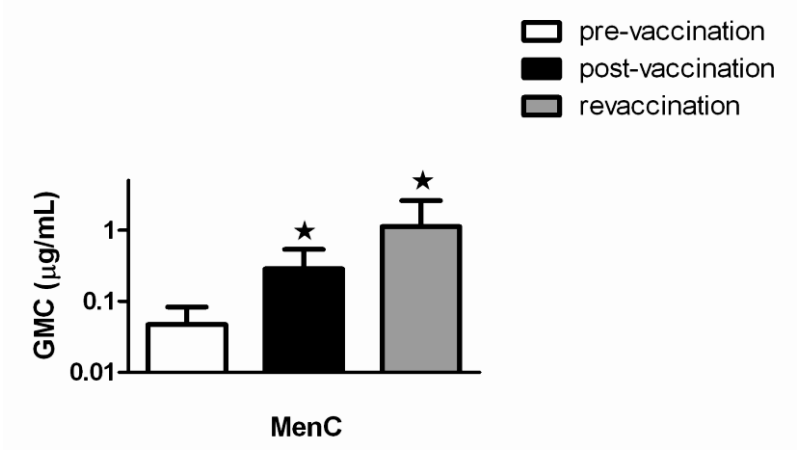
Sera after revaccination were available of 22 patients. In this patient group, GMC pre-vaccination was 0.05 µg/mL, rising after the first vaccination to 0.29 µg/mL. After the second vaccination, the GMC of MenC-specific IgG rose significantly to 1.12 µg/mL (Figure 3). Thirteen (59%) patients reached the threshold of ≥ 2.0 µg/mL.

Table 3. Characteristics of responders and hyporesponders to one dose of MenC conjugate vaccine

	responders	**hyporesponders**
Patients (n)	78	38
Male gender	39 (50)	20 (53)
Reason for Sx		
Trauma	31 (40)	4 (11)
Haematological malignancy	12 (15)	6 (16)
Other reasons	35 (45)	28 (74)
Age at Sx in years	27 (5-76)	30 (3-78)
Age at vaccination in years	52.0 (23-86)	53.5 (29-83)
Time from Sx to vaccination in years	25 (0-55)	23 (2-50)
Previously vaccinated	14 (18)	3 (8)

Sx= splenectomy. For gender, reason for Sx and previously vaccinated, data are given in numbers of patients with percentages recorded in parentheses. For age at Sx, age at vaccination and time from Sx to vaccination, data are given in mean number of years, with the range in parentheses

Figure 3. Geometric mean concentrations after MenC revaccination (n=22)



GMC: geometric mean concentration in µg/mL with upper 95% confidence interval
★ statistically significant increase ($p < 0.05$) in antibody concentration post-vaccination compared to pre-vaccination

Antibody avidity

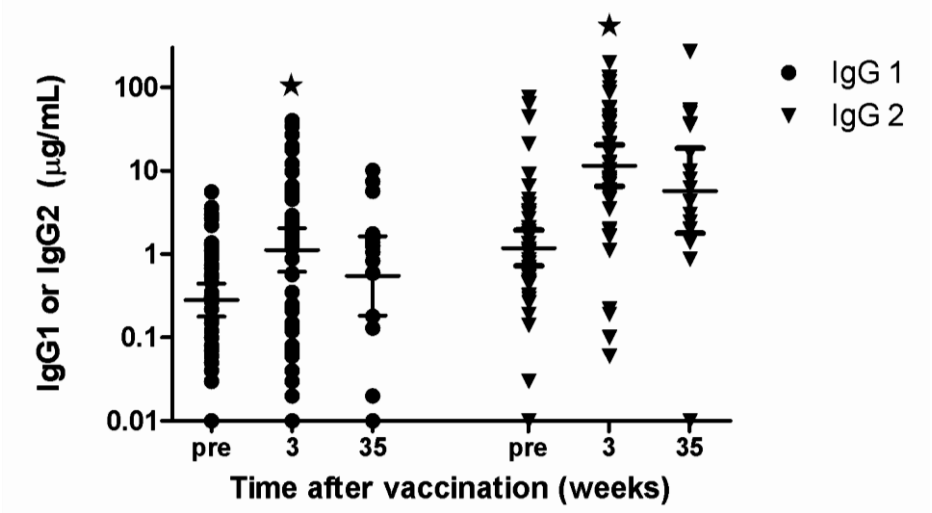
The avidity index (AI) of the MenC antibodies was measured using a grading system to discriminate between sera with low AI (0-33%), intermediate AI (34-66%) and high AI (67-100%). Only sera that contained ≥ 0.25 µg/mL of MenC-specific IgG were selected for AI determination. In the overall cohort, the geometric mean AI (GMAI) was 27% (95% CI 19-35%) pre-vaccination (n=48), 25% (95% CI 20-30%) 3 weeks post-vaccination (n=104) and 32% (95% CI 23-41%) 35 weeks post-vaccination (n=34), respectively. In patients who were revaccinated, the mean AI rose from 14% after the first dose of vaccine to 26% after the second vaccination (n=13; $p < 0.01$). No associations were found between the AI pre- and post-vaccination and the underlying disease resulting in asplenia or previous vaccination with meningococcal vaccines.

IgG subclasses

In Figure 4a, the concentrations of MenC IgG1 and IgG2 are given before, three weeks and 35 weeks after vaccination in patients with ≥ 0.25 µg/ml of MenC PS-specific IgG pre-vaccination. In Figure 4b, the development of the IgG1/IgG2 ratio in this patient group is given in relation to the changes in avidity indices. Great inter-individual differences between antibody responses were seen, with IgG1/IgG2 ratio's increasing in 7 patients and decreasing in 26 patients. In only 7 patients the IgG1/IgG2 ratio after one conjugated vaccination was > 1 and in only 2 patients the ratio changed from < 1 to > 1 post-vaccination. The mean IgG1 concentration after vaccination was 1.17 µg/mL versus 9.52 µg/mL IgG2. The ratio of IgG1/IgG2 did

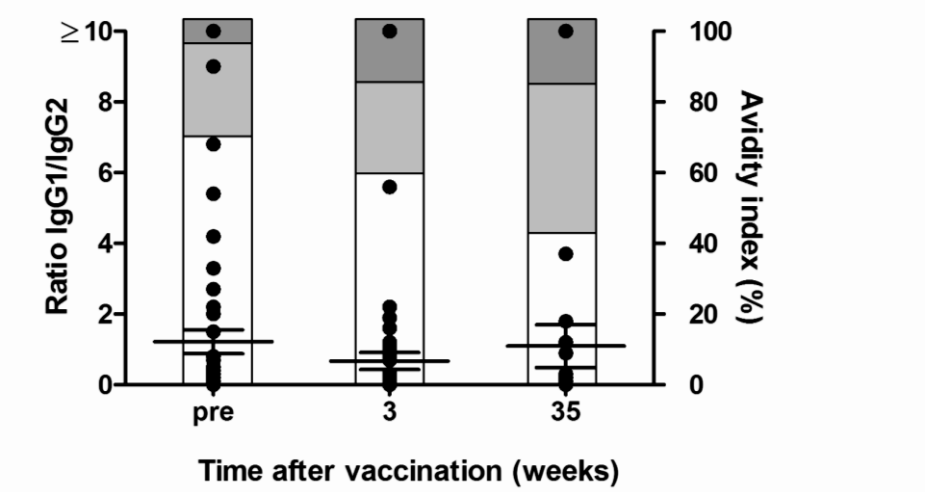
not change by vaccination (0.23 pre-vaccination, 0.25 3 weeks post-vaccination, and 0.20 35 weeks post-vaccination).
After revaccination, the mean IgG1 concentration rose from 0.93 to 3.25 µg/mL (Figure 4c). The concentration of IgG2 rose from 0.76 to 1.72. The ratio of IgG1/IgG2 was 1.43 post-vaccination, which is significantly higher ($p < 0.01$) than after primary vaccination.

Figure 4a. Concentrations of MenC IgG1 and IgG2 in relation to time after vaccination



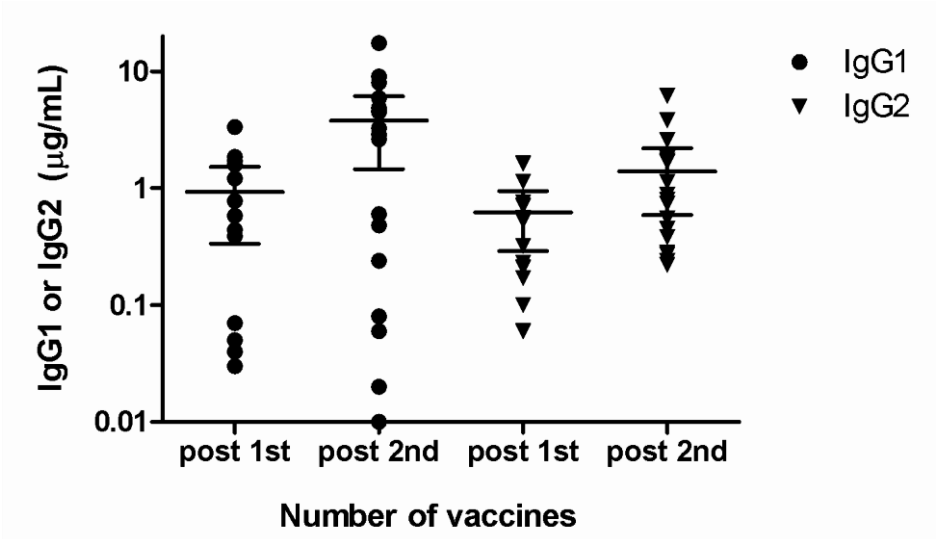
IgG with lines at mean with 95% confidence interval
★ statistically significant increase ($p < 0.05$) in antibody concentration post-vaccination compared to pre-vaccination

Figure 4b. Development of MenC IgG1/IgG2 ratio compared to changes in avidity



Dots: IgG with lines at mean with SEM
Columns: Avidity indices: dark gray 67-100%, light gray 34-66%, white 0-33%

Figure 4c. Concentrations of MenC IgG1 and IgG2 after the first and second conjugate vaccine in hypo-responders



IgG with lines at mean with 95% confidence interval

DISCUSSION

The immune response to the MenC conjugate vaccine in a group of 116 asplenic patients was found to be of poor quality and quantity. After a single dose of MenC conjugate vaccine, only 67% of the asplenic patients reached the chosen threshold antibody concentration of ≥ 2.0 µg/mL, with an increase in GMC from 0.16 µg/mL to 3.69 µg/mL post-vaccination. In healthy children and adults, including our own series, 5-10 times higher GMC's were reached after one dose of this MenC conjugate vaccine.[28-31] Previously, Balmer *et al.,* using a different MenC conjugated vaccine, found three times higher GMC's in asplenic patients after vaccination compared to our asplenic patients.[19] In that study, no difference was found between IgG GMC's in asplenic patients and an age-matched control group. However, functionality of the antibodies (based on bactericidal antibody in serum (SBA)) was significantly lower in the asplenic patient group. In our asplenic patient group, the functionality of the antibodies in terms of antibody avidity was also low. As indicated above we used a different MenC conjugated vaccine (MenC coupled to tetanus toxoid (TT)) than Balmer *et al.* (CRM_{197} conjugated). The vaccine we used has turned out to be more immunogenic in healthy subjects than other MenC conjugated vaccines.[31] Yet, in our patients lower antibody concentrations were observed than in the study of Balmer *et al*.[19]

Balmer *et al.* recommend a second dose of vaccine offered to the patients if their post-vaccination SBA titre is below the proposed protective threshold, or routinely offering two doses of MenC conjugate vaccine. In our study, after revaccination still 41% of the revaccinated patients did not achieve protective serum levels. This percentage is in accordance with the percentage of hypo-responders found by Balmer *et al*.[19] We therefore can conclude that hypo-responsiveness to MenC remained in a considerable fraction of patients and cannot be overcome by a more potent conjugate MenC vaccine.

In healthy adults, IgG GMC's fell by 50% from 33.3 to 16.7 µg/mL at six months after a single dose of MenC-TT vaccine.[30] In the asplenic patients of whom sera were available eight months after vaccination, GMC dropped only slightly from 3.69 to 3.10 µg/mL. The asplenic state of the patients may be a factor which determines the only marginal decrease in GMC in the 8 months post-vaccination. In accordance with our findings, in the study of Smets *et al.*, 6 months after pneumococcal conjugate vaccination in asplenic children, GMC's had not decreased significantly.[9] High antibody concentrations months after vaccination can be explained by ongoing production of antibodies and/or lower rate of removal from the circulation. In asplenic individuals, alternative homing of peripheral B-cells

takes place, with memory B-cells homing in peripheral organs and bone marrow in stead of in the spleen. These specific niches may be favourable for long lived plasma cells. Another argument for longer persistence of antibodies in asplenic patients is the absence of the reticulo-endothelial system (RES) function of the spleen. Immune complexes can longer remain in circulation and cause prolonged B-lymphocyte activation.
Antibody persistence following pneumococcal polysaccharide or conjugate vaccination of healthy adults (50-80 years) with an intact spleen has been studied by Goldblatt *et al*.[32] One year after one dose of pneumococcal conjugate vaccine, GMC's for 4 out of the 7 pneumococcal serotypes were at least 80% of the GMC's at the peak of the response, i.e. 1 month after vaccination. It should therefore be concluded that polysaccharide (conjugated) vaccines have the ability to induce antibody levels which persist for periods up to 1 year after vaccination, in the presence or absence of a functional spleen.

Interestingly, the antibody concentrations against serogroup A and W135 also rose statistically significant after vaccination with MenC. In a previous study of Trotter *et al.* the seroprevalence of antibodies against MenC and MenA was measured after introduction of the MenC conjugate vaccine.[33] In that study GMC's of serogroup A-specific IgG were higher than GMC's of serogroup C. This phenomenon is attributed to the presence of cross-reacting antigens like polysaccharides of *Escherichia coli* strains and the genus *bacillus*.[34-37] Moreover, due to similarities in the bacterial capsular polysaccharide structures, a low level of cross-reactivity between the four meningococcal serogroups is not unexpected.[22,23] However, in multiplex immunoassay of meningococcal serogroup A, C, W135 and Y IgG antibodies, no cross-inhibition has been found.[20,22]

Ultimately, the protection against bacterial infections depends on the quality of the produced antibodies more than on the quantity. High antibody avidity indicates high functional activity of the meningococcal antibodies.[12] In the asplenic patient group, the GMAI rose from 19% 3 weeks post-vaccination to 29% eight months after vaccination. The rise in antibody avidity is consistent with earlier studies on affinity maturation of antibodies after conjugated vaccines.[12,14,30,38] However, in our study, most patients do not develop antibodies with intermediate or high avidity; most antibodies are of low avidity.

In adults, natural exposure to meningococci and exposure to cross-reactive agents can lead to the development of memory B-cells (priming). These memory B-cells will be stimulated after polysaccharide and conjugate vaccination, leading to higher avidity indices in adults compared to children.[16,39] For this reason, measurement

of AI's in adults may be considered to be of less importance.[16,39,40] In our adult asplenic group however, most adults had low antibody avidity indices before vaccination. In this patient group therefore, an antibody AI measurement may be an adequate way of determining the development of immunological memory.

In general, IgG1 antibodies have a higher affinity than IgG2.[38] The ability to induce an IgG1 T-cell dependent (TD) response is among others dependent on age at vaccination, type of vaccine and type of adjuvant.[40-43] In the asplenic patients studied, after one meningococcal conjugate vaccination, both IgG1 and IgG2 increase, with higher concentrations of IgG2 (mean concentration 9.52 µg/mL 3 weeks after vaccination) than IgG1 (mean concentration 1.17 µg/mL). There is a large variability in the IgG1/IgG2 ratio in individual patients. This is consistent with the findings of Findlow *et al.*, who detected significant increases in IgG1 and IgG2 with large differences in IgG1/IgG2 ratio's after vaccination with one dose of quadrivalent meningococcal conjugated vaccine in healthy adults.[41] After revaccination, the amount of IgG1 in the asplenic population rises more than IgG2, suggesting that after revaccination, the T-cell dependent response is more adequate.

In contrast with the study of Balmer *et al.*, in the current study a number of patients received *Hib* and pneumococcal vaccines concomitant with the MenC vaccine, dependent on the vaccination status before start of the study.[19] The response to *Hib* vaccine was much better than to MenC vaccine; 97% of the patients reached a protective antibody concentration after vaccination (Meerveld-Eggink, manuscript submitted). Theoretically, the diminished response to MenC could be due to the simultaneous administration of different vaccines, thereby overloading the immune system. Although the MenC-specific IgG GMC post-vaccination in patients receiving concomitant vaccines was 10% lower than in patients solely immunised with the MenC vaccine, this difference was not statistically significant.
In both the current study in asplenic patients and the study of Balmer *et al.*, 15-30% of patients had received prior MenC polysaccharide vaccination.[19] These previously vaccinated patients had higher MenC GMC's pre-vaccination than patients not vaccinated with meningococcal vaccines before. In contrast to Balmer *et al.*, we found that previous MenC vaccination also led to higher GMC's post-vaccination.

CONCLUSIONS

We conclude that a single dose of MenC conjugated vaccine does not result in an adequate antibody response in 33% of asplenic patients. A second dose of MenC vaccine leads to an additional increase in antibody concentration and avidity, suggesting that asplenic patients benefit from a second dose of vaccine. This conclusion should be limited to older, asplenic patients, who did not receive MenC conjugated vaccines as part of the Dutch National Vaccination Programme. The relevance of a second dose of vaccine in countries where all children are vaccinated against MenC and circulation of MenC therefore is low, should be considered.

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Chapter 5

Response to influenza virus vaccination during chemotherapy in patients with breast cancer

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ABSTRACT

We determined the overall efficacy of influenza virus vaccination, measured as protective serum antibody titres, during chemotherapy in breast cancer patients compared to healthy adults. Patients were randomized for early (day 4 +/- 1 day) or late (day 16 +/- 1) vaccination during the chemotherapy cycle. The design of this study was a randomised trial. In the period October/November 2009 the regular influenza vaccination was given to breast cancer patients receiving FEC (5-fluorouracil, epirubicin and cyclophosphamid) -containing, tri-weekly chemotherapy regimens and a control group of healthy female adults. Patients were randomised for vaccination early in the chemotherapy cycle (day 4) or late in the chemotherapy cycle (day 16). Sera before and three weeks after vaccination were obtained and influenza virus specific antibody titres were determined by haemagglutination inhibition (HI) assay. We included 38 breast cancer patients, of which 20 patients were vaccinated at day 4 and 18 patients on day 16. Data were compared to the response to vaccination in 21 healthy controls. The overall patient group had statistically significant lower responses to the influenza virus vaccine compared to healthy controls in terms of seroconversion rates, seroprotection rates and mean increase in GMT's. Patients vaccinated early in the chemotherapy cycle tended to have higher antibody titres and seroconversion rates as compared to patients vaccinated at day 16. HI GMT's post-vaccination for day 4 versus day 16 were 63.7 versus 29.5 (H3N2), 28.2 versus 19.6 (H1N1) and 29.8 versus 16.0 (B/Brisbane), respectively. Patients on chemotherapy have significantly lower response to influenza virus vaccination compared to healthy controls. Vaccination early during the chemotherapy cycle induces better responses than does vaccination in the last week of the cycle. Follow-up studies in larger patient groups are needed to confirm this effect.

INTRODUCTION

Influenza virus infections are an important cause of acute respiratory disease with a seasonal character. Rates of infection are highest in children, but serious morbidity and mortality are also found in so-called high risk patients like the elderly and patients with cancer.[1]

The immune response to natural viral infections is impaired in patients receiving chemotherapy or immunosuppressive drugs; these patients are especially at risk for serious post-influenza complications.[2] Two immunosuppressive factors influence the response to viral infection in patients with cancer: their disease state and the treatment (i.e. chemotherapy). Chemotherapy is used to destroy rapidly growing tumour cells, but white blood cells, including lymphocytes, are also adversely affected. Therefore, cancer patients are eligible for influenza vaccination, although their response, for reasons outlined above, may be suboptimal.[3]

Yearly, 441 per 100.000 oncology patients are hospitalised in the United States because of influenza virus infections, which is 3-5 times higher than in the general population. Moreover, the mortality rate is 9% in oncology patients (relative risk of four compared to the general population).[4] As a consequence, yearly influenza vaccination is strongly recommended for patients with reduced function of their immune system because of chemotherapy or immunosuppressive drugs.[5]

Especially in patients receiving adjuvant chemotherapy in potentially curable disease, it is important to initiate treatment promptly and to complete the chemotherapy course without interruptions. Intercurrent infections, including seasonal influenza, could lead to postponement of chemotherapy. Thus, vaccination against influenza A and B virus infections is also important to prevent delay in the treatment of the underlying malignancy.

In general, it is advised to vaccinate patients before start of the chemotherapy.[6,7] However, administration of the influenza vaccination is scheduled for the months October and November on the Northern hemisphere. Because a full chemotherapy course takes 18 weeks, a large number of patients will have to be vaccinated while on chemotherapy. Limited data are available on the efficacy and optimal moment of vaccination during ongoing chemotherapy regimens and guidelines are lacking.

This study is designed to evaluate the efficacy (in terms of serological response) of influenza vaccination during chemotherapy and to assess the effect of the timing of influenza vaccination during chemotherapy in patients with breast cancer.

PATIENTS AND METHODS

We included patients who received adjuvant chemotherapy because of breast cancer. All patients were treated with FEC (5-fluorouracil 500 mg/m^2, epirubicin 100 mg/m^2 and cyclophosphamid 500 mg/m^2) 6 cycles or FEC 3 cycles followed by docetaxel (100 mg/m^2) 3 cycles.
Exclusion criteria were fever at time of vaccination (defined as a temperature of ≥ 38.5 °C), previous or known allergic reaction to any of the components of the vaccine (for example hypersensitivity to egg protein), low platelet count (< 50 * 10^9/L) at moment of vaccination or treatment with prednisone at the moment of vaccination.
This study was approved by the central committee on research involving human subjects of the Netherlands. All patients signed informed consent. The study was registered at Clinical Trials.Gov with ID number NCT01000246.

This multicenter randomised trial was executed in seven hospitals in the Netherlands. Eligible patients were randomised for vaccination early (day 4) or late (day 16) during a chemotherapy cycle. A randomisation schedule was used in which patients were randomised 50% / 50%, with randomisation in blocks of 10 patients. Day of vaccination was allocated to patients in order of notification of the principal investigator. The results of vaccination in the patient group were compared to those of vaccinated female employees of one of the participating hospitals. These employees participated voluntarily in the influenza vaccination campaign, which is a standard yearly program for health care personnel.

Vaccination

In the period October/November 2009, patients received one of two available seasonal influenza virus vaccines (one dose of 0.5 mL), dependent of which vaccine was available in the participating hospitals. These two vaccines (Influvac® 2009/2010, Solvay Biologicals B.V, the Netherlands and Vaxigrip® 2009/2010, Sanofi Pasteur MSD, Belgium) each contain 15 microgram haemagglutinin of the following influenza strains: A/Brisbane/10/2007 (H3N2)-like strain, A/Brisbane/59/2007 (H1N1)-like strain, B/Brisbane/60/2008-like strain.

After vaccination, patients were asked to keep a diary for three days to determine any adverse advents after vaccination. In particular, patients were asked about pain, stiffness, fever and haematoma post-vaccination. Furthermore, the patients were asked to note the intensity (minor, moderate or severe) and duration of this side-effect.

Laboratory investigations

Serum samples were collected before and three weeks after vaccination and stored at -70°C until use. Before vaccination, blood cell counts including leukocyte differentiation were performed. Antibodies to the haemagglutinin of all 3 vaccine strains were measured by the haemagglutination inhibition (HI) test, according to standard procedures using four haemagglutinating units of virus and turkey erythrocytes.[8,9] Two-fold serial dilutions of patients sera were tested with a starting dilution of 1:20. Sera were pre-treated with receptor-destroying enzyme (cholera filtrate) to remove non-specific inhibitors and then tested for HI antibodies specific for the viruses A/Brisbane/10/2007 (H3N2), A/Brisbane/59/2007 (H1N1) and B/Brisbane/60/2008. The antibody titre is expressed as the reciprocal value of the highest dilution that still inhibits agglutination.

Data are expressed as geometric mean HI titre (HI GMT), seroprotection rate (SPR; i.e. the percentage of vaccine recipients with a serum HI titre ≥ 40 after vaccination) and seroconversion rate (SCR; i.e. the percentage of vaccine recipients with a fourfold increase or more in post-vaccination titre).[10,11] According to the criteria of the EMEA (The European Agency for the Evaluation of Medicinal Products), in healthy adults ≤ 60 years of age, an adequate response to vaccination includes one of the following three requirements of the serological assessments: seroprotection rate > 70%, seroconversion rate ≥ 40% and mean increase in GMT > 2.5.[12]
In persons above 60 years, the criteria for an adequate response are SPR > 60%, SCR > 30% and GMT > 2.0, respectively.

Statistical analysis

For statistical purposes, HI titres < 10 were assigned an arbitrary value of five. Comparisons of GMT before and after vaccination (paired samples) were performed using the Wilcoxon signed rank test. For independent samples, the Mann-Whitney U-test was used. Pearson's χ^2 or Fisher's exact test were used to compare groups. A p-value of < 0.05 was considered statistically significant.

RESULTS

A total of 44 patients with breast cancer fulfilled the inclusion criteria. Five of the 44 patients were excluded at time of vaccination due to intercurrent infectious diseases at the moment of vaccination (n=4) or preference for the (at that moment released) vaccine against the (H1N1)2009 ('Mexican flu') influenza virus strain (n=1). One patient received the H1N1 vaccination, just before the second serum sample was taken and was excluded from analysis due to interference of this second vaccine with determination of antibody responses to the seasonal H1N1 vaccine. Of the resulting 38 breast cancer patients, 20 patients had been randomised for vaccination on day 4 of the chemotherapy cycle and 18 for vaccination on day 16 of the chemotherapy cycle.
The response to vaccination was compared to the serologic response in 24 healthy female employees of one of the participating hospitals. In three persons of the control group, the serum agglutinated the erythrocytes non-specifically which prevented measurement of HI activity. Therefore, these patients were excluded. The mean age of the 21 controls used in this study was 35.6 years, range: 24-62 years. Two healthy controls (10%) were > 60 years. Nine (43%) controls had been vaccinated against the influenza virus previously, with a mean of 2 previous vaccinations.

In Table 1, baseline characteristics of the breast cancer patients are given in both groups. Co-morbidity consisted of diabetes (n=1), hypertension (n=5), hypercholesterolemia (n=2), rheumatoid arthritis (n=1), hypothyroidism (n=1), glaucoma (n=1), arrhythmia (n=1) and COPD (n=1). Three patients had multiple co-morbidities. The two groups did not differ in age, type of chemotherapy (i.e. FEC or FEC-docetaxel), previous influenza vaccination or co-morbidity (Table 1). Haemoglobin, leukocytes and neutrophil numbers were significantly lower in the late vaccination group (day 16 of chemotherapy cycle; Table 1).

Table 1. Baseline characteristics of breast cancer patients

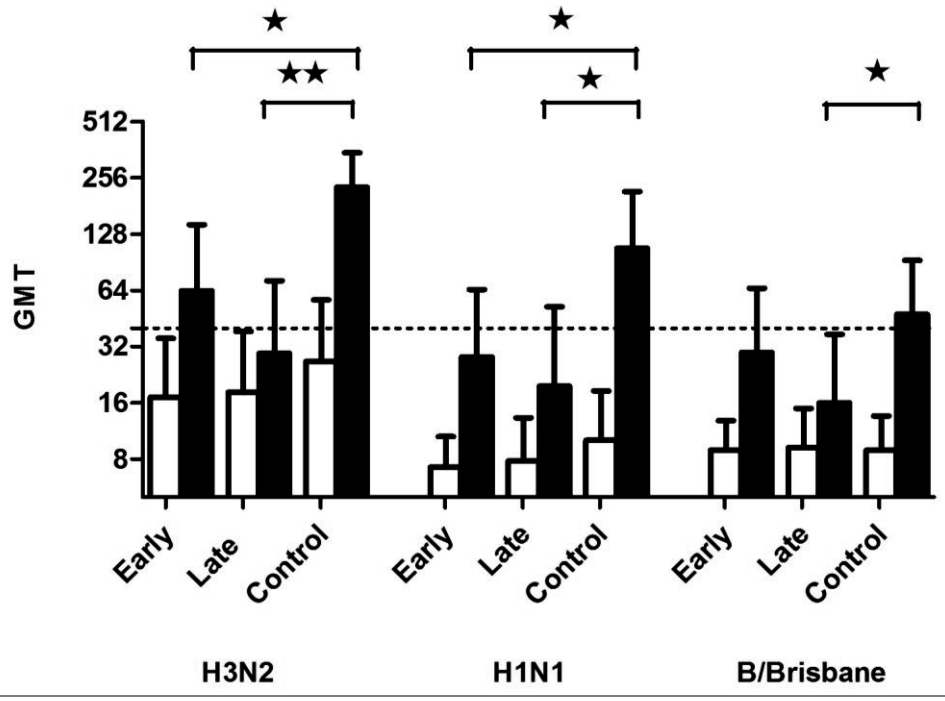
Characteristics		**Day 4**	**Day 16**	***p-value***
Patients (n)		20	18	
Mean age[1]		54.5 (38-69)	54.2 (43-66)	*n.s.*
Patients > 60 years[2]		5 (25)	4 (22)	*n.s.*
Chemotherapy[2]	FEC	10	9	*n.s.*
	FEC/Doc	10	9	*n.s.*
Previous vaccination[2]		6 (30)	8 (44)	*n.s.*
Co-morbidity[2]		5 (25)	4 (22)	*n.s.*
Haemoglobin[3]		7.6 (6.4-8.9)	7.2 (6.2-8.1)	*0.04*
Leukocytes[4]		6.2 (2.4-9.7)	3.4 (1.7-9.4)	*<0.01*
	Neutrophils	4.9 (0.7-8.4)	1.7 (0.2-7.3)	*<0.01*
	Lymphocytes	1.3 (0.2-4.4)	1.0 (0.4-1.52)	*n.s.*
Thrombocytes[4]		309 (167-532)	272 (127-419)	*n.s.*

FEC: 5-fluorouracil, epirubicin and cyclophosphamid
n.s. = non-significant
[1] data given in mean years with range in parentheses
[2] data given in numbers with percentages in parentheses
[3] data given in mean mmol/L with range in parentheses
[4] data given in numbers $*10^9$/L with range in parentheses

Haemagglutination inhibition titres were measured pre- and post-vaccination. For each influenza strain in the vaccine, antibody titres were determined separately (Figure 1).
In the early patient group and control group, GMT post-vaccination increased significantly for all three virus strains. In the late patient group, a statistically significant increase in GMT post-vaccination was only seen for the H1N1 virus strain.

No statistically significant difference was seen between the pre-vaccination titres in the early and late patient group versus the control group. Numbers of patients with HI titres ≥ 40 pre-vaccination are given in Table 2. No differences in GMT's post-vaccination were seen between the two different vaccines that were used (data not shown).

Figure 1. HI antibody titres in patients and controls



GMT = geometric mean titre
Open bars show pre-vaccination GMT, closed bars post vaccination GMT
In all cases, vaccination induced a significant increase in GMT, except for the late patients group for H3N2 and influenza B. Statistical significance of differences between the patient groups and the controls are indicated as ★ $p < 0.05$, ★★$p < 0.01$

Table 2. Seroprotection rates pre-vaccination

		Patients all	Early	Late	Controls
HI antibodies ≥ 40 (n (%))					
	H3N2	13 (34)	7 (35)	6 (33)	9 (43)
	H1N1	2 (5)	1 (5)	1 (6)	5 (24)
	B/Brisbane	4 (11)	1 (5)	3 (17)	2 (10)

HI = haemagglutination inhibition

Previous influenza vaccination had a statistically significant influence on the pre-vaccination HI titre of H1N1 in the early patient group (GMT 14.4 in the previous vaccinated group versus 5.4 in the vaccine-naïve group, p=0.01). In the control group, previous vaccination had a statistically significant influence on the antibody levels pre-vaccination to both H3N2 and H1N1 strains (GMT 65.5 versus 13.6, p = 0.03 and GMT 20.3 versus 5.9, p = 0.05, respectively).

As can be seen in Table 3, for all three virus strains the control group meets all three criteria of the EMEA for defining an adequate response. In the early patient group, SPR criteria are not met, and SCR > 40% is only met for H3N2. In the late patient group, SPR and SCR criteria are not met for any virus strain. A GMT increase > 2.5 is found for all three virus strains in the early patient group, but not in the late patient group. Although not significant, the post-vaccination titres in the early patient group were overall higher for each virus strain compared to the late patient group.

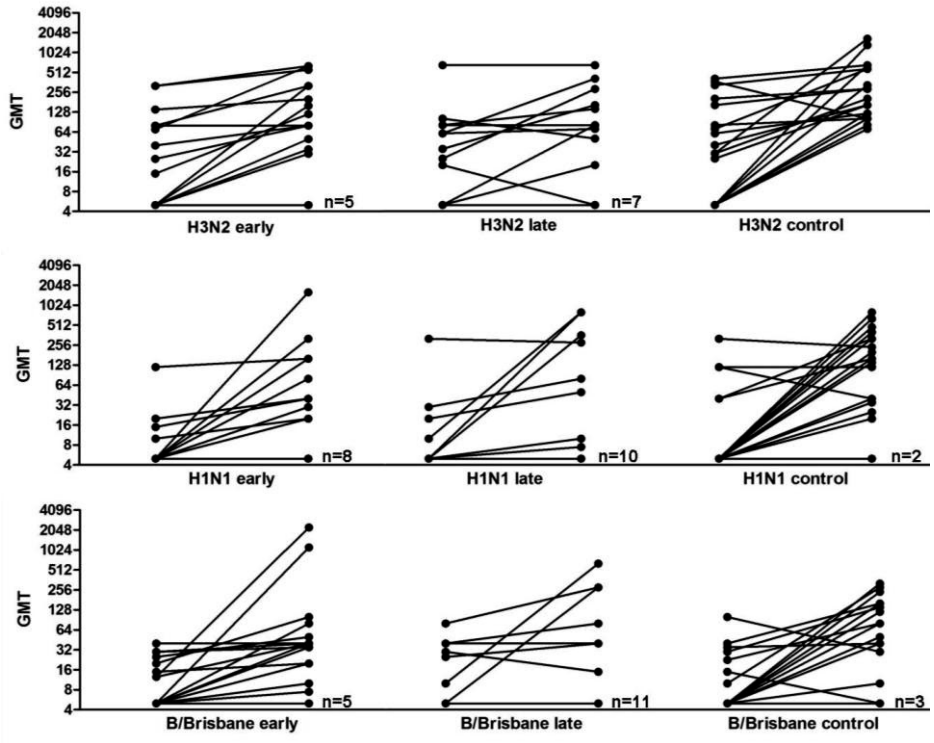
Table 3. Percentages of patients and healthy controls reaching the protective threshold of ≥ 40 HI antibody titres or reaching seroconversion

		Patients all	**Early**	**Late**	**Controls**
HI antibodies ≥ 40[1]					
	H3N2	22 (58)	13 (65)	9 (50)	21 (100)
	H1N1	15 (40)	9 (45)	6 (33)	16 (76)
	B/Brisbane	15 (40)	9 (45)	6 (33)	14 (67)
Seroconversion[1]					
	H3N2	14 (37)	9 (45)	5 (28)	14 (67)
	H1N1	10 (26)	7 (35)	3 (17)	16 (76)
	B/Brisbane	9 (24)	7 (35)	2 (11)	12 (57)
Mean increase in GMT	H3N2	2.5	3.7	1.6	8.6
	H1N1	3.2	3.9	2.5	10.7
	B/Brisbane	2.4	3.3	1.7	5.3

HI = haemagglutination inhibition, GMT = geometric mean titre
[1] data given in numbers with percentages in parentheses

In Figure 2, the individual responses to vaccination for all three virus strains are shown. According to the EMEA criteria, in patients > 60 years, lower thresholds are used to define an adequate antibody response. In our total patient group (n=38), patients > 60 years had higher pre-vaccination HI titres for the H3N2 virus strain than patients ≤ 60 years (GMT 43.6 versus 13.3, p = 0.04). The HI titre for the B/Brisbane strain, however, was lower in patients > 60 years (GMT 5.6 versus 10.5, p=0.02).
In the overall patient group, all patients > 60 years had received prior vaccination compared to 17% of the patients ≤ 60 years of age (p < 0.01).

Figure 2. Individual responses to vaccination with three different virus strains



GMT = geometric mean titre
Numbers of patients with no response post-vaccination are indicated by 'n='

Patients were asked to keep records of any side-effects during the first three days post-vaccination. One patient, vaccinated at day 16, did not return the diary, so side-effects could not be determined. Side-effects of vaccination in both breast cancer groups are given in Table 4. No serious adverse events of vaccination were reported. One patient, vaccinated at day 4, documented severe pain and stiffness in the arm after vaccination, but this resolved without further problems. No statistically significant differences in the occurrence of side-effects was seen between the two groups or between the two different influenza vaccines that were used.

Table 3. Side-effects of vaccination during chemotherapy*

	Day 4	Day 16
Side-effect (n (%))		
None	14 (70)	13 (72)
Pain	1 (5)	1 (6)
Fever	2 (10)	0
Stiffness	4 (20)	4 (22)
Haematoma	0	0

*All but one side-effect were reported 'minor'

DISCUSSION

We studied the response to the seasonal influenza virus vaccination in patients receiving FEC-containing chemotherapy for breast cancer. Compared to healthy controls, the response to vaccination was significantly lower in the cancer patients. When comparing serological response in both patient groups (early versus late), a trend was seen towards relative better responses in the early vaccination group.

In healthy adults, inactivated influenza vaccine has a 70–90% efficacy in preventing influenza in case of a sufficient antigenic match between the vaccine and the epidemic virus.[1,13] To study the effect of vaccination, beside the clinical evaluation of prevention of infection, the serological response to vaccination can be used as a surrogate marker for efficacy and protection. Serum HI antibody titres of ≥ 40 are associated with at least a 50% reduction in risk for influenza infection or disease in populations and this threshold is often used to define seroprotection rates.[14-16] Beside seroprotection rate, the EMEA has defined two additional criteria to define an adequate response: the seroconversion rate and the mean increase in GMT. In our healthy adult group, immunogenicity requirements as stated by the EMEA were met for all three virus strains and for all indicators.
In the overall breast cancer patient group, the requirements of EMEA were not met in terms of seroconversion rates and seroprotection rates. A mean increase in GMT of > 2.5 was met in two of the three tested virus strains. Patients vaccinated early in the chemotherapy cycle (day 4) showed a trend for higher GMT's and higher percentages of SPR and SCR than the late patient group. Breast cancer patients had significantly lower responses in SPR, SCR and GMT's compared to our healthy control group. This is consistent with other studies on the effect of chemotherapy in cancer patients on the response to influenza vaccines.[17,18]

In breast cancer patients, treatment consists, among others, of chemotherapy with FEC-containing regimens.[6] These chemotherapeutics are given in cycli of three

weeks and consist of myelosuppressive agents, interfering with the response to viral and bacterial infection.[19,20] Vilar-Compte *et al.* showed that during chemotherapy, patients with breast cancer indeed had a lower response to vaccination (24-48 hours prior to cytotoxic treatment) than patients who did not receive chemotherapy.[21] Brydak *et al.* found that the immune response to influenza vaccination in breast cancer patients with or without chemotherapy was as effective as vaccination of healthy adults. The patient group however was small (n=9) and heterogeneous.[2]

In general, it is advised to vaccinate patients before start of the chemotherapy. Due to the seasonal character of the influenza virus and the restricted period of time in which vaccines are available, this is not always feasible. Limited data are available on the effect of vaccination during cycles of current chemotherapy regimens. To determine whether the antibody response is influenced by the moment of vaccination during the chemotherapy, we chose two different time-points for vaccination during a chemotherapy cycle: day 4 versus day 16 of a three weekly cycle.

The myelosuppression that is induced by the chemotherapy is transient. The duration of myelosuppression varies between agents due to variable drug distribution of the chemotherapeutics and different pharmacokinetic patterns in patients. In general, acute myelosuppression starts 7-14 days after chemotherapy, continues from 10 to 14 days and will recover in 3-4 weeks post-chemotherapy administration.[6] In our study, we therefore chose to vaccinate patients before (day 4) or after (day 16) the nadir, in order to reduce the change of bleeding complications in the patients. No bleeding complications occurred during this study.

Ortbals *et al.* vaccinated oncology patients (both haematological and solid tumours) at the day of chemotherapy or at the time of their white blood cell count nadir.[22] Only 50% of the patients mounted an adequate antibody response at the day of chemotherapy in terms of seroconversion, whereas 93% of the patients vaccinated in their nadir showed seroconversion. No difference in response was seen between patients with haematological or solid tumours. It was concluded that vaccines should not be given at the day of chemotherapy. The study of Ortbals *et al.* was conducted in 1977. Since that time, considerable changes have been made in vaccine formulations and chemotherapy regimens, giving a possible explanation for the difference in response compared to our patient group.

In the current study a trend was seen towards better responses to vaccination in patients vaccinated early (day 4) in the chemotherapy cycle compared to

vaccination in the last week of the chemotherapy cycle (day 16). This may be explained by the differences in the relative sensitivity for chemotherapy of distinct phases of the humoral immune response. As can be seen in Table 1, lymphocyte numbers did not differ significantly between day 4 and day 16. The chemotherapy therefore may not influence the total lymphocyte count, although it does affect the proliferative phase of the immune response. In the late group, the next chemotherapy dose is given approximately 5 days after vaccination. This chemotherapy is toxic for proliferating lymphocytes and therefore greatly impairs the antibody response. In the early patient group, 17 days will pass before the next dose of chemotherapy is given, enabling the proliferative phase of the humoral response to complete. In patients who were vaccinated during the sixth chemotherapy cycle, no further cycles followed. In the patients vaccinated at day 16 in this last cycle (n=4), antibody responses indeed were higher compared to patients vaccinated at day 16 of other cycles (n=14). For H1N1, this difference was statistically significant, with a GMT HI post-vaccination in cycle 6 of 101.9 versus 12.3 in other cycles (p=0.035). This suggests that the subsequent chemotherapy dose is responsible for suppression of the antibody response in patients vaccinated at day 16 during chemotherapy.

Overall, patients were better motivated for late vaccination due to the physical inconveniences of the chemotherapy in the first week. Despite these physical complaints, the number and severity of the side-effects of vaccination in our breast cancer patient group were mild, with ≥ 70% of patients in both groups who did not notice any side-effects. This is consistent with other studies, reporting mild adverse effects to vaccination in oncology patients.[6,7] In general, side-effects of influenza vaccination are pain at the injection site and, less commonly, fever, malaise, myalgia, arthralgia, or both, starting 6–12 hours after vaccination and lasting less than 48 hours.

Despite international recommendations, vaccine coverage in high-risk patients (with an indication for influenza vaccination, like diabetes, chronic bronchitis and cancer) remains low. In the United States, vaccine coverage is about 25% for these high-risk patients aged 18–64 years, increasing with the patient's age (18% in patients aged 18-49 years, 32% in patients of 50-64 years old.[23,24] In the Netherlands, the vaccine coverage of high-risk patients is approximately 70% in patients under the age of 65 compared to 80% in patients above the age of 65.[25] Reasons for physicians not to offer vaccination to all their patients were lack of awareness of the recommendations and concern about the efficacy of the influenza vaccination in patients with solid tumours treated with chemotherapy.[23] In the current study we show that although the response to influenza vaccination was

lower in breast cancer patients than in healthy controls, influenza vaccination does lead to adequate HI titres in 45-65% of the early patients and in 33-50% of the patients vaccinated late in the chemotherapy cycle.

During the design of our study, an epidemic rise in infections with the so-called 'Mexican flu' (H1N1) worldwide appeared. Only two months after the start of our study with the 'regular' seasonal influenza vaccine, vaccines against the influenza strain H1N1 became available and were introduced for all participating patient groups in this study. Therefore, the inclusion of patients in this study had to be stopped, because priority was given to the H1N1 vaccination and seasonal influenza vaccination was postponed. For this reason, only 38 patients could be included in this study. Although we were only able to include a small number of patients, a trend towards better responses in patients vaccinated early in the chemotherapy cycle is seen.

Recently, seasonal influenza virus vaccines with adjuvants became available. The use of an adjuvant enhances immune stimulation, with higher GMT's post-vaccination, maintaining after subsequent immunisations.[26] In this high risk group, in which vaccination before chemotherapy is not always feasible, we suggest a follow-up study to determine the best moment of vaccination during chemotherapy. Moreover, the response to adjuvanted vaccines compared to non-adjuvanted vaccines should be studied to determine whether adjuvanted vaccines in this patient group could improve response to vaccination. For these two purposes, larger groups of patients have to be included.

CONCLUSIONS

Influenza virus vaccination induces significantly lower antibody responses in patients with breast cancer on FEC-containing chemotherapy regimens compared to healthy adults, but a substantial proportion of these patients develops protective antibody responses. When comparing early (day 4) versus late (day 16) vaccination during a chemotherapy cycle, a trend was seen towards higher responses in the early vaccination group. Follow-up studies with larger patient numbers have to be conducted to confirm this effect and to test the efficacy of adjuvanted vaccines.

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Chapter 6

Antibody response to polysaccharide conjugate vaccines after nonmyeloablative allogeneic stem cell transplantation

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ABSTRACT

After allogeneic stem cell transplantation with reduced-intensity conditioning regimens (allo-RIST), patients are susceptible to bacterial and viral infections for a period that may last several years. The efficacy of the recommended vaccination schedules, in terms of induction of a protective antibody response, is unknown. In this study, the reconstitution of humoral immunity after allo-RIST is determined by measuring the vaccination-induced antibody response against *Streptococcus pneumoniae*, *Haemophilus influenzae* type b (*Hib*), and tetanus toxoid (TT) 1 year post-transplantation. Patients who underwent allo-RIST were vaccinated according to a schedule starting at 12 months following transplantation with conjugated vaccines against *S. pneumoniae*, *Hib*, and TT. Of twenty-six patients both pre- and post-vaccination sera were available. Patients were required to be off immunosuppression at the time of vaccination, and, therefore, all but 9 of the 26 patients did not start vaccination at 12 months post-stem cell transplantation but rather at a median range of 15 months (12-36 months) post-transplantation. Except for pneumococcal serotype 6B, more than 73% of the patients developed antibody levels ≥ 0.35 µg/mL for all pneumococcal serotypes included in the vaccine. For *Hib* and TT, protective antibody levels were found in 77% and 96% of the patients, respectively. Vaccination of patients at a median of 15 months post-allo-RIST leads to significant rise in concentrations of pneumococcal, *Hib*, and TT antibodies in the majority of patients.

INTRODUCTION

Non-myeloablative (NMA) allogeneic stem cell transplantation (or allogeneic stem cell transplantation with reduced-intensity conditioning regimens (allo-RIST)), has become the therapy of choice for a number of haematologic malignancies. After allo-RIST, immunosuppressive therapy is required to prevent severe graft versus host disease (GVHD). Because of this immunosuppressive therapy, the pre-transplant chemotherapy, and the underlying haematologic disease, patients are susceptible to bacterial and viral infections for a period that may last up to several years.[1] Defects in B cell function can last for 12 to 24 months, causing a significant impairment of the humoral immune system of the host and thereby increasing the susceptibility to infections after transplantation.[2] The humoral immunodeficiency involves defects in the response to T-cell independent antigens, including the capsular polysaccharides of *Streptococcus pneumoniae* and *Haemophilus influenzae* type b (*Hib*), as well as T-cell dependent protein antigens.[2,3] Therefore, immunisation against these pathogens is recommended by the Centers for Disease Control (CDC) and the Infectious Diseases Working Party of the European Group for Blood and Marrow Transplantation (EBMT).[1,4] Despite the recommendations that have been implemented widely, limited information is available on (the kinetics of) the functional reconstitution of the cellular and humoral immune system following allo-RIST. Although there is consensus on the need for a vaccination program, the efficacy of the recommended vaccination schedules, in terms of induction of a protective antibody response, is unknown in the NMA setting.[1] In this study, the reconstitution of humoral immunity after allo-RIST is determined by measuring the antibody response to vaccination against *S. pneumoniae*, *Hib*, and tetanus toxoid (TT) 1 year post-transplantation.

PATIENTS AND METHODS

From May 2005 until January 2008, patients who entered the standard vaccination protocol (see the following section) of the Vrije Universiteit (VU) University Medical Centre after allo-RIST were included. All patients gave informed consent. In case of recurrence of malignant disease that required chemotherapy, patients were not vaccinated.

All patients received a NMA stem cell transplantation (SCT); the conditioning regimen consisted of Fludarabine (Flu; 25 mg/m^2 i.v. for 5 days) and cyclophosphamide (Cy; 500 mg/m^2 i.v. for 5 days), Flu (30 mg/m^2 for 3 days) and total body irradiation (TBI; 2 Gy once) or TBI alone (2 Gy once). GVHD prophylaxis consisted of mycophenolate mofetil (MMF) and cyclosporine (CsA) until 3 months post-transplantation. Thereafter, MMF was stopped in 2 weeks. The CsA was

tapered approximately 25% a week if GVHD had not developed. In case of GVHD, an alternative scheme of an 8% decrease a week was used.
The standard vaccination procedure after allogeneic SCT starts 1 year after transplantation and consists of 5 vaccination moments with vaccines against *Haemophilus influenzae* type b (Act-HIB®, Sanofi Pasteur MSD, Brussels, Belgium), *Streptococcus pneumoniae* (Prevnar®, Wyeth Lederle Vaccines S.A., Louvain- la-Neuve, Belgium and Pneumovax-23®, Sanofi Pasteur MSD, Brussels, Belgium), diphtheria, tetanus and poliomyelitis (DTP, Nederlands Vaccin Instituut (NVI), Bilthoven, The Netherlands), and *Neisseria meningitidis* serogroup C (Meningitec®, Wyeth Pharmaceuticals B.V., Hoofddorp, the Netherlands).[1,4] The schedule for this vaccination procedure is given in Table 1. Blood samples were drawn 1 year after SCT, that is, prior to the first vaccination, and 3 weeks after the second vaccination with DTP.

Table 1. Vaccination protocol after allogeneic stem cell transplantation

Time since transplantation	**1 year**	**+ 2 wk**	**+ 8 wk**	**+ 12 wk**	**+ 26 wk**
Vaccines	DTP		DTP	DTP	
	Hib			*Hib*	*Hib*
		PCV-7	PCV-7		PPV-23
			MenC		

DTP = diphtheria, tetanus and poliomyelitis vaccine; DTP, Netherlands Vaccine Institute (NVI), Bilthoven, The Netherlands.
Hib = *Haemophilus influenzae* type b vaccine; Act-HIB®, Sanofi Pasteur MSD, Brussels, Belgium
PCV-7 = pneumococcal seven-valent conjugate vaccine; Prevnar®, Wyeth Lederle Vaccines S.A., Louvain-la-Neuve, Belgium
MenC = *Neisseria meningitidis* serogroup C vaccine; Meningitec®, Wyeth Pharmaceuticals B.V., Hoofddorp, the Netherlands
PPV-23 = pneumococcal 23-valent Polysaccharide Vaccine; Pneumovax-23®, Sanofi Pasteur MSD, Brussels, Belgium

Laboratory investigations

Serum samples were collected and stored at -20°C until use. Regularly scheduled laboratory investigations included complete blood count with leukocyte differentiation and chimerism determination. Chimerism was determined on days +28, +56, +84, +182, and +364 after SCT and thereafter every year for 5 years by means of short tandem repeat (STR) assay. Full chimerism is defined as > 95% of donor origin. T-cell or B-cell chimerism were not examined separately.

Antibody concentrations against *Hib* and TT were determined by ELISA as described previously.[5-7] Briefly, microtitre plates coated with *Hib* or TT were incubated with serial dilutions of patient sera. Antibody levels were determined with goat anti-human IgG conjugated to alkaline phosphatase, and developed with *p*-nitrophenylphosphate. Data are reported in terms of proportions of patients who

showed response (defined as a ≥ 4-fold increase in antibody concentrations) and who attained protective antibody concentrations. For *Hib*, an antibody concentration of ≥ 1.0 µmg/mL is considered to correlate with long-term protection.[8,9] For tetanus, antibody concentrations of 0.1 IU/mL are correlated with long-term protection.[10-12] Antibody levels to 8 pneumococcal serotypes (3, 4, 6B, 9V, 14, 18C, 19F, and 23F) and cell wall polysaccharide (CWPS) were determined simultaneously with fluorescent bead-based assay (Luminex®). Patient sera were pre-absorbed with pneumococcal serotype 22F to remove CWPS2.[13,14] Patient sera diluted 1:100 or 1:1000 were incubated with the mixture of polysaccharide-coated beads. Goat anti-human IgG conjugated to phycoerythrin was added and samples were measured on a Bio-plex 100 system (Bio-Rad, Hercules, CA). The pneumococcal reference serum 89SF was kindly provided by Dr. C.E. Frash, Division of Bacterial Products, Center for Biologics Evaluation and Research, Rockville, MD.

The chosen anti-pneumococcal antibody threshold for protection against invasive disease was ≥ 0.35 µg IgG/mL, in accordance with recommendations by the World Health Organisation (WHO).[15] However, for immunocompromised patients, and for invasive pneumococcal infections, this threshold is often not considered to be protective. Therefore, we also calculated response rates using a higher threshold of ≥ 1.0 µg IgG/mL. All antibody concentrations were log-transformed and geometric mean concentrations (GMC) were calculated.

Statistical analysis

Comparisons of GMC before and after vaccination (paired samples) were performed using the Wilcoxon signed rank test. A p-value of < 0.05 was considered statistically significant. For independent samples, the Mann-Whitney U-test was used.

RESULTS

From the total cohort of 41 patients who underwent an allo-RIST and were vaccinated, pre- and post-vaccination sera were available from 26 patients. The patient group consisted of 15 men (58%) and 11 women. Mean age at vaccination was 56.5 years. Baseline characteristics of these patients, the donors, and the transplantation are given in Table 2. Of the total 15 patients that could not be included in this analysis, baseline characteristics could be traced for 9 patients. Of 6 patients no further details are known.

Table 2. Baseline characteristics of patients included in this study and patients not included in the study (although receiving an allo-RIST SCT)

		Included	**Not included**
Patients: n		26	9
Male[1]		15 (58)	3 (33)
Age at vaccination (years)[2]		56.5 (44-67)	59.1 (42-67)
Underlying disease[1]	MM	11 (42)	3 (33)
	NHL	5 (19)	2 (22)
	CLL	5 (19)	0
	AML	3 (12)	3 (33)
	MCL	1 (4)	0
	CML	1 (4)	0
	MW	0	1 (11)
Time SCT to vaccination (months)[2]		15 (12-36)	14.9 (12-21)
Vaccination > 24 months post SCT[1]		5 (19)	0
Immunosuppressive free period (months)[2]		3.5 (0-9)	4.7 (0-10)
Leukocytes ($*10^9$/L)[1]		5.5 (3.2-11.9)	6,1 (2.3-12.4)
Donor chimerism > 95%[1]		24 (92)	9 (100)
Conditioning regimen[1]	Flu/Cy	15 (58)	3 (33)
	TBI	7 (27)	3 (33)
	Flu/TBI	4 (15)	3 (33)
Male donor[1]		11 (42)	5 (56)
Age donor (years)[2]		56 (33-76)	57.9 (47-67)
AB0 compatible[1]		23 (89)	7 (78)
CMV status[1]	Patients CMV+	13 (50)	4 (44)
	Donors CMV+	14 (54)	6 (67)
	Compatibel	18 (69)	5 (56)
GvHD prophylaxis[1]	CSA	26 (100)	9 (100)
	MMF	21 (81)	9 (100)
Infection prophylaxis[1]	co-trimoxazol	26 (100)	9 (100)
	valaciclovir	26 (100)	9 (100)
	penicillin/cipro	26 (100)	9 (100)
Transplant[2]	CD 34+ cells ($*10^6$/kg)	5.7 (2.7-12.2)	5.0 (4.1-6.3)
	T lymphocytes ($*10^7$/kg)	30.1 (0.4-62.1)	35.1 (0.3-72.7)

MM= multiple myeloma, NHL= non-Hodgkin lymphoma, CLL= chronic lymphocytic leukaemia, AML= acute myeloid leukaemia, MCl = mantle cell lymphoma, CML= chronic myeloid leukaemia, MW = morbus Waldenström, SCT = stem cell transplantation, Flu= fludarabine, Cy= cyclophosphamide, TBI= total body irradiation, CMV= cytomegalovirus, compatibel= patient and donor both CMV positive or CMV negative, CSA= cyclosporin A, MMF= mycophenolate mofetil, Cipro = ciprofloxacin

[1] data are given in numbers with percentages in parentheses

[2] data are given in medians with range in parentheses

The vaccination schedule was designed to start at 1 year post-transplantation, provided the immunosuppressive therapy had ended at that time. As can be seen from Table 2, the mean time between the SCT and start of the vaccination in practice was 15 months. The reason for postponing the vaccinations in individual patients was indeed the ongoing use of immunosuppressive therapy because of active GVHD. Nine patients (35%) did actually start with the vaccination schedule at 12 months post-transplantation. All patients received NMA SCT for treatment of a haematological malignancy. Complete replacement of the host haematopoietic system with donor cells (full donor chimerism) had occurred in 92% of all patients at start of vaccination.

During the follow-up of the study, several infectious complications occurred in the patient group. Furthermore, in 20 patients (77%) GVHD was diagnosed. In this cohort, no acute GVHD (aGVHD) was diagnosed. All cases of GVHD started > 100 days post-transplantation (late onset). According to the Seattle classification of chronic GVHD (cGVHD), the number of patients with limited and extended GVHD was determined. A summary of the complications, causative organisms, and (additional) treatments for GVHD and relapse of the haematological malignancy is given in Table 3. None of the patients received intravenous immunoglobulins post-transplantation.

One year after allo-RIST, patients started with a vaccination schedule that involved DTP and protein conjugated polysaccharide vaccines for *S. pneumoniae*, *Hib*, and *N. meningitidis* serotype C (see also Table 1). The conjugated pneumococcal vaccine Prevnar® after 2 doses yielded a significant antibody response in the majority of patients (Table 4).

Table 3. Complications during follow-up

Infection[1]	HZV		3 (12)
	Pneumonia	*Aspergillus spp.*	2 (8)
		Legionella spp.	1 (4)
		S. aureus	1 (4)
		H. influenzae	1 (4)
		S. viridans	1 (4)
	Upper airway infection e.c.i.		17 (65)
	Sepsis	*P. aeruginosa*	1 (4)
	Other		19 (73)
CMV reactivation[1]			3 (12)
GvHD[1]	Total		20 (77)
	Limited		4 (15)
	Extensive		16 (62)
Additional treatment GvHD[1]	Prednisolon		4 (15)
	Thalidomide		1 (4)
Relapse[1]	Multiple myeloma		4 (15)
	Non-Hodgkin lymphoma		1 (4)
Treatment relapse[1]	DLI		3 (12)
	Thalidomide		4 (15)
	Rituximab		2 (8)
	Lenalidomide		2 (8)
	Other		3 (12)

HZV= herpes zoster virus, CMV= cytomegalovirus, DLI= donor lymphocyte infusion. The use of thalidomide as additional treatment for GvHD or as treatment for relapse of malignancy did not occur in the same patient

[1] data are given in numbers with percentages in parentheses

Table 4. IgG antibody seroconversion response after conjugate pneumococcal, *Hib* and TT vaccination[1]

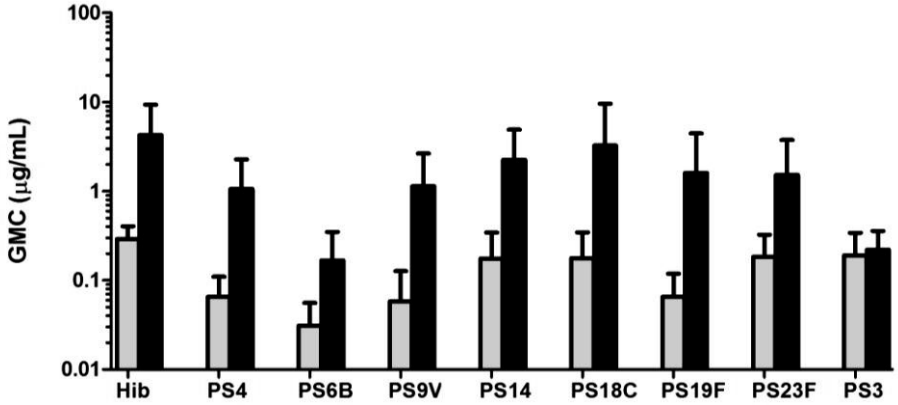
Hib	**TT**	**P 4**	**P 6B**	**P 9V**	**P 14**	**P 18C**	**P 19F**	**P 23F**	**P 3**
20 (78)	22 (85)	22 (85)	12 (46)	19 (73)	18 (69)	18 (69)	18 (69)	16 (62)	3 (12)

Hib = *Haemophilus influenzae* type b, TT = Tetanus toxoid, P = pneumococcal polysaccharide serotype
Seroconversion: ≥ 4-fold increase in antibody concentration after vaccination

[1] data are given in numbers of patients with percentages in parentheses

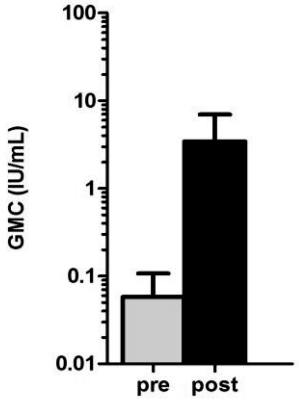
Post-vaccination GMC rose above the level of 0.35 µg/mL for all vaccine serotypes except polysaccharide 6B (Figure 1). Seven patients (27%) achieved this chosen threshold antibody concentration for all 7 vaccine serotypes. The fraction of patients with pre- and post-vaccination pneumococcal serotype-specific IgG concentrations of ≥ 0.35 µg/mL and ≥ 1.0 µg/mL, respectively, are shown in Figure 2.

Figure 1a. Geometric mean concentations before and after *Hib* and pneumococcal vaccination



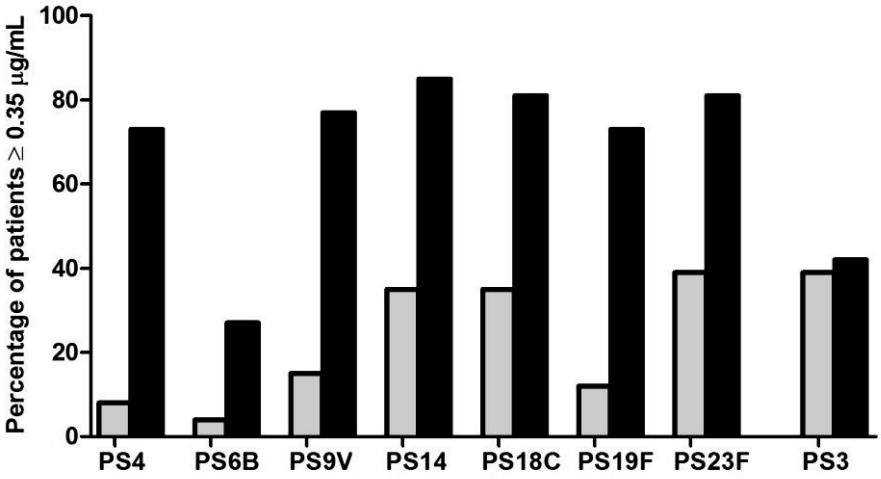
Geometric mean concentrations (in µg/mL) with 95% confidence interval pre-vaccination (gray columns) and post-vaccination (black columns). PS = pneumococcal serotype

Figure 1b. Geometric mean concentrations before and after tetanus vaccination



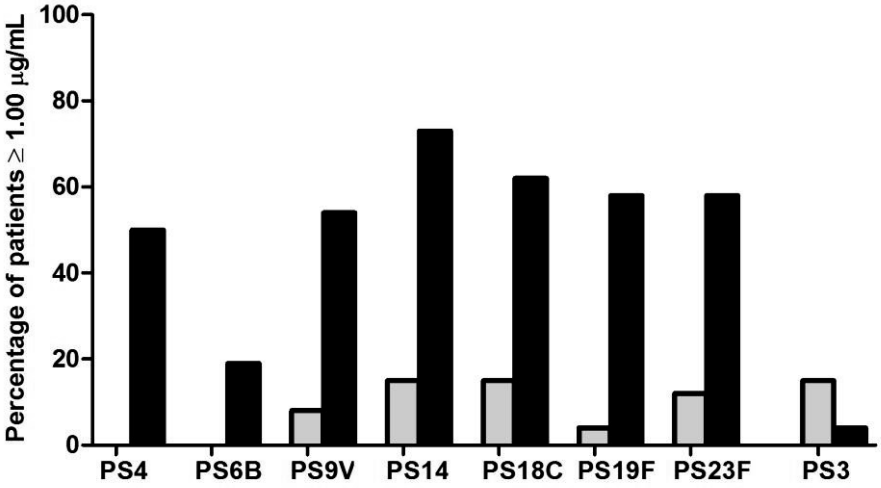
Geometric mean concentrations (in IU/mL) with 95% confidence interval pre-vaccination (gray column) and post-vaccination (black column)

Figure 2a. Percentage of patients with pneumococcal serotype-specific IgG concentrations ≥ 0.35 µg/mL pre- and post-vaccination



Percentage of patients with pneumococcal serotype-specific IgG concentrations ≥ 0.35 µg/mL before (gray columns) and after (black columns) vaccination. PS=pneumococcal serotype

Figure 2b. Percentage of patients with pneumococcal serotype-specific IgG concentrations ≥ 1.0 µg/mL pre- and post-vaccination

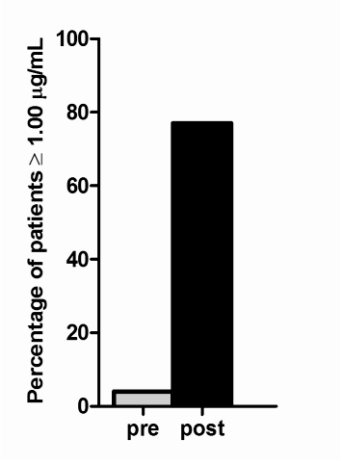


Percentage of patients with pneumococcal serotype-specific IgG concentrations ≥1.00 µg/mL before (gray columns) and after (black columns) vaccination. PS=pneumococcal serotype

Geometric mean anti-*Hib* IgG antibody concentrations significantly increased from 0.29 to 4.30 µg/mL after vaccination. For TT, geometric mean antibody concentrations significantly increased from 0.06 IU/mL pre-vaccination to 3.42 IU/mL post-vaccination (Figure 1).

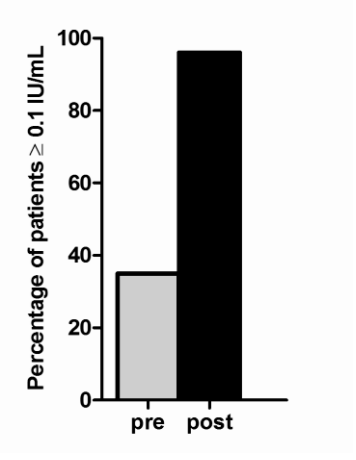
Except for pneumococcal polysaccharide serotype 6B, ≥ 73% of the patients obtain antibody levels considered to be in the protective range (≥ 0.35 µg/mL) after 2 doses of pneumococcal conjugate vaccine. Before vaccination, 31% had serotype-specific IgG concentrations of ≥ 0.35 µg/mL for 3 or more pneumococcal serotypes. After vaccination, this percentage had risen to 85%. If the threshold of ≥ 1.0 µg/mL was chosen, these percentages were 12% before vaccination and 62% after vaccination. For *Hib* conjugate vaccine, 77% of the patients reached the threshold of ≥ 1.0 µg/mL.

Figure 3. Percentage of patients with anti-*Hib* IgG concentrations ≥ 1.0 µg/mL pre- and post-vaccination



Percentage of patients with anti-*Hib* IgG concentrations ≥ 1.0 µg/mL before (gray columns) and after (black columns) vaccination

Figure 4. Percentage of patients with anti-tetanus toxoid IgG concentrations ≥ 0.1 IU/mL pre- and post-vaccination



Percentage of patients with anti-tetanus toxoid IgG concentrations ≥ 0.1 IU/mL before (gray columns) and after (black columns) vaccination

Pre-vaccination, 1 patient (4%) had an *Hib* antibody concentration of ≥ 1.0 µg/ mL (Figure 3). After vaccination with 2 doses of TT, all but 1 patient reached the protective threshold of 0.1 IU/ mL; prior to vaccination, the percentage of patients with protective antibody concentrations for tetanus was 35% (9 patients). These results are given in Figure 4.

For *Hib*, 6 patients did not reach the chosen threshold antibody concentration of 1.0 µg/mL. This group of hypo-responders consisted of 4 men and 2 women. Reasons for transplantation were non-Hodgkin lymphoma (NHL, n=3), multiple myeloma (MM, n=2) and chronic lymphocytic leukaemia (CLL, n=1). Five patients were conditioned with a regimen of Flu and Cy prior to vaccination, 1 patient received TBI. The time from SCT to vaccination varied between 12 and 34 months, with a mean of 17 months. To gain more insight into the effect of immunosuppressive therapy on the vaccination response, the time between the end of the immunosuppressive therapy and the first vaccination (the immunosuppressive free period) was calculated. The mean immunosuppressive free period before start of the vaccination schedule was 4 months. GVHD was seen in 4 patients. No statistically significant relation was found between the response to vaccination and age of the patient, age of the donor, sex of the donor, conditioning regimen, time from SCT to vaccination, or immunosuppressive free period. One patient developed a pneumonia caused by *Hib*, despite an otherwise adequate response to *Hib* vaccination. This was a female acute myeloid leukaemia (AML) patient, treated with a conditioning regimen of Flu and TBI who developed GVHD.

For the pneumococcal conjugate vaccine, 7 patients did not reach the chosen threshold antibody concentration of 0.35 µg/mL for 3 or more vaccine serotypes. This group consisted of 4 men and 3 women with NHL (n=4), CLL (n=1), MM (n=1), and mantle cell lymphoma (n=1). All patients were conditioned with a Flu/Cy regimen. Mean time from SCT to vaccination was 16 months, the mean time between end of the immunosuppressive therapy and start of the vaccination was 3 months. In 4 patients GVHD occurred. Of the 7 pneumococcal hypo-responders, 4 patients were also a hypo-responder for *Hib*. These 4 patients all were conditioned with the Flu/Cy regimen.

Of the 17 patients who developed an upper airway infection during the follow-up, 11 patients were good responders to *Hib* and/or pneumococcal vaccination.
As for the *Hib* hypo-responders, also for pneumococcal polysaccharide conjugate vaccination, no statistically significant relation was found between the outcome of vaccination and age of the patient, age of the donor, sex of the donor, immunosuppressive free period or time from SCT to vaccination. However, the conditioning regimen used was a determining factor on the outcome of vaccination ($p=0.025$).
Hyporesponse to *Hib* or pneumococcal vaccines thus cannot statistically significantly be attributed to either disease, age, time from SCT to vaccination, or immunosuppressive free period. It should be noted, however, that most of the hypo-responders received Flu/Cy as conditioning regimen and, of the

pneumococcal conjugate vaccine failures, 5 patients had an NHL, so there might be a trend toward a relationship between disease or conditioning regimen and the response to vaccination.

DISCUSSION

In the current study, patients who underwent an NMA SCT were vaccinated with *Hib*, pneumococcal, and DTP vaccines in a schedule starting 1 year after transplantation. Because of the conditioning regimens used before SCT, antibody concentrations decline and B-cell function deficiencies last for 12-24 months.[2] The impairment of humoral immune function after SCT and the additional effects of immunosuppressive therapy lead to susceptibility to bacterial and viral infections that may last several years. To prevent these (bacterial) infections, vaccination is recommended. However, the (temporary) B-cell function deficiencies may lead to an impaired response to immunisation. Therefore, timing of vaccination is crucial for allowing for an adequate antibody response to develop in patients who are treated by SCT.

The use of less intensive (NMA) conditioning regimens has reduced toxicity of the treatment itself, but immune recovery after such conditioning regimens is not studied extensively.[16-18] In this study, the vaccination-induced antibody response to vaccination against *S. pneumoniae*, *Hib*, and TT is measured at 1 year post-transplantation. The response rate for *Hib* in this study was 77% after 1 vaccination. By comparison, van der Velden *et al.* found that 6 months after autologous SCT, only 56% of the patients respond to anti-*Hib* after a first dose of vaccine.[19] In a study with myeloablative (MA) transplant recipients, 55% of the patients reached protective *Hib* antibody concentrations after the first vaccination at 3 months post-transplantation.[20] In the group of van der Velden *et al.*, the percentage of patients with anti-TT antibody concentrations of ≥ 0.1 IU/mL rose from 50% to 86% after 2 vaccinations starting at 6 months post-transplantation.[19] In our allo-RIST group, all but 1 patient received protective tetanus antibody concentrations after 2 vaccinations. Parkkali *et al.* found that after MA allogeneic SCT, the percentage of patients with protective anti-TT antibody concentrations of ≥ 0.1 IU/mL was approximately 95% after 2 doses of vaccine, regardless of the time between the SCT and the start of the vaccination schedule (6 versus 18 months).[21]

The heptavalent conjugated pneumococcal vaccine Prevnar® contains pneumococcal polysaccharides of serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F. Serotype 3 is not included in the vaccine. Therefore, it was to be expected that the

patients would not develop an antibody response against this pneumococcal polysaccharide. Pneumococcal serotype 6B is known for its poor antigenicity and post-vaccination antibody concentrations against 6B were likewise significantly lower as to other vaccine serotypes. Twenty-seven percent of the allo-RIST patients achieved antibody concentrations above the threshold of ≥ 0.35 mg/mL for all 7 serotypes of the conjugated pneumococcal vaccine after 2 doses. Three months following myeloablative SCT, Molrine *et al.* found that 36% of the patients reached a concentration of ≥ 0.50 mg/mL for all 7 serotypes after the first dose of vaccine.[22] After the second dose of vaccine, at 6 months post-transplantation, this percentage was 35%. At 12 months post-transplantation, and after receiving 3 doses of pneumococcal vaccine, 64% reached this threshold. In the study of vaccination after autologous transplantation, the response rate to a single dose of Prevnar® was only 6% at 6 months post-transplantation. Pneumococcal antibody concentrations ≥ 0.35 mg/mL increased from 6% to 39% to 72% after a second conjugate pneumococcal vaccine (Prevnar®) and a polysaccharide vaccine (Pneumovax-23®) at 8 and 14 months post-transplantation, respectively. In the immunocompromised allo-RIST population, it was shown that, except for pneumococcal polysaccharide serotype 6B, ≥ 73% of the patients obtain antibody levels considered to be in the protective range after 2 doses of pneumococcal conjugate vaccine. The complete vaccination protocol consists of a 3-dose pneumococcal vaccine schedule. Blood samples of these patients at later time points, however, were not available. Therefore, it is possible that patients who did not develop an adequate antibody response after 2 doses of pneumococcal vaccine, will develop protective antibody concentrations after the third dose of vaccine.

In approximately 25% of the patients, protective antibody concentrations were not obtained after a first dose of *Hib* and 2 doses of pneumococcal conjugate vaccines. In this group, time from transplantation to vaccination varied between 12 to 34 months. All but 1 patient had undergone the conditioning regimen with Flu and Cy. The prevalent underlying disease was NHL. Based on this dataset, it is suggested that the lymphoproliferative disease and the strong immunosuppressive effect of the Flu that is used in the conditioning regimen determine the outcome of vaccination.

The effect of donor age on post-vaccination antibody levels was variable in different studies.[2,22,23] In accordance with Storek *et al.*, in this study, donor age did not affect post-vaccination concentrations.[23]

Only 35% of the patients started the vaccination schedule at 12 months post-transplantation. In the majority of patients, vaccination was postponed because of the use of immunosuppressive therapy at that time. In some patients, vaccination was started despite ongoing immunosuppressive therapy. In these patients, the response to vaccination was variable, not completely negative. Two patients received thalidomide during vaccination and had good responses to all given vaccines. One patient received prednisolone and did not reach protective antibody concentrations after the given vaccines.

It can be concluded that a vaccination schedule starting at 12-17 months after allo-RIST yields antibody concentrations above the chosen threshold(s) in the majority of patients after vaccination with TT and conjugate pneumococcal and *Hib* vaccines. In comparison to autologous transplantation, antibody response rates are higher in this patient group treated with an NMA conditioning regimen after 1 dose of conjugate *Hib* and 2 doses of pneumococcal vaccine. A possible explanation might be that, in allogeneic SCT's, a fully immunocompetent stem cell donor is used, whereas in autologous transplantation, the humoral immune status of the transplant may be impaired because of the haematological disease and the pre-transplant therapy. Second, in the study of van der Velden *et al.*, autologous stem cell recipients were vaccinated at 6 months post-transplantation.[19] A more relevant and interesting comparison can be made to MA allotransplant patients. The allo-RIST patients in this study started at ≥ 12 months post-transplantation. For MA allogeneic SCT, it was recently shown that there was no significant difference in response rate to pneumococcal vaccination when starting at 3 or 9 months post-transplantation, respectively.[24] Further studies will have to show whether earlier start of the vaccination schedule induces equally sufficient antibody responses in the allo-RIST patients, as well as in the increasing number of allo-RIST transplanted with matched unrelated donor (MUD).

CONCLUSION

In summary, vaccination of patients at a median range of 15 months after NMA SCT leads to significant rise in concentrations of pneumococcal, *Hib*, and TT antibodies in the majority of patients.

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Chapter 7

Summary & General discussion

SUMMARY

This thesis concerns the response to vaccination with pneumococcal, *Hib*, meningococcal, DTP and influenza virus vaccines in two major groups of immunocompromised patients: asplenic (functional or anatomical) patients and patients with a malignancy (especially breast cancer and haematological malignancies). Both groups of patients have increased susceptibility for infection and vaccination is therefore recommended to prevent life-threatening infections. In the asplenic population, this risk of infection is life-long, whereas in patients with breast cancer and patients with a haematological malignancy who underwent stem cell transplantation, deficiencies in the immune system are transient with a slow but mostly complete recovery of the immune function after cessation of the immunosuppressive therapy.
Efficacy of vaccination is determined by the clinical protection against corresponding disease. Long-term follow-up of large numbers of patients is required to determine this true efficacy. For relative small patient groups, such as the ones described in this thesis, efficacy of vaccination cannot be determined in this way. This thesis is based on studies towards the amount (and quality) of antibodies produced after administration of a vaccine, which is a surrogate marker for response to vaccination.[1,2] Increase in antibody concentrations is available in the weeks following vaccination and can be obtained in individual patients. Antibody concentrations before and after vaccination are therefore frequently used, also in this thesis, to determine the efficacy of the several different vaccine-schedules.

In **Chapter 1**, a general introduction is given with an overview of the innate and acquired immune system. In patients with asplenia, patients treated with chemotherapy and after stem cell transplantation, deficiencies in both arms of the immune system lead to increased infection rates. In asplenic patients, the absence of marginal zone B-lymphocytes and macrophages leads to increased risk of infections with encapsulated bacteria like *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* type b (*Hib*). After both chemotherapy and stem cell transplantation, humoral and cellular immune functions are compromised during a prolonged period of time, with increased risk of both bacterial and viral (for example influenza virus) infections. Vaccination can reduce the risk of infections and differences in both polysaccharide and conjugated vaccines are discussed.

In **Chapter 2**, an inventory is made of the awareness of the infectious risks and vaccination status of asplenic patients in the Netherlands. By means of

questionnaires and blood samples, we assessed the awareness and vaccination status of a group of 184 asplenic patients in the Utrecht area of the Netherlands. Less than 50% of the patients did receive adequate information about the infectious risks of their asplenic state and the urgency to contact a physician immediately in case of fever. For *S. pneumoniae*, 88% of patients has received a vaccination, although 21% did not receive a revaccination in the past five years. For *Hib* and *N. meningitidis*, vaccination rates are considerably lower: 32% and 27% respectively are vaccinated. Only 17% of the patients had received all three vaccines (i.e. pneumococcal, meningococcal and *Hib*), in accordance with international recommendations. It can be concluded that the preventive care in asplenic patients in the Netherlands is not optimal and can be improved substantially.

In **Chapter 3**, antibody responses to the *Hib* conjugated vaccine and both pneumococcal 23-valent polysaccharide and 7-valent conjugated vaccines were analysed in asplenic patients.
In 92 patients, anti-*Hib* IgG geometric mean concentrations (GMC) rose significantly from 1.3 to 16.8 µg/mL. After a single dose of vaccine, 97% of the patients reached the chosen threshold of ≥ 1.0 µg/mL. After one dose of conjugate pneumococcal vaccine, 82% of 54 patients reached the chosen threshold of ≥ 1.0 µg/mL for 5 out of 7 vaccine-serotypes. This percentage rose to 85% and 92% after a second dose of conjugate vaccine and one dose of polysaccharide vaccine, respectively. Thus, both *Hib* and pneumococcal conjugate vaccines induce adequate antibody concentrations in the majority of asplenic patients.

In **Chapter 4**, the response to the meningococcal serogroup C (MenC) conjugate vaccine was studied in asplenic patients. After a single dose of vaccine, 67% of the patients reached the chosen antibody threshold of ≥ 2.0 µg/mL, with MenC IgG GMC rising from 0.16 to 3.69 µg/mL. The quality of the antibodies (expressed as avidity maturation) was poor; the increase in IgG2 was more prominent as compared to IgG1. After revaccination of patients with a suboptimal response, the response to vaccination rose from 67% to 78%, but again, with only marginal rise in avidity maturation. In healthy children and adults, 5-10 times higher GMC's were reached after one dose of this MenC conjugate vaccine. In conclusion, the MenC conjugate vaccine is less immunogenic in asplenic patients than in healthy adults and children. Revaccination of patients with a suboptimal response leads to higher IgG GMC's, but avidity of the formed antibodies remains low.

Chapter 5 deals with another patient group at increased risk for infectious diseases because of an impaired immune system, namely patients with breast cancer on chemotherapy. The response to influenza virus vaccination was determined in 38 patients vaccinated at day 4 or at day 16 of a chemotherapy cycle of 21 days. The results in the breast cancer patients were compared to the responses in 21 healthy adults. It appeared that patients on chemotherapy have significant lower antibody responses than healthy adults. When comparing the two different moments of vaccination during a chemotherapy cycle, vaccination early during the chemotherapy cycle induces better responses than does vaccination in the last week of the cycle. HI geometric mean titres (GMT's) post-vaccination for day 4 versus day 16 were 63.7 versus 29.5 (H3N2), 28.2 versus 19.6 (H1N1) and 29.8 versus 16.0 (B/Brisbane), respectively. Follow-up studies in larger patient groups are needed to confirm this effect.

In **Chapter 6**, the response to vaccination after allogeneic stem cell transplantation with reduced intensity conditioning regimens (allo-RIST) was studied. Patients were vaccinated with pneumococcal, *Hib*, meningococcal and DTP (diphtheria, poliomyelitis and tetanus) vaccines starting at 12 months post-transplantation. After two doses of conjugated pneumococcal vaccines, more than 73% of the patients developed antibody levels ≥ 0.35 µg/mL for all pneumococcal serotypes included in the vaccine, except for pneumococcal serotype 6B. For *Hib* and tetanus toxoid (TT), protective antibody levels were found in 77% and 96% of the patients respectively, after one dose of *Hib* vaccine and two doses of DTP vaccine. In conclusion, vaccination of allo-RIST patients starting at approximately 12 months post-vaccination induces significant antibody responses in the majority of patients.

GENERAL DISCUSSION

PATIENTS WITH ASPLENIA

Few organs have been the subject of such an elaborated discussion of its function as the spleen.[3] In antiquity, the spleen was among the 'ten organs that minister to the soul' because it was said to produce laughter (Talmud/Midrash). The writer Galen of Pergamon (AD 131-200) however, inspired by Hippocrates, posed that the spleen received nourishment from black bile, passed from the liver. Black bile also was called 'melancholia'. So, in contrast to the production of laughter, Galen implied that the spleen had a melancholic function. These different functions of the spleen led to a definition of the spleen as 'the seat of emotions and passions'.[4] Since the early 1900s, the role of the spleen in the immune system (part of the reticulo-endothelial system, with efficient phagocytic action of splenic leukocytes), haematopoiesis and erythrophagocytosis became known. Despite these important functions, it was thought that removal of the spleen did not lead to 'permanent abnormal disturbance'.[5,6] Only decades later, the infectious risks that were associated with the removal of the spleen were recognized.[7,8] An overwhelming post-splenectomy infection (OPSI) has a life-time risk of 5% with high mortality rates and is mostly caused by encapsulated bacteria; with *Streptococcus pneumoniae* as the prevalent causative organism.[3,9,10]

Age at splenectomy, time since splenectomy and underlying disease are of importance for the risk of infection. Young children tend to have higher rates of infection than adult asplenic patients.[11] In children < 5 years, the incidence of infection was 10.4% after splenectomy, with a mortality rate of 4.5%. In adults, these percentages were 0.9% and 0.8%, respectively.[12] At the age of 5 years, the immune system has (almost) completely matured. Under the age of 5 years therefore more infections are expected. Moreover, splenectomy for trauma in young children is uncommon; an underlying disease is most often the reason for splenectomy. Patients splenectomised due to a trauma are otherwise immunologically competent. In contrast, patients splenectomised for haematological malignancies are immunocompromised due to the underlying disease and the immunosuppressive therapy. It was found that after splenectomy for trauma, patients have the least risk of infection; patients suffering from haematological malignancies and treatment with chemo- or radiotherapy have the highest risk of infection.[11,13] Most infections occur in the first two years after splenectomy, although infections can occur many years after splenectomy.[14]

To prevent the life-threatening complications of an OPSI, patients should be aware of these infectious risks and should receive immunisation against the encapsulated bacteria *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* type b (*Hib*). In case of fever, the patient has to start immediately with antimicrobial therapy.[15-19] In several countries, the vaccination status of asplenic patients has been studied and the results give reason for concern.[10,20-22] In the Netherlands, however, an inventory of the vaccine coverage of asplenic patients had not been made before. We gathered a group of 184 asplenic patients in the Utrecht area of the Netherlands mostly by addressing general practitioners. In Chapter 2, a subset of these patients are described. For this study, 384 GP's were approached. In the Netherlands, one GP serves about 2000 patients. Our domain of approached GP's thus consisted of 750,000 patients. A total of 263 asplenic patients were identified and approached by their GP, of whom 130 patients were eligible. When extrapolating these data to the total Dutch population (approximately 16 million inhabitants), in the Netherlands, approximately 5600 asplenic patients should be found. Whether this is an adequate estimation, is hard to establish. Data on the prevalence of asplenia in the Netherlands are not readily available. The absolute incidence of splenectomy in the Netherlands is about 1000 per year.[23] In our cohort the mean age of the patients was 50 years, with a mean age at splenectomy of 30 years. Splenectomies for spherocytosis are often performed in children. In our study, we included patients ≥ 18 years of age. Therefore, the total number of splenectomised patients in the Netherlands could be substantially higher than our proposed 5000-6000. On the other hand, the number of splenectomies for non-Hodgkin lymphoma (staging) have dropped during the last decades due to the improvement of radiological imaging techniques. The number of functional asplenic patients is not known. Exact data on the prevalence of asplenia can therefore not be given.

Asplenic patients that were not immunised according to international guidelines, were offered catch-up vaccination. The vaccination schedule used in these patients is given in Table 1. Patients were vaccinated with 2 doses of pneumococcal conjugate vaccine, followed by a single dose of 23-valent pneumococcal polysaccharide vaccine. Patients were also given one dose of *Hib* and MenC vaccine, in accordance with recommendations of the British Committee for Standards in Haematology (BCSH).[24]

Table 1. Vaccination schedule in asplenic patients

Time	**Day 0**	**+ 6 weeks**	**+ 6 months**
Vaccine	PCV-7	PCV-7	PPV-23
	Hib	MenC	

PCV-7 = 7-valent pneumococcal conjugate vaccine, *Hib* = conjugate *Hib* vaccine, MenC = conjugate meningococcal serogroup C vaccine, PPV-23 = 23-valent polysaccharide pneumococcal vaccine

Up till recently, the vaccination schedule for asplenic patients only included polysaccharide pneumococcal vaccines. In asplenic patients, however, antibody concentrations after polysaccharide vaccination decline in 5-10 years, with a decrease of 30% in the first year.[25] Another disadvantage of polysaccharide vaccines is the absence of induction of immunological memory. Therefore, revaccination is advised; in case of pneumococcal polysaccharide vaccines immunisation should be repeated every 3-5 years.[23,26]
To improve the response to pneumococcal vaccination and to induce immunological memory, we changed the schedule and started with two doses of conjugate 7-valent pneumococcal vaccine. This conjugated vaccine proved to be immunogenic in asplenic patients.[27] In the current study, 82% of the patients reached the highest chosen antibody threshold (≥ 1.0 µg/mL) for five or more of the seven pneumococcal vaccine-serotypes after one dose of vaccine.

In our asplenic patient group, six months after the second dose of PCV-7, one dose of PPV-23 was administered. PCV-7 contains the polysaccharides of seven pneumococcal serotypes (4, 6B, 9V, 14, 18C, 19F and 23F). These seven serotypes together are responsible for 59-67% of all invasive pneumococcal disease (IPD) in the Netherlands.[28] Moreover, the vaccine-serotypes are the causative organism in 60% of all pneumococcal bacterial meningitis in children under the age of 5 in the Netherlands.[29] In young children in Europe, the percentage of IPD caused by serotypes included in the 7-valent vaccine is estimated to be 75%.[30] In older children and adults, this percentage is approximately 50%. Several serotypes not included in the conjugate vaccines are each responsible for approximately 2% of invasive disease in older children and adults, especially serotypes 8 and 12.[30] In asplenic patients, many different pneumococcal serotypes can cause OPSI.[12,22,31] The distribution of pneumococcal serotypes responsible for IPD appears to be comparable with that in other risk groups.[12] The coverage of PCV-7 in our patient cohort therefore is an estimated 50%. By adding the PPV-23 vaccine to the immunisation schedule, we had the intention to cover an additional 16 pneumococcal serotypes (1, 2, 3, 5, 7F, 8, 9N, 10, 11A, 12F, 15B, 17F, 19A, 20, 22F and 33F) by vaccination.

The combined use of conjugate and polysaccharide pneumococcal vaccines has been studied in splenectomised rats. In these rats, admixture of a polysaccharide vaccine with a protein-conjugated vaccine did not lead to higher antibody concentrations against the non-conjugated pneumococcal serotypes.[32] This can be explained by differences in location and help provided by T-lymphocytes: the response to PPV-23 takes place especially in marginal zone B-lymphocytes in the spleen and is dependent of complement.[33] The response to PCV-7 is not specifically restricted to the splenic marginal zone and T-cell mediated, implying a better possible result to PCV-7 vaccines in splenectomised patients.

Beside admixture of both PPV-23 and PCV-7 vaccines, booster vaccination with PPV-23 after PCV-7 might extend the coverage of PCV-7 to more serotypes. This schedule too was studied in splenectomised rats.[32] After priming with PCV-7, a booster vaccination with PCV-7 led to higher secondary responses. Vaccination with PPV-23, after priming with PCV-7 as well as priming with PPV-23, did not lead to an additional increase in antibody responses.
In a subgroup of our asplenic patients (n=16), the rise in antibodies against serotypes solely included in the PPV-23 vaccine was studied after vaccination with the PPV-23 vaccine. Boosting with PPV-23 six months post PCV-7 led to significant rises in antibody concentrations for five out of seven tested pneumococcal serotypes included in the PPV-23 vaccine. This observation is at variance with the experiments performed in splenectomised rats. One explanation might be the high percentage of patients that had been immunised with PPV-23 in the past (more than 5 years before the vaccination study). In the sixteen patients in which this booster response was studied, 81% had been vaccinated before. For five out of the seven tested serotypes, the GMC pre-vaccination already exceeded the 0.35 µg/mL threshold, indicating the presence of pre-existing immunity against these pneumococcal serotypes. This pre-existing immunity may be caused by previous polysaccharide vaccination, as well as by natural exposition to pneumococci. Nasopharyngeal carriage of pneumococci may be a sufficient stimulus for the human immune system to cause a gradual increase in pneumococcal antibody levels, also in the absence of any obvious pneumococcal disease. In rats, as well as other rodents, nasopharyngeal carriage of pneumococci does not occur.

In previously unvaccinated children with a mean age of 5 years, addition of PPV-23 to the immunisation schedule with two doses of PCV-7 did not lead to high antibody responses to serotypes included in the PPV-23 vaccine.[34] In previously unvaccinated elderly adults, administration of a dose of PCV-7 one year after one dose of PPV-23 led to approximately 3-fold lower anti-polysaccharide and

opsonophagocytic assay (OPA) responses compared to a single dose of PCV-7.[35] Two doses of PCV-7 or one dose of PCV-7 followed by one dose of PPV-23 led to higher antibody concentrations for most pneumococcal serotypes. De Roux *et al.* suggest that this immunological hyporesponsiveness of the PPV-23/PCV-7 vaccination sequence might be explained by a depletion of polysaccharide-specific memory B-lymphocytes by the initial dose of PPV-23. The response to a booster dose of PCV-7 thus would be diminished due to the B-lymphocyte depleted environment.[35,36] In earlier studies, hyporesponsiveness after multiple doses of PPV-23 was found in almost all cases.[37-40] The hyporesponsiveness is defined as lower antibody concentrations after the second dose of vaccine, although GMC's did increase significantly after the second dose of vaccine. This phenomenon is important considering the discussion about life-long, 5-yearly repeated vaccination schedule with polysaccharide pneumococcal vaccines in asplenic patients.

In the asplenic population studied in this thesis, 76% of the 54 patients had received polysaccharide pneumococcal vaccination in the past (≥ 5 years before revaccination). PPV-23 vaccination was given six months after two doses of PCV-7. The PPV-23 did not lead to a further increase in conjugate vaccine-serotypes, but did lead to significant increases in serotypes solely included in the PPV-23 vaccine. The response to PCV-7 and PPV-23 of the previous vaccinated patients was not lower than that of the pneumococcal vaccination naïve patients. It thus can be concluded that the phenomenon of polysaccharide vaccine induced hyporesponsiveness discussed above is not maintained for a period of 5 years. Therefore, from this study no major arguments against 5-yearly repeated pneumococcal polysaccharide vaccination can be made.
Whether a vaccination schedule including one dose of PCV-7 followed by PPV-23 does lead to comparable antibody concentrations in asplenic patients compared to the schedule used in this thesis with two doses of PCV-7, has to be determined in further studies.

The conjugated vaccine currently used in the Netherlands consists of seven pneumococcal serotypes. Recently, 11-valent and 13-valent vaccines have been produced. In December 2009, the 13-valent conjugate pneumococcal vaccine from Wyeth Pharmaceuticals, Philadelphia PA, USA, received European marketing authorisation. This vaccine, which includes the pneumococcal serotypes of the 7-valent pneumococcal vaccine plus six additional serotypes (1, 3, 5, 6A, 7F and 19A), is indicated for active immunisation in infants and children from 6 months to 5 years of age. In this vaccine, the same carrier protein (CRM_{197}) is used as in the 7-valent vaccine. Of the 90 known pneumococcal serotypes, approximately 15 cause the majority of IPD.[30] The most invasive serotypes are least commonly carried in

children, whereas the most commonly carried serotypes were least likely to cause IPD.[41] The most invasive pneumococcal serotypes are type 1, 5 and 7.[41] Serotype 1 for example accounts for > 6% of IPD in Europe in young children.[30] These serotypes 1, 5 and 7 are not included in the 7-valent vaccine. The 9-valent vaccine includes type 1 and 5, and the serogroups in this 9-valent vaccine are responsible for 90% of IPD in Europe in young children. In the 11-valent vaccine, serogroups 3 and 7F are added, boosting the potential IPD coverage in Europe with approximately 6% in young children. For older children and adults, the 9-valent vaccine-serotypes account for ≤ 60% of IPD and the 11-valent serotypes for 80% of IPD in Europe.[30] The 9-valent and 11-valent vaccine-serotypes cause an estimated 68% and 78%, respectively, of IPD in children age 0-10 years in the Netherlands.[28,42]
In the Netherlands, currently the use of the 13-valent pneumococcal conjugate vaccine in the prevention of community-acquired pneumonia (CAP) in the elderly population is studied.[43] Studies to determine the efficacy of these 11-valent and 13-valent vaccines in asplenic patients should be initiated as soon as possible.

For *Hib*, 97% of the patients reached the threshold of ≥ 1.0 µg/mL antibody concentration after one dose of vaccine. When applying a higher threshold of ≥ 2.5 µg/mL, vaccination responses were adequate in 82%. This implies that in our asplenic patient group, the immunogenicity of the used *Hib* vaccine is high.

The meningococcal serogroup C (MenC) vaccine did turn out to be not as immunogenic as the pneumococcal and *Hib* conjugate vaccines, with 67% of the patients reaching the chosen antibody concentration of ≥ 2.0 µg/mL after one dose of vaccine. Beside a diminished increase in MenC antibody concentration compared to healthy individuals, the avidity of the antibodies was low. Antibody avidity is defined as the overall antigen-binding capacity of polyclonal antibodies with different affinities.[44-47] The polysaccharide conjugate vaccines have the ability to elicit higher avidity antibodies with increased functional activity than do plain polysaccharide vaccines.[44,45] There are no absolute threshold values for adequate avidity; the magnitude of the avidity index is dependent on the type of conjugate vaccine.[48] In our asplenic patients, the response to a single dose of meningococcal conjugated vaccine was low in quantity and also in quality.

In general, IgG1 antibodies have higher affinity than IgG2.[49] Loss of bactericidal activity may correlate with lower IgG1 antibody levels. After immunisation with a conjugated vaccine, the T-cell dependent (TD) response may lead to an increase of the IgG1/IgG2 ratio, e.g. an increase in the amount of IgG1.[50] The ability to induce a IgG1 TD response is among others dependent on age at vaccination, type

of vaccine and type of adjuvant.[50-52] In individual patients however, large variability exists in the ratio of IgG1/IgG2 after vaccination with a conjugated vaccine.[51] This may be explained by the fact that the response to a conjugated vaccine can originate from a restricted number of committed B-cell clones. Dependent on the dominating clones within a patient, more IgG1 or IgG2 will be produced.[52]
In patients with a suboptimal response, after a second dose of vaccine an additional 59% of the patients reached the threshold concentration. Moreover, after revaccination the amount of IgG1 in the asplenic population increased more than IgG2, suggesting that after revaccination, the T-cell dependent response is more adequate. These findings indicate that a second dose of MenC vaccine may be of additional value in asplenic patients.

An important factor in the discussion about the necessity of vaccination is the prevalence of the bacteria in the general population. Since the start of this century, conjugated vaccines against *Hib*, *N. meningitidis* and *S. pneumoniae* are incorporated in the Dutch National Vaccination Programme for children and infants, implying that (almost) all children are vaccinated against these bacteria. After vaccination, the nasopharyngeal carriage of vaccine-associated pneumococcal serotypes reduces significantly, whereas the carriage of non-vaccine-associated serotypes increases.[53] The reduction in the rate of colonisation by vaccine serotypes in the general population decreases the risk of infection in vulnerable patients; a so-called 'herd effect' of vaccination.[54] This herd effect of vaccination might have implications for the number of vaccines and vaccination doses that need to be given to patients. For MenC in particular, the incidence of infection in asplenic patients is much lower than the incidence of pneumococcal infections. Although we showed in this thesis that a second dose of meningococcal vaccine does lead to better quality and quantity of the produced antibodies, with the decreased circulation of the meningococcal bacteria in the general population, the necessity of a second dose of vaccine can be the subject of debate.

In earlier studies, it was found that patients splenectomised due to a trauma had better responses to vaccination than do patients splenectomised for other reasons.[55-57] Two explanations can be given: first, patients splenectomised because of a haematological malignancy suffer from an underlying condition that directly has impact on the function of the leukocytes and therefore the lymphocytes. As a consequence, the underlying condition itself might reduce the response to vaccination.[58] Second, in case of rupture of the spleen, splenic tissue may disperse throughout the abdominal cavity (splenosis). This splenic tissue can develop to form an accessory spleen. Although additional splenic tissue cannot fully take over the function of the original spleen, it is suggested that they can partly

execute immunological function.[59,60] For MenC vaccination, patients splenectomised due to trauma indeed had a higher antibody response after vaccination than did patients splenectomised for other reasons. For both pneumococcal and *Hib* vaccination, this phenomenon was not found.

Prior MenC polysaccharide vaccination is thought to induce immunological hyporesponsiveness to MenC capsular polysaccharide.[61,62] In both the current study in asplenic patients and the study of Balmer *et al.*, 15-30% of patients had received prior MenC polysaccharide vaccination.[63] These previously vaccinated patients had higher MenC GMC's pre-vaccination than patients not vaccinated with meningococcal vaccines before. In contrast to Balmer *et al.*, we found that previous MenC vaccination also led to higher GMC's post-vaccination. In a study of Borrow *et al.*, in children with a history of MenC polysaccharide vaccination and a low SBA (bactericidal antibody in serum) titre after the first MenC conjugate vaccine, a satisfactory response was found after the second dose of MenC conjugate vaccine, including avidity maturation. Borrow *et al.* concluded that the hyporesponsiveness induced by prior MenC polysaccharide vaccination can be overcome by additional doses of MenC conjugate vaccine.[64]

An ongoing debate for vaccination against encapsulated bacteria deals with the determinants of clinical protection: are prolonged antibody levels above a certain threshold level required in order to remain protected or would it suffice to induce immunological memory. It should be kept in mind that polysaccharide vaccines do not induce classical immunological memory in the sense that repeated vaccination does not induce higher antibody responses. After pneumococcal polysaccharide vaccination, Giebink *et al.* found a 30% decrease in IgG in the first year post-vaccination in splenectomised children.[25]
Furthermore, in adult splenectomised patients, antibody levels declined to pre-treatment levels within 3 years post-pneumococcal polysaccharide vaccination.[65] In contrast, after vaccination with a conjugate vaccine, we found an only marginal decrease in MenC IgG six months post-vaccination in our asplenic adult patients. In accordance with our findings, in the study of Smets *et al.*, 6 months after pneumococcal conjugated vaccination in asplenic children, GMC's had not decreased significantly.[66] The asplenic state of the patients itself may influence the marginal decrease in GMC post-vaccination. However, in healthy adults (50-80 years), one year after one dose of pneumococcal conjugated vaccine, GMC's do not decrease drastically either.[67] Although this suggests that in patients who have a functional spleen, IgG levels can remain considerably high too, the decrease in GMC in these elderly healthy patients does seem to be more pronounced than in asplenic patients. From our data we cannot extrapolate or predict antibody levels to

any of the vaccine antigens at 5 years after vaccination. Long-term management of asplenic patients therefore will require regular measurement of antibody levels.

Informing the asplenic patient about infectious risks and (re)vaccination is a combined responsibility of the surgeon who performs the splenectomy, the internist who is involved in the treatment of a haematological cause for the splenectomy and the general practitioner (GP). A nationwide protocol for management of asplenia did not exist in the Netherlands. Often, GP's and medical specialists were not aware of the preventive measures they had to take for their patients. Therefore, in 2009 a working party initiated by the RIVM (National Institute of Public Health and the Environment) was established in the Netherlands to propose such a protocol for asplenia. Recommendations were formulated so that the vaccine schedule and antimicrobial prophylaxis will be uniform in the whole country. This is especially important since the recently developed conjugated (pneumococcal) vaccines have not been integrated yet in the existing vaccination schedules in many hospitals.
The new vaccination schedule for asplenic adults will consist of one dose of conjugated *Hib* and MenC vaccine if patients have not been vaccinated with these conjugated vaccines before. For *S. pneumoniae*, one dose of PCV-7 is recommended, followed after two months by a dose of PPV-23. The PPV-23 must be repeated every 3-5 years. For young children an age-dependent schedule will be proposed.
With the implementation of these new guidelines, it would be advisable to determine antibodies before and after vaccination. We showed in our asplenic patient group that the response to the meningococcal vaccine is lower compared to the response to pneumococcal and *Hib* vaccines. A nationwide, routine measurement of the antibody response after conjugated vaccines is a valuable database for evaluation of the response to vaccination in asplenic patients. Furthermore, a long-term follow-up of asplenic patients to define the number of infectious periods is of utmost importance. Rise in antibody concentration is only a surrogate marker for the effectiveness of vaccination. A decrease in infections with the bacteria against which vaccination has taken place is the ultimate goal of these vaccinations.

Currently, differences in reimbursement of the costs of vaccination by health care insurance companies exist for asplenic patients. Most health care insurance companies are willing to pay the costs of the pneumococcal polysaccharide vaccine and the *Hib* conjugated vaccine. At the moment however, despite international guidelines and recommendations, in general the costs of the pneumococcal conjugated vaccine are not reimbursed. After introduction of the recently developed nationwide vaccination schedule with integration of

meningococcal and pneumococcal conjugated vaccines in the Netherlands, reimbursement of the costs of all the vaccines is expected to take place by all health care insurance companies.

Future perspectives

With the introduction of a nationwide vaccination schedule with conjugated vaccines for asplenic patients, a long-term follow-up of asplenic patients vaccinated according to this schedule is warranted. Blood samples pre- and post-vaccination have to be taken to determine rise in antibody concentrations post-vaccination. However, as mentioned above, rise in antibody concentrations is only a surrogate marker of protection. To study the effect of vaccination on reduction of overwhelming post-splenectomy infections (OPSI's), long-term follow-up with registration of all infections occurring in asplenic patients has to take place. This requires the combined effort of general practitioners, surgeons and internists. A nationwide database of asplenic patients can be a useful instrument for this purpose.[68]

Due to the introduction of pneumococcal and meningococcal conjugate vaccines in the Dutch National Vaccination Programme, shifts in carriage of serotypes will occur from vaccine serotypes to non-vaccine serotypes. Circulation of prevalent non-vaccine serotypes in asplenic patients can be monitored and may even lead to adjustments in serotypes added to the conjugated vaccines.

With the introduction of a 13-valent pneumococcal vaccines in the Netherlands in December 2009, studies to determine the efficacy of this vaccine in asplenic patients should take place as soon as possible.

Although vaccination can drastically reduce the incidence of life-threatening infection, the coverage of all mentioned vaccines is not 100%. Therefore, the most important task is the education of both medical practitioners and patients about the risk of infections and the necessity of immediate contact in case of fever. We suggest a unique, voluntarily, national register of splenectomised patients, from which patients are being informed on a regular base about infectious risks and the need for revaccination. Splenectomised patients should be registered by the surgeon who performed the operation or the internist involved in the follow-up of a splenectomised or functionally asplenic patient. Catch-up registration of patients receiving a splenectomy in the past and currently not being treated by an internist, should be performed by the general practitioners.

CHEMOTHERAPY

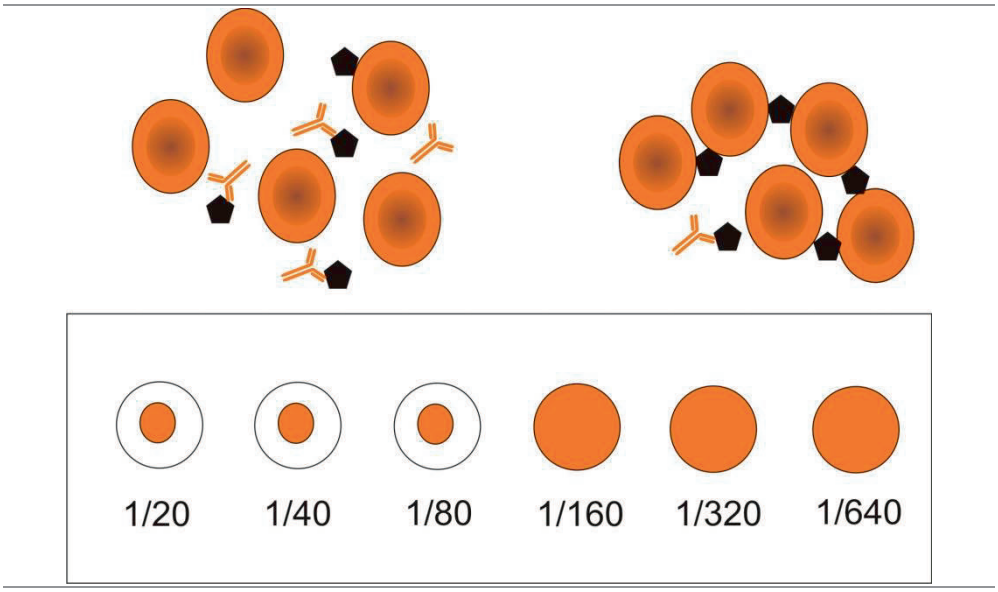
Protective immunity to influenza virus infections depends on the combined activity of haemagglutinin- and neuraminidase-specific antibodies, cytotoxic T-lymphocytes and mucosal antibodies.[69,70] The haemagglutinin-specific antibodies are capable of neutralising the virus and therefore can prevent illness. By reducing the release of virus from infected cells, the duration of the infection is shortened. Moreover, cytotoxic T-lymphocytes are able to prevent influenza-related complications.[69] Neutralising antibodies at mucosal surfaces prevent invasion and inhibit replication of the virus.[71]

In patients with cancer treated with chemotherapy or immunosuppressive drugs, the immune response to natural viral infections is impaired, and this patient group can be considered a high-risk group who are at risk of particularly serious post-influenza complications.[72] Hospitalisation because of infection with the influenza virus is 3-5 times higher in oncology patients than in the general population, with mortality rates four times higher.[73,74] Two factors influence the response to virus infection in patients with cancer: their disease state may be directly immunosuppressive and the treatment for cancer can be immunosuppressive.[75]

In healthy adult volunteers, protective antibody levels (≥ 40 haemagglutination inhibition antibodies) against influenza strains develop in more than 80% after vaccination with one dose of inactivated influenza virus vaccine (Figure 1).[69,76,77] Of the patients on chemotherapy, a substantial part is able to generate an immune response after influenza vaccination.[78] However, these responses are most often lower than in healthy controls.[79-82]

Because of the increased risk of infection and despite the fact that oncology patients generate lower immune responses, vaccination against the influenza virus in patients immunocompromised due to chemotherapy is recommended.[83-85] Vaccine coverage in high-risk patients remains suboptimal. Our data show that influenza vaccination can induce protective antibody responses in a substantial fraction of breast cancer patients on chemotherapy but that timing of the vaccination is important. Communication of these data to the professionals responsible for the clinical care for this patient category could lead to better awareness and ultimately higher vaccination coverage.

Figure 1. Determination of haemagglutination inhibition antibodies



The concentration of haemagglutination inhibition (HI) antibodies is expressed as the reciprocal of the highest dilution in which the serum HI antibodies prevent agglutination of turkey red blood cells by influenza virus particles (black pentagons). In this example, the HI antibody titre is 80.

Many studies on efficacy of influenza vaccination in patients on chemotherapy were performed in the 1970/1980's. The most cited study on vaccination during chemotherapy comes from Ortbals *et al* in 1977.[86] It was found that vaccination at the time of chemotherapy administration induced a response in 50% of the patients, whereas vaccination between chemotherapy cycles (e.g. in the nadir) induced responses in 93% of the patients. Based on this result, it is in general advised to vaccinate between chemotherapy cycles.[87] This study however consisted of only 36 patients, with 15 different haematological or oncological diseases. Of these patients, only 7 patients were treated for breast cancer and these patients were split over both groups. Therefore, extrapolation of these study results and generalisation to all cancer patients and, more specific, to breast cancer patients is based on only very limited evidence.

Currently given chemotherapy cycles differ from the ones used twenty/thirty years ago. Moreover, only recently monoclonal antibodies (MoAb's) were added to the therapeutic regimen for oncology patients. These MoAb's, like rituximab, have immunosuppressive effects and induce lower responses to the influenza virus vaccine.[88] Beside differences in treatment, many previous studies have included small numbers of patients or a mixture of patients with different haematological or oncological diseases, making it difficult to establish the efficacy of vaccination in

specific oncological patient categories.[72,80] For these reasons, it remained opportune to conduct a study in a specific patient group who could benefit from vaccination: patients with breast cancer treated with FEC-containing chemotherapy.

To determine the efficacy of vaccination during chemotherapy, we chose to vaccinate at two different time points in a 21-days chemotherapy cycle; at day 4 of the cycle (patients were before the nadir), and at day 16 of the cycle (the nadir had passed). We found a better response to vaccination in the patients vaccinated at day 4 compared to day 16. An explanation for this phenomenon might be the effect of the next dose of chemotherapy on proliferating B- and T-lymphocytes. In patients vaccinated at day 16 of a chemotherapy cycle, the next dose of chemotherapy is given 5 days after the vaccination. This chemotherapy is toxic for proliferating lymphocytes and therefore greatly impairs the antibody response. In the early patient group, 17 days will pass before the next dose of chemotherapy is given, enabling the proliferative phase of the humoral response to complete. Indeed, in patients vaccinated at day 16 of their last course of the 6-cycle chemotherapy regimen (e.g. the humoral response will not be impaired by a next dose of chemotherapy), antibody responses were higher compared to patients vaccinated at day 16 of chemotherapy cycles 1-5.

Although patients vaccinated at day 4 generated a better immune response than patients vaccinated at day 16, the response in both patient groups was significantly lower than in the control group. This is consistent with previous findings.[79-81]
Strategies to improve the response to vaccination in immunocompromised patients can be divided into three categories: administration of a higher dose of vaccine, booster vaccination or addition of adjuvants to the vaccine.
In both healthy volunteers and residents of long-term care facilities higher doses of antigen lead to only a modest increase in antibody levels post-vaccination.[89,90] In patients immunocompromised due to systemic lupus erythematosus and elderly residents of long-term care facilities, booster influenza vaccination was only of very limited additional effect.[89,91] However, booster vaccination in immunocompromised liver transplant patients led to HI serum antibody titres ≥ 40 for all three vaccine strains in 80% of patients compared to 68% after the primary vaccination.[92] The responses in these transplant patients were lower than the healthy controls. In patients immunocompromised due to chemotherapy, booster vaccination may therefore also be of additional benefit.

Addition of an adjuvant to a subunit vaccine facilitates the delivery of the antigen to the immune system and enhances the cell-mediated immune response to the

vaccine.[93,94] In case of a limited amount of antigen (either for a single vaccinee or for the population at large) adjuvants can enhance the immune response and thus generate an equally effective response with a lower dose of vaccine.
The first influenza vaccines consisted of inactivated or killed whole-pathogen vaccines. These vaccines have a self-adjuvanting capacity caused by the presence of Toll-like receptor (TLR) agonists, like lipopolysaccharides on the outer viral surfaces.[93]
Since the discovery of the specific antigen of influenza that generates an immune response, subunit vaccines were developed. These subunit vaccines are composed of only a few antigenic proteins of the influenza virus instead of the whole pathogen, thereby reducing the reactogenicity of the vaccine. These subunit vaccines however reduced the potency of the vaccine by excluding the TLR's from the vaccine.[93] As mentioned above, the addition of an adjuvant can overcome this shortcoming.

For human influenza vaccines, in the Netherlands up till recently only aluminium salts were allowed as adjuvant in subunit vaccines. Other adjuvants, for example the oil-in-water emulsions, led to adverse reactions in humans and the first oil-in-water adjuvants were not licensed.[94] Since the influenza A H1N1 ('Mexican flu') pandemia of 2009, the oil-in-water adjuvants AS03 and MF59, both containing squalene, were registered for use in human vaccines in the Netherlands. Addition of the oil-in-water adjuvant squalene to an inactivated influenza subunit vaccine induces higher post-immunisation geometric mean titres, seroconversion and seroprotection rates in healthy adults and elderly patients compared to non-adjuvanted influenza vaccines.[95,96] Vaccination with adjuvanted influenza vaccines might be of additional benefit to patients on chemotherapy too.

In our study in breast cancer patients, we used healthy female healthcare workers as a control group. Our control group however was almost 2 decades younger than the patient group (mean age 35.6 versus 54 years, respectively). Moreover, in the patient groups 22-25% of the patients was > 60 years old, whereas in the control group 10% was above 60. In a previous study on the effect of influenza vaccination in healthy adults and elderly living in nursing homes, a decline in HI titres post-vaccination was seen with increasing age.[97] Due to the small group of patients that was included, any confounding effect of age on the results of vaccination could not be studied. Therefore, a follow-up study with larger numbers of included patients may be performed to study this effect. The EMEA (The European Agency for the Evaluation of Medicinal Products) criteria for adequate response to influenza vaccination are designed for a minimal group size of 50 patients.[98] Thus,

follow-up studies on the effects of vaccination should include at least 50 persons in every study subgroup.

In the present thesis a HI titre ≥ 40 is considered to correlate with protection, a threshold that is used in immunocompetent persons. However, it is not clear whether a HI titre ≥ 40 also provides protection in immunocompromised individuals.[92] To determine the true protective effect of vaccination, long-term follow-up of vaccinated patients with registration of influenza infections should take place.

Future perspectives

This study on the effect of influenza vaccination at different moments during chemotherapy was conducted in a small group of patients with a specific oncological disease (breast cancer). Although it only was a small group, an obvious trend towards better responses during chemotherapy cycles was seen in patients that were vaccinated at day 4 of the chemotherapy cycle. We advise a follow-up study in which a larger group of patients is included and in which long-term follow-up of the vaccinated patients is performed to determine the incidence of clinical influenza infections in the vaccinated patients. Furthermore, the use of adjuvanted influenza virus vaccines might lead to a better immune response and should therefore be studied in these immunocompromised patients.

The results in breast cancer patients cannot easily be extrapolated to other oncological patient groups due to differences in chemotherapy schedules and cytotoxic medication. Two other, relative large patient groups in which the moment of vaccination during chemotherapy deserves to be studied are patients with colorectal cancer and patients with haematological diseases on treatment with biologicals like rituximab (anti-CD20 monoclonal antibodies), lenalidomide or other monoclonal antibodies. The design and validation of effective vaccination schedules for these patient groups can have major clinical impact for all physicians and others concerned with the care for these patients.

ALLOGENEIC STEM CELL TRANSPLANTATION

Patients undergoing an allogeneic stem cell transplantation (alloSCT) are immunocompromised for a prolonged period of time. The susceptibility for bacterial and viral infections is caused by the underlying haematological disease, by the chemotherapy given pre-transplantation, and the immunosuppressive therapy that is necessary to prevent graft versus host disease (GvHD).[99-103]

After a myeloablative alloSCT, defects in B- and T-lymphocyte function can last for 12-24 months.[104-107] In general, T-lymphocyte numbers are reduced during the first three months post-transplantation. The recovery of the $CD4^+$ cells is influenced by the age at which transplantation takes place and whether or not GvHD does develop.[107] Due to toxicity of the treatment, only relatively young patients without serious co-morbidity are eligible for myeloablative stem cell transplantation.
To reduce the risk of complications after an alloSCT, non-myeloablative (or reduced intensity, allo-RIST) conditioning regimens have been developed. In these regimens, the pre-transplantation chemo- or radiotherapy that is used is of less intensity, reducing the transplantation-related morbidity and mortality. This chemotherapy of the allo-RIST regimen reduces the tumour load of the patient less adequately. T-lymphocytes of the donor, however, recognise alloantigens on malignant cells of the patient that are still present, thus enabling an additional anti-tumour effect. Moreover, not all T-lymphocytes of the patient are destroyed by reduced intensity therapy (resulting in a state of so-called mixed chimerism), enabling these residual patient T-lymphocytes to prevent infections. Overall, these characteristics of the allo-RIST SCT make this type of SCT favourable in the elderly patient population.

The prevalent causative organisms of infection post-SCT are *S. pneumoniae* en *Hib*. In the United States, the incidence rate of invasive pneumococcal disease in adults with haematological cancer is 503.1/100.000 persons, with a relative risk of 38.3 (adjusted RR, 95% confidence interval 15.9-92.2) compared to healthy adults.[108] The overall incidence of any bacteraemia post-alloSCT was 0.96 episodes per patient.[102] In the late phase post-transplantation, i.e. more than 100 days after SCT, chronic GvHD is a major determinant for the still increased infection risk. Immune restitution is slowed down in patients with chronic GvHD. Together with the immunosuppressive medication necessary for managing the GvHD this leads to vulnerability for infections. Moreover, chronic GvHD is associated with functional asplenia and deficiency of immunoglobulin IgG2 and IgG4.[109-112] The incidence of an invasive pneumococcal infection in the late phase

post-transplantation was 19/1000 patients in case of GvHD versus 8/1000 patients without GvHD.[113]

Without vaccination, antibody concentrations decline in the first year post-transplantation.[114,115] The loss of immunity post-transplantation in the myeloablative setting depends on the degree of pre-transplantation immunity of the patient, the immune status of the donor and the occurrence of GvHD.[113,115,116] As described above, in patients with chronic GvHD the incidence of invasive pneumococcal infections is higher.[113] Loss of immunity post-transplantation in recipients appears to occur more frequently in vaccinated patients as compared to patients who have experienced natural infection pre-transplantation.[117] Recipients transplanted from donors with high tetanus and *Hib* antibody concentrations have higher tetanus and *Hib* antibody levels post-transplantation, irrespective of the vaccination status of the donor.[118]

In order to reduce the risk of infection, vaccination schedules for stem cell transplant recipients have been designed. These schedules include pneumococcal, *Hib*, meningococcal and DTP vaccines starting at 6-12 months post-transplantation.[99,119,120] For autologous and allogeneic SCT, the guidelines of the CDC and the EBMT differ with respect to the starting point of the vaccination.[99,120] In the guidelines of the EBMT, the first vaccines should be administered at six months post-transplantation, to minimise the time during which patients are unprotected (Table 2).[99]

Table 2. Vaccination schedule after allogeneic stem cell transplantation according to the EBMT

Time since SCT	**6 months**	**8 months**	**10 months**	**12 months**
Vaccine	*Hib*	*Hib*	*Hib*	
	DTP	DTP	DTP	
	PCV-7	PCV-7		PPV-23
	MenC			

SCT = stem cell transplantation, DTP = diphtheria, tetanus and poliomyelitis vaccine
PCV-7 = 7-valent pneumococcal conjugate vaccine, *Hib* = conjugate *Hib* vaccine, MenC = conjugate meningococcal serogroup C vaccine, PPV-23 = 23-valent polysaccharide pneumococcal vaccine

Pneumococcal polysaccharide vaccines (PPV-23) induce low response rates in patients following SCT.[115] In case of chronic GvHD, response rates were only 25%, whereas in the absence of GvHD the response rate was approximately 50%. The response to the conjugated pneumococcal vaccine (PCV-7) is better. After three doses of vaccine, the response rate in SCT patients is approximately 60-70%.[116,121]

In the non-myeloablative (allo-RIST) setting, the efficacy of these vaccines and the timing of start of vaccination after the transplantation had not been studied before and guidelines for vaccination are not yet available. Therefore, we conducted a study in 26 allo-RIST patients to determine the efficacy of pneumococcal, *Hib* and DTP conjugated vaccines in terms of rise in antibody concentrations, in a vaccination schedule starting at 12 months post-transplantation (Table 3). We chose to study the response 12 months post-transplantation because by that time, most patients have stopped using immunosuppressive medication. Due to the long-term use of immunosuppressive therapy for prevention of GvHD, median time from transplantation to immunisation in our patient group was 15 months. By then, more than 70% of the patients reached adequate antibody levels for pneumococcal and *Hib* vaccines. For tetanus toxoid (TT), the response rate was 96% after two doses of vaccine.

Table 3. Vaccination protocol after allo-RIST stem cell transplantation

Time since SCT	**12 months**	**14 months**	**15 months**	**18 months**
Vaccine	*Hib*		*Hib*	*Hib*
	DTP	DTP	DTP	
	PCV-7	PCV-7		PPV-23
		MenC		

SCT = stem cell transplantation, DTP = diphtheria, tetanus and poliomyelitis vaccine
PCV-7 = 7-valent pneumococcal conjugate vaccine, *Hib* = conjugate *Hib* vaccine, MenC = conjugate meningococcal serogroup C vaccine, PPV-23 = 23-valent polysaccharide pneumococcal vaccine

In the studied patient group, 77% suffered from limited or extensive chronic GvHD. Whether a vaccination schedule starting at 6 months post-allo-RIST would be as effective as a schedule starting at 12 months post-transplantation, has to be studied. Recently, a study was published in myeloablative SCT patients in which vaccination as early as 3 months post-transplantation was compared to vaccination from 6 months post-SCT onwards.[122] In the total patient group, 34% of patients suffered from acute GvHD and 44% of chronic GvHD. After three doses of PCV-7, 79% and 82% of the patients reached the chosen threshold of ≥ 0.15 µg/mL pneumococcal IgG after vaccination at 3 or 6 months post-transplantation, respectively. When applying a higher threshold of ≥ 0.5 µg/mL, the percentage of patients reaching this threshold was 56% in the early group and 54% in the late group. In both groups the response rates were lower than in our study. The functional activity of the formed antibodies was studied and found to be adequate.[123] The decline in antibody concentrations was significantly higher in the early vaccination group compared to the late group. At six months after the last dose of PCV-7 vaccine, a trend was seen towards higher antibody concentrations

in the patient group that was vaccinated at 6 months post-transplantation. The risk of early vaccination is that in the non-responding patients, tolerance may be induced, rather than immunological memory.
When receiving a PPV-23 vaccination at 12 months post-transplantation, in the late patient group, the percentage of patients with response was significantly higher (88%) compared to the early patient group (69%, p=0.02).[123] This difference might be explained by the induction of tolerance in the early group. In the allo-RIST patient group, we therefore suggest further studies to evaluate the response at six months post-transplantation, rather than at three months post-transplantation.

In the overall patient group, chronic GvHD was associated with a significantly impaired response to vaccination. Six months after the last dose of PCV-7, one dose of PPV-23 was given. The response in the late group was significantly better than in the early group (88% versus 69%, p=0.02).[123] Furthermore, in patients not responding to PCV-7, after the PPV-23 immunisation 41% did have an adequate rise in antibody concentrations. This implies a booster effect of PPV-23 on the response to PCV-7 serotypes. Unfortunately, in our study, no sera were available after PPV-23 vaccination. A potential booster effect of PPV-23 in the non-myeloablative setting thus remains to be demonstrated.

It is suggested that both vaccination of the donor and vaccination of the patient pre-transplantation could improve the response to vaccination post-transplantation.[116,124] The mechanism would be that memory B- and T-lymphocytes homing to the bone marrow could be transplanted to the recipient. Parkkali *et al.* vaccinated 111 alloSCT donors with DTP and *Hib* vaccines in a period of 2-10 weeks before (full graft) bone marrow harvest.[118] Patients were vaccinated at 3, 6 and 12 months post-transplantation with the same vaccines. At 3 and 6 months post-transplantation, all patients used immunosuppressive therapy (cyclosporine A and/or methylprednisolone). At 12 months post-transplantation, approximately 60% of the patients in both groups still used immunosuppressive medication. The percentage of patients suffering from acute GvHD was approximately 30%, as was the percentage of patients with chronic GvHD. Stem cell recipients of an immunised donor had higher antibody levels than did recipients of unimmunised donors from 6 months post-transplantation onwards. The response of the immunised donor itself also had an effect on the response of the recipient: a higher antibody response after vaccination in the donor led to higher responses in the recipient. It has to be noted that enrichment of $CD34^+$ stem cells negatively selects for memory lymphocytes and thus counteracts the benefits of donor vaccination. From the study of Storek *et al.* it can be concluded that for protein-conjugated vaccines, a combination of vaccination of the donor and vaccination of

the patient pre- and post-transplantation induces the best response rates.[124] Whether these immunisation strategies are effective in the allo-RIST setting too, will have to be studied in the future.

As for asplenic patients described above, the conjugated polysaccharide vaccines that are currently being developed are of clinical importance. The conjugated pneumococcal vaccine used in the Netherlands is the heptavalent vaccine with polysaccharides of the pneumococcal serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. These seven serotypes are responsible for 64% of the invasive pneumococcal infections in young children (< 2 years) in the Netherlands. In adults however, invasive pneumococcal infections are often caused by the serotypes 1, 3, 7, 8 and 12, all of which are not included in the heptavalent vaccine. At the moment, 11-valent and 13-valent vaccines are being developed that contain the latter pneumococcal serotypes. The 13-valent vaccine has received authorisation for the European market. Whether these vaccines are effective in SCT patients will have to be determined.

Future perspectives

The study in allo-RIST patients was conducted with a vaccination schedule starting at 12 months post-transplantation. Based on data from literature, immunisation schedules starting at 3 and 6 months post-transplantation could already be effective and therefore should also be studied in these allo-RIST patients. If patients reach adequate antibody levels earlier post-transplantation, the period in which patients are prone to infections may be shortened and infectious complications reduced.

In our allo-RIST population, the effect of vaccination of the donor was not studied. Studies have shown that immunisation of the donor before harvest of stem cells leads to higher antibody concentrations in recipients. Therefore, the efficacy of vaccination of the donor in terms of antibody levels in allo-RIST recipients warrants investigation. Beside donor vaccination, vaccination of the recipient pre-transplantation can induce higher antibody concentrations post-transplantation. This vaccination strategy can be performed together with donor vaccination in upcoming allo-RIST vaccinations.

As for asplenic patients, the new 13-valent pneumococcal conjugate vaccine might be of additional value in the allo-RIST patient group. The response to this vaccine in terms of rise in antibody concentrations against pneumococcal vaccine-serotypes can be determined as soon as this vaccine is introduced on the Dutch market. We propose long-term follow-up of these patients to determine the incidence of (pneumococcal) infections in these patients post-vaccination and thus the efficacy of these vaccines in this patient group.

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Chapter 8:

Nederlandse samenvatting

INLEIDING

Dit proefschrift beschrijft het effect van vaccinatie bij patiënten met verschillende afweerstoornissen. De patiëntengroepen die in dit proefschrift worden beschreven, zijn mensen zonder milt (asplenie; a=niet, spleen=milt), borstkankerpatiënten die een behandeling met chemotherapie krijgen en patiënten die een allogene stamceltransplantatie (van een donor) hebben ondergaan. Patiënten uit al deze groepen hebben meer kans op een infectie en deze infecties verlopen ernstiger dan bij gezonde volwassenen.
Het beschreven onderzoek speelt zich af op drie onderdelen van de interne geneeskunde: de hematologie (bloedziekten), de infectiologie (infectieziekten) en de oncologie (tumoren).

Eerst zal een korte inleiding gegeven worden over de werking van het menselijk afweersysteem en de werking van vaccins. Daarna worden de verschillende ziektebeelden besproken. Ten slotte zullen de resultaten van de verschillende onderzoeken beknopt worden weergegeven.

HET IMMUUNSYSTEEM

Het afweersysteem (ook wel: immuunsysteem) van een menselijk lichaam heeft als belangrijkste taak het verdedigen van het lichaam tegen infecties en bestaat uit twee systemen: een aangeboren en een verworven systeem. Het aangeboren immuunsysteem is al meteen bij de geboorte aanwezig en kan direct functioneren. Het verworven immuunsysteem reageert op specifieke infecties. De daardoor opgebouwde immuniteit wordt dus verworven.
Wanneer bacteriën of virussen het lichaam binnendringen (een infectie), treedt het aangeboren systeem als eerste in werking. Dit systeem bestaat uit verschillende soorten (witte) bloedcellen en eiwitten, die de bacterie kunnen doden. De primaire functie van het aangeboren systeem is het maken van onderscheid tussen ‘schadelijk’ en ‘niet schadelijk’. In de darm bijvoorbeeld zitten meer dan duizend miljard bacteriën, maar de meeste daarvan zijn niet schadelijk voor het lichaam. Het aangeboren immuunsysteem kan, door middel van het herkennen van grove patronen (structuren) op de bacterie of het virus, bepalen of het gaat om een schadelijke indringer en deze indringer vervolgens doden.

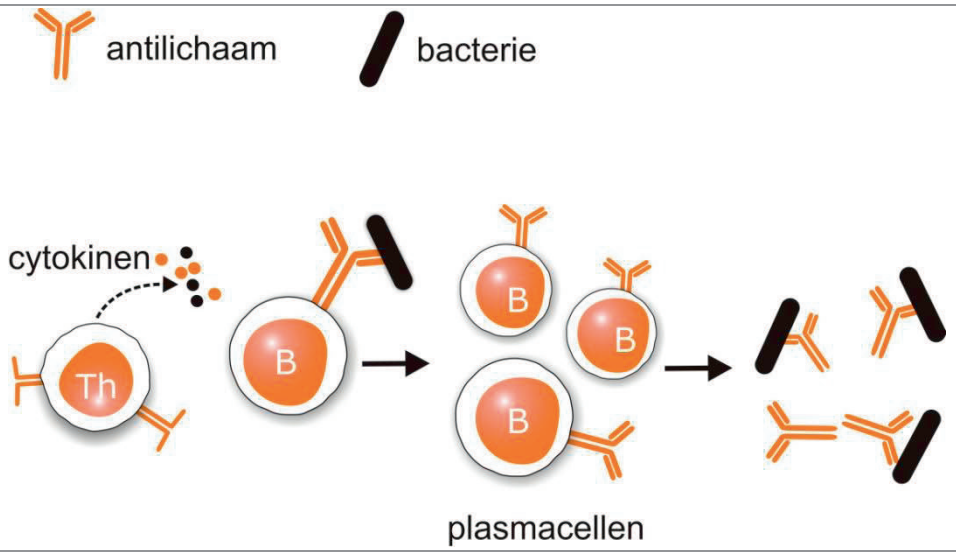
Het verworven immuunsysteem komt iets later op gang. Dit systeem bestaat voornamelijk uit twee soorten witte bloedcellen (lymfocyten) die B-cellen en T-cellen worden genoemd. De B-cellen worden gemaakt in het beenmerg (de B van Beenmerg); de T-cellen rijpen uit in de thymus (de T van Thymus, ook wel

zwezerik genoemd). De afweer verzorgd door de B-cel is vooral gericht tegen bacteriën, de afweer tegen virussen gaat met name via T-cellen. Iedere B- of T-cel kan heel specifiek één klein stukje van één bacterie of virus (het antigeen) herkennen. Elk mens komt in zijn/haar leven in aanraking met honderden verschillende bacteriën en virussen. Om zich hiertegen te kunnen beschermen, zijn dan ook miljoenen verschillende lymfocyten nodig.

Het aangeboren en verworven immuunsysteem werken nauw samen. Het aangeboren immuunsysteem bestaat onder andere uit een complex van eiwitten dat complementsysteem wordt genoemd. Het complementsysteem kan micro-organismen direct doden. Micro-organismen kunnen ook makkelijker worden 'opgegeten' (fagocyteren) wanneer er complementeiwitten op zitten. Het complement kan ook B-cellen, en dus het verworven immuunsysteem, activeren.

De B-cel kan een bacterie herkennen via een eiwit op het oppervlak van de B-cel, de antistof (ook wel het antilichaam genoemd). Iedere B-cel heeft een eigen, unieke antistof op zijn oppervlak. Deze antistof herkent een klein deel van de buitenste celwand van de bacterie. Wanneer een B-cel zijn specifieke bacterie 'ziet', hecht de antistof van de B-cel aan de celwand van de bacterie (zie Figuur 1). Door de hechting gaat er een signaal naar de kern van de B-cel. Dit signaal zorgt ervoor, samen met de hulp van T-helper cellen, dat de B-cel zich gaat delen. Door deze deling worden grote aantallen speciale B-cellen, zogenaamde plasmacellen, gemaakt. Deze plasmacellen produceren op hun beurt grote hoeveelheden van de specifieke antistof. Deze antistoffen blijven niet op het oppervlak van de plasmacel, maar worden uitgescheiden en verspreiden zich in de bloedbaan. De antistoffen hechten zich vervolgens aan de bacteriën die zich in de bloedbaan bevinden, waarna andere cellen van het immuunsysteem zich aan de antistof kunnen hechten en de bacterie kunnen opruimen.

Figuur 1. De B-cel produceert antistoffen met behulp van een helper-T-cel



Een B-cel herkent met zijn specifieke antistof een bacterie. Met de hulp van signaalmoleculen (cytokinen) van een T-cel (een T-helper-cel, Th), kan de B-cel zich gaan delen. Hierdoor worden er grote hoeveelheden antilichamen gemaakt, die zich aan andere bacteriën kunnen hechten. Zo kunnen deze bacteriën worden gedood.

Bij de mens komen 5 verschillende soorten antistoffen voor: IgM, IgG, IgA, IgD en IgE. Ig is de afkorting voor immuunglobuline (antistof). Elke antistof heeft een eigen specifieke functie. IgM en IgG spelen een belangrijke rol bij de afweer tegen bacteriën. IgA is de antistof die zich in speeksel, tranen en moedermelk bevindt. De functie van IgD is nog niet goed opgehelderd. IgE komt vrij als er sprake is van een worminfectie of bij een allergische reactie.
De eerste keer dat een B-cel in aanraking komt met zijn specifieke bacterie, duurt het een aantal dagen voordat de cel zich gedeeld heeft en er genoeg antistoffen zijn geproduceerd. Dit heet de primaire antistofrespons. Er wordt in de primaire antistofrespons voornamelijk IgM geproduceerd. Nadat deze respons voorbij is, blijft een aantal geheugen B-cellen over, die in het lichaam blijven circuleren. Bovendien neemt tijdens de primaire respons de gevoeligheid van de antistof voor de bacterie nog toe; dit heet toename van de affiniteit en daardoor kan de antistof nog beter binden aan de bacterie. Wanneer het lichaam voor een tweede keer wordt geïnfecteerd met dezelfde bacterie en de B-cellen opnieuw in actie moeten komen (de zogenaamde secundaire antistofrespons, waarbij vooral IgG wordt gemaakt), verloopt dit proces veel sneller door de al aanwezige geheugen B-cellen. Ook hechten de antistoffen nóg beter aan de bacterie (affiniteit). Hierdoor kan een infectie sneller en beter opgeruimd worden.

In de meeste gevallen heeft de B-cel hulp nodig van T-cellen om een bacterie op te ruimen; dit heet de T-cel afhankelijke respons. Er zijn echter bacteriën die om de celwand heen nog weer een kapsel van suikers hebben, waartegen de B-cel antistoffen kan maken zónder de hulp van T-cellen. Dit heet de T-cel onafhankelijke respons. De B-cellen die deze T-cel onafhankelijke respons kunnen veroorzaken bevinden zich vooral in de milt.

Het afweersysteem dat bestaat uit B-cellen en antistoffen, heet het humorale afweersysteem. De afweer tegen virussen wordt verzorgt door T-cellen en heet de cellulaire afweer. T-cellen kunnen worden onderverdeeld in T-helper cellen (zij helpen de B-cellen bij het produceren van antistoffen) en cytotoxische T-cellen. Deze laatste groep T-cellen is verantwoordelijk voor het doden van cellen die geïnfecteerd zijn met een virus. Dit proefschrift richt zich vooral op de humorale afweer.

VACCINATIE

Het principe van vaccinatie berust op het inspuiten van een klein onderdeel van een bepaald virus of bacterie. Dit onderdeel kan een suiker (polysaccharide) of een eiwit (proteïne) zijn. Doordat het slechts een klein deel van de bacterie of het virus is, kan een mens er niet ziek van worden, maar het afweersysteem wordt wel gestimuleerd om antistoffen en geheugencellen te maken. Er is een aantal vaccins waarbij een levend verzwakt virus of bacterie wordt ingespoten, dit is bijvoorbeeld het geval bij het BMR (bof, mazelen, rode hond) vaccin. Wanneer een gevaccineerde patiënt later besmet wordt met de bacterie of het virus waartegen hij is gevaccineerd, worden snel specifieke antistoffen gemaakt, waardoor de infectie geen kans meer heeft om ziekte te veroorzaken.

De daadwerkelijke, klinische effectiviteit van vaccinatie kan alleen worden bepaald wanneer in een grote groep gevaccineerde patiënten jarenlang wordt gevolgd hoeveel infecties er optreden. Wanneer in de gevaccineerde groep minder infecties optreden dan in een vergelijkbare groep patiënten die niet is gevaccineerd, kan worden geconcludeerd dat vaccinatie effectief is. Omdat dergelijk onderzoek veel tijd vergt en er grote groepen patiënten nodig zijn, is dit niet altijd mogelijk. Daarom zijn er surrogaat-maten voor effectiviteit van vaccinatie (bescherming) gedefinieerd. Voor elk vaccin is een drempelwaarde vastgesteld voor de antistofconcentratie die bereikt moet worden. Wanneer een patiënt na vaccinatie een antistofconcentratie heeft die groter of gelijk is aan de drempelwaarde, gaat men ervan uit dat de patiënt beschermd is.

De meeste bacteriën hebben alleen een celwand om zich heen. Er zijn echter bacteriën die buiten de celwand, als extra bescherming tegen het immuunsysteem, nog een kapsel van suikers om zich heen hebben. Belangrijke gekapselde bacteriën zijn de pneumococ (officiële naam: *Streptococcus pneumoniae*), de *Haemophilus* bacterie (*Hib,* officiële naam: *Haemophilus influenzae* type b) en de meningococ (officiële naam: *Neisseria meningitidis*). Deze drie bacteriën veroorzaken onder andere longontsteking, oorontsteking en hersenvliesontsteking.

Er bestaan twee soorten vaccins die gebruikt worden voor bescherming tegen gekapselde bacteriën. De oudste vaccins bestaan uit de suikermoleculen van het kapsel van de bacteriën. Deze vaccins kunnen voldoende antistoffen opwekken, maar ze wekken geen geheugen B-cellen op. Dat betekent dat na een aantal jaren de antistoffen uit het lichaam zijn verdwenen en er opnieuw moet worden gevaccineerd. Een ander belangrijk nadeel is dat deze vaccins niet werken bij kinderen jonger dan 2 jaar en ook minder goed bij patiënten zonder milt.
Daarom zijn er vaccins ontwikkeld waarbij een suiker van de bacterie is gekoppeld aan een eiwit. Dit proces heet conjugatie en het vaccin heet dan ook een geconjugeerd vaccin. Door de toevoeging van dit eiwit worden er wél geheugen B-cellen gevormd. Deze vaccins zorgen ook voor voldoende antistofvorming in kleine kinderen. Het Rijksvaccinatieprogramma (RVP) in Nederland bevat dan ook een groot aantal geconjugeerde vaccins.

De pneumococ is één van de belangrijkste verwekkers van een longontsteking. Ter bescherming tegen de pneumococ zijn zowel ongeconjugeerde als geconjugeerde vaccins op de markt. Er zijn meer dan 90 verschillende soorten (serotypen) van de pneumococ bekend. Het ongeconjugeerde vaccin biedt bescherming tegen 23 serotypen, maar heeft als nadeel dat het niet werkt in kleine kinderen en mensen zonder milt en er wordt geen geheugen opgebouwd. Het conjugaatvaccin biedt slechts bescherming tegen de 7 belangrijkste serotypen, maar biedt, in tegenstelling tot het ongeconjugeerde vaccin, wel bescherming in kleine kinderen en miltlozen. Bij vaccinatie met een conjugaatvaccin wordt wel geheugen opgebouwd. Vanwege de verschillende voordelen van de geconjugeerde en ongeconjugeerde pneumococcenvaccins, wordt bij patiënten met een afweerstoornis steeds vaker een combinatie van beide vaccins gebruikt.

AFWEZIGHEID VAN DE MILT (ASPLENIE)

De milt is vroeger als de zetel van emoties en passies omschreven. In de Talmud bijvoorbeeld, de Joodse uitleg van de eerste bijbelboeken, wordt de milt

omschreven als de plek waar de lach ontstaat. In latere eeuwen werd de milt vooral gezien als de zetel van de zwaarmoedigheid.
Strikt medisch gesproken is lange tijd gedacht dat de milt geen enkele belangrijke functie in het lichaam vervulde. Vanaf het begin van de 20ste eeuw zij er echter steeds meer aanwijzingen gekomen dat de milt een aantal belangrijke functies heeft in de regulatie van rode bloedcellen en in de afweer tegen infecties.
Deze functies zijn:

1) het filteren van oude en vervormde rode bloedcellen uit de bloedcirculatie
2) het filteren van bacteriën uit de bloedbaan
3) productie van antistoffen tegen bacteriën met een kapsel om zich heen

Het ontbreken van de milt leidt dus met name tot een verhoogde gevoeligheid voor gekapselde bacteriën. De meest belangrijke bacterie waar mensen zonder milt gevoelig voor zijn, is de pneumococ. Twee andere bacteriën die een grote rol spelen, zijn *Hib* en de meningococ. Wanneer een patiënt zonder milt een infectie met één van deze bacteriën oploopt, kan hij/zij binnen enkele uren overlijden. Het is dan ook van het grootste belang dat een patiënt zonder milt zich bij koorts onmiddellijk wendt tot een arts en dat direct wordt gestart met antibiotica om de infectie te bestrijden. Om echter te voorkómen dat de patiënt een infectie krijgt, zal hij/zij tegen deze drie bacteriën moeten worden gevaccineerd. De geconjugeerde vaccins, die in het RVP worden gebruikt, zijn ook voor deze patiënten van belang.

De redenen, waarom een patiënt zijn/haar milt kwijtraakt, zijn heel divers. Dit kan onder andere gebeuren door een trauma, bijvoorbeeld een auto-ongeluk. Wanneer de milt beschadigd raakt en hevig bloedt, moet deze verwijderd worden. Er zijn verschillende andere aandoeningen waarbij de milt verwijderd wordt als onderdeel van de behandeling. Bij patiënten die antistoffen maken tegen hun eigen bloedplaatjes (idiopathische trombocytopenische purpura, oftewel ITP) of die afwijkende rode bloedcellen hebben (sikkelcelziekte, sferocytose), wordt de milt zo groot dat het klachten veroorzaakt. Operatieve miltverwijdering is dan noodzakelijk.

Het heeft de voorkeur om patiënten voorafgaand aan de miltverwijdering te vaccineren. Wanneer dat niet mogelijk is, bijvoorbeeld bij een trauma, dan moet de patiënt vanaf twee weken na de operatie worden gevaccineerd. Kinderen krijgen daarnaast tot hun 16e of 18e jaar een onderhoudsbehandeling met antibiotica om infecties te voorkómen.

CHEMOTHERAPIE

Patiënten met kanker die behandeld worden met chemotherapie hebben een groter risico op een infectie met het griepvirus (influenza virus). Ze moeten vaker opgenomen worden in het ziekenhuis en de kans om te overlijden aan de gevolgen van de griep is 4x groter dan bij andere volwassenen. Daarom is de griepvaccinatie (de 'griepprik') van groot belang bij deze patiëntengroep. Helaas worden deze patiënten vaak niet gevaccineerd tegen de griep. Hun behandelend arts is soms niet op de hoogte van de noodzaak van vaccinatie, de patiënt is bang voor bijwerkingen van de vaccinatie en de arts weet niet goed op welk moment de vaccinatie moet worden geven tijdens de chemotherapie.
In het algemeen wordt geadviseerd om de griepvaccinatie te geven voorafgaand aan de start van de chemotherapie. Omdat de griepvaccinatie seizoensgebonden is, is dit niet altijd mogelijk.
Er is nog weinig onderzoek gedaan naar het effect van griepvaccinatie tijdens chemotherapie en deze studies zijn vaak al meer dan 30 jaar oud. In de laatste jaren zijn zowel de chemotherapie en het griepvaccin veranderd. Ook zijn er nieuwe middelen aan de behandeling van kanker toegevoegd, die invloed hebben op de afweer. Een voorbeeld hiervan is Rituximab®, een middel dat de B-cellen van een mens vernietigt.
Er bestaan geen landelijke richtlijnen waarin wordt beschreven wat het beste tijdstip is om patiënten tijdens de chemokuren te vaccineren.

STAMCELTRANSPLANTATIE

Patiënten met bloed- of lymfklierkanker (leukemie of lymfoom) worden vaak behandeld met een stamceltransplantatie (SCT).
Het principe van een transplantatie berust op het vernietigen van het beenmerg van de patiënt door middel van chemotherapie, eventueel in combinatie met bestraling, waarna stamcellen van de patiënt zelf of van een donor worden teruggegeven aan de patiënt. Deze stamcellen kunnen vervolgens weer uitrijpen tot alle verschillende bloedcellen van het lichaam: de rode bloedcellen, de witte bloedcellen en de bloedplaatjes.
Het beenmerg van de patiënt wordt vernietigd door chemotherapie en bestraling om de kankercellen van de patiënt te verwijderen. Als bijwerking worden ook alle andere, gezonde, bloedcellen vernietigd. Hierdoor heeft de patiënt zelf nauwelijks tot geen afweer meer en is dus vatbaar voor infecties.
Een SCT kan worden verricht met cellen van de patiënt zelf, dit heet een autologe transplantatie (autos = zelf in het Grieks). Een SCT kan ook worden gedaan met cellen van een donor (meestal een eerstegraads familielid). Dit heet een allogene SCT (allos = ander).

In dit proefschrift wordt onderzoek gedaan bij patiënten die een allogene stamceltransplantatie hebben ondergaan.

Nadat de patiënt de stamcellen van de donor heeft ontvangen, moet worden voorkómen dat het immuunsysteem van de patiënt de cellen van de donor afstoot. Deze afstotingsreactie heet Host versus Graft Disease (HvGD, letterlijk vertaald: gastheer versus transplantaat ziekte). Bij stamceltransplantatie zal dit niet zo gauw voorkomen, omdat het immuunsysteem van de patiënt ernstig beschadigd is door de bestraling en chemotherapie. De omgekeerde reactie (Graft versus Host Disease, GvHD, transplantaat versus gastheer) is minstens zo erg: de B- en T-cellen in het transplantaat herkennen de patiënt als “lichaamsvreemd” en gaan daarop reageren. Om een dergelijke reactie tegen te gaan, moet de patiënt maanden tot jaren worden behandeld met afweeronderdrukkende medicijnen. Door het onderdrukken van de afweer wordt de werking van B- en T-cellen geremd, waardoor deze patiënten een grotere kans hebben op het krijgen van een infectie.
Relatief jonge patiënten met bloedkanker kunnen een allogene transplantatie, voorafgegaan door een zware chemokuur, aan en de bijwerkingen zijn acceptabel. Bij oudere patiënten zijn de bijwerkingen van de behandeling zo ernstig dat een zware chemokuur eigenlijk niet meer toegepast kan worden. Daarom zijn er de afgelopen jaren behandelingen ontwikkeld waarin de chemotherapie voorafgaand aan de transplantatie minder intensief is. Dit heet reduced-intensity stamceltransplantatie (allo-RIST transplantatie). Met deze vorm van transplantatie kunnen ook oudere patiënten worden behandeld.

Vanwege het risico op infecties zijn er internationale richtlijnen opgesteld waarin wordt beschreven welke vaccins dienen te worden gegeven aan patiënten na SCT. De eerste maanden na transplantatie heeft het weinig zin om te starten met vaccinatie, omdat vanwege de onderdrukte afweer er nog geen respons zal kunnen optreden. Over het algemeen wordt er 6-12 maanden na transplantatie gestart met vaccinatie. Patiënten worden, net als mensen zonder milt, gevaccineerd tegen de pneumococ, *Hib* en de meningococ. Daarnaast wordt ook het DTP-vaccin (difterie, tetanus en polio) aan deze patiënten gegeven.

SAMENVATTING VAN DIT PROEFSCHRIFT

Na een inleidend hoofdstuk, wordt de respons op vaccinatie beschreven bij mensen zonder milt (hoofdstuk 2 t/m 4), bij patiënten die chemotherapie ondergaan (hoofdstuk 5) en na stamceltransplantatie (hoofdstuk 6).

MENSEN ZONDER MILT

Mensen zonder milt hebben een levenslang verhoogd risico op infecties met gekapselde bacteriën. Deze infecties kunnen bij de afwezigheid van de milt in enkele uren fataal verlopen. Daarom is het van belang om deze mensen te vaccineren, van antibiotica te voorzien en te wijzen op de noodzaak om zich bij koorts te melden bij een arts. In **Hoofdstuk 2** wordt beschreven hoe het is gesteld met de voorlichting en vaccinatie van patiënten zonder milt in de regio Utrecht. Hiervoor zijn alle huisartsen verbonden aan het Sint Antonius Ziekenhuis benaderd om hun patiënten zonder milt te laten deelnemen aan dit onderzoek. Aan deze patiënten is gevraagd of ze op de hoogte waren van de maatregelen die zij moeten nemen bij koorts, of ze gevaccineerd waren en of ze antibiotica in huis hadden. In andere landen zijn dergelijke studies al eerder verricht; de resultaten zijn niet bemoedigend. In Nederland heeft dit onderzoek echter nog niet eerder plaatsgevonden. Het blijkt dat 43% van de ondervraagde patiënten zonder milt niet op de hoogte is van het feit dat ze een verhoogd risico hebben op het krijgen van ernstige infecties. Ook weet 50% niet dat ze zich bij koorts onmiddellijk tot een arts moeten wenden.
Het pneumococcenvaccin is van de drie benodigde vaccins het langst op de markt. Het blijkt dat 88% van de patiënten in het verleden is gevaccineerd tegen de pneumococ. Slechts 34% van de patiënten heeft echter ooit een *Hib*-vaccin gehad en 27% een meningococcenvaccin. Wanneer er wordt gekeken of patiënten alle drie benodigde vaccins hebben gekregen volgens internationale richtlijnen, blijkt dat maar 22% van de patiënten volgens deze richtlijnen is behandeld. Er wordt dan ook geconcludeerd dat de zorg voor patiënten zonder milt in Nederland kan (en moet) worden verbeterd.

Na deze inventarisatie is een inhaal vaccinatieschema opgesteld voor iedere miltloze patiënt. Voorafgaand en na vaccinatie worden antistofconcentraties gemeten. Er wordt onderzocht of patiënten na vaccinatie een antistofconcentratie in het bloed hebben die boven een bepaalde drempelwaarde ligt. Deze afkapwaarde wordt internationaal aangehouden als maat voor bescherming. In **Hoofdstuk 3** wordt onderzoek naar het effect van pneumococcen- en *Hib*-vaccinatie beschreven. Patiënten worden gevaccineerd volgens het schema in

Tabel 1. In Nederland werden tot nu toe alleen ongeconjugeerde pneumococcenvaccins geadviseerd aan patiënten zonder milt. Zoals te zien in Tabel 1 wordt er in deze studie tweemaal een geconjugeerd vaccin toegediend, waarna er een éénmalige vaccinatie met het ongeconjugeerde vaccin volgt.

Tabel 1. Vaccinatieschema voor patiënten zonder milt

Tijdstip	**0**	**+ 6 weken**	**+ 6 maanden**
Vaccin	PCV-7	PCV-7	PPV-23
	Hib	MenC	

PCV-7 = conjugaat pneumococcenvaccin
Hib = *Hib* conjugaat vaccin
MenC = conjugaat meningococcenvaccin
PPV-23 = ongeconjugeerd pneumococcenvaccin

Van de 54 patiënten die de pneumococcenvaccins krijgen toegediend, behaalt 82% een goede antistofrespons (drempelwaarde voor een voldoende antistofconcentratie ≥ 1.0 microgram IgG per milliliter bloed (µg/mL)) na 1 pneumococcen conjugaatvaccin. Na het tweede conjugaatvaccin en het ongeconjugeerde vaccin stijgen deze percentages naar respectievelijk 85% en 92%. Het vaccinatieschema met pneumococcenvaccins blijkt dus effectief te zijn in patiënten zonder milt. Het *Hib*-vaccin wordt éénmalig gegeven aan 92 patiënten. Na vaccinatie haalt 97% de drempelwaarde van ≥ 1.0 µg/mL. Dit vaccin is dus effectief in het behalen van hoge antistofconcentraties bij patiënten zonder milt.

In **Hoofdstuk 4** wordt het effect van het meningococcen serogroep C (MenC) conjugaatvaccin in patiënten zonder milt onderzocht. Na één vaccinatie behaalt slechts 67% van de 117 gevaccineerde patiënten de gekozen drempelwaarde van ≥ 2.0 µg/mL MenC antistoffen. Dit percentage ligt dus lager dan het percentage behaald na pneumococcen- en *Hib*-vaccinatie. De patiënten die een onvoldoende antistofrespons hebben, worden nogmaals gevaccineerd met hetzelfde vaccin. Na deze revaccinatie haalt 59% alsnog de drempelwaarde. Behalve naar de hoeveelheid antistoffen, kijken we in deze studie ook naar de kwaliteit van de antistoffen. Het blijkt dat het conjugaat meningococcenvaccin in patiënten zonder milt leidt tot kwalitatief minder goede antistoffen dan in gezonde volwassenen. De redenen hiervoor zijn niet helemaal duidelijk. Na revaccinatie neemt de affiniteit van de antistoffen iets, hoewel niet veel, toe. Het is te overwegen om patiënten die na 1 vaccinatie de drempelwaarde niet halen, te revaccineren.

In **Hoofdstuk 5** wordt de respons op het influenza virus (griepvirus) vaccin beschreven in vrouwen met borstkanker die ten tijde van de vaccinatie worden behandeld met in totaal 6 chemokuren die telkens 3 weken duren. Patiënten

worden ingedeeld in een groep die vroeg tijdens de chemokuur een vaccinatie krijgt (dag 4 van de kuur) en een groep die laat tijdens de kuur wordt gevaccineerd (dag 16). In totaal wordt de respons gemeten in 38 vrouwen met borstkanker. Dit wordt vergeleken met de respons in 24 gezonde vrouwelijke vrijwilligers. Het blijkt dat de respons op vaccinatie in borstkanker patiënten veel minder goed is dan in gezonde vrijwilligers.

Het griepvaccin bevat 3 verschillende influenza-stammen. De gezonde vrijwilligers halen de drempelwaarde in 67-100% van de gevallen, terwijl de borstkankerpatiënten in 40-58% van de gevallen deze drempelwaarde halen. Het blijkt dat de patiënten die vroeg worden gevaccineerd een betere respons hebben dan de patiënten die op dag 16 worden gevaccineerd. Omdat de aantallen patiënten klein zijn, kan er slechts gesproken worden van een trend die zichtbaar is; de resultaten zijn niet statistisch significant. Graag hadden we een grotere groep vrouwen onderzocht, maar ten tijde van het onderzoek kwam het vaccin tegen de Mexicaanse griep beschikbaar. Dat laatste vaccin diende zo snel mogelijk gegeven te worden en daardoor stopte onze studie. Het onderzoek zal daarom moeten worden herhaald in een grotere groep patiënten om te zien of deze conclusies dan ook kunnen worden getrokken. Tot nu toe was de standaard dat patiënten voorafgaand aan start van de chemotherapie werden gevaccineerd. Vaccinatie tijdens de chemotherapie werd nauwelijks toegepast, en indien het wel werd toegepast dan was dat met name in de derde (en laatste) week van een chemotherapiecyclus. Wanneer in een grotere groep patiënten zal blijken dat vaccinatie in de eerste week van een chemotherapiecyclus daadwerkelijk leidt tot een betere respons, zal moeten worden overwogen of het huidige vaccinatie-advies (in de derde week van de kuur) moet worden aangepast.

In **Hoofdstuk 6** worden de antistofconcentraties voor en na vaccinatie gemeten in 26 patiënten die een allo-RIST stamceltransplantatie hebben ondergaan. Antistoffen tegen pneumococcen, *Hib* en tetanus zijn gemeten. Het vaccinatieschema van deze studie, zoals vermeld in Tabel 2, start 12 maanden na transplantatie. Antistoffen worden bepaald voorafgaand aan de eerste vaccinatie en na de tweede DTP vaccinatie. In de eerste maanden na transplantatie is de afweer dusdanig verminderd (er zijn dan zeer weinig B-cellen aanwezig) dat men verwacht dat vaccinatie niet effectief zal zijn. Daarom wordt in het algemeen geadviseerd om 6-12 maanden na transplantatie te starten met vaccinatie.
Na vaccinatie met twee conjugaat pneumococcenvaccins haalt 62% van de patiënten de drempelwaarde van ≥ 1.0 µg/mL. Vaccinatie met het *Hib*-vaccin leidt in 77% van de patiënten tot het behalen van de drempelwaarde van ≥ 1.0 µg/ml *Hib* antistoffen. Voor tetanus wordt een andere maat gehanteerd; de drempel ligt

daarbij op 0.1 internationale eenheid per milliliter bloed (IU/mL). Deze drempel wordt op één patiënt na door alle patiënten gehaald (96%). Ongeveer een kwart van de patiënten heeft dus een onvoldoende respons op de pneumococcen- en *Hib*-vaccinatie. Het blijkt dat een groot deel van deze patiënten voorafgaand aan transplantatie werd behandeld met hetzelfde middel dat de afweer sterk onderdrukt. Het lijkt er dus op dat dit middel, fludarabine, en het onderliggende ziektebeeld waarvoor dit middel wordt gebruikt, de uitkomst van vaccinatie kan beïnvloeden. In het algemeen kan worden geconcludeerd dat vaccinatie vanaf 12 maanden na allo-RIST transplantatie leidt tot een voldoende antistofrespons in de meerderheid van deze patiënten.

Tabel 2. Vaccinatieschema na allo-RIST stamceltransplantatie

Tijdstip	**1 jaar**	**+ 2 weken**	**+ 8 weken**	**+ 12 weken**	**+ 26 weken**
Vaccin	DTP		DTP	DTP	
	Hib			*Hib*	*Hib*
		PCV-7	PCV-7		PPV-23
			MenC		

DTP = difterie, tetanus, polio vaccin
Hib = conjugaat *Hib*-vaccin
PCV-7 = conjugaat pneumococcenvaccin
PPV-23 = ongeconjugeerd pneumococcenvaccin
MenC = conjugaat meningococcenvaccin

AANBEVELINGEN VOOR TOEKOMSTIG ONDERZOEK:

1) In 2010 zal een landelijke richtlijn voor patiënten zonder milt worden gepubliceerd zodat er een éénduidig vaccinatiebeleid in Nederland kan worden gevoerd. Het meten van antistofwaarden na vaccinatie is een surrogaat-maat voor het meten van bescherming; de daadwerkelijke bescherming van mensen zonder milt kan worden vastgesteld wanneer patiënten die gevaccineerd zijn minder infecties oplopen in de jaren daarna dan patiënten die niet zijn gevaccineerd. Het is dus zaak om vanaf nu een goed registratiesysteem te ontwikkelen waarin alle infecties bij mensen zonder milt kunnen worden vastgelegd.
2) Sinds december 2009 is er een nieuw conjugaat pneumococcenvaccin op de markt. Dit vaccin biedt bescherming tegen 13 pneumococcen serotypen (het oudere vaccin bevat 7 serotypen). Het is nog niet onderzocht of dit vaccin effectief is bij mensen zonder milt en bij patiënten na stamceltransplantatie. Dit zal de komende tijd uitgezocht moeten worden. Indien dit vaccin effectief is, zal moeten worden overwogen of het in het vaccinatie schema kan worden opgenomen.
3) De studie bij borstkankerpatiënten zal moeten worden herhaald in een grotere groep patiënten om te zien of het effect van vaccinatie daadwerkelijk beter is in patiënten die vroeg tijdens de chemotherapie worden gevaccineerd.
4) Het effect van vaccinatie kan in sommige gevallen worden verhoogd door toevoeging van een zogenaamd ‘adjuvans’ (een stof die ervoor zorgt dat het afweersysteem extra wordt geactiveerd). Of het gebruik van adjuvantia in griepvaccins bij patiënten die chemotherapie ondergaan leidt tot hogere antistofconcentraties na vaccinatie zal moeten blijken uit nader onderzoek.
5) Voor patiënten na stamceltransplantatie geldt: er zijn aanwijzingen dat het vaccineren van de donor voorafgaand aan het afnemen van stamcellen ervoor zorgt dat er hogere antistofconcentraties bij de patiënt worden gevonden. Ook vaccinatie van de patiënt zelf voorafgaand aan transplantatie leidt tot hogere antistofconcentraties na vaccinatie. Deze gegevens komen voort uit studies bij transplantaties waarbij het beenmerg volledig is uitgeschakeld. Of vaccinatie van de donor en patiënt voorafgaand aan transplantatie ook effectief is bij een allo-RIST transplantatie, zal moeten worden onderzocht.