Pneumococcal vaccination in inflammatory rheumatic disease and in splenectomy patients. From antibody response to memory cells.

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LUND
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DOCTORAL DISSERTATION

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Faculty opponent
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Abstract

Objectives:

The overall aim of the dissertation is to examine antibody response to immunization with pneumococcal vaccine in patients with inflammatory rheumatic disease (IRD), in relation to disease-modifying antirheumatic drug (DMARD) treatments, and in postsplenectomy patients.

Methods:

(I) Splenectomized patients without previous pneumococcal conjugate vaccine (PCV) immunization were invited to receive one dose 13-valent PCV (PCV13). Blood was drawn before and 4-6 weeks after PCV13. Serotype-specific antibody responses were determined using a multiplex fluorescent microsphere immunoassay (MFMI). **(II and III)** Consecutive patients with systemic vasulitis, rheumatoid arthritis (RA), and primary Sjögren's syndrome (pSS), and healthy controls (HC) were invited to receive immunization with one dose PCV13. Serotype 6B and 23F IgG were determined before and 4-6 weeks after PCV13 using enzyme-linked immunosorbent assay (ELISA) and functionality of antibodies (23F) with an opsonophagocytic activity (OPA) assay. Positive antibody response (AR) was defined as ≥2-fold rise in pre- to postvaccination IgG. **(IV)** Patients with RA or systemic vasculitis and HC were invited to receive PCV and a booster dose with 23-valent pneumococcal polysaccharide vaccine (PCV23) after at least 8 weeks. IgG was determined before PCV and PPV23 and 4-6 weeks after using MFMI and OPA assay. **(V)** RA patients planned to start methotrexate (MTX) treatment, patients without DMARD and HC were included. Blood was obtained at inclusion, at immunization with PCV13 (after at least 6 weeks on MTX) and 7 days after for flow cytometric phenotyping of lymphocytes, and 4-6 weeks after for MFMI.

Results:

Splenectomy patients (n=24) with previous PPV23, received a dose of PCV13, and geometric mean concentration (GMC) increased for 9/12 serotypes. Patients with systemic vasculitis (n=49) and ongoing standard of care therapy received one dose of PCV13, IgG GMC for serotypes 6B and 23F increased, and there was no significant difference in antibody response (≥2-fold rise in IgG) compared to HC. Although OPA increased after PCV13, it was lower in patients compared to HC (p=0.001). In patients with RA (n=50) and pSS (n=15) without ongoing DMARD treatment IgG GMC for 6B and 23F and OPA increased, and the proportions with positive antibody responses for RA (52%) were similar to HC (55%, n=49). Patients with IRD treated with rituximab (RTX, n=30), abatacept (n=23), conventional DMARD (cDMARD, n=27) and HC (n=28) received immunization with PCV+PPV23. Antibody response improved after PPV23 in cDMARD (both 2-fold AR and OPA), and ABT (2-fold AR but not OPA), but no improvement was seen in RTX treated patients. Start of MTX treatment in RA patients resulted in decreased Th17 cells, and impaired memory B cell and plasmablast responses after PCV13.

Conclusions:

PCV is immunogenic as a booster dose in splenectomized patients with previous PPV23 immunization. PCV is immunogenic in systemic vaculitis patients with ongoing standard of care treatment, although functionality is lower compared to HC. Antibody response is not impaired in RA and pSS patients without DMARD treatment compared to HC. A PPV23 booster could be recommended in IRD patients with cDMARD, and ABT, but vaccination needs to be completed before starting RTX. MTX treatment can have negative effects on memory B cells following PCV13.

Key words: inflammatory rheumatic disease, pneumococcal conjugated vaccine, pneumococcal polysaccharide vaccine, antibody response, memory cells, splenectomy

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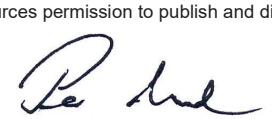
Signature  Date 2021-03-23

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Abbreviations

AAV	ANCA-associated vasculitis
ABT	Abatacept
ANCA	Anti-neutrophil cytoplasmic antibody
Anti-CCP	Antibodies against cyclic-citrullinated peptides
ARR	Antibody response ratio
AZA	Azathioprine
CD	Cluster of differentiation
cmTfh	Circulating memory T follicular helper
CYC	Cyclophosphamide
CXCR	C-X-C motif chemokine receptor
DAS28	28-joint disease activity score
DMARD	Disease-modifying antirheumatic drug
ELISA	Enzyme-linked immunosorbent assay
GC	Germinal center
GMC	Geometric mean concentration
HC	Healthy controls
ICOS	Inducible T cell costimulatory
Ig	Immunoglobulin
IL	Interleukin
IPD	Invasive pneumococcal disease
Mabs	Monoclonal antibodies
MFMI	Multiplex fluorescent microsphere immunoassay
MHC	Major histocompatibility complex
MMFt	Mycophenolate mofetil
MTX	Methotrexate
MZ	Marginal zone
OPA	Opsonophagocytic activity

OPSI	Overwhelming postsplenectomy infection
PCV	Pneumococcal conjugate vaccine
PD-1	Programmed cell death protein 1
PPV23	23-valent pneumococcal polysaccharide vaccine
pSS	Primary Sjögren's syndrome
RA	Rheumatoid arthritis
RF	Rheumatoid factor
RTX	Rituximab
TCR	T cell receptor
TD	T cell dependent
Th	T helper
Tfh	T follicular helper
TI	T cell independent
Treg	T regulatory

Populärvetenskaplig sammanfattning

Bakgrund

Reumatoid artrit (RA) och andra inflammatoriska reumatiska sjukdomar är förenade med en ökad risk för allvarliga infektioner. Den förhöjda infektionsrisken beror dels på sjukdomen i sig, samsjuklighet såsom hjärt- eller lungsjukdom, och till sist kan den vara en följd av den antireumatiska behandlingen. Behandlingen som syftar till att dämpa inflammationsaktiviteten, förbättra livskvaliteten och på sikt minska skador och förbättra överlevnaden för patienten, kan också innebära ett nedsatt immunförsvar med ökad mottaglighet för infektioner. En stor andel av dessa infektioner utgörs av lunginflammationer, och inom denna grupp är infektioner orsakade av pneumokockbakterier vanligast. Pneumokocker är också den vanligaste orsaken till allvarlig hjärnhinneinflammation, öroninflammation och bihåleinflammation men kan även orsaka blodförgiftning, infektioner i leder, skelett m.m. Även personer som till följd av skador eller sjukdom behövt operera bort mjälten löper ökad risk för pneumokockinfektioner.

Pneumokocker omges av en kolhydratkapsel, och denna utgör bakteriens viktigaste skyddsmekanism för att undkomma att bli upptäckt och omhändertagen av kroppens immunförsvar. Pneumokockinfektioner kan potentiellt förebyggas genom vaccination. Det finns i dagsläget två olika typer av pneumokockvacciner. Det äldre vaccinet Pneumovax® består av kolhydrater och skyddar mot 23 vanliga typer av pneumokocker. I det modernare vaccinet Prevenar 13® har kolhydrater från 13 pneumokocktyper på kemisk väg bundits till ett bärarprotein. Troligen leder det till en ökad stimulering av immunförsvarets T-celler med bildning av minnesceller och förhoppningen är att det ger ett mer långvarigt skydd mot pneumokockinfektioner. Prevenar 13® har en skyddseffekt på ca 75 % mot allvarliga pneumokockinfektioner (orsakade av vaccintyper) hos äldre personer. Liknande resultat har tidigare visats i studier av Pneumovax®. På senare år rekommenderas patienter med nedsatt immunförsvar att vaccineras med en kombination av Prevenar 13® och en dos Pneumovax® efter minst 8 veckor.

Det övergripande syftet med denna avhandling var att undersöka effekten av pneumokockvaccin på immunförsvarets antikroppar vid inflammatorisk reumatisk sjukdom och till patienter som opererat bort mjälten.

Pneumokockvaccination vid avsaknad av mjälte

Patienter som opererat bort mjälten, bjöds in till att delta i en studie av tidigare vaccinationer och vaccinerades med Prevenar 13® om de inte fått det tidigare. Det visade sig att de flesta av patienterna hade fått pneumokockvaccin (81 %), men betydligt färre var vaccinerade mot s.k. meningokocker (23 %). Patienter som

tidigare fått Pneumovax®, vaccinerades i studien med en dos Prevenar 13® och antikroppssvar analyserades. Relativt höga nivåer av antikroppar sågs redan vid inklusion i studien (efter tidigare Pneumovax®), och Prevenar 13® hade en förstärkande effekt på immunförsvarets antikroppar. Resultatet innebär att patienter som opererat bort mjälten och tidigare fått grundvaccination med Pneumovax®, kan ha nytta av att rekommenderas vaccination med Prevenar 13® som en påfyllnadsdos. Patienter som grundvaccineras idag bör dock få Prevenar 13® först och följt av en dos Pneumovax®.

Pneumokockvaccination vid reumatiska sjukdomar

I en andra delstudie av patienter med inflammatorisk kärlsjukdom (vaskulit) under uppföljning vid reumamottagning, genomfördes vaccination med Prevenar 13® under pågående inflammationsdämpande behandling. Antikroppssvaret hos dessa patienter jämfördes med svaret hos en grupp av friska försökspersoner efter en dos av samma vaccin. Vaccination visade sig leda till en stegring av antikroppar som nästan var i nivå med kontrollerna men immuncellers upptag av pneumokocker (s.k. opsonofagocytos) var nedsatt. Det innebär att patienter med vaskulit som behöver inleda behandling tidigt, kan vaccineras och få effekt mot pneumokocker trots immundämpande behandling.

I en tredje delstudie inkluderades patienter med RA och patienter med s.k. primärt Sjögrens syndrom (pSS) som inte hade någon aktiv antireumatisk behandling. I studien fanns även en mindre grupp av RA patienter behandlade med methotrexate (MTX, den vanligaste antireumatiska medicinen) samt friska kontroller. Alla vaccinerades med en dos Prevenar 13®. Det visade sig att antikroppssvar vid RA och pSS är i samma nivå som hos friska kontroller även om opsonofagocytos var något nedsatt. Patienter med MTX uppvisade i likhet med tidigare studier ett tydligt nedsatt antikroppssvar efter vaccination. Det innebär att det är mycket viktigt att patienter vaccineras innan de påbörjar antireumatisk behandling.

I delstudie fyra jämfördes antikroppssvaret efter en dos Prevenar 13® med svaret efter påfyllnad (boosting) med en dos Pneumovax® hos patienter med reumatisk sjukdom och friska kontroller. Det fanns tre grupper av antireumatisk behandling: (1) B-cellshämmande rituximab, (2) T-cellshämmande abatacept och (3) konventionell antireumatisk behandling (MTX m.fl.). Patienter med rituximab uppvisade mycket nedsatt antikroppssvar efter Prevenar 13®, och en dos Pneumovax® förbättrade inte detta. Patienter med abatacept svarade något bättre på Prevenar 13® och det sågs en liten tilläggseffekt av Pneumovax®. Däremot sågs tydlig förbättring av antikroppssvar, hos patienter med konventionella antireumatiska mediciner (MTX m.fl.), när de vaccinerades med Prevenar 13® följt av Pneumovax®, jämfört med bara en dos Prevenar 13®. Studien understryker vikten av att RA patienter vaccineras före start av den kraftigt B-cellshämmande medicinen

rituximab. RA patienter som behandlas med MTX, och tidigare fått en dos Prevenar 13®, kan ha nytta av en påfyllnadsdos Pneumovax®.

Immunceller vid RA, effekt av MTX och pneumokockvaccination

Syftet med den femte studien var att undersöka effekten av MTX på immunförsvarets B och T celler, och effekt av immunstimulering med konjugerat pneumokockvaccin. Detta för att förstå mer om mekanismerna bakom den välkända effekten av MTX att minska antikroppssvar efter pneumokockvaccin. I studien inkluderades patienter med RA som planerades att påbörja behandling med MTX, RA patienter utan behandling och friska kontroller. Immunsystemets B- och T-celler analyserades i blodet vid olika tidpunkter. Alla vaccinerades med en dos Prevenar 13®, men RA patienter som startade med MTX fick vänta minst 6 veckor för att behandlingen skulle börja verka före vaccination. Efter start av behandling med MTX sågs en minskning av s.k. T-hjälparceller 17. Efter vaccination av RA patienter utan behandling och kontroller sågs tydliga ökningar av förstadier till antikroppsbildande celler (plasmablaster) och minnes-B-celler. Dessa ökningar uteblev hos patienter med MTX. Vi tolkar det som att MTX har effekter på både T celler och B celler, vilket kan få negativa effekter på det immunologiska minnet, dvs den långvariga skyddseffekten mot pneumokockinfektioner.

Introduction

The history of vaccination begins in 16th century China with the practice of variolisation, i.e. the inoculation of human smallpox material to prevent disease (1). In 1796, British physician Edward Jenner conducted a famous experiment based on the observation that milkmaids previously exposed to the mild cowpox disease were protected against smallpox (2). With an arm-to-arm transfer technique, he inoculated cowpox material, thus introducing the concept of live attenuated vaccination. About 80 years later, Louis Pasteur developed methods to attenuate microorganisms in his Paris laboratory, and the work resulted in the first human rabies virus vaccine (3).

In 1881, Pasteur, and George Sternberg in the United States, independently discovered the pneumococcus, and both described its surrounding capsule (4). Three years later, Hans Christian Gram while examining deceased pneumonia patients, developed a technique, known as Gram staining, to differentiate the Gram-positive pneumococci from Gram-negative *Klebsiella pneumoniae*. Sir Almroth Wright conducted the first trial with a killed pneumococci vaccine in South African gold miners in 1910, but subsequent analysis questioned its efficacy (4). The presence of distinct pneumococcal serotypes was first demonstrated by Neufeld and Händel in 1910, and in 1917, Avery reported that pneumococcal capsules were composed of polysaccharide (4). In 1927, Schiemann and Casper described the immunogenicity of pneumococcal polysaccharides in mice (5), and in 1930, Francis and Tillett reported similar immune responses in humans (6). During a pneumonia epidemic in the military in 1945, recruits were either immunized with capsular polysaccharides of serotypes 1, 2, 5 and 7, or saline, and Macleod and Heidelberger reported that this 4-valent vaccine was efficacious in the prevention of pneumococcal pneumonia (7). Alexander Fleming's discovery of penicillin and its efficacy in the treatment of pneumococcal infections led to a decline in the field of pneumococcal serotyping and vaccine development. In the 1970s, Eli Lilly & Co and Merck & Co conducted a trials of polyvalent pneumococcal polysaccharide vaccines (PPVs) in South Africa, with around 80% efficacy in the prevention of vaccine-type pneumococcal infection and bacteremia (4). Merck & Co licensed a 14-valent PPV in 1977, and the 23-valent vaccine (PPV23, Pneumovax®) followed in 1983. PPV23 was formulated to cover 90% of serotypes causing IPD worldwide at the time (8).

In recent years, worldwide measles vaccine coverage has reached 85% (9), and conversely, the annual number of deaths from measles has decreased from an

estimated 2.6 million in 1963, to 110.000 in 2017. However, the most notable success story in the history of vaccination was the eradication of smallpox disease in the late 1970s, through large immunization campaigns coordinated by the World Health Organization (WHO). Child immunization with 7-valent protein-conjugate pneumococcal vaccine (PCV7) was introduced in the United States in 2000, and it was followed by a great reduction of pneumococcal disease in young children. Through herd immunity, pneumococcal disease rates decreased in the elderly population (10). 13-valent pneumococcal conjugate vaccine (PCV13) was licensed late in 2011, and has further reduced pneumococcal disease, but continued serotype replacement with emerging infections of non-vaccine type is a great problem. The immunocompromised population is growing, e.g. more patients undergo life-saving transplantations, and others are getting efficient treatments for debilitating chronic inflammatory disease. The immunocompromised are at risk of severe pneumococcal infections, but knowledge regarding pneumococcal vaccine immunogenicity, efficacy, and safety in this populations is scarce.

Pneumococcal disease

The bacterial pathogen *Streptococcus pneumoniae* (the pneumococcus) has a high invasive potential. Pneumococcal disease is the leading cause of mortality among infectious diseases worldwide. On the other hand, pneumococci are frequent colonizers of the upper airways in up to 60% of asymptomatic small children (11). The pneumococcus attaches to the nasopharyngeal epithelium, and from there it can spread locally causing sinusitis or otitis, aspiration of the bacteria may lead to pneumonia, and invasion of the blood stream or blood-brain barrier leads to septicaemia or meningitis. Invasive pneumococcal disease (IPD) is defined by the isolation of pneumococci from a normally sterile bodily compartment, such as blood or cerebrospinal fluid. Before the introduction of penicillin, the mortality associated with pneumococcal pneumonia, bacteraemia and meningitis was 20%, 50%, and 80-100%, respectively. With modern antibiotic treatment and intensive care, the mortality rates of pneumococcal disease have improved to 5% in pneumonia, 20% in bacteraemia, and 30% in meningitis (12, 13).

Streptococcus pneumoniae

Microbiology

The genus streptococcus consists of Gram-positive coccoid shaped bacteria that are catalase-negative, facultative anaerobes, and grow in pairs or chains. They grow optimally on blood agar, a source of catalase, and can be further classified based on their ability to cause lysis of red blood cells (14). On blood agar, colonies of *S.pyogenes,* the archetypal β-hemolytic streptococci, are surrounded by a clear zone of hemolysis. In contrast, the green discoloration around *S. pneumoniae* colonies, classically termed α-hemolysis, is caused by oxidation of haemoglobin to methemoglobin, and the red blood cell membranes are left intact. Pneumococci characteristically arrange in pairs, as diplococci. The bacteriological identification of pneumococci, and differentiation from other α-hemolytic commensal (viridans) streptococci, requires two additional reactions: growth inhibition by ethyl hydrocupreine (Optochin) and finally solubility in bile (15).

Cellular anatomy.

Outside the pneumococcal cytoplasmic membrane is the cell wall, which surrounds a thin periplasmic space. The main building blocks of the cell wall are the polymeric carbohydrates peptidoglycan and teichoic acid. Teichoic acid covalently linked to peptidoglycan on the surface of the cell wall constitutes the C-polysaccharide (15). The polysaccharide capsule has covalent links to peptidoglycan and C-polysaccharide, and it covers the external surface of the pneumococcus.

Virulence factors

The capsular polysaccharide is the pneumococcus' most important virulence factor, because it inhibits complement binding and phagocytosis, unless anticapsular antibody is present. So far, 97 capsular serotypes have been described (16). The serotypes are numbered, and similar polysaccharide structures are grouped together, e.g. 6A, 6B and 6C. Although almost all clinical isolates of pneumococci are encapsulated strains, non-encapsulated strains have been described as cause of both non-invasive (mainly in outbreaks of conjunctivitis) and rarely invasive disease (17). The binding of anticapsular antibodies leads to swelling of the capsule, the quellung reaction, which enables microscopic visualization of the pneumococcal capsule (18).

Pneumococci produce pneumolysin, which is cytotoxic for phagocytes and respiratory epithelial cells, and proinflammatory by activating complement and inducing tumor necrosis factor-α and interleukin-1 (15).

Autolysin cuts the peptidoglycan cross-links of the cell wall, resulting in autolysis of the pneumococcus, release of pneumolysin and other cell components and subsequent tissue inflammation.

Pneumococcal surface protein A (PspA) inhibit phagocytosis by blocking deposition and activation of complement.

Choline-binding protein A (CbpA) can bind to the epithelium of both the nasopharynx and the blood-brain barrier, facilitating invasion of the blood stream or central nervous system.

Immune response to pneumococcal infection

Pneumococci are poorly opsonized bacteria, and in the natural course of pneumococcal infection, the resolution of fever after 5-8 days is accompanied by the appearance of anti-capsular antibody (15). Anti-capsular antibody greatly increases phagocytosis and killing of pneumococci in vitro (19).

Pneumococcal antibody levels increase with age and inversely the incidence of IPD decreases, except for the elderly, although antibody levels remain high in the population above age 65 years they are at an increased risk of IPD (20).

Role of the spleen in the immune response to pneumococcal infection

Unopsonized pneumococci in the circulation are mainly cleared by the spleen (21). The marginal zone is located between the erythrocyte-rich red pulp and the lymphocyte-dominated white pulp of the spleen. The marginal zone contains a unique population of memory B cells which produce natural immunoglobulin M (IgM) antibodies against encapsulated bacteria, such as pneumococci (22). Marginal zone (MZ) B cells are reduced in young children (<2 years), patients with common variable immunodeficiency (CVID), human immunodeficiency virus (HIV) infection, asplenia, and elderly people (22).

Risk factors for invasive pneumococcal disease

Populations with impaired antibody responses, such as infants, elderly, bone marrow transplant recipients, and patients with hypogammaglobulinemia are at increased risks of serious pneumococcal infections (23). Before the introduction of pneumococcal conjugate vaccine (PCV) in child immunization programs, the highest incidence of IPD was observed in children under age 5 years (24). In the PCV era, peak IPD incidence has shifted to the elderly population, above age 65 years, and a smaller peak is seen in young children (25).

Patients with chronic respiratory disease, chronic heart failure, and smokers are at increased risks of pneumococcal pneumonia and IPD (26). The risk of IPD is also increased in diseases with interference in the function of polymorphonuclear phagocytes, such as diabetes mellitus, chronic renal disease and cirrhosis of the liver (23).

Immunocompromised patients are at increased risk of pneumococcal disease, mainly because of impaired abilities to generate antibodies to new antigens (23). In a meta-analysis, the pooled incidence of IPD was 318-331/100,000 person years in HIV patients, 812/100,000 person years following allogeneic stem cell transplant, and 465/100,000 in solid organ transplant recipients (27), compared to 10/100.000 person years in healthy controls. Based on few studies, and relatively small samples sizes, pooled incidence of IPD was 65/100.000 person years in patients with chronic inflammatory disease. Retrospective cohort studies of IPD in England have demonstrated increased rates of IPD in patients with rheumatoid arthritis (RA, incidence rate ratio [IRR] 2.5), systemic sclerosis (SSc, IRR 4.2) and systemic lupus erythematosus (SLE, IRR 5.0) (28). In a Swedish retrospective cohort study, IRR

was 4.9 for RA and 14.2 for SLE patients (29). Hematologic malignancies are associated with high risks of IPD, especially multiple myeloma (29, 30).

Asplenia or hyposplenic states

Functional or anatomical asplenia are well-known risk factors for pneumococcal disease. Splenectomy causes a substantial reduction in numbers of circulating MZ $IgM^{+}CD27^{+}$ memory B cells, which are important in the defence against encapsulated bacteria (22). Overwhelming post-splenectomy infection (OPSI) is characterized by fulminant sepsis, pneumonia or meningitis after surgical removal of the spleen (22). The classic causes of OPSI from the prevaccination era, are encapsulated bacteria such as pneumococcus (50-90%), *Haemophilus influenzae* type B, or *Neisseria meningitidis* (22). Other important pathogens are Escherichia coli, Pseudomonas aeruginosa, Capnocytophaga canimorsus, babesia and malaria in endemic areas (31). The reported mortality in OPSI is high, 50-70% (32), although no studies have addressed this question in the recent years.

Post-traumatic surgery accounts for about a quarter of splenectomies, but these procedures are decreasing in favour of alternative treatments (31). Another quarter of splenectomies are performed due to hematological disease, such as idiopathic thrombocytopenic purpura, sickle cell disease and hereditary spherocytosis. The remaining half of these surgeries are associated with solid tumors, or the result of accidental injury to the spleen during laparotomy (31).

Hyposplenia or splenic atrophy can be congenital, or associated with a wide range of disorders, including coeliac disease, hepatic cirrhosis, haematological disease, and autoimmune disease (22).

Pneumococcal vaccination

At the present, two principally different pneumococcal vaccines are in use, the 23-valent polysaccharide vaccine (PPV23, Pneumovax®, MSD), and the 13-valent protein conjugate vaccine (PCV13, Prevenar 13®, Pfizer). In PCV13, the polysaccharides are covalently linked to carrier protein CRM197, i.e. recombinant diphtheria toxoid. Twelve serotypes are included in both vaccines, with additional 11 serotypes in PPV23, and serotype 6A is only included in PCV13 (Table 1). Their principally different effects on the immune system, advantages and disadvantages will be addressed in this section.

Table 1.
Serotypes included in PPV23 and PCV13.

	1	2	3	4	5	6A	6B	7F	8	9N	9V	10A	11A	12F	14	15B	17F	18C	19A	19F	20	22F	23F	33F
PPV23	+	+	+	+	+	-	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
PCV13	+	-	+	+	+	+	+	+	-	-	+	-	-	-	+	-	-	+	+	+	-	-	+	-

Vaccine immunology

Antigen-specific antibodies generally mediate the early protective efficacy of vaccines, but long-term immunity can depend on both persistence of antibodies and/or memory cells (33). B cells can differentiate into antibody-secreting plasma cells but the production of high-affinity antibody and long-lasting memory B cells generally requires help from T cells. Cell surface markers, called cluster of differentiation (CD), are used to identify immune cells. Important subgroups of T lymphocytes are the $CD4^+$ T helper cells, and $CD8^+$ cytotoxic T cells. The $CD4^+$ T helper (Th) cells are further divided into different phenotypes with specific roles within the immune system (table 2). The T helper 1 (Th1) cells express the chemokine receptor CXCR3 (34), produce cytokines such as interleukin(IL)-2, interferon-γ (IFN-γ) and tumor necrosis factor (TNF), and support $CD8^+$ T cells and macrophages in immune responses against intracellular pathogens, e.g. virus and mycobacteria. The T helper 2 (Th2) cells secrete signature cytokines IL-4 and IL-5, IL-10 and IL-13, providing support to B cells in the defence against parasites (33). The T helper 17 (Th17) cell and its signature cytokine interleukin-17 (IL-17) was

first described in 1995 (35). The Th17 cells express the chemokine receptor CCR6 (36), and their main function is in the defence against extracellular bacteria, e.g. pneumococci, and fungi on mucosal surfaces (33). Another subset, the Th9 cells secrete IL-9, and are involved in the response to extracellular pathogens. Regulatory T cells (Tregs) control effector Th cells and mediate immune tolerance (33). The follicular T helper (Tfh) cells express CXCR5 (37), and specialize in providing help to B cells in germinal centre reactions in secondary lymphoid organs, resulting in the formation of plasma cells and memory B cells.

Table 2.
Relevant $CD4^+$ T helper cell subsets, markers, cytokines and functions.

Subset:	Surface markers:	Signature cytokines:	Function in immune system:
Th1	$CD183^+$ ($CXCR3^+$)	IL-2, IFN-γ, TNF	Response to intracellular microbes
Th2	$CD183^-$, $CD196^-$	IL-4, 5, 10, 13	Response to parasites
Th9	$CD183^-$, $CD196^+$ ($CCR6^+$)	IL-9	Response to extracellular pathogens
Th17	$CD183^-$, $CD196^+$ ($CCR6^+$)	IL-17, 21, 22	Response to extracellular bacteria and fungi
T regulatory cell (Treg)	$CD25^+$	IL-10	Regulatory function, maintaining tolerance/preventing autoimmunity
T follicular helper (Tfh)	$CXCR5^+$, $PD\text{-}1^+$, $ICOS^+$	IL-21	Specialized B cell helper in germinal center reaction

T cell independent response to polysaccharide vaccine

The polysaccharide (PS) antigens of PPV23 activate B cells in the absence of T cell help, thus the immune response is termed T cell independent (TI). After immunization, PS antigens in the circulation reach the marginal zone of the spleen or lymph nodes, where cross-linking of surface Ig-receptors activates MZ IgM^+CD27^+ memory B cells in extrafollicular foci (22, 38). Within a week the MZ B cells differentiate to antibody-secreting plasma cells producing intermediate-affinity IgG antibodies, but absent or small numbers of memory cells (33). Repeated pneumococcal PS immunizations cause hyporesponsiveness, and gradual depletion of the memory B cell pool (39).

T cell dependent response to protein conjugate vaccine

Protein conjugate vaccines, as well as protein, toxoid, inactivated, or live attenuated viral vaccines, elicit T cell dependent (TD) immune responses resulting in high-affinity antibodies and immune memory (33).

The extrafollicular reaction

After injection of PCV, the repetitive structure of PS antigen cross-links the surface Ig receptors on naïve B cells, resulting in their activation. Activated B cells express

chemokine receptor CCR7, which causes homing of the cells towards the T cell zone of secondary lymphoid organs (33). The extrafollicular reaction with help from T cells causes B cells to differentiate into antibody secreting plasma cells that rapidly produce low-affinity unmutated germ-line IgM and small amounts of IgG within days of immunization. These plasma cells have a short life ended by in-situ apoptosis (40).

T follicular cells and the germinal centre reaction

In the first phase (days 0-3) after vaccine injection, the protein-PS conjugate is internalized by tissue dendritic cells (DC), and the protein part of antigen is processed for display on the major histocompatibility complex (MHC) class II surface receptor (41). Activated DCs migrate to the lymph nodes and express costimulatory surface molecule B7 for T cell activation. Activation of naïve T cells requires at least two signals, T cell receptor (TCR) – MHC class II-antigen interaction, and co-stimulatory signal from CD28-B7 interaction. T cell activation initiates the T follicular helper cell (Tfh) differentiation program, starting with the pre-Tfh cell, which express high levels of surface molecules PD-1 and inducible co-stimulator (ICOS) (41). Further, pre-Tfh cells gain expression of CXCR5 and loose CCR7, resulting in migration towards the T-B-cell junction.

The second phase (days 4-5), is the interaction between the pre-Tfh cell with antigen-presenting B cells which finalizes the $CD4^{+}CXCR5^{+}ICOS^{+}PD\text{-}1^{+}$ Tfh cell phenotype, and result in the migration of both Tfh and B cells toward the follicle (41).

In the third phase (days 6-10) B-cells proliferate in the primary follicle to form the germinal center (GC) (41). In the GC, B cells circulate between a dark zone (DZ) and a light zone (LZ) (42). In the DZ, B cells undergo somatic hypermutation (SHM) in the variable regions of light and heavy chain genes. The next step is affinity selection in the LZ, where B cells with higher affinity B cell receptors (BCRs) are able to retrieve peptide antigen from follicular DCs. The antigen is internalized, and B cells present it on MHC class II to Tfh cells. Successful presentation results in B cell survival, proliferation, and recycling in a new round of SHM in the DZ. When affinity maturation is complete, B cells leave GC and differentiate into high-affinity plasma cells and switched memory B cells (42).

Principal differences in the immune response to pneumococcal conjugate compared to polysaccharide vaccines

Pneumococcal conjugate vaccines elicit strong antibody responses in infants, in contrast to PS vaccines which are poorly immunogenic during the first 2 years of life, possibly due to immaturity of the splenic marginal zone (33). Theoretically, PCV has the advantage of the TD immune response with the formation of high affinity antibodies and memory B cells. Further, conjugate vaccines can induce neutralising antibody responses at mucosal surfaces, thus preventing bacterial colonisation (33). In contrast to PPV23, PCV seems to be independent of splenic marginal zone tissue, and therefore hypothesized to be more immunogenic in asplenic populations (22). Pneumococcal conjugate vaccines might be more immunogenic in healthy adults, but the evidence is inconclusive (43).

Efficacy

Pneumococcal polysaccharide vaccine efficacy for the prevention of IPD is around 50-80 %, in immunocompetent adult and elderly populations (44). The evidence regarding PPV efficacy against non-bacteremic pneumococcal pneumonia is inconclusive (45). A double-blind placebo-controlled randomized clinical trial (RCT) did not demonstrate efficacy of PPV23 in the prevention of all-cause or pneumococcal pneumonia in RA patients (46). In contrast, the 10-year relative risk of pneumonia was 9.7 in non-vaccinated compared to PPV23 vaccinated RA patients treated with MTX, in a retrospective study (47).

In a large trial (CAPITA) in the Netherlands, about 85,000 adults aged 65 years or older were randomized to receive either PCV13 or placebo. The CAPITA trial demonstrated 45.0% efficacy of PCV13 against vaccine-type non-bacteremic pneumococcal pneumonia, and 75.0% efficacy against vaccine-type IPD, but there was no effect on mortality (48). The CAPITA study has been critized because the protective effect of PCV13 was compared to placebo, instead of PPV23 (49). In a meta-analysis by Moberley et al., the efficacy of PPV23 against IPD was 80%, which is similar to the efficacy of PCV13 reported in the CAPITA study (50).

A study conducted in Malawi, randomized 496 HIV infected persons to immunization with either two doses of PCV7 or placebo four weeks apart, and demonstrated 74 % efficacy against IPD (51). In a placebo controlled RCT in Uganda, PPV23 was ineffective for prevention of IPD in HIV infected adults (52). To the author’s knowledge, pneumococcal vaccine efficacy studies in other immunocompromised populations are largely lacking.

Epidemiology of IPD in the era of PCV

In the United States, one year after the introduction of PCV7 in the childhood immunization program in 2000, IPD incidence decreased by 69 % in children < 2 years (53). In 2007, overall IPD incidence had dropped by 76 % in children < 5 years, and 45 % in all age groups, compared to the prevaccine era (54). Similar indirect protective effects in non-vaccinated populations due to herd immunity have been observed with PCV13 (45).

In England and Wales, the post-PCV13 overall IPD incidence in 2016/17 was 37 % lower in all age groups, compared to the pre-PCV7 period (55). Although PCV7-type IPD incidence had decreased by 97 %, and additional PCV13-type by 64 %, rapid increases in IPD caused by non-PCV13 serotypes have been observed in the adult population (55). Serotype replacement reduces the effect of PCV13 vaccination in the immunocompromised population. In Sweden, PCV13-type IPD only accounted for about 30 % of all IPD cases in 2019 (56).

Combination of PCV13 and PPV23

Since 2012, the Centers for Disease Control and Prevention (CDC) Advisory Committee on Immunization Practices (ACIP) recommendations for adults with immunocompromising conditions is to receive immunization with a dose of PCV13, followed after at least 8 weeks by a dose of PPV23, because of the wider serotype coverage (57). The European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Vaccine Study Group (EVASG) also recommends that at-risk adults receive this vaccine schedule (58), which is referred to as the prime-boost pneumococcal vaccination strategy (59).

In a RCT by Lesprit et al., 212 HIV patients either received PCV7 followed by PPV23 after 4 weeks or PPV23 alone at week 4 (59). No differences in antibody response were found 4 weeks after PCV or PPV, but patients who received the prime-boost vaccination strategy were more likely to achieve 2-fold increase in serotype-specific IgG and ≥1 µg/mL in IgG level at week 8, and the differences remained significant at week 24. Similar results have been reported for PCV13 followed by PPV23 in HIV patients (60). In contrast, in liver transplant or renal transplant recipients the prime-boost strategy did not improve immunogenicity, compared with single dose PPV23 (61, 62). In a trial by Nguyen et al., RA patients treated with biologics were randomized to single dose PCV13 followed by PPV23 after 16 or 24 weeks or double dose PCV13 followed by PPV23 after 16 weeks, without differences in early antibody response (63).

Measuring antibody response to pneumococcal vaccines

Antibody responses following pneumococcal vaccination are evaluated through IgG quantification, most commonly using enzyme-linked immunosorbent assay (ELISA), and functionality of antibodies, i.e. opsonophagocytic activity (OPA).

Enzyme-linked immunosorbent assay

The first step in the pneumococcal enzyme-linked immunosorbent assay (ELISA) is to coat microtiter plates with specific pneumococcal capsular PS. In a preabsorption step, pneumococcal C PS is added to patient serum to reduce cross-reacting antibodies, and a WHO validated protocol also uses preabsorption with 22F PS (64). Patient serum is then added to the ELISA plates, and specific IgG antibodies bind to their corresponding antigen. In the next step, goat anti-human IgG antibodies conjugated with alkaline phosphatase are added to the plates, followed by addition of the substrate, nitrophenyl phosphate. The enzyme causes the substrate to change color, and the measured optical density is proportional to the IgG concentration. The method is calibrated using a reference serum provided by the United States Food and Drug Administration (FDA) (65). Reference serum 89SF has been replaced by 007Sp in the standard WHO validated method (64).

Opsonophagocytic activity

The most important defence mechanism against pneumococci is opsonisation by anticapsular antibodies, followed by phagocytosis and killing of the bacteria. Therefore functional assays measuring OPA are considered more biologically relevant, compared to IgG quantification methods. The killing-type OPA measures the titer of sera that reduce live bacteria by more than half (65), and such a method described by Romero-Steiner has become the standard in pneumococcal vaccine evaluations (66). Opsonophagocytosis can also be measured with flow cytometric assays, using fluorescent-labeled bacteria and phagocytes (67).

Putative protective thresholds

Optimal serotype-specific correlates of protection against IPS in adults are unknown, and licensure of new pneumococcal vaccines rely on demonstration of non-inferiority for each of the common serotypes compared to a licensed PCV (65). Higher levels of antibody might be required to confer protection against mucosal colonization or acute otitis media, compared to IPS. The World Health Organization (WHO) has recommended a protective threshold ≥0.35 μg/mL of serotype-specific

IgG following conjugate immunization in infants (68). However, immunogenicity varies between different serotypes, e.g. for serotype 3 it is high, while serotypes 6B and 23F have lower immunogenicity (69). A study in England and Wales used an indirect cohort method to determine serological correlates of protection in infants, and suggested the aggregate threshold ≥0.8 μg/mL for all serotypes in PCV13, except serotype 3 for which the correlate was higher and protection was non-significant (70). In the same study protective thresholds for serotypes 6B and 23F were lower (0.16 and 0.2 μg/mL). Serotype-specific IgG ≥1.0 μg/mL is commonly used as a threshold of putative protection in adults. Chronic arthritis patients with antibody responses ≥1.0 μg/mL after vaccination with pneumococcal conjugate vaccine were less likely to suffer from serious infections (71). The American Academy of Allergy, Asthma & Immunology (AAAAI) has recommended a putative protective IgG level for each serotype ≥1.3 μg/mL (69). It is not uncommon for healthy nonimmunized adults to have protective antibody levels to one or a more serotypes after previous clinical or subclinical infections (69). The higher the preimmunization serotype-specific IgG level, the less likely the serotype will increase significant after vaccination (72), but most patients with preimmunization IgG ≥ 1.3 μg/mL will be able to mount a 2-fold increase after vaccination (73).

Autoimmune inflammatory rheumatic disease

Rheumatoid arthritis

Rheumatoid arthritis (RA) is an autoimmune disease characterized by chronic inflammation primarily engaging the joints of the hands and feet in a symmetric pattern, and if untreated it will eventually lead to destruction of the joints.

Epidemiology

The prevalence of RA is 0.5-1.0 % in western populations (74, 75). Similarly, in a study from the Democratic Republic of the Congo, the reported prevalence was 0.6 % (76). Native American populations have the highest prevalence of RA, 6-8 % (77).

Classification criteria

The American College of Rheumatology (ACR) classification criteria for RA from 1987 (78) have been used as inclusion criteria in many studies. The revised classification criteria by ACR and European League Against Rheumatism (EULAR) from 2010 are shown in table 3 (79).

Disease activity, DAS28

The 28-joint disease activity score (DAS28) has been widely used to evaluate disease activity of RA patients in clinical trials since it was introduced in 1995 (80). The following formula is used for calculation of DAS28:

$$DAS28 = 0.56 \times \sqrt{TEN28} + 0.28 \times \sqrt{SW28} + 0.70 \times \ln(ESR) + 0.014 \times SA$$

TEN28 = number of tender joints (0-28), SW28 = number of swollen joints (0-28), ESR = erythrocyte sedimentation rate, SA = self-assessment of disease activity during the preceding 7 days (0-100).

Disease activity can be categorized by DAS28 as follows: <2.6 remission; 2.6-3.2 low; >3.2-5.1 moderate; >5.1 high.

Table 3.
The ACR/EULAR classification criteria for RA.

	Score
Target population (Who should be tested?): Patients who	
1) have at least 1 joint with definite clinical synovitis (swelling)	
2) with the synovitis not better explained by another disease	
Classification criteria for RA (score-based algorithm: add score of categories A–D;	
a score of ≥6/10 is needed for classification of a patient as having definite RA)‡	
A. Joint involvement	
1 large joint*	0
2−10 large joints	1
1−3 small joints** (with or without involvement of large joints)	2
4−10 small joints (with or without involvement of large joints)	3
>10 joints (at least 1 small joint)	5
B. Serology* ** (at least 1 test result is needed for classification)	
Negative RF *and* negative ACPA	0
Low-positive RF *or* low-positive ACPA	2
High-positive RF *or* high-positive ACPA	3
C. Acute-phase reactants (at least 1 test result is needed for classification)	
Normal CRP *and* normal ESR	0
Abnormal CRP *or* abnormal ESR	1
D. Duration of symptoms	
<6 weeks	0
≥6 weeks	1

* "Large joints" refers to shoulders, elbows, hips, knees, and ankles.
** "Small joints" refers to the metacarpophalangeal joints, proximal interphalangeal joints, second through fifth metatarsophalangeal joints, thumb interphalangeal joints, and wrists.
*** Negative refers to IU values that are less than or equal to the upper limit of normal (ULN) for the laboratory and assay; low-positive refers to IU values that are higher than the ULN but ≤3 times the ULN for the laboratory and assay; high-positive refers to IU values that are >3 times the ULN for the laboratory and assay. Where rheumatoid factor (RF) information is only available as positive or negative, a positive result should be scored as low-positive for RF. ACPA = anti−citrullinated protein antibody.
CRP = C-reactive protein; ESR = erythrocyte sedimentation rate.

Immunopathology of RA

The CD4+ T helper cells arguably have central roles in the immunopathology of RA. First, memory CD4+ T cells are increased in the inflamed synovial tissue of RA patients (81). Second, therapy inhibiting the activation of T cells, i.e. co-

stimulation blockade at the CD80/86–CD28 interaction is an efficient therapy in RA (82). Third, RA is associated with several risk alleles of the human leukocyte antigen (HLA)-DRB1 gene within the MHC class II region (83). The HLA-DRB1 risk alleles encode a specific amino acid sequence, the shared epitope (84). About 2/3 of RA patients have antibodies against cyclic-citrullinated peptides (anti-CCP), associated with HLA-DRB1 risk alleles (85).

B cells

Although RA is often considered a T cell-driven disease, several aspects of the disease are mediated by B cells. Rheumatoid factor (RF), an autoantibody directed against the Fc part of the IgG molecule, is a disease marker with sensitivity and specificity in RA around 70% and 85% respectively (86). Anti-CCP antibodies are more specific, 95% (86), and high titers are associated with poor prognosis (87). Perhaps the most important argument for an important role of B cells in the pathogenesis of RA is the efficacy of rituximab anti-CD20 B cell depletion therapy in RA refractory to TNF-blockade (88). This effect is not directly related to autoantibody production, as plasma cells lack expression of CD20.

Accumulation of pre-switch memory B cells ($CD19^+CD27^+IgD^+$) in synovial tissue might explain that frequencies of these cells are decreased in peripheral blood of RA patients (89). In contrast, switched memory B cells ($CD19^+CD27^+IgD^-$) are increased in peripheral blood and correlate with disease duration in RA (89).

T helper 1 cells

RA has been described as a Th1-driven disease, with an imbalance of Th1/Th2 cytokines (90). Th1 cells are present in the synovial fluid and tissue in RA, and these cells activate macrophages to produce the pro-inflammatory cytokine tumor necrosis factor (TNF) (36). TNF-blockade is an efficient therapy in RA (91).

T helper 17 cells

The Th17 cells produce the cytokine IL-17 which contributes to recruitment of neutrophils, in the defence against extracellular pathogens. In patients with RA, Th17 cells were found to be either increased or in normal numbers in the blood, compared to healthy subjects (36). Production of IL-17 can induce neutrophil inflammation in the synovial tissue and bone resorption in RA patients, but clinical studies have not shown efficacy of treatments targeting IL-17A or IL-17 receptor in RA (92, 93).

Follicular T helper cells

It has been shown that Tfh cells are able to exit GCs, and develop into memory Tfh cells (94), and blood $CXCR5^+$ Th cells are thought to represent a circulating memory compartment of Tfh lymphocytes (cmTfh) (95). Further, cmTfh cells consist of

subsets cmTfh1 ($CXCR3^+CCR6^-$), cmTfh2 ($CXCR3^-CCR6^-$), and cmTfh17 ($CXCR3^-CCR6^+$) (95). Blood cmTfh2 and cmTfh17 can efficiently induce naïve B cells to produce class-switched immunoglobulins, but cmTfh1 cells lack this capacity (96). Several studies have shown increased percentages of cmTfh cells in RA patients compared to controls (97-100). In contrast, other studies found no difference in cmTfh, cmTfh1, cmTfh2 or cmTfh17 (101, 102), or lower percentages of cmTfh (103) in RA patients compared to healthy controls.

Regulatory T cells

Regulatory T cells and proinflammatory Th17 cells have opposite functions in the immune system. In mice, Tregs ($CD4^+CD25^+$) have the ability to prevent several autoimmune diseases (104). Tregs accumulate in synovial tissue and synovial fluid of RA patients, and proinflammatory cytokines such as TNF inhibit Treg suppressive function in vitro (36). In blood, $CD45RA^+$ Tregs represent naïve cells, whereas $CD45RO^+$ Tregs are previously activated cells (105). In RA patients responding to TNF-inhibitors, the treatment was shown to increase percentages of Tregs in blood (106).

Systemic vasculitis

The vasculitides are diseases characterized by inflammation and necrosis of different blood vessels, causing damage to the vessels, ischemia or aneurysm formation. Vasculitis is a heterogenous group of disorders with a diverse symptomatology, depending on which part of the blood vessel tree and organs are involved. Vasculitides can be divided into two main groups, i.e. infectious vasculitis, caused by direct invasion of microbes, e.g. syphilitic aortitis, rickettsial vasculitis or aspergillus arteritis, and noninfectious vasculitis (107). Noninfectious vasculitis is categorized by the 2012 revised nomenclature of the International Chapel Hill Consensus Conference (CHCC2012) (107). The primary categorization is based on the size of engaged blood vessels, i.e. large, medium or small vessels, although there are significant overlaps between these categories (Figure 1).

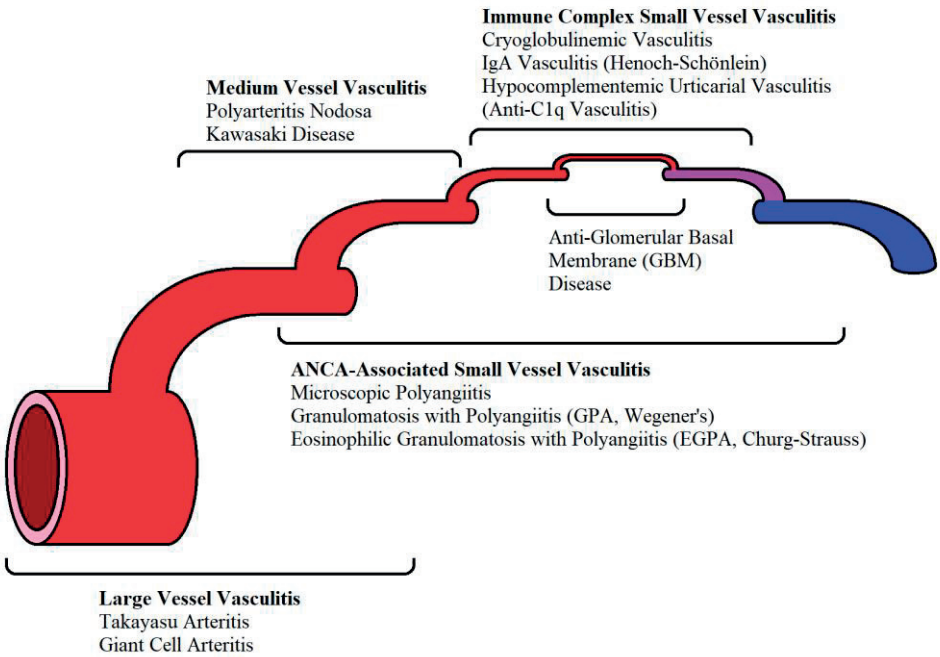

Figure 1. Vasculitides categorized on size of blood vessel involvement. Modified after Jennette et al. (107).
ANCA = Anti-neutrophil cytoplasmic antibody.

Diagnosis and classification

Before classification, three criteria must be fulfilled for the clinical diagnosis of primary systemic vasculitis, as described by Watts et al (108). The first criteria is symptoms or signs characteristic or compatible with ANCA-associated small vessel vasculitis (AAV) or polyarteritis nodosa (PAN) (e.g. upper or lower airway symptoms in granulomatosis with polyangitiis [GPA], or hematuria with red cell casts in renal vasculitis). Second, there should be histological proof of vasculitis and/or granuloma, positive serology for ANCA, specific investigations strongly suggestive of vasculitis (e.g. magnetic resonance angiography in PAN), or eosinophilia (>10% or $>1.5\times10^9$/l). The third criteria is exclusion of malignancy, infection (hepatitis B and C, HIV, tuberculosis and subacute bacterial endocarditis), drugs (e.g. cocaine), secondary vasculitis (RA, SLE, Sjögren's syndrome), other vasculitides (e.g. Behcet's, Takayasu's arteritis, giant cell arteritis), vasculitis mimics (e.g. cholesterol embolism), sarcoidosis and other granulomatous diseases. In analogy, diagnoses of large vessel vasculitides, i.e. Takayasu's arteritis and giant cell arteritis, are made on the basis of typical symptoms, typical histological or angiographic findings, elevated ESR and in the absence of findings suggestive of

infection, malignancy or other rheumatic disease. After clinical diagnosis, patients can be classified using the respective ACR 1990 criteria for eosinophilic granulomatosis with polyangiitis (EGPA), GPA, giant cell arteritis or Takayasu's arteritis (109).

Primary Sjögren's syndrome

Primary Sjögren's syndrome (pSS) is an autoimmune disease characterized by dryness of the eyes and mouth, hypofunction of salivary and lacrimal glands, and possible systemic manifestations. In patients diagnosed with another systemic inflammatory disease, usually RA or SLE, symptoms of dry eyes and mouth are referred to as secondary Sjögren's syndrome. The ACR/EULAR 2016 criteria for pSS are shown in table 4 (110).

Table 4. American College of Rheumatology/European League Against Rheumatism classification criteria for primary Sjögren's syndrome.

The classification of primary Sjögren's syndrome applies to any individual who meets the inclusion criteria,* does not have any of the conditions listed as exclusion criteria,† and has a score of ≥4 when the weights from the 5 criteria items below are summed.

Item	Weight/score
Labial salivary gland with focal lymphocytic sialadenitis and focus score of ≥1 foci/4 mm2	3
Anti-SSA/Ro positive	3
Ocular Staining Score ≥5 (or van Bijsterveld score ≥4) in at least 1 eye	1
Schirmer's test ≤5 mm/5 minutes in at least 1 eye	1
Unstimulated whole saliva flow rate ≤0.1 ml/minute	1

* These inclusion criteria are applicable to any patient with at least 1 symptom of ocular or oral dryness, for the definition see Shiboski et al. (106).

† Exclusion criteria include prior diagnosis of any of the following conditions, which would exclude diagnosis of SS and participation in SS studies or therapeutic trials because of overlapping clinical features or interference with criteria tests: 1) history of head and neck radiation treatment, 2) active hepatitis C infection (with confirmation by polymerase chain reaction, 3) AIDS, 4) sarcoidosis, 5) amyloidosis, 6) graft-versus-host disease, 7) IgG4-related disease.

Antirheumatic treatment

Corticosteroids

The corticosteroids have variable degrees of glucocorticoid and mineralocorticoid effects. The glucocorticoids have strong anti-inflammatory and immunosuppressive effects, mediated through both genomic and nongenomic mechanisms (111). Glucocorticoids bind to cytosolic glucocorticoid receptors, which act as ligand-

inducible transcriptional factors to activate anti-inflammatory and repress pro-inflammatory genes, such as nuclear factor κB. The following effects are reduced cytokine production (e.g. IL-1, IL-6 and TNF-α), decreased chemotaxis and adhesion of leukocytes, impaired phagocytosis and lymphocyte anergy (112). Higher doses of glucocorticoids suppress antibody responses. Because of the quick onset of action and strong anti-inflammatory effects glucocorticoids are essential in the treatment of rheumatic diseases. However, their long-term use results in serious adverse effects, such as bacterial, viral and fungal infections, diabetes mellitus, hypertension, osteoporosis, psychosis, depression, skin atrophy and bruising. Severe organ involvement in AAV is often treated with intravenous pulses of methylprednisolone 1000 mg daily for three days, or oral prednisolone 1 mg/kg daily, which is tapered to 30-40 mg by one month, and 10-20 mg by three months (113).

Conventional disease-modifying antirheumatic drugs (DMARDs)

Methotrexate (MTX) has been the predominant treatment for rheumatoid arthritis patients since three decades (114). In the oncological setting, high doses (up to 1000 mg) of MTX antagonizes folate at the enzyme tetrahydrofolate reductase which blocks purine synthesis, resulting in cell cycle arrest at S phase and subsequent apoptosis of malignant cells (114). Low dose (15-25 mg/week) MTX has a half-life of 6 hours (115). Its positive antirheumatic effect consist of slow onset anti-inflammatory action, which is usually first noted after 4-8 weeks (116). The efficacy of MTX in RA is well-documented, a Cochrane Review concluded that the pooled numbers-needed-to-treat (NNT) to achieve 50 % improvement of disease parameters was 7 patients (117). The mechanisms behind the anti-inflammatory effects of low-dose MTX are still not fully understood (114). Methotrexate modulates cell-signalling pathways, which regulates the functions of most cells involved in inflammation (118). One of the most important mechanisms is MTXs ability to promote release of adenosine, which binds to cell surface receptors and exerts strong inhibitory effects on neutrophils, macrophages, T cells and other inflammatory cell types (119). When therapeutic monoclonal antibodies (Mabs) are used in combination with MTX, interaction between MTX and B-cell activation factor (BAFF) promotes adenosine release from regulatory B-cells which reduce immunization against Mabs (120).

Other antimetabolite DMARDs include the purine antagonist azathioprine (AZA), which in oral doses 50-200 mg daily have anti-inflammatory actions used mainly in treatment of SLE and systemic vasculitis (116). Mycophenolate mofetil (MMF) inhibits de novo synthesis of purines, leading to selective inhibition of lymphocyte proliferation, and is used in transplantation and treatment of AAV (121).

Cyclophosphamide (CYC) is an alkylating chemotherapeutic agent causing broad immunosuppression and B cell depletion, used in combination with glucocorticoids as induction therapy in severe cases of systemic vasculitis with internal organ involvement (113). It is either used in monthly or biweekly intravenous pulses or in daily oral doses. Adverse effects include bone marrow suppression, hemorrhagic cystitis, infertility and increased cancer risk.

Sulfasalazine (SSZ) is a slow acting anti-inflammatory drug, which is an alternative to MTX in treatment of RA patients. The anti-malaria drugs, chloroquine and hydroxychloroquine (HCQ), have moderate effects in combination with MTX or SSZ in RA patients, but are important treatments in SLE patients (116).

Biological DMARDs

Tumor necrosis factor (TNF) inhibitors, i.e. infliximab, etanercept, adalimumab, golimumab and certolizumab pegol, are inhibitors of the cytokine TNF-alpha. TNF-inhibitors are used in combination with MTX in RA patients not responding to MTX monotherapy.

Abatacept (ABT) is an efficacious treatment in RA patients not responding to TNF inhibitors (122). The mechanism of ABT is modulation of immune responses by binding to CD80/CD86 on antigen-presenting cells, thus preventing costimulatory binding of CD28 on naïve T cells and attenuating T-cell activation (Figure 2) (123). Treatment with ABT reduces levels of switched memory B cells (124). Abatacept is administered as a 500-1000 mg intravenous infusion, repeated after 2, 4 weeks, and followed by a dose every 4 weeks.

B-cell depletion therapy with anti-CD20 rituximab (RTX) causes almost total reduction of B-cells (>95%) in the circulation. The mechanisms of action of RTX are shown in Figure 3. It is used as step-up therapy in combination with MTX in RA patients who have failed to respond to TNF-inhibitors or abatacept. Rituximab is also used as an alternative to CYC for induction of remission in AAV patients, with comparable efficacy and adverse events (125, 126). In RA, the RTX regimen is 1000 mg twice over 2 weeks, and followed by treatment every 6 months. Common regimens in AAV are 375 mg/m^2 weekly for four weeks or 1000 mg twice in 2 weeks (113).

Expression of CD20 starts with pre-B cells in the bone marrow, but when naïve B cells differentiate into plasmacells, expression of CD20 is lost, which explains why long-lived plasmacells are resistant to RTX. If RTX treatment is discontinued, B-cells start replenishing after 6-9 months, but these are mostly naïve or transitional B-cells (127). One year after RTX treatment, 80% of CD27+ memory B cells were still depleted and their recovery can be delayed up to 5 years (128).

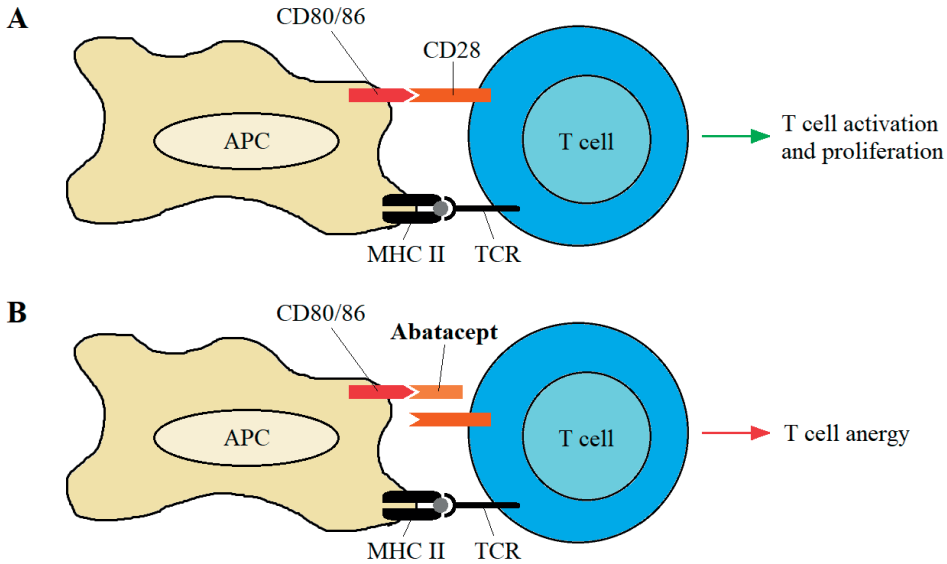

Figure 2. Mechanism of action of abatacept.
(A) Activation of naïve T cells requires at least two signals, T cell receptor (TCR) – MHC class II-antigen interaction, and co-stimulatory signal from CD28 – CD80/86 interaction. (B) Abatacept binds to CD80/CD86 on antigen-presenting cells (APC), thus preventing costimulatory binding of CD28 on naïve T cells and attenuating T-cell activation.

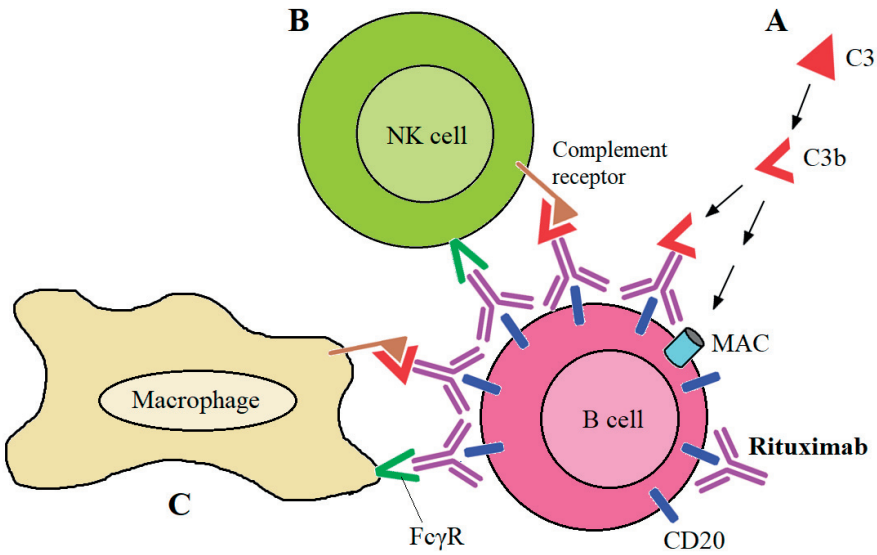

Figure 3. Mechanisms of action of rituximab.
(A) Complement-mediated cytotoxicity, (B) Direct lysis, or (C) FcγR/complement receptor-mediated opsonic phagocytosis or antibody-dependent cellular cytotoxicity. Modified after Taylor and Lindorfer (129). FcγR = Fcγ receptor. MAC = membrane attack complex.

Risk of infections in inflammatory rheumatic diseases

RA

Serious infections contribute to the increased overall mortality in patients with RA (130). The susceptibility to infections is likely due to a combination of immune pathology associated with underlying disease mechanisms in RA, comorbidities, and the use of immunocompromising drugs. Innate immune responses might be compromised by immune complex formation leading to impaired function and decreased numbers of neutrophils (131). Further, adaptive immune responses are impaired by limited T cell receptor repertoire (132), and reduced clonal expansion of naïve T cells in response to new antigen stimulation in RA patients (133).

In a retrospective cohort study by Doran et al., increased risk of infection requiring hospitalization (hazard ratio [HR]=1.8), was seen in RA patients after adjustment for confounders, e.g. glucocorticoid use, compared to age- and sex-matched non-RA controls (134). A second publication by the same authors reported age, extraarticular manifestations, leukopenia, and comorbidities such as diabetes mellitus, and corticosteroid use, to be strong predictors of infection in patients with RA (135). As previously described, the risk of IPD was more than doubled in RA patients compared to controls (28).

George et al. recently reported increased risk of hospitalized infections even with low dose glucocorticoids (≤5 mg/day) compared to no treatment in RA patients, and with higher doses a dose-dependent risk increase was observed (136). Corticosteroid treatment is a well-known risk factor for the opportunistic pneumocystis jiroveci pneumonia (137). Use of conventional (non-biologic) DMARDs without glucocorticoids has not been associated with increased risk of infectious complications in RA patients (138).

Treatment with TNF-inhibitors is well known to increase the risk of tuberculosis in RA patients (139-141), and is associated with reactivation of hepatitis B (142). In a meta-analysis, treatment with standard or high dose biological drugs with or without DMARDs was associated with increased risks of serious infections compared with traditional DMARDs (OR 1.3 and 1.9, respectively) (143). In a 2009 meta-analysis, RTX or ABT were not associated with significantly increased risks of serious infections compared with placebo, but this could have been due to the study being underpowered (144). Repeated courses of RTX are associated with hypogammaglobulinemia (145), and may also be associated with a higher rate of serious infections (146). A British biologics registry study, reported a higher risk of pneumocystis jiroveci pneumonia but lower risk of tuberculosis in RTX treated RA patients, compared with patients receiving TNF-inhibitors (147).

Systemic vasculitis

While immunosuppressive treatments are necessary in patients with systemic vasculitis, they come at the expense of life-threatening infectious complications (148). In AAV patients, infections are the most common causes of death during the first year after diagnosis, and contributes to increased morbidity and mortality for several years (149, 150). Lymphopenia, renal impairment and high-dose corticosteroids are known risk factors for severe infections in patients with AAV (151, 152).

The first year following diagnosis, patients with giant cell arteritis had doubled rates of severe infections, compared to age- and sex-matched controls, and treatment with prednisolone doses >10 mg/day one year after diagnosis was associated with increased infection related mortality (153).

Primary Sjögren's syndrome

Infections and cardiovascular disease are leading causes of mortality in pSS (154). Increased risk of pulmonary tuberculosis has been described in patients with pSS in Taiwan, and was associated with glucocorticoid use and higher age (155).

Pneumococcal vaccine response during antirheumatic treatment

Methotrexate

Our group and several others have reported impaired antibody response to pneumococcal and influenza vaccines in RA patients treated with MTX (156-160). However, the number of antigen specific plasmablasts in peripheral blood was not decreased (161).

Abatacept

Our group has previously demonstrated reduced antibody response to serotypes 6B and 23F after PCV7 in patients treated with ABT, and concomitant MTX in 76%, compared to healthy controls (162). Migita and colleagues reported similar reductions of antibody response following immunization with PPV23 in patients treated with ABT plus MTX, compared to MTX alone or RA with prednisolone monotherapy (163).

Rituximab

Previous studies have shown reduced antibody responses of either single dose PPV23 or PCV13 in RA patients treated with RTX (162, 164, 165).

Aims

The overall aims were to investigate:

I. The adherence to vaccination guidelines and the immunogenicity to PCV13 in splenectomized individuals.

II. The effect of standard of care therapy on antibody response following immunization using 13-valent pneumococcal conjugate vaccine (PCV13) in patients with systemic vasculitis compared to healthy controls.

III. If antibody response and functionality of antibodies following immunization with PCV13 is impaired in patients with RA and pSS without active anti-rheumatic treatment compared to RA patients treated with methotrexate (MTX), or to healthy controls.

IV. If the combined schedule of PCV13 followed by PPV23 improved antibody response compared with single dose PCV13 in patients with inflammatory rheumatic diseases (IRD) during various immunosuppressive therapies and in healthy controls.

V. The underlying mechanisms by which MTX exerts its effect on the antibody response using vaccination with PCV as a model for antigen challenge.

Patients and methods

Patient inclusion and pneumococcal immunization

Paper I

All patients who underwent splenectomy at the Central Hospital Kristianstad, Sweden, during the period January 2000 to October 2012 were identified using ICD-10 procedure codes for splenectomy (JMA00 or JMA10). Underlying diagnoses leading to splenectomy were noted. All patients received an invitation letter with a questionnaire regarding prior vaccinations, current medications or chronic illnesses affecting the immune system. Clinical records were scrutinized for additional information. Patients were enrolled in the study with an opt-out method, and after collection of informed consent. Patients were excluded if they had a history of allergic reaction at previous vaccinations, were pregnant, or had an ongoing infection. Participants completed all study visits at the Department of Infectious Diseases, Central Hospital Kristianstad, Sweden.

All participants who had neither received PCV13 previously nor PPV23 within the last year, and without contraindications, were immunized with one 0.5 mL dose of PCV13 (Prevenar 13®, Pfizer). Participants with a PPV23 vaccination within the last year also received dose of PCV13 if ≥2 pneumococcal serotype-specific IgG concentrations were below 0.35 μg/mL. PCV13 was administered as an intramuscular injection in the deltoid muscle.

Papers II and III

Adult patients with established systemic vasculitis (paper II), and RA or primary Sjögren's syndrome (pSS, paper III) who were regularly monitored at the Department of Rheumatology in Lund and Malmö at Skåne University Hospital were eligible for this study. In paper II, patients had to fulfil the American College of Rheumatology criteria for different systemic vasculitides (109). In paper III, patients had to fulfil the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria for RA or pSS (79, 110). Ongoing treatment at the time of vaccination was noted as a basis for later patient stratification. Patients were excluded from the study if anti-rheumatic treatment had

been changed within 4 weeks before and 6 weeks after vaccination, if they had been previously vaccinated with PPV23 within 1 year, had a history of allergic reaction at previous vaccinations, were pregnant, or had an ongoing infection. Healthy control subjects were recruited from the staff and relatives at the department of Rheumatology in Lund.

Paper IV

Adult patients with inflammatory rheumatic disease, regularly monitored at the Departments of Rheumatology at Skåne University Hospital in Lund and Central Hospital in Kristianstad, were eligible for this study. Patients had to fulfil the American College of Rheumatology (ACR)/European League Against Rheumatism (EULAR) criteria for RA or ACR criteria for systemic vasculitides (79, 109). Ongoing treatment at the time of vaccination was noted as a basis for later patient stratification. Patients were eligible for prime-boost pneumococcal vaccination if they had not previously received pneumococcal conjugate vaccine and they had not received PPV23 within the last 5 years. Patients previously immunized with one dose pneumococcal conjugate vaccine but no PPV23 within the VACCIMIL (Vaccination in Inflammatory Rheumatic Disease) study (166) were eligible for immunization with a PPV23 booster dose within the present study. Patients were excluded from the study if antirheumatic treatment had been changed within 4 weeks before vaccination, had a history of allergic reaction at previous vaccinations, were pregnant, or had an ongoing infection. Healthy control subjects were recruited from the staff and relatives at the Department of Rheumatology in Lund.

All participants fulfilling criteria for prime-boost pneumococcal vaccination were immunized with a single 0.5 mL dose of PCV13 (Prevenar 13®, Pfizer), followed after 8 weeks by a single 0.5 mL dose of PPV23 (Pneumovax®, MSD), administered as intramuscular injections in the deltoid muscle by a physician or nurse. Participants previously immunized with a single dose of PCV13 within the VACCIMIL study received a single 0.5 mL dose of PPV23 in the present study. In the RTX group ($n = 30$), a subgroup of 10 patients had previously received a single 0.5 mL dose of PCV7 (Prevenar®, Pfizer), and they were immunized with a 0.5 mL dose of PPV23.

For all participants receiving prime-boost pneumococcal vaccination, serum samples were collected immediately before administration of PCV13 and PPV23 vaccines and 4–6 weeks after PPV23. For participants previously included in the VACCIMIL study, serum samples were drawn immediately before administration of PPV23 vaccine and 4–6 weeks after, and frozen serum samples taken immediately before and 4–6 weeks after prior PCV vaccination were re-analyzed

Paper V

Adult RA patients either planned to start methotrexate treatment (MTX-group), or without ongoing/planned DMARD treatment (0DMARD-group), at the Department of Rheumatology, Skåne University Hospital Lund were eligible for this study. Patients had to fulfil the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) criteria for RA (79, 109). At the time of inclusion, a rheumatologist performed a clinical examination and data were collected on disease and treatment characteristics and previous vaccinations using a structured protocol. Patients were excluded from the study if they had been treated with DMARD within 6 months, were treated with prednisolone >15 mg/day, if they had previously received pneumococcal vaccine, had a history of allergic reaction at previous vaccinations, were pregnant, or had an ongoing infection. Healthy controls were recruited from the staff and relatives at the department of Rheumatology in Lund.

All participants received a single 0.5 mL dose of PCV13 (Prevenar 13®, Pfizer) administered as an intramuscular injection in the deltoid muscle. Patients in the 0DMARD-group and healthy controls received immunization at time of inclusion. Patients in the MTX-group were immunized 6-12 weeks after start of methotrexate treatment and being on stabile MTX dose for at least 4 weeks. At time of vaccination, a clinical examination was performed, and data was collected on disease activity.

Pneumococcal serology

Multiplex fluorescent microsphere immunoassay (MFMI) - Papers I, IV and V

Sera were frozen at -80° C and subsequently analyzed at Statens Serum Institut (SSI), Copenhagen, Denmark. Pneumococcal serotype-specific IgG concentrations were determined for the 12 capsular serotypes (1, 3, 4, 5, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) common to both PCV13 and PPV23, using an in-house MFMI (Luminex) based on the procedure previously described by Lal et al. (167). This method permits simultaneous measurement of antibodies to all 12 serotypes in a single sample.

In short, pneumococcal PS purchased from SSI Diagnostica were conjugated to poly-l-lysine (PLL), and the PLL-modified PS were covalently bound to carboxyl microspheres (Luminex). Serum samples were first incubated with the PLL-PnPS-microspheres, and then with R-phycoerythrin conjugated anti-human IgG (Jackson ImmunoResearch Laboratories). The microspheres were analysed using a Bio-Plex

200 system (Bio-Rad). The assay was calibrated with international reference serum 89SF. Serotype-specific IgG concentrations were determined from a standard curve of median fluorescent intensity against expected IgG concentration for 89SF and converted to μg/ml.

In paper V, determination of pneumococcal serotype-specific IgG was performed using a different in-house MFMI at the Department of Clinical Immunology, Lund, Sweden. This method is also based on the method by Lal et al., but executed with some minor modifications. Pneumococcal polysaccharides (SSI Diagnostica A/S) were modified using 4-(4,6-dimethoxy[1,3,5]triazin-2-yl)-4-methyl-morpholinium chloride (DMTMM, Sigma Aldrich), and conjugated to magnetic carboxylated microspheres (COOH-DMTMM-method) (168). Cell wall PS (CWPS Multi, SSI Diagnostica A/S) was used for preadsorption. The assay was calibrated using pneumococcal reference serum 007Sp (NIBSC, Potters Bar, UK), according to the WHO standard protocol for pneumococcal ELISA (64).

Enzyme-linked immunosorbent assay (ELISA) – Papers II and III

Serum samples were collected immediately before and 4–6 weeks after vaccination. Serotype-specific IgG antibody concentrations for pneumococcal serotypes 6B and 23F, both included in PCV13, were quantified using enzyme-linked immunosorbent assay (ELISA) meeting World Health Organization (WHO) standard, described previously (64). The method was executed with minor modifications. In short, ELISA plates were coated with 1 μg pneumococcal capsular polysaccharides (PnPS) 6B or 23F. In order to diminish nonspecific binding, dilutions of human sera were adsorbed with pneumococcal cell wall PS, and then added to the ELISA plates. In contrast to the WHO protocol, 22F PnPS was not used for absorption. Goat anti-human IgG antibodies, conjugated with alkaline phosphatase, followed by addition of the substrate, nitrophenyl phosphate, were used for the detection of serotype-specific antibodies (anti-6B and anti-23F IgG). The optical density, proportional to the amount of anti-6B and anti-23F IgG present in the serum, was measured with an ELISA plate reader at 405 nm. The assay was calibrated with international reference serum 89SF, that was kindly provided by Dr. C. Frasch, Bethesda, MD, USA (169). This is also a modification to the latest WHO protocol which utilizes reference serum 007sp (64). The lower limit of detection was 0.01 mg/L.

Opsonophagocytic activity (OPA) assay – Papers II, III and IV

OPA assay was performed for pneumococcal serotype 23F (paper II and III), and serotypes 6B and 23F (paper IV). The method has been described by Martinez et al. (67) and was executed with some modifications.

Pneumococci of serotypes 6B and 23F obtained from Statens Serum Institut in Copenhagen, were cultured, killed by addition of glutaraldehyde and subsequently frozen in aliquots as described previously (170). Killed bacteria were thawed and incubated for 20–30 min in the dark with FITC (fluorescein isothiocyanate; F7250, Sigma-Aldrich, St. Louis, MO, USA) in sodium carbonate buffer, subsequently washed 3 times in VBS-CaMg (veronal-buffered saline with 0.15 mM Ca^{2+} and 0.5 mM Mg^{2+}).

FITC-labeled bacteria (5 × 107/mL), 20 µL suspended in VBSCaMg were incubated with 10 µL of heat-inactivated patient or control serum (prediluted 1/16 in VBS-CaMg) for 30 min at 37 °C. Subsequently 20 µL of baby rabbit serum (CL3441, Cedarlane, USA) was added and incubation was continued for 30 min at 37 °C. Polymorphonuclear leukocytes from healthy donors, obtained as previously described (170) were preincubated with PE (Phycoerythrin)-labeled anti-CD66 (551480, BD Biosciences, Franklin Lakes, NJ, USA) and subsequently added to the opsonized bacteria at a final concentration of 800 cells/mL. After incubation for 30 min at 37 °C, cells were analysed with BD Accuri C6 flow cytometry (BD Biosciences). Results were expressed as percentage of cells (PE positive events) with significant uptake of bacteria (events positive for both PE and FITC). Inter-assay variation was compensated for by adjusting values to the mean value of a serum with high opsonizing ability, included in each analysis. A negative control consisting of bacteria preincubated only with BSA and no serum was also included in each analysis.

Phenotyping of lymphocytes with flow cytometry

Venous blood was obtained in heparin tubes, 4-6 ml (Becton Dickinson, BD, Vacutainer ref 369622). The tubes were stored dark at room temperature and all samples were analyzed within 24 h. The red blood cells were lysed by adding the blood to 45 ml 0.84% ammonium chloride for 10 min in room temperature. The lysed blood was then centrifuged for 10 min at 250×g. The cells were washed once with 50 ml PBS (without Mg2+ and Ca2+) and centrifuged for 10 min at 250×g. After centrifugation, the cell pellet was resuspended in 100 µl FACS buffer (PBS + 0.5% BSA). The cells were divided in three tubes, 50 µl cell suspension to each tube. 50 µl of antibody mix was added to their respective FACS tube (for antibody mix, see supplemental table 1). The cells were incubated for 20 min, dark in room temperature. The cells were then washed by adding 3 ml PBS and centrifuged for 3 min at 250×g and resuspended in 250 µl PBS. The analyses were performed on FACS Aria Fusion (BD Bioscience) using FACS Diva software. Cell populations were identified according to the gating strategies described by Maecker et al. (171).

Statistical methods

Differences between groups were analysed using the Chi-square test and the Mann-Whitney U test when appropriate.

Serotype-specific IgG concentrations as determined by ELISA or MFMI were log-transformed to calculate geometric mean concentrations (GMC) with 95% confidence intervals (CI). Pre- to postvaccine changes in specific IgG were compared using nonparametric (Wilcoxon's matched pairs signed rank test) or parametric paired statistics (paired T-test) as appropriate.

Antibody response ratio (ARR, i.e., the ratio of post- to prevaccination serotype-specific IgG concentration) was calculated, and positive antibody response was defined as ARR ≥2. Because there is no international consensus definition of putative protective IgG concentration after pneumococcal vaccination, different thresholds were examined: ≥0.35 μg/mL (paper I), ≥1.0 μg/mL (papers I and II), ≥1.3 μg/mL (papers III and IV), and ≥5.0 μg/mL (paper I). Proportions of individuals with positive antibody responses and putative protective IgG were calculated with 95% confidence intervals (95% CI). Proportions of patients with putative protective levels pre- and postvaccination (matched pairs) were compared using McNemars test.

In paper IV, sums of serotypes with positive antibody responses and putative protective levels, respectively, were calculated for each study participant. Pre- to postvaccine differences within groups were tested using Wilcoxon matched-pairs signed-ranks test. We used multivariate linear regression to examine the possible influence of different exposure variables on outcome, i.e. number of serotypes with positive antibody response. The following variables were included in a multivariate regression model: gender, age (years), C-reactive protein (CRP, mg/L), ongoing rituximab (yes/no), abatacept (yes/no), conventional DMARD (cDMARD) (yes/no) and prednisolone dose (mg/day). In a stepwise selection procedure; (1) each variable was omitted in turn, *P*-value for each likelihood ratio test was recorded, and (2) the model was fitted with all variables except for the one with highest *P* value in step one. Step (1) and (2) were repeated until only variables with $P < 0.10$ were left to be included in the final model.

Possible monotonic associations between two variables were examined using Spearman's rank correlation test.

Results

Splenectomy patients (paper I)

In total, 78 patients underwent splenectomy at the Central Hospital Kristianstad between January 2000 and October 2012. The median age at the time of splenectomy was 61.5 years (range 11–88 years). Thirty-one individuals were deceased at the start of the study, and of these 5 patients (6.4%) had died within 14 days post-splenectomy. Twelve patients had died at the hospital, and the following causes of death were identified in records: pneumonia or sepsis of unknown etiology (n = 4), terminal cancer (n = 3), hemorrhage (n = 2), pneumonia and septic shock caused by non-encapsulated *Haemophilus influenzae* (n = 1), multitrauma (n = 1), and cardiac failure (n = 1). Nineteen patients had died in a hospice, nursing home or at home, and the final causes of death in this group is unknown to the authors but ten of these patients had been diagnosed with metastatic cancer.

Adherence to vaccination guidelines

Regarding vaccination against pneumococci, 64 individuals (81.0%) had received pneumococcal vaccine, with the following primary schedules: 1 dose PPV23 (n=59), and PCV13 followed by PPV23 after 8 weeks (n=5). The number of patients immunized against *Haemophilus influenzae* type B and meningococci (A+C polysaccharide or conjugated A, C, Y, and W-135 vaccine) were 41 (51.9%) and 18 (22.8%), respectively.

Immunogenicity of PCV13 in asplenic individuals with previous PPV23

Twenty-four splenectomized individuals received immunization with one dose PCV13. In this group, all had previously received PPV23, with the following doses (numbers of individuals; range of time since the last dose of PPV23): 1 dose (n=12; 0.8–13.0 years), 2 doses (n=10; 0.5–6.5 years) and 3 doses (n=1; 1.3 years). Mean years since vaccination was 4.6 (range 0.5–13.0 years). Nine individuals were classified as immunocompromised for the following reasons: hematological malignancies (n = 5), ongoing immunosuppressive treatment (n = 3, methotrexate or rituximab) and generalized solid organ malignancy (n = 1).

High levels of specific IgG were observed before immunization with PCV13, with GMC ≥0.35 µg/mL for 10/12 serotypes (1, 4, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F), and GMC ≥1.0 µg/mL for 8/12 serotypes (1, 6B, 7F, 9V, 14, 19A, 19F, and 23F), in all study participants. In the immunocompromised subgroup, GMC ≥0.35 µg/mL and ≥1.0 µg/mL, were observed respectively for 9/12 and 7/12 serotypes.

Serotype-specific IgG concentrations increased significantly pre- to post-PCV13 for pneumococcal serotypes 1, 3, 4, 5, 7F, 18C, 19A, 23F ($p \leq 0.001$), and 19F ($p = 0.01$, Figure 4). After PCV13, the following levels of IgG GMCs were observed: ≥0.35 µg/mL (all serotypes), and ≥1.00 µg/mL (all except serotype 3).

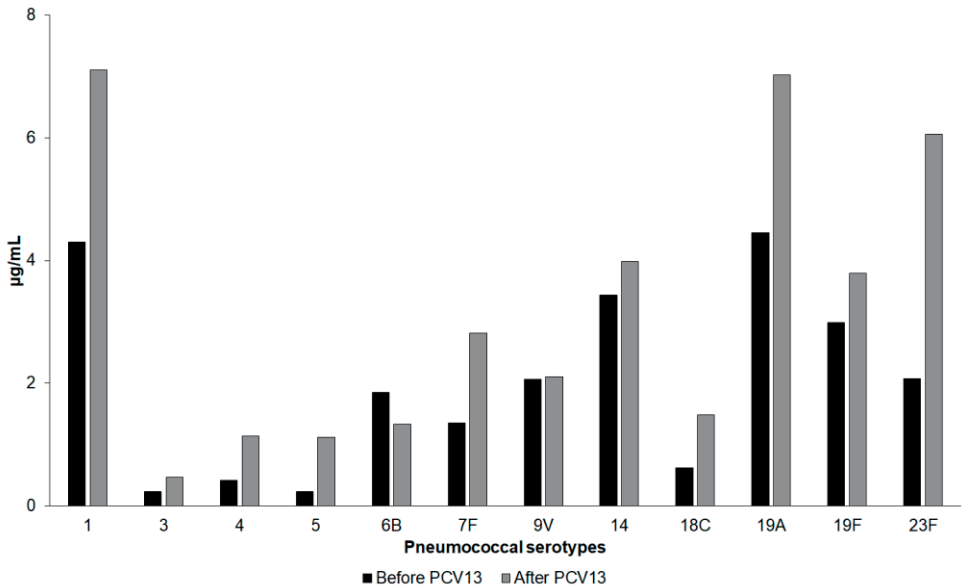

Figure 4. Geometric mean concentrations before and after PCV13 immunization in 24 patients who previously had received PPV23 .

Systemic vasculitis patients (paper II)

Forty-nine patients diagnosed with systemic vasculitides and 49 controls participated in the study. Diagnoses, disease characteristics, ongoing treatments and prior vaccinations are shown in Table 5. Patients receiving biologics were excluded from subgroup analyses because of small sample size. Remaining patients were stratified into two groups based on their treatment, as follows: Patients with MTX, AZA or CYC (n = 26); Patients with prednisolone monotherapy (n = 15). There was no age difference between MTX/AZA/CYC group and controls, but the prednisolone monotherapy group was older than controls (p = 0.003). In group 1, 81% of patients were diagnosed with GPA/EGPA, in contrast to group 2, in which 87% of patients were diagnosed with giant cell arteritis.

Table 5.
Demographic, diagnoses, disease characteristics, treatments and previous pneumococcal vaccination at the time of vaccination.

	All patients (*n*=49)	**MTX/AZA/CYC (*n*=26)**	**Prednisolone (*n*=15)**	**Controls (*n*=49)**
Age, median (range) years	65 (22-85)	61 (26-85)	70 (56-85)	57 (17-85)
Sex, % females	51	50	40	63
Diagnoses, *n* (%):				
Granulomatosis with polyangiitis (GPA)	21 (43)	16 (62)	1 (7)	-
Eosinophilic granulomatosis with polyangiitis (EGPA)	8 (16)	5 (19)	1 (7)	-
Giant cell vasculitis	13 (27)	0	13 (87)	-
Takayasu arteritis	4 (8)	2 (8)	0	-
Other vasculitis	3 (6)	2 (8)	0	-
Disease characteristics:				
Disease duration, mean (range) years	5.1 (0-42)	5.8 (0-42)	2.5 (0-18)	-
C-reactive protein, median (range) mg/L	2.3 (0-141)	2.2 (0-84)	5.4 (0-141)	0.8 (0-12)
Erythrocyte sedimentation rate, median (range) mm/h	10 (2-80)	10 (2-44)	14.5 (6-80)	-
Ongoing treatments, *n* (%):				
Azathioprine (AZA)	11 (22)	11 (42)	0	-
Cyclophosphamide (CYC)	6 (12)	6 (23)	0	-
Methotrexate (MTX)	9 (18)	9 (35)	0	-
Mycophenolate mofetil	2 (4)	0	0	-
Prednisolone	42 (86)	22 (85)	15 (100)	-
Rituximab	3 (6)	0	0	-
TNF inhibitor	2 (4)	0	0	-
No active treatment	2 (4)	0	0	-
Prednisolone dose, median (range) mg/day	7.5 (0-65)	5 (0-60)	12,5 (2.5-65)	-
Previous vaccinations:				
PPV23, *n* (%)	4 (8)	2 (8)	1 (7)	3 (6)

Safety

Localized pain or redness at the injection site, or headache was reported by 13 patients and 16 controls. A few patients experienced slightly increased body temperature and one patient reported general muscle pain and difficulties to move the upper arm where the vaccine was injected. All adverse reactions were considered mild and resolved within a few days.

Serotype-specific anti-pneumococcal antibody

Serotype-specific IgG increased for serotypes 6B and 23F, in both patient and control groups ($p < 0.001$, Figure 5). Postvaccination GMC (95% CI) for 6B and 23F were respectively in patients: 1.7 (0.9–2.9) µg/mL and 1.8 (1.2–2.8) µg/mL, and in controls: 3.1 (1.9–5.0) µg/mL and 3.3 (2.0–5.5) µg/mL. In patient subgroups 1 and 2, specific antibody increased for both serotypes.

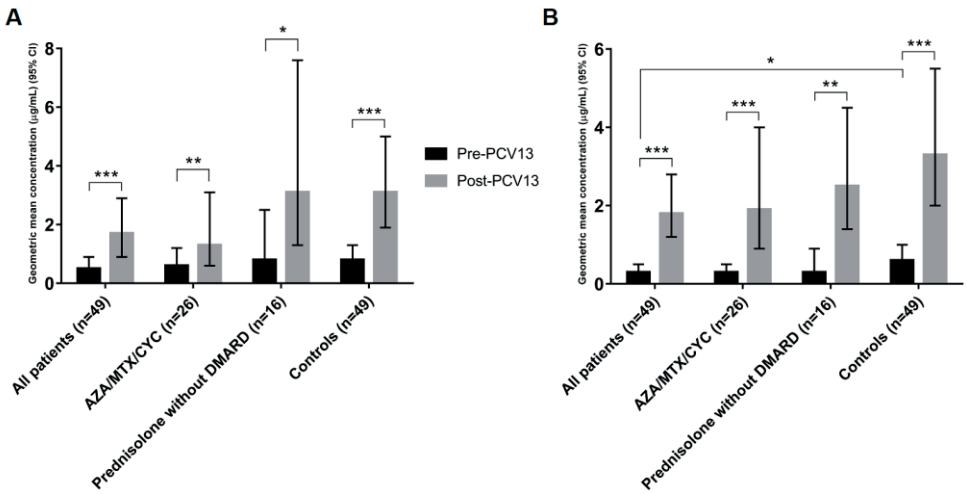

Figure 5. Pneumococcal serotype 6B (A) and 23F (B) geometric mean concentrations before and after immunization with PCV13 in systemic vasculitis treatment groups and healthy controls.
*p≤0.05, ** p≤0.01, *** p≤0.001.

Antibody response

No significant differences were found in the proportions of individuals with a positive antibody response (i.e. ARR ≥2) to serotype 6B, 23F or both serotypes in all patients, or subgroups, compared to controls (Figure 6).

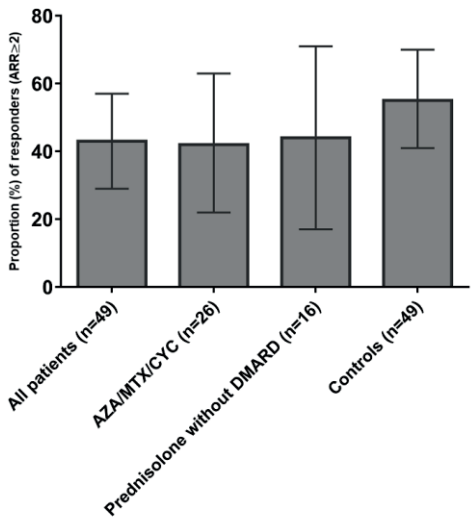

Figure 6. Responders (% with ARR ≥2 for 6B and 23F) to immunization with PCV13.

Opsonophagocytic activity (OPA)

Phagocytosis of serotype 23F pneumococci as measured by OPA assay increased in both controls (n = 36) and patients (n = 48) after vaccination (both $p < 0.001$, Figure 7). Prevaccination OPA was numerically lower in patients (0.0%) compared to controls (6.2%, ns). Postvaccination OPA was lower in patients (6.7%) compared with controls (31.9%, $p = 0.001$). Patients on DMARDs (group 1) had lower postvaccination OPA compared with controls ($p = 0.043$). Patients treated with prednisolone monotherapy (group 2) had lower both pre- and postvaccination OPA compared with controls ($p = 0.007$ and $p = 0.002$).

Correlations between ELISA IgG and OPA

After vaccination, there were significant correlations between serotype 23F IgG measured by ELISA and phagocytosis (%), in both patients (correlation coefficient = 0.33, $p = 0.02$) and controls (correlation coefficient = 0.54, $p = 0.001$).

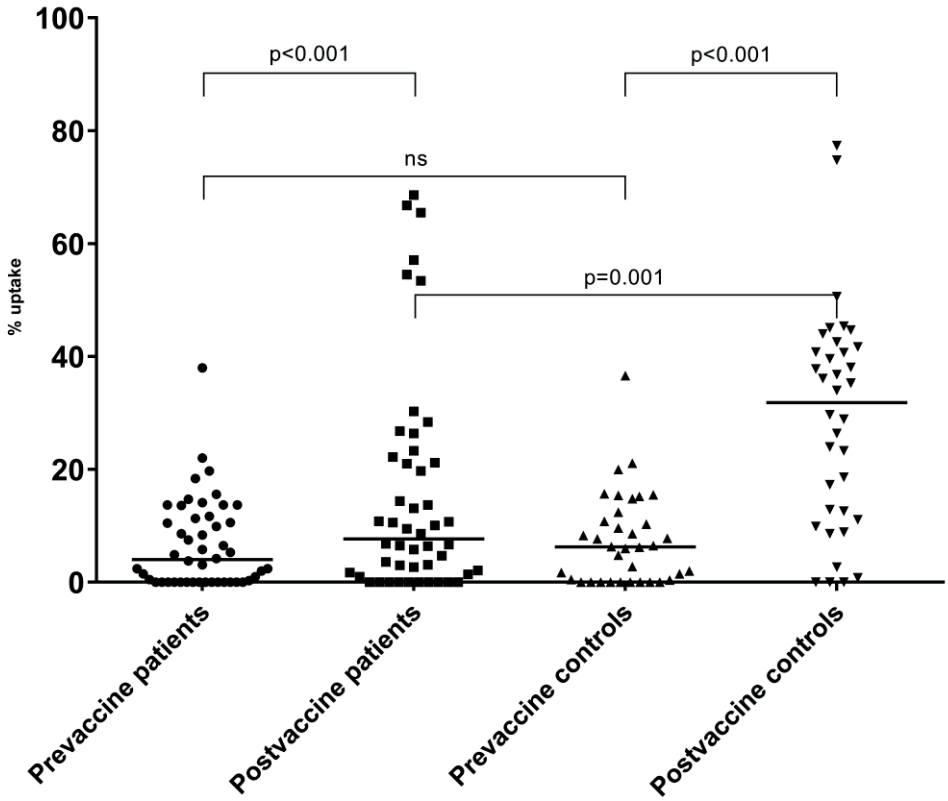

Figure 7. Proportion of phagocytes (%) with uptake of pneumococcal serotype 23F.

Patients with rheumatoid arthritis or primary Sjögren's syndrome without disease modifying treatment (paper III)

A total of 60 patients with RA (50 without DMARD and 10 on MTX), 15 patients with pSS without DMARD and 49 controls were vaccinated. The demographic details, disease characteristics, pre- and postvaccination geometric mean antibody concentrations of the participants are shown in table 6.

Table 6. Demographic and patient characteristics in treatment groups and controls.

	RA 0DMARD (*n*=50)	RA MTX (*n*=10)	pSS 0DMARD (*n*=15)	Controls (*n*=49)
Age, median (range) years	66.9 (35-87)	67.4 (39-79)	62.3 (25-89)	57.2 (17-85)
Sex (% female)	78.0	70.0	87.0	63.3
Disease duration, mean (range) years	5.6 (0-36)	13.1 (2-40)	7.0 (0-23)	-
CRP, median (range) mg/L	7 (0-78)	3 (0-11)	1.7 (0.7-38)	-
ESR, median (range) mm/h	21 (4-71)	16 (5-42)	12 (7-66)	-
RF positive, %	78	80	43	-
Anti-CCP positive, %	69	70	8	-
DAS28, mean (range)	4.4 (1.8-6.2)	2.4 (1.7-3.1)	-	-
ANA positive, %	-	-	73	-
Anti-ENA positive, %	-	-	73	-
Anti-SSA (anti-Ro) positive, %	-	-	67	-
Anti-SSB (anti-La) positive, %	-	-	40	-
Prednisolone, %	58	0	13	0
Dose, median (range) mg/day	5 (0-15)	0	0 (0-10)	-
Previous PPV23, *n* (%):	1 (2)	0	0	3 (6)

DMARD = Disease modifying anti rheumatic drugs, RA = Rheumatoid arthritis, pSS = primary Sjögren's syndrome; CRP = C-reactive protein, ESR = Erythrocyte sedimentation rate; Anti-CCP = antibodies against cyclic-citrullinated peptides, ANA = Antinuclear antibodies, Anti-ENA = Antibodies against extractable nuclear antigens, Anti-SSA (anti-Ro)= Anti-Sjögren's-syndrome-related antigen A, Anti-SSB (anti-La) = Anti-Sjögren's-syndrome-related antigen B, RF = Rheumatoid factor.

Antibody response

Proportions of responders (i.e. ARR≥2) to serotypes 6B and 23F was lower in patients with RA on MTX treatment (both $p<0.01$), but not in RA without DMARD or pSS without DMARD, compared to controls (Figure 8). Proportions of antibody responders to both serotypes did not differ between groups RA without DMARD (52 %), pSS without DMARD (40 %) and controls (55 %).

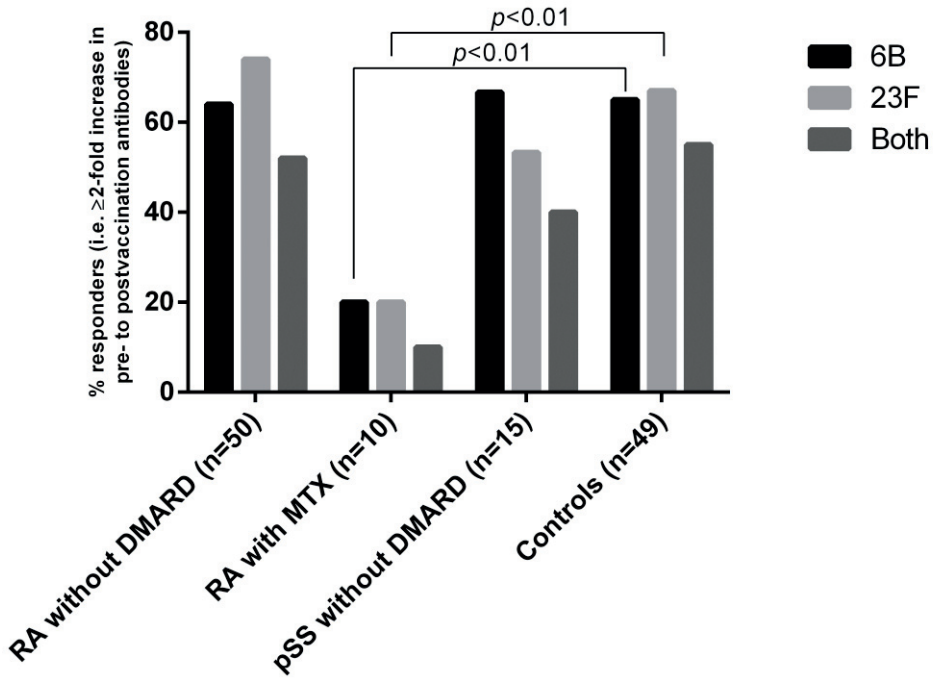

Figure 8. Proportions of responders to immunization with PCV13.

Putative protective levels

The proportions of participants with specific IgG ≥1.3 µg/mL for serotype 6B increased after vaccination in RA without DMARD ($p=0.001$) and controls ($p<0.001$) but not in RA with MTX and pSS without DMARD. For 23F, increases were found in RA without DMARD ($p<0.001$), pSS without DMARD ($p=0.05$) and controls ($p<0.001$) but not in RA with MTX. The pre- to postvaccination percentage increase for serotype 6B and 23F respectively were 24% and 42% in RA without DMARD, 10% and 20% in RA with MTX, 14% and 26% in pSS without DMARD, and 28% and 40% in controls.

Opsonophagocytic activity (OPA)

After vaccination, phagocytosis measured with OPA assay (% uptake) increased in groups RA without DMARD (p<0.001), pSS without DMARD (p=0.03) and controls (p<0.001) but no increase was seen in patients with RA on MTX. Positive correlations between percentage change in OPA and pre- to postvaccination increase in ELISA were found for patients with RA without DMARD (ρ=0.28, p=0.03) and controls (ρ=0.45, p=0.001).

Predictors of positive antibody response

In a multivariate logistic regression model neither RA diagnosis (OR=1.2, 95% CI 0.5-2.6, p=0.7) nor age (OR=0.99, 0.96-1.0, p=0.3), but treatment with MTX was associated with a lower probability (OR=0.10, 0.01-0.87, p=0.037) of positive antibody response to both serotypes.

The higher the prevaccination serotype-specific IgG levels the less likely a significant rise in specific IgG will occur after immunization (72). Therefore, two separate models of the respective responses to 6B and 23F was made, using the same covariates but with added adjustment for prevaccine IgG level. Both models yielded similar results regarding the effect of MTX treatment on antibody response: 6B (OR=0.15, 0.03-0.89, p=0.036), and 23F (OR=0.09, 0.02-0.51, p<0.006), and for 6B prevaccination IgG was negatively associated with response (OR=0.56, 0.42-0.74, p<0.001).

Prime-boost vaccination strategy in patients receiving conventional DMARDs, abatacept and rituximab (paper IV)

Patients treated with rituximab (RTX, n=30), abatacept (ABT, n = 23), or monotherapy with conventional DMARD (cDMARD, n = 27, i.e. MTX/AZA/MMF), and 28 healthy controls participated in the study. All patients in the RTX group (n = 30) had been started on treatment with RTX (at least 2 doses) before receiving PCV immunization (PCV13 n = 20; PCV7 n = 10), and had ongoing RTX at the time of PPV23 immunization. In the RTX group, 16 patients (53%) had concomitant treatment with MTX. In the ABT group (n=23), patients had ongoing treatment with ABT since at least 6 months, and 11 patients (48%) were also treated with MTX. Patients and controls were included in this study in accordance with Figure 9. Demographic data, diagnoses, disease characteristics, and medication details in the treatment groups and controls are summarized in Table 7.

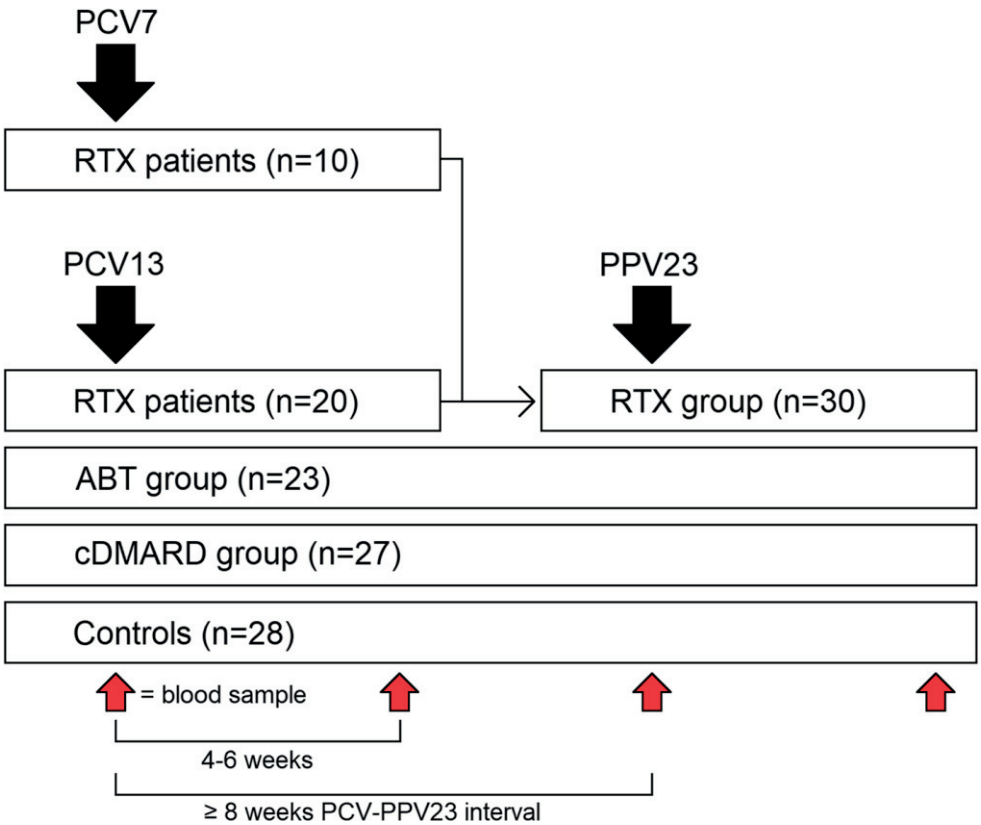

Figure 9. Schematic of PCV and PPV23 immunizations and blood samples in treatment groups and controls.

Table 7.
Demographic, diagnoses, disease characteristics, and treatment at inclusion in study, in treatment groups and controls.

	Rituximab	Abatacept	cDMARD[1]	Controls
N	30	23	27	28
Female gender, %	53%	83%	74%	64%
Age, median (range) years	69 (31-88)[2]	64 (42-78)[2]	68 (25-87)[2]	55 (18-84)
Rheumatoid arthritis, n (%)	27 (90%)	23 (100 %)	14 (52%)	0
RF-positive (% of RA patients)	100%	79%	90%	-
Anti-CCP-positive (% of RA patients)	92%	68%	80%	-
Granulomatosis with polyangiitis, n (%)	3 (10%)	0	7 (26%)	0
Eosinophilic granulomatosis with polyangiitis, n (%)	0	0	3 (11%)	0
Other systemic vasculitis, n (%)	0	0	3 (11%)	0
Disease duration, median (range) years	20 (2-57)	15 (4-45)	5 (2-46)	-
DAS28 in RA patients, median (range)	2.7 (0.5-6.5)[3]	3.2 (1.5-5.5)	2.3 (1.6-4.3)	-
CRP, median mg/L	3.0	2.3	3.1	0.7
Total IgG, median (range) g/L	7.4 (4.0-13.9)	-	-	-
RTX duration, median (range) years	6.3 (0.7-10.9)	-	-	-
ABT duration, median (range) years	-	3.7 (0.7-10.2)	-	-
cDMARD duration, median (range) years	12.9 (3.3-22.4)	10.3 (4.2-20.3)	3.5 (1.4-15.3)	-
MTX, n (%)	16 (53%)	11 (48%)	19 (70%)	0
MTX mg/week, median	15	20	20	0
Azathioprine, n (%)	1 (3%)	0	5 (19%)	0
Azathioprine mg/day, median	150	0	100	0
Mycophenolate mofetil, n (%)	0	0	3 (11%)	0
Mycophenolate mofetil mg/day, median	0	0	1500	0
Prednisolone, n (%)	10 (33%)	10 (43%)	15 (56%)	0
Prednisolone mg/day, median (range)	5 (2.5-15)	5.6 (2.5-20)	5 (2.5-15)	0
Previous treatment with TNFα-inhibitor (%)	72.4	80.0	11.1[4]	0

1. Conventional disease-modifying antirheumatic drugs: methotrexate, azathioprine or mycophenolate mofetil.
2. All treatment groups were older than controls (all $p<0.05$).
3. DAS28 did not differ between treatment groups.
4. In the cDMARD group, compared to other treatment groups, a lower proportion of patients had previously received TNFα-inhibitor treatment.

Positive antibody responses

The numbers of serotypes with positive antibody responses (ARR ≥ 2) in treatment groups and controls after PCV and PPV23 are shown in Figure 10. PCV13 + PPV23 compared to single-dose PCV13 resulted in an increased number of serotypes with positive responses in the ABT ($p = 0.016$), cDMARD ($p = 0.013$), and control groups ($p = 0.007$). Compared to controls, the numbers of serotypes with positive response after PCV + PPV23 were reduced in all patients groups ($p < 0.001$). Antibody responses after PCV13 and PCV13+PPV23 were lower in RTX group, compared to ABT and cDMARD groups ($p<0.001$). There was no difference in antibody response in ABT compared to cDMARD group.

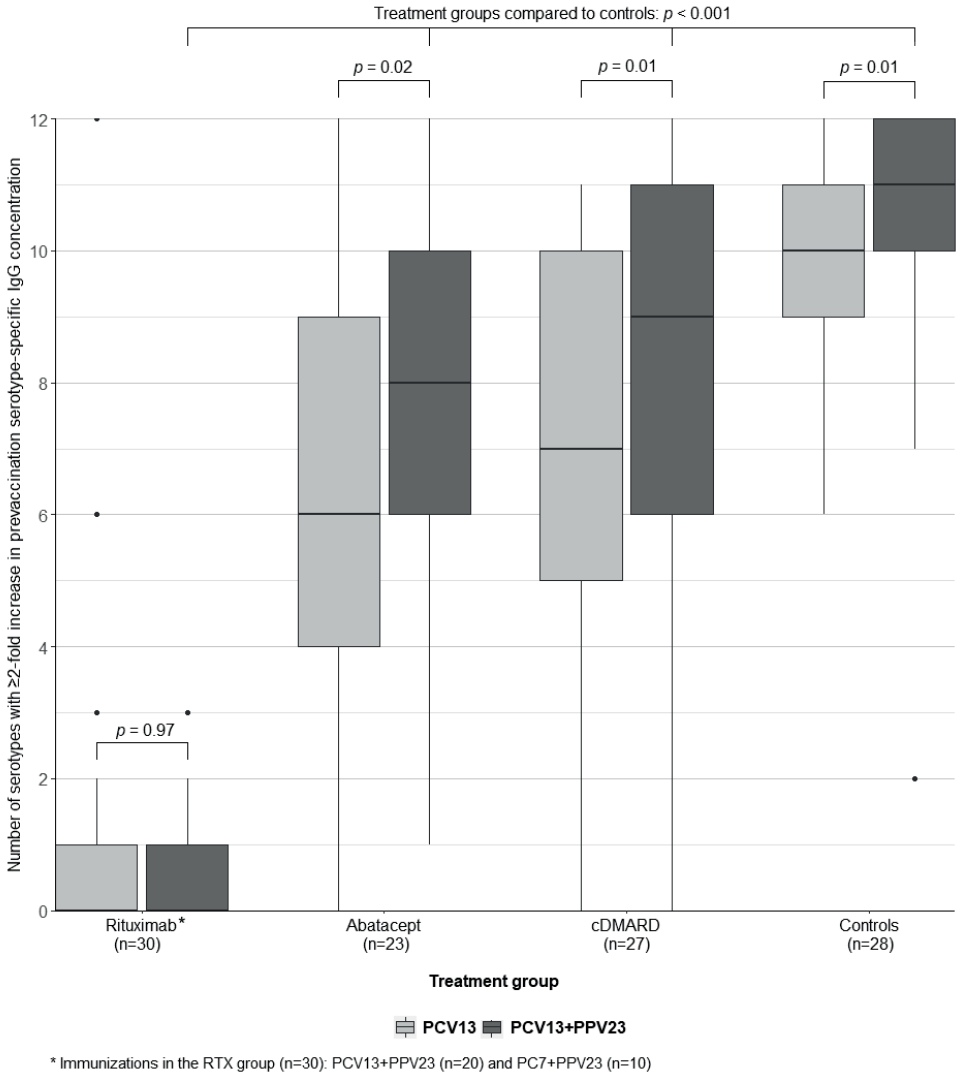

Figure 10. Antibody response to PCV and PPV23 in treatment groups and controls.
Median number of serotypes with positive antibody response after PCV13 and PCV13 + PPV23 in treatment groups and controls.

Putative protective levels

In the RTX group, the number of serotypes with specific IgG concentration ≥ 1.3 µg/mL increased slightly pre- to post-PCV (median 2 to 3, $p = 0.03$), but no further increase was seen post-PPV23 ($p = 0.98$, Figure 11). In the ABT group, there was a pre- to post-PCV13 increase from median 2 to 6 serotypes with protective

level ($p < 0.001$), but no changes were seen post-PPV23 ($p = 0.63$). The number of serotypes ≥ 1.3 μg/mL in the cDMARD group increased pre- to post-PCV13 (median 1 to 4, $p < 0.001$) and post-PPV23 (median 4 to 7, $p = 0.03$). Comparing treatment groups with controls, no significant differences in the number of serotypes with protective levels before vaccination were found. Post-PCV protective levels in the RTX, ABT, and cDMARD groups were reduced compared to controls ($p < 0.001$, $p = 0.02$, and $p = 0.002$). Post-PPV23 protective levels were reduced in all groups compared with controls (RTX, $p < 0.001$; ABT, $p < 0.001$; and cDMARD, $p < 0.001$). Post-PPV23 protective levels were lower in RTX group, compared to ABT and cDMARD groups ($p<0.001$), but there was no difference between ABT and cDMARD groups.

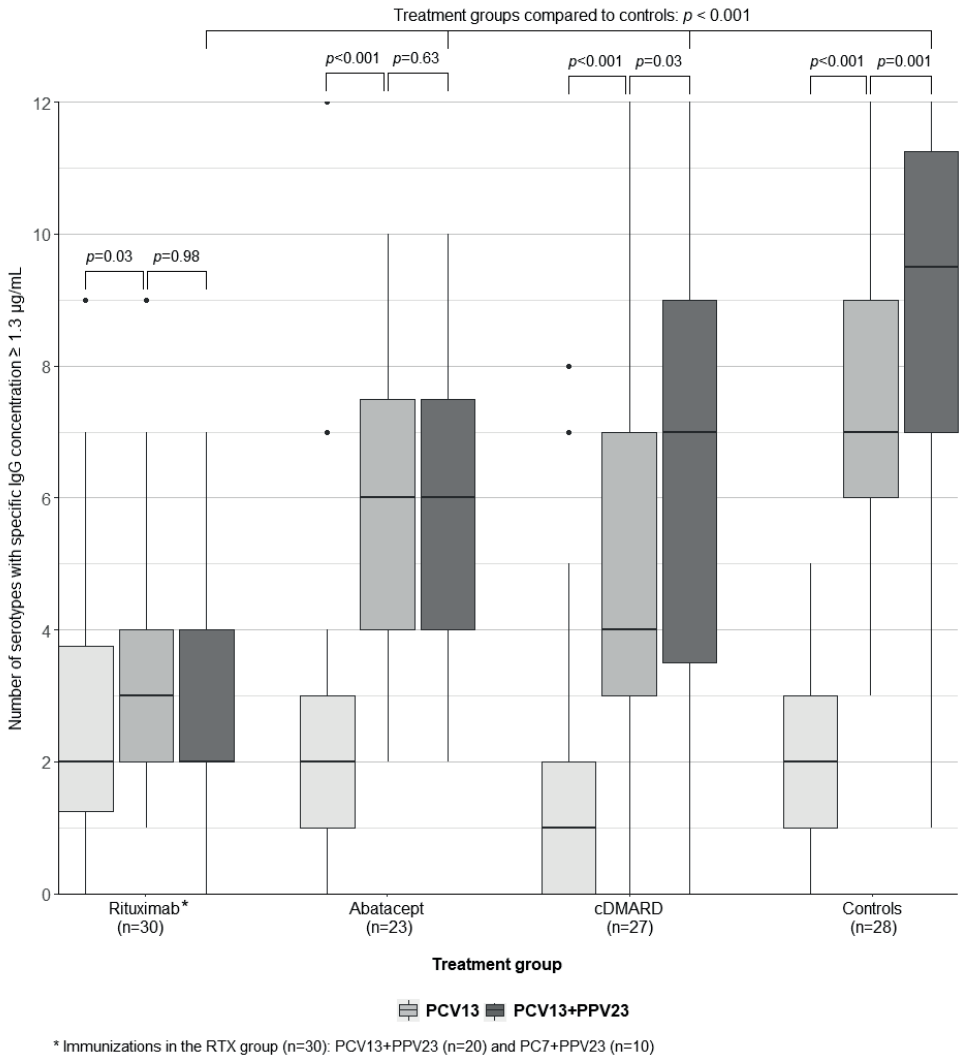

Figure 11. Number of serotypes with specific IgG concentration ≥1.3 µg/mL after PCV13 and PCV13+PPV23 in treatment groups and controls.

Predictors of positive antibody response to prime-boost pneumococcal vaccination

A multivariate linear regression model was derived in a stepwise selection procedure, where gender, age, CRP, and prednisolone dose were omitted each in turn because of no association with the outcome (likelihood test, all $p \geq 0.10$), i.e., the number of serotypes with positive antibody response (Table 7). Rituximab was found to be an independent risk factor associated with a large reduction and abatacept and cDMARD with a moderate reduction in number of serotypes with positive antibody response (Table 8). Within the RTX group, the number of responding serotypes was not associated with rituximab treatment duration (data not shown). In a separate, multivariate regression model of positive antibody response in RA patients, DAS28 was not associated with the outcome ($p = 0.61$).

Table 8.
Predictors of number of serotypes (0-12) with positive antibody response, i.e. ≥2-fold increase from prevaccination serotype-specific [IgG], after prime-boost vaccination.

	Stepwise selection of exposure variables, *p* of likelihood ratio test				**Multivariate linear regression model**		
Predictors:	1	2	3	4	Coefficient estimate	95 % CI	*P*
Intercept (control)					11.2	10.3, 12.1	<0.001
Rituximab (yes/no)	<0.001	<0.001	<0.001	<0.001	-8.6	-9.8, -7.4	<0.001
Abatacept (yes/no)	0.008	0.009	0.009	0.007	-1.9	-3.2, -0.6	0.005
cDMARD (yes/no)	<0.001	<0.001	<0.001	<0.001	-1.8	-2.8, -0.8	<0.001
Gender	0.11	0.13	0.13	0.10	Goodness of fit:		
Age (years)	0.59	-	-	-	Multiple $R^2 = 0.69$		
CRP (mg/L)	0.38	0.39	0.36	-			
Prednisolone dose (mg/day)	0.56	0.63	-	-			

Opsonophagocytosis of pneumococcal serotypes 6B and 23F

In the RTX group, functionality of antibodies for pneumococcal serotypes 6B (Pn6B) and 23F (Pn23F), as measured by OPA assay, neither increased after PCV prime nor PPV23 boost immunization, and post-PPV23 OPA was reduced compared to controls (both serotypes $p < 0.001$). In the ABT group, OPA increased after immunization with PCV (Pn6B, $p = 0.002$ and Pn23F, $p = 0.008$) but did not increase further after PPV23, and post-PPV23 OPA for Pn23F was reduced compared to controls ($p = 0.020$). In the cDMARD group, OPA increased after PCV for Pn6B ($p = 0.017$) but did not increase further after PPV23. In this group, PCV13 + PPV23 resulted in increased OPA ($p = 0.003$) for Pn23F, and post-PPV23 OPA for Pn23F was similar to controls. There were no differences between post-PCV13 and post-PPV23 OPA in the control group.

Methotrexate reduced Th17 cells and impaired plasmablast and memory B cell responses after PCV immunization in RA patients (paper V)

Rheumatoid arthritis patients without ongoing or planned DMARD treatment (0DMARD group, n=12), RA patients planned to start MTX treatment (MTX group, n=11), and healthy controls (HC, n=13) were enrolled in the study. Disease activity was higher in the MTX group compared to the 0DMARD group (p=0.01). Demographics, laboratory and clinical data are summarized in Table 9.

Table 9. Demographic, clinical and laboratory characteristics and treatment.

	Healthy controls	RA 0DMARD	RA MTX
N	13	12	11
Age years, median (range)	40.0 (32.1-62.7)	56.6 (29.7-74.3)	63.1 (39.5-82.1)[1]
Gender, female	67 %	67 %	90 %
RF positive	-	75 %	100 %
ACPA positive	-	92 %	45 %[2]
DAS28 at inclusion, median (range)	-	4.7 (2.9-7.0)	5.7 (4.6-7.5)[3]
DAS28 at vaccination, median (range)	-	4.7 (2.9-7.0)	4.6 (2.2-6.0)
CRP at vaccination, mg/L	0.7 (0.6-5.3)	3.9 (0.6-9.1)[4]	2.8 (0.6-14.0)[4]
ESR at vaccination, mm	5 (2-19)	25.5 (5-66)[5]	35 (4-64)[5]
Disease duration years, median (range)	-	5.4 (0.1-54)	0.8 (0.1-29)
Prednisolone dose, median (range) mg/day	0 (0)	0 (0-5)	0 (0-15)
MTX dose at vaccination, median (range) mg/week	0 (0)	0 (0)	20 (15-25)

1. RA with MTX-start group were older than controls (p=0.002).
2. Percentage of ACPA positives were lower in RA with MTX-start compared to RA without DMARD (p=0.02).
3. DAS28 at inclusion was higher in group RA with MTX-start compared to RA without DMARD (p=0.01).
4. CRP at vaccination was higher in both RA groups compared to controls (p≤0.001)
5. ESR at vaccination was higher in both RA groups compared to controls (p<0.01)

B cell and T cell subsets at baseline

Different stages of B cells ($CD19^+$) were determined using a panel described by Maecker at al. (171). Total lymphocytes, B cell and T cell subsets are shown in Table 10. Switched memory B cells were higher in RA patients compared to HC (p=0.034 and p=0.010, Figure 12D), but did not correlate with disease activity (DAS28). Exhausted (IgD^-CD27^-) B cells, i.e. an antigen experienced non-functional phenotype (172), correlated with disease duration (R=0.53, p=0.017), and age of patients (R=0.44, p=0.05).

There were no differences in T cell subsets at baseline.

Table 2. Total lymphocytes and B and T cells with subsets at baseline.

	Healthy controls	RA 0DMARD	RA MTX
Lymphocytes, $\times 10^9$ cells/L[1]	1.4 (1.0-2.4)	1.8 (0.9-2.5)	1.7 (0.7-2.5)
B cells, % of lymphocytes	5.7 (2.6-11.2)	4.8 (1.3-14.7)	10.2 (1.8-15.7)
Naïve, % of B cells	52.4 (40.9-69.2)	51.3 (18.9-74.8)	55.6 (24.7-82.5)
Transitional, % of naïve	7.9 (1.5-15.0)	6.5 (0.2-14.2)	3.3 (0.3-14.7)
Preswitch memory, % of B cells	7.0 (2.2-25.9)	10.0 (2.0-21.3)	6.0 (1.1-14.6)
Switched memory, % of B cells	14.8 (6.1-27.7)	27.0 (9.4-57.4)[2]	23.4 (4.5-56.6)
Plasmablasts, % of switched	9.5 (2.4-30.9)	6.2 (1.9-14.3)	6.8 (1.6-11.3)
Exhausted, % of B cells	22.5 (4.9-36.1)	11.5 (4.0-24.9)	12.2 (6.1-30.4)
T cells, % of lymphocytes	72.7 (54.2-86.8)	75.4 (55.7-87.4)	72.0 (44.4-85.5)
HLA-DR$^+$, % of T cells	1.9 (0.4-7.5)	1.8 (0.4-3.5)	1.7 (0.6-6.5)
CD38$^+$, % of T cells	9.3 (2.5-31.3)	14.9 (3.1-28.9)	8.5 (2.5-37.1)
NKT cells, % of T cells	7.5 (0.8-42.2)	12.2 (0.9-48.0)	9.4 (2.2-20.7)
CD4$^+$ Th, % of T cells	66.4 (46.1-81.5)	51.6 (31.0-83.6)	66.6 (48.6-77.7)
Naïve Th, % of CD4$^+$	49.0 (10.5-58.5)	38.9 (11.6-73.3)	42.5 (13.7-64.3)
TEMRA, % of CD4$^+$	20.8 (8.4-62.0)	20.2 (11.9-67.3)	16.3 (4.2-43.0)
Central memory, % of CD4$^+$	8.3 (0.1-22.1)	9.1 (3.4-21.8)	10.5 (4.5-47.8)
Effector memory, % of CD4$^+$	18.4 (0.1-32.6)	20.2 (10.9-54.8)	19.7 (14.1-48.2)
Th1, % of CD4$^+$	10.1 (5.0-23.5)	8.4 (4.0-15.6)	11.6 (2.2-19.5)
Th2, % of CD4$^+$	18.7 (6.0-24.7)	16.8 (0.9-26.4)	15.2 (6.1-65.4)
Th17, % of CD4$^+$	18.7 (8.6-28.7)	17.1 (8.7-33.7)	18.4 (7.4-56.0)
Treg, % of CD4$^+$	2.8 (0.3-5.7)	1.2 (0.5-5.1)	2.0 (0.3-9.6)
Activated Treg, % of Treg cells	19.6 (7.4-32.3)	20.6 (8.4-43.0)	21.7 (1.9-52.2)
cmTfh, % of CD4$^+$CD45RO$^+$ T cells	21.3 (10.2-30.9)	22.7 (9.5-29.0)	14.3 (11.7-74.9)
cmTfh1, % of cmTfh cells	16.8 (9.8-24.5)	17.6 (11.3-23.6)	16.5 (9.7-29.4)
cmTfh2, % of cmTfh cells	17.5 (11.4-24.3)	18.8 (10.1-35.1)	22.9 (7.6-32.6)
cmTfh17, % of cmTfh cells	45.8 (35.8-60.5)	42.7 (27.0-57.5)	41.8 (26.4-58.5)
PD1$^+$ICOS$^+$ cmTfh, % of cmTfh cells	1.3 (0.3-5.1)	2.2 (0.1-7.5)	1.4 (0-3.8)
Tph, % of CD4$^+$	0.7 (0.1-0.9)	0.8 (0.2-2.0)	0.9 (0.3-2.6)

Definitions of lymphocyte subsets and abbreviations are shown in Paper 5, Supplementary Table S2.

1. All results are presented as median (range).
2. Switched memory B cells were higher in RA 0DMARD and all RA patients compared to HC ($p<0.01$ and $p=0.02$).

Switched memory B cells and plasmablasts after immunization

Switched memory B cells and plasmablasts increased after pneumococcal vaccination in HC and in the 0DMARD group ($p \leq 0.03$), but no changes were seen in the MTX group (Figures 12A and 12B). After vaccination, plasmablasts were

lower in the MTX group compared to the 0DMARD group (p=0.002), and HC (p<0.001, Figure 12C).

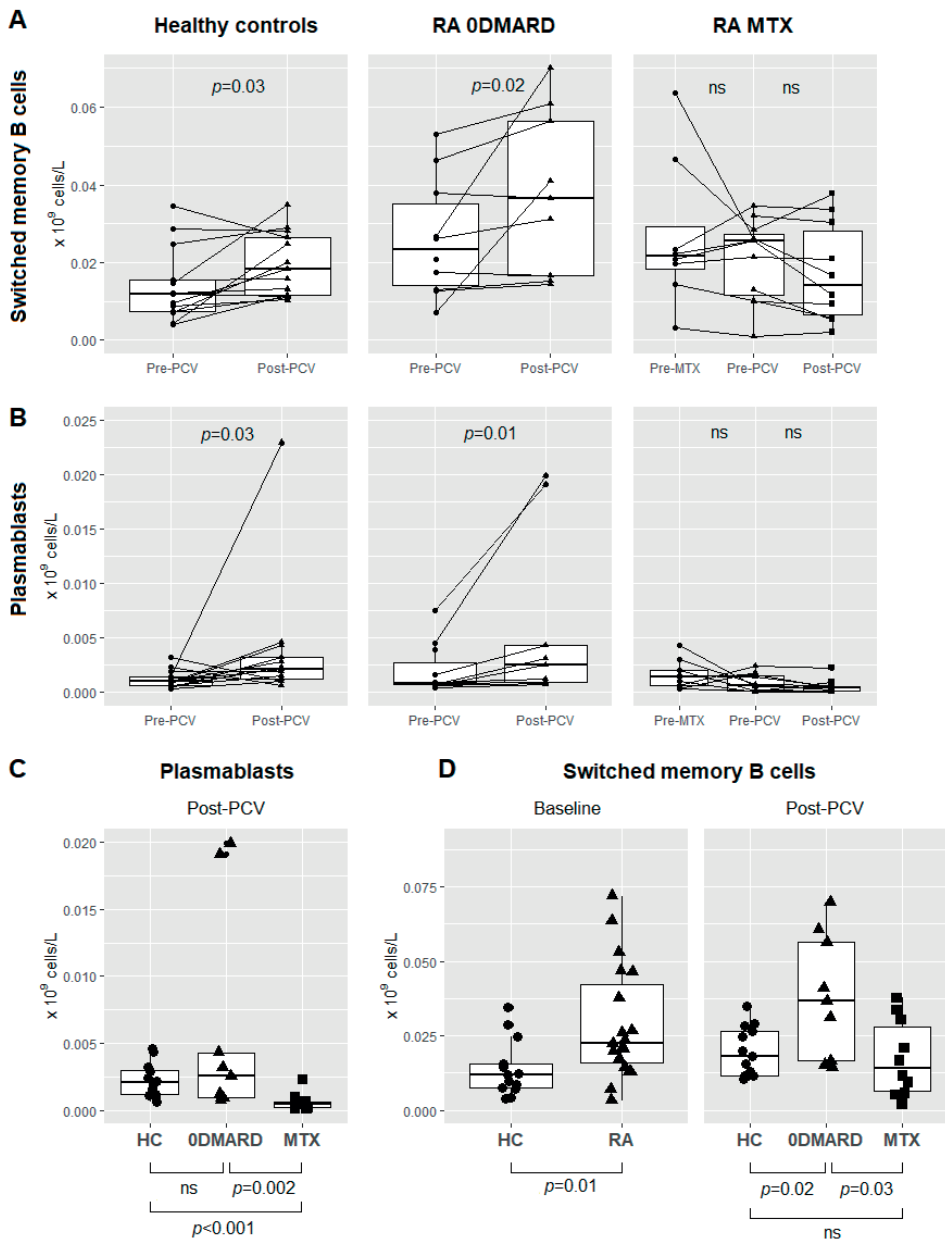

Figure 12. Circulating switched memory B cells and plasmablasts in RA groups and controls.
Concentrations of (A) switched memory B cells, and (B) plasmablasts in HC and RA 0DMARD groups: before and after PCV, and in RA MTX group: before start of MTX, before and after PCV. (C) Group comparison of plasmablast concentrations after PCV. (D) Group comparison of switched memory B cells at baseline and after PCV.

Effect of MTX treatment on Th17 cells in blood

Initiation of methotrexate treatment resulted in decreased Th17 cell frequencies (% of $CD4^+$) and concentrations (p=0.02 and p=0.03, Figure 13). In HC, vaccination resulted in increased Th17 cell frequencies (% of CD4+, p=0.03) and concentrations (p=0.01, Figure 13), but no changes were seen in the RA groups.

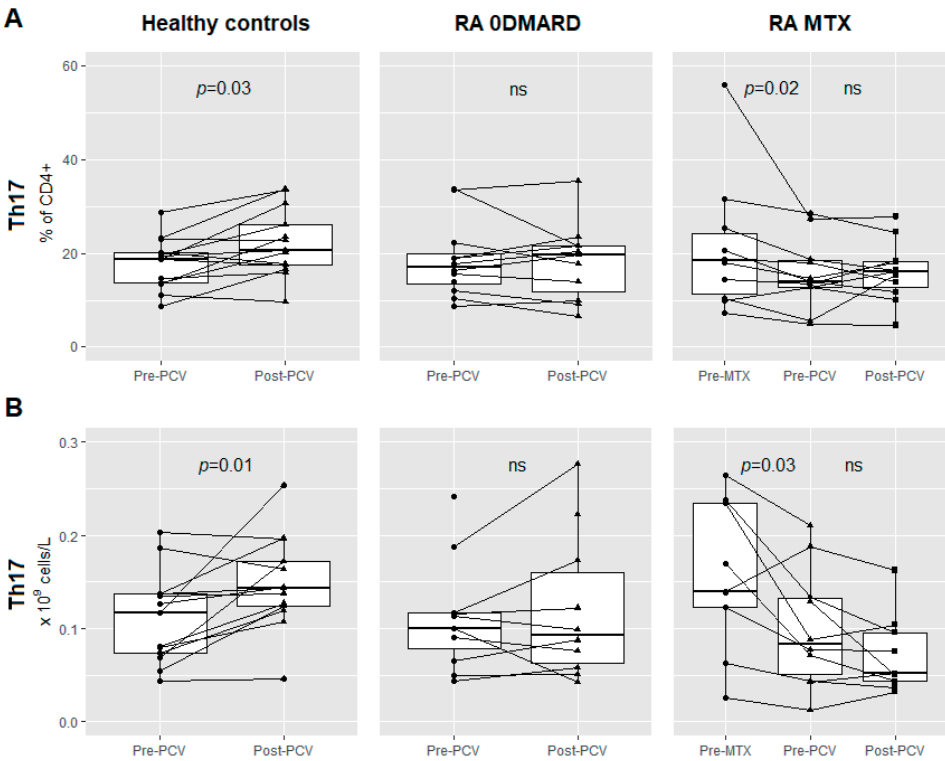

Figure 13. Circulating T helper 17 cells in RA groups and controls.
T helper 17 cells in % of CD4+ (A), and concentrations (B), in HC and RA 0DMARD groups: before and after PCV, and in MTX group: before start of MTX, before and after PCV.

Circulating T follicular helper cell subsets in relation to methotrexate and immunization

No effects of MTX on total or activated ($ICOS^+$ $PD\text{-}1^+$) cmTfh cells were found. Frequencies of total cmTfh cells (% of CD4+ cells) were unchanged after immunization in all groups (Figure 3A). Although non-significant, activated cmTfh cells (% of CD4+) increased after vaccination in 8 of 11 patients in the 0DMARD group (p=0.10), 7 of 11 patients in the MTX group (p=0.14), and in 7 of 12 HC (p=0.20).

Pneumococcal antibody concentrations

Positive antibody responses (≥2-fold increase in ≥6 of 11 serotypes) were seen in 90% of HC participants, 87.5% of the 0DMARD group, and 56% of the MTX group. Mean fold change in pneumococcal IgG concentrations correlated with plasmablast concentrations in all participants (R=0.52, p=0.011), and all RA patients (R=0.57, p=0.035).

Discussion

Immunocompromised patients are at increased risk of serious infections, and some of these infections are vaccine-preventable. While immunizations with live vaccines are contraindicated in the immunocompromised host, non-live vaccines can often safely be administered but there is a risk of impaired immune responses and low vaccine efficacy. The population of immunocompromised patients is growing, and the patients with secondary immunodeficiency (SID) by far outnumbers those with a genetic, primary immunodeficiency (112). A SID is defined by an extrinsic cause of the compromised immune system, such as malnutrition, HIV infection or treatment with glucocorticoids or immunomodulatory drugs. Splenectomized patients constitute a SID subpopulation with a specific defect in the immune defence against encapsulated bacteria. The use of immunosuppressive drugs in western populations is increasing, as more patients receive solid organ or bone marrow transplants, and as a consequence of higher proportions of patients with autoimmune inflammatory diseases who receive biological treatments. Among the B cell directed biological treatments, rituximab is used in the treatment of patients with RA or AAV, as well as in patients with haematological malignancies.

Post splenectomy patients

Adherence to guidelines for primary pneumococcal immunization in splenectomized patients was adequate. In contrast, there was poor adherence to recommendations regarding meningococcal immunization, as only one fourth of patients had documented immunization with meningococcal vaccine. Studies from France and Netherlands have shown similar results (173, 174).

High levels of pneumococcal serotype-specific IgG concentrations were seen in previously PPV23 immunized asplenic patients, and PCV13 was immunogenic as a booster dose with increases in specific IgG for 9 of 12 serotypes (1, 3, 4, 5, 7F, 18C, 19A, 23F, and 19F). To the authors knowledge this was the first study investigating immunogenicity of PCV13 in the asplenic population. In a study from the UK, Stanford et al. reported similar results regarding immunization with PCV7 in asplenic individuals (175).

The clinical implication of our results is that PCV13 can elicit adequate antibody responses in asplenic patients previously immunized with PPV23. Optimally PCV should be administered before PPV23, since several studies in adults have shown reduced immunogenicity of PCV 6-12 months after an initial PPV23 dose (176-179). The CDC/ACIP recommendation for asplenic patients is immunization with one dose PCV13 followed in ≥8 weeks by a PPV23 dose, and a PPV23 booster dose after 5 years (57). Patients with previous PPV23 are recommended immunization with a PCV13 dose ≥1 year after PPV23.

Due to small sample size, and heterogeneity of underlying reason for splenectomy, our results should be interpreted with some caution. Larger studies are needed to further investigate immunogenicity of PCV and optimal immunization schedules in asplenic patients.

Systemic vasculitis patients receiving standard of care therapy

We found that PCV was safe and immunogenic with increased IgG concentrations (6B and 23F), OPA (23F), and improvement of antibodies above threshold 1.0 µg/mL, after immunization of patients with systemic vasculitis. There were no differences in IgG after immunization or proportion of responders between all patients, subgroups and controls. However, treatment with cytotoxic drugs might impair antibody responses, as illustrated by the trend toward fewer patients in the AZA/MTX/CYC group (85% diagnosed with AAV) reaching IgG ≥ 1.0 µg/mL, compared to controls. We did not find an association between glucocorticoid dose and antibody response, but most patients were treated with low or moderate doses.

Although functionality of antibodies improved after immunization in all groups, post-PCV OPA was lower and correlation with ELISA was weaker in patients, compared to controls. In the glucocorticoid monotherapy group (87% GCA), both pre- and post-PCV OPA were lower compared to controls, probably due to higher age and a negative effect of glucocorticoids on phagocytosis.

Our results are in line with a study from the UK, which showed preserved immunogenicity to PCV7 in patients with AAV in remission (180). In a case series of AAV patients from France, decreased response to PCV±PPV23 was seen in 9 patients on induction therapy but not in 8 patients with maintenance therapy (181).

To the author's knowledge, this is the first study of both quantitative and functional antibody response after PCV immunization in patients with systemic vasculitis. Since this patient group is at high risk of severe infections, such as IPD, pneumococcal immunization should be a priority. Optimally immunizations should be performed before initiating any immunosuppressive treatment, but for patients

with rapidly progressive necrotizing vasculitis this is seldom possible. The clinical implication of this study is that immunization with PCV is safe and elicits adequate antibody responses in patients with systemic vasculitis and ongoing immunosuppressive therapy.

Patients with RA or primary Sjögren's syndrome patients without DMARD treatment

Antibody responses to PCV in RA or pSS patients without DMARD treatment were comparable to those of healthy controls. However, functionality of antibodies (OPA) after immunization was reduced in RA patients without DMARD, compared to controls. Similarly, correlation between change in OPA and ELISA was weak in RA without DMARD, and moderately strong in healthy controls. Higher age in RA patients and glucocorticoid treatment could probably explain reduced phagocytosis compared to healthy controls. In line with previous studies, the response in MTX treated patients was reduced (182).

The underlying autoimmune pathologies of RA and pSS are known to increase the risk of serious infections (28, 134, 155). Our findings, i.e. that pneumococcal conjugate vaccine was immunogenic in both RA and pSS without DMARD, support the use of this vaccination in newly diagnosed patients before starting immunosuppressive DMARD treatment.

Prime-boost pneumococcal vaccination in relation to rituximab, abatacept and cDMARD

In RTX treated patients, antibody response was not improved by prime-boost vaccination compared to single PCV dos, and the response was markedly impaired compared to healthy controls. This is in line with previous studies which have shown reduced antibody response to single dose PCV or PPV23 in RTX treated RA patients (162, 164, 165). Reductions in antibody responses could be expected since rituximab causes almost total depletion of B cells in the circulation. After RTX treatment is discontinued, it takes 6-9 months before B cells start replenishing, and most of these are naïve or transitional B cells (127), and most of the memory B cells (80%) are still depleted one year after RTX treatment (128). Our findings strongly support the recommendation to complete pneumococcal immunization before initiating RTX treatment.

We found reduced antibody response after the prime-boost vaccination strategy in ABT treated patients, for serotypes in common to PCV13 and PPV23, compared to controls. Although antibody response improved with PCV13+PPV23, compared to PCV13 alone, no change was seen in putative protective levels. A previous study from our group has shown reduced antibody response to PCV7 in ABT treated RA patients (76% with concomitant MTX), compared to healthy controls (162). In a study by Migita et al., antibody response to PPV23 was reduced in RA patients with ABT+MTX treatment compared to MTX or glucocorticoid monotherapy (163). Abatacept was also reported to reduce levels of switched memory B cells (124).

Our results support the recommendation to immunize against pneumococci before starting ABT treatment, but in patients with ongoing ABT and concomitant MTX, prime-boost vaccination could have a potential benefit over single dose PCV. Prime-boost vaccination strategy improved the number of serotypes with positive antibody response and IgG ≥1.0 μg/mL compared to single-dose PCV, in IRD with conventional DMARD treatment. Several groups have reported that MTX treatment reduce antibody response to PCV or PPV23 in arthritis patients (182). Our results suggest that the prime-boost vaccination strategy is more likely to elicit a strong antibody response compared to single-dose PCV13 when initiation of conventional DMARD treatment can't be delayed.

Strengths and limitations with the pneumococcal serological assays

In papers II and III, pneumococcal IgG antibodies to serotypes 6B and 23F were quantified using an ELISA assay. Determining antibody levels to two of thirteen vaccine serotypes is an obvious limitation, and serotypes 6B and 23F are considered less immunogenic compared with other PCV13-types (69). However, since the aforementioned studies aimed to study the effects of immunosuppressive treatments and rheumatic disease on antibody response, rather than describing the response to different serotypes in detail, the authors believe this was a minor limitation.

Although the MFMI (Luminex) assay, used in papers IV and V, is a validated method, it has not been as extensively used and evaluated as the standard WHO ELISA (64). The advantages of MFMI over ELISA is that it allows for the rapid simultaneous quantification of antibodies to many serotypes.

Regardless of which serological assay is used, the quantification of antipneumococcal antibodies is only a surrogate marker for protection, in the absence of efficacy studies. Different protective thresholds have been described, e.g., 0.35 μg/mL for children, 1.0 μg/mL for adults, and 1.3 μg/mL for immunocompromised adults (69). While antibodies in the range of 0.20 to

0.35 µg/mL can protect against bacteremia, ten times higher antibody levels may be required to prevent pneumonia, otitis media and nasopharyngeal carriage (70, 183).

In general, functionality of antibodies measured by an OPA assay offers a more biologically relevant picture, compared to IgG quantifications. We used an in house flow cytometry based OPA assay, and although it is a validated method (67), it differs from the killing type OPA method which has become a gold standard for pneumococcal vaccine evaluations (184),.

Methotrexate reduced circulating Th17 cells and impaired memory B cell and plasmablast responses after immunization

Immunization with PCV was associated with increases in the concentrations of circulating plasmablasts and switched memory B cells in HC and RA patients without DMARD treatment, but these changes were not seen in MTX treated patients. These subsets of B cells are considered antigen experienced after TD germinal centre reactions in secondary lymphoid organs (42). Although nonspecific B cells were measured in this study, the results are in line with previous findings of reduced antibody responses to PCV in MTX treated arthritis patients (158). Methotrexate is known to increase extracellular levels of adenosine, which has potent inhibitory effects on many inflammatory cell types (118). Interaction between MTX and BAFF, promoting adenosine release, has been proposed as the mechanism behind reduced immunization against therapeutic Mabs in patients receiving a combination of MTX and TNF-inhibitors (120).

Initiation of MTX treatment in RA patients lead to a reduction in Th17 cells in blood, which is similar to the findings of Szalay et al. (185), and consistent with an in-vitro study by Guggino et al. (186). Shen et al. previously demonstrated increased frequencies of IL-17 producing T cells in PBMCs from RA patients and strong correlation with disease activity (187). T helper 17 cells are defenders against extracellular bacteria, e.g. pneumococci, on mucosal surfaces (33), and these cells could be critical for vaccine induced memory immune responses (188).

The effects of MTX on Th17 cells, switched memory B cells, and plasmablasts could have implications for both vaccine-induced antibody and long-term immune memory responses.

Future perspectives

The limitations of vaccine type protection and serotype replacement in the population drives the development of new pneumococcal conjugate vaccines with wider spectra of serotype coverage. For example, in Sweden only 32 % of IPD cases in 2019 were caused by serotypes included in PCV13, while PPV23 covered 64 % of invasive isolates (56). A 15-valent PCV (PCV15) developed by Merck & Co, adds two additional serotypes: 22F and 33F, and immunogenicity of the shared serotypes were non-inferior to PCV13 in a phase 2 trial of healthy older adults (189). Further, a 20-valent PCV (PCV20) which is under development by Pfizer, adds seven additional serotypes (8, 10A, 11A, 12F, 15B, 22F, and 33F) compared to PCV13. Immunogenicity of the new serotypes in PCV20 were similar to PPV23, and the remainder comparable to PCV13 in 60-64 years old adults (190), and this vaccine is currently in phase 3 clinical trials. While PCV15 is developed for use in both infants and adults, PCV20 is intended for protection of adults against IPD. The gap between PCV and PPV23 is obviously closing, but PPV23 will still confer the broadest serotype protection, 37 years after it licensure in 1983.

With higher valency PCVs, the prime-boost pneumococcal immunization (PCV+PPV23) will probably become outdated as a strategy for broader protection, which was the original intention with the recommendation (57). However, in immunosuppressed populations with impaired response to PCV, a PPV23 booster dose could have the potential to enhance immunogenicity, as our study suggested for cDMARD treated IRD patients. Whether improvement of immunogenicity leads to higher efficacy for prevention of IPD is unknown. It has been hypothesized that while a PCV booster stimulates and expands the memory B cell pool, a PPV23 booster may results in depletion of these cells (39). Future studies are needed to better understand the immunological differences with repeat conjugate versus PS vaccine doses in immunocompromised populations.

The main problem with PCVs is that they only confer protection against certain capsular PS types. The optimal pneumococcal vaccine should be serotype independent, which principally could be achieved with a cross protective protein antigen, or a combination of protein antigens. Several pneumococcal protein antigen, e.g. virulence factors pneumococcal surface protein A, C, and pneumolysin have been studied in animal and to some extent in human models (65). Perhaps a more promising vaccine platform for the future is the whole-cell pneumococcal vaccine (WCV), which was ironically also the first type of pneumococcal vaccines that were developed in the early 20th century. The WCVs can be produced at a low cost and express a wide range of surface pneumococcal protein antigens for serotype independent cross protection, and an inactivated WCV is currently in phase 1/2 clinical trials (191).

At the time of writing this dissertation, we are amidst a historic pandemic of the novel disease coronavirus disease 2019 (Covid-19) caused by severe acute respiratory syndrome coronavirus 2 (SARS-COV-2) (192). SARS-COV-2 causes a wide spectrum of disease from mild or asymptomatic infection to acute respiratory distress syndrome and multiorgan failure (193). The pandemic has put millions of people under lockdown, and immense pressure on healthcare and intensive care units worldwide. The pandemic has led to significant advances in the science of vaccinology, with several vaccines against Covid-19 now licensed in USA and Europe, including messenger RNA based platforms. The restrictions imposed due to the pandemic has been associated with historically low incidences of seasonal influenza virus infection in many countries (194) and a large decline in the incidence rate of IPD was observed in the UK (195). Coinfection of Covid-19 and IPD is rare (195).

The antirheumatic treatment arsenal is continuously growing, improving control of inflammation, leading to remission and enhanced quality of life in IRD patients. Biological treatments aimed at targeting important disease mechanisms, may have more or less predictable adverse effects on the physiological functions of immune cells or cytokines. Some priorities for research and development in this field can be identified. First, there will be an ongoing need of studies addressing immunogenicity of pneumococcal vaccines in relation to new classes of antirheumatic drugs, e.g. Janus kinase (JAK) inhibitors in RA patients. The importance of vaccinations early in the course of IRD, ideally before initiation of immunosuppressive treatment, cannot be stressed enough. Second, efforts are needed to obtain more knowledge on the efficacy of pneumococcal vaccines. As discussed earlier, PCVs and PPV23 have principal differences in their abilities to elicit TD or TI immune responses, but it is not clear how this translates in terms of efficacy, i.e. protection against pneumococcal infections. Third, with the challenges in obtaining efficacy data for pneumococcal vaccines in these patient groups, better understanding of immunological correlates of protection and long-term immune memory is vital. Fourth, epidemiological surveillance of serotypes is important to gain knowledge regarding differences in the ability to cause IPD and to tackle issues with serotype replacement.

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