
**SAHLGRENSKA ACADEMY**

# Immune Function in Patients with Invasive Pneumococcal Disease

Degree Project in Medicine

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# 1. Abstract


***Degree project, Programme in Medicine***

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***Background*:** Invasive pneumococcal disease (IPD) is a serious condition caused by the bacteria *Streptococcus pneumoniae.* It is most common in children and elderly. People with chronic diseases and immunodeficiency are especially vulnerable to IPD.

***Aim*:** To determine whether IPD patients differed in immune function compared to healthy controls.

***Method:*** Immunoglobulin (Ig) levels and IgG subclass levels were measured in 60 patients during the acute phase of the infection, and an extensive immune function analysis was performed in 18 healthy controls and 16 IPD patients 2 – 4 months after recovery in the convalescent phase. The immune results from the acute phase and convalescent phase were compared, as were the results between the patients and the controls. Clinical data was collected from the patient's journals.

***Results*:** The patients had significantly higher IgM levels in the convalescent phase than the controls, and the IgG2 and IgG4 levels were significantly lower in the patients in the acute phase than in the controls. The patients also had significantly lower levels of total lymphocytes, total T cells, helper T cells, and B cells in the convalescence phase, compared to the control, and the patient's monocyte function was also significantly lower than the controls'. No difference in B cell function could be seen, and no correlation could be found between the immune tests and the severity of the patients' illness.

***Conclusions*:** This study suggests that several of the immune functions of IPD patients differ from that of healthy controls. Although several results coincided with those obtained by other studies, several results were also inconsistent with other studies. This indicates that the knowledge on the subject remains incomplete, and further research is necessary.



***Keywords:*** IPD; Invasive pneumococcal disease; Immune deficiency, Immunoglobulin deficiency, *Streptococcus pneumoniae*

# 2. Background

## 2.1. *Streptococcus pneumoniae*

*Streptococcus pneumoniae* is a Gram positive bacterium with 100 known serotypes [1]. It is the most common cause of community acquired pneumonia [2, 3], but can also cause a more serious condition called invasive pneumococcal disease (IPD). IPD is defined as a condition in which *S. pneumoniae* is isolated from a sterile environment, such as the blood, cerebrospinal fluid, or synovial fluid.

### 2.1.1. Polysaccharide capsule

*S. pneumoniae's* most important virulence factor is its polysaccharide capsule, which is integral to its ability to adhere to host cells and cause inflammation. It also helps protect the bacterium from the host by inhibiting phagocytosis by innate immune cells, protecting the bacterium from recognition by host receptors and complement factors, and avoiding neutrophil traps[4].

Different serotypes have different polysaccharides on the outer coat of their capsule, which affects their virulence and likelihood of causing an invasive disease [4]. The serotypes that have been linked to a higher likelihood of causing IPD include serotype 1, 4, 5, 7F, 8, 12F, 14, 18C and 19A [5]. All of these, with the exception of serotype 8 and 12F, are included in the 13-valent pneumococcal conjugate vaccine (PCV13) [6]. Since the introduction of the PCV13 a decrease in PCV13 associated cases as well as overall IPD cases has been documented in several countries [7-11]. However, some studies have reported an increase of non-PCV13 serotypes among IPD cases, somewhat offsetting the decrease in IPD cases since the introduction of PCV13, thus raising the question of serotype replacement [8-11]. Despite the PCV13, the burden of pneumococcal disease remains high [12]. The data is inconclusive on whether pneumococcal serotype has any correlation with mortality in IPD. Some studies have found no significant association [13], while others seem to indicate an association between certain serotypes and an increased mortality [14].

## 2.2. Incidence of IPD

The overall incidence rate of IPD varies, and is dependent on many factors such as geographic location, socioeconomic status and vaccination status [15]. The World Health Organization reports an incidence rate that ranges from 8 to 34 cases per 100,000 people in industrialized countries [16]. A study conducted between the years 1995 and 2002 calculated the annual incidence rate of IPD in Finland to 11 cases per 100,000 persons [17]. Another study calculated the yearly incidence rate in Ontario, Canada between the years 2007-2017 to 7-10 cases per 100,000 persons [11]. A Swedish study from 2016 calculated the mean yearly incidence between 1996 and 2008 to 15 per 100,000 [18]. According to the Public Health Agency of Sweden (Folkhälsomyndigheten) the incidence of IPD in Sweden was 13 per 100,000 in the year 2019, which is similar to the incidence in previous years [19].

## 2.3. Host Risk Factors

### 2.3.1. Age

There are many host factors that have been linked to an increased risk of acquiring IPD. Most prominent among them is young (<5 years) and old (>65 years) age [14]. Several studies have found a link between older age and the incidence of IPD, as well as between older age and IPD mortality [10, 11, 20-23]. One study estimated the annual incidence of IPD amongst people aged 65 years and older to be 57 per 100,000, which was 7 times higher than the estimated incidence amongst people aged 16-64 [24]. The same study also found an association between older age and a higher mortality rate [24]. Similarly, a Swedish study found an incidence rate of 45 per 100,000 for people 65 years and older, as well as a case fatality rate (CFR) that increased with rising age [18]. This correlation between age and IPD incidence and mortality is shown in several other studies as well [10, 11, 20-23].

### 2.3.2. Sex

The impact of sex on IPD incidence and mortality is somewhat unclear. There have been studies that have found a significant association between the male sex and IPD [25], as well as studies that show the male sex as an independent risk factor for death in IPD [18, 23].

There have also been several studies that have not found any significant correlation between sex and IPD. A Canadian study consisting of 2,435 patients found no significant difference in incidence rates between male and female subjects [10]. Another large international study comprised of approximately 2,900 patients from 17 European countries also found no association between sex and an increased IPD mortality rate [14].

### 2.3.3. Lifestyle risk factors

***Smoking.*** Smoking has been shown to increase the risk of community acquired pneumonia, but its connection to IPD is more unclear [26]. Data suggests there may be a link between smoking and risk of IPD, but more research on the subject is necessary [27]. One study, which included immunocompetent adults between 18-64 years, found a strong dose-response association between smoking and pneumococcal disease. The risk of IPD decreased by 14% for every year since the patient stopped smoking, reaching the same level as a non-smoker after about 13 years [25].

***Alcohol abuse.*** Alcohol abuse might also be linked to an increased IPD risk [27]. A Danish study consisting of almost 19,000 patients conducted over the course of 30 years found a significant association between alcohol abuse and an increased 30-day mortality from IPD [23]. Alcohol abuse and its connection to increased mortality has been shown in other studies as well [17, 21, 22].

### 2.3.4. Manifestation

Pneumonia with bacteremia is the most common among IPD manifestations, constituting 40-80% of overall IPD cases [15, 21, 28]. However, there appears to be a difference in manifestation between age groups, where both a Swedish and a Canadian study have reported a decrease in meningitis with age, as well as an increase in bacteremic pneumonia [10, 18]. Although the Swedish study found the highest case fatality rate (CFR) among patients with septicemia with unknown focus, meningitis was still shown to have an increased mortality compared to pneumonia [18]. Several other studies have also found that meningitis as a manifestation is significantly associated with increased mortality in IPD [13, 14, 17, 20, 21, 23].

### 2.3.5. Underlying conditions

It has been shown that many chronic diseases increase the risk of acquiring IPD, as well as increase the mortality rate among IPD patients [13, 15, 20, 23, 25]. A review article which covered several chronic diseases and their connection to IPD found that not only were individuals with conditions such as diabetes mellitus, chronic heart disease and chronic respiratory diseases at an increased risk of acquiring IPD, but they also had a higher mortality rate. Furthermore, a cumulative effect of multiple risk factors on both incidence and mortality has been shown [26, 29].

***Chronic heart disease.*** Chronic heart conditions such as congestive heart failure have also been associated with an increased IPD risk, and coronary artery disease has been identified to be a predictor of mortality [17, 21, 26, 28].

***Chronic respiratory conditions.*** Chronic respiratory conditions such as chronic obstructive pulmonary disease (COPD) and chronic bronchitis have been linked to an increased risk of IPD [24, 26]. However, some studies have not found an association with increased mortality amongst COPD patients [21]. Asthma has been considered a risk factor for acquiring IPD ever since the release of a US study conducted in Tennessee in 1994, which found a significant association between asthma and IPD [30]. A systematic literary review on the subject that was published in 2013 concluded that there may exist a positive association between asthma and IPD, as all eight articles included in the review found this association [31]. However, a Swedish study from 2016 found no association, which the authors suggest might be due to differences between their population and the population in the Tennessee study [18].

***Chronic liver disease***. Chronic liver disease has also been shown to increase the risk of IPD [21, 24]. One study showed that, among people aged 16-64, liver disease was the most prominent risk factor for contracting IPD. Liver disease was also associated with the highest CFR among elderly patients (aged ≥65 years) [24]. An increased CFR among IPD patients with liver disease has been reported by other studies as well [17, 21].

***Chronic kidney disease***. Patients with chronic kidney disease, especially those treated with dialysis, have been shown to have an increased incidence, mortality, and morbidity in IPD [18, 21, 32].

***Autoimmune diseases***. Several autoimmune diseases such as systemic lupus erythematosus (SLE) and rheumatoid arthritis have been linked to a higher incidence of IPD [18, 33]. Diabetes mellitus has also been connected to an increased rate of IPD, and appears to be an especially strong risk factor among younger adults (<40 years) [26].

### 2.3.6. Immunosuppression

Immunocompromised individuals have been shown to be one of the groups with the highest risk of IPD, as well as having an increased mortality rate. The proportion of immunocompromised patients among those diagnosed with IPD has been reported to lie somewhere in-between 20-40% [13, 15, 17, 20, 24, 28, 33]. The most common conditions among younger adults with IPD are human immunodeficiency virus (HIV), hepatic cirrhosis and organ/bone marrow transplantation [33]. However, the elder population are more likely to suffer from multiple myeloma, leukemia, and lymphoma [28, 33].

***HIV.*** HIV, in particular, has been linked to an increased risk of IPD [20], with one study showing that patients with HIV have a much higher likelihood of contracting IPD compared to healthy individuals [24]. As previously mentioned, the overall incidence of IPD in industrialized countries have been reported between 8-34 cases per 100,000 [16]. A meta-analysis conducted to assess the incidence of IPD among immunocompromised groups found that the pooled IPD incidence among HIV patients in non-African countries in the era of late-antiretroviral treatment was 330 per 100,000. Furthermore, the incidence among solid organ transplant patients was 470 per 100,000, and 700 and 800 per 100,000, respectively, in autologous and allogenic stem cell transplant patients [34].

***Asplenia.*** Asplenia has also been linked to an increased risk of IPD [35, 36]. A Korean study found patients with asplenia to be almost 18 times more likely of contracting IPD compared to the general population [37].

## 2.4. Host response against *S. pneumoniae* and primary immunodeficiencies

### 2.4.1. Phagocytes

Neutrophils make up a large portion of the white blood cells, and have the ability to both phagocyte bacteria and to release granules filled with enzymes that help to break down the bacteria's cell wall [4]. They can also eject DNA to the extracellular matrix in order to create traps for *S. pneumoniae*, thus facilitating the killing of the bacterium [4]. Macrophages are another type of phagocytic cell that are activated by their pattern recognition receptors (PRRs) when they come into contact with and engulf *S. pneumoniae*. Their activation allows them to signal and recruit other immune cells as well as to phagocyte pneumococci that have been opsonized by antibodies and complement factors [4]. Phagocyte defects do not appear to be strongly linked to an increased risk of IPD [38].

### 2.4.2. B and T cells

B and T cells belong to the adaptive immune response, and are thus not fully activated until a few days after the initial infection. $CD4^+$ T cells have been shown to play an important role in the immune response to *S. pneumoniae* during infection [4]. Some T cell subclasses produce cytokines that stimulate a cellular-mediated immune response by recruiting other immune cells to the site of infection. Macrophages, monocytes, and neutrophils are commonly recruited, as they are needed in the defence against *S. pneumoniae.* Other subclasses aid B cells in their production of antibodies, also known as immunoglobulins (Ig) [4].

One study found that patients with IPD had a systemic decrease in T cells during the acute phase of the infection, which resolved itself in the convalescent phase of the infection [39]. The study found no similar decrease in B cells [39].

The importance of $CD4^+$ T cells in IPD is made clear in patients with HIV, a virus which destroys $CD4^+$ T cells and causes a significantly increased risk of IPD [20]. A decrease in $CD4^+$ T cells will result both in a decrease in cytokine response, but it will also indirectly cause B cell dysfunction as proper B cell function is linked to activation by $CD4^+$ T cells. This will result in decreased Ig levels and memory B cell depletion [40].

B cell disorders are closely linked to antibody deficiencies, and are the most common type of immunodeficiency [41]. Hypogammaglobulinemia and agammaglobulinemia are defined as an impaired ability to produce Ig, resulting in lowered Ig levels in the bloodstream. Several types of these conditions exist with varying severity, but they have been linked to an increased risk of *S. pneumoniae* infection [38, 41].

### 2.4.3. Antibodies and complement factors

Type-specific opsonizing antibodies directed to the polysaccharides and proteins in the pneumococcal capsule is one of the body's most important defenses against IPD. The bacteria's natural protection against phagocytosis is circumvented by the antibodies binding to polysaccharides and proteins in the pneumococcal capsule [36, 42-44]. A study reported that over a third of their patients had a nonfunctional opsonic antibody response, and found it to be significantly associated with IPD [45]. This might also explain why people with hypo- and agammaglobulinemia are more susceptible to infections caused by encapsulated bacteria [35, 44, 46]. It is in fact common for IPD patients to have antibody deficiencies, either primary or secondary to other diseases. Studies have found that up to one third of IPD patients also have hypogammaglobulinemia [44, 47].

Immunoglobulin A (IgA) antibodies make up about one third of serum antibodies that are reactive to the polysaccharide capsule of *S. pneumoniae*. A study has shown that IgA antibodies, together with complement factors and phagocytes, play a role in the incapacitation of the *S. pneumoniae* bacteria [48]. Complements are important for IgA to mediate phagocytosis, whereas although they increased the binding and uptake of *S. pneumoniae* into the phagosome, are not required for IgG to function [48]. IgA opsonization is mostly reliant on the alternate pathway in the complement cascade. However, it has been shown that IgA can mediate killing of *S. pneumoniae* without complement factors, as long as the neutrophils have been activated beforehand by certain proinflammatory cytokines [48].

Although studies suggest that there might be a link between IgA deficiency and IPD, IgG antibodies have been shown to be more important for preventing IPD. The IgG2 subclass, in particular, appears to play an important role in the defence against *S. pneumoniae,* as it is involved in the response against polysaccharide antigens [35, 38, 46, 47, 49]. A study on IgG

and IgG subclasses in the acute phase and convalescent phase of IPD found that overall IgG as well as all IgG subclasses were significantly lower in the acute phase compared to the convalescent phase [43]. Several studies have also noted that normal total IgG levels can hide an IgG2 subclass deficiency due to a compensatory increase of other IgG subclasses [43, 46, 50, 51].

### 2.4.4. Summary

Immunodeficiencies have been shown to be linked to an increased risk of IPD. Present knowledge suggest that B and T cell defects and some complement factor deficiencies convey a high risk of IPD, whereas phagocyte deficiencies and IgA deficiency pose low or no risk of IPD [38]. However, there are few studies on the subject, and more research needs to be done before any conclusions can be made about which immunodeficiencies increase the risk of IPD. This knowledge is important for identifying IPD risk groups that could benefit from preventative measures such as vaccination or immunoglobulin replacement therapy.

# 3. Aim

The aim of this study was to determine whether a difference in immune function between patients with invasive pneumococcal disease and healthy controls exists. More specifically, whether patients with invasive pneumococcal disease and healthy controls differ in antibody levels, total lymphocyte population, number and proportion of immune cells, phagocyte function, T cell function, and B cell function. The study also aimed to determine whether the patients antibody levels differed between the ongoing infection (acute phase) or after recovery from the infection (convalescent phase).

If a difference between patients and controls could be established, the study aimed to seek a correlation between deviating immune function and the severity of the disease.

# 4. Method

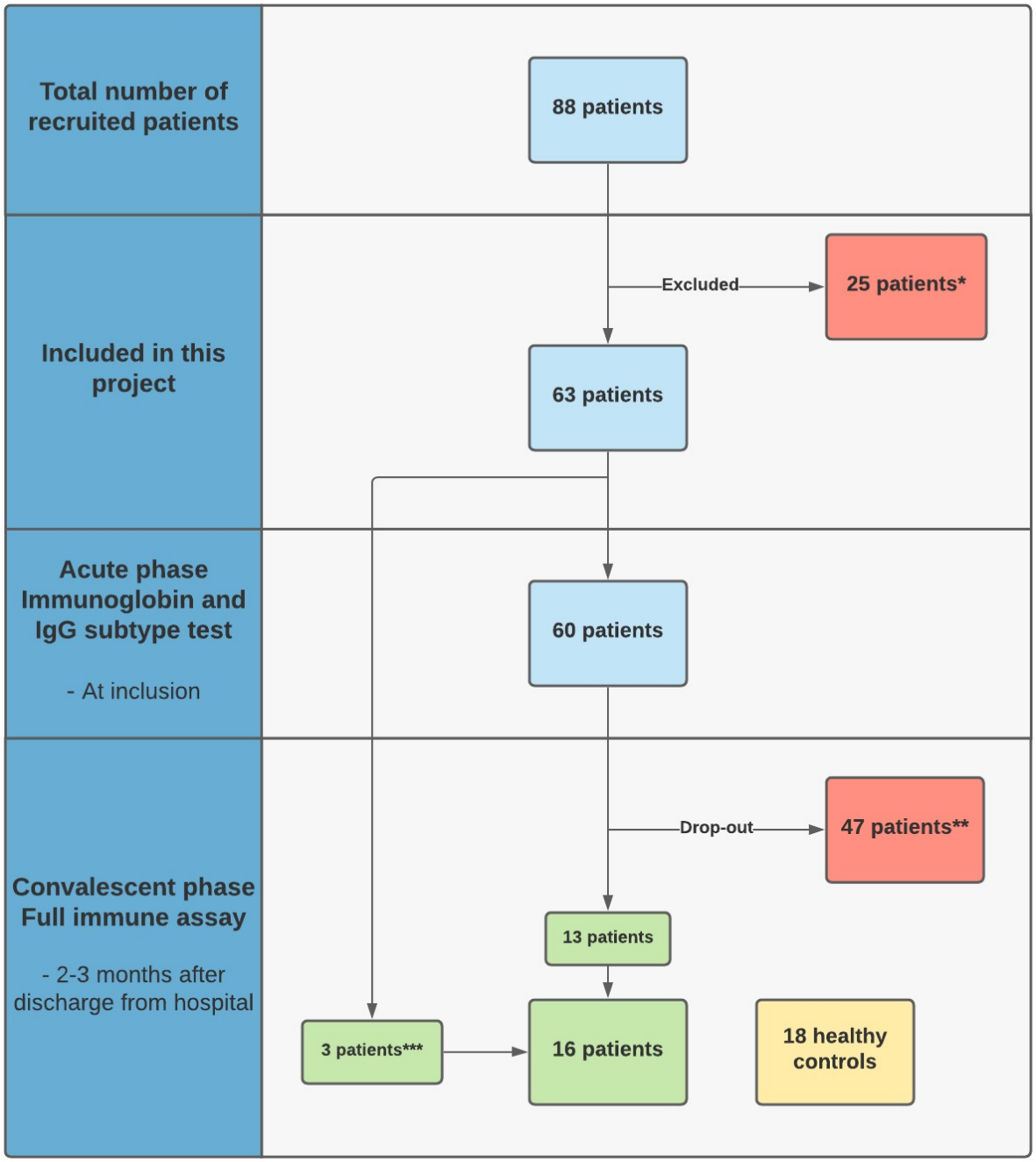
## 4.1. Study population and inclusion

This project was part of a larger prospective study which runs between 2018 and 2022. Up till this project in the autumn of 2020, 88 participants had been recruited to the overall study from two hospitals in the Region Västra Götaland of Sweden. Forty-two patients were recruited from Northern Älvsborg County Hospital (NÄL) and 46 from Södra Älvsborg Hospital (SÄS). All participants were adults (>18 years) and had a blood culture positive for *S. pneumoniae.* The recruitment to the study was conducted either by a study doctor or nurse working at the hospitals' infection clinics, or the patients were contacted after discharge and recruited policlinically.

As is shown in *Figure 1,* 63 of the 88 patients included in the overall study were included in this project. Twenty-five patients were excluded because of a lack of acute phase antibody tests, which was due to policlinical inclusion or inclusion post-mortem. 60 of the 63 included patients underwent Ig isotype and IgG subclass testing at inclusion in the study, during the acute phase of their illness (Figure 1). The three remaining patients were included policlinically, and thus only underwent testing during the convalescent phase. Of the 60 patients that underwent antibody testing during the acute phase, 13 went on to do further testing of their full immune function 2-3 months after being discharged from the hospital (Figure 1). The testing during the convalescent phase included the same antibody tests as during acute phase testing, as well as mapping of the total lymphocyte population, testing of phagocyte function, T cell function, and B cell function.

The reason for the high drop-out rate was that many patients were unable to come to a follow-up appointment because of bad health conditions or death, as well as many participants declining further testing. In total, 16 patients went on to participate in the testing of their full immune function, and 18 healthy age-matched subjects were recruited as a control group.

**Figure 1: Flow chart of inclusion and exclusion at the projects' different stages**




**Excluded due to absence of tests caused by policlinical or post-mortem inclusion.*
***Did not participate in follow up testing due to poor health condition, death or unwillingness to participate.*
****Included policlinically, thus underwent only convalescent phase testing.*

## 4.2. Laboratory methods

### 4.2.1. Laboratory data

All immune analyses in the study were conducted at the department of Clinical Immunology and Transfusion Medicine at Sahlgrenska University Hospital. The data results of the immune analyses were collected from the consultation responses sent from the laboratory to the doctor that ordered the tests. Each consultation response also included a comment with an interpretation of the test results by a medical specialist in Clinical Immunology. The test values and their related comments were documented in an excel file, and the specialists' interpretations were used as a guideline to mark the deviating results.

### 4.2.2. Ig isotype and IgG subclass analysis

The Ig isotype- and IgG subclass levels were quantified using turbidimetry. Anti-IgG, IgA and IgM antibodies, as well as anti-IgG1, IgG2, IgG3 and IgG4 were added to serum samples derived from the subjects, which caused formation of antibody-complexes. The serum was then analyzed using turbidimetry, where light was shone through the sample. The complexes caused the light to scatter, resulting in a decrease in light intensity which was detected by a photoreceptor. The resulting curves were then compared to curves caused by known antibody concentrations, by which the concentration (g/l) of IgG, IgA, and IgM as well as IgG1, IgG2, IgG3 and IgG4 were determined. See *Appendix 1* for the reference values used in this study.

### 4.2.3. Lymphocyte population

Lymphocyte populations were measured using a type of flow cytometry called fluorescence-activated cell sorting (FACS). By adding antibodies specific to certain cell surface proteins that are labeled with fluorescent molecules to the patients sample and then analyzing it using flow cytometry, the different types of lymphocytes were distinguished and separated from one another. Using this method, the total number of lymphocytes ($x10^9$/l) was determined, as well as total number of T cells (CD3+), T helper cells (CD3+CD4+), cytotoxic T cells (CD3+CD8+), B cells (CD19+) and NK cells (CD3-CD16+CD56+). Using the obtained values, the different immune cells proportion of the total number of lymphocytes was calculated. A quotient of CD4+/CD8+ cells was also calculated. See *Appendix 1* for the reference values used in this study.

### 4.2.4. T cell stimulation

T cell proliferation after antigen stimulation was measured using radioactive thymidine. Three peripheral blood mononuclear cell (PBMC) cultures were established, to which a solution of radioactive thymidine was added. Antigens were then added to stimulate T cell proliferation; one culture was stimulated using phytohaemagglutinin P (PHA), one using Concanavalin A (ConA), and one acted as a negative control. Both PHA and ConA are selective T cell mitogens. After the cells had been incubated, the unbound excess thymidine was washed away and the T cells were isolated. Lastly, the radioactivity of the cells (counts per minute, cpm) was measured and used as an indication of T cell proliferation. Samples from healthy blood donors were analyzed at the same time as the patient and control samples, to ensure the machine was correctly calibrated. The T cell proliferation values obtained from the patients and the controls were also compared against the levels of the blood donor samples which were analyzed simultaneously, and a patient/blood donor or control/blood donor quota was calculated for each test subject. See *Appendix 1* for the reference values used in this study.

### 4.2.5. Phagocyte function

Phagocyte function was measured using two different tests:

Phagotest was used to measure the ability of the monocytes and granulocytes to phagocyte live bacteria. Opsonized *E. coli* bacteria marked with fluorescence were added to the subjects' blood samples, and to another sample from the same subjects a negative control stimuli was added instead. After lysing the red blood cells the samples were washed and thereafter analyzed using flow cytometry. By this method the proportion of monocytes and granulocytes that were marked with fluorescein and thus had engulfed a bacteria were measured. See *Appendix 1* for the reference values used in this study.

The oxidative burst test was used to measure the ability of granulocytes to produce reactive oxygen species (ROS). The subjects' blood sample was mixed with opsonized *E. coli* (particulate stimulant of ROS production), formyl-methionyl-leucyl-phenylalanine (fMLP) (extracellular stimulant), or Phorbol 12-myristate 13-acetate (PMA) (intracellular stimulant). Dihydrorhodamine 123 was added to the mixture, as it becomes fluorescent when it comes into contact with reactive oxygen species. After lysing red blood cells and washing the

samples were analyzed using FACS, which presented the proportion of neutrophils which produced reactive oxygen species after stimulation. See *Appendix 1* for the reference values used in this study.

#### 4.2.6. B cell stimulation

B cell activation was quantified using enzyme linked immunospot (ELISPOT) to measure the cells' ability to activate and differentiate into Ig-producing cells. Tree PBMC cultures were established, to which mitogens were added to stimulate cell division. Epstein-Barr Virus (EBV) was added as a T cell-independent mitogen to one culture, poke weed mitogen (PWM) was used as a T cell-dependent mitogen in the second culture, and the last culture was used as a negative control. These cultures were then added to wells coated with anti-Ig antibodies. After incubation, the cells were washed off from the wells, and biotinylated anti-IgG, anti-IgA and anti-IgM antibodies were added. Streptavidin-enzyme conjugate, which binds to the biotinylated antibodies, was then added. In the last step a substrate was added, which was catalyzed by the streptavidin-enzyme conjugate, creating coloured spots. As each coloured spot represents one antibody-secreting plasma cell, the sample was analyzed and used to determine the number of Ig-producing B cells. See *Appendix 1* for the reference values used in this study.

## 4.3. Clinical data collection

The patients were given a questionnaire at inclusion, where they were asked about symptoms preceding IPD, if they had previously had a pneumococcal infection, pneumococcal vaccination status and smoking and alcohol habits.

Additional clinical data was collected using a standardized form that was filled in using information in the patients' journals. The data collected pertaining to this project was length of hospital stay, manifestation of IPD, if the patient was treated at an intensive care unit (ICU) ward, 30-day mortality, complications, and predisposing risk factors.

Manifestations were divided into four categories: pneumonia with bacteremia, meningitis, bacteremia with unknown focus and other manifestations (such as septic arthritis, otitis, and

sinusitis). A complication was defined as a condition caused by IPD or as a result of its symptoms. The complication categories defined were empyema, pleural fluid >2 cm, heart infarction, and other complications. The predisposing risk factors accounted for in this study were if the patient was a current smoker or quit smoking <10 years ago, alcohol and substance abuse, systemic autoimmune disease, malignancy, liver disease, kidney disease, chronic pulmonary disease, diabetes mellitus, asplenia and current pregnancy.

## 4.4. Statistics

The data was entered in an excel file and sorted into three groups: Acute phase testing, convalescent phase testing, and controls. The data was then transferred to the statistical program Graphpad Prism (version 9.0.0 for Windows) which was used for data analysis in this project. All data was assumed to be nonparametric. When comparing the two patient groups group with the control group the Mann-Whitney test was used (unpaired, nonparametric t-test). When comparing the data from the acute phase Ig-tests with their respective convalescent phase Ig-tests (paired, nonparametric t-test) the Wilcoxon test was used. Graphpad Prism was also used to calculate the descriptive statistics of the project.

A Spearman correlation test (nonparametric) was used to determine whether a correlation was present between the variables that showed a statistical significance between the patients and the control group as well as the severity of the patients' illness. The severity of the illness was defined as length of hospital stay (measured in days), if the patients were admitted to the ICU ward and if they had complications.

## 4.5. Ethics

This project was a part of an ongoing study that has acquired ethical permission from the Swedish Ethics Review Authority for inclusion of patients and controls in the study (Dnr: 894-17 and T616-18). All patients were given information about what participation in the study would entail before giving their informed consent. In those cases where the patient was

unable to give consent due to the severity of their illness or death, the consent was acquired by informed family members. To decline or accept participation in the study did not affect the quality of the patients' obtained healthcare.

# 5. Results

## 5.1. Descriptive statistics

During 2018-2020, 63 IPD patients between 29-90 years were included in this study. Baseline characteristics of the patients are shown in *Table 1*. In total, 52% were female, and the mean and median age was 68 and 71 years, respectively. The average length of hospital stay was 13 days. The acute phase testing was done on average 5 days (range 1 – 11 days) after hospital admittance, and the convalescent phase testing was done on average 75 days (range 55 – 107 days) after the acute phase testing.

Eighty-four percent manifested with pneumonia, 13% with meningitis, 3% with bacteremia without focus and 11% with other manifestations (Table 1). Ten percent of the patients presented with more than one manifestation. Almost a third of the patients (29%) spent time in the ICU unit, but only 5% died within 30 days. Forty-nine percent experienced some type of complication, with acute renal failure (16%) being the most common (Table 1).

Eighty-one percent of the patients had at least one predisposing risk factor, the most common one being current smoking (48%), followed by cardiovascular disease (40%) and pulmonary disease (29%) (Table 1).

***Table 1: Baseline characteristics of patients with invasive pneumococcal disease (IPD) by patient group***

| Characteristics | Patient group, n (%) | | |
|---|---|---|---|
| | Acute phase patients (n=60) | Convalescent phase patients (n=16) | All patients (n=63) |
| Sex | | | |
| Female | 30 (50) | 11 (69) | 33 (52) |
| Male | 30 (50) | 5 (31) | 30 (48) |
| Manifestations | | | |
| Pneumonia | 51 (85) | 13 (81) | 53 (84) |
| Meningitis | 7 (12) | 2 (13) | 8 (13) |
| Bacteremia with unknown focus | 2 (3) | 0 (0) | 2 (3) |
| Other* | 7 (12) | 2 (13) | 7 (11) |
| Comorbidities** | | | |
| Any | 50 (83) | 7 (44) | 51 (81) |
| Smoking | 30 (50) | 5 (31) | 30 (48) |
| Addicion | 2 (3) | 0 (0) | 2 (3) |
| Systemic Autoimmune Disease | 5 (8) | 0 (0) | 5 (8) |
| Haematological malignancy | 4 (7) | 1 (6) | 4 (6) |
| Solid malignancy | 4 (7) | 1 (6) | 4 (6) |
| Cardiovascular disease | 25 (42) | 5 (31) | 25 (40) |
| Liver disease | 1 (2) | 0 (0) | 1 (2) |
| Kidney disease | 3 (5) | 0 (0) | 3 (5) |
| Pulmonary disease | 17 (28) | 5 (31) | 18 (29) |
| Diabetes mellitus | 8 (13) | 1 (6) | 8 (13) |
| Complications | | | |
| Any | 30 (50) | 5 (31) | 31 (49) |
| Empyema | 7 (12) | 1 (6) | 7 (11) |
| Pleural fluid | 6 (10) | 2 (13) | 7 (11) |
| Heart infarction | 1 (2) | 0 (0) | 1 (2) |
| Acute renal failure | 10 (17) | 0 (0) | 10 (16) |
| Atrial fibrillation | 9 (15) | 2 (13) | 9 (14) |
| Other | 8 (13)*** | 1 (6)**** | 8 (13)***** |

** Seven patients presented with other manifestations either as the sole manifestation or in combination with other manifestations. Two patients presented with acute otitis media, one with mastoiditis, two with septic arthritis, one with mastitis and one patient presented with spondylodiscitis and an epidural abscess.*
*** Asplenia and pregnancy were excluded from the table as none of the patients had either condition.*
**** Two patients (3%) experiences hearing loss, two patients (3%) had a falling accident caused by cognitive failure as a result of their IPD, two patients (3%) had pulmonary embolisms, one patient (2%) had pericarditis and one patient (2%) ad hyperosmolar syndrome.*
***** One patient (6%) experienced hearing loss.*
****** In total, two patients (3%) presented with hearing loss, two (3%) with pulmonary embolisms, two (3%) patients had a falling accident caused by cognitive failure as a result of their IPD, one (2%) with liver failure, one (2%) with pericarditis and one (2%) with hyperosmolar syndrome.*

## 5.2. Ig analysis

The testing of Ig and IgG subclasses was performed on 60 patients during the acute phase of the illness, on 16 patients in the convalescent phase of the illness (2-3 months after recovery), and on 18 age matched controls (Figure 1). The rate of Ig abnormalities was high, with 26 acute phase patients (43%), 5 convalescent phase patients (31%), and 4 controls (22%) having abnormal Ig levels. The number of acute phase patients, convalescent phase patients and controls which had at least one Ig test below the threshold was 14 (23 %), 2 (13%), and 3 (17%), respectively (Figure 2).

The IgM levels were significantly higher for patients tested in the convalescent phase compared to the control group (median 1.0 and g/l, respectively, $p = 0.03$) (Figure 2c). Although the median IgM value of the controls was significantly lower than the convalescent phase groups' values, it still stayed within the established reference values. Two of the controls (11%) had IgM values below the lower reference limit (Figure 2c). The proportion of acute phase patients with at least one Ig test below the reference threshold was 23%, compared to 13% in convalescent phase patients, and 17% in controls (Figure 2). However, none of the other Ig analyses resulted in a statistically significant result, neither when patients and controls were compared, nor when the acute and convalescent phase patients were compared (Figure 2).

The IgG subclass analysis demonstrated that the patients' IgG2 levels were significantly lower during the acute phase of the illness compared to their convalescent phase (median 2.1 and 2.5 g/l, respectively, $p=0.01$) (Figure 3b). The patients also had significantly lower IgG2 levels in the acute phase compared to the control group (median 2.1 and 3.5 g/l, respectively, $p<0.0001$) (Figure 3b). Similarly, a significant difference was found in IgG4 levels between the acute phase group and controls (median 0.25 and 0.43, respectively, $p=0.03$) (Figure 3d). The proportion of acute phase patients with at least one IgG subclass deficiency was 30%, compared to 0% in convalescent phase patients, and 17% in controls (Figure 3). However, none of these comparisons showed any significant difference (Figure 3).

The rate of IgG subtype abnormalities was high, with 31 acute phase patients (52%), 4 convalescent phase patients (25%), and 5 controls (28%) having at least one abnormal IgG

subtype test. The number of acute phase patients, convalescent phase patients and controls which had at least one IgG subtype test below the threshold was 18 (30 %), 0 (0%), and 3 (17%), respectively (Figure 3).

## 5.3. Lymphocyte population

Next, the lymphocyte population was measured in the convalescent phase (approximately 2 to 3 months after recovery from the IPD episode) in 16 patients and 18 controls (Figure 1). The frequency of all lymphocytes, total T cells, helper T cells, cytotoxic T cells, B cells and NK-cells were measured using flow cytometry.

Several significant differences between the patients and controls were found in the frequencies of each cell type, which can be seen in *Figure 4*. Although the majority of the patients' lymphocyte frequency values were within the reference limits, the patient group demonstrated a significantly lower total lymphocyte count compared to the controls ($p=0.002$) (Figure 4a).

Similarly, the patient group had significantly lower frequencies of both total T cells (CD3+) and helper T cells compared to the control group ($p=0.01$ and $p=0.01$, respectively) (Figure 4b and c). Patients also had a significantly lower B cell frequency compared to the controls ($p=0.01$). However, whereas none of the controls had a B cell count below the reference limit, almost one third (31%) of the patients had values below the limit (Figure 4e). No significant differences were found in cytotoxic T cell or NK cell frequencies (Figure 4), neither could a significant difference be established in any of the cell proportion comparisons.

## 5.4. T cell stimulation

T cell stimulation was tested in 15 convalescent phase patients (due to technical malfunction, it was not possible to analyze one of the patient's samples) and 18 healthy controls. It is measured by exposing the T cells to a radioactive nucleotide, which when T cells are simulated with different antigens, are incorporated into the T cell's DNA during replication. By measuring the T cells' radioactive activity (counts per minute, cpm), it is possible to quantify the T cell's ability to get activated by antigens. The cpm of unstimulated T cells, and

T cells stimulated with the selective T cell mitogens PHA and ConA were measured for both patients and controls. These values were also compared as a proportion of blood donor values which were analyzed at the same time as the study samples to act as a reference value.

The only significant difference found was that the patients had lower values in the PHA stimulated test when compared to the control group as a proportion of the blood donor value (median 87% and 112%, respectively, $p=0.03$) (Figure 5b).

## 5.5. Phagocyte function

The phagocyte function was tested in 16 patients in the convalescent phase (approximately 2 to 3 months after recovery from their IPD episode) and in 18 healthy controls. The phagocyte function was measured by adding opsonized bacteria (*E.coli*) to monocytes and granulocytes, and then measuring the percentage of phagocytes that have engulfed bacteria. Another test, which measured the percentage of granulocytes that produced reactive oxygen species after being stimulated by different substances, was also performed. Although a large proportion of the data of both patients (88%) and controls (88%) were above the established reference values, the patient group still had significantly lower values for the monocytes compared to the controls ($p<0.0001$) (Figure 6a). However, the median of the patients and controls only differed by one percentage point (median 98 and 99 %, respectively). None of the other tests were statistically significant (Figure 6).

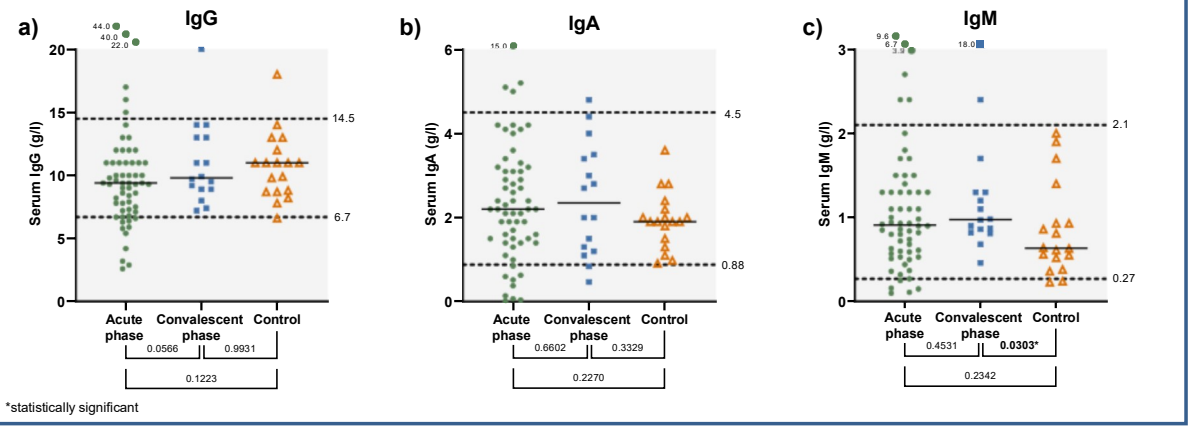
## 5.6. B cell stimulation

The B cells' ability to produce antibodies after being simulated by different mitogens was tested in 16 patients in the convalescent phase (approximately 2 to 3 months after recovery from their IPD episode) and in 18 healthy controls. Two different stimuli were used; one T cell independent (EBV), and one T cell dependent (PWM). One test was also left unstimulated. The patients and controls were then compared to each other. However, none of the results were statistically significant.

## 5.7. Correlation between diverging test levels and illness severity

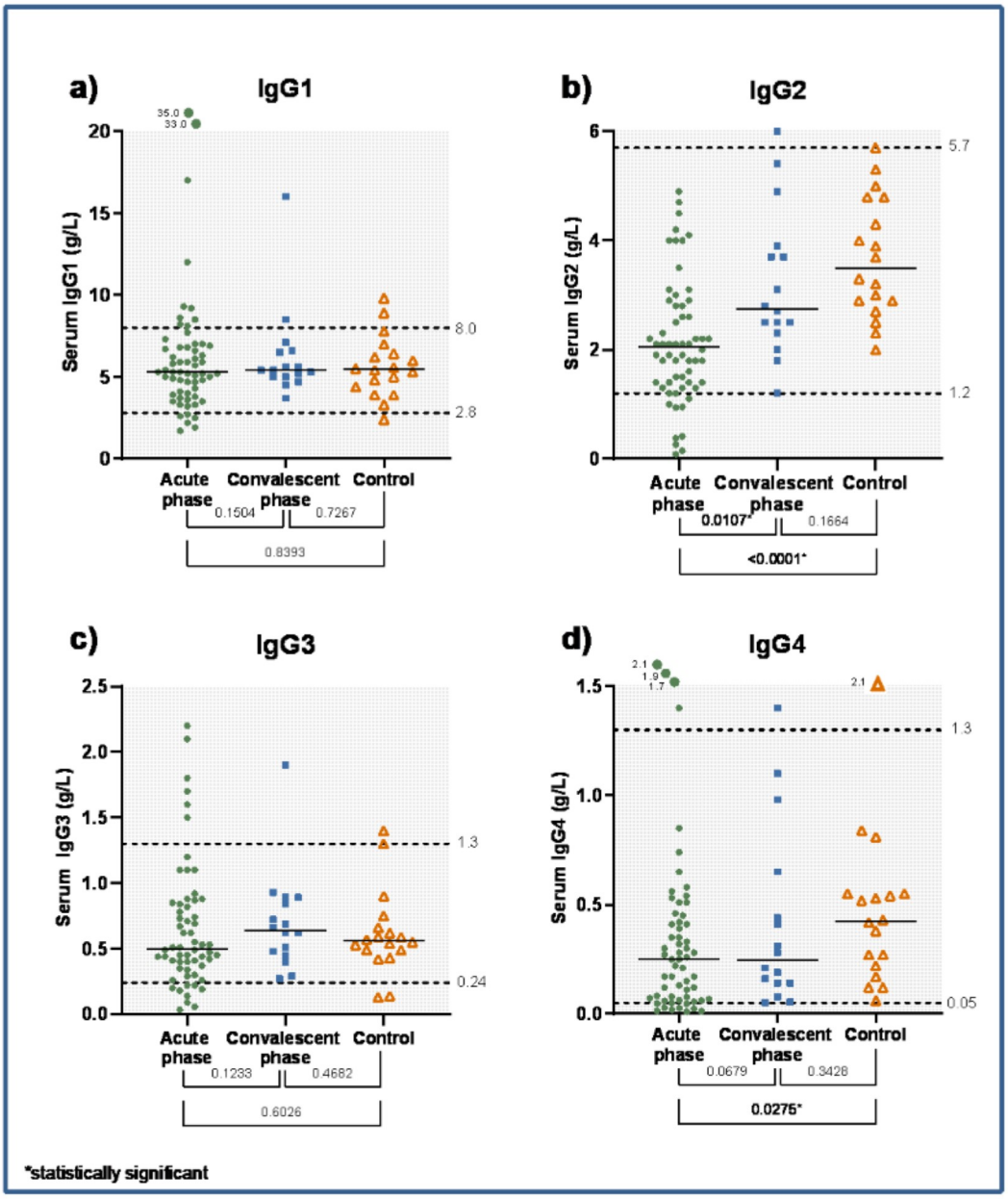
All tests which were statistically significant in this study (monocytic phagocytosis, PHA T cell stimulation in proportion to blood donor value, lymphocyte cell count, T cell count, helper T cell count, B cell count, IgG2, IgG4 and IgM levels) were statistically analyzed to find a correlation with the severity of the patients' illness. Length of hospital stay, ICU admittance and if the patient experienced any complications were used to measure the illness' severity. No significant correlation was found for any of the above mentioned tests, and the results are therefore not presented in a graph.

***Figure 2: Serum IgG, IgA and IgM levels for patients tested in the acute and convalescent phase, and of the control group.***




*Both the acute (n=60) and convalescent (n=16) phase groups were individually comparied against the control (n=18), and a paired comparison was also made between the acute phase values against the same patients' values in the convalescent phase. The median values of the groups are presented as a black line. The dotted line represents the reference interval for each Immunoglobulin (Ig) analysis, and their exact values are shown to the right of the line. Some extreme values did not fit in the graph, and are displayed slightly above the graph next where their exact values are written.*
***a) Serum IgG levels.*** *The median values for acute phase, convalescent phase and controls' IgG levels were 9.4, 9.8 and 11.0 g/l, respectively.*
***b) Serum IgA levels.*** *The median values for acute phase, convalescent phase and controls' IgA levels were 2.2, 2.4 and 1.9 g/l, respectively.*
***c) Serum IgM levels.*** *The median values for acute phase, convalescent phase and controls' IgM levels were 0.9, 1.0 and 0.6 g/l, respectively.*

***Figure 3: Serum IgG subclass levels for patients tested in the acute and convalescent phase, and of the control group.***




*Both the acute (n=60) and convalescent (n=16) phase groups were individually comparied against the control (n=18), and a paired comparison was also made between the acute phase values against the same patients' values in the convalescent phase. The median values of the groups are presented as a black line. The dotted line represents the reference interval for each Ig analysis, and their exact values are shown to the right of the line. Some extreme values did not fit in the graph, and are displayed slightly above the graph next where their exact values are written.*

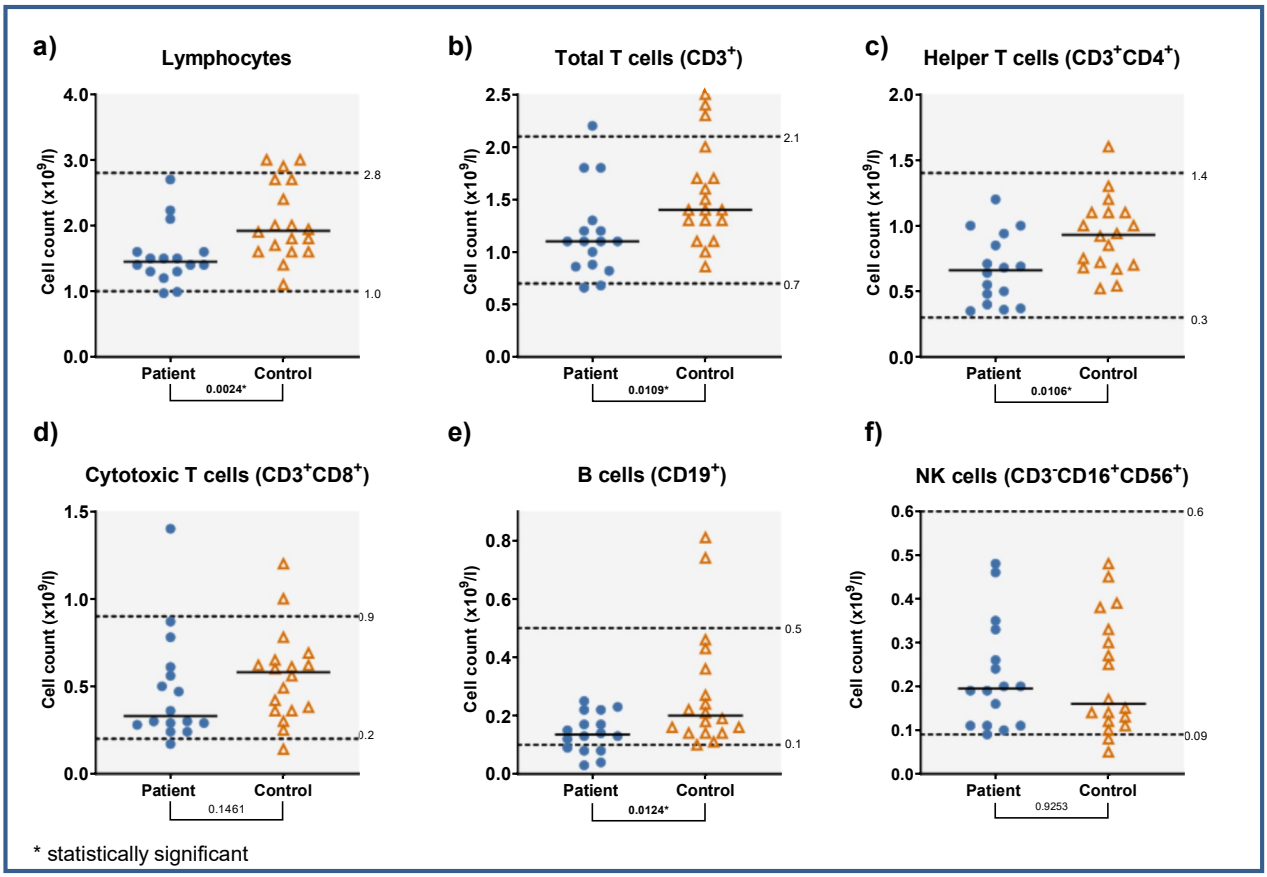
***a) Serum IgG1 levels.*** *The median values for acute phase, convalescent phase and controls' IgG1 levels were 5.3, 5.4 and 5.5 g/l, respectively.*

***b) Serum IgG2 levels.*** *The median values for acute phase, convalescent phase and controls' IgG2 levels were 2.1, 2.8 and 3.5 g/l, respectively.*

***c) Serum IgG3 levels.*** *The median values for acute phase, convalescent phase and controls' IgG3 levels were 0.5, 0.6 and 0.6 g/l, respectively.*

***d) Serum IgG4 levels.*** *The median values for acute phase, convalescent phase and controls' IgG4 levels were 0.25, 0.25 and 0.43 g/l, respectively.*

*Figure 4: Serum levels of total lymphocyte population and its different cell types*




*The patients (n=16) were tested in the convalescent phase and their values were comparied against the controls (n=18). The median values of the groups are presented as a black line. The dotted line represents the reference interval for each cell type, and their exact values are shown to the right of the line.*

***a) Lymphocyte levels in serum.*** *The median values of total lymphocyte population for the patients and controls were 1.5 and 1.9 cells x10$^9$cells/l, respectively.*

***b) Total T cell levels in serum.*** *The median values of total T cell population for the patients and controls were 1.1 and 1.4 cells x10$^9$cells/l, respectively.*

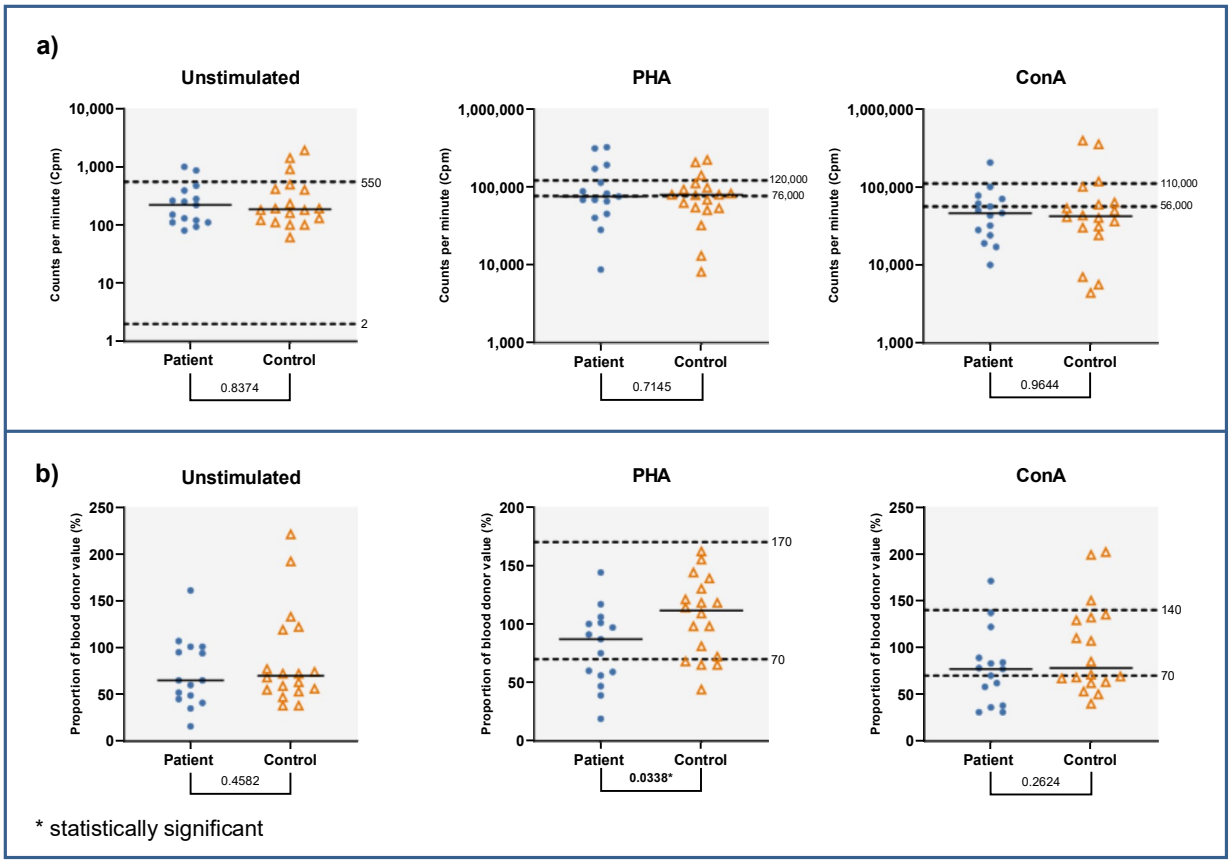
***c) Helper T cell levels in serum.*** *The median values of helper T cell population for the patients and controls were 0.7 and 0.9 x10$^9$cells/l, respectively.*

***d) Cytotoxic T cell levels in serum.*** *The median values of cytotoxic T cell population for the patients and controls were 0.3 and 0.6 x10$^9$cells/l, respectively..*

***e) B cell levels in serum.*** *The median values of B cell population for the patients and controls were 0.14 and 0.20 x10$^9$cells/l, respectively.*

***f) NK cell levels in serum.*** *The median values of NK cell population for the patients and controls were 0.20 and 0.16 x10$^9$cells/l, respectively.*

*Figure 5: T cell stimulation.*




*The patients (n=15) were tested in the convalescent phase and their values were compared against the controls (n=18). Due to a technical malfunction, one patient sample was unable to be analyzed. The median values of the groups are presented as a black line. The dotted line represents the reference interval for each cell type, and their exact values are shown to the right of the line.*

***a) T cell proliferation after stimulation in patients and controls.***

*The median unstimulated T cell proliferation for the patients and controls were 220 and 185 counts per minute (cpm), respectively.*

*The median phytohaemagglutinin P (PHA) stimulated T cell proliferation for the patients and controls were 75,000 and 78,500 cpm, respectively.*

*The median Concanavalin A (ConA) stimulated T cell proliferation for the patients and controls were 46,000 and 42,000 cpm, respectively.*

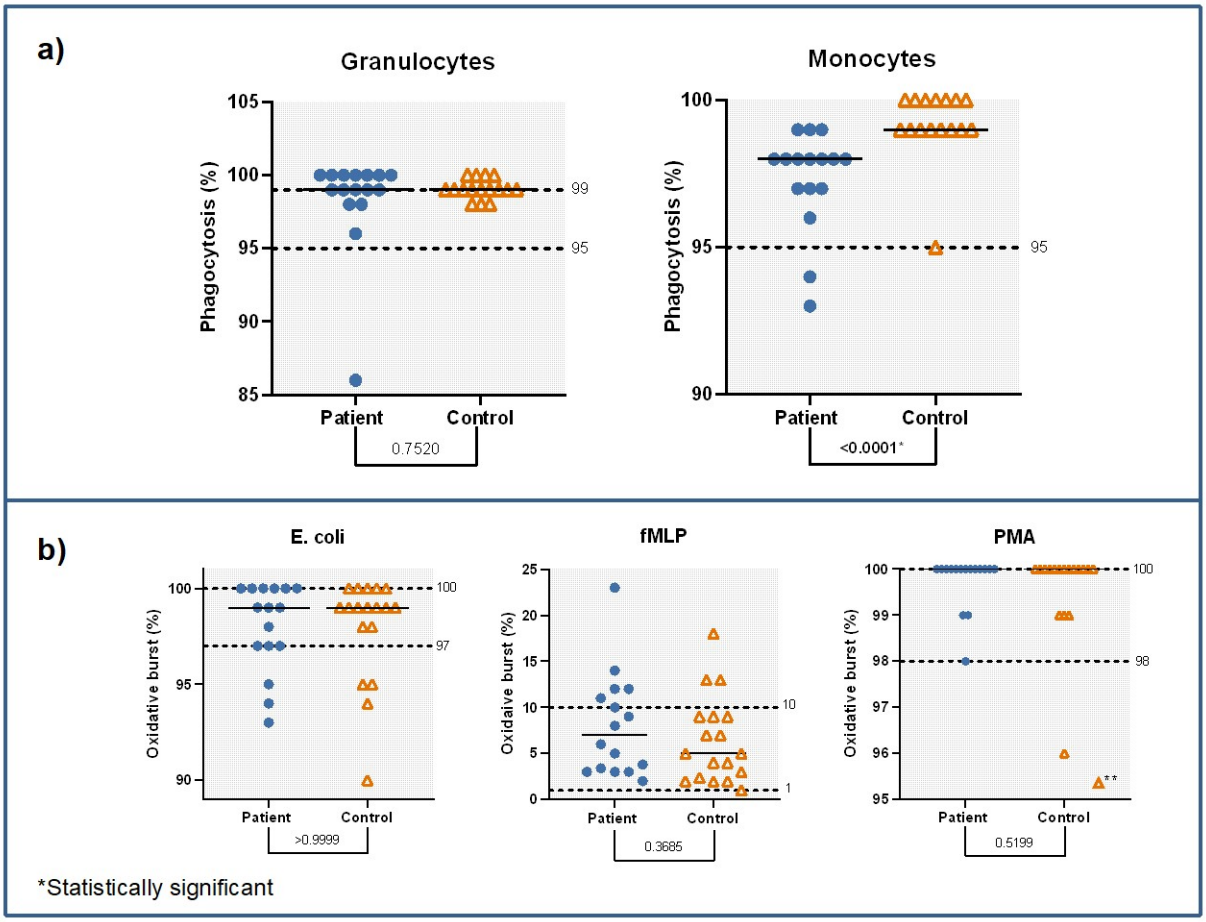
***b) T cell proliferation in patients and controls measured as a proportion to blood donor values.***

*The median unstimulated proportion of blood donor for the patients and controls were 65 and 70 %, respectively.*

*The median PHA stimulated proportion of blood donor for the patients and controls were 87 and 112 %, respectively.*

*The median ConA stimulated proportion of blood donor for the patients and controls were 77 and 78 %, respectively.*

*Figure 6: Phagocyte function*




***The control group of the PMA test had one extreme value (78) which did not fit in the graph.*
*The patients (n=16) were tested in the convalescent phase and their values were compared against the controls (n=18). Due to a machine error during the analysis of the samples, only 16 control samples were able to be analyzed for the tests depicted in part* ***a)*** *of this figure. The median values of the groups are presented as a black line. The dotted line represents the reference interval for each cell type, and their exact values are shown to the right of the line.*
***a) Percentage of granulocytes and monocytes which had engulfed opsonized E.coli in patients and controls.***
*The median granulocyte values for patients and controls were both 99%.*
*The median monocyte values for the patients and controls were 98 and 99 %, respectively.*
***b) Percentage of granulocytes that produced reactive oxygen species (ROS) after different stimulants.***
*All 18 controls and 16 patients were included in this part of the test.*
*The median E. coli (particulate stimulant of ROS) values for patients and controls were both 99%.*
*The median formyl-methionyl-leucyl-phenylalanine (fMLP, extracellular stimulant) values for patients and controls were 7 and 5 %, respectively.*
*The median phorbol 12-myristate 13-acetate (PMA, intracellular stimulant) values for patients and controls were both 100%.*

# 6. Discussion

This study aimed to determine whether a difference in immune function exists between IPD patients and healthy controls, as well as which parts of the immune system that are affected. Several significant results were found in this study, the most prominent being in IgG2 and IgG4 subclass levels as well as in the frequency of the cells in the lymphocyte population.

## 6.1. Immunoglobulin deficiencies

Some studies suggest a possible association between decreased IgA or IgM levels and IPD [44, 47], however, it is widely accepted that a deficiency in IgG has the closest connection with risk of IPD [35, 38, 43, 44, 46]. IgG subclass deficiencies, with IgG2 deficiency in particular, have also been connected to an increased risk of IPD [35, 38, 43, 47]. Although our study did not show any significant difference in total IgG levels between the patients and the controls, nor between the acute phase and the convalescent phase (Figure 2), we found several significant differences in the IgG subclass levels (Figure 3). Both IgG2 and IgG4 were significantly lower in acute phase patients compared to the controls, and IgG2 was also significantly lower in the acute phase compared to the convalescent phase (Figure 3b and d).

In a study published by *Ekdahl et al*, both total IgG level and all IgG subclass levels were lower in the acute phase compared to the convalescent phase in IPD patients [43]. Our study only found a significant decrease of IgG2 levels when comparing the levels in the acute phase with those in the convalescent phase (Figure 3). The differing results of the two studies might be partially caused by our study's low number of patients that underwent convalescent phase testing (16 patients, compared to *Ekdahl et al's* 68 patients [43]).

As to why a significant difference in IgG levels could not be demonstrated, even though both IgG2 and IgG4 was significantly lowered in the acute phase patients compared to the controls (Figure 3b and d), it has previously been suggested by some studies that an IgG2 deficiency can be masked by a compensatory increase in other IgG subclass levels, causing the deficiency not to show up in total IgG tests [43, 50, 51]. This might be one explanation as to why only the IgG subclass tests showed significant differences in our study. Interestingly,

IgM values were significantly higher in convalescent phase patients compared to the controls (Figure 2c).

It has been suggested that selective IgM deficiency might be associated with an increased infection risk [52]. *Martinot et al* found a high rate of selective IgM deficiencies among *S. pneumoniae* and *H. influenzae* patients [44]. However, there does not appear to be any previous findings that show IPD patients having higher IgM values than the controls. *Barton et al* found that patients with IgG2 deficiency had a significantly lower prevalence of IgM deficiency, compared to patients with a total IgG deficiency or an IgG subclass deficiency other than IgG2 [49]. This correlation needs to be studied further, but might offer a partial explanation to the findings of this study.

The prevalence of IgG deficiency has been reported to be approximately one third of IPD patients [43]. In our study, 10 of 60 acute phase IPD patients (17%) had total IgG levels below the reference threshold. This is lower than the percentage reported by *Ekdahl et al* [43], but coincides with the numbers reported by *Martinot et al* [44], where 18% of patients tested in the acute phase of an infection caused by *S. pneumoniae* or *Haemophilus influenzae* had reduced IgG levels. The prevalence of IgG deficiency in convalescent phase patients was 0% in our study, which could suggest that IgG levels normalize when patients recover from their illness. However, several studies have reported considerably higher proportions ranging between 10 and 32 % of IgG deficiency in convalescent phase patients [43, 47]. This calls our study's result into question, particularly as no significant difference could be established. The prevalence of IgG subclass deficiencies also differed from those reported by *Ekdahl et al* [43]. However, these differences can mostly be explained by the fact that the reference limits used by *Ekdahl et al* [43] differed from those used in this study.

Although a significant increase between the patients in the acute and convalescent phase could only be demonstrated for IgG2 (Figure 3b), all acute phase patients with decreased IgG subclass levels who underwent convalescent phase testing had normalized IgG subclass levels (Figure 3). Albeit not statistically significant, it demonstrates a trend which could suggest that values normalize in most patients with low IgG subclass levels during the acute phase of IPD.

## 6.2. Lymphocyte population

We found that convalescent phase IPD patients had a significantly lower frequency of total lymphocytes, total CD3+ T cells, CD3+CD4+ helper T cells, and CD19+ B cells compared to healthy controls (Figure 4). While the proportion of patients with total lymphocyte, CD3+ T cell, and CD3+CD4+ helper T cell levels below the lower reference threshold was fairly low (13, 13 and 0 %, respectively), approximately one third (31%) of convalescent phase IPD patients had CD19+ B cell levels below the limit (Figure 4). This contrasts the results of *Baril et al*, who found a significant difference between acute phase IPD patients and controls in all lymphocyte cell types, except in CD19+ B cells [39]. *Baril et al* also report that the deviating lymphocyte levels during the acute phase returned to normal in the convalescent phase of the infection [39], which contradicts our findings [39]. One explanation as to why *Baril et al* had normal lymphocyte levels during the convalescent phase, is that they excluded all patients with an already known immunodeficiency [39], thus making it likelier for their IPD patients to have normal lymphocyte levels in their habitual state. Our study, on the other hand, included all patients diagnosed with IPD regardless of comorbidities.

*Kemp et al* [53] report similar findings to those of *Baril et al*[39], with decreased T cell levels during the acute phase of pneumococcal disease, which after one week had increased significantly. *Kemp et al* also reported that CD19+ B cell levels were unaffected in the acute phase [53]. However, *Kemp et al* did not test the patients in the convalescent phase of the disease (after the patients had fully recovered) [53], and it is therefore arguable whether their results are comparable to those in this study.

The decrease in serum T cell levels during the acute phase of the infection which was described by *Baril et al* [39] and *Kemp et al* [53], is theorized to be caused by an increased T cell migration and apoptosis during the acute phase of infection, which explains why the lymphocyte levels normalize as the patients recover [53]. However, it does not offer an explanation as to why this study's convalescent phase results differ from the other studies. More research on IPD patient's lymphocyte cell counts during the convalescent phase of the infection necessary to be able to draw any conclusions, but our data suggests IPD patients might have lower levels of certain lymphocytes during the convalescent phase. It might also be helpful to analyze the possible correlations between lowered lymphocyte levels and patient

characteristics such as age, sex, and comorbidities. CD3+ T cell levels have been shown to decrease with older age [54], thus making it important to ensure the significant differences found are not due to patient characteristics.

## 6.3. Phagocyte function

Although the difference in monocyte function between the convalescent phase patients and controls was small, it still showed the patients had significantly lower values compared to the controls (Figure 6a). This finding is contradicted by that of *Picard et al* [38], who conclude in their literature review that defects in mononuclear or polymorphonuclear phagocytes are not associated with an increased risk of pneumococcal disease. However, *Picard et al* [38] do admit that is a subject that has not been greatly studied. Our findings indicate that a connection between monocyte dysfunction and IPD could exist. Monocytes have, after all, been shown to play an important part in eliminating *S. pneumoniae*, as they prevent CD3+ T cell necrosis by inducing apoptosis in the CD3+ T cells with the effect of reduced levels of invasive pneumococcal disease [55].

## 6.4. Limitations

This study has several limitations. Most prominent among them being the low number of patients which participated in the convalescent phase testing. This could also have caused an inadvertent selection bias in the convalescent phase group, as the reason many of the acute phase patients declined further testing was a poor health condition. As the conditions behind the patient's poor health could either be caused by or cause a lowered immune function, there is a possibility that the immune function of the convalescent phase group in this study was not representative for the entire IPD patient population. Because of the low number of patients and controls there is also a risk that a multiple testing problem has occurred. That is, since the p-value limit for significance was set at $\leq 0.05$, and this study analyzed a large number of different tests, there is a possibility that some of the significant results in this study are false (type 1 error). Therefore, this study should be viewed as explorative, and any conclusions from the results ought to be drawn tentatively.

If this study were to be performed again, it would be preferable to include a larger number of patients tested in the convalescent phase as well as a larger number of controls. If possible, all immune function tests should be conducted in the acute phase as well as the convalescent phase. A multivariable analysis also ought to be performed, to verify that none of the statistical significances are due to factors other than being an IPD patient/control (such as age, sex, and comorbidities).

## 6.5. Conclusions

In conclusion, this study suggests that a difference in immune function is present between patients with IPD and healthy controls. As expected, IgG2 and IgG4 were significantly lower in acute phase patients compared to controls, and IgG2 was also significantly lower in the patients during the acute phase of the illness compared to after recovery. As these results coincide with those in previous studies, it suggest the presence of a correlation between lowered IgG subclass levels and IPD. In contrast, we found that convalescent phase patients had significantly lower levels of total lymphocytes, T cells, helper T cells and B cells compared to controls, which differs from data reported by previous studies. This indicates the need for further research on the subject, specifically on the immune function of convalescent phase IPD patients.

It is also important to note that for most significant patient values, although lower than the controls, were still within the reference limits. Whether this suggests that the established reference limits are inaccurate, or that the results of this study lacks clinical value is hard to say. The fact that our study found no correlation between the patient's lower test values and the severity of their illness suggests the latter. However, the fact that no correlation could be found is not necessarily indicative that no correlation exists. The low number of patients in our study could also be a cause.

Although this study might give an indication as to which immune deficiencies are most commonly associated with IPD, the contradictory findings of this study makes it evident that our knowledge is incomplete.

By identifying which specific immune function deficiencies that are most commonly associated with an increased risk of IPD, we could recognize at-risk individuals earlier, and take earlier preventative measures. For example, knowledge about which lymphocyte levels that are associated with an increased IPD risk can be used to create new reference limits for pneumococcal vaccination protocols. We can thus identify and protect individuals who have low enough lymphocyte levels to increase the risk of IPD, but not low enough to pass the lower reference limit. Further study on Ig deficiencies and their connection to risk of IPD could help us better identify individuals in need of immunoglobulin replacement therapy.

# 7. Populärvetenskaplig sammanfattning

***Examensarbete i Medicin***

Amanda Nilsson, 2020

## *Immunfunktionen hos patienter med invasiv pneumokocksjukdom*

***Bakgrund:*** Pneumokocker är en sorts bakterie som ofta orsakar lunginflammation. Ibland kan bakterien dock ge upphov till en allvarligare typ av sjukdom: invasiv pneumokocksjukdom. Det betyder att bakterien har tagit sig in i delar av kroppen där det vanligtvis inte bör finnas bakterier, i till exempel blodet eller vätskan som omsluter hjärnan. Denna infektion är lyckligtvis ganska ovanlig, men vissa personer har högre risk att drabbas än andra. Barn under fem år och äldre personer över 65 år blir oftare sjuka i invasiv pneumokocksjukdom, och om man samtidigt har vissa andra sjukdomar såsom diabetes (sockersjuka) och lungsjukdomar har man också ökad risk att drabbas. Särskilt känsliga är personer som har nedsatt immunförsvar. Detta kan vara på grund att de tar mediciner som sänker immunförsvaret (till exempel cellgifter), att man har en medfödd brist på någon del av immunförsvaret eller att man har en sjukdom som påverkar immunförsvaret (till exempel HIV som attackerar en viss typ av vita blodkroppar). De vita blodkropparna cirkulerar i blodet och vävnaderna och letar efter främmande ämnen som kan vara farliga, till exempel bakterier och virus. Det finns många olika sorters vita blodkroppar; vissa oskadliggör bakterier genom att äta upp och bryta ner dem, och vissa bildar antikroppar som fastnar på bakterien och gör det lättare för de andra vita blodkropparna att hitta och attackera bakterien.

***Syfte:*** Syftet med studien var att undersöka om personer som insjuknat i invasiv pneumokocksjukdom har ett immunförsvar som fungerar sämre än hos personer som inte blivit sjuka.

***Metod:*** Detta gjorde vi genom att ta blodprover på patienter som insjuknat i invasiv pneumokocksjukdom där vi mäter hur bra de olika vita blodkropparna fungerar. Vi tar också samma prover på friska kontrollpersoner, och använder dem för att jämföra med patienternas resultat.

***Resultat:*** Det vi upptäckte var att patienterna under den akuta fasen hade lägre nivåer av den antikroppstyp som är särskilt viktig i kroppens försvar mot invasiv pneumokocksjukdom, jämfört med några månader senare då de hunnit tillfriskna. Patienterna som testades efter att ha tillfrisknat hade också lägre nivåer av många sorters vita blodkroppar jämfört med friska kontrollpersoner som aldrig haft sjukdomen.

***Slutsats:*** Det talar för att personer med låga nivåer av dessa typer av vita blodkroppar kan ha ökad risk att insjukna i invasiv pneumokocksjukdom jämfört med någon som har normala värden. Kunskap om vilka typer av brister i immunförsvaret som ger ökad risk att insjukna i invasiv pneumokocksjukdom kan hjälpa oss att identifiera personer som kan behöva extra skydd mot sjukdomar orsakade av pneumokockbakterien. Man kan då erbjuda dem vaccin, och vissa typer av brister i immunsystemet kan man behandla och på så sätt minska risken för personen att insjukna.

# 8. Acknowledgements

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# *Appendix 1: Reference values*

| Immunoglobulins | |
|---|---|
| **Ig isotype** | **Reference values (g/l)** |
| IgG | 6.7 – 14.5 |
| IgA | 0.88 – 4.5 |
| IgM | 0.27 – 2.1 |
| **IgG subclass** | **Reference values (g/l)** |
| IgG1 | 2.8 – 8.0 |
| IgG2 | 1.2 – 5.7 |
| IgG3 | 0.24 – 1.3 |
| IgG4 | 0.05 – 1.3 |
| **Lymphocyte population** | |
| **Cell count** | **Reference values ($x10^9$/l)** |
| Total lyphocytes | 1.0 – 2.8 |
| CD3+ T cells | 0.7 – 2.1 |
| CD3+CD4+ T cells | 0.3 – 1.4 |
| CD3+CD8+ T cells | 0.2 – 0.9 |
| CD19+ B cells | 0.1 – 0.5 |
| CD3-CD16+CD56+ NK cells | 0.09 – 0.6 |
| **Proportion of total lymphocytes** | **Reference values (%)** |
| CD3+ T cells | 55 – 83 |
| CD3+CD4+ T cells | 28 – 57 |
| CD3+CD8+ T cells | 10 – 39 |
| CD19+ B cells | 6 – 19 |
| CD3-CD16+CD56+ NK cells | 7 – 31 |
| CD3+CD4+/CD3+CD8+ quota | 1.0 – 3.6 |
| **T cell stimulation** | |
| **Counts per minute** | **Reference values (cpm)** |
| Unstimulated | 2 – 550 |
| PHA stimulated | 76,000 – 120,000 |
| ConA stimulated | 56,000 – 110,000 |
| **Blood donor quota** | **Reference values (%)** |
| Unstimulated | No reference values |
| PHA stimulated | 70 – 170 |
| ConA stimulated | 70 – 140 |
| **Phagocyte function** | |
| **Phagotest** | **Reference values (%)** |
| Monocytes | 65 – 95 |
| Granulocytes | 95 – 99 |
| **Oxidative burst** | **Reference values (%)** |
| *E.coli* stimulated | 97 – 100 |
| fMLP stimulated | 1 – 10 |
| PMA stimulated | 98 – 100 |
| **B cell stimulation** | |
| **IgM producing B cells** | **Reference values (number of cells)** |
| Unstimulated | 50 – 1,000 |
| EBV stimulated | 1,000 – 10,000 |
| PWM stimulated | 500 – 15,000 |
| **IgG producing B cells** | **Reference values (number of cells)** |
| Unstimulated | 50 – 800 |
| EBV stimulated | 1,000 – 10,000 |
| PWM stimulated | 1,000 – 25,000 |
| **IgA producing B cells** | **Reference values (number of cells)** |
| Unstimulated | 10 – 500 |
| EBV stimulated | 150 – 2,500 |
| PWM stimulated | 200 – 8,000 |