The Role of IgG and its Subclasses in Byssinosis

by

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I declare the dissertation herewith submitted for the Master of Medical Science degree at the University of Natal, has not been previously submitted by me for a degree at any other University and is my own unaided work.

Signed: 

Garth Andrew Hunter

Dedicated to:

God, Nina, Dr Coertze and Mom, who all supported and encouraged me.

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ABSTRACT

A case control study was performed in 6 cotton mills in KwaZulu-Natal, South Africa. The study used questionnaire and pulmonary function testing results to categorise respiratory symptoms in 52 exposed symptomatic, 30 exposed asymptomatic and 46 unexposed control subjects. These categorisation results were used to explore the relationship between serum IgG subclasses and cotton-specific IgG to byssinosis.

No definitive relationships between the serum IgG subclasses and clinical and functional symptoms of byssinosis were found. Whereas, exposed symptomatic (22.72 mg $_A$/l) subjects had significantly higher ($P = 0.01$) mean specific IgG concentrations than exposed asymptomatic (15.02 mg $_A$/l) or unexposed control (13.08 mg $_A$/l) subjects. A patho-aetiological or marker-aetiological role is indicated for specific IgG in the development of byssinosis.

The findings of this research challenged the status quo in terms of the accepted aetiological pathways of byssinosis. In turn the acceptance of a different aetiological pathway provided a possible answer to the varying presentation of the disease and by implication contested the current definition of byssinosis.

TABLE OF CONTENTS

FIGURES

TABLES

ABBREVIATIONS

ACDE	Aqueous Cotton Dust Extracts
ATS	American Thoracic Society
COPD	Chronic Obstructive Pulmonary Disease
C	Complement
CD	Cluster of Differentiation
CMT	Cut Make Trim
CV_t	Total Coefficient of Variation
ELISA	Enzyme-Linked Immunosorbent Assay
Fc	Fragment crystalisable
FEF_{25-75}	Forced Expiratory Flow from 25 to 75 percent of volume
FEIA	Fluoroenzymeimmunoassay
FEV_1	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
HIV	Human Immunodeficiency Virus
IL	Interleukin
IFN	Interferon
LPS	Lipopolysaccharide
mAb	Monoclonal Antibody
NCOH	National Centre for Occupational Health
OSHA	Occupational Safety and Health Administration
ODTS	Organic Dust Toxic Syndrome

PBMC	Peripheral Blood Mononuclear Cells
RAST	Radio Allergo Sorbent Technique
RTI	Respiratory Tract Irritation
SORDSA	Surveillance of work related and Occupational Respiratory Diseases in South Africa
THF	5,7,3,4 tetrahydroxyflavan 3-4 diol
TNF	Tumor Necrosis Factor
WHO	World Health Organisation

DEFINITIONS

Asthma:

Asthma is commonly defined as a narrowing of the airways that is reversible over short periods of time, either spontaneously or as a result of treatment (Newman Taylor, 1998: 997).

Asthma-like syndrome:

An acute nonallergic airway response arising from inhalation of various agents in the agricultural environment. The symptoms consist of chest tightness, wheeze, and / or dyspnoea and can be associated with cross-shift decline in forced expiratory volume in one second (FEV_1) (usually less than 10%), which is dose related (American Thoracic Society, 1998: S23).

Atopy:

Is a hereditary feature manifested by abnormal immediated-type hypersensitivity to a certain allergen or a group of allergens (Slavkovsky, 1995:1).

Black(s):

Of the human race, having dark coloured skin of African decent (The Concise Oxford Dictionary, 1992: 112).

Byssinosis (classical):

The disease (byssinosis) has a characteristic history. At first the (cotton) worker notices tightness of the chest and breathlessness on Mondays or on the first day back at work after an absence. On the next day he is completely recovered. These symptoms may get worse and occur on Tuesdays and then on all working days. Eventually there is severe and permanent disability (Schilling *et al.*, 1955: 217).

Chronic Bronchitis:

Productive cough for at least three months per year for at least two years (Rylander, 1997a: 154).

Δ FEV_1

Post-exposure FEV_1 -pre-exposure FEV_1 (White, 1989: 437).

Smoking habits: (Ferris, 1978: 38).

Current smoker:

A person who has smoked at least 20 packs of cigarettes or at least 1 cigarettes per day for at least 1 year (or cigarettes rolled from 12oz tobacco) in a lifetime.

Ex-smoker:

A person who has stopped smoking 1 month prior to the interview, spirometric testing and venipuncture.

Never smoker:

A person who has never smoked as much as one cigarette a day for as long as one year at the time of interview and testing.

Organic dust toxic syndrome (ODTS):

Is an acute inflammatory condition affecting airways and alveoli. It is caused by the inhalation of one or more agents in organic dusts. With heavy exposure, it includes such nonspecific symptoms as fever and malaise (American Thoracic Society, 1998: S31).

Pack years:

$$\frac{\text{No of cigarettes / day}}{20} \text{ X years of smoking (White, 1985: 4-24).}$$

Symptom status (expanded definition of byssinosis):

In addition to the clinical symptoms present in the classical definition, the functional symptoms of declines in Δ FEV_1 and baseline FEV_1, are included in the expanded definition of byssinosis termed 'symptom status'. Within this dissertation 'symptom status' refers to the case control groupings of exposed symptomatic, exposed a-symptomatic and unexposed control. 'All symptom status' includes all three of these groupings, whereas 'exposed status' refers to the exposed symptomatic and exposed a-symptomatic groups, but excludes the unexposed control group (The use of this definition is specific to this study).

White (s):

Of the human race having light coloured skin (The concise Oxford dictionary, 1992: 1399).

CHAPTER ONE

INTRODUCTION

1.0 INTRODUCTION

The development of respiratory symptoms after exposure to cotton was noted as early as 1713 by Ramazzini. The advent of the industrial revolution in England followed by America dramatically changed both the severity and number of workers affected [1](Jacobs, 1997). Respiratory problems through inhalation of cotton continued through and beyond the industrial revolution. The magnitude of the problem was such that in the 1980's, Cotes and co-workers (1987) noted that byssinosis was likely to be the world's most prevalent occupational lung disease. Although this is no longer the case in developed countries (Fishwick *et al.,* 1994) byssinosis remains a substantial problem in developing countries (Christiani *et al.,* 1994). Thus research relating to byssinosis has relevance both nationally and internationally.

There are many potential aetiological agents of byssinosis (Schachter, 1994), with endotoxin being implicated as the primary aetiological agent (Rylander, 1981; Fisher *et al.*, 1986; Kennedy *et al.,* 1987; Castellan *et al.,* 1987 and Rylander *et al.,* 1989). However, byssinosis is only one of a number of respiratory disorders associated with exposure to cotton dust (Rylander *et al.,* 1987).

[1] An abridged method of the Harvard system was used for referencing, for the purpose of making the text more readable. For publications with up to two authors, both authors were noted in the text, whereas for publications with more than two authors, only the first author was noted in the text followed by *et al.*

Additionally, byssinosis does not present itself as a simplistic disease entity. There is substantial disagreement and confusion as to what symptoms of cotton dust exposure constitute byssinosis (Haglind *et al.,* 1984; Ferguson, 1998).

There is substantial agreement among researchers, that cotton dust is able to induce respiratory symptoms and disease that do not meet the criteria of the classical definition of byssinosis, known as Schilling's classification (Hughes, 1981; WHO, 1981; Wegman *et al.,* 1983; Rylander *et al.,* 1987 and Christiani *et al.,* 1994). There is not a lack of understanding of the aetiological agents or pathways of the disease byssinosis, as classified by the well-defined and tightly structured Schilling's classification system. There is less understanding and consensus relating to peripheral respiratory disorders closely associated to byssinosis, also induced by cotton dust. The aetiological agents and pathways of these peripheral disorders have largely been ignored or sidelined in an effort to determine the aetiology of classical byssinosis.

The postulate of both a single aetiological agent and pathway mechanism, for all respiratory disorders induced by cotton dust, is unlikely to be correct. The diverse presentation of disorders suggests varying or overlapping of aetiological agents and pathways. It is likely that there are both specific and non-specific immune mechanisms responsible for respiratory disorders induced by cotton dust. Yet at present the humoral specific immune response is not accepted as inducing or playing any part in the development of byssinosis or any respiratory disorder, aside from occupational asthma, induced by cotton dust.

The potential specific immune response includes the role of the IgG class of antibodies. A number of potential roles has been attributed to specific IgG antibodies formed against extracts of cotton dust. Specific IgG antibodies were attributed as a marker of exposure in guinea pigs (Olaniran and Karol, 1988; Karol and Lemp, 1990) and have been found to be raised in cotton workers (Karol *et al.*, 1992). The only studies that investigated byssinotic effects in cotton workers with specific IgG antibodies against aqueous cotton dust extract (ACDE) produced conflicting results. Noweir (1981) demonstrated an association between specific IgG antibodies and byssinosis grades, whereas Sigsgaard and Karol (1993) did not. Thus the role of specific IgG antibodies in the development of byssinosis, has remained untested by corroborating research.

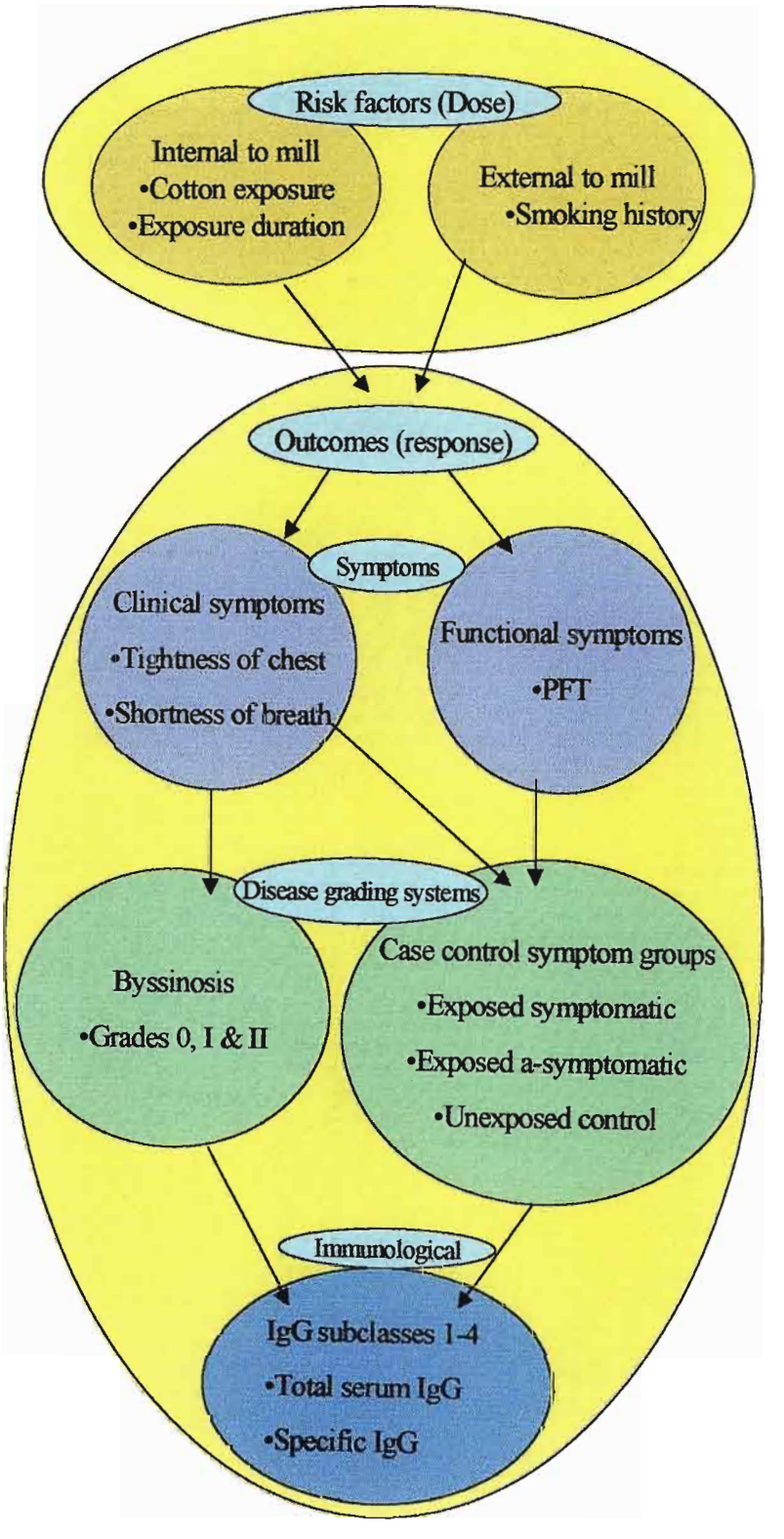
As far as can be ascertained the association between byssinosis and specific or serum IgG subclasses has not been determined. The IgG isotype response to respiratory antigens is predominantly IgG_1 (Kemeny *et al.,* 1989 as cited by Kay, 1997), whereas exposure to endotoxin is associated with raised IgG_2 concentrations (Mayumi *et al.*, 1983). Raised IgG_4 titres have been found in atopic individuals (Michils *et al.,* 1999), with atopy being a known risk factor for byssinosis.

Noting changes in individual IgG subclasses in cotton workers using a case control model, should provide a tool to partially profile the IgG humoral response to cotton dust. Together with the specific IgG data it should be possible to determine whether the IgG class of antibodies plays a protective, exposure marker, pathological outcome marker, or aetiological mechanism role in the development of byssinosis.

The classical definition of byssinosis only makes use of the clinical symptoms of tightness of chest and shortness of breath (Schilling *et al.,* 1955). Although both of these symptoms are subjective measures which are unverifiable, the classical definition of byssinosis is still widely accepted and used (Sigsgaard *et al.,* 1989; Baratawidjaja, 1991; Fletcher *et al.,* 1991; Warburton *et al.,* 1992; Sigsgaard and Karol, 1993; Fletcher *et al.,* 1993 and Warburton *et al.,* 1993). However, a dose-response relationship has been shown between classical symptoms (Roach and Schilling, 1960; Elwood *et al.,* 1966; Merchant *et al.,* 1972; Merchant *et al.,* 1973; Fox *et al.,* 1973; Berry *et al.,* 1973 and Kamat *et al.,* 1981), as well as between functional symptoms (Batawi *et al.,* 1964; Merchant *et al.,* 1973; Castellan *et al.,* 1987; Glindmeyer *et al.,* 1991, Glindmeyer *et al.,* 1994 and Fletcher *et al.,* 1997) of byssinosis and exposure to cotton dust.

Consequently it is questionable to view clinical symptoms of byssinosis in isolation to functional changes in pulmonary function. Supporting this view Schachter (1994) holds that implicit in the Schilling grading system, is the fact that byssinosis progresses from Monday morning cross-shift changes in pulmonary function to chronic, irreversible lung disease. For this reason a symptom status model was used as the primary classification system for disease in the case control study. The classical definition of byssinosis was used as a secondary categorisation system for disease, and was applied when appropriate. The symptom status classification included cotton-exposed workers with either classical symptoms of byssinosis and / or those with acute as well as chronic changes in pulmonary function.

Figure 1.1 Conceptual study design



1.1 STATEMENT OF THE PROBLEM

The aim of this investigation was to evaluate byssinosis in black workers exposed to cotton dust in textile mills with reference to IgG subclass concentrations and specific IgG levels, in order to determine the possible protective, marker or aetiological mechanism roles of these immunological factors, in the development of byssinosis.

Objective 1

To evaluate byssinosis in black workers exposed to cotton dust in textile mills with reference to individual and total IgG subclass concentrations, in order to establish how changes in the IgG subclasses relate to the disease.

Objective 2

To evaluate byssinosis in black workers exposed to cotton dust in textile mills with reference to specific IgG concentrations, in order to determine the nature of the relationship between specific IgG concentrations and clinical, as well as functional physiological outcome indicators of byssinosis.

Objective 3

To integrate how changes in individual IgG subclass as well as total serum IgG levels relate to byssinosis, together with the relationship between specific IgG concentrations and clinical as well as functional physiological outcome indicators of byssinosis, in order to determine the possible protective, marker or aetiological mechanism roles of these immunological factors in the development of the disease.

1.2 HYPOTHESIS TO BE TESTED

It was uncertain how IgG_1, IgG_3 and IgG_4 would be associated, whereas IgG_2 as well as specific IgG would be positively associated with a patho-aetiological role indicated, and total IgG not associated, with clinical and functional outcome indicators of byssinosis.

CHAPTER TWO

REVIEW OF THE RELATED LITERATURE

2.0 INTRODUCTION

Literature is reviewed which directly focuses on byssinosis, as well as literature that does not directly investigate byssinosis, but is of relevance to the current research focus. The related literature is reviewed as follows: socio-demographic (2.1), respiratory disorders (2.2), pulmonary function (2.3), aetiological agents and mechanisms (2.4), immunological mechanisms (2.5) and summary (2.6).

2.1 SOCIO-DEMOGRAPHIC FACTORS

Socio-demographic factors include both those that relate to the cotton industry and risk factors that are external as well as internal to the mill environment.

2.1.1 Cotton industry

Byssinosis is a respiratory disease that affects workers both internationally and nationally, with prevalence rates varying between countries and localised cotton mills.

a) International cotton industry

In developed countries, there is a decreasing prevalence of byssinosis (Fischer and Dommelsmith, 1997). Prevalence rates of 60% were recorded in the 1950's, (Schilling *et al.*, 1955) but had decreased to 3.7% in the United Kingdom by 1994 (Fishwick *et al.*, 1994). The United Kingdom is considered a developed country. It was argued that the lower number of workers required to operate more modern automated cotton-processing equipment (Fishwick

et al., 1990) and the better control of dust by this equipment (Abdel-Kader *et al.,* 1987) had led to this decline.

Concomitantly, much of the world's cotton processing has shifted from developed to developing countries. The countries included in the following studies are considered developing countries. Prevalence rates of 9% in Guangzhou (Jiang *et al.,* 1995) and 9.7% in Shanghai, China (Christiani *et al.,* 1994), were recorded in fairly recent studies. Prevalence rates of 27% were recorded in Egypt (El Batawi *et al.,* 1964), 28% in Cameroon (Takam and Nemary, 1988) and 37% in a mill which processed rough cotton and 1% in a mill which processed fine cotton, which were both cotton mills in Sudan (Awaad Elkarim *et al.*, 1987). Prevalence rates in Ethiopia, were recorded as follows: blowing 43%, carding 38%, drawing 24%, simplex 24%, ringframe 17%, preparatory 11% and weaving 4% (Woldeyohannes *et al.*, 1991). The results from these aforementioned studies show that byssinosis prevalence rates are higher in developing countries than in developed countries, indicating that byssinosis is still a substantial problem in developing countries.

b) The South African cotton industry

The only comprehensive study South African cotton industry found in the literature had been conducted by White (1989). He recorded the prevalence of byssinosis as 11.2 % in spinning, 6.1 % in winding, 6.4 % in weaving, 13.6 % in cleaning, and 43.8 % in bale opening as well as in blow room workers. In a national register, surveillance of work related and occupational respiratory diseases in South Africa (SORDSA) (1999), recorded nationally 19 (0.47 %), industry reported cases of byssinosis, which were all from KwaZulu-Natal. This was out of a total of 4049 SORDSA recorded cases of occupational disease for the period 1996 to 1999.

From 1994-1997, the Department of Labour had 23 industry reported cases of byssinosis on their records in KwaZulu-Natal alone (Holder, 1999). In June 1999 a total of 14 237 workers were employed in 21 mills that processed cotton lint into yarns and/or fabrics in South Africa. Out of these 14 237 workers, 2 707 were employed in the Western Cape; 5 810 in KwaZulu-Natal, 5 416 in the Eastern Cape, and 304 in Mpumalanga (Brink, 1999).

It is arguably impossible that byssinosis only occurs in cotton workers in KwaZulu-Natal. This lack of reported cases is almost certainly due to generally inadequate medical surveillance programs. Consequently, both the SORDSA and Department of Labour records are considered to be gross underestimates of the true byssinotic prevalence in the South African cotton industry.

2.1.2 Risk factors

Risk factors to developing byssinosis exist. These factors are both external and internal to the cotton mill environment.

2.1.2.1 Risk factors, external to mill

Risk factors outside of the cotton mill environment include domestic smoke exposure (Graham and Dutkiewicz, 1999), socio-economic status (Kanner *et al.*, 1979), familial susceptibility (WHO, 1981) and smoking history (Huib *et al.*, 1996).

Smoking and its history are the most important contributory factors to the increase in the rate of loss of pulmonary function (Huib *et al.*, 1996). Similarly, higher pack years of smoking are associated with raised levels of decline in FEV_1, (Postma *et al.*, 1988), with smokers

experiencing larger decreases in FEV_1 than non-smokers (Krzyzanowski *et al.*, 1991). Berry and co-workers (1974) record cigarette smoking as interacting with cotton dust exposure, to increase both the prevalence and severity of byssinosis. Yet smoking does not affect the cross-shift decline in ventilatory function (Jones *et al.*, 1979 as reviewed by Tockman *et al.*, 1984). In his study on byssinosis in the South African textile industry White (1989), notes that of a total of 2 411 workers included in his study, 62 % of males were smokers as opposed to only 3.4 % of females. He attributes the trend of fewer black female than black male smokers as largely being related to culture.

2.1.2.2 Risk factors, internal to mill

A number of factors in the internal mill environment impact on the respiratory response of cotton workers.

a) Classical symptoms

A dose-response relationship has been shown between classical (Kamat *et al.*, 1981) as well as functional (Fletcher *et al.*, 1997) symptoms of byssinosis and exposure to cotton dust. Whereas both Cinkotai *et al.*, (1977) and Cinkotai and Whitaker (1978) found a highly significant correlation between gram-negative bacteria and byssinotic symptoms, no such correlation was found for cotton dust. Similarly, Haglind *et al.* (1981, as cited by Rylander 1981a) found that the levels of gram-negative bacteria showed a stronger correlation to byssinosis than cotton dust levels did.

b) Functional symptoms

Endotoxin levels have also been shown to correlate with functional symptoms. Strong correlations have been found between gram-negative bacteria (Rylander *et al.*,1979b) as well as between endotoxin (Haglind *et al.,* 1984; Kennedy *et al.,* 1987; Fischer *et al.*, 1986; Castellan *et al.,* 1987; Rylander *et al.,* 1989) and Δ FEV_1 declines. However, a poor correlation was noted between cotton dust levels and Δ FEV_1 declines (Rylander *et al.*, 1979b; Haglind *et al.,* 1984; Kennedy *et al.,* 1987).

Importantly, Rylander *et al.* (1989) found fever and a decrease in FEV_1 approximately six to eight hours after exposure. This pattern and timing of lung function decline are typical of byssinosis.

c) Organic dust toxic syndrome (ODTS)

Endotoxin in cotton dust is certainly the most likely cause of ODTS (mill fever) (Fischer *et al.*, 1986).

d) Work department

In a cross sectional study conducted in the English cotton industry, Simpson and co-workers (1995) found that endotoxin concentrations were highest in spinning. Skadhauge and Sigsgaard (1995), also found mean endotoxin levels to be higher in spinning than in winding. Measurement of endotoxin levels was outside of the scope of this research investigation. However, the literature reviewed above indicates that spinning, followed by winding and then

weaving departments, are likely to have the highest endotoxin concentrations. Consequently, for the purposes of this study it is taken that endotoxin exposure is likely to be highest in spinning, followed by winding and then weaving.

e) Working hours

Another risk factor internal to the cotton mill is extended working hours[2]. If working hours are extended from 8 to 12 hours, it may result in a pulmonary response that is equivalent of 150 % of that induced by the shorter exposure time (Cloutier and Guernsey, 1998).

2.2 RESPIRATORY DISORDERS

The respiratory disorders induced by cotton dust exposure are varied and include the following:

- ODTS is also known as 'mill fever' or 'chills', and describes the clinical syndrome of toxic pneumonitis (Rylander, 1993). Endotoxin contained in cotton dust has been found to correlate most strongly with ODTS (American Thoracic Society, 1998).

- Weaver's cough is said to originate from exposure to mouldy cotton, and manifests itself in the form of extrinsic allergic alveolitis, also known as 'farmer's lung' (Crofton and Douglas, 1968, as cited in Lacey and Lacey, 1987).

[2] Variability in working hours exists in South African cotton mills, and this is reflected in the study sample, with certain mills working 8 hours and others 12 hours.

- Cough, like wheeze, is described by Pickering (1999) as an additional symptom (of byssinosis). The cough associated with dust exposure is usually unproductive at first, and does not follow the classical byssinosis cyclical timing.

- Chronic bronchitis, which may, at least in part, be induced by endotoxin (Rylander, 1997a).

- 'Monday dyspnoea' is the term used to describe tightness of the chest that is associated with shortness of breath, and describes the classic byssinotic response that occurs amongst some cotton workers (Beck *et al.*, 1982, as cited in Witek *et al.*, 1988).

- Chest tightness on the first day of the working week is accepted as one of the classical defining indicators of byssinosis, and is known as 'Monday tightness' (Schilling *et al.* 1955). This classical periodicity of chest tightness may be present in some, but not all, workers who experience chest tightness as a result of exposure to cotton (Hughes, 1981; WHO, 1981; Wegman *et al.*, 1983).

- Wheeze is the sound made whilst breathing, and may be present due to the symptom of chest tightness and/or functional obstruction that may be acute or chronic in nature. Wheeze is not a classical symptom of byssinosis. Instead it is an additional symptom of cotton exposure without the classical periodicity of byssinosis (Pickering, 1999).

- Hyperreactivity was documented in the 'Manchester Criteria' as one of the symptoms of cotton dust exposure (Rylander *et al.,* 1987).

- In the 'Manchester Criteria' document, occupational asthma as a distinct respiratory disorder to byssinosis, is alluded to as being induced by cotton (Rylander *et al.* 1987). Both mould (Salvaggio *et al.,* 1986) and endotoxin can induce asthma (Hollander *et al.*, 1994). However, byssinosis is not a form of occupational asthma (Edwards, 1981), although Baratawidjaja (1991) found a high correlation between byssinosis and asthma.

- Ocular and nasal irritation is experienced by a high percentage of cotton workers (Raza *et al.,* 1990). In a follow-up study, Fishwick and co-workers (1994) found no relationship between symptoms of ocular or nasal irritation and atopy, symptoms of byssinosis or dust concentrations.

- 'Asthma like syndrome' is a name termed by the American Thoracic Society (ATS) (1998) for a non-allergic airway response. The symptoms of byssinosis approximate those stipulated for asthma-like syndrome.

As byssinosis is the focus of this study, the pathology and diagnosis of this disorder are discussed in more detail.

2.2.1 Pathology of byssinosis

Byssinosis has been found to induce significantly more goblet cell metaplasia and smooth muscle hypertrophy (Pratt *et al.*, 1981; Edwards *et al.*, 1975, as cited in WHO, 1981; Schachter, 1994). Mild byssinosis has not been found to produce significant changes in small airways (Haglind *et al.*, 1983).

2.2.2 Diagnosis of byssinosis

Confirming the application of the classical definition in the diagnosis of byssinosis, Niven *et al.* (1991:200) note: "It [byssinosis] is diagnosed purely on the basis of symptoms, their unusual periodicity being the essential feature". However, not all cotton dust researchers agree. Schachter (1994) argues that no single test will verify the diagnosis of byssinosis. Rather, the diagnosis should be made on the basis of worker symptoms and signs, in conjunction with lung function data, which together should be used to characterise degree of impairment. Pickering (1998) sanctions this approach, noting that spirometric testing can be used to verify the presence of chest tightness.

Since the classical diagnosis of byssinosis is based only on the qualitative clinical symptoms of shortness of breath and tightness of chest (Niven et al, 1991), the diagnosis is subjective, problematic, controversial and not verifiable by quantitative techniques. This problem has been a hallmark of cotton dust research with replicate studies producing dissimilar and often conflicting results (Christiani et al, 1994).

Both clinical manifestations and lung function changes are recognised within the World Health Organisation (WHO) (1981) classification system as being induced by cotton dust.

However, only clinical manifestations are recognised as byssinotic symptoms, with lung function changes being limited to the assessment of effects of cotton dust exposure. Importantly, lung function changes are not recognised as being byssinotic symptoms. The WHO (1981) recognise the following assessment tools as being appropriate for the measurement of clinical manifestations and lung function changes:

- A standardised cotton questionnaire, validated by an accepted authority, should be used for the assessment of respiratory symptoms (byssinosis) and;
- Standardised spirometric testing should be used to measure forced vital capacity (FVC) and FEV_1, using appropriate predictive values. WHO (1981) stipulates minimum standards for spirometric tests taken from the 1978 Occupational Safety and Health Administration (OSHA) regulations, which are based on ATS standards.

2.3 PULMONARY FUNCTION

Byssinotic symptoms are associated with both acute (McKerrow *et al.,* 1958; Schachter, 1994) and chronic (Christiani *et al.,* 1994; Fishwick *et al.,* 1996) declines in pulmonary function.

- Cross-shift declines in FEV_1 were found to be present in a small number of workers with byssinotic symptoms (Imbus *et al.*, 1973, as cited by Tockman *et al.*, 1984). These acute declines are linked to longitudinal declines in both FVC and FEV_1 (Zuskin *et al.,* 1991); and

- Chronic lung function declines induced by cotton dust are associated with acute cross-shift declines (Zuskin *et al.,* 1991; Christiani *et al.,* 1994).

2.4 AETIOLOGICAL AGENTS AND MECHANISMS

There is general agreement that the aetiological agent of byssinosis is not the cotton lint itself, but rather a contaminant thereof (Weill 1981, as cited by Rylander and Morey, 1982).

Cotton dust contains more than 50 antigenic or biologically active substances, among which are the aetiological agents responsible for the development of byssinosis (Butcher *et al.,* 1983). Amongst these toxic chemical components are aromatic and alicyclic carbonyl compounds, aromatic alcohols, phenols, esters, latones, pyrans, epoxides, pyrazines, terpenoids, tannins, primary amines, endotoxins, glucans, and lacinelenes (de Rochemonteix *et al.,* 1991; Schachter, 1994; WHO, 1981). These substances originate from plant components such as terpenoid aldehydes, applied toxic material including pesticides, desiccants, defoliants and micro-organisms like gram-positive bacteria and fungi with associated metabolites. Such metabolites include peptides, mycotoxins and proteases (Jacobs, 1997).

Only the more important substances that have been implicated in the development of byssinosis or respiratory disorders induced by cotton dust as possible aetiological agents, are discussed below. It is likely that there is substantial additive or synergistic interplay, not only between the substances reviewed, but also between those not reviewed. Moreover, if a relationship has been found to exist between exposure to a particular substance and an effect, this in itself does not mean that the particular substance is causing the effect. The substance

may be acting as a marker for other substances present in the cotton dust that are actually causing the effect (Rylander, 1997b).

2.4.1 Micro-organisms

There are various types of gram-negative bacteria, gram-positive bacteria, actinomycetes and fungi that have been isolated in the air of cotton mills (Lacey and Lacey, 1987).

Several species of these isolated fungi and actinomycetes are known to be potentially pathogenic. These include *Absidia corymbifera, Aspergillus fumigatus, Mucor puillus,* and *Nocardiopsis dassonvillei. Alternaria* spp, *Cladosporium* spp, *Thermoactinomyces* spp and *Faenia rectivirgul* are potentially allergenic. Those that produce carcinogenic or toxic metabolites, include *Aspergullus flavus and A. ochraceus* (Lacey and Lacey, 1987). Additionally, *A. fumigatus* is known to induce allergic asthma (Trompelt *et al.,* 1994).

Gram-negative bacteria are found in high numbers on cotton; approximately 10^6-10^8 per gram of dry weight cotton fibre (Morey *et al.*, 1983). At least nine morphologically distinct gram-negative colony-forming types of bacteria on cotton dust have been identified. Specifically, *Enterobacter agglomerans* and *Pseudomonas syringae* have been found to be most plentiful on cotton lint and associated trash (Akinwunmi *et al.*, 1989 as cited by Akinwunmi and Heintz, 1997).

The effects of endotoxin in particular are discussed in detail.

2.4.2 Endotoxin

Endotoxin comprises of a complex amalgamation of associated lipopolysaccharide (LPS) molecules, which forms part of the outer shell of gram-negative bacteria. There are both smooth and rough types of LPS molecules that make up the cell wall of gram-negative bacteria. The different bacterial species and strains of gram-negative bacteria exhibit different structures and composition (Couturier *et al.*, 1991). The purified derivative of endotoxin is LPS (Michel *et al.*, 1997). Literature on the subject uses the terms endotoxin and LPS interchangeably. Thus, in this text endotoxin and LPS indicate the same moiety.

2.4.2.1 Biological impact of endotoxin

The biological effects of LPS are varied. It is important to examine these effects of endotoxin in relation to the effects of inhaled cotton dust in isolation, in order to be able to compare the two. Michel and co-workers (1997) record that LPS produces a systemic and a bronchial inflammatory response, involving mast cells, neutrophils and macrophages. This response is dose related.

a) Humoral response

Importantly, at low concentrations endotoxin is able to initiate a specific antibody response. At high concentrations LPS has been found to be a polyclonal B cell activator that impacts on almost all B cells, stimulating growth and differentiation, without binding to the membrane immunoglobulin. However, neither the B cell receptor nor the mechanism of B cell activation has been clearly established (Abbas *et al.*, 1994). Splawski *et al.* (1989), citing Levitt *et al.* (1981), support these findings, noting that endotoxin is able to weakly stimulate murine B cells, leading to low level proliferation and differentiation.

b) Inflammatory response

Endotoxin further activates several pro-inflammatory mediators, such as arachidonic acid, neutrophil enzymes and complement (C). In addition, LPS is able to induce the release of histamine (Pernis *et al.*, 1961; Hinshaw *et al.*, 1960; Davis *et al.*, 1963, as cited by Rylander, 1981a).

c) Bronchial response

Endotoxin induces a number of respiratory disorders including acute respiratory tract infection, hypersensitivity pneumonitis, asthma, and chronic bronchitis (Hollander *et al.*, 1994). Additionally, chronic exposure to endotoxin increases the risk of developing non-atopic chronic obstructive airways disease (Schwartz *et al.*, 1995), as well as conventional asthma (Michel *et al.*, 1996). LPS induced bronchoconstriction has not been associated with atopic status (Michel, *et al.*, 1992a; Michel *et al.,* 1995).

Challenging the lung vascalature with endotoxin primes the lung capillary system to respond with a severe increase in vascular permeability to a relatively low dose of exotoxin (Schutte *et al.*, 1997). In addition, harmful biological effects of endotoxin on the lung include lung microvasculature disturbances, with subsequent edema resulting in loss of gas exchange function. Direct cellular toxicity of endotoxin has also been noted (Esbenshade *et al.*, 1982; Brigham *et al.*, 1986; Meyrick *et al.*, 1986, as cited by Schutte *et al.*, 1997).

d) Systemic response

A statistically significant increase in neutrophils and total white blood cell count has been noted in normal patients exposed to 20μg LPS after 120 minutes (Michel *et al.*, 1995). This increase has been attributed to the migration of cells from the bone marrow (Espevik *et al.*, 1983 as cited by Michel *et al.*, 1995).

LPS induces monocytes and macrophages to produce many different cytokines, including InterLeukin (IL) 2 (Chensue *et al.*, 1995 as cited by Schuyler *et al.*, 1998; Burrell 1997). Endotoxin also stimulates monocytes to produce IL 8 (Dentener *et al.*, 1993). Macrophages in turn produce lysomal enzymes and different cytokines. Included in the cytokines produced are 1, platelet activating factor and tumour necrosis factor α (TNF) (de Rochemonteix *et al.*, 1991; Schachter, 1994; WHO, 1981). Wright *et al.* (1991) note that TNF α, Granulocyte Colony Stimulating Factor (G-CSF), Granulocyte-Macrophage Colony Stimulating Factor (GM-CSF) and formyl peptide have been independently found to lead to a doubling in expression of cluster of differentiation (CD) 14 on the surface of neutrophils.

e) Dose-response

Furthermore, a dose of 50μg may induce symptoms of fever, increasing both circulatory and respiratory rate (Michel, 1998). In normal patients exposure to 20μg challenge with endotoxin does not lead to a significant decrease in FEV_1, although it does induce a systemic inflammatory response (Michel *et al.*, 1995). However, in asthmatic patients the same inhalational challenge exposure of 20μg pure endotoxin, has been found to have caused a significant decrease in FEV_1 that has lasted for five hours (Michel *et al.*, 1995).

The no-response threshold to an acute exposure of endotoxin via the respiratory pathway is less than 0.5 µg (Michel *et al.*, 1997). However, exposure to endotoxins has caused alveolar macrophages to release cytokines TNF α and ILs 1 and 6 at concentrations as low as <1ng.mL (Michel, 1998; Couturier *et al.*, 1992). Furthermore, threshold doses for fever reactions have been reported at 0.002 µg /kg bodyweight in humans, and pulmonary function changes have been found to occur at exposures of 0.01 µg /kg bodyweight. Typical exposures within a cotton mill over an eight hour shift render an approximate endotoxin dose of 3.8µg or 0.05 µg /kg bodyweight (Wolf, 1973; Muittari *et al.*, 1980 as cited by Rylander and Morey, 1982). An endotoxin threshold for no Δ FEV_1 effect has been established at 0.05 µg/m^3 (Haglind and Rylander, 1984).

This clearly shows the potential for a diverse physiological response in cotton workers exposed at both high and low concentrations of endotoxin.

2.4.2.2 Endotoxin in Cotton dust

The LPS[3] found in cotton dust similarly forms part of the cell envelope of gram-negative bacteria, which are present in cotton mills and commonly liberated due to cell lysis (Rylander and Morey, 1982; American Thoracic Society, 1998).

[3] The effects of endotoxin as a risk factor, internal to mill were reviewed in Chapter 2, Section 2.1.2: Risk factors.

2.4.2.3 Summary

In 1981(a), Rylander noted that there was enough evidence to support a causal relationship between endotoxin and byssinosis. The status quo remains, albeit reinforced since the time of writing. There is also compelling evidence that endotoxin is in large part responsible for the Δ FEV_1 declines noted in cotton workers. Yet in endotoxin exposed workers in the cotton mill environment, only some workers develop byssinosis. In contrast, human subjects exposed to pure endotoxin in clinical trials show a uniform increase in pharmacological inflammatory agents and resultant inflammation in all specimens exposed to endotoxin (Michel *et al.*, 1995).

The different responses evidenced between cotton workers and subjects in clinical trials may be as a result of antigenic substances other than endotoxin in cotton dust. When Buck (1981) removed both endotoxin and lacinelene from the ACDE, the extract retained its bronchoconstriction activity (Schacter, 1994). This finding indicates that endotoxin is not the only agent in cotton dust able to induce bronchoconstriction. The poor correlation that exists between cotton dust and endotoxin (Haglind *et al.*, 1984; Rylander *et al.*, 1985; Castellan *et al.*, 1987) further supports this hypothesis. Endotoxin may act as a marker for a number of other agents contained in cotton dust. Accordingly, it is highly likely that the effects noted in endotoxin environments are as a result of the additive or synergistic interplay between biologically potent agents (Rylander, 1997b).

2.4.3 (1→3) β-D-Glucan

Fungi grow on cotton dust and have been isolated in the air of cotton mills (Lacey and Lacey, 1987). (1→3) β-D-Glucan is a compound that is found in the cell walls of fungi, various types

of bacteria and plants (Millins, 1990; Tsuchiya *et al.*, 1996 as cited by Thorn and Rylander, 1998).

a) Biological activity

The marked neutrophilia, as well as the inflammatory response induced by LPS in the airway, is different to the inflammatory response produced by (1→3) β-D-Glucans (Fogelmark *et al.*, 1998; Thorn and Rylander, 1998). Jones and Nichols (1998) however, showed that (1→3) β-D-Glucans induced specific hypo-responsiveness, and not inflammation, promoting a decrease in airway reactivity, which may be dose dependent. Ohno *et al.* (1998) note that soluble β-Glucans are of very low toxicity. Activities such as inflammatory cytokine synthesis, nitric oxide synthesis, vascular permeability and limulus factor G activation are dependent on the structure of the single helix formation.

b) LPS interaction

Certain (1→3) β-D-Glucans have been found to have the ability to activate the complement cascade (Ohno *et al.*, 1998). Furthermore, glucans can induce the secretion of many different cytokines, including IL 1 α, IL 2 and IL 6, which are secreted by monocytes as well as macrophages (Schuyler *et al.*, 1998; Adachi *et al.*, 1998). Both glucans and LPS activate macrophages to secrete cytokines in a synergistic effect via interaction with CD 14. The release of IL 12 and TNF α, but not IL 10, is compatible with the induction of a Th1 type response (Adachi *et al.*, 1994 as cited by Schuyler *et al.*, 1998). Schuyler *et al.* (1998) found that glucans bind to macrophages using LPS binding sites such as CD14, thus inhibiting or limiting the LPS binding on the macrophage. Once bound to the macrophage, glucans

activate the macrophage to kill and phagocytize (Browder *et al.*, 1988; Williams *et al.*, 1987 as cited by Schuyler *et al.*, 1998). Fogelmark and Rylander (1994) note that glucans act synergistically with LPS.

c) Conclusion

The literature on the role and mechanisms of (1→3) β-D-Glucans is conflicting with regard to the role and mechanism of activation. The common thread that appears in the more recent literature is that (1→3) β-D-Glucan induces a hyporesponsive reaction rather than an inflammatory one. There appears to be evidence to indicate an interaction between (1→3) β-D-Glucan and LPS, although the interactive mechanisms are not clearly evident.

2.4.4 Tannins

Tannins are distributed widely in plants, especially in their roots, leaves and fruits. Both cotton dust and ACDE contain tannin, and it makes up 4.2% of mill dust and 5.1% of respirable cotton dust (Schachter, 1994; Johnson *et al.*, 1986).

Condensed tannin induces time and dose dependent damage to endothelial cells, induces neutrophil release in the airways and causes the aggregation of platelets with the associated release of serotonin and thromboxane. However, tannins are not able to produce smooth muscle contraction in dog airways or guinea pig ileum. Additionally, tannins have not been found to produce cross-shift pulmonary function changes in cotton workers (Schachter, 1994). Thus, clinical evidence to support tannin as the sole etiologic agent of byssinosis is lacking.

Yet this does not mean that tannins are not involved in the byssinotic response. Cloutier and Guernsey (1998) hypothesise that the reduction in FEV_1 over the first day of the working shift is in part caused by exposure to tannin. Tannin exposure uncouples the β-adrenergic receptor, restricts the availability of cAMP and results in a decrease in airway inhibition factors. This creates an imbalance among the contracting factors available, with a subsequent reduction in FEV_1. Exposure to tannin via the respiratory route, in cotton workers over the work shift, results in a reversible down-regulation of the β-adrenergic receptor, which leads to a "cycle of desensitization of the airway" (Cloutier and Guernsey, 1998:189). This produces a state of relative hyposensitivity that limits the number of β-adrenergic receptors available for interaction with tannin. This effect increases as the work week goes by, and leads to the gradual increase in FEV_1 that is associated with the byssinotic response.

Giving consideration to the varying viewpoints, it seems likely that tannin is involved as a possible co-aetiological agent of byssinosis and other respiratory disorders induced by cotton dust.

2.4.5 Lacinilene

Lacinilene is a component of the cotton plant (Jacobs, 1997). Kilburn *et al.* (1981) investigated lacinilene as an aetiological agent of byssinosis. Although the authors were able to establish the toxicological effects of lacinilene, their results were inconclusive in establishing it as the primary aetiological agent of byssinosis. However, even when both endotoxin and lacinelene were removed from ACDE, the extract was found to retain its bronchoconstriction activity (Schachter, 1994).

In conclusion, an examination of the current literature has not revealed whether lacinilene plays either an additive or synergistic role as a co-aetiological agent, or whether it is involved at all in the development of byssinosis.

2.5 IMMUNOLOGICAL MECHANISMS

Only biological mechanisms which impact in some way on the IgG class of antibodies, are reviewed in this section.

2.5.1 Histamine release

Raised levels of histamine have been found in cotton workers (Schachter, 1994). Concentrations have been found to be significantly higher on the first day of work after an absence from work of 36 to 48 hours. Certain studies have noted that histamine levels are related to cotton dust levels (Noweir, 1981).

Rylander (1981a) records that histamine release is a classic reaction of the byssinotic response. A metabolite of histamine, 1-methylimidazole-4-acetic acid, was found to be raised in workers subjected to an inhalation challenge of cotton dust. This finding indicates that endogenously formed histamine is released after exposure to cotton dust, indicating that agents within cotton dust induce the formation of histamine (Schachter, 1994). Several authors (Pernis *et al.,* 1961; Hinshaw *et al.,* 1960; Davis *et al.,* 1963, as cited by Rylander 1981a) have recorded that LPS is able to induce the release of histamine. The results of a study conducted by Watson *et al.* (1997) have supported the finding that histamine production

is independent of a specific IgE mechanism, indirectly supporting the finding that endotoxin is likely to be responsible for raised histamine levels.

2.5.1.1 IgG and histamine

It has been well established that IgG can act as a 'blocking' antibody on basophils, preventing the release of histamine (May *et al.*, 1972; Schumacher *et al.*, 1979; Lichtenstein *et al.*, 1986; Clinton *et al.*, 1989 as cited by Witteman *et al.*, 1996).

In addition, specific IgG has been found to block histamine release from basophils, without the use of IgE and IgG with identical epitope specificity (van Ree *et al.*, 1995). These results indicate the potential of utilising an IgG mechanism to block the release of histamine in cotton workers, pointing to a protective role for specific IgG.

2.5.2 Cytokines

Cytokines as a group have a wide range of biological impact. IL 3 has been found to be an activating factor for eosinophils and not neutrophils (Clark and Kamen, 1987). Endotoxin has been found to cause alveolar macrophages to release cytokines TNF α and ILs 1 and 6 (Michel, 1998). In addition, research has also shown that (1→3) β-D-Glucans can induce the secretion of cytokines IL 1 α, 2 and 6 (Schuyler *et al.*, 1998; Adachi *et al.*, 1998).

2.5.2.1 Cytokines and IgG

Cytokines have a substantial effect on IgG and its subclasses. Accordingly, a review of this relationship follows.

a) IFN-γ

It has been shown that interferon (IFN)-γ has the ability to specifically induce IgG_2a, although it suppresses the production of IgG_1, IgG_2b and IgG_3 (Kawano *et al.*, 1994). Snapper and Paul (1987) also found that IFN-γ induces IgG_2a in resting B cells stimulated with endotoxin. Unstimulated peripheral blood mononuclear cells (PBMC) spontaneously produce IgG_2, dependent upon IFN-γ. IFN-γ suppresses the production of IgE (Kawano *et al.*, 1994). Although it was found that IFN-γ secreted by activated natural killer cells was a switching factor for IgG (Snapper *et al.*, 1992; Kitani *et al.*, 1993 as cited by Snapper *et al.*, 1993; Snapper and Paul, 1987), this finding was not supported by the findings of Kawano and co-workers (1994).

Snapper and Paul (1987) found that IFN-γ caused endotoxin activated B cells to increase IgG_2a production which led to a decrease in IgG_1 and IgG_3 concentrations. Amigorena *et al.* (1990) and Michel *et al.* (1991, as cited by Snapper *et al.*, 1993) showed that NK cells can act as the source for IFN-γ active in the induction of IgG_2a class switching produced by endotoxin stimulated B cells.

b) Interleukins general

IL 2, IL 6 and IL 8 have been shown to be released as part of the biological response to endotoxin[4].

[4] For details of the Cytokine biological response to endotoxin, see Chapter 2, Section 2.4.2.1: Biological impact of endotoxin.

IL 2

IL 2 has been shown to increase synthesis of the IgG subclasses (Splawski *et al.,* 1989; Flores-Romo *et al.,* 1990; Splawski *et al.,* 1991; Kitani *et al.,* 1993 and Kawano *et al.,* 1995, as cited by Kawano *et al.,* 1995; Calvert *et al.,* 1990). The researchers (Kawano *et al.,* 1995) found that IL 2 stimulated the secretion of all four of the IgG subclasses *in vitro*. IL 2 dependent secretion in the four subclasses was shown to be highest in the IgG_2 subclass. The amount of IgG subclasses secreted showed inter-variability which was dependent on the tissue source of the B cells. In addition, IL 2 has shown the ability to stimulate the differentiation of B cells.

The aforementioned research provides a possible mechanism of IgG production induced by endotoxin.

IL 6

Kawano *et al.* (1994) have found IL 6 to enhance all four subclasses of IgG. IgG_2 production has also been found to be enhanced by IL 6, which acts as a late differentiation factor, requiring from zero to nine days. Moreover, it has been determined that pokeweed mitogen is required to be present for IgG_2 to be released by lymphocytes. Other researchers have found both pokeweed mitogen and *S. aureas* Cowan I strain to exhibit the capability to induce IgG production (Muraguchi *et al.*, 1988; Bertolini *et al.*, 1990, as cited by Kawano *et al.*, 1995). Kawano *et al.* (1995) reached the conclusion that the main function of IL 6 was to aid in the differentiation of committed B cells. This involves the direct action of IL 6 on B cells, which is partly mediated by T cells. The authors (Kawano *et al.,* 1995) further comment that IL 6 seems to upregulate the synthesis of the IgG subclasses per cell, as opposed to increasing precursor frequency.

After exposure to pokeweed mitogen and *S. aureas,* PBMC cause the release of IL 6, which results in production of IgG. In explanation, the simultaneous exposure of PBMC to IFN-γ and IL 6 has been found to have a negative effect on the production of IgG_1, but a positive effect on IgG_2 production (Kawano *et al.*, 1994). Further, Michel (1998) has noted that IL 6 was one of the cytokines that were released upon exposure to endotoxin.

In the light of this, it is reasonable to postulate that the same mechanism of IgG_2 production would be likely to be observed if PBMC were exposed to LPS. No study was found in the review of the literature, in which the effects of IL 6 production by PBMC upon exposure to endotoxin were observed.

IL 8

IL 8 is a major neutrophil chemotactic. Sekido *et al.* (1993), using a rabbit model, found that monoclonal IgG antibody prevented IL 8 induced neutrophil infiltration and related lung injury.

Neutrophil infiltration has been noted as one of the consistent effects of cotton dust exposure (Niven and Pickering, 1996; Rylander, 1997b). This is probably linked to endotoxin, which causes the release of cytokine chemotactants, including IL 8. The findings by Sekido and colleagues (1993) highlights the potential impact of IgG deficiencies. The findings also indicate a possible mechanism of preventing neutrophilia in the lungs, thereby reducing the physiological impact of byssinosis in those affected by the disease. Sakamaki *et al.* (1996) demonstrated that a non-IgG mechanism, namely ONO-5046, was able to reduce concentrations of neutrophil elastase, but was not able to reduce neutrophilia in the lungs. This mechanism

could attenuate endotoxin induced lung injury, but would not appear to be as effective at achieving a reduction in lung injury as the monoclonal IgG antibody tested by Sekido *et al.* (1993).

Kimate *et al.* (1995) observed that IL 8 caused selective inhibition of spontaneous IgE and IgG_4 production in atopic subjects. This finding is potentially important, as it may provide a possible reason as to why byssinosis is not IgE mediated. IL 8 is also able to induce decreases in the production of the cytokines, IL 6 and TNF $\propto$. This is despite the fact that IL 6 and TNF $\propto$ induce IL 8 (Stanidiford *et al.*, 1990; Ziesche *et al.*, 1994 as cited by Kimata *et al.*, 1995).

c) Summary

The studies reviewed above have shown the importance of the relationship between cytokines and IgG. It is clear that very little cognisance has been taken of this research in cotton dust related literature, as well as the potential impact of these findings on research into the humoral mechanisms of byssinosis. The role of IgG as a potential 'blocker' of the disease pathways of byssinosis does not appear to have been examined.

2.5.3 Complement

The complement system contains at least 25 different glycoproteins (Abbas *et al.*, 1994). The classical pathway is activated via antigen-antibody complexes, and the alternate pathway is induced immunologically by aggregated IgA or IgG_4, or non-immunologically, by a number of microbial cell products (Hyde, 1995).

a) Alternate and classical pathways

Endotoxin extracts were found by Wilson *et al.* (1980) to be ten times more effective in activating the alternative complement pathway than cotton dust. These authors did not investigate the effect of the extracts on the classical pathway. As opposed to the findings of Wilson *et al.* (1980), Mundie *et al.* (1983a) found that crude cotton dust is able to activate the classical and alternate complement pathways *in vitro*, although the same is not true for endotoxin. These authors (Mundie *et al.*, 1983a) conclude that endotoxin is not responsible for the activation of the complement pathway, but rather that other plant constituents are.

b) Byssinosis

Activation of the classical pathway should not lead to the supposition that byssinosis or other respiratory disorders induced by cotton dust is mediated via the humoral pathway. Ainsworth *et al.* (1981) found insignificant differences in complement concentrations between 28 byssinotic and 29 non-byssinotic workers. As a result they concluded that byssinosis was not mediated via an immunological mechanism. Further, in 78 cotton workers, all groups, including smokers, non-smokers, workers with allergies and those without, as well as byssinotics, showed decreases in complement C3 concentrations after exposure (Mundie *et al.*, 1983b). The findings of this study have been substantiated by a follow-up study which was undertaken by some of the same authors. Mundie *et al.* (1985) also found that complement C 3 concentrations decreased in both byssinotic and non-byssinotic workers, after exposure from Monday to Friday.

The findings of Baker *et al.* (1988) may provide an explanation for decreases in complement concentrations that have been found by Mundie and colleagues (1985). LPSs are comprised

of a lipid-A structure together with a polysaccharide portion. Baker *et al.* demonstrated that the polysaccharide portion activates the alternative complement cascade, whereas the diphosphoryl-lipid-A portion of LPS activates the classical cascade.

c) Summary

As has been shown, there is substantial literature to support the theory that both classical and alternate complement pathways are activated in exposed cotton workers, and that endotoxin is able to activate both the classical and alternate complement pathways. The findings of Ainsworth *et al.* (1981), Mundie *et al.* (1983c) and Mundie *et al.* (1985), indicate that complement is activated in workers with and in those without byssinosis. What is less clear is the role of complement activation in relation to various aetiological agents, the IgG response and the range of other respiratory disorders induced by cotton dust.

2.5.4 IgG, non-cotton

The humoral response is part of the specific immune response. In turn, the production of antibodies by B cells is part of the humoral response (Hyde, 1995).

2.5.4.1 Introduction to IgG and its subclasses

There is a growing awareness of the importance of both increases and decreases in the IgG subclasses in relation to disease, specifically in the development of recurrent infections, community-acquired pneumonia, cystic fibrosis, interstitial lung disease, as well as in forms of obstructive lung disease (Feldman and Wadee, 1998). Yet, cotton dust researchers have not yet investigated the role of the IgG subclasses in the development of byssinosis. It is therefore

pertinent to examine and determine how non-cotton IgG subclass related findings in the literature can be applied to byssinosis.

Although there is substantial interest in IgG and its subclasses, the role of this antibody class in relation to the consequences of changes in subclass levels, is poorly understood (Naegel *et al.*, 1984; Madassery *et al.*, 1988; Ruefenacht *et al.*, 1991; Feldman *et al.*, 1992; Igea *et al.*, 1993; Jennin *et al.*, 1994; Tame *et al.*, 1996; Smith *et al.*, 1998). This is despite a period in excess of 30 years of research conducted into IgG (Feldman *et al.*, 1992). It is important to bear this in mind when reading through the issues relating to IgG covered in this literature review.

a) Percentage breakdown

Normative percentage breakdown of the different IgG subclasses in human serum is as follows: 70% IgG_1, 20% IgG_2, 6% IgG_3 and 4% IgG_4 (Schur, 1987). Subclasses are named in order of decreasing concentrations in serum (Fahey *et al.*, 1967).

b) Biological differences

IgG subclasses have been found to have relatively similar protein structures, and yet are encoded by four separate genes with differing biological functions (Flanagan *et al.*, 1982 as cited by Madassery *et al.*, 1988). These differences include how readily they fix complement, their ability to cross the placental barrier, and their ability to bind to fragment crystalisable (Fc) receptors (Spiegelberg, 1974 as cited by Madassery *et al.*, 1988). The various IgG subclasses are able to bind to the Fc monocyte or macrophage receptor with different binding strengths as follows: starting from the highest binding potential and decreasing in order IgG_1,

IgG_3, IgG_4 and IgG_2; in their ability to bind to C1q: IgG_3, IgG_1, IgG_2 and IgG_4; and in complement-mediated cell lysis IgG_1, IgG_3, IgG_2 and IgG_4 (Waldmann, 1989, as cited by Jayne *et al.,* 1991).

c) Isotype expression

The data collected by Eriksson *et al.* (1994) in the investigation of Sjogrens syndrome suggest that the nature of the stimulating antigen plays a role in IgG isotype expression. IgG_1 and IgG_4 are reported to be the predominant allergen specific subclasses (Van der Giessen *et al.*, 1976; Devey *et al.*, 1976; Aalberse *et al.*, 1983; Djurup, 1985 as cited by Schuurman *et al.*, 1997). Mellbye *et al.* (1994) record that IgG_1 and IgG_3 are the most operative in inflammation, through their capacity to bind and activate complement. However, IgG_4 cannot activate or fix complement. An IgG_1/IgG_2 imbalance may be indicative of an autoimmune disease (Eriksson *et al.* 1994).

d) Deficiencies and disease

The socio-demographic factor of gender has an impact in IgG subclass deficiencies, where deficiencies in IgG subclasses are 2.4 times more likely to occur in females than males (Soderstrom *et al.*, 1988 as cited by Bradwell *et al.*, 1995). However, the relationship between socio-demographic factors and IgG subclass deficiencies is not uniform. This is shown where chronic bronchitis rather than smoking was found to be responsible for IgG subclass deficiencies in smokers who had chronic bronchitis (Popa, 1994).

The first time that a deficiency was positively linked to a clinical disease was by Schur and colleagues in 1970 (Schur *et al.*, 1970 as cited by Feldman *et al.*, 1992). Bjorkander *et al.*

(1985) indicate that there is a causal relationship between IgG subclasses and reductions in pulmonary function. Further, Bjorkander *et al.* (1986, as cited by Madassery *et al.*, 1988), propose that IgG subclass deficiency is the most common immunodeficiency. However, Lefranc *et al.* (1982, as cited by Eriksson *et al.*, 1994) note that deficiencies in IgG subclasses were detected in totally asymptomatic subjects. Deficiencies in IgG_2 and IgG_4 are considered to hold the most relevance when the development of respiratory disease is considered (Oxelius, 1974; Beck *et al.*, 1981 as cited by Feldman *et al.*, 1992).

e) IgG and lung disease

There is a diversity of IgG subclass response between varying respiratory disorders. Evidence of this is that patients with chronic obstructive pulmonary disease (COPD) who are on steroid therapy have lowered IgG subclass levels (O'Keeffe *et al.*, 1991). However, patients with acute or chronic respiratory tract infection were found to have IgG_2 as well as IgG_4 deficiencies, but raised IgG_3 levels (Stanley *et al.*, 1984).

Further, workers with pigeon fanciers' lung, a type three or four immunologically mediated disease, were found to have raised titres of IgG_1 and IgG_2, with a reduction in IgG_3 titres (Baldwin *et al.*, 1998). Workers with Wegener's granulomatosis, however, evidenced IgG_1 and IgG_4 as the dominant subclasses. These IgG subclass antibodies are cytoplasmic autoantibodies formed in response to neutrophil cytoplasmic antigens (Mellbye *et al.*, 1994). Neutrophilia is accepted as an important part of the byssinotic response (Niven *et al.*, 1996).

The research presented below shows both the diverse IgG subclass response in disease as well as having relevance to cotton dust research. *A. fumigatus* is present in the air of cotton mills (Lacey and Lacey, 1987), and is able to induce IgE mediated asthma, extrinsic allergic alveolitis, as well as allergic bronchopulmonary aspergillosis (Trompelt *et al.*, 1994). Raised IgG_1 and IgG_2 were found in subjects with allergic alveolitis (Trompelt *et al.*, 1994), and raised IgG_4 subclass appeared in subjects with bronchopulmonary aspergillosis (Leung *et al.*, 1988). Patients with allergic asthma showed specific IgE antibodies in serum, and the IgG subclass response that did appear was found to be slight and unconvincing (Trompelt *et al.*, 1994). Further, *A. fumigatus* -specific IgG, total serum IgG, IgG_2 and IgG_4 titres of antibodies were increased in those subjects that showed a hypersensitivity response to *A. fumigatus* (Igea *et al.*, 1993). The varying IgG response in different disease outcomes, triggered by the same antigen (*A. fumigatus*), is of interest here. It indicates that the same or different antigens in cotton dust may result in both different respiratory disorders and differing IgG subclass responses.

2.5.4.2 IgG, socio-demographic

The socio-demographic variables of race, gender, age and smoking in relation to IgG, are reviewed below.

a) Race

Race may significantly influence baseline immunologic parameters. Blacks have been found to have approximately 30% higher IgG serum concentrations than whites. This has been attributed to blacks having increased B cell activity when compared with whites (Tollerud *et al.*, 1995).

When Tollerud and co-workers (1995) measured total serum IgG levels in 173 subjects from the black race group, they found the mean concentrations to be 1,587 ± 35 mg/dl; $P<0.001$. Mean IgG subclass concentrations for black children 3.05-3.50 years of age were recorded by Ambrosino and colleagues (1991) as follows: [IgG_1] 4.86, [IgG_2] 0.366, [IgG_3] 0.3.40 and [IgG_4] 0.32 (results are in g/ml).

A study was carried out on the inhabitants of the Kavango territory of SouthWest Africa/Namibia, geographically close to South Africa. Hesseling and colleagues (1986) recorded the following total serum IgG concentrations among black, onyalai relatives - 262.8 (139.4 - 459.6); scholars - 264 (141 – 418.4); and scholars and relatives - 262 (139.4 – 459.6) (results recorded in IU/ml).

b) Gender

Females have more frequent IgG subclass deficiencies, with males having slightly higher IgG_4, but lower IgG_3 levels (Bradwell, 1995).

c) Age

When Aisworth *et al.* (1981) measured total serum IgG levels in 57 cotton workers, they found no significant differences between age groupings. This finding was in contrast to Bradwell's (1995) conclusion that serum IgG subclasses differ between age groups.

d) Smoking

Smoking has been found to cause a decrease in IgG concentrations after antigenic challenge (Gruchow *et al.*, 1981; McSharry *et al.*, 1985; Kusaka *et al.*, 1989 as cited by Tollerud *et al.*,

1995). These authors linked this effect to the lower incidence in hypersensitivity pneumonitis among smokers, when compared with non-smokers. However, contrasted with this finding, Mundie *et al.* (1983b) found no significant differences for serum IgG levels between smokers and non-smokers, within a sample group of 78 cotton workers.

e) IgG and HIV

All IgG subclasses have been found to be involved in the human immunodeficiency virus (HIV) infection response[5]. The most active IgG subclass response to HIV, in descending order, is IgG_1, IgG_3, IgG_2 and IgG_4 (Klasse *et al.*, 1987; Lal *et al.*, 1991). Infection with HIV-1 may be linked to either an increase or a decrease in IgG subclasses (Bartmann *et al.*, 1991, as cited by Bradwell, 1995; Roilides *et al.*, 1991), although total serum IgG concentrations are normally raised in HIV positive patients (Roilides *et al.*, 1991). With progression of HIV infection to manifestation of an HIV related disease, those with disease exhibit a restricted IgG subclass response when compared with those who are HIV positive and without disease (Klasse *et al.*, 1987).

2.5.4.3 IgG_1

The IgG isotype response to protein/polypeptide antigens is mainly IgG_1 (Mellbye *et al.*, 1994). IgG_1 binds strongly to monocytes, but less strongly to alveolar macrophages (Fick *et al.*, 1986). In cystic fibrosis patients exposed to a polysaccharide antigen, IgG_1% values made up 37.9% of total IgG in serum, as opposed to 66.9% make-up in the normal (non-cystic

[5] Although it was noted as a confounding variable, the investigation of the relationship between HIV, IgG and byssinosis was outside of the scope of the current study.

fibrosis) controls (Fick *et al.*, 1986). The IgG_1 subclass is the dominant IgG subclass in non-atopics (Michils *et al.*, 1999). Smokers were found to have comparatively raised levels of IgG_1 in their serum (Fick *et al.*, 1986). Ruefenacht *et al.* (1991) examined the relationship between IgG subclasses and streptococcal group A. They concluded that IgG_1 antibodies have a stronger preferential recognition by the Fcγ receptors of monocytes/macrophages than do IgG_2 subclass antibodies.

2.5.4.4 IgG_2

IgG_2 provides the dominant response to polysaccharide antigens (Freijd *et al.*, 1984). Yet IgG_2 was not found to have strong bonds to monocytes or to alveolar macrophages (Fick *et al.*, 1986). Interestingly, an association between decrease in IgG_2 and smoking was noted (Fick *et al.*, 1986; Popa, 1994). Further, Soiffer *et al.* (1995) showed infusions of recombinant IL 2 to decrease IgG_2 concentrations, yet this may have been as a result of the increased number of NK cells which were caused as a result of the IL 2 infusions.

A significant correlation was found in subjects with COPD and deficiencies in IgG_2 (O'Keeffe *et al.*, 1991). IgG_2 antibody concentrations were also found to be statistically reduced in patients with pneumococcal capsular polysaccharide antigen, in community acquired pneumonia of bacterial or unknown cause (Herer *et al.*, 1990). The findings of Smith *et al.* (1990) support the findings of Herer and his colleagues. They found that in children with chronic chest symptoms, IgG_2 titres were related to exposure to bacterial polysaccharide. Raised IgG_2 subclass titres were also found in patients with cystic fibrosis, which may however have been due to chronic antigen stimulation (Fick *et al.*, 1986). The formation of IgG_2 antibodies to the LPS portion of *P. aeruginosa* is important to note. This

indicates that there is the potential for antibodies of the IgG_2 class to be formed against other LPSs, including LPS from *E. agglomerans*. In addition, a significant correlation was also found between reduction in FEV_1, FEV% and deficiencies in IgG_2 (O'Keeffe *et al.*, 1991).

Despite the evidence for an association between IgG_2 and respiratory disease, which has been presented above, not all research confirms this relationship. Asymptomatic children were noted by Shackelford *et al.* (1990, as cited by Feldman *et al.*, 1992) to have IgG_2 deficiencies. The authors found no apparent explanation for this finding. Similarly, Madassery *et al.* (1988) found that a small sub-population of 8 015 blood donor adults had IgG_2 levels that were approximately half those of the general population. However, these authors did not characterise their study population or analyse results in terms of race, sex, age or any environmental parameters that are known to affect IgG levels.

2.5.4.5 IgG_3

IgG_3 provides a good response to protein/polypeptide antigens that are highly T-cell dependent (Teale *et al.*, 1984), which is at times of higher affinity than the response provided by IgG_1 (Bradwell, 1995). Strong bonds were reported by Fick *et al.* (1986), between monocytes, alveolar macrophages and IgG_3. Smokers were also found to have significantly elevated IgG_3 concentrations as opposed to non-smokers (Merrill *et al.*, 1985; O'Keeffe *et al.*, 1991; Popa, 1994).

Patients with cystic fibrosis, with and without respiratory carriage of *P. aeruginosa* with associated endotoxin, were found to have significantly raised IgG_3 levels (Fick, 1986). Yet, in an investigation of severe inflammatory chest disease in children, Bernatowska-

Matuszkiewcz *et al.* (1991) found significant deficiencies in IgG_3. These authors do not favour the hypothesis that the subclass deficiency is of pathological significance, but rather believe that it represents a marker of disease.

Chronic antigen stimulation is put forward by Seaton *et al.* (1989, as cited by Feldman *et al.*, 1992) and Stanley *et al.* (1984) as a postulate for raised serum levels of IgG_3.

2.5.4.6 IgG_4

IgG_4 does not bind to polysaccharide antigens, and the production of subclass requires chronic antigen stimulation (Mellbye *et al.*, 1994). Fick *et al.* (1986) report that IgG_4 does not have strong bonds between itself and phagocytic cells, alveolar macrophages and monocytes. As IgG_4 is not considered to be capable of binding or fixing complement Mellbye *et al.* (1994) do not consider the subclass of pathogenic importance. This hypothesis is in conflict with some of the studies presented below, where the pathogenesis of IgG_4 was examined in non-complement activated pathways. Both smokers and smokers with chronic respiratory tract infection were found to have deficiencies in IgG_4 by Popa (1994).

A number of roles have been attributed to IgG_4. Specific IgG_4 was shown to act as a blocking agent in immunotherapy to insect venom and house dust mite (Urbanek *et al.*, 1986; Ohashi *et al.*, 1987; Nakagawa *et al.*, 1987, as cited by Yokota *et al.*, 1998). IgG_4 titres were also found to be raised in atopic individuals (Michils *et al.*, 1999). Researchers (Yokota *et al.*, 1998; Jimeno *et al.*, 1992) hypothesised that IgG_4 may constitute a non-IgE mechanism that sensitises both mast cells and basophils to release histamine, thus initiating the allergic or asthmatic response.

Consequently, IgG_4 is implicated as an anaphylactic antibody. An alternate hypothesis is that IgG_4 may be a marker of chronic antigen stimulation (Feldman and Wadee, 1998).

However, Jimeno and colleagues (1992) note that the induction of histamine release from basophils has been questioned by a number of authors, namely Malley, *et al.* (1974), van Toorenenbergen, *et al.* (1982), van Toorenenbergen, *et al.* (1981) and Devey, *et al.* (1975) (as cited by Jimeno *et al.*, 1992), whose research has not concurred with the findings of Yokota *et al.* (1998) and Jimeno, *et al.* (1992). Further, Jimeno *et al.* (1992) found that the monoclonal binding site specific anti IgG_4 antibodies were only able to induce histamine release from basophils if they were directed against the Fab region of IgG_4, and not the Fc region. This may form part of an explanation for the negative findings in the studies recorded above, as different researchers use different antibodies with ill-defined specificities. Thus, if the antibody used was not able to trigger the Fab region, it would be incorrectly concluded that IgG_4 was not an anaphylactic antibody.

The literature indicates that the IgG_4 dependent on the inducing antigen, may act as a blocking (Urbanek *et al.*, 1986; Ohashi, *et al.*, 1987; Nakagawa *et al.*, 1987, as cited by Yokota *et al.*, 1998) or anaphylactic (Jimeno *et al.*, 1992) antibody. Given this, IgG_4 must be considered to play an important role in the allergic response, including asthma. Byssinosis is not a form of occupational asthma (Edwards, 1981) and consequently not a disease with allergic aetiology [6].

[6] Since byssinosis is not a form of occupational asthma, a full exploration of the relationship between allergy including asthma and the relationship to IgG_4 was not undertaken, as it was outside of the scope of this study.

2.5.4.7 Specific IgG

Determination of specific IgG as a parameter is a measurement of total specific IgG. Researchers have measured IgG antibodies specifically formed against a variety of antigens present in the work environment.

Ewan *et al.* (1993) used the radio allergo sorbent technique (RAST) with bee and wasp venom antigen to determine the relationship between venom IgG levels and protection from stings. They found a differing specific IgG response in bee and wasp allergic subjects, indicating that different antigens may produce a differing IgG antigenic response. Results suggested that specific IgG formed against venom is related to exposure to stings and not to protection against stings.

An investigation was also conducted using enzyme-linked immunosorbent assay (ELISA), to determine the relationship between 'farmer's lung' and specific IgG formed against moulds and actinomycetes. Increased titres were associated with an increase in exposure (Katila *et al.*, 1986). Eduard (1995) reviewed research studies also examining the relationship between specific IgG antibodies and 'farmer's lung'. He similarly concluded that specific IgG levels were probably related to the number of exposure hours per day, and not to accumulated exposure years.

The role in subclasses of total specific IgG and specific IgG in the development of pulmonary aspergilloma was determined by ELISA, CAP- fluoroenzymeimmunoassay (FEIA) and immunoblotting techniques (Tomee *et al.*, 1996). These authors found elevated total specific IgG levels in periods of exacerbation of the disease.

The studies reviewed have indicated the role of specific IgG predominantly as an indicator of exposure. However, in order to obtain a more accurate picture of specific IgG, it is necessary to examine more detailed studies relating to the role of specific IgG in the individual subclasses.

2.5.4.8 Specific IgG in IgG subclasses

In addition to measurement of total specific IgG, it is possible to measure specific IgG against a particular antigen in each of the individual IgG subclasses (Tomee *et al.*, 1996). In essence, it divides the specific IgG antibodies against an antigen into individual subclasses.

The specific IgG in individual subclasses test was used in varying *in vivo* study applications. Shakib *et al.* (1986) used the ELISA method to determine IgG subclass specific antibodies against cow milk proteins. They found that determination of IgG subclass specific antibodies to milk proteins was useful in diagnosing milk intolerance, particularly in subjects with eczema. Other authors, namely Pressler *et al.* (1990), Pressler *et al.* (1992) and Cowan and Winnie (1993), used the ELISA technique to determine specific antibodies against *P. aeruginosa* in the individual IgG subclasses.

Earlier, Pressler and co-workers (1990) found that elevated levels of IgG_2 and IgG_3 corresponded with a poor prognosis of cystic fibrosis. The findings of Pressler *et al.* (1992) confirmed the findings of this earlier study. It is also of interest that *P. aeruginosa* contains endotoxin within the cell wall of the organism, in the same way that *Ent. agglomorans* and other gram-negative bacteria found in the air of cotton mills do.

In addition to measurement of specific IgG antibodies to the antigen *P. aeruginosa*, Cowan and Winnie (1993) also measured serum IgG subclass concentrations. They noted that use of the anti-*P. aeruginosa* IgG subclass test produced more definitive results between colonised and non-colonised patients than use of the serum IgG subclass test. The ELISA method was also used by Tomee and colleagues (1996) to determine specific IgG subclass concentrations against *A. fumigatus* antigen. The authors concluded that the IgG_4 response that was noted, was as a result of chronic antigen stimulation.

Of particular interest in these studies, is the differing role of each of the individual subclasses in relationship to each disease. The role of the subclasses varies from being protective, an indicator of negative disease outcomes, or an indicator of pathology. In addition, the results outlined above, particularly those of Cowan and Winnie (1993) indicate the potential use of a modified ELISA method in cotton dust research, to measure the cotton specific IgG antibodies in the individual IgG subclasses. However, measurements using this test parameter had not previously been carried out in the arena of cotton dust research and as a consequence an analysis method had not been developed. The development of the method with accompanying measurements of specific IgG in subclasses was outside of the scope of the current research study.

2.5.4.9 IgG subclasses and endotoxin

From the review of IgG and its subclasses, it is apparent that IgG is of potential aetiological mechanism interest in the development of byssinosis. That endotoxin has the strongest aetiological agent association with byssinosis has already been firmly established Rylander

(1981a). Consequently, it is of interest and relevance to examine the relationship between IgG and endotoxin.

a) Effects of endotoxin on IgG expression

It is generally accepted that antibodies to polysaccharide antigens are of the IgG_2 subclass (Chudwin *et al.*, 1987). Endotoxin is a polysaccharide.

In both human and mouse models, endotoxin showed the ability to stimulate resting B cells (Kearney *et al.*, 1975; Melchers *et al.*, 1975; Andersson *et al.*, 1981; Kuritani (unpublished results), as cited by Mayumi *et al.*, 1983). IgG plasma cell increases in response to endotoxin are T cell dependent. That being so, endotoxin induces a highly selective IgG subclass distribution response, with dominance of the IgG_2 subclass (Levitt *et al.*, as cited by Mayumi *et al.*, 1983; Bich-Thy *et al.*, 1984 as cited by Kawano *et al.*, 1994). This finding is corroborated by Snapper and Paul (1987), who observed IFN-γ causing B cells, activated by LPS, to increase IgG_2a production, whilst decreasing titres of IgG_1 and IgG_3. This finding is important as it suggests the pattern of response that may be present in cotton workers exposed to endotoxin in the work environment. In addition, Mayumi *et al.* (1983) observed the expression of the IgG subclasses on endotoxin stimulated PBMC. The subclass distribution was found to be as follows: IgG_2>80%, IgG_1<20%, IgG_3<1% and IgG_4<1%.

Similarly, it was found that less stimulation was required by IL 2 to induce an IgG_2 response in humans, in comparison to a response by IgG_1 in spleen B cells cultures. The spleen plays an important part in the production of antibodies in response to exposure to polysaccharide antigens (Amlot *et al.*, 1985 as cited by Calvert *et al.*, 1990). Further, Calvert *et al.* (1990)

demonstrated that endotoxin can act as a polyclonal activator, leading to the release of IL 2, which in turn acts on human B cells and causes the secretion of the IgG subclasses, mainly IgG_2. Moreover, the IgG / albumin ratio was shown to be a parameter of human intravenous endotoxin exposure (Burrell, 1997).

b) IgG immune complex lung injury

In the rat model, the physiological mechanism by which neutrophils are recruited into the lungs, with evolving IgG immune complex-induced lung injury, was found to be linked to the requirements for a number of cytokines. These cytokines are TNF α and IL 1 (Warren *et al.*, 1991a and Warren *et al.*, 1991b), CD11a/CD18 (Mulligan *et al.*, 1993a), ICAM-1 (Mulligan *et al.*, 1993b) and E-selectin (Shanley *et al.*, 1995 as cited by Shanley *et al.*, 1997). The physiological response exhibited by the human immune system upon exposure to endotoxin, and the requirements for induction of an IgG immune complex lung injury, share a number of common immunological parameters. Consequently, these research findings may have application in the human model.

c) Inhibition potential of IgG on endotoxin

Researchers have aimed to determine whether monocytes and macrophages produce cytokines IL 6 and IL 8 as a result of exposure to LPS, through a CD14 mediated pathway. Five monoclonal antibodies (mAb), namely MEM-15 (IgG_1), MEM-18 (IgG_1), mAb FMC17 (IgG_2b), Cris6 (IgG_1) and UCHMI (IgG_2a), were used to determine the extent of their 'blocking' potential on the expression of CD14. It was found that upon exposure to LPS, the mAbs were able to largely inhibit cytokine release by mononuclear phagocytes, in both serum and serum-free conditions (Dentener *et al.*, 1993).

Wright *et al.* (1991) note that mAbs (IgG subclasses) are able to block the CD18 receptor that is expressed on neutrophils. This causes cessation of neutrophilia in endotoxin exposed animals. The human physiological response to endotoxin depends, at least in part, on the CD11b/CD18 receptor. The macrophage migration inhibitory factor is a pro-inflammatory cytokine, and is able to potentiate the effects of endotoxin, which is upregulated in gram-negative bacteria sepsis. There is an anti-migration inhibitory factor antibody (IgG fraction; 3.9~8.6 mg/kg) which is capable of reducing the LPS induced neutrophilia into the lungs, thus exhibiting a protective effect (Makita *et al.*, 1998). These studies indicate the potential role that the IgG subclasses play in inhibiting the release of cytokines from LPS stimulated mononuclear phagocytes and neutrophils, thus potentially reducing or influencing the impact of LPS.

d) Summary

Exposure to endotoxin induces inflammation in both the blood and lung in which neutrophils and macrophages are involved (Sandstrom *et al.*, 1992 and Michel *et al.*, 1995 as cited by Michel *et al.*, 1997). Neutrophils are the major cell population in the acute inflammatory response, and they express receptors for both IgG and complement proteins. Neutrophils move to and accumulate at sites of complement activation. Activated macrophages produce TNF α and IL 1, and this may recruit leukocytes to sites of antibody deposition (Abbas *et al.*, 1994). In addition, Michel *et al.* (1998) record that exposure to LPS induces the release of the cytokines TNF α, IL 1 and IL 6 at exposures as low as <1ng/mL.

In the light of the findings of the studies detailed above, it seems reasonable to hypothesise that exposure to endotoxin may induce a specific antigenic response, through the IgG mechanism. Endotoxin may act as a precursor for other biologically and chemically active substances that are present in cotton, creating both a non-specific and a specific immunologic synergistic effect.

2.5.4.9.1 Cystic fibrosis

The bacteria *P. aeruginosa,* which is associated with cystic fibrosis in respiratory carriage, contains a surface LPS (Fick *et al.*, 1986).

It has been recorded that patients with chronic *P. aeruginosa* infection have comparatively raised IgG_2 subclass concentrations (Fick *et al.* 1986, Cowan and Winnie, 1993). The pattern of IgG_2 formation in patients with cystic fibrosis indicates that antibodies have been formed in response to chronic antigenic stimulation, by *P. aeruginosa* surface LPS (Fick *et al.*, 1986).

These findings were of potential interest in this study, as it was expected that since cotton dust contains endotoxin, an IgG_2 subclass response might be seen in cotton workers.

2.5.4.10 IgG and atopy

Mechanisms of specific IgE and IgG_4 induction are independent. In turn, allergen specific IgG_4 antibody increases independently of the antibodies of the other subclasses of IgG (Tame *et al.*, 1996).

However, Jeannin *et al.* (1994) observed that subjects developed an IgG_4 response to allergens they were not sensitive to, as a result of an accompanying IgE response to an allergen. Similarly Eysink and colleagues (1999) found a correlation between raised IgG titres to foods, and increased probability of having raised IgE to dog, cat, mite milk or egg antigens. Nakagawa *et al.* (1983, as cited by Tame *et al.* 1996) found a significant relationship between specific IgG_4 to mite antigen Der p and total serum IgG. The postulate offered by Tame *et al.* (1996) for these seemingly conflicting results is that the crude extracts used by Nakagawa *et al.* offered more epitope binding sites than the single component extract Der p 2. Although the presence of a correlation between IgG and IgE, as found by Eysink *et al.* (1999), does not necessarily indicate a dependent relationship between the variables, an explanation for the findings by Jeannin *et al.* (1994) is unknown.

In addition, atopics show raised levels of IgG_4, whereas non-atopics show relatively higher concentrations of IgG_1. However, specific IgG antibodies were found in the serum of atopic and non-atopic patients, making IgG titres unsuitable for classification of atopic status (Michils, 1999). Atopic subjects with bronchial asthma, who were exposed to Der p 2, showed a doubling in IgG_4 titres (Oshika *et al.*, 1992 as cited by Smith *et al.*, 1998).

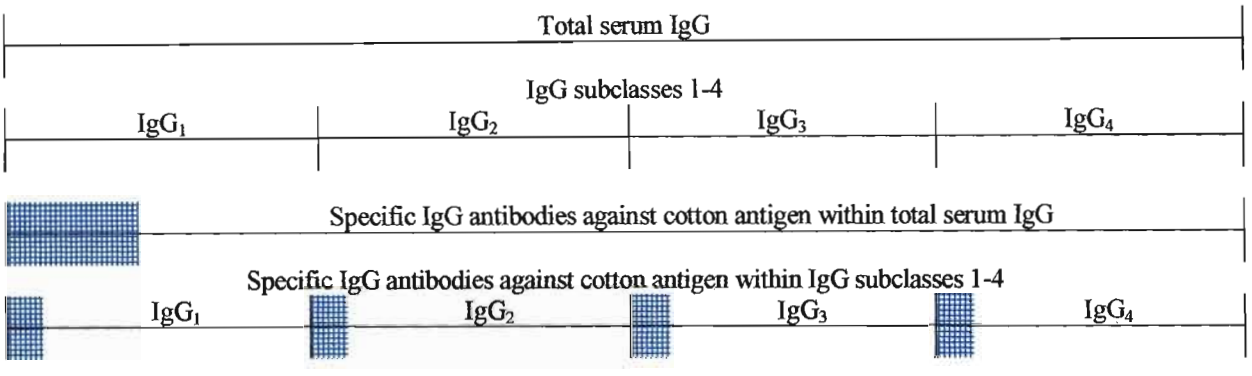
In summary, the studies that examined the relationship between atopy and IgG, at times produced conflicting results. This may be due to the different antigen exposures and resultant disease outcomes examined by the various researchers.

2.5.5 IgG, cotton

The hypothesis that antigen/s in cotton elicits an antibody response is not new. Proponents of this hypothesis include Bramwell and Ellis (1932), Van Leeuwen (1932) and Prausnitz (1936, as cited by Massoud and Taylor, 1964).

The segment of the IgG class that is measured is dependent on the test procedure.

Figure 2.1 Division of the IgG class of antibodies



(Figure is original)

Figure 2.1 gives an indication of the manner in which the IgG class of antibodies is divided for purposes of cotton dust research. All immunological research testing that was reviewed, measured at least one of the IgG parameters shown in Figure 2.1, sometimes in conjunction with a parameter from another immunoglobulin class.

2.5.5.1 Introduction to IgG and cotton

There is a well-established link between the IgG class of antibodies and respiratory disease [7]. Some of the agents and disease patterns that were present in studies relating to IgG and other respiratory disease are mirrored in byssinosis. However, this truism cannot lead to the supposition that the same mechanisms exist between IgG and byssinosis.

In order to determine the exact nature of the relationship between IgG and byssinosis, it is also necessary to examine studies that relate to the diverse effects of cotton dust exposure on IgG. The studies reviewed do not represent a homogenous subset of research investigations into the relationship of IgG exposure to cotton dust. Test results conducted on the IgG parameter may be more or less comparable for a number of reasons, including:

- Test methods used for the measurement of the IgG class of antibodies measure either all or a varying specificity of antibodies;
- When specific antibodies are measured, the specificity of the antibodies is dependent in part upon the antigens used. Different antigens are used in an attempt to measure a varying specificity of antibodies;
- Antigen preparations, used to measure the same specificity of antibodies, may have different sources of origin or preparation make up methods;
- Test methods employed, which are used to measure the same specificity of antibodies, may be either more or less quantitative, thus having different coefficients of variation; and

[7] The relationship between IgG and respiratory disease has been covered comprehensively in Chapter 2, Section 2.5.4: IgG non-cotton.

- Differences exist in the serum of both cotton and non-cotton workers, for reasons of variations in demographic make up or exposure.

In order to introduce comparability between results, it is appropriate to examine literature relating the IgG class of antibodies to byssinosis, by measurement method.

2.5.5.2 IgG subclasses and cotton

After an extensive literature search, the only study that was found which related to measurement of the IgG subclasses and cotton dust, was the study conducted by Edwards and Jones (1973). In this study the authors did not measure specific or total individual IgG subclass concentrations in the sera of cotton exposed workers, but rather determined the reactions of 5,7,3,4 tetrahydroxyflavan 3-4 diol (THF) and cotton dust extracts with myeloma IgG. The myelomas (three IgG_1, one IgG_2 and two IgG_3) were not from cotton workers. The THF reacted with the myelomas to precipitate antibodies from each of the six myelomas used in the study. The authors concluded that the THF was responsible for the precipitation, as the reaction of a single antigen with more than one IgG subclass was improbable.

As cotton workers sera were not used in this study, it was not possible to determine how the IgG subclass profiles differed from those in workers with and without byssinosis.

2.5.5.3 IgG, total serum and cotton

Total serum IgG measurement, as the name implies, is a direct measurement of all serum IgG antibodies. A number of researchers have measured total serum IgG and have attempted to determine the effect of cotton dust on the IgG class of antibodies.

These authors include Kamat *et al.* (1979), who included 86 textile workers and 17 control workers in their study. Results indicated that total serum IgG concentrations, measured by radial immunodiffusion assay, were higher in cotton workers than in non-exposed controls. Those workers with byssinosis and a-typical byssinosis had comparatively higher IgG levels, whereas exposed workers with bronchitis did not. Kamat and co-workers state that this indicated a causative link between IgG and byssinosis. Following the publication of these results, a number of studies were carried out to confirm or reject the findings of these researchers.

One of the studies carried out in this era investigated the relationship of total serum IgG to cotton exposure in 11 volunteers, four of whom had previously worked in a cotton mill. These volunteers were exposed to cotton dust for six hours in a model cardroom. Pre- and post-exposure IgG levels were found to be inside the normal range (Butcher *et al.*, 1981). However, a single exposure of six hours duration, in previously unexposed individuals and a small sample of non-byssinotic workers, is not a suitable research model to determine stimulation of the specific immune system, a fact which the authors acknowledge.

In an attempt to determine if byssinosis is mediated by an immunological mechanism, Ainsworth *et al.* (1981) measured total serum IgG, pre- and post-shift on a Monday and Friday afternoon. Levels of total serum IgG were determined by use of the radial immunodiffusion technique. Total serum IgG levels were found to be within the normal range for both byssinotics and non-byssinotics. The authors thus concluded that these results did not support an antigen-antibody reaction as the pathogenesis of byssinosis.

In another study carried out in the same period, both total serum IgG concentrations, using a single radial diffusion method, and total precipitating antibodies, using the agar double diffusion (Oucherlongy) technique, were measured. The researchers found that total serum IgG total levels were slightly elevated in exposed cotton workers. Cotton mills with the highest levels of cotton dust had workers with the highest levels of total serum IgG. Yet, total serum IgG concentrations did not correlate positively with total precipitating antibodies (O'Neil *et al.*, 1981).

The last of these studies reviewed in this period was a comprehensive research initiative carried out by Noweir (1981). All five classes of total serum immunoglobulins were measured using the radial immunodiffusion technique. Increase in total serum IgG was found to be highly significant in those workers that had had less than ten years exposure. This effect was reduced in workers that had been exposed for periods of longer than 20 years. The author comments that the high level of total serum IgG in these workers indicated the presence of precipitating antibodies. The effect of raised total serum IgG was not limited to workers with respiratory symptoms / disease, including byssinosis, or related to levels of cotton dust exposure. While the effect of comparatively raised total serum IgG in exposed workers may have been related to exposure, the exposure-response relationship was undetermined.

When pulmonary lavages were performed on 15 rabbits exposed to cotton dust extract, total serum IgG levels were measured at 15 minutes, 1 hour, and 6 hours post exposure. No changes in total serum IgG were noted with the effect of time (Mundie *et al.*, 1983c).

In addition to this work on rabbits, Mundie *et al.* (1983b) measured immunoglobulin levels in cotton workers. The findings published by Mundie *et al.* (1983b), in addition to new work, were reported in a single publication by Mundie *et al.* (1985). Thus, the paper by Mundie *et al.* (1985) reported on two distinct studies of immunoglobulin levels measured in cotton workers. The first study, which was carried out in 1974, measured total serum concentrations of IgG, IgM and IgA. The second study, conducted in 1984, measured total serum IgG, IgM, IgA, C3 and C4. The first study collected sera from 19 byssinotic and 17 non-byssinotic workers, and measured total serum IgG using a commercial radial immunodiffusion test. Concentrations of total serum IgG decreased significantly over the week but no significant differences were noted between byssinotic and non-byssinotic workers. The second study collected sera from 8 byssinotic and 67 non-byssinotic cotton mill workers. The authors noted a trend of decreased serum C3 and C4 concentrations over shift in byssinotic workers, to a greater extent than in non-byssinotic workers. This is a sign of complement activation. Total serum IgG concentrations did not change significantly over shift.

Although the two studies had different findings, the authors concluded that the data did not support an immune complex mechanism for byssinosis. Since IgG levels were not measured in control workers in the first and second studies, the researchers were not able to determine if cotton workers had higher serum concentrations than non-cotton workers. The first study had low statistical reliability, as there were few byssinosis cases (Mundie *et al.*, 1983b). Consequently, both the study design together with the immunological test parameters measured formed an inappropriate platform from which to draw such wide extrapolations.

Researchers (Olenchock *et al.*, 1986) exposed 37 human volunteers, divided into lesser reactor and greater reactor groups, to controlled levels of cotton dust during carding of cotton, over a three-day interval. Results indicate that no group differences from published normal levels, were noted for total serum IgG measured by solid phase fluorescence assay. However, the authors found that serum IgG levels were higher in the greater reactor group than in the lesser reactor group on the third day of exposure. The tentative conclusion was reached that cotton exposure had not affected the immunological status of the workers. This finding is hindered by the lack of an external control reference group.

Further, total serum IgG was measured in the sera of 381 cotton workers, with results indicating that smokers had significantly higher total serum IgG levels than non-smokers, in all mill types. Workers in the cotton and wool mills were found to have higher total serum IgG concentrations than those in the man-made fibre mill. No significant differences were found among non-smokers between the different mills, whereas the two highest combined exposure groups of non-smokers had significantly elevated IgG concentrations (Sigsgaard and Gravesen, 1991).

The research investigations reviewed above were all carried out with approximately the same aim, yet they reveal widely differing outcomes. Kamat and co-workers (1981) argue that raised total serum IgG concentrations in symptomatic cotton workers were as a result of IgG precipitating antibodies, whereas O'Neil *et al.* (1981) state that the presence of precipitating antibodies to cotton only comprises of a small fraction of the total IgG antibodies. Thus, increases or decreases in total IgG serum levels evidenced in cotton workers, are unlikely to be as a result of precipitating antibodies. This is substantiated by the finding by O'Neil *et al.*

(1981), that specific precipitating antibodies against ACDE are not correlated to total IgG serum concentrations.

Bradwell (1995:3), after having reviewed "all published literature in the English language" on IgG, notes that total serum IgG measurements are not a sensitive enough marker of changes in each of the IgG subclasses. It is likely that precipitating IgG antibodies to cotton would be of a dominant IgG subclass. Thus total serum IgG measurements are likely to provide a crude measurement parameter for precipitating IgG antibodies to cotton. If this assumption is made, the studies reviewed above, which only measured total serum IgG in cotton workers, should not be viewed as being adequate in producing a quantitative assessment of the effect of cotton dust on the IgG class of antibodies.

2.5.5.4 Precipitation of antibodies against ACDE

Precipitation of antibodies by ACDE involves precipitation of serum antibodies, which may be more or less specific for a single class or classes of antibodies. The specificity is dependent on the testing technique utilised.

It appears that Massoud and Taylor (1964), who determined antibody titres by the gel-diffusion method, were the first authors who noted that unexposed workers had antibodies against ACDE. Importantly, they found that those symptomatic and asymptomatic workers who were exposed to cotton, had higher titres than unexposed workers. Workers who were exposed and symptomatic for byssinosis, had significantly higher concentrations of antibodies against cotton, than those who were exposed and asymptomatic for byssinosis. The authors note that the

findings provide evidence in favour of the hypothesis of an antigen-antibody complex formation in cotton workers.

Using the agar double diffusion (Ouchterlony) and the tanned red cell agglutination methods in 82 cotton workers, 83 hemp, flax and jute workers, and 41 byssinotic workers, Popa *et al.* (1969) found opposing results. The agar double diffusion (Ouchterlony) method produced negative results in all workers, whilst the tanned red cell agglutination method indicated the presence of antibodies against all allergen types among all worker groups.

Taylor *et al.* (1971) followed up on Massoud and Taylor's (1964) earlier study, with a large case control study involving 177 byssinotic workers, 196 non-byssinotic workers and 203 controls. Antibody titres were determined by dilution methods. These researchers found that a condensed tannin reacted with serum components, both by precipitation and by passive agglutination with human IgG. This reactivity varied from individual to individual. Confirming the findings of Massoud and Taylor (1964), Taylor *et al.* (1971) found that titres were higher in exposed workers than in unexposed control workers, and that exposed byssinotic workers had significantly higher titres than exposed non-byssinotic workers.

The authors proposed a number of hypotheses, which relate the findings to the disease byssinosis. These include the hypothesis that byssinotics are individuals who have greater antibody forming potential, and the hypothesis that raised IgG titres could be the result and not the cause of byssinosis. The hypothesis that raised IgG titres are the result and not the cause of byssinosis is unlikely, as raised IgG titres are only seen in some workers with byssinosis, but

not in all. Although the authors noted substantial differences in presentation between byssinosis and other type III mechanism diseases, they state that this mechanism cannot be ruled out.

In a follow-up study to the two studies reviewed above, Edwards and Jones (1973) utilised the technique of radial diffusion with Hyland immunoplates This was done to determine the reaction of THF and cotton dust extracts with human serum from eight workers with 'farmer's lung'. They found that THF precipitated 58% of the IgG. The researchers concluded that the THF, a tannin-like polymer, was responsible for the precipitation of IgG, and thus concluded that byssinosis was not an immune-complex lung disease, terming the occurrence a 'pseudo-immune' reaction. Because tannin is able to precipitate IgG antibodies it does not mean that specific IgG antibodies against cotton dust antigens do not exist in the serum of cotton workers.

Edwards and Jones (1973) investigated a single aspect of the antigen-antibody reaction. However, they made extrapolations beyond what they had investigated. They did not determine if exposed cotton workers had raised IgG titres or if exposed byssinotic workers had higher IgG concentrations than exposed non-byssinotic workers. Thus, their study design was not suitable to confirm or refute the findings of Massoud and Taylor (1964) or those of Taylor *et al.* (1971).

The next research investigation aimed at determining the agent responsible for the reaction described by Taylor *et al.* (1971), and termed a 'pseudo-immune' reaction by Edward and Jones (1973), was conducted by Kutz *et al.* (1981). This investigative research included 59 cotton exposed workers and 35 non-exposed workers. The authors conjectured that the substance responsible for the 'pseudo-immune' reaction was a cotton plant polyphenolic tannin. The researchers were not able to demonstrate true precipitating antibodies to aqueous

extract of cotton carpels, bracts, stems, lint leaves or cardroom dust, in the sera of the 59 exposed cotton workers. They suspect that the reason for this is that the plant tannin complexed with the antibodies. When the tannin was removed with polyvinylpolypyrrolidone, the antibodies were also removed. They note further that the ACDE precipitated mainly the IgG class of antibodies.

O'Neil *et al.* (1981) did not find precipitating antibodies in the sera of the exposed cotton workers, and this reported 'pseudo-immune' reaction was therefore in stark contrast to their findings. Out of 11 subjects who were exposed in a model cardroom for 6 hours, none had precipitating antibodies when measured by the Oucherlongy technique. However, only 4 out of the 11 subjects had previous cotton work exposure (Butcher *et al.*, 1981). In a more rigorous follow-up study, Butcher and co-workers (1983) also assayed serum samples for the presence of precipitating specific IgG or IgM antibodies, measured by the agar double diffusion (Oucherlongy) technique. A total of 343 workers were included in the study, with 254 cotton workers and 89 synthetic fibre workers. The researchers found that only 5 out of 341 workers showed positive precipitin lines. Four of the 5 workers that tested positive to IgG or IgM to ACDE, were cotton workers and all 5 were atopic. The authors do not state whether these workers had clinical or functional byssinotic symptoms. Because of this, it is difficult to draw any definitive conclusions from the findings of the study.

However, the studies of Popa *et al.* (1969), O'Neil *et al.* (1981) and Butcher *et al.* (1983), who all used the agar double diffusion technique, clearly show no 'pseudo-immune' precipitation. Whether studies show a 'pseudo-immune' precipitation or not, may depend on the test technique used. These studies all produced strongly negative results, which are in

contradiction to results obtained by other methods. Antigenic make-up of the particular ACDE used, may also be of importance. In the same study, using the tanned red cell agglutination method, Popa and co-workers (1969) found that all workers had antibodies against every type of antigen. The results obtained with this method diametrically opposed those obtained using the agar double diffusion method, and were likely to be due to a 'pseudo-immune' precipitation of antibodies.

From the findings of Massoud and Taylor (1964) and Taylor *et al.* (1971), it seems that even when a 'pseudo-immune' precipitation is observed, there is also a true precipitation of antibodies. Due to a possible complexing of the plant tannin and antibodies, it may not be possible by precipitation to eliminate or divide the 'pseudo-immune' precipitation from the true precipitation of antibodies. Rohrbach and colleagues (1983) found that cotton bracts, tannin and IgG act synergistically at low tannin concentrations to enhance the release of 5-hydroxytryptamine from human platelets. These findings further indicate the interaction between the IgG class of antibodies and tannin. However, the true extent or full nature of the interaction between IgG and tannin is not known.

2.5.5.5 IgG, specific against endotoxin and other antigens

Specific IgG antibodies formed against endotoxin, as well as other specific antigens contained in cotton dust, limit the range of antibodies measured to only those specified. These antibodies do not relate to antibodies formed against the collective antigens contained in ACDE. As the endotoxin antigen or other non-tannin antigen preparations used eliminate any cotton plant polyphenolic tannins, it is unlikely that these studies recorded 'pseudo-immune' precipitation.

One of the earliest studies that examined specific IgG, IgA and IgM antibody levels against endotoxin antigen, was conducted by Rylander *et al.* (1979a). The researchers used the ELISA technique when determining antibody levels in rats and cotton workers. The results produced were both varied and conflicting. Approximately half of the rats had IgG antibodies against endotoxin in their serum, but not in their bronchial fluid. In the sera taken from English cotton workers, IgG antibodies were significantly lower than those for IgM and IgA. This was in contrast to the results obtained from sera collected from Swedish cotton workers where IgG antibody levels against endotoxin were comparatively raised. The authors note that it is difficult to draw conclusions on the humoral response of the respiratory tree by measurement of antibody levels in blood.

Rylander and colleagues (1981b) followed up the earlier study detailed above, by measuring nasal antibody titres of IgG, by ELISA technique, to LPS extracted from *E. agglomerans* and *P. syringae*. The study was conducted on 12 cotton workers with grade ½ byssinosis, 10 asymptomatic cotton workers and 24 control workers from a wood-processing factory. Antibodies to LPS were reported in both exposed cotton workers and wood exposed control workers. LPS exposure occurs via naturally occurring gram-negative bacteria in the gut or in wood dust, potentially stimulating an IgG response in the wood processing workers. As a result, these workers may have been an inappropriate control group. Similarly, the use of only grade ½ byssinotics may have resulted in the insignificant IgG levels that were noted between byssinotics and non-byssinotics.

Apart from conducting total serum IgG testing, Noweir (1981) carried out titration of symptomatic and asymptomatic cotton worker sera with endotoxins, using both the radial diffusion technique and the cutaneous anaphylaxis test in rabbits. The results produced were negative, and no positive tests were recorded. These findings are questionable, as all other studies investigating the presence of IgG antibodies to endotoxin have shown the presence of antibodies in the sera of exposed cotton workers. It is possible that the differing make-up of the endotoxin antigen used or the measurement technique employed, may have affected the results that Noweir (1981) produced.

Tuxford *et al.* (1989) used *Bacillus pumilus*, *B. subtilis*, *B. cerus* and *E. agglomerans* as bacterial antigen extracts, against the antibodies in the sera of 6 byssinotic and 6 non-byssinotic workers. The ELISA technique was used to determine antibody titres. The highest mean titres were obtained using *E. agglomerans*, which contains endotoxin in its cell wall, as the bacterial antigen. The authors determined that the cotton workers had antibodies to the bacterial antigens, which were isolated from the air of cotton mills. Despite this, the findings of the study were inconclusive, as the study sample was small and no unexposed control workers were included in the study sample.

In a follow-up to the study of Tuxford *et al.* (1989), where the less specific ELISA method was used, Hoult and Tuxford (1991) used the technique of Western blotting. This method was used to evaluate IgG, IgM and IgA antibody levels in the sera of 20 textile workers, against gram-negative bacteria and *Bacillus* species. Some of the negative sera and grade 1 byssinosis sera contained IgG antibodies against *Bacillus* species and *E. agglomerans* antigen. The researchers state that isolation of the bacterial antigens used may have been problematic,

or that the correct antigens were not identified. They concluded that further experimentation was needed.

Guinea pigs exposed to cotton dust produce the cytokine TNF α (Karol and Lemp, 1990). In a follow-up study, Ruppel-Kerr *et al.* (1991) immunised rabbits with recombitant murine TNF α. Rabbit sera were found to contain high titres of IgG antibody (measured by the ELISA technique) to the TNF α immunogen. The antibodies were found to neutralise the cytotoxicity of the TNF α. The authors concluded that TNF α was immunogenic in rabbits. The findings of this study indicate a potentially protective mechanism for IgG.

Finally, it is widely accepted that endotoxin elicits an IgG_2 subclass response. Researchers who have recorded this include Yount *et al.* (1968), Perlmutter *et al.* (1978), Siber *et al.* (1980), Mayumi *et al.* (1983) and Chudwin *et al.* (1987). It is possible that the differing make-up of the endotoxin antigen used or measurement technique employed may have affected the results of Noweir (1981). The studies of Rylander *et al.* (1979a), Rylander *et al.* (1981b), Tuxford *et al.* (1989) and Hoult and Tuxford (1991), indicate that it is likely that specific antibodies against endotoxin are formed in cotton workers. There is no evidence to show that there are differences in specific IgG levels against endotoxin, between byssinotic and non-byssinotic workers. The potentially protective mechanism of IgG in the study of Ruppel-Kerr *et al.* (1991), indicates the diverse role of IgG

2.5.5.6 IgG, specific against ACDE

The measurement of specific IgG antibodies formed against ACDE, relates to the measurement of IgG antibodies formed against the collective antigens contained in ACDE. In theory, measurement of specific IgG antibodies in the sera of exposed cotton workers, formed against a single antigen, or a number of antigens contained in cotton dust, should indicate if the humoral pathway is activated via the IgG class of antibodies. However, reaching a definitive conclusion has proved not to be a simplistic task.

Noweir (1981), in addition to the analysis of total serum IgG and specific IgG against endotoxin, conducted specific IgG line precipitation testing. This testing was carried out on all antibody classes using the techniques of gel diffusion and counterelectrophoresis. The serologic test that this researcher used equates to a specific IgG test against ACDE. The highest precipitation of IgG antibodies was evidenced in those workers with grade II byssinosis. In comparison, asymptomatic workers or those workers with grade III byssinosis had less positive reactions.

In an *in vivo* study Olaniran and Karol (1988), guinea pigs were exposed over a six-month period to airborne cotton dust at a concentration of 21 mg/m^3. These researchers found that titres of specific IgG, measured by Zone Electrophoresis and ELISA techniques, increased significantly over time. This indicated that the immune system was stimulated as a result of cotton exposure. The authors further proposed that the presence of specific IgG antibodies in all cotton exposed guinea pigs, demonstrated the potential use of specific IgG antibodies as a cotton dust exposure indicator.

Karol and Lemp (1990) followed up on the 1988 study, by exposing 27 guinea pigs to cotton dust concentrations of 30 mg/m^3, over a six-week exposure period. They did this in order to determine, amongst other things, an antibody exposure response and the uniformity and sensitivity of the response. A modified ELISA technique was used for quantification of specific IgG titres. The researchers determined that IgG appeared to be a sensitive, long lasting marker of cotton dust exposure, with antibody titres being dependent on the cotton dust concentration and not on the number of exposures.

After the relative success of specific IgG measurements in guinea pigs, Karol *et al.* (1992) assayed the immunoglobulin levels of 425 workers within two Danish cotton mills, one wool mill and one man-made fibre mill. Workers in the two cotton mills were found to have produced antibodies specific for a glycoprotein component of the cotton dust. Results showed that 13% of exposed cotton workers produced specific IgG antibodies. Although results were low, they contrasted with the absence of antibodies in the serum of surveyed wool and man-made fibre workers. However, no attempt was made to determine if clinical or functional symptoms of byssinosis correlated with increased specific IgG titres.

Sigsgaard and Karol (1993) followed up on the study conducted by Karol *et al.* in 1992. They used the same immunological data on specific IgG produced by Karol *et al.* in 1992, in conjunction with questionnaire and pulmonary function data. The serum sample, questionnaire and pulmonary function data were linked to individual cotton workers. They found a negative association between specific IgG antibodies and pulmonary function data. The pulmonary function data consisted of Δ FEV_1, baseline FEV_1 and baseline FVC results.

The researchers involved in the studies on specific IgG mentioned above, put forward a number of hypotheses relating to the role of specific IgG in cotton workers. These include the hypothesis that IgG is a marker of chronic cotton dust exposure, and that it has a possible aetiological mechanism role in the development of byssinosis. The studies reviewed show that there is a high level of agreement between authors that specific IgG antibodies are formed against cotton, yet there is a lack of consensus on the role of these antibodies.

As far as could be ascertained, only two studies were performed that linked the byssinotic outcomes of human cotton workers to specific IgG immunological responses against ACDE. Noweir's (1981) study showed a correlation between specific IgG and byssinosis, whereas Sigsgaard and Karol's (1993) study did not. Thus, characterisation of the role of specific IgG antibodies remained unresolved. The current study aimed to corroborate either the findings of Noweir (1981) or those of Sigsgaard and Karol (1993), and accordingly to provide the required characterisation.

2.5.5.7 IgG summary

The unsuitability of total serum IgG as a marker of IgG antibodies formed against antigens in cotton dust, is reflected in the inter-variability of findings between research initiatives. As recorded above, a number of studies have investigated the role of precipitating antibodies against ACDE. These studies show that it is likely that in addition to the 'pseudo-immune' precipitation of antibodies, there is also a true precipitation of antibodies by the cotton dust extracts. The studies reviewed on specific IgG formed against endotoxin indicate that there is an association between endotoxin and formation of antibodies, but no evidence of an aetiological association exists. Studies relating to specific IgG levels against ACDE show the

formation of antibodies against the extracts, but the role of these antibodies as markers of exposure or as an aetiological mechanism, is uncertain.

As a whole, studies relating to IgG and cotton dust indicate that IgG antibodies are formed in cotton workers as a result of cotton exposure. The evidence that IgG antibodies are raised in byssinotic as opposed to non-byssinotic workers is distinctly shown in some studies but not in others.

2.5.5.8 Problems in antibody testing

Popa *et al.* (1969) suggest that differences in study results obtained between researchers, are probably due to differences in methods of extracting antigens contained in cotton. This is problematic, as there are no internationally accepted methods for standardisation of antigen extraction in cotton dust research.

2.5.5.9 Humoral pathway

There are two possible reasons why the byssinotic response could be attributed to the humoral pathway. Firstly, there is a variable latency period between initial exposure to cotton dust and onset of symptoms. This is suggestive of a sensitisation period. Secondly, not all cotton workers who are exposed develop byssinosis, and this suggests genetic influences such as possible differences in host response of the cotton worker, or differences in immunoglobulin class-specific antibody-forming potential (Butcher *et al.*, 1983).

Evidence against an antigen-antibody response is that the exposure concentrations are directly correlated with the degree of airway response (American Thoracic Society, 1998). This type of

dose response exposure relationship is not synonymous with an antigen-antibody-mediated reaction.

However, there are numerous substances in cotton dust with known but diverse toxicological effects. Moreover, the byssinosis syndrome has a dissimilar presentation of symptoms among cotton workers (Salvaggio *et al.*, 1986). Literature comprehensively indicates that agents such as endotoxin, in addition to exerting a direct toxicological action, stimulate an IgG humoral response. It is both relevant and necessary to determine the role of these IgG antibodies that are formed in response to cotton exposure.

2.6 SUMMARY

Literature consistently notes that byssinosis is poorly understood in terms of which symptoms constitute byssinosis (Rylander *et al.*, 1987). However, a standardised cotton dust questionnaire and cross-shift spirometry (WHO, 1981) are accepted as the standard measurement tools for the evaluation of byssinosis and its symptoms. Accordingly, these measurement tools were used in the current study.

The relationship of IgG and its subclasses to disease in general is poorly understood (Feldman *et al.*, 1992). Although it is not recorded in literature, there is a profound uncertainty of the relationship of the IgG class of antibodies to byssinosis and other disorders induced by cotton dust. This is evidenced in the conflicting results and interpretations among studies, which investigated this relationship (Noweir, 1981; Sigsgaard and Karol, 1993).

It can be inferred from a review of the pertinent literature that the measurement of specific IgG (Massoud and Taylor, 1964; Taylor *et al.*, 1971; Noweir, 1981) and the IgG subclasses (Mayumi *et al.*, 1983; Snapper and Paul, 1987; Fick *et al.*, 1986; Cowan and Winnie, 1993; Bradwell, 1995) is appropriate in measuring the IgG humoral response to cotton dust exposure.

CHAPTER THREE

MATERIALS AND METHODS

3.0 INTRODUCTION

As revealed by the literature, there is a limited understanding of the role of IgG and its subclasses in the development of byssinosis (Noweir, 1981; Sigsgaard and Karol, 1993). Appropriate research methods have been informed by the literature review, in order to provide clarity on the association between IgG and byssinosis. The methodology is presented as follows: selection of the mill sample (3.2), case definitions (3.3), use of symptom group, case control model (3.4), traceability and blinding of data (3.6), instruments and their administration (3.7), ethical compliance (3.8), approach to data analysis (3.9) and data analysis (3.10).

3.2 SELECTION OF THE MILL SAMPLE

Information taken from the product index of the Textile Federation members and constituent associations revealed that there were ten cotton mills processing cotton yarn from raw cotton in KwaZulu-Natal (Textile Federation, 1997). These mills are shown in the table that follows.

Table 3.1 Cotton mills included in study sample

Mill (Study name)	Geographical location	Selected	Annual lung function test	Cross-shift lung function
A	New Germany	Yes	Yes	Yes
B	Ladysmith	Yes	Yes	No
C	Mooi River	Yes	Yes	No
D	Pietermaritzburg	Yes	Yes	No
E	Pinetown	Yes	Yes	Yes
F	Tongaat	Yes	Yes	Yes
-	*Hammarsdale*	*Not*	*No*	*No*
-	*Hammarsdale*	*Not*	*Yes*	*Yes*
-	*New Germany*	*Not*	*No*	*No*
-	*Jacobs*	*Not*	*No*	*No*

The results of medical surveillance programs run at the selected cotton mills were used to select the subject participant sample. Consequently, the cotton mill sample inclusion criteria were specified, in order to facilitate the identification of subject participants. These criteria were:

- The medical surveillance program had to include an annual lung function testing program; and
- There had to be identified cases of byssinosis at the mill.

The six cotton mills selected complied with these criteria.

3.3 CASE DEFINITIONS

The case definitions included the classical definition of byssinosis, but the primary case definition used was an expanded definition of byssinosis. The relationship between clinical symptoms and functional symptoms in relation to the diagnosis of byssinosis was reviewed in Chapter 2.2.2. The definitions of byssinosis (classical) and symptom status (expanded definition of byssinosis) are given in Definitions page XIV. The use of these definitions in

analysis is clarified in Chapter 3.9(a). Importantly when the terms 'byssinosis' or 'symptom status' are used in Chapter 4 and Chapter 5, use of the terms relate specifically to the study definitions.

The case definition of 'byssinosis' was taken from the classification of clinical manifestations as specified in Table 3.2. The case definition of 'symptom status' was based on the inclusion of workers with byssinosis (clinical symptoms) and lung function changes (functional symptoms) as specified in Table 3.2.

Table 3.2 Classification of clinical manifestations and lung function changes in respiratory disorders from exposure to vegetable dusts causing byssinosis or respiratory tract irritation (RTI).

World Health Organisation grading system		
	Classification	Symptoms
Byssinosis	*Grade 0*	No symptoms
	Grade B1	Chest tightness and/or shortness of breath (SOB) on most of first days back at work.
	Grade B2	Chest tightness and/or SOB on the first and other days of the working week.
Respiratory tract irritation	*Grade RTI1*	Cough associated with dust exposure.
	Grade RTI2	Persistent phlegm (i.e. on most days during 3 months of the year) initiated or exacerbated by dust exposure.
	Grade RTI3	Persistent phlegm initiated or made worse by dust exposure either with exacerbation's of chest illness or that persists for 2 years or more.
Lung function	*Acute changes*	
	No effect	A consistent decline (decline occurs in at least 3 consecutive tests made after an absence from dust exposure of 2 days or more) in forced expiratory volume in 1 second (FEV_1) of <5% or an increase in FEV_1 during the work shift.
	Mild effect	A consistent decline of between 5 and 10% in FEV_1 during the work shift.
	Moderate effect	A consistent decline of between 10 and 20% in FEV_1 during the work shift.
	Severe effect	A decline of 20% or more in FEV_1 during the work shift.
	Chronic changes	
	No effect	FEV_1 value by a pre-shift test after an absence from dust exposure of 2 days or more) - 80% of predicted (predicted values should be based on data obtained form local populations or similar ethnic and social class groups)
	Mild to moderate effect	FEV_1 (as above) -60-79% of predicted value (as above).
	Severe effect	FEV_1 (as above) - <60% of predicted value (as above).

WHO (1987)

3.4 USE OF SYMPTOM GROUP CASE DEFINITION

A gold standard for the diagnosis of byssinosis does not exist. This can be inferred by the substantial disagreement as to the symptoms of cotton dust exposure that constitutes byssinotic symptoms (Rylander et al., 1987). Consequently, all cotton dust research studies have been influenced, to varying extents, by the incorrect categorisation of byssinosis. Since no gold standard exists to differentiate between clinical or functional symptoms induced by cotton or induced by other non-cotton influences, the responsibility rests on the research team

to control these potential problems, within a suitable research model.

This problem was dealt with in two ways:

- Firstly subjects with either clinical symptoms of byssinosis or functional symptoms of cotton dust exposure or subjects with both clinical symptoms of byssinosis and functional symptoms of cotton dust exposure were included in a single group definition. This group definition was termed the expanded definition of byssinosis. This infers that within this group there were some workers with byssinosis (clinical symptoms) but no lung function changes (functional symptoms) and workers with lung function changes (functional symptoms) but without byssinosis (clinical symptoms).

- Secondly the unexposed control group was included, which had subjects with both normal and abnormal clinical and functional symptoms unrelated to the influence of cotton dust. Within this model the unexposed control group provided a valid immunological comparison to compare the exposed symptomatic and exposed asymptomatic groups against. For all clinical and functional symptoms, the same logic applied when determining whether differences in immunological parameter concentrations were due to exposure to cotton, non-cotton specific pathology, or pathology induced by cotton.

Table 3.3 provides a concept interpretation model for determining the meaning of effects within the symptom group case control model used in this study. For the interpretation of Table 3.3, an effect means any variable which may relate to pathology, collected in this

study. These effect variables include respiratory disorder variables, pulmonary function variables, and immunological variables.

Table 3.3 Interpretation of effect within the symptom group case control model

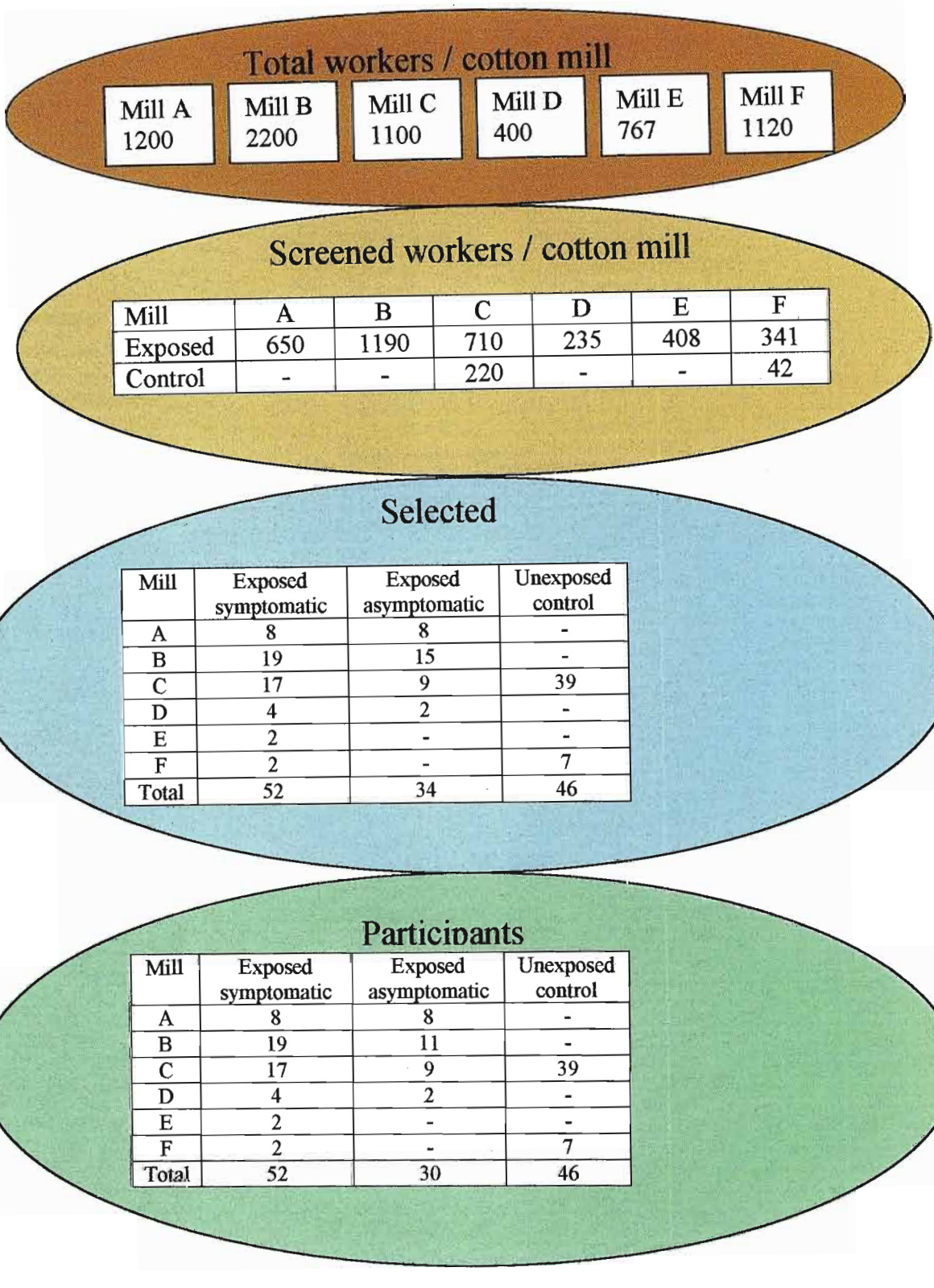
Effect	Exposed symptomatic	Exposed a-symptomatic	Unexposed control
A	Same	Same	Different
B	Same	Different	Same
C	Different	Same	Same

A. Effect may be due to cotton exposure, not related to pathology.
B. Effect may be due to non-cotton specific pathology. That is the general effect of disease.
C. Effect may be due to pathology, directly induced by cotton exposure.

(Model is original)

It is understood that the explanation of Table 3.3 is only applicable when a logical pattern is shown for any effect between symptom groups. That is, increases or decreases in the effect between symptom groups should comply with a norm for that parameter. For example, higher FEV_1 values (effect variable) may be noted in exposed symptomatic workers and comparatively decreased values evident in the exposed a-symptomatic and unexposed control groups. In this example it cannot be concluded that the difference seen in the exposed symptomatic group is due to a disorder related to exposure. There would have to be a comparative decrease in FEV_1 values in the exposed symptomatic group in order to conclude that the effect may be due to exposure.

Figure 3.1 Research architecture for selection of participants [8]



Mill	A	B	C	D	E	F
Exposed	650	1190	710	235	408	341
Control	-	-	220	-	-	42

Mill	Exposed symptomatic	Exposed asymptomatic	Unexposed control
A	8	8	-
B	19	15	-
C	17	9	39
D	4	2	-
E	2	-	-
F	2	-	7
Total	52	34	46

Mill	Exposed symptomatic	Exposed asymptomatic	Unexposed control
A	8	8	-
B	19	11	-
C	17	9	39
D	4	2	-
E	2	-	-
F	2	-	7
Total	52	30	46

[8] 'Selected' was the total number of subjects selected for participation, whereas 'participants' were the actual study subjects. Four selected subjects at Mill B chose not participate in the study.

3.5 SELECTION CRITERIA FOR SYMPTOM GROUPS

Inclusion and exclusion selection criteria were set for symptom groups in terms of socio-demographic profiling, sample size and selection, and criteria for the exposed symptomatic, exposed asymptomatic and unexposed control symptom groups. To be included in the study each of the subjects had to agree to being subjected to the administration of all study tools. Four subjects were excluded from participation in the study, from mill B, on the basis of not wanting to provide blood for serum samples. These were the only workers who objected to the administration of any of the study tools.

3.5.1 Socio-demographic profiling

Literature revealed (Bradwell, 1995) that it was necessary to attain a specific socio-demographic profile for the sample group to ensure that differences between groups did not impact or influence immunological parameter concentrations. Consequently, certain inclusion and exclusion criteria had to be set relative to socio-demographic factors. These socio-demographic factors and criteria are dealt with as follows.

a) Race

Serum IgG values differ by as much as 30% between blacks and whites (Tollerud *et al.*, 1995). To control for the confounding influence of race, the study sample was limited to subject participants from the black race group.

b) Smoking

Smokers were excluded from the original study design as smoking had the potential to act as a confounding variable, impacting on lung function (Huib *et al.*, 1996) independent of byssinosis and serum IgG_2 concentrations (Fick *et al.*, 1986). However, inadvertently 6% of the study sample were current smokers, as shown in Table 4.1. This was due to the clinic sisters having no knowledge of the smoking habits of these workers. Spirometry testing had been conducted and the serum samples drawn before the true smoking status of these workers was determined.

c) Age and gender

Age and gender affect IgG subclass concentrations (Bradwell, 1995). As a result, symptom groups were approximately group matched for both age and gender.

d) Work department

The research that Karol and Lemp (1990) conducted on the role of specific IgG in guinea pigs, suggested that specific IgG antibodies against ACDE acted as markers of exposure. As a consequence it was deemed appropriate to group match symptom groups for work departments.

3.5.2 Sample size and selection

Identification of the subject sample included issues relating to size of sample, selection criteria and mill origin.

a) Size

The proposal included a sample size of 120 subjects, with an equal split of 40 exposed symptomatic, 40 a-symptomatic and 40 unexposed control subjects.

b) Selection

It proved far more difficult to identify workers who met all the criteria for the exposed symptomatic and exposed a-symptomatic groups, than had originally been anticipated. In the proposal, it was put forward that the researcher would screen existing pulmonary function testing data that formed part of the medical surveillance program at each of the three selected cotton mills (mills A, B, C). From the total number of subjects who met the criteria, a random sample was to be taken that would fulfil the proposed number requirements. An amended method had to be adopted with an increase of cotton mills included in the study sample (mills D, E, F), and the sampling selection method had to be modified. Instead of randomly selecting a sample of exposed symptomatic and exposed a-symptomatic subjects from those who met the criteria, all subjects who met the criteria were included in the study sample.

In addition, the need to conduct symptom group matching for various demographic factors made it difficult to exactly match the symptom groups in terms of numbers. Additional subjects had to be included in the unexposed control group in order to achieve acceptable group matching.

c) Mill origin

The exposed symptomatic population was translated in practice into the population of workers with byssinotic symptoms at mills A, B, C, D, E and F, whereas the exposed a-symptomatic population was randomly selected from mills A, B, C and D. The unexposed control population was randomly selected from mills C and F. The unexposed control workers were selected from areas outside of the cotton mill. At mill C the workers who formed part of the unexposed control group were taken from two areas. The first area was called Grey Cloth and was a final inspection area where no raw cotton dust was present. This Grey Cloth area was in a separate building outside the cotton mill. The second area at mill C was cut make trim (CMT), where after printing or dyeing, the final cloth was taken and cut and sewn into finished products such as curtains or bedding apparel. Some unexposed control workers also worked in the despatch department of CMT. The second mill from which participating control workers were sourced was mill F. The control workers in mill F all worked in the despatch area of the mill. This area was totally free from raw cotton and was in a separate building.

3.5.3 Selection criteria for exposed symptomatic group

The specific criteria, taken from the WHO (1987) grading system (Table 3.1), were:

- Non-smokers;
- Clinical symptoms of breathlessness[9] or chest tightness in accordance with WHO grading system; and / or
- Functional changes of baseline FEV_1 or ΔFEV_1 declines in accordance with WHO grading

system.

3.5.4 Selection criteria for exposed a-symptomatic group

Pulmonary function testing, administration of the questionnaire[10] and immunological testing were performed in the same manner as specified for the exposed symptomatic group. In order to be defined as exposed a-symptomatic, a worker was required to meet the following criteria:

- Non-smokers;
- At least 10 years exposure to cotton dust in a mill environment;
- A baseline FEV_1 value of greater than 90% of predicted value[11];
- Δ FEV_1 change of not greater than 5% of predicted value; and
- No clinical symptoms of byssinosis. That is breathlessness[9] or tightness of chest.

Tightness of chest is a subjective symptom, but it is not associated with unfitness due to obesity, lack of exercise or old age, whereas breathlessness often is. The symptom of breathlessness, as asked in the standard cotton questionnaire, is fairly open to biased interpretation, particularly grade A and B breathlessness. As a result, a differential decision was made related to chest tightness and breathlessness, as follows:

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[9] The classification of breathlessness was subject to the criteria specified in pages 85 & 86.
[10] See Annexure 1.

[11] Since an FEV_1 of <80% is considered in the WHO (1981) classification system as mild lung function impairment it was decided to build in a margin of error of 10% above the cut of point for mild impairment. Hence an FEV_1 of >90% was chosen as an arbitrary cut-off for the inclusion into the exposed asymptomatic group.

Any person who reported work related chest tightness was excluded from the exposed a-symptomatic group and included in the exposed symptomatic group, while workers with breathlessness were treated differently. Workers with grade C or D breathlessness were excluded from the exposed a-symptomatic group and included in the exposed symptomatic group, even in the absence of functional symptoms of byssinosis. If the worker had grade A or B breathlessness without any functional decline in baseline FEV_1, or changes in ΔFEV_1, or any other clinical changes, gathered either in the questionnaire or by a doctor's diagnosis, the subject was included in the exposed a-symptomatic group. In addition, the worker must have complied with the other criteria specified for the exposed a-symptomatic group.

In the absence of a gold standard for the diagnosis of byssinosis, all cotton dust research studies base the diagnosis of byssinosis solely on the subjective classical symptoms. The very nature of subjective symptoms is that they are open to bias. Thus, researchers should apply logic before blindly accepting the reported subjective symptoms at face value. What is described above, relating to symptoms of breathlessness, is the application of such logic.

3.5.5 Selection criteria for unexposed control group

Cross-shift spirometry testing, administration of the questionnaires and immunological testing were conducted in the same manner as for the other groups. A number of inclusion criteria were specified for this group:

- Approximate the socio-economic profile of the exposed symptomatic and exposed a-symptomatic groups;

- Never have worked in an area where there was exposure to raw cotton dust, or have been exposed to an occupation which could induce immunological or impair pulmonary function; and
- Non-smokers.

3.6 TRACEABILITY AND BLINDING OF DATA

Each subject participant was given an ascending study identification number regardless of allocation of symptom group or cotton mill environment. The number was allocated to the worker when the pre-shift spirometry test was performed and written in a book the researcher kept for record purposes. The study identification number, the name of the subject and the name of the cotton mill where the subject worked were all entered into the book. The number was then entered into the spirometer, in addition to the subject's name, and appeared on the spirometry printout.

When the post-shift lung function test was performed, the number was re-entered into the spirometer and thus appeared on both the pre- and post-shift spirometry printouts. Once the post-shift lung function test had been completed, a blood sample was taken in two vacuum test tubes on which the study identification number was written. After the blood sample had been drawn and then centrifuged, it was poured into four cryogenic tubes. Each of these tubes had unique identification of which the first part comprised the study identification number. After the questionnaires had been handed to the administrators, both the name of the subject participant, and the study identification number were written on the questionnaire.

This numbering system ensured that the questionnaires, lung function results, and immunological results were linked by the same study identification number. Since there was also no link between the study identification number and cotton mill, the immunological laboratories were blinded to both mill origin and symptom status of the serum samples.

3.7 INSTRUMENTS AND THEIR ADMINISTRATION

Literature aided in the identification of suitable instruments for categorisation of the byssinotic response (White, 1985; WHO, 1981), and instruments for determination of the IgG humoral response (Feldman *et al.*, 1996; Tomee *et al.*, 1996) to cotton dust exposure.

A standard cotton dust questionnaire (3.6.1) and pulmonary function tests (3.6.2) were identified as suitable categorisation instruments for the byssinotic response. The immunological test measurements (3.6.3) of IgG subclasses and specific IgG against ACDE, were noted as suitable instruments for determination of the IgG humoral response to cotton dust exposure.

3.7.1 Questionnaires

Both socio-demographic and respiratory disorder data were collected by means of a questionnaire. The standard format for a physical examination was attached to the standard cotton questionnaire[12].

[12] See Annexure 1.

3.7.1.1 Selection

There are different variations of the standard cotton dust questionnaire, including:

- NIOSH (1974) questionnaire;
- The ATS 1978 questionnaire, as used by Christiani *et al.* (1994);
- The Department of Labour, OSHA 1978 questionnaire, as used by White (1985); and
- The WHO 1981 questionnaire, which was based on several earlier versions of a respiratory questionnaire drafted by the Medical Research Council of Great Britain (WHO, 1981).

These questionnaires are in essence all the same, and include questions related to clinical symptoms contained in Schilling's classical definition of byssinosis (Schilling *et al.*, 1955). In the only comprehensive published South African study into byssinosis, White (1985) used the OSHA 1978 questionnaire, translated the English version of this questionnaire into Zulu and had it validated. As a result, the English and Zulu questionnaires used by White were considered appropriate for use in the current study, and were administered to all participating subjects.

3.7.1.2 Administration

As the researcher was not able to speak Zulu and not all the study participants could speak English, it was necessary to identify a mechanism to effectively administer the questionnaire.

Due to potential intra-mill bias with the use of different personnel, an attempt was made to utilise one person alone at each mill. This approach was not possible at two mills. At mill A the staff nurse who was going to administer the questionnaires died approximately two weeks before the data collection at the mill was due to begin. An interim replacement nurse was trained and completed the majority of the questionnaires at mill A. However, towards the end of data collection at this mill a permanent nurse was placed at the clinic, who completed the questionnaire administration.

At mill B the nurse who was trained to complete the questionnaire was severely injured towards the end of the data collection process. Her injuries were not related to her work, or to her involvement in the study. She was in hospital for a number of weeks and after discharge from hospital spent a number of months recuperating at home. The second staff nurse who worked at mill B was trained in questionnaire administration and completed the questionnaire administration process.

The researcher conducted initial training at each of the mills ensuring that the questionnaire administrator understood the purpose and functioning of the questionnaire. Once the training had been completed the researcher left blank questionnaires to be completed with the administrator. As shown in Table 3.4, the majority of clinic personnel who administered the questionnaires were staff nurses.

Table 3.4 Personnel utilised in administration of questionnaire

Cotton mill	Personnel used	Number of people used
A	Staff nurses	2
B	Staff nurses	2
C	Staff nurse	1
D	Personnel staff	1
E	Staff nurse	1
F	Researcher	1
Total		8

There was no staff nurse or other Zulu speaking personnel available at mill F to administer the questionnaires. Mill F was situated in a peri-urban area and although all study participants at this mill were native Zulu speakers, they spoke English well enough for the researcher to administer the English questionnaires.

3.7.1.3 Quality assurance

When a number of questionnaires had been completed, the researcher went back to each of the individual mills and checked the manner in which they had been completed. Where questionnaires had been filled in poorly, with blank or inconsistent answers, the researcher asked the questionnaire administrator to schedule an appointment with the study participant. During these appointments, questions or sections of questions were re-visited, in order to ensure the proper completion of all questionnaires. At the same time, the researcher re-trained the questionnaire administrator, with emphasis on questions or sections of questions that were problematic to that administrator.

3.7.1.4 Physical examination

The format for the physical examination was taken from the standard OSHA 1978 questionnaire. All subjects participating in the study underwent a physical examination. A medicine practitioner or occupational medicine practitioner conducted the physical examination.

3.7.2 Pulmonary function testing

WHO (1981) notes that spirometry is used for the assessment of baseline FEV_1 and ΔFEV_1. Consequently, spirometry was used in this study. There were a number of issues relating to these tests that included validation of subject selection by spirometry, the role of the spirometric technician, the spirometer and its use and conducting the test, which will be elaborated on in the following sections.

3.7.2.1 Validation of subject selection by spirometry

All of the participating cotton mills had some form of spirometric testing program in place, with annual spirometric data available for all study participants. However, to ensure both the accuracy and the reliability of the pulmonary function testing data used in this study, it was necessary to independently validate the annual spirometric data of all study participants.

In order to validate the sample selection, the researcher performed independent cross-shift spirometric testing on the exposed symptomatic, exposed a-symptomatic and unexposed control groups. The testing was performed in the morning, before the workers went into the work environment, and again after 6 hours of exposure within the work environment.

3.7.2.2 Role of spirometric technician

The researcher, who conducted all spirometric testing, filled the role of spirometric technician. This ensured that the large degree of subjectivity that can occur through different encouragement techniques, training methods and problem solving techniques, was avoided. The WHO (1981) specifies that 16 hours of training (including theoretical and practical) on spirometry is necessary to qualify a person as a spirometric technician. The researcher met these training criteria.

3.7.2.3 The spirometer and its use

The pulmonary function recordings were made using a SP 200/AT6 Schiller spirometer. The spirometer met ATS criteria (Baxter, 1998). The instrument requires weight, height, age and race[13] (Caucasian or non-Caucasian) data to be entered. The spirometer used was portable and was transported by car from mill to mill. The spirometer included a built-in battery pack and printer. The results were printed after the performance of each test. In order to produce standardised results, only one Schiller spirometer, with a Schiller manufactured calibration syringe, was used throughout the entire study. A number of potential sources of error existed, which were addressed in this study as specified below.

a) Spitting in flow-head

Spitting in the flow-head of a spirometer produces erroneous results. On the Schiller spirometer this led to higher than actual values. When spirometry results were noted to climb

[13] The Schiller uses the Schoenberg *et al* (1978) predicted values for race.

consistently in a linear fashion, with larger similarly shaped curves, spitting was checked as a potential cause of error. If the flow-head gauze had spittle or any solid on the surface, the flow-head was dismantled, the gauze washed with a soap solution, dried, reassembled, and the spirometer re-calibrated. From time to time, depending on the condition of the gauze, it was changed to avoid any variance caused by non-consistency.

b) Cold starts

When the spirometer was cold in the early morning, problems were sometimes encountered with large changes in values over, on average, the first two tests. Importantly, the spirometer was calibrated before and after testing on every day it was used, even when the number of workers tested did not exceed one worker. Thus, it was always possible to determine if the variance exceeded three percent. There were two occasions when the variance between calibrations exceeded three percent. Both occasions occurred on cold mornings and involved the first workers tested. As the workers went into the cotton mill immediately after the first lung function test had been performed, it was not possible to retest the workers on the same day. This problem was overcome by re-testing the workers on a new workweek, which was the first day of the work shift. On the first occasion 13 workers had to be re-tested and on the second occasion 10 workers had to be re-tested.

3.7.2.4 Conducting the test

After a break of 48 hours, all subject participants were required to perform three maximal expiratory manoeuvres. ATS (1995) standard procedures of calibration and measurement were used to measure FVC and FEV_1. These spirometric procedures and measurement

parameters are uniformly employed in cotton research investigations (WHO, 1981; Jiang *et al.*, 1995). The spirometer automatically selected the best exhalation curve. Only the best curve was used with no composite make-up of curves utilised. Tests were done in the standing position.

3.7.3 Immunological testing

Immunological parameters measured were the IgG subclasses by nephelometric assay and specific IgG by FEIA.

3.7.3.1 Obtaining the serum samples

Both nephelometry and FEIA methods require serum samples. Serum samples were therefore collected from subject participants, as outlined below.

a) Collection and preparation of serum samples

Either a qualified doctor or nurse conducted the venipuncture, obtaining the blood for the serum samples. The blood was collected in evacuated tubes and allowed to clot. Serum was separated by centrifugation, and each sample divided into four separate 1.8ml cryogenic test tubes[14].

[14] Standard methods were used for all procedures described (Satoh *et al.*, 1998).

b) Transportation and storage

The tubes were transported at - 10°C using dry ice and bulk stored at –80 °C in a freezer at Allerton Laboratories in Pietermaritzburg. The tubes were then transported at -18°C in a refrigerated truck to the National Centre for Occupational Health (NCOH) laboratory in Johannesburg, and were stored at -70°C in a freezer, where they remained until analysis.

3.7.3.2 Quality assurance

The need to ensure quality assurance relating to: handling, storage and analysis, traceability, and the researcher's training and oversight, was paramount to achieving valid and reliable immunologic assay results.

a) Handling transport storage and analysis

There was no difference in the way the symptomatic, a-symptomatic or control serum samples were collected, handled, transported, stored or analysed. Therefore the results of these case groups can be reliably compared. There was no differentiation between the serum test tube series used for the nephelometric assay and FEIA.

b) Traceability

Each serum sample taken from an individual worker was split into four cryogenic test tubes[15]. There were 131 serum samples collected in total, each of which was allocated a research identification number. In addition to being labelled with the research identification number, the four series which contain the individual workers serum were allocated a series number

[15] As described in the Chapter 3, Section 3.7.3.1: Obtaining the serum samples.

[1], [2], [3] or [4]. This ensured that every tube was allocated a unique identification number. For example, the first serum sample (split into four test tubes) of an individual worker was allocated the number 1[1], 1[2[, 1[3] and 1[4].

c) Researcher's training and oversight

The researcher travelled to the Department of Serology in Pretoria in order to gain a thorough practical understanding of the conduction as well as a theoretical understanding of the principals of both the nephelometric assay and FEIA. Whilst being guided through the procedures adopted by the laboratories in practice, he ascertained compliance by the laboratories with the documented assay methods.

3.7.3.3 The immunological test parameters

Suitable measurement techniques were sought for measurement of the IgG subclasses and total IgG, as well as for specific IgG.

a) IgG subclasses and total IgG

The literature reviewed (Feldman *et al.*, 1996) indicated that nephelometry, as a laboratory test method for measuring the IgG subclasses, was used in a South African study. Feldman and co-workers' (1996) study included subjects with a comparable socio-economic background to the current study. Further, nephelometry is recorded (Behring, 1997) as the best-proven immunochemical method for the determination of protein in serum. Consequently, it was adjudged the most suitable method for the determination of IgG in

subclasses. The addition of the IgG subclasses constituted the total serum IgG[16] parameter and therefore it was not necessary to conduct separate total serum IgG measurements.

b) Specific IgG

RAST is accepted as the most reliable form of specific antibody measurement technique in screening for allergy (ALLSA, 1994). RAST has been used to measure the specific IgG response against bee venom (Ewan *et al.,* 1993), and FEIA has been used to measure the response to antigens inducing pulmonary aspergilloma (Tomee *et al.,* 1996). However, neither RAST nor FEIA has been used to measure the specific IgG response against cotton dust. The Phadebas ® was the first generation RAST test from Pharmacia & Upjohn Diagnostics, and the ImmunoCAP ® RAST the second generation (ALLSA, 1994). Although the name RAST is still used, it is used as an accepted generic name for the test technique, which has largely been replaced by an equivalent test technique called the FEIA (Larson, 2002). The second generation Pharmacia CAP System, specific IgG FEIA, was used in the current study. Despite, the experimental use of the FEIA in this research arena, was likely to provide a suitable measure of the IgG response against ACDE.

3.7.3.4 IgG subclasses (Nephelometric assay)

The nephelometric assay was conducted in the University of Pretoria's Department of Serology laboratory. As part of a quality assurance proficiency testing program, the University of Pretoria's Department of Immunology laboratory ran inter-laboratory analysis comparisons with NCOH and SAIMR (SANAS approved).

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[16] See Chapter 2, Figure 2.1: Division of the IgG class of antibodies. The total serum IgG measurement

The IgG subclass levels were determined[17] using standard laser nephelometry[18], using the appropriate Binding Site, human IgG subclass liquid reagent BNII test kits, code LK001.TA (Binding Site Ltd, Birmingham, England). The nephelometer used was the Behring Nephelometer II (Behring Diagnostika, Germany). Both the make of instrument and the manufacturer of the IgG subclass kits used in the current study were the same as those used by Feldman and colleagues (1996) in a South African study.

The primary outcome measures for this immunological parameter were the IgG subclass results generated from the nephelometric assay. The secondary outcome measures were normative immunological reference values, for the IgG subclasses, set by The Binding Site Limited.

3.7.3.5 Specific IgG (FEIA)

The FEIA[19] was conducted in the Department of Health's NCOH laboratory, in Johannesburg. As part of a quality assurance proficiency testing program, the NCOH laboratory ran inter-laboratory analysis comparisons with the SAIMR (SANAS approved) and the University of Pretoria's Department of Immunology.

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quantified the antibodies entitled 'Total serum IgG".

[17] Since standard test instruments, kits and techniques, including calibration techniques, were used with no deviations, it was not appropriate to detail these aspects in the methods section.

[18] See Chapter 2, Figure 2.1: Division of the IgG class of antibodies. Nephelometry analysis measured the antibodies entitled "IgG subclasses 1-4".

[19] See Chapter 2, Figure 2.1: Division of the IgG class of antibodies. The FEIA measured the antibodies entitled 'Specific IgG antibodies against cotton antigen within total serum IgG".

The Pharmacia & Upjohn UniCAP^{TM} specific IgG FEIA, article number 10-9331-01, was used to quantify the potential specific IgG antibody response. The antigen used was cotton crude fibres (immunoCAP), article number 14-4476-01. This specific IgG measurement was a total specific IgG measurement. The analysis was performed on the Pharmacia & Upjohn, UniCAP 100 instrument (Pharmacia & Upjohn Diagnostics AB, Uppsala, Sweden).

Standard test instruments and techniques, including calibration techniques, were used with no deviations. Although the use of the UniCAP^{TM} specific IgG kits was standard, use of the crude cotton fibres immunoCAP, in conjunction with the UniCAP^{TM} specific IgG, was not. Pharmacia & Upjohn (1998) notes that if immunoCAP is not provided specifically for UniCAP^{TM} specific IgG, then the results generated should not be used for diagnostic purposes, but rather for research purposes only. The crude cotton antigen, which is an immunoCAP product, is not provided specifically for UniCAP specific IgG. Therefore the proviso that the results generated by this particular assay should be used for research purposes only, is applicable.

The primary outcome measures for this immunological parameter were the specific IgG results generated from the FEIA. No normative immunological reference values, set by Pharmacia & Upjohn, exist for the specific IgG response against the crude cotton antigen. Consequently, the secondary outcome measure for specific IgG was a statistical analysis of concentration differences between the symptom groups.

3.8 ETHICAL COMPLIANCE

Ethical approval was obtained from the Ethics Committee, Faculty of Medicine, University of Natal, for the use and application of the study tools that were detailed in the original proposal. Each subject participant provided individual informed consent. In addition, briefings were conducted with appropriate unions, clinic staff and employer representatives, ensuring transparency and identification with the study objectives.

3.9 DATA ANALYSIS

Data from the study was captured, cleaned and analysed using the SPSS®, version 9.0 statistical package for Windows™. The 95% confidence interval ($P< 0.05$) was considered statistically significant. As the 90% ($P< 0.10$) confidence interval was not considered statistically significant, but rather of borderline significance, it was mentioned when considered of interest.

a) Byssinosis (classical) versus symptom status (expanded definition of byssinosis)

Use of the expanded definition of byssinosis termed 'symptom status' partly, but not fully, addressed the problems associated with the classical definition of byssinosis. These problems relate to disagreement over whether functional symptoms occur in isolation to clinical symptoms. The issues with the classical and expanded definitions of byssinosis were dealt with by making use of both definitions in the analysis of data, with a bias towards the expanded definition, which was used in the comparative case control model. Use of both the classical and expanded definitions of byssinosis in this study can be considered 'best practice' in terms of byssinosis research.

b) Rules for the presentation of P values

Non-significant exact P values were restricted to two decimal places and significant P values taken to three decimal places for exact values in tables, and the notations $P< 0.05$ or $P< 0.01$ used in text. When highly significant P values were obtained ($P< 0.000$), the annotation $P< 0.005$ was used in text. Standard rounding-up and rounding-down rules were used for interpretation of the P values at the third decimal place.

a) Distribution of data

Study results for all the pulmonary function and immunological variables displayed distributions acceptably close to the normal distribution. No bimodality was found to exist and coefficient of skewness values were within acceptable limits. Application of parametric statistical test techniques was thus appropriate.

b) Categorical data

Chi-square test: Frequencies and percentages were obtained by generating crosstabulations for analysis of categorical data, including both ordinal and nominal level measurements. The Chi-square test was used as the test of significance for testing the difference between two population proportions, when analysing categorical data.

c) Measurement data

A variety of different statistical procedures was used for the analysis of measurement data including both interval and ratio level measurements.

Paired-Samples t-test: This particular t-test procedure compares the independent means of two continuous variables for a single group. An example of where the Paired-Samples t-test procedure was used was on pre- and post-pulmonary function tests, where the pre- and post-tests were independent of one another but were conducted on the same group of individuals. The test compared the group pre-test mean with the group post-test mean.

Independent-Samples t-test: This procedure accommodates analysis of multiple quantitative variables, analysed as an individual variable against the means for two groups of cases (categorical grouping variable).

One-Way ANOVA: This statistical test produces a one-way analysis of variance for a continuous dependent variable by a single factor (independent) discrete variable. Analysis of variance is used to test the hypothesis that several means are equal. However, the One-Way ANOVA procedure does not provide information on the means of the dependent variable split into grouping categories contained in the independent variable. Consequently, the Duncan, post-hoc range test and General Linear Model, Univariate test, as statistical techniques, were used to provide further clarity of the relationships between variables.

Duncan, post- hoc range test: was used for determining what differences existed between the means of the grouping categories in homogenous subsets. This provided clarity on the means in a grouping category that was statistically different enough to form a homogenous subset, enabling meaningful discussion.

General Linear Model, Univariate: Interactions between a dependent continuous variable (such as IgG_1) and two random factors (such as chest tightness and all symptom status) were investigated. These analyses circumvented limitations of the One-Way ANOVA.

Bivariate Correlations: The correlation procedure was used to measure the degree of the relationship between continuous variables. Pearson's correlation coefficient was used as the test of correlation in conjunction with its significance level.

CHAPTER FOUR

RESULTS

4.0 INTRODUCTION

A total of 128 workers were investigated in six cotton mill study sites, ranging from peri-urban to rural locations in KwaZulu-Natal, South Africa[20]. The results of socio-demographic factors (4.1), respiratory disorders (4.2) and pulmonary function testing (4.3), provided indicator variables for the analysis of immunological status (4.4). Serum IgG subclass and specific IgG data were analysed simultaneously. This allowed for comparison of trends between the IgG subclasses and specific IgG.

Importantly, the format in which the results have been presented [21], requires an understanding of the definition of byssinosis (classical) and expanded definition of byssinosis (symptom status) [22] as defined in Definitions, page XV.

4.1 SOCIO-DEMOGRAPHIC FACTORS

There were 52 subjects (40.6%) in the exposed symptomatic group, 30 subjects (23.4%) in the exposed asymptomatic group and 46 subjects (35.9%) in the unexposed control group. Each of the study sites, apart from the mill in Tongaat, processed cotton from raw cotton

[20] All subjects included in the study were from the black race group.
[21] The primary definition of byssinosis used in the data analysis was the expanded case definition (symptom status). Where appropriate, another definition of byssinosis (classical) was included in the analysis, for comparison of the expanded and classical definitions of byssinosis. See Definitions page XIV.
[22] 'Symptom status' refers to the case control groupings of exposed symptomatic, exposed asymptomatic and unexposed control. 'All symptom status' includes all three of these groupings, whereas 'exposed' refers to the exposed symptomatic and exposed asymptomatic groups, but excludes the unexposed control group.

bales to process winding or beyond. Questionnaire reported socio-demographic factor variables are shown in Table 4.1.

Table 4.1 Questionnaire reported socio-demographic factors by symptom status – Numbers (%)

*Socio-demographic factors	Exposed				Unexposed		Total		P
	Exposed symptomatic group		Exposed asymptomatic group		Unexposed control group				
	Male n=30 (23.4)	Female n=22 (17.2)	Male n=18 (14.1)	Female n=12 (9.4	Male n=21 (16.4)	Female n=25 (19.5)	Male n=69 (53.9)	Female n=59 (46.1)	
Age (yrs)									**
Mean	45.80	42.91	42.17	44.17	40.14	44.32	43.13	43.76	0.34
SD	8.82	3.01	6.53	5.97	7.41	9.53	8.13	6.93	
Exposure duration (yrs)									**
Mean	22.55	18.91	16.36	18.67	16.26	17.72	19.02	18.356	0.032
SD	10.77	5.67	5.69	3.23	8.58	8.48	9.43	6.609	
Geographical area									
Ladysmith	5 (16.7)	14 (63.6)	4 (22.2)	7 (58.3)	-	-	9 (13.0)	21 (35.6)	
Mooi River	17 (56.7)	-	9 (50.0)	-	14 (66.7)	25 (100)	40 (58.0)	25 (42.4)	
Pietermaritzburg	2 (6.7)	2 (9.1)	2 (11.1)	-	-	-	4 (5.8)	2 (3.4)	**
New Germany	2 (6.7)	6 (27.3)	3 (16.7)	5 (41.7)	-	-	5 (7.2)	11 (18.6)	0.000
Pinetown	2 (6.7)	-	-	-	-	-	2 (2.9)	-	
Tongaat	2 (6.7)	-	-	-	7 (33.3)	-	9 (13.0)	-	
% of gender total	30 (100)	22 (100)	18 (100)	12 (100)	21 (100)	25 (100)	69 (100)	59 (100)	
Department									
Spinning	22 (73.3)	20 (90.9)	15 (83.3)	10 (83.3)	-	-	37 (53.6)	30 (50.8)	
Winding	4 (13.3)	1 (4.5)	-	1 (8.3)	-	-	4 (5.8)	2 (3.4)	*
Weaving	4 (13.3)	1 (4.5)	3 (16.7)	1 (8.3)	-	-	7 (10.1)	2 (3.4)	0.53
Unexposed	-	-	-	-	21 (100)	25 (100)	21 (30.4)	25 (42.4)	
% of gender total	30 (100)	22 (100)	18 (100)	12 (100)	21 (100)	25 (100)	69 (100)	59 (100)	
Occupations									
Operator	22 (73.3)	22 (100)	14 (77.8)	11 (91.7)	-	-	75.0	97.1	
Cleaner	2 (6.7)	-	-	-	-	-	4.2	-	
Mechanic	2 (6.7)	-	-	-	-	-	4.2	-	*
Supervisor	4 (13.3)	-	3 (16.7)	-	-	-	14.6	-	0.32
Fork lift driver	-	-	1 (5.6)	-	-	-	2.1	-	
Tester	-	-	-	1 (8.3)	-	-	-	2.9	
Unexposed	-	-	-	-	21 (100)	25 (100)	21 (30.4)	25 (42.4)	
% of gender total	30 (100)	22 (100)	18 (100)	12 (100)	21 (100)	25 (100)	69 (100)	59 (100)	
Smoking status									**
Current	2 (6.7)	-	5 (27.8)	1 (8.3)	-	-	7 (10.1)	1 (1.7)	0.001
Ex	13 (43.3)	-	3 (16.7)	-	7 (33.3)	-	23 (33.3)	-	0.19
Never	15 (50)	22 (100)	10 (55.6)	11 (91.7)	14 (66.7)	25 (100)	39 (56.5)	58 (98.3)	0.22
Total	30 (100)	22 (100)	18 (100)	12 (100)	21 (100)	25 (100)	69 (100)	59 (100)	

P values reflect differences between socio-demographic variables and *exposed status or **all symptom status

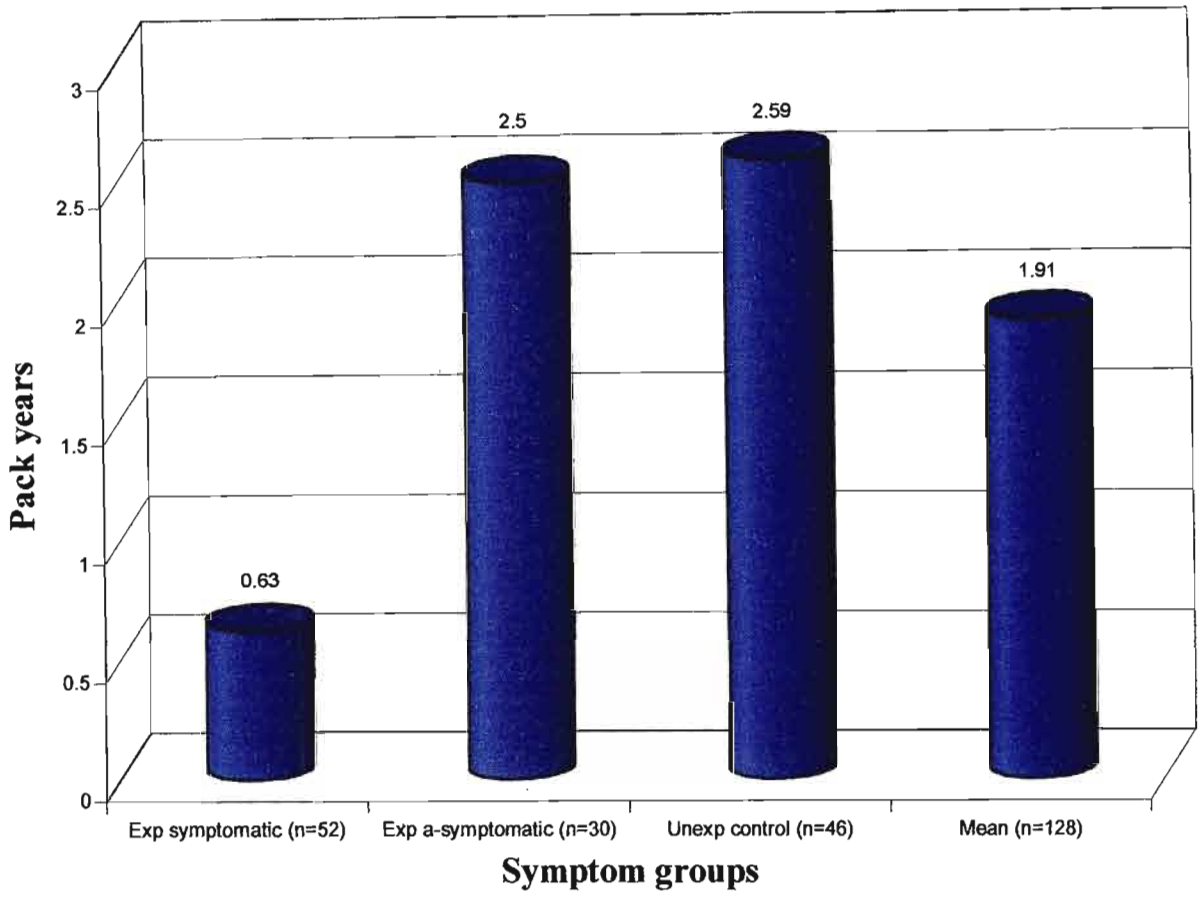
Although age was approximately group matched without consideration of gender, duration of exposure was not group matched and was significantly different ($P< 0.05$), with the longest exposure histories being evidenced by the exposed symptomatic males. Departments and occupations, but not geographical area ($P< 0.005$), were assessed as equivalent. However, as approximately half of the subject participants from each of the symptom groups originated from the Mooi River area, a common measure of socio-economic consistency was provided.

Smoking categories, apart from current smoking ($P< 0.005$), were assessed to be of even distribution, with more exposed asymptomatic current smokers.

Although these results are not shown in Table 4.1, only 29 workers had worked in another department within the cotton mill, and only six subjects had worked in a dusty work environment other than a cotton mill. Females were found to have less job variation in the cotton mills investigated than males.

The pack years of all symptom groups are shown in Figure 4.1.

Figure 4.1 Pack years of symptom groups



The pack years of symptom groups reflect the mean pack years of both the current smokers and ex-smokers. As is shown in Table 4.1, the exposed symptomatic group had higher numbers of ex-smokers than the exposed asymptomatic or unexposed control groups. However, Figure 4.1 indicates that the current-smokers and ex-smokers in the exposed symptomatic group smoked for fewer pack years than the exposed asymptomatic or unexposed control groups.

4.2 RESPIRATORY DISORDERS

The findings with regard to questionnaire reported respiratory disorders[23] are detailed in Table 4.2.

Table 4.2 Questionnaire reported respiratory disorders across all symptom groups – Numbers (%)

Respiratory disorders		Exposed		Unexposed	P
		Exposed symptomatic group	Exposed asymptomatic group	Unexposed control group	
Cough		8 (15.4)	2 (6.7)	3 (6.5)	0.26
Phlegm		8 (15.4)	4 (13.3)	-	0.023
*Grade resp. tract irritation	0	42 (80.8)	26 (86.7)	43 (93.5)	0.018
	1	2 (3.8)	-	2 (4.3)	
	2	5 (9.6)	3 (10.0)	1 (2.2)	
	3	3 (5.8)	1 (3.3)	-	
Grade of breathlessness	Nil	11 (22.0)	16 (53.3)	24 (53.3)	0.34
	A	15 (30.0)	9 (30.0)	12 (26.7)	
	B	20 (40.0)	5 (16.7)	9 (20.0)	
	C	2 (4.0)	-	-	
	D	2 (4.0)	-	-	
Tightness of chest	No tightness	36 (69.2)	28 (93.3)	43 (93.5)	0.085
	Tight at work	4 (7.7)	1 (3.3)	-	
	Tight on Monday	2 (3.8)	-	-	
	Tight general	2 (3.8)	1 (3.3)	-	
	Tight other days	7 (13.5)	-	3 (6.5)	
	Tight more days	1 (1.9)	-	-	
Wheeze		11 (21.2)	3 (10.0)	2 (4.3)	0.038
Grade of byssinosis	0	22 (42.3)	30 (100)	46 (100)	0.000
	B1	21 (40.4)			
	B2	9 (17.3)			

[23] The grading categorisation systems used for the various respiratory disorders are shown in the questionnaire, attached as Annexure 1.

No significant differences were observed between symptom groups for cough, but significant differences between symptom groups were shown for both phlegm ($P< 0.05$) and RTI ($P< 0.05$). Breathlessness, tightness of chest and byssinosis were not independent to symptom grouping. Grade of byssinosis ($P< 0.005$) was highly significant, tightness of chest was significant at the borderline level ($P< 0.10$) and grade of breathlessness was not significant between symptom groups. As byssinosis was not independent to symptom status, the significantly higher ($P< 0.005$) frequency of byssinosis in the exposed symptomatic group was expected. As an additional symptom of byssinosis, wheeze was partly dependent on the classical symptoms of tightness of chest and shortness of breath. Consequently, due to the inclusion of these classical symptoms in the symptom status definition [24] a significantly higher percentage ($P< 0.05$) of subjects in the exposed symptomatic group were found to have wheeze.

4.3 PULMONARY FUNCTION TESTING

The following section details the relationship of pulmonary function testing results [25] to various parameters. These parameters include effects of work department, smoking, byssinosis grades and symptom status as shown in Table 4.3. Results in this section were generated using, pulmonary function measurements over predictive values expressed as a percentage, as the pulmonary function input variables[26].

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[24] See Definitions, page XV.

[25] Percentages of predicted values were used in pulmonary function analyses, as they were normalised values, which Fishwick *et al.* (1996) identify as the most clinically relevant in studies of cotton workers.

[26] Detailed analyses of multiple pulmonary function variables to symptom status are shown in Annexure 2.

Table 4.3 Percentage of predicted baseline FEV_1 and ΔFEV_1 across varying symptom groups - Numbers

Pulmonary function		Exposed symptomatic		Exposed asymptomatic				P
		*Baseline FEV_1 %	*ΔFEV_1 %	*Baseline FEV_1 %	*ΔFEV_1 %	*Baseline FEV_1 %	*ΔFEV_1 %	
Work departments						Total		
	Mean	86.31	-4.14	111.00	-0.04	95.52	-2.61	
Spinning	n	42	42	25	25	67	67	
	SD	22.75	6.07	12.34	2.75	22.84	5.44	
	Mean	82.60	-5.20	124.00	1.00	89.50	-4.17	*0.83
Winding	n	5	5	1	1	6	6	**0.55
	SD	34.05	5.17	.	.	34.83	5.27	
	Mean	84.20	-9.40	108.25	1.50	94.89	-4.56	
Weaving	n	5	5	4	4	9	9	
	SD	13.90	6.88	16.11	5.20	18.83	8.17	
***Smoking status						Unexposed controls		
	Mean	92.00	-5.00	113.67	-0.33			*0.076
Current	n	2	2	6	6	-	-	**0.77
	SD	26.87	1.41	15.44	4.23			
	Mean	77.38	-2.23	106.00	2.00	95.71	-0.00	*0.014
Ex	n	13	13	3	3	7	7	**0.31
	SD	18.68	5.04	24.33	1.00	11.15	3.83	
	Mean	88.56	-5.71	111.05	0.10	98.16	-.3947	*0.33
Never	n	34	34	21	21	38	38	**0.43
	SD	24.84	6.72	10.35	2.90	14.18	4.28	
RTI grades						Unexposed controls		
	Mean	86.64	-4.14	112.00	0.46	98.84	-0.81	
0	n	42	42	26	26	43	43	
	SD	24.00	5.87	13.19	3.15	12.47	3.92	
	Mean	92.50	-5.00			79.50	5.50	
1	n	2	2	-	-	2	2	*0.31
	SD	13.44	4.24			28.99	3.54	**0.035
	Mean	76.60	-6.00	108.00	-0.67	86.00	6.00	
2	n	5	5	3	3	1	1	
	SD	23.07	6.96	2.00	0.58	.	.	
	Mean	84.00	-11.00	96.00	-4.00			
3	n	3	3	1	1	-	-	
	SD	10.54	9.17	.	.			
Byssinosis grades						Unexposed controls		
	Mean	93.14	-6.09	111.07	0.20	97.72	-0.39	
0	n	22	22	30	30	46	46	
	SD	21.93	6.26	12.64	3.06	13.52	4.15	*0.000
	Mean	84.00	-2.76					**0.035
B1	n	21	21	-	-			
	SD	23.72	5.89					
	Mean	71.78	-6.11					
B2	n	9	9	-	-			
	SD	17.03	5.86					
Symptom status						Unexposed controls		
Symptom groups	Mean	85.75	-4.75	111.07	0.20	97.72	-0.39	
	n	52	52	30	30	46	46	*0.000
	SD	22.88	6.16	12.64	3.06	13.52	4.15	**0.000

*Baseline FEV_1 P, values, **ΔFEV_1 P, values

***P values, shown in Table 4.3 of the previous page, were derived from group mean comparisons of the following groups: For current smokers the group mean differences between current- and ex-smokers / never smokers combined. For ex-smokers the group mean differences between ex-smokers and current smokers / never smokers combined. For never smokers the group mean differences between never- and current smokers /ex-smokers combined

The majority of cotton exposed subjects worked in spinning, the department in a cotton mill with the highest respiratory risk. Differences between work departments within the cotton mill for both baseline FEV_1 and ΔFEV_1 were found not to be significant.

The only significantly different ($P< 0.01$) values that were shown for smoking categories were between ex-smokers and current smokers /never smokers combined. In this analysis the ex-smokers had comparatively lowered baseline FEV_1 values. The trend of lower baseline FEV_1 values in ex-smokers when compared to current smokers or never smokers was apparent to a smaller extent in the exposed asymptomatic and unexposed control groups. An apparent anomaly was evidenced, with current smoking exposed asymptomatic subjects having shown higher mean baseline FEV_1 values than any group of ex-smokers or never smokers.

Exposed symptomatic current smokers had strong ΔFEV_1 declines, although these were still not as strong as the declines seen in the never smokers in this group. However, numbers of current smokers in this group were small.

For grades of byssinosis, baseline FEV_1 ($P< 0.005$) and ΔFEV_1 ($P< 0.05$) were shown to be significant between groups. Exposed subjects with the most severe grade of byssinosis (B2) had both the lowest baseline FEV_1 and the highest declines in ΔFEV_1. Since symptom group

status and baseline FEV_1 ($P< 0.005$) and ΔFEV_1 ($P< 0.005$) were not independent, the highly significant relationship seen between these variables was expected.

Baseline FEV_1 and ΔFEV_1 results[27], together with a frequency correlation of the variables, are shown in Table 4.4.

Table 4.4 Correlation of baseline decreases in FEV_1 against ΔFEV_1 – Numbers (%)

Pulmonary function	Exposed and unexposed				
	ΔFEV_1				
Baseline FEV_1	<5% decrease in FEV_1	5-10% decrease in FEV_1	10-20% decrease in FEV_1	>20% decrease in FEV_1	Total
	*Exposed symptomatic group				
>80%	9 (34.6)	14 (73.7)	3 (50.0)	1 (100.0)	27 (51.9)
60-79%	12 (46.2)	5 (26.3)	1 (16.7)	-	18 (34.6)
<60%	5 (19.2)	-	2 (33.3)	-	7 (13.5)
Total	26 (100.0)	19 (100.0)	6 (100.0)	1 (100.0)	52 (100.0)
	**Exposed asymptomatic group				
>80%	30 (100.0)	-	-	-	30 (100.0)
60-79%	-	-	-	-	-
<60%	-	-	-	-	-
Total	30 (100.0)	-	-	-	30 (100.0)
	***Unexposed control group				
>80%	34 (87.2)	5 (100.0)	2 (100.0)	-	41 (89.1)
60-79%	4 (10.3)	-	-	-	4 (8.7)
<60%	1 (2.6)	-	-	-	1 (2.2)
Total	39 (100.0)	5 (100.0)	2 (100.0)	-	46 (100.0)

*P = 0.087, Pearson's r = -0.212, significance P = 0.13
** Chi-square or Pearson's correlation cannot be computed, as there is only a single category
*** P = 0.90, Pearson's r = -0.130, significance P = 0.38

Although the Chi-square statistic (P) or Pearson's correlation (r) do not show any significant differences or associations for any of the symptom groups, the data reveal an interesting visual pattern. In the exposed symptomatic group there were a number of subjects who had both acute (decline in ΔFEV_1) and chronic (decline in baseline FEV_1) respiratory symptoms.

[27] According to WHO (1987) categorisation.

The exposed asymptomatic group had no workers with acute or chronic respiratory symptoms, due to the inclusion and exclusion criteria[28]. Subjects with acute or chronic respiratory symptoms in the unexposed control group had either acute or chronic respiratory symptoms with no overlapping of acute and chronic symptoms in subjects as seen in the exposed symptomatic group.

4.4 IMMUNOLOGICAL TESTING

The comparison of measured mean to normal reference concentrations is shown in sub-section 4.4.1. The results of the relationship between inter-correlation of immunological parameters are detailed in sub-section 4.4.2 and the influence of demographics[29] on immunological parameters is outlined in sub-section 4.4.3. Further, the influences of clinical symptoms on immunological parameters are shown in sub-section 4.4.4, and immunological findings relative to pulmonary function is provided in sub-section 4.4.5. An analysis of the immunological parameters linked to both byssinosis grading and pulmonary function is shown in sub-section 4.4.6. The differences in immunological parameter concentrations for exposure and symptom groups are detailed in sub-section 4.4.7. Lastly, more detailed analyses of the specific IgG parameter are shown in sub-section 4.4.8.

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[28] These criteria were specified, in Chapter 3, Section 3.5: Selection criteria for symptom groups

[29] As the symptom groups were approximately matched for age and gender and work department, it was not appropriate or necessary to control for these variables in the analysis of immunological data.

4.4.1 Comparison of measured mean to normal reference concentrations

A comparison of adult normal IgG means, as well as normal ranges to study means for males and females, are shown in Table 4.5.

Table 4.5 Exposed study mean concentrations compared to adult normal IgG mean and range concentrations – Numbers (%)

Immunological parameters		Study mean (Exposed) n=69	Normal mean n=64	% Study mean higher than normal mean	95% Range (Mean ± 2 SD)	
					Normal lower range	Normal upper range
Males	*IgG_1 (g/l)	13.34 (66.08)	6.74	49.45	4.56	8.93
	*IgG_2 (g/l)	4.46 (22.20)	3.63	18.65	1.99	5.27
	*IgG_3 (g/l)	1.80 (8.96)	0.58	67.61	0.17	1.00
	*IgG_4 (g/l)	0.51 (2.54)	0.41	19.80	0.14	0.74
	*Total IgG (g/l)	20.09 (100)	12.45	38.03	8.21	16.69
Females	*IgG_1 (g/l)	13.85 (66.08)	6.66	51.95	4.56	8.75
	*IgG_2 (g/l)	4.86 (23.19)	3.24	33.37	1.89	4.59
	*IgG_3 (g/l)	1.80 (8.59)	0.55	69.22	0.20	0.91
	*IgG_4 (g/l)	0.45 (2.15)	0.36	20.44	0.72	0.64
	*Total IgG (g/l)	20.96 (100)	12.23	41.65	8.19	16.27

*Normal ranges obtained from the manufacture of the kit: The Binding Site LTD (1999), values are for European populations

The study mean in males for IgG_2 and IgG_4 was higher than the normal mean but below the upper normal range 95% CI, while for IgG_1 and IgG_3 the study mean for males was higher than the upper normal range 95% CI. For total IgG the study mean for males was higher than the upper normal range 95% CI.

In females the study mean for IgG_4 was shown to be higher than the normal mean, but below the upper normal range 95% CI. For IgG_1, IgG_2 and IgG_3 for females the study mean was higher than the upper normal range 95% CI. The study mean recorded for total IgG in

females was higher than the upper normal range 95% CI. No reference ranges[30] were available for specific IgG.

4.4.2 Correlation among immunological parameters

Before establishing what relationships exist between immunological variables and non-immunological variables, it is appropriate to examine the correlations that exist between the immunological variables themselves. Table 4.6 shows these correlations.

Table 4.6 Correlation among immunological parameters – Numbers (%)

Immunologial parameters		Exposed and unexposed					
		IgG_1 (g/l)	IgG_2 (g/l)	IgG_3 (g/l)	IgG_4 (g/l)	Total IgG (g/l)	Specific IgG (mg A/l)
IgG_1 (g/l)	r	1.000	-0.117	0.449**	0.333**	0.970**	-0.073
	P	-	0.18	0.000	0.000	0.000	0.41
IgG_2 (g/l)	r	-0.117	1.000	-0.023	0.060	0.102	0.161
	P	0.18	-	0.80	0.50	0.25	0.071
IgG_3 (g/l)	r	0.449**	-0.023	1.000	0.298**	0.534**	-0.065
	P	0.000	0.80	-	0.001	0.000	0.46
IgG_4 (g/l)	r	0.333**	0.060	0.298**	1.000	0.398**	0.160
	P	0.000	0.50	0.001	-	0.000	0.073
Total IgG (g/l)	r	0.970**	0.102	0.534**	0.398**	1.000	-0.034
	P	0.000	0.25	0.000	0.000	-	0.70
Sp IgG (mg A/l)	r	-0.073	0.161	-0.065	0.160	-0.034	1.000
	P	0.41	0.071	0.46	0.073	0.70	-

r is Pearson's correlation (bivariate)
** Correlation is significant at the 0.01 level (2-tailed)

Specific IgG showed the strongest correlation to IgG_2 and IgG_4, bordering on significance ($P< 0.10$). Highly significant ($P< 0.005$) correlation relationships were shown between IgG_1, IgG_3 and IgG_4. Since total IgG was not independent to the IgG subclasses, inter-correlation relationships between these variables were not meaningful.

[30] No range for specific IgG against antigens contained in ACDE was available from the supplier of the kit, namely Pharmacia & Upjohn. Both Noweir (1981) and Sigsgaard *et al.* (1993) used methods that did not produce results measured as specific IgG concentrations.

4.4.3 Influence of demographics on immunological parameters

The influence of demographic parameters on the specified IgG parameters are detailed in Table 4.7.

Table 4.7 Relationship between demographic and immunological parameters – Numbers

Demographic parameter	Category		Immunoassays					
			IgG_1 (g/l)	IgG_2 (g/l)	IgG_3 (g/l)	IgG_4 (g/l)	Total IgG (g/l)	Sp IgG (mg A/l)
	P (ANOVA)		0.094	0.21	0.14	0.043	0.12	0.31
	*P (Interaction)		0.000	0.80	0.15	0.42	0.001	0.99
Age (years)	<45	Mean	14.51	4.48	1.89	0.53	21.41	18.64
	(n=81)	SD	8.48	1.83	1.01	0.43	9.02	18.19
	>45	Mean	11.97	4.91	1.64	0.39	18.90	15.51
	(n=47)	SD	7.65	1.93	0.81	0.27	8.39	14.22
	P (ANOVA)		0.278	0.111	0.392	0.910	0.621	0.539
	*P (Interaction)		0.422	0.592	0.498	0.677	0.506	0.152
Exposure duration (years)	<20	Mean	13.31	4.36	1.72	0.44	19.84	21.00
	(n=49)	SD	7.95	1.73	0.96	0.42	8.26	22.90
	>20	Mean	11.67	5.04	1.91	0.43	19.04	18.26
	(n=33)	SD	4.08	2.04	1.01	0.27	5.47	13.66
	P (ANOVA)		0.72	0.22	0.95	0.36	0.58	0.83
	*P (Interaction)		0.56	0.37	0.57	0.73	0.72	0.33
Gender	Male	Mean	13.34	4.46	1.80	0.51	20.09	17.76
	(n=69)	SD	8.42	1.68	0.98	0.43	8.98	18.98
	Female	Mean	13.85	4.86	1.80	0.45	20.96	17.14
	(n=59)	SD	8.10	2.06	0.91	0.31	8.73	14.02
	P (ANOVA)		0.26	0.92	0.49	0.086	0.28	0.096
	*P (Interaction)		0.85	0.15	0.71	0.27	0.89	0.57
Department (Only exposed subjects)	Spinning	Mean	12.78	4.69	1.84	0.42	19.72	19.80
	(n=67)	SD	7.01	1.95	1.00	0.34	7.62	16.35
	Winding	Mean	14.75	4.14	2.04	0.29	21.21	13.73
	(n=6)	SD	6.4857	1.82	0.57	0.17	5.86	7.85
	Weaving	Mean	10.30	4.59	1.36	0.63	16.88	24.73
	(n=9)	SD	3.26	1.38	1.00	0.56	4.47	40.16
	P (ANOVA)		0.54	0.22	0.50	0.43	0.35	0.69
Smoking status	Current	Mean	11.87	3.87	1.58	0.38	17.70	15.24
	(n=8)	SD	6.07	1.36	1.03	0.20	6.60	10.83
	P (ANOVA)		0.17	0.85	0.28	0.65	0.23	0.39
	Ex	Mean	11.44	4.58	1.99	0.51	18.50	14.78
	(n=23)	SD	5.77	1.46	1.06	0.43	7.48	9.57
	P (ANOVA)		0.29	0.59	0.82	0.99	0.28	0.62
	Never	Mean	14.23	4.74	1.78	0.48	21.23	18.31
	(n=97)	SD	8.97	2.02	0.93	0.39	9.38	18.88

*P is [31]interaction between immunoassays - demographic variable - all symptom status

[31] P values shown relate to the variable of concern and to the immunological variable, specifically excluding symptom status from analysis. Cont pg 119

Only IgG_4 was found to be significantly different ($P< 0.05$) for age category above and below 45 years of age, but significant interactions between IgG_1 – age – symptom status ($P< 0.005$) and total IgG – age – symptom status ($P< 0.005$) were shown. Work departments were significant at the borderline level of significance ($P< 0.10$) for specific IgG. No significant associations or interactions were noted for gender or smoking categories. Although a slight trend of decreasing total IgG concentrations in ex- and current smokers was noted, numbers of current smokers were small.

4.4.4 Influence of respiratory disorders on immunological parameters

Analyses of the association shown between the clinical symptoms of RTI, grade of breathlessness, tightness of chest and byssinosis grades are shown in Table 4.8.

Cont from pg118. The interaction P values show the relationship between the variable of concern and the immunological variable, but with the inclusion of symptom status in the analysis.

Table 4.8 Relationship between respiratory disorders and immunological parameters – Numbers

Clinical symptoms	Grade		Immunoassays					
			IgG_1 (g/l)	IgG_2 (g/l)	IgG_3 (g/l)	IgG_4 (g/l)	Total IgG (g/l)	Sp IgG (mg A/l)
	P (ANOVA)		0.68	0.99	0.46	0.18	0.67	0.11
	*P (Interaction)		0.79	0.28	0.49	0.18	0.62	0.17
Respiratory tract irritation	Grade 0 (n=111)	Mean	13.89	4.62	1.83	0.47	20.81	16.78
		SD	8.66	1.92	0.98	0.37	9.23	13.43
	Grade RTI1 (n=4)	Mean	9.56	4.59	1.24	0.41	15.80	13.85
		SD	3.07	2.37	0.66	0.14	5.16	9.86
	Grade RTI2 (n=9)	Mean	12.19	4.83	1.91	0.40	19.29	18.86
		SD	4.81	1.28	0.67	0.32	5.73	17.50
	Grade RTI3 (n=4)	Mean	12.06	4.75	1.33	0.87	19.01	37.28
		SD	5.01	1.74	0.54	0.80	5.41	62.26
	P (ANOVA)		0.29	0.29	0.29	0.52	0.050	0.20
	*P (Interaction)		0.57	0.57	0.57	0.24	0.60	0.45
Grade of breathlessness	Nil (n=51)	Mean	12.29	4.88	1.68	0.55	19.40	17.54
		SD	6.27	2.02	0.73	0.41	7.38	20.22
	A (n=36)	Mean	13.54	4.28	1.70	0.37	19.89	15.24
		SD	9.45	1.55	0.87	0.27	9.83	11.28
	B (n=34)	Mean	16.20	4.61	2.17	0.51	23.47	16.65
		SD	9.58	1.98	1.22	0.44	9.86	11.88
	C (n=2)	Mean	10.24	5.20	0.09	0.19	16.51	41.69
		SD	2.77	1.79	0.01	0.04	4.54	50.78
	D (n=2)	Mean	12.47	5.92	2.53	0.50	21.41	40.80
		SD	9.53	1.92	0.89	0.01	6.73	36.80
	P (ANOVA)		0.93	0.79	0.014	0.64	0.91	0.30
	*P (Interaction)		0.47	0.52	0.18	0.48	0.32	0.93
Tightness of chest	No tightness (n=108)	Mean	13.86	4.62	1.74	0.47	20.69	17.55
		SD	8.73	1.94	0.87	0.38	9.28	17.06
	Tight at work (n=4)	Mean	14.13	4.92	3.00	0.80	22.85	21.60
		SD	4.92	1.48	1.98	0.61	7.31	13.52
	Tight on Monday (n=2)	Mean	12.47	5.92	2.53	0.50	21.41	40.80
		SD	9.53	1.92	0.89	0.01	6.73	36.77
	Tight general (n=3)	Mean	12.60	3.59	2.97	0.53	19.69	10.63
		SD	2.31	0.46	1.57	0.53	4.40	9.21
	Tight other days (n=10)	Mean	11.17	4.92	1.46	0.43	17.94	14.20
		SD	4.83	1.56	0.60	0.30	6.12	10.96
	Tight more days (n=1)	Mean	9.18	3.79	1.69	0.66	15.32	0.00
		SD	.	.	.	.	.	.
	P (ANOVA)		0.81	0.47	0.066	0.57	0.98	0.028
**Byssinosis grades	B0 (n=98)	Mean	13.83	4.53	1.71	0.46	20.54	15.37
		SD	9.09	1.91	0.83	0.36	9.69	11.17
	B1 (n=21)	Mean	12.79	4.93	2.24	0.56	20.51	22.74
		SD	4.73	1.84	1.36	0.51	4.92	27.99
	B2 (n=9)	Mean	12.57	5.16	1.78	0.44	19.90	27.99
		SD	5.69	1.44	0.82	0.30	6.48	27.56

*P is interaction between immunoassays - clinical symptom - all symptom status

**Unexposed control subjects excluded from analysis, as workers not exposed to cotton, cannot have byssinosis

No statistically significant differences were found between grades of RTI. However, total IgG was found to be significantly different ($P < 0.05$) between grades of breathlessness, IgG_3 was significantly different for chest tightness ($P < 0.01$) and specific IgG was significantly different for grades of byssinosis ($P < 0.05$). IgG_3 was at the borderline level of significance ($P < 0.10$) for byssinosis grades.

For purposes of later discussion it is necessary to show a further breakdown of IgG_3 and specific IgG for the 10 subjects (shown in Table 4.8) with tightness of chest on other days, within symptom groupings. Seven of the 10 subjects were from the exposed symptomatic group with mean IgG_3 concentrations of 1.53 g/l and specific IgG concentrations of 17.21 mg A/l. The remaining 3 of the 10 subjects were from the unexposed control group with mean IgG_3 concentrations of 1.30 g/l and specific IgG concentrations of 7.17 mg A/l.

Analyses of homogenous subsets for these clinical symptoms between immunoassays reveal a number of additional relationships as well as trends within those relationships which are mentioned as being significantly different. These relationships are shown in Table 4.9.

Table 4.9 Mean concentrations of immunoassay results in homogenous subsets of clinical symptom grades - Numbers

Immunoassays	Clinical symptoms	Exposed and unexposed		
		n	Subset for alpha = 0.05	
			Subset 1	Subset 2
	RTI grade			
IgG_4 (g/l)	2	8	0.40	-
	1	4	0.41	-
	0	111	0.47	0.47
	3	4	-	0.873
HM = 6.309	P		0.74	0.06
Specific IgG (mg_A/l)	1	4	13.85	-
	0	111	16.78	-
	2	8	18.86	-
	3	4	-	37.28
HM = 6.450	P		0.61	1.00
	Grade of breathlessness			
IgG_3 (g/l)	C	2	0.88	-
	Nil	51	1.68	1.68
	A	36	1.70	1.70
	B	34	2.17	2.17
	D	2	-	2.53
HM = 4.643	P		0.054	0.21
Specific IgG (mg_A/l)	A	36	15.24	-
	B	34	16.65	-
	Nil	51	17.54	-
	D	2	-	40.80
	C	2	-	41.69
HM = 4.642	P		0.85	0.94
	Grade of byssinosis			
Specific IgG (mg_A/l)	B0	97	15.37	
	B1	21	22.74	22.74
	B2	9		27.99
HM = 17.75	P		0.18	0.34

HM is harmonic mean
Post-hoc Duncan test

Those workers with grade 3 RTI had significantly raised IgG_4 and significantly raised specific IgG concentrations. Specific IgG showed a fairly logical progression, with subjects with the most severe RTI grades having the highest concentrations. Significantly raised concentrations of specific IgG were shown in those workers with the two most severe grades of breathlessness. Only those workers with the most severe grade of breathlessness had significantly raised IgG_3 concentrations. Those subjects with grade B2 byssinosis had significantly higher mean specific IgG values.

4.4.5 Influence of pulmonary function on immunological parameters

Results of the association between baseline FEV_1 and Δ FEV_1 declines to specified immunological parameters are detailed in Table 4.10. Specific IgG homogenous subset concentrations are detailed in baseline FEV_1 groupings in Table 4.11.

Table 4.10 Influence of pulmonary function on immunological parameters – Numbers

Pulmonary function	Category		Immunoassays					
			IgG_1 (g/l)	IgG_2 (g/l)	IgG_3 (g/l)	IgG_4 (g/l)	Total IgG (g/l)	Sp IgG (mg A/l)
	P (ANOVA)		0.62	0.29	0.25	0.50	0.77	0.019
	*P (Interaction)		0.65	0.35	0.94	0.51	0.52	0.49
Baseline FEV_1	FEV_1>80%	Mean	13.82	4.72	1.75	0.49	20.77	16.12
	(n=98)	SD	8.57	1.94	0.94	0.40	9.31	15.88
	$FEV_1$60-79%	Mean	13.48	4.12	1.84	0.47	19.90	17.68
	(n=22)	SD	8.06	1.37	1.02	0.35	8.13	16.63
	FEV_1<60%	Mean	10.88	5.16	2.33	0.33	18.70	33.40
	(n=8)	SD	3.57	2.09	0.77	0.19	3.67	22.39
	P (ANOVA)		0.031	0.94	0.65	0.51	0.049	0.097
	*P (Interaction)		0.015	0.65	0.33	0.025	0.022	0.101
Δ FEV_1 declines	<5%	Mean	13.10	4.64	1.78	0.46	19.98	16.12
	(n=95)	SD	7.76	1.71	0.96	0.34	8.39	13.14
	5-10%	Mean	17.02	4.71	1.79	0.50	24.02	18.67
	(n=24)	SD	10.26	2.50	0.88	0.44	10.89	12.01
	>10->20%	Mean	9.38	4.45	2.08	0.61	16.51	28.59
	(n=9)	SD	3.29	1.72	1.05	0.61	3.76	43.35

*P is interaction between immunoassays - clinical symptom - all symptom status

Specific IgG emerged as the only immunological parameter that was statistically significant ($P< 0.05$) between baseline FEV_1 groupings. Both IgG_1 ($P< 0.05$) and total IgG ($P< 0.05$) were significantly different between Δ FEV_1 decline groupings. The total IgG result was influenced by the IgG_1 result, as the parameters were not independent of each other. Specific IgG was at the borderline level of significance ($P< 0.10$) for Δ FEV_1. Significant interaction effects were shown between IgG_1 - Δ FEV_1 - symptom status ($P< 0.05$), IgG_4 - Δ FEV_1 - symptom status ($P< 0.05$) and total IgG - Δ FEV_1 - symptom status ($P< 0.05$). Borderline significance ($P< 0.10$) interaction effects were noted between specific IgG - Δ FEV_1 – symptom status.

Table 4.11 Mean concentrations of immunological parameters in homogenous subsets of baseline FEV_1 and Δ FEV_1 declines - Numbers

Immunoassays	Pulmonary function	Exposed and unexposed		
		N	Subset for alpha = 0.05	
			Subset 1	Subset 2
	Baseline FEV_1			
	>80%	97	16.12	-
Specific IgG (mg_A/l)	60-79%	22	17.68	-
	<60%	8	-	33.40
HM = 16.60	P		0.79	1.00
	Δ FEV_1 declines			
	>10->20%	9	9.38	-
IgG_1 (g/l)	<5%	95	13.10	13.10
	5-10%	24	-	17.02
HM = 18.37	P		0.16	0.14
	>10->20%	9	16.51	-
Total IgG (g/l)	<5%	95	19.98	19.98
	5-10%	24	-	24.02
HM = 18.37	P		0.23	0.16
	<5%	94	16.12	-
**Specific IgG (mg_A/l)	5-10%	24	18.67	18.67
	>10->20%	9	-	28.59
HM = 18.36	P		0.64	0.071

HM is harmonic mean
Post-hoc Duncan test

Analyses by homogenous subsets gave a more detailed breakdown of the means of the individual baseline FEV_1 groupings for specific IgG. Subjects with the lowest baseline FEV_1 values had significantly higher concentrations of specific IgG.

Only specific IgG follows a logical pattern of increase in concentration with decrease in Δ FEV_1. IgG_1 is not independent of total IgG, which is part of the reason for the Δ FEV_1 intervals following the same pattern in the respective parameters.

4.4.6 Immunological parameters linked to both byssinosis grading and pulmonary function

Analyses of the interaction between the clinical grades of byssinosis, baseline FEV_1, as well as Δ FEV_1 declines to specified immunological parameters, are shown in Table 4.12.

Table 4.12 Interaction of immunological parameters between grades of byssinosis and baseline FEV_1 as well as Δ FEV_1 declines – Numbers

Immunoassays		Exposed, excludes unexposed controls Clinical symptoms of byssinosis									*P
		Grade B0			Grade B1			Grade B2			
		Baseline FEV_1									
		>80%	60-79%	<60%	>80%	60-79%	<60%	>80%	60-79%	<60%	
n		43	8	1	11	6	4	3	4	2	
IgG_1 (g/l)	Mean	12.88	11.30	11.25	12.21	14.60	11.70	15.71	11.41	10.19	0.83
	SD	7.38	9.29	.	4.27	6.28	3.67	8.27	3.48	6.31	
IgG_2 (g/l)	Mean	4.48	3.96	5.72	5.50	3.82	5.00	4.31	5.07	6.62	0.54
	SD	2.09	1.02	.	1.50	1.85	2.39	1.28	1.41	0.92	
IgG_3 (g/l)	Mean	1.61	1.59	2.47	2.10	2.54	2.16	1.55	1.45	2.76	0.55
	SD	0.80	0.59	.	1.47	1.50	1.074	0.72	0.72	0.57	
IgG_4 (g/l)	Mean	0.40	0.33	0.19	0.70	0.44	0.36	0.39	0.52	0.34	0.85
	SD	0.31	0.26	.	0.61	0.41	0.24	0.17	0.42	0.22	
Total IgG (g/l)	Mean	19.37	17.18	19.62	20.50	21.40	19.21	21.83	18.45	19.91	0.95
	SD	8.07	9.41	.	5.06	5.94	3.61	10.19	5.39	4.61	
Sp IgG (mg A/l)	Mean	17.46	12.64	50.30	24.64	15.15	28.90	16.53	30.92	39.30	0.52
	SD	12.60	8.60	.	36.54	8.10	21.95	3.54	35.85	38.89	
		Δ FEV_1									
		<5%	5-10%	>10->20%	<5%	5-10%	>10->20%	<5%	5-10%	>10->20%	
n		36	14	2	15	4	2	5	1	3	
IgG_1 (g/l)	Mean	12.24	13.76	11.15	12.51	16.58	7.37	15.06	8.28	9.86	0.71
	SD	7.82	7.43	5.16	3.89	6.25	0.96	6.07	.	4.50	
IgG_2 (g/l)	Mean	4.25	5.14	2.62	5.07	4.41	4.92	5.10	3.94	5.68	0.38
	SD	1.51	2.78	0.26	1.68	2.70	2.16	1.41	.	1.76	
IgG_3 (g/l)	Mean	1.53	1.75	2.52	2.44	1.77	1.66	1.58	0.87	2.40	0.21
	SD	0.59	0.93	2.05	1.48	0.94	1.12	0.73	.	0.74	
IgG_4 (g/l)	Mean	0.40	0.38	0.04	0.49	0.38	1.40	0.50	0.21	0.45	0.010
	SD	0.33	0.20	0.04	0.42	0.27	0.92	0.39	.	0.24	
Total IgG (g/l)	Mean	18.42	21.02	16.33	20.50	23.14	15.35	22.14	13.30	18.38	0.71
	SD	8.07	8.63	7.52	4.90	4.62	1.40	7.50	.	4.20	
Sp IgG (mg A/l)	Mean	15.66	21.84	16.37	19.15	12.07	71.00	33.50	5.78	26.20	0.008
	SD	12.77	13.03	12.07	14.43	12.02	83.72	26.83	.	35.65	

*P is interaction between immunological parameter - byssinosis grades - baseline FEV_1 / Δ FEV_1 groupings

Interaction effects were only noted for Δ FEV_1 groupings. Subjects with grade B1 byssinosis had increasing concentrations of IgG_4 ($P< 0.01$) and specific IgG ($P< 0.01$) for increasing severity of Δ FEV_1 groupings, which were both statistically significant. However, numbers of subjects with grade B1 byssinosis were small and the same trends, noted above, were not seen in subjects with grade B2 byssinosis.

4.4.7 Differences in immunological parameter concentrations for exposure and symptom groups

Differences in immunological parameter concentrations between various combinations of symptom/exposure groups are shown in Table 4.13.

Table 4.13 Comparison of immunological parameters between exposed / unexposed groups and symptomatic / asymptomatic / unexposed groups – Numbers

Immunoassays		Includes two groups exposed and unexposed				
		Exposed			Unexposed	P
N		82			46	
IgG_1 (g/l)	Mean	12.65			15.22	0.14
	SD	6.68			10.35	
IgG_2 (g/l)	Mean	4.64			4.65	0.96
	SD	1.88			1.88	
IgG_3 (g/l)	Mean	1.80			1.80	0.99
	SD	0.98			0.90	
IgG_4 (g/l)	Mean	0.43			0.56	0.073
	SD	0.37			0.39	
Total IgG (g/l)	Mean	19.51			22.23	0.14
	SD	7.24			11.02	
Specific IgG (mg_A/l)	Mean	19.90			13.08	0.007
	SD	19.65			8.37	
		Symptomatic	Asymptomatic	*P	Unexposed	**P
N		52	30		46	
IgG_1 (g/l)	Mean	12.46	12.98	0.736	15.22	0.23
	SD	5.58	8.35		10.35	
IgG_2 (g/l)	Mean	4.85	4.27	0.181	4.65	0.41
	SD	2.00	1.61		1.88	
IgG_3 (g/l)	Mean	1.96	1.51	0.020	1.80	0.12
	SD	1.11	0.60		0.90	
IgG_4 (g/l)	Mean	0.44	0.42	0.872	0.56	0.20
	SD	0.39	0.34		0.39	
Total IgG (g/l)	Mean	19.70	19.1	0.760	22.23	0.24
	SD	6.44	8.56		11.02	
Specific IgG (mg_A/l)	Mean	22.72	15.02	0.046	13.08	0.012
	SD	22.65	11.72		8.37	

*P values between the exposed symptomatic and exposed asymptomatic groups, excluding unexposed control group

**P values between all groups including exposed symptomatic, exposed asymptomatic and unexposed control group

The only parameter that was found to be statistically different ($P< 0.01$) between exposed and unexposed groups was specific IgG, aside from IgG_4 which was at the borderline level of significance ($P< 0.10$). For both parameters, concentrations and standard deviations were higher in the exposed group than in the unexposed group. The higher standard deviation in the exposed group indicates a greater distribution spread of immunological parameter results. Interestingly, total IgG concentrations were lower in the exposed group than in the unexposed group.

Significant differences were noted for IgG_3 ($P< 0.05$) and specific IgG ($P< 0.05$) between the exposed symptomatic and exposed asymptomatic group, excluding the unexposed control group.

Only specific IgG was found to be statistically significant ($P< 0.01$) between all symptom groups, including the unexposed control group. Specific IgG showed a progressive increase trend, with the lowest concentrations being recorded in the unexposed controls, followed by the exposed asymptomatic and the highest concentrations in the exposed symptomatic workers. The standard deviation for specific IgG showed the same trend as the concentrations, indicating an increasing variability in concentrations between subjects with exposure and symptoms.

Figure 4.2 and Figure 4.3 provide IgG subclass percentage composites, which are based on the results shown in Table 4.13.

Figure 4.2 Differences in IgG subclass percentage composites between exposed and unexposed symptom groups - %

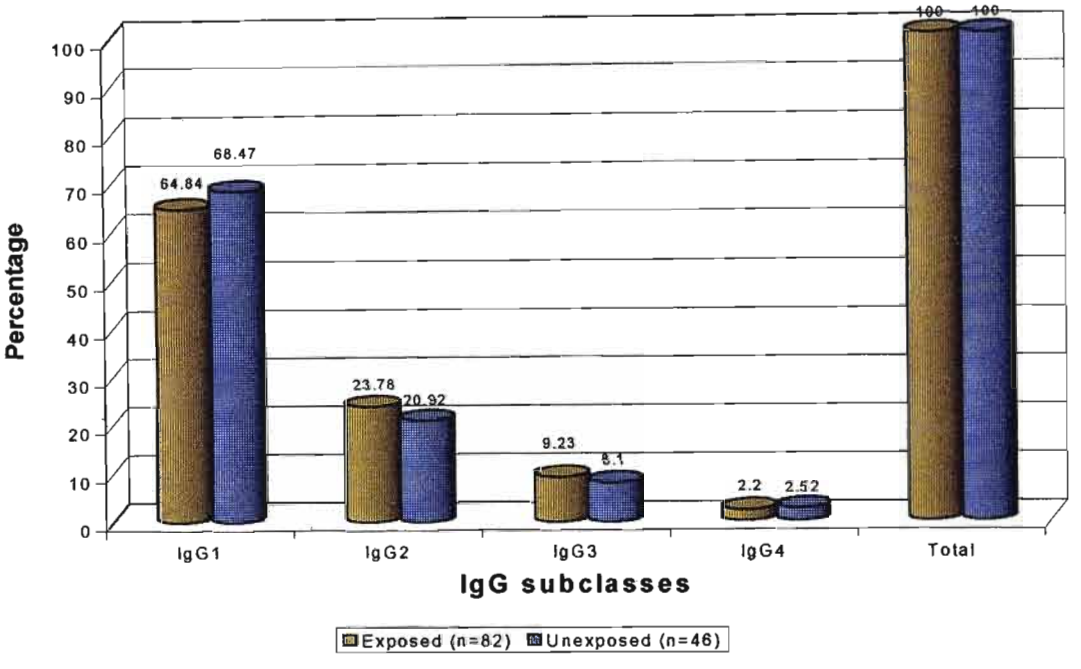
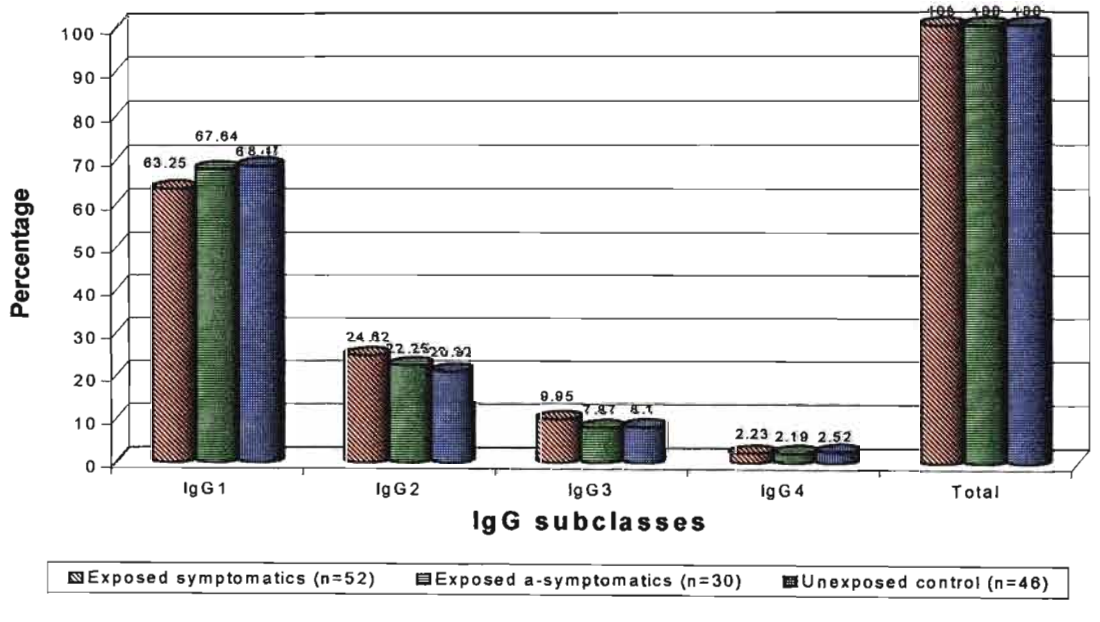


Figure 4.3 Differences in IgG subclass percentage composites between all symptom groups - %



Results of IgG subclass percentage composites shown in Figure 4.2 and Figure 4.3 indicate slight but important trends. It can be seen in Figure 4.2 that IgG_1 comprised a comparably lower and IgG_2 a comparably higher percentage of the total IgG subclass make-up in the exposed group. A further breakdown of symptom group results, as evidenced in Figure 4.3, followed a logical trend with the exposed symptomatic group having the lowest IgG_1 and highest IgG_2 concentrations.

4.4.8 Specific IgG

Results of mean concentrations of specific IgG are detailed in Table 4.14, with the aim of providing greater clarity on differences between symptom group concentrations, using the Duncan post-hoc test. Figure 4.4 shows a frequency distribution of specific IgG among all symptom groups.

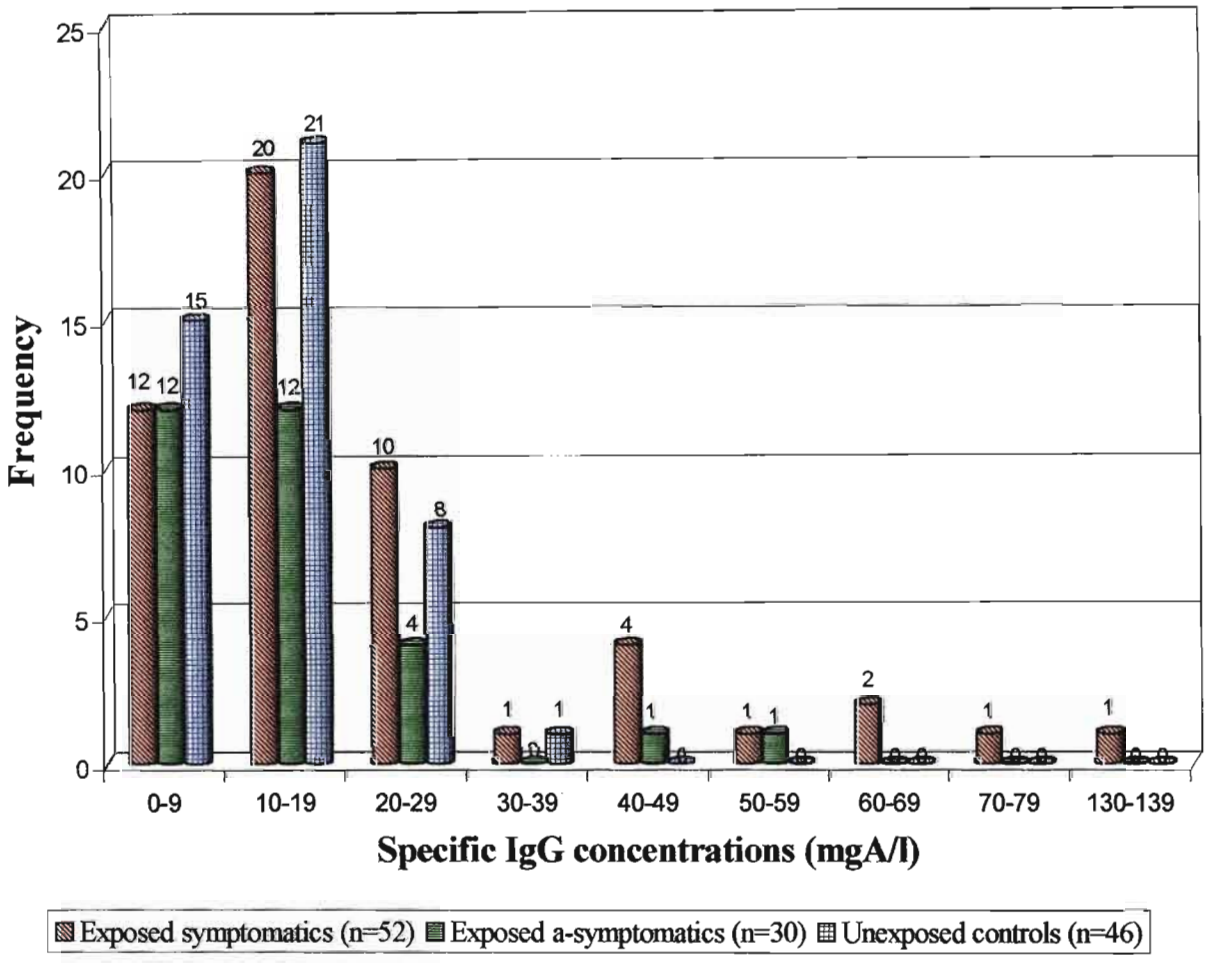
Table 4.14 Mean concentrations of specific IgG in homogenous subsets - Numbers

Specific IgG (mg_A/l)	n	Subset for alpha = 0.05	
All symptom status		Subset 1	Subset 2
Unexposed control	45	13.08	-
Exposed asymptomatic	30	15.02	-
Exposed symptomatic	52	-	22.72
P	P	0.60	1.00

Uses harmonic mean sample size 40.11
Post-hoc Duncan test

The exposed symptomatic group evidences specific IgG concentrations, which were sufficiently high to form an individual homogenous subset.

Figure 4.4 Frequency distribution of specific IgG interval concentrations among all symptom groups – Numbers (%)



The graph indicates that there is a logical interval dispersion frequency, for specific IgG within each of the individual symptom groups. As indicated in Figure 4.4, 9 subjects had raised specific IgG concentrations in the five top concentration intervals in comparison to 2 subjects from the exposed asymptomatic group and nil from the unexposed control group.

4.4.9 Summary

Analysis of homogenous subsets ($P< 0.05$) showed that workers with the most severe grades of breathlessness had raised IgG_3 levels and IgG_4 was raised in those workers with the most severe grade of RTI[32]. Further, specific IgG was raised in those subjects with the most severe grade of RTI, the most severe grades of breathlessness, the most severe grade of byssinosis[32] and tightness of chest on a Monday[33].

Analysis of homogenous subsets ($P< 0.05$) showed that subjects with the lowest baseline FEV_1 values and highest Δ FEV_1 declines had significantly higher concentrations of specific IgG[34].

Higher[35] and logically distributed[36] concentrations of specific IgG, were shown among symptom groups.

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[32] See Table 4.9.
[33] See Table 4.8.
[34] See Table 4.11.
[35] See Table 4.14.
[36] See Figure 4.4.

CHAPTER FIVE

DISCUSSION

5.0 INTRODUCTION

This comparative case control study of exposed symptomatic, exposed asymptomatic and unexposed control workers performed according to design, as was anticipated in Table 3.3. In terms of the symptom status case definition[37], all exposed subjects with clinical or functional symptoms of byssinosis, were categorised as exposed symptomatics. Therefore, no subjects in the exposed asymptomatic symptom group[38] had symptoms of breathlessness or tightness of chest or baseline FEV_1 values of <80% or Δ FEV_1 value declines of >5% of predicted value. Consequently, it is highly likely that some workers with clinical or functional respiratory symptoms, were given the classification of 'exposed symptomatic' status not because of their exposure to cotton, but rather as a result of non-cotton influences. This increased the chance of rejecting the operational hypotheses, as immunological variability due to cotton exposure between the exposed symptomatic and exposed asymptomatic symptom groups was decreased by this effect.

The results shown in Chapter four are discussed as follows: socio-demographic profile (5.1); respiratory disorders (5.2); pulmonary function (5.3); and immunological testing (5.4).

[37] See Definitions page XIV.
[38] See Table 4.4.

5.1 SOCIO-DEMOGRAPHIC FACTORS

When the study was initiated, cognisance was taken of the fact that subjects employed within the cotton industry came from differing localities and cotton processing factories. To ensure that the measurement of immunological parameters took place within a validated context, socio-demographic profiling of each subject was performed. This profiling included a description of study subjects, the duration of exposure, the geographical area of the cotton mill, the department within the mill, occupation within the mill and smoking status.

a) Description of subjects in the study, including race and age

Since all subjects were from the black race group, race was not a possible confounding variable for any study parameter. Additionally, a racially homogenous sample group working in the same industrial sector provided a measure of socio-economic consistency.

The mean subject age recorded in this study[39] was far higher than that recorded by White (1985), who conducted the largest, and perhaps only, prevalence rate study carried out in South Africa. This can be largely attributed to group matching for age in exposed asymptomatic and unexposed control workers against symptomatic workers. This case control study design differed from the prevalence study design selected and used by White.

[39] See Table 4.1.

b) Exposure duration

Exposure years for cotton workers in this study [40], in comparison to White's (1985) study (6.2% > 20yrs), are shown to be high. In his 1985 study White predicted the advent of longer exposure durations for the South African textile industry. The exposure duration results are therefore not unexpected. Results of this study indicate stable employment, with little change in either employer or department within the company within which workers were employed. The primary reason that this workforce appears to be so stable is that in the year 2000 the cotton industry in South Africa, despite it being an established industrial sector, was declining, due for the most part to the advent of free market globalization (Textile Federation, 1997). In 1985 the cotton industry was still being established as an industrial growth sector of the economy that was being established.

c) Geographical area

Since a comparative case control study design was used, subjects were not chosen randomly from the geographical areas. However, it was desirable to obtain a sample group that was socio-economically homogenous. This was attained as approximately half of the study subjects from each symptom group came from a single cotton mill in Mooi River [40], providing a common measure of socio-economic consistency.

[40] See Table 4.1.

d) Work department

It is well established that exposure risk varies by work departments within the cotton mill environment, with the highest exposure risk found in spinning and declining risk in winding and weaving (Simpson *et al.*, 1995). Since the distribution of subjects in work departments across the exposed symptomatic and exposed asymptomatic groups was equivalent[41], effects seen between these groups were unlikely to be as a result of differing exposures.

e) Occupations

There were no selection criteria placed on the occupations of study participants. The finding of males having more job variety in relation to females[41] was similar to the trend seen in White's (1985) study. This is probably due to socially sanctioned gender role assignment.

f) Smoking

The low number of smokers included in the current study[41], was unlikely to have had any effect on the allocation of symptom group status. The smokers in White's (1985) study had pack years for males of 4.38 and 0.75 for females, with comparatively lower pack years being recorded in the current study[42].

An overview of the various elements relating to demographic profile indicates that the study design was able to be carried out as anticipated, inferring validity to the strategy.

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[41] See Table 4.1.
[42] See Figure 4.1

5.2 RESPIRATORY DISORDERS

This study explored questionnaire reported symptoms of respiratory disorders, as discussed below. Phlegm, RTI, wheeze and grade of byssinosis were significantly different among symptom groups[43].

a) Cough, phlegm and RTI

Since RTI is associated with byssinosis (Pratt, 1981), it was expected that the exposed symptomatic group would have more subjects with RTI. When White (1985) reported a mean prevalence of bronchitis symptoms of 34.2% in males and 13.6% in females, he attributed the differences between sexes to smoking habits. A far lower frequency of RTI[43] was shown in the current study, which can be attributed to the low number of smoker participants.

b) Breathlessness, tightness of chest, wheeze and byssinosis

Work related breathlessness and tightness of chest are classical symptoms of byssinosis (Rylander *et al.*, 1987), with wheeze being an additional symptom of byssinosis (Pickering, 1999). The symptom status definition included classical symptoms of byssinosis as inclusionary or exclusionary criteria into the current study sample. Therefore, it is not appropriate to compare prevalence rates of byssinosis recorded in this case control study with those recorded in prevalence rate studies.

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[43] See Table 4.2.

c) Symptom groups

The exposed symptomatic group had higher frequencies of all clinical symptoms[44]. This was due to the comparative case control study design.

5.3 PULMONARY FUNCTION TESTING

Pulmonary function testing by spirometry was used for classification of symptom group status but not for classification of classical byssinosis. Ex-smoking, byssinosis and symptom status were significant between baseline FEV_1 groupings, whereas only symptom status was significant between groupings for ΔFEV_1.

a) Work departments

Although differences were not significant between ΔFEV_1 and work department, the ΔFEV_1 values between work department groupings of spinning, winding and weaving were not found in the expected order[45]. It had been expected that the largest ΔFEV_1 declines would be found in spinning, followed by winding and then weaving. Within the exposed symptomatic group, declines in ΔFEV_1 were reversed, whereas increases and decreases in the exposed asymptomatic group were found to be in the expected order. This was probably due to individual susceptibility among symptomatic subjects, or inter-mill exposure variability, or the small number of subjects in winding and weaving when compared to spinning.

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[44] See Table 4.2.
[45] See Table 4.3

b) Smoking

The inclusion of 8 current smokers in the study[46] was unlikely to have negatively impacted on mean baseline FEV_1 as the current smokers had higher baseline FEV_1 values than the ex- or never smokers. The significantly lower mean baseline FEV_1 percentage recorded for ex-smokers was probably due to the residual effects of cigarette smoking. Although the exposed symptomatic ex-smokers had the lowest baseline FEV_1 values, White (1989) noted that chronic irreversible lung disease of non-occupational origin is indistinguishable from chronic byssinosis. Thus it was not possible to differentiate within this group, between the effects of past smoking habits and the effects of cotton exposure on baseline FEV_1 values. This is one of the problematic hallmarks of byssinosis research, and is not unique to this study.

c) RTI

Chronic bronchitis is associated with declines in baseline FEV_1 (Huib *et al.*, 1996). This finding was approximately confirmed in the current study. Although no significant difference was found for baseline FEV_1, exposed symptomatic subjects with grades 2 and 3 RTI were shown to have lower values than those with grade 0 or 1 RTI[46]. Additionally, exposed symptomatic subjects with grade 3 RTI had significantly sharper declines in Δ FEV_1, than workers with grade 0, 1 or 2 RTI, confirming White's (1985) findings. White found significantly different results between baseline FEV_1 as well as Δ FEV_1 values and bronchitis in men, but not in women.

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[46] See Table 4.3.

d) Byssinosis

The finding that decreased baseline FEV_1 is associated with increasing severity grades of byssinosis[47] is confirmed by White (1985); Christiani *et al.* (1994) and Fishwick *et al.* (1996). Similarly, the finding that subjects with grade 2 byssinosis had similar strong declines in Δ FEV_1 is in line with the research results of Christiani (1994) and Imbus *et al.* (1973 as cited by Christiani, 1994). However, these results are not in line with the findings of White (1985), McKerrow *et al.* (1958) or Schachter (1994). Christiani (1994) suggests that acute cotton induced bronchoconstriction or respiratory disorders other than byssinosis are likely to be responsible for declines in Δ FEV_1, in cotton workers without classical subjective symptoms of byssinosis. This provides an explanation for declines in Δ FEV_1, with exposed workers with grade 0 byssinosis.

e) Symptom groups

Both baseline FEV_1 and Δ FEV_1, the two standard pulmonary function criteria used for chronic as well as acute classification of byssinosis, were not independent of symptom group status.

All three groups had normal or above normal mean FVC results[48], indicating that restrictive lung disease was not present in any of the groups. This was expected in cotton exposed workers and was almost certainly due to the low number of subjects ever having been exposed in previous high-risk employment. Additionally, above normal mean FVC values in

[47] See Table 4.3.
[48] See tables A2.1, A2.2 and A2.3 in Annexure 2.

symptom groups indicated that subjects blew into the spirometer for a sufficiently long time, providing verification of subject effort.

The slight decline of – 17.61 ml (–0.39 %) in Δ FEV_1 in the unexposed control group[49] was unexpected, but within measurement error. Randolph (1997), who conducted pulmonary function tests on a group of exposed and unexposed workers in the paint industry in KwaZulu-Natal, found that the unexposed control group in his study had an increase of + 17 ml in Δ FEV_1. It is quite probable that a number of subjects within the unexposed control group may have been placed in a 'non-cotton' environment due to pre-existing respiratory disorders at the time of employment. These disorders would have rendered them unsuitable for employment in the high-risk cotton environment. Legitimate comparisons between the exposed symptomatic and unexposed control groups were only possible because there were subjects in the unexposed control group both with and without respiratory disorders. However, as the mean decline in Δ FEV_1 recorded in the unexposed control group was within measurement error the slight decline as stated above was of no real consequence.

Of all the pulmonary function variables, Forced Expiratory Flow from 25 to 75 percent of volume (FEF_{25-75}) showed the most variability over shift, with the exposed symptomatic group displaying the strongest percentage declines[50]. FEF_{25-75} is a predictor of lung function change in small airways. However, Haglind *et al.* (1983) concluded that the most severe changes occurring in the byssinotic lung most likely did not occur in the small airways.

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[49] See Table A2.3 in Annexure 2.
[50] See Table A2.1 in Annexure 2.

The substantially lower mean FEV_1/FVC ratio present in the exposed symptomatic group[51] indicated lowered baseline FEV_1 values relative to baseline FVC values in these subjects. This pattern is synonymous with byssinosis.

Christiani *et al.* (1994) found that those subjects with ΔFEV_1 declines of greater than five percent had the greatest longitudinal losses in baseline FEV_1. These findings confirm those of the present study[52], which indicate that a number of subjects with ΔFEV_1 declines had decreased baseline FEV_1 values. This pattern was clearly absent in the unexposed control group.

5.4 IMMUNOLOGICAL TESTING

The manner in which immunological status is discussed differs from the discussion of socio-demographic profile or pulmonary function testing data. Each of the sub-aims of the study[53] are used to provide a structure for discussion of the majority of the immunological data.

Study results to normal reference ranges are discussed in section 5.4.1 and correlation between the immunological parameters in section 5.4.2. In both section 5.4.1 and section 5.4.2 the IgG subclasses, total IgG and specific IgG are dealt with simultaneously. Whereas the first sub-aim relating to IgG subclasses and total IgG is dealt with in section 5.4.3, followed by a discussion on specific IgG in section 5.4.4, the focus of the second sub-aim.

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[51] See Table A2.1 in Annexure 2.
[52] See Table 4.4.
[53] See Chapter 1, Section 1.1: Statement of the problem.

As proposed in the third sub-aim, the IgG subclasses, total IgG and specific IgG are integrated in section 5.4.5.

5.4.1 Comparison of measured mean, to normal reference concentrations

Some authors (Tollerud *et al.*, 1995) find that blacks have approximately 30% higher IgG serum concentrations than whites. Since The Binding Site (1999) values were based on European populations, the higher IgG subclass than reference range concentrations recorded in the current study[54] were expected. This study thus supports the findings that blacks have higher subclass and serum concentrations than whites.

Measured study results for percentage breakdown of the IgG subclasses[54], are very close to the normative percentage breakdown given by Schur *et al.* (1987). These authors record the IgG subclass percentage breakdown as 70% IgG_1, 20% IgG_2, 6% IgG_3 and 4% IgG_4. The mirrored closeness of the study results to those obtained by Schur *et al.* point to the accuracy of IgG subclass measurements in the study.

5.4.2 Correlation among immunological parameters

Correlations relating to the IgG subclasses, total serum IgG and specific IgG are discussed below where relevant.

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[54] See Table 4.5.

a) IgG subclasses and total serum IgG

IgG_1 showed significantly strong correlation with IgG_3, IgG_4 and total IgG. IgG_2 was found to be correlated at the borderline level of significance to specific IgG. IgG_3 was significantly correlated to IgG_1, IgG_4 and total IgG[55]. The correlation's between IgG_4 to IgG_1, IgG_3 and total IgG were all highly significant. Whereas IgG_4 was correlated to specific IgG at the borderline level of significance. The significance of inter-IgG subclass correlation was not found in literature and consequently the relevance of these findings is uncertain. Although total IgG was found to be highly correlated with IgG_1, IgG_3 and IgG_4, this finding has limited meaning as the total serum IgG parameter was obtained by adding the individual subclass concentrations. It is therefore logical that total IgG would be correlated with the IgG subclasses, as it is not independent of the subclasses.

b) Specific IgG

Specific IgG was not correlated with any of the IgG subclasses at the 0.05 level, but it was correlated with IgG_2 and IgG_4 at the borderline level of significance[55]. The hypothesis relating the interrelationship of specific IgG to the IgG subclasses stated[56] that a relationship would be seen between specific IgG and IgG_2. As is noted in the discussion on IgG_2, no relationship between IgG_2 and byssinosis was demonstrated. Ewan *et al.* (1993), who investigated venom-specific IgG antibodies in bee and wasp allergy, proposes that the IgG immune response may be complex, and may possibly involve specific IgG antibodies within the individual IgG subclasses. This may be the case in this study where a relationship

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[55] See Table 4.6.
[56] See Chapter 1, Section 1.2: Hypotheses to be tested.

between specific IgG and IgG_2 as well as IgG_4 was indicated, in that these parameters were correlated at the borderline level of significance. Yet, a clear parallel relationship between the parameters was not demonstrated. Noweir (1981) measured total serum IgG and specific IgG in cotton workers, but did not report a correlation between the parameters.

5.4.3 IgG subclasses 1-4 and total serum IgG

The relationship of the IgG subclasses to respiratory disease is generally poorly understood (Feldman *et al.*, 1992). This lack of comprehension is magnified between the IgG subclasses and byssinosis, with the current study exploring an original study arena. Thus, the discussion of byssinosis in relation to the IgG subclasses should be viewed as an exploratory discourse. Consequently, conclusions in this arena are made with the understanding that they are at best tenuous until substantiated by further research, which relates cotton exposure to the IgG subclasses.

The relationship of total serum IgG to byssinosis was understood from the planning stages of the study[57] to be a poor indicator of the relationship between the IgG class of antibodies and byssinosis.

5.4.3.1 Influence of demographics on IgG subclasses 1-4 and total IgG

The socio-demographic characteristics of age, gender, work department and smoking are discussed.

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[57] See Chapter 2, Section 2.5.5.3: IgG, total serum and cotton.

a) Age

IgG subclass concentrations vary with age (Bradwell, 1995) and the current study results[58] reflected significant changes for age in IgG_4. The interaction effect between age, IgG_1, as well as total IgG and symptom status, suggests a relationship between these variables which is likely to have been due to the group matching of symptom groups for age[59]. The significant interaction effect seen in total IgG were as a result of the non-independent relationship between IgG_1 and total IgG. The insignificant differences found between age categories for total IgG support the findings of Ainsworth *et al.* (1981), who report no significant differences between age groupings.

b) Exposure duration

Whereas Noweir (1981) found that total serum IgG titres were comparatively raised in subjects with increasing exposure years, the current study findings[58] concur with those of Ainsworth *et al.* (1981) and Mundie *et al.* (1985). These authors also found that differences in exposure duration were not significant for the total IgG parameter.

c) Gender

The finding in this study that IgG serum concentrations were not significantly different between black males and females[58] is in line with the findings of Ambrosino *et al.* (1991) and Tollerud *et al.* (1995).

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[58] See Table 4.7.

[59] Since symptom groups were group matched for age in the current study, it was not necessary to control for age in the analysis.

d) Work department

Although Yokota *et al.* (1998) found that IgG_4 titres reflected the intensity of exposure, the results in this study[60] showed no significant differences or trends between work departments.

e) Smoking

A number of studies in the cotton (Mundie *et al.*, 1983b; Mundie *et al.*, 1985; Sigsgaard and Gravesen, 1991) and non-cotton (Tollerud *et al.*, 1995) environments have noted that smoking leads to decreases in total serum IgG. The results of this study[60] indicate that total IgG concentrations were not significantly decreased in current smokers.

The results of Merrill *et al.* (1985) showed that smokers had an increase in IgG_1 levels, whereas IgG_1 was not significantly different in smokers in the present study. However, Popa (1994) found that 3/26 smokers had reduced IgG_2 levels, supporting the finding in the current study that current smokers had reduced, although not significant, levels of IgG_2. Similarly, IgG_3 was not significantly reduced between smoking groups in the current study. In contrast, both Merrill *et al.* (1985) and O'Keeffe *et al.* (1991) found that smokers had raised IgG_3 levels when compared with non-smokers. IgG_4 levels were also not significantly reduced in current smokers in the present study.

Tollerud and colleagues (1995) noted that people from the black race group tended to smoke less than those from the white race group. The findings of White (1989) confirm that South African cotton workers smoke a low number of cigarettes. Smoking fewer cigarettes per day

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[60] See Table 4.7.

is likely to result in less impact on IgG concentrations than smoking many cigarettes, and this could provide an explanation for the present study findings as discussed above.

5.4.3.2 Influence of respiratory disorders on IgG subclasses 1-4 and total IgG

a) RTI

The only IgG subclass that showed significant differences between homogenous subsets for RTI was IgG_4, where subjects with grade 3 RTI had distinctly higher concentrations[61] than subjects with other grades of RTI. Although these results indicate that severity of RTI may raise IgG_4 concentrations, O'Keeffe *et al.* (1991) and Popa (1994) found that subjects with chronic RTI had deficiencies in IgG_4. Thus, the findings in this study relating to IgG_4 and RTI grades may support an alternative hypothesis proposed by Feldman and Wadee (1998). These authors hypothesise that IgG_4 may be a marker of chronic antigen stimulation.

Kamat *et al.* (1981) found that byssinosis but not RTI was related to total serum IgG. Since no significant difference was found in total serum IgG between RTI grades[62], the present results support those of Kamat *et al.* (1981) which relate to total IgG and RTI.

b) Breathlessness

Popa (1994) noted that IgG_3 deficiencies were related to chronic respiratory illness, while Bernatowska-Matuszkiewcz *et al.* (1991) in their study attributed deficiencies in IgG_3 as a

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[61] See Table 4.9.
[62] See Table 4.8.

marker of respiratory disease with no pathological significance. Bradwell (1995) notes that IgG_3 provides a good response to protein/polypeptide antigens; at times showing a higher affinity to the antigen than IgG_1, which provides the largest immune response to these antigens. Theoretically, it is possible that one or more of the numerous antigens in cotton dust were responsible for inducing the IgG_3 response seen in the current study[63]. However, the picture is mixed[64] as the lowest IgG_3 concentrations were found in two workers, who reported Grade C breathlessness, while the highest concentrations were found in two workers with Grade D breathlessness. Since numbers were low and there was a conflicting pattern of IgG_3 response, no definitive conclusions can be drawn.

c) Tightness of chest

No literature could be found which dealt with the relationship between tightness of chest and IgG_3. This relationship was found to be significant between groupings in the current study[63]. Those subjects who reported tightness of chest at work, either on Monday or in general, were found to have higher concentrations of IgG_3 than those subjects with no tightness or tight on other days or tight on more days. This pattern may relate to the association between IgG_3 and respiratory disease in general, rather than to a specific mechanism that exists between IgG_3 and tightness of chest.

[63] See Table 4.8.
[64] See Table 4.9.

d) Byssinosis

Grades of byssinosis are dependent on the clinical symptoms of reported breathlessness and tightness of chest. In the current study[65], only IgG_3 was significantly different between groups at the borderline significance level, with no distinct patterns evident for IgG_3 between byssinosis grades.

The finding that there were no significant differences between byssinotic grades relative to total IgG, approximates that of Mundie *et al.* (1985). These authors found that total serum IgG concentrations decreased over the work week independently of byssinotic status.

5.4.3.3 Influence of pulmonary function on IgG subclasses 1-4 and total IgG

O'Keeffe *et al.* (1991), who examined patients with chronic obstructive pulmonary disease, found a significant correlation between reduction in baseline FEV_1% and deficiencies in IgG_2. The current study found[66] that IgG_2 concentrations were not significantly different between categories of baseline FEV_1, presenting a mixed picture that does not allow for definitive extrapolation.

Those workers with Δ FEV_1 groupings of 5-10% had significantly raised IgG_1 concentrations and those workers with decreased IgG_1 titres had Δ FEV_1 groupings of 10-20%[67]. This pattern was repeated for total IgG, which is substantially dependent on IgG_1. Kemeny *et al.* (1989 as cited by Kay 1997), note that the IgG isotype response to respiratory antigens is

[65] See Table 4.8.
[66] See Table 4.10.
[67] See Table 4.11.

mainly IgG_1. As the IgG_1 response between respiratory groupings was significantly different but not linear within decreasing groupings of Δ FEV_1, it is uncertain whether the spread of the IgG_1 response is related to respiratory antigen challenge.

5.4.3.4 IgG subclasses 1-4 and total IgG linked to byssinosis grading and to pulmonary function

A significant interaction effect[68] was noted for IgG_4, byssinosis and Δ FEV_1. Although there was a trend of decreasing concentrations of IgG_4 for increasing severity of Δ FEV_1 groupings in grade B0 byssinotics, the same pattern was not evident for grade B1 and B2 byssinotics. Therefore, it is not possible to reach any determination on the relevance of the interaction.

5.4.3.5 Differences in IgG subclasses 1-4 and total IgG concentrations for exposure and symptom groups

As a function of exposure to cotton, IgG_4 was at the borderline level of significance[69], with comparatively lower IgG_4 concentrations in exposed workers than in unexposed workers. This finding contrasts with the results of the analysis between IgG_4 and RTI[70], where increases in IgG_4 were noted in those workers with the most severe RTI grading. It is therefore difficult to interpret whether the finding of comparatively lowered IgG_4 concentrations in the exposed group has relevance in the absence of other studies.

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[68] See Table 4.12.
[69] See Table 4.13.
[70] See Table 4.9.

Kamat *et al.* (1979) and O'Neil *et al.* (1981) both found serum IgG levels to be increased as a result of cotton exposure. Total IgG concentrations were not significantly different between the exposed group and unexposed group[71], although they were slightly lower in the exposed group. Contrasting with the findings of the authors stated above, but supporting the findings of the current research initiative, are the results of Mundie *et al.* (1985) and Olenchock *et al.* (1986). These authors found insignificant differences in total IgG as a result of exposure. The reason for conflicting results, between studies, is almost certainly a reflection of the non-specific measure of the IgG response provided by total serum IgG.

As both the exposed symptomatic and exposed asymptomatic subjects were exposed to cotton dust, the significant differences in IgG_3 concentrations[71] between these groups were unlikely to be due to cotton dust exposure, but rather non-specific respiratory disease. Bernatowska-Matuszkiewcz *et al.* (1991) found that patients with cystic fibrosis, with and without respiratory carriage of *P.aeruginosa* with associated endotoxin, had significantly raised IgG_3 levels. In essence this is the same effect seen in the current study, indicating the uncertainty of the finding of raised IgG_3 concentrations shown in the exposed symptomatic or unexposed control groups. This result is therefore unlikely to indicate for or against the effect of endotoxin in the cotton exposed subjects.

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[71] See Table 4.13.

5.4.3.6 Summary, IgG subclasses 1-4 and total IgG

The expected association between IgG_2 in response to the lippolysaccharide antigen present in cotton dust was not seen either in clinical symptom[72] or pulmonary function[73] presentation. A fairly weak association between IgG_2 and byssinosis was seen in its correlation[74] to specific IgG.

Oxelius (1974, as cited by Feldman *et al.*, 1992), and Beck *et al.*. (1981), note that IgG_2 and IgG_4 deficiencies are considered to be of importance in the development of respiratory disease. Yet Mayumi *et al.* (1983) found that endotoxin stimulated blood mononuclear cells or plasma cells, expressed the IgG subclass distribution as follows: IgG_2>80%, IgG_1<20%, IgG_3<1% and IgG_4<1%. Although the results[75] indicate no difference in mean IgG_2 response between exposed and unexposed workers, as well as between symptom groups, there was a trend between groups in percentage subclass distribution[76]. It is evident[77] the IgG_2 subclass made up a comparatively higher percentage of total serum IgG in exposed subjects when compared with unexposed subjects. As shown[78], in the exposed symptomatic group, IgG_2 constituted a higher percentage followed by the exposed asymptomatic and then unexposed control group. This trend is slight when compared with the results of Mayumi *et al.* (1983), but is corroborated by the findings of Snapper and Paul (1987). These authors report that IFN-γ

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[72] See Table 4.8.
[73] See Table 4.10.
[74] See Table 4.6.
[75] See Table 4.13.
[76] See Figure 4.2 and Figure 4.3.
[77] See Figure 4.2.
[78] See Figure 4.3.

causes B cells that have been activated by lipopolysaccharide to increase IgG_2a production, whilst decreasing titres of IgG_1 and IgG_3.

HIV status may have acted as a confounding variable depressing IgG_2 levels across all symptom groups. However, this is unlikely as Klasse *et al.* (1987) record that the IgG_2 response constitutes only a minor part of the HIV total antibody response.

Typical exposures in cotton mill environments render concentrations capable of inducing physiological effects in ΔFEV_1 (Wolf, 1973; Muittari *et al.*, 1980 as cited by Rylander and Morey, 1982). Accordingly, a concomitant IgG_2 response was expected in cotton exposed workers. Consequently, results[79] from IgG_2 subclass measurements allows for the following extrapolations:

- Typical endotoxin exposures in the cotton mill environment are not high enough, to generate a significant percentage change in the IgG subclass distribution; and

- Serum IgG subclass measurements are not sufficiently sensitive to accurately quantify the IgG_2 response to antibodies formed against endotoxin contained in ACDE.

Mundie *et al.* (1983.b) support the argument that the amount of specific IgG antibodies against ACDE, within total serum or even total serum in the individual subclasses, is very low. The argument is further substantiated by the results of Cowan and Winnie (1993) who were not able

[79] See Table 4.6, Table 4.8, Table 4.10, Table 4.13, Figure 4.2 and Figure 4.3.

to clearly distinguish the effects of colonisation by *P.aeruginosa* in cystic fibrosis patients using serum IgG subclass tests. Therefore, it is likely that low concentrations of specific IgG against antigens contained in ACDE and not HIV infection status was responsible for the lack of clarity in the IgG subclass results, generated in this study[80].

There are a number of possible explanations for the comparatively raised IgG_3 concentrations in subjects with grade D breathlessness and in those subjects with tightness of chest on a Monday or tightness of chest generally[81]. Protein/polypeptide antigens, likely to be present in ACDE, were capable of inducing this IgG_3 response (Bradwell, 1995). Alternatively, the IgG_3 response may have been the result of chronic respiratory illness (Popa, 1994).

No associations of significance or relevance were determined between respiratory disorders[81], pulmonary function testing[82] and immunological parameter variables[83], to total serum IgG. With the analysis of the IgG subclasses, it became obvious why total IgG is a poor indicator of the effect of cotton dust on the IgG class of antibodies. Total serum IgG at best reflects large increases or decreases in any of the individual subclasses. The smaller the percentage make-up of the subclass, the smaller is the impact of the subclass on total serum IgG concentrations. This research confirms that both total IgG and serum IgG subclass measurements are inappropriate measures of the IgG response in cotton dust research.

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[80] See Table 4.6 and Table 4.8.
[81] See Table 4.8.
[82] See Table 4.10.
[83] See Table 4.6.

5.4.4 Specific IgG

Specific IgG antibodies formed against ACDE were measured in the current study by FEIA in order to evaluate the role of these antibodies in the development of byssinosis. There were only two research studies found in the literature (Noweir, 1981; Sigsgaard and Karol, 1993) that related specific IgG concentrations to byssinotic effects. The reason the research of Sigsgaard and Karol (1993) is consistently quoted is that these authors record substantial detail of the relationship between specific IgG and clinical, as well as functional, symptoms of byssinosis. In contrast, Noweir (1981) does not provide the same level of detail.

The precipitating antibodies test against ACDE may include classes of antibodies other than IgG. The test therefore provides lower specificity for IgG than the specific IgG test. Notwithstanding this, it is still of relevance to compare results of studies which used the precipitating antibodies test against those that used the specific IgG test.

5.4.4.1 Influence of demographics on specific IgG

The finding that specific IgG concentrations were not significantly different by age, gender or smoking[84], is substantiated by the results of Sigsgaard and Karol (1993). Confirming these findings, Taylor *et al.* (1971) found that precipitating IgG titres were unrelated to age.

Karol *et al.* (1992) proposed that specific IgG antibody response to ACDE could act as a potential biomarker of cotton dust exposure. The results of Sigsgaard and Karol (1993) and the current study[84] indicate that specific IgG levels are not statistically different for exposure

[84] See Table 4.7.

duration. Additionally, specific IgG concentrations in the current study, did not appear to be related to work department[85], although a trend of lower concentrations in the winding department, which was at the borderline level of significance, in both the exposed symptomatic and the exposed asymptomatic groups was noted. Since numbers of subjects were low in the winding and weaving departments, it is not prudent to draw any definitive conclusions from the results. Although, differences in smoking status were not significant[85], marginally higher concentrations were found in never smokers when compared with current and ex-smokers. This follows the trend recorded by Sigsgaard and Karol (1993).

5.4.4.2 Influence of respiratory disorders on specific IgG

The relationship of respiratory disorders to specific IgG is examined as follows.

a) RTI

Although specific IgG was raised in subjects with grade 3 RTI[86], a general trend of raised specific IgG in either exposed or unexposed subjects was not seen. Noweir (1981) and Sigsgaard and Karol (1993) found a similar trend, with no significant differences relating to RTI respectively found between the exposure subgroups. Additionally, it is difficult to determine if RTI was responsible for the raised levels of specific IgG, as specific IgG was also significantly raised for the most severe grades of breathlessness and byssinosis[86].

[85] See Table 4.7.
[86] See Table 4.9.

b) Breathlessness

Whereas Sigsgaard and Karol (1993) found no significant differences between exposure groups, in the current study those subjects with grade C as well as grade D breathlessness had significantly raised specific IgG concentrations[87]. Although Noweir (1981) does not specifically note an association between specific IgG and breathlessness as one of the symptoms of byssinosis, it is reasonable to assume this relationship. Noweir notes a statistically significant association between specific IgG and byssinosis. Thus Noweir's assumed findings approximate those of the current study, indicating an association between specific IgG and breathlessness.

c) Tightness of chest

Differences between groupings of tightness of chest were not significant[88], with the results being difficult to interpret. Subjects with classical byssinotic symptoms of tightness of chest on a Monday had the highest specific IgG concentrations, with no other results noticeably raised. Workers without tightness of chest had relatively raised specific IgG concentrations, indicating that factors other than chest tightness were responsible for raised specific IgG concentrations in some subjects within the exposed symptomatic group. Sigsgaard and Karol (1993) also found that specific IgG was not significantly different between groups for tightness of chest.

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[87] See Table 4.9.
[88] See Table 4.8.

As tightness of chest is also a symptom of byssinosis, it is reasonable to assume that Noweir (1981) noted a significant association between tightness of chest and specific IgG. Consequently the results relating to tightness of chest in the present study lie somewhere between those of Sigsgaard and Karol (1993) and Noweir (1981). The current results were not as definitive as the assumed results of Noweir (1981), but also not negative, as were the results of Sigsgaard and Karol (1993).

d) Byssinosis

The finding that grade B2 byssinotics had significantly raised antibody levels[89], is in line with the findings of a number of authors, including Massoud and Taylor (1964) and Taylor *et al.* (1971). These authors measured precipitating antibodies, which are mainly of the IgG class of antibodies. In addition, Noweir (1981) showed that grade B2 byssinotics had significantly raised specific IgG titres. Contrasted with this, Sigsgaard and Karol (1993) found no significant relationship between specific IgG antibodies and grades of byssinosis. In the light of these findings, it can be concluded with a level of certainty that grade B2 byssinotics have higher specific IgG levels.

5.4.4.3 Influence of pulmonary function on specific IgG

Although Noweir (1981) measured pulmonary function, he did not note whether an association between specific IgG and any of the pulmonary function parameters was present or not. Neither Massoud and Taylor (1964) nor Taylor *et al.* (1971) measured pulmonary

[89] See Table 4.9.

function. As a result, only the findings of Sigsgaard and Karol (1993) can be discussed in relation to the current study.

While subjects with baseline FEV_1 values of less than 60% had significantly higher specific IgG concentrations, subjects with baseline FEV_1 groupings of 60-79% did not[90]. Those subjects with the most severe declines in Δ FEV_1 had correspondingly raised specific IgG concentrations. Although this difference was only at the borderline level of significance by ANOVA[91] analysis, Duncan's post-hoc test[92] indicates that subjects with Δ FEV_1 declines of >10->20% had significantly raised specific IgG concentrations.

This pattern supports the clinical symptoms finding[93], that only subjects with more severe symptoms have raised specific IgG levels, suggesting a patho-physiological relationship associated with increased specific IgG values. In contrast, Sigsgaard and Karol (1993) found a significant negative association between baseline FEV_1, as well as Δ FEV_1 and specific IgG values, suggesting a protective effect for specific IgG antibodies formed against antigens contained in ACDE. In the absence of additional corroborating research either for or against the relationship between pulmonary function and specific IgG, it is not appropriate to draw any definitive conclusion on this relationship.

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[90] See Table 4.10 and Table 4.11.
[91] See Table 4.10.
[92] See Table 4.11.
[93] See Table 4.9.

5.4.4.4 Specific IgG linked to byssinosis grading and pulmonary function

A significant interaction effect[94] was noted in the present study between specific IgG, byssinosis and Δ FEV_1. Since the nature of this interaction effect was not evident, since there was no clear trend, and since the literature does not report on the interaction of these variables, it is not possible to derive any conclusions.

5.4.4.5 Differences in specific IgG concentrations for exposure and symptom groups

Significantly higher concentrations of specific IgG were found in exposed workers than in unexposed workers, with the highest concentrations found in subjects within the exposed symptomatic group[95]. Massoud and Taylor (1964) and Taylor *et al.* (1971), carried out precipitating antibody testing. In addition, Noweir (1981) and Karol *et al.* (1992) conducted specific IgG testing on unexposed controls and exposed cardroom workers. All these researchers found that exposed symptomatic and asymptomatic workers, displayed higher concentrations of IgG than workers who were not exposed to cotton.

These findings are supported by the extensive research of Karol and Lemp (1990), who demonstrated a uniform increase in specific IgG levels in exposed guinea pigs over a six month and eight-week period, when compared with unexposed animals. The researchers noted that the increase in specific IgG titres was attributed to the immune system being

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[94] See Table 4.12.
[95] See Table 4.13.

stimulated. This background increase in antibody levels is unlikely to reflect a sensitisation to cotton dust antigen/s, but rather acts as a marker of antigen exposure.

Exposure to cotton dust produces uniform specific IgG concentrations in guinea pigs (Karol and Lemp, 1990), but as shown in the current study[96] and substantiated by other studies (Noweir, 1981; Karol *et al.*, 1992; Sigsgaard and Karol, 1993), cotton dust produces non-uniform specific IgG levels in cotton workers. This pattern can be explained by the differences in exposure duration and exposure concentration experienced by guinea pigs and cotton workers. The exposure variables of duration and concentration remain constant among guinea pigs under laboratory conditions, but vary among cotton workers in the cotton mill environment.

Despite a significantly higher specific IgG exposed symptomatic group mean[96], a number of workers in the exposed asymptomatic and unexposed control group had specific IgG concentrations that were relatively but not highly raised[97]. The finding of unexposed workers having relatively raised specific IgG concentrations against ACDE was reflected in the studies of Massoud and Taylor (1964); Taylor *et al.* (1971) and O'Neil *et al.* (1981). This indicates that a certain constant level of serum proteins, including immunoglobulin, may be complexed to the phenolic tannins, contained in ACDE, and precipitated on immunological testing, irrespective of exposure to cotton or not. This finding was first proposed by Edward and Jones (1973), and later supported by the findings of Kutz *et al.* (1981).

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[96] See Table 4.13.
[97] See Figure 4.4.

As has been mentioned, specific IgG was raised in exposed symptomatic subjects[98]. There was also a uniform interval dispersion frequency of specific IgG within each of the symptom exposure groups[99]. Only subjects from the exposed symptomatic group appeared within the top three intervals of specific IgG, indicating the specificity of the results. The trend of lower specific IgG concentrations was lessened in the exposed asymptomatic group, and a fairly normal distribution was also evidenced. This decreasing trend was then continued in the unexposed control group, with no subjects falling into the top five intervals of specific IgG. This clear trend is in line with the original study hypothesis[100] for specific IgG, and is certainly one of the most significant findings of the study. The research of Massoud and Taylor (1964); Taylor *et al.* (1971) and Noweir (1981) corroborates the finding of exposed symptomatic workers having higher specific IgG concentrations than exposed asymptomatic workers. This indicates that the phenolic tannins contained in ACDE, may only be able to precipitate out a uniform amount of serum proteins, including IgG. However, specific IgG concentrations above this uniform precipitation level signify true specific antibodies to ACDE.

The only negative findings in a study, which used appropriate methodology to determine the association between specific IgG and byssinosis, were those of Sigsgaard and Karol (1993). Given that the findings of the current study[101] indicate that mean specific IgG concentrations were raised in byssinotic workers, and since this finding is supported by other human studies, it is theorised that grade B2 byssinotic workers have raised specific IgG concentrations.

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[98] See Table 4.13.
[99] See Figure 4.4.
[100] See Chapter 1, Section 1.2: Hypotheses to be tested.
[101] See Table 4.9 and Table 4.11.

5.4.4.6 Summary, specific IgG

The results of the current study indicate that only subjects with more severe clinical symptoms are likely to have raised specific IgG levels[102], with large variations between subjects. The uniform increase in specific IgG concentrations noted in guinea pigs exposed to cotton dust by Olaniran and Karol (1988) and Karol and Lemp (1990), is not present in cotton workers. This observation is supported by the results of the current study[103], as well as the studies of Noweir (1981) and Sigsgaard and Karol (1993).

A statistically significant association was noted between specific IgG and both reported clinical symptoms of byssinosis[102] determined with a questionnaire, and for functional symptoms of byssinosis[104] determined by independent spirometry. The studies of Massoud and Taylor (1964); Taylor *et al.* (1971) and Noweir (1981) confirm the relationship found between clinical symptoms of byssinosis and raised specific IgG levels. These studies do not note an association, either for or against, a relationship between functional symptoms and raised specific IgG levels. Sigsgaard and Karol's (1993) study do not support either the clinical or functional findings of this study. Hence the study of Sigsgaard and Karol stands alone in the light of the finding of a negative association between specific IgG antibodies and byssinotic symptoms.

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[102] See Table 4.9.
[103] See Figure 4.4.
[104] See Table 4.11.

Exposed workers had higher specific IgG levels than unexposed workers, and exposed subjects that were symptomatic had higher specific IgG levels than exposed asymptomatic subjects[105]. The relatively raised specific IgG levels in exposed workers indicates that specific IgG levels are affected by chronic antigen stimulation. In addition, the non-uniform pattern of specific IgG results shown in the current study[106] indicates that this immunological parameter may be influenced by individual susceptibility or other environmental influences including exposure variability. There are a number of possible explanations for the raised specific IgG concentrations seen in the exposed symptomatic subjects. Both *Aspergillus fumigatus* (AF) and *Enterobacter agglomorans* are present in the air of cotton mills (Lacey and Lacey, 1987). Igea *et al.* (1993) demonstrated that exposure to AF leads to increases in AF-specific IgG. *Enterobacter agglomorans* is a gram-negative bacterium, which contains endotoxin (ATS, 1998). Endotoxin stimulates an IgG_2 response (Freijd *et al.*, 1984). Thus the specific IgG antibodies measured in the current study may have been of the IgG_2 subclass.

The pattern of only certain exposed workers developing raised antibody concentrations is synonymous with a true hypersensitivity pattern. It is not however purported that humoral sensitisation is responsible for all byssinosis. Many researchers, including Wegman *et al.* (1983); Hughes (1981) and WHO (1981), have reported on the variability in the presentation of symptoms induced by cotton exposure. It is feasible that endotoxin in addition to its direct toxicological action, is able to stimulate a humoral response. The toxicological effect of endotoxin is likely to occur independently of the humoral response, which may arise

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[105] See Table 4.13.
[106] See Figure 4.4.

simultaneously in some, but certainly not all, exposed workers. This provides a plausible explanation for the variability in presentation of respiratory disorders[107], both clinical and functional, seen in exposed cotton workers.

5.4.5 Integration of immunological parameters

The relationship of the IgG subclasses, together with total serum IgG to specific IgG, is integrated in this section. IgG and its subclasses have been positively linked to respiratory disease[108]. Yet, an integration of measurement results between specific IgG antibodies formed against ACDE and serum IgG subclasses, is exploratory discussion in the research context. Noweir (1981) is the only author who measured both total and specific IgG against ACDE, but he does not offer comment on any interrelationship between the variables. This limits the discussion to non-cotton literature, which partially or indirectly examines interrelationships of the aforementioned parameters.

5.4.5.1 Influence of demographics on immunological parameters

There were no clear trends of interaction between immunological parameters relating to any of the socio-demographic characteristics[109].

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[107] See Table 4.2 and Table 4.3 for results of the current study and Chapter 2, Section 2.2: Respiratory disorders, for results of variability of respiratory disorders in other studies conducted in the cotton dust research arena.
[108] As detailed extensively in Chapter 2, Section 2.5.4: IgG, non-cotton.
[109] See Table 4.7.

5.4.5.2 Influence of respiratory disorders on immunological parameters

When integrated, a number of interesting relationships were shown between the IgG subclasses, specific IgG, and respiratory disorders.

a) RTI

In the current study, those workers with grade 3 RTI had raised IgG_4 and specific IgG concentrations[110]. Neither Noweir (1981) nor Sigsgaard and Karol (1993) found any relationship between specific IgG and RTI. Additionally, since only those workers with grade 3 RTI in the exposed symptomatic group had raised specific IgG[111], it is unlikely that a relationship exists between specific IgG and RTI. The most probable reason for increased IgG_4 concentrations is that it is not due to RTI, but can be explained by an hypothesis proposed by Feldman and Wadee (1998), viz. that IgG_4 is a marker of chronic antigen stimulation.

Giving cognisance to the discussion of the literature stated above, it is unlikely that there is any relevant interaction between RTI, IgG_4 and specific IgG.

b) Breathlessness

Although IgG_3 was significantly different between grades of breathlessness in homogenous subsets[110], definitive trends were not seen. It is possible that the IgG_3 response is, as Bradwell (1995) suggests, against protein/polypeptide antigens. These antigens may exist in

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[110] See Table 4.9.
[111] See Table 4.14.

cotton dust. Specific IgG was found to be significantly raised in those subjects with the most severe grades of breathlessness[112]. Although subjects with the most severe grade of breathlessness had both raised IgG_3 and specific IgG concentrations, IgG_3 concentrations between groupings of breathlessness were not definitive, consequently it is unlikely that this interactive effect has relevance.

c) Tightness of chest

Comparatively raised concentrations of IgG_3 were found in those workers with tightness of chest on a Monday and those with general tightness of chest[113]. Although exposed symptomatic subjects with tightness of chest on Monday had the highest concentrations of specific IgG, tightness of chest was not isolated to these subjects. There were 3 subjects in the unexposed control group who had tightness of chest on other days[114], with comparatively low IgG_3 and specific IgG concentrations. The difference between the exposed symptomatic and unexposed control group was that the exposed symptomatic group had been exposed to cotton dust whereas the unexposed control group had not.

It is possible that cotton dust exposure is responsible for raised specific IgG and IgG_3 concentrations as a response to protein/polypeptide antigens, but there is no confirmation in the literature to support such a relationship. It should be noted that the findings of Sigsgaard and Karol (1993) fail to support an association between specific IgG and tightness of chest.

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[112] See Table 4.9.
[113] See Table 4.8.
[114] See Section 4.4.4, pg 121: Influence of respiratory disorders on immunological parameters.

An association between tightness of chest, IgG_3 and specific IgG should be seen as possible but speculative.

d) Byssinosis grades

Specific IgG was found to be significantly raised in exposed symptomatic workers with increasing grades of byssinosis[115]. No relationship was noted in the current study between total IgG and byssinosis grades. The findings related to specific IgG, but not total IgG, closely approximate the findings of Noweir (1981). He noted a potential pathogenic or marker of pathogenesis role for specific IgG against ACDE and a cotton dust exposure response relationship for total IgG, which was not related to respiratory disease or byssinosis. Consequently, both the current study[116] and that of Noweir (1981) indicate that there is no association between specific IgG against antigens in ACDE and total IgG.

The potential relationship between IgG_3 and breathlessness[117], and IgG_3 and tightness of chest[118], as classical symptoms of byssinosis, has been discussed. Byssinosis was significant at the borderline level[115] between grades for IgG_3, although the absence of a logical progression of change in IgG_3 concentration for byssinosis grades was noted. Based on the results of Noweir (1981), it appears that there is a firm relationship between byssinosis and specific IgG, but with the results of the current study showing a tentative but uncertain link to IgG_3.

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[115] See Table 4.8.
[116] See Table 4.6.
[117] See 5.4.5.2 (b).
[118] See 5.4.5.2 (c).

5.4.5.3 Influence of pulmonary function on immunological parameters

Increases in specific IgG were seen in those subjects with the most severe lung function impairment for baseline FEV_1 and Δ FEV_1 [119]. Those subjects who experienced the most severe declines in baseline FEV_1 had insignificant although slightly increased IgG_2 values, between baseline FEV_1 groupings[120]. In contrast subjects with the most severe declines in Δ FEV_1 showed significantly decreased IgG_1 levels[119]. The results showed that the trend effect for subjects with severe lung function impairment between specific IgG and IgG_2 and IgG_1 was not comprehensible[120]. Thus, it is inappropriate to form conclusions on interactions between the variables.

5.4.5.4 Immunological parameters linked to both byssinosis grading and pulmonary function

Although the interaction between byssinosis grading and Δ FEV_1 for both IgG_4 and specific IgG was significant[121], on examination, there appeared to be no logical relationships among the variables.

5.4.5.5 Differences in immunological parameter concentrations for exposure and symptom groups

Subjects in the unexposed group had higher IgG_4 levels, at the borderline level of significance level, than those in the exposed group[122]. The opposite was shown for specific

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[119] See Table 4.11.
[120] See Table 4.10.
[121] See Table 4.12.
[122] See Table 4.13.

IgG, with subjects in the exposed group having higher concentrations than subjects in the unexposed group. As a consequence, the parameters of IgG_4 and specific IgG are unlikely to be related.

As extensively discussed [123], comparatively raised IgG_3 concentrations were related to non-specific disease which could not be specifically isolated to cotton exposure. However, specific IgG was shown to be significantly higher in exposed symptomatic subjects than in either exposed asymptomatic or unexposed control subjects[124]. This pattern indicates that specific IgG is associated specifically with cotton induced disease, and is not associated with non-specific disease or exposure. As a clear correlation as well as association with disease between specific IgG and the IgG subclasses was not present[125] it was not possible to determine the predominant subclass associated with specific IgG.

5.4.5.6 Summary, integration

The only interaction between the IgG subclasses and specific IgG was in the relationship between byssinotic symptoms, IgG_3 and specific IgG[126]. A tentative but uncertain link was noted between these variables.

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[123] See Chapter 5, Section 5.4.3.5: Differences in IgG subclasses 1-4 and total IgG concentrations for exposure and symptom groups.
[124] See Table 4.13.
[125] See Table 4.6.
[126] See Table 4.8.

5.5 LIMITATIONS

The limitations and the manner in which they were addressed follows.

a) Definition of byssinosis

In excess of 50 antigenic substances (Butcher *et al.*, 1983) have been isolated in cotton dust, which have been found to induce a number of respiratory disorders, including byssinosis. Since the classical diagnosis of byssinosis is based only on the qualitative clinical symptoms of shortness of breath and tightness of chest (Niven *et al.*, 1991), the diagnosis is subjective, problematic and not verifiable by quantitative techniques. This problem was not unique to the current study, but had been a hallmark of cotton dust research with replicate studies producing dissimilar and often conflicting results (Christiani *et al.*, 1994).

Use of the expanded symptom status definition of byssinosis[127] partly, but not fully, addresses the problems associated with the classical definition of byssinosis. These problems relate to disagreement over whether functional symptoms occur in isolation to clinical symptoms. The issues with the classical and expanded definitions of byssinosis were dealt with by taking cognisance of both definitions in the analysis of data, with a bias towards the expanded definition, which was used in the comparative case control model. Use of both the classical and expanded definitions of byssinosis in this study can be considered 'best practice' in terms of byssinosis research.

[127] See Definitions page XIV.

b) Questionnaire administration

Answers provided by subject participants during the administration of the questionnaires were substantially subjective. Moreover, because different questionnaire administrators were used, there was potential inter-mill variability in administrator style. Careful choice of the questionnaire and stringent administration procedures [128] ensured that quality and standardised data were obtained.

c) Pulmonary function testing

Potentially both subject performance and the spirometer itself are subject to variability whilst conducting spirometry. The researcher conducted rigorous quality assurance testing procedures [129] to remedy these potential problems.

d) Immunological testing

Inherent in the measurement of the IgG subclasses and specific IgG is variability in the collection and analysis methods, expressed by the Total Coefficient of Variation (CV_t). Although strict quality control procedures were adopted, including the utilisation of recognised laboratories, some variation was inevitable. As the CV_t values recorded for analyses were within acceptable limits, this variability is not considered to have unduly influenced results.

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[128] See Chapter 3, Section 3.7.1: Questionnaires.
[129] See Chapter 3, Section 3.7.2: Pulmonary function testing.

e) Serum IgG subclass measurements

Serum IgG subclass measurements are a measure of all IgG antibodies in each of the four individual subclasses. The current study used this measurement technique for the first time in the arena of cotton dust research. Examination of results revealed that this measurement technique was an insensitive measure of the IgG response in exposed cotton workers. Consequently, use of the serum IgG subclass measurement technique is identified as a limitation to interpretation of results.

f) HIV

Infection with HIV causes increases or decreases in IgG subclasses (Bradwell, 1995). It is recognised that the limitation of not knowing the HIV status of any of the subject participants may have acted as a confounding influence on both IgG subclasses 1-4 and total IgG concentrations. However, as discussed[130], HIV status is unlikely to have influenced results as a major determining confounding variable. HIV infection status does not influence the specific IgG parameter (Mattsson, 2001). Therefore, HIV status did not act as a confounding influence on the specific IgG results.

5.6 SUMMARY AND CONCLUSIONS

This overview summarises the findings and conclusions relating to IgG in subclasses, specific IgG and an integration of these immunological parameters.

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[130] See Chapter 5, Section 5.4.3.6: Summary, IgG subclasses 1-4 and total IgG.

a) IgG subclasses and total IgG

The expected distribution of the IgG subclasses with considerably raised IgG_2 levels stimulated by endotoxin, was not observed. These findings indicate that cotton dust exposures in the cotton mill environment are not high enough to generate a significant percentage change in the IgG subclass distribution, indicating that the IgG response to antigens in ACDE is not as strong as expected. Consequently, serum IgG subclass measurements are not sufficiently sensitive to accurately quantify the IgG_2 response to antibodies formed against endotoxin contained in ACDE. Moreover, low concentrations of specific IgG antibodies formed against antigens in ACDE in serum IgG subclasses, is far more likely to have played a confounding role than HIV infection status.

No significant or relevant associations were noted between total serum IgG and any of the variables measured in this study. This study confirms Bradwell's (1995) observation that total serum IgG is a crude and insensitive measure of the IgG class of antibodies. It is concluded that total IgG has little value in the arena of determining the role of the IgG class of antibodies to byssinosis or any other respiratory disorder induced by cotton dust.

b) Specific IgG

A significant relationship was shown between specific IgG and reported clinical symptoms of byssinosis determined with a questionnaire as well as functional symptoms of byssinosis determined by independent spirometry.

It is concluded that over and above the pseudo-immune precipitation of IgG noted by Edwards and Jones (1973) and Kutz *et al.* (1981), there is a distinct trend related to byssinotic

symptoms in the specific IgG parameter. The results of the current study support those of Massoud and Taylor (1964); Taylor *et al.* (1971) and Noweir (1981), and imply an association between clinical symptoms of byssinosis, and raised specific IgG levels. In addition to an association between clinical symptoms of byssinosis, this study shows an association between functional symptoms of byssinosis and specific IgG antibodies against ACDE. These findings indicate that part of the byssinotic physiological response to cotton dust involves the humoral immune response. A patho-aetiological or marker-aetiological role for specific IgG is indicated in the development of byssinosis.

5.7 RECOMMENDATIONS FOR FURTHER STUDY

Two major areas of further study are identified. The first area of study will supply a new construct framework for byssinosis and other disorders induced by cotton dust. The second area is a measurement of specific IgG in subclasses, which will provide data to evaluate the IgG response in cotton workers.

a) Definition of byssinosis

The definition of byssinosis is problematic, with uncertainty among cotton dust researchers as to how the different effects of cotton dust exposure relate to one another, including what symptoms constitute byssinosis. That this problem remains after more than fifty years of intensive cotton dust research confirms the difficulty of the problem.

Within the Manchester Criteria document Rylander and co-authors (1987) note that a number of large population studies have shown reactions to cotton dust that do not fit the accepted

classical description of byssinosis. The panel asserts that these findings led to the use of confusing terms such as 'acute byssinosis', 'atypical byssinosis' or 'bronchitis byssinosis'. However, they do not state what terms should be used for cotton dust induced respiratory disease that presents atypically or respiratory disease induced by low concentrations of cotton dust where the classical byssinosis criteria are not met.

Taken to its logical conclusion, cotton dust induced respiratory disease presenting with atypical clinical symptoms or acute or chronic functional symptoms, not accompanied by classical symptoms of byssinosis, is cotton dust induced respiratory disease but not byssinosis. A dose-response relationship has been shown between classical symptoms (Roach and Schilling, 1960; Elwood *et al.*, 1966; Merchant *et al.*, 1972; Merchant *et al.*, 1973; Fox *et al.*, 1973; Berry *et al.*, 1973; Kamat *et al.*,1981), as well as functional symptoms (Batawi *et al.*, 1964; Merchant *et al.*, 1973; Castellan *et al.*, 1987; Glindmeyer *et al.*, 1991; Glindmeyer *et al.*, 1994; Fletcher *et al.*, 1997) of byssinosis and exposure to cotton dust. The same dose-response relationship noted between cotton dust and byssinosis has been noted between endotoxin and classical symptoms (Cinkotai *et al.*, 1977; Cinkotai and Whitaker, 1978) and functional symptoms (Haglind *et al.*, 1984; Rylander *et al.*, 1985, Castellan *et al.*, 1987) of byssinosis.

Pickering (1999) notes that the longitudinal studies of Glindmeyer *et al.* (1991) and Glindmeyer *et al.* (1994) showed that cotton workers presented with grade 2 or 3 byssinosis without having passed through the earlier grades of byssinosis. These reports provide substantiation for the possibility that the threshold of disease is induced at lower concentrations than required for Monday morning chest tightness, dyspnoea, or even acute

functional declines. In addition, some workers experience clinical symptoms as a result of cotton dust exposure a-typically, thus not meeting the criteria for the classical periodicity of symptoms (WHO, 1981; Rylander *et al.*, 1987). Although Michel *et al.* (1995) reports that a dose of endotoxin induces fever at 50µg, the no-response threshold via the respiratory pathway is less than 0.5µg (Michel *et al.*, 1997). Furthermore, a dose of <1 ng/ml endotoxin caused alveolar macrophages to release cytokines TNF and interleukins 1 and 6 (Michel, 1998). Rylander and Morey (1982) predicted that cotton workers are exposed to an approximate concentration of 3.8µg endotoxin, over an eight-hour shift, with concentrations varying substantially between work areas and mills.

Thus, there is little understanding of how low cotton dust with associated endotoxin concentrations (dose) relate to a-typical clinical symptoms (response). Considering that a dose response relationship has been shown between cotton dust exposure as well as between endotoxin exposure and byssinosis, it is likely that the disease mechanism pathway remains subdued but constant at low concentrations, rendering it still capable of producing disease, albeit reduced and dependent on biological susceptibility. Yet, in the early stages of this respiratory disorder, it cannot be termed byssinosis, as it does not fulfil the symptom requirements in terms of Schilling's classical definition of byssinosis.

This dilemma is unlikely to be new; only the presentation of the classical periodicity of byssinotic symptoms has become rare. As byssinosis has been shown to be dose related, there would always have been workers exposed to cotton dust in sufficient concentrations to induce chronic respiratory disease, but not to induce the classical periodicity of symptoms. If the threshold of disease were initiated by concentrations that were at least as high as those

required initiating the threshold of Monday morning chest tightness or dyspnoea, use of the classical definition of byssinosis would be appropriate. If however, the reverse holds true, the classic definition is shown to be inadequate. El Batawi noted as far back as 1969 the problems with the classical clinical grading system. He noted that the system did not account for the irritative effects of dust exposure, nor did it address those cases where workers had acute changes in lung function but no clinical symptoms (El Batawi, 1969).

A change in both the framework of disease induced by cotton dust and the definition of byssinosis seems appropriate. Thus, further conceptual work needs to be conducted, as a foundation for establishing a new construct framework for byssinosis and other respiratory disorders induced by cotton dust.

b) Specific IgG in subclasses

The clinical outcome of specific IgE antibodies formed against allergens in the occupational work environment is occupational asthma. With specific IgG there are no confirmatory clinical diagnoses such as asthma to validate the test results against. This is partly due to a lack of understanding of the role of specific IgG antibodies to disease outcomes. If non-cotton literature on total specific IgG is examined against the specific IgG in subclasses, it becomes apparent that the role of total specific IgG may differ between antigen exposure and disease outcome. Ewan *et al.* (1993) measured specific IgG by RAST against bee and wasp venom antigen, and found it was not useful in predicting protection against stings. Katila *et al.* (1986) measured specific IgG by ELISA against environmental micro-organisms, and found that results for certain antigens appeared to be associated with exposure, but concluded that the test did not appear to have much worth as a diagnostic tool. When Tomee *et al.* (1996) measured

total specific IgG and specific IgG in subclasses in patients with pulmonary aspergilloma, they found elevated total specific IgG levels in periods of exacerbation of the disease. In a review of the role of specific IgG in 'farmer's lung', Eduard (1995) noted that specific IgG concentrations were related to exposure hours per day. Similarly, it is not known if specific IgG antibodies formed against ACDE act as protective, marker or aetiological mechanisms, or if they have any clinical significance.

Although it was not investigated in the present study, it is possible to further analyse total specific IgG within specific IgG in subclasses[131]. The advantage of conducting this testing is that instead of alluding to a potential or likely aetiological mechanism, it is possible to discuss the potential mechanism pathway of the finding. Shakib *et al.* (1986); Pressler *et al.* (1990); Pressler *et al.* (1992); Cowan and Winnie (1993) and Tomee *et al.* (1996) all used ELISA measurements of specific IgG in subclasses to substantial effect in the investigation of various disease conditions. This method could be adapted to accommodate the cotton antigen in place of the other antigens used, thus rendering it suitable to determine the cotton-specific antigen response in the individual IgG subclasses. Although the specific IgG in subclasses test is a breakdown measurement of the total specific IgG in serum, literature reveals that researchers have had improved clarification of results using the specific IgG in subclasses test. Therefore, application of this test is likely to provide a more detailed and substantial quantification of the cotton-specific IgG antigen response.

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[131] See Chapter 2, Figure 2.1: Division of the IgG class of antibodies. The antibodies entitled 'Specific IgG antibodies against cotton antigen within IgG subclasses 1-4' should be measured in further studies.

ANNEXURE ONE

STANDARD [132] COTTON DUST QUESTIONNAIRE, ENGLISH
*MARK ALL APPROPRIATE QUESTIONS WITH A CROSS "**X**"*

A. IDENTIFICATION DATA

Study identification number (1-120)

1. **Name** .....
2. **Company Number**
3. **Age in years** (Estimate age if it is not known)
4. **Sex** Male ☐ Female ☐
5. **Race** Black ☐ Indian ☐ Colored ☐ White ☐
6. **Telephone number** H:
7. **Address** .....

.....

8. **Name of interviewer** .....
9. **Date of interview** d d m m y y y y

B. WORK HISTORY OF WORKER

10. **Company**

Frame textiles New Germany:

a. Frametex ☐ 1
b. Seltex ☐ 2
c. Frame Knitting Mill (FKM) ☐ 3
d. Frame Fibers ☐ 4

Frame textiles Ladysmith

e. Mill 1 ☐ 5
f. Mill 2 ☐ 6
g. Mill 3 ☐ 7
h. Mill 4 ☐ 8

i. David Whiteheads ☐ 9
j. Mooi River Textiles ☐ 10

11. **Shift**

AX BX CX ☐ 3x12 ('144)
AX BX CX ☐ 3x9.45 (132)
A B C Special ☐ 4x12 (168)

A B C D shift ☐ 4x8 Ladysmith
Day normal ☐ 5x12
D W 5 ☐ 5x9hr 45min

12. **Day / night shift**

Which shift do you work? Day ☐ Night ☐ Both day and night ☐

Over the years that you have been at this company have you worked more day shift or more night shift? Day ☐ Night ☐ Same day & night ☐

13. **Which department do you work in?** *Mark appppropriate block with an "**X**"*

a. Bale opening &blending ☐
b. Blow room ☐
c. Carding ☐
d. Draw frame ☐
e. Spinning ☐
   i. Draw frame ☐
   ii. Speed frame ☐
   iii. Ring frame ☐
f. Doubling ☐
g. Winding ☐
h. Weaving preparation ☐
   i. Warping ☐
   ii. Sizing ☐
i. Weaving ☐
j. Dye house ☐

What type of work do you do in the department you work in?

a. Operator ☐
b. Doffer ☐
c. Cleaner ☐
d. Mechanic ☐
e. Supervisor or foreman ☐
f. Other ☐

Air conditioning worker ☐
Waste collector ☐
Waste press ☐
Hyster driver ☐
Dye house ☐

If other, write any other job .....

[132] Coding boxes removed for dissertation printing, due to margin constraints.

14. **Date of birth** d d m m y y y y ☐☐☐☐☐☐☐☐

15. **How many years have you worked for the present company?** ..... Yrs

16. **How many years have you worked in your present department?** .....

17. **Have you ever worked in another department for a long tim** Yes ☐ No ☐

a. If yes which department did you work in ? .....

.....

b. How many years did you work in that department? .....

c. What type of job did you do in that department? .....

18. **Have you worked for any other textile companies?** Yes ☐ No ☐

a. If the answer is yes what is the name of that company? .....

b. How long did you work for this company? .....

c. In which department did you work? .....

C. HISTORY OF DUST / SMOKE AT PREVIOUS WORKPLACE

19. **Have you ever worked in any other factory or mine where there was dust or smoke?** Yes ☐ No ☐

a. If the answer is yes what type of industry was it? .....
b. What was the companies name? .....
c. How long did you work there? .....
d. What type of dust or smoke was in this work environment? .....
e. What type of work did you do? .....
f. Did you have any type of chest problems whilst working at this company,
Yes ☐ No ☐ If yes, specify .....

D. TOBACCO SMOKING

20. **Do you smoke?** Yes ☐ No ☐
a. Have you ever smoked Yes ☐ No ☐
(only record no if the worker has never smoked as much as much as one ciggarette a day for as long as one year)
b. If you smoked how many years ago did you stop smoking? ..... Years
If you presently smoke complete question c
c. For how many years have you smoked? ..... Years

21. **Type of cigarrette/pipe smoked**

a.	Cigarrette	Yes ☐	No ☐	
aa.	Hand rolled	Yes ☐	No ☐	
ab.	Bought in packet	Yes ☐	No ☐	
b.	Pipe	Yes ☐	No ☐	
c.	Dagga / marijuana	Yes ☐	No ☐	

22. **How many cigarettes/pipes do you smoke in a day?** *Put "X" in box*

a. Less than 20 cigarettes. If less give number ☐ Number of cig's ☐☐
b. 1Packet (20 cigarettes) ☐
c A packet and a half ☐
d. More than a packet and a half ☐

23. Do/did your parents smoke, in the house or around you? Yes ☐ No ☐

24. Do/did your children smoke, in the house or around you? Yes ☐ No ☐

E. CHEST SYMPTOMS

I am going to ask you some questions about your health, especially about your chest. Please answer Yes or No.

Coughing

25. Do you normally cough when you wake up in the morning? Yes ☐ No ☐

26. Do you cough usually during the day or night? Day ☐ Night ☐
a year?

27. Do you cough like this on most days and weeks for as much as three months
Yes ☐ No ☐

28. Is there any day of the week that your cough is worse? Yes ☐ No ☐

a. If the answer is yes, indicate which on shift in the week it is worst?

First shift ☐ Second ☐ Third ☐ Fourth ☐ Fifth ☐ Sixth ☐
Seventh ☐

If there is more than one shift that your cough is bad, mark all the appropriate shifts

b. Is that the shift that your cough is always worst on? Yes ☐ No ☐

29. Your coughing does not become better because of dust ? Yes ☐ No ☐

30. Do you ever cough so much that you become breathless? Yes ☐ No ☐

Phlegm

31. If you cough in the morning when you wake up do you cough up phlegm?
Yes ☐ No ☐

32. During the day or night do you bring up any phlegm? Yes ☐ No ☐
Day ☐ Night ☐

33. Have you brought up phegm like this on most days for as much as three months in a year Yes ☐ No ☐

34. For how many years have you been coughing up this amount of phlegm?

a. Less than one year ☐ <1year
b. More than one year but less than three years ☐ > 1year but < 3 years
c. More than three years ☐ > 3 years

Breathlessness

35. Do you suffer from any shortness of breath? Yes ☐ No ☐

If the answer is No then proceed to the following symptom - Tightness. If the answer is Yes talk to the employee and find out how severe the breathlessness is. Breathlessness is determined according to how badly it limits a person's ability to do work or do normal exercises. Find out which of the following paragraphs, a, b, c or d describes the workers condition the most accurately. Mark one of the boxes with an "X"

a. Are you able to keep to the same pace when you walk on level ground with a person the same age as you, but are unable to keep to the same pace when you walk up stairs or a hill with a person the same age as you. ☐ a ☐ b ☐ c ☐ d

b. Can you walk for a mile and not become breathless if you are walking on your own but when you walk with a person the same age as you cannot walk the mile with them without becoming breathless.

c. Do you become breathless if you walk down the road or if you walk around the cotton mill.

d. Do you become breathless when getting dressed or when talking.

Yes ☐ No ☐

Tightness

36. Does your chest ever feel tight or your breathing become difficult?

Yes ☐ No ☐

37. Are there some days which you experience this more than other days

Yes ☐ No ☐

38. If your answer is Yes, indicate which shift in the week it is most tight.

First shift ☐ Second ☐ Third ☐ Fourth ☐ Fifth ☐ Sixth ☐ Seventh ☐

39. Is your chest always tight on this shift or is your chest only sometimes tight?

Always tight on this shift ☐ Tight only sometimes ☐

40. Do you experience this at home or at work? Home ☐ Work ☐

41. Do you experience this chest tightness when you start working in the mill after a break having days off or after a few days of working in the mill.

Wheezing

42. Does your chest feel wheezy or whilstling? Yes ☐ No ☐

43. Do you suffer from attacks of shortness of breath with wheezing?

Yes ☐ No ☐

44. How many years ago did these attacks start?

☐ 0-1 years
☐ 2-4 years
☐ 5-10 years
☐ 11 years and longer

45. Do you still get these attacks? Yes ☐ No ☐

46. Did you get these attacks before going into a cotton mill? Yes ☐ No ☐

47. Is your breathing normal between these attacks? Yes ☐ No ☐

Chest illnesses

48.How many days sick leave have you had in the past year?

- ☐ 0 days
- ☐ 1-7 days
- ☐ 8-20 days
- ☐ More than 20 days

49.How many of these days were for chest illnesses?

- ☐ 0 days
- ☐ 1-7 days
- ☐ 8-20 days
- ☐ More than 20 days

50.**Have you ever suffered from?**

a. High blood pressure	Yes	☐	No	☐	Uncertain	☐	102
b. Bronchitis	Yes	☐	No	☐	Uncertain	☐	103
c. Asthma	Yes	☐	No	☐	Uncertain	☐	104
d. Tuberculosis	Yes	☐	No	☐	Uncertain	☐	105

51.If worker has had T.B. then ask the following:

a. What year did the T.B start?

- 1940-1950 ☐ 106
- 1951-1960 ☐
- 1961-1970 ☐
- 1971-1980 ☐
- 1981-1990 ☐
- 1990-1999 ☐

If year is known put year .....

b. How many months were you treated in hospital/clinic?

- ☐ 0-1 months
- ☐ 2-6 months
- ☐ 7-12 months
- ☐ 12 months and longer

c. What hospital/clinic were you treated in? .....

d. How many months treatment were you given altogether, both in hospital and the clinic?

- ☐ 0-1 months
- ☐ 2-6 months
- ☐ 7-12 months
- ☐ 12 months and longer

e. Have you been treated more than once for T.B.? Yes ☐ No ☐

ANNEXURE TWO

Baseline lung function testing and the Δ pulmonary function tests for different symptom groups.

Table A2.1 Pulmonary function of exposed symptomatic group – Numbers (%).

Socio-demographic variables		Exposed symptomatic (n=52)		
Age (yrs)	Mean	44.58		
	SD	7.07		
Height (cm)	Mean	163.94		
	SD	8.64		
Weight (kg)	Mean	74.44		
	SD	14.80		
Pulmonary function (litres) or (%)		Pre / baseline	Δ pulmonary function	P
FVC measurement (l)	Mean	3.53	-0.12	0.003
	SD	0.74	0.28	
FVC prediction (l)	Mean	3.59	0	
	SD	0.65	-	
FVC %	Mean	99.56	-3.65	0.007
	SD	19.19	9.26	
FEV_1 measurement (l)	Mean	2.48	-0.12	0.000
	SD	0.65	0.18	
FEV_1 prediction (l)	Mean	2.93	0	
	SD	0.48	-	
FEV_1%	Mean	85.75	-4.75	0.000
	SD	22.88	6.16	
FEV_1/FVC measurement %	Mean	70.53	-1.57	0.012
	SD	13.03	5.34	
FEV_1/FVC prediction %	Mean	79.91	0	
	SD	1.62	-	
FEV_1/FVC %, %	Mean	88.15	-2.33	0.013
	SD	15.70	6.51	
FEF_{25-75}% measurement	Mean	2.03	-0.31	0.000
	SD	1.22	0.54	
FEF_{25-75}% prediction	Mean	3.78	0	
	SD	0.44	-	
FEF_{25-75}%, %	Mean	54.18	-8.39	0.000
	SD	33.68	15.59	
PEF measurement (l)	Mean	6.70	-0.22	0.086
	SD	1.74	0.91	
PEF prediction (l)	Mean	7.59	0	
	SD	1.24	-	
PEF %	Mean	89.83	-3.00	0.070
	SD	25.02	11.58	

The post lung function value can be obtained by adding the Δ lung function value to baseline value.
P values only relate to Δ pulmonary function values and were obtained by paired t-test.

Table A2.2 Pulmonary function of exposed a-symptomatic group – Numbers (%).

Socio-demographic variables		Exposed a-symptomatic group (n=30)		
Age	Mean	42.97		
	SD	6.28		
Height (cm)	Mean	164.93		
	SD	11.61		
Weight (kg)	Mean	73.87		
	SD	12.40		
Pulmonary function (litres) or (%)		Pre / baseline	Δ pulmonary function	P
FVC measurement (l)	Mean	4.21	0.02	0.70
	SD	0.96	0.21	
FVC prediction (l)	Mean	3.71	0	
	SD	0.83	-	
FVC %	Mean	114.30	0.43	0.68
	SD	12.31	5.83	
FEV_1 measurement (l)	Mean	3.35	0.003	0.87
	SD	0.77	0.10	
FEV_1 prediction (l)	Mean	3.03	0	
	SD	0.61	-	
FEV_1%	Mean	111.07	0.20	0.72
	SD	12.64	3.06	
FEV_1/FVC measurement %	Mean	79.98	-0.27	0.73
	SD	7.45	4.38	
FEV_1/FVC prediction %	Mean	80.07	0	
	SD	1.25	-	
FEV_1/FVC %, %	Mean	99.83	-0.43	0.66
	SD	9.06	5.47	
FEF_{25-75}% measurement	Mean	3.42	-0.002	0.98
	SD	1.34	0.62	
FEF_{25-75}% prediction	Mean	3.89	0	
	SD	0.52	-	
FEF_{25-75} %, %	Mean	87.77	-0.23	0.93
	SD	30.71	16.10	
PEF measurement (l)	Mean	8.86	0.13	0.52
	SD	2.05	1.08	
PEF prediction (l)	Mean	7.80	0	
	SD	1.51	-	
PEF %	Mean	113.50	1.97	0.50
	SD	12.90	15.84	

The post lung function value can be obtained by adding the Δ lung function value to baseline value.
P values only relate to Δ pulmonary function values and were obtained by paired-samples t-test.

Table A2.3 Pulmonary function of unexposed control group – Numbers (%).

Socio-demographic variables		Unexposed control (n=46)		
Age	Mean	42.41		
	SD	8.79		
Height (cm)	Mean	162.96		
	SD	8.62		
Weight (kg)	Mean	77.35		
	SD	17.46		
Pulmonary function (litres) or (%)		Pre / baseline	Δ pulmonary function	P
FVC measurement	Mean	3.68	0.05	0.063
	SD	0.91	0.19	
FVC prediction	Mean	3.48	0	
	SD	0.73	-	
FVC %	Mean	105.65	1.76	0.037
	SD	15.45	5.55	
FEV_1 measurement	Mean	2.83	-0.02	0.31
	SD	0.73	0.12	
FEV_1 prediction	Mean	2.88	0	
	SD	0.55	-	
FEV_1%	Mean	97.72	-0.39	0.52
	SD	13.52	4.15	
FEV_1/FVC measurement %	Mean	77.18	-1.39	0.008
	SD	6.39	3.83	
FEV_1/FVC prediction	Mean	80.33	0	
	SD	1.56	-	
FEV_1/FVC %, %	Mean	96.13	-1.93	0.007
	SD	7.80	4.65	
FEF_{25-75}% measurement	Mean	2.60	-0.15	0.064
	SD	1.09	0.54	
FEF_{25-75}% prediction	Mean	3.78	0	
	SD	0.56	-	
FEF_{25-75} %, %	Mean	67.83	-3.91	0.084
	SD	24.03	15.04	
PEF measurement	Mean	7.72	-0.18	0.17
	SD	1.55	0.89	
PEF prediction	Mean	7.39	0	
	SD	1.40	-	
PEF %	Mean	105.57	-3.41	0.083
	SD	17.47	13.06	

The post lung function value can be obtained by adding the Δ lung function value to baseline value.
P values only relate to Δ pulmonary function values and were obtained by paired-samples t-test.

Table A2.4 Pulmonary function of all symptom group – Numbers (%).

		All subject group (n=128)			
Age (yrs)	Mean	43.42			
	SD	7.58			
Height (cm)	Mean	163.82			
	SD	9.36			
Weight (kg)	Mean	75.35			
	SD	15.28			
Pulmonary function (litres) or (%)		Pre / baseline	P	Δ pulmonary function	P
FVC measurement	Mean	3.74	0.003	-0.03	0.001
	SD	0.89		0.25	
FVC prediction	Mean	3.58		0	
	SD	0.73		-	
FVC %	Mean	105.20	0.001	-0.75	0.001
	SD	17.31		7.69	
FEV_1 measurement	Mean	2.81	0.000	-0.06	0.000
	SD	0.78		0.16	
FEV_1 prediction	Mean	2.93		0	
	SD	0.54		-	
FEV_1%	Mean	95.98	0.000	-2.02	0.000
	SD	20.23		5.35	
FEV_1/FVC measurement %	Mean	75.14	0.000	-1.20	0.45
	SD	10.54		4.61	
FEV_1/FVC prediction	Mean	80.10		0	
	SD	1.52		-	
FEV_1/FVC %, %	Mean	93.76	0.000	-1.74	0.33
	SD	12.76		5.66	
FEF_{25-75}% measurement	Mean	2.56	0.000	-0.18	0.063
	SD	1.31		0.57	
FEF_{25-75}% prediction	Mean	3.81		0	
	SD	0.50		-	
FEF_{25-75} %, %	Mean	67.06	0.000	-4.84	0.067
	SD	32.30		15.73	
PEF measurement	Mean	7.57	0.000	-0.13	0.25
	SD	1.94		0.95	
PEF prediction	Mean	7.57		0	
	SD	1.36		-	
PEF %	Mean	101.03	0.000	-1.98	0.17
	SD	22.21		13.28	

The post lung function value can be obtained by adding the Δ lung function value to baseline value.
P values were obtained by ANOVA testing, and represent differences in baseline or Δ pulmonary function values between symptom groups.

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