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Retrospective analysis of pulmonary tuberculosis epidemiology in early phase clinical trial patients in the Western Cape of South Africa from 1991 to 2008

Dissertation

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ACTH	adrenocorticotropic hormone
AFB	acid-fast bacilli
AIDS	acquired immunodeficiency syndrome
ALP	alkaline phosphatase
APTT	activated partial thromboplastin time
AST	aspartate aminotransferase
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
bpm	beats per minute
C°	degrees centigrade
CD4	CD4 T-lymphocytes
CFU	colony forming unit
cm	centimetres
cm^2	cubic centimetres
dl	decilitre
Diast.RR	diastolic blood pressure
DNA	deoxyribonucleic acid
DMP	data management plan
DOTS	directly observed treatment short-course
E	ethambutol
EBA	early bacterial activity
fl	femtolitre
g	gram
GGT	gamma-glutamyl transferase
H	isoniazid
HAART	highly active antiretroviral therapy
HCT	hematocrit
HGB	hemoglobin
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography

ID	identification
INR	international normalized ration
IUATLD	International Union against Tuberculosis & Lung disease
l	litre
kg	kilogram
LDH	lactate dehydrogenase
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDR	multi drug-resistant
mm^3	cubic millimetre
mmHg	millimeter of mercury
mmol	millimol
M. tuberculosis	Mycobacterium tuberculosis
umol	micromol
n	number of observations
N	number of patients
ng	nanogram
nmol	nanomol
NP	national party
PA	posterior-anterior
PCR	polymerase chain reaction
pg	picogram
R	rifampicin
RBC	red blood cell
RR	blood pressure
S	streptomycin
SAS	statistical analysis software
SBP	systolic blood pressure
SD	standard deviation
sec	second

STI	sexually transmitted infection
Syst.RR	systolic blood pressure
TB	tuberculosis
U	unit
USD$	US Dollars (currency)
WBC	white blood cell
WHO	World Health Organization
XDR	extensively drug resistant
Z	pyrazinamid
ZAR	South African Rand (currency)

1 Study objective

The primary objective of this retrospective study was to analyze and describe the profiles of pulmonary tuberculosis patients from the Cape Town metro area of South Africa in the time period from 1991-2008. Scope of the analysis included the specific clinical appearance, biochemical and bacteriological parameters and radiological scores of patients.

The second objective was to compare two distinct cohorts from the time periods 1991-2001 and 2002-2008 in order to:

a. Examine if significant differences in pulmonary tuberculosis severity and/or disease representation existed between the two cohorts/time periods.
b. Investigate whether specific variations in clinical, biochemical and radiological patient parameters occurred over time and how they may relate to differences in disease severity between the two cohorts.
c. Describe the discovered clinical, biochemical and radiological key parameters that differ between the two cohorts and outline possibilities in which their variation may have influenced changes in pulmonary tuberculosis severity over time.

The study applied a strong focus on historic and cultural aspects of the Republic of South Africa and the Western Cape Province and took unique environmental factors of country and region into consideration.

2 Introduction

2.1 Pulmonary tuberculosis in South Africa

2.1.1 Historic background

It is assumed that tuberculosis was introduced to South Africa by European immigrants from Britain and the Netherlands in the 17th century who were infected with the disease during the historically documented tuberculosis epidemics in Europe at the time (Edginton 2000, South African History Timelines 2000).

These first settlers arrived at the Cape of Good Hope and encountered the only inhabitants of the region, the native KhoiKhoi and San tribes, whose presence in the Cape leads back to the 5th century AD. The native people were forced to work on the settlers' quickly expanding farms and it is likely that first transmissions of tuberculosis between Europeans and South Africans took place (South African History Timelines 2000, Coovadia 2009). As more settlers arrived, colonies expanded eastwards into the territories of the amaXhosa, a 'black' South African tribe located along the country's East coast (Edginton 2000, South African History Timelines 2000) and supposedly the infection was spread further.

Between the 17th and 18th century, additional slaves were imported to the Cape from East Asia who may have introduced an additional tuberculosis genotype into Western Cape communities (van Helden et al. 2002, Mokrousov et al. 2005). These slaves of Asian background represent the ancestors of South Africans who were later classified as 'Coloureds' under apartheid and are described with the terminology 'mixed ethnic background' in the further context of this paper (Coovadia 2009, South African History Timelines 2000). Today, this specific ethnic group is almost exclusively represented in the Western Cape and forms 9% of South Africa's total population (Statistics South Africa 2012).

In the early 19th century South Africa's economic focus rapidly shifted from agriculture to mining, based on the discovery of diamonds and gold. This resulted in societal changes which also affected the transmission patterns of

infectious diseases (Houghton 1976, Shaw 1977). The growing mining industry significantly contributed to the infection of large numbers of ‘black’ South Africans with tuberculosis (TB) which was fundamentally based on poor working and living conditions such as meagre nutrition, overcrowded accommodation and physical exhaustion of ‘black’ workers. Sick miners who were sent home for recovery spread the disease among their families and rural communities and it is estimated that over 60% of the ‘black’ South African population was infected with *Mycobacterium tuberculosis* by the 1930’s (Coovadia 2009, Edginton 2000, Packard 1989).

Since the first settlers arrived, a key characteristic of health care in South Africa had been fragmentation and even in the first colonies, health facilities and health services were separated according to ethnic background, as the Public Health Amendment Act of 1897 states (Coovadia 2009, Ilbert 1899). From 1948, when the National Party came to power, apartheid, a system of social and racial separation of ‘non-whites’ and ‘whites’ was implemented and the health system was further divided. ‘Black’ people’ were denied citizenship and the government created ‘black’ homelands or ‘bantustans’ which had their own health department and to which many ‘black’ people were bound (Coovadia 2009, Marks & Andersson 1987, World Health Organization 1983). In this way, the apartheid government established a dual health system, with ‘’a superior service for urban white people and a basic health center system for the majority ‘black’ population’’ as Jinabhai 1986 points out.

Until the 1970s main pillars of the health system in ‘black’ homelands were non-profit, missionary hospitals and after the take-over by the state in the 1980s, the official per capita health budget in Western Cape homelands ranged from ZAR 23 to ZAR 200 (USD$ 11), (McIntyre 1990, Coovadia 2009, World Health Organization 1983). Coovadia et al. 2009 described this as ‘’underfunded’’ and according to Karim et.al 2009 this ‘’unstructured and poorly funded sector was not equipped or committed to tackle the tuberculosis epidemic among the by tuberculosis affected indigent population’’.

The fact that particularly citizens of rural areas had to travel long distances to reach health care facilities and to receive treatment, prevented easy access to

TB medication and made it impossible for many (Edginton 2000). Although apartheid regulations restricted ‘black’ people to the ‘bantustans’, the ‘black’ population in South African cities grew by 94% between 1921 and 1936 (Coovadia 2009, Rooiyard 1948). This enhanced the spread of tuberculosis and incidence reached its first peak with over 350 cases per 100.000 population in the 1960s (Salim et al. 2009).

A human immunodeficiency virus (HIV) epidemic in the early 1990s caused an alarming rise in new tuberculosis cases. HIV incidence in pregnant women attending prenatal clinics grew from 0.7% in 1990 to 30.2% in 2005. By 2006 almost every fifth South African was infected with HIV and with 0.7% of the world’s population the country had 19% of all cases of TB in HIV-positive adults (AVERT 1986, Government of South Africa 2001, Government of South Africa 2007, Government of South Africa 2008, Nattrass 2008, Wulfsohn 1985).

In 1996 the World Health Organization conducted a survey to assess the status of TB in South Africa. The Joint Programme Review was based on population estimates of 41.4 million and 130.000 new TB cases for 1995 and showed that South Africa had one of the highest annual TB incidence rates (311 per 100.000) in the world (World Health Organization and Department of Health South Africa 1996). Key findings of the report showed differences in annual TB incidence rates for different ethnic groups among the population, led by people of mixed ethnic background with 713 TB cases per 100.000 and variations by region with the Western Cape being most affected with 737 TB cases per 100.000 inhabitants (World Health Organization and Department of Health South Africa 1996).

Despite initiatives of the South African government to implement tuberculosis prevention measures, TB incidence continued to rise and an increase from 109.328 to 224.420 notified cases was reported between 1996 and 2002. The TB cure rate (49.7%) and TB treatment completion rate (60.5%) as measures for treatment outcome reached a low in 2001 (Government of South Africa 2006). At the same time multi drug-resistant TB (MDR-TB) emerged, defined by *M. tuberculosis* resistance to isoniazid and rifampicin with or without an additional resistance to other first-line drugs (Schaaf et al. 1996, Weyer et al.

1995, World Health Organization 2012b). A countywide survey of TB drug resistance in 2002 showed, that all South African provinces had already documented TB cases of this multi drug-resistant type (World Health Organization 2004, Weyer et al. 2004).

In 2003 the free distribution of antiretroviral drugs in public hospitals was initiated after the connection of HIV/AIDS and tuberculosis had been recognized, but TB incidence grew further to 720/100.000 and case notifications reached 341.165 by 2006 (Compion 2008).

In 2005 tuberculosis had been declared a national crisis by the South African Minister of Health (Government of South Africa 2006) and in the same year the World Health Organization declared TB an emergency in the AFRO Region. As a result the National TB Crisis Management Plan was introduced in South Africa whose goal was to address the districts with the highest TB disease burden and poor treatment results and to improve the situation by increasing smear conversion and TB cure rates by more than 10% within one year (Government of South Africa, 2006).

Several TB control plans followed and in 2011 the current National Strategic Plan on HIV, STIs and TB 2012-2016 was announced (Government of South Africa 2011, Mail & Guardian 2011). This plan states, that about 1% of South Africans develop tuberculosis every year and the highest incidence of latent TB, estimated at 88%, occurs among the age group of 30–39 year olds in township situations and poor living conditions (Government of South Africa 2011).

2.1.2 Current situation

Latest 2014 World Health Organization (WHO) figures show that South Africa is still among the six countries with the largest number of tuberculosis incident cases (410.000–520.000) together with India (2.0 million‑2.3 million), China (0.9 million‑1.1 million), Nigeria (340.000–880.000), Pakistan (370.000–650.000) and Indonesia (410.000–520.000) whereby India and China alone accounted for 24% and 11% of global cases respectively (World Health Organization 2014a).

2013 figures suggesting 450.000 incidences of TB (850 per 100.000), indicate a slight decreased in new TB cases in comparison to 2011 with 500.000 cases (993 per 100.000) but remain higher than in the years 2010 and 2006 with 484.000 and 341.165 new TB cases respectively (World Health Organization 2007, World Health Organization 2011, World Health Organization 2012a).

In 2011 an estimated 330.000 among the 500.000 estimated cases of tuberculosis were HIV-positive (65%) (World Health Organization 2012a) and in 2012 HIV was shown to be one of the most significant contributors to maternal mortality in South Africa (Chweneyagae et al. 2012, National Department of Health South Africa 2012). Prevalence of tuberculosis in HIV-infected pregnant women in SA was just slightly below that of the general population with approximately 795 per 100.000 in 2012 (World Health Organization 2012a).

The WHO stated that South Africa currently ranks amongst the 4 countries with the highest estimated burden of MDR-TB together with China, India and the Russian Federation, these countries together almost hold 60% of the world's cases of MDR-TB. There were almost 10.085 notified MDR-TB cases out of which 5.643 were enrolled in treatment programs in 2011. This shows a steady increase from the year 2010 in which 7.386 MDR-TB cases were notified (World Health Organization 2011, World Health Organization 2012a).

The rising global numbers of drug-resistant TB cases represents a growing threat. In 2011 the number MDR-TB cases among pulmonary TB notifications reached 320.000 worldwide, in 2012 an estimated 450.000 people developed MDR-TB and an estimated 170.000 people died from MDR-TB (World Health Organization 2012a). In 2011 extensively drug-resistant tuberculosis (XDR-TB), defined as MDR-TB plus additional resistance to any flourchinolone and at least one of three injectable second-line drugs, had been identified in 84 countries globally. By using reported data from 65 countries the proportion of MDR-TB cases with XDR was identified to be 9.0% by the WHO in 2011 (World Health Organization 2012a). By the end of 2012 at least one case of XDR- TB had been reported by 92 countries and on average, an estimated 9.6% of MDR-TB cases had XDR-TB (World Health Organization 2012a).

MDR-TB today is quickly spreading among the HIV-positive population in sub-Saharan Africa (World Health Organization 2008a, Weyer 2007). By the end of 2012, 15 countries in the African region had identified and reported at least one case of XDR-TB and South Africa remains the country, which reports most XDR-TB cases in the world (World Health Organization 2012a).

In 2006 the WHO had announced that XDR-TB was detected in the rural village of Tugela Ferry in Kwazulu-Natal, South Africa (Singh & Padayatchi 2007). Of the 544 patients studied in the area in 2005, 221 had MDR-TB. Of these 221 cases, 53 were identified as XDR-TB and therefore represented almost one-sixth of all known XDR-TB cases reported worldwide. All of the patients who were tested for HIV were found to be infected (Wright 2006, World Health Organization 2006). In 2010, 741 laboratory-confirmed cases of XDR-TB in South Africa were counted (Government of South Africa 2006). In 2012 this had increased to 1596 confirmed cases of XDR-TB (World Health Organization 2012a).

Lastly, it is important to note that the tuberculosis cure rate in South Africa has been improving over the years from 54% of notified new smear-positive tuberculosis cases in 2000 (Government of South Africa 2011) to 74% in 2011 (National Department of Health South Africa 2014, Barron et al. 2007). The treatment success rate of new TB cases is estimated to be 79% for the years 2013/2014, however the global target of >85% has not yet been reached (World Health Organization 2012a, National Department of Health South Africa 2014).

The established estimate that about one-third of the global population is currently infected with latent tuberculosis remains applicable (World Health Organization 2014b). 1.3 million people died from tuberculosis in 2012, including almost one million deaths among HIV-negative and 300.000 among HIV-positive individuals. Tuberculosis continued to be one of the top killers of women, with 240.000 deaths amongst HIV-negative and 160.000 deaths amongst HIV-positive women in 2012 (World Health Organization 2012a).

2.2 Risk factors for pulmonary tuberculosis

2.2.1 Poverty

There are clearly poverty related risk factors of pulmonary tuberculosis. Several studies show that a populations' nutritional status is strongly connected with the degree of poverty, defined by societal, economic and environmental development of a country and has a strong influence on TB incidence (Lönnroth et al. 2009, Cegielski & McMurray 2004). Malnutrition, according to the World Health Organization 2008b, is linked to an increased risk to develop active disease from TB infection, which is based on the negative effects of a lack in micro-and macronutrients on the immune system, as Lönnroth et al. 2010 and Onwubalili 1988 described.

The relationship between nutritional status and infectious diseases has been known for centuries (Scrimshaw 2003) and the ancient Greeks already described the weight loss in TB patients as 'consumption' (Davies 2000). In this context, multiple studies have suggested that poor nutritional status may increase susceptibility to tuberculosis (Macallan 1999, van Lettow et al. 2003) and being underweight at the time of TB diagnosis has been associated with increased relapse rates (Khan et al. 2006) and faster time to death (Zachariah et al. 2002).

Crowded living conditions, badly ventilated rooms and co-habitation between animals and humans are additional risk factors of tuberculosis susceptibility and progression of disease (Vynnycky et al. 1999, Chan-Yeung et al. 2005, Munch et al. 2003, Lienhard et al. 2003, Radhakrishna et al. 2007).

2.2.2 Demographics

Demographic predispositions such as gender and age of individuals represent important risk factors for TB.

Men and women are exposed to different environmental risk factors and show different health-seeking behavior and treatment compliance (Uplekar et al. 2001). Women can experience longer delays and greater barriers to early

tuberculosis detection than men (Karim et al. 2007). Particularly in the developing world, women are often disadvantaged with regards to financial means, education and access to information and their health status is often decided on by their husbands or partners (Mukherjee et al. 2012).

In addition to cultural and socioeconomic aspects, studies in India suggest that variations in cellular immune response between genders and different hormonal backgrounds may give females an advantage and reduce TB incidence (Mukherjee et al. 2012). The male to female ratio in patients with pulmonary tuberculosis has been shown to be as much as 2:1 by a number of studies and among women, more teenagers were diagnosed with tuberculosis whilst among men, more elderly were infected (Vynnycky 1996, Hudelson 1996, Salim et al. 2004, Rao 2009). Particularly the category of young females between the ages 15-44 appears to be more at risk of active disease after TB infection than men (Rieder 1999, Borgdorff et al. 2000, Radhakrishna et al. 2003). Children, on the other hand, are less likely to develop primary disease but strongly tend to develop non-pulmonary forms of tuberculosis (Vynnycky, E. & Fine, P.E.M. 1997, Sutherland et al. 1982).

2.2.3 Self-inflicted risk factors

So-called ‘self-inflicted risk factors’ include extreme alcohol consumption and smoking of tobacco and increase the possibility of developing primary pulmonary tuberculosis and the reactivation of latent tuberculosis infection (Bates et al. 2007, Lönnroth et al 2007).

Explanations for the impact of smoking on pulmonary tuberculosis such as reduced immune response are outlined in several studies and meta-analysis have shown that smoking almost doubles the risk of a negative TB outcome (Bothamley 2005, Slama et al. 2007, Lin et al. 2007). In studies on the effect of smoking cessation, it was shown that TB mortality significantly dropped, after smoking was given up (Wen et al. 2010).

2.2.4 Obesity

It is currently under discussion whether or not inflammatory reactions, which are obesity specific, increase protection and survival from tuberculosis. As suggested by Roth 2009, the fact that obese individuals produce more pro-inflammatory cytokines could result in a stronger immune system and better reaction towards TB infection.

This is supported by a cohort study of 42.116 individuals of 65 years or older in Hong Kong, China which showed a risk-limiting effect of high BMI that was specific for pulmonary tuberculosis. Obese (BMI >30) and overweight (BMI: 25-30) individuals of the study were at significantly lower risk of developing active tuberculosis than individuals of normal weight (BMI: 18.5-25), (Leung et al. 2007).

A South African study concluded that HIV-infected individuals with obesity and overweight BMIs have a significantly reduced risk of TB infection and mortality after adjustment to highly active antiretroviral therapy (HAART), (Hanrahan et al. 2010). Studies conducted in Tanzania showed similar results and stated that low BMI and falling BMI predict HIV-associated TB (Maro et al. 2010).

2.2.5 Diabetes

Diabetes seems to have an effect on the severity and infectiousness of tuberculosis, and Stevenson et al. 2007 stated, that once TB treatment has started, diabetes increases the possibility of treatment failure and relapse. Multiple studies show that glucose intolerance in pulmonary TB can occur as a result of the infection and improve or reverse after TB therapy (Jawad et al. 1995, Basoglu et al. 1999, Oluboyo & Erasmus 1990). Other studies have identified Diabetes mellitus as a significant predisposition for active pulmonary tuberculosis (Kim et al. 1995, Coker et al. 2006).

2.3 Clinical relevance of TB and HIV co-infection

The mortality risk is significantly higher in TB/HIV-co-infected patients than in patients with just one of the two diseases (Tabarsi, P. et al. 2008, Stoneburner, R. et al. 1992, Perriens, J.H. 1991, Sande, M. et al. 2004) and there is a known synergistic effect in co-infected patients, whereby the two diseases enhance each other's progress (Lawn & Bekker 2009, Whalen, C. 1995, del Amo, J. 2003).

The clinical manifestation of tuberculosis in HIV-positive patients can differ significantly and is strongly related to the progression level of the HIV infection and the patient's CD4 count (Gilks et al. 1990, Elliot et al. 1993, Batungwanyao et al. 1992). In early HIV infection, patients' present characteristics of post-primary tuberculosis with typical symptoms such as severe lung damage with cavitation and upper lobe involvement (De Cock et al. 1992). Features of HIV-infected individuals with normal CD4 counts are often not different from those of HIV-negative individuals (Klein et al.1989) but in a further progressed HIV infection and with falling CD4 counts, patients usually present an atypical pulmonary disease similar to primary pulmonary tuberculosis (de Cock et al. 1992, Richter et al. 1994).

HIV-positive individuals then show AFB (acid-fast bacilli) negative sputum smears and can present extra-pulmonary infection sites (Harries et al. 1998, Jones et al. 1997, Klein et al. 1989, Mugusi et al. 2006). Classical patterns of tuberculosis on chest X-rays can be entirely missing (Perlman et al. 1997) and pulmonary infiltrates without cavities and lower lobe involvement are more frequently found (Harries et. Al 1998). In cases with latent tuberculosis it is known that the rate of progression to active disease or reactivation of tuberculosis is much higher in HIV-positive than in HIV-negative patients, independent of CD4 cell counts (Ackah et al. 1995).

Because of the various appearances of TB in HIV co-infected patients, standard diagnostic methods in the developing world such as chest X-ray and sputum smears frequently fail to detect TB. Particular awareness of the high possibility

of HIV-positivity and standard HIV testing of TB patients are key preventative measures (Harries et al. 1998).

2.4 Diagnosis of pulmonary tuberculosis

2.4.1 Clinical profile and hematology

In regions of high TB incidence, pulmonary tuberculosis should be considered if a patient presents himself with chronic cough (> 3 weeks) hemoptysis or acute pneumonia that has not responded to penicillin. Fever, night sweats, fatigue, loss of appetite and weight loss are common symptoms (Enarson et al. 1994).

HIV-infected patients have an increased risk of pneumonia, mostly caused by *Streptococcus pneumonia*. In cases of known HIV infection, the failure to respond to a broad spectrum antibiotic should be an additional criterion for the suspicion of pulmonary tuberculosis (Hirschtick et al. 1995, Gilks et al. 1996).

Wasting is an important symptom of tuberculosis and particularly in the developing world many patients present a significant degree of wasting by the time they are diagnosed with TB (Kennedy et al. 1996, Harries et al. 1988, Zachariah et al. 2002). Tuberculosis related wasting is believed to be caused by a combination of reduced appetite and changes in metabolism, resulting from inflammatory and immune responses (Paton et al. 2003, Macallan 1999).

The relationship of lean tissue mass to fat mass is an accurate measure for the assessment of a TB patient's wasting status (Van Itallie et al. 1990, Kyle et al. 2003, Evans et al. 2008). When the weight loss exceeds 5% within 3-12 months combined with signs of fatigue, loss of skeletal muscle and abnormal blood parameters such as anemia, inflammation and low serum albumin this status can be classified as 'cachexia' (Evans et al. 2008). Other typical laboratory findings that can be found but are not specific for the diagnosis of tuberculosis are anemia (often normochromic, normocytic) and leukocytosis (Morris et al. 1989, Lee et al. 2006, Ramakrishnan et al. 2008).

2.4.2 Sputum microscopy and smear grading

Sputum microscopy for AFB is the most common diagnostic method for pulmonary tuberculosis in developing countries and recommended by the World Health Organization as initial technique in the diagnosis of pulmonary tuberculosis (World Health Organization & Stop TB Partnership 2009). It is usually recommended that pulmonary TB suspects submit three sputum specimens for microscopy (Toman 1979), however recent studies have shown that the additional value of a third smear examination after two negative examinations is relatively small (Harries et al. 1996, Ipuge et al. 1996). Most laboratories screen sputum smears for AFB using light microscopy and Ziehl-Neelsen staining. Sputum smear microscopy is considered positive when there are 10.000 organisms per ml of sputum (Krysl et al. 1994). Positive smears are graded from 1-9 AFB per 100 high-power fields to >10 AFB per 20 fields. Therefore the examination of 100 fields is required before a smear can be pronounced negative. Disadvantage of the technique is its reduced sensitivity in HIV-positive patients, especially in individuals with severely compromised immune status (Harries et al. 1998, Mugusi et al. 2006).

2.4.3 Chest radiography

The radiological appearance of tuberculosis can have many different patterns and particularly in primary pulmonary tuberculosis it can be found to be normal in about 15% of cases (Daley, C.L. & Gotway, M. 2009). Regular radiographic findings are represented by lymphadenopathy and resulting hilar enlargement with or without perihilar infiltrate and pleural infusions (Krysl et al. 1994, Daley, C.L. & Gotway, M. 2009, Leung, A.N. 1999, Stead et al. 1968, Choyke et al. 1983). The right lung is usually more commonly affected, which Leung, A.N. 1999 related to the larger capacity of ventilated lung. Other typical radiological findings are miliary disseminations, atelectasis, pleural effusions and focal nodules. Their manifestations, however, strongly vary depending on factors such as age and immune status of the individual (Daley, C.L. & Gotway, M. 2009).

In post-primary tuberculosis most patients show abnormal chest radiographs even in absence of respiratory symptoms (Barnes et al. 1988). Classic findings in post-primary pulmonary tuberculosis are cavitation and alveolar or interstitial infiltrates, mainly in the lung apex or upper areas of the lower lobes (Hoffmann & Churchyard 2009). Patients with HIV infection can present atypical radiographic findings such as infiltrates without cavitation, particularly in the lower lobes, and additional hilar lymphadenopathy (Harries et al. 1998).

2.4.4 Tuberculosis culture

Culture of tuberculosis bacilli in liquid or on solid media is known as the 'goldstandard' technique for tuberculosis diagnosis. With this method, the growth of *M. tuberculosis* is initially confirmed by a Ziehl-Neelsen stain and contamination is excluded. Afterwards, a variety of procedures i.e. phenotypic methods (Niacin test), mycolic acid analysis (high-performance liquid chromatography (HPLC)) or genotypic analysis (nucleic- acid-based assays) can be used to identify acid-fast bacilli in the culture (Whitelaw & Sturm 2009). The TB culture requires safety facilities that are expensive to build and maintain and laboratory technicians to perform the procedure. The procedures are time intensive and diagnosing TB with culture can take weeks based on slow growth rates of TB bacilli (World Health Organization & Stop TB Partnership 2009).

2.4.5 GeneXpert MTB/RIF

GeneXpert is an automated and cartridge based rapid polymer chain reaction (PCR) for the combined detection of *M. tuberculosis* and rifampicin susceptibility. The technique detects deoxyribonucleic acid (DNA) sequences specific for tuberculosis and rifampicin resistance, by isolating and concentrating *M. tuberculosis* bacilli from sputum samples and separating their genomic DNA. The technique uses unprocessed sputum samples and results are on average obtained in 90 minutes. Minimal technical training to be able to operate the GeneXpert and limited biohazard are described advantages of the

method (Van Rie et al. 2010, Helb et al. 2010). In 2010 the WHO endorsed GeneXpert for the use in tuberculosis endemic countries; it was therefore not yet available in the time range from which our study population stems (Steingart et al. 2014).

2.5 Treatment of pulmonary tuberculosis

The so-called ‘first line’ anti-tuberculosis drugs which are commonly used for treatment of pulmonary tuberculosis include isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E). Streptomycin (S) as addition to the ‘first line’ treatment regimen in patients with TB relapse has no more significance today. The most common regimen today is an initial HRZE treatment phase of 2 months which is followed by a HR continuation phase of another 4 months.

The same regimen applies to TB/HIV co-infected individuals (Grzemska 2009, World Health Organization 2010) but optimal treatment periods in these cases are difficult to determine as re-infection with a different TB strain can cause recurrence in the follow-up phase (Nahid et al. 2007). This is an important aspect of TB treatment schemes, especially for HIV-positive patients in areas with high TB incidence (Charalambous et al. 2008, Verver et al. 2005).

Since the discovery of rifamycins and the introduction of rifampicin into standard anti-tuberculosis regimens, no new anti-tuberculosis agent was evaluated or formally registered for several decades (Maggi et al. 1966). In 2012 bedaquiline, a diarylquinoline was the first drug in over 40 years that has been approved for cases of pulmonary MDR-TB in which no other effective treatment regimen is possible. The approval of bedaquiline for the treatment of MDR-TB is perceived as a critical step forward in the fight against TB (Sundare 2014).

3 Methodology

3.1 Background of retrospective data

3.1.1 EBA studies

The bactericidal effect of anti-tuberculosis agents can be measured by studying the drop in numbers of colony forming units (CFU) of *M. tuberculosis* in the sputum of patients with smear-positive pulmonary tuberculosis. In the pioneering EBA studies anti-tuberculosis agents of the time were studied alone and later in combination during the first 14 days of treatment. Study results showed significant differences between the various drugs, and since these differences were most obvious during the first 2 days of treatment, this specific period of activity was named ‘early bactericidal activity’ (EBA) or ‘standard EBA’ (Donald & Diacon 2008, Jindani et al. 1980).

EBA studies are conducted to assess bacterial activity, pharmacokinetics, safety and drug tolerability in early phase II (proof of concept) trials. The testing of a range of doses allows to establish a top dose beyond which no increase in EBA occurs and a lowest dose at which bactericidal activity can still be found. Pharmacokinetic studies allow the understanding of relationships between bactericidal activity and different pharmacokinetic parameters and the toxicity in patients can be studied under close hospital observation in which toxic events are related to the pharmacokinetic specifications of the agent (Donald et al. 1997).

The bactericidal activity of a drug or a drug combination on bacteria is measured in serialized 16-hour overnight sputum by means of fall in log10 of CFUs of *M. tuberculosis* per ml sputum per day on agar plates. The duration of such trials can range from 2 days to 14 days and discovered suitable doses can be tested in additional, more extensive clinical trials (Donald & Diacon 2008).

Several EBA studies have been conducted by the private research institute Task Applied Science in the Western Cape of South Africa. Studies are mostly conducted over 2 day periods based on the initial findings by Jindani et al 1980 but recent studies have also been carried out over 5, 7 and 14 days and it is

expected that valuable additional information can be discovered from these ‘extended EBA’ studies, (Johnson et al. 2006, Dietze et al. 2001, Donald et al. 2003, Donald et al. 1997).

3.2 Study population

The patient population, which formed the basis of our study, consisted of a total cohort of N=1178 patients of mixed genders, age groups and ethnic backgrounds.

All patients had initially been diagnosed with a case of uncomplicated, drug-susceptible, smear-positive pulmonary tuberculosis by one of the primary care clinics that collaborated with the Institute of Task Applied Science at the time and volunteered to participate in an early bacterial activity (EBA) study. N=670 patients were recruited for EBA trials conducted during the period of 1991-2001 (in the following referred to as ‘old’ patients) and n=508 patients participated in EBA trials during the period of 2002 to 2008 (in the following referred to as ‘new’ patients).

In order to assess the suitability of these patients to enter any EBA trials, an initial assessment and ‘baseline screening’ of each individual had been conducted. In cases of newly diagnosed and previously untreated, uncomplicated pulmonary TB, individuals were admitted and required to have posterior-anterior (PA) chest X-rays taken, which had to be compatible with TB. Furthermore, a sputum smear had to show positive on direct microscopy for acid-fast bacilli on the scale of the International Union against Tuberculosis and Lung Disease (IUATLD), see section 2.3.3 for details, and a general anamnesis and HIV test had to be conducted. Only in ‘new’ patients after 2002 detailed blood tests were performed.

In addition participants were required to meet the following key inclusion criteria prior to randomization for EBA studies between 1991 and 2008:

a. Treatment naive subjects (or no received treatment in the last 3 years) with *M. tuberculosis* infection and willing to start anti-TB therapy.
b. HIV-negative status or HIV-positive status without HIV related complications and CD4 T-lymphocyte count of 300 cell/ mm^3 or above.
c. Male or female gender and age range from 18 to 64 years.
d. Ability to produce an adequate volume of sputum as estimated from a spot assessment (estimated 10 ml or more overnight production).
e. Negative pregnancy test in female participants of childbearing potential and agreement to use a highly effective method of birth control.
f. Agreement to use an adequate method of contraception (double barrier) by male participants throughout participation in the trial and for 10 weeks after last dose.

Additional in-and exclusion criteria may have varied in each EBA study protocol, however, these were not retrievable as part of this study.

3.3 Data collection and evaluation

The collection of patient folders to provide relevant data for this study took place at Tygerberg Hospital and the Institute of Task Applied Science on Karl Bremer Hospital Campus in the Western Cape of South Africa between February and December 2010. All of the collected data represent selected ‘baseline’ data of the before mentioned patients, who had been found suitable and selected for participation in EBA trials. Thus, our study data represent parameters of treatment naïve patients with diagnosed pulmonary tuberculosis.

3.3.1 Demographics, biometrics, bacteriology and hematology

3.3.1.1 'Old' patients

The 'old' patient data of EBA studies between 1991 and 2001 were retrieved from the original patient folders at Tygerberg Hospital as well as in the format of comprehensive Excel files.

The Excel files had been previously designed from the original patient folders for the purpose of better data storage and safety and contained identical patient information in a compiled electronic format. Each Excel file contained the demographic, biometric and microbiological information on the entire patient population who had participated in the same, anti-tuberculosis agent specific EBA trial.

In order to achieve coherency of all patient data from 'old' EBA studies, the separate Excel files were compiled by us and entered into one comprehensive 'old' master Excel table. This table was then divided into primary sub-groups of the tested EBA anti-tuberculosis acting agent and each sub-group was further detailed into the individual patient numbers and all related patient data of each EBA study in descending order. Errors were avoided by using explicit Excel functions such as VLOOKUP during the transfer of individual Excel files into one master table.

In order to control the correct data transfer an equivalent of 150 random crosschecks of data between the master Excel table and individual EBA study excel files as well as between master Excel table and original, handwritten patient folders were performed and showed a data transfer correctness of 100%.

3.3.1.2 'New' patients

Patient data of 'new' EBA studies from 2002 onwards were received in electronic format from the database archives of the Global Alliance for TB drug development. An official written permission was granted to us to collect, save and further process all data received through them.

The ‘new EBA’ data-files were received and reviewed in SAS (Statistical Analysis Software) format. All patient data per individual ‘new’ EBA study were primarily listed in a separate SAS table named after the individual EBA study in which they had participated.

These SAS tables were then transferred into Excel 2007 individually and, in a second step, by means of Excel VLOOKUP functions, transferred and compiled into one comprehensive ‘new EBA’ master Excel table.

3.3.2 Radiology

3.3.2.1 ‘Old’ patients

The original chest X-rays of the EBA patient cohort from 1991-2001 could not be retrieved for scoring and evaluation. However, n=523 patient folders (78%) of 670 ‘old’ patients contained the original primary chest X-ray assessment and scoring of each patient, following the original X-ray score sheet by Simon which in the further process of this study was used as the basis of a purpose designed X-ray evaluation form (see 10.Appendix) for the scoring of the ‘new’ patients’ chest X-rays. Each one of the n=523 patients had chest X-ray scores that assessed the parameters of ‘extent of disease’ and ‘cavitation’. ‘Cavity wall thickness’ had been measured for n=491 patients.

3.3.2.2 ‘New’ patients

The original chest X-rays of n=349 (69%) of 508 ‘new’ patients were received from ‘Metrofile’, a private medical archive by which information from past EBA studies is stored.

Name, date of birth and patient identification (ID) number of each X-ray folder were compared with the patient information on the database to ensure matching patient information. This procedure was repeated by two independent individuals (Schmidt-Rhode, Diacon) in order to minimize errors.

The chest X-rays of all n= 349 ‘new’ patients were scored according to the specifically designed evaluation form, assessing the radiological parameters ‘extent of disease’, ‘cavitation’ and ‘cavity wall thickness’ (see Radiology 3.4.2). The two additional parameters, ‘numbers of lobes involved’ and ‘lung quadrant involvement’ were introduced by us and added to the score sheet. These parameters had not been assessed for the ‘old’ patient cohort. The retrieved information was then registered in a specifically designed Excel 2007 spreadsheet named ‘X-rays new EBA’.

3.3.3 Sputum microscopy and smear grading

One of the screening tests for all ‘old’ and ‘new’ patients was the microscopic examination of early morning sputum. In order to participate in the EBA studies, patients had to be positive for acid-fast bacilli on direct smear examination.

The IUATLD scale proposes five groups for reporting the results of reading smears under x1000 magnification for acid fast bacilli and ‘old’ and ‘new’ patients had been recorded accordingly, see Table 1.

Table 1: IUATLD[1] scale for smear grading

Microscopy findings	Grading classification
No acid-fast bacilli found in at least 100 fields	negative
1 to 9 acid-fast bacilli per 100 fields	scanty positive
10 to 99 acid-fast bacilli per 100 fields	+
1 to 10 acid-fast bacilli per field in at least 50 fields	++
More than 10 acid-fast bacilli per field in at least 20 fields	+++

[1] (Rieder et al. 1998)

Out of a total ‘old’ patient population of N=670, detailed sputum grading results were available for n=563 (84%) patients. In the 'new’ patient population of N= 508, sputum grading results were found for n=481 (95%) patients.

3.3.4 Set-up of a Microsoft Access ® patient database

In order to design one consistent and comprehensive database that could hold all ‘old’ and ‘new’ EBA patient and X-ray data from 1991-2008, the template for an ‘all data’ Excel table 2010 was designed. In this template, column headlines were allocated to measurable demographic, biometric, microbiological and hematological features and X-ray measures and rows were categorized into ‘old’ and ‘new’ EBA studies and further into individual EBA studies and the related patient names and ID numbers.

This template was then imported into Microsoft Access 2010 to serve as the ‘blueprint’ structure into which all further data could be imported.

In a second step the individual master Excel files of ‘old EBA data’, ‘new EBA data’, ‘old EBA X-rays’ and ‘new EBA X-rays’ were converted from Excel 2007 into Excel 2010 and were saved as individual text files.

Lastly, a link for each patient number of each EBA study was created in Microsoft Access 2010 between the individual EBA study master Excel files and the Microsoft Access template and used to import all patient information and X-ray parameters from the individual Excels text file into one comprehensive Microsoft Access 2010 database.

Following the data transfer to Microsoft Access 2010, n=150 crosschecks were undertaken between the original Excel tables and the Microsoft Access database and no data transfer related errors could be found.

3.4 Patient data in detail

3.4.1 Demographics, biometrics, bacteriology, hematology

The spectrum of ‘old’ patient parameters was significantly smaller than the spectrum of ‘new’ patient parameters. Out of a total population of N=1178 patients, the cohort of n= 670 ‘old’ patients contained significantly less detailed blood and microbiology results than the cohort of n=508 ‘new’ patients. The responsible laboratories and applicable ranges were not identified in several of the original EBA study documents; hence hematology data were transferred to identical measuring units by us and compared to internationally approved ranges.

The analyzed parameters per ‘old’ and ‘new’ patient population can be found in Table 2, parameters with exclusive availability to ‘new’ patients can be found in Table 3.

Table 2: Demographic, biometric and bacteriological parameters assessed for ‘old’ and ‘new’ patients

Parameters	Category/Unit	Definition
Demographics		
Ethnic background	Mixed ethnicity, ‘black’, white	
Gender	male, female	
Age	years	
Smoking	yes/no	≥1 cigarette/ day: yes
Biometrics		
BMI	kg/m^2	<18.5: underweight[2] 18.5-25.0: normal[2] >25.0: overweight[2]
Weight	kg	
Height	cm	
HIV status	positive, negative	
Bacteriology		
Smear grading	scanty, 1+, 2+, 3+	see IUATLD scale, Table 1.

[2] World Health Organization (2006). *Global Database on Body Mass Index.* Retrieved January 2014, from World Health Organization: http://www.assessmentpsychology.com/icbmi.htm.

Table 3: Biometric, bacteriological and hematological parameters exclusive to 'new' patients

Parameters	Unit	Normal range
Bacteriology		
TTP	hrs	
Biometrics		
Temperature	C°	orally: 36.8
Systolic RR	mmHg	90-119[3]
Diastolic RR	mmHg	60-79[3]
Pulse	bpm	60-100[4]
CD4 count	cells/mm^3	500-1200[5]
Hematology		
Blood chemistry		
Glucose	mmol/l	3.05 – 6.1[6]
Sodium	mmol/l	134-145[7]
Potassium	mmol/l	3.4-5,2[8]
Chlorine	mmol/l	95-112[8]
Calcium	mmol/l	2.15-2.65[8]
Phosphate	mmol/l	0.8-1.5[8]
Triglycerides	mmol/l	0.83-1.7[7]
Cholesterol	mmol/l	3.1-6.5[7]
Creatinine	umol/l	44-106[7]
Uric Acid	mmol/l	0.18-0.48[8]
Complete blood cell count		
RBC count *male*	10^{12}/l	4.3-6.2[8]
RBC count *female*	10^{12}/l	3.8-5.5[8]
WBC count	10^9/l	4.1-10.9[8]
Platelets	10^9/l	150-350[6]
MCV	fl	85-98[6]
MCHC	g/dl	30-36[6]
MCH	pg	27-34[6]
HCT *male*	l/l	0.4-0.52[7]
HCT *female*	l/l	0.35-0.47[7]
HGB male	g/dl	14.0-18.0[6]
HGB female	g/dl	12.0-16.0[6]

[3] American Heart Association. (2014). *Understanding Blood Pressure Readings.* Retrieved 2014,online.
[4] American Heart Association. (2014). *Target Heart Rates.* Retrieved 2014, online.
[5] Bofill M. et al. 1992
[6] Hahn, J. M. (2006). *Checkliste Innere Medizin. Normalwerte in der Labordiagnostik.* Thieme. Retrieved 2014, online.
[7] Hagemann, O. (2004). *Wichtige Laborparameter und Referenzbereiche.* Berlin: Labor für Laboratoriumsmedizin und Pathobiochemie (Charité-Universitaetsklinik Berlin). Retrieved 2014, online.
[8] University of Texas Southwestern Medical Center Dallas. *Normal Reference Range Table from Interactive Case Study Companion to Pathologic basis of disease.* Retrieved 2012, online.

Table 3 continued: Biometric, bacteriological and hematological parameters exclusive to 'new' patients

Parameters	Measures/Unit	Normal range
Differential blood cell count		
Monocytes	%	2.0-8.0[6]
Neutrophils (seg.)	10^9/l	50-70[6]
Neutrophils	%	2.0-8.0[9]
Lymphocytes	%	25-40[6]
Lymphocytes absolute	10^9/l	1.0-4.8[9]
Reticulocytes	%	0.5-1.5[8]
Reticulocytes absolute	cells/l	0.05-0.1[8]
Enzymes		
AST *male*	U/l	up to 50[9]
AST *female*	U/l	up to 35[8]
ALP *male*	U/l	40-129[8]
ALP *female*	U/l	35-104[8]
Amylase	U/l	up to 100[8]
GGT *male*	U/l	up to 66[8]
GGT *female*	U/l	up to 39[8]
LDH *male*	U/l	up to 248[8]
LDH *female*	U/l	up to 247[8]

3.4.2 Radiology

The posterior anterior (PA) chest X-rays of n=349 'new' patients were examined by our study team (C. Schmidt-Rhode, Prof. P .Donald & Prof. A. Diacon) and described and scored by means of a purpose designed X-ray evaluation form by Simon (see 10.Appendix) which describes 5 aspects of a chest X-ray as explained below.

3.4.2.1 Extent of disease

For 'extent of disease' the overall surface of the tuberculosis affected area of lung tissue was described by means of 6 categories in ascending order.

Abbreviation key: ACTH: adrenocorticotropic hormone, GGT: gamma-glutamyl-transferase, HCT: hematocrit, HGB: hemoglobin, LDH: lactate dehydrogenase, RBC count: red blood cell count, WBC count: white blood cell count

Categories of ‘extent of disease’ evaluation scores:

I. **Trivial** - on radiological grounds not active
II. **Minima**l - on radiological grounds active
III. **Limited** - more than above but involving an area less than the right upper lobe
IV. **Moderate** - more than above but an area less or equal to one lung
V. **Extensive** - a total of more than one lung, it is healthy lung tissue visible
VI. **Massive** - extensive bilateral disease, no healthy lung tissue visible

In III: If the right upper lobe (RUL) fissure was not visible on the PA radiograph or the lobe was abnormal in size an imaginary horizontal line from mid hilus to the right chest wall was drawn and the area above it chosen as the representative size of a RUL in the sense of this scale.
In IV: If both lungs were affected the area of one lung was defined as the area of a normal lung.

3.4.2.2 Cavitation

For the identification of cavities, we applied the definition of Hansell et al. 2008 as "gas-filled spaces, seen as a lucency or low-attenuation area, within pulmonary consolidation, a mass, or a nodule" and added the criterion of ‘1cm in diameter or above’. Based on this, cavities were categorized as shown below. In cases of several visible cavities, the diameter of the largest cavity was considered for scoring.

Categories of ‘cavitation’ evaluation scores:

i. No cavities
iia. One single cavity with a diameter <2cm
iib. One single cavity with a diameter between 2cm-4cm
iic. One single cavity with a diameter >4cm
iiia. Multiple cavities, the largest one having a diameter of <2cm
iiib. Multiple cavities the largest one having a diameter between 2cm-4cm
iiic. Multiple cavities the largest one having a diameter >4cm

3.4.2.3 Cavity wall thickness

The wall thickness of the largest identified cavity was measured at the thickest part of the wall. The thickest wall portion had to be at least 25% of the total circumference to count for the respective category, see Fig.1.

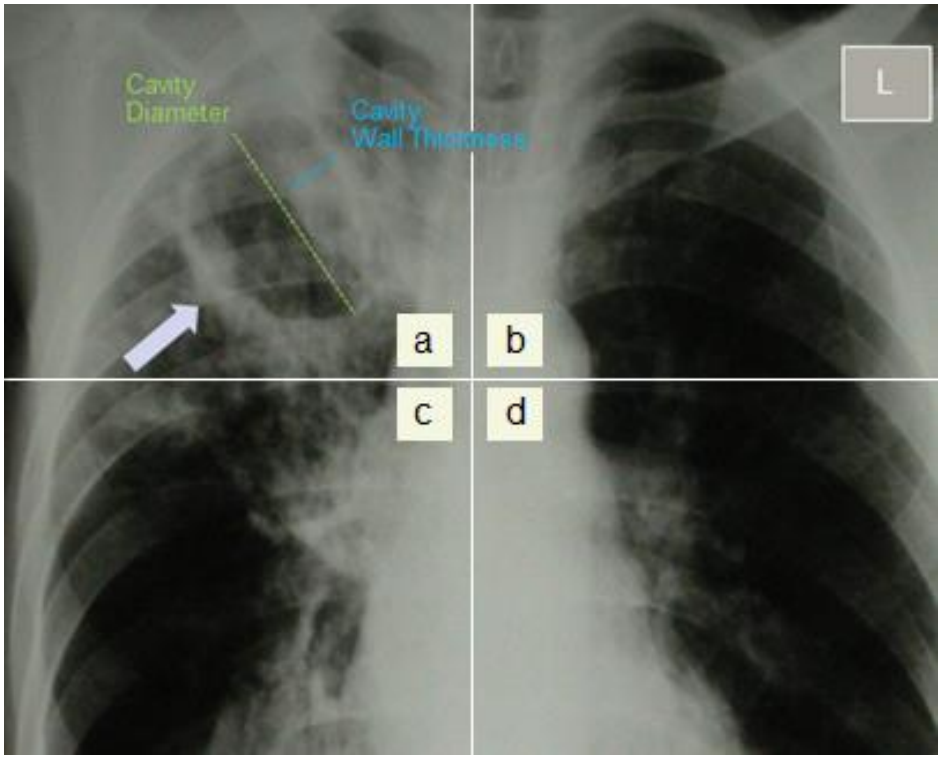

Figure 1: Cavitation and quadrant assessment[10] in posterior anterior chest X-ray

Options for categorization were as follows:

i. Hairline thickness
ii. Thickness <0.5cm
iii. Thickness ≥0.5cm

If a cavity was surrounded by an infiltrate of such extent that no wall could be identified it was scored as category iii. ≥0.5cm.

[10] PA erect chest X-ray of anonymous EBA study patient. (2009). Karl Bremer Hospital, Radiology department, CT.

3.4.2.4 Number of lobes involved

In accordance with the definition of pulmonary lobes outlined below, the number of involved lobes was assessed and quantities were scored in Arabic numbers from 0 to 6 (0 = no lobes affected, 6 = all lobes affected).

Classification of lobes:

i. Right upper lobe (RUL): Tissue from the horizontal fissure and higher
ii. Right middle lobe (RML): Mid zone affecting the contour of the heart (heart border not clearly visible)
iii. Right lower lobe (RLL): Affecting mid and/or lower zones without involving the heart shadow
iv. Left upper lobe including lingula (LUL): Upper and mid zone affecting the contour of the heart (heart border not clearly visible)
v. Left lower lobe (LLL): Mid zone affecting the contour of the heart (heart border not clearly visible)

3.4.2.5 Lung quadrant involvement

In each scoring process, the X-ray of the lung was divided into 4 equally sized quadrants of: ‘upper right’ (UR=a) and ‘upper left’ (UL=b) and ‘lower right’ (LR=c) and ‘lower left’ (LL=d) quadrant. Each quadrant was then evaluated individually in order to describe the exact location and extent of the affected lung tissue, see Fig.1.

The percentage of disease involvement of lung tissue of each quadrant was described as:

i. 0% involvement
ii. Less than 50% involvement
iii. More than 50% involvement

3.4.3 Transfer of radiology scores into Arabic numbers

In order to calculate numerical mean radiology scores as part of the statistical analysis the ranking of ‘extent of disease’ (I.-VI.), ‘cavitation’ (i.-iiic.) and ‘cavity wall thickness’ (i.-iii.) were translated into Arabic numbers. A one-way analysis of variance (ANOVA) was used for the analysis, see Table 4.

Table 4: Translation of radiology scores into Arabic numbers

Radiological Parameters	Score	Arabic number
Extent of disease		
Trivial	I	1
Minimal	II	2
Limited	III	3
Moderate	IV	4
Extensive	V	5
Massive	VI	6
Cavitation		
No cavities	i	1
Single cavity, diameter <2cm	iia	2
Single cavity, diameter 2cm-4cm	iib	3
Single cavity, diameter >4cm	iic	4
Multiple cavities, largest diam. <2cm	iiia	5
Multiple cavities, largest diam. 2-4cm	iiib	6
Multiple cavities, largest diam. >4cm	iiic	7
Cavity wall thickness		
Hairline thickness	i	1
Thickness <0.5cm	ii	2
Thickness ≥0.5 cm	iii	3

3.5 Data management and statistical analysis

For the descriptive and comparative statistical analysis of all EBA patient data, the ‘all data’ Excel table 2010 (see section 2.3.4) was transferred into the statistical software ‘STATISTICA’ Version 11.

3.5.1 Descriptive statistics of ‘all’ patients

In a first step, descriptive statistics of the total patient population (‘all’ patients) were calculated for the specific patient parameters that were equally available in ‘old’ and ‘new’ patients and could be added to form one large study population.

Results of the descriptive statistics were demonstrated in the format of simple histograms of data status in order to illustrate the frequencies of patient parameters occurring in a total of N=1178 patients. Further detail on the described parameters can be found in Table 5.

3.5.2 Descriptive and comparative statistics of ‘old’ and ‘new’ patients

In a second step, ‘old’ patients were compared against ‘new’ patients by applying a separate descriptive statistical evaluation to each of the two cohorts. Results were demonstrated in categorized histograms, allowing direct comparison between frequencies of the same parameters in both cohorts.

Each histogram in this case had its numerical basis in a *2-way summary table*, illustrating parameter counts of both cohorts as well as *Chi-squared -v* and *p-value*s of the comparison of the two. Quantitative data were investigated by one-way analysis of variance (ANOVA), based on descriptive statistics analyzing mean, standard deviation and standard error of each set of parameters for each cohort. From the one-way ANOVA’s, F-test p-values were calculated for each parameter, and the non-parametric Mann-Whitney U-test was also calculated to cater for deviations from the ANOVA assumptions.

In general, sample sizes were large enough which negates any possible effects of deviations from assumptions. Tests were performed at a 95% confidence interval. Further detail on the compared parameters of ‘old’ and ‘new’ patients can be found in Table 5. Hematological parameters that were exclusively available for ‘new’ patients were analyzed by means of descriptive statistics see Table 6.

Table 5: Data sets available for 'all' patients

Parameters	Unit	'All' data N/n	'Old' data N/n	'New' data N/n
Total population		1178	670	508
Demographics				
Ethnic background		969	593	376
'Black'		182	86	96
Mixed ethnicity		785	506	279
White		2	1	1
Gender		1046	595	451
Male		662	391	271
Female		384	204	180
Age	years	1027	581	446
Smoking		305	248	57
Yes		194	149	45
No		111	99	12
Biometrics				
BMI	kg/cm^2	869	478	391
Weight	kg	992	557	435
Height	cm	913	478	435
HIV status		674	277	397
Bacteriology				
Smear grading		1044	563	481
Radiology				
Extent of disease		872	523	349
Cavitation		872	523	349
Cavity wall thickness		840	491	349

Table 6: Data sets exclusively available for 'new' patients

Parameters	N/n	Parameters	N/n
Total population	**508**		
Biometrics		**Differential blood cell count**	
CD4 count	362	Monocytes (%)	414
TTP	222	Neutrophils (%)	414
Urine Tests		Neutrophils absolute	414
Urine glucose	330	Lymphocytes (%)	414
Radiology		Lymphocytes absolute	414
Lung quadrant involvement		Reticulocytes (%)	72
Lung quadrant upper right	349	Reticulocytes absolute	72
Lung quadrant lower right	349	**Enzymes**	
Lung quadrant upper left	349	AST	417
Lung quadrant lower left	349	ALP	417
Number of lobes involved		Amylase	306
5	349	LDH	378
4	349	GGT	417
3	349	**Protein**	
2	349	Protein absolute	417
1	349	Albumin	417
0	349	**Endocrinology**	
Laboratory		Cortisol	72
Blood Chemistry		ACTH	70
Glucose	126		
Sodium	417		
Potassium	417		
Chlorine	417		
Calcium	416		
Phosphate	378		
Triglycerides	417		
Cholesterol	417		
Creatinine	417		
Uric Acid	347		
Complete blood cell count			
RBC count	414		
WBC count	414		
Platelets	413		
MCV	72		
MCHC	72		
MCH	72		
HCT	275		
HGB	426		

4 Study Results

4.1 Descriptive statistics of 'all data'

4.1.1 Demographics

Information on ethnic background was available for 83% of the total patient population. The majority of those patients were found to be of mixed ethnic background, followed by a 'black' ethnic background and a minority of white individuals (<1%), see Table 7.

Patients were mostly of young age within the first half of their 30s and predominantly of male gender. Men were significantly older than women ($p<0.01$), see Table 7 and Table 8.

Table 7: Evaluation of 'all' patient data

Parameters	Unit	N/n	%	Median	Mean	SD
Total population		**1178**	**100**			
Demographics						
Ethnic background		**969**	**83**			
'Black'		182	19			
Mixed ethnicity		785	81			
White		2	0			
Gender		**1046**	**89**			
Male		662	63			
Female		384	37			
Age	years	1027	87	31.0	32.7	10.4
Smoking		**305**	**26**			
Yes		194	64			
No		111	36			
Biometrics						
BMI	kg/m^2	**869**	**74**	18.5	18.9	2.8
<18.5		435	50			
18.5-25.0		412	47			
>25.0		22	3			
Weight	kg	992	84	52.0	52.3	7.9
Height	cm	913	78	167.0	166.1	8.7
Systolic RR	mmHg	435	37	114.5	115.3	13.6
Diastolic RR	mmHg	435	37	73.0	73.9	11.0
Pulse	bpm	435	37	94.0	94.8	18.3
Body temperature	C°	390	33	36.9	37.0	0.8
HIV status		**674**	**57**			
Negative		602	89			
Positive		72	11			

Table 7 continued: Evaluation of 'all' patient data

Parameters	Unit	N/n	%	Median	Mean	SD
Total population		**1178**	**100**			
Bacteriology						
Smear grading		1044	89			
Radiology						
Extent of disease		**872**	**74**			
Grade I		8	1			
Grade II		13	13			
Grade III		130	15			
Grade IV		329	38			
Grade V		283	32			
Grade VI		109	13			
Cavitation		**872**	**74**			
Grade i		9	1			
Grade iia		9	1			
Grade iib		26	3			
Grade iic		26	3			
Grade iiia		140	16			
Grade iiib		313	36			
Grade iiic		349	40			
Cavity wall thickness		**840**	**71**			
Hairline thickness		8	1			
< 0.5 cm		328	39			
≥0.5 cm		504	60			

Of N=305 patients who had given information on their smoking behavior n=194 considered themselves as 'smokers', whereby 90% were of mixed ethnic background and 73% were males. A correlation (p=0.01) between smoking and BMI as well as patient weight (p<0.01) could be found, with non-smokers weighing on average 2 kg more than smokers, see Table 9.

Table 8: 'All' patients: correlation of gender with demographics, biometrics, bacteriology and radiology

Parameters	Unit	N/n			Males			Females			Correlation
		Male	Female	Total	Range	Mean	SD	Range	Mean	SD	p-value
Demographics											
Age		651	376	1027		33.58	10.44		31.21	10.21	**<0.01**
Biometrics											
BMI	kg/m^2	583	334	917		18.31	2.15		20.03	3.30	**<0.01**
Weight	kg	627	363	990		52.99	7.06		50.89	7.94	**<0.01**
Bacteriology											
Smear grading		591	331	922		3.02	1.08		2.98	1.07	**0.62**
Radiology											
Extent of disease		515	286	801		4.44	0.97		4.28	1.02	**0.03**
Cavitation		515	286	801		5.91	1.35		5.76	1.44	**0.13**
Cavity wall thickness		515	280	801		2.55	0.63		2.48	0.66	**0.14**

Table 9: 'All' patients: correlation of smoking with demographics, biometrics, bacteriology and radiology

Parameters	Unit	Smoking					Smokers		Non-Smokers		Correlation
		Yes (N/n)	%	No (N/n)	%	Total	Mean	SD	Mean	SD	p-value
Demographics											
Ethnic background		**194**	**100**	**111**	**100**	**305**					**0.37**
'Black'		19	10	17	15	36					
Mixed ethnicity		175	90	94	85	269					
White		0		0		0					
Gender		**194**	**100**	**111**	**100**	**305**					**0.16**
Male		141	73	83	75	224					
Female		53	27	28	25	81					
Biometrics											
BMI	kg/m^2	111		193		304	17.29	0.13	17.83	0.17	**0.01**
Weight	kg	177		110		287	48.38	5.81	50.38	6.64	**<0.01**
Bacteriology											
Smear grading		178		95		273	2.97	1.10	3.02	1.06	**0.69**
Radiology											
Extent of disease		150		108		258	4.63	0.91	4.56	0.89	**0.96**
Cavitation		150		108		258	6.20	0.81	6.21	1.07	**0.59**
Cavity wall thickness		148		106		254	2.53	0.55	2.59	0.51	**0.32**

4.1.2 Biometrics

BMI information was available for 74% of ‘all’ patients. Half of those patients were classified as ‘underweight’, a minority of patients was considered obese (3%)’ and the remaining patients were within the normal BMI range, according to the WHO Global Database on Body Mass Index 2006, see Table 7.

BMI was found to correlate with the sputum smear grading as well as the chest radiology parameters ‘extent of disease’ and ‘cavitation’, see Table 10.

A significant difference in BMI relative to gender and ethnic background could be shown, with females having a higher BMI than males and ‘black’ patients having the highest BMI out of all ethnic groups. A significant correlation between gender and weight was found, with males weighing on average 2 kg more than females; see Table 8 and Table 10.

Table 10: ‘All’ patients: correlation of BMI with demographics, biometrics, bacteriology and radiology

Parameters	Unit	BMI			Correlation
		N/n	Mean	SD	p-value
Demographics					
Ethnic background		843			**<0.01**
‘Black’		152	19.58	0.22	
Mixed ethnicity		691	18.77	0.10	
White					
Gender		917			**<0.01**
Male		583	18.31	2.15	
Female		334	20.03	3.30	
Biometrics					
Weight	kg	899			**<0.01**
Bacteriology					
Smear grading		841			**0.03**
Radiology					
Extent of disease		689			**<0.01**
Cavitation		689			**0.01**
Cavity wall thickness		664			**0.14**

Systolic and diastolic blood pressure readings were found to be normal, according to the standards of the American Heart Association 2014. Measures falling outside the normal ranges were pulse and body temperature which were both elevated.

57% (n= 674) of ‘all’ patients had HIV status information available of who close to 90% presented negative HIV test results. For n=70 HIV-positive patients gender information was available and the male:female ratio was found to be 53%:47%. A correlation was found between HIV status and ethnic background (p=0.07) whereby 69% of HIV-positive and 80% of HIV-negative patients for who ethnic background information had been available were of a mixed ethnic background, see Table 7 and Table 12.

Table 11: 'All' patients: correlation of ethnic background with demographics, biometrics, bacteriology and radiology

Parameters	Unit	N/n						'Black'		Mixed ethnicity		White		Correlation
		'Black'	%	Mixed ethnicity	%	White	%	Total	Mean	SD	Mean	SD	Mean	SD	p-value
Demographics															
Gender		**182**	**19**	**785**	**81**	**2**	**0**	**969**							**0.31**
Male		121	20	490	80	2	0	613							
Female		61	17	295	83	0	0	356							
Biometrics															
Weight	kg	181		736				917	55.23	7.68	51.30	7.72			**<0.01**
Bacteriology															
Smear grading		154		700		3		854	2.86	1.19	3.05	1.03	1.33	0.61	**<0.01**
Radiology															
Extent of disease		146		603				749	4.41	1.07	4.38	0.96	4.33	0.58	**0.94**
Cavitation		146		603				749	5.79	1.56	5.89	1.31	6.33	0.79	**0.60**
Cavity wall thickness		141		579				720	2.45	0.64	2.53	0.63	2.0	0.0	**0.15**

Table 12: 'All' patients: Correlation of HIV status with demographics, biometrics, bacteriology and radiology

Parameters	Unit	HIV					HIV-positive		HIV-negative		Correlation
		pos.	%	neg.	%	total	Mean	SD	Mean	SD	p-value
Demographics											
Ethnic background		**55**	**100**	**478**	**100**	**533**					**0.07**
'Black'		17	31	95	20	112					
Mixed ethnicity		38	69	383	80	421					
White		0	0	0	0	0					
Gender		**70**	**100**	**539**	**100**	**609**					**0.09**
Male		37	53	342	63	379					
Female		33	47	197	37	230					
Smoking		**9**	**100**	**149**	**100**	**158**					**0.57**
Yes		6	67	85	57	91					
No		3	33	64	43	67					
Biometrics											
BMI	kg/m^2	69	13	505	87	574	19.579	2.418	19.16	2.80	**0.24**
Weight	kg	67	12	514	88	581	53.98	7.44	52.98	8.16	
Bacteriology											
Smear grading		70		563		633	2.91	1.16	2.94	1.11	**0.84**
Radiology											
Extent of disease		56		546		602	4.32	0.99	4.32	1.01	**0.96**
Cavitation		56		546		602	5.89	1.42	5.83	1.46	**0.74**
Cavity wall thickness		56		546		602	2.50	0.76	2.54	0.66	**0.68**

4.1.3 Sputum smear grading

90% of N=1044 available patient data showed a positive sputum smear grade 1+ or more. N=218 patients (20%) showed a grade 1+, n=268 (25%) a grade 2+ and n=457(45%) a grade 3+. The remaining n=101 patients (10%) were found to be in the 'scanty' grading category.

Patients of mixed ethnic background were found to have a higher smear grading than any other ethnic group, showing a significant correlation of $p<0.01$ between the two parameters, see Table 11.

4.1.4 Radiology

The radiological information to assess the 'extent of disease' was available for 74% of 'all' patients, see Table 7. A minority of those patients (16%) had limited lung tissue damage of Grade II and III and only 1% presented no visible damage of their lungs on X-ray (Grade I). With 83% the majority of patients showed changes to their lungs with an 'extent of disease' equal to or larger than Grade IV. 'Extent of disease' results in detail are shown in Fig.2.

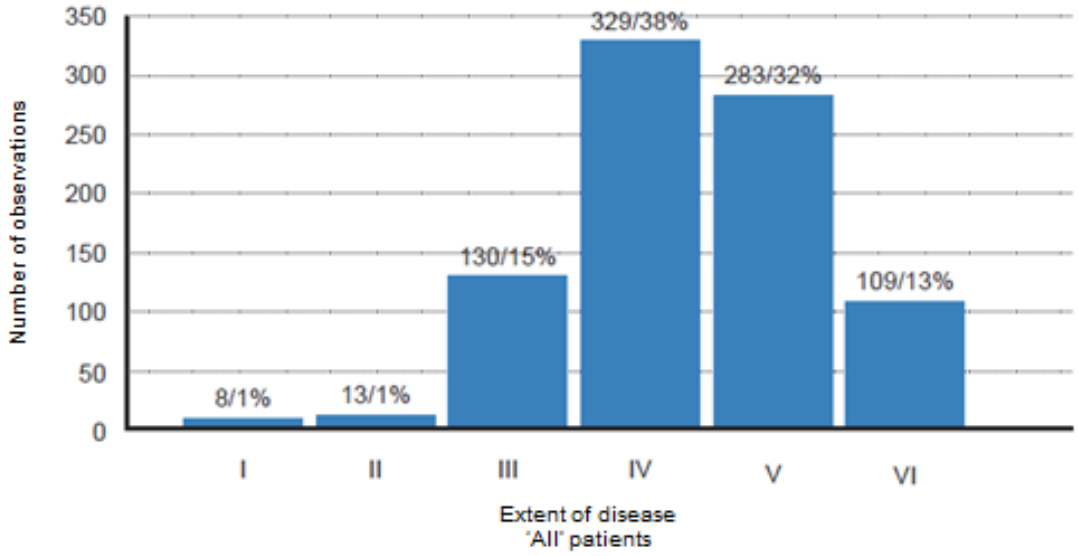

Figure 2: 'All patients: Total number of observations of radiological 'extent of disease'

For 74% of all patients, radiological information on ‘cavitation’ was available. 92% of those patients were found to have multiple lung cavities of which 40% presented a largest cavity with diameter of >4cm, 36% presented a largest cavity of 2cm-4cm and the remaining patients presented a largest cavity below 2cm. A minority of 8% of patients had one single cavity of any size or no cavities at all.

For 71% of all patients radiological information on their ‘cavity wall thickness’ was available. 60% of those patients showed thick cavity walls of ≥0.5 cm, the remaining patients presented thinner walls. Hairline thickness of walls was not commonly found, see Table 7.

A significant correlation between gender and the ‘extent of disease’ could be found (p=0.03) with females having slightly less affected lung tissue, see Table 8.

4.1.5 Hematology

Hematological results were exclusively available for the ‘new’ patient population and are shown in Table 13. Mean values for hemoglobin, hematocrit and MCV were lower than the normal range, platelets were found to be elevated.

Mean neutrophils (absolute) and lymphocytes (%) were outside the normal range whereby neutrophils were elevated and lymphocytes reduced. GGT was found to be elevated in females as well as LDH in both gender groups. Serum protein was found to be within the normal range for adults, the mean value of albumin however was slightly reduced. Cortisol levels were within the normal (8am/morning) range.

65% of n=362 available CD4 count results were found to be >500 cells/mm^3 and therefore within the normal range, the remaining results were found to be below 500 cells/$mm^{3.}$

Table 13: 'New' patients: exclusive hematology

Parameters	Unit	Normal Range	N/n	%	Median	Mean	SD	Value Range	
								Lowest	Highest
Blood Chemistry									
Glucose	mmol/l	3.05 – 6.1	128		4.90	5.45	2.52	3.00	22.80
Sodium	mmol/l	134-145	429		137.00	135.96	4.03	119.00	146.00
Potassium	mmol/l	3.4-5.2	429		4.40	4.48	0.50	3.00	6.00
Chlorine	mmol/l	95-112	429		98.00	97.71	4.11	85.00	108.00
Calcium	mmol/l	2.15-2.65	428		2.31	2.31	0.17	1.91	2.97
Phosphate	mmol/l	0.8-1.5	390		1.20	1.20	0.24	0.56	2.47
Triglycerides	mmol/l	0.83-1.7	78		0.87	0.96	0.42	0.20	4.77
Cholesterol	mmol/l	3.1-6.5	429		3.40	3.52	0.96	1.50	7.80
Creatinine	umol/l	44-106	429		62.00	62.35	13.22	33.00	104.00
Uric Acid	mmol/l	0.18-0.48	347		0.24	0.25	0.07	0.09	0.58
Complete blood cell count									
RBC count m*ale*	10^{12}/l	4.3-6.2	252			4.48	0.59		
RBC count *female*	10^{12}/l	3.8-5.5	174			4.20	0.53		
WBC count	10^{9}/l	4.1-10.9	426		9.90	10.36	3.55	4.00	25.60
Platelets	10^{9}/l	150-350	425		506.00	511.77	162.86	140.00	1137.00
MCV	fl	85-98	78		83.00	82.65	7.53	68.00	104.20
MCHC	g/dl	30-36	78		33.05	33.44	1.00	31.30	36.00
MCH	pg	27-34	78		28.00	27.65	2.90	21.40	35.20
HCT *male*	l/l	0.4-0.52	172			0.38	0.05		
HCT *female*	l/l	0.35-0.47	115			0.34	0.05		
HGB *male*	g/dl	14.0-18.0	252			12.47	1.84		
HGB *female*	g/dl	12.0-16.0	174			11.05	1.70		
Differential blood cell count									
Monocytes	%	2.0-8.0	426		5.10	5.55	2.03	1.80	14.20
Neutrophils (sag.)	10^{9}/l	50-70	426		75.00	73.95	8.86	31.00	91.70
Lymphocytes	%	25-40	426		17.10	18.40	7.75	4.60	64.00
Neutrophils	%	2.0-8.0	426		7.31	7.81	3.22	2.24	20.17
Lymphocytes absolute	10^{9}/l	1.0-4.8	426		1.66	1.78	0.75	0.58	6.85
Reticulocytes	%	0.5-1.5	78		1.34	1.42	0.56	0.38	2.80
Reticulocytes absolute	cells/l	0.05-0.1	78		0.06	0.06	0.02	0.02	0.12

Colour key: grey = above normal value range, blue= below normal value range

Table 13 continued: 'New' patients: exclusive hematology

Parameters	Unit	Normal Range	N/n	%	Median	Mean	SD	Value Range	
								Lowest	Highest
Enzymes									
AST *male*	U/l	up to 50	253			30.68	21.59		
AST *female*	U/l	up to 35	176			26.59	19.71		
ALP *male*	U/l	40-129	253			104.78	56.57		
ALP *female*	U/l	35-104	176			99.80	46.86		
Amylase	U/l	up to 100	306		61.00	69.04	36.36	22.00	226.00
GGT *male*	U/l	up to 66	253			58.04	51.36		
GGT *female*	U/l	up to 39	176			46.83	44.24		
LDH *male*	U/l	up to 248	230			279.37	111.05		
LDH *female*	U/l	up to 247	160			292.18	121.81		
Protein									
Protein absolute	g/l	65-87	429		82.00	82.53	8.65	58.00	120.00
Albumin	g/l	36-50	429		35.00	35.20	5.90	19.00	54.00
Endocrinology									
Cortisol	nmol/l	140-690	78		441.50	463.92	157.30	137.00	885.00
ACTH	pmol/l	2.0-11.0	76		6.50	11.06	10.80	0.20	51.70
CD4 Count									
<500	cells/mm^3		124	34					
500-1000	cells/mm^3		183	51					
>1000	cells/mm^3		55	15					

Colour key: grey = above normal value range, blue= below normal value range

4.2 Comparative statistics of ‘old’ and ‘new’ patients

4.2.1 Standard comparison of ‘old’ and ‘new’ patient parameters

4.2.1.1 Demographics

The ethnic composition of the ‘new’ cohort differed significantly from the ‘old’ cohort, by showing a lower percentage of mixed ethnic background and a higher percentage of ‘black’ patients, see Fig.3.

With regards to gender and age, both patient cohorts were made up similarly with a majority of male patients and a mean age of 32 years. A trend towards more females among the ‘new’ patient population was observed.

A significant difference could be found in patients’ smoking behavior, showing the ‘old’ study population to have relatively fewer smokers (60% of n=248 available data sets) in comparison to the ‘new’ cohort with 79% smokers of n=57 available data sets, see Table 14.

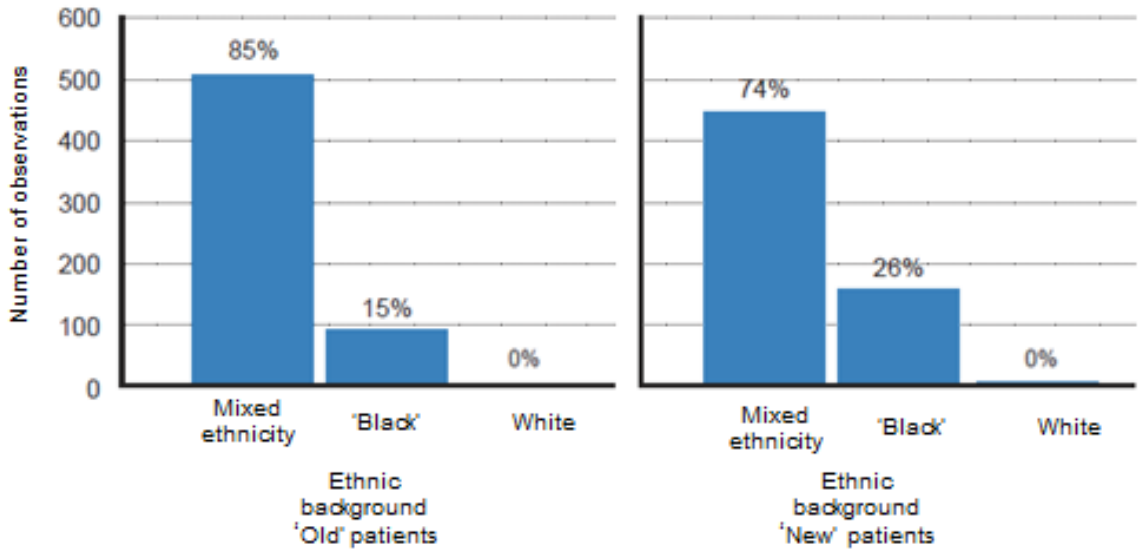

Figure 3: Ethnic background: ‘old’ vs. ‘new’ patients

Table 14: 'Old' vs. 'new' patients': demographics, biometrics, bacteriology

Parameters	Unit	'Old' data				'New' data				Correlation
		N/n	%	Mean	SD	N/n	%	Mean	SD	p-value
Total population		670	57			508	43			
Demographics										
Ethnic background		**593**	**100**			**376**	**100**			**0.00005**
'Black'		86	15			96	26			
Mixed ethnicity		506	85			279	74			
White		1	0			1	0			
Gender		**595**	**100**			**451**	**100**			**0.06186**
Male		391	66			271	60			
Female		204	34			180	40			
Age	years	581	87	32.88	10.33	446	88	32.49	10.53	**0.56**
Smoking		**248**	**100**			**57**	**100**			**0.00576**
Yes		149	60			45	79			
No		99	40			12	21			
Biometrics										
BMI	kg/m^2	**478**	**100**			**391**	**100**			**0.001**
<18.5		261	55			174	45			
18.5 - 25.0		211	44			201	51			
>25.0		6	1			16	4			
Weight	kg	557	83	51.11	6.67	435	86	53.7	9.00	**< 0.01**
Height	cm	478	71	165.94	8.70	435	86	166.2	8.79	**0.61**
HIV status		**277**	**100**			**397**	**100**			**0.000001**
Negative		271	98			331	83			
Positive		6	2			66	17			
Bacteriology										
Smear grading		563	100			481	100			**0.000001**

4.2.1.2 Biometrics

The investigation of BMI distribution between the two cohorts showed 'new' patients with a lower share of 'underweight' and larger percentage of 'normal' and 'overweight' individuals in comparison to the 'old' cohort, see Table 14. This was related to a significant difference in body weight ($p< 0.01$) between the two patient populations with similar mean height, see Fig.4. HIV positivity between the two patient populations differed significantly.

The ‘old’ patient cohort showed a low (2%) HIV positivity rate, compared to the ‘new’ cohort who showed a 17% HIV positivity rate, see Table 14.

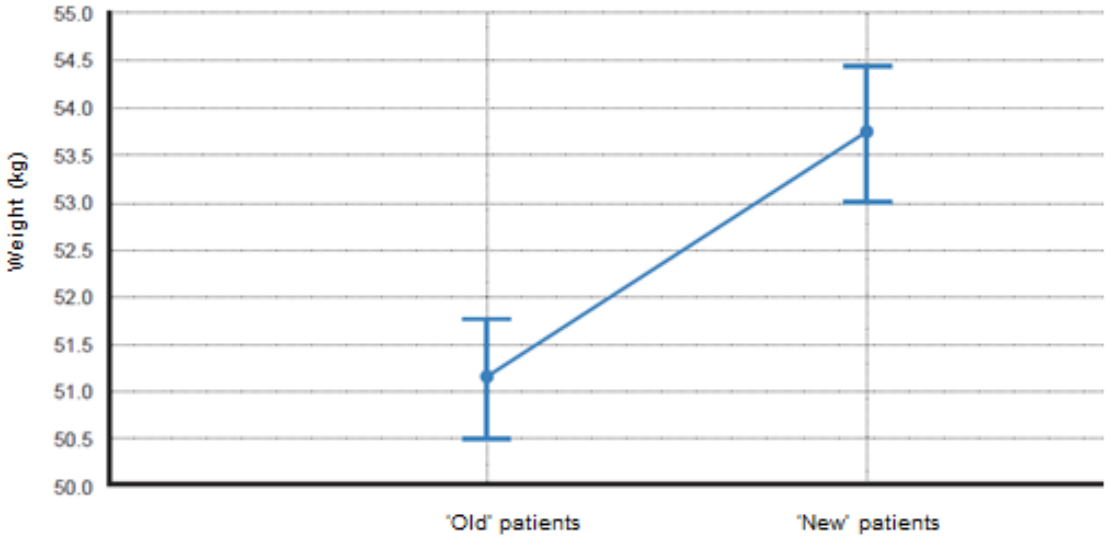

Figure 4: Body weight: ‘old’ vs. ‘new’ patients

4.2.1.3 Sputum smear grading

‘Old’ patients showed a larger tendency towards higher smear grades (54% of grade 3+) compared to ‘new’ patients, who showed more evenly distributed smears across the grading spectrum, see Fig.5.

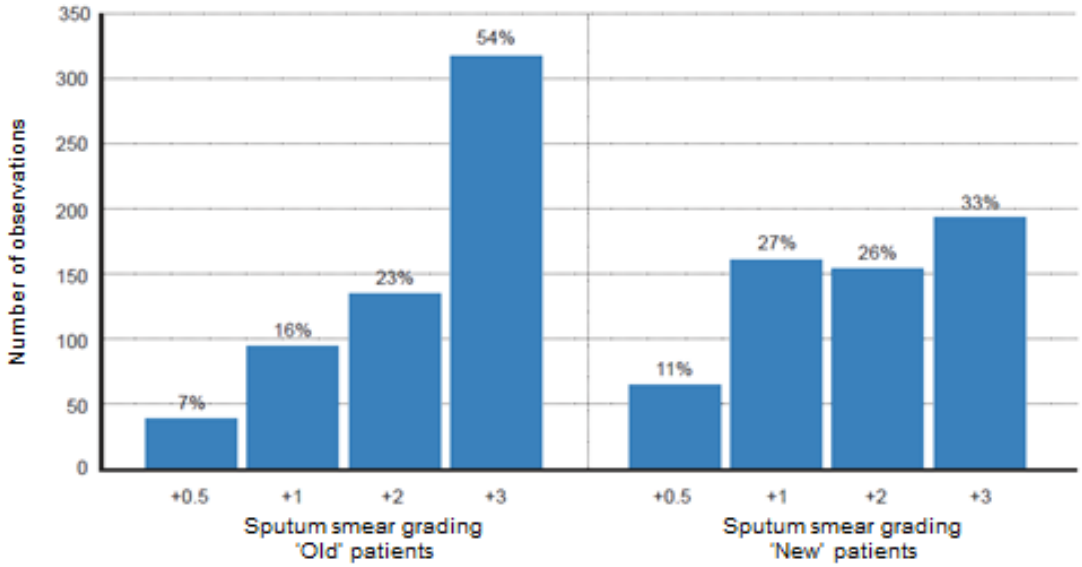

Figure 5: Sputum smear grading: ‘old’ vs. ‘new’ patients

4.2.1.4 Radiology

The extent to which lung tissue was affected by tuberculosis was significantly more severe in ‘old’ patients. Higher frequencies of scores larger or equal to Grade IV and a particularly high percentage (17%) of Grade VI characterized ‘old’ patients whereas ‘new’ patients showed limited frequencies of severe scores such as Grade VI (6%), see Fig.6.

95% of ‘old’ patients presented multiple lung cavities compared to ‘new’ patients who had a multiple cavity score of 82% and showed more single cavity scores.

‘Old’ patients had thicker cavity walls with 62% of scores being of the thickest type 3 (≥0.5cm). ‘New’ patients showed 59% of type 3 and 40% of type 2 (<0.5cm) wall thickness, 0.9% showed hairline thickness cavity walls.

In the ‘new’ cohort the upper quadrants were affected more than the lower quadrants with the ‘right upper quadrant’ showing the most radiological changes. 2 or 3 lobes were involved in more than 50% of all ‘new’ patients, see Table 15 and Table 16.

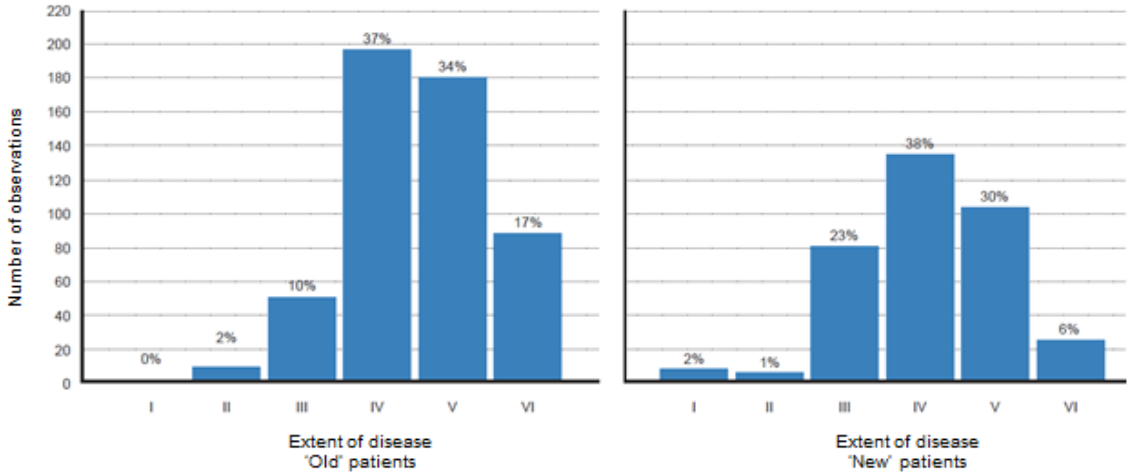

Figure 6: ‘Extent of disease’: ‘old’ vs. ‘new’ patients

Table 15: ‘Old’ vs. ‘new’ patients: radiology

Parameters	‚Old’ data		‚New’ data		Correlation
	N/n	%	N/n	%	p-value
Total population	670	57	508	43	
Extent of disease	523	100	349	100	**0.000001**
Grade VI	87	17	22	6	
Grade V	180	34	103	30	
Grade IV	196	37	133	38	
Grade III	50	10	80	23	
Grade II	8	2	5	1	
Grade I	2	0	6	2	
Cavitation	523	100	349	100	**0.000001**
Grade iiic	202	39	131	38	
Grade iiib	198	38	105	30	
Grade iiia	93	18	50	14	
Grade iic	10	2	19	5	
Grade iib	5	1	22	6	
Grade iia	3	1	5	1	
Grade i	12	2	17	5	
Cavity wall thickness	491	100	349	100	**0.000001**
Hairline	8	2	3	1	
< 0.5 cm	181	36	140	40	
≥0.5 cm	302	62	206	59	

Table 16: New' patients: exclusive radiology

Parameters	‚Old' data		‚New' data	
	N/n	%	N/n	%
Total population			508	100
Lobes involved			**349**	**69**
5			34	10
4			35	10
3			91	26
2			107	31
1			75	21
0			7	2
Lung quadrants				
Lung quadrant UR			**349**	**69**
0%			85	24
1-50%			48	14
>50%			216	62
Lung quadrant UL			**349**	**69**
0%			111	32
1-50%			68	19
>50%			170	49
Lung quadrant LR			**349**	**69**
0%			173	49
1-50%			86	25
>50%			90	26
Lung quadrant LL			**349**	**69**
0%			146	42
1-50%			89	26
>50%			114	32

4.2.2 Correlation of ethnic background with ‘old’ and ‘new’ patient parameters

4.2.2.1 Biometrics

In both cohorts a strong correlation was found between ethnic background and weight ($p<0.01$) whereby each cohort’s ‘black’ patient population was more than 3 kg heavier than patients of mixed ethnic background, see Table 17. Independent from ethnic background, the ‘new’ patient cohort showed a mean weight which was 2.5 kg above the ‘old’ patient cohort, see Table 14.

4.2.2.2 Radiology

A correlation was found between ‘extent of disease’ and ethnic background among the ‘new’ patient cohort ($p=0.03$). ‘Black’ patients showed larger areas affected by disease than patients of mixed ethnic background. All other radiological parameters were found to be more severe in the ‘old’ patient group, independent from ethnic background, see Table 17.

4.2.3 Correlation of gender with ‘old’ and ‘new’ patient parameters

4.2.3.1 Demographics

In both cohorts a strong correlation was found between gender and weight ($p<0.01$) whereby males and females of the ‘new’ patient cohort weighed about 3 kg more than their equivalents of the ‘old’ patient cohort, see Table 18.

4.2.3.2 Radiology

A correlation was found between ‘cavity wall thickness’ and gender in the ‘old’ patient cohort and the ‘extent of disease’ and gender in the ‘new’ patient cohort. Radiological data comparison illustrated that males and females of the ‘old’ patient population had larger ‘extent of disease’, ‘cavitation’ and ‘cavity wall thickness’ than the ‘new’ comparison group. In particular females of the ‘old’

patient population had an ‘extent of disease’ (4.59) significantly above the ‘new’ female comparison group (3.95), see Table 18.

4.2.4 Correlation of BMI with ‘old’ and ‘new’ patient parameters

4.2.4.1 Demographics

BMI and gender showed a positive correlation in both patient cohorts. The BMI of ‘new’ males was significantly higher (18.67 kg/m^2) than the BMI of ‘old’ males (18.02 kg/m^2). This also applied to ‘new’ females, who presented a higher BMI (20.43 kg /m^2), than their comparative group (19.62 kg/m^2). Furthermore females of both cohorts had significantly higher BMIs than males within the same cohort.

‘New’ patients of mixed ethnic background presented a significantly higher BMI (19.27 kg/m^2) than their equivalents of the ‘old’ cohort (18.47 kg/m^2). The mean BMI among the ‘new’ ‘black’ population (19.89 kg/m^2) showed a limited increase compared to the ‘old’ cohort (19.14 kg/ m^2).

BMI and ethnic background showed a positive statistical correlation within the ‘old’ cohort, no correlation could be found in the ‘new’ cohort.

The comparison of ‘old’ and ‘new’ patient data, independent from the applied population split, showed a lower BMI of ‘old’ patients in all analyzed categories, see Table 19.

4.2.4.2 Biometrics

BMI and HIV status did not statistically correlate in either of the two cohorts. HIV-positive patients within the ‘new’ cohort presented themselves with a significantly higher BMI (19.64 kg/m^2) than HIV-positive patients of the comparative group (18.22 kg/m^2). The ‘old’ and ‘new’ patient cohort showed a statistically significant correlation between BMI and weight ($p<0.01$), see Table 19.

4.2.4.3 Sputum smear grading

BMI and sputum smear grading showed a positive correlation within the ‘old’ cohort (p=0.06), within the ‘new’ cohort no correlation was found, see Table 19.

4.2.4.4 Radiology

‘Extent of disease’ in ‘new’ patients showed a strong negative correlation with BMI (p< 0.01), the same was found for ‘cavitation’ (p<0.01). No correlation was found between BMI and radiological parameters in ‘old’ patients. ‘Cavity wall thickness’ did not correlate with BMI in either of the two patient populations, see Table 19.

Table 17: Correlation of ethnic background with 'old' and 'new' patient parameters

Parameters	Unit	'Old' data												Correlation	'New' data												Correlation
		'Black'				Mixed ethnicity				White					'Black'				Mixed ethnicity				White				
		N/n	%	Mean	SD	N/n	%	Mean	SD	N/n	%	Mean	SD	p-value	N/n	%	Mean	SD	N/n	%	Mean	SD	N/n	%	Mean	SD	p-value
Demographics																											
Gender		86	100			506	100			1	100			**0.39**	96	100			279	100			1	100			**0.92**
Male		60	70			329	65			1	100				61	64			161	58			1	100			
Female		26	30			177	35			0	0				35	36			118	42			0	0			
Biometrics																											
Weight	kg	84		54.3	5.58	472		50.5	6.68					**<0.01**	97		56.1	9.07	264		52.7	9.14					**<0.01**
Bacteriology																											
Smear grading		62		2.97	1.21	427		3.2	0.99	1		2.0	0	**0.12**	92		2.79	1.18	273		2.82	1.05	0				**0.84**
Radiology																											
Extent of disease		68		4.56	1.06	381		4.60	0.92	1		4.0	0.94	**0.79**	78		4.28	1.07	222		4.01	0.92	0				**0.03**
Cavitation		68		6.00	1.42	381		6.03	1.09	1		6.0	1.15	**0.98**	78		5.60	1.65	222		5.65	1.59	0				**0.83**
Cavity wall thickness		63		2.56	0.53	357		2.59	0.53	1		2.0	0	**0.50**	78		2.37	0.70	222		2.45	0.75	0				**0.45**

Table 18: Correlation of gender with 'old' and 'new' patient parameters

Parameters	Units	'Old' data								'New' data							
		Male				Female			Correlation	Male				Female			Correlation
		N/n	%	Mean	SD	N/n	%	Mean	SD	p-value	N/n	%	Mean	SD	N/n	%	Mean	SD	p-value
Biometrics																			
Weight	kg	371		51.91	6.28	186		48.52	7.13	**<0.01**	256		54.57	7.82	177		52.54	10.34	**<0.01**
Bacteriology																			
Smear grading		332		3.19	1.05	160		3.14	0.97	**0.62**	259		2.80	1.07	171		2.84	1.14	**0.60**
Radiology																			
Extent of disease		306		4.60	0.94	146		4.59	0.92	**0.93**	209		4.22	0.97	140		3.95	1.01	**0.01**
Cavitation		306		6.06	1.17	146		5.97	1.09	**0.17**	209		5.70	1.56	140		5.54	1.71	**0.60**
Cavity wall thickness		306		2.61	0.53	146		2.51	0.53	**0.09**	209		2.46	0.73	140		2.44	0.77	**0.99**

Table 19: Correlation of BMI with 'old' and 'new' patient parameter

Parameters	Units	'Old' data			Correlation		'New' data			Correlation	
		N/n	Mean BMI	SD	p-value	r	N/n	Mean BMI	SD	p-value	r
Demographics											
Ethnic background		477	18.55	2.35	**0.03**		366	19.40	3.20	**0.10**	
Mixed ethnicity		414	18.47	2.36			277	19.24	3.19		
'Black'		63	19.14	2.22			89	19.89	3.21		
White		0					0				
Gender		478	18.56	2.35	**<0.01**		439	19.36	3.09	**<0.01**	
Male		317	18.02	1.96			266	18.67	2.31		
Female		161	19.62	2.67			173	20.43	3.76		
Age		477			**0.80**	(-0.01)	439			**0.13**	0.07
Smoking		247	17.24	1.31	**0.16**		57	18.59	2.95	**<0.01**	
Yes		148	17.14	1.42			45	17.81	1.83		
No		99	17.39	1.11			12	21.52	4.35		
Biometrics											
Weight	kg	478			**<0.01**	0.67	421			**<0.01**	0.71
HIV status		182	18.99	2.07	**0.51**		391	19.31	3.02	**0.33**	
Negative		179	19.00	2.08			326	19.24	3.12		
Positive		3	18.22	1.20			65	19.64	2.45		
Bacteriology											
Smear grading		418			**0.06**	(-0.09)	481			**0.50**	(-0.03)
Radiology											
Extent of disease		342			**0.66**	0.02	347			**<0.01**	(-0.20)
Cavitation		342			**0.32**	0.05	347			**<0.01**	(-0.17)
Cavity wall thickness		317			**0.60**	(-0.03)	347			**0.25**	(-0.06)

4.2.5 Correlation of HIV with ‘old’ and ‘new’ patient parameters

4.2.5.1 Biometrics

In the ‘old’ patient cohort a correlation was found between HIV status and weight (p=0.08). HIV-positive patients weighed on average 5 kg less than HIV-negative patients. No such correlation was found among the ‘new’ patient cohort. Data further showed that HIV-positive patients of the ‘new’ patient cohort were on average 7 kg heavier than ‘old’ HIV-positive patients, see Table 20.

4.2.5.2 Sputum smear grading

A correlation could not be found between sputum smear grading and HIV status in either of the two cohorts. However, data evaluation showed that smear grading was highest in the ‘old’ HIV-positive patient group, followed by the ‘old’ HIV-negative patient group. In ‘new’ patients smear grading was lower than in the ‘old’ patients, regardless of HIV status, see Table 20.

4.2.5.3 Radiology

No correlation could be found between HIV status and radiological parameters within ‘old’ and ‘new’ patient groups. Despite that, data showed that mean values of ‘extent of disease’, ‘cavitation’ and ‘cavity wall thickness’ were highest in the HIV-positive patients of the ‘old’ cohort. HIV-positive patients of the ‘new’ cohort showed ‘extent of disease’ and ‘cavitation’ scores that were slightly above those of the ‘new’ HIV-negative patients, see Table 20.

Table 20: Correlation of HIV status with 'old' and 'new' patient parameters

Parameters	Units	'Old' data									'New' data								
		HIV-positive				HIV-negative				Correlation	HIV-positive				HIV-negative				Correlation
		N/n	%	Mean	SD	N/n	%	Mean	SD	p-value	N/n	%	Mean	SD	N/n	%	Mean	SD	p-value
Demographics																			
Ethnic background		4	100			206	100			**0.44**	51	100			274	100			**0.14**
Mixed ethnicity		4	100			167	81				34	67			216	79			
'Black'		0	0			38	19				17	33			57	21			
White		0	0			1	0				0	0			1	0			
Gender		4	100			208	100			**0.86**	65	100			331	100			**0.32**
Male		1	25			140	67				36	54			202	61			
Female		3	75			68	33				30	45			129	39			
Smoking		3	100			98				**0.66**	6				51				**0.78**
Yes		1	33			45	46				5	83			40	78			
No		2	67			53	54				1	17			11	22			
Biometrics																			
Weight	kg	4		47.0	6.37	198		52.6	3.46	**0.08**	63		54.43	7.14	316		53.25	9.09	**0.33**
Bacteriology																			
Smear grading		6		3.83	0.41	246		3.15	1.09	**0.12**	64		1.91	1.0	317		1.87	0.7	**0.77**
Radiology																			
Extent of disease		6		5.17	0.75	265		4.55	0.97	**0.13**	50		4.22	0.97	281		4.09	1.00	**0.39**
Cavitation		6		6.33	0.52	265		6.03	1.19	**0.54**	50		5.84	1.49	281		5.63	1.65	**0.41**
Cavity wall thickness		6		2.67	0.52	258		2.63	0.52	**0.86**	50		2.46	0.76	281		2.48	0.79	**0.83**

4.2.6 Correlation of smoking with ‘old’ and ‘new’ patient parameters

4.2.6.1 Biometrics

A strong correlation ($p<0.01$) was found between smoking behavior and weight within the ‘new ‘patient cohort, whereby non-smokers were on average 9 kg heavier than smokers. Within the ‘old’ patient cohort a weak correlation was found between the same parameters ($p=0.07$) with a difference of 1 kg in weight between smokers and non-smokers, see Table 21.

4.2.6.2 Radiology

A correlation between ‘cavitation’ and smoking behavior was found within the ‘new’ patient population ($p=0.03$), no correlation was seen in the ‘old’ patient cohort. Data evaluations further showed, that ‘extent of disease’ was more severe in smokers than non-smokers of both patient cohorts. Particularly radiological parameters of ‘non-smoker’ among the ‘old’ patients were more severe than those of non-smokers of the ‘new’ cohort, see Table 21.

4.2.6.3 Sputum smear grading

No correlation could be found between sputum smear grading and smoking behavior in either patient group. However, data evaluation showed that independent from smoking behavior ‘new’ patients’ presented sputum smear positivity close to one grade below the ‘old’ patients, see Table 21.

Table 21: Correlation of smoking with 'old' and 'new' patient parameters

Parameters	Unit	'Old' data									'New' data								
		Non-smoking				Smoking				Correlation	Non-smoking				Smoking				Correlation
		N/n	%	Mean	SD	N/n	%	Mean	SD	p-value	N/n	%	Mean	SD	N/n	%	Mean	SD	p-value
Demographics																			
Ethnic background		99	100			149	100			**0.52**	12	100			45	100			**0.5**
Mixed ethnicity		85	86			135	91				9	75			40	89			
'Black'		14	14			14	9				3	25			5	11			
White		0				0					0				0				
Gender		99	100			149	100			**0.28**	12	100			45	100			**0.49**
Male		76	77			110	74				7	58			31	69			
Female		23	23			39	26				5	42			14	31			
Biometrics																			
Weight	kg	95		49.48	5.42	149	5.68	48.17		**0.07**	11		58.5	10.61	28		49.5	6.48	**<0.01**
Bacteriology																			
Smear grading		83		3.12	1.05	137		3.11	1.10	**0.96**	12		2.42	0.99	41		2.46	0.98	**0.89**
Radiology																			
Extent of disease		96		4.63	0.89	119		4.69	0.89	**0.60**	12		4.08	0.79	31		4.39	0.92	**0.32**
Cavitation		96		6.27	1.04	119		6.14	0.81	**0.31**	12		5.75	1.24	31		6.45	0.77	**0.03**
Cavity wall thickness		94		2.64	0.50	117		2.56	0.55	**0.31**	12		2.25	0.45	31		2.39	0.56	**0.45**

5 Discussion

5.1 Main findings of the study

The ethnic composition of the ‘new’ patient cohort had changed significantly in favor of ‘black’ individuals although the majority of both cohorts comprised of patients from mixed ethnic origin.

BMIs of ‘old’ patients were significantly lower, with lower body weight and same mean height as ‘new’ patients. BMIs were lowest in ‘old’ cohort males of mixed ethnic background and associated with increased TB severity; identified by higher sputum smear grades, radiological greater ‘extent of disease’, more lung ‘cavitation’ and thicker cavity walls. In both cohorts BMIs of female patients were above male BMIs and BMIs of ‘black’ patients were significantly higher than BMIs of mixed ethnic background patients.

The ‘new’ patient cohort showed a larger proportion of smokers. In both cohorts smoking was a predominant habit of males from mixed ethnic background and BMIs of non-smokers were found to be significantly higher than BMIs of smokers.

A larger proportion of HIV-positive patients was found in the ‘new’ patient population with a majority of affected males. BMIs of HIV-positive ‘new’ patients were above the BMIs of HIV-positive ‘old’ patients and HIV-positive ‘new’ patients presented the heaviest weight out of all HIV status related subcategories.

5.2 Impact of ethnic background and gender distribution on the manifestation of pulmonary tuberculosis

The change of the ethnic distribution within our two patient populations seems to reflect the overall changes in the ethnic distribution of the Western Cape population between 1994 (end of apartheid) and 2008.

In 1994 the ethnic split of the Western Cape was documented by the SA Health Review to be 17.4 % 'black', 57.8% mixed ethnic background, 24.0% white and 0.8% Indian (out of 3.6 million people), (Health Systems Trust 1995).

Our data of the time period 1991-2001 were very much in line with those statistics and showed a mix of 15% 'black' and 85% mixed ethnic background patients. Due to the applicable political regulations during apartheid the white population was mainly separated from the rest of South Africa's population and not treated in the same hospitals. This could therefore explain their absence in our patient cohorts, who were recruited in government hospitals and clinics. The incidence of TB in the white population was and still is in any event very low.

Today, the population of the Western Cape is still comprised of a mixed ethnic background majority (53.9%) but there has been a shift towards a larger 'black' population with 26.7%, 18.4% white and 0.9% Indian/Asian (out of 4.6 million total population, (Statistics South Africa 2008). Our 'new' patient cohort was made up from 25% 'black' patients, 74% patients of mixed ethnic background and 1% whites. These findings seem to reflect the composition and changes of Cape Town's official ethnic distribution patterns.

Despite a change in migration patterns and the progression of change in the ethnic population distribution in and around Cape Town after 1994, it must also be taken into consideration that selection of patients for EBA studies might have changed over the years. The network of clinics from which Task Applied Science has been recruiting trial patients has grown and might have expanded from previously predominantly mixed ethnic background living areas into predominantly 'black' areas, which would have a significant impact on the ethnic composition of our patient cohorts and might have favored a larger percentage of 'black' patients in the ‚new' cohort.

Even before South Africa was affected by the HIV epidemic in the 1990s, people of mixed ethnic background had a higher incidence of TB than any other ethnic group. Particularly in the Western Cape incidence among this population has been rising since the mid-1970s (Department of Health and Welfare 1984). In 1986, the calculated annual case load among 'mixed ethnic' people of the Western Cape was 68% of the total case load in the region (Collie & Küstner 1989). Several explanations for this have been in discussion, stating alcohol and smoking as risk factors for disease and overcrowding as a risk factor for infection (Comstock 1982, Friedman et al. 1987, Feingold 1976, Brown & Campbell 1961). The mixed ethnic population has the highest risk for dual substance use in South Africa, as several studies confirm (Peltzer et al. 2008, Peltzer 2008), however living conditions do not significantly differ from those of the 'black' population. The aspect of patient compliance to treatment has also been taken under consideration, however a broad-based study by Bell & Yach 1984 on patient compliance to supervised short-course therapy across the entire Western Cape showed that 'black' patients had significantly worse compliance (73%) than patients of 'mixed ethnic background (86%), (Bell & Yach 1984).

Our study confirms an increased smoking habit among patients of mixed ethnic background, however any other conclusions with regards to ethnic differences in TB incidence should be subject to further investigation.

The much larger percentage of male pulmonary tuberculosis patients in our 'old' and 'new' study cohort is not surprising as it was demonstrated in previous studies that, in almost every TB affected population, more male than female cases of tuberculosis are notified each year (World Health Organization 2000). Globally there are 70% more male than female smear-positive TB notifications (Diwan & Thorson 1999). The consistency of the excess of TB notifications among males suggests that they represent epidemiological patterns of disease (World Health Organization 2000), however, the reasons for gender differences in notification rates is unclear as stated by Diwan & Thorson 1999. Research has not yet determined whether the higher rate of TB notifications among males

reflects a true higher incidence of disease among men, the under-detection or under-reporting of cases among women, or both (Uplekar et al. 2001).

5.3 Impact of BMI on the manifestation of pulmonary tuberculosis

A study on the impact of malnutrition on tuberculosis by van Lettow et al. 2003 found a lower BMI in TB diseased males in comparison to TB diseased females and in keeping with outcomes from our evaluations, allows speculation that the often higher BMI of females could be the distinguishing factor of tuberculosis manifestation between genders.

In addition, our results showed a strong negative correlation between BMI and the radiological scores ‘extent of disease’ and ‘cavitation’ as well as sputum smear positivity (applicable for the ‘old’ population). Our heavier ‘new’ patient population therefore appeared to be less ‘sick’, which may be an indicator that BMI acts as a preventive factor on TB progression and disease severity.

Van Lettow et al. 2004 demonstrated that the extent of pulmonary disease, as assessed by chest radiographs, is associated with the severity of malnutrition and similar observations were made by Thorson et al. 2007. The study analyzed chest X-rays of men and women with smear-positive pulmonary TB and concluded that radiological findings were more advanced in men than women, despite similar disease onset and time to diagnosis (Thorson et al. 2007).

To confirm a correlation of low BMI and malnutrition with radiological severity in TB, Deniz et al. 2007 established proof that serum total cholesterol, HDL-C and LDL-C concentrations correlate with the radiological ‘extent of disease’ in patients with pulmonary tuberculosis. The study suggests that serum TC, HDL-C and LDL-C concentrations are generally lower in patients with pulmonary tuberculosis than those in healthy controls (Deniz et al. 2007). TB is known to be a wasting disease and as Schwenk & Macallan 2000 explain, the link between malnutrition and TB is ‘’bi-directional’’; tuberculosis results in secondary wasting whereas primary malnutrition is a risk factor for tuberculosis.

A more strongly pronounced manifestation of this ‘’bidirectional’’ effect among the ‘old’ patient population is likely to have been the cause of their lower BMIs and consequently a more severe appearance of tuberculosis, as outlined below.

‘Old’ patient participated in EBA studies from 1991 onwards and according to the Cape Town TB Progress report 1997-2003 case detection rates for new smear-positive TB had been drastically increased from 1997 onwards (Provincial Administration of the Cape Metropole Region and City of Cape Town 2003).

Before 1996, delayed and missed diagnosis of pulmonary TB happened to a significantly larger extent which was often associated with more extensive disease, a poorer clinical condition and worse prognosis. Delayed diagnosis and treatment of TB affects individuals by increasing morbidity and mortality, and society overall by increasing transmission of disease (Rodger et al. 2003, Greenaway et al. 2002, Pablos-Méndez et al. 1996, Zahar et al. 2001, Chin et al. 2000, Golub et al. 2006). Based on the time period during which the ‘old’ patients were recruited for EBA studies it can be assumed that, in them, diagnosis of pulmonary tuberculosis was more delayed and had happened at a later stage of the infection than for the ‘new’ patients. Therefore, secondary wasting may have had already progressed further in the ‘old’ patient cohort at admission to EBA studies than in ‘new’ patients.

In a recent study Virenfeldt et al. 2014 found a clear association between treatment delay and clinical severity at inclusion. The study showed that the proportion of severely ill was higher among those with a long delay; in the long diagnostic delay groups a third of patients were severely ill compared with a fifth in the short delay group. This may translate into mortality risk; patients with a TB score classified as severe have a 60% higher mortality risk as previously shown by Wejse et al. 2008, a six fold higher risk of treatment failure and an increase in infection transmission (Rudolf et al. 2013). Furthermore, a higher risk of hospitalization for patients with a longer treatment delay has also been previously described by Lawn et al. 1998.

Severe malnutrition has been described as an effect of tuberculosis in various different international settings, i.e. England, India and Japan (Onwubalili 1988, Tsukaguchi et al. 1991) and US studies showed weight loss as a presenting symptom in 45% of TB patients of whom 26% suffered from persistent anorexia (Miller et al. 2000). Decreased appetite and weight loss are common symptoms in chronic disease and have a negative impact on quality of life and mortality. As Braun & Marks 2010 showed, weight loss in chronic disease is linked to fat and lean mass, which is then referred to as cachexia. Alterations in appetite and body weight loss occur in a wide variety of chronic diseases (Braun & Marks 2010), including tuberculosis.

Karyadi et al. 2000 demonstrated that the nutritional status of patients with active pulmonary TB was poor compared with healthy subjects. The poorer nutritional status may be due to anorexia based lack of nutrient absorption or catabolism. As Macallan et al. 1998 described, the body's response to chronic inflammation, i.e. metabolic changes such as increased energy expenditure, are considered deregulatory and contribute to the negative patient condition, rather than resulting in improvement.

Changes in protein metabolism appear to be counter-regulatory in inflammatory states, as first described by Cuthbertson 1930 and then further explained by Desborough 2000 on the example of surgery and trauma. Generally, patients lose weight and protein-energy wasting is diagnosed if three characteristics are present (low serum levels of albumin, transthyretin or cholesterol), reduced body mass (low or reduced body or fat mass or weight loss with reduced intake of protein and energy and reduced muscle mass (Fouque, et al. 2008).

Secondly, the aspect of primary malnutrition as a risk factor for TB must be taken into consideration. Low body mass has been shown to be a distinct risk factor for tuberculosis and a greater Body Mass Index has been shown to be protective by several studies (Lönnroth et al. 2010). This may contribute to our findings that the 'old' study cohort and particularly its subcategory of males from mixed ethnic background were most severely malnourished and therefore developed a more severe manifestation of pulmonary tuberculosis than heavier, better nourished individuals of the 'new' patient cohort.

A 1995 survey by the Department of National Health indicated that children of mixed ethnic background in the Northern, Eastern, Western Cape and Free State had the poorest nutritional status (Health Systems Trust 1995), whereby 'black' children showed moderate levels of underweight. The susceptibility of mixed ethnic background children to stunting and underweight shown in this study was further supported by numerous studies of low birth weight. Key determinants of low birth weight among this group included both nutritional factors (of mother and/or child) and the smoking status of pregnant women (Health Systems Trust 1995). According to the same study, between 40 and 50% of pregnant mixed ethnic background women were smokers at the time.

Malnutrition is associated with an increased risk of progression from TB infection to active disease, because of the negative impact of micro-and macronutrient deficiencies on the cell-mediated immune system (Cegielski & McMurray 2004, Academy of Science of South Africa 2007). Experimental studies also found that hyperglycemia may affect a patient's immune response to TB (Rayfield et al. 1982, Stalenhoef et al. 2008). BMI <18.5 kg/m2 is a possible indicator for under-nutrition (British Association for Parenteral and Enteral Nutrition Malnutrition Advisory Group 2000, Cegielski et al. 2012) which can reduce the immune response of a patient either by interfering with macrophage - T-lymphocyte interaction and cytokine release (Rook & Hernamndez-Pando 1996) or by secondary immune deficiency that increases susceptibility to infection (Chan et al. 1997). Low BMI has also been shown to be an independent and strong predictor of mortality in HIV-positive adults, even for those participating in HAART (Macallan et al. 1998, Cegielski, & McMurray 2004).

Conversely, the determining factor of a 'new' cohort with higher BMI and lower disease severity and an 'old cohort' with lower BMI and higher disease severity may not be secondary wasting or malnutrition but the difference in obesity rates. There has been a large increase in obesity in South Africa in recent years, which is mainly linked to changes in diet that move away from traditional towards westernized products with more animal fats and carbohydrates. In addition, South Africans' alcohol consumption is amongst the highest in the

world (Self Medication Association South Africa 2013). In 2010 a survey conducted by the pharmaceutical company GlaxoSmithKline found that 61% of the South African population was overweight, obese or morbidly obese (Smith 2010). A 2013 investigation of the SA Medical Research Council stated that 70% of all South African women over the age of 35 were overweight or obese with 33% of 'black' women exposed to the greatest risk, closely followed by a quarter of mixed ethnic background, white and Indian women. In contrast, 18% of white men over the age of 35 are obese, followed by 9% percent of Indian, 8% percent of mixed ethnic background, and 6% percent of 'black' men (Ogunbanjo 2013).

Overweight and obese BMIs have been shown to be protective against mortality and disease progression in HIV-infected individuals (Schwenk et al. 2004, Schwenk & Macallan 2000, Shuter et al. 2001) and a recently conducted study found a 43% and 17% prevalence of overweight and obesity in HIV-infected individuals in the KwaZulu-Natal Province of South Africa (Bärnighausen, et al. 2008).

Leung et al. 2007 examined a large cohort study involving elderly health-center patients in Hong Kong and found that obese and overweight BMI patients are at a lower risk of TB, as compared to those with normal and underweight BMI. This finding confirmed studies conducted by Tverdal 1986 in Norway showing a decreased risk of TB with increasing BMI and by Edwards et al. 1971, who found a reduced progression to active TB disease among overweight US naval recruits compared to those who were underweight. In a recent Indian study by Pednekar et al. 2008, overweight men and women in Mumbai were shown to be at decreased risk for death from tuberculosis.

The controversial theory of Roth 2009 on the evolutionary advantage that obesity might have had in the past regarding infectious diseases further confirms this point. Roth 2009 stated the opinion that ''fat is a part of the innate immune system. Visceral fat, which is stored in the abdomen, tends to cause more inflammation than subcutaneous fat, which is stored closer to the skin, on the arms and legs. It is possible that survival during the tuberculosis era favored those who stored excess visceral fat''. Ferrante 2007 described that visceral

adipose tissue contains more macrophages which produce TNF and that subcutaneous adipose tissue produces more leptin. A release of leptin and other pro-inflammatory cytokines was described by van Crevel et al. 2002. Other studies suggest that loss of body fat mass may lead to a decrease in plasma leptin concentration, which may be further suppressed by chronic inflammation in TB (Otero et al. 2006). This is supported by recent finding that outline how leptin participates in immune homeostasis and inflammatory processes as a modulator of T-cell activity, but is also involved in autoimmune diseases such as autoimmune encephalomyelitis, diabetes type 1, bowel inflammation, osteoarthritis and rheumatoid arthritis (van Crevel et al. 2002). An advantage in immune response based on increased weight and BMI and resulting less severe clinical and radiological manifestation of tuberculosis may therefore be taken into consideration for the ‘old’ patient cohort of our study.

5.4 Impact of HIV infection on the manifestation of pulmonary tuberculosis

5.4.1 HIV infection and radiology

When dividing the ‘old’ and ‘new’ patient population of our study into HIV-positive and -negative subcategories, the ‘old’ HIV-positive patients showed the most severe radiological score. In both cohorts it was the HIV-positive subcategory that showed more severe radiology than HIV-negative patients.

According to previous studies on HIV-positive patients with pulmonary tuberculosis the clinical pattern of disease and histological characteristics of the lesions correlate with the patient’s immune status (de Cock et al. 1992). In early HIV infection, patients present characteristics of post-primary tuberculosis and often have typical symptoms such as extensive lung destruction, cavitation and upper lobe involvement. Sputum smears at this stage are AFB positive and classic tuberculous lesions are found in histology assessments. With more advanced HIV infection and declining CD4 counts patients usually present with primary pulmonary tuberculosis like atypical pulmonary disease (de Cock et al. 1992, Richter et al. 1994).

According to Elliott et al.1990 and Harries et al. 1990 productive cough and hemoptysis are less frequent and chest radiographs often show pulmonary infiltrates without cavities, lower lobe involvement, intrathoracic lymphadenopathy and sometimes a radiologically normal appearance. The radiographic presentation is often related to the patient's CD4 count. A study by Keiper et al.1995 in Canada found that the mean CD4 count in HIV-positive pulmonary tuberculosis patients was 323 cells/mm^3 when the chest radiograph was 'typical' and 69/mm^3 cells when it was 'atypical' (de Cock et al. 1992). Similar findings have been reported from Ivory Coast and South Africa (Abouya et al. 1995, Post et al. 1995).

Since CD4 counts of our 'old' and 'new' HIV-positive patients were both >300 cells/mm^3 (EBA inclusion criterion), it can be assumed that 'old' and 'new' HIV patients behaved in accordance with the findings of Keiper et al.1995 and de Cock 1992 and showed 'typical' tuberculosis lesions and chest radiographs of a post-primary tuberculosis. The greater radiological severity of 'old' HIV-positive patients compared to 'new' HIV-positive patients may be related to additional critical factors, such as variances in individual CD4 counts in the range above the 300 cells/ mm^3 limit and/or the significantly lower BMIs which the 'old' HIV-positive patients presented. The larger extent of radiological manifestation in HIV-positive patients over HIV-negative patients should be subject to further investigation.

It must be noted that HIV-positive patients in the 'old' population were strongly underrepresented in terms of numbers and in neither of the two patient cohorts a correlation between HIV and radiological parameters was found. This is likely to have influenced the outcome of our analysis.

5.4.2 HIV infection and sputum smear grade

An important observation of our study is the slightly higher sputum smear grading amongst HIV-positive patients in both, 'old' and 'new' cohorts in comparison to the respective HIV-negative subcategories. In order to participate in EBA studies all patients, independent from their HIV status, had to be at least

‘scanty’ positive for acid-fast bacilli on direct smear examination and present CD4 counts >300 cells/mm^3 (EBA inclusion criterion). Therefore a preselection had been applied to our study population which is likely to have influenced our results. De Cock et al. 1992 concluded that in HIV-positive patients the extent of radiological manifestation and sputum smear positivity correlate with a patient’s immune status. In early HIV infection with an intact immune system, patients show ‘typical’ chest radiographs and positive sputum smears whereas during advanced HIV infection with reduced CD4 counts, sputum smears are often negative and radiographs ‘atypical’ (Colebunders et al. 1989, De Cock et al.1992, Elliott et al. 1990, Richter et al. 1992). Based on the applicable EBA inclusion criteria we can assume that our patients presented TB ‘typical’ radiological lung destruction which hence resulted in positive sputum smears. Our patient population is therefore in line with findings by Klein et al. 1989, that ‘’features of HIV-infected individuals with close to normal CD4 counts are not distinguishable from those of HIV-negative individuals’’. This also confirms findings by Mugusi et al. 2006, showing that the amount of bacillary density in the sputum of patients diagnosed with AFB-positive pulmonary tuberculosis is significantly affected by the patient’s HIV status and the degree of immune suppression. A positive correlation between CD4 count and bacillary density was shown whereby bacillary density increased with increase in CD4 counts. A CD4 cell count > 500 cells/ mm^3 was found to correlate with a high bacillary density compared with <200 cells/mm^3 and a rise in CD4 count was linked to an increasing probability of high bacillary density. Harries et al. 1990 outlined that sputum smears tend to be negative more in patients with advanced HIV infection because of limited presence of tubercle bacilli in sputum based on reduced pulmonary inflammation fewer cavities at that stage.

Especially in cases of low CD4 count, clinical, radiological and histological signs of tuberculosis infection can be transformed by HIV co-infection. In such cases HIV-positive individuals show negative sputum smears and present ‘atypical’ chest radiology and extrapulmonary infection sites (Harries et al. 1998, Jones et al. 1997, Klein et al. 1989, Mugusi et al. 2006). Studies showed that the proportion of sputum smear-negative patients can then be greater among HIV –

positive patients than among HIV-negatives. A study conducted in Zambia by Elliott et al. 1993 which included over 100 patients found that 24% of HIV-negative individuals had a negative sputum smear, compared with 43% of those who were HIV-positive. Necrosis, frequent absence of granulomata, and large numbers of acid-fast bacilli inside macrophages can be observed histologically (Harries et al. 1990) and lower AFB positivity rates in bronchoalveolar lavage of HIV-positive patients can be found (Kivihya-Ndugga et al. 2004, Harries et al 1998, Raviglione et al. 1992).

The finding of HIV-positive patients having more positive sputum smears than HIV-negative patients seems to relate to the previously described, more severe radiological manifestation of pulmonary TB in these patients. However this should be subject to further investigation. It must be noted that in neither of the two patient cohorts a correlation between HIV and smear grading was found.

5.4.3 HIV infection and nutritional status

Several studies on the nutritional status of HIV-infected adults with tuberculosis have focused on the assessment of body weight and serum albumin concentrations (Scalcini et al. 1991) and showed that albumin levels are lower in HIV-co-infected patients than singularly TB infected patients and that low albumin and weight can be predictors of mortality in HIV-co-infection (Tabarsi et al. 2012). A recent study from Burundi on patients with pulmonary, extrapulmonary and disseminated TB showed that the HIV-positive patients had significantly lower weight, BMI and fat-free mass compared with HIV-negative patients (Niyongabo et al. 1999). Moreover, an epidemiological study in Uganda by Whalen et al. 2000 suggested that low BMI in TB/HIV-co-infected patients can act as a marker for advanced HIV disease, comparable to low CD4 counts and Maro et al. 2010 described that low BMI and falling BMI predicted HIV-associated TB in a study population in Tanzania.

In a study by Shah et al. 2001, the nutritional status of 261 HIV-positive adults was compared to that of 278 HIV-negative adults with pulmonary tuberculosis. Among 163 HIV-positive and 199 HIV-negative male and 98 HIV-positive and

79 HIV-negative female patients, Shah et al. 2001 found no significant differences in BMI, body cell mass, fat mass or fat-free mass between HIV-positive and HIV-negative adults. Shah et al. 2001 outlined that among their HIV-positive patients, ‘’BMI, body cell mass, fat mass and phase angle were significantly lower in patients with CD4 lymphocytes ≤200 cells/mm^3 compared with those who had > 200 cells/ mm^3,’’. These findings raise the possibility that low CD4 counts under HIV positivity have stronger impact on BMI.

CD4 counts of our ‘old’ and ‘new’ patient cohorts were > 300 cells/mm^3 (EBA inclusion criterion), however data suggested a strong correlation between HIV status and weight in the ‘old’ population with HIV-positive patients being of significantly lower weight. Among the ‘new’ population HIV-positive patients were heavier than HIV-negative patients and no correlation between the two parameters was found. This and the fact that HIV-positives of the ‘new’ cohort were on average 7 kg heavier than their equivalents of the ‘old’ cohort could confirm our theory that the ‘old’ patient population was primarily malnourished at the point of infection or TB diagnosis was much delayed and weight had already been compromised. However it could also lead to the assumption that patients with pulmonary TB and HIV/Aids co-infection of the ‘new’ cohort benefitted from their initially heavier body weight and increased BMI and therefore presented less severe signs of tuberculosis.

It has been shown that a cholesterol-rich diet might increase the sterilization rate of sputum cultures in pulmonary TB patients, indicating that cholesterol could be an additional part of anti-tuberculosis treatment schemes (Pérez-Guzmán et al. 2005). Furthermore, low total cholesterol concentrations might limit lymphocyte and macrophage activity which facilitates progression of pulmonary tuberculosis and additional cholesterol might enhance cellular immunity (Pérez-Guzmán 2005, Cooper 1977, Drabowsky et al. 1980, Heiniger & Marshall, Gatfield & Pieters 1982). The decrease in serum high-density lipoprotein cholesterol (HDL-C) concentrations was shown during infection and inflammation in several studies (Sammalkorpi et al. 1988, Cabana et al. 1989, Feingold et al. 1993, Deniz et al. 2006).

5.5 Impact of smoking on the manifestation of pulmonary tuberculosis

In our findings, smoking was a predominantly male habit. Smoking correlated with patients' weight and particularly among the 'new' patients, the non-smokers were found to be significantly heavier than the smokers ($p<0.01$). Smoking and BMI correlated within the 'new' patient population. Rradiological scores, such as 'extent of disease' and 'cavity wall thickness' as well as sputum smear grading were more severe in the 'new' smokers. Despite the fact that these parameters showed no correlation with smoking, these findings confirmed results of previous studies on the relationship between smoking and manifestation of pulmonary tuberculosis. Trifunovic et al. 2009 analyzed the impact of smoking on tuberculosis patients and found significantly more males in the smoking group and more females in the non-smoking group. Cavitary pulmonary lesions were more frequently found in the smokers than the non-smokers, positive sputum smears were more frequent in the smokers and BMI was lower in the smokers than the non- smokers (Trifunovic et al. 2009). Our observations also partially confirm the findings of Chiang et al. 2007 that smokers are more likely to present radiological involvement of the upper lungs, cavity lesions and positive sputum cultures. Racil et al. 2010 analyze the seriousness of the radiological lesions by rating them according to severity and extent and found that smoking was associated with much more extensive and severe radiological TB lesions and increased risk of morbidity and mortality in TB patients. Although the smokers in both of our study cohorts did not show as significantly increase radiological severity of TB compared to the non-smokers as Racil et al. 2010 described for their study, we can however confirm a trend towards a more distinct radiological manifestation of TB in our smoking population. Patra et al. 2014 conducted a large meta-analysis in 14 high tuberculosis burden countries which demonstrated that smoking, heavy alcohol use, diabetes, and BMI <18.5 kg/m^2 represent individual risk factors but in combination can significantly increase the risk of TB development. According to findings of Patra et al. 2014, drinking and smoking were closely correlated in men; and among women, such habits were less common.

It can therefore be assumed that because the added number of risk factors in the male mixed ethnic background population of our ‘old’ patient population such as malnutrition, low BMI as well as the smoking habit was highest, it may have significantly contributed to a more sever manifestation of tuberculosis in the ‘old’ cohort than in any other sub-population of the study. The fact that the ‘new’ patient population showed a larger percentage of smokers but a less severe manifestation of TB might be due to an initially higher BMI of patients and a reduced impact of additional risk factors on the disease status in general.

5.6 Study strengths and limitations

A clear strength of this study is its large amount of pulmonary tuberculosis patient data from one of the regions with the highest TB prevalence worldwide. Patient data have a high level of detail and include clinical patient descriptions (i.e. age, gender, weight, ethnic background, HIV/AIDS status and extensive general health status), radiological data with structured evaluations of chest X-rays, sputum microbiology and detailed hematological data (the latter not discussed).

Although study staff was different in the later years, patient parameters were measured and collected in a highly standardized manner. This includes the taking and scoring of chest X-rays, which were scored by a standardized score sheet across the various studies that fall into the ‘old EBA cohort’ and continued by us for the ‘new’ patient cohort.

The EBA studies from which patient data were retrieved encompass a significant time period from early1990’s until 2008, studies were all performed within the same area of the Western Cape of South Africa and analyses were based on standardized clinical and laboratory methods.

Our study had several limitations. Most significant limitations stem from the study’s retrospective observational design with its inherent biases.

Patients in our population were recruited for an early phase clinical trial with strict in- and exclusion criteria. Results may therefore not be representative for

the entire population of the Western Cape of South Africa but only a select subgroup of Cape Town's urban and direct suburbia population.

There was an inconsistency of data availability. Not all parameters were available for all patients, which may affect the results of the statistics analysis as some statistical results were calculated from only very few patient, furthermore the sizes of the comparative 'old' and 'new' patient cohorts were often not the same.

The patient recruiting network, i.e. the clinics which collaborate with Task Applied Science and therefore the areas and communities might have changed from which patients were recruited. In the areas around Cape Town this could have had an impact on the ethnic background of patients which participated in studies, depending on the suburbs from which patients were predominantly recruited.

5.7 Conclusion

In conclusion, this retrospective study gives a detailed overview of how the manifestation of pulmonary tuberculosis in patients on early phase clinical trials has changed in the Western Cape of South Africa in the time period from 1991 to 2008. The key observation across this population is that lower BMI is associated with male gender, mixed ethnic background, greater disease severity such as higher smear grading, radiologically higher ‘extent of disease’, more ‘cavitation’ and a stronger smoking habit.

It should be further investigated whether more body fat and a higher BMI result in less severe disease and ultimately better response to treatment, also taking gender differences in BMI into consideration. The consideration of a higher female BMI as critical factor, may help to answer why, in several drug trial studies, women have had better responses to tuberculosis treatment than men. Furthermore, the known phenomenon of age and gender related differences in TB infection, with predominance of females up to the age of 30 and predominance of males in older age groups may also be explained with the basic physiological aspects of BMI.

Future studies should investigate, to what extend better nutrition and ‘weight gain’ may be of benefit in the treatment of pulmonary tuberculosis and the prevention of TB recurrence in tuberculosis and tuberculosis/HIV co-infected patients.

6 Summary

Background: For decades South Africa has been a TB high-burden country and recent WHO figures show that today it is still among the six nations with highest TB incidence (World Health Organization 2014). We surveyed how the manifestation of pulmonary tuberculosis in early phase clinical trial patients has changed in the Western Cape of South Africa between 1991 and 2008 and compared two independent pulmonary TB patient cohorts of the time periods 1991-2001 (’old patients’) and 2002-2008 (‘new patients’). **Results**: The ethnic composition of the ‘new’ cohort had changed significantly in favor of ‘black’ individuals, despite a majority of ‘mixed ethnicity’ patients in both cohorts. BMIs in ‘old’ patients were significantly lower, with lower body weight and same mean height as ‘new’ patients. BMIs were lowest in ‘old’ cohort males of mixed ethnic background and associated with increased TB severity; higher sputum smear grades, radiologically more severe ‘extent of disease’, more lung ‘cavitation’ and thicker cavity walls. In both cohorts BMIs of female patients were above male BMIs and BMIs of ‘black’ patients were significantly higher than of mixed ethnic background patients. Smoking, predominant in mixed ethnic background males, was associated with lower BMIs in both cohorts. The ‘new’ cohort was found to have a larger proportion of HIV-positive patients with a majority of affected males, the ‘old’ cohort showed a larger female share of HIV-positive patients. BMIs of HIV-positive patients in the ‘new’ cohort were significantly higher than BMIs of HIV-positive ‘old’ patients. **Conclusion:** Future studies should investigate, to what extend better nutrition and ‘weight gain’ may be of benefit in the treatment of pulmonary tuberculosis and the prevention of TB recurrence in tuberculosis and tuberculosis/HIV co-infected patients.

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This paper is dedicated to my love Edoardo M. Vitali.

9 Curriculum Vitae

10 Appendix

10.1 Tuberculosis chest X-ray evaluation forms

Date of radiograph: _____

Scorer: _____

Pa: ☐ Lat: ☐

Extent of disease:

I	Minimal or trivial-on radiological grounds not active	☐
II	Minimal or slightly more extensive –on radiological grounds active	☐
III	Limited- more than above but involving an area less or equal to R upper lobe	☐
IV	Moderate- more than above but involving an area less or equal to one lung	☐
V	Extensive- involving a total of more than one lung, healthy lung tissue visible	☐
VI	Massive- extensive bilateral disease, no healthy lung tissue visible	☐

Cavitation:

☐	i.	None	
☐	i.	Single cavity	A. 2cm or smaller ☐ B. Between 2-4cm ☐ C. Greater than 4cm ☐
☐	iii.	Multiple cavities	A. Largest 2cm or smaller ☐ B. Largest between 2-4cm ☐ C. Largest greater 4cm ☐

Cavities Wall thickness (measured at thickest part of wall):

1. Hairline thickness ☐
2. Less than 0.5cm ☐
3. More than 0.5cm ☐

Number of lobes involved:

1 ☐ 2 ☐ 3 ☐ 4 ☐ 5 ☐

Lung quadrants involved:

Upper Left

	0
	<= 50%
	>50%

Upper Right

	0
	<= 50%
	>50%

Lower Left

	0
	<=50%
	>50%

Lower Right

	0
	<=50%
	>50%

11 Affirmation in lieu of oath (Eidesstattliche Versicherung)

Ich versichere ausdrücklich, dass ich die Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die aus den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen einzeln nach Ausgabe (Auflage und Jahr des Erscheinens), Band und Seite des benutzten Werkes kenntlich gemacht habe.

Ferner versichere ich, dass ich die Dissertation bisher nicht einem Fachvertreter an einer anderen Hochschule zur Überprüfung vorgelegt oder mich anderweitig um Zulassung zur Promotion beworben habe.

Ich erkläre mich einverstanden, dass meine Dissertation vom Dekanat der Medizinischen Fakultät mit einer gängigen Software zur Erkennung von Plagiaten überprüft werden kann.

Unterschrift: